Evaluation of the relative importance of *Ascochyta pisi* in the Ascochyta blight complex of pea in Saskatchewan

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By

Nimllash Thangam Sivachandra Kumar

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ABSTRACT

Ascochyta blight may be caused by the fungal pathogens *Peyronellaea pinodes*, *Ascochyta pisi*, *Phoma medicaginis* var. *pinodella*, and *Phoma koolunga*. While *P. pinodes* has been the most important pathogen in Saskatchewan, *A. pisi* has become more prevalent in southern and southern-western parts of the province. Conidial germination of *A. pisi* and *P. pinodes* on glass slides and infection of plants of pea cultivar CDC Cooper by both pathogens were evaluated under controlled conditions at temperatures ranging from 10 to 30°C and wetness periods of 0 to 12 h to determine whether they had different optimal environmental requirements. For both pathogens, conidial germination and disease severity increased with increasing temperature and leaf wetness period up to the optimum of 20 to 25°C. Overall, *P. pinodes* had consistently higher germination and disease severity compared to *A. pisi*. For conidial germination, these differences became obvious starting at 20°C after more than 4 h incubation, and at 25 and 30°C *P. pinodes* had consistently higher germination after 2 h of incubation. Similarly, disease severity caused by *P. pinodes* was consistently higher at 20 and 25°C compared to that caused by *A. pisi*. The role of infected seed in the epidemiology of *A. pisi* in pea was studied with naturally infected seeds under field conditions in 2012, 2013 and 2014 at two locations where low natural inoculum were expected. Results revealed a significant effect of *A. pisi* seed infection on emergence of seedlings ($P < 0.05$). Seed infection levels of 10 and 14.5% resulted in reduced emergence compared to 0.5% seed infection, but the level of seed infection at planting had no impact on *A. pisi* disease severity, seed infection levels of harvested seed or seed yield. No visible symptoms caused by *A. pisi* appeared on the aerial parts of the seedlings. Results suggest that disease did not progress from seeds, or contributed to infection of aerial parts of the plants, hence infected seeds cannot be regarded as a source of inoculum in the epidemiology of this pathogen. Assessing seed components of seeds with varying levels of *A. pisi* infection and seed staining revealed that the pathogen was present in all components of the seed, regardless of the severity of seed staining. Field studies were conducted to assess yield loss caused by *A. pisi* between 2012 and 2014 at Swift Current and Stewart Valley where a high incidence of *A. pisi* had been reported in the past, and under irrigation with inoculation at Saskatoon (2014). Two (2012, 2013) or three (2014) fungicides (pyraclostrobin, chlorothalonil, or pyraclostrobin and boscalid) were applied to create plots with low levels of *A. pisi* infection

that were compared with a non-sprayed treatment. The more susceptible pea cultivars Cooper and SW Midas were compared with the more resistant cultivars CDC Bronco and CDC Golden. Disease pressure was low in all three years and no differences in yield between fungicide treated and non-sprayed treatments were observed. Nevertheless, the incidence of *A. pisi* isolated from harvested seeds of the fungicide treatments was lower than that of the non-sprayed treatment, despite similar *A. pisi* severity on plants before harvest.

The response of the recombinant inbred line (RIL) pea population PR-10 developed from a cross between the susceptible variety Cooper and the partially resistant variety CDC Bronco to *A. pisi* infection was evaluated in 2012, 2013 and 2014 under field conditions. RILs were inoculated with *A. pisi* in 2014 at Saskatoon under irrigation. Under low disease pressure in all three years, no difference in *A. pisi* severity was observed between the parents and among RILs. Yields differed among RILs and were attributed to genetic differences. Considering that the parents did not differ in resistance to *A. pisi* it was concluded that this population might not be suitable for the study of genetic control of resistance to *A. pisi*.

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1.0 Introduction

Field pea (*Pisum sativum*, 2n = 14) is a dicotyledonous, self-pollinated annual cool season crop, belonging to the family Fabaceae that is cultivated throughout the world. Canada is the leading producer and exporter of field pea (Government of Canada, 2016). Field pea seeds are a good source of complex carbohydrates, minerals, vitamins, and proteins and have some medicinal properties. This crop is usually used as a rotational crop, as its roots have the ability to fix atmospheric nitrogen through a symbiotic relationship with rhizobial bacteria. Pea is sensitive to flooding during the seedling stage. Pea uses less water and can tolerate drought stress well, whereas excess moisture leads to disease development and death of the plant.

Ascochyta blight is one of the major diseases affecting field pea production. Ascochyta blight of field pea can be caused by four pathogens with anamorphs in the genus *Ascochyta*, which are collectively known as the Ascochyta blight complex. The pathogens causing Ascochyta blight are *Peyronellaea pinodes*, *Ascochyta pisi*, *Phoma medicaginis* var. *pinodella*, and *Phoma koolunga*. Ascochyta blight is a major disease of field pea in western Canada that has caused 25-30% yield loss (Wallen, 1965). *Peyronellaea pinodes* is considered to cause most economic damage worldwide (Basu et al., 1973; Beasse et al., 1999; Bretag and Ward, 2001; Lawyer, 1984), and can cause yield loss of 50-75 % when the conditions are favorable (Wallen, 1974; Xue et al., 1997).

Ascochyta pisi is a seed-borne pathogen that can cause leaf and stem blight and pre-emergence damping off in pea (Smith et al, 1988). In the early 1950's *A. pisi* was the most commonly isolated pathogen from pea seeds originating from different parts of Canada (Skolko et al., 1954). After 1967, once resistance to *A. pisi* had been incorporated into pea cultivars, *P. pinodes* developed into the most destructive pathogen in pea growing areas of Canada (Wallen et al., 1967a). Seed testing results and field observations showed that in the last 10 years *A. pisi* has been more commonly seen in the southern and south-western parts of Saskatchewan than *P.*

pinodes (Morrall et al., 2011; Warkentin et al., 2012), which may have reduced the quality of seed produced by growers.

The Ascochyta blight pathogens are usually transmitted through soil, air and infected seeds. The pathogens can survive on infested stubbles for long periods of time (Carter and Moller, 1961; Ali et al., 1982). When compared to *A. pisi, P. pinodes* and *Ph. medicaginis* var. *pinodella* are more destructive. *Peyronellaea pinodes* and *Ph. medicaginis* var. *pinodella* can infect the emerging seedlings and the grown plants at the lower and higher nodes increasing the problem of lodging that can cause severe yield loss (Snoad, 1985). Ascospores of *P. pinodes* are transmitted through air, making it difficult to control the infection. Sometimes the symptoms produced by *Ph. medicaginis* var. *pinodella* are not easily distinguishable from *P. pinodes*, but *Ph. medicaginis* var. *pinodella* tends to cause less infection than *P. pinodes*.

A variety with complete resistance to *P. pinodes* has not been found, and most pea cultivars are either partially resistant or fully susceptible (Ali et al., 1978; Kraft et al., 1998a, Xue and Warkentin, 2001). Management of the disease can be achieved by spraying chemical fungicides, but this is often not economically viable (Davidson and Kimber, 2007). A study conducted by the Danish Research Centre for Organic Farming in 2005 showed that intercropping of pea with barley resulted in a 40% reduction in seed infection (Wolffhechel and Bodker, 2005**)**. Burying of infected stubble through tillage resulted in a decline of survival of the pathogen in the field (Sheridan, 1973). Long-term crop rotation of 3 - 5 years has also proven to control the blight caused by *P. pinodes* (Bailey et al, 1992). The severity of disease is promoted by wet and warm conditions (Warkentin et al., 1996; Xue et al., 1996). Late sowing practices carried out in some parts of Australia reduced infection by *P. pinodes*. Davidson and Kimber (2007) showed that sowing of peas after the autumn rain showed reduced infection of plants with *P. pinodes*. However, the success of these practices can only be achieved by fully understanding the epidemiology of the pathogens and their interaction with environmental factors.

As *A. pisi* appears to be more commonly found than *P. pinodes* on harvested seeds from pea fields in parts of Saskatchewan, research is warranted to determine the reasons for this re-emergence. The effect of this fungus on pea plants, as they develop, and its epidemiology has not been studied under field conditions in Saskatchewan. There is a lack of data to determine whether a more conducive climate in the southern and southwestern parts of Saskatchewan is

causing the predominance of *A. pisi* in this region. Research is also needed to determine whether this disease is of economic importance in Saskatchewan.

1.1 Research hypotheses

1. *Ascochyta pisi* and *P. pinodes* have different optimum moisture and temperature requirements for conidial germination and infection.
2. *Ascochyta pisi* seed infection plays a major role in disease development in pea.
3. *Ascochyta pisi* infection will result in significant yield loss in pea.
4. Resistance to *A. pisi* in a Recombinant Inbred Line (RIL) population is conferred by a single gene.

1.2 Objectives

1. To determine and compare temperature and moisture optima of *A. pisi* and *P. pinodes*;
2. To assess the role of seed infection with *A. pisi* on disease development in field pea through a seed component study and through field experiments;
3. To assess yield loss caused by *A. pisi* under field conditions;
4. To determine the genetic control of resistance to *A. pisi* in the field using a RIL population.

2.0 Literature Review

2.1 Economic importance of pea

Humans have known field pea production for more than 10,000 years. Pea is said to have originated in the southwestern part of Asia (Makasheva, 1983). French settlers introduced pea to Canada in 1535 (Slinkard 1994; Ali et al., 1995). Field cultivation of pea first occurred in Ontario and Manitoba at a large scale, and it became the most popular crop in eastern Canada in 1902. Pea cultivation in Canada is now mostly restricted to the Prairie Provinces. Pea production in Canada began to increase in 1985 due to the opening of the European market to pea (Slinkard, 1994). Lower prices for cereal grains also promoted pea as a field crop (Slinkard, 1994; Ali et al., 1995). Saskatchewan became the major producer of pea in 1986, followed by Alberta and Manitoba.

In 2010, Canada produced 32% of world pea production (Pulse Canada, 2016). Pea production in Canada had gradually increased to 3.8 million tonnes by 2014, but the production decreased to 3.2 million tonnes by 2015 due to 8% reduction in yield and 7% reduction in harvested area compared to 2014 (Canadian Grain Commission, 2015). Canadian pea exports increased to 3.2 million tonnes in 2014 (Government of Canada, 2016). Saskatchewan produced about 56% of the total Canadian pea production, while Alberta and Manitoba produced about 42% and 2%, respectively, (Canadian Grain Commission, 2015, Saskatchewan Pulse Growers, 2016). More than 80% of the pea grown in Canada are exported, with India, China, and Bangladesh being the major importers (Government of Saskatchewan, 2012; Kissinger and Consulting, 2016).

Field pea cultivation in Canada is well suited to the Black, Dark Brown and Brown soil zones. Pea can tolerate a pH of up to 8, but the ideal pH for cultivation is 6 - 6.5. In Canada, pea is planted in late April to early May and harvested in August or September, depending on environmental conditions during the growing season. Most Canadian pea varieties are semi-determinate, early maturating and high yielding (Cousin, 1997). The semi leafless varieties have additional tendrils that support the plants to grow upright, which reduce the problem of lodging

thereby reducing the incidence of fungal diseases in the crop (Bretag and Brouwer, 1995a). The average yield in Saskatchewan is 2500 kg per ha (Ali-Khan and Zimmer, 1989).

2.2 Ascochyta Blight complex in Canada

2.2.1 *Peyronellaea pinodes* (Berk. & A. Bloxam)

Peyronellaea pinodes can cause Ascochyta blight of pea and is one of the most important endemic Ascomycete pathogens of pea, causing heavy yield losses and reduction in the quality of seeds. *Peyronellaea pinodes* is the sexual stage of *A. pinodes* (Kraft et al., 1998a). *Peyronellaea pinodes* was originally named *Mycosphaerella pinodes* (Punithalingham and Holliday, 1972a), but based on DNA sequence data of the internal transcribed spacer region the isolates were shown to cluster with *Didymella exigua* (type species for *Didymella*) and not with *Mycosphaerella* spp. (Chilvers et al., 2009). This species was recently named as the new combination *Peyronellaea pinodes* (Berk. & A. Bloxam) Aveskamp, Gruyter & Verkley (Aveskamp et al., 2010).

The pathogen *P. pinodes* produces two types of fruiting bodies. Asexual reproduction of the fungus results in the production of pycnidia in which pycnidiospores develop that are easily transported by rain splash (Lawyer, 1984). Pycnidia produced by *P. pinodes* are globose or pear shaped and dark brown with an opening at the top (ostiole), through which pycnidiospores are released. Pycnidiospores of *P. pinodes* are hyaline and two septate, similar to those of *Ph. medicaginis* var. *pinodella*, but the spores of *P. pinodes* are smaller than those of *Ph. medicaginis* var. *pinodella* (Bowen et al., 1997; Fig 2.1). Pycnidia production increases with long light periods and decreases with lowered temperature under controlled conditions (Hare and Walker, 1994). Pycnidiospores are produced throughout the growing period when conditions are favorable in the field. Sexual reproduction of this fungus produces pseudothecia, which release ascospores. Ascospores are hyaline, 2-celled in nature and constricted at the septum (Punithalingham and Holliday, 1972a). Ascospores are easily transmitted by wind (Bretag, 1991). The rupture of asci within pseudothecia and the release of ascospores will be triggered by rainfall, high humidity or heavy dew (Wallen, 1965). Chlamydospores produced by *P. pinodes* are dark brown, spherical or irregular in shape, and are produced in single or in chains (Punithalingham and Gibson, 1976).

2.2.2 *Ascochyta pisi*

In 1830, Libert (reviewed in Skoglund et al., 2011) first described *Ascochyta pisi* in Europe. *Ascochyta pisi* causes leaf and pod spots of pea (Wallen, 1974; Lawyer, 1984). Halste (1893, as reviewed in Van Hook, 1906) was the first to describe *A. pisi* as a seed borne pathogen. The sexual stage of *A. pisi* was found to be *Didymella pisi*, but was observed only in laboratory conditions (Chilvers et al., 2009). During its asexual reproduction *A. pisi* produces spherical or globose brown pycnidia. Pycnidiospores produced inside pycnidia are septate with a slight constriction at the septum, hyaline, slightly curved and with rounded tips (Punithalingham and Gibson, 1972b; Fig 2.2). Colonies on potato dextrose agar produce abundant pycnidia and pycnidial exudates are carrot red, which, in addition to the light colored mycelium, distinguished it from *P. pinodes.* Chlamydospores are absent in *A. pisi. Ascochyta pisi* produces a metabolic toxin called ascochytin, which is not produced by any other pathogen associated with the Ascochyta blight complex (Marcinkowska et al., 1991). *Ascochyta pisi* is easily transferred from one field to another by infected seeds, debris and rain splash (Smith et al., 1988).

2.2.3 *Phoma medicaginis var. pinodella*

In 1987 White et al (reviewed in Davidson et al., 1999) was first to identify *Ph. medicaginis* var. *pinodella* in 1927 as *Ascochyta pinodella*, but based on morphological features it was renamed as *Ph. medicaginis* var. *pinodella* (Punithalingham and Gibson, 1976). During its asexual reproduction the fungus produces pycnidia. Pycnidiospores are transmitted through diseased seeds and by rain splash. Cool temperatures favour the growth of spores and the severity of the infection. Pycnidia produced are brown to black with a sub-globose to globose shape and are usually larger than those of *P. pinodes* (Punithalingham and Gibson, 1976). Colonies grown on agar media look darker than *P. pinodes* due to the production of chlamydospores, which are dark-brown and are produced in single or in chains (Punithalingham and Gibson, 1976).

The teleomorphic stage of *Ph. medicaginis* var *pinodella* was first reported in 1997 from infected material collected from the field (Bowen et al., 1997). Originally, it was not described morphologically since other researchers were unable to reproduce the teleomorphic stage of *Ph. medicaginis* var. *pinodella* (Tivoli and Banniza, 2007). The teleomorphic stage has been named *P. pinodella* (Aveskamp et al., 2010). Pseudothecia produced by this fungus are globose, dark brown, cylindrical and larger than those of *P. pinodes* and asci produced are cylindrical to

sub-clavate. Ascospores are hyaline, bicellular and have round ends at the septum. *Phoma medicaginis* var *pinodella* differs from *P. pinodes* in producing larger pycnidiospores (Bowen et al., 1997).

Phoma medicaginis var *pinodella* causes diseases in pea as well as other crops of the Leguminaceae (Punithalingham and Gibson, 1976). The disease caused by *Ph. medicaginis* var *pinodella* is considered secondary to Ascochyta blight caused by the *P. pinodes*. This pathogen has been isolated from pea fields throughout the world (Hare and Walker, 1944; Knappe and Hoppe, 1995). The severity of foot rot disease increases when the fields are waterlogged (Barbetti and Khan, 1987). Crop rotation is found to be one of the successful practices to control *Ph. medicaginis* var *pinodella.*

2.3 Epidemiology and life cycle of *Peyronellaea pinodes* and *Ascochyta pisi*

2.3.1 Infection process

The spread of inoculum to other plants occurs through wind, water, rain and debris. The penetration of a plant surface by a fungal pathogen is accomplished either by direct penetration through the leaf cuticle or through natural openings or wounds. Cutin present on the outer plant cell wall acts as a physical barrier to protect the cell from pathogens. In addition to cutin, xylan and pectin are also present in the cell wall of the host plant. The secretion of cell wall degrading enzymes contributes both, to the attachment of, and penetration by the pathogen. Cutinase produced by the pathogen degrades the plant cuticle into cutin monomers, which help in the attachment of spores to the host. Nasraoui et al (1990) observed inhibition of penetration and subsequent infection due to a cutinase inhibitor, suggesting that a cutinase-mediated process of penetration across the cuticle was involved in successful infection by *P. pinodes* and *A. pisi*. Six hours after inoculation, pycnidiospore of *P. pinodes* germinate to produce one or more germ-tubes, which form appressorium-like structures that attach themselves to the host (Roger et al., 1999a). *Peyronellaea pinodes* penetrates 8 h after inoculation (Nasir et al., 1992) without disturbing the stomatal region of the host plant (Hare and Walker, 1944). An infection vesicle is formed partly in the cell lumen and partly in the epidermal cell wall, which then forms penetration and inter-cellular hyphae (Nasir et al., 1992). With the help of cell wall degrading enzymes, penetration and rapid colonization by *P. pinodes* occurs within 24 h of inoculation. The

colonization of pea leaves by *P. pinodes* and *A. pisi* leads to the death of the tissue, visible as browning of the infected cells; pycnidia are formed after three days (Heath and Wood, 1969). Nasir et al (1992) found that in resistant genotypes the formation of infectious vesicles and hyphal growth is retarded.

The size of lesions produced by *P. pinodes* was shown to be 3 - 6 times larger than those of *A. pisi* (Heath and Wood, 1969; Makasheva, 1984). *Peyronellaea pinodes* develops faster than *A. pisi*, but the rate of germination of pycnidiospores for both pathogens is the same. However, penetration of the host by *P. pinodes* was 26% higher than *A. pisi* (Heath and Wood, 1969). Pea plants protect themselves through the production of phytoalexins such as pisatin that was shown to reduce the growth of germ tubes (Paxton, 1980). When the environmental conditions are favorable and when there is enough moisture on the surface of the leaves, pisatin produced by the plant is easily detoxified by *P. pinodes* (Delserone et al., 1992) and certain pathogenic strains of *A. pisi* (Heath and Wood, 1971) through a demethylation process causing severe damage to the plants.

2.3.2 Life cycle and disease symptoms

Pseudothecia of *Ascochyta* spp. can overwinter in soil, seeds, or infected plant debris and release ascospores in spring as primary inoculum. Initially visible symptoms may start to appear within 24 hours of inoculation, after which lesions spread and coalesce to kill the entire leaf. Within 3 - 5 days pycnidia are formed on the infected leaves (Heath and Wood, 1969). As the concentration of the inoculum of *P. pinodes* increases in the field, disease severity also increases by expansion of small lesions to affecting the entire leaf, but it does not affect the number of lesions produced on the leaf (Heath and Wood, 1969; Roger et al., 1999a).

Symptoms produced by *P. pinodes* and *Phoma* spp. are similar, but *A. pisi* produces very distinctive lesions. *Peyronellaea pinodes* produces small purplish irregular lesions without a well-defined margin on the pods, stems and the leaves (Punithalingham and Holiday, 1972a). Later the purple spots enlarge to form brownish black lesions with defined margins (Fig 2.3; Roger and Tivoli, 1996). The infected leaves and stems senescence but remain attached to the plant (Hegedorn, 1985). Punithalingham and Holiday (1972a) described that stem lesions initially start as small streaks, which later coalesce, leading to the girdling of stems that weaken

the crown region, eventually causing lodging of pea plants. Allard et al (1993, as reviewed in Garry et al., 1996) reported that the flowers infected with *P. pinodes* wilt and drop, which leads to yield loss. The shape of pycnidiospores can be used to identify each Ascochyta blight pathogen.

Ascochyta pisi produces pale brown spots of varying size on leaves, which are round and encircled by dark brown margins (Fig 2.3 A, B, C). During favorable conditions the lesions enlarge and the pathogen spreads to other plants by rain splash. Recently tested samples in Canada indicated that *A. pisi* is increasing in frequency in some areas of Saskatchewan (Morrall et al., 2007).

Pea plants can get infected with *P. pinodes* at any growth stage from seeds to mature plants (Fig 2.3 D, E, F). Infection on plants can be seen on all plant parts except the roots. After maturation of pea plants, *P. pinodes* produces pycnidia and perithecia, spores of which act as the secondary inoculum in the field. The spread of infection on the stem starts from the soil and crop residues. Lesions expand, which can lead to girdling of the stem, resulting in lodging and the death of plants (Punithalingham and Gibson, 1976). Research conducted by Heath and Wood (1971) showed that the spreading of a lesion by *P. pinodes* was the result of more cell wall degrading enzymes compared to *A. pisi*. The pathogenicity and survival of these Ascochyta blight pathogens as saprophytes is due to the production of enzymes, which allow them to digest cellulose (Rattan, 1974), providing them with a readily available carbon source when the host plant tissue is dead.

The planting of infected seeds can cause foot rot or death of seedlings (Hare and Walker, 1944; Tivioli et al., 1996). Infection of flowers results in spotted lesions, which lead to flower abortion. Affected pods are shrivelled and distorted. The pathogen can survive in infected stubble and seeds for a long period of time. During adverse conditions, *P. pinodes* produce chlamydospores, but they are rarely seen on plant debris (Wallen et al., 1967a).

2.3.3 Primary inoculum

All three fungi can be transferred through infected seed and infected stubble from the field, but *A. pisi* does not survive in the soil for long compared with other pathogens causing Ascochyta blight (Gossen et al., 2011). Halste (1893, as reviewed in Van Hook, 1906) reported that infected

seed is the primary source of transmission for *A. pisi*. Ascospores are the primary source of inoculum for *P. pinodes*. Large amounts of ascospores are released from the infected stubble during the first spring rain in Australia (Carter and Moller, 1961). Abundant pseudothecia develop in lesions on senescent plant parts, at any time of the season. Moisture is the important factor for the release of ascospores into the air. When the moisture level is high pseudothecia become turgid and due to the turgoid pressure the walls of asci rupture at the tip releasing ascospores into the air (Hare and Walker, 1944; Roger and Tivoli, 1996). Generally, pseudothecia develop 3 - 4 weeks after senescence, but this varies depending on the availability of moisture. The released ascospores travel long distance and spread the pathogen to other fields (Bretag and Ward, 2001). Carter and Moller (1961) reported that dewdrops can also trigger ascospores to be released into the air, but a larger amount is released at the time of rain due to high humidity in the air and in the plant canopy. Schoeny et al (2007) in France observed that there were low numbers of ascospore released in early spring, compared to the release of ascospore in southern Australia at that time. Experiments conducted under controlled conditions showed that optimum temperatures of 15-20˚ C with 4-6 h of leaf wetness favour the release of ascospores in diurnal intervals. The release of high amounts of ascospores was triggered during the late afternoon but was lower at night. The production of pseudothecia happens after the senescence of the crop parts, when environmental conditions are favourable.

2.3.4 Secondary Inoculum

The secondary spread of ascospores and pycnidiospores causes the greatest damage in pea (Bretag, 1991). The severity of the secondary infection depends upon the abundance of primary inoculum, spacing between plants and the prevailing environmental conditions in the region. Both pycnidia and pseudothecia are produced on the same plant. Pycnidia and pseudothecia are only produced after the senescence of the crop parts resulting in greater disease expression on those tissues (Roger and Tivoli, 1996). This secondary inoculum usually spreads through wind or rain splash, and infection is also promoted by heavy dew, or by any other cultural practices done during wet periods (Punithalingham et al., 1972b). These secondary infection cycles can occur many times in the field throughout the growing season (Roger and Tivoli, 1996).

In France, during wet periods pycnidia may be formed within 11 days after the initial symptom appeared, and can continue to form until the end of the vegetative cycle of the crop (Roger and

Tivoli, 1996). Pseudothecia may be formed 13 days after the appearance of the first disease symptom. Under Australian conditions, ascospores of *P. pinodes* are released in larger amounts during spring rains (Davidson et al., 1999, McDonald and Peck, 2009). Ascospores are dispersed through wind causing the uniform spread of disease throughout the field. The amount of ascospore dispersal decreases as the canopy in the field increases until the end of the season (Roger and Tivoli, 1996). Hare and Walker (1944) showed that in case of secondary infection ascospores are more important for an epidemic than pycnidiospores for spread and dissemination of the fungus to other fields. An important part of Ascochyta blight management is to delay the process of senescence in plants in order to lower pseudothecia production (Roger and Tivoli, 1996).

2.4.3 Mode of infection through soil

Few studies have been conducted to study the behaviour of *P. pinodes* in the soil, although this fungus has the ability to survive in the soil for many years (Bretag and Ward, 2001). The soil-borne infection of this pathogen can cause foot rot of pea at the base of the stem and rain splash can cause the spread of pycnidiospores to the leaves (Sakar et al., 1982). The survival of *P. pinodes* in the soil is due to the production of chlamydospores. The survival of chlamydospores is based on the presence of thick protective cell walls. Chlamydospores act as partial saprophytes.

Peck et al (2001) in the southern part of Australia observed that soil-borne inoculum of *P. pinodes* began to decline in the field after three years without pea, whereas the stubble-borne inoculum in the soil started to decline in the soil after one year. When the infected stubble is buried, the survival of *P. pinodes* is limited to less than 12 months (Zhang et al., 2005), probably due to the absence of oxygen and other enzymatic activity. Wallen and Jeun (1968) showed that *Ph. medicaginis* var. *pinodella* and *P. pinodes* are strongly antagonistic to *A. pisi* in the soil, which reduced the population of *A. pisi* in the soil under controlled condition.

2.4.4 Mode of infection through seeds

Hare and Walker (1944) showed that the transmission of Ascochyta pathogens through seeds is one of the major survival mechanisms of these pathogens. Infected seed can act as the carrier and transfer *P. pinodes* and *A. pisi* from diseased fields to uninfected fields of pea in the next

growing season. Severely infected seeds may not emerge from the soil. While the seeds germinate, the pathogen can infect the hypocotyl and epicotyl region of the seedlings, potentially leading to the death of the seedling (Wallen, 1965; Wallen et al., 1967). A study conducted by Skolko in 1954 in western Canada showed that out of 5000 seed lots tested, 37% of isolates were *A. pisi*, 13% *P. pinodes* and 5% *Ph. medicaginis* var. *pinodella*. A similar study conducted in Ottawa showed that 85% of the seed lots tested were infected, of which 40% were infected with *A. pisi*, 28% with *P. pinodes*, and 10% with *Ph. medicaginis* var. *pinodella* (Wallen et al., 1965). In Australia, out of 691 seed lots tested 463 lots were infected with the Ascochyta blight complex pathogens, of which 98% of the isolates were *P. pinodes*, 5% *Ph. medicaginis* var. *pinodella* and 1% *A. pisi* (Bretag and Brouwer, 1995a). Only a few studies have been conducted to study the spread of the disease from seeds to stems and other plant parts. Under controlled condition, seed-to-seedling transmission was 100% for *P. pinodes* (Xue, 2000) and 40% for *A. pisi* leading to seedling death (Maude, 1966), but it has been shown that seed infection of *Ascochyta* spp. is not destructive when compared to other sources of inoculum (Moussart et al., 1998).

Seed-borne inoculum is influenced by factors including rainfall, temperature and seeding date (Bretag and Brouwer, 1995a; Bretag et al., 1995b). Areas with low rainfall often produce disease-free seeds in the field (Cruickshank, 1957; Bretag et al., 1989; Bathgate et al., 1989). *Peyronellaea pinodes* can survive on pea seed coats for several years (Bretag et al., 1995b). Surface sterilization of seeds before sowing resulted in a reduction of seed infection by 60%, which indicated that most of *P. pinodes* was carried on the seed coat of pea (Bathgate et al., 1989). When seed infection levels with *P. pinodes* are higher than 15% and the environmental conditions are conducive, the pathogen can cause severe economic damage to the crop (Xue, 2000; Moussart et al., 1998). Seed-borne infection of other species of the Ascochyta blight complex such as *Phoma* spp., has not been identified as important in causing Ascochyta blight in the field. The storage of pea seed for several years showed a reduction of *A. pisi* on the seed, but this technique is not of practical use (Wallen, 1955).

