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Sensitivity of *Mycosphaerella pinodes* to pyraclostrobin and optimizing fungicide application in field pea

by

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ABSTRACT

Mycosphaerella pinodes caused by mycosphaerella blight, a destructive disease of field pea is primarily managed with foliar fungicides. Development of fungicide insensitivity in *M. pinodes* could severely reduce management options. The objectives of this study were to assess insensitivity to pyraclostrobin fungicide in *M. pinodes* populations from western Canada and the U.S.A., and to determine the optimum fungicide delivery system to manage mycosphaerella blight. Over 300 *M. pinodes* isolates collected in 2010-11 were tested for insensitivity and 19 isolates were found to be insensitive to the fungicide, suggesting the need for judicious use of pyraclostrobin. Sprayer technology trials under field conditions revealed that double nozzles and water volumes up to 400 L ha^{-1} improved fungicide efficacy relative to control treatments. Above 400 L ha^{-1}, disease was higher and yield was lower in all trials, suggesting that higher volumes can over-saturate the leaves and cause fungicide run-off.

PREFACE

Although Robyne Bowness was the author of this thesis, many individuals contributed to the research work described in Chapters 2 and 3. In Chapter 2, Dr. Bruce Gossen provided a collection of *Mycosphaerella pinodes* isolates from Saskatoon, and Dr. Ruebella Goswami provided a collection of *M. pinodes* isolates from North Dakota that were used to create a baseline that made the sensitivity testing possible. During the summers of 2010 and 2011, many researchers and agronomists from across Saskatchewan and Alberta provided freshly collected *M. pinodes* isolates with which to test the adaptation of the pathogen to the fungicide. Mrs. Trina Dubitz, Ms. Lynne Schnepf and Ms. Lindsay Patterson provided technical support, especially in the laboratory and growth chambers. Dr. Kan-Fa Chang provided the laboratory, other facilities, chemicals, fungicides and equipment necessary to perform the testing. Dr. Christian Willenborg and Dr. Rong-Cai Yang provided statistical advice. Drs. Chang, Willenborg, Gossen and Strelkov, as members of my committee provided advice, feedback and critical review of the draft chapter.

In Chapter 3, Dr. Bruce Gossen and Dr. Robert Conner provided data from research studies conducted in Saskatchewan and Manitoba that is included in this thesis. Mrs. Trina Dubitz, Ms. Lynne Schnepf and Ms. Lindsay Patterson provided technical support in the field and growth chamber. Dr. Kan-Fa Chang provided the equipment, seed and products necessary to perform the testing. Dr. Tom Wolf provided advice on water volumes and fungicide application. Dr. Rong-Cai Yang and Dr. Bruce Gossen provided statistical advice. Drs. Chang, Willenborg, Gossen and Strelkov, as members of my committee provided advice, feedback and critical review of the draft chapter. As my supervisor, Dr. Strelkov provided feedback on Chapter 1 and 4.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. THE CROP: PEA

1.1.1. Introduction

Field pea (*Pisum sativum*) is a member of the *Fabaceae* (formerly *Leguminosae*) family of cool season legume crops commonly known as pulse crops, which include lentil, faba bean, bean and chickpea (Park et al. 1999). Worldwide, field pea is the second most widely grown grain legume (FOASTAT 2009). Although the exact origin of pea is unknown, it is most widely accepted that the crop originated in northwest Asia, spread to the temperate zones of Europe, and from there spread to other parts of the world. Field pea was first cultivated about 9000 years ago, making it one of the oldest cultivated crops (Government of Saskatchewan 2009).

Field pea was introduced into North America by the early European explorers. In Canada, indigenous people started growing peas in the Montréal region in the early 1900's (Canadian Encyclopedia 2011). By 1970, there were about 25,000 hectares grown, mostly in Manitoba. Since then, an increased emphasis on crop diversification and crop rotation has led to dramatic increases in pea and other pulse production across western Canada. This growth was further driven in the 1980s by the increased export of field pea to the European feed pea market, and by the higher net economic return to growers from pea than from red spring wheat (Canadian Encyclopedia 2011).

1.1.2. Adaptation

Field pea is considered a cool season crop with a relatively shallow root system that requires timely periodic moisture during its rapid vegetative growth phase and throughout flowering. Pea are best suited to the dark brown and brown soil zones of the Canadian prairies, but also do very well on the black soils, and with proper management are successful on grey wooded soils as well (Agriculture and Agri-Food Canada 2009). Pea should be seeded as early in the spring as practical when the soil is 4-5°C at a seeding depth of approximately 5 cm below the soil surface. The timing of seeding must be precise to avoid flower blast during the prolonged heat of July, which results in reduced yield. The crop tolerates spring frosts and is relatively drought tolerant, but does not like waterlogged soils, high salinity or pH outside of the 5.5-7.0 range (Agriculture and Agri-Food Canada 2009).

1.1.3. Production

Worldwide

The leading pea producing countries are: Canada, Russia, China, India, USA, France, and Ukraine, with the main exporting countries consisting of Canada, France, Australia, Ukraine and the USA. Total world pea production averaged 10.3 million metric tonnes from 2004-2008. The main importing countries are:

India, Spain, China, Bangladesh, Netherlands, and Pakistan (United States Department of Agriculture 2010).

Western Canada

Since 1998, Canada has been the largest dry pea producer and exporter in the world (Pulse Canada 2010). The average harvested area of dry pea in Canada over the past 5 years has been 1.5 million hectares producing 2.9 million metric tonnes of pea (Alberta Agriculture and Rural Development 2011). Currently, dry pea represents the fourth largest field crop in Canada after wheat, barley and canola. The majority of dry pea in western Canada is produced in Saskatchewan, followed by Alberta and Manitoba (Canadian Encyclopedia 2011). Saskatchewan produces 90% of the Canadian pea crop (Saskatchewan Pulse Growers 2010).

1.1.4. Utilization

Field pea is used primarily for human consumption, secondarily for animal feed, and is an important source of protein, particularly in developing countries. Pulse crops, including pea, have two to three times the protein content of cereal grains and provide about 10% of the world's dietary protein (Goodwin 2003). Uses include split pea for soup, ground pea for flour and noodles, cooked pea for salads, baked pea for snack foods, crushed pea for paste, pea sprouts for salads, pea mixed with chickpea flour for dhal, split or whole pea and silage for feed (Park et al. 1999).

1.1.5. Diseases of field pea

Field pea is subject to a number of soil and stubble-borne diseases that can increase in severity as pea cropping intensifies. Disease prior to flowering reduces the potential number of pods which a plant may carry, whereas disease after flowering reduces the ability of a plant to fill those pods (Tivoli et al. 1996). Diseases that attack the plant before flowering are usually root diseases caused by various species of fungi, including *Pythium ultimum* (Trow)*, Rhizoctonia solani* (Kuhn), *Fusarium solani* (Mart.) Sacc. f. sp*. pisi* (Jones) Snyder & Hans, *Fusarium oxysporum (*Schlechtend:Fr.) f.*sp. pisi* (Hall) Synder & Hans. and *Fusarium avenaceaum* (Corda:Fr.) Sacc. *(*Davidson and Ramsay 2000). Diseases that attack the plant just before, at, or after flowering are usually foliar in nature and include the ascochyta blight complex ((*Mycosphaerella pinodes* (Berk. & Blox.) Vestergr*, Ascochyta pisi* Lib*., and Phoma pinodella* (L.K.Jones) Morgan-Jones & K.B. Burch)), downy mildew ((*Peronospora viciae (Berk.*) Gaum)) and powdery mildew (*Erysiphe pisi DC.*). The most problematic of all foliar pathogens of pea, worldwide, is the fungus *M.pinodes* (Davidson and Ramsay 2000; Bretag et al. 2006; Banniza and Vandenberg 2003; Beasse et al. 1999).

1.2. THE PATHOGEN: *MYCOSPHAERELLA PINODES*

Mycosphaerella blight, caused by *Mycosphaerella pinodes (*anamorph *Ascochyta pinodes* (Berk & Blox) Jones), is the most destructive foliar disease of pea and the most important necrotrophic disease of pea crops in western Canada and around the world (Davidson and Ramsay 2000; Bretag et al. 2006;

Banniza and Vandenberg 2003; Beasse et al. 1999). All commercial cultivars are susceptible and severe epidemics can result in total crop failure. Yield losses from this disease vary considerably from year to year and region to region. In Canada, losses were reported to be as high as 50% (Conner et al. 2007). However, most of the information on mycosphaerella blight associated yield loss is qualitative or anecdotal and not quantitative, i.e., actual data on percentage of negative yield loss in the literature are not given and usually only estimates are presented. Bretag et al. (1995a) identified this problem and conducted a study to develop a yield loss model. They found that disease severity was closely correlated with reductions in grain yield, and that for most varieties, there was a 5–6% reduction in grain yield for every 10% of stem area affected by disease. As an average, in wet areas, yield losses were estimated to be at a maximum of around 30%, with losses dropping to about 20% in drier areas. This depended on variety, as some varieties showed more susceptibility than others. The very susceptible varieties showed losses as high as 70%.

Mycosphaerella pinodes is part of the ascochyta blight complex involving three fungal pathogens: *A. pisi* (Chilvers et al. 2009) causing leaf, stem and pod spot, *M. pinodes* (anamorph *Ascochyta pinodes*, causing leaf, stem and pod spot as well as foot rot, and *P. pinodella* causing leaf spot, stem lesions and foot rot (Bretag et al. 2006). Of the three, *M. pinodes* is by far the most abundant, causing 90% of ascochyta blight infections (Ali et al. 1982). Lawyer (1984) also reported that *M. pinodes* is the most damaging of the ascochyta blight fungi and

can reduce grain yields by up to 75%. In contrast, *P. pinodella* is of least importance because of its low pathogenicity and variability relative to the other two (Ali et al. 1978). Distinguishing the three pathogens from each other in the field situation is difficult as the symptoms are not readily discernible (Kraft and Pfleger 2001). Chen et al. (2010) developed a selective starch-casein medium that can be used to easily identify *M. pinodes* present in soil samples, but which is not able to distinguish this pathogen from *A. pisi* or *P. pinodella*. The three pathogens can be differentiated on agar medium by observing colony morphology and spore color, or by examination under the microscope (Bowen et al. 1996). Visual identification using morphological characteristics such as the size and shape of conidia and appressoria, pycnidial size, presence of chlamydospores, teleomorphic state and cultural characteristics such as colony color, growth rate and texture is accurate if done correctly (Punithalingam and Holliday 1972a; Punithalingam and Holliday 1972b; Punithalingam and Gibson 1976), but also can be unreliable as morphological characteristics of the three pathogens may overlap as a result of phenotypic variability and growing conditions (Taylor and Ford 2007). The most accurate pathogen diagnoses rely on biochemical and molecular techniques. Faris-Mokaiesh et al. (1996) used polyacrylamide gel electrophoresis (PAGE), enzyme staining, and polymerase chain reaction techniques and determined that *M. pinodes* and *P. pinodella* are very similar while *A. pisi* is quite distinguishable. Wang et al. (2000) used a similar approach and reached the same conclusions.

1.2.1. Taxonomy and Classification

Mycosphaerella pinodes is a heterothallic ascomycete fungus, with sexual reproduction requiring two alternate alleles at a single mating type locus, each encoding a single regulatory gene (Chilvers et al. 2009). For sexual reproduction to occur, the two isolates must be of different mating types (Taylor and Ford 2007). Stone (1912) considered the anamorph of *M. pinodes* to be *A. pisi*. However, Jones (1927) showed that the anamorph of *M. pinodes* was actually *A. pinodes*. Van Warmerlo (1966) placed the perfect stage of *A. pinodes* in *Mycosphaerella*, but more recently (Peever et al. 2007) showed that *M. pinodes* fits better within the *Didymella* taxon and proposed that the accepted name be changed *D. pinodes*. As literature on the *D. pinodes* taxon as a proposed new classification is extremely limited, for the purposes of this thesis, the teleomorphic stage will be referred to as *M. pinodes* and the asexual stage or the conidial form as *A. pinodes* as per Ali et al. (1982).

The genus *Mycosphaerella* was originally known as *Sphaeria* or *Sphaerella*, literally meaning a sphere or spherical fruiting body, due to the circular nature of the signs and symptoms of this disease (Crous 2009). However, since the name "*Sphaerella*" was already in use to denote a genus of green algae, all fungal taxa classified as *Sphaerella* were moved into *Mycosphaerella*.

1.2.2. Symptoms

Mycosphaerella pinodes causes necrotic spots on all aerial portions of the pea plant, including leaves, stems, flowers and pods (Roger et al. 1999a). Symptoms of infection are always more severe on the lowest parts of the plant (Beasse et al. 2000). Jones (1927) provided an excellent description of symptoms of *M. pinodes* infection as follows:

> Infection of the leaves by ascospores of *M. pinodes* results in many small purple spots on the leaves. Under drier conditions these remain small and without definite margins, while under moist conditions they enlarge, turning brown to black, assuming definite margins and often are arranged in distinct zones. Affected leaves may die but remain attached to the plant. Stem lesions are similar in colour and elongate, often extending upward and downward from the point of attachment of an infected leaf. These areas become progressively longer and often join together to completely girdle stems and give the entire lower plant a blue-black appearance. When the blossoms or flowers are infected, small, pinpoint lesions appear on the flower parts causing the blossom or small pods to drop, distorting the surviving pod and limiting seed production. Infected seed may be symptomless, or there may be shrinkage and dark brown discolouration. Spread of infection from seeds to emerging seedlings results in foot rot, starting at the point of seed attachment and advancing up the stem and down the taproot. Severe infection may kill or stunt young plants. As plants approach maturity, severe general infection causes senescence of all lower leaves and blackening of the stems at the base of the plant.

1.2.3. Disease cycle

Mycosphaerella pinodes survives in the soil, on the seed coat, in the seed, and on the pea residue (Bretag et al. 2006; Zhang et al. 2005b). The pathogen survives as mycelium on pea plant residues or in the soil as thick-walled mycelia (sclerotia) and chlamydospores. The mycelia walls thicken and darken, forming resting spores that survive up to 18 months, and the pycnidiospores transform into chlamydospores when buried (Dickinson and Sheridan 1968; Sheridan 1973; Bretag et al. 2001).

There is evidence to suggest that sporulation is higher during the afternoon and that it is also stimulated by light (Leach 1959). Temperature does not appear to be a critical factor, as spores can be released anywhere from 0 – 37^0C. Early in the season, when ascospores are carried into a new crop from infected pea debris nearby, a disease gradient may develop away from the source of infected stubble (Bretag 1991; Zhang et al. 2004). Release of ascospores also seems to be gradual, with a high concentration early in the season, followed by a slow decline in numbers as the season progresses. This suggests that the supply of ascospores gradually becomes exhausted. Plants may be attacked at any growth stage and all plant parts are susceptible. If moist conditions prevail throughout the growing season, there can be considerable infection of pods resulting in high levels of seed infection (Bretag et al. 2006).

Mycosphaerella blight can spread and develop through rain splash and air-borne spores as well as by commercial distribution of infected plant material or seed. Primary infection usually occurs during wet weather, when *M. pinodes* forms pseudothecia that produce air-borne ascospores that can be carried from infected fields over 1.6 km into new fields (Lawyer 1984). In this way, the pathogen can be disseminated over relatively large areas, including throughout an entire crop and even to nearby fields in which pea may not have previously been grown. After primary infection, symptoms of disease appear within 2–4 days. Secondary infection occurs when the pathogen produces pycnidia from which pycnidiospores are dispersed primarily by rain splash to lower leaves and adjacent plants. A high number of pycnidiospores can be liberated by rain splash from an infected plant, although most of these spores are deposited nearby and usually result in the disease spreading over a small area (Roger and Tivoli 1996). When sufficient moisture is available, pycnidia develop in new lesions and produce additional secondary inoculum (Bretag et al. 2006). Moist conditions are required for release, spread, and infection by both pycnidiospores and ascospores. Pycnidiospores are released from mature pycnidia during periods of rainfall or heavy dew (Walker 1969; Roger and Tivoli 1996; Zhang et al. 2005a), but can remain viable for up to 21 days under dry conditions (Banniza and Vandenberg 2003). Ascospore release was studied by Zhang et al. (2005a), who found that while moisture from dew was sufficient to cause the release of a few ascospores, the largest air-borne concentrations occurred early in periods of

rainfall. Roger et al. (1999a) showed that the amount of leaf wetness on host plants was the most important factor in determining whether or not infections developed. When plants were exposed to the same temperatures, symptoms developed faster on leaves with surface water as opposed to leaves under high relative humidity. Roger et al. (1999b) suggested that dry periods could be vital in hampering disease development and contributing to the formation of only small lesions. While infection and disease development appear to be stopped or reduced by a lack of moisture, the role of temperature in the infection process cannot be dismissed. Disease development takes longer under cooler conditions, and prolonged wetness is required (Roger et al. 1999a).

1.2.4. Epidemiology

Seed

Seed to seedling transmission

The fungus *M. pinodes* has been found on field pea seed in Canada since intensive seed examination was started in 1939 (Wallen et al. 1967), and is found as dormant mycelium in and on the seed in most pea growing areas of the world. Disease can result from the use of infected seed and this has been responsible for the introduction of *M. pinodes* into new regions (Ali et al. 1978; Bretag et al. 1995b: Xue et al. 1996). However, it is unclear from the literature exactly how long the pathogen can survive on pea seed, although anywhere from 3 – 6 years has been suggested (Bretag et al. 1995b). Fungal mycelium is, nevertheless, consistently found and may be confined to the exterior of the seed or may

penetrate the cotyledons, depending on infection conditions. Bretag et al. (1991) reported that fungal spores or mycelium are not only present on the surface of the seed, but can be harboured deep inside where they infect the hypocotyl. In contrast, Abd El Rehim et al. (1997) reported that *M. pinodes* was mostly located in the seed coat, and embryo infection was uncommon. Moussart et al. (1998) observed that if less than 25% of the seed coat was discoloured, the pathogen was only present on the surface of the seed, but if the amount of discolouration exceeded 25%, then it was found deep within the embryo tissues. Infection levels on seed will vary depending on weather conditions, but the amount of rainfall during the spring seems to be the most important factor, followed by temperature. There is a great deal of information in the literature about seed transmission of *M. pinodes*, but no correlation between seed infection and grain yield loss has been reported (Xue 2000). The key seems to be whether or not the infection is severe enough to affect germination, rather than whether it can spread within the plant to cause aerial symptoms (Moussart et al. 1998; Betag et al. 1995b).

Infection of seed

Whether or not *M. pinodes* is able to infect the seed and cause problems in subsequent years will depend on a variety of factors. Because the disease is favoured by wet conditions, seed produced under wet conditions is usually more highly infected than seed produced under drier conditions (Walker 1969; Bretag et al. 1995b; Jones 1927; Maude 1966). Seed infection is determined by weather

conditions between flowering and maturity. Warm humid conditions during this time usually result in heavy pod and seed infection. Chen et al. (1994) found that the fungus penetrates into the seeds through infected pods, and that younger pods are more susceptible to infection than older pods. Beasse et al. (1999) found that pod infection decreased the weight of individual seeds by 20%. Despite the occurrence of seed infection, however, it seems that *M. pinodes*-infected pea stubble or airborne ascospores usually represent a more important source of inoculum for crop infection.

Soil and pea residues

When pea are grown in repeated years in an area, the severity and incidence of *M. pinodes* is increased. This is due to the fact that the infected pea stubble and soil-borne spores remaining from the previous crops constitute a reservoir of primary inoculum that can infect new crops (Davidson and Ramsey 2000). Pathogen population levels in the soil and on crop residue are related to the severity of the epidemic in the last pea crop grown and so will vary from field to field (Zhang et al. 2005a).

Infected pea debris appears to be the main source of primary inoculum (Roger and Tivoli 1996; Walker 1969; Bretag and Ramsey 2001). This primary inoculum could be spread by wind or rain-splash from the residues onto new plants, or through contact of emerging seedlings with infected residues from previous years. Pycnidia (asexual spores) and perithecia (sexual spores) develop throughout the growing season on infected plants and after harvest on the pea

stubble. The formation of both perithecia and pycnidia seems to be triggered by senescence of the green tissue. Moisture is required for formation of both, and dry conditions delay their development and maturation (Roger and Tivoli 1996). Pycnidia can form within 3 days, whereas perithecia form within 14 days of initial infection (Roger et al. 1999b). As the minimum period from infection to formation of mature sporocarps in the field is only 3 days for pycnidia and 13–14 days for perithecia (Jones 1927), there are likely to be many generations per growing season.

Survival of *M. pinodes* in the soil, and the resulting infection of new plants, can be very important in pea crops as this pathogen competes well with saprophytes, enabling it to survive for years under optimal environmental conditions as dormant mycelium or chlamydospores. The ability of this pathogen to tolerate temperatures ranging from -20 to 25^{0}C (Wallen and Jeun 1968) also contributes to its success. When infected straw is buried, pycnidiospores and ascosprores contained within the mature fruiting bodies are transformed into thick-walled vegetative chlamydospores and can survive in the soil this way for long periods (Carter and Moeller 1961). When the debris is buried, there is restricted growth of the fungus and infection can only occur if there is close contact between the plant and the inoculum. Zhang et al. (2005a) studied *M. pinodes* residue, comparing surface residue with buried residue, and found that the pathogen survives better on surface residue because it is drier and less decomposed. With the widespread adoption of minimum and zero tillage

techniques across western Canada, the severity of residue-borne pathogens would be expected to increase (Bailey et al. 2000b), as at least 30% of the soil surface is left covered with plant residue (Bailey et al. 2000a). However, research done by Bailey et al. (2000a; 2000b; 2001) and Gossen (1997) showed that tillage systems did not affect foliar incidence or severity of disease in field pea. Tillage plays a minor role, as these studies have shown that the environment has a much larger impact than any other factor (Bailey et al. 2001).

Host – pathogen interactions

Mycosphaerella pinodes is a necrotrophic fungus that grows using nutrients in the apoplast of the host cells (Garry et al. 1996). Spores of *M. pinodes* germinate on the leaf surface and produce germ tubes that either directly penetrate the cuticle or enter through the stomata (Wroth 1998). According to Clulow et al. (1991), the pathogen enzymatically penetrates the cuticle via an infection peg formed under an appressorium, then grows through the outer wall of the epidermal cells, penetrating and growing within the cell walls predominantly aligned with the longitudinal axis of the epidermis, occasionally without causing necrosis (Clulow et al. 1991).This is followed by a typical nectrophobic phase involving progressive necrosis without entering the mesophyll of the plant. This fungus is capable of producing cell-wall-degrading enzymes, including amylase, aminopeptidases and invertases, and appears to also have high cellulase and pectinase activity (Agrios 2005). Heath and Wood

(1969) showed that *M. pinodes* was able to produce enzymes that enabled digestion of cellulose and suggested that this may be important both in pathogenicity and survival of this fungus as a saprophyte. The ability to digest cellulose ensures that the fungus has an available source of carbon even when some of the host tissue is dead or the entire host plant dies.

Mycosphaerella pinodes has been reported to attack species of *Pisum*, *Lathyrus*, *Vicia*, *Vigna*, *Medicago*, *Melilotus*, *Lens*, *Trifolium*, *Lupinus*, *Cicer*, and *Phaseolus* (Bretag 1991). However, the importance of other hosts is not clear, because many of these species were easily infected by spray inoculation under greenhouse conditions and often will escape infection in the field, where conditions are less favourable. Alternative hosts are generally considered of minor importance in the epidemiology of *M. pinodes* and are unlikely to be an important source of primary inoculum (Lawyer 1984). The pathogens of the larger complex known as ascochyta blight (which includes *M. pinodes*) are host-specific and so can be easily distinguished from other *Ascochyta* species attacking other pulse crops causing similar symptoms (Hernandez et al. 2006).

Impact of host canopy structure

Canopy structure will modify the microclimate of the pea crop and these modifications will affect mycosphaerella blight severity and progression. Le May et al. (2009) reported yield losses of 7–23% in different pea varieties and showed that canopy structure or morphological differences between varieties could influence grain yield, susceptibility and severity of mycosphaerella blight. This

work was related to stem density, which changed the structure of the canopy and affected the leaf area index (LAI) of the plant. The distance between internodes was just as important as the LAI. Cultivars with shorter internodes favour splash dispersal of conidia, whereas cultivars with longer internodes favour ascospore capture (Le May et al. 2009; Schoney et al. 2008). Dispersal is crucial in the lifecycle of a plant pathogen, ensuring disease progression. Plant canopy structures that favour capture of spores could play an important role in enhancing disease severity. Leaf size, leaf roughness and/or leaf flexibility could also have an impact on the capture of spore-containing water droplets. Other plant structures may contribute to the attraction of vectors or trapping of air-borne spores (Schoeny et al. 2008). Some authors have suggested that semi-leafless cultivars would promote air movement and lower disease severity, but this effect was not observed by Conner et al. (2007).

Lodging

Lodging has been associated with an increase in infection by *M. pinodes*. Lesions girdle and weaken the stems, which then break easily, leading to premature lodging and yield loss (Wang et al. 2006). It is estimated that lodging of pea causes yield reductions of approximately 10% on average, as a result of harvest losses and poorer quality of seed. Warkentin et al. (1996) observed that cultivars that were not prone to lodging had lower disease severity. Results from experiments conducted over three years by Banniza et al. (2003) confirmed that there is a strong link between lodging and disease development.

Injury

Banniza and Vandenberg (2003) showed that plant injury within 24 hours of infection increases mycosphaerella blight severity as well. Plants that come in contact with the pathogen shortly after injury (24-48 hours) develop symptoms more readily and severity is higher than plants that come in contact with the pathogen later (4-8 days).

Plant density

Seeding rate, and as a result plant density, is an important consideration in field pea crops (Gan 2003) and will affect mycosphaerella blight severity. Humidity will rise as canopy density increases, especially in the lower canopy where dense foliage reduces air movement. Humidity has a major influence on the rate of disease development (Roger et al. 1999a). Tivoli et al. (1996) found that epidemics were more severe in higher density canopies as compared to lower densities, because the movement of air and increased light penetration were less favourable for the disease in the latter. Roger et al. (1999b) presented data showing that without humidity, disease development is stopped or significantly reduced, so plant density can potentially have a large impact on humidity levels and as a result, disease levels. In addition, a dense canopy hinders the penetration of foliar applied fungicides to the lower levels, where disease severity is highest. Finally, competition among plants in a dense canopy may reduce stem diameter and a lesion on a thin stem may restrict water and nutrient transport more so than on a thicker stem. A thin stem may also break

and lodge easier. A study by Hwang et al. (2006) confirmed this. Disease severity increased with an increase in seeding rate, and yields were reduced by about 20% in the plots with higher plant densities. However, the levels of mycosphaerella blight were not high enough to overcome the increase in yield due to higher plant populations (Hwang et al. 2006).

Planting depth

Soil conditions, such as temperature and moisture level, will modify the efficacy of transmission of the pathogen from infected seeds to seedlings (Xue 2000). As seeding depth will affect both temperature and moisture, depth can be varied to affect the environment surrounding the seed. It is hypothesized that cooler, drier conditions will reduce pathogen spread. However, a study by Hwang et al. (2006) showed that depth of seeding did not affect seedling density, severity of disease, or yield in mature plants. The unexpected results of this study could possibly be explained by dry soil conditions and delays in seeding. During seed germination the environment was warmer than usual, promoting faster emergence, and moisture levels were lower than usual, masking the effects of seedling blight symptoms.

Planting and harvest date

Bretag et al. (2000) showed that in areas of Australia where planting date can be varied by as much as 3 months, planting the pea crop as late as possible will result in lower mycosphaerella blight disease severity. This is due to the fact that the young plants are able to avoid the high levels of primary inoculum that occur

earlier in the season when crops are planted later. The trade-off is that as a result of the higher temperatures and lower rain-fall amounts later in the growing season, yields tend to be lower. Nevertheless, in years where there is adequate moisture, planting the crop later may be an option, especially if a premium is paid for pathogen-free seed. This is very beneficial in areas where seeding dates can be varied, but in many areas around the world, including the Canadian prairies, this practice is not feasible due to existing limitations in the growing season.

Harvest date is another contributing factor of disease development. Dew formation at night on the mature plants later in the growing season can provide enough moisture for further disease development. Pea crops should be harvested as soon as practical once they reach physiological maturity in order to minimize the levels of seed infection by the fungus (Bretag et al. 2000).

Photosynthesis, biomass, compound translocation, seed weight and number

Studies by Garry et al. (1998a) showed that infection by *M. pinodes* caused a significant reduction in the photosynthetic leaf area of the plant, and a decrease in the photosynthetic efficiency of the remaining green leaf area. Beasse et al. (2000) confirmed this and suggested that the effect of *M. pinodes* on leaf photosynthesis is solely responsible for the decrease in plant growth, seed filling and lower yields. The negative effect of lower photosynthetic ability translates directly into other problems observed in many other studies. Measurement of the aerial biomass of diseased pea plants showed that the disease reduces plant

growth at all stages of development, from the start of flowering to seed filling (Tivoli and Banniza 2007), and that the decrease in the photosynthetic rate is proportional to the reduction in biomass production (Garry 1996, Garry et al. 1998b). Studies by Le May et al. (2005) and Tivoli et al. (1996) provided clues as to why this happens. These showed that the reduction in crop growth is due to the lower radiation use efficiency of diseased leaves, and to a limited degree, radiation interception efficiency. This affects translocation of carbohydrates and nitrogenous compounds from the leaf and hull into the seed or conversion to dry matter. They found that the levels of sucrose and starch were lower in infected plants, and so the seeds did not fill as well. This was attributed to water loss leading to pre-mature seed desiccation. Loss of these compounds also leads to lower protein quality in the seed. Many studies have been conducted showing that *M. pinodes* affects yield by reducing seed number and individual seed weight (Tivoli and Banniza 2007; Xue et al. 1997; Garry et al. 1998b; Beasse et al. 1999). High levels of infection before seed formation affects seed number, whereas once seed number is fixed, individual seed weight is affected.

1.2.5. Management of *M. pinodes*

Management of *M. pinodes* is best achieved by first reducing the amount of available primary inoculum and secondly suppressing the subsequent epidemic. To accomplish the first, pea debris must be destroyed, clean seed must be used, and soil-borne inoculum must be managed. To accomplish the second, careful selection of cultivars and application of foliar fungicides as needed should be

considered. Options for reducing the amount of infected pea stubble include crop rotation and burying plant debris by tilling the soil. Alternating the sowing date (if possible) may minimize exposure to inoculum. To get clean seed, an initial, disease-free seed source must be identified, and/or the seed must be treated to eliminate any seed-borne inoculum. The best long-term strategy would be the development of cultivar resistance, but attempts to achieve this have met with only limited success (Xue 2003). The current most utilized strategy for management of this disease is the application of fungicidal sprays in a preventative and systematic schedule (Beasse et al. 2000). A combination of the above strategies in an integrated management system would offer the most reliable approach for management of mycosphaerella blight, but the specific type of combination would be determined by economics, available options and epidemiological considerations.

Problems may arise with some of these management strategies, as in no-till or reduced till cropping systems, where burial of pea residue is not compatible with the tillage regime, or in areas where crop residue breakdown is slow (and cropping rotations may need to be longer) (Davidson and Kimber 2007). In some areas where the ascospores are the major source of infection, crop rotation would be less effective, so burial of the trash could be considered to accelerate residue decomposition, and fungicide application would have to be considered as the most effective option. In practice, residues remain on the surface in zero-till operations, and crop rotation periods may be adjusted to take advantage of

commodity price fluctuations (Hwang et al. 2006). Studies by Davidson and Ramsay (2007) showed that under certain conditions basic practices, like ensuring proper nutrient levels and the avoiding any type of additional plant stress, can contribute to decreased mycosphaerella blight severity as well.

Genetic resistance

At this time, even the most resistant field pea cultivars or breeding lines available are moderately susceptible to *M. pinodes* (Warkentin et al. 1996; Kraft et al. 1998), and their deployment alone is inadequate to control the disease (Fondevilla et al. 2005). Variability in the cultivars and differences in the virulence of different strains of *M. pinodes* complicates the selection for improved resistance (Ali et al. 1978; Clulow et al. 1992; Wang et al. 2000). Wroth (1998) found negative agronomic traits, such as days to flower, when transferring mycosphaerella blight resistance from another *Pisum* species to *P. sativum.* According to Conner et al. (2007), a combination of resistance in all three tissue types, leaves, stems and pods, is necessary to slow the build-up and spread of *M. pinodes*. A study by Xue and Warkentin (2001) showed that there appears to be a link between the resistance in the leaves and pods, but that resistance in the stems could be an independently-inherited trait. Clulow et al. (1992) concluded the same, reporting that resistance to *M. pinodes* in the stems and leaves of field pea involves different mechanisms. The resistance of stem tissue may be most important because the stem lesions are particularly damaging and contribute to lodging of the crop**.** Ali et al. (1978) stated that

resistance at the seedling stage is not correlated at all to expression of resistance in adult plants, but Fondevilla et al. (2005) found that pea lines that showed some resistance at the seedling stage also showed some resistance as adult plants. In later work, Fondevilla et al. (2007) reported that susceptibility is a dominant trait whereas resistance is a recessive trait, and suggested that resistance is controlled by multiple genes whose expression is highly influenced by the environment. Zhang et al. (2006) and Wroth (1999) also found resistance to be quantitative, highly dependent on the environment and moderately inheritable. This may help explain why resistance to this pathogen has been difficult to achieve.