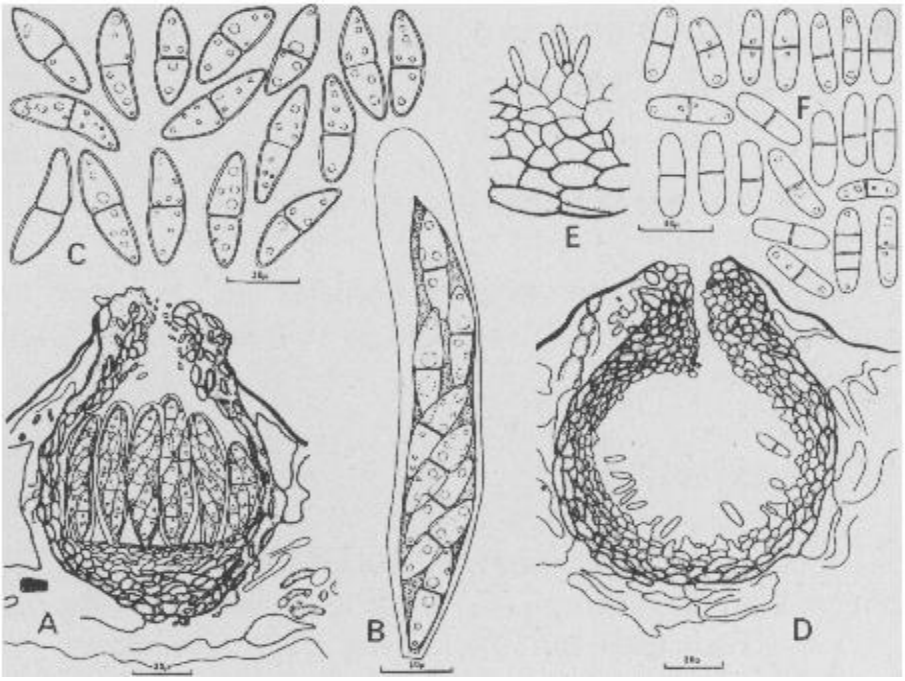


Fig 2.1 *Peyronellaea pinodes* **A.** Pseudothecia containing asci **B.** asci (sacs) containing ascospores **C.** Ascospores **D.** Pycnidium **E.** Conidiophore on substratum **F.** Conidia (secondary spores) are produced on leaves (Punithalingham and Holliday 1972a)

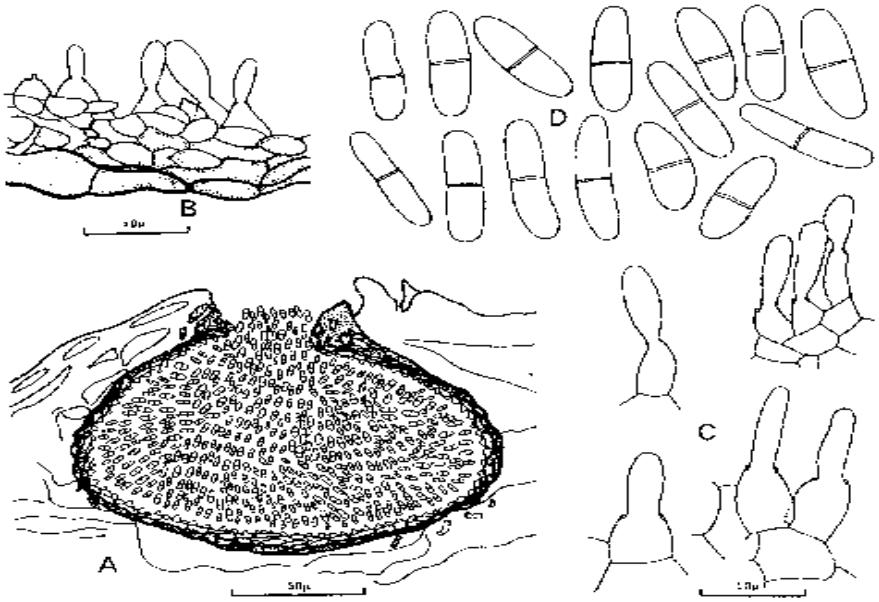


Fig 2.2 *Ascochyta pisi* **A.** Immersed pycnidium **B.** Colony of conidiophore on substratum **C.** Conidiophore **D.** Conidia (Punithalingham and Gibson 1972b)

Fig 2.3 A. *Ascochyta pisi* lesions on leaves **B.** *A. pisi* lesions on stem **C.** *A. pisi* pod infection **D.** *P. pinodes* lesions on plants **E.** *Peyronellaea pinodes* symptoms on leaves **F.** *P. pinodes* girdling of stem

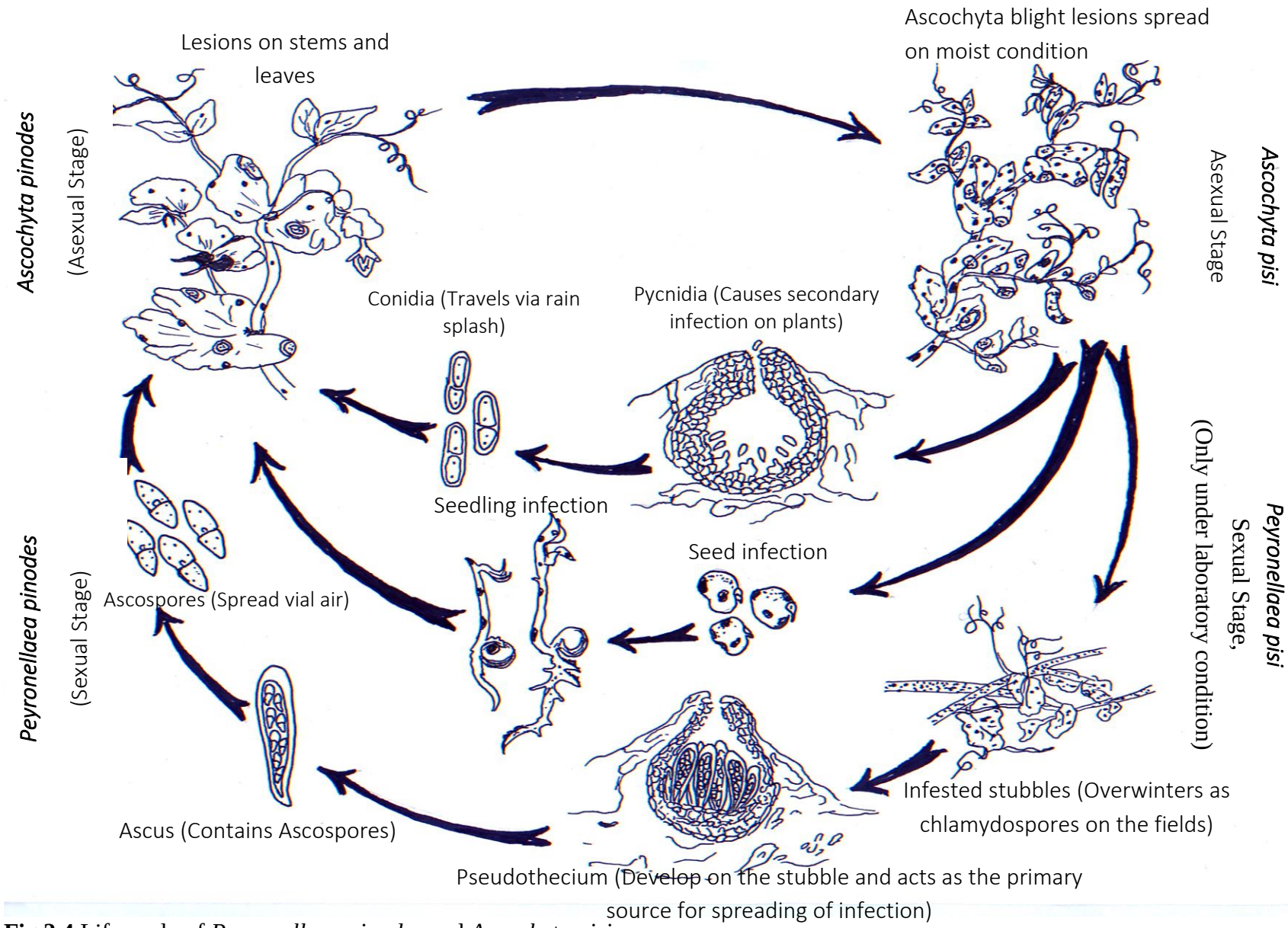


Fig 2.4 Life cycle of *Peyronellaea pinodes* and *Ascochyta pisi*

2.5 Effects of temperature and moisture on disease development

2.5.1 Effects of temperature

Temperature is one of the important factors that limits or encourages the infection process of the pathogens. Studies on relationships among temperature, moisture, and various stages of the infection processes have been conducted under controlled environments for *P. pinodes* (Roger et al., 1999a, 1999b). Conidial germination began 2 h after inoculation at 18˚C, appressoria were observed after 4 h, and hyphal penetration began after 8 h at 15 to 30˚C. The optimum temperature required for the pathogen to develop was found to be 20˚C (Roger et al., 1999a; Wallen et al., 1967a). Chen et al (1994, as reviewed in Bretag et al., 2004) reported that pod infection by *P. pinodes* is stimulated at temperatures between 24-30˚C. In India, research conducted by Pushpinder et al (2005) revealed that *A. pisi* have an optimum temperature of 25˚C and maximum temperature of 32˚C with 67% disease severity when wetness periods ranged from 12-24 h. Temperatures of 5 and 10˚C delayed infection processes, and temperatures of 5, 10, and 30˚C resulted in limited pycnidial formation by *P. pinodes* (Roger et al., 1999a).

Wallen et al (1967a) assessed the growth and infection of the *P. pinodes* on plants, and evaluated the effect of temperature (10, 15, 18, 25 and 30˚C). Lesions on plants appeared smaller at 10˚C and 30˚C. At 15˚C and 25˚C, small streaks were observed on the epicotyl region of the plants. At 18˚C, *P. pinodes* caused severe infection and lesions were coalesced leading to the development of black girdling lesions in the epicotyl region. *Peyronellaea pinodes* can survive at temperatures between -20˚C to 25˚C under sterile conditions (Wallen and Jeun, 1968). Chlamydospores can withstand 100˚ C for 12-15 h and also survive in sterile soil for more than 12 months (Wallen and Jeun, 1968). The formation of pycnidia and perithecia is induced at 20˚C under controlled conditions (Roger and Tivoli, 1996). *Peyronellaea pinodes* can adapt to a wider range of temperatures than *A. pisi* (Makasheva, 1984).

2.6.2 Effects of moisture

Moisture is another important factor for initiation and development of Ascochyta blight. Moisture is introduced into the environment in different forms such as rainfall, dew, irrigation and humidity due to evaporation. Moisture is essential for conidial germination and penetration of germ tubes into the host plant. Viability of the pycnidiospores is lower during a prolonged dry period during infection, specifically during germination. However, once the hyphae penetrate

into the plant cell, leaf wetness conditions become less relevant (Roger et al., 1999b). A minimum of 2 h of moisture is needed for conidia of *P. pinodes* to germinate (Roger et al., 1999b). The viability of conidia was reduced to 2% if moisture was not available until 12 days after inoculation. The canopy structure of `plants can also preserve moisture, particularly in the bottom of the canopy, promoting infection at the bottom of the plants. Studying the development of disease and fruiting bodies of the *P. pinodes* at constant temperatures of 5, 10, 15, 20, 25 or 30˚C with 60 to 70% RH revealed that 20˚C resulted in the most severe infection on plants (Roger et al., 1999b). If moisture was removed before 6 h post inoculation, disease development was severely limited, whereas longer wetting periods of 12-72 h promoted higher disease severity. In France, frequent rain in the spring season often results in optimum temperature and moisture conditions creating disease epidemics (Roger et al., 1999b).

2.6 Ascochyta blight management

2.6.1 Crop rotation and tillage

Peyronellaea pinodes and *A. pisi* are able to survive in infected stubble for only a few years in the absence of an appropriate host plant, therefore a three-year crop rotation can reduce the incidence or fully eradicate the inoculum from the field (Zhang et al., 2005). In Australia, crop rotation intervals of five years and delayed sowing are recommended to control Ascochyta blight (Davidson and Ramsey, 2000) However, Holm et al (2006) argued that crop rotation has very little or no effect on the severity of the disease caused by *P. pinodes,* because airborne ascospores from a nearby field may be a major source of inoculum of the pathogen. A diversified rotation of crops generally resulted in a low disease incidence of *P. pinodes* and also improved the health of the soil (Nayyar et al., 2009). Wallen and Jeun (1968) showed that *P. pinodes* infection in pea plots was much higher in continuous pea rotations, when compared to rotations that included corn and vegetables. Tillage is also one of the major practices for control of Ascochyta blight. Bailey et al (2001) observed that burying infected stubble after harvest of pea led to a reduction in the severity of *P. pinodes* in the early growing season. However in western Canada, burying of infected crop residue by tillage is not a common practice, since most of the growers have switched to the zero tillage crop production to reduce soil erosion. As a consequence, the infected stubble is left on the field surface and increases the disease potential.

2.6.2 Cultural method and biocontrol agents

Reducing the amount of primary inoculum in the form of seed-borne inoculum can control Ascochyta blight. Commercial seed production in low rainfall areas reduces seed infection by *Ascochyta* spp. (Walker, 1961). If harvested seeds are used for seeding a new crop, the Saskatchewan Pulse Growers and the Government of Saskatchewan recommend a threshold level of 10% for seed infection with Ascochyta blight pathogens; as long as the percentage of germination is high and seedlings have good vigor. Cleaners and color sorters can be used to remove some diseased or damaged seed to improve the seed quality for planting (Saskatchewan Ministry of Agriculture, 2015). Wallen (1948) showed that the percentage of *A. pisi* in pea seed was 60% lower after three years of storage compared to freshly stored seed, suggesting the viability of *A. pisi* on seed is reduced to nearly half over a period of three years. Gadd (1950) showed that hot water treatment at 100˚C for 10 min without damaging seeds reduced viability of pathogens on the seed coat, but the treatment of larger quantities of seed is not practical.

In most pea growing regions there are seasonal fluctuations in the amount of air-borne inoculum. In Western Australia, Salam et al (2011a, b) developed G1 Blackspot Manager Software, which can predict the release of ascospores of *P. pinodes*. Farmers use this information to delay the sowing date until 50% of the ascospores have been released and landed on the soil. However, sometimes-late sown crops can be severely affected due to air-borne spores produced from early sown pea crops on nearby infected plants (Walker and Hare, 1943). In India research conducted by Reddy and Singh (1993) showed that sowing in early winter might increase yield by 50%, when compared to sowing in spring.

Many studies conducted on biological control of Ascochyta blight indicated that it might be possible to reduce disease severity by pre-inoculation either with non-pathogenic fungi or non-pathogenic bacteria. Pre-inoculation with *A. fabae*, *A. pisi*, avirulent strains of *P. pinodes*, *Pseudomonas phaseolicola* and pea lectin are said to effectively protect pathogen infection sites or reduce the size of the lesions (Lepoivre, 1979, as reviewed in Bretag et al., 2006). The mechanism underlying the suppression of disease severity is still unclear. Boy (1981, as reviewed in Bretag et al., 2006) showed that pea plants inoculated with *A. fabae* prior to inoculating with *P. pinodes* showed reduced disease severity. However, at present, biological control for *P. pinodes* pathogen is not economical for larger scale control in Saskatchewan.

2.6.3 Seed treatment and foliar fungicides

Peyronellaea pinodes is a seed-borne pathogen that can be partly controlled by seed treatment with fungicides. The first fungicidal seed treatments used for seed dressing against Ascochyta blight pathogens were mainly copper and mercury based (Walker, 1961). Maude (1966) suggested that infected seeds could be treated with captan or thiram at a concentration of 0.2% for 24 h before sowing at 30˚C to control *P. pinodes* and *A. pisi* infection. Maude (1966) showed that chemical fungicides could be applied through slurries, instead of powder seed treatment. Soaking seeds in fungicide suspension of captan and thiram had better penetration and effective control of Ascochyta blight (Maude, 1966). Both fungicides were equally effective to control these two pathogens, and better than methyl mercuric dicyanamide (Wallen et al., 1967a). Hwang et al (1991) showed that there was no significant difference between captan and manganese ethylene-bis-dithio carbonate in controlling Ascochyta blight pathogens. Thiram and iprodine were shown to control *A. pisi* by controlling the pathogen's growth, resulting in 28% increased emergence of seedlings (Wallen et al., 1966). In Australia, few growers use seed dressing to control *P. pinodes* in the field. A potential problem with application of fungicidal seed treatment is that it can be harmful to *Rhizobium*, which affects nodulation of legume crops (Rennie et al., 1985).

The use of chemical fungicides is common to control Ascochyta blight on foliar parts of the plants. In Australia, *P. pinodes* is controlled by applications of chlorothalonil and mancozeb in the field (Bretag et al., 2000). Warkentin et al (1996) showed that chlorothalonil and benomyl reduced the severity of *P. pinodes* blight and increased pea seed yield and weight in Canada. Although the incidence of Ascochyta blight in Canadian field pea can be reduced by application of foliar fungicides during flowering, applications of fungicides are often not cost effective. Environmental conditions also play a major role and need to be considered before an application of fungicides (Xue et al., 2003; Warkentin et al., 2000). In France, plants are sprayed with fungicides from flowering to maturity at intervals of 10 - 15 days (Schoeny et al., 2007). In Australia, recent evidence showed that pea cultivars Kaspa and Parafield produced an economic crop yield of at least 2.5 t/ha after spraying fungicides (Davidson et al., 2004). The correct time of identification of pycnidia release can help to optimize the time of spraying of a fungicide to reduce the number of maturing pycnidia (Roger et al., 1996; 1999a). Several fungicides have

been registered for control of Ascochyta blight in Canada and it is documented that one or two applications of fungicides per season can reduce Ascochyta blight severity (Warkentin et al., 1996).

2.6.4 Host plant resistance

2.6.4.1 Inheritance of resistance to *Ascochyta pisi*

The inheritance of resistance to *Ascochyta* spp. in pea is poorly understood. The introduction of varieties resistant to *A. pisi* reduced the incidence of *A. pisi* blight in Canada (Wallen and Jeun, 1968). Resistance to *A. pisi* were found to be governed by three dominant genes, two complementary genes (Wallen and Jeun, 1968) and a single dominant and recessive gene (Darby et al., 1985). However, Brittain (1987) found a wide range of resistance in *Pisum* lines to infection by *A. pisi*. Three new genes, *Rap*-1, *Rap-3* and *Rap-4*, were identified for control of resistance to *A. pisi*. Rap-1 appeared to confer general resistance while Rap-3 and Rap-4 appeared to be race-specific. However, Csizmadia (1995) suggested that a single dominant gene governs resistance to *A. pisi*.

The presence of physiological races for each pathogen species makes it more difficult to identify resistance and to develop resistant varieties. Jones (1927) reported on physiological specialisation in *A. pisi* and differences in pathogenicity among isolates of *A. pisi* from different countries. In Canada at least four distinct races of *A. pisi* were found, each of which had a geographically limited distribution. It was speculated that this may be due to similar pea varieties in each region and different climatic conditions in those regions (Wallen, 1957). After the introduction of varieties with resistance to leaf and pod spot caused by *A. pisi*, this disease was really observed in Canada (Wallen and Jeun, 1968) until recently (Warkentin et al., 2012). Sakar et al. (1982) suggested that the method used for resistance screening to Ascochyta blight and the scoring methods can influence whether accessions are considered resistant or susceptible.

2.6.4.2 Inheritance of resistance to *Peyronellaea pinodes*

Screening for resistance to *P. pinodes* have been carried out in many countries, but only minor resistance genes have been reported to control *P. pinodes* in Canada (Xue and Warkentin, 2001) and the United Kingdom (Clulow et al., 1991). Resistance in plants to *P. pinodes* is only partial, and heritability and the expression of resistance can be affected by temperature, the amount of

inoculum in the field and also depends on the age of the plants (Zhang et al., 2006). A single recessive gene in *P. fulvum* could be used to transfer resistance to *P. sativum* by conventional breeding (Wroth, 1998a). Gurung et al (2002) reported that the good sources of resistance to *P. pinodes* have been found in primitive *Pisum* and *Lathyrus* species, but attempts to transfer this resistance to conventional pea types have been unsuccessful. Dolgikh et al (1971, as reviewed in Bretag et al., 2004) showed increased resistance to *P. pinodes* in mutant pea varieties developed through radiation.

Kraft et al (1998b) reported that they found no pea lines with major gene resistance to *P. pinodes*. Xue and Warkentin (2001) tested 335 pea lines from different countries for resistance to *P. pinodes* and identified seven lines that showed partial resistance. Kraft et al (1998b) screened 2,936 accessions of *Pisum sativum* from the USDA collection under field conditions in Ireland from 1991 to 1994 for resistance to *P. pinodes* with no replicated trial due to limitation in seeds, but results showed that high level of resistance to *P. pinodes* did not exist in this collection. Cultivar Radley (commercially grown variety in Canada in the 1990s) was among the group of accessions with best resistance and Radley was used as a check in the western Canadian pea registration trials. A total of 151 lines of *P. sativum* were found promising and further screening was done in replicated trials in Ireland and New Zealand in 1995. *P. sativum* accessions PI 142441 (Peru), PI 142442 (Peru), PI 381132 (Ethiopia), PI 404221 (Russia) and PI 413691 (Hungary) were resistant in both Ireland and New Zealand, but none were more resistant than check cultivar Radley. Foliar screening with *P. pinodes* showed that most lines were moderately to extremely susceptible as the age of the plant increased. No correlation was found between foliar disease and foot rot scorings based on disease incidence (Nasir and Hoppe, 1991; Ali et al., 1978). In Turkey, PI173052 of *P. sativum* was found to be resistant to different pathotypes of *A. pisi* and *P. pinodes* (Ali, 1986). The resistance in varieties should be confirmed with the local pathotype (Bretag and Ward, 2001, Darby et al., 1986).

Fondevilla et al (2005) observed that *Pisum sativum* ssp. *syriacum* accession P665 had good resistance to five different isolates of *P. pinodes* obtained from different countries. Fondevilla et al (2007) suggested that resistance to *P. pinodes* was controlled by a complex system. Clulow et al (1991) classified genotypes based on a qualitative approach, where disease scoring of 0 - 2 indicated resistance and 3 - 5 susceptibility. He found four genes that conditioned resistance to *P.*

pinodes at the seedling stage (*Rmp1* and *Rmp2*) and stem resistance (*Rmp3* and *Rmp4*). Recent screenings for resistance to *P. pinodes* has been focused on identifying molecular markers and Quantitative Trait Loci (QTL). Timmerman-Vaughan et al (2002, 2004) identified thirteen QTLs for resistance to *P. pinodes*, which were scattered across the seven linkage groups of pea. Timmerman-Vaughan et al (2004) also found that the QTLs for resistance to *P. pinodes* were co-located with those for plant height and flowering date. Three QTLs were also identified for resistance to *P. pinodes* under field condition (Tar' a et al., 2003). Prioul et al (2004) identified six QTLs for resistance to *P. pinodes* at the seedling stage under controlled conditions and ten QTLs for resistance at the adult stage under field conditions.

Fondevilla et al (2008, 2010) derived a genetic map from a cross between *P. sativum* ssp. *syriacum* accession P665 and *P. sativum* ssp. *sativum* cv. Messire, which was used to identify QTLs controlling resistance to *P. pinodes* at different developmental stages and under different environmental conditions. Even though these studies have increased knowledge of the genetic architecture of partial resistance in pea to *P. pinodes*, when screening pea varieties for disease resistance the resistance to *P. pinodes* varied between individual plants (Skolko et al., 1954). Sometimes this can be caused by genetic heterogeneity within varieties but it might also be due to environmental variations (Ali-Khan et al., 1973). Further research is being carried out to identify the biochemical reactions taking place that control resistance, which will provide an alternative or supplemental method for evaluating disease resistance in controlling *P. pinodes* (Hwang et al., 2004, Jha et al., 2012, Jha et al., 2013).

3. Evaluation of optimum temperature and moisture requirements for *Ascochyta pisi* and *Peyronellaea pinodes*

3.1 Introduction

Pea is an important crop in Canada. Saskatchewan is the major Canadian pea producer, followed by Alberta and Manitoba. Successful pea cultivation, to a large part, depends on climatic conditions. Pea can tolerate drought stress better than lentil but excess moisture leads to disease development. Diseases are a major constraint to pea production in Saskatchewan. Ascochyta blight, root rot, powdery mildew, Sclerotinia stem rot, rust and Septoria leaf blotch are the common diseases of pea that have been associated with yield losses, but epidemic disease development always depends on prevailing conditions. Ascochyta blight caused by *Peyronellaea pinodes* is considered a major pathogen in Saskatchewan and causes economic damage worldwide (Bretag, 2001; Lawyer, 1984). In the last decade, *Ascochyta pisi* has been more commonly found on the seed lots obtained from southern and south-western parts of Saskatchewan than *P. pinodes* (Morrall et al., 2011; Warkentin et al., 2012).

The ability of *A. pisi* and *P. pinodes* to infect pea is dependent on the pathogen's ability to adapt to the environmental conditions prevalent in Saskatchewan. As with all host-pathogen interactions, temperature and moisture are primary environmental factors influencing both conidial germination, and disease development caused by *A. pisi* (Pushpinder et al., 2005) and *P. pinodes* (Roger et al., 1999a, 1999b). Allard et al (1992, as reviewed in Garry et al., 1996) reported that the presence of free water on the leaf surface at optimal temperature promotes germination, disease and fruiting body development of *P. pinodes.* The optimum temperature for *P. pinodes* infection was determined to be between 15-20˚C (Bretag, 1991; Wallen, 1965). A study on the effects of dry periods during the infection process of *P. pinodes* revealed that if plant surfaces dried up within 6 h after inoculation, disease development was severely limited whereas longer wetness periods of 12-72 h promoted higher disease severity (Roger et al., 1999b). In India research conducted by Pushpinder et al (2005) observed that *A. pisi* had an optimum temperature of 25˚C and maximum temperature of 32˚C with 67% disease severity

when wetness periods ranged from 12-24 h. Temperatures of 5 and 10˚C delayed the infection process, and temperatures of 5, 10, and 30˚C resulted in limited pycnidial formation by *P. pinodes* (Roger et al., 1999a). Heath and Wood (1971) found that the efficiency of disease development by *P. pinodes* depended on the number of pycnidiospores present at the time of inoculation. Lawyer (1984) observed an increase in pycnidia development under high levels of humidity. The secretion of cell wall degrading enzymes contributes both to the attachment of fungal structures on the plant surface, and to penetration of the host tissue. A cutinase-mediated process is involved in the penetration across the cuticle and is required for successful infection by *P. pinodes* and *A. pisi* (Nasraoui et al., 1990).

Specific effects of environmental factors on *A. pisi* under a semi-arid climate are not known. There is a lack of data to determine whether a more conducive climate in the southern and south-western parts of Saskatchewan may be contributing to the predominance of *A. pisi* in this region. The objective of this study was to evaluate the effects of temperature and wetness periods on the development of *A. pisi* and *P. pinodes*, which may help to explain why *A. pisi* has been observed more frequently in southern and south-western parts of Saskatchewan compared to *P. pinodes*. Two experiments were established, a) germination of conidia in well slide, and b) inoculation of pea seedlings incubated at different temperatures and with different leaf wetness periods.

3.2 Materials and Methods

3.2.1 Isolate selection and preparation of conidial suspensions

Single-spored field isolates of *A. pisi* and *P. pinodes* were retrieved from the culture collection of the Pulse Crop Pathology Program of the Crop Development Centre, University of Saskatchewan. Isolates were tested for their ability to sporulate after 7 days of incubation at room temperature. *Ascochyta pisi* isolate AP6 (collected from Canora, 2006) and *P. pinodes* MP25 (collected from Lake Lenore, 2006) showed good conidial production and higher conidial germination and were selected for further study. Refreshed single-spored isolates of AP6 and MP25 were obtained from stem lesions of artificially inoculated pea (cv. Cooper). Small pieces of mycelium from both cultures were used to prepare the stock cultures, which were stored in Cryoprotective media (Cryoinstant TM) and all cultures used in experiments were derived from a single stock. Conidia of *A. pisi* and *P. pinodes* were obtained by culturing the fungi in 9 mm Petri dishes containing oatmeal agar and were incubated at

room temperature under fluorescent light with a photoperiod of 16 h light per day for 10 days to achieve optimum sporulation of the cultures. Conidia were harvested by flooding the dish with sterile tap water and scraping the culture gently using a sterile glass slide before filtering the suspension through cheese-cloth to remove culture residues. The number of conidiospores was determined using a hemocytometer and adjusted by adding sterile tap water.

3.2.2 Effects of temperature and incubation period on conidial germination using well slides

Experiments using a randomized complete block designs with four replications were conducted. All germination tests were carried out on well slides. Conidial suspensions (1 x 10^4 conidia ml^{-1}) of *A. pisi* AP6 and *P. pinodes* MP25 were prepared from 10-day-old cultures for this study. An aliquot of 100 µl of conidial suspension was evenly spread into the well of a well slide, which was placed on top of moist filter paper in a labeled Petri dish. Petri dishes were incubated at 10, 15, 20, 25 or 30˚C under continuous fluorescent light (Panasonic cooled incubator MIR 254 series). Slides were sampled destructively, so separate slides were prepared for 0, 2, 4, 6, 8, 10 and 12 h incubation periods. Conidial germination was determined by evaluating 100 conidia per slide, selected randomly from left to right across the slides. Conidia were considered to have germinated when the germ-tube was half the width of the conidium. The total number of germinated conidia was expressed as percentage of conidia germinated. The experiment was conducted twice.

3.2.3 Effects of temperature and leaf wetness periods on disease development

3.2.3.1 Planting and maintenance of pea

Pisum sativum L. cv. Cooper, a cultivar susceptible to *A. pisi* infection was used in the experiment. Disease-free seeds were grown at room temperature under fluorescent light with a photoperiod of 16 h light per day. The seeds were planted four seeds per 4" pot filled with commercial potting mixture (Sunshine mix # 4 - Peat, Dolmite lime and Perlite) and plants were fertilized with a complete fertilizer solution (PlantProd ® 20-20-20 plus micronutrients) 11 days after planting at a rate of 3 g l^{-1} water. Pots were randomly assigned to treatments.

3.2.3.2 Plant inoculation

Conidial suspensions of isolates AP6 and MP25 were prepared as described in Section 2.1.1 and adjusted to 1 x 10^5 conidia ml^{-1}. One drop of the surfactant Tween 20 was added to the

conidial suspension. Sixteen-day-old plants were inoculated with 5 ml of conidial suspension per plant using a CO_2-air brush sprayer (model RUH8210, Oxygen regulator, Uniweld, U.S.A). The inoculated plants were staked and covered with plastic bags to ensure high humidity and leaf wetness periods of the desired length. Pots were placed in incubators kept at daytime (16 h) temperatures of 10, 15, 20, 25 and 30˚C and a night temperature of 10˚C with relative humidity of 90% ± 5%. Bags were removed 0, 2, 4, 6, 8, 10, and 12 h after inoculation and the plants were dried using the cold air setting of a hair dryer. Pea plants remained in the incubator at respective temperature for a total of 14 days and were watered as required. Non-inoculated plants were included as controls.

3.2.3.3 Disease assessment

Assessment of disease severity was carried out 7 and 14 days after inoculation using a 0-10 scale (grading system) based on 10% incremental increases in the percentage of disease severity on leaves and stems. Plants were individually assessed and disease scores were converted to percentage of infected plant tissue using the class midpoint values. The average of four plants per pot was used for data analysis.

3.3 Statistical analysis

Data were analyzed using the mixed model procedure of the SAS program (9.3 SAS Institute Inc., 2010). Prior to analysis, data from each repeat and pooled data were tested for homogeneity of variances using the Levene's test. Heterogeneous variances were modeled with the repeated statement as required.

Initially, separate data analyses for each pathogen were conducted for each repeat separately and for pooled data to determine the effect of temperature and incubation period or wetness period on conidial germination of, and disease severity caused by *A. pisi* and *P. pinodes*. Temperature and incubation period or wetness period were considered fixed effects, whereas blocks and repeats (for pooled data) were considered random effects. Incubation period or wetness period were identified as repeated measures and were modeled with an autoregressive covariance structure. Non-inoculated controls as well as conidia and plants with 0 h incubation period or wetness period had no sporulation or disease development, and were not included in the analysis of variance.

Differences in conidial germination and disease progress between *A. pisi* and *P. pinodes* at each temperature were determined with repeated measures analysis of combined data from both repeats using the mixed model procedure. Pathogens and incubation period or leaf wetness period were considered fixed effects, whereas repeats and block nested in repeat were considered random effects. Incubation period or leaf wetness period were considered the factors for repeated measures. An autoregressive covariance structure was used to model repeated measures.

Regression analyses were performed on combined data to evaluate the germination rate of conidia and the rate of disease increase in response to incubation period at different temperatures. All regression analyses were performed separately for each pathogen. As an initial step, data were transformed using linear forms of logistic, Gompertz and exponential models. Linear regression analyses were conducted with the regression procedure of SAS. Goodness-of-fit for each data set was examined by plotting residuals versus predicted values and examining coefficients of determination (R^2), Mean Square Errors (MSE) and Standard Errors (SE) of the parameter estimates from linear regression analysis (Campbell et al., 1990). After selection of appropriate models based on statistical fit, conidial germination and disease progress data were further analyzed with the nonlinear regression procedure of SAS with Marquardt's compromise method. Initial parameters for non-linear regression for each pathogen were given as $y_0 = 0.1$ and the rate parameters generated through linear regressions analyses.

3.4 Results

3.4.1. Germination of conidia in well slide

Conidial germination was examined at temperatures of 10, 15, 20, 25 and 30˚C on glass slides containing conidial suspensions. Conidia of *A. pisi* and *P. pinodes* germinated at all temperatures. Conidial germination progressed similarly in both repeats (Appendices 1 and 2); so only results from combined data analyses are presented. As an initial step, the effects of temperature and incubation period were explored for each pathogen (Fig A1.1 of Appendix 1), followed by a comparison of the pathogens at the different temperatures (Fig 3.1). For both pathogens, conidial germination in general increased with increasing incubation period, and temperature up to the optimum (Fig A1.1 of Appendix 1).

For *A. pisi*, analysis of combined conidial germination data revealed that temperature, incubation period and the incubation period by temperature interaction had significant effects on percentage of conidial germination ($P < 0.0001$). At 10˚C, conidial germination did not change from 2 to 6 h, but increased after 8, 10 and 12 h of incubation period reaching an average maximum of 25% (Fig 3.1). At 15˚C conidial germination did not change from 2 to 4 h, significantly increased after 6 h, and remained at a similar level up to 8 h of incubation. Germination increased further after 10 h incubation period and remained similar up to 12 h reaching 43% on average. At 20 and 25˚C conidial germination had significantly increased at each sampling point up to 12 h of incubation, reaching 62 and 51%, respectively. At 30˚C conidial germination steadily increased up to 10 h of incubation and remained at a similar level after 12 h of incubation period with a mean of 41%.

For *P. pinodes*, analysis of combined conidial germination data showed that temperature, incubation period and incubation period by temperature interaction had significant effects on percentage of conidial germination ($P < 0.0001$). At 10˚C, conidial germination did not change from 2 to 4 h, but increased steadily after that up to 12 h of incubation reaching a mean of 31%. At 15˚C conidial germination steadily increased up to 10 h of incubation and remained at a similar level after 12 h of incubation period with a mean germination of 52%. At 20, 25 and 30˚C conidial germination steadily increased after 2 h up to 12 h of incubation period reaching 87, 79 and 76%, respectively.

Comparison of conidial germination between both pathogens at 10˚C through repeated measures analysis based on combined data revealed that pathogens ($P = 0.0630$) and the pathogens by incubation period interaction ($P = 0.0510$) had no effect, whereas incubation period had a significant effect on conidial germination ($P < 0.0001$). At 15˚C, incubation period ($P < 0.0001$) and the pathogens by incubation period interaction ($P = 0.0045$) had significant effects on percentage of conidial germination, but the pathogen effect was non-significant ($P = 0.0815$).

At 20˚C, pathogens, incubation period (both $P < 0.0001$) and the pathogens by incubation period interaction ($P = 0.0002$) had significant effects on percentage of conidial germination. There was no difference in conidial germination between *A. pisi* and *P. pinodes* up to 4 h after

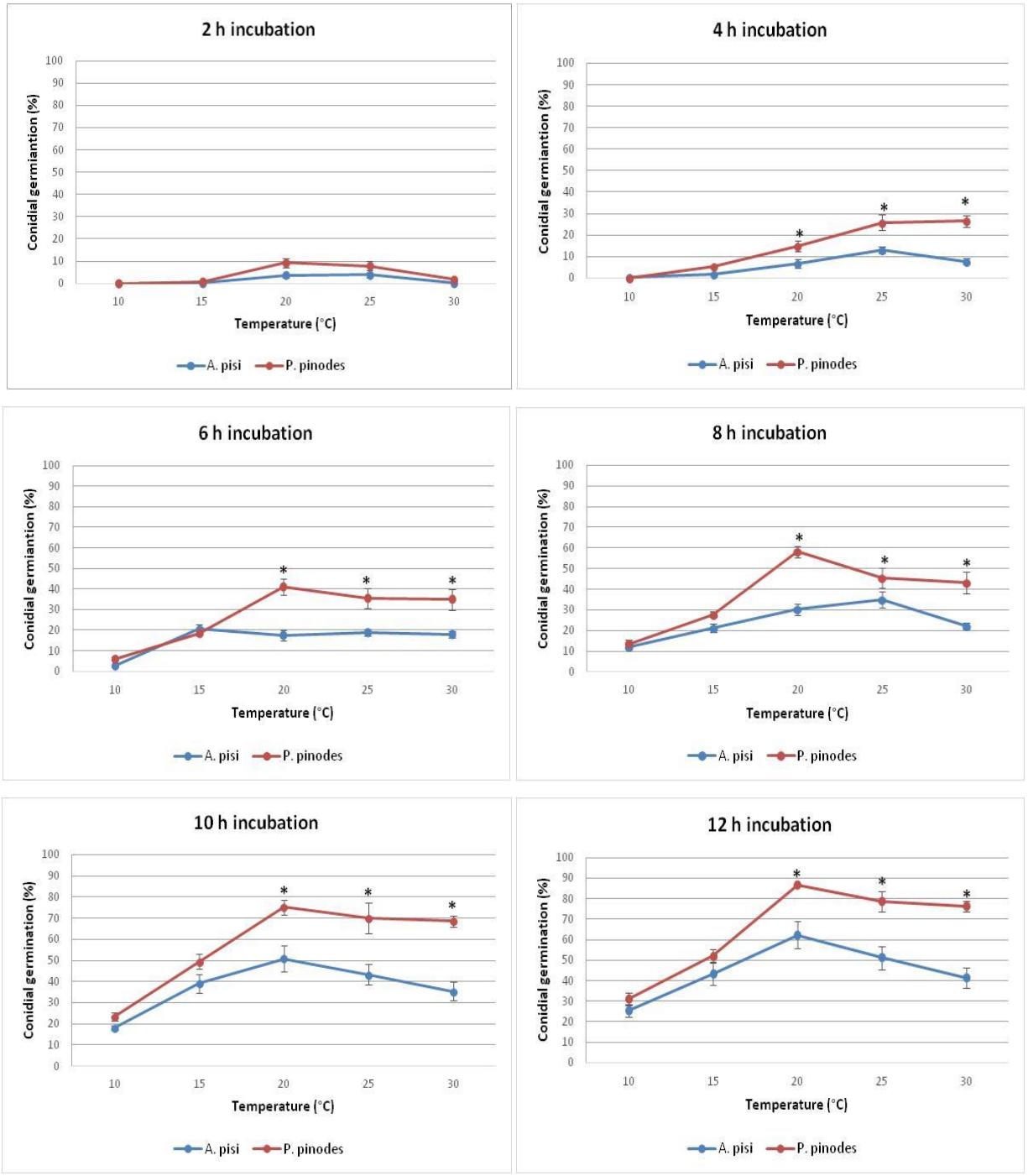


Fig 3.1. Effect of incubation period on percentage of conidial germination of *Ascochyta pisi* and *Peyronellaea pinodes* at 10 to 30˚C. Error bars represent standard errors of the means. * Indicates significant differences between *A. pisi* and *P. pinodes* at $P \leq 0.05$

which *P. pinodes* had higher conidial germination compared to *A. pisi*. At 25 and 30˚C, pathogens, incubation period and pathogens by incubation period interaction had highly significant effects on percentage of conidial germination ($P < 0.0001$), and with the exception of 2 h of incubation, *P. pinodes* had consistently higher germination compared to *A. pisi*.

The Gompertz model best described conidial germination at each temperature over a period of 12 h in linear regression analyses, and was subsequently used for describing the progression of germination through non-linear regression modeling (Table 3.1 & Appendix 3). As analyses were done separately for each temperature and pathogen, comparisons of the rate of germination and intercepts are only descriptive. The rate of germination increased from 10 to 20°C and then declined towards 30°C for both pathogens. The intercepts were lowest at 10°C and highest at 25°C for both pathogens.

Table 3.1. Rate parameter estimates and intercepts generated through non-linear regression analysis using the Gompertz model to describe conidial germination of *Ascochyta pisi* isolate AP6 and *Peyronellaea pinodes* isolate MP25 in response to incubation periods of 0, 2, 4, 6, 8, 10 and 12 h at temperatures of 10 to 30°C. The analyses are based on data from 2 repeats of the experiment.

	Ascochyta pisi		***Peyronellaea pinodes***	
Temperature (°C)	**Intercept**	**Rate parameter**	**Intercept**	**Rate parameter**
10	0.000809	0.1401	0.00105	0.1510
15	0.00666	0.1570	0.00398	0.1895
20	0.00139	0.2210	0.00408	0.2956
25	0.0196	0.1533	0.0218	0.2230
30	0.0127	0.1281	0.0171	0.2292

3.4.2 Disease severity on inoculated plants

Disease severity was examined at temperatures of 10, 15, 20, 25 and 30˚C on pea plants inoculated with spore suspensions. Disease progressed at all temperatures for both *A. pisi* and *P. pinodes* with increasing wetness periods. Disease severity progressed similarly in both repeats, so only combined data analyses are presented. Results from single repeats are presented in Appendices 4 and 5. As an initial step, the effects of temperature and leaf wetness were explored for each pathogen (Fig A4.2 of Appendix 4), followed by a comparison of the pathogens at the different temperatures (Fig 3.2). For both pathogens, disease severity in general increased with increasing leaf wetness periods, and temperature up to the optimum (Fig A4.2 of Appendix 4).

Analysis of combined *A. pisi* severity data revealed that temperature, leaf wetness period and the leaf wetness period by temperature interaction had significant effects on percentage of disease severity caused by *A. pisi* ($P < 0.0001$). At 10˚C, disease severity did not change from 2 to 8 h but increased after 10 h and remained at a similar level after 12 h of leaf wetness reaching a final mean severity level of 23%. At 15˚C disease severity did not change from 2 to 4 h, significantly increased after 6 h, and was at a similar level after 8 h of leaf wetness. Disease severity at 10 and 12 h after inoculation was higher than that observed at 8 h with a mean level of 42% after 12 h of leaf wetness. At 20 and 25˚C disease severity did not change from 2 to 4 h, and then steadily increased further up to 12 h reaching average severity levels of 66 and 71%, respectively. At 30˚C, disease severity did not change from 2 to 8 h, but increased after 10 h up to 12 h of leaf wetness, but only reached on average 20% after 12 h.

Combined data analysis for *P. pinodes* revealed that temperature, leaf wetness period and the leaf wetness period by temperature interaction had significant effects on percentage of disease severity ($P < 0.0001$). At 10˚C, disease severity did not change from 2 to 6 h, but increased after 8h. Disease severity increased further after 10 h and then remained constant after 12 h of leaf wetness with a final mean of 26%. At 15˚C, disease severity did not change from 2 to 4 h, and then steadily increased further up to 12 h reaching 42%. At 20 and 25˚C, disease severity had significantly increased after each sampling point up to 12 h when disease severity means reached 85 and 83%, respectively. At 30˚C, disease severity increased after 6 h,

and remained constant up to 8 h. Disease severity increased further after 10 and 12 h of leaf wetness, but only reached 27%.

Comparing both pathogens through repeated measures analyses revealed that at 10 and 15˚C pathogens ($P > 0.1294$) and the pathogens by leaf wetness period interaction ($P > 0.2180$) had no effects on disease severity. Leaf wetness period had significant effects on disease severity ($P < 0.0001$). At 20 and 25˚C, pathogens and leaf wetness periods (both $P < 0.0001$) had significant effects on disease severity, whereas the pathogens by leaf wetness period interaction was non-significant ($P > 0.0598$). At 20˚C, there was no difference in disease severity caused by *A. pisi* and *P. pinodes* up to 2 h of leaf wetness, but after 4 h, *P. pinodes*-infected plants had higher disease severity than those infected with *A. pisi* (Fig. 3.2). At 25˚C, there was no difference in disease severity caused by the pathogens up to 4 h of leaf wetness, whereas thereafter *P. pinodes* caused higher disease severity than *A. pisi*. At 30˚C, pathogens ($P = 0.0050$) and the pathogens by leaf wetness period interaction ($P < 0.0001$) had significant effects on disease severity. Leaf wetness period ($P = 0.4131$) had no effect on disease severity. There were no significant differences in disease severity caused by *A. pisi* and *P. pinodes* up to 8 h, but *P. pinodes* induced higher disease severity at 10 and 12 h leaf wetness compared to *A. pisi*.

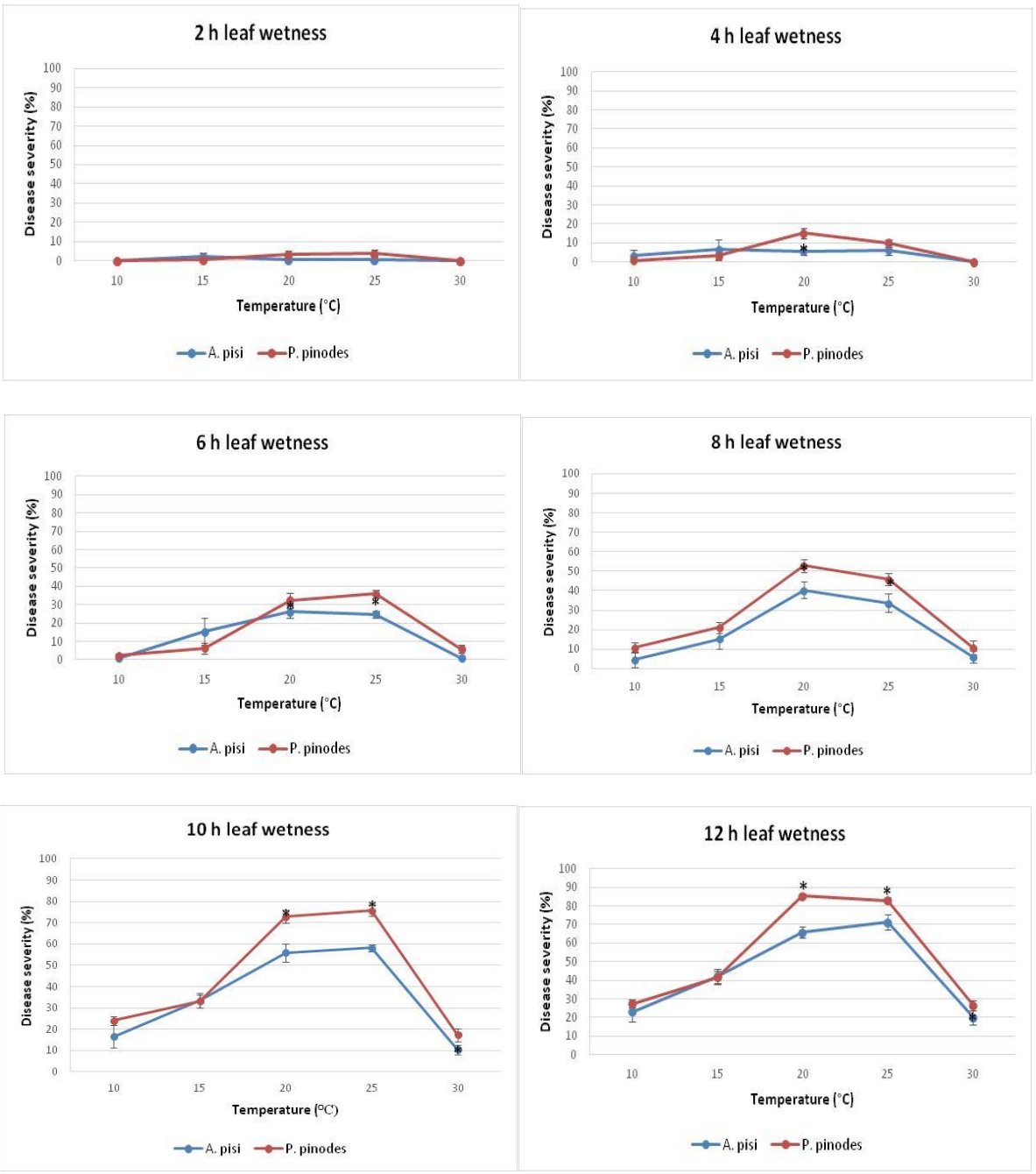


Fig 3.2. Effect of leaf wetness period on percentage of disease severity caused by *Ascochyta pisi* and *Peyronellaea pinodes* at 10 to 30˚C. Error bars represent standard errors of the means. * Indicates significant differences between *A. pisi* and *P pinodes* at $P \leq 0.05$.

In linear regression analysis, the Gompertz model also best described disease severity at each temperature over a period of 12 h, and was subsequently used for describing the progression of disease through non-linear regression modeling (Table 3.2 & Appendix 6). As before, only descriptive comparisons are possible. The rate of disease increase and disease severity increased with temperature. The rate of disease increase increased from 10 to 20˚C and then declined as temperature increased to 30˚C for both pathogens. The intercepts were lowest at 10˚C and was highest at 25˚C for both pathogens.

Table 3.2. Rate parameter estimates and intercepts generated through non-linear regression analysis using the Gompertz model to describe disease severity cause by *Ascochyta pisi* isolate AP6 and *Peyronellaea pinodes* isolate MP25 in response to leaf wetness periods of 0, 2, 4, 6, 8, 10 and 12 h at temperatures from 10 to 30˚C. The analyses are based on data of 2 experiments.

	Ascochyta pisi		***Peyronellaea pinodes***	
Temperature (°C)	**Intercept**	**Rate parameter**	**Intercept**	**Rate parameter**
10	0.000058	0.1493	0.000528	0.1536
15	0.00561	0.1607	0.00116	0.1753
20	0.00301	0.2285	0.00133	0.3005
25	0.000776	0.2527	0.000800	0.3036
30	0.000036	0.1539	0.00117	0.1360

3.5 Discussion

In Saskatchewan, summers are usually warm and dry with daytime temperatures ranging from 15°C in May to the mid 30°C in July and August and cool nights. To mimic the mean temperature observed in the field, temperatures of 10, 15, 20, 25 and 30˚C for day time (16 h) and a constant 10°C at night (8 h) were maintained in the incubators. Conidial germination and disease severity induced by *A. pisi* and *P. pinodes* on pea increased with increasing temperatures up to 20 to 25˚C. Overall, *P. pinodes* had higher conidial germination and induced higher disease severity compared to *A. pisi*. The differences in conidial germination between the two pathogens became obvious starting at 20˚C with more than 4 h incubation, and at 25 and 30˚C *P. pinodes* had consistently higher germination after 2 h of incubation. The rates of increases in germination based on regression analyses were at least numerically also higher for *P. pinodes* than for *A. pisi*. Differences in disease severity caused by the two pathogens at 20 and 25˚C were similar to those observed in conidial germination. At 30˚C *P. pinodes* only induced higher disease severity with 10 and 12 h of leaf wetness. The rate of disease increase based on regression analysis was also higher for *P. pinodes* up to 25˚C, but then declined sharply. From the results of this study, it appears that both pathogens have their temperature optimum between 20 and 25˚C.

Earlier studies suggested optimum temperatures for conidial germination and disease development of 15 to 18˚C for *P. pinodes* (Wallen, 1965) and 20˚C (Bretag, 1991). Sattar (1934) also reported that optimum range for *P. pinodes* spore germination is 20-25˚C. Bretag (1991) reported that at lower temperature, longer leaf wetness period are needed by *P. pinodes* for spore germination and disease severity. He also reported that at 10˚C, a 12 h leaf wetness period was required for infection, but at 20˚C, 6 h leaf wetness period was sufficient. The incubation periods reported for initial disease development vary in length from 2 to 4 days for *P. pinodes* to 6 to 8 days for *A. pisi* (Schroeder, 1953). Periods of moisture of at least 4 to 8 h were required for the pathogens at the lowest and highest temperatures for the appearance of disease symptoms compared to 2 h for temperatures of 15 to 25˚C. Brewer (1960) reported that the temperature after infection also influences disease development. He observed increases in lesion size and the number of lesions caused by *P. pinodes* at 5 to 10˚C when compared with 15 to 25˚C. Carter and Moller (1961) reported that spores of *P. pinodes* were able to survive

interrupted leaf wetness period and have the ability to infect when the conditions were favourable. Roger et al (1999a) observed that conidiospores of *P. pinodes* were able to survive dry periods of up to 21 days after inoculation. The effects of wet and dry periods depended on when the dry period occurs during the infection and appressorium formation and hyphal penetration was continued throughout the dry period. Infection remained asymptomatic until the leaves were re-wetted.

Ascochyta pisi has become more prominent in Saskatchewan over the past 10 years. Results here confirm that while *P. pinodes* is the more aggressive pathogen, as previously reported, the temperature optimum of both pathogens is the same, hence rejecting the hypothesis that the prevalence of *A. pisi* in south and south-western Saskatchewan is due to different temperature and moisture optima for the two pathogens. However, results obtained under controlled conditions for conidial germination of, and disease severity caused by, *A. pisi* and *P. pinodes* on pea plants in response to wetness periods and temperature may not reflect the complex situations encountered in the field. For example, in the field pathogens are exposed to daily temperature fluctuations. Also, prolonged periods of wetness are not common in Saskatchewan, particularly in the southern and south-western regions. Thus, there is a need to investigate the effect of interrupted leaf wetness on *A. pisi* and *P. pinodes*.

4. Assessment of the role of seed infection with *Ascochyta pisi* on disease development in peas

4.1 Introduction

Ascochyta blight is caused by a complex of pathogens. *Peyronellaea pinodes*, *Ascochyta pisi* and *Phoma medicaginis* var *pinodella* are the major pathogens of this disease complex in Canada. Ascochyta blight is widespread in major pea growing areas around the world (Wallen, 1965). The transmission of *Ascochyta* spp. including *A. pisi* through seeds is one of the major survival mechanisms of these pathogens. Deneufbourg et al (1994, as reviewed in Moussart et al., 1998) showed that infected seed can act as carriers and transfer *P. pinodes* from diseased fields to uninfected fields of pea in the next growing season with varying levels of seed infection from year to year, depending on local climatic conditions. While the seeds germinate *P. pinodes* can infect the hypocotyl and epicotyl region of the seedlings, resulting in poor crop establishment (Wallen, 1965). Very little information is available on the importance of transmission of *A. pisi* from seed to stem and other parts of the plant. Under controlled condition, seed-to-seedling transmission was 100% for *P. pinodes* (Xue, 2000) and 40% for *A. pisi* (Maude, 1966) which led to the death of the seedlings, but it has been observed that seed inoculum of *P. pinodes* is not very destructive when compared to other sources of inoculum (Moussart et al., 1998).

The impact of seed-borne inoculum is influenced by many factors including rainfall, temperature and seeding date (Bretag and Brouwer, 1995a; Bretag et al., 1995b). Areas with low rainfall often produce disease free seeds in the field (Bretag et al., 1989; Bathgate et al., 1989). Studies on seed-borne inoculum of *P. pinodes* and disease development revealed a negative correlation indicating that seed infection appears to be more important as a cause of poor emergence than as a source of inoculum for aerial infection (Bretag and Brouwer, 1995a). *Peyronellaea pinodes* can survive on pea seed coats for several years (Bretag et al., 1995b).

Surface sterilization of seed before sowing resulted in a reduction of seed infection by 60%, which indicated that *P. pinodes* was carried on the seed coat of pea (Bathgate et al., 1989).

Under suitable environmental conditions, and when the proportion of *P. pinodes* infected seed is more than 15% the pathogen can cause severe economic damage to the crop (Xue, 2000; Moussart et al., 1998). Seed-borne infection of other species of the Ascochyta blight complex such as *Phoma* spp. has not been identified as important in causing Ascochyta blight in the field. Wallen (1955) reported reduction of *A. pisi* on the seed when the harvested seed was stored for several years, but this is not practical.

The recent increase in *A. pisi* occurrence on seeds from southern and south-western parts of Saskatchewan (Morrall et al., 2011; Warkentin et al., 2012) has warranted research to determine the economic importance of this pathogen for pea production in Canada. The objective of this study was to assess the role of seed infection in the epidemiology of *A. pisi* in order to understand the importance of seed-to-seedling transmission of this pathogen under field conditions, and to determine the nature of *A. pisi* seed borne infection. Two experiments were established: a) seed-to-seedling transmission experiments under field conditions, and b) a seed component study from naturally *A. pisi* infected pea seeds under laboratory condition.

4.2 Materials and Methods

4.2.1 Experimental design and assessments for field experiments

CDC Patrick seeds, a green cotyledon field pea cultivar, were used for this experiment. Commercial seed lots with natural *A. pisi* seed infection levels of 0.5% and 14.5% and low or no *P. pinodes* infection, confirmed by a commercial seed testing lab, were obtained from a seed grower. Samples were combined to obtain *A. pisi* infection levels of 0.5, 5, 10 and 14.5%, which were confirmed through seed testing. The experiment were established on 25 May, 2012 at Outlook; on 30 May, 2012, 10 May, 2013 and 12 May, 2014 at Saskatoon; and on 7 May, 2013 and 11 May, 2014 at Milden where levels of *A. pisi* infection had been low in previous years. Experiments were designed as randomized complete blocks with 4 replicates; plots size was 1.2 x 3.7 m with a seeding density of 86 seeds per m^2.

During the growing season, plant emergence was assessed by counting the number of seedlings per meter in an arbitrarily selected row of each plot. Disease severity caused by *A. pisi* and *P. pinodes* were assessed at the seedling stage, during flowering, at the podding stage and at maturity using the 0-10 scale described in Section 3.2.3.3. Five arbitrarily selected plants were

rated in each plot and data were transformed to percentage disease severity using the class mid points. The averages per plot were calculated for further data analyses. At harvest, seed yield were determined for each plot, and seed assessments of size, thousand seed weight (TSW), and disease seed testing were performed.

For seed testing, harvested pea seeds were evaluated for *A. pisi* and *P. pinodes* infection by plating 100 seeds per plot onto Potato Dextrose Agar (PDA). Seeds were surface-sterilized by soaking in 0.6% sodium hypochlorite (NaOCl) for 3 min with constant agitation, rinsing with sterile distilled water for 2 min, and drying on a sterile distilled paper towel before being placed on PDA. Seeds were incubated at 20˚C for 7 days under continuous fluorescent light on the bench top. Each seed were assessed for infection and fungal growth was identified by spore evaluation under the microscope.

4.2.2 Seed component study

For this experiment, seeds of CDC Patrick naturally infected with 14.5% *A. pisi* infection were used. Assuming that seed coat staining was caused primarily by *A. pisi* infection, the seeds were visually sorted into four categories based on the amount of seed coat staining: 0% (clean seeds without any staining), 1 to 25%; 26 to 50%; 51 to 75%; 76 to 100% (Fig 4.1). Components of the infected seed were tested for infection with *A. pisi*. For each category, seven replicates of 50 seeds were soaked in sterile distilled water for 2 h. Seeds were dissected into seed coat, cotyledon and embryo. Seed components were surface-sterilized by soaking in 0.6% sodium hypochlorite (NaOCl) for 3 min with constant agitation, rinsing with sterile distilled water for 2 min, dried on a sterile distilled paper towel before being placed on PDA. Seeds were incubated at 20˚C for 7 days under continuous fluorescent light inside bench top incubators. Each plate was assessed for infection and fungal growth were identified to the species level with a compound microscope.

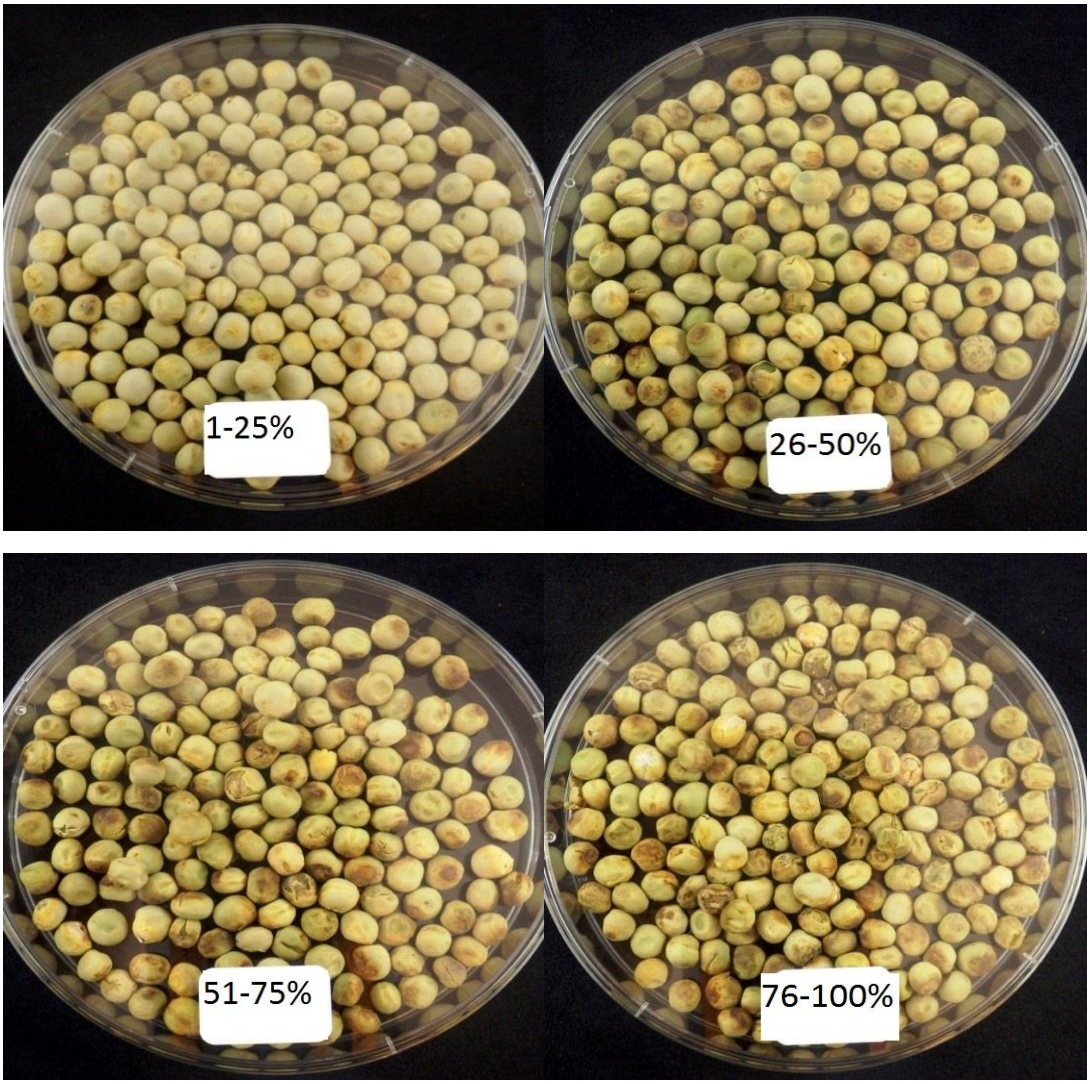


Fig 4.1. Visual categorization of pea seeds of cv. CDC Patrick into four categories based on seed coat staining due to *Ascochyta pisi*.