1.3. THE USE OF FUNGICIDES

Increases in pea yields of 15-75% have been reported in field experiments where *M. pinodes* has been managed using fungicides (Bretag et al. 2006; Xue et al. 2003). Results of studies show that preventative sprays are more effective than curative sprays, that it is important that fungicides be applied before the disease becomes established, and that several sprays are required to effectively control the disease (Bretag 1985). Multiple sprays, initiated at early to mid-flowering, provide effective disease control and yield gain (Warkentin et al. 2000). Repeated fungicide applications, however, must be carefully considered to reduce the risk of insensitivty development to the fungicides by the pathogen.

The selection pressure imposed by the use of fungicides is the leading contributor to fungicide insensitivity (Gisi et al. 1997).

1.3.1. Importance of accurate placement of fungicides

It is extremely important to deliver and retain the active ingredients of a fungicide on critical sites at high enough rates to inhibit the target pathogen and protect the plant (Gossen et al. 2008). Most agrochemical systems are set up to deliver herbicides to the crop. The focus when using a herbicide system is to get coverage and contact, both horizontal and vertical, when plants are young. While this is an effective strategy for systemic herbicides that are applied to small plants, horizontal surfaces at the top of the canopy are not the ideal targets for a fungicide. Fungicides must be targeted to specific areas on a plant and are usually applied later to older plants, when the density of the crop canopy is much thicker and harder to penetrate. This makes delivery of the fungicide to the target tissues more difficult. Most fungicides are not translocated throughout the plant, and if they are, they travel only a short distance. Newer products penetrate into and redistribute throughout the leaf, but do not move in the xylem or phloem, and so do not translocate from the leaf where they are applied (Karadimos et al. 2005). Any products that do move systemically do so only upwards (Edgington 1981), and so need to be applied to lower areas of the plant or the base of the leaf in order to be distributed in that tissue. They will not translocate from upper leaves to lower portions of the plant.

1.3.2. Efficacy and cost effectiveness

The efficiency with which fungicides are utilized in agriculture is, in general, extremely poor (Chapple et al. 1997). In part, this is because these compounds are usually applied to large hectarage crops that are treated as a whole, even when only small areas of the crop are actually infected with the pathogen (Hislop 1987). To add to the problem is the fact that some areas of the field are more heavily infected than others, and unless the crop is very uniform the growth stages may vary. Ebert et al. (1999) noted that effective dose requirements necessary to manage the diseases being sprayed for are difficult to know and so producers tend to over-apply to ensure successful results.

Efficacy is determined by the uptake and effectiveness of the active ingredient and the degree of coverage of the target plant. Good plant coverage depends on the architecture of the plant, its leaf surface characteristics, the characteristics of the spray mixture, water volume, spray quality and spray angle. Armstrong et al. (2008) suggest that a narrow droplet size distribution that eliminates both small easily drifting droplets and large poorly retained droplets can increase fungicide efficacy.

1.3.3. Fungicide insensitivity management

Fungicides are essential for the maintenance of healthy crops and reliable yields in environments where disease pressure exists. However, the effectiveness of fungicides can be seriously affected in some situations by the development of fungicide insensitivity in target pathogens (Brent and Holloman, 2007a).

Fungicide insensitivity is an adjustment by a fungus due to selection by a fungicide, resulting in reduced sensitivity of the fungus to the fungicide. Eventually over the years of fungicide use, isolates of the target pathogen may arise that are no longer sensitive to the active ingredient (Brent and Holloman 2007b). Insensitivity may result from single or multiple gene mutations. Insensitive isolates typically arise through naturally occurring genetic mutations, but can be induced by delivering sub-lethal doses to the pathogen as well. The induction would be similar. These mutations confer insensitivity (or reduced sensitivity) to fungicidal compounds, particularly when applied at the recommended rates. Since the fungicide can still effectively control the sensitive isolates, insensitive isolates become more common under the selection pressure imposed by continued fungicide application; as the frequency of fungicide insensitive isolates continues to increase, application of the product may not be sufficient to control the disease (Ma and Michailides 2005). Insensitivity problems arise in cropping systems if some areas of the crop do not receive enough fungicide to kill all isolates of the pathogen. If the concentration is not high enough, there is selection for the most insensitive isolates of the pathogen.

Café-Filho and Ristaino (2008) showed how successive exposure of a pathogen to sub-lethal doses of a fungicide induced insensitivity to that fungicide.

Two problems that contribute to the development of fungicide insensitivity are spray retention and insufficient penetration into the crop canopy. Gossen et al. (2008) state that uniform spray coverage, able to penetrate the canopy and that delivers a consistent dose, could help alleviate this problem as well as other problems associated with spray application. In contrast, Ebert et al. (1999) state that sub-lethal doses of fungicides can result from uniform coverage and create tolerance to the fungicide. Uniform coverage gives time for the plant to grow, the pathogen to compensate and the active ingredient to break down, ultimately giving poorer results. Ebert et al. (1999) also suggest that more research is needed on effectively targeting the problem areas as opposed to targeting the whole crop. Wirth et al. (1991) attribute some of the insensitivity problems to the surface characteristics of the target crop plants. Some plant surfaces tend to have reflective properties that affect the amount of active ingredient retained, which in turn affects whether a dose is lethal or not. Improved fungicide spray application techniques focused on optimum product delivery, retention and efficacy based on changes to nozzles, orientation and droplet size have been shown to improve coverage and enhance crop health (Gossen et al. 2008).

Fungicide insensitivity, resulting from the repeated use of active ingredients belonging to the same group of fungicides, represents an additional problematic concept related to fungicide insensitivity management. When fungicides from

the same group, using the same mode of action, are applied repeatedly in the same growing season, the phenomenon of fungicide insensitivity in a pathogen population can be accelerated considerably than if the modes of action were different (Brent and Holloman 2007b).

1.3.4. Considerations for optimal fungicide delivery

When discussing fungicides, it is imperative to remember that there is an important difference between protectant and systemic fungicides. Contact fungicides must build up a uniform and stable deposit on the leaf surface in order to deflect fungal attack, whereas systemic fungicides can also prevent infection largely by redistribution inside the plant itself (Steurbaut 1993).

In the current review of the literature, no studies were found that defined the best spray coverage, droplet size, and water volumes to optimize the efficacy of these different kinds of fungicides. Researchers have anticipated that protectant fungicides will be most effective if applied as small droplets that evenly cover both sides of the leaf surface. Systemic fungicides that can be translocated within leaf tissue only need to target one side of the leaf, and so may be effective when applied as larger droplets (Bateman 1993; Elliot and Mann 1997). Nonetheless, small droplets tend to improve the efficacy of fungicides due to increased spray coverage, placement of the droplets on the underside of the leaf, and the increased frequency with which the pest

encounters the droplet (Spillman 1984). The negative aspects, however, include more susceptibility to drift and evaporation loss.

With both protectant and systemic fungicides, conditions that allow the droplets to remain on the leaf for a longer period of time before drying, like large droplets, and application under conditions of low wind, low temperature, and high humidity, will likely increase uptake (Gossen et al. 2008).

1.3.5. Droplet size

Droplet size is one of the most researched topics in sprayer application studies. There are benefits to the use of both small and large droplets in a spray application system. In general, the smaller the droplet size, the better leaf coverage and retention that can be expected. Using a fine spray quality provides alarge number of smaller droplets that are easily carried by the air flow of the sprayer, so smaller droplets give greater coverage and because of their size are less likely to drip off. It has been shown with both many herbicides and fungicides that smaller droplets give better efficacy than larger droplets (Knoche 1994; Armstrong et al. 2008). On crops, the smaller droplets are easily caught by the leaves at the top of the canopy and are likely to stay there unless either the surface is suddenly moved, causing the droplet to be thrown off, or a large number of droplets impact in the same area, and the surface becomes so saturated that run-off occurs (Cross et al. 2001). The movement of small droplets is largely dependent on meteorological conditions and the plant canopy itself.

However, small droplets do not penetrate the canopy as well and are readily displaced by wind, constituting the majority of off-target drift (Spillman 1984; Wolf et al. 1993). Smaller droplets also result in faster evaporation rates. Large droplets are likely to get caught near the top of the canopy, as well as penetrate into the lower parts of the crop. Feng et al. (2003) showed that spray absorption is improved with larger droplets as well. The absorption of fungicides used in that study increased with an increase in droplet size.

There are so many factors affecting the efficacy of droplet size that it is difficult to make general statements as to which size is more effective. Some data show larger droplets to be more effective, whereas other data show smaller droplets to be superior. When reviewing herbicide studies, Knoche (1994) found that in 71% of studies, spray retention increased as droplet size decreased, in 21% of the studies there was no difference, and in 9% of studies smaller droplets negatively affected spray retention. For each spray system, the best droplet size to use for optimum retention will depend on the desired outcome. Most studies would indicate, however, that penetration into the canopy and a high level of spray coverage on the leaves tend to be the most important factors with respect to retention. Larger droplets penetrate the canopy better, are more readily absorbed and seem to be more effective overall because of the lower risk of drift (Feng et al. 2003; Maybank et al. 1991; Armstrong-Cho et al. 2008).

1.3.6. Nozzle types and configurations

There are many different types of nozzles available to deliver spray products to the crop. Hydraulic nozzles, usually a flat-fan or hollow-cone type, are the primary means of applying fungicides. A tapered flat-fan nozzle design is most common because it provides a uniform spray pattern and minimum spray drift (Gossen et al. 2008; Elliot et al. 1997). Hydraulic flat fan nozzles are superior in terms of the evenness of the spray delivered to the crop canopy. Replacing hydraulic nozzles with venture style improves spray distribution under certain crop and weather conditions, and increases crop penetration (Nordbo et al. 1993). As a result, air-induced nozzles that produce coarser sprays are becoming more widely used (Wolf et al. 2000).

Higher fungicide depositions on lower leaves were observed by Armstrong-Cho et al. (2008) when sprayed with an air induction nozzle compared to conventional flat fan nozzles. Nozzle choice had no significant effect on disease or yield in canola, as long as the spray pressure on air-induced nozzles was sufficiently high (Kutcher and Wolf 2006).

There has been considerable research directed toward improving sprayer technology to overcome some of the shortcomings of conventional nozzle systems, but hydraulic nozzles continue to dominate commercial applications because of their versatility in delivering all classes of agrochemicals (Gossen et al. 2008).

1.3.7. Double nozzles and nozzle orientation

Some research was done by Hall et al. (1996) comparing the use of double nozzles to single nozzles for spray coverage. They found that a double nozzle system improved the spray pattern for finer sprays, provided greater pesticide efficacy, and reduced the dilution of the product on the leaf surface. The system involves two nozzles working together. One nozzle produces a coarse spray, delivering water directly down, while the second nozzle produces a fine spray delivering the active ingredient into the water spray cloud (Downer 2009). Using this system, Chapple et al. (1997) found that application amounts could be reduced to 30-50% of the label rate.

For application to vertical targets such as wheat heads or grassy weeds, Wolf (2009) recommends double nozzles, with one pointed forward and the other backward, for better coverage. This could easily apply to other vertical targets such as the growing tip of pulses. No data were found using double nozzles indicating whether or not the coverage translated to lower disease ratings or increased yield using this nozzle system.

Different orientation of nozzles to change the angle at which the spray formulation hits the target can have an effect on the amount of penetration there is into the crop canopy. As a rule, nozzles should face backward when fungicides are being applied because canopy penetration is best with this orientation (Wolf 2009). Armstrong-Cho et al. (2008) found that angling spray nozzles backward resulted in better fungicide coverage of the middle and lower leaves compared to straight nozzles.

1.3.8. Carrier volumes

Increasing the water volumes used in sprayer systems substantially improves penetration into the crop and increases the frequency of droplets at all levels of the canopy. Larger volumes have the added benefit of decreasing the potential for drift and increasing nozzle performance (Wirth 1991). Most research suggests that both fungicides and herbicides work best with higher carrier volumes (Wolf et al. 1993). Work by Cross et al. (2000) showed that an increase in fungicide carrier volume significantly reduced the disease severity under moderate to high disease pressure. Disease severity data taken by Armstrong-Cho et al. (2008) revealed the same result.

While weeds can be a problem in a wide variety of crops, fungal diseases tend to be very crop-specific. Fungicide formulations as well as carrier volumes often must be customized to obtain effective results (Steurbaut 1993). Despite the apparent importance of coverage in fungicide application, the scientific literature contains few studies on the effects of carrier volumes on plant pathogens. A review of 110 studies, done by Knoche (1994), on the effects of carrier volumes ranging from 5 to 2200 L/ha on the efficacy of herbicides revealed that coverage was not the only critical factor determining herbicide efficacy. In 25% of these studies, herbicide efficacy was increased by lowering carrier volumes, in 32% no effect was reported for carrier volumes, and in 44% increasing carrier volumes increased herbicide efficacy. It therefore appears as

though the effect of carrier volume on herbicide performance is dependent, at least in part, upon other factors.

If large carrier volumes create a challenge, Wolf (2009) and Cross et al. (2001) state that smaller droplet sizes can be used to compensate as long as drift can be managed. Jensen et al. (2001) agree, but caution that if water volumes are not high enough to maintain adequate droplet densities, aspects of spray targeting may be compromised. Most fungicides, but not all, will work effectively on crops when applied at lower volumes. Some fungicides require high volumes to work.

1.4. STROBILURIN FUNGICIDES

Strobilurin fungicides are an important class of fungicides that has been widely used since 1996 (AgroPages 2011). They provide disease control and also produce favorable effects on the physiology of the plant. These fungicides were initially isolated from the mycelium of a Basidiomycete wood-rotting mushroom fungi called *Strobilurus tenacellus*, which led to these compounds being known as strobilurins (Anke et al. 1977). The popularity of this chemical family as a fungicide grew very quickly, as the binding site was novel at the time and insensitivity issues crossing over between the strobilurins and other fungicides was not likely at the time of introduction. Beginning with temperate cereals and expanding to include a wide variety of crops around the world, strobilurins can now be considered to be one of the most valuable classes of fungicides ever

discovered (AgroPages 2011). They are single-site mode of action fungicides classified as quinine outside inhibitors (Qol's) because they work by binding at the Qo site of cytochrome b in the cytochrome bc1 enzyme complex found in the inner mitochondrial membrane of fungi. With the inhibitor bound, electron transfer between cytochrome b and cytochrome c1 cannot occur, resulting in an energy deficiency due to a lack of ATP (Bartlett et al. 2002).

1.4.1. Insensitivity issues with strobilurin fungicides

Some classes of fungicides are more prone to elicit insensitivity issues in the target organisms than others. Although the strobilurins do not exhibit insensitivity issues crossing over between them and other fungicide groups, there are at least eight different natural and synthetic fungicidal formulations available where this has happened (Bartlett et al. 2002; Sierotzki et al. 2000). The site-specific mode of action of the strobilurins put them at a high risk for development of fungicide insensitivity within the pathogen they manage. These fungicides are very widely used and fungicide insensitivity resulting from the repeated use of active ingredients belonging to the same group represents a problem for fungicide insensitivity management.

There are two types of insensitivity: quantitative and qualitative. In quantitative insensitivity, the pathogen becomes less sensitive to the fungicide in comparison to the wild-type, but it can still be controlled with higher rates and/or more fungicide applications. With qualitative insensitivity, the pathogen

becomes completely insensitive to the active ingredient and control is no longer possible at field rates. Naturally occurring insensitivity to the strobilurin fungicides has been shown to be qualitative (Ypema and Gold 1999). Insensitivity to strobilurin fungicides usually results from an alteration at the fungicidal binding site in the target pathogen, so that the fungicide cannot inhibit respiration. Specifically, most pathogen insensitivity is conferred by a single nucleotide change in the mitochondrial cytochrome b gene, leading to a substitution of amino acid residue 143 from glycine to alanine (G143A) (Torriani et al. 2008) or of amino acid residue 129 from phenylalanine to leucine (F129L). Populations with the F129L mutation are moderately insensitive to strobilurins, and effective control with QoI is still possible, whereas the G143A mutation results in complete loss of sensitivity to the fungicide (Gisi et al. 2000). Results from a study conducted by Torriani et al. (2008) showed that the development of strobilurin insensitivity in one *Mycosphaerella* species was due to independent mutation of G143 in isolates from different geographical areas and genetic backgrounds. The frequency of the mutation increased as a result of fungicide selection and was spread by wind-borne ascospores.

Mutational changes of the cytochrome *b* target site are responsible for several cases of insensitivity to QoI fungicides (Avila-Adame et al. 2003), and there are many documented cases of pathogen insensitivity to the strobilurins. Within the *Mycosphaerella* genus, there are three commonly studied species, (*M. fijiensis (*Morelet), *M. citri* (Whiteside) and *M. graminicola* (Fuckel) Schrot),

in the literature, causing considerable economic losses (Grasso et al. 2006; Gisi et al. 1997; Keinath 2009; Miguez et al. 2003; Mondahl et al. 2005). The highest Ec_{50} value (the effective concentration to inhibit 50% of the pathogen growth) of the three *Mycosphaerella* pathogens tested has been identified in *M. citri*. The Fungicide Resistance Action Committee (FRAC) lists 48 pathogens insensitive to this fungicide group, which occur on many crops ranging from fruit and vegetables to pasture and field crops, that impact many agricultural areas. It is important to remember that these pathogens are always changing and insensitivity in one geographical area does not necessarily mean insensitivity in other areas, but it does mean that insensitivity management strategies must be used (FRAC 2011).

1.4.2. Pyraclostrobin

Pyraclostrobin is a broad-spectrum fungicide controlling major plant pathogens from four classes of fungi or fungal-like microorganisms: ascomycetes, basidiomycetes, deuteromycetes, and oomycetes. This product has protective, curative, eradicative, translaminar and locosystemic properties, depending on the crop on which it is used (AgroPages 2011). This fungicide became commercially available in 2002 (Barlett et al. 2002) and is used on a wide variety of crops. It is rapidly absorbed by the plant and retained in the waxes of the leaf cuticle. It has good translaminar movement from one side of the leaf to the other, resulting in disease control on both sides of the leaf surface. The

fungicide works by inhibiting spore germination, giving it preventative qualities, and halting mycelial growth, giving it curative or eradicative qualities. Due to its rapid uptake by plants, the product has a short rainfast period, adding to its effectiveness. Pyraclostrobin is also reported to have beneficial effects on plant growth and yield as a result of enhanced nitrate reductase activity, which leads to improved nitrogen assimilation, and decreased ethylene production, which delays senescence. According to BASF, this fungicide provides unprecedented control of *Ascochyta* / *Mycosphaerella* species in pulse crops (BASF 2011).

Significant amounts of insensitivity research have already been carried out on pyraclostrobin, with insensitivity having been documented in many pathogens (FRAC 2011). In Canada, the research into pyraclostrobin insensitivity in field crop pathogens has focussed on *Ascochyta rabiei* on chickpea, which has developed qualitative insensitivity to this product (Gossen and Anderson 2004; Thaher 2011; Chang et al. 2007). Field pea, like chickpea, is a high value, drought resistant crop that helped to increase cropping system diversity in the dry regions of the Canadian prairies (Chang et al. 2007). The similarities between the pathogens affecting chickpea and field pea have led to interest on the question of fungicide insensitivity in pathogens associated with the latter.

1.4.3. Baseline sensitivity work

In order to properly monitor and detect the possible development of fungicide insensitivity in a pathogen species, the baseline sensitivity of the

pathogen to the fungicide must be established. This is done by collecting isolates of the pathogen through field surveys prior to fungicide use and testing these for sensitivity to the product. Another way would be to test a population "A" that has never been exposed to the fungicide, and compare it against a population "B" that has been exposed (Avenot and Michailides 2007). Brent and Holloman (2007a) state that it is important to undertake these types of studies for three reasons: (1) to develop and test an accurate, rapid, reproducible method for determining the degree of sensitivity of large numbers of field samples of major target fungi, so that such a method is readily available for any future monitoring that may be required; (2) to obtain initial data regarding the range of sensitivity that exists in major target pathogens and major areas of use, to serve as a baseline against which any future measurements of sensitivity can be compared in order to reveal any possible shifts in sensitivity; and (3) to detect any differences in sensitivity among samples that might, through the build-up of the less sensitive components, lead to future insensitivity problems. Jutsum et al. (1998) and Russell (2004) also stressed the importance of determining the range of sensitivities present in target pathogen populations. They indicated that this work should be conducted prior to the commercialization of any product. Gisi et al. (2002) adds that these studies must be determined separately for each fungicide/pathogen combination. A pathogen with a very narrow range of baseline sensitivities might be easily monitored using only a few discriminating

doses, whereas a pathogen with a wide baseline might need to be monitored with a much broader range of doses (Jutsum et al. 1998).

1.4.4. Radial growth and conidial germination testing

In order to test the fungicide sensitivity levels of a fungal pathogen, one of two *in vitro* Petri dish techniques are generally used. The first technique is a radial growth or colony growth assay. Isolates of the fungus are grown on an appropriate culture medium. Small mycelial plugs are transferred onto fresh medium containing various concentrations of the fungicide being tested (and a control plate containing no fungicide for comparison). The fungicide-amended plates and controls are incubated at temperatures and light conditions conducive for optimal growth for a prescribed amount of time or until the control colonies have grown to cover 50-75% of the plate. Colony growth on the fungicide-amended plates is then compared to the controls and a percent growth (PG) or relative growth (RG) is calculated (Mondahl et al. 2005; Wise et al. 2008).

The second technique consists of conidial germination assays. Isolates of the fungus are cultured on appropriate medium as above. The colonies are then flooded with deionized water and gently scraped to release conidia. The conidial suspensions are adjusted to an appropriate concentration and placed onto medium containing various concentrations of the fungicide being tested. Controls in which no fungicide is included are also prepared for comparison. The

spore containing plates are incubated for a prescribed period (usually 24 h) under conditions conducive for optimal germination of the spores, and then examined for rates of germination. Typically, 100 conidia are examined per plate, and a spore is considered germinated if the germ tube is as long as the conidium itself (Olaya and Koller 1999). Germination on the fungicide-amended plates is then compared to the control plates and a percent germination (PG) or relative germination (RG) is calculated (Keniath 2009 and Wise et al. 2009).

The sensitivity of fungal isolates evaluated with either technique can be assessed by estimating the effective concentration of the amount of fungicide active ingredient required to inhibit radial growth or conidial germination by 50%, which is calculated using a statistical analysis appropriate for the dose response curve (Keniath 2009; Mondahl et al. 2005; Wise et al. 2008). Of the two approaches described above, radial growth is the less labor-intensive and time-dependent method, but conidial germination must also be assessed to verify radial growth results, since the primary growth stage targeted by protectant fungicides is the germination of conidia. An advantage of the conidial germination procedure is that it produces information in a short period of time, which may help guide insensitivity management strategies with possible implementation within a growing season (Seyran et al. 2010).

1.4.5. Saliclyhydroxamic acid (SHAM)

In vitro research on fungal respiration in the presence of respiration-inhibiting fungicides, such as the strobilurins, has shown that some of these microorganisms have the ability to use an alternative respiration pathway, which involves the production of a cyanide-insensitive alternative oxidase (AOX) (Miguez et al. 2003). This enables the bc complex in the mitochondrial respiration chain to be by-passed and allows mycelial growth and spore germination even in the presence of the fungicide (Oyla and Koller 1999; Vincelli and Dixon 2002; Ziogas et al. 1997). While this phenomenon has been observed *in vitro*, it is hypothesized that plant-produced flavones prevent the induction of AOX in nature, thus inhibiting alternative respiration on crops in the field (Oyla and Koller 1999; Vincelli and Dixon 2002). The three large protein complexes of mitochondrial nicotinamide adenine dinucleotide (NADH) oxidation are complex I, III, and IV in the mitochondrial respiration chain. AOX allows electron flow through ubiquinol to complex III (Wood and Hollomon 2003; Ypema and Gold 1999). The induction of this alternative pathway of respiration can serve as a highly effective rescue mechanism when pathogen sensitivities are tested in the absence of a host, but thus far, alternative respiration has not been directly affiliated with selection of QoI-insensitive pathogens (Avila-Adame et al. 2003). However, alternative respiration may have an impact on results of *in vitro* assays, leading to incorrect assessments of fungicide sensitivity. Two chemicals can be used to inhibit AOX, salicylhydroxamic acid (SHAM) or propyl gallate (3,4,5-trihydroxybenzoic acid propyl ester). Wise et al. (2009) and Miguez et al.(2003)

state that an AOX inhibitor should be included in any QoI *in vitro* fungicide sensitivity assessments, to prevent fungal pathogens from using this alternative respiration mechanism that could confound the results.

1.4.6. Fitness of isolates

In the absence of the fungicide, some fungicide-insensitive strains are less fit than sensitive ones (Al-Mughrabi and Gray 1995). This may affect the rate at which the pathogen population develops insensitivity to a fungicide. The relative fitness of insensitive versus sensitive strains will depend on the particular fungal species, the nature of the mutations conferring insensitivity, and the mode of action of the fungicide. Without the fungicide present, the fungicidial insensitivity trait can be accompanied by poor germination or reduced fungal growth, which will contribute to a lack of fitness, poor viability and a resulting decline in the rate at which a fungal population will stop responding to a fungicide group (Ziogas et al. 2002). However, Gisi et al. (2002) report that the mutations at G143 and F129 conferring insensitivity to pyraclostrobin or any of the strobilurin fungicides do not impact isolate fitness. Dekker (1976) states that even though insensitive isolates are identified in the laboratory, this does not necessarily mean that insensitivity will arise in a field situation, but in some cases it is a very strong indication of this possibility. Dekker (1976) goes on to say that insensitive fungi that sporulate abundantly on aerial parts of the plant may spread very rapidly after the sensitive populations are eliminated by the

fungicide. It is possible also that an insensitive population becomes sensitive when use of the fungicide is terminated, but as soon the fungicide is re-introduced the insensitivity shows up again immediately. This suggests that fungicide insensitivity issues associated with strobilurins are irreversible (Dekker 1976).

1.5. PRESENT RESEARCH AND OBJECTIVES

1.5.1. Strobilurin sensitivity testing

The strobilurin group of fungicides has been identified by FRAC, and repeatedly throughout the literature, as being at high risk for the development of pathogen insensitivity. Indeed, many fungal pathogens have already developed insensitivity to these compounds. Pyraclostrobin is a strobilurin fungicide widely used in Canada since 2002. It is commonly and repeatedly applied to field crops on the Canadian prairies and the northwestern United States. Upon the initiation of this study there had been a shift to strobilurin-insensitivity in western Canadian populations of the chickpea pathogen *A. rabiei*. The asexual form of *M. pinodes* is *A. pinodes*, a close relative of *A. rabiei*. Given that these two similar fungi occur on similar crops, which are grown in the same geographical areas and are treated with the same fungicide group, we hypothesized that *M. pinodes* may also be at risk for the development of fungicide insensitivity. Therefore, as one component of this thesis, an evaluation of the fungicide sensitivity of *M. pinodes* was carried out *in vitro*.

Specifically, the objectives of this component of the thesis were to: (1) determine the baseline sensitivity level and EC_{50} value of *M. pinodes* populations from field pea to pyraclostrobin fungicide using isolates collected before the use of this fungicide; and (2) test *M. pinodes* isolates collected in 2010 and 2011 to determine if the sensitivity level of the pathogen to the fungicide is changing on the Canadian prairies and the northwestern United States.

1.5.2. Sprayer technology testing

Factors contributing to whether or not a pathogen develops fungicide insensitivity include: the properties of the fungicide and/or its mode of action, variability within the pathogen population, and the way in which the fungicide is delivered. Each time a fungicide is applied there is selection pressure on the target fungus, which may result from insufficient canopy coverage and the potential for non-lethal doses that contribute to insensitivity. There are many factors to consider when applying a fungicide in order to obtain the maximum coverage with minimum product at the correct time. Different nozzle and spray types, angles, water volumes, application rates and timings are all important. Research on the delivery of pyraclostrobin to field pea for optimal *M. pinodes* control is lacking. Therefore, a second component of this thesis focused on the evaluation of sprayer technologies, with the specific objective of determining the most effective fungicide delivery system for optimal crop coverage for reducing mycosphaerella blight severity on field pea and increasing seed yield.

1.6. LITERATURE CITED

Abd El Rehim, M.A., Abo Taleb, E.M., Michail, S.H. and Metwally, S.M. 1997. Seed health testing of peas for *Ascochyta* spp. and their impact on foliage seed health and seed yield. Acta Phytopathol. et Entomol. Hung. 32: 89–95.

Agriculture and Agri-food Canada. 2009. [Online]. Available: http://www4.agr.gc.ca/AAFC-AAC/display-afficher.do?id=1247678986193&lang=eng [accessed September 2010].

Agrios, G. 2005. Plant Pathology. 5th ed. Elsevier Academic Press. Page 202.

AgroPages. 2011. Strobilurin fungicides. [On-line]. Available: http://www.agropages.com/AgroData/AgroDataList-2-3699. [accessed December 2011].

Alberta Agriculture and Rural Development. 2011. Canadian Peas Supply and Disposition. [On-line]. Available: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/sdd12672 [accessed December 2011].

Al-Mughrabi, K.I. and Gray, B.A. 1995. Competition between triadimefon-sensitive and triadimefon-resistant isolates of *Erysiphe graminis* f. sp. *tritici*. Plant Dis. 79: 709–712.

Ali, S.M.,Nitschke, L.F., Dube, A.J., Krause, M.R. and Cameron, B. 1978. Selection of pea lines for resisance to pathotypes of *Ascochyta pinodes, A. pisi* and *Phoma medicaginis* var *pinodella.* Aust. J. Agric. Res. 29: 841–849.

Ali, S.M., Paterson, J., and Crosby, J. 1982. A standard technique for detecting seed-borne pathogens in peas, chemical control and testing commercial seed in South Australia. Aust J. Agric. Res. 22: 348−352.

Anke, T., Oberwinkler, F., Steglich, W., and Schramm, G. 1977. Strobilurins – New antifungal antibiotics from the basidiomycete *Strobilurus tenacellus* (PERS ex FR) Sing. J. Antibiot. 30: 806−810.

Armstrong-Cho, C., Chongo, G., Wolf, T., Hogg, T., Johnson, E., and Banniza, S. 2008. The effect of spray quality on ascochyta blight control in chickpea. Crop Prot. 27: 700–709.

Avenot, H.F. and Michilaids, T.J. 2007. Resistance to boscalid fungicide in *Alternaria alternata* isolates from pistachios in California. Plant Dis. 91: 1345–1350.

Avenot, H.F. and Michailides, T.J. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Prot. 29: 643−651.

Avila-Adame, C., Olya, G. and Koller, W. 2003. Characterization of *Colletotricum graminicola* isolates resistant to strobilurin-related QoI fungicides. Plant Dis. 87: 1426–1432.

Bailey, K.L., Gossen, B.D., Derksen, D.A. and Watson, P.R. 2000a. Impact of agronomic practices and environment on diseases of wheat and lentil in southeastern Saskatchewan. Can. J. Plant Sci. 80: 917–927.

Bailey, K.L., Johnston, A.M., Kutcher, H.R., Gossen, B.D. and Morrall, R.A.A. 2000b. Managing crop losses from foliar diseases with fungicides, rotation, and tillage in the Saskatchewan Parkland. Can. J. Plant Sci. 80: 169–175.

Bailey, K.L., Gossen, B.D., Lafond, G.P., Watson, P.R. and Derksen, D.A. 2001. Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: Univariate and multivariate analyses. Can. J. Plant Sci. 81: 789−803.

Banniza, S. and Vandenberg, A. 2003. The influence of plant injury on development of *Mycosphaerella pinodes* in field pea. Can. J. Plant Pathol. 25: 304−311.

Banniza, S., Warkentin, T., Vandenberg, A., and Davis, A., 2003. Investigation into the relationship between lodging, stem anatomy, degree of stem lignifications and susceptibility to *Mycosphaerella pinodes* in field pea. Final Report for project 19990342.

Bartlett, D.W., Clough, J.M., Godwin, J.R.,Hall, A.A., Hamer, M., and Parr-Dobranzki, B. 2002. The strobilurin fungicides. Pest Manage. Sci. 58: 649-662.

Bateman, R. 1993. Simple, standardized methods for recording droplet measurements and estimation of deposits from controlled droplet applications. Crop Prot. 12: 201–206.

BASF. 2011. Headline. [On-line] Available: https://agro.basf.ca/West/Products/HEADLINE.html [accessed December 2011].

Beasse, C., Ney, B. and Tivoli, B. 1999. Effects of pod infection by *Mycosphaerella pinodes* in yield components of pea (*Pisum sativum*). Ann. Appl. Biol. 135: 359−367.

Beasse, C., Ney, B., and Tivoli, B. 2000. A simple model of pea (*Pisum sativum*) growth affected by *Mycosphaerella pinodes*. Plant Pathol. 49: 187−200.

Bowen, J.K., Peart, J., Lewis, B.G., Cooper, C., and Matthews, P. 1996. Development of monoclonal antibodies against the fungi of the Ascochyta complex. Plant Pathol. 45: 393−406.

Brent, K.J. and Holloman, D.W. 2007a. Fungicide resistance: The assessment of risk. Fungicide Resistance Action Committee, Monograph 2 (revised), Global Crop Protection Federation, Brussels. Available: http://www.frac.info/frac/index.htm [accessed March 2011].

Brent, K.J. and Holloman, D.W. 2007b. Fungicide resistance in crop pathogens: How can it be managed? [Online]. Fungicide Resistance Action Committee, Monograph 1, Global Crop Protection Federation. Brussels. Available: http://www.frac.info/frac/index.htm [accessed March 2011].