4.2.2 Data analysis

All data were analyzed using the mixed model procedure of the SAS program (9.3 SAS Institute Inc., 2010). Prior to analysis, the data were tested for homogeneity of variance using the Levene's test. Heterogeneous variances were modeled with the repeated statement in SAS as required. Data from the field experiment were analyzed for each location separately and also pooled for analysis to determine the effect of *A. pisi* seed infection on pea plants. Seed infection levels were considered fixed effects, whereas blocks, years and locations were considered random effects.

For the seed component study, seed staining categories and seed components were considered fixed effects, whereas replications were considered random effects. Initially, other pathogens detected in seed samples were used as covariates. Final modeling of *A. pisi* data was done with the significant covariate(s) and means were separated by Fisher's least significant difference test.

4.3 Results

4.3.1 Seedling emergence in field experiments

Data analysis revealed that except at Saskatoon, 2012 and for the combined data analysis, seed infection levels with *A. pisi* had no effect on the emergence of the seedlings ($P > 0.3$). At Saskatoon, 2012 and when all data were pooled, seed infection levels of 10 and 14.5% resulted in reduced emergence compared to 0.5% seed infection (Fig 4.2).

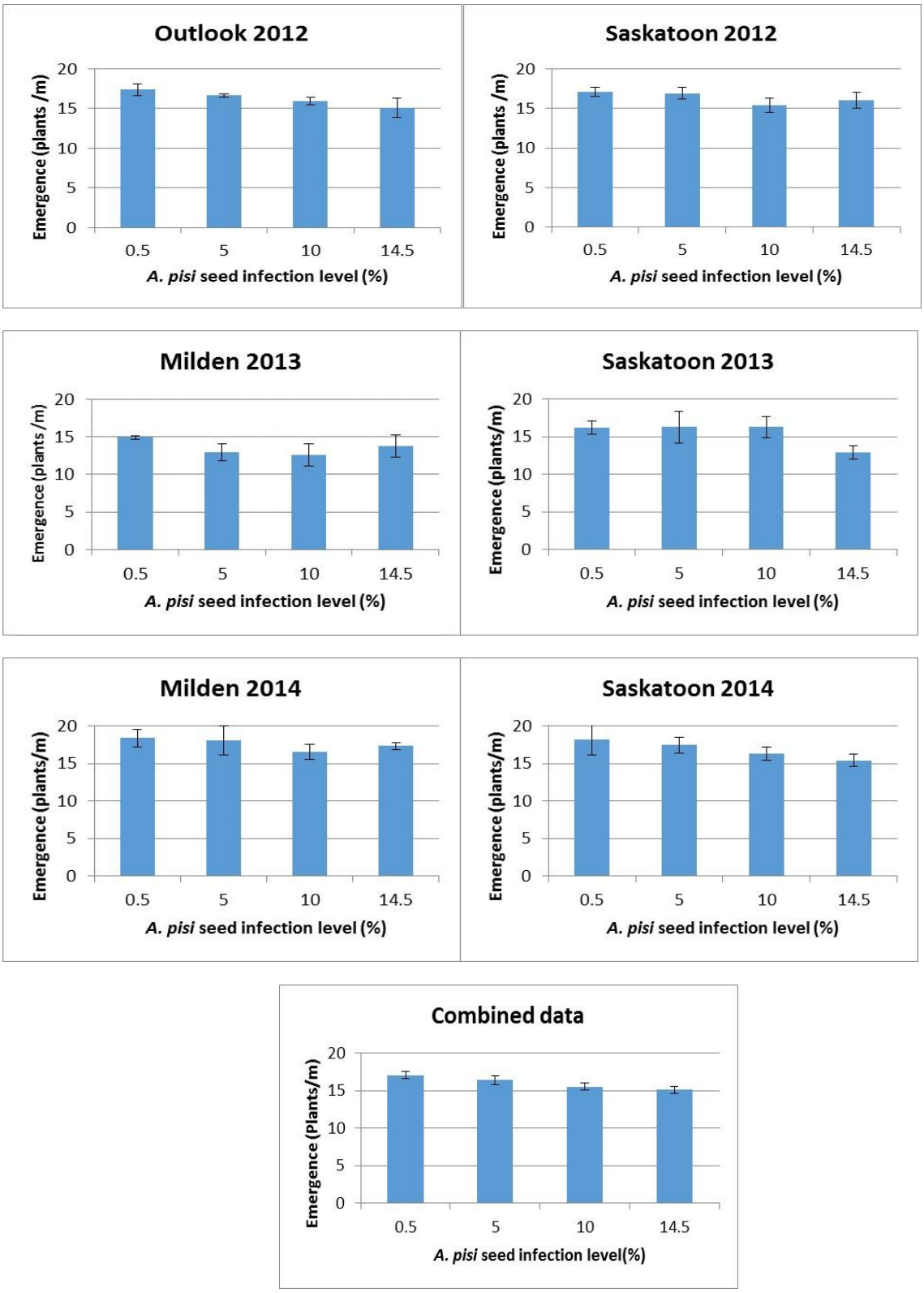


Fig 4.2 Seedling emergence of cv. CDC Patrick field pea from seed with different levels of infection with *Ascochyta pisi* from Outlook, Milden and Saskatoon in 2012, 2013 and 2014.

4.3.2 Disease severity

Disease severity caused by *A. pisi* and *P. pinodes* was very low at Outlook, Milden and Saskatoon in all six location-years. *Ascochyta pisi* lesions and fruiting bodies were seen on the older leaves at the end of the growing season. With the exception of Saskatoon, 2013, seed infection with *A. pisi* had no effect on *A. pisi* severity on plants at maturity, and pooling of data did not change that ($P > 0.8$; Fig 4.3). At Saskatoon, 2013, seed infection levels had a significant effect on *A. pisi* severity on the plants ($P = 0.0372$). Plots seeded with a 14.5% seed infection level had higher *A. pisi* disease severity on plants at maturity compared to those with 0.5 and 5% seed infection. As expected, seed infection levels had no effect on disease severity of *P. pinodes* ($P > 0.1$; Fig 4.3).

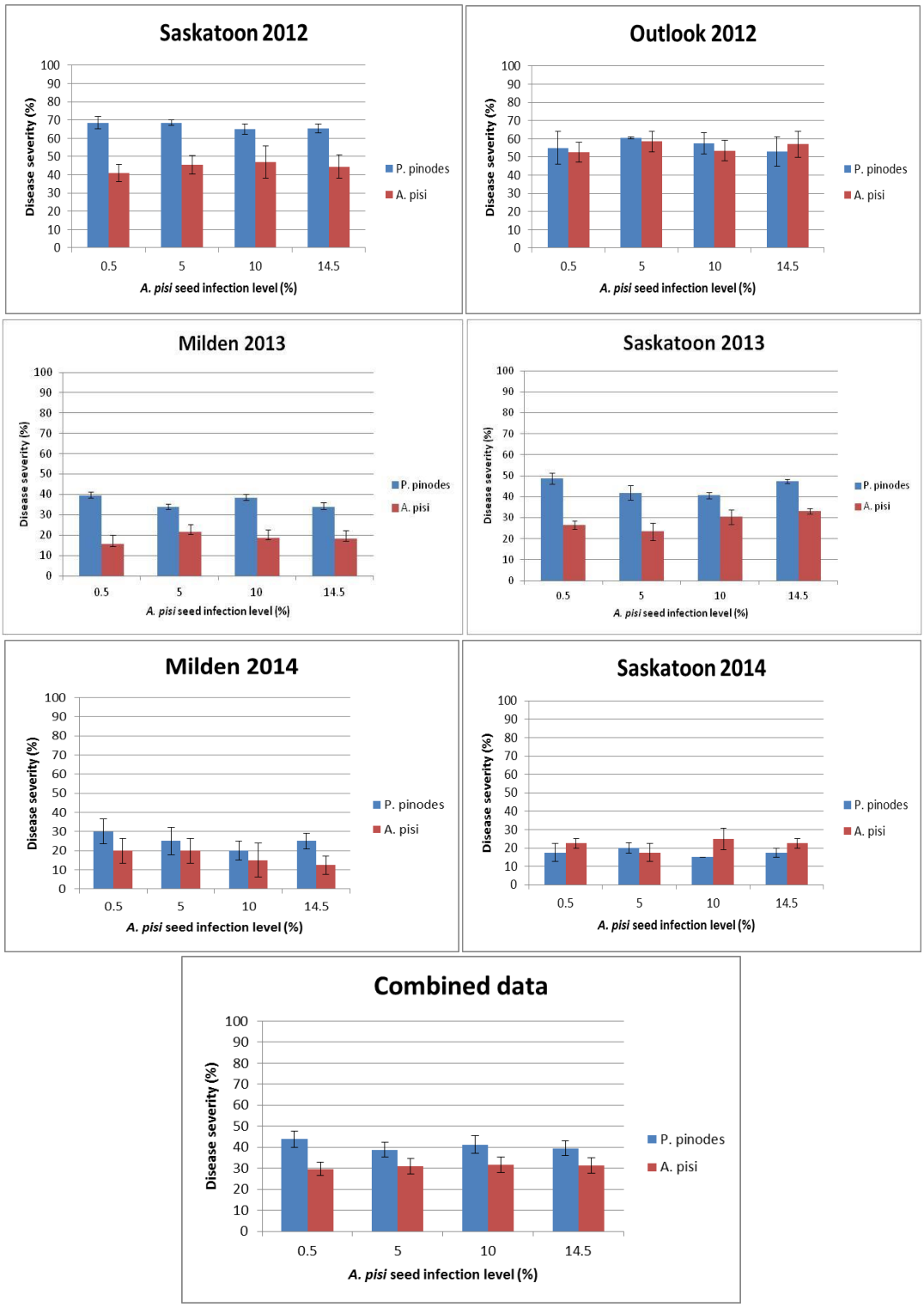


Fig 4.3 Ascochyta blight severity caused by *Ascochyta pisi* and *Peyronellaea pinodes* on pea plants cv. CDC Patrick developing from seed with different levels of infection with *Ascochyta pisi* from Outlook, Milden and Saskatoon in 2012, 2013 and 2014.

4.3.3 Yield

Seed yield at Saskatoon, 2014 were higher than at Milden, 2014, but were similar at both locations in 2012 and 2013. Analysis of 2012, 2013 and 2014 data, irrespective of whether they were analyzed separately or pooled, revealed that seed infection levels had no effect on yield ($P > 0.5$; Fig 4.4).

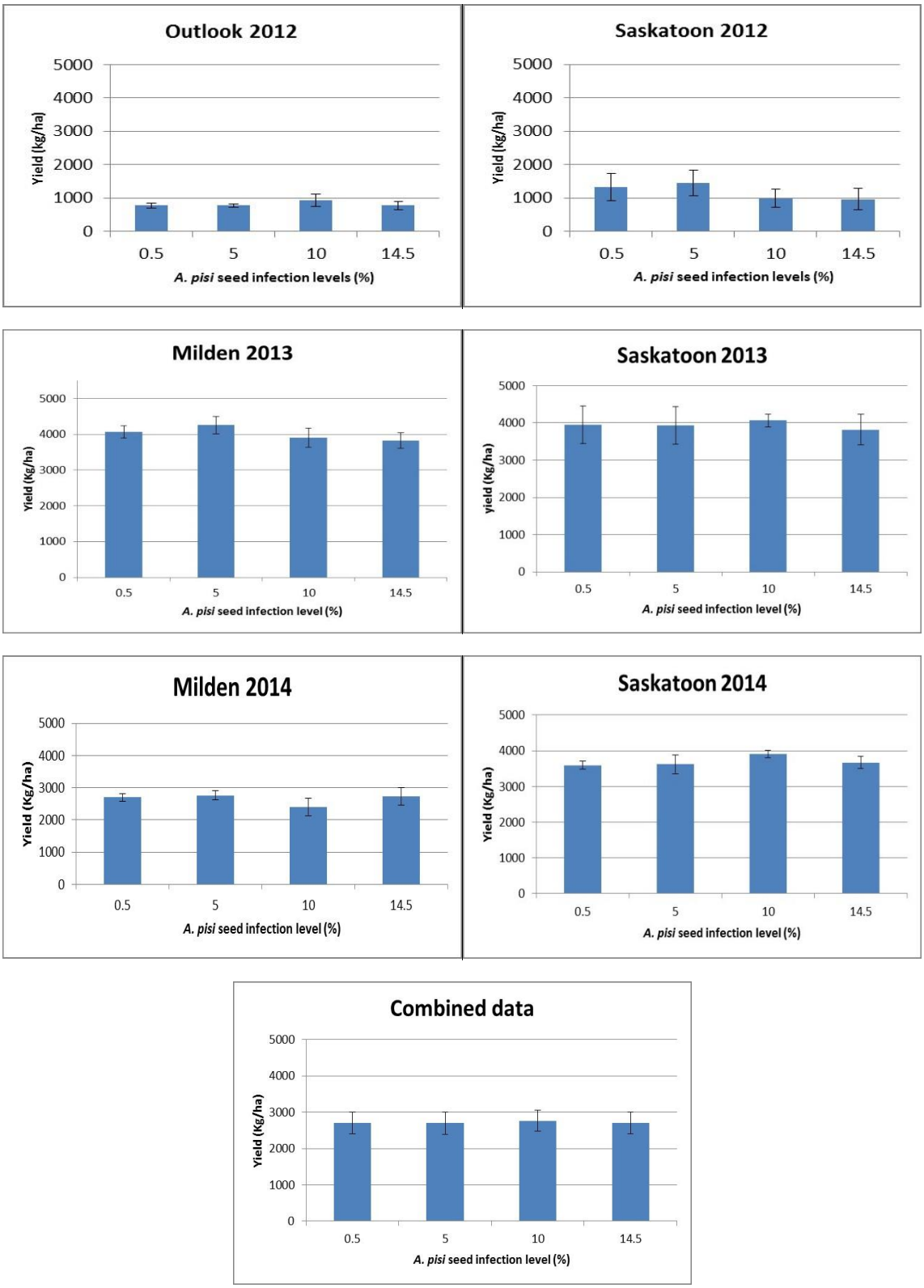


Fig 4.4 Seed yields of pea cultivar CDC Patrick grown from seeds with different levels of *Ascochyta pisi* infection from Outlook, Milden and Saskatoon in 2012, 2013 and 2014.

4.3.4 Seed infection of harvested seed

Seed testing of harvested CDC Patrick seeds revealed infections with *A. pisi*, *P. pinodes*, *Colletotrichum* spp, *Fusarium* spp., *Alternaria* spp., *Epicoccum* spp., unidentified green moulds and bacteria. Only *A. pisi* and *P. pinodes* infection data were analyzed. Individual site-year analyses revealed that *A. pisi* seed infection levels of 0.5, 5, 10 and 14.5% had no effect on infection levels with *A. pisi* on harvested seeds ($P > 0.1$). Seed infection after harvest with *P. pinodes* was higher at Milden 2014 than other sites. No differences in *P. pinodes* seed infection of seeds harvested were observed at Outlook, Milden and Saskatoon, 2012, 2013 and 2014 ($P > 0.2$). Pooled data from 2012, 2013 and 2014 revealed that seed infection levels had significant effects on infection levels with *A. pisi* from harvested seeds ($P < 0.0001$; Fig 4.5). Seed infection levels of 10 and 14.5% used for planting resulted in higher *A. pisi* infection from harvested seeds compared to 0.5% seed infection. There was also a significant difference in *P. pinodes* seed infection of harvested seeds ($P < 0.0001$).

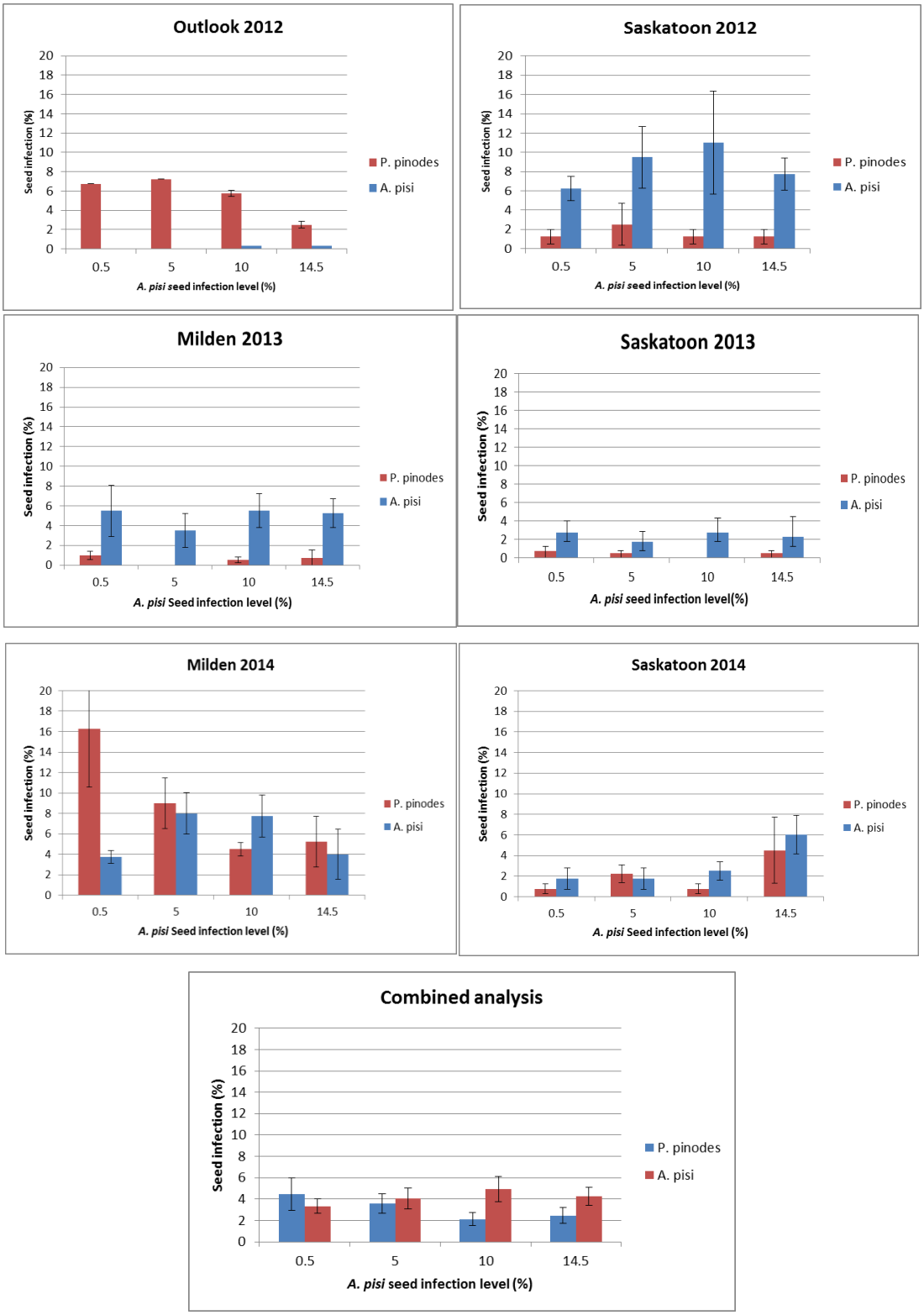


Fig 4.5 Incidence of seed infection with *A. pisi* and *P. pinodes* of seeds harvested from CDC Patrick pea plots seeded with different levels of infection with *Ascochyta pisi* from Outlook, Milden and Saskatoon in 2012, 2013 and 2014.

4.3.5 Seed component study

Results from the seed component study revealed that components without staining were not infected with *A. pisi*. Seeds of all other four seed staining categories revealed infection of the three seed components with *A. pisi*. In addition to *A. pisi*, other organisms, such as *Colletotrichum* spp, *Fusarium* spp, *Alternaria* spp, *Epicoccum* spp, unidentified green moulds and bacteria were also identified on the stained seed components (Fig 4.6). Only *Epicoccum* spp had a significant effect on the model as a co-variate (P = 0.0212) and were included in the model. Analysis of *A. pisi* infection data showed that seed staining category, seed components, and the seed staining category by seed component interaction (P < 0.0001) had significant effects on *A. pisi* seed infection. Seed staining categories 51-75% and 76-100 % had higher seed coat infection compared to that in staining category of 26-50%. Seeds staining categories 1-25% and 76-100 % had higher cotyledon infection compared to staining categories of 26-50% and 51-75%. The analysis showed that there was no difference in embryo infection among the seed staining categories (Fig 4.7).

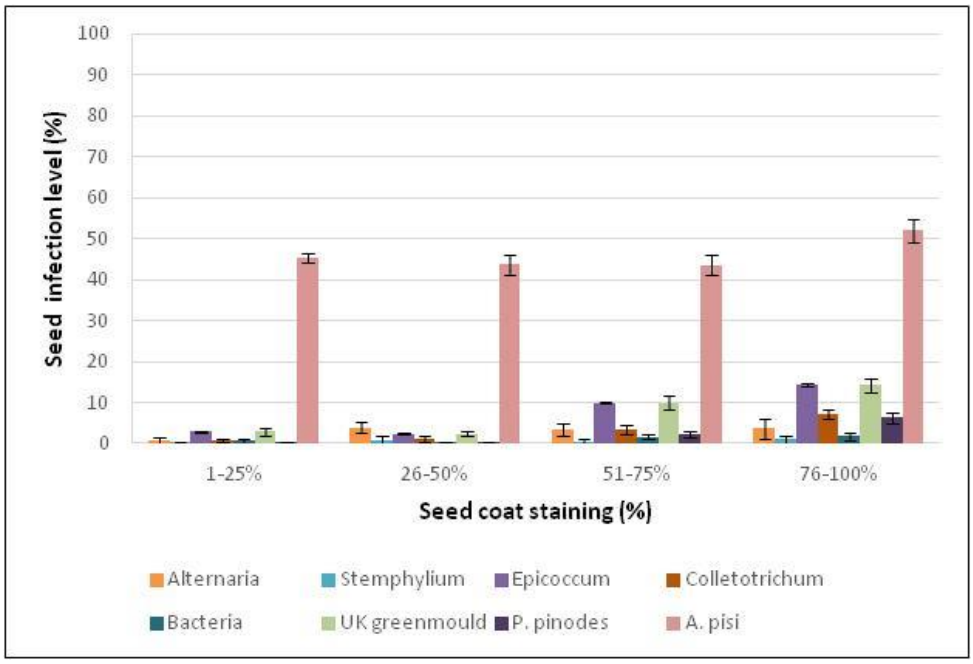


Fig 4.6 Infection levels of seed with *Ascochyta pisi* (mean of 3 seed components) on seed coat, cotyledon and embryo from CDC Patrick field pea seeds naturally infected with *Ascochyta pisi* and separated into four seed coat staining categories

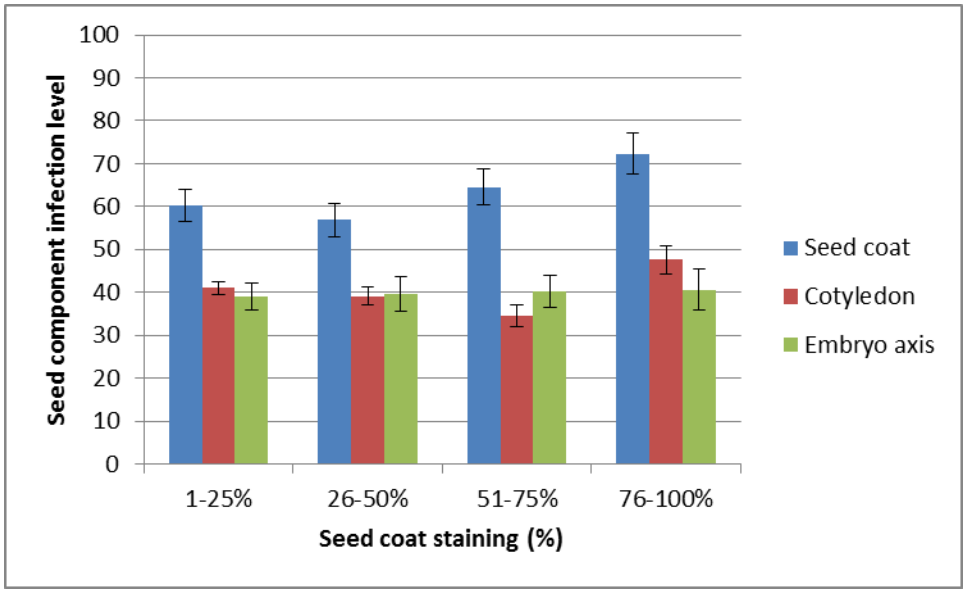


Fig 4.7 Percentage of seed infected with *Ascochyta pisi* on the seed coat, cotyledon and embryo from CDC Patrick field pea seeds naturally infected with *Ascochyta pisi* and separated into four seed coat staining categories.

4.4.4 Discussion

The aim of this study was to determine the importance of seed-borne inoculum of *A. pisi* in seed-to-seedling transmission by using naturally infected field pea seed. Field experiments revealed that seedling emergence from seeds with 10 % or higher *A. pisi* infection was lower than from seeds with 0.5 or 5 % infection. However, this was of minor importance in the epidemiology of the disease under field conditions considering that final *A. pisi* severity was low. Similar to a study of *P. pinodes* (Moussart et al., 1998), no visible symptoms caused by *A. pisi* appeared on the aerial parts of the seedlings. At physiological maturity, however, older leaves were visibly infected and had fruiting bodies of both *A. pisi* and *P. pinodes*. Overall, disease severity at harvest in plots seeded with seeds that had 0.5% infection levels with *A. pisi* was not different from those in plots seeded with 14.5% seed infection, which suggests that the disease did not progress from seed, or contributed to infection of aerial parts of the plants. This also agrees with the study conducted on *P. pinodes,* which showed that seed infection can have negative effects on seedling emergence and vigor, but seed-to-seedling transmission of these pathogens in the field is low (Moussart et al., 1998).

A seed component study conducted on *P. pinodes* showed that in seeds with less than 25% seed coat staining, *P. pinodes* was only detected within the tissues of the seed coat, and when the infection reached 25% the pathogen could be found in all seed components (Moussart et al., 1998). However, *A. pisi* was present in all components of the seed, regardless of the staining categories. When compared to the study conducted on *P. pinodes,* the field study and the seed component study on *A. pisi* had lower seed infection (Moussart et al., 1998). Unlike *P. pinodes*, *A. pisi* did not have visible fruiting bodies on the seed coat which makes it difficult to identify it on the seed.

At harvest, there was no difference in yield among plots planted with seeds that had different levels of seed infection. In spite of only low to moderate *A. pisi* symptoms on the pods and top nodes at physiological maturity of the plants, seed infection with *A. pisi* of more than 3% were observed. If harvested seed is used for seeding a crop, the Saskatchewan Pulse Growers and the Government of Saskatchewan recommends a threshold level of 10% of seed infection with Ascochyta blight pathogens, as long as the percentage of germination is high and seedlings have good vigor (Saskatchewan Pulse Growers, 2016). Diseased seeds are often shrunken or discolored, but from the seed component studies, it was also evident that visual categorization of seeds based on seed staining was a challenge, because sometimes seeds might be shrunken or discolored in the absence of *A. pisi* infection or there were other pathogens on the seeds. Cleaners and color sorters can be used to remove some diseased or damaged seed to improve the seed quality for planting (Saskatchewan Ministry of Agriculture, 2015).

Wallen (1948) showed that the percentage of pea seed infected with *A. pisi* was 60% lower after three years of storage compared to when it was fresh; suggesting the viability of *A. pisi* on seed is greatly reduced over a period of three years. Experiments here indicate that 14.5% seed infection with *A. pisi* gave rise to less than 10 % infection from the harvested seeds, which is the threshold level for Ascochyta blight pathogen. Although there was no seed-to-seedling transmission or reduction in yield as a result of such seed infection levels, there was a reduction in emergence with the treatments seeded with 10 and 14.5% infection level. Maude (1966) suggested that infected seeds can be treated with captan or thiram at a concentration of 0.2% for 24 h before sowing at 30˚C to control *P. pinodes* and *A. pisi* infection in seeds. Maude (1966) showed that chemical fungicides could be applied through slurries, instead of a seed treatment as

a powder. Better penetration of captan and thiram into seeds can be achieved by soaking seeds in the fungicides suspension (Maude, 1966). Thiram and iprodine were shown to control *A. pisi* by controlling the pathogen's growth, resulting in increased emergence of 28 % (Wallen et al., 1966). However, due to the relatively low economic impact of *A. pisi*, seed treatment does not appear to be warranted here.

5. Yield loss caused by *Ascochyta pisi* in four field pea cultivars

5.1 Introduction

Field pea is one of the major crops grown in western Canada. Pea production in Canada has gradually increased to 3.8 million tonnes by 2014, but decreased to 3.2 million tonnes in 2015 due to a 7% reduction in harvested area compared to 2014 (Canadian Grain Commission, 2015). Ascochyta blight of pea is widespread throughout the world. *Peyronellaea pinodes*, one of several fungal species that can cause Ascochyta blight, is considered to cause the most economic damage worldwide (Bretag and Ward 2001; Lawyer, 1984), and can cause yield loss of 50-75 % under favorable conditions (Wallen, 1974; Xue et al., 1997). In Canada, *P. pinodes* has caused yield loss up to 25-30% by affecting leaves, stems and also pod weight (Wallen, 1965). Studies to develop a crop model by Le May et al (2005) for spring pea cultivation in France showed that all cultivars used, despite differences in their canopy structure, were affected by Ascochyta blight, which caused a reduction in photosynthesis resulting in growth and yield reductions and early senescence.

Most research on Ascochyta blight of pea since 1927 has focused on *P. pinodes*. In Canada, field samples collected from 1939 to 1950 identified that most of the Ascochyta blight was spread through seed (Skolko et al., 1954). Severe yield losses were noted in Alberta, Manitoba and Saskatchewan. Surveys conducted during the early 1970's showed *P. pinodes* was the major cause of Ascochyta blight in Canada (Xue and Burnett, 1994). Infection of nodes during the flowering stage leads to a reduction in the number of pods and mean seed weight of harvested seeds (Garry et al., 1996; Xue et al., 1997). Yield loss due to *P. pinodes* was 34 % when infections occurred at the seed filling stage, however, infection after the seed filling stage reduced seed weight by 40% (Garry et al., 1998; Xue et al., 1997). Control of Ascochyta blight is largely dependent on fungicide treatment and cultural practices. Fungicide applications of mancozeb, chlorothalonil, benomyl, have been used to control Ascochyta blight and increase yield (Warkentin et al., 1996, 2000). However, fungicide application timing and frequency need to be optimized for different fields in different regions. In France, chemical sprays currently

manage Ascochyta blight caused by *P. pinodes* as a preventive and are applied on a systematic schedule. However, fungicide application is questionable due to various factors such as high fungicide cost, variable yield potential of the cultivars, early harvest and late onset of disease in the field.

Much less is known about the relationship between disease severity and yield losses caused by *A. pisi*. Wallen (1964) found that greater loss was caused by *P. pinodes* and *Phoma medicaginis* var. *pinodella* than *A. pisi*. He also found that plots seeded with seeds infected with *P. pinodes* and *Ph. medicaginis* var. *pinodella* had foot rot symptom, but seeds infected with *A. pisi* did not. Similar results were obtained in research conducted in 1972, when yield loss up to 50% was recorded in plots inoculated with *P. pinodes* and *Ph. medicaginis* var. *pinodella*, whereas less yield loss was observed in plots inoculated with *A. pisi* (Wallen, 1974). Premature defoliation and high infection was also observed in the plots inoculated with *P. pinodes* and *Ph. medicaginis* var. *pinodella,* when compared to *A. pisi* (Wallen, 1974).

An understanding of the relationship between disease severity and yield loss is essential for implementing a disease management strategy. The aim of the present study was to examine the effect of *A. pisi* on yield components under field conditions, and to identify the relationship between disease severity and yield loss in trials using four different pea cultivars. Fungicides were applied to keep the disease levels in control plots as low as possible.

5.2 Materials and Methods

5.2.1 Experimental design and agronomic management

For this study, the AAFC Swift Current Research Center and a field at Stewart Valley were chosen in 2012 and 2013 where there had been a high incidence of *A. pisi* in the past. In 2014, experiments were established under natural conditions at Swift Current and under irrigation at Saskatoon.

The experiment was designed as a split plot with four replications where the main plots were treated twice in 2012, 2013 at the 5-node to early flowering stages, and three times in 2014 at the 5-node, early flowering and pod filling stages with fungicides pyraclostrobin (Headline), chlorothalonil (Bravo), or pyraclostrobin/ boscalid (Headline Duo) or were not sprayed to create plots with different levels of *A. pisi* infection (Appendix 8). Sub-plot treatments were four

cultivars of which two were considered more susceptible to *A. pisi* (Cooper and SW Midas) and two to have partial resistance to *A. pisi* (CDC Bronco and CDC Golden), based on previous data, with a subplot size of 2 x 6 m. The experiment was established on 11 May, 2012, 7 May, 2013 and 5 May, 2014 at Swift Current, on 9 May, 2012 and 8 May, 2013, and on 10 May, 2014 at Stewart Valley. At Saskatoon, 2014, pyraclostrobin/boscalid (Headline Duo) and chlorothalonil (Bravo) were sprayed on 17 June, 3 July, and 17 July, 2014 (podding stage) respectively. Plots were irrigated immediately after inoculum was spread and as needed. Information on agronomic management and the timings of fungicide application at each site-year and the rate of active ingredient used during the field season is given in Appendix 8.

5.2.2 Preparation of artificial inoculum

In 2014 at Saskatoon, plots were artificially inoculated with *A. pisi* seed inoculum. Faba beans were soaked overnight in cold water, then autoclaved in Nalgene bottles for 2 cycles with a cooling period between cycles. Once cooled, faba beans were inoculated with a spore suspension of 2 x 10^5 spores ml^{-1} of *A. pisi* prepared as previously described. Nalgene bottles were placed under fluorescent lamps and shaken every third day to ensure even growth of the *A. pisi* inoculum on seeds. After 1 to 2 weeks, beans were covered with mycelium and were dried inside a bio-safety cabinet before storage under cool and dry conditions. The seeds were roughly ground before being spread in the field at approximately 65 g m^2 on 15 June and 13 July, 2014. All plots were inoculated with *A. pisi* including the unsprayed plots.

5.2.3 Data collection and statistical analysis

Severity of *A. pisi* and *P. pinodes* were scored at the flowering, podding stage and at maturity of pea plants, using the disease rating scale of 0-10 described in Section 3.2.3.3. Seed quality and infection levels were assessed at the end of the season through seed testing as previously described (Section 4.1.1.4). The experiments were harvested on 13 August, 2012, 19 August, 2013, and 2 September, 2014 at Swift Current, on 16 August, 2012 and 19 August, 2013 at Stewart Valley, and on 21 August, 2014 at Saskatoon.

Statistical analyses were carried out for individual years and locations and the data were also pooled for analysis to assess yield loss caused by *A. pisi*. Data were tested for homogeneity of variance using the Levene's test. Heterogeneous variances were modeled with the repeated

statement in SAS as required. Data were analyzed using the mixed model procedure of the SAS program (9.3 SAS Institute Inc., 2010). Foliar fungicide treatments and pea cultivars were considered fixed effects. Block and block by fungicide effects were considered as random effects for data from each location and year, whereas for pooled data location-year, replication nested in location-year and replication by fungicide treatments nested in location-year were considered random effects. At Swift Current 2014, there was irregular germination of seeds, so data analysis was done using germination percentage as a covariate in the analysis. The result revealed that the co-variate emergence had no effect, so was dropped from the model.

5.3 Results

5.3.1 Disease severity

Disease severity caused by *A. pisi* and *P. pinodes* were very low to moderate in all three years ranging between 5 and 45 % (Fig 5.1), primarily because of warm and relatively dry weather in the second half of each growing season (Appendix 7). Except for Swift Current 2013, Saskatoon 2014 and the pooled data from all three years, fungicide treatments had no significant effects on the *A. pisi* severity on the plants ($P > 0.2$). At Swift Current 2013 ($P = 0.0287$), Saskatoon 2014 ($P = 0.0075$) and pooled data from all three years ($P = 0.0004$), the fungicide treatment had a significant effect on the *A. pisi* severity on plants; unsprayed treatments had higher *A. pisi* severity compared to fungicide treatments. Except for Swift Current 2013 ($P < 0.0001$), cultivars had no significant effect on the *A. pisi* severity on the plants at any location ($P > 0.3$). At Swift Current 2013, CDC Bronco had higher *A. pisi* severity compared to CDC Golden, Cooper and SW Midas. Except for Swift Current 2013 ($P < 0.0001$) and on the pooled data ($P = 0.0113$), the fungicide by cultivar interaction was not significant in any of the experiments ($P > 0.4$). In Swift Current 2013, unsprayed treatments of Cooper and CDC Bronco had higher disease when compared to fungicide treatments of Cooper and CDC Bronco, but there was no difference between unsprayed and fungicide treatments of CDC Golden and SW Midas. In pooled data, Cooper, SW Midas CDC Golden and CDC Bronco unsprayed treatments had higher disease severity when compared to fungicide treatments of Cooper, SW Midas CDC Golden and CDC Bronco (Fig 5.1).

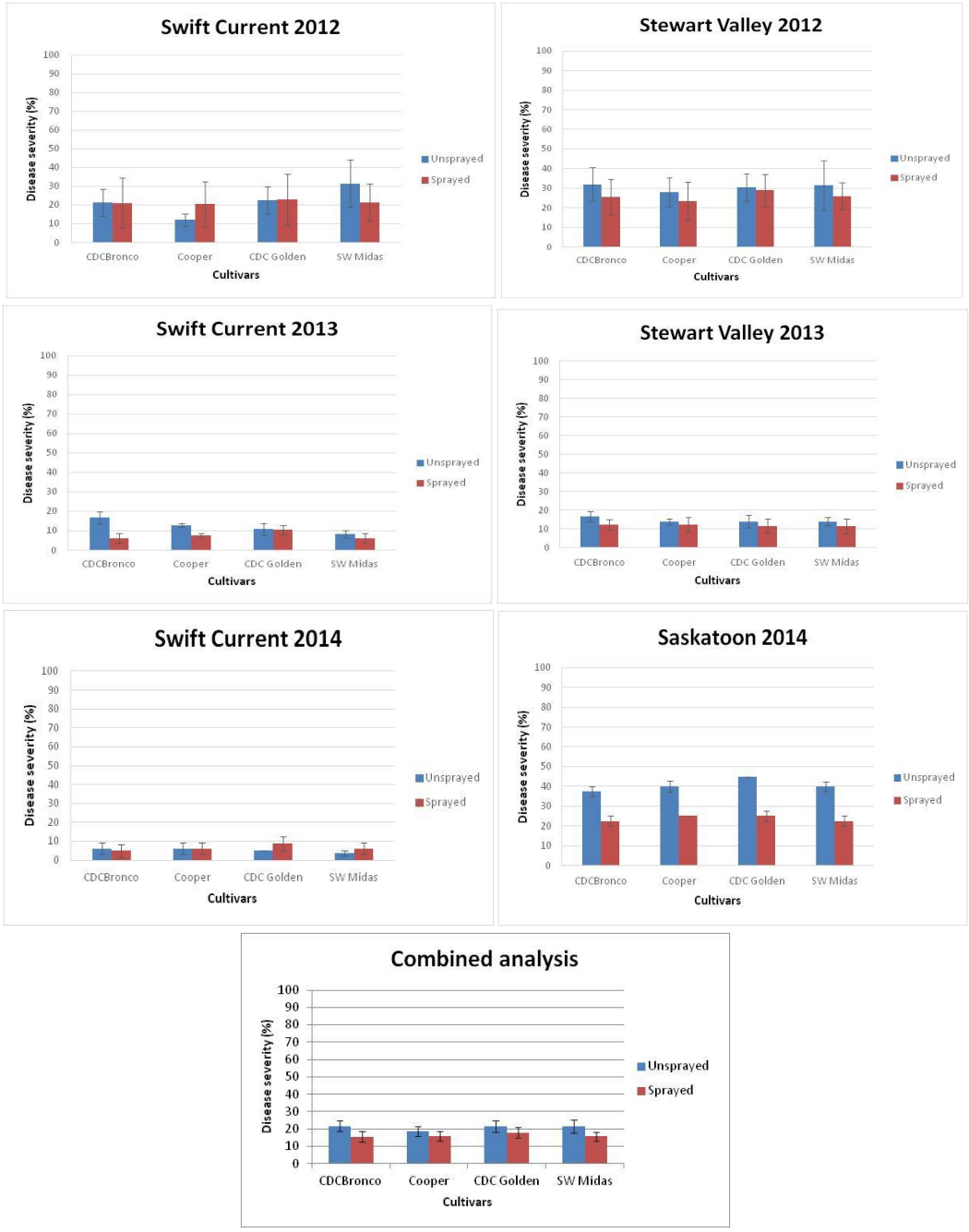


Fig 5.1 Disease severity caused by *A. pisi* in plots treated with fungicides to create low diseased plots, and unsprayed plots to create highly diseased plots of pea cv. CDC Bronco, Cooper, CDC Golden, SW Midas under natural inoculum conditions at Stewart Valley 2012, 2013, 2014 and Swift Current 2012, 2013 and 2014, and in an inoculated experiment at Saskatoon in 2014.

Except at Stewart Valley 2012 and for the pooled data (P = 0.0156), fungicide treatments had no significant effects on the *P. pinodes* disease severity on the plants (P > 0.2). At Stewart Valley 2012 and pooled data, unsprayed treatments had higher disease severity when compared to fungicide treatments. Except for Swift Current 2014, cultivars had significant effects on the *P. pinodes* severity (Swift Current 2012: P = 0.0002; Stewart Valley 2012: P = 0.0356; Swift Current 2013: P = 0.0016; Stewart Valley 2013: P = 0.0045; Saskatoon 2014: P = 0.0042; Pooled data: P < 0.0001). In Swift Current 2012, 2013 and Stewart Valley 2012 CDC Golden and SW Midas had higher *P. pinodes* severity compared to CDC Bronco and Cooper, whereas at Saskatoon 2014, CDC Golden had higher *P. pinodes* disease severity compared to CDC Bronco, Cooper and SW Midas. Pooled data analysis showed that SW Midas and CDC Golden had higher disease severity compared to Cooper and CDC Bronco. At Stewart Valley 2013, CDC Bronco had high *P. pinodes* severity when compared to Cooper and SW Midas. Pooled analysis done for *P. pinodes* showed that the fungicide by cultivar interaction (P = 0.8359) had no significant effect on *P. pinodes* severity (Fig A8.1).

5.3.2 Yield

Except for Swift Current 2012 and Saskatoon 2014 (P < 0.4), cultivars had significant effects on yield (Stewart Valley 2012: P = < 0.0001; Swift Current 2013: P = 0.0234; Stewart Valley 2013: P = 0.0031; Swift Current 2014: P < 0.0001; Pooled data: P = 0.0134). SW Midas had significantly lower yield compared to that of Cooper, CDC Golden and CDC Bronco at Stewart Valley 2013 and Swift Current 2014, whereas at Swift Current 2013 CDC Golden had lower yield compared to CDC Bronco (Fig 5.2). At all locations and for the pooled data fungicide treatments and the fungicide by cultivar interaction had no significant effect on yield (P > 0.2) (Fig 5.2).

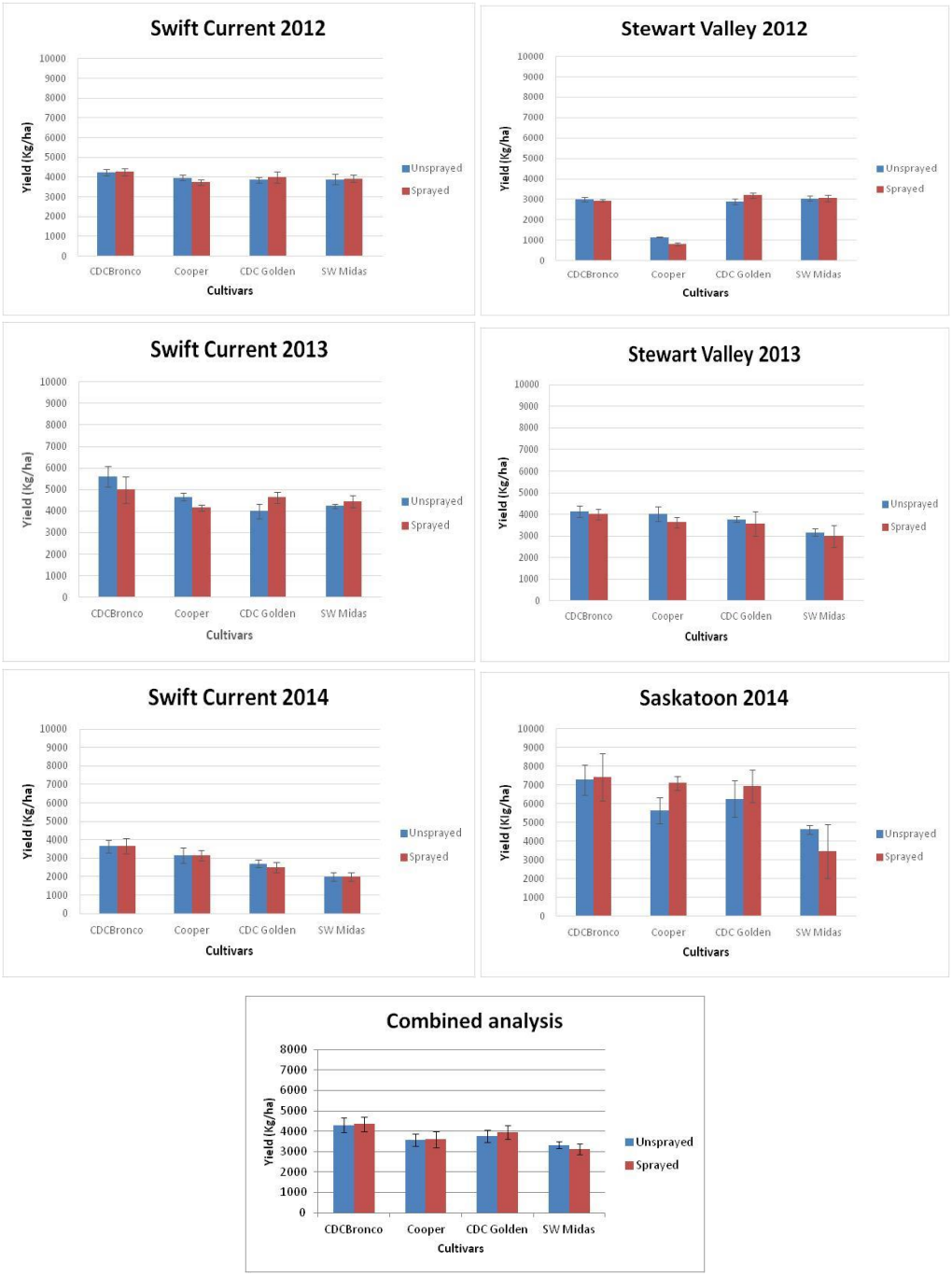


Fig 5.2 Seed yield from plots treated with fungicides to create low diseased plots, and unsprayed plots to create highly diseased plots of pea cv. CDC Bronco, Cooper, CDC Golden, SW Midas under natural inoculum conditions at Swift Current 2012, 2013, 2014 and at Stewart Valley 2013 and in an *A. pisi* inoculated experiment at Saskatoon in 2014.

5.3.3 Seed Infection of harvested seeds

In 2012, disease severities caused by *A. pisi* on plants were extremely low, so testing for seed infection was not carried out. In other years, the incidence of seed infection ranged from 5 to 13%. Seed infection levels of *A. pisi* and *P. pinodes* from the harvested seeds from 2013 were very low. Cultivars, fungicide treatments and fungicide by cultivar interaction had no significant effect on the seed infection with *A. pisi* ($P > 0.2$). At Saskatoon 2014, Swift Current 2014 and for pooled data (Fig 5.3) from 2013 and 2014 fungicide treatments had a significant effect on the *A. pisi* seed infection ($P < 0.03$). The unsprayed treatments had higher *A. pisi* seed infection from the harvested seeds when compared with fungicide-treatments. Cultivar or fungicide by cultivar interaction had no effect on *A. pisi* seed infection.

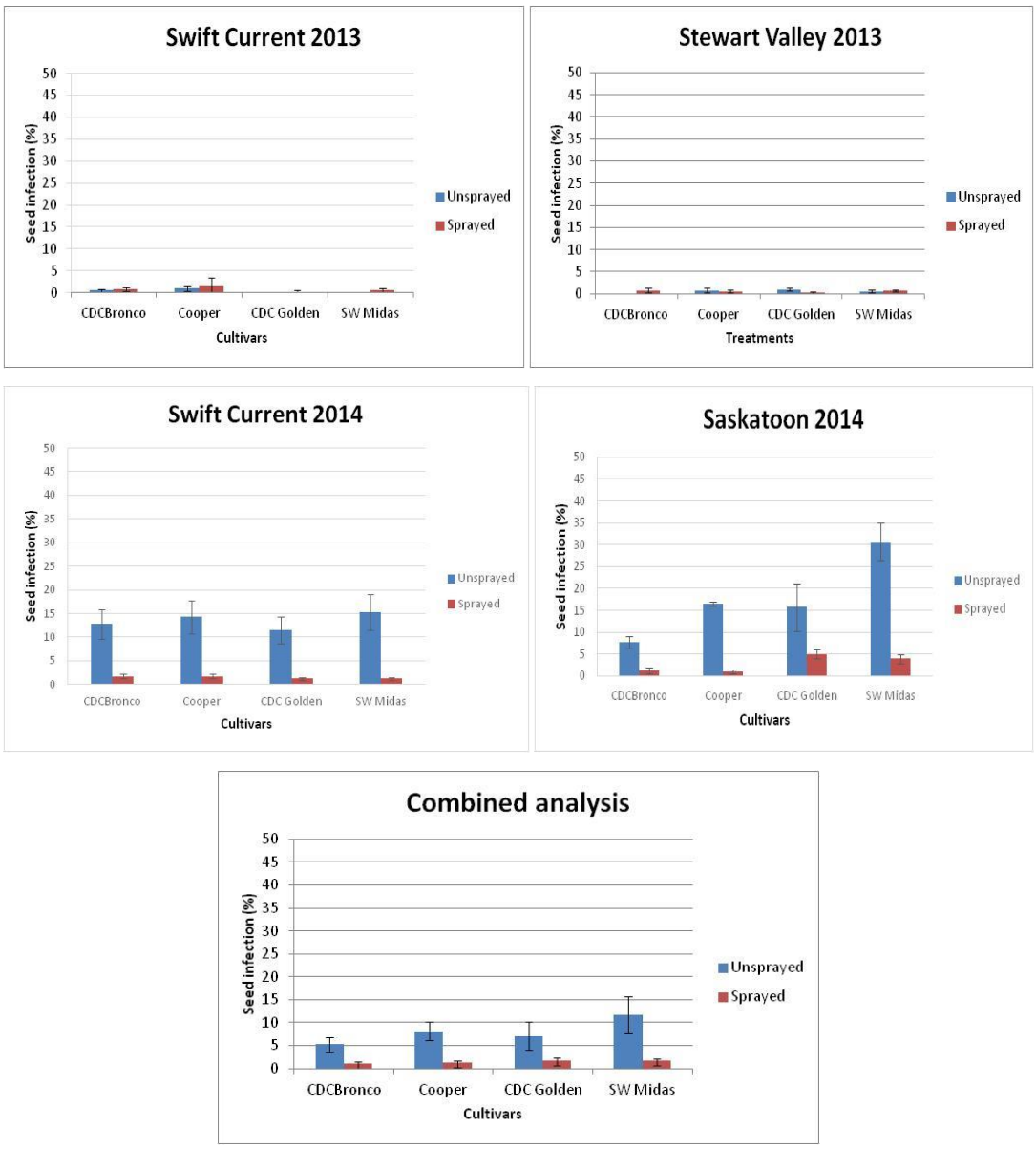


Fig 5.3 Incidence of seed infection with *A. pisi* of seeds harvested from plots treated with fungicides to create low diseased plots, and unsprayed plots to create highly diseased plots of pea cv. CDC Bronco, Cooper, CDC Golden, and SW Midas under natural inoculum conditions at Swift Current 2012, 2013, 2014 and at Stewart Valley 2013 and in an *A. pisi* inoculated experiment at Saskatoon in 2014.

5.4 Discussion

Ascochyta pisi has been reported on pea in Saskatchewan since the early 1960's, but the introduction of resistant varieties during that period controlled Ascochyta leaf spot caused by this pathogen (Lyall and Wallen, 1958). No quantification of yield loss due to this pathogen has been done in recent decades; therefore, this study were conducted to determine the relationship of *A. pisi* symptom severity and yield losses using two susceptible and two partially resistant cultivars. There was no difference in yield between fungicide treatments and non-treated controls. This may have been because disease severity was very low in all three years. Indeed, results from the 2012 and 2013 showed that there were no differences in *A. pisi* severity between the fungicide treated and non-treated plots or among cultivars. In both years, fungicides were applied at the 5-node to early flowering stages, at which time the canopy were not yet closed facilitating spray penetration, but not disease development. It is possible that this was too early, which may explain the lack of fungicide treatment effects in those years. In 2014 at Saskatoon, fungicide treatments were applied during the 5-node stage, early flowering and pod setting stages resulted in a significant reduction of disease under irrigation with artificial inoculation of *A. pisi*.

The level of *P. pinodes* infection on the plants can vary according to the micro-climate inside the canopy or the inoculum concentration (Roger et al., 1998). He also reported that lower leaves on the plant were heavily infected due to the longer wetness period, which creates a more favourable environment for these pathogens than on the upper leaves. Gent (1988) reported that crops watered by overhead sprinklers had high *A. pisi* infection because the foliage remained wet for long periods. In an experiment conducted in 2014 at Saskatoon it was observed that artificial grain inoculation and with overhead sprinkler irrigation did not increase the disease level in the field. Despite low disease severity in the field, *A. pisi* was isolated from harvested seed. Seed from the fungicide treatments in 2014 and for pooled data from 2013 and 2014 had lower seed infection than seed from the unsprayed treatment, although fungicides had no effect on *A. pisi* severity on the plants at all sites and in either years. Therefore, the mechanism of spread to seeds is unclear considering that plants had low *A. pisi* symptoms. Unlike for *P. pinodes*, sexual fruiting structures that could release airborne ascospores of *A. pisi* have only been observed under controlled condition. Nevertheless observations here indicate that late season ascospore showers may be the cause of seed infection. Seed infection data indicate that an application of a

foliar fungicide can reduce *A. pisi* seed infection. However, whether a fungicide treatment to protect seed is economical is questionable considering that experiments on seed-to-seedling transmission showed that although seed infection of *A. pisi* can reduce seedling emergence, this has no effect on disease severity on plants or on seed yields.

It was previously shown that the impact of fungicide to control *P. pinodes* on seed yield is less consistent than its effect on disease severity (Gossen et al., 2001). Experiments conducted to evaluate the effect of different races of *A. pisi* on yield showed that Race 3 and 4 reduced yield and plots inoculated with the combined races reduced yield by 11%, but only in cases of very severe infection with *A. pisi* (Wallen, 1964). Wallen (1964) reported that spraying or dusting pea crops with fungicides had little value in controlling *A. pisi.* He also found that one antibiotic and cycloheximide controlled *A. pisi*, but only at high concentrations, which resulted in phytotoxic effects. From this experiment he also observed that only a high incidence of *A. pisi* seed infection resulted in yield loss whereas much lower seed infection of *P. pinodes* and *A. pinodella* was sufficient to cause yield loss in pea. From my experiment, three fungicide applications had a positive effect on pea seed infection with *A. pisi*, but due to the relatively low economic impact of *A. pisi*, application of a fungicide for disease control was likely not cost-effective.

6. Genetic control of *Ascochyta pisi* resistance

6.1 Introduction

Pea is an important source of vegetable protein and plays a major role in fixing atmospheric nitrogen in the soil. Pea production in Canada began to increase in 1985 due to the opening of the European market and is now widely cultivated in Saskatchewan, Alberta and Manitoba. (Slinkard, 1994). The average yield of pea in Saskatchewan is 2500 kg per ha. Pea yields are unstable due to adverse effects of biotic and abiotic stresses. One of the greatest biotic stresses reducing potential yield is Ascochyta blight, caused by a complex of pathogens that is known to cause yield losses 50% to 75% in severely affected fields (Wallen, 1974; Xue et al., 1997). Progress in pea resistance breeding to the Ascochyta blight pathogens has been slow due to the complex nature of resistance (Skolko et al., 1954) and the suggestion that resistance to foliar, stem, seed and root infection appears to be controlled by different genes for each pathogen (Clulow et al., 1992). Resistance to *A. pisi* was found to be governed by three dominant genes, two complementary genes and a single dominant and recessive gene (Wallen and Jeun, 1968; Darby et al., 1985), while no genes have been identified conveying good resistance to *P. pinodes.* Another difficulty in breeding resistance to Ascochyta blight pathogens is the potential presence of physiological races for each species. In Canada, at least four distinct races of *A. pisi* were identified, each with a geographically limited distribution (Jones, 1927). Studies of *P. pinodes* suggests that pathotypes exist among *P. pinodes* in Canada (Xue et al., 1998) and Germany (Nasir and Hoppe, 1991), but in Western Australia (Wroth, 1998b) and France (Onfroy et al., 1999) no pathotypic groups among isolates are identified.

In Canada (Xue and Warkentin, 2001) and the United Kingdom (Clulow et al., 1991), potential resistance sources were found to convey moderate resistance to *P. pinodes*. After the introduction of varieties with resistance to leaf and pod spot caused by *A. pisi*, this disease was rarely observed in Canada (Wallen and Jeun, 1968), until the recent report in 2012 (Warkentin et al., 2012). Resistance identified in pea against *P. pinodes* so far provides only partial resistance. The heritability and the expression of resistance to *P. pinodes* can be affected by temperature, the

amount of inoculum in the field and also depends on the age of the plants (Zhang et al., 2006). Quantitative Trait Loci (QTL) for developing resistant genotypes are increasingly used in breeding programs.

Thirteen QTLs have been identified for resistance to *P. pinodes*, which are scattered across the seven linkage groups of pea (Timmerman-Vaughan et al. 2004). QTLs for resistance to *P. pinodes* pathogens were co-located with those are associated with plant height and flowering date (Timmerman-Vaughan et al., 2002; 2004). Six QTLs for resistance to *P. pinodes* were identified at the seedling stage under controlled conditions and ten QTLs for resistance at the adult stage under field conditions were identified (Prioul et al., 2004). Study of these QTLs to improve resistance has increased knowledge of the genetic architecture of partial resistance in pea to *P. pinodes*, but the exact genes controlling resistance are still unknown. At present, there are breeding efforts for *P. pinodes*, but the recent re-emergence of *A. pisi* has warranted the need to increase the genetic resistance to *A. pisi*.

The aim of this study was to determine the genetic control of *A. pisi* by testing the Recombinant Inbred Line (RIL) population PR-10 developed by single seed descent method from a cross between the susceptible variety Cooper and the partially resistant variety CDC Bronco. The susceptibility of Cooper and partial resistance in CDC Bronco had previously been determined in greenhouse experiments when screening 18 pea cultivars commonly grown in Saskatchewan for resistance to this *A. pisi*. Disease severity reached 6% on CDC Bronco and 29% on Cooper (Banniza et al., 2007).

6.2 Materials and Methods

6.2.1 Experimental design and agronomic management

The experiments were seeded at the AAFC Swift Current Research Center and at Stewart Valley in 2012 and 2013, where there had been a high incidence of *A. pisi* in the past and at Saskatoon under irrigation and at the AAFC Swift Current Research Center in 2014. Due to limitations in space, only the 29 most resistant and 29 most susceptible RILs and the parents were selected for evaluation in this field trial. The RILs in the F_7 generation had been selected based on their response to *A. pisi* in a replicated greenhouse experiment. The field experiments were conducted using an RCBD with three replications and were established on 25 April, 2012, 8 May, 2013 and

1 May, 2014 at Swift Current, on 9 May, 2013 at Stewart Valley and on 10 May, 2014 at Saskatoon. Agronomic data collected during the field seasons is listed in Appendix 9. Inoculum of *A. pisi* was prepared as described above, and was spread in the field on 15 June, 2014 and 13 July, 2014 at Saskatoon, and 20 June, 2014 at Swift Current (Appendix 9).

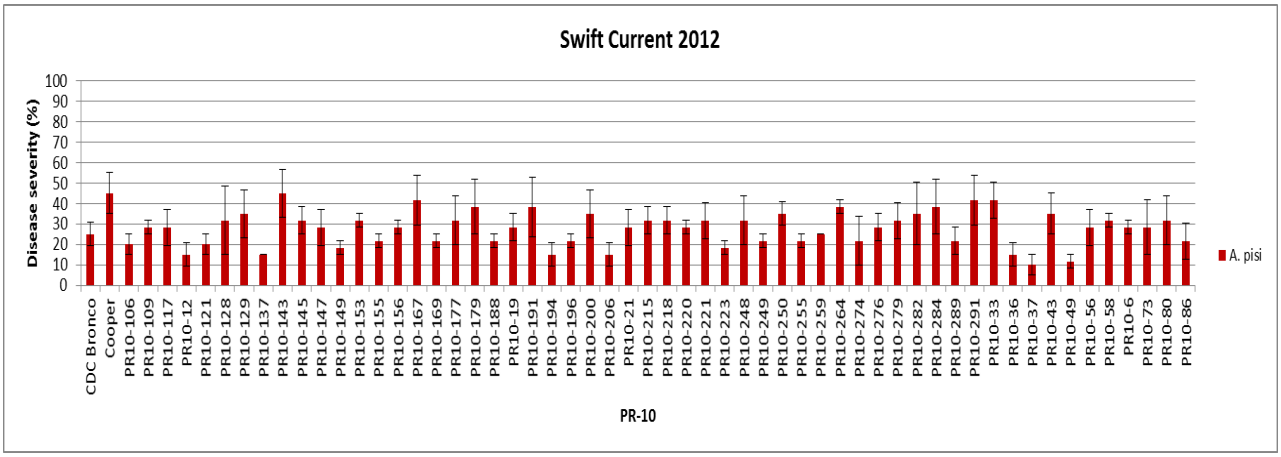
6.2.2 Plant assessment and statistical analyses

Plants were assessed for severity of *A. pisi* and *P. pinodes* infection at the flowering stage and at maturity using the 0-10 scale as previously described. All statistical analyses were carried out using SAS (SAS 9.3 Institute Inc., 2010). The data from each location were tested for homogeneity of variance. Heterogeneous variances were modelled with the repeated statement as required. Means of disease severity calculated from five plants per plot and yield from entire plots were analyzed with the mixed model procedure where RILs were considered fixed effects, whereas block was considered a random effect. For pooled data, location-year and block nested in location-year were considered random effects. Linear contrast analysis was done to compare responses of the parents (CDC Bronco and Cooper) to *A. pisi*.