Bretag, T.W. 1985. Chemical control of ascochyta blight of field peas. Aust. Plant Path. 14: 42–43.

Bretag, T.W. 1991. Epidemiology and control of Ascochyta blight of field peas. Ph.D. thesis, La Trobe University, Victoria, Australia.

Bretag, T.W., Keane, P.J. and Price, T.V. 1995a. Effect of ascochyta blight on the grain yield of field peas (*Pisum sativum* L.) grown in southern Australia. Aust. J. Exper. Agric. 35: 531–536.

Bretag, T.W., Price, T.V. and Keane, P.J. 1995b. Importance of seed-borne inoculum in the etiology of the ascochyta blight complex of field peas (*Pisum sativum* L.) grown in Victoria. Aust. J. Exper. Agric. 35: 525–530.

Bretag, T.W., Keane, P.J. and Price, T.V. 2000. Effect of sowing date on the severity of ascochyta blight in field peas (*Pisum sativum* L.) grown in the Wimmera region of Victoria. Aust. J. Exper. Agric. 40: 1113–1119.

Bretag, T.W. and Ramsey, M.D. 2001. Foliar diseases caused by fungi: *Ascochyta* spp. In Compendium of pea diseases and pests. APS Press: The American Phytopathological Society: St. Paul, MN. Edited by Kraft, J.M. and Pfleger, F.L. pp. 24–28.

Bretag, T.W., Keane, P.J. and Price, T.V. 2006. The epidemiology of ascochyta blight in field peas: A review. Aust. J. Agric. Res. 57: 883−902.

Café-Filho, C.C. and Ristaino, J.B. 2008. Fitness of isolates of *Phytophthora capsisci* resistant to mefenoxam from squash and pepper field from North Carolina. Plant Dis. 92: 1439–1443.

Canadian Enclyclopedia. 2011. [Online]. Available: http://www.thecanadianencyclopedia.com/index.cfm?PgNm=TCE&Params=A1ARTA0006160 [accessed January 2012].

Carter, M.V., and Moller, W.J. 1961. Factors affecting the survival and dissemination of *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr. in South Australian irrigated pea fields. Aust. J. Agric. Res. 12: 878–888.

Chang, K.F., Ahmed, H.U., Hwang, S.F., Gossen, B.D., Strelkov, S.E., Blade S.F. and Turnbull, G.D. 2007. Sensitivity of field populations of *Ascochyta rabiei* to chlorothalonil, mancozeb, and pyraclostrobin fungicides, and effects of strobilurin fungicides on the progress of ascochyta blight of chickpea. Can. J. Plant Sci. 87: 937–944.

Chapple, A.C., Wolf, T.M., Downer, R.A., Taylor, R.A.J., and Hall, F.R. 1997. Use of nozzle-induced air-entrainment to reduce active ingredient requirements for pest control. Crop Prot. 16: 323–330.

Chen, M.H., Huang, J.W., and Yein, C.F. 1994. The pathway and influence factors in pod and seed infections of garden peas by *Mycosphaerella pinodes*. Plant Pathol. Bull. 3: 133–139.

Chen, M.H., Chung, W.C., Huang, H.C. and Huang, J.W. 2010. Development of a semi-selevtive meium for detection of *Mycosphaerella pinodes* in soil, plant debris and seed. Can. J. Plant Pathol. 32: 342−350.

Chilvers, M.I., Rogers, J.D., Dugan, F. M., Stewart, J.E., Chen, W. and Peever, T. 2009. *Didymella pisi* sp. nov., the teleomorph of *Ascochyta pisi*. Mycol. Res. 113: 391−400.

Chongo, G. and Gossen, B.D. 2003. Diseases of Pea. Pages 201-213 *in* K. L. Bailey, B. D. Gossen, R. Gugel, and R. A. A. Morrall, eds. Diseases of field crops in Canada. Canadian Phytopathological Society, Saskatoon, SK. 290 pp.

Clulow, S.A., Lewis, B.G., and Matthews, P. 1992. Expression of resistance to *Mycosphaerella pinodes* in *Pisum sativum*. 41: 362−369.

Clulow, S.A., Lewis, B.G., Parker, M.L., and Matthews, P. 1991. Infection of pea epicotyls by *Mycosphaerella pinodes* in pea seedlings. Mycol. Res. 95: 817−820.

Conner, R.L., Hwang, S.F., Woods, S.M., Chang, K.F., Bing, D.J., Dongfang, Y., Su, H., McAndrew, D.W. and Yager, L.M. 2007. Influence of agronomic traits on the expression of tissue-specific resistance to mycosphaerella blight in field pea**.** Can. J. Plant Sci. 87: 157–165.

Cross, J.V., Walklate, P.J., Murray, R.A., and Richardson, G.M. 2001. Spray deposits and losses in different sized apple trees from an axial fan orchard sprayer. 2. Effects of spray quality. Crop Prot. 20: 333–343.

Crous, P.W. 2009. Taxonomy and phylogeny of the genus *Mycosphaerella* and its anamorphs. Fungal Div. 38: 1−24.

Davidson, J.A. and Kimber, R.B. 2007. Integrated disease management of ascochyta blight in pulse crops. Eur. J. Plant Pathol. 119: 99–110

Davidson, J.A., and Ramsey, M.D. 2000. Pea yield decline syndrome in South Australia: the role of diseases and the impact of agronomic practices. Aust. J. Agric. Res., 51, 347–354.

Dekker, J. 1976. Acquired resistance to fungicides. Annu. Rev. Phytopathol. 14: 405−428.

Demirci, F., Bayraktar, H., Babaliogullu, I., Dolar,F.S., and Maden, S. 2003. *In vitro* and *in vivo* effects of some fungicides against the chickpea blight pathogen, *Ascochyta rabiei*. J. Phytopathol. 151: 519−524.

Dickinson, C.H. and Sheridan, J.J. 1968. Studies on the survival of *Mycosphaerella pinodes* and *Ascochyta pisi*. Ann. Appl. Biol. 62: 473–483.

Downer, R.A. 2009. The double nozzle sprayer. Laboratory for Pest Control Application Technology. [On-line] Available: www. ordc.ohio-state.edu/LPCAT/default.htm#transfer [accessed December 2009].

Ebert, T.A., Taylor, R.A.J., Downer, R.A. and Hall, F.R. 1999. Deposit structure and efficacy of pesticide application. 1: Interactions between deposit size, toxicant concentration and deposit number. Pestic. Sci. 55: 783−792.

Edgington, L.V. 1981. Structural requirements of systemic fungicides. Ann. Rev. Phytopathol. 19: 107–124.

Elliott, R.H., and Mann, L.W. 1997. Control of wheat midge, *Sitodiplosis mosellana* (Gehin), at lower chemical rates with small-capacity sprayer nozzles. Crop Prot. 16: 235–242.

Faris-Mokaiesh, S., Boccara, M., Denis, J.B., Derrien, A., and Spire, D. 1996. Differentiation of the Ascochyta complex fungi of pea by biochemical and molecular markers. Curr. Genet. 29: 182−190.

Feng, P.C.C., Chiu, T., Sammons, R.D., and Ryerse, J.S. 2003. Droplet size affects glyphosate retention, absorption, and translocation in corn. Weed Sci. 51: 443–448.

FOASTAT. 2009. Food and Agriculture Organization. [On-line]. Available: http://faostat.fao.org/site/567/default.aspx [accessed March 2011].

FRAC. 2011. Pathogens with field resistance towards QoI fungicides. [On-line]. Available: http://www.frac.info/frac/index.htm. [accessed December 2011].

Fondevilla, S., Avila, C.M., Cubero, J.I. and Rubiales, D. 2005. Response to *Mycosphaerella pinodes* in a germplasm collection of *Pisum* spp. Plant Breed. 124: 313−315.

Fondevilla, S., Cubero, J.I. and Rubiales, D. 2007. Inheritance of resistance to *Mycosphaerella pinodes* in two wild accessions of *Pisum*. Eur. J. Plant Pathol. 119: 53−58.

Gan, Y.T., Miller, P.R., McConkey, B.G., Zentner, R.P., Liu, P.H. and McDonald, C. L. 2003. Optimum plant population density for chickpea and dry pea in a semiarid environment. Can. J. Plant. Sci. 83: 1–9.

Garry, G., Jeuffroy, M.H., Ney, B., and Tivoli, B. 1998a. Effects of Ascochyta blight (*Mycosphaerella pinodes*) on the photosynthesizing leaf area and the photosynthetic efficiency of the green leaf area of dried-pea (*Pisum sativum*). Plant Pathol. 47: 473–479.

Garry, G., Jeuffroy, M.H., and Tivoli, B. (1998b). Effects of ascochyta blight (*Mycosphaerella pinodes* Berk. & Blox.) on biomass production,seed number and seed weight of dried-pea (*Pisum sativum* L.) as affected by plant growth stage and disease intensity. Ann. Appl. Biol. 132: 49–59.

Garry ,G., Tivoli, B., Jeuffroy, M.H., and Citharel, J. 1996. Effects of ascochyta blight caused by *Mycosphaerella pinodes* on the translocation of carbohydrates and nitrogenous compounds from the leaf and hull to seed of dried pea. Plant Pathology 45: 769–777.

Gisi, U., Hermann, D., Ohl, L., and Steden, C. 1997. Sensitivity profiles of *Mycosphaerella graminicola* and *Phytophthora infestans* populations to different classes of fungicides, Pestic. Sci. 51: 290−298.

Gisi, U., Chin, K.M., Knapova, G., Farber, Kung, R., Mohr, U., Parisi, S., Sierotzki, H., and Steinfeld, U. 2000. Recent developments in elucidating modes of resistance to phenylamide, DMI and strobilurin fungicides. Crop Prot. 19: 863–872.

Gisi, U., Sierotzki, H., Cook, A., and McCaffery, A. 2002. Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. Pest Manage. Sci. 58: 859−867.

Goodwin, M. 2003. Crop profile for pea (*Pisum sativum*). Pulse Canada. [Online] Available: http://www.pulsecanada.com. [accessed February 2012].

Gossen, B.D. and Anderson, K.L. 2004. First report of resistance to strobilurin fungicides in *Didymella rabiei*. Can. J. Plant. Pathol. 26: 411.

Gossen, B.D., Bailey, K.L. and Lafond, G.P. 1997. Impact of tillage management on disease severity in field pea in a four year rotation. Can. J. Plant Pathol. 19: 324–325 (Abstr.).

Gossen, B.D., Peng, G., Wolf, T.M., and McDonald, M.R. 2008. Improving spray retention to enhance the efficacy of foliar-applied disease and pest management products in field and row crops. Can. J. Plant Pathol. 30: 505−516.

Government of Saskatchewan. 2009. Dry Pea. [Online]. Available: http://www.agriculture.gov.sk.ca/pea [accessed September 2010].

Grasso, V., Palermo, S., Sierotzki, H. Garibaldi, A., and Gisi, U. 2006. Cytochrome *b* gene structure and consequences for resistance to Qo inhibitor fungicides in plant pathogens. Pest Manage. Sci. 62: 465–472.

Hall, F.R., Downer, R.A., Wolf, T.M., and Chapple, A.C. 1996. The "double nozzle"– a new way of reducing drift and improving dose transfer? *In* Pesticide formulations and application system–16th Symposium. Edited by M.J. Hopkinson, H.M. Collins, and R. Goss. Amer. Soc. Testing Materials. Philadephia, PA. pp. 114–125.

Heath, M.C. and Wood, R.K.S. 1969. Leaf spots induced by *Ascochyta pisi* and *Mycosphaerella pinodes.* Ann. Bot. 33: 657−670.

Hernandez-Bello, M.A., Chilvers, M.I., Akamatsu, H., and Peever, T.L. 2006. Host specificity of *Ascochyta* spp. infecting legumes of the *Viciae* and *Cicerae*

tribes and pathogenicity of an interspecific hybrid. Phytopathology. 96: 1148−1156.

Hislop, E.C. Can we define and achieve optimum pesticide deposits? Aspect. of Appl. Biol. 14: 153−172.

Hwang, S.F., Conner, R.L., Chang, K.F., Gossen, B.D., Su, H., Howard, R.J. and Turnbull, G.D. 2006. Impact of seeding rate and depth on mycosphaerella blight and seed yield of field pea. Can. J. Plant Sci. 86: 854-853.

Jones, .LK. 1927. Studies on the nature and control of blight, leaf and pod spot, and foot-rot of peas caused by species of *Ascochyta*. New York State Agric. Exper. Stn., Research Report 547: 1–46.

Jensen, P.K., Jorgensen, L.N., and Kirknel, E. 2001. Biological efficacy of herbicides and fungicides applied with low-drift and twin-fluid nozzles. Crop Prot. 20: 57–64.

Jutsum, A.R., Heaney, S.P., Perrin, B.M. and Wege, P.J. 1998. Pesticide resistance: Assessment of risk and the development and implementation of effective management strategies Pestic. Sci. 54: 435−446.

Karadimos, D.A., Karaoglanidis, G.S. and Tzavella-Klonari, K. 2005. Biological activity and physical modes of action of the Q_o inhibitor fungicides trifloxystrobin and pyraclostrobin against *Cercospora beticola*. Crop Prot. 24: 23–29.

Keinath, A. 2009. Sensitivity to azoxystrobin in *Didymella bryoniae* isolates collected before and after field use of strobilurin fungicides. Pest Manage. Sci. 65: 1090−1096.

Knoche, M. 1994. Effect of droplet size and carrier volume on performance of foliage-applied herbicides. Crop Prot. 13: 163–178.

Kraft, J. M., Dunne, B., Goulden, D., and Armstrong, S. 1998. A search for resistance in peas to *Mycosphaerella pinodes.* Plant Dis. 82: 251−253.

Kraft, J.M. and Pfleger, F.L. 2001. Compendium of pea diseases. American Pathological Society Press. St.Paul, Minnesota.

Kutcher, H.R. and Wolf, T.M. 2006. Low-drift fungicide application technology for Sclerotinia stem rot control in canola. Crop Prot. 25: 640–646.

Lawyer, A.S. 1984. Foliar diseases caused by fungi: diseases caused by *Ascochyta* spp. In Compendium of pea diseases. edited by Hagedorn, D.J. pp. 11–15. APS Press, The American Phytopathological Society: St. Paul, MN.

Leach, C.M. 1959. Effect of visible and ultraviolet radiations on the sporulation of *Ascochyta pisi* and other seed-borne fungi. Phytopathology. 49: 543.

Le May, C. Ney, B. Lemarchand, E., Schoeny, A. and Tivoli, B. 2009. Effect of pea plant architecture on spatiotemporal epidemic development of ascochyta blight (*Mycosphaerella pinodes*) in the field. Plant Pathol. 58: 332−343.

Le May, C., Schoeny, A., Tivoli, B., and Ney, B. 2005. Improvement and validation of a pea crop growth model to stimulate the growth of cultivars infected with Ascochyta blight (*Mycosphaerella pinodes*) Eur. J. Plant Pathol. 112: 1−12.

Ma, Z. and Michailides, T.J. 2005. Advances in understanding molecular mechanisims of fungcided resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Prot. 24: 853−863.

Maude, R.B. 1966. Pea seed infection by *Mycosphaerella pinodes* and *Ascochyta pisi* and its control by seed soaks of thiram and captan suspensions. Ann. App. Biol. 57: 193−200.

Maybank, J., Shewchuk, S.R. and Wallace, K. 1991. The use of shielded nozzles to reduce off target herbicide spray drift. Can. Agric. Eng. 32: 235−241.

Miguez, M., Reeve,C., Wood, P.M., and Holloman, D.W. 2003. Rapid report. Alternative oxidase reduces the sensitivity of *Mycosphaerella graminicola* to QOI fungicides. Pest Manage. Sci. 60: 3−7.

Mondahl, S.N., Bhatia, A., Shilts,T. and Timmer,L.W. 2005. Baseline sensitivities of fungal pathogens of fruit and foliage of citrus to azoxystrobin, pyraclostrobin and fenbuconazole. Plant Dis. 89: 1186−1194.

Moussart, A., Tivoli, B., Lemarchand, E., Deneufbourg, F., Roi, S., and Sicard, G. 1998. Role of seed infection by the ascochyta blight pathogen of dried pea (*Mycosphaerella pinodes*) in seedling emergence, early disease development and transmission of the disease to aerial parts. Eur. J. Plant Path. 104: 93–102.

Nordbo, E., Kristensen, K., Kirknel, E., 1993. Effects of wind direction, wind speed and travel speed on spray deposition. Pestic. Sci. 38: 33–41

Olaya, G., and Köller, W. 1999. Diversity of kresoxim-methyl sensitivities in baseline populations of *Venturia inaequalis*. Pestic. Sci. 55: 1083−1088

Park, B., Lopetinsky, K., Bjorlund, R., Buss, T., Eppich, S., Laflamme, P., Miller, N., Olson, M., and Piquette, K. 1999. Field Pea. *In* Pulse Crops in Alberta. Edited by B. Park and K. Lopetinsky. Alberta Agriculture and Food. Edmonton, AB. Pg. 37−88.

Peever, T.L., Barve, M.P., Stone, L.J., Kaiser, W.J. 2007. Evolutionary relationships among *Ascochyta* species infecting wild and cultivated hosts in the legume tribes *Cicereae* and *Vicieae*. Mycologia. 99: 59–77.

Punithalingam, E. and Gibson, I.A.S. 1976. *Phoma medicaginis* var. *pinodella*. C.M.I. Descriptions of Pathogenic Fungi and Bacteria. 518: 1-2. The Eastern Press Ltd., London. UK.

Punithalingam, E. and Holliday, P. 1972a. *Ascochyta pisi*. C.M.I. Descriptions of Pathogenic Fungi and Bacteria. 334: 1−2. The Eastern Press Ltd., London.

Punithalingam, E. and Holliday, P. 1972b. *Mycosphaerella pinodes*. C.M.I. Descriptions of Pathogenic Fungi and Bacteria. 340: 1−2. The Eastern Press Ltd., London. UK.

Pulse Canada. 2010. Pulse Crops. [On-line] Available: http://www.pulsecanada.com

Roger, C. and Tivoli, B. 1996. Spatio-temporal development of pycnidia and perithecia and dissemination of spores of *Mycosphaerella pinodes* on pea (*Pisum sativum*). Plant Pathol. 45: 518–528.

Roger, C., Tivoli, B. and Huber, L. 1999a. Effects of temperature and moisture on disease and fruit body development of *Mycosphaerella pinodes* on pea (*Pisum sativum*). Plant Pathol. 48: 1–9.

Roger, C., Tivoli, B. and Huber, L. 1999b. Effects of interrupted wet periods and different temperatures on the development of ascochyta blight caused by *Mycosphaerella pinodes* on pea (*Pisum sativum*) seedlings. Plant Pathol. 48: 10–18.

Russell, P.E. 2004. Sensitivity baselines in fungicide resistance research and management. [Online]. Fungicide Resistance Action Committee, Monograph 3, Global Crop Protection Federation. Brussels. Available from: http://www.frac.info/frac/index.htm.

SAS Institute Inc. 2011. Cary, N.C: SAS Institute Inc. [Online]. Available: http://support.sas.com/documentation/ [accessed September 2010].

Saskatchewan Pulse Growers. 2010. [Online]. Available: http://www.saskpulse.com/producer/growing/index.php?page=18. [accessed September 2011].

Seyran, M., Brenneman. T.B., and Stevenson, L. 2010. A rapid methid to monitor fungicide sensitivity in the pecan scab pathogen, *Fusicladium effusum*. Crop Prot. 1257−1263.

Schoeny, A., Menat, J., Darsonval, A., Rouault, F., Jumel, S., and Tivoli, B. 2008. Effect of pea canopy architecture on splash dispersal of *Mycosphaerella pinodes* conidia. Plant Pathol. 57: 1073−1085.

Sheridan, J.J. 1973. The survival of *Mycosphaerella pinodes* in pea haulm buried in soil. Ann. Appl. Biol. 75: 195−203.

Sierotzki, H., Parisi, S., Steinfeld, U., Tenzer,I., Poirey, S., and Gisi, U. 2000. Mode of resistance to respiration inhibitors at the cytochrome bc1 enzyme complex of *Mycospherella fijiensis* field isolates. Pest Manage. Sci. 56: 833−841.

Spillman, J.J. 1984. Spray impaction, retention and adhesion: An introduction to basic characteristics. Pestic. Sci. 15: 97–106.

Stone, R.E. 1912. The life history of Ascochyta on some leguminous plants. Ann. Mycol. 10: 564−592.

Steurbaut, W. 1993. Adjuvants for use with foliar fungicides. Pestic. Sci. 38: 85–91.

Taylor, P.W.J. and Ford, R. 2007. Diagnostics, genetic diversity and pathogenic variation of ascochyta blight of cool season food and feed legumes. Eur. J. Plant Pathol. 119: 127−133.

Tivoli, B. and Banniza, S. 2007. Comparison of the epidemiology of ascochyta blights on grain legumes. Eur. J. Plant Pathol. 119: 59–76.

Tivoli, B., Beasse, C., Lemarchand, E., and Masson, E. 1996. Effect of Ascochyta blight *(Mycosphaerella pinodes)* on yield component of single pea *(Pisum sativum)* under field conditions. Ann. Appl. Biol. 129: 207−216.

Thaher, N. 2011. Fungicide insensitivity in *Ascochyta rabiei* in Saskatchewan. Masters thesis. University of Guelph. Ontario, Canada.

Torriani, S. F.F., Brunner, P.C., McDonald, B.A., and Sierotzki, H. 2008. QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*. Pest Manage. Sci. 65: 155−162.

United States Department of Agriculture. 2010. Dry Peas and Lentils. [On-line]. Available: http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1578 [accessed November 2012].

Vincelli, P., and Dixon, E. 2002. Resistance to QoI (strobilurin-like) fungicides in isolates of *Pyricularia grisea* from perennial ryegrass. Plant Dis. 86: 235−240.

van Warmelo, K.T. 1966. The identity of a pea blight fungus in South Africa. Bothalia 9: 183–194.

Walker, J.C. 1969. Diseases incited by Ascomycetes. V. *Mycosphaerella* and *Ascochyta* diseases of pea. In Plant Pathology. McGraw-Hill Inc. pp. 369–376.

Wallen, V.R., Cuddy, T.F., and Grainger, P.N. 1967. Epidemiology and control of *Ascochyta pinodes* on field peas in Canada. Can J. Plant Sci. 47: 395–403.

Wallen, V.R., and Jeun, J. 1968. Factors limiting the survivalof *Ascochyta* spp. of peas in soil. Can J. Bot. 46: 1279–1286.

Wang, H., Hwang,S.F., Chang, K.F., Turnbull, G.D., and Howard, R.J. 2000. Characterization of Ascochyta isolates and susceptibility of pea cultivars to the ascochyta disease complex in Alberta. Plant Pathol. 49: 540−545.

Wang, T.F., Gossen, B.D. and Slinkard, A.E. 2006. Lodging increases severity and impact of mycosphaerella blight on field pea. Can J. Plant Sci. 86: 855−863.

Warkentin, T.D., Rashid, K.Y. and Xue, A.G. 1996. Fungicidal control of Ascochyta blight of field pea. Can J. Plant Sci. 76: 67–71.

Warkentin, T.D., Xue, A.G., and McAndrew, D.W. 2000. Effect of mancozeb on the control of mycosphaerella blight of field pea, Can J. Plant Sci. 80: 403−406.

Wirth, W., Storps, S., and Jacobsen, W. 1991. Mechanisms controlling leaf retention of agricultural spray conditions. Pestic. Sci. 33: 411–420.

Wise, K.A., Bradley, C.A., Pasche, J.S., Gudmestad, N.C., Dugan, F.M., and Chen, W. 2008. Baseline sensitivity of *Ascochyta rabiei* to azoxystrobin, pyraclostrobin, and boscalid. Plant Dis. 92: 295−300.

Wise, K.A., Bradley, C.A., Pasche, J.S., and Gudmestad, N.C. 2009. Resistance to QoI fungicides in *Ascochyta rabiei* from chickpea in the northern great plains. Plant Dis. 93: 528−536.

Wolf, T.M., Grover, R., Wallace, K., Shewchuk, S.R., and Maybank, J. 1993. Effect of protective shields on drift and deposition characteristics of field sprayers. Can J. Plant Sci. 73: 1261–1273.

Wolf, T.M., Harrison, S.K., Hall, F.R., and Cooper, J. 2000. Optimizing postemergence herbicide deposition and efficacy through application variables in no-till systems. Weed Sci. 48: 761–768.

Wolf, T. M. 2009. Pesticide application and choosing the right nozzles. FactsSheet. Saskatchewan Ministry of Agriculture. [On-line] Available: www.agriculture.sk.ca. [accessed November 22 2009].

Wood, P.M. and Hollman, D.W. 2003. Review: A critical evaluation of the role of alternative oxidase in the performance of strobilurin and related fungicides acting at the Qo site of complex III. Pest Manage. Sci. 59: 499−511

Wroth, J.M. 1998. Variation in pathogenicity among and within *Mycosphaerella pinodes* populations collected from field pea in Australia. Can. J. Bot. 76: 1955–1966.

Wroth, J.M. 1999. Evidence suggests that *Mycosphaerella pinodes* infection of *Pisum sativum* is inherited as a quantitative trait. Euphytica. 107: 193–204.

Xue, A. G. 2000. Effect of seed-borne *Mycosphaerella pinodes* and seed treatments on emergence, foot rot severity, and yield of field pea. Can J. Plant Path. 22: 248–253.

Xue, A.G., Charest, J., Davidson, C.G., McAndrew, D.W., Bing, D.J. and Warkentin, T.D. 2003. Response of field pea cultivars to chlorothalonil in the control of mycosphaerella blight. Can. J. Plant Sci. 83: 313–318.

Xue, A.G. and Warkentin, T.D. 2001. Partial resistance to *Mycosphaerella pinodes* in field pea. Can. J. Plant Sci. 81: 535–540.

Xue. A.G., Warkentin, T.D. Greeiaus, M.T. and Zimmer, R.C. 1996. Genotypic variability in seedborne infection if field pea by *Mycosphaerella pinodes* and its relation to foliar disease severity. Can. J Plant Pathol. 18: 370−374.

Xue, A.G., Warkentin, T.D. and Kenaschuk, E. O. 1997. Effects of timings of inoculation with *Mycosphaerella pinodes* on yield and seed infection of field pea. Can. J. Plant Sci. 77: 685–689.

Ypema, H.L. and Gold, R.E. 1999. Kresoxim-methyl. Plant Dis. 83: 4-19.

Zhang, J.X., Fernando, W.G.D., and Xue, A.G. 2004. Temporal and spatial dynamics of mycosphaerella blight [*Mycosphaerella pinodes*] in field pea. Can. J. Plant Pathol. 26: 522–532.

Zhang, J.X., Fernando, W.G.D., and Xue, A.G. 2005a. Daily and seasonal spore dispersal by *Mycosphaerella pinodes* and development of mycosphaerella blight of field pea. Can. J. Bot. 83: 302–310.

Zhang, J. X., Xue, A. G., and Fernando, W. G. D. 2005b. Effect of residue type and burial depth on survival of *Mycosphaerella pinodes* in Manitoba. Can J. Plant Path. 27: 132–136.

Zhang, R. Hwang, S.F., Chang, K.F., Gossen, B.D., Strelkov, S.E., Turnbull, G.D. and Blade, S.F. 2006. Crop Sci. 46: 2409−2414.

Ziogas, B. N., Baldwin, B. C. and Young, J. E. 1997. Alternative respiration: a biochemical mechanism of resistance to azoxystrobin (ICIA 5504) in *Septoria tritici*. Pestic. Sci. 50: 28−34.

Ziogas, B.N., Markoglou, A.N., and Tzima, A. 2002. A non-Mendelian inheritance of resistance to strobilurin fungicides in *Ustilago maydis*. Pest Manage. Sci. 58: 908–916.

CHAPTER 2: SENSITIVITY OF *MYCOSPHAERELLA PINODES* IN FIELD PEA TO PYRACLOSTROBIN FUNGICIDE.

2.1. INTRODUCTION

Mycosphaerella blight, caused by *Mycosphaerella pinodes*, is the most destructive foliar disease of field pea, and the most important disease of pea crops in western Canada and around the world (Davidson and Ramsay 2000; Bretag et al. 2006; Banniza and Vandenberg 2003; Beasse et al. 1999). Yield losses from this disease vary considerably from year to year and region to region. In Canada, pea crop losses were reported to be as high as 50% (Conner et al. 2007). *Mycosphaerella pinodes* is part of the ascochyta blight complex, which is comprised of three fungal pathogens: *Ascochyta pisi* Lib., (Chilvers et al. 2009), *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr (anamorph *Ascochyta pinodes* (Berk. & Blox.) Jones), and *Phoma pinodella* (L.K. Jones) Morgan-Jones & K.B. Burch, which between them causes leaf, stem and pod spot as well as foot rot. Of the three, *M. pinodes* is by far the most abundant, causing 90% of reported ascochyta blight infections (Ali et al. 1982).

Mycosphaerella pinodes is best managed by reducing the amount of available inoculum and suppressing the subsequent epidemic. To reduce available inoculum, pea debris must be destroyed, clean seed must be used, and soil-borne inoculum must be reduced. To supress the epidemic, careful selection of cultivars should be considered and foliar fungicides should be applied as needed. The best long-term management strategy would be the development of cultivar

resistance, but attempts to achieve this have met with only limited success (Conner et al. 2007). The most commonly utilized strategy for management of mycosphaerella blight is the application of fungicide sprays in a preventative and systematic schedule (Beasse et al. 2000). Multiple sprays, initiated at early to mid-flowering, provide effective disease control and improve yield (Warkentin et al. 2000). However, the selection pressure imposed by the repeated use of fungicides is the leading contributor to fungicide insensitivity in pathogen populations (Gisi et al. 1997). This risk must be taken into consideration when applying a single fungicide repeatedly, or those with the same mode of action (Brent and Holloman 2007b).

Strobilurin fungicides have been widely used since 1996. They have activity against a broad range of plant pathogens, and have added positive effects on the physiology of the crop under some circumstances. Strobilurins are single-site mode of action fungicides classified as quinine outside inhibitors (Qol's), as they bind the Qo site of cytochrome b in the cytochrome bc1 enzyme complex (found in the mitochondrial membranes of fungi), so that electron transfer between cytochrome b and cytochrome c1 cannot occur. This causes an energy deficiency due to a lack of ATP (Bartlett et al. 2002). The Fungicide Resistance Action Committee (FRAC 2011) has identified strobilurins as a high risk for insensitivity due to their site-specific mode of action, especially with repeated use.

There are two types of fungicide insensitivity: quantitative and qualitative. Quantitative insensitivity results in the pathogen becoming less sensitive to the

fungicide, but higher rates and/or more fungicide applications are still effective. Qualitative insensitivity causes the pathogen to become completely insensitive to the active ingredient and control is no longer possible at field rates. Naturally occurring insensitivity to the strobilurin fungicides is generally qualitative (Ypema and Gold 1999). Within the *Mycosphaerella* genus, there are three species, *M. fijiensis*, *M. citri* and *M. graminicola,* which are currently known to be insensitive to strobilurins (Grasso et al. 2006; Gisi et al. 1997; Keinath 2009; Miguez et al. 2003; Mondahl et al. 2005). Results of a study conducted by Torriani et al. (2008) showed that insensitivity in *M. graminicola* was due to a mutation in isolates from different geographical areas and genetic backgrounds, was spread by wind-borne ascospores and was a result of fungicide selection.

Pyraclostrobin is a strobilurin fungicide that has been widely used in Canada since 2002, and is the active ingredient in the product Headline™. It provides broad-spectrum control of many plant pathogens. It is rapidly absorbed by the plant and retained in the leaf cuticle. The fungicide works by inhibiting spore germination, and by halting mycelial growth. Due to its rapid uptake by plants, the product has a short rainfast period, adding to its effectiveness. This fungicide provides excellent control of *Ascochyta* / *Mycosphaerella* species on pulse crops (BASF 2011), however, an insensitivity response due to the use of pyraclostrobin has been documented in many pathogens (FRAC 2011). In Canada, the research into pyraclostrobin insensitivity in field crop pathogens has focused on *Ascochyta rabiei* on chickpea, which has developed qualitative insensitivity to this product

(Gossen and Anderson 2004; Chang et al. 2007; Thaher 2011). Field pea, like chickpea, is a high value, drought resistant crop that helped to increase cropping system diversity in many regions of the Canadian prairies (Chang et al. 2007). The similarities between the pathogens affecting chickpea and field pea have led to interest in the area regarding fungicide insensitivity in pathogens associated with the latter.

In vitro research on fungal respiration in the presence of respiration-inhibiting fungicides, such as the strobilurins, has shown that some microorganisms have the ability to use an alternative respiration pathway, involving the production of a cyanide-insensitive alternative oxidase (AOX) (Miguez et al. 2003). This enables the bc complex in the mitochondrial respiration chain to be by-passed and allows mycelial growth and spore germination even in the presence of the fungicide (Oyla and Koller 1999; Vincelli and Dixon 2002; Ziogas et al. 1997). Although alternative respiration has not been directly identified in QoI-insensitive pathogens (Avila-Adame et al. 2003), the induction of this alternative pathway of respiration can serve as a highly effective rescue mechanism when pathogen sensitivities are tested in the absence of a host. Salicylhydroxamic acid (SHAM) is one of two chemicals used to inhibit AOX. This chemical is routinely included in QoI *in vitro* fungicide sensitivity assessments, as alternative respiration may impact the results of these assays, leading to incorrect assessments of fungicide sensitivity (Wise et al. 2009; Miguez et al. 2003).