6.3 Results

6.3.1 Disease Severity

Disease levels on pea were moderately high in Swift Current 2012, but low in 2013 and 2014. The lowest *A. pisi* level of 5% was observed in cv. Cooper peas at Swift Current and Stewart Valley 2013 and the highest level of 45 % in Cooper at Swift Current 2012 (Fig 6.1). Except for Swift Current 2014 ($P < 0.0001$), RILs did not differ significantly in *A. pisi* severity ($P > 0.3$). For all three years, locations and for pooled data contrast analysis revealed no difference between the parents in *A. pisi* severity ($P > 0.3$). Except for pooled data analysis ($P = 0.0023$), RILs also had no significant effect on *P. pinodes* severity ($P > 0.2$). Contrast analysis between the parents showed that they did not differ in *P. pinodes* severity ($P > 0.1$).


Swift Current 2012
Disease severity (%)
100
90
80
70
60
50
40
30
20
10
0
CDC Bronco
Cooper
PR10-106
PR10-109
PR10-117
PR10-12
PR10-121
PR10-128
PR10-129
PR10-137
PR10-143
PR10-145
PR10-147
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PR10-167
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PR10-177
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PR10-188
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PR10-276
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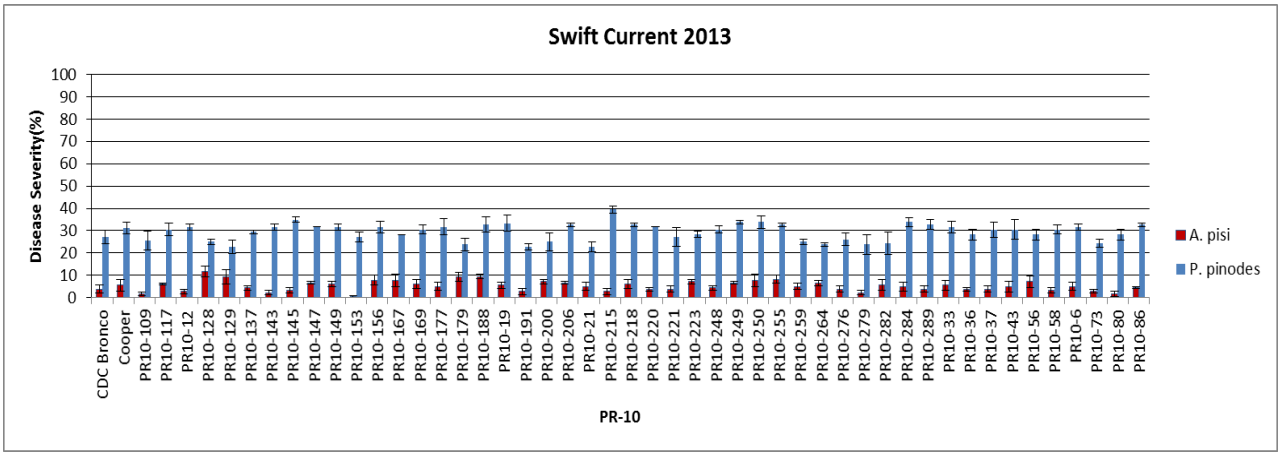
PR10-284
PR10-289
PR10-291
PR10-33
PR10-36
PR10-37
PR10-43
PR10-49
PR10-56
PR10-58
PR10-6
PR10-73
PR10-80
PR10-86
PR-10
A. pisi


Swift Current 2013
Disease Severity(%)
100
90
80
70
60
50
40
30
20
10
0
CDC Bronco
Cooper
PR10-109
PR10-117
PR10-12
PR10-128
PR10-129
PR10-137
PR10-143
PR10-145
PR10-147
PR10-149
PR10-153
PR10-156
PR10-167
PR10-169
PR10-177
PR10-179
PR10-188
PR10-19
PR10-191
PR10-200
PR10-206
PR10-21
PR10-215
PR10-218
PR10-220
PR10-221
PR10-223
PR10-248
PR10-249
PR10-250
PR10-255
PR10-259
PR10-264
PR10-276
PR10-279
PR10-282
PR10-284
PR10-289
PR10-33
PR10-36
PR10-37
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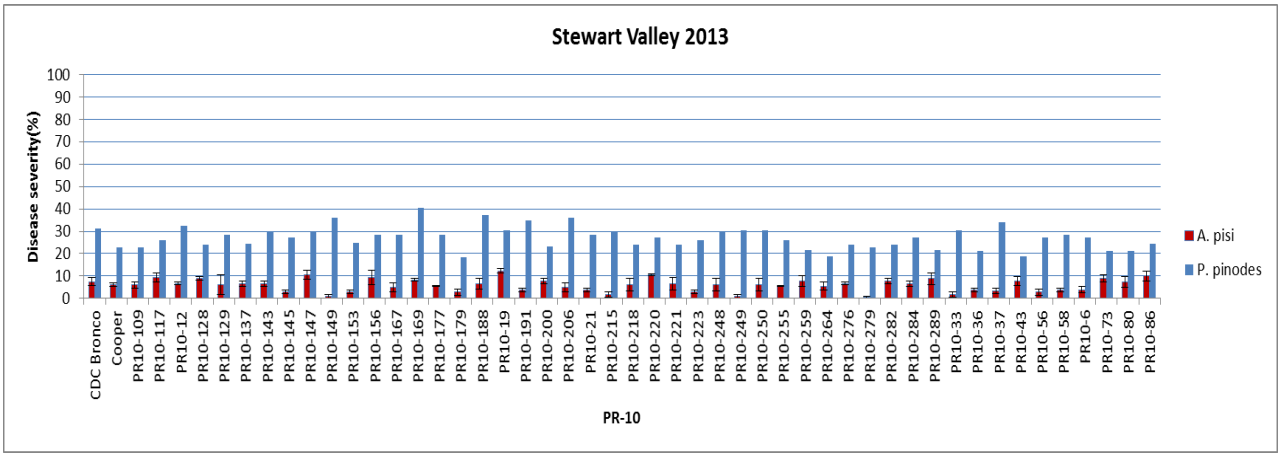
PR10-73
PR10-80
PR10-86
PR-10
A. pisi
P. pinodes


Stewart Valley 2013
Disease severity(%)
100
90
80
70
60
50
40
30
20
10
0
CDC Bronco
Cooper
PR10-109
PR10-117
PR10-12
PR10-128
PR10-129
PR10-137
PR10-143
PR10-145
PR10-147
PR10-149
PR10-153
PR10-156
PR10-167
PR10-169
PR10-177
PR10-179
PR10-188
PR10-19
PR10-191
PR10-200
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PR10-264
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PR10-284
PR10-289
PR10-33
PR10-36
PR10-37
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PR10-58
PR10-6
PR10-73
PR10-80
PR10-86
PR-10
A. pisi
P. pinodes

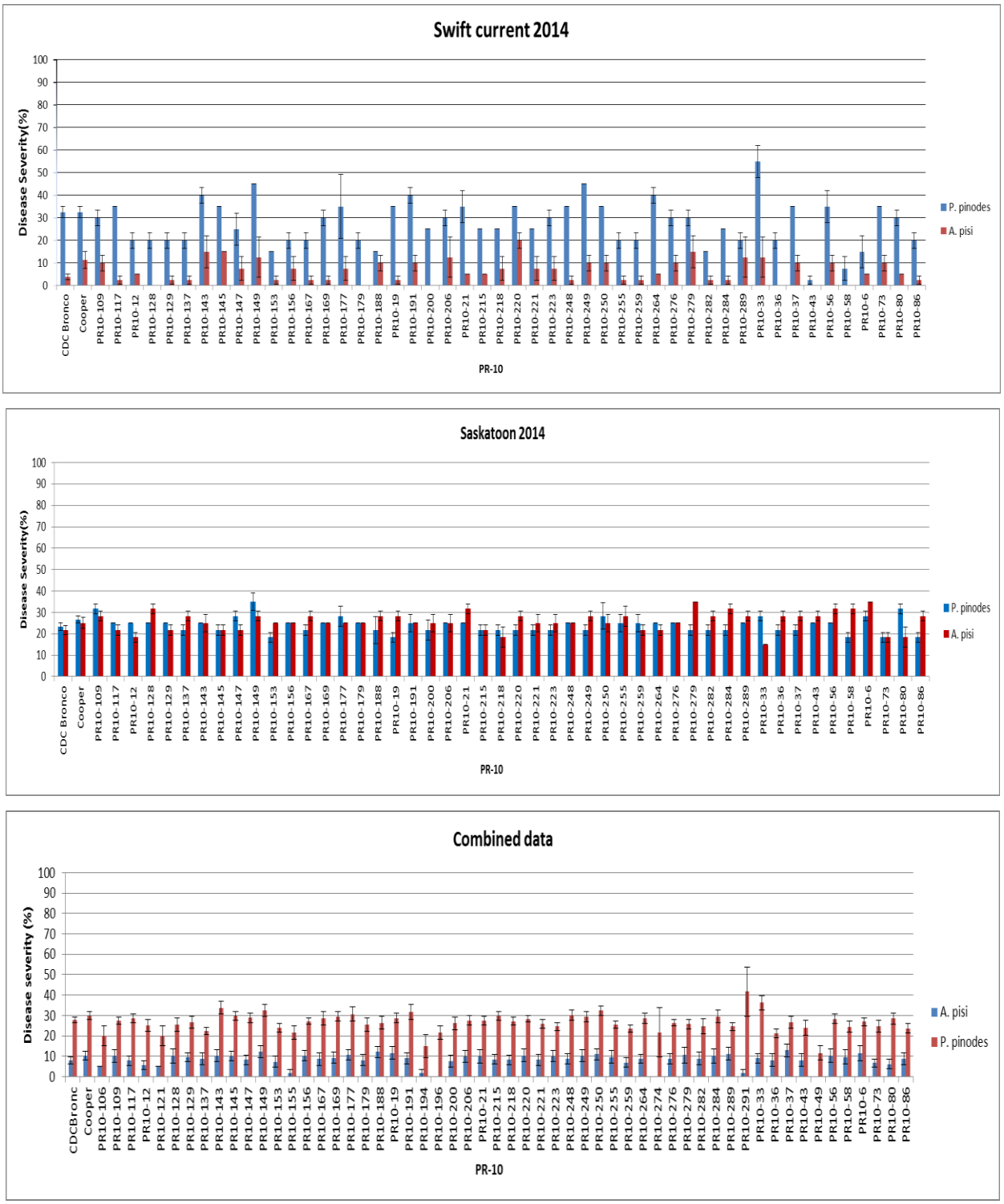
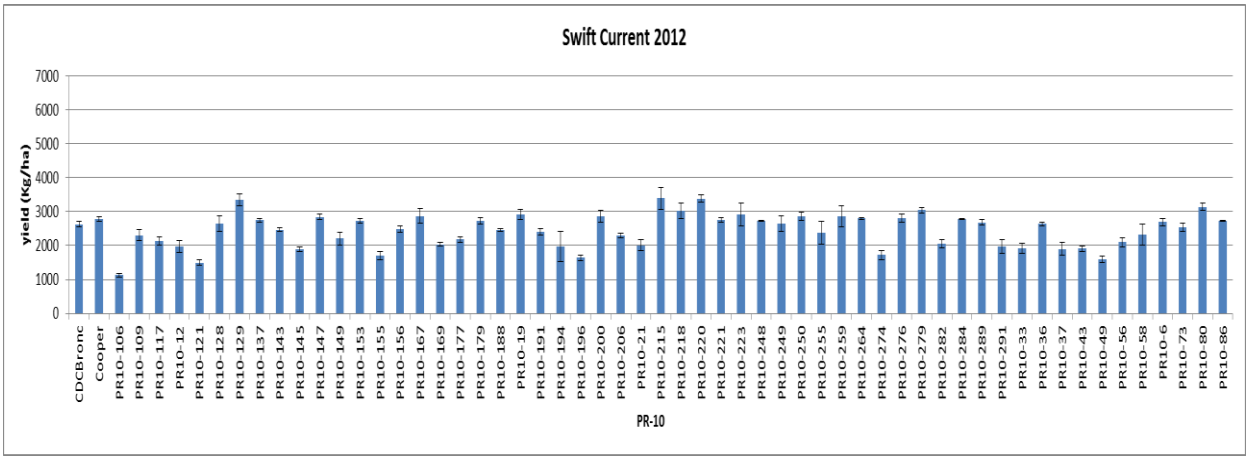


Fig 6.1. Disease severity caused by *Ascochyta pisi* and *Peyronellaea pinodes* on PR-10 RILs developed from a cross of pea cv. CDC Bronco and Cooper tested under natural condition at Swift Current and Stewart Valley in 2012 and 2013, and under irrigation at Saskatoon and natural condition in Swift Current 2014.

6.3.2 Yield

There was variability in seed yields among the RILs at Swift Current 2013 ($P < 0.0001$), Stewart Valley 2013 ($P < 0.0001$), Saskatoon 2014 ($P = 0.0142$) and Swift Current 2014 ($P < 0.0001$) that appeared to be unrelated to *A. pisi* and *P. pinodes* severity. Contrast analysis revealed that the parents differed in yield (Swift Current 2012: $P = < 0.0001$; Stewart Valley 2013: $P < 0.0001$; Saskatoon 2014: $P = 0.0043$; Swift Current 2014: $P = 0.0338$) and CDC Bronco yielded more than Cooper**,** except for Swift Current 2013 and the pooled data ($P < 0.2$)**.** However**,** pooled data analysis shows that RILs had no significant effect on yield ($P > 0.5$).


Swift Current 2012
yield (Kg/ha)
7000
6000
5000
4000
3000
2000
1000
0
CDCBronc
Cooper
PR10-106
PR10-109
PR10-117
PR10-12
PR10-121
PR10-128
PR10-129
PR10-137
PR10-143
PR10-145
PR10-147
PR10-149
PR10-153
PR10-155
PR10-156
PR10-167
PR10-169
PR10-177
PR10-179
PR10-188
PR10-19
PR10-191
PR10-194
PR10-196
PR10-200
PR10-206
PR10-21
PR10-215
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PR10-250
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PR10-259
PR10-264
PR10-274
PR10-276
PR10-279
PR10-282
PR10-284
PR10-289
PR10-291
PR10-33
PR10-36
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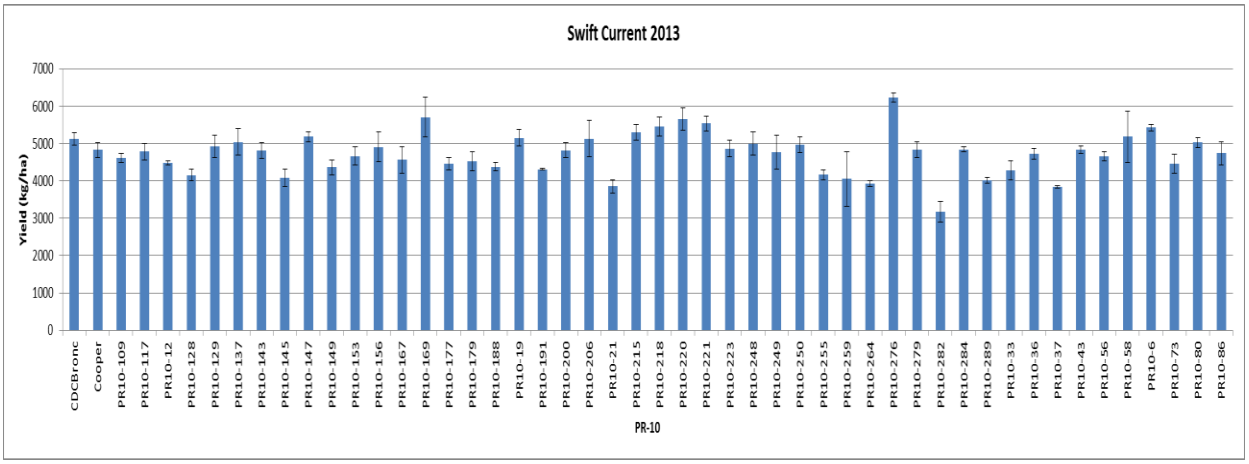
PR10-56
PR10-58
PR10-6
PR10-73
PR10-80
PR10-86
PR-10


Swift Current 2013
Yield (kg/ha)
7000
6000
5000
4000
3000
2000
1000
0
CDCBronc
Cooper
PR10-109
PR10-117
PR10-12
PR10-128
PR10-129
PR10-137
PR10-143
PR10-145
PR10-147
PR10-149
PR10-153
PR10-156
PR10-167
PR10-169
PR10-177
PR10-179
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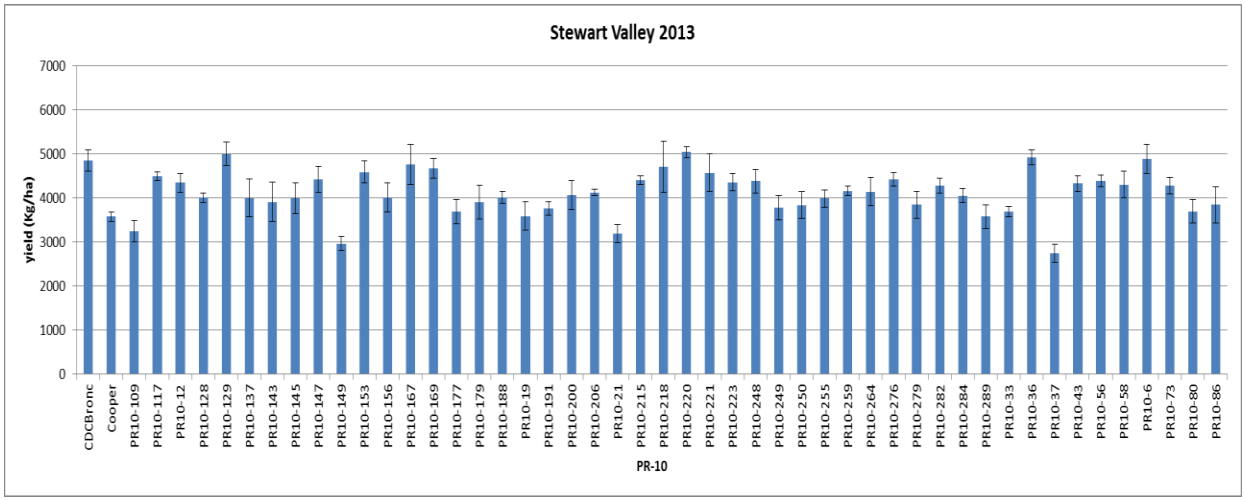
PR10-6
PR10-73
PR10-80
PR10-86
PR-10


Stewart Valley 2013
yield (Kg/ha)
7000
6000
5000
4000
3000
2000
1000
0
CDCBronc
Cooper
PR10-109
PR10-117
PR10-12
PR10-128
PR10-129
PR10-137
PR10-143
PR10-145
PR10-147
PR10-149
PR10-153
PR10-156
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PR10-6
PR10-73
PR10-80
PR10-86
PR-10

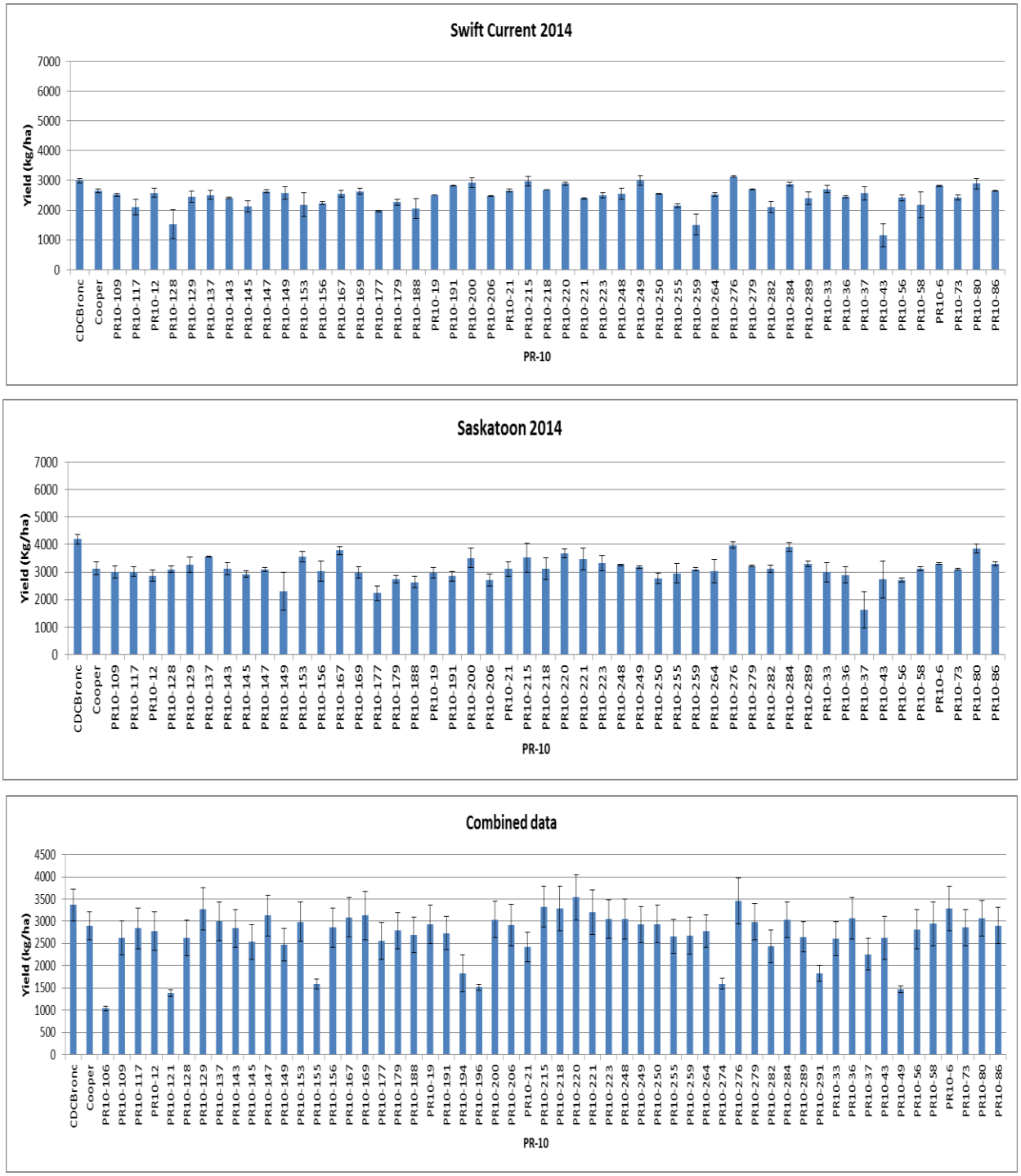

Fig 6.2 Seed yields (averaged across locations and years) for PR-10 RILs developed from a cross of pea cv. CDC Bronco and Cooper tested under natural condition at Swift Current and Stewart Valley in 2012, 2013 and under irrigation at Saskatoon and natural condition in Swift Current 2014.

6.4 Discussion

The inheritance of resistance to *Ascochyta* spp. in pea is poorly understood. The introduction of varieties resistant to *A. pisi* reduced the incidence of *A. pisi* blight in Canada (Wallen and Jeun, 1968). Screening of RIL population PR-10 developed from a cross between the susceptible variety Cooper and the partially resistant variety CDC Bronco showed there was not enough disease caused by *A. pisi* to differentiate between the parents and RILs. Disease severity on RILs was generally consistently low over three years, even with artificial inoculation of *A. pisi* under irrigated conditions at Saskatoon in 2014. There were differences in yield among the RILs, however these may not have been due to disease severity caused by *A. pisi* as the disease level was very low, but rather due to genetic differences.

Resistance to *A. pisi* were previously found to be governed by three dominant genes, two complementary genes (Wallen and Jeun, 1968) and a single dominant and recessive gene (Darby et al., 1985). Brittain (1987) found a wide range of resistance in *Pisum* lines to infection by *A. pisi* and identified three new genes, *Rap*-1, *Rap-3* and *Rap-4*. *Rap-1* appeared to confer general resistance while *Rap-3* and *Rap-4* appeared to be race-specific. However, Csizmadia (1995) suggested that a single dominant gene governs resistance to *A. pisi*. The presence of physiological races for each pathogen species will makes it more difficult to identify resistance and to develop resistant varieties. Jones (1927) was the first to report on physiological specialisation in *A. pisi* and differences in pathogenicity between isolates of *A. pisi* from different countries. Since then, many studies have reported the distinction of races in *P. pinodes, A. pisi* and *Ph. medicaginis* var *pinodella*. In Canada, at least four distinct races of *A. pisi* were found and each race had a geographically limited distribution. It was speculated that this might be due to similar pea varieties in each region and different climatic conditions in those regions (Wallen, 1954). Vladimirseva et al (1989) isolated and identified 31 races of *A. pisi* based on the pathogenic behavior on pea. Matthews and Dow (1974) from the John Innes Institute (UK) reported that the resistance to Race 3 of *A. pisi* is controlled by a single recessive gene. At present, the resistance to *P. pinodes* is studied around the world, but efforts to improve the resistance to *A. pisi* are needed if its frequency increases. Ali Raza Jamali et al (2005) found that eight pea genotypes inoculated with ten isolates of *A. pisi* collected from different locations in

Pakistan showed a significant variability in disease severity. All genotypes were susceptible to the Race-1 the most virulent one, whereas the least virulent was Race-6.

Field evaluation of PR-10 suggests that disease resistance levels in the parents did not differ as reported previously, which has raised the question whether they may not be suitable to study the genetic control of resistance to *A. pisi*. Understanding the control of resistance in pea is still of relevance and breeders will continue to search for better parents to study the inheritance of resistance.

7. General discussion and conclusion

Global food production must increase by 50% to meet the demand of the world's population by 2050. Research on the impact of climate change on plant diseases is limited (Coakley et al., 1999) due to the predictability of climate change and knowledge of its impacts on disease epidemics (Chakraborty et al., 1998). Saskatchewan is the heart of the Canadian pulse industry with 64% of the total production of field pea. Researchers at the Crop Development Center of the University of Saskatchewan are focused on meeting the overall goals of higher yield, better quality and disease resistance in pea. Ascochyta blight is the most important biotic constrain affecting the Saskatchewan pea industry. *Peyronellaea pinodes* is one of the major pathogen, which cause Ascochyta blight, but *A. pisi* has also increasingly been observed during the last decade in southern and south-western Saskatchewan. In Saskatchewan, the shift in the regional predominance of *Ascochyta* spp. may be due to a loss of resistance in the existing cultivars, climate change or variation in the timing of *A. pisi* ascospore production.

Development of plant diseases is determined by the availability of moisture and optimal temperature. The present study shows that differences in conidial germination between the two pathogens became obvious at 20°C with more than 4 h incubation, and at 25 and 30°C *P. pinodes* had consistently higher germination after 2 h of incubation. Differences in disease severity caused by the two pathogens at 20 and 25°C were similar to those observed in conidial germination. The rate of disease increase based on regression analysis was also higher for *P. pinodes* up to 25°C, but then declined sharply. From the results of this study, it appears that for both pathogens the temperature optimum is between 20 to 25˚C. Wallen (1965) reported that optimum temperature for *P. pinodes* is between 15 and 18°C. Similar studies have reported that optimum temperature for disease development was at 20°C (Bretag, 1991). Longer leaf wetness period of 8 to 12 h were required for *P. pinodes* at lower and higher temperature of 10 and 30°C for spore germination and disease severity. Roger et al (1999b) observed that for *P. pinodes* severe disease development during dry periods were only observed if conidial germination and

hyphal development occurred before they dry periods at incubation temperature of 15 to 25°C with a leaf wetness period of 6 to 8 h. Fewer disease symptoms developed when appressorium and penetration occurred after dry periods. At high temperature (30°C), an initial leaf wetness of 72 to 96 h was insufficient for severe disease development during subsequent dry periods. He also observed that the spores resumed infection when there were favourable conditions, but disease development on the plants was delayed. The tolerance of *A. pisi* to interrupting dry periods, repeated wetness periods and their survival mechanisms under these conditions are poorly understood.

Seeds are often an important source of primary inoculum. Therefore, the seed-to-seedling transmission rate of a pathogen is important, because the number of infected seeds may determine how many infected seedlings develop. Studies in sterile grit showed that *A. pisi*-infected seeds gave rise to 40% infected seedlings with lesions on the stem and on the first two leaves, whereas seeds infected with *P. pinodes* had infection developing from below and above ground symptoms (Maude 1966; Moussart et al., 1988). The present study on *A. pisi* showed 14.5% *A. pisi* infected seed used for planting had slightly reduced emergence. However, an effect of seed-to-seedling transmission of this pathogen on disease development on the plants and yield loss was not observed under field conditions. A seed component study conducted on *P. pinodes* showed that in seeds with less than 25% seed coat staining, *P. pinodes* was only detected within the tissues of the seed coat, and when the infection reached 25% the pathogen could be found in all seed components (Moussart et al., 1998). In the current study, *A. pisi* were present in all components of the seed, regardless of the staining categories. When compared to the study conducted on *P. pinodes*, the field study and the seed component study on *A. pisi* had lower seed infection (Moussart et al., 1998). Unlike *P. pinodes*, *A. pisi* did not have visible fruiting bodies on the seed coat which makes it difficult to identify it on the seed. The frequencies of transmission of *P. pinodes* increased at lower temperatures (Moussart et al., 1998) and were 100%, 61%, and 70% at 15°C, 18°C and 25°C, respectively (Corbiere et al., 1994). Even though there were a high *P. pinodes* frequency on pea seeds in Australia, a negative correlation between seed infection and Ascochyta blight severity were observed (Bretag and Brouwer, 1995a). Incubation of seed components showed that seeds with or without discoloration showed infection with *A. pisi* of all seed components. It is therefore difficult to visually distinguish between

infected and non-infected seeds. Considering that disease levels in the field were low, the mechanism of seed infection for this pathogen is mysterious and further studies are needed in this area. There is the potential for airborne ascospores to play a role in the epidemiology of *A. pisi* in Saskatchewan. Seed infection with *A. pisi* showed minimal effect on crop establishment, and no effect on disease development or seed yield.

Breeding for disease resistance is time-consuming and expensive, so it needs to focus on economically important diseases. There are many reports on yield loss caused by *P. pinodes*, whereas there are few reports on yield loss caused by *A. pisi*. Trials in Denmark showed that seed-borne *Ascochyta pisi* infection causes 0.059 kg/ha yield reduction, and sowing of seeds with 20% infected seeds were expected to reduce yield by 1.18 kg/ha (Wolffhechel and Bodker, 2005). Fungicide application was shown to control *P. pinodes* severity in pea and to increase yield by 15 to 75% (Bretag 1989b; Warkentin et al., 1996). A study by Bretag and Brouwer (1995a) showed different pea cultivars had different levels of tolerance to *P. pinodes* severity and yield loss under the same level of disease pressure. In the present study, fungicide application at low and moderate disease levels was of no benefit. When *A. pisi* infection reached 45%, it had minimal effect on pea performance. Fungicide applications may therefore not be profitable considering the fungicide cost, variable yield potential of the cultivars, early harvest and late onset of disease in the field.

Resistance to *P. pinodes* has been assessed under field conditions, but has only been done in controlled conditions for *A. pisi* (Ali et al., 1978; Kraft et al., 1998b). Resistance breeding in pea to the Ascochyta blight pathogens has progressed slowly due to the complex nature of resistance (Skolko et al., 1954) and the suggestion that resistance to foliar, stem, seed and root infection appears to be controlled by different genes for each pathogen (Clulow et al., 1992). In the present study, screening of different RILs for resistance to *A. pisi* revealed no difference in reaction of the parents, possibly due to the low disease pressure. Brittain (1987) found that three genes controlled resistance. Gene *Rap-1* controlled general resistance to *A. pisi* while *Rap-3* and *Rap-4* appeared to be race specific. However, resistance to *A. pisi* were found to be governed by three dominant genes, two complementary genes and a single dominant and recessive gene (Wallen and Jeun (1968); Darby et al., (1985)). Csizmadia (1995) found that resistance to *A. pisi* is governed by single dominant gene. For *P. pinodes,* Clulow et al (1991) suggested that single

dominant genes controlled stem and foliar resistance for *P. pinodes*. Field evaluation of PR-10 here suggested that disease resistance levels in the parents may not be as different as previously reported, which has raised the question of the suitability of this population to study the genetic control of *A. pisi* resistance.

In conclusion, experiments here showed that *P. pinodes* were more aggressive than *A. pisi*, but both pathogens had a temperature optimum of 20 to 25°C. Thus it cannot explain why *A. pisi* is more prevalent in south and south-western Saskatchewan. However, results obtained under controlled conditions for conidial germination, and disease severity caused by *A. pisi* and *P. pinodes* on pea plants in response to wetness periods and temperature may not reflect the complex situations encountered in the field and also prolonged wetness period is not common in Saskatchewan. Seed-to-seedling transmission were not observed, but seed infection was common even under low disease development on plants. No yield losses were observed, but *A. pisi* severity were only moderate at best. Results indicate that *A. pisi* does not appear to pose a major risk to pea production in Saskatchewan.

Further studies should be focused on the search for more suitable parents with differential resistance to *A. pisi* in order to study the inheritance of resistance to this pathogen. In spite of low disease severity, seed infection with *A. pisi* was observed from the harvested seeds, which warrants further research on the potential for latent infections of flowers by *A. pisi*, differences in the production of airborne ascospores of *P. pinodes* and *A. pisi*, and also to assess the possibility of an erosion of resistance to *A. pisi* in south and south-western Saskatchewan.

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Appendix

Appendix 1 Progression of conidial germination for *Ascochyta pisi* and *Peyronellaea pinodes*

For *A. pisi*, data analysis for repeat 1 revealed that incubation period ($P < 0.0001$) and the incubation period by temperature interaction ($P = 0.0009$) had significant effects on percentage of conidial germination of *A. pisi*. Temperature had no effect on conidial germination ($P = 0.0596$). At 10˚C, conidial germination did not change from 2 to 6 h, but increased after 8 and 10 h and then remained at a similar level after 12 h of incubation (Fig A1.1). At 15˚C, conidial germination did not change from 2 to 4 h, significantly increased after 6 h, and remained at a similar level up to 8 h of incubation. Germination at 10 h after inoculation had increased further and remained at a similar level after 12 h. At 20˚C, conidial germination did not change from 2 to 4 h, and increased after 6 h, up to 10 h and remained similar level up to 12 h. At 25˚C, conidial germination increased after 4 h and remained steady up to 6 h of incubation before increasing further after 8 h of incubation. Germination after 10 and 12 h was similar to that at 8 h. At 30˚C, conidial germination increased after 4 h and 6 h and then remained steady up to 8 h of incubation. Germination after 10 and 12 h of incubation was similar, but higher compared to that at 8 h.

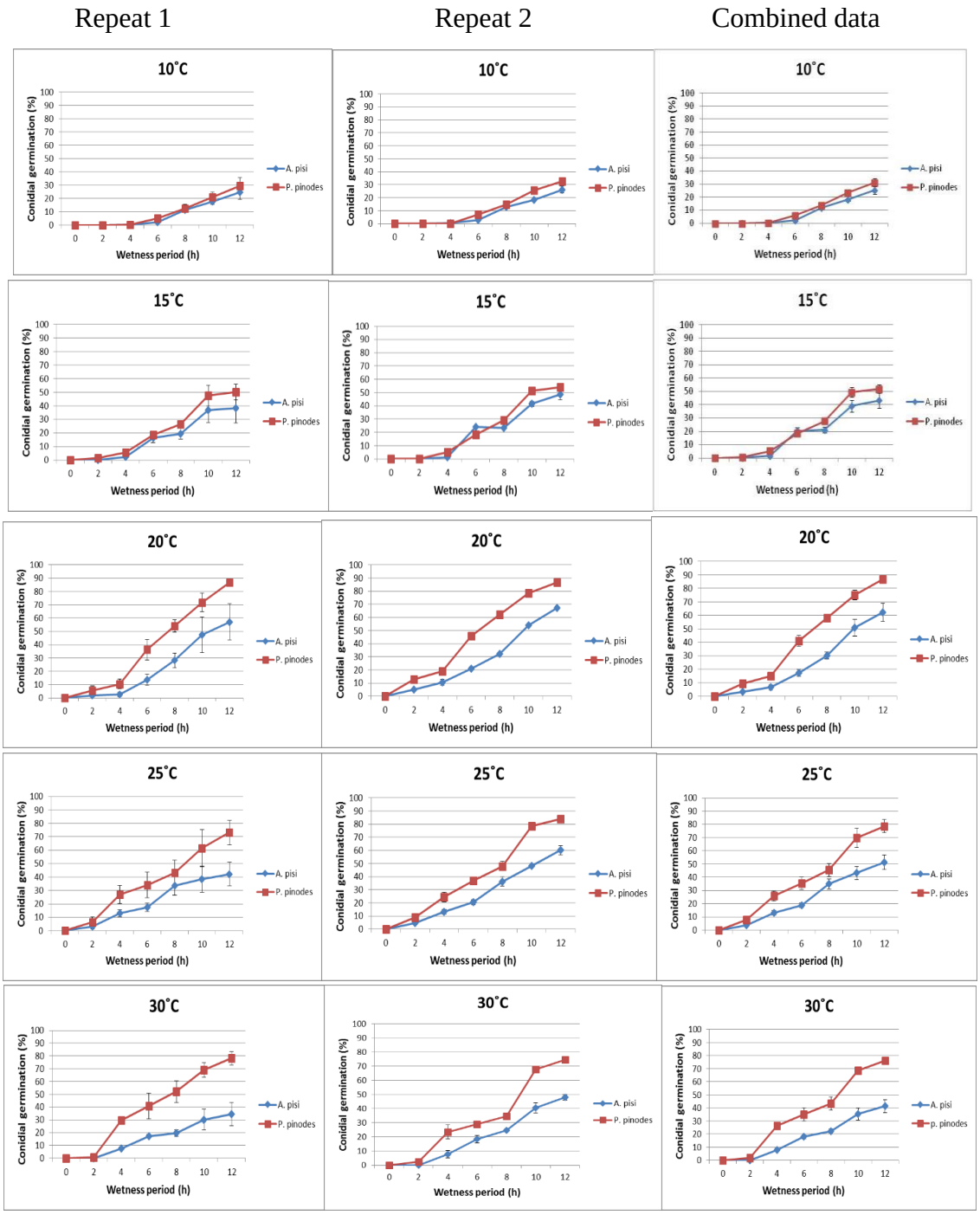
Data analyses for repeat 2 revealed that temperature, incubation period and the incubation period by temperature interaction had significant effects on percentage of conidial germination of *A. pisi* ($P < 0.0001$). At 10˚C, conidial germination did not change from 2 to 6 h, but increased after 8 h, 10 and 12 h of incubation. At 15˚C conidial germination did not change from 2 to 4 h, significantly increased after 6 h, and remained at a similar level after 8 h of incubation. Germination at 10 and 12 h after inoculation was higher to that observed at 6 and 8 h. At 20, 25 and 30˚C conidial germination had significantly increased after at each sampling up to 12 h.

For *P. pinodes*, data analysis for repeat 1 revealed that temperature ($P = 0.0002$), incubation period and the incubation period by temperature interaction (both $P < 0.0001$) had significant effects on percentage of conidial germination of *P. pinodes*. At 10˚C, conidial germination did not change from 2 to 6 h, but increased after 8 and 10 h and then remained at a similar level after 12 h of incubation (Fig A1.1). At 15˚C, conidial germination did not

change from 2 to 4 h and increased after 6 h and 8 h of incubation. Germination after 10 and 12 h of incubation was similar, but higher compared to that at 8 h. At 20˚C conidial germination did not change from 2 to 4 h, and then steadily increased up to 12 h. At 25˚C conidial germination significantly increased after 4 h and remained at a similar level up to 8 h of incubation. Germination had increased further by 10 and 12 h of incubation. At 30˚C conidial germination steadily increased up to 10 h of incubation and remained at a similar level after 12 h of incubation period.

Data analyses for repeat 2 revealed that temperature, incubation period and the incubation period by temperature interaction had significant effects on percentage of conidial germination of *P. pinodes* ($P < 0.0001$). At 10˚C, conidial germination did not change from 2 to 4 h, but steadily increased thereafter up to12 h of incubation (Fig A1.1). At 15˚C, conidial germination did not change from 2 to 4 h, then increased steadily up to 10 h and remained at a similar level at 12 h. At 20, 25 and 30˚C conidial germination steadily increased from 2h of incubation up to 12 h.

Fig A1.1 Effect of temperature (˚C) and incubation period (h) on percentage of conidial germination of *Ascochyta pisi* and *Peyronellaea pinodes.* Error bars represent standard errors of the means.



Appendix 2 Comparison of conidial germination of *Ascochyta pisi* and *Peyronellaea pinodes* at different temperatures

Analysis for repeat 1 revealed that at 10 and 15˚C, pathogens and the pathogens by incubation period interaction had no significant effect on conidial germination ($P > 0.05$). Incubation period had significant effects on conidial germination at both temperatures ($P < 0.0001$). At 20˚C, pathogens ($P = 0.0138$) and incubation period ($P < 0.0001$) had significant effects on percentage of conidial germination, whereas the pathogens by incubation period interaction was non-significant ($P = 0.2201$). There was no difference in conidial germination between *A. pisi* and *P. pinodes* up to 4 h, but after that *P. pinodes* conidia had consistently higher germination compared to *A. pisi* conidia. At 25˚C, pathogens ($P = 0.0015$) and incubation period ($P < 0.0001$) had significant effects on percentage of conidial germination. Here too, the pathogens by incubation period interaction had no effect on conidial germination ($P = 0.0682$). There were no significant differences in conidial germination between *A. pisi* and *P. pinodes* at 2, 4 and 8 h of incubation. *Peyronellaea pinodes* had higher germination at 6, 10 and 12 h compared to *A. pisi*. At 30˚C, pathogens, incubation period (both $P < 0.0001$) and the pathogens by incubation period interaction ($P = 0.0036$) had significant effects on percentage of conidial germination. With the exception of 2 h incubation period, *P. pinodes* had consistently higher germination compared to *A. pisi*.

Analysis from repeat 2 revealed that at 10˚C pathogens ($P = 0.0016$) and incubation period ($P < 0.0001$) had significant effects on percentage of conidial germination of *A. pisi* and *P. pinodes. The* pathogens by incubation period interaction had no effect on conidial germination ($P = 0.0537$). There was no significant difference in conidial germination between *A. pisi* and *P. pinodes* at 2, 4 and 8 h of incubation period. *Peyronellaea pinodes* had higher germination at 6, 10 and 12 h compared to *A. pisi*. At 15˚C, pathogens, incubation period and the pathogens by incubation period interaction had significant effects on percentage of conidial germination ($P < 0.01$). There was no difference in conidial germination between *A. pisi* and *P. pinodes* up to 4h after which *P. pinodes* had consistently higher germination up to 12h compared to *A. pisi*. At 20, 25 and 30˚C pathogens, incubation period and the pathogens by incubation period interaction had very highly significant effects on percentage of conidial germination ($P < 0.0001$). At 20˚C, *Peyronellaea pinodes* conidia had higher germination

from 2h up to 12h compared to *A. pisi* whereas at 25 and 30˚C that was the case for 4h up to 12h of incubation.

Table A2.1 Analysis of variance of the effect of temperature (˚C) and incubation period (h) on the percentage of conidial germination of *Ascochyta pisi*.

Type 3 Tests of Fixed Effects

Experiment	Effect	Num DF	Den DF	F Value	Pr > F
Repeat 1	**temperature**	4	16.5	2.81	0.0596
	incubation period	5	22.9	37.20	<.0001
	temperature*incubation period	20	22.9	4.03	0.0009
Repeat 2	**temperature**	4	22.7	72.84	<.0001
	incubation period	5	28.6	530.53	<.0001
	temperature*incubation period	20	28.6	14.84	<.0001
Repeat 1& 2	**temperature**	4	37.5	13.80	<.0001
	incubation period	5	62.7	151.88	<.0001
	temperature*incubation period	20	62.7	9.54	<.0001

Table A2.2 Analysis of variance of the effect of temperature (˚C) and incubation period (h) on the percentage of conidial germination of *Peyronellaea pinodes.*

Type 3 Tests of Fixed Effects

Experiment	Effect	Num DF	Den DF	F Value	Pr > F
Repeat 1	**temperature**	4	16.3	10.42	0.0002
	incubation period	5	33.4	137.46	<.0001
	temperature*incubation period	20	33.4	8.42	<.0001
Repeat 2	**temperature**	4	20.2	154.11	<.0001
	incubation period	5	31	978.80	<.0001
	temperature*incubation period	20	31	25.09	<.0001
Repeat 1& 2	**temperature**	4	39.1	39.83	<.0001
	incubation period	5	78.8	427.47	<.0001
	temperature*incubation period	20	78.8	16.17	<.0001

Table A2.3 Analysis of variance of comparison of conidial germination of *Ascochyta pisi* and *Peyronellaea pinodes* at different temperatures.

Type 3 Tests of Fixed Effects

Experiment	Temperature	Effect	Num DF	Den DF	F Value	Pr > F
Repeat 1	**10˚C**	**pathogens**	1	33	0.39	0.5381
		incubation period	5	33	32.11	<.0001
		incubation period*pathogens	5	33	0.52	0.7615
	15˚C	**pathogens**	1	33	1.25	0.2715
		incubation period	5	33	30.84	<.0001
		incubation period*pathogens	5	33	0.54	0.7415
	20˚C	**pathogens**	1	33	6.76	0.0138
		incubation period	5	33	30.14	<.0001
		incubation period*pathogens	5	33	1.49	0.2201
	25˚C	**pathogens**	1	33	12.03	0.0015
		incubation period	5	33	23.72	<.0001
		incubation period*pathogens	5	33	2.29	0.0682
	30˚C	**pathogens**	1	33	20.71	<.0001
		incubation period	5	33	25.39	<.0001
		incubation period*pathogens	5	33	4.38	0.0036
Repeat 2	**10˚C**	**pathogens**	1	33	11.86	0.0016
		time	5	33	120.30	<.0001
		incubation period*pathogens	5	33	2.45	0.0537

Type 3 Tests of Fixed Effects

Experiment	Temperature	Effect	Num DF	Den DF	F Value	Pr > F
	15˚C	**pathogens**	1	33	7.52	0.0098
		incubation period	5	33	304.05	<.0001
		incubation period*pathogens	5	33	8.41	<.0001
	20˚C	**pathogens**	1	33	939.34	<.0001
		incubation period	5	33	1390.80	<.0001
		incubation period*pathogens	5	33	40.44	<.0001
	25˚C	**pathogens**	1	33	185.10	<.0001
		incubation period	5	33	307.78	<.0001
		incubation period*pathogens	5	33	10.74	<.0001
	30˚C	**pathogens**	1	33	192.04	<.0001
		incubation period	5	33	269.95	<.0001
		incubation period*pathogens	5	33	12.11	<.0001
Repeat 1&2	**10˚C**	**pathogens**	1	77	3.56	0.0630
		incubation period	5	77	104.25	<.0001
		incubation period*pathogens	5	77	2.32	0.0510
	15˚C	**pathogens**	1	77	3.12	0.0815
		time	5	77	121.75	<.0001
		incubation period*pathogens	5	77	3.73	0.0045
	20˚C	**pathogens**	1	77	31.00	<.0001
		incubation period	5	77	125.12	<.0001

Type 3 Tests of Fixed Effects

Experiment	Temperature	Effect	Num DF	Den DF	F Value	Pr > F
		incubation period*pathogens	5	77	5.50	0.0002
	25˚C	**pathogens**	1	77	39.25	<.0001
		incubation period	5	77	92.66	<.0001
		incubation period*pathogens	5	77	6.05	<.0001
	30˚C	**pathogens**	1	77	44.19	<.0001
		incubation period	5	77	79.27	<.0001
		incubation period*pathogens	5	77	8.51	<.0001

Appendix 3. Rate parameters and coefficients of determination (R^2) of Gompertz models describing germination of *Ascochyta pisi* isolate AP6 and *Peyronellaea pinodes* isolate MP25 conidia at 10, 15, 20, 25 and 30°C through linear regression modeling.

		Ascochyta pisi			***Peyronellaea pinodes***		
Temperature (°C)	**Rep**	**Intercept**	**Rate parameter**	**R^2**	**Intercept**	**Rate parameter**	**R^2**
10	**1**	-2.17163	0.15188	0.8500	-2.18064	0.16441	0.8404
	2	-2.13979	0.15563	0.9134	-2.19293	0.17868	0.9035
	1 & 2	-2.15571	0.15375	0.8797	-2.18679	0.17154	0.8697
15	**1**	-2.00481	0.18427	0.7937	-1.86577	0.20172	0.9191
	2	-2.15075	0.21899	0.8780	-2.06209	0.22953	0.9568
	1 & 2	-2.07778	0.20163	0.8338	-1.96393	0.21563	0.9358
20	**1**	-2.08038	0.22706	0.7742	-2.04745	0.32518	0.9096
	2	-1.75857	0.22246	0.9748	-1.66864	0.30779	0.9782
	1 & 2	-1.91947	0.22476	0.8415	-1.85804	0.31648	0.9298
25	**1**	-1.72397	0.17638	0.7546	-1.73905	0.25801	0.7737
	2	-1.69864	0.20548	0.9523	-1.71487	0.29550	0.9435
	1 & 2	-1.71131	0.19093	0.8399	-1.72696	0.27675	0.8485
30	**1**	-1.89559	0.17042	0.8130	-1.88690	0.29872	0.8491
	2	-1.95561	0.20162	0.9274	-1.85231	0.26370	0.9221
	1 & 2	-1.92560	0.18602	0.8644	-1.86960	0.28121	0.8716

Appendix 4. Progression of disease development caused by *Ascochyta pisi* and *Peyronellaea pinodes* on inoculated plants

Analysis of *A. pisi* severity data for repeat 1 revealed that temperature, leaf wetness period and the leaf wetness period by temperature interaction had no significant effects on percentage of disease severity caused by *A. pisi* ($P > 0.05$).

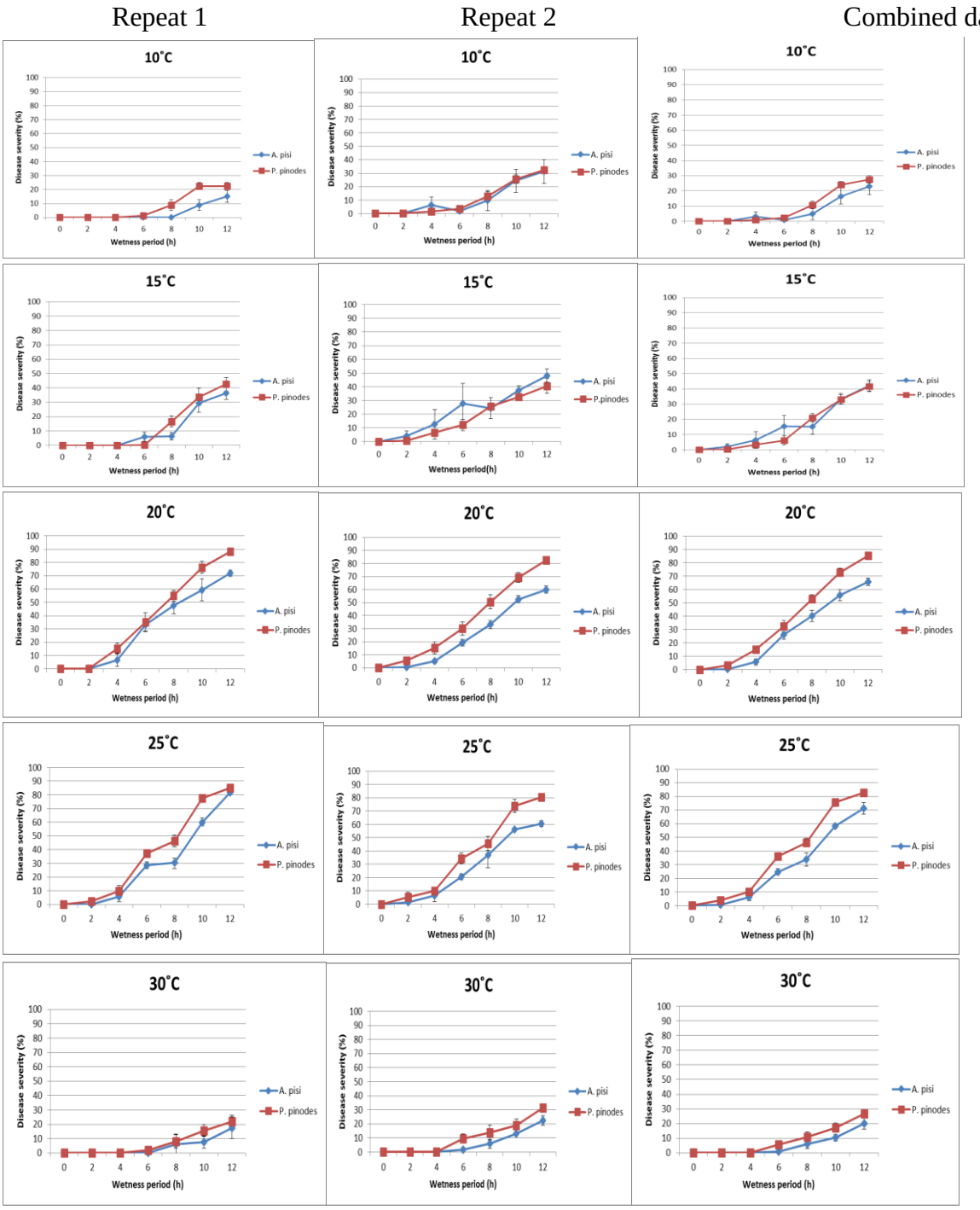
Data analyses for repeat 2 revealed that temperature, leaf wetness period and the leaf wetness period by temperature interaction had significant effects on percentage of disease severity caused by *A. pisi* ($P \leq 0.0001$). At 10˚C, disease severity did not change from 2 to 8 h but increased after 10 h and remained at similar level after 12 h of leaf wetness period. At 15˚C disease severity did not change from 2 to 4 h, significantly increased after 6 h, and remained at a similar level after 8 h of leaf wetness period. Disease severity increased further by 10 and 12 h of leaf wetness period. At 20 and 25˚C disease severity did not change from 2 to 4 h, increased after 6, 8 and 10 h and then remained steady up to 12 h of leaf wetness period. At 30˚C, disease severity did not change up to 8h, but increased at 10 and again at 12h of leaf wetness period.

For *P. pinodes*, analysis for repeat 1 revealed that temperature, leaf wetness period and the leaf wetness period by temperature interaction had significant effects on percentage of disease severity caused by *P. pinodes* ($P < 0.0001$). At 10 and 15˚C disease severity did not change from 2 to 6 h, disease severity increased further after 8 and 10 h leaf wetness period and remained similar up to 12 h (Figure 3.2). At 20˚C disease severity had significantly increased after at each sampling point up to 12 h. At 25˚C, disease severity increased after 4 and 6 h, and remained at a similar level up to 8 h. Disease severity at 10 and 12 h was similar but higher to that of observed at 6 and 8 h of leaf wetness period. At 30˚C, disease severity did not change from 2 to 8 h, but increased after 10 h and then remained at a similar level after 12 h of leaf wetness period.

Data analysis for repeat 2 revealed that temperature, leaf wetness period and the leaf wetness period by temperature interaction had a significant effects on percentage of disease severity caused by *P. pinodes* ($P < 0.0001$). At 10˚C, disease severity remained similar from 2 to 6 h and increased after 8 and 10h (Figure 3.2). Disease severity at 10 and 12h remained

similar but higher to that of observed at 2 to 8h of leaf wetness period. At 15˚C, disease severity increased after 4 h and remained at similar level up to 6 h of leaf wetness period. Disease severity increased after 8 and 10h of incubation and remained at a similar level up to 12 h of leaf wetness period. At 20˚C disease severity had significantly increased after at each sampling up to 12 h. At 25˚C, disease severity did not change from 2 to 4 h, and increased after 6, 8 and 10 h and remained at a similar level up to 12 h. At 30˚C, disease severity did not change from 2 to 4 h, but increased after 6 h and again after 12 h.

Fig. A4.1 Effect of temperature (˚C) and leaf wetness periods (h) on percentage disease severity caused by infection with *Ascochyta pisi* and *Peyronellaea pinodes.* Error bars represent standard errors of the means.



Appendix 5 Comparison of *Ascochyta pisi* and *Peyronellaea pinodes* in their ability to cause disease at different temperatures

Analysis for repeat 1 revealed that at 10˚C pathogens (P = 0.0027), leaf wetness period (P < 0.0001) and the pathogens by leaf wetness period interaction (P = 0.0155) had significant effects on percentage of disease severity caused by *A. pisi* and *P. pinodes*. There was no difference in disease severity between *A. pisi* and *P. pinodes*-infected plants exposed to leaf wetness periods up to 6h, but and after 8 h of leaf wetness percentage of disease severity caused by *P. pinodes* was higher than *A. pisi* (Figure 3.2). At 15˚C, pathogens (P = 0.1992) and the pathogens by leaf wetness period interaction (P = 0.2249) had no effects on disease severity caused by *A. pisi* and *P. pinodes*. Leaf wetness periods had significant effects on disease severity (P < 0.0001). At 20˚C, pathogens (P = 0.0050) and leaf wetness period interaction (P < 0.0001) had significant effects on disease severity caused by *A. pisi* and *P. pinodes*. The pathogens by leaf wetness period had no effect on disease severity (P = 0.4051). There was no difference in disease severity between *A. pisi* and *P. pinodes*-infected plants up to 8h of leaf wetness periods. After 10 h of leaf wetness, *P. pinodes* had caused higher disease severity than *A. pisi*. At 25˚C, it revealed that pathogens, leaf wetness period (both P < 0.0001) and the pathogens by leaf wetness period interaction (P = 0.0175) had significant effects on disease severity induced by *A. pisi* and *P. pinodes*. After 6 to 10 h of leaf wetness, *P. pinodes*-infected plants had higher disease severity than *A. pisi*, but not when leaf wetness periods were shorter or longer. At 30˚C, pathogens (P = 0.2709) and the pathogens by leaf wetness period interaction (P = 0.7963) had no effects on disease severity caused by *A. pisi* and *P. pinodes*. Leaf wetness period had significant effect on disease severity (P < 0.0001).

Analysis of repeat 2 data revealed that at 10 and 15˚C pathogens (P > 0.2519) and the pathogens by leaf wetness period interaction (P > 0.6042) had no effects on disease severity cause by *A. pisi* and *P. pinodes*. Leaf wetness period (P < 0.0001) had significant effect on disease severity. At 20˚C, pathogens and the pathogens by leaf wetness period interaction (both P < 0.0001) had significant effects on disease severity. Leaf wetness period (P = 0.1555) had no effect on disease severity. There was no difference in disease severity caused by *A. pisi* and *P. pinodes* after 2 h of leaf wetness, but after longer wetness periods *P. pinodes*-infected plants had higher disease severity than *A. pisi*. At 20 and 25˚C, pathogens (P <

0.0001) and the pathogens by leaf wetness period interaction (P < 0.0014) had significant effects on disease severity. Leaf wetness period (P > 0.2013) had no effect on conidial germination. There was no difference in disease severity caused by *A. pisi* and *P. pinodes* up to 4 h, but with leaf wetness periods of 6 h and higher, *P. pinodes* had higher disease severity than *A. pisi*.