To properly monitor and detect the possible development of fungicide insensitivity in a pathogen species, the baseline sensitivity of the pathogen to the active ingredient in the fungicide must be established. This is done by collecting isolates of the pathogen through field surveys prior to fungicide use and testing these for sensitivity to the product. Another way would be to test a population that has never been exposed to the fungicide, and compare it against a population that has had exposure (Avenot and Michailides 2007). It is important to undertake baseline and further insensitivity testing for three reasons: (1) to obtain initial data regarding the range of sensitivity that exists in major target pathogens (2) to develop an accurate, rapid, reproducible method for determining the degree of sensitivity of major target fungi and (3) to detect differences in sensitivity levels between samples that might lead to future insensitivity concerns (Brent and Holloman 2007a).

Work done by researchers on the Canadian prairies and the northwestern United States has identified insensitivity to pyraclostrobin in a fungal pathogen of chickpea, an important pulse crop grown in these areas. The lack of available control measures for this pathogen and the repeated product application have contributed to the problem. However, studies on pyraclostrobin insensitivity in *M. pinodes*, a field pea pathogen, have not been conducted. Considering the widespread use of this fungicide, the site-specific nature of its mode of action, and the identification of insensitivity issues in a similar pathogen affecting these crops, it was suspected that similar insensitivity issues may have already evolved.

An assessment of sensitivity to pyraclostrobin of a random sample of many isolates from across the Canadian prairies and the northern Great Plains of the United States was carried out *in vitro*. While some researchers have stated that insensitivity in the laboratory does not necessarily imply insensitivity in the field (Al-Mughrabi and Gray 1995), others suggest that the identification of insensitive isolates *in vitro* could point to expected issues in the field environment (Gisi et al. 2002).

2.1.1. Objectives

The objectives in this portion of the study were to: i) quantify the baseline sensitivity of *M. pinodes* to pyraclostrobin fungicide using radial growth assessments, ii) determine if using the formulated product (Headline) affected baseline sensitivity, as assessed using technical grade product, and if inhibiting the AOX with salicylichydroxamic acid affected these assessments, iii) determine if baseline assessments using conidial germination produced the same pattern of response as radial growth assessments, and iv) compare the reaction of *M. pinodes* isolates collected in 2010 and 2011 with this baseline to determine if sensitivity to pyraclostrobin is changing in western Canada and the Northern Great Plains of the United States.

2.2. MATERIALS AND METHODS

2.2.1. Baseline sensitivity

Preparation of isolates

Forty *M. pinodes* isolates were obtained from long-term storage at the Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada. Thirty isolates were obtained from the long-term *M. pinodes* collection at the United States Department of Agriculture in Pullman, Washington, USA. These cultures were collected prior to 2003, before the registration of QoI fungicides in those geographical areas, and so represent a true baseline collection of isolates that have not been exposed to QoI fungicides. The isolates were confirmed to be *M. pinodes* by plating onto oatmeal agar (OA), mass transferred onto commercial potato dextrose agar (PDA) (Difco Laboratories, Detroit, IL) and incubated for 14 days. They were then transferred onto water agar (WA) (Difco Laboratories), allowed to grow for 5 days and purified by isolation from hyphal tips. Each isolate was confirmed to be *M. pinodes* based on colony and spore morphology. The cultures were then transferred back onto PDA and grown for 14 days under white fluorescent light at 20 °C (±2 °C) under 16 hr light / 8 hr dark. After 2 weeks, ten 5-mm-diameter cores were removed from each colony, placed into separate cryogenic vials containing 2.5 mL of 20% glycol solution and placed into liquid nitrogen at -80 °C for long-term storage. Another set of the cultures was mass-transferred onto PDA slants and placed at 4 °C for short-term storage.

Radial growth assessments

Pure cultures of each of the 70 isolates of *M. pinodes* prepared for the baseline assessment were inoculated onto 15 mm × 100 mm PDA Petri dishes and grown for 1 week at 16 h/8 h (light/dark) under white fluorescent light at 20 °C +/- 2 °C. After 1 week, 5-mm-diameter cores of each actively growing culture were transferred, mycelium side down, onto the center of each of four replicate PDA dishes per treatment using a cork borer. The agar was amended with concentrations of pyraclostrobin fungicide at 0 (control), 0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, or 50 mg L^{-1} active ingredient (ai) using the formulated fungicide product Headline 250 EC (BASF Canada, Mississauga, ON) as the source of the active ingredient. To obtain the required pyraclostrobin concentrations, serial dilutions of Headline were made using sterilized deionized water, and then added to sterilized PDA growth medium after it had cooled to 55 °C. The amended media were poured into 10-cm-diameter Petri dishes. After inoculation, the dishes were placed on a light table under white fluorescent light (16 h light / 8 h dark) at 20 °C +/- 2 °C for 7 d. The mean diameter (radial growth) of the culture was measured with a digital calliper at the widest point and a second measurement, perpendicular to the first, was taken. The diameter of the 5-mm-core was subtracted from each measurement and the two measurements were averaged. The dishes were then returned to the table for another 7 days under the same growing conditions, when each colony was measured again. The radial growth measurements were converted to a percentage of the growth of an un-amended control using the following equation: [1- (growth on amended

medium/ growth on un-amended medium) x 100]. The measurements taken 14 days after inoculation are presented because they showed more pronounced and consistent differences among the isolates than the assessments at 7 days. The experiment was arranged in a completely randomized design with one dish per replicate and four replicates per treatment. This design was used in all subsequent trials. The EC_{50} value for each isolate was estimated by fitting the data to a non-linear equation and using non-linear regression as described below. The EC_{50} value represents the effective concentration of the amount of fungicide active ingredient required to inhibit growth of the pathogen by 50%. A discriminatory dose of pyraclostrobin was set by observation of the dose response of the isolates to the fungicide and analyses of the obtained EC_{50} values. This discriminatory dose was used for further sensitivity testing of *M. pinodes* isolates.

To determine the effect of salicylhydroxamic acid (SHAM, 99%; Sigma-Aldrich, St. Louis, MO) on radial growth measurements in *M. pinodes*, a trial using formulated product was conducted using pyraclostrobin-amended media with and without SHAM. The trial was conducted using the layout, methods, and pyraclostrobin treatment concentrations described previously. SHAM was prepared by dissolving 100 mg of SHAM into 1 mL of methanol and adding the mixture to the PDA medium at a concentration of 0.01% by volume (100 µg ml^{-1}) as described by Wise et al. (2008). SHAM was filter-sterilized and added to the autoclaved medium after cooling to 55 °C. The two response curves (with and

without SHAM) for each isolate were tested against each other independently and graphed to determine if the individual dose response curves were different.

To determine if formulation affected sensitivity to pyraclostrobin in *M. pinodes*, a test was conducted to compare a commercial formulation of pyraclostrobin (Headline) to the technical grade product (89% ai; BASF). Technical grade pyraclostrobin was prepared by dissolving the powdered product into 1 mL of acetone to obtain a concentration of 100 mg mL^{-1}. Serial dilutions using acetone were made to obtain the pyraclostrobin concentrations described above. The final concentration of acetone in the medium was 0.01% by volume and was filter-sterilized before adding to the cooled, autoclaved medium. The 70 unexposed isolates of *M. pinodes* were prepared, transferred and measured using the same radial growth procedure described above. The two response curves (formulated product and technical grade product) for each isolate were compared as described above.

Conidial germination assessments

Pure mass-transfer cultures of 50 isolates, chosen at random (using the RAND function in Microsoft Excel 2007, Microsoft Corp. Redmond WA) from the original unexposed isolates, were inoculated onto PDA-filled Petri dishes and grown for 1 week under fluorescent light (16 h light/8 h dark) at 20° C ± 2 °C. After 7 days, 2 ml of sterilized deionized water containing 0.05% (v/v) Tween 20

(5 drops in 100 ml of water) was added to the plate of the actively growing culture and the conidia were gently dislodged with a glass rod and/or small transfer loop. The resulting conidial suspension was adjusted with a haemocytometer to 2 x 10^5 spores mL^{-1} and 100 µL of suspension was pipetted onto each of four replicate PDA dishes. The PDA was amended with concentrations of pyraclostrobin fungicide at 0 (control), 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5 or 10 mg L^{-1} ai. Based on the results of the radial growth trials above (formulated product versus technical grade formulation; with and without SHAM) formulated product was used and SHAM was added (ensuring no alternative respiration) to the PDA medium following the procedures outlined above. The dishes were incubated under fluorescent white light (16 h light/8 h dark) at 20 °C ± 2 °C for 24 hours. Following incubation, 100 conidia per dish were assessed for germination under a compound microscope using a 20× objective lens (200× magnification). A conidium was considered germinated if the germ tube was at least as long as the conidium itself (Pasche et al. 2004). The germination counts were converted to percentage germination relative to the un-amended control with the following equation: [1- (germination on amended medium/germination on un-amended medium) x 100]. The EC_{50} value based on the response of the conidia was determined, and a discriminatory dose chosen as described above.

2.2.2. Sensitivity of *M. pinodes* isolates collected in 2010 and 2011

Preparation of isolates

Over 300 isolates of *M. pinodes* from Alberta, Saskatchewan, North Dakota and Washington State were obtained from infected plant samples collected during the summers of 2010 and 2011. These cultures represented isolates of *M. pinodes* that had (potentially) been exposed to pyraclostrobin fungicide use for up to 8 years, given the registration of the fungicide in those geographical areas in 2003. Samples of field pea plants with symptoms of mycosphaerella blight were collected from commercial pea fields and brought into the laboratory for isolation. Sections of the leaf containing disease lesions were removed and surface-sterilized in 5% NaOCl for 25 s, rinsed three times with deionized water and allowed to dry for 15 min in a laminar flow hood. The leaf sections were transferred onto PDA plates and the fungus was allowed to grow for 7 d. Putative cultures of *M. pinodes* were then transferred onto WA plates, allowed to grow for 5 d and purified by transferring hyphal tips onto PDA plates. The cultures were then grown for 14 d under white fluorescent light (16 h light/8 h dark) at 20 °C (± 2 °C), their identity was confirmed based on culture and spore morphology, and they were transferred onto PDA slants and stored at 4 °C for long-term storage.

Testing isolates for sensitivity

All 300 isolates collected in 2010 and 2011 were tested using the radial growth procedure described previously at a discriminatory dose of 5.0 mg L^{-1}. Based on the results of previous trials comparing methodologies, the cultures

were tested using formulated product (Headline) and 0.1% SHAM. The discriminatory dose was chosen based on the baseline assessments of radial growth; where 5.0 mg L^{-1} consistently reduced colony growth by more than 70%, but some growth still occurred. The dose was deliberately chosen above the mean EC_{50} value to identify highly insensitive isolates. The isolates were classified as sensitive if growth was reduced by more than 70%, intermediate if growth was reduced between 30% and 70%, and as insensitive if growth was reduced by less than 30%. The response of the exposed isolates was compared to the response of the unexposed isolates to see if the sensitivity level of the isolates had changed in the respective geographical areas.

Any isolates that were determined to be insensitive in the radial growth procedure were confirmed to be insensitive using the conidial germination procedure as described previously. A random selection of 25 isolates, which were determined to be sensitive in the radial growth procedure, was confirmed to be sensitive using this procedure at a discriminatory dose of 0.1 mg L^{-1} ai. This dose was also chosen above the mean EC_{50} value of the unexposed isolates to identify highly insensitive isolates. The isolates were classified as sensitive, intermediate, or insensitive as described above.

EC_{50} of insensitive isolates

To determine the degree of insensitivity, it was necessary to determine the EC_{50} values for the insensitive isolates. Using the same radial growth procedure as described for the unexposed baseline isolates, the insensitive isolates were

plated onto PDA plates containing 0, 5, 10, 20, 40, 80, 160 or 320 mg L^{-1} ai of pyraclostrobin. The colonies were measured after 14 d and the EC_{50} values were calculated. The differences between the sensitive and insensitive isolates from all geographical areas were tested and treatment means were compared as described below. An insensitivity factor was calculated for the insensitive isolates using the equation: (mean EC_{50} value of insensitive isolates) / (mean EC_{50} value of sensitive isolates).

Response of insensitive isolates to fungicide application on plant material

A randomized complete block design (RCBD) experiment was conducted in a growth chamber at AARD in Lacombe, Alberta in 2012 to observe the response of insensitive isolates inoculated onto plants of the field pea cv. CDC Meadow to the formulated product of pyraclostrobin, with four replicate blocks and 10 pots per experimental unit. Five randomly selected insensitive isolates were combined into a single-spore suspension with the same procedure used to prepare the isolates for the conidial germination trial. This was also done for five randomly selected sensitive isolates. Sixty 15-cm-diameter pots containing soil-less mix were prepared, planted to 'CDC Meadow' at a rate of 12 seeds per pot, placed into the growth chamber (16 h light/8 h dark at 20 °C and 15 °C, respectively), and subsequently thinned to 10 plants per pot. The treatments were as follows: 1) pyraclostrobin application, then inoculation with insensitive isolates; 2) pyraclostrobin application, then inoculation with sensitive isolates; 3) inoculation with insensitive isolates only (inoculated control); 4) inoculation with

sensitive isolates only (inoculated control); 5) pyraclostrobin application (fungicide control); and 6) nontreated control (not sprayed or inoculated). The plants were sprayed with the recommended rate of pyraclostrobin (0.04 mL per 10 mL water) at 21 d after planting and inoculated 1 day later by spraying plants with a spore suspension (2×10^5 spores mL^{-1}) until run-off. A hand-held spray bottle containing a pre-determined amount of water was used for both fungicide application and inoculation. After inoculation, the pots were transferred into a transparent plastic moisture chamber at high humidity for 48 h under 16 h light at 18 °C / 8 h dark at 15 °C. After removal from the moisture chamber, the plants were returned to the growth chamber where the humidity was kept as high as possible with the use of a humidifier. Each plant was rated for mycosphaerella blight lesion development at 7 d after inoculation using the scale developed by Xue et al. (1996).

2.2.3. Statistical analysis

All statistical analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC) and differences were considered significant at $P \leq 0.05$. Each test of radial growth or conidial germination was arranged in a completely randomized design with one dish per replicate and four replicates per treatment. The EC_{50} values for each isolate, for both radial growth and conidial germination, were determined by fitting the data to a non-linear equation and using non-linear regression (PROC NLIN). The residual data were tested for normality using PROC

UNIVARIATE (Shapiro-Wilk test), and did not follow a normal curve because of the non-linear dose response of the pathogen. To normalize the residuals, the data were transformed using a square root transformation. To compute variance and eliminate outliers, mean EC_{50} values that were 3, 2 and then 1 standard deviation from the mean were removed from the data set and the data were tested using PROC NLIN in SAS 9.2 to obtain the individual Analysis of Variance (ANOVA) tables for each data set. The ANOVA tables used to obtain the EC_{50} values were then compared against each other. F-tests were conducted on the values obtained from the ANOVA tables (MSE, SSE and df) to ensure homogeneity of the data (Wise et al. 2009). Once the data were homogeneous, the value was recorded and a discriminatory dose chosen from the homogeneous data set (R.C. Yang, University of Alberta, *personal communication*).

To assess the impact of SHAM and product formulation on expression of sensitivity, the two response curves (with and without SHAM; formulated vs. technical grade product) for each isolate were tested against each other independently to compare the EC_{50} values and graphed. The data were tested to ensure the assumptions of ANOVA were met, means were separated using Fisher's protected LSD and declared significant at $P \leq 0.05$. The data were then log-transformed to linearize the logarithmic curve and compared using the general linear model (PROC GLM). Although the isolates would be considered a random effect, as each isolate was tested individually isolates were not included

in the model statement. The same log-transformation and statistical analysis was used to assess the two procedures in subsequent trials.

The EC_{50} values for the insensitive isolates were determined using non-linear regression (PROC NLIN). Treatment means between the insensitive and sensitive isolates from all geographical areas were compared using the general linear model (PROC GLM) with Tukey's multiple range test at $P \leq 0.05$. and orthogonal contrast statements with 1 degree of freedom.

2.3. RESULTS

2.3.1. Baseline Sensitivity

Radial growth measurement

The radial growth response of the isolates to pyraclostrobin varied slightly but was not significantly different. Observation of the non-analyzed data indicated that the EC_{50} value was between 0.05 and 0.1 mg L^{-1} ai (Fig. 2-1). From the analysis of the baseline isolates from Alberta, Saskatchewan, North Dakota and Washington, the EC_{50} values of each isolate were determined. The individual isolate EC_{50} values ranged from 0.03 mg/L to 0.29 mg L^{-1}, with a mean of 0.12 mg L^{-1} of pyraclostrobin (Table 2-1).

In companion trials comparing the effects of SHAM addition to the media, the calculated EC_{50} values were not significantly different (Table 2-1, 2-2). The EC_{50} values were also not significantly different in the trials comparing media

made with formulated product versus technical grade product (Table 2-1, 2-3). When graphed and tested against each other, there was no significant difference between the response curves of the two data sets for each specific isolate in the two comparison trials (Figs. 2-2, 2-4). The data were then $\log_{10}$ transformed to linearize the non-linear curve, graphed and tested again (Tables 2-2, 2-3). As before, the two curves were compared against each other, and there was no significant difference between the response curves of the two data sets in the two comparison trials (Figs. 2-3, 2-5).

Conidial germination assessment

The conidial germination response of the isolates to pyraclostrobin varied slightly but did not differ significantly between isolates. Observation of the non-analyzed data indicated that the EC_{50} value was approximately 0.01 mg L^{-1} ai (Fig. 2-6). From the analysis of the baseline isolates from Alberta, Saskatchewan, North Dakota and Washington, the EC_{50} values of each isolate were determined. The individual isolate EC_{50} values ranged from 0.008 mg L^{-1} to 0.041 mg L^{-1}, with a mean of 0.015 mg L^{-1} of pyraclostrobin (Table 2-4). Although the EC50 values were lower than for the radial growth procedure, the classification of the isolates using this procedure was the same.

2.3.2. Sensitivity of isolates collected in 2010 and 2011

Testing isolates for insensitivity

A total of 324 isolates were tested from four geographical areas at a discriminatory dose of 5.0 mg L^{-1} using the radial growth procedure. Of those isolates, 19 were determined to be insensitive, 304 were determined to be sensitive and one isolate was of intermediate sensitivity based on the classification system outlined above (Table 2-5, Fig. 2-7). The 19 isolates that were identified as insensitive were assessed using the conidial germination procedure at a discriminatory dose of 0.1 mg L^{-1}. The isolates were confirmed to follow the same response pattern and were again classified as insensitive (Table 2-5).

EC_{50} value of the insensitive isolates

The radial growth response of the insensitive isolates to the high concentrations of the pyraclostrobin fungicide varied for each isolate. The data were analyzed and the EC_{50} value for each isolate was determined. The raw data indicated EC_{50} values as high as 260 mg L^{-1}, and as low as 120 mg L^{-1}, but once analyzed the individual EC_{50} values for each isolate ranged from 80 mg L^{-1} to 261 mg L^{-1}, with a mean of 180 mg L^{-1} of pyraclostrobin (Table 2-5).

The dose response curve and EC_{50} values for the insensitive isolates were significantly different from the dose response curve and EC_{50} values of the sensitive isolates (Fig. 2-8), according to Tukey's Multiple Range Test (Table 2-6) and contrast statements at $P \leq 0.05$ (Table 2-7). When comparing the average EC_{50} value of the insensitive isolates to the average EC_{50} value of the sensitive

isolates, the insensitive isolates were nearly 1500 times more insensitive to the fungicide than the sensitive isolates.

Response of insensitive isolates to fungicide application on plant material

As expected, the fungicide had no effect on the insensitive isolates, and the pathogen response was similar to that in the unsprayed control plants that were inoculated with the insensitive isolates (Fig. 2-9). The sensitive isolates in contrast responded very well to the fungicide (Fig. 2-9). The mean mycosphaerella blight rating for the sensitive isolates sprayed with the fungicide was 0.2 (out of 9), while the mean rating of the insensitive isolates was 6.4.

2.4. DISCUSSION

Because of the very site-specific mode of action, strobilurins have been identified as being at high risk for the evolution of insensitive biotypes in the pathogen population. Repeated application of strobilurin fungicides during the growing season is the most effective management option for *M. pinodes* on field pea (Bretag 1985; Warkentin et al. 2000). However, repeated applications increase selection pressure for fungicide-insensitivity in pathogen populations (Gisi et al. 1997; Ma and Michailides 2005). Insensitivity to the strobilurins has already been reported in western Canadian populations of *A. rabiei*, (Gossen et al. 2004; Chang et al. 2007; Thaher 2011), highlighting the need to determine the

baseline sensitivity to this chemistry in *M. pinodes*, as well as the need to identify changes in sensitivity since strobilurin fungicides were registered in 2003.

To determine if the response of a pathogen to a fungicide has changed or not, a baseline sensitivity level is established and an EC_{50} value for further testing is ascertained. In this study, to identify an accurate EC_{50} value, a large number of isolates from four geographical areas were assessed. Insensitivity to a particular fungicidal mode of action may occur in a pathogen population that has not been previously exposed to that fungicide, as a consequence of naturally occurring mutations in some isolates (Brent and Holloman 2007b). In the current study, there was a concern that such isolates could be represented in the samples analyzed and could therefore affect the baseline measurement. However, all of the isolates in the baseline sensitivity assessment had a consistent and similar response to the fungicide, so we conclude that all of the isolates in the baseline group were sensitive to strobilurin fungicides.

There are two main *in vitro* methodologies used to test the sensitivity of a pathogen to a fungicide; radial growth and conidial germination. Several studies indicate that conidial germination is a more effective method for assessing pathogen insensitivity than radial growth assessments (Demirci et al. 2003, Wise et al. 2008, 2009, Vincelli and Dickson 2002). In this study of the *M. pinodes* pathosystem, there were no significant differences between the response to either procedure used to test the isolates for sensitivity response. However, the EC_{50} values of the isolates did differ depending on the procedure, ranging from

0.008 – 0.041 mg L^{-1} in the conidial germination assay, and from 0.031 – 0.294 mg L^{-1} in the radial growth assay. Nonetheless, the classification of isolates based on percentage growth relative to the control showed the same pattern of response.

Previous research involving *in vitro* testing indicates that salicylhydroxamic acid (SHAM) is necessary to inhibit alternative respiration in some pathogen systems. Wise et al. (2008, 2009) found differences in the EC_{50} values of *A. rabiei*, between trials with and without SHAM and recommended that it be included in *in vitro* assays. In contrast, Thaher (2011) found that these differences were not significant and concluded that SHAM need not be used. In the current study, differences were not observed in the baseline isolates of *M. pinodes*. There were no significant differences in EC_{50} values or response curves between trials where SHAM was used and trials where SHAM was not used. Although alternative respiration was not detected, it may still occur. Therefore, SHAM was included in the insensitivity testing of isolates collected in 2010 and 2011 as a precaution, to ensure that alternative respiration did not occur in *M. pinodes* and that an accurate response to the fungicide was obtained. Adding SHAM to the *in vitro* procedure is simple, as the chemical is readily available, dissolves easily in methanol, and it provides confidence in the resulting data.

Both formulated and technical grade product have been used in previous *in vitro* studies testing the insensitivity of pathogens to fungicides. Use of formulated product ensures access to the active ingredient, but may create

complication since the additives in the formulated product might affect the results. In this study, however, there was no significant difference between trials using formulated or technical grade product. As the technical grade product is not readily available, the formulated product is easier and more cost effective to use when testing the sensitivity of *M. pinodes.*

Choosing an appropriate discriminatory dose to test the insensitivity of pathogens to a fungicide can be challenging. It is important to choose a dose that is high enough to differentiate sensitive and insensitive isolates, but not so high as to excessively reduce growth and the accuracy of the sensitivity data. When isolates are found that are insensitive to strobilurins, there usually has been a qualitative response and so these isolates tolerate very high levels of fungicide (Avila-Adame 2003; Mondal et al. 2005; Wise et al. 2009).

In this study, the sensitivity to pyraclostrobin of 324 isolates of *M. pinodes* representing four geographical regions in Canada and the United States was assessed. Radial growth assessments at a discriminatory dose of 5.0 mg L^{-1} demonstrated that 19 of the 324 isolates were insensitive to pyraclostrobin and one isolate had an intermediate sensitivity. Of the *M. pinodes* isolates that were insensitive to pyraclostrobin, nine were collected from an area in central Alberta where field pea is grown intensively and fungicides are applied every year as a preventative measure. Four of the insensitive isolates were collected from more northern areas of Alberta, where field pea cultivation is also widespread and

pyraclostrobin is frequently used. The remaining five isolates were collected from random areas across central and southern Saskatchewan (Fig. 2-10).

The response of these isolates is not unexpected considering the total breakdown in response of *A. rabiei* to the strobilurin group of fungicides (Gossen and Anderson 2004; Chang et al. 2007; Thaher 2011). This lack of response in chickpea happened quickly, forcing producers to utilize a fungicide with an alternative mode of action. This type of abrupt change in the sensitivity to strobilurin fungicides has not been observed with *M. pinodes*, likely because of lower selection pressure. Pyraclostrobin has not been applied as intensively onto pea as it was applied to chickpea. With the high costs associated with the application of fungicides and the low price of pea compared to chickpea, many producers do not consider it economically feasible to apply fungicide to the pea crop in an average crop year.

Selection of an appropriate discriminatory dose to test insensitivity can be challenging. Discriminatory doses are specific to the pathogen, fungicide and research being conducted. Doses can be as high as 200 times the EC_{50} and as low as 4 times the EC_{50} (Avila-Adame 2003, Mondal et al. 2005, Rebollar-Alviter et al. 2007, Wise et al. 2009), but in all cases are chosen based on previous observations of the growth response of the pathogen at various fungicide doses. The discriminatory dose of 5 mg L^{-1} used in this study to classify the isolates collected in 2010 and 2011 was a single dose deliberately chosen to distinguish sensitive isolates from insensitive isolates. It was sufficiently low to permit

growth, but high enough to noticeably reduce growth. The discriminatory dose was roughly 50 times the EC_{50} value of the baseline isolates, and roughly 2.5 times the recommended rate of the fungicide. We cannot, with complete accuracy, compare the rate used in the laboratory to the rate used in the field because there are a number of factors that preclude such a comparison, including different environments and application procedures. Calculating this number does, however, provide an estimate of what the fungicide concentration would be if a comparison could be made.

The degree of insensitivity in the insensitive isolates suggests a G143 mutation, which is a single nucleotide change in the mitochondrial cytochrome b gene, leading to a substitution of amino acid residue 143 from glycine to alanine (G143A) (Torriani et al. 2008). This substitution results in qualitative insensitivity and is evident by the complete lack of response by the pathogen. The alternative mutation of amino acid residue 129 from phenylalanine to leucine (F129L) causes only moderate insensitivity to strobilurins, and effective control with QoI fungicides is still possible (Gisi et al. 2000). Results from a study conducted by Torriani et al. (2008) showed that the development of strobilurin insensitivity in *M. graminicola* resulted from an independent mutation of G143 in isolates from different geographical areas and genetic backgrounds. The frequency of the mutation increased as a result of strong fungicide selection, and the insensitivity trait was spread by wind-borne ascospores. Pyraclostrobin has been used very intensively on the Canadian prairies since 2003, which would have contributed to

a high level of selection pressure. Since the G143 mutation results in qualitative insensitivity, increasing the amount of fungicide used or the frequency of fungicide application will not result in disease suppression. Indeed, in the current study, the average EC_{50} value of the insensitive isolates was determined to be 179.6 mg L^{-1}, representing an unreasonable application rate since it corresponds to about 65 times the label rate.

None of the isolates collected from North Dakota or Washington State appeared to be insensitive. This may have reflected lower selection pressure in those areas. While field pea cultivation in the United States has remained stable since 2003, the Canadian hectarage has increased (USDA 2010). Canada now grows approximately seven times as many hectares of field pea than the United States. This more intensive cultivation of field pea in Canada has resulted in a more intense application of pyraclostrobin for disease control, along with a corresponding increase in the intensity of selection pressure for insensitive strains of the pathogen. Nevertheless, the prevalence of insensitive strains may eventually increase even in areas where selection pressure is not high, because of the air-borne nature of the sexual spores of *M. pinodes*. Fungicidal insensitivity can be spread over long distances through the dissemination of air-borne ascospores (Torianni et al. 2008).

A group of five insensitive isolates were inoculated onto pea plants in a growth chamber and sprayed with the fungicide to observe the response *in planta*. A similar group of sensitive isolates was also tested for comparison. As

expected, the insensitive isolates caused high disease severity and did not respond to fungicide application, while almost no disease developed following the inoculation of fungicide-treated plants with the sensitive isolates. These results suggest that the pathogenicity of the insensitive isolates remained high, and may provide an indication of the response of the plant material to the isolates in the field. Ma and Michailides (2005) note that under field conditions, insensitive isolates in general become more common because of the selection pressure imposed by continued fungicide application. Thus, as long as the isolates remain pathogenic, and given the sexual reproduction of *M. pinodes*, the frequency of fungicide insensitive isolates will likely continue to increase, and application of the fungicide will no longer be sufficient to control the disease. This will mean that strobilurins will not be effective in controlling *M. pinodes* in field pea and other fungicides will have to be used.

Only about 6% of the 324 isolates of *M. pinodes* tested from across the Nothern Great Plains region were insensitive to the pyraclostrobin, while the overwhelming majority were sensitive to the fungicide. This is good news for producers, as fungicide application is the most effective strategy for managing mycosphaerella blight. Nonetheless, the identification of 19 insensitive isolates is a cause for concern, as it indicates that a larger insensitivity problem may be emerging. Based on the current fungicide application frequency and industry response of rotating fungicides, reduced applications and new fungicide options, the problem is likely to emerge more slowly than was observed in the *A. rabiei*

population. However, it is critically important to continue to monitor *M. pinodes* populations for decreases in strobilurin sensitivity. The agricultural industry must ensure the prudent use of this fungicide chemistry in the future and continue to work diligently to develop other management strategies, such as cultivar resistance for the control of mycosphaerella blight in field pea.

2.5. LITERATURE CITED

AgroPages. 2011. Strobilurin fungicides. [On-line]. Available: http://www.agropages.com/AgroData/AgroDataList-2-3699.htm. [accessed January 2013].

Ali, S.M., Paterson, J., and Crosby, J. 1982. A standard technique for detecting seed-borne pathogens in peas, chemical control and testing commercial seed in South Australia. Aust. J. Agric. Res. 22: 348–352.

Al-Mughrabi, K.I. and Gray, B.A. 1995. Competition between triadimefon-sensitive and triadimefon-resistant isolates of Erysiphe graminis f. sp. tritici. Plant Dis. 79: 709–712.

Avenot, H.F. and Michilaids, T.J. 2007. Resistance to boscalid fungicide in *Alternaria alternata* isolates from pistachios in California. Plant Dis. 91: 1345–1350.

Avila-Adame, C., Olya, G. and Koller, W. 2003. Characterization of *Colletotricum graminicola* isolates resistant to strobilurin-related QoI fungicides. Plant Dis. 87: 1426–1432.

Banniza, S., Armstrong-Cho, C.L., Gan, Y. and Chongo, G. 2011. Evaluation of fungicide efficacy and application frequency for the control of ascochyta blight in chickpea. Can. J. Plant Pathol. 33: 135−149.

Banniza, S. and Vandenberg, A. 2003. The influence of plant injury on development of *Mycosphaerella pinodes* in field pea. Can. J. Plant Pathol. 25: 304–311.

Bartlett, D.W., Clough, J.M., Godwin, J.R.,Hall, A.A., Hamer, M., and Parr-Dobranzki, B. 2002. The strobilurin fungicides. Pest Manage. Sci. 58: 649–662.

BASF. 2011. Headline. [On-line] Available: https://agro.basf.ca/West/Products/HEADLINE.html [accessed December 2011].

Beasse, C., Ney, B. and Tivoli, B. 1999. Effects of pod infection by *Mycosphaerella pinodes* in yield components of pea (*Pisum sativum*). Ann. Appl. Biol. 135: 359–367.

Beasse, C., Ney, B., and Tivoli, B. 2000. A simple model of pea (*Pisum sativum*) growth affected by *Mycosphaerella pinodes*. Plant Pathol. 49: 187–200.

Brent, K.J. and Holloman, D.W. 2007a. Fungicide resistance: the assessment of risk. Fungicide Resistance Action Committee, Monograph 2 (revised), Global Crop Protection Federation, Brussels. Available: http://www.frac.info/frac/index.htm [accessed March 2011].

Brent, K.J. and Holloman, D.W. 2007b. Fungicide resistance in crop pathogens: How can it be managed? [Online]. Fungicide Resistance Action Committee, Monograph 1, Global Crop Protection Federation. Brussels. Available: http://www.frac.info/frac/index.htm [accessed March 2011].

Bretag, T.W. 1985. Chemical control of ascochyta blight of field peas. Aust. Plant Path. 14: 42–43.

Bretag, T.W., Keane, P.J. and Price, T.V. 2006. The epidemiology of ascochyta blight in field peas: a review. Aust. J. Agric. Res. 57: 883–902.

Chang, K.F., Ahmed, H.U., Hwang, S.F., Gossen, B.D., Strelkov, S.E., Blade S.F. and Turnbull, G.D. 2007. Sensitivity of field populations of *Ascochyta rabiei* to chlorothalonil, mancozeb, and pyraclostrobin fungicides, and effects of strobilurin fungicides on the progress of ascochyta blight of chickpea. Can. J. Plant Sci. 87: 937–944.

Chilvers, M.I., Rogers, J.D., Dugan, F. M., Stewart, J.E., Chen, W. and Peever, T. 2009. *Didymella pisi* sp. nov., the teleomorph of *Ascochyta pisi*. Mycol. Res. 113: 391–400.

Chongo, G. and Gossen, B.D. 2003. Diseases of Pea. Pages 201-213 *in* K. L. Bailey, B. D. Gossen, R. Gugel, and R. A. A. Morrall, eds. Diseases of field crops in Canada. Canadian Phytopathological Society, Saskatoon, SK. 290 pp.

Conner, R.L., Hwang, S.F., Woods, S.M., Chang, K.F., Bing, D.J., Dongfang, Y., Su, H., McAndrew, D.W. and Yager, L.M. 2007. Influence of agronomic traits on the expression of tissue-specific resistance to mycosphaerella blight in field pea**.** Can. J. Plant Sci. 87: 157–165.

Davidson, J.A., and Ramsey, M.D. 2000. Pea yield decline syndrome in South Australia: the role of diseases and the impact of agronomic practices. Aust. J. Agric. Res. 51: 347–354.

Demirci, F., Bayraktar, H., Babaliogullu, I., Dolar,F.S., and Maden, S. 2003. *In vitro* and *in vivo* effects of some fungicides against the chickpea blight pathogen, *Ascochyta rabiei*. J. Phytopathol. 151: 519–524.

FRAC. 2011. Pathogens with field resistance towards QoI fungicides. [On-line]. Available:

http://www.frac.info/frac/index.htm. [accessed December 2011].

Gisi, U., Hermann, D., Ohl, L., and Steden, C. 1997. Sensitivity profiles of *Mycosphaerella graminicola* and *Phytophthora infestans* populations to different classes of fungicides. Pestic. Sci. 51: 290–298.

Gisi, U., Chin, K.M., Knapova, G., Farber, Kung, R., Mohr, U., Parisi, S., Sierotzki, H., and Steinfeld, U. 2000. Recent developments in elucidating modes of resistance to phenylamide, DMI and strobilurin fungicides. Crop Prot. 19: 863–872.

Gisi, U., Sierotzki, H., Cook, A., and McCaffery, A. 2002. Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. Pest Manage. Sci. 58: 859–867.

Gossen, B.D. and Anderson, K.L. 2004. First report of resistance to strobilurin fungicides in *Didymella rabiei*. Can. J. Plant Pathol. 26: 411.

Grasso, V., Palermo, S., Sierotzki, H. Garibaldi, A., and Gisi, U. 2006. Cytochrome *b* gene structure and consequences for resistance to Qo inhibitor fungicides in plant pathogens. Pest Manage. Sci. 62: 465–472.

Keinath, A. 2009. Sensitivity to azoxystrobin in *Didymella bryoniae* isolates collected before and after field use of strobilurin fungicides. Pest Manage. Sci. 65: 1090–1096.

Ma, Z. and Michailides, T.J. 2005. Advances in understanding molecular mechanisims of fungcide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Prot. 24: 853–863.

Miguez, M., Reeve,C., Wood, P.M., and Holloman, D.W. 2003. Rapid report. Alternative oxidase reduces the sensitivity of *Mycosphaerella graminicola* to QoI fungicides. Pest Manag. Sci. 60: 3–7.

Mondahl, S.N., Bhatia, A., Shilts, T. and Timmer, L.W. 2005. Baseline sensitivities of fungal pathogens of fruit and foliage of citrus to azoxystrobin, pyraclostrobin and fenbuconazole. Plant Dis. 89: 1186–1194.

Olaya, G., and Köller, W. 1999. Diversity of kresoxim-methyl sensitivities in baseline populations of *Venturia inaequalis*. Pestic. Sci. 55: 1083–1088.

Pasche, J.S., Wharman, C.M. and Gudmestad, N.C. 2004. Shift in insensitivity of Alternaria solani in response to QoI fungicides. Plant Dis. 88: 181-187.

Rebollar-Alviter, A., Madden, L. V., Jeffers, S. N., and Ellis, M. A. 2007. Baseline and differential sensitivity to two QoI fungicides among isolates of *Phytophthora cactorum* that cause leather rot and crown rot on strawberry. Plant Dis. 91: 1625−1637.

SAS Institute Inc. 2011. Cary, N.C: SAS Institute Inc. [Online]. Available: http://support.sas.com/documentation/ [accessed September 2010].

Thaher, N. 2011. Fungicide Insensitivity in *Ascochyta rabiei* in Saskatchewan. M.Sc. thesis. University of Guelph. 145 pages.

Torriani, S. F. F., Brunner, P.C., McDonald, B.A., and Sierotzki, H. 2008. QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*. Pest Manage. Sci. 65: 155–162.

United States Department of Agriculture. 2010. Dry Peas and Lentils. [On-line]. Available: http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1578

[accessed November 2012].

Vincelli, P., and Dixon, E. 2002. Resistance to QoI (strobilurin-like) fungicides in isolates of *Pyricularia grisea* from perennial ryegrass. Plant Dis. 86: 235–240.

Warkentin, T.D., Xue, A.G., and McAndrew, D.W. 2000. Effect of mancozeb on the control of mycosphaerella blight of field pea, Can J. Plant Sci. 80: 403–406.

Wise, K.A., Bradley, C.A., Pasche, J.S., Gudmestad, N.C., Dugan, F.M., and Chen, W. 2008. Baseline sensitivity of *Ascochyta rabiei* to azoxystrobin, pyraclostrobin and boscalid. Plant Dis. 92: 295–300.

Wise, K.A., Bradley, C.A., Pasche, J.S., and Gudmestad, N.C. 2009. Resistance to QoI fungicides in *Ascochyta rabiei* from chickpea in the northern great plains. Plant Dis. 93: 528–536.

Xue. A.G., Warkentin, T.D. Greeiaus, M.T. and Zimmer, R.C. 1996. Genotypic variability in seedborne infection if field pea by *Mycosphaerella pinodes* and its relation to foliar disease severity. Can. J. Plant Path. 18: 370–374.

Yang, R.C. 2012. Statistician. Alberta Agriculture and Rural Development, University of Alberta, Edmonton, Alberta. Personal communication.

Ypema, H.L. and Gold, R.E. 1999. Kresoxim-methyl. Plant Dis. 83: 4–19.

Ziogas, B. N., Baldwin, B. C., and Young, J. E. 1997. Alternative respiration: a biochemical mechanism of resistance to azoxystrobin (ICIA 5504) in *Septoria tritici*. Pestic. Sci. 50: 28–34.

2.6. TABLES AND FIGURES

Table 2-1. The baseline effective concentration to inhibit 50% growth (EC_{50}) using the formulated product and technical grade formulations of pyraclostrobin fungicide in a collection of *Mycosphaerellea pinodes* isolates from Saskatchewan and Washington, as determined by measuring radial growth on potato dextrose agar (PDA) with and without the addition of salicylhydroxamic acid (SHAM).

	No SHAM FP*	with SHAM FP**	with SHAM TG***
Isolate	EC_{50} (µg mL^{s1})	EC_{50} (µg mL^{-1})	EC_{50} (µg mL^{-1})
R010R	0.1043	0.0744	N/A
Sep0002	0.0723	0.1873	0.1159
Sep0004	0.0764	0.1647	0.1364
Sep0008	0.0774	0.1144	0.0589
Mar0405	0.2162	0.1520	0.0590
Mar0412	0.0746	0.0900	0.1176
Mar0413	0.1312	0.1367	0.1420
Mar0419	0.1205	0.0914	0.1691
Mar0425	0.1988	0.1074	0.0920
Mar0426	0.1104	0.1006	0.0985
Mar0427	0.1211	0.0903	0.0843
Mar0435	0.0705	0.0647	0.0497
Mar0448	0.1217	0.1731	0.1374
Mar0452	0.1281	0.1789	0.1472
Mar0458	0.1847	0.1670	0.1251
Mar0460	0.1513	0.1660	N/A
Mar0463	0.0523	0.1486	0.0998
Mar0464	0.1389	0.0843	0.1023
Apr0404	0.0781	0.1689	0.0628
Apr0405	0.1329	0.1494	0.1421
Apr0409	0.0999	0.0887	0.0954
Aug0002	0.0650	0.0794	0.0335
Aug0004	0.0313	0.0922	0.0370
Aug0006	0.0911	0.0823	0.0358
Nov0456	0.2945	0.1284	0.1244
MP01	0.2087	0.2033	0.1168
MP03	0.0914	0.0943	0.0660
MP04	0.1208	0.2162	0.1463
MP05	0.0928	0.0885	0.0773
MP06	0.1172	0.1195	0.1244
MP07	0.1854	0.1476	0.1504
MP08	0.1773	0.2062	N/A
MP09	0.1504	0.1422	N/A
MP10	0.0999	0.1800	0.1178
MP11	0.1384	0.1525	0.1095
MP12	0.1581	0.1288	0.1421
MP14	0.1475	0.1564	0.1878
MP15	0.0900	0.1039	0.0934

MP17	N/A	0.1644	0.2407
MP18	N/A	0.1885	0.1229
MP22	0.0813	0.1280	0.1759
MP23	0.1451	0.1300	N/A
MP24	0.2483	0.1850	0.2404
MP26A	0.0571	0.1075	0.1586
MP26B	0.1061	0.1209	0.1256
MP27	0.0872	0.1042	0.1504
MP28	0.1445	0.1040	0.1192
MP30	0.1734	0.1366	0.1483
AP10	N/A	0.0663	0.1245

N/A - indicates missing data

EC_{50} calculated using PROC NLIN is SAS 9.2

* No SHAM FP – SHAM not added to PDA, formulated product of pyraclostrobin

** With SHAM FP – SHAM added to PDA, formulated product of pyraclostrobin

*** With SHAM TG – SHAM added to PDA, technical grade formulation of pyraclostrobin

Table 2-2. Analysis of variance (ANOVA) results for radial growth response of *Mycosphaerella pinodes* isolates from Saskatchewan and Washington grown on potato dextrose agar (PDA) amended with pyraclostrobin fungicide with and without the addition of salicylhydroxamic acid (SHAM).

Isolate	Pr > F	F-value	R-Square	Coeff of Var.	Log_{10} mean growth*
R010R	0.1755	2.04	0.91	24.79	1.246
Sep0002	0.1382	2.44	0.90	45.75	1.023
Sep0004	0.0094	9.05	0.94	26.89	1.112
Sep0008	0.0416	5.03	0.97	12.45	1.274
Mar0405	0.1662	2.13	0.94	17.04	1.326
Mar0412	0.1855	1.94	0.90	34.24	1.135
Mar0413	0.9981	0.00	0.95	16.04	1.268
Mar0419	0.6161	0.26	0.97	12.26	1.262
Mar0425	0.2983	1.17	0.92	34.43	1.104
Mar0426	0.5221	0.43	0.96	11.81	1.347
Mar0427	0.2851	1.24	0.95	19.87	1.167
Mar0435	0.0279	6.01	0.92	29.51	1.133
Mar0448	0.1010	3.08	0.95	15.65	1.293
Mar0458	0.0805	3.55	0.91	26.61	1.214
Mar0460	0.9362	0.01	0.96	13.74	1.283
Mar0464	0.1172	2.79	0.92	31.21	1.151
Apr0404	0.0521	4.50	0.96	15.58	1.245
Apr0405	0.2455	1.47	0.96	14.98	1.279
Apr0409	0.1231	2.69	0.92	34.90	1.088
Aug0002	0.1292	2.62	0.86	42.92	1.116
Aug0004	0.0397	5.14	0.85	52.00	1.067
Aug0006	0.4132	0.71	0.81	60.64	1.086
Nov0456	0.1777	2.01	0.93	16.38	1.376
MP01	0.1594	2.21	0.96	12.25	1.327
MP03	0.4926	0.50	0.95	18.01	1.225
MP04	0.8018	0.07	0.95	13.72	1.332
MP05	0.0668	3.87	0.77	27.19	1.338
MP06	0.0068	10.02	0.96	17.13	1.198
MP07	0.6600	0.20	0.95	10.31	1.449
MP08	0.2122	1.72	0.92	12.27	1.506
MP09	0.0273	6.07	0.96	15.26	1.240
MP10	0.2609	1.38	0.92	17.76	1.385
MP11	0.7421	0.11	0.90	24.32	1.298
MP12	0.7770	0.08	0.92	18.89	1.310
MP14	0.2300	1.59	0.94	14.66	1.380
MP15	0.1458	2.37	0.95	15.01	1.247
MP22	0.0198	6.92	0.97	14.64	1.201
MP23	0.0328	5.53	0.72	45.47	1.229
MP24	0.1562	2.27	0.87	24.07	1.405
MP26A	0.6370	4.05	0.97	21.16	0.995
MP26B	0.5243	0.43	0.98	8.15	1.352
MP27	0.1197	2.75	0.96	19.91	1.103
MP28	0.5820	0.40	0.94	16.62	1.280
MP30	0.5551	0.37	0.96	8.63	1.446

Analyzed using PROC GLM in SAS 9.2.

*Dependent variable was log_{10} transformation of % growth response with 1 degree of freedom and $P \leq 0.05$.

Table 2-3. Analysis of Variance (ANOVA) results for radial growth response of *Mycosphaerella pinodes* isolates from Saskatchewan and Washington grown on potato dextrose agar (PDA), comparing the formulated product and technical grade formulation of pyraclostrobin fungicide.

Isolate	Pr > F	F-value	R-Square	Coeff of Var.	Log_{10} mean growth
Sep0002	0.2800	1.28	0.95	9.70	1.470
Sep0004	0.7145	0.14	0.95	9.86	1.463
Sep0008	0.0851	3.52	0.91	20.61	1.343
Mar0405	0.2826	1.25	0.72	25.65	1.403
Mar0412	0.1624	2.22	0.97	11.92	1.330
Mar0413	0.6586	0.21	0.95	12.74	1.443
Mar0419	0.4232	0.69	0.92	19.71	1.352
Mar0425	0.3721	0.87	0.95	14.24	1.391
Mar0426	0.1803	2.02	0.96	9.72	1.419
Mar0427	0.2971	1.19	0.95	14.91	1.308
Mar0435	0.0346	5.68	0.97	10.43	1.335
Mar0448	0.5617	0.36	0.96	8.25	1.492
Mar0452	0.7910	0.07	0.95	10.55	1.503
Mar0458	0.4759	0.54	0.95	11.67	1.469
Mar0463	0.9728	0.00	0.95	8.85	1.481
Mar0464	0.2743	1.29	0.65	46.31	1.257
Apr0404	0.1264	2.70	0.94	11.98	1.447
Apr0405	0.3731	0.86	0.94	12.45	1.473
Apr0409	0.9941	0.00	0.95	11.98	1.412
Aug0002	0.0562	4.55	0.97	11.85	1.250
Aug0004	0.9642	0.00	0.80	53.14	1.122
Aug0006	0.0432	5.11	0.94	22.64	1.182
Nov0456	0.3732	0.86	0.89	17.43	1.480
MP01	0.1461	2.42	0.93	19.21	1.344
MP03	0.3400	0.99	0.95	20.50	1.245
MP04	0.6414	0.23	0.96	11.30	1.425
MP05	0.0221	7.09	0.98	7.82	1.436
MP06	0.2649	1.37	0.95	13.38	1.372
MP07	0.2463	1.50	0.95	11.19	1.479
MP10	0.2501	1.47	0.92	19.82	1.392
MP11	0.5549	0.37	0.89	24.50	1.319
MP12	0.3426	0.98	0.92	18.63	1.386
MP14	0.8701	0.03	0.94	17.99	1.329
MP15	0.0837	3.56	0.96	11.83	1.352
MP17	0.2253	1.65	0.92	13.41	1.520
MP18	0.2028	1.83	0.95	12.43	1.441
MP22	0.9658	0.00	0.93	18.47	1.327
MP24	0.6560	0.21	0.85	35.71	1.331
MP26A	0.0888	3.48	0.94	21.37	1.264
MP26B	0.1147	2.89	0.96	10.40	1.403
MP27	0.7788	0.08	0.95	20.10	1.237
MP28	0.8161	0.06	0.93	14.76	1.364
MP30	0.0346	5.68	0.95	13.83	1.404
AP10	0.0926	3.34	0.97	11.01	1.348

Analyzed using PROC GLM in SAS 9.2. Dependent variable was log_{10} transformation of % growth response with 1 degree of freedom and $P \leq 0.05$.

Table 2-4. The effective concentration to inhibit 50% growth (EC_{50}) of the formulated product of pyraclostrobin fungicide in a collection of *Mycosphaerellea pinodes* isolates from Saskatchewan and Washington, as determined by measuring conidial germination on potato dextrose agar (PDA) with the addition of salicylhydroxamic acid (SHAM).

	with SHAM FP		with SHAM FP**
Isolate	EC_{50} (µg mL^{-1})*	Culture	EC_{50} (µg mL^{-1})
Sep0002	0.0147	MP01	0.0165
Sep0004	0.0132	MP03	0.0107
Sep0008	0.0112	MP04	0.0153
Mar0405	0.0110	MP05	0.0181
Mar0412	0.0108	MP06	0.0166
Mar0413	0.0138	MP07	0.0097
Mar0419	0.0410	MP08	0.0194
Mar0426	0.0117	MP09	0.0197
Mar0427	0.0106	MP11	0.0199
Mar0435	0.0083	MP14	0.0235
Mar0448	0.0147	MP17	0.0086
Mar0452	0.0207	MP18	0.0156
Mar0458	0.0167	MP19	0.0164
Mar0463	0.0217	MP22	0.0111
Mar0464	0.0236	MP24	0.0134
Apr0404	0.0099	MP26A	0.0212
Apr0405	0.0111	MP26B	0.0135
Apr0409	0.0195	MP27	0.0115
Aug0002	0.0093	MP28	0.0118
Aug0004	0.0166	MP30	0.0146
Aug0006	0.0080	AP10	0.0161

*EC_{50} calculated using PROC NLIN is SAS 9.2

**With SHAM FP – SHAM added to PDA, formulated product of pyraclostrobin

Table 2-5. Isolates of *Mycosphaerella pinodes* collected in 2010 and 2011 from Saskatchewan and Alberta that were classified as insensitive or intermediately sensitive to pyraclostrobin fungicide, based on <30% or 30-70% reduction in radial growth and percent conidial germination, respectively, on potato dextrose agar (PDA) amended with the fungicide.

Year	Culture	% growred*	% germred**	EC_{50} (µg mL^{-1})	Classification
2010	Barrhead 8	28.57	6.33	144.4	insensitive
	Barrhead 7	25.33	9.76	260.7	insensitive
	Barrhead 6	16.89	9.12	186.0	insensitive
	Mannville F11A-2	16.86	1.68	211.5	insensitive
	Mannville F11A-4	15.18	11.33	255.2	insensitive
2011	Kelsey 5	30.74	4.18	176.0	insensitive
	Hwy 611 11	25.31	2.71	226.8	insensitive
	Red Deer F1-3	20.95	16.60	149.0	insensitive
	Lacombe F2-1	15.54	5.19	148.3	insensitive
	Red Deer F2-3	15.14	19.63	161.3	insensitive
	New Norway 1	10.41	2.51	216.5	insensitive
	Hwy 611 1	-5.29	1.00	185.8	insensitive
	Hwy 611 5	-10.61	7.88	230.5	insensitive
	Hwy 611 8	-35.29	6.86	136.4	insensitive
2010	Swift Current 3	29.30	13.98	80.3	insensitive
	Wingard Ferry 4	21.06	6.62	179.9	insensitive
	Swift Current 6	16.22	17.72	189.2	insensitive
	Wolsley 3	12.57	3.05	97.6	insensitive
	Saskatoon 1	-23.95	-0.40	176.8	insensitive
2011	Purdue 17	50.19	.	5.0	intermediate

EC_{50} value using radial growth methodology analyzed with PROC NLIN in SAS 9.2.
All of the other 304 isolates collected were sensitive to pyraclostrobin
*% growred = % growth reduction using the radial growth methodology
**% germred = % germination reduction using the conidial germination methodology

Table 2-6. Comparison of percent radial growth reduction and statistical classification of sensitive and insensitive *Mycosphaerella pinodes* isolates collected in 2010 and 2011 in Alberta and Saskatchewan.

Isolate	% growth reduction*	Classification**
Kuans 2	98.37 a	sensitive
Tramping Lake 8	97.76 a	sensitive
Nokomis F4-1	97.71 a	sensitive
Mariposa 1	96.97 a	sensitive
Sask 10	96.71 a	sensitive
Lacombe 24	96.66 a	sensitive
Kinley 5	96.64 a	sensitive
Sask 6	96.55 a	sensitive
Graff F2-5	96.43 a	sensitive
Biggar 2	96.35 a	sensitive
Kelsey 6	96.31 a	sensitive
Lacombe 3	96.30 a	sensitive
Kutcher F4-2	96.28 a	sensitive
Purdue 8	96.18 a	sensitive
Graff F2-3	96.17 a	sensitive
Mariposa 5	96.16 a	sensitive
Kelsey 3	96.12 a	sensitive
Mannville F12-4	96.10 a	sensitive
Purdue 17	50.19 b	intermediate
Kelsey 5	30.74 c	insensitive
Krikkie 3	29.30 c	insensitive
Barrhead 8	28.57 c	insensitive
Barrhead 7	25.33 c	insensitive
Hwy 611 11	25.31 c	insensitive
Wingard Ferry 4	21.06 cd	insensitive
Kuans F1-3	20.95 d	insensitive
Barrhead 6	16.89 d	insensitive
Mannville F11A-2	16.86 d	insensitive
Wierenga F2-1	15.54 d	insensitive
Mannville F11A-4	15.18 d	insensitive
Kuans F2-3	15.14 d	insensitive
Wolsley 3	12.57 de	insensitive
New Norway 1	10.41 de	insensitive
Krikkie 6	5.46 e	insensitive

Means followed by the same letter are not significantly different according to Tukey's Multiple range test at P≤0.05.

* % radial growth reduction on potato dextrose agar (PDA) amended with pyraclostrbin fungicide at 5.0 µg ml^{-1}

** classification groups based on >70% (sensitive), 30-70% (intermediate), or <30% (insensitive) radial growth reduction

Table 2-7. Analysis of Variance (ANOVA) comparing the EC_{50} value of pyraclostrobin-insensitive, intermediate and sensitive groupings of *Mycosphaerella pinodes* isolates using contrast statements in SAS 9.2.

Contrast*	DF	Contrast SS	Mean square	F value	Pr > F
insensitive vs intermediate	1	4316.6	4316.6	91.2	< .0001
insensitive vs sensitive	1	340558.7	340558.7	7195.1	< .0001
intermediate vs sensitive	1	5206.6	5206.6	110.0	< .0001

* classification groups based on >70% (sensitive), 30-70% (intermediate), or <30% (insensitive) radial growth reduction on potato dextrose agar amended with the fungicide

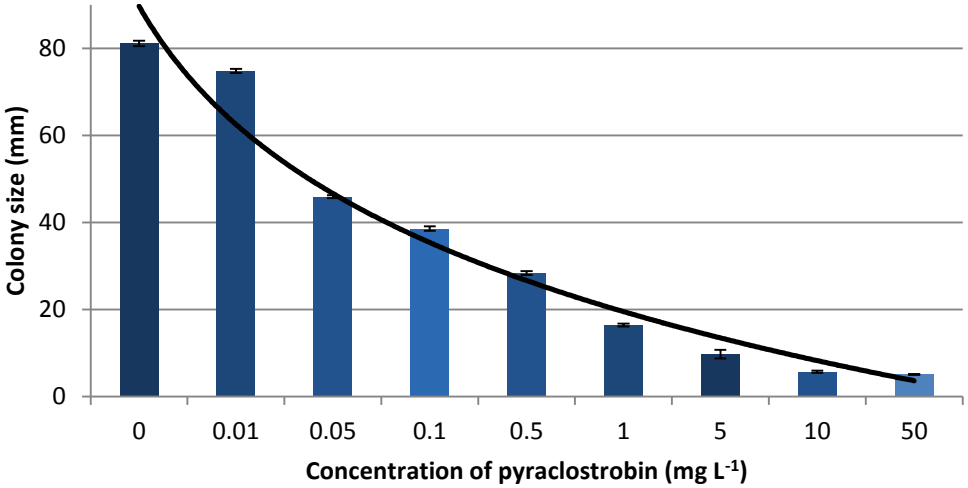

Figure 2-1. Radial growth response of a representative *Mycosphaerella pinodes* isolate R0101R to concentrations of the formulated product of pyraclostrobin fungicide grown on potato dextrose agar (PDA) medium without the addition of salicylhydroxamic acid (SHAM).

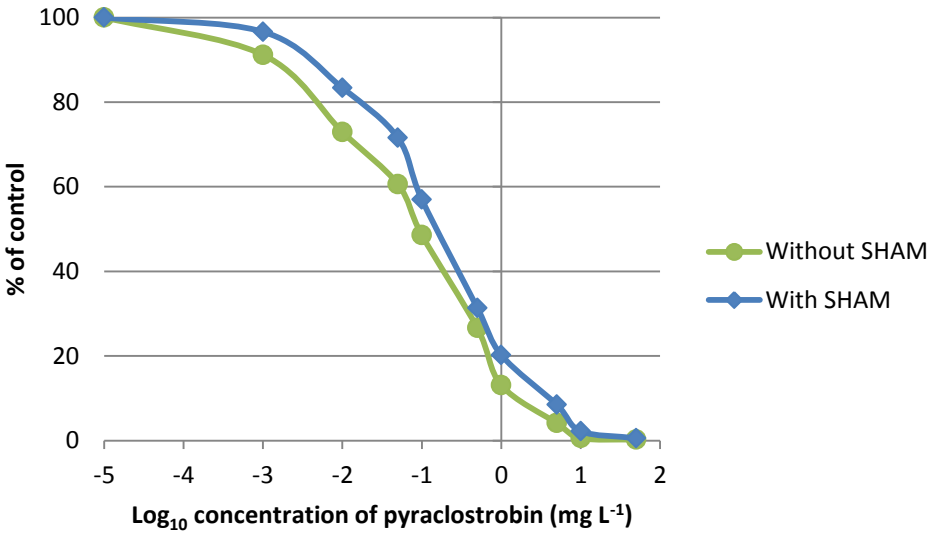

Figure 2-2. Comparison of the logistic data curves of a representative isolate of *Mycosphaerella pinodes* Apr0404 from Saskatchewan tested for radial growth response to pyraclostrobin fungicide on potato dextrose agar (PDA) medium, with and without the addition of salicylhydroxamic acid (SHAM). The control plate was pure PDA which did not contain any pyraclostrobin fungicide.

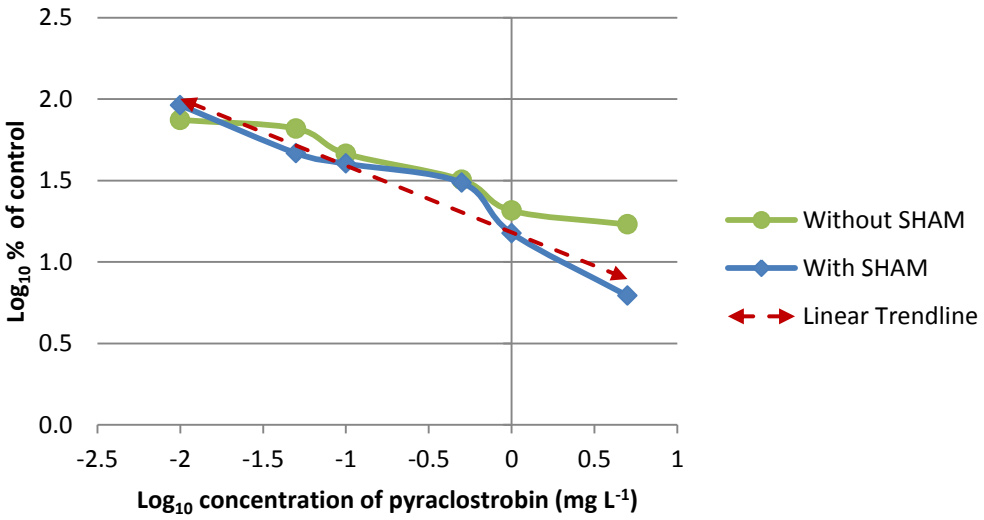

Figure 2-3. Comparison of the $\log_{10}$ transformed data curves of the radial growth response of a representative *Mycosphaerella pinodes* isolate Apr0404 from Saskatchewan to pyraclostrobin fungicide, tested on potato dextrose agar (PDA) medium with and without the addition of salicylhydroxamic acid (SHAM). The control plate was pure PDA which did not contain any pyraclostrobin fungicide.

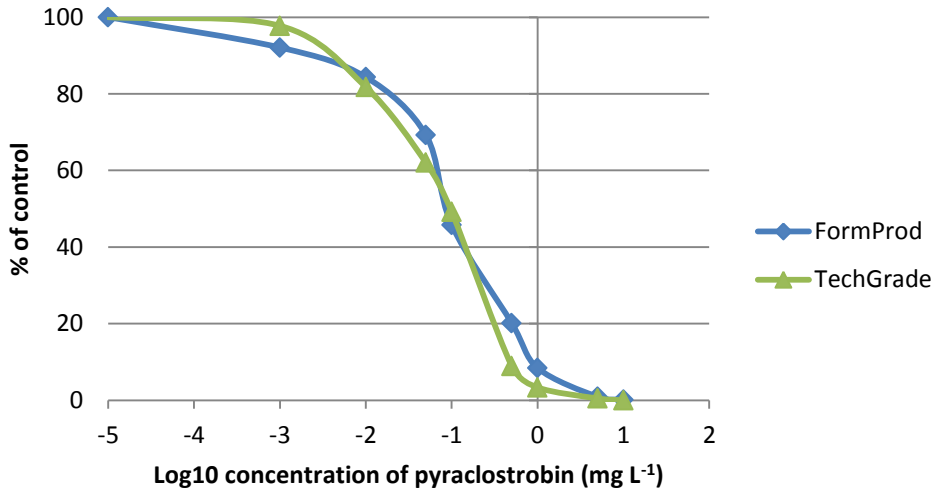

Figure 2-4. Comparison of the logistic data curves of a representative isolate of *Mycosphaerella pinodes* MP03 from North Dakota tested for radial growth response to pyraclostrobin fungicide on potato dextrose agar (PDA) medium using the formulated product versus the technical grade formulation of the fungicide. The control plate was pure PDA which did not contain any pyraclostrobin fungicide.

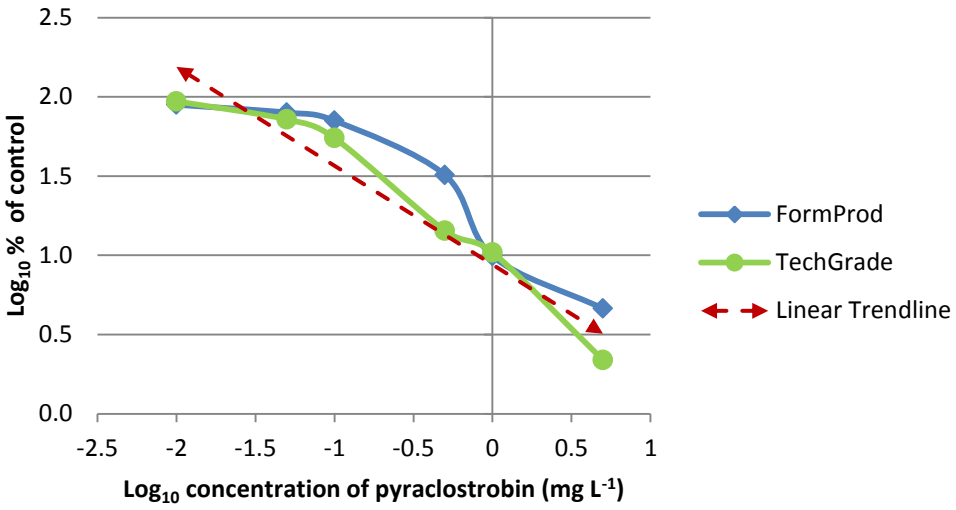

Figure 2-5. Comparison of the $\log_{10}$ transformed data curves of the radial growth response of a representative *Mycosphaerella pinodes* isolate MP03 from North Dakota to pyraclostrobin fungicide, tested on potato dextrose agar (PDA) medium using the formulated product versus the technical grade formulation of the fungicide. The control plate was pure PDA which did not contain any pyraclostrobin fungicide.

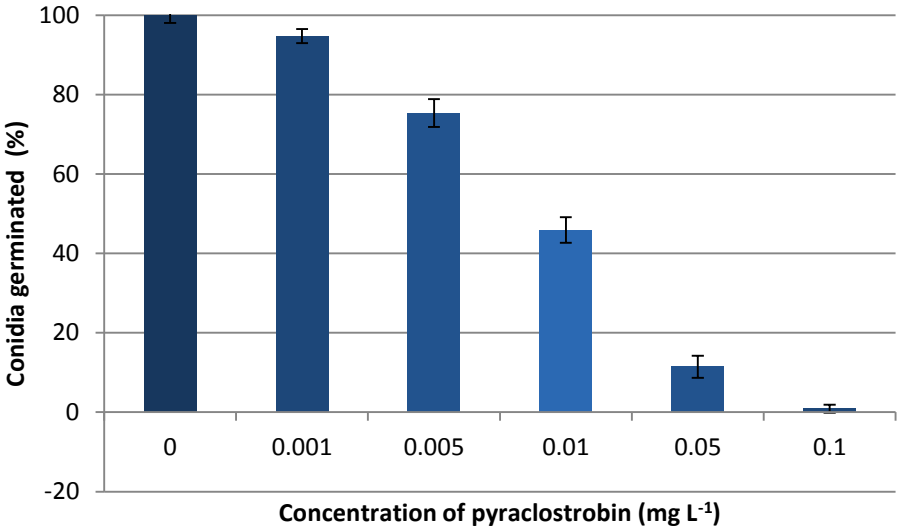

Figure 2-6. Conidial germination response of a representative *Mycosphaerella pinodes* isolate to concentrations of the formulated product of pyraclostrobin fungicide grown on potato dextrose agar (PDA) with the addition of salicylhydroxamic acid (SHAM).

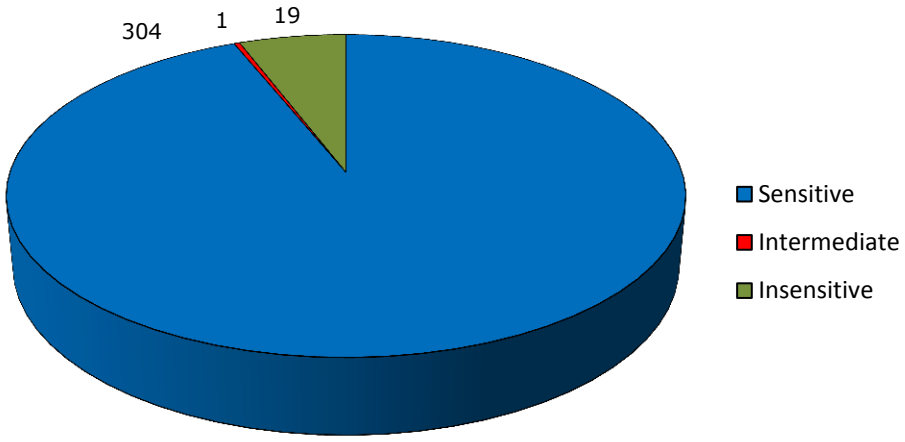

Figure 2-7. Sensitivity to pyraclostrobin fungicide, evaluated based on radial growth and conidial germination assays, of 324 *Mycosphaerella pinodes* isolates collected in 2010 and 2011 in Alberta, Saskatchewan, North Dakota and Washington. Isolates were classified as sensitive if growth was reduced >70%, intermediate if growth was reduced between 30-70%, or insensitive if growth was reduced by <30%, relative to a control treatment.

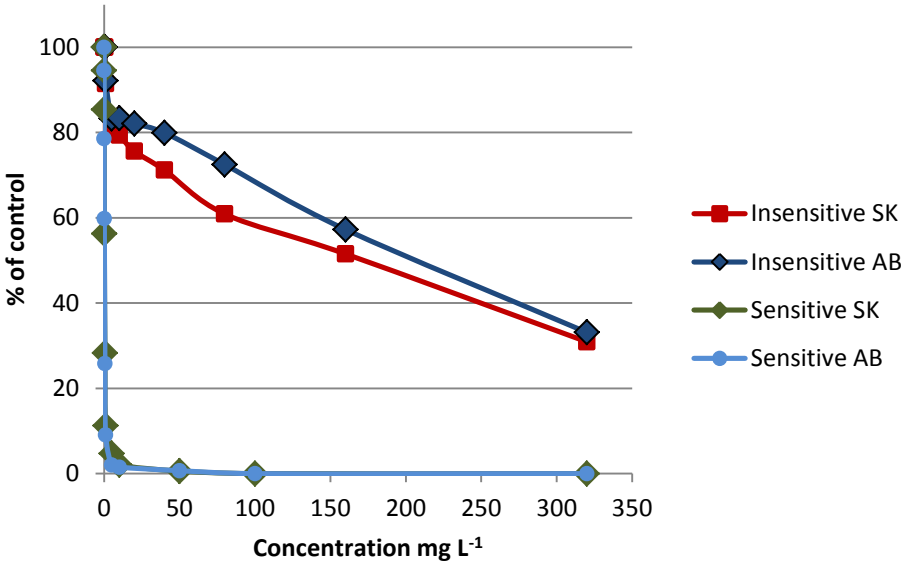

Figure 2-8. Dose response curve of the average of all *Mycosphaerella pinodes* isolates that were insensitive and sensitive to pyraclostrobin fungicide collected from Alberta and Saskatchewan in 2010 and 2011.

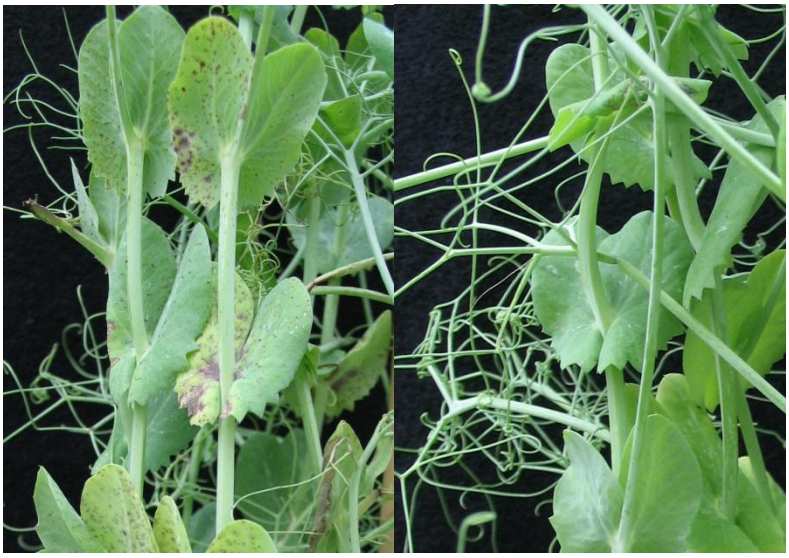

Figure 2-9. Symptoms on pea cv. CDC Meadow seven days after inoculation with a pyraclostrobin-insensitive (left) or sensitive (right) isolate of *Mycosphaerella pinodes.* The plants were sprayed with pyraclostrobin fungicide 1 day prior to inoculation.

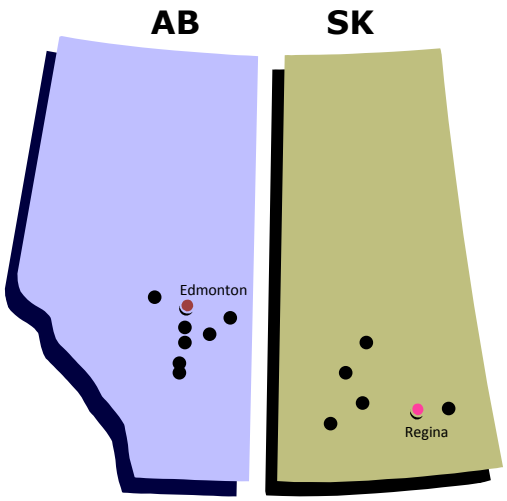

Figure 2-10. Origin of pyraclostrobin insensitive *Mycosphaerella pinodes* isolates collected in Saskatchewan and Alberta in 2010 and 2011.

CHAPTER 3: OPTIMIZING SPRAYER APPLICATION TECHNOLOGY FOR MANAGEMENT OF *MYCOSPHAERELLA PINODES* IN FIELD PEA

3.1. INTRODUCTION

Field pea (*Pisum sativum* L.) offers producers an alternative to growing cereal and canola crops in cropping rotations, with the added benefit of lower input costs due to the plants ability to fix atmospheric nitrogen. An increased emphasis on crop rotation and crop diversification on commercial farms over the last 30 years has led to substantial increases in field pea and other pulse crop production across the Canadian prairies. In 1980 there were approximately 50,000 hectares of field pea grown in this region, compared to 1.3 million hectares in 2012 (Statistics Canada 2013).

Mycosphaerella pinodes (Berk. & Blox.) Vestergr (anamorph *Ascochyta pinodes* (Berk. & Blox.) Jones) causes mycosphaerella blight, a serious disease that can limit field pea production in western Canada. The fungus survives as a saprophyte on infected crop residues, producing inoculum to initiate infection in subsequent years. Mycosphaerella blight has generally been found in all surveyed commercial fields in western Canada, and the disease severity is dependent on environmental conditions (Banniza and Vandenberg 2003). The fungus attacks the leaves, stems and pods from pre-flower through to plant maturity. At early growth stages it interferes with photosynthesis, while at later stages it affects seed quality and yield (Bretag 1991).

Management of *M. pinodes* is best achieved by reducing the amount of available primary inoculum and by suppressing the subsequent epidemic. Avoidance strategies such as crop rotation are key to the management of plant pathogens, especially those that are residue-borne (Peairs et al. 2005). To suppress epidemics of mycosphaerella blight, careful selection of cultivars and application of foliar fungicides should be considered. The best long-term strategy to manage the disease would be the development of resistant cultivars, but attempts to identify strong sources of resistance have, so far, met with very limited success (e.g., Zhang and Gossen 2007). Currently, the most frequently utilized strategy for management of this disease in many parts of the world is the application of fungicidal sprays on a preventative schedule (Beasse et al. 2000). For the most reliable management of mycosphaerella blight, the above strategies should be combined as components of an integrated management system. The specific combination would be determined by economics, available options and epidemiological considerations such as weather and disease level. Fungicide application would have to be considered as the most effective of the currently available management options. Multiple applications from early to mid-flowering, provide effective disease severity reduction and yield gain (Warkentin et al. 2000). Increases in pea yields of 15-75% have been reported in field experiments where *M. pinodes* has been managed with fungicides (Bretag et al. 2006; Xue et al. 2003).

Foliar fungicides are applied to protect the crop from plant pathogens, to increase crop yield, and to obtain high quality seed. In some instances, the use of foliar fungicides is critical for the growth of certain crops because without them, production of those crops would be impossible. The objective for application of any crop protection agent is the placement of just enough active ingredient on the target plant or tissue to achieve the desired biological result safely and economically (Ebert 1999). There are a range of factors affecting the safe and effective application of pesticides, from the properties of the spray solution to the action of the active ingredient in the target system (Wirth et al. 1991).

One aspect of safe and effective application of pesticides is efficacy. Efficacy is determined by the uptake and effectiveness of the active ingredient and the degree of coverage of the target plant (Armstrong-Cho et al. 2008a). Superior plant coverage is affected by the architecture of the plant, its leaf surface characteristics, the characteristics of the spray mixture, carrier water volume, spray quality and spray angle.

There have been no published studies that define the best spray coverage, droplet size, and water volumes needed to optimize the efficacy of various kinds of fungicides to manage mycosphaerella blight in field pea. Fungicides applied later to older plants, when the crop canopy is dense and difficult to penetrate makes delivery of the fungicide to the target tissues a challenge. Droplet size is

an important topic of sprayer application research. There are benefits to the use of both small and large droplets in a spray application system. Protectant fungicides are generally most effective when applied as small droplets that evenly cover both sides of the leaf surface. In contrast, systemic fungicides move within leaf tissue and so may be relatively more effective when applied in larger droplets (Bateman 1993; Elliot and Mann 1997). However, so many factors interact with droplet size that generalizations as to which droplet size is most effective are very difficult.

A double-nozzle system for spray application can improve the spray pattern for finer sprays, provide greater pesticide efficacy, and reduce the dilution of the product on the leaf surface (Hall et al. 1996). The system involves two nozzles working together, one nozzle producing a coarse spray and the second nozzle producing a fine spray. Use of this system can reduce pesticide application amounts by 30-50% in many host-pathogen systems as a consequence of better coverage (Chapple et al. 1997). Double nozzles have been recommended to optimize coverage on vertical targets, such as the growing tips of many pulse crops (Wolf 2009). Different orientation of the two nozzles can also affect penetration of the active ingredient into crop canopies. In general, nozzles should face backward when fungicides are being applied to optimize vertical canopy penetration and horizontal crop coverage (Wolf 2009).

Increasing the water carrier volumes used in sprayer systems can substantially improve penetration into the crop and increases the frequency of

droplets at all levels of the canopy. Higher volumes also have the added benefit of decreasing the potential for drift and increasing nozzle performance (Wirth 1991). An increase in fungicide carrier volume significantly increased leaf and fruit coverage in apple orchards (Cross et al. 2000) and reduced ascochyta blight severity in chickpea under moderate to high disease pressure (Armstrong et al. 2008).

It is extremely important to deliver and retain the active ingredient to critical sites at high enough rates to inhibit the target pathogen and protect the plant (Gossen et al. 2008). It is also important to consider environmental consequences and not over apply these products as insurance for effective results (Ebert 1999). Fungicides need to target specific sites on the plant, and due to the timing of fungicide application into dense canopies, effectively contacting these target sites presents a challenge. Altering nozzle orientation and carrier water volumes may increase the efficacy of fungicides, but there are disagreements as to which system will be most effective in particular host-pathogen systems.

3.1.1. Objective

The objective of this study was to identify a fungicide delivery system to improve penetration and coverage of the crop canopy to manage *M. pinodes* on field pea by: i) determining the most effective spray quality delivery method, and ii) examining the potential benefits of increasing fungicide carrier volumes.

3.2. MATERIALS AND METHODS

3.2.1. Field Studies

Effect of nozzle configurations and angles on fungicide delivery

Field trials were conducted at the Agriculture and Agri-Food Canada research farms in Morden, MB, Saskatoon, SK, and Lacombe, AB, and at the Crop Diversification Centre North, Alberta Agriculture and Rural Development, Edmonton, AB, from 2008 to 2011, to examine the effect of various nozzle configurations and angles for the management of *M. pinodes* in field pea. Treatments varied between locations and evolved over the study years as described in Table 3-1. The treatments were as follows: 1) an untreated control; 2) a single nozzle, producing a fine spray, mounted in a vertical position; 3) a single nozzle, producing a coarse spray, mounted in a vertical position; 4) double nozzles, producing a fine spray, mounted in a vertical position; 5) double nozzles, producing a coarse spray, mounted in a vertical position; 6) double nozzles, vertical coarse spray, 60° fine spray (Y-adapter fitted with two nozzles, using a cap with 60° elbow to make fine spray point forward); 7) single nozzle, fine spray applied in a carrier volume of 100 L ha^{-1}; 8) single nozzle, producing a fine spray, mounted at a high angle (using a cap with a 60° elbow to make the spray point forward); 9) single nozzle, coarse spray, high angle; 10) double nozzles, fine spray, high angle, and 11) double nozzles, coarse spray, high angle.

In 2008, 2009 and 2010, trials to examine treatments 1 to 7 were conducted in Morden and Saskatoon (three trials in 2009, none in 2010 due to flooding

after seeding) (Table 3-1). In 2009, two trials examining treatments 1-5 and 8-11 were conducted at Lacombe and two trials were conducted at Edmonton (Table 3-1). In 2011, one trial examining treatments 1-5 and 8-11 was conducted at Saskatoon and two trials were conducted at Lacombe (Table 3-1). The nozzles used were ComboJet™ ER (extended range), MR (mid-range) and DR (drift reduction) with a flat fan pattern (Westward Parts, Red Deer, AB) spraying with an 80° angle range from nozzle tip.

The cultivars used in these trials differed among years to allow for adaptation of cultivars depending on location. 'Topper' was used at Morden in 2008, 2009 and 2010; at Saskatoon in 2011; at Edmonton in 2009 and at Lacombe in 2009 and 2011. **'**Topper' is a yellow, early maturing cultivar with poor mycosphaerella blight and powdery mildew resistance (Ali-Khan 1991). 'Delta' was used in Saskatoon in 2008. 'Delta' is a yellow pea that was chosen because it has fair mycosphaerella blight resistance, poor powdery mildew resistance and fair resistance to lodging (Saskatchewan Seed Guide 2007). 'Nitouche' was used at Saskatoon in 2009, at the same site as a trial using the 'CDC Montero'. This provided a comparison of treatments in an upright crop canopy compared to one that was more likely to lodge. 'Nitouche' is a green, semi-leafless, medium maturing cultivar with fair mycosphaerella blight resistance, poor powdery mildew resistance, and with a good resistance to lodging (Saskatchewan Seed Guide 2007). 'CDC Montero' was also assessed at Saskatoon in 2009. 'CDC Montero' is a green, semi-leafless, medium maturing cultivar with fair

mycosphaerella blight resistance, very good powdery mildew resistance, and fair lodging resistance (Saskatchewan Seed Guide 2009). 'Carneval' was used at Edmonton in 2009, and Lacombe in 2009 and 2011. 'Carneval' was chosen because it is a yellow, semi-leafless, medium maturing cultivar with good mycosphaerella blight resistance and fair lodging resistance (Alberta Seed Guide, 2009).

In 2011 the treatment structure was amended, based on the results from the previous years, to detect a treatment which may have clear significant differences in disease severity and yield. The data collected from the original treatments 1-7 (Table 3-1) indicated that higher angles may be beneficial for fungicide coverage, so additional treatments 8-11 (Table 3-1) were added. Trials were conducted in Saskatoon, Lacombe and Edmonton.

Trials were laid out in a randomized complete block design (RCBD) with four replications. Plots consisted of either four rows (Morden, Lacombe and Edmonton) or eight rows (Saskatoon), and varied in size from 7.5 m^2 in Morden to 9 m^2 in Edmonton and Lacombe, to 15 m^2 in Saskatoon. Barley guard rows were used in Saskatoon as a precaution to prevent fungicide drift between plots. Row spacing varied from 25 to 30 cm, depending on seeding equipment, and plots were seeded to establish a target plant density of 85-90 plants m^{-2}. Initial weed control was obtained by application of soil applied trifluralin (Loveland Products, Loveland, CO) or ethalfluralin (Dow AgroSciences Canada Inc., Calgary,

AB) at the recommended rates for the area in the previous fall or early spring. In-crop applications of bentazon (BASF Canada, Mississauga, ON), sethoxydim (BASF Canada) or imazethapyr (BASF Canada) were applied at the recommended rates for the target weeds at the recommended timing and the plots were then hand weeded as necessary. Overhead irrigation was applied at the Saskatoon location in 2009 to initiate disease infection due to very dry environmental conditions.

Seedling emergence was counted to determine plant density at each location to ensure uniformity within each experimental unit. Plots were artificially inoculated using one of two methods depending on location. In Morden, Saskatoon, Lacombe and Edmonton, crop residue was collected from a previous field pea crop that had been heavily infested with mycosphaerella blight and was applied evenly to the pea trials to initiate an epidemic. In addition, plots at Edmonton and Lacombe were inoculated with a *M. pinodes* spore suspension sprayed onto plants at the early flower stage with a hand pump sprayer. The cultures for this procedure were isolates that had been collected the previous year from surveys conducted in Alberta, and were grown on Petri dishes containing potato dextrose agar (PDA) (Difco Laboratories, Detroit, IL) for 14 days under white fluorescent light at 20 °C (±2 °C) for 16 h light / 8 h dark. The dishes were then flooded with 5 mL of water and the spores dislodged with a glass rod and/or a small transfer loop. The supernatant from each Petri dish was filtered through two layers of commercial cheesecloth and combined into a

single spore suspension. The spore suspension was adjusted to 2 x 10^5 spores mL^{-1} and 0.05% Tween 20 was added. The spore solution was sprayed onto the plants until the leaves were wet but not dripping.

The foliar fungicide pyraclostrobin (Headline EC, BASF Canada) was applied at a rate of 100 g a.i. ha^{-1} with a backpack sprayer in a carrier volume of 250 L ha^{-1} of water at Saskatoon, Edmonton and Lacombe, and at a rate of 200 L ha^{-1} at Morden (unless otherwise stated). Each nozzle combination was calibrated to 210-275 kPa before initial application. The fungicide was applied either once (at the early flowering stage when symptoms were noted) or twice (once at the early flowering stage and once 10-14 d later at the mid-flower to early pod stage). The timing of applications depended on mycosphaerella blight severity at each location and fungicide was not applied if disease was not present.

Ten pea plants were selected at random from each plot and assessed for severity of mycosphaerella blight symptoms on foliage and stems on a 0–9 scale, where 0 = no disease symptoms and 9 = the plant was completely covered with lesions (Xue, 1996). Disease ratings were taken just before fungicide application and again approximately every 7 d until plant senescence. Plots were harvested at physiological maturity using a small plot combine. Seed was air-dried, cleaned and weighed, and yield was determined.

Effect of carrier volume and nozzle configuration on fungicide delivery

Field trials were conducted at Saskatoon, SK, and Lacombe, AB, Canada in 2010 and 2011 to examine the effect of selected water volumes and nozzle configurations for the management of *M. pinodes* in field pea. The treatments were as follows: 1) an untreated control; 2) a single nozzle with a 50 L ha^{-1} output; 3) a single nozzle with a 100 L ha^{-1} output; 4) a single nozzle with a 200 L ha^{-1} output; 5) a single nozzle with a 400 L ha^{-1} output; 6) a single nozzle with a 800 L ha^{-1} output; 7) double nozzles with a 50 L ha^{-1} output; 8) double nozzles with a 100 L ha^{-1} output; 9) double nozzles with a 200 L ha^{-1} output; 10) double nozzles with a 400 L ha^{-1} output; and 11) double nozzles with a 800 L ha^{-1} output. Treatments varied slightly between locations (Table 3-2).

The cultivar used in these trials was 'Cutlass', a yellow, semi-leafless, medium maturing variety with fair mycosphaerella blight resistance, very good powdery mildew resistance, and good lodging resistance (Saskatchewan Seed Guide 2011).

Trials were laid out in a RCBD with four replications. Plots consisted of four rows and plot size ranged from 7.5 m^2 in Saskatoon to 9 m^2 in Lacombe. Row spacing varied from 25 to 30 cm depending on seeding equipment, and plots were seeded to achieve a target density of 85-90 plants m^{-2}. Seedling emergence was counted to determine plant density at each location to ensure uniformity within each experimental unit. Plots were artificially inoculated using one of the two methods as described above. Initial weed control was obtained by

application of soil applied ethalfluralin (Dow AgroSciences Canada Inc., Calgary, AB) at the recommended rate and timing (fall or early spring) for the area. In-crop applications of bentazon (BASF Canada), sethoxydim (BASF Canada) or imazethapyr (BASF Canada) were applied at the recommended rate for the target weeds at the recommended timing, and the plots were then hand weeded as necessary.

The foliar fungicide pyraclostrobin was applied at a rate of 100 g a.i. ha^{-1} with a backpack sprayer at the various water volume treatment rates. Each nozzle combination was calibrated to 210-275 kPa before initial application. The fungicide was applied twice; once at the early flowering stage and once 10-14 d later at the mid-flower to early pod stage.

Ten pea plants per plot were selected at random and assessed for mycosphaerella blight disease severity on foliage and stems of field pea on the 0–9 scale of Xue (1996). Disease ratings were taken just before fungicide application and again approximately every 7 d until plant senescence. Plots were harvested at maturity using a small-plot combine. Seed was air-dried, cleaned, and weighed, and yield was determined.

3.2.2. Growth chamber study - carrier volume and nozzle configuration

A RCBD experiment was conducted in a growth chamber in 2012 to assess the effect of a range of water volumes and selected nozzle configurations on

levels of *M. pinodes* severity on field pea plants under controlled environmental conditions (Table 3-2).

To be consistent with the field assessments of the same treatments, 'Cutlass' was used in this study. Each experimental unit consisted of a single pot containing 10 plants, with four replications per treatment. Pure mass-transfer cultures of randomly selected pathogenic isolates of *M.pinodes* were inoculated onto 50 Petri dishes containing commercial potato dextrose agar (PDA) medium and grown for 1 week under a 16 h photoperiod under fluorescent light at 20 °C ± 2 °C. The isolates used were collected the previous year from surveys conducted in Alberta and Saskatchewan. After 7 days, conidia were harvested from each Petri dish as described above. Approximately 12 pea seeds were sown into each of 44 15-cm-diameter pots containing soil-less mix (Premier Horticultural Canada Inc., Riviere-de-Loupe, QC), which were placed in a growth chamber with a 16 h photoperiod at 15 °C ± 2°C. After emergence, the seedlings were thinned to 10 plants per pot. Plants were sprayed with the recommended rate of pyraclostrobin (0.04 mL per 10 mL water) at 21 d after planting and inoculated 1 d later by spraying plants with a spore suspension of 2×10^5 spores mL^{-1} until run-off. A hand-held spray bottle containing a pre-determined amount of water (enough to cover the leaves and stems until run-off) was used for both fungicide application and inoculation. After inoculation, the pots were transferred into a clear plastic humidity chamber and maintained at high relative humidity for 48 h with a 16 h photoperiod at 15 °C ± 2°C. After removal from the

humidity chamber, the plants were returned to the growth chamber where the humidity was kept as high as possible with the use of a cool air electrostatic humidifier. Each plant was rated for lesions of *M. pinodes* at 7, 14 and 21 d after inoculation using the scale developed by Xue et al. (1996). Yield data were not collected.

3.2.3. Statistical analysis

All of the statistical analyses were conducted using the mixed model analysis of variance (PROC MIXED) in SAS 9.2 (SAS Institute Inc., Cary, NC), and differences were considered significant at $P \leq 0.05$. Residuals were tested for normality using PROC UNIVARIATE (Shapiro-Wilk test), and homogeneity prior to analysis. Minor heterogeneous variances were modelled using the mixed procedure in SAS (Gomez and Gomez, 1984). Models were structured to look only for differences among treatments and did not consider treatment differences as they pertained to individual locations. Replication, year and location, as well as interactions with fixed effects were considered random terms in the model. Application treatment, cultivar and interactions between the two were considered fixed terms.

Each field and growth chamber trial was laid out as an RCBD with four replications. As a result of the variation in treatments and cultivars over the years of the trials, the data were initially analyzed within site year. Data were

pooled for analysis across locations and years whenever treatment structure was similar and statistical analysis was valid. The variation in the number of treatments in each of the trials made it inappropriate to combine data from all of the treatments across all of the sites, since blocks of different sizes would have different inter- and intra-block variation, affecting proper allocation of error in PROC MIXED analysis. Similar treatments were pooled across all years and locations where possible and random error could be properly allocated. In these analyses, treatments were examined in a combined analysis across all of the site years; the effect of fungicide application was compared with the untreated control using a single degree of freedom orthogonal contrast. Where this contrast was significant in the nozzle orientation study, treatments 2–5 were analyzed as a factorial design with nozzle number (single vs. double) and droplet quality (spray vs. coarse) as the fixed effects. Similarly, sites that included treatments 8–11 were combined for locations where fungicide reduced blight severity and analysed as a factorial design with nozzle number, droplet quality and orientation (vertical vs. high angle) as the fixed effects. In the carrier volume and nozzle configuration trials, treatments 2-11 were analysed as a factorial design with nozzle number (single vs. double) and water volume (50–800 L ha^{-1}) as the fixed effects.

Plant density data at seedling establishment was assessed to verify that the stands were uniform and that differences among treatments were not confounded by plant population. Initial analyses of mycosphaerella blight

severity and seed yield were tested for differences among fungicide application treatments with different nozzle configurations and water volumes. The treatment means were then compared using the differences between least square means method (Steel et al., 1997). Single degree of freedom contrasts (although not always orthoganol) were used to compare the seed yield of each treatment to the control. Pre-determined contrasts were used to identify differences among logically corresponding treatments for seed yield. The pre-determined contrasts for the nozzle configuration trials were: each treatment compared to the control, single nozzle-fine droplet size compared to double nozzles-fine droplet size, single nozzle-coarse droplet size compared to double nozzles-coarse droplet size, double nozzles-fine droplet size compared to double nozzles with both fine and coarse droplet sizes (one of each), double nozzles-coarse droplet size compared to double nozzles-both fine and coarse droplet sizes, single nozzle-fine droplet size compared to single nozzle-fine droplet size at low carrier volume, single nozzle-fine droplet size compared to single nozzle-fine droplet size at high angle, single nozzle-coarse droplet size compared to single nozzle-coarse droplet size at high angle, double nozzles-fine droplet size compared to double nozzles-fine droplet size at high angle, and double nozzles-coarse droplet size compared to double nozzles-coarse droplet size at high angle. The pre-determined contrasts for the carrier volume and nozzle configuration trials were: each individual treatment compared to untreated control, and each

of the single nozzle treatments at a specified carrier volume compared to the double nozzle treatment at the same carrier volume.

The data from the carrier volume trials and growth chamber trials were tested for first and second degree polynomial response (linear and quadratic, respectively) for disease ratings and yield (carrier volume trials only) using orthogonal polynomial coefficient contrast (Steel et al. 1997) to assess the response to application rates.

3.3. RESULTS

3.3.1. Field Studies

Effect of nozzle configurations and angles on fungicide delivery

In the trials at Morden using the pea 'Topper', there were no differences in 2008 and 2010, fungicide application did not affect disease severity and there were no significant differences in yield. Growing conditions in 2008 were abnormally dry, and although mycosphaerella blight was present, it did not reach levels severe enough to cause treatment differences (Appendix, Table A3-1). In 2009, despite dry conditions, there were differences among treatments with the double nozzle system. The double nozzle - fine spray treatments resulted in the lowest blight severity, which was reflected in seed yield. The untreated control treatment yielded 4505 kg ha^{-1}, which was significantly lower than the double nozzle – fine spray (5588kg ha^{-1}), double nozzle – coarse spray (5415 kg ha^{-1}), and

double nozzle – both fine and coarse spray (5487 kg ha^{-1}) treatments (Table 3-3; Appendix Table A3-2). The double nozzles - coarse spray treatment also yielded significantly higher than the single nozzle – coarse spray (4678 kg ha^{-1}) treatment (Table 3-3). In 2010, blight severity was very high as a result of very wet conditions and differences between treatments for yield were small and not statistically significant (Table 3-4). As in 2009, the lowest final disease ratings were observed on the double nozzle – fine spray treatment (Table 3-4). When all three years at the Morden site were combined, the double nozzle treatment combining a fine and coarse spray nozzle was the only significantly different treatment, and only when compared to the untreated control.

In 2008 at Saskatoon, as at Morden, conditions were dry and not conducive for disease establishment and spread. Using the pea 'Delta' mycosphaerella blight was most severe in the untreated control and lowest in the treatments containing a double nozzle with a fine spray (Appendix, Table A3-3). Yield was lowest in the control plot (1902 kg ha^{-1}), and the double nozzle with a fine spray was significantly higher than the control (2150 kg ha^{-1}), as was the single nozzle treatment with a fine spray (2221 kg ha^{-1}) (Table 3-3). In 2009, there were three trials conducted under dry conditions. The pea 'CDC Montero' was seeded at two sites (Site 1 and Site 2) and 'Nitouche' was seeded at Site 1. In the trials where 'CDC Montero' was used, the double nozzle – fine spray and double nozzle - coarse spray treatments had lower final mycosphaerella blight ratings than the other treatments, except for the untreated control (Table 3-5), but there were

no significant differences in yield. In the trial with 'Nitouche', there was a decrease in mycosphaerella blight severity and a significant increase in yield for the double nozzle – coarse spray treatment at 4057 kg ha^{-1} compared to the untreated control at 3600 kg ha^{-1} (Table 3-3; Appendix, Table A3-4). A single nozzle using a fine spray (4005 kg ha^{-1}) and double nozzles – combining a fine and a coarse spray (3899 kg ha^{-1}) were both more effective than double nozzles with a fine spray (3530 kg ha^{-1}) for both disease severity and yield (Table 3-3). The two trials conducted at the same site, one with 'CDC Montero' and the other with 'Nitouche', were pooled and analyzed together. There was a significant cultivar effect, no significant sprayer application treatment effect and no interaction between the two variables (Table 3-6). The results of pre-determined contrasts supported the findings of the trial with 'Nitouche', where the double nozzle – coarse spray treatment was more effective than the untreated control, but in addition, the former treatment was also more effective than the single nozzle – coarse spray treatment (Appendix, Table A3-5).

In 2011 at Saskatoon, conditions were quite wet and mycosphaerella blight severity was moderate to high throughout the season. A trial using 'Topper' was conducted with a modified treatment structure that added a high angle (60°) to the existing single and double, coarse and fine spray treatments. Disease ratings were lowest in the double nozzle – coarse spray with a high angle treatment, but differences between treatments were small and not statistically significant for disease severity and seed yield (Appendix, Table A3-6).

In 2009 at Edmonton, conditions were quite dry, which resulted in low disease severity. Consequently, no differences were observed between treatments for disease severity or yield in the trial with 'Carneval'. In the trial with 'Topper', however, significant differences were found for both disease severity and yield. The double nozzle- fine spray - high angle treatment had the lowest level of disease of all treatments, particularly the untreated control (Appendix, Table A3-7). With respect to yield, there were significant differences between the single nozzle – fine spray (2064 kg ha^{-1}), double nozzle – fine spray (2165 kg ha^{-1}), single nozzle – fine spray with a high angle (2116 kg ha^{-1}), single nozzle – coarse spray with a high angle (2100 kg ha^{-1}), double nozzle – fine spray with a high angle (2066 kg ha^{-1}) and double nozzle – coarse spray with a high angle (2199 kg ha^{-1}) when each was compared to the untreated control at 1745 kg ha^{-1} (Table 3-3). The results of the trial with 'Topper' indicate that increasing the angle of the spray increased the effectiveness of the fungicide. The trials were combined for analysis to compare the cultivars and the results confirmed the findings that the double nozzle – fine spray treatment increased yield when compared to the control. There was a significant effect of cultivar but no interaction between cultivar and treatment (Table 3-7). This indicates that increasing the angle of fungicide application may be especially effective on a plant stand that has a tendency to lodge.

In 2009 and 2011 at Lacombe, there were no significant differences in mycosphaerella blight severity or yield in the trial using the pea 'Carneval'.

Similar results were obtained with this cultivar in Edmonton under the same dry conditions in 2009. The environmental conditions in 2011, however, were substantially different. Despite the large amount of moisture after planting, conditions became drier and there were no differences in disease severity or yield under moderate disease pressure at Lacombe. As was observed in Edmonton, significant differences were found in both years (2009 and 2011) in the trials using 'Topper'. Mycosphaerella blight severity was lower for all treatments in 2009 when compared to the control, with the lowest ratings being in the double nozzle – fine spray – high angle treatment (Table 3-8). There were significant differences between the untreated control and all other treatments for yield in both years, except for the double nozzles – coarse spray in 2011 (Table 3-8; Table 3-3; Appendix, Table A3-8). The Lacombe data obtained with 'Topper' were combined across both years for analysis to compare growing conditions under contrasting disease pressures. When combined, all treatments were significantly better than the control (Figure 2-1). Contrary to the results observed at Edmonton, the use of higher angles on the spray nozzle combinations did not improve fungicide efficacy at this site. Treatments were not significantly different than the same treatment applied at the lower angle (Table 3-9). The data from the trials at Lacombe in 2009 were combined with the data from the trials at Edmonton (in the same year) and analyzed to determine the effect of cultivar. The results confirmed the observations of the individual site years. The effect of treatment was not significant, but the effect of cultivar

was significant with no interaction between the two (Table 3-10). Once again, this suggests that cultivar reaction to fungicide application was associated with the tendency of the plant stand to lodge.

The factorial analysis of pooled selected sites resulted in no significant difference among treatments. There was no treatment differences found for nozzle number, spray quality, or angle of spray application. In addition, there were no significant interactions found between any of the techniques tested. This result indicates that combining individual spray application techniques does not result in a favorable response in disease severity or yield (Table 3-11).

Effect of water volume and nozzle configuration on fungicide delivery

In 2010 at Lacombe, the environmental conditions were very wet and differences were observed among treatments for mycosphaerella blight severity and yield. The lowest disease ratings were observed in the double nozzle – 200 L ha^{-1} treatment, which resulted in differences in final yield (Table 3-12). When compared to the untreated control (1021 kg ha^{-1}), yield for the single nozzle – 100 L ha^{-1} (1705 kg ha^{-1}), single nozzle – 400 L ha^{-1} (1551 kg ha^{-1}) and double nozzle – 200 L ha^{-1} (1450 kg ha^{-1}) treatments were significantly higher (Table 3-13). There were no other significant differences among any of the treatments. The double nozzles showed a 2nd degree polynomial (quadratic) response to increasing carrier volumes, where treatment yields increased up to the 200 L ha^{-1} rate and then decreased for the 400 L ha^{-1} rate and 800 L ha^{-1} rate (Figure 2-2).

In 2011, environmental conditions in Lacombe were again quite wet. The only treatment where significant differences in yield were detected was the single nozzle – 800 L ha^{-1} (3457 kg ha^{-1}) treatment (Appendix, Table A3-9), which was significantly higher than the untreated control (2323 kg ha^{-1}) (Table 3-13). No differences were observed in disease severity and no polynomial trends were detected in the data. When the data for the two years of trials in Lacombe were combined, the results were similar to the results from the individual years. The single nozzle - 800 L ha^{-1} treatment yielded significantly higher than the untreated control, as was observed in 2011 (Table 3-13). The double nozzles once again showed a quadratic response to increasing volumes, where treatment yields increased between the 50 and 100 L ha^{-1} rate, decreased slightly, levelled off between 100 and 400 L ha^{-1} and then decreased substantially at the 800 L ha^{-1} rate (Figure 3-3).

At the trials at Saskatoon in 2010, as in Lacombe, the environmental conditions were wet and mycosphaerella blight severity was moderate to high early in the year. However, unlike Lacombe, conditions became drier later in the season and the disease did not progress. There were no differences between any of the treatments for *M. pinodes* severity, and no significant differences between treatments for yield. In 2011, despite wet conditions during seeding of the trial, conditions were dry during crop establishment from pre-bloom to harvest. As a result, disease pressure was very low and there were no differences observed

between treatments for disease severity and no significant differences between treatments for yield (Appendix, Table A3-10).

The factorial analysis of pooled selected sites resulted in no significant difference among treatments. There were no treatment differences found for nozzle number or water carrier volume. In addition, there were no significant interactions found between the two techniques tested. This result indicates that combining nozzle number with carrier increasing water volumes does not result in a favorable response with respect to disease severity or yield (Table 3-14).

3.3.2. Growth Chamber Study

The trial conducted in the growth chamber at Lacombe revealed significant differences among the treatments for mycosphaerella severity. When tested for a polynomial response to increasing water volume rates, both single and double nozzles showed a negative quadratic response to increasing volumes, where disease severity decreased up to the 200 L ha^{-1} rate and then increased for the 400 L ha^{-1} rate and 800 L ha^{-1} rate (Figure 2-4).

3.4. DISCUSSION

Collectively, the current studies examined 11 different nozzle combinations, using three different angles, over 4 years on six cultivars, resulting in 17 station years of data. As is often the case with large data sets such as this, the variation among all of the locations, years, treatments and cultivars made it difficult to identify the most effective treatment for the application of pyraclostrobin

fungicide on field pea. To limit conflicting results and test for statistical differences, the statistical significance level chosen for data analysis was set at $P \leq 0.05$. Since the changes in fungicide application techniques would involve only small additional costs to producers, the economic feasibility of changing producer practices and investing in new sprayer techniques was considered when presenting the results of these studies.

The majority of sprayer systems on commercial farms utilize single hydraulic nozzles that are usually either a flat-fan or hollow-cone type. Fungicides on commercial farms are often applied at the minimum possible water volume. A tapered flat-fan nozzle design is most common because it provides a uniform spray pattern and minimum spray drift (Gossen et al. 2008; Elliot et al. 1997). Most agrochemical systems are set up to deliver herbicides to the crop. The focus when using herbicide systems is to get good coverage of horizontal surfaces at the top of the canopy, and is effective for systemic herbicides that are applied to small plants. However, fungicidal products are most effective when delivered throughout the entire canopy rather than just on the horizontal surfaces at the top. For many farm operations, pesticides, including fungicides, constitute a significant portion of their variable input costs. If it were possible to modify spray application technology to reduce this expenditure, it could significantly change the economics of farming for many operations while reducing the potential for detrimental environmental impacts of pesticide use. Fungicides are very important in crop disease management and represent about

21% of pesticide use in the world (Steurbaut, 1993). It is important to apply these products to protect the crops, but it is also important to use the appropriate amount of active ingredient to apply them efficiently, safely and economically.

The double nozzle system involves two nozzles working together. The nozzle combinations may vary to include one nozzle delivering water directly down with a second nozzle spraying at an angle, both nozzles spraying vertically, or both nozzles spraying at an angle (one pointed forward and one backward). Within each of these combinations, fine and coarse nozzles can be incorporated depending on the desired effect. The active ingredient can be carried by either of the nozzles or both, depending on applicator preference. Downer (2009) found that when nozzle qualities are combined, the results are more effective if the fine spray nozzle contains the active ingredient and delivers it into the water spray cloud. The trials conducted in this study combined single and double nozzles with coarse and fine droplet sizes. In 10 of the 13 nozzle combination trials conducted, configurations containing double nozzles consistently gave 15% better control of mycosphaerella blight than the untreated control (Tables 2-4, 2-5, 2-8, 2-12). The impact of single nozzle treatments was generally intermediate, but often not significantly different from the control. The exceptions were in 2009 at Saskatoon on 'Montero' and at Lacombe and Edmonton on 'Carneval' where single nozzles had a positive impact on yield. In many cases, the differences in disease severity were small and did not affect seed yield. However,

at all four locations in 2009 and at Lacombe in 2011, treatments containing double nozzles were significantly higher in yield than the untreated control (Table 3-3). Across the 17 station years assessed and compared, there were three instances where significant differences occurred between treatments when the control was removed. Two of those exceptions involved the use of double nozzles versus a single nozzle and occurred in 2009 at Morden on 'Topper' and in the same year at Saskatoon on 'Nitouche'. In Morden, double nozzles with a coarse spray were significantly better than a single nozzle with a coarse spray, and in Saskatoon, double nozzles with a fine spray were significantly better than single nozzles with a fine spray (Table 3-3). In both instances, the double nozzle treatment significantly increased yield when compared to the single nozzle treatment with the same droplet size. In the trials where single and double nozzles were combined with water volume, there were no differences between the single nozzles and double nozzles in any of the trials, regardless of volume being applied, and there was no interaction between them. Hall et al. (1996) reported that the use of double nozzles instead of a single nozzle for spray coverage improved the spray pattern for finer sprays, provided greater pesticide efficacy, and reduced the dilution of the product on the leaf surface. In a recent report, double nozzles combined with coarse sprays and lower boom height provided better coverage for fungicides on canola and wheat crops (Wolf and Dietz, 2013). The present study indicates that a double nozzle configuration may be beneficial if it could be incorporated into fungicide sprayer

application methodology on commercial farms. The drawback would be the minimal financial cost, compared to other farm purchases, of investing in new sprayer techniques, but that would be offset by the yield increases over the following years.

Droplet size is one of the most important components of fungicide application. There are benefits to the use of both small and large droplets in a spray application system depending on the target crop, pesticide being applied, environmental conditions and other influences. In general, the smaller the droplet size, the better leaf coverage and retention that can be expected. Using a finer spray quality allows for a greater number of smaller droplets, which are more easily carried by the air flow of the sprayer, so smaller droplets give greater coverage and because of their size are less likely to drip off. It has been demonstrated with both herbicides and fungicides that smaller droplets give better efficacy than larger droplets (Knoche, 1994). The smaller droplets are easily caught by the leaves at the top of the canopy and are likely to stay in that place unless either the surface is accelerated strongly, causing the droplet to be thrown off, or a large number of droplets impact in the same area and the surface becomes so saturated that run-off occurs (Cross et al. 2001). Therefore, the movement of small droplets is largely dependent on meteorological conditions and the plant canopy itself. However, small droplets do not penetrate the canopy as well and are readily displaced by wind, constituting the majority of off-target drift (Spillman, 1984; Wolf et al., 1993; Wilson 2007). Smaller droplets

also mean faster evaporation rates. When spraying in crops, penetration into the canopy is increased. In contrast, large droplets are more likely to get caught near the top of the canopy, penetrate better into the lower parts of the crop and be more readily absorbed (Feng et al., 2003).

There are many factors affecting the efficacy of droplet size, making it difficult to make recommendations as to which size is more effective. Producers are always interested in spraying techniques that will improve the productivity of the farm. Some data show larger droplets to be more effective whereas other data show smaller droplets to be superior. For each spray system and target crop, this will depend on the desired outcome. In the present study, because the target pathogen initiates infection of the crop in the lowest portion of the crop canopy, penetration into the bottom areas of the canopy was important. The droplet size that effectively accomplished that goal was investigated by measuring disease severity and seed yield, but the use of fine or coarse spray nozzles did not result in significant differences among treatments. Consistently across all of the station years, both fine and coarse nozzle treatments resulted in disease reduction when compared to the control. (Table 3-3; Table 3-9; Figure 2-1). There were no consistent trends for spray quality efficacy. These findings are similar to results from research trials conducted by Armstrong-Cho et al. (2008) with *A. rabiei* on chickpea and Kutcher and Wolf (2006) with *Sclerotinia sclerotiorum* (Lib.) de Bary on canola, where spray quality had no effect on disease levels. Most reports are consistent in that penetration into the canopy

and a high level of spray coverage on the leaves tend to be the most important factors. Larger droplets penetrate the canopy better, are more readily absorbed, have a significant positive impact on disease levels and seem to be more effective overall because of the lower risk of drift (Feng et al. 2003; Maybank et al. 1991).

Initial results in 2009 at Morden and Edmonton, under dry conditions, indicated that higher nozzle orientation angles of 60° decreased mycosphaerella blight severity and improved yield (Table 3-3), particularly on 'Topper' that has a tendency to lodge. The use of the nozzle combinations containing a nozzle that sprayed the crop from a more horizontal angle appeared to have improved results over the same nozzle combination at the lower angle. However, the data from Lacombe and Saskatoon in the same year (2009), as well as in those locations in 2011, when conditions were wetter, did not support that result. Applications at the lower angles were just as effective as the applications at the higher angles (Table 3-8). When the 2009-2011 Lacombe data were pooled for analysis, the double nozzles producing a fine droplet size at a higher angle increased yield relative to the lower angle, but the difference was not significant (Table 3-9). In 2011, double nozzles at a high angle effectively reduced disease severity but differences were not reflected in final yield. Previous studies indicate that at least one nozzle should be placed at an angle when fungicides are being applied because canopy penetration is best with that orientation (Wolf 2009; Wolf and Dietz 2013). However, the current study does not support that

result. Based on the results of the present study, it is not clear as to which nozzle orientation would provide the best management. These data indicate that increasing angles on sprayer equipment does not improve management of *M. pinodes* on field pea. Vertical applications appear to be just as effective as angled applications and other factors such as disease pressure and cultivar characteristics seem to be more important to fungicide efficacy for management of mycosphaerella blight.

In contrast to the results on chickpea (Armstrong-Cho et al. 2008), cultivar had a significant effect on spray efficacy in the current study. Six different field pea cultivars were assessed in this study to compare differences between individual cultivar characteristics, particularly the cultivar's tendency to lodge. When data sets were combined relative to cultivar and compared, cultivar was always highly significantly different, even though treatments were not (Table 3-6; Table 3-7; Table 3-10). Similar results were seen at Saskatoon in 2009 with 'CDC Montero' and 'Nitouche' as were seen at Lacombe and Edmonton with 'Carneval' and 'Topper' in 2009. Consistently, when the same treatments were applied in the same year under the same conditions, 'Carneval' did not show any differences between treatments whereas 'Topper' consistently did. The lack of interaction between the treatments and cultivars shows that the differences were because of cultivar regardless of the treatment applied. 'Carneval' has a good resistance to *M. pinodes* and resists lodging, which may explain why in these studies there were no differences seen between the treatments in any

year. ‘CDC Montero’, ‘Delta’ and ‘Nitouche’ all have fair resistance to the pathogen, which explains why differences were sometimes seen in station years but not consistently throughout the study. Fungicide efficacy depended more on environmental conditions than nozzle combinations or volumes. ‘Topper’ has poor resistance to *M. pinodes* and therefore treatment differences were generally seen. The exception was under dry conditions where differences were not observed because of low mycosphaerella blight severity, such as at Morden in 2008 and Saskatoon in 2011. Plant architecture or position relative to the application of fungicide appeared to play an important role in these studies. With a cultivar such as ‘Topper’, penetration into the canopy is not the most critical factor. The cultivar has long stems and tendrils, lodges easily, stays wet longer and therefore, leaf coverage at lower levels of a standing canopy is not as important. There are no cultivars completely resistant to *M. pinodes*, but cultivar selections made based on reduced susceptibility and better lodging resistance may influence the need to adjust nozzle combinations for best efficacy in pyraclostrobin fungicide applications in field pea. A plant that is lying on the ground, or has a greater amount of horizontal surface may be easier to target than a standing crop. This is important, as some cultivars tend to lodge more than others, but in general, all pea crops lodge to some extent. The negative impacts of lodging on crop yields is exacerbated by *M. pinodes* infection, making disease management even more important, especially in wet years or when growing a cultivar that has only a fair to moderate lodging resistance.

Under low to moderate disease pressure at Saskatoon in 2010 and 2011, water carrier volume for pyraclostrobin fungicide did not affect the level of mycosphaerella blight or impact the yield of field pea. In contrast, at Lacombe under higher disease pressure, increasing the carrier volume up to 200 L ha^{-1} reduced disease severity and increased yield (Tables 2-12, 2-13; Figure 2-3). However, larger volumes (up to 800 L ha^{-1}) had the opposite effect, progressively increasing severity and reducing yield. The results of the present study are consistent with previous reports by Cross et al. (2001) and Armstrong et al. (2008), which demonstrated that an increase in fungicide carrier volume up to a certain level significantly reduced the disease severity under moderate to high disease pressure. Increasing the water volumes used in sprayer systems improves penetration into the crop and increases the frequency of droplets at all levels of the canopy. Larger volumes have the added benefit of decreasing the potential for drift and increasing nozzle performance (Wirth, 1991). Most research indicates that both fungicides and herbicides work best with higher carrier volumes (Wolf et al., 1993). While weeds can be a problem in a wide range of crops, fungal diseases tend to be crop or cultivar specific. Fungicide formulations coupled with carrier volumes often must be customized to obtain the desired results (Steurbaut, 1993). If water volumes are not high enough to maintain adequate droplet densities, aspects of spray targeting may be compromised (Jensen et al., 2001). Some fungicides will work effectively on

crops using lower volumes, but it appears that with pyraclostrobin on pulse crops, higher volumes are required, as long as they are not too high.

A large number of sprayer technology studies have been conducted to understand the processes involved in fungicide application, from formulation right through to fungicidal mode of action. There are numerous factors involved in optimizing the application of fungicidal products, since complicated systems are often involved, but advances have been made in understanding the basis for effective delivery of these compounds. There are many things that can be done to improve fungicide application efficacy for management of mycosphaerella blight in field pea. The results of this study show that double nozzles may provide an advantage, but the trend indicates that as long as application rates are not too low (below 100 L ha^{-1}) there should be effective management resulting in economic benefit. More important is applying the correct fungicide at the correct timing and rate under the correct conditions, to achieve optimal disease management. Ultimately, superior fungicide application still depends on the knowledge, care and judgment of the sprayer operator.

3.5. LITERATURE CITED

Ali-Khan, S.T. 1991. Topper field pea. Can. J. Plant Sci. 70: 189−190.

Armstrong-Cho, C., Chongo, G., Wolf, T., Hogg, T., Johnson, E., and Banniza, S. 2008a. The effect of spray quality on ascochyta blight control in chickpea. Crop Prot. 27: 700–709.

Armstrong-Cho, C., Chongo, G., Wolf, T., Hogg, T., Johnson, E., and Banniza, S. 2008b. The effect of carrier volume on ascochyta blight (*Ascochyta rabiei*) control in chickpea. Crop Prot. 27: 1020−1030.

Banniza, S. and Vandenberg, A. 2003. The influence of plant injury on development of *Mycosphaerella pinodes* in field pea. Can. J. Plant Pathol. 25:304−311.

Bateman, R. 1993. Simple, standardized methods for recording droplet measurements and estimation of deposits from controlled droplet applications. Crop Prot. 12: 201–206.

Beasse, C., Ney, B., and Tivoli, B. 2000. A simple model of pea (*Pisum sativum*) growth affected by *Mycosphaerella pinodes*. Plant Pathol. 49: 187–200.

Bouse L.F., Carlton, J.B. and Merkle, M.G. 1976. Spray recovery from nozzles designed to reduce drift. Weed Sci. 24: 361−365.

Bretag, T.W., Keane, P.J., and Price, T.V. 2006. The epidemiology of ascochyta blight in field peas: A review. Aust. J. Agric. Res. 57: 883−902.

Chapple, A.C., Wolf, T.M., Downer, R.A., Taylor, R.A.J., and Hall, F.R. 1997. Use of nozzle-induced air-entrainment to reduce active ingredient requirements for pest control. Crop Prot. 16: 323–330.

Chongo, G. and Gossen, B.D. 2003. Diseases of pea. Pages 201-213 in K. L. Bailey, B. D. Gossen, R. Gugel, and R.A.A. Morrall, eds. Diseases of field crops in Canada. Canadian Phytopathological Society, Saskatoon, SK. 290 pp.

Cross, J.V., Walklate, P.J., Murray, R.A., and Richardson, G.M. 2001. Spray deposits and losses in different sized apple trees from an axial fan orchard sprayer. 2. Effects of spray quality. Crop Prot. 20: 333–343.

Davidson, J.A., and Kimber, R.B. 2007. Integrated disease management of ascochyta blight in pulse crops. Eur. J. Plant Pathol. 119: 99–110.

Downer, R.A. 2009. The double nozzle sprayer. Laboratory for Pest Control Application Technology. [On-line] Available: www. ordc.ohio-state.edu/LPCAT/default.htm#transfer [accessed December 4 2010].

Ebert, T.A., Taylor, R.A.J., Downer, R.A., and Hall, F.R. 1999. Deposit structure and efficacy of pesticide application. 1: Interactions between deposit size, toxicant concentration and deposit number. Pestic. Sci. 55: 783−792.

Edgington, L.V. 1981. Structural requirements of systemic fungicides. Ann. Rev. Phytopathol. 19: 107–124.

Elliott, R.H., and Mann L.W. 1997. Control of wheat midge, *Sitodiplosis mosellana* (Gehin), at lower chemical rates with small-capacity sprayer nozzles. Crop Prot. 16: 235–242.

Feng, P.C.C., Chiu, T., Sammons, R.D., and Ryerse, J.S. 2003. Droplet size affects glyphosate retention, absorption, and translocation in corn. Weed Sci. 51: 443–448.

Gomez, K.A. and Gomez, A.A. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley and Sons. Pg. 298−306.

Gossen, B.D., Peng, G., Wolf, T.M., and McDonald, M.R. 2008. Improving spray retention to enhance the efficacy of foliar-applied disease and pest management products in field and row crops. Can. J. Plant Pathol. 30: 505−516.

Grover, R., Maybank, J., Caldwell, B.C., and Wolf, T.M. 1997. Airborne off-target losses and deposition characteristics from a self-propelled, high speed and high clearance ground sprayer. Can. J. Plant Sci. 77: 493–500.

Hall, F.R., Chapple, A.C., Downer, R.A., Kirchner, L.M., and Thacker, J.R.M. 1993. Pesticide application as affected by spray modifiers. Pest. Sci. 38: 123−133.

Hall, F.R., Downer, R.A., Wolf, T.M., and Chapple, A.C. 1996. The "double nozzle"– a new way of reducing drift and improving dose transfer? *In* Pesticide formulations and application system–16th Symposium. *Edited by*

M.J. Hopkinson, H.M. Collins, and R. Goss. Amer. Soc. Testing Materials. Philadephia, PA. pp. 114–125.

Jensen, P.K., Jorgensen, L.N., and Kirknel, E. 2001. Biological efficacy of herbicides and fungicides applied with low-drift and twin-fluid nozzles. Crop Prot. 20: 57–64.

Karadimos, D.A., Karaoglanidis, G.S., and Tzavella-Klonari, K. 2005. Biological activity and physical modes of action of the Q_o inhibitor fungicides trifloxystrobin and pyraclostrobin against *Cercospora beticola*. Crop Prot. 24: 23–29.

Knoche, M. 1994. Effect of droplet size and carrier volume on performance of foliage-applied herbicides. Crop Prot. 13: 163–178.

Kromann, P., Leon, D., Taipe, A., Andrade-Piedra, J.L., and Forbes, G.A. 2008. Comparison of two strategies for use of translaminar and contact fungicide in the control of potato late blight in the highland tropics of Ecuador. Crop Prot. 27: 1098–1104.

Kutcher, H.R., and Wolf, T.M. 2006. Low-drift fungicide application technology for sclerotinia stem rot control in canola. Crop Prot. 25: 640–646.

Maybank, J., Shewchuk, S.R., and Wallace, K. 1991. The use of shielded nozzles to reduce off target herbicide spray drift. Can. Agric. Eng. 32: 235−241.

Peairs, F.B., Bean, B., and Gossen, B.D. 2005. Pest management implications of reduced fallow periods in dryland cropping systems in the Great Plains. Agron. J. 97: 373–377.

SAS Institute Inc. 2011. Cary, N.C: SAS Institute Inc. [Online]. Available: http://support.sas.com/documentation/ [accessed April 2013].

Spillman, J.J. 1984. Spray impaction, retention and adhesion: An introduction to basic characteristics. Pestic. Sci. 15: 97–106.

Statistics Canada. 2013. [On-line]. Available: http://www5.statcan.gc.ca/cansim/a26?lang=eng&retrLang=eng&id=0010010&tabMode=dataTable&srchLan=-1&p1=-1&p2=9.

Steel, R.D.G., Torrie, J.H., and Dickey, D.A. 1997. Principles and procedures of statistics: A biometrical approach. 3rd ed. WCB McGraw-Hill. Boston, MA.

Steurbaut, W. 1993. Adjuvants for use with foliar fungicides. Pestic. Sci. 38: 85–91.

Warkentin, T.D., Xue, A.G., and McAndrew, D.W. 2000. Effect of mancozeb on the control of mycosphaerella blight of field pea. Can. J. Plant Sci. 80: 403−406.

Wilson, J. 2007. Pesticide drift. South Dakota State University publication. Brookings, SD.

Wirth, W., Storps, S., and Jacobsen, W. 1991. Mechanisms controlling leaf retention of agricultural spray conditions. Pestic. Sci. 33: 411–420.

Wolf, T.M. 2009. Pesticide application and choosing the right nozzles. FactsSheet. Saskatchewan Ministry of Agriculture. [On-line] Available: www.agriculture.sk.ca. [accessed November 22 2012].

Wolf, T.M., and Dietz, J. 2013. Asymmetric nozzles better for fungicides. *In* Top Crop Manager. Western edition. March. Pg. 34−41.

Wolf, T.M., Grover, R., Wallace, K., Shewchuk, S.R., and Maybank, J. 1993. Effect of protective shields on drift and deposition characteristics of field sprayers. Can J. Plant Sci. 73: 1261–1273.

Xue. A.G., Warkentin, T.D., Greeiaus, M.T. and Zimmer, R.C. 1996. Genotypic variability in seedborne infection of field pea by *Mycosphaerella pinodes* and its relation to foliar disease severity. Can. J. Plant Path. 18: 370–374.

Xue A.G., Charest, J., Davidson, C.G., McAndrew, D.W., Bing, D.J. and Warkentin, T.D. 2003. Response of field pea cultivars to chlorothalonil in the control of mycosphaerella blight. Can. J. Plant Sci. 83: 313–318.

Yang, R.C. 2012. Statistician. Alberta Agriculture and Rural Development, University of Alberta, Edmonton, Alberta.

Zhang, R., and Gossen, B.D. 2007. Heritability estimates and response to selection for resistance to mycosphaerella blight in pea. Crop Sci. 47: 2303–2307.

3.6. TABLES AND FIGURES

Table 3-1. List of nozzle treatments used to apply pyraclostrobin fungicide to pea in field trials at Morden, MB, Saskatoon, SK, Lacombe, AB and Edmonton, AB, from 2008 to 2011.

Trt #[*]	Nozzle	Spray	Angle	Type 1[**]	Type 2[**]	Comment
1	None	.	.	.	.	
2	Single	fine	vertical	ER8002	.	
3	Single	coarse	vertical	MR8002	.	
4	Double	fine	vertical	ER8001	ER8001	
5	Double	coarse	vertical	DR8001	DR8001	
6	Double	fine/coarse	both	ER8001	DR8001	vertical coarse spray, 60° fine spray
7	Single	fine	vertical	ER8001	.	applied at 100 L ha^{-1}
8	Single	fine	high	ER8002	.	60° angle
9	Single	coarse	high	MR8002	.	60° angle
10	Double	fine	high	ER8001	ER8001	60° angle
11	Double	coarse	high	DR8001	DR8001	60° angle

[*]Trt# = treatment. 1 = untreated control; 2 = a single nozzle, producing a fine spray, mounted in a vertical position; 3 = a single nozzle, producing a coarse spray, mounted in a vertical position; 4 = double nozzles, producing a fine spray, mounted in a vertical position; 5 = double nozzles, producing a coarse spray, mounted in a vertical position; 6 = double nozzles, vertical coarse spray, 60° fine spray; 7 = single nozzle, fine spray applied with a water volume of 100 L ha-1; 8 = a single nozzle, producing a fine spray, mounted at a high angle; 9 = single nozzle, coarse spray, high angle; 10 = double nozzles, fine spray, high angle, and 11 = double nozzles, coarse spray, high angle.

[**]ComboJet ™ nozzles - ER (extended range), MR (mid-range) and DR (drift reduction) with a flat fan pattern spraying at an 80° angle from the nozzle tip. Nozzles ending in -02 have higher output than nozzles ending in -01.

Table 3-2. List of nozzle treatments and carrier volumes used to apply pyraclostrobin fungicide in field and growth chamber trials at Saskatoon, SK, and Lacombe, AB, in 2010 and 2011.

Trt #[*]	Nozzle	Volume (L ha^{-1})	Type 1[**]	Type 2[**]
1	None	.	-	-
2	Single	50	ER8002	-
3	Single	100	ER8002	-
4	Single	200	ER8004	-
5	Single	400	ER8008	-
6	Single	800	ER8025	-
7	Double	50	ER8001	ER8001
8	Double	100	ER8001	ER8001
9	Double	200	ER8002	ER8002
10	Double	400	ER8004	ER8004
11	Double	800	ER8015	ER8015

[*] Trt# = treatment. 1 = untreated control; 2 = a single nozzle with a 50 L ha^{-1} output; 3 = a single nozzle with a 100 L ha^{-1} output; 4 = a single nozzle with a 200 L ha^{-1} output; 5 = a single nozzle with a 400 L ha^{-1} output; 6 = a single nozzle with a 800 L ha^{-1} output; 7 = double nozzles with a 50 L ha^{-1} output; 8 = double nozzles with a 100 L ha^{-1} output; 9) double nozzles with a 200 L ha^{-1} output; 10) double nozzles with a 400 L ha^{-1} output; and 11) double nozzles with a 800 L ha^{-1} output.

[**] ComboJet ™ nozzles - ER (extended range) with a flat fan pattern spraying at an 80° angle from the nozzle tip. Nozzles ending in -01 have lowest output and output increases as number increases with nozzles ending in -25 having the highest output.

Table 3-3. Differences of least square means values for orthogonol contrast analysis to test yield differences of pyraclostrobin fungicide application treatments at Morden, MB, Saskatoon, SK, Lacombe, AB, and Edmonton, AB, from 2008 to 2011.

Year	Location	Cultivar	Treatmentt A	Treatment B		Std		
				Nozzle	Angle	Error	t value	Pr > |t|
2009	Morden	Topper	control	double-fine	vertical	204.58	-3.18	0.0052
			control	double-coarse	vertical	204.58	-2.67	0.0156
			control	double-both	both	204.58	-2.88	0.0100
			single- coarse	double-coars	vertical	204.58	-2.16	0.0442
2008	Saskatoon	Delta	control	single-fine	vertical	153.84	-3.12	0.0060
			control	double-fine	vertical	153.84	-2.42	0.0265
2009	Saskatoon	Nitouche	control	double-coarse	vertical	254.17	-2.69	0.0148
			single-fine	double-fine	vertical	254.17	2.80	0.0118
			double-fine	double-both	vertical	254.17	-2.18	0.0431
2009	Edmonton	Topper	control	single-fine	vertical	116.32	-2.47	0.0212
			control	double-fine	vertical	116.32	-3.25	0.0034
			control	single-fine	high	116.32	-2.87	0.0084
			control	single-coarse	high	116.32	-2.75	0.0112
			control	double-fine	high	116.32	-2.48	0.0205
			control	double-coarse	high	116.32	-3.51	0.0018
2009	Lacombe	Topper	control	single-fine	vertical	196.97	-2.60	0.0161
			control	single-coarse	vertical	196.97	-4.33	0.0002
			control	double-fine	vertical	196.97	-2.43	0.0231
			control	double-coarse	vertical	214.07	-3.69	0.0012
			control	single-fine	high	196.97	-4.12	0.0004
			control	single-coarse	high	196.97	-3.86	0.0008
			control	double-fine	high	196.97	-4.16	0.0004
			control	double-coarse	high	196.97	-3.39	0.0025
2011	Lacombe	Topper	control	single-fine	vertical	235.91	-3.74	0.0012
			control	single-coarse	vertical	235.91	-3.38	0.0029
			control	single-fine	high	218.40	-3.87	0.0009
			control	single-coarse	high	218.40	-2.53	0.0196
			control	double-fine	high	218.40	-4.36	0.0003
			control	double-coarse	high	218.40	-3.72	0.0013

Single nozzle either mounted in a vertical position from the boom (vertical) or mounted at a 60° angle using a cap with a 60° elbow to make the spray point forward (high). Double nozzles (two nozzle) fitted using a Y-adapter either mounted in a vertical position from the boom (vertical) or mounted at a 60° angle using a cap with a 60° elbow to make the spray point forward (high). Nozzles either delivered a small droplet size (fine) or a large droplet size (coarse).

Table 3-4. Mycosphaerella blight disease severity ratings and yield under different pyraclostrobin fungicide application treatments at Morden, MB, in 2010 on pea 'Topper'.

Treatment	Severity rating*				Yield**
	1-Aug	9-Aug	16-Aug	22-Aug	(T ha^{-1})
Control	3.3	5.5	6.0	9.0	3.52
Single Nozzle					
- fine spray	2.3	4.3	5.8	8.4	3.50
- coarse spray	2.0	4.3	5.3	8.3	4.17
- fine spray / 100 L ha^{-1}	2.0	3.0	5.0	8.3	4.52
Double Nozzle					
- fine spray	2.3	4.0	4.5	8.2	3.84
- coarse spray	2.0	3.8	5.3	8.2	4.13
- vertical coarse, 60° fine	2.0	4.0	5.5	8.1	4.56

*Visual ratings using a 0-9 scale (Xue 1996).

**No significant differences between treatments at $P \leq 0.05$ using Proc Mixed in SAS 9.2

Table 3- 5. Mycosphaerella blight disease severity ratings and yield under different pyraclostrobin fungicide application treatments at Saskatoon, SK, in 2009 on pea 'CDC Montero' at Site 1.

Treatment	Severity rating*				Yield**
	31-Jul	16-Aug	22-Aug	9-Sep	(T ha^{-1})
Control	2.7	***	5.4	7.9	4.43
Single Nozzle					
- fine spray	2.6	***	5.1	6.0	4.48
- coarse spray	2.5	***	4.7	5.9	4.36
- fine spray / 100 L ha^{-1}	2.6	***	4.9	6.0	4.47
Double Nozzle					
- fine spray	2.6	***	4.6	5.6	4.48
- coarse spray	2.5	***	4.7	5.7	4.70
- vertical coarse, 60° fine	2.6	***	4.7	5.9	4.57

* Visual ratings using a 0-9 scale (Xue 1996).
** No - significant differences between treatments at $P \leq 0.05$ using Proc Mixed in SAS 9.2
*** Data is not available.

Table 3-6. Analysis of variance (fixed effects) of pyraclostrobin fungicide application treatments and cultivar effects with pea 'CDC Montero' and 'Nitouche' at Saskatoon, SK, in 2009.

Effect	Num DF	Denom DF	F value	Pr > F
Treatment (T)	6	39	1.89	0.1072
Cultivar (C)	1	39	81.27	< .0001
T*C	6	39	0.67	0.6766

Treatments including single and double nozzles, vertical and high angle, fine and coarse spray particles.

Table 3- 7. Analysis of variance (fixed effects) of pyraclostrobin fungicide application treatments and cultivar effects with pea 'Carneval' and 'Topper' at Edmonton, AB, in 2009.

Effect	Num DF	Denom DF	F value	Pr > F
Treatment (T)	8	51	1.52	0.1748
Cultivar (C)	1	51	22.44	< .0001
T*C	8	51	1.02	0.4349

Treatments including single and double nozzles, vertical and high angle, fine and coarse spray particles.

Table 3-8. Mycosphaerella blight disease severity ratings and yield under different pyraclostrobin fungicide application treatments at Lacombe, AB, in 2009 on pea 'Topper'.

Treatment	Severity rating[a]				Yield
	31-Jul	10-Aug	20-Aug	26-Aug	(T ha^{-1})
Control	6.5	7.6	8.5	8.8	1.47
Single Nozzle					
- fine spray	5.5	6.9	7.9	8.2	2.04*
- coarse spray	5.0	6.9	8.1	8.1	2.42*
- fine spray high angle	6.2	7.0	8.1	8.4	2.37*
- coarse spray high angle	6.0	7.0	8.0	8.2	2.32*
Double Nozzle					
- fine spray	5.7	7.1	7.6	8.0	2.00*
- coarse spray	5.7	7.0	8.0	8.3	2.33*
- fine spray high angle	5.8	6.9	7.7	7.7	2.38*
- coarse spray high angle	6.0	6.9	8.0	8.5	2.21*

[a] Visual ratings using a scale of 0-9 (Xue 1996).

* Significantly different than untreated control at $P \leq 0.05$ using Proc Mixed in SAS 9.2

Table 3-9. Orthoganol contrast analysis of least square means values of yield response to pyraclostrobin fungicide application treatments in field pea at Lacombe, AB, in 2009 and 2011 (pooled data).

Cultivar	Treatmeant A	Treatment B	Estimate	Standard Error	t value	Pr > |t|
Topper	single fine - low	single fine - high	-163.54	147.06	-1.11	0.2984
	single coarse - low	single coarse - high	167.77	147.06	1.14	0.2869
	double fine - low	double fine - high	-407.83	147.60	-2.88	0.0605
	double coarse - low	double coarse - high	-171.04	147.02	-1.16	0.2782

Table 3-10. Analysis of variance (fixed effects) of pyraclostrobin fungicide application treatments and cultivar effects with pea 'Carneval' and 'Topper' at Lacombe and Edmonton, AB, in 2009.

Effect	Num DF	Denom DF	F value	Pr > F
Treatment (T)	8	8	1.56	0.2717
Cultivar (C)	1	109	124.49	< .0001
T*C	8	109	0.64	0.7402

Treatments including single and double nozzles, vertical and high angle, fine and coarse spray particles.

Table 3-11. Factorial analysis of variance (fixed effects) of pyraclostrobin fungicide application treatment effects on pea 'Topper' at Morden, MB, Saskatoon, SK, Lacombe and Edmonton, AB, in 2008-2011 (pooled data).

Treatments[*]	Effect[**]	Num DF	Denom DF	F value	Pr > F
2 - 5	Nozzle number (N)	1	70	0.01	0.9306
	Spray quality (S)	1	70	0.01	0.9036
	N*S	1	70	0.01	0.9951
2-5 and 8-11[***]	Nozzle number (N)	1	82	0.16	0.6945
	Spray quality (S)	1	82	0.07	0.7955
	N*S	1	82	0.00	0.9512
	Angle (A)	1	82	3.63	0.0603
	N*A	1	82	1.49	0.2264
	S*A	1	82	1.16	0.2844
	N*S*A	1	82	0.29	0.5913

[*]Treatments: 2 = a single nozzle, producing a fine spray, mounted in a vertical position; 3 = a single nozzle, producing a coarse spray, mounted in a vertical position; 4 = double nozzles, producing a fine spray, mounted in a vertical position; 5 = double nozzles, producing a coarse spray, mounted in a vertical position; 8 = a single nozzle, producing a fine spray, mounted at a high angle; 9 = single nozzle, coarse spray, high angle; 10 = double nozzles, fine spray, high angle, and 11 = double nozzles, coarse spray, high angle.

[**]Nozzle number including single and double nozzles, spray quality including fine and coarse spray particles, and angles including vertical and high (60°).

[*]Treatments 8-11 were included in analysis at sites tested and when sdf contrast was significant at P≤0.05.

Table 3-12. Mycosphaerella blight disease severity ratings and yield under different pyraclostrobin fungicide carrier volume application treatments at Lacombe, AB, in 2010 on pea 'Cutlass'.

Treatment	Severity rating*				Yield
	26-Jul	3-Aug	11-Aug	16-Aug	(T ha^{-1})
Control	5.8	7.2	8.4	8.4	1.02
Single Nozzle					
- 50 L ha^{-1}	6.1	7.1	7.7	7.9	1.36
- 100 L ha^{-1}	6.1	6.8	7.4	7.7	1.71**
- 200 L ha^{-1}	6.3	7.1	7.5	8.0	1.21
- 400 L ha^{-1}	6.3	6.9	7.6	7.7	1.56**
- 800 L ha^{-1}	6.4	6.7	7.2	7.8	1.44
Double Nozzle					
- 50 L ha^{-1}	6.3	6.8	7.3	7.6	1.26
- 100 L ha^{-1}	6.6	7.0	7.6	7.8	1.44
- 200 L ha^{-1}	6.2	6.9	7.2	7.4	1.45**
- 400 L ha^{-1}	6.5	7.2	7.6	7.8	1.17
- 800 L ha^{-1}	6.3	7.0	7.7	7.9	1.10

* Visual ratings using a scale of 0-9 (Xue 1996).

** Significantly different than untreated control at $P \leq 0.05$ using Proc Mixed in SAS 9.2.

Table 3-13. Orthogonol contrast analysis of least square means for yield response to pyraclostrobin fungicide carrier volume application treatments at Lacombe, AB, in 2010 and 2011.

Year	Treatment A	Treatment B		Std			
		Nozzle	Volume (L ha^{-1})	Estimate	Error	t value	Pr > |t|
2010	control	single nozzle	100	-615.70	180.45	-3.41	0.0028
	control	single nozzle	400	-477.70	180.45	-2.65	0.0155
	control	double nozzles	200	-474.53	179.41	-2.64	0.0155
2011	control	single nozzle	800	-1020.75	369.39	-2.76	0.0097
2010/2011	control	single nozzle	800	-746.31	225.76	-3.31	0.0079

Table 3-14. Factorial analysis of variance (fixed effects) of pyraclostrobin fungicide carrier volume application treatment effects on pea 'Cutlass' at Lacombe, AB, in 2010 and 2011 (pooled data).

Treatments*	Effect**	Num DF	Denom DF	F value	Pr > F
2 - 11	Nozzle number (N)	1	58	0.13	0.7165
	Volume (V)	4	58	0.19	0.9408
	N*V	4	58	0.12	0.9762

*Treatments are: 2 = a single nozzle with a 50 L ha^{-1} output; 3 = a single nozzle with a 100 L ha^{-1} output; 4 = a single nozzle with a 200 L ha^{-1} output; 5 = a single nozzle with a 400 L ha^{-1} output; 6 = a single nozzle with a 800 L ha^{-1} output; 7 = double nozzles with a 50 L ha^{-1} output; 8 = double nozzles with a 100 L ha^{-1} output; 9) double nozzles with a 200 L ha^{-1} output; 10) double nozzles with a 400 L ha^{-1} output; and 11) double nozzles with a 800 L ha^{-1} output.

**Nozzle number including single and double nozzles, volume including water volumes from 50-800 L ha^{-1}.

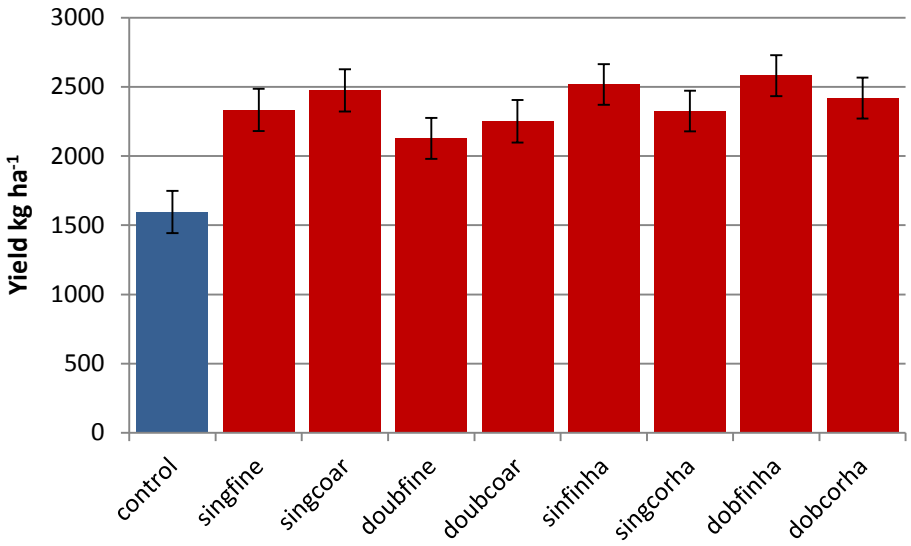

Figure 3-1. Yield response of field pea 'Topper' to pyraclostrobin fungicide application treatments at Lacombe, AB, in 2009 and 2011.
Control= untreated; singfine=single nozzle with a fine droplet size; singcoar=single nozzle with a coarse droplet size; doubfine=double nozzles with a fine droplet size; doubcoar=double nozzles with a coarse droplet size; sinfinha=single nozzle with a fine droplet size at a 60° angle; sincorha=single nozzle with a coarse droplet size at a 60° angle; dobfinha=double nozzles with a fine droplet size at a 60° angle; and dobcorha=double nozzles with a coarse droplet size at a 60° angle.

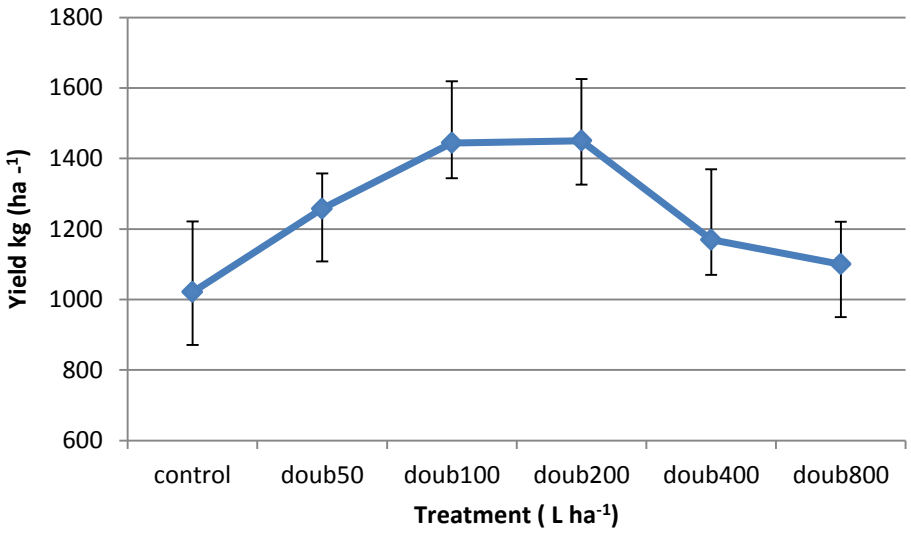

Figure 3-2. Quadratic yield response of pea ' Cutlass' to water carrier volumes of pyraclostrobin fungicide applied with double nozzles in a field trial at Lacombe, AB, in 2010.
Control = untreated; doub50 = double nozzles at 50 L ha^{-1}; doub100 = double nozzles at 100 L ha^{-1}; doub200 = double nozzles at 200 L ha^{-1}; doub400 = double nozzles at 400 L ha^{-1}; and doub800 = double nozzles at 800 L ha^{-1}.

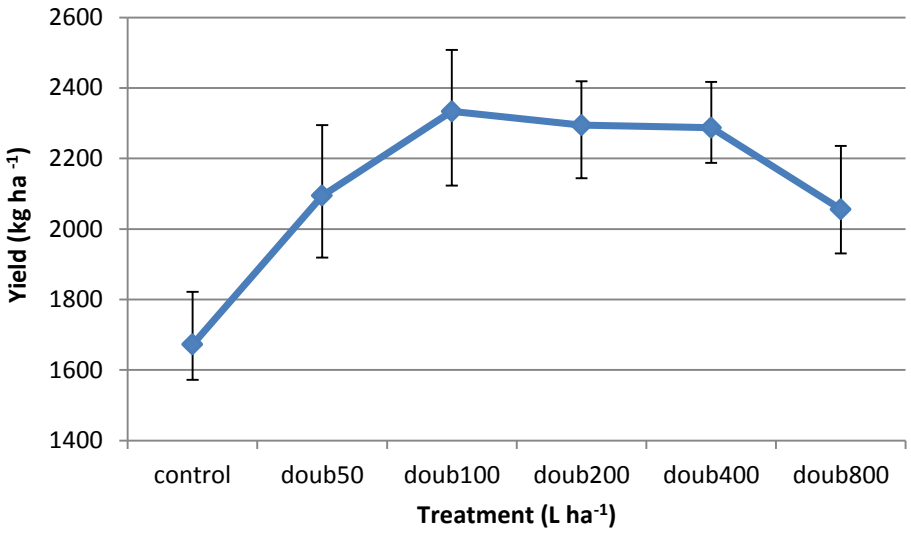

Figure 3-3. Quadratic yield response of field pea ' Cutlass' to water carrier volumes of pyraclostrobin fungicide applied with double nozzles in a field trial at Lacombe, AB, in 2010 and 2011 (pooled data).Control = untreated; doub50 = double nozzles at 50 L ha^{-1}; doub100 = double nozzles at 100 L ha^{-1}; doub200 = double nozzles at 200 L ha^{-1}; doub400 = double nozzles at 400 L ha^{-1}; and doub800 = double nozzles at 800 L ha^{-1}.

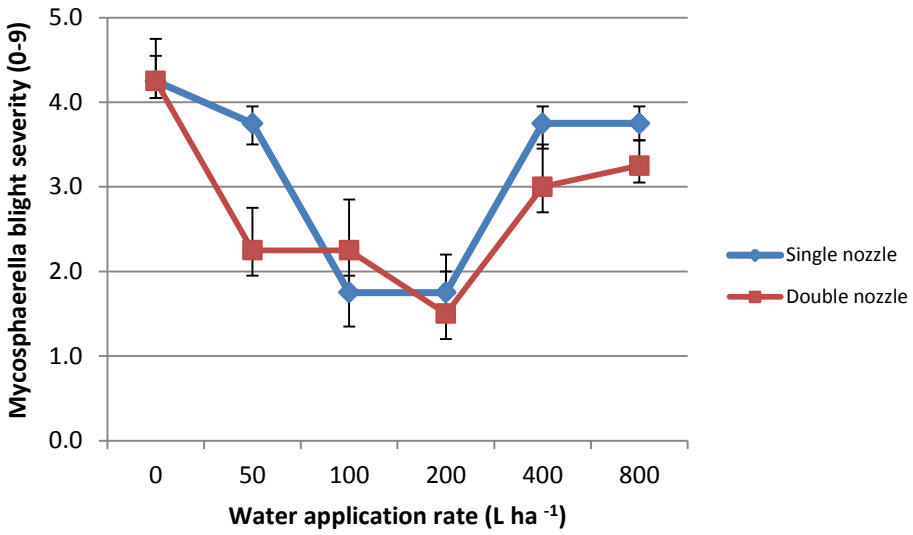

Figure 3-4. Negative quadratic effect of nozzle orientation and water carrier volumes with single and double nozzles of fungicide applications on mycosphaerella blight severity (0-9 scale) on pea 'Cutlass' in a growth chamber experiment (pooled across repetitions).
Pyraclostrobin was applied to 21 day old plants at a rate of 0.04ml per 10ml of water until run-off.

CHAPTER 4: GENERAL CONCLUSIONS

Mycosphaerella blight, caused by *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr (anamorph *Ascochyta pinodes*), is the most destructive foliar disease of field pea and the most important disease of pea crops in western Canada and around the world (Davidson and Ramsay 2000; Bretag et al. 2006; Banniza and Vandenberg 2003; Beasse et al. 1999). Mycosphaerella blight has generally been found in all surveyed commercial pea crops in western Canada, with the disease severity dependent on environmental conditions (Banniza and Vandenberg 2003). The fungus attacks the leaves, stems and pods from pre-flower through until plant maturity. At early growth stages, it interferes with photosynthesis, while at later stages it affects seed quality and yield (Bretag 1991).

Mycosphaerella pinodes is best managed by first reducing the amount of available inoculum, and secondly by suppressing the subsequent epidemic. The best long-term management strategy would be the development of cultivar resistance, but no sources of strong resistance have been identified (Zhang and Gossen 2007). Currently, the most widely utilized strategy for management of mycosphaerella blight is the application of fungicidal sprays in a preventative and systematic schedule (Beasse et al. 2000). Multiple sprays, initiated at early to mid-flowering, would have to be considered as the most effective of management options available for effective disease control and yield gain (Warkentin et al. 2000).

The objectives of this thesis were to: (1) determine the baseline sensitivity of *M. pinodes* to pyraclostrobin using isolates collected prior to registration of this fungicide in Canada and the United States; (2) test isolates recently collected from Alberta, Saskatchewan, Washington State and North Dakota, USA for sensitivity to pyraclostrobin to evaluate whether there have been shifts in fungicide sensitivity since registration of this product; and (3) determine the most effective fungicide delivery system to decrease mycosphaerella blight severity on field pea and increase seed yield.

Fungicides are essential for the maintenance of healthy crops and reliable yields in environments where disease pressure exists. However, the selection pressure imposed by the repeated use of fungicides can seriously affect the effectiveness of the products being applied. Strobilurin fungicides are one of the most important classes of fungicides and have been widely used since 1996 (AgroPages 2011). The site-specific mode of action of the strobilurins has put them at a high risk for of development of fungicide insensitivity in high risk pathogens. Pyraclostrobin is a broad-spectrum strobilurin fungicide that has been widely used since registration in 2003. Significant amounts of insensitivity research have already been carried out on pyraclostrobin. In Canada, the research into pyraclostrobin insensitivity in field crop pathogens has focussed on *Ascochyta rabiei* on chickpea, which has developed qualitative insensitivity to this product (Gossen and Anderson 2004; Thaher 2011; Chang et al. 2007). Field pea, like chickpea, is a high value, drought resistant crop that helped to increase

cropping system diversity in the dry regions of the Canadian prairies (Chang et al. 2007). The similarities between the pathogens that affect chickpea and field pea led to interest on the question of fungicide insensitivity in pathogens associated with the latter.

4.1. FUNGICIDE SENSITIVITY

In the sensitivity study (Chapter 2), 70 isolates of *M. pinodes* were obtained from long-term storage at Saskatoon, Saskatchewan, Canada and Pullman, Washington. These cultures were collected prior to registration of QoI fungicides in those geographical areas in 2003, and represented a baseline collection of isolates with no exposure to QoI fungicides. Using radial growth and conidial germination assessments on pyraclostrobin amended medium, the level of sensitivity to the fungicide was determined for the 70 isolates. During the summers of 2010 and 2011 over 300 isolates of *M. pinodes* from Alberta, Saskatchewan, North Dakota and Washington were obtained from infected plant samples. These isolates represented populations of *M. pinodes* that had been exposed to pyraclostrobin fungicide for up to 8 years. Using a discriminatory dose obtained by comparing the two sets of cultures, 19 isolates were found to be insensitive to pyraclostrobin and another was found to have intermediate sensitivity. When comparing the average EC_{50} value of the insensitive isolates to the average EC_{50} value of the sensitive isolates, the insensitive isolates were nearly 1500 times more insensitive than the sensitive isolates (Chapter 2). The high degree of insensitivity in the insensitive isolates indicates that they carry the

G143 mutation, a single nucleotide change in the mitochondrial cytochrome b gene (Gisi et al. 2000). This substitution results in qualitative insensitivity and is evident by the almost complete lack of response by the pathogen. As a result of this type of mutation, increasing the amount of fungicide used or the frequency of fungicide application will not result in disease suppression. Insensitivity to one strobilurin fungicide means insensitivity to all strobilurin fungicides so no other products found within the strobilurin fungicide group can be used either (Brent and Hollowman 2007a).

Of the *M. pinodes* isolates that were insensitive to pyraclostrobin, nine were collected from an area in central Alberta where field pea is grown intensively and fungicides are applied every year as a preventative measure. Four of the insensitive isolates were collected from more northern areas of Alberta, where field pea cultivation is also widespread and pyraclostrobin is frequently used. The remaining five isolates were collected from random areas across central and southern Saskatchewan. The response of these isolates is not unexpected considering the total breakdown in response of *A. rabiei* on chickpea to the strobilurin group of fungicides (Gossen and Anderson 2004; Thaher 2011; Chang et al. 2007).

Based on the results of this study, there has not been a total breakdown of response of the *M. pinodes* pathogen population to the strobilurin group of fungicides. Only about 6% of the isolates tested were insensitive to the fungicide.

This is positive news for producers across western Canada, since the application of pyraclostrobin is the most effective strategy to manage mycosphaerella blight. However, the identification of 19 insensitive isolates is a cause for concern, as it indicates that an insensitivity problem may be emerging and more years of exposure may increase the insensitivity. In 2012, BASF Canada released a new fungicide called Priaxor®. This fungicide contains two active ingredients with different modes of action: pyraclostrobin and fluxapyroxad. Fluxapyroxad is a succinate dehydrogenase inhibitor (SDHI) and belongs to the carboxamide class of fungicides that inhibit fungal respiration by blocking the ubiquinone-binding sites in the mitochondrial complex II (Avenot and Michailides 2010). This is a different mode of action than for pyraclostrobin. Mixing the modes of action of the fungicides during application for disease management is an effective way to alleviate the problems caused by fungicide insensitivity (Brent and Holloman 2007), and may be a useful approach to slowing the development of fungicide insensitivity in *M. pinodes*. Indeed, based on the current application frequency and the introduction of new active ingredients, complete insensitivity to pyraclostrobin by *M. pinodes* is likely to emerge more slowly than anticipated. An increase beyond 6% is very possible within five years if strategies are not adopted to mitigate this response by the pathogen. It is therefore, critically important to continue to monitor populations of this pathogen for decreases in sensitivity.

4.2. SPRAYER TECHNOLOGY

In the sprayer technology study (Chapter 3), various nozzle configurations and angles for the management of *M. pinodes* in field pea were tested. Treatments compared single and double nozzles, fine and coarse spray quality and low and high angles. Pea plants were selected and assessed for disease severity at flowering and seed yield was collected at physiological maturity. In 10 of the 13 nozzle combination trials conducted, configurations containing double nozzles consistently gave 15% better control of mycosphaerella blight. In 2009 at Saskatoon, double nozzles were significantly better than single nozzles when applying with a fine droplet size. *M. pinodes* initiates infection of the crop at the base of the plant, and for that reason, penetration into the lower areas of the canopy is important. The droplet size that most effectively accomplishes that goal was investigated, but the use of fine or coarse spray nozzles did not result in significant differences between treatments (Chapter 3). Consistently throughout the trials at all locations in all years, both fine and coarse nozzles worked very well when compared to the control treatment to reduce mycosphaerella blight severity and increase yield. The effect of higher angles of application was also investigated. The treatments applied at the lower angles were just as effective and there were no significant differences between them, regardless of angle. A third theory investigated was that higher water volumes would provide better coverage and crop canopy penetration. At Lacombe, water volumes up to 200 L ha^{-1} decreased disease and increased yield, but larger

volumes (up to 800 L ha^{-1}) had the opposite effect. After 400 L ha^{-1}, disease was higher and yield was lower in all trials. This suggests that application of fungicide with too high a water volume may over-saturate the leaves and cause fungicide run-off.

The results presented in Chapter 3 indicate that a double nozzle configuration may be beneficial if it could be incorporated into fungicide sprayer application methodology. Double nozzles appear to have an advantage with respect to coverage and penetration, reaching the lower levels of the canopy. In terms of droplet size, larger droplets also penetrate the canopy better, and are more readily absorbed. Considering those factors, and the lower tendency for larger droplets to drift, larger droplets may be more suitable when trying to manage fungal pathogens such as *M. pinodes* in field pea. When tested, vertical applications appear to be just as effective as higher angles, suggesting that adjusting the angles on sprayer systems may not be worth the effort. When deciding on sprayer technology techniques, factors such as disease pressure, environmental conditions and cultivar characteristics seem to be just as, if not more, important.

4.3. A RELATIONSHIP BETWEEN SPRAYER TECHNOLOGY AND FUNGICIDE INSENSITIVITY?

Comparing the phenomenon of fungicide insensitivity with the sprayer techniques employed to apply the fungicide raises the question of whether or not particular application techniques are contributing to the problem. Consideration of the results presented in Chapters 2 and 3 suggests that application techniques may in fact be contributing to fungicide insensitivity. While factors such as the over-application of fungicides and the use of products with a single site-specific mode of action are undoubtedly the primary reasons for the development of fungicide insensitivity, another contributing factor is the delivery of sub-lethal fungicide doses. If the sprayer technology techniques being used are not delivering adequate doses to all areas of the crop canopy, the pathogen could possibly mutate and survive, accelerating the development of insensitivity in fungal populations. The results of the current studies indicate that a shift towards insensitivity is occurring, but they do not provide any information as to where in the canopy the insensitive isolates are originating. It would be interesting to investigate this as part of future research.

4.4. FUTURE RESEARCH

This thesis represents an initial study to understand the nature of *M. pinodes* insensitivity to pyraclostrobin in field pea, but additional research is required. The degree of insensitivity of the insensitive isolates suggests a G143 mutation in the mitochondrial cytochrome b gene. The complete lack of response by the pathogen to the fungicide supports this conclusion, but this needs to be confirmed. The isolates that demonstrated this lack of response need to be

examined at a molecular level. A nucleotide sequence analysis of the cytochrome b gene, where the mutation is found, should be performed, followed by comparison of the sequenced regions in pyraclostrobin sensitive and insensitive isolates. It may also be informative to examine the single isolate that appeared to give an intermediate reaction, to see if a different basis for insensitivity was involved in that particular case.

While only 6% of the 324 isolates of *M. pinodes* tested were insensitive to pyraclostrobin, the identification of these isolates indicates that a larger insensitivity problem may be emerging, therefore further research is required. A program to monitor the *M. pinodes* sensitivity level to the strobilurin group of fungicides needs to be implemented in western Canada and the Great Plains of the United States to determine if the problem is increasing. Isolates of the pathogen should be collected from the affected regions at least every two years and tested for response. This would involve collection from as many fields as possible from each of the major growing areas within these geographical regions to ensure adequate representation. However it is important to ensure that sampling is un-biased as this may cause either an under- or over-estimation of sensitive isolates. The isolates should be tested *in vitro* using formulated product and radial growth measurements (as per the results of this study). Regular monitoring of the pyraclostrobin sensitivity levels in *M. pinodes* populations from Canada and the U.S. would allow the industry to respond to any changes in a timely manner.

Further to monitoring the sensitivity of *M. pinodes* to the strobilurins, it would be advantageous to monitor the response of this pathogen to the new fungicide, Priaxor®, released in 2102. This fungicide contains pyraclostrobin, as well as fluxapyroxad. Studies into how the use of this added active ingredient affects changes in the fungicide sensitivity of *M. pinodes* may be interesting. In addition, fluxapyroxad is a succinate dehydrogenase inhibitor (SDHI) that also affects fungal respiration, using a different mode of action. The SDHI fungicides are also identified by FRAC as being at high risk for fungicide insensitivity development (FRAC 2011). The level of sensitivity of *M. pinodes* to this fungicide is likely to become an important question in the future.

Given the numerous factors involved in optimizing the application of fungicidal products, the current studies were unable to conclusively identify the best sprayer technology and carrier volumes to provide optimal canopy coverage and penetration. Additional studies in this area could be very beneficial. More studies conducted in controlled environments (such as a spray chamber) may show that some technologies actually do provide enhanced results. These studies could then be confirmed in the field situation, preferably on a larger scale using farm-sized equipment.

Whether or not the development of insensitivity to pyraclostrobin in *M. pinodes* populations becomes an issue in the field is difficult to predict. Nevertheless, a prudent strategy to minimize this possibility would be advisable

for farmers. Such a strategy should include the use of strobilurin-based products according to the manufacturer's recommendations, avoiding over-application of any strobilurin fungicide, and utilizing products with different modes of action for disease management. The industry must focus on the sustainable use of pyraclostrobin and other strobilurins, including the development of improved and economical application techniques. Perhaps most importantly, the application of fungicides for mycosphaerella blight control should be viewed as only part of an integrated disease management strategy, in which these products are used judiciously and in combination with practices such as good crop rotation. To this end, additional research aimed at developing cultivars with improved tolerance or resistance to mycosphaerella blight, and formulating disease forecasting systems to help farmers make informed spray decisions, will be particularly important.

4.5. LITERATURE CITED

AgroPages. 2011. Strobilurin fungicides. [On-line]. Available: http://www.agropages.com/AgroData/AgroDataList-2-3699. [accessed December 2011].

Avenot, H.F. and Michailides, T.J. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Prot. 29: 643–651.

Banniza, S. and Vandenberg, A. 2003. The influence of plant injury on development of *Mycosphaerella pinodes* in field pea. Can. J. Plant Pathol. 25:304−311.

Beasse, C., Ney, B. and Tivoli, B. 1999. Effects of pod infection by *Mycosphaerella pinodes* in yield components of pea (*Pisum sativum*). Ann. Appl. Biol. 135: 359−367.

Beasse, C., Ney, B., and Tivoli, B. 2000. A simple model of pea (*Pisum sativum*) growth affected by *Mycosphaerella pinodes*. Plant Pathol. 49: 187−200.

Brent, K.J. and Holloman, D.W. 2007. Fungicide resistance in crop pathogens: How can it be managed? [Online]. Fungicide Resistance Action Committee, Monograph 1, Global Crop Protection Federation. Brussels. Available: http://www.frac.info/frac/index.htm [accessed March 2011].

Bretag, T.W. 1991. Epidemiology and control of Ascochyta blight of field peas. Ph.D. thesis, La Trobe University, Victoria, Australia.

Bretag, T.W., Keane, P.J. and Price, T.V. 2006. The epidemiology of ascochyta blight in field peas: A review. Aust. J. Agric. Res. 57: 883−902.

Chang, K.F., Ahmed, H.U., Hwang, S.F., Gossen, B.D., Strelkov, S.E., Blade S.F. and Turnbull, G.D. 2007. Sensitivity of field populations of *Ascochyta rabiei* to chlorothalonil, mancozeb, and pyraclostrobin fungicides, and effects of strobilurin fungicides on the progress of ascochyta blight of chickpea. Can. J. Plant Sci. 87: 937–944.

Davidson, J.A., and Ramsey, M.D. 2000. Pea yield decline syndrome in South Australia: the role of diseases and the impact of agronomic practices. Aust. J. Agric. Res. 51, 347–354.

Gisi, U., Chin, K.M., Knapova, G., Farber, Kung, R., Mohr, U., Parisi, S., Sierotzki, H., and Steinfeld, U. 2000. Recent developments in elucidating modes of resistance to phenylamide, DMI and strobilurin fungicides. Crop Prot. 19: 863–872.

Gossen, B.D. and Anderson, K.L. 2004. First report of resistance to strobilurin fungicides in *Didymella