Table A5.1 Analysis of variance of effect of temperature (˚C) and leaf wetness periods (h) on percentage disease severity caused by infection with *Ascochyta pisi.*

Type 3 Tests of Fixed Effects

Experiment	Effect	Num DF	Den DF	F Value	Pr > F
Repeat 1	**temperature**	4	1	72.35	0.0879
	wetness period	5	1	146.46	0.0626
	temperature* wetness period	20	1	23.92	0.1599
Repeat 2	**Temperature**	4	17.9	11.08	0.0001
	wetness period	5	37.3	99.36	<.0001
	temperature* wetness period	20	35.4	4.29	<.0001
Repeat 1& 2	**temperature**	4	30.7	38.79	<.0001
	wetness period	5	79.2	156.88	<.0001
	temperature* wetness period	20	79.1	11.66	<.0001

Table A5.2 Analysis of variance of effect of temperature (˚C) and leaf wetness periods (h) on percentage disease severity caused by infection with *Peyronellaea pinodes.*

Type 3 Tests of Fixed Effects

Experiment	Effect	Num DF	Den DF	F Value	Pr > F
Repeat 1	**temperature**	4	1	155.14	0.0601
	wetness period	5	1	400.21	0.0379
	temperature* wetness period	20	1	38.23	0.1269
Repeat 2	**temperature**	4	21.1	72.17	<.0001
	incubation wetness	5	30.2	240.95	<.0001
	temperature* wetness period	20	30.2	11.54	<.0001
Repeat 1& 2	**temperature**	4	52.7	192.24	<.0001
	wetness period	5	74.3	486.91	<.0001
	temperature* wetness period	20	74.3	32.53	<.0001

Table A5.3 Analysis of variance of comparison of *Ascochyta pisi* and *Peyronellaea pinodes* in their ability to cause disease at different temperatures

Type 3 Tests of Fixed Effects

Experiment	Temperature	Effect	Num DF	Den DF	F Value	Pr > F
Repeat 1	**10˚C**	**pathogens**	1	33	10.56	0.0027
		wetness period	5	33	27.46	<.0001
		wetness period*pathogens	5	33	3.32	0.0155
	15˚C	**pathogens**	1	33	1.72	0.1992
		wetness period	5	33	52.74	<.0001
		wetness period*pathogens	5	33	1.47	0.2249
	20˚C	**pathogens**	1	32	9.09	0.0050
		wetness period	5	32	82.49	<.0001
		wetness period*pathogens	5	32	1.05	0.4051
	25˚C	**pathogens**	1	30	27.59	<.0001
		wetness period	5	30	278.26	<.0001
		wetness period*pathogens	5	30	3.29	0.0175
	30˚C	**pathogens**	1	33	1.25	0.2709
		wetness period	5	33	10.17	<.0001
		wetness period*pathogens	5	33	0.47	0.7963
Repeat 2	**10˚C**	**pathogens**	1	33	0.01	0.9106
		wetness period	5	33	13.90	<.0001
		wetness	5	33	0.26	0.9310

Type 3 Tests of Fixed Effects

Experiment	Temperature	Effect	Num DF	Den DF	F Value	Pr > F
		period*pathogens				
	15˚C	**pathogens**	1	32	1.36	0.2519
		wetness period	5	32	13.11	<.0001
		wetness period*pathogens	5	32	0.73	0.6042
	20˚C	**pathogens**	1	33	35.58	<.0001
		wetness period	5	33	115.35	<.0001
		wetness period*pathogens	5	33	1.73	0.1555
	25˚C	**pathogens**	1	32	26.65	<.0001
		wetness period	5	32	106.32	<.0001
		wetness period*pathogens	5	32	1.55	0.2013
	30˚C	**pathogens**	1	33	12.13	0.0014
		wetness period	5	33	34.05	<.0001
		wetness period*pathogens	5	33	1.20	0.3287
Repeat 1&2	**10˚C**	**pathogens**	1	77	1.54	0.2180
		wetness period	5	77	27.58	<.0001
		wetness period*pathogens	5	77	0.94	0.4607
	15˚C	**pathogens**	1	76	0.31	0.5817
		wetness period	5	76	38.45	<.0001
		wetness period*pathogens	5	76	1.77	0.1294

Type 3 Tests of Fixed Effects

Experiment	Temperature	Effect	Num DF	Den DF	F Value	Pr > F
	20˚C	**pathogens**	1	76	34.85	<.0001
		wetness period	5	76	174.81	<.0001
		wetness period*pathogens	5	76	2.08	0.0767
	25˚C	**pathogens**	1	73	45.49	<.0001
		wetness period	5	73	271.56	<.0001
		wetness period*pathogens	5	73	2.23	0.0598
	30˚C	**pathogens**	1	77	8.37	0.0050
		wetness period	5	77	36.79	<.0001
		wetness period*pathogens	5	77	1.02	0.4131

Appendix 6. The rate parameters and coefficients of determination (R^2) of Gompertz models describing disease severity of *Ascochyta pisi* isolate AP6 and *Peyronellaea pinodes* isolate MP25 conidia at 10, 15, 20, 25 and 30°C through linear regression modeling.

		Ascochyta pisi			***Peyronellaea pinodes***		
Temperature (°C)	**Replicate**	**Intercept**	**Rate parameter**	**R^2**	**Intercept**	**Rate parameter**	**R^2**
10	1	-2.18509	0.11687	0.5408	-2.25451	0.15199	0.7436
	2	-2.15817	0.15664	0.6990	-2.17540	0.17084	0.8988
	1 & 2	-2.18509	0.12918	0.5408	-2.21331	0.16225	0.8013
15	1	-2.00481	0.18427	0.7937	-2.37513	0.20200	0.8049
	2	-1.85098	0.17768	0.5721	-1.94471	0.17859	0.7947
	1 & 2	-2.04861	0.18246	0.6824	-2.17390	0.19283	0.7881
20	1	-2.14990	0.28511	0.8968	-2.17282	0.35123	0.9493
	2	-1.97828	0.23113	0.9503	-1.84157	0.28407	0.9524
	1 & 2	-2.08247	0.26006	0.9073	-2.00212	0.31899	0.9426
25	1	-2.24522	0.30091	0.9261	-2.03336	0.32234	0.9604
	2	-2.07970	0.25116	0.9161	-1.99517	0.29703	0.9269
	1 & 2	-2.13413	0.27172	0.9048	-1.98956	0.31049	0.9459
30	1	-2.19580	0.09969	0.4293	-2.22895	0.14077	0.7120
	2	-2.20455	0.13811	0.7870	-2.08688	0.15969	0.8238
	1 & 2	-2.20018	0.11890	0.5780	-2.15791	0.15023	0.7421

Appendix 7. Rainfall data, irrigation dates and temperature data for the period of field trials, from May to August in Saskatoon, Milden and Outlook (http://climate.weather.gc.ca/).

Location	Year	Month	Rainfall (mm)	Temperature data (°C)		
				Max	Min	Mean
Saskatoon	2012	May	108	16.4	3.8	10.1
		June	121.1	21.5	10.0	15.8
		July	80.9	25.3	14.1	19.7
		August	48.5	23.9	10.7	17.3
Outlook	2012	May	99.7	16.9	4.7	10.8
		June	137.3	22.2	10.5	16.3
		July	55.7	25.9	14.1	20.0
		August	51.0	25.2	11.1	18.1
Saskatoon	2013	May	15.2	20.8	5.1	13.0
		June	115.9	20.9	10.0	15.5
		July	35.2	23.6	11.1	17.4
		August	14.7	26.4	11.3	18.9
Milden	2013	May	12.7	20.8	5.0	12.9
		June	73.5	21.8	10.0	15.9
		July	28.0	23.7	11.4	17.5
		August	28.8	26.3	11.3	18.8
Saskatoon	2014	May	61.1	17.3	2.8	10.1
		June	94.8	19.4	8.7	14.1
		July	44.5	24.5	12.0	18.3
		August	18.5	24.6	11.2	17.9
Milden	2014	May	81.2	18.0	3.5	10.8
		June	98.2	19.8	9.4	14.7
		July	28.4	24.7	12.0	18.4
		August	26.5	24.7	11.7	18.2

Table A7.1 Analysis of variance of seedling emergence of cv. CDC Patrick field pea from seed with different levels of infection with *Ascochyta pisi*. Pooled data from Outlook, Milden and Saskatoon in 2012, 2013 and 2014.

Year	Location	*A. pisi* infection levels	Num DF	Den DF	F Value	Pr > F
2012	**Saskatoon**	**Seed infection**	3	9	5.61	0.0191
	Outlook	**Seed infection**	3	9	1.66	0.2447
2013	**Milden**	**Seed infection**	3	9	0.74	0.5546
	Saskatoon	**Seed infection**	3	9	1.45	0.2925
2014	**Milden**	**Seed infection**	3	9	0.69	0.5802
	Saskatoon	**Seed infection**	3	9	1.04	0.4191
2012, 2013 &2014	**Pooled data**	**Seed infection**	3	69	3.96	0.0116

Table A7.2 Analysis of variance of Ascochyta blight severity caused by *Ascochyta pisi* on pea plants cv. CDC Patrick developing from seeds with different levels of infection with *Ascochyta pisi*. Pooled data from Outlook, Milden and Saskatoon in 2012, 2013 and 2014.

Type 3 Tests of Fixed Effects

Year	Location	*A. pisi* infection levels	Num DF	Den DF	F Value	Pr > F
2012	**Saskatoon**	**Seed infection**	3	9	0.16	0.9210
	Outlook	**Seed infection**	3	9	0.59	0.6378
2013	**Milden**	**Seed infection**	3	9	0.36	0.7859
	Saskatoon	**Seed infection**	3	9	4.36	0.0372
2014	**Milden**	**Seed infection**	3	9	0.30	0.8224
	Saskatoon	**Seed infection**	3	9	0.37	0.7737
2012, 2013 &2014	**Pooled data**	**Seed infection**	3	69	0.17	0.9168

Table A7.3 Analysis of variance of Ascochyta blight severity caused by *Peyronellaea pinodes* on pea plants cv. CDC Patrick developing from seeds with different levels of infection with *Ascochyta pisi*. Pooled data from Outlook, Milden and Saskatoon in 2012, 2013 and 2014.

Type 3 Tests of Fixed Effects

Year	Location	*A. pisi* infection levels	Num DF	Den DF	F Value	Pr > F
2012	**Saskatoon**	**Seed infection**	3	9	0.88	0.4851
	Outlook	**Seed infection**	3	9	0.31	0.8200
2013	**Milden**	**Seed infection**	3	9	2.81	0.1005
	Saskatoon	**Seed infection**	3	9	2.94	0.0914
2014	**Milden**	**Seed infection**	3	9	0.30	0.8224
	Saskatoon	**Seed infection**	3	9	1.60	0.2577
2012, 2013 &2014	**Pooled data**	**Seed infection**	3	69	2.64	0.0566

Table A7.4 Analysis of variance of seed yields from pea cultivar CDC Patrick grown from seeds with different levels of infection with *Ascochyta pisi*. Pooled data from Outlook, Milden and Saskatoon in 2012, 2013 and 2014.

Type 3 Tests of Fixed Effects

Year	Location	*A. pisi* infection levels	Num DF	Den DF	F Value	Pr > F
2012	**Saskatoon**	**Seed infection**	3	9	1.01	0.4338
	Outlook	**Seed infection**	3	9	0.29	0.8291
2013	**Milden**	**Seed infection**	3	9	0.72	0.5651
	Saskatoon	**Seed infection**	3	9	0.00	0.9998
2014	**Milden**	**Seed infection**	3	9	0.74	0.5558
	Saskatoon	**Seed infection**	3	9	0.65	0.6004
2012, 2013 &2014	**Pooled data**	**Seed infection**	3	67	0.59	0.6212

Table A7.5 Analysis of variance of incidence of seed infection with *A. pisi* of seeds harvested from CDC Patrick pea plots seeded with different levels of infection with *Ascochyta pisi*. Pooled data from Outlook, Milden and Saskatoon in 2012, 2013 and 2014.

Type 3 Tests of Fixed Effects

Year	Location	*A. pisi* infection levels	Num DF	Den DF	F Value	Pr > F
2012	**Saskatoon**	**Seed infection**	3	9	0.17	0.9162
	Outlook	**Seed infection**	3	9	0.01	0.9975
2013	**Milden**	**Seed infection**	3	9	0.37	0.7785
	Saskatoon	**Seed infection**	3	9	0.15	0.9248
2014	**Milden**	**Seed infection**	3	9	1.45	0.2915
	Saskatoon	**Seed infection**	3	9	2.59	0.1176
2012, 2013 &2014	**Pooled data**	**Seed infection**	3	69	1.241E7	<.0001

Table A7.6 Analysis of variance of Incidence of seed infection with *P. pinodes* of seeds harvested from CDC Patrick pea plots seeded with different levels of infection with *Ascochyta pisi*. Pooled data from Outlook, Milden and Saskatoon in 2012, 2013 and 2014.

Type 3 Tests of Fixed Effects

Year	Location	*A. pisi* infection levels	Num DF	Den DF	F Value	Pr > F
2012	**Saskatoon**	**Seed infection**	3	9	0.06	0.9819
	Outlook	**Seed infection**	3	9	0.97	0.4480
2013	**Milden**	**Seed infection**	3	9	1.87	0.2044
	Saskatoon	**Seed infection**	3	9	3.02	0.0865
2014	**Milden**	**Seed infection**	2	9	2.16	0.1711
	Saskatoon	**Seed infection**	3	9	1.44	0.2952
2012, 2013 &2014	**Pooled data**	**Seed infection**	3	69	9.21	<.0001

Table A7.7 Analysis of variance of percentage of seed infection with *Ascochyta pisi* on seed coat, cotyledon and embryo from CDC Patrick field pea seeds naturally infected with *Ascochyta pisi* and separated into four seed coat staining categories.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Seed staining category	4	83	626.60	<.0001
Seed component	2	83	100.60	<.0001
Seed staining category * Seed component	8	83	31.81	<.0001
Epicoccum	1	83	5.52	0.0212

Appendix 8. Agronomic management of the field experiments in 2012, 2013 and 2014 in Swift Current, Stewart Valley and Saskatoon.

Chemical treatments	2012		2013		2014	
	Swift Current	**Stewart Valley**	**Swift Current**	**Stewart Valley**	**Swift Current**	**Saskatoon**
Pre-seeding herbicide	3May, Roundup weathermax 660ml/ac	3 May, Roundup weathermax 660ml/ac	3May, Roundup weathermax 660ml/ac 1 May, Edge granular17kg/ha	No application	2 May, Roundup weathermax 660ml/ac 9May, Weathermax+ Heat (500 ml/ac & 10.4 gr/ac)	No application
Post-seeding herbicide	Glycophosphate (Post seeding burnoff) 4 June, Solo 11.7g/ac	Glycophosphate (Post seeding burnoff) 4 June, Solo 11.7g/ac	4 June, Odyssey 17.3gm/ac	6 June, Odyssey 17.3gm/ac	10 June, Odyssey 17.3gm/ac	No application
Weevil pesticide	No application	No application	18 June, Metador 33ml/ac	5 June, Metador 33ml/ac	30 May, Metador 33ml/ac	No application
Fungicide treatment	June 22, Bravo 500 1.6L/ac July 5, Headline Duo 240ml/ac & Lance 170g/a	June 25, Bravo 500 1.6L/ac July 6, Headline Duo 240ml/ac & Lance 170g/a	1 June, Bravo 500 1.6L/ac 21 June, Headline Duo 240ml/ac & Lance 170g/a	6 June, Bravo 500 1.6L/ac 4 July, Headline Duo 240ml/ac & Lance 170g/a	1 June, Bravo 500 1.6L/ac 24 June & 17 July Headline Duo 240ml/ac & Lance 170g/a	17 June, Bravo 500 1.6L/ac 3 July & 17 July Headline Duo 240ml/ac & Lance 170g/a
Artificial *A. pisi* inoculum	No application	No application	No application	No application	25 June 2014	15 June &13 July 65g m-2
Disease ratings						
1st Rating	5th July	5th July	13th June	13th June	25th June	26th June
2nd Rating	31st July	31st July	16th July	17th July	15th July	16th July
3rd Rating	-	-	7th August	9th August	30th July	31th July
Desiccant Spray	1 August, Reglone 700ml/ac	7 August, Reglone 700ml/ac	14 August, Reglone 700ml/ac	17 August, Reglone 700ml/ac	19 August, Reglone 700ml/ac	No Application

Fig A8.1 Disease severity caused by *P. pinodes* in plots treated with fungicides to create low diseased plots, and unsprayed plots to create highly diseased plots of pea cv. CDC Bronco, Cooper, CDC Golden, SW Midas under natural inoculum conditions at Stewart Valley 2012, 2013, 2014 and Swift Current 2012, 2013 and 2014, and in an inoculated experiment at Saskatoon in 2014.

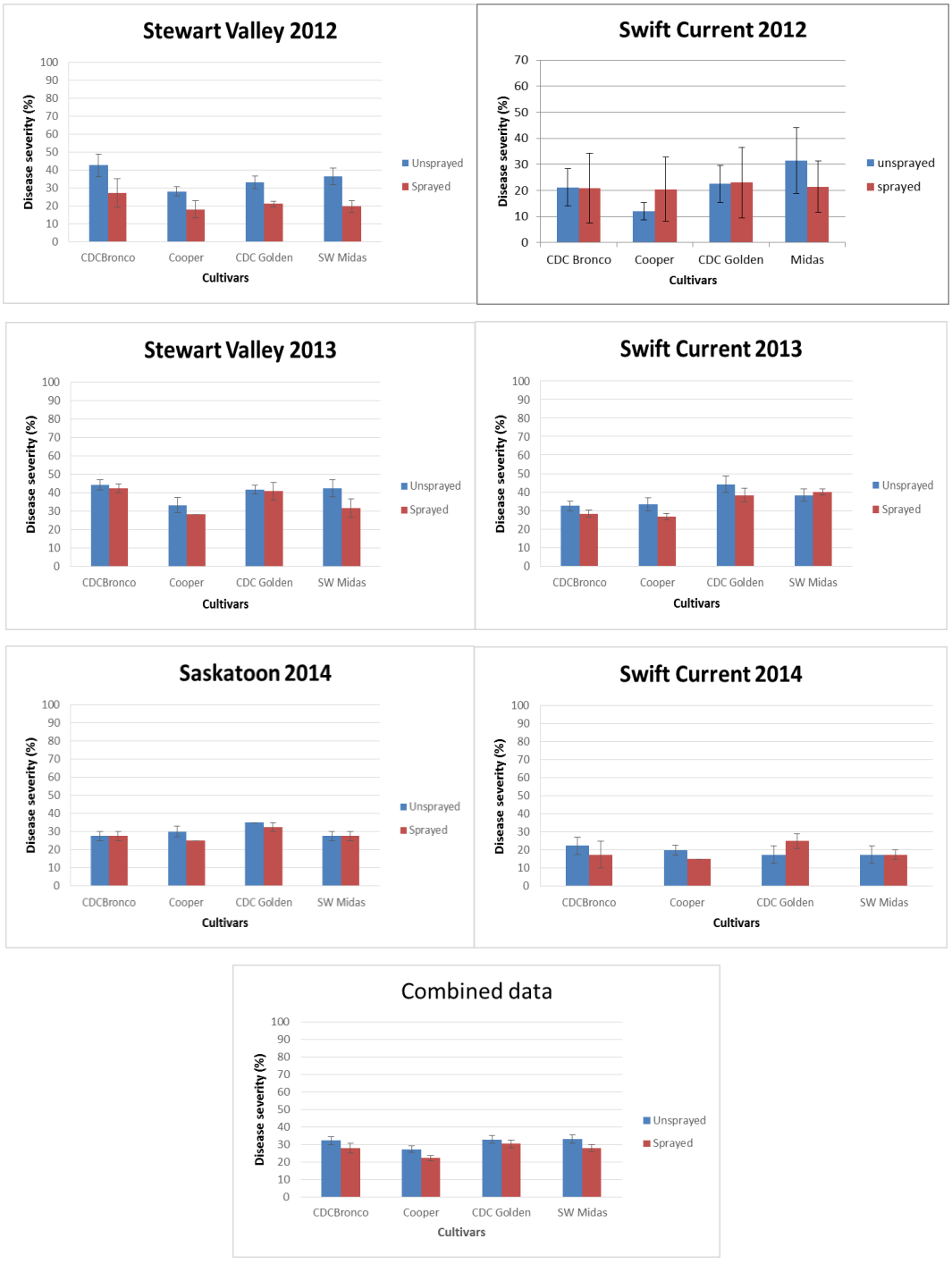


Table A8.1 Analysis of variance of disease severity caused by *A. pisi* in plots treated with fungicides to create low diseased plots, and unsprayed plots to create highly diseased plots of pea cv. CDC Bronco, Cooper, CDC Golden, SW Midas under natural inoculum conditions at Stewart Valley 2012, 2013 and Swift Current 2012, 2013 and 2014, and in an inoculated experiment at Saskatoon in 2014.

Type 3 Tests of Fixed Effects

Year	Location	Effect	Num DF	Den DF	F Value	Pr > F
2012	**Swift Current**	**Splot**	3	18	0.68	0.5782
		Mplot	1	3	0.00	0.9625
		Mplot*Splot	3	18	0.58	0.6368
	Stewart Valley	**Splot**	3	18	0.38	0.7657
		Mplot	1	3	1.34	0.3301
		Mplot*Splot	3	18	0.14	0.9350
2013	**Swift Current**	**Splot**	3	18	1634337	<.0001
		Mplot	1	3	15.69	0.0287
		Mplot*Splot	3	18	1634337	<.0001
	Stewart Valley	**Splot**	3	18	0.39	0.7615
		Mplot	1	3	2.19	0.2353
		Mplot*Splot	3	18	0.17	0.9136
2014	**Swift Current**	**Splot**	3	18	0.15	0.9258
		Mplot	1	3	0.37	0.5863
		Mplot*Splot	3	18	0.31	0.8195
	Saskatoon	**Splot**	3	18	0.75	0.5384
		Mplot	1	3	41.81	0.0075
		Mplot*Splot	3	18	0.23	0.8711
2012,2013 & 2014	**Pooled data**	**Splot**	3	138	1.70	0.1706
		Mplot	1	23	17.15	0.0004
		Mplot*Splot	3	138	3.83	0.0113

*Splot - Subplot (Cultivars); Mplot - Main plot (Fungicide treated and non-fungicide treated plots)

Table A8.2 Analysis of variance of disease severity caused by *P. pinodes* in plots treated with fungicides to create low diseased plots, and unsprayed plots to create highly diseased plots of pea cv. CDC Bronco, Cooper, CDC Golden, SW Midas under natural inoculum conditions at Stewart Valley 2012, 2013 and Swift Current 2012, 2013 and 2014, and in Saskatoon at 2014.

Type 3 Tests of Fixed Effects

Year	Location	Effect	Num DF	Den DF	F Value	Pr > F
2012	**Swift Current**	**Splot**	3	18	11.55	0.0002
		Mplot	1	3	0.02	0.8915
		Mplot*Splot	3	18	0.68	0.5778
	Stewart Valley	**Splot**	3	18	3.54	0.0356
		Mplot	1	3	17.87	0.0242
		Mplot*Splot	3	18	0.84	0.4912
2013	**Swift Current**	**Splot**	3	18	7.70	0.0016
		Mplot	1	3	3.18	0.1727
		Mplot*Splot	3	18	0.80	0.5113
	Stewart Valley	**Splot**	3	18	6.18	0.0045
		Mplot	1	3	4.27	0.1307
		Mplot*Splot	3	18	1.05	0.3958
2014	**Swift Current**	**Splot**	3	18	0.73	0.5464
		Mplot	1	3	0.05	0.8368
		Mplot*Splot	3	18	1.90	0.1665
	Saskatoon	**Splot**	3	18	6.29	0.0042
		Mplot	1	3	3.74	0.1486
		Mplot*Splot	3	18	1.66	0.2116
2012,2013 & 2014	**Pooled data**	**Splot**	3	138	19.56	<.0001
		Mplot	1	23	6.83	0.0156
		Mplot*Splot	3	138	0.29	0.8359

*Splot - Subplot (Cultivars); Mplot - Main plot (Fungicide treated and non-fungicide treated plots)

Table A8.3 Analysis of variance of seed yield from plots treated with fungicides to create low diseased plots, and unsprayed plots to create highly diseased plots of pea cv. CDC Bronco, Cooper, CDC Golden, SW Midas under natural inoculum conditions at Swift Current 2012, 2013, 2014 and at Stewart Valley 2013 and in an *A. pisi* inoculated experiment at Saskatoon in 2014.

Type 3 Tests of Fixed Effects

Year	Location	Effect	Num DF	Den DF	F Value	Pr > F
2012	**Swift Current**	**Splot**	3	18	1.41	0.2712
		Mplot	1	3	0.09	0.7858
		Mplot*Splot	3	18	0.51	0.6820
	Stewart Valley	**Splot**	3	17	293.58	<.0001
		Mplot	1	3	0.15	0.7236
		Mplot*Splot	3	17	3.75	0.0310
2013	**Swift Current**	**Splot**	3	18	4.03	0.0234
		Mplot	1	3	0.07	0.8121
		Mplot*Splot	3	18	1.59	0.2271
	Stewart Valley	**Splot**	3	18	6.72	0.0031
		Mplot	1	3	0.55	0.5135
		Mplot*Splot	3	18	0.12	0.9475
2014	**Swift Current**	**Splot**	3	18	19.36	<.0001
		Mplot	1	3	0.03	0.8778
		Mplot*Splot	3	18	0.11	0.9547
	Saskatoon	**Splot**	3	18	4.47	0.0164
		Mplot	1	3	0.17	0.7043
		Mplot*Splot	3	18	0.67	0.5834
2012,2013 & 2014	**Pooled data**	**Splot**	3	136	3.70	0.0134
		Mplot	1	23	0.02	0.8816
		Mplot*Splot	3	136	0.13	0.9394

*Splot - Subplot (Cultivars); Mplot - Main plot (Fungicide treated and non-fungicide treated plots)

Table A8.4 Analysis of variance of incidence of seed infection with *A. pisi* of seeds harvested from plots treated with fungicides to create low diseased plots, and unsprayed plots to create highly diseased plots of pea cv. CDC Bronco, Cooper, CDC Golden, and SW Midas under natural inoculum conditions at Swift Current 2013, 2014 and at Stewart Valley 2013 and in an *A. pisi* inoculated experiment at Saskatoon in 2014.

Type 3 Tests of Fixed Effects

Year	Location	Effect	Num DF	Den DF	F Value	Pr > F
2013	**Swift Current**	**Splot**	3	18	0.57	0.6429
		Mplot	1	3	1.99	0.2529
		Mplot*Splot	3	18	0.65	0.5935
	Stewart Valley	**Splot**	3	18	0.27	0.8443
		Mplot	1	3	0.00	1.0000
		Mplot*Splot	3	18	1.82	0.1800
2014	**Swift Current**	**Splot**	3	18	0.85	0.4842
		Mplot	1	3	52.78	0.0054
		Mplot*Splot	3	18	1.16	0.3535
	Saskatoon	**Splot**	3	18	2.21	0.1224
		Mplot	1	3	15.57	0.0290
		Mplot*Splot	3	18	1.47	0.2575
2013 & 2014	**Pooled data**	**Splot**	3	90	0.51	0.6796
		Mplot	1	15	13.41	0.0023
		Mplot*Splot	3	90	0.64	0.5917

*Splot - Subplot (Cultivars); Mplot - Main plot (Fungicide treated and non-fungicide treated plots)

Appendix 9. Agronomic management of the field experiments in 2012, 2013 and 2014 in Swift Current, Stewart Valley and Saskatoon for genetic control of *Ascochyta pisi*.

Agronomic information	2012	2013		2014	
	Swift Current	Swift Current	Stewart Valley	Swift Current	Saskatoon
Planting date	25 April	08 May	09 May	01 May	10 May
Pre-seeding herbicide	23 April, Edge granular 17kg/ac	01 May, Edge granular 17kg/ha	No application	25 April, 09 May Edge 17kg/ha, weathermax+heat 500ml/ac &10.54 g/ac	No application
Post emergence	No Application	28 May. Odyssey 17.3 g/ac	06 June, Odyssey 17.3 g/ac	No application	No application
Pea weevil treatment	30 May, Matador 33ml/ac	01 June & 18 June Metador 33ml/ac	05 June, Metador 33ml/ac	27 may & 02 June, Metador 33ml/ac	No application
Seed Inoculum	No application	No application	No application	June 20,2014	June 15, 2014 July 13, 2014
Disease rating					
1st disease rating	05 July	16 July	17 July	25 June	26 June
2nd Disease rating	31 July	07 August	09 August	15 July	16 July
3rd Disease rating	-	15 August	-	30 July	31 July
Dessicants	07 August, Reglone 700 ml/ac	17 August, Reglone 700 ml/ac	17 August, Reglone 700 ml/ac	16 August, Reglone 700 ml/ac	No application
Harvest date	13 August	26 August	23 August	02 September	25 August

Table A9.1 Analysis of variance and linear contrast analysis of disease severity caused by *Ascochyta pisi* on PR-10 RILs developed from a cross of pea cv. CDC Bronco and Cooper tested under natural condition at Swift Current and Stewart Valley in 2012 and 2013, and under irrigation at Saskatoon and natural condition in Swift Current 2014.

Type 3 Tests of Fixed Effects

Year	Location	Effect	Num DF	Den DF	F Value	Pr > F
2012	**Swift Current**	**RILs**	57	1	0.82	0.7252
2013	**Swift Current**	**RILs**	49	1	8.00	0.2748
	Stewart Valley	**RILs**	49	1	4.49	0.3611
2014	**Swift Current**	**RILs**	49	53	1.00	0.4978
	Saskatoon	**RILs**	49	5.28	0.56	0.8697
2012, 2013& 2014	**Pooled data**	**RILs**	57	1	Infty	<.0001

Contrasts

Year	Location	Label	Num DF	Den DF	F Value	Pr > F
2012	**Swift Current**	**CDCBronco vs Cooper**	1	4	0.21	0.6680
2013	**Swift Current**	**CDCBronco vs Cooper**	1	1	0.43	0.6312
	Stewart Valley	**CDCBronco vs Cooper**	1	5.72	0.01	0.9424
2014	**Swift Current**	**CDCBronco vs Cooper**	1	53	1.24	0.2705
	Saskatoon	**CDCBronco vs Cooper**	1	10.2	0.95	0.3517
2012, 2013& 2014	**Pooled data**	**CDCBronco vs Cooper**	1	1	Infty	<.0001

Table A9.2 Analysis of variance and linear contrast analysis of disease severity caused by *Peyronellaea pinodes* on PR-10 RILs developed from a cross of pea cv. CDC Bronco and Cooper tested under natural condition at Swift Current and Stewart Valley in 2012 and 2013, and under irrigation at Saskatoon and natural condition in Swift Current 2014.

Type 3 Tests of Fixed Effects

Year	Location	Effect	Num DF	Den DF	F Value	Pr > F
2013	**Swift Current**	**RILs**	49	97.1	0.99	0.5087
	Stewart Valley	**RILs**	49	1	4.43	0.3633
2014	**Swift Current**	**RILs**	49	53	3.60	<.0001
	Saskatoon	**RILs**	49	8.26	0.40	0.9776
2012, 2013& 2014	**Pooled data**	**RILs**	58	461	1.67	0.0023

Contrasts

Year	Location	Label	Num DF	Den DF	F Value	Pr > F
2013	**Swift Current**	**CDCBronco vs Cooper**	1	97.1	1.29	0.2582
	Stewart Valley	**CDCBronco vs Cooper**	1	1	5.90	0.2487
2014	**Swift Current**	**CDCBronco vs Cooper**	1	53	0.00	1.0000
	Saskatoon	**CDCBronco vs Cooper**	1	11	2.09	0.1760
2012, 2013& 2014	**Pooled data**	**CDCBronco vs Cooper**	1	235	2.08	0.1506

Table A9.3 Analysis of variance and linear contrast analysis of seed yields (averaged across locations and years) for PR-10 RILs developed from a cross of pea cv. CDC Bronco and Cooper tested under natural condition at Swift Current and Stewart Valley in 2012, 2013 and under irrigation at Saskatoon and natural condition in Swift Current 2014.

Type 3 Tests of Fixed Effects

Year	Location	Effect	Num DF	Den DF	F Value	Pr > F
2012	**Swift Current**	**RILs**	57	1	31.51	0.1408
2013	**Swift Current**	**RILs**	49	13	8.74	<.0001
	Stewart Valley	**RILs**	49	104	3.66	<.0001
2014	**Swift Current**	**RILs**	49	53	6.05	<.0001
	Saskatoon	**RILs**	49	106	408.03	<.0001
2012, 2013& 2014	**Pooled Data**	**RILs**	5	1	259121	0.0015

Contrasts

Year	Location	Label	Num DF	Den DF	F Value	Pr > F
2012	**Swift Current**	**CDCBronco vs Cooper**	1	3.67	2.06	0.2308
2013	**Swift Current**	**CDCBronco vs Cooper**	1	11	1.44	0.2556
	Stewart Valley	**CDCBronco vs Cooper**	1	104	21.70	<.0001
2014	**Swift Current**	**CDCBronco vs Cooper**	1	53	4.75	0.0338
	Saskatoon	**CDCBronco vs Cooper**	1	106	361.68	<.0001
2012, 2013& 2014	**Pooled data**	**CDCBronco vs Cooper**	1	1	Infty	<.0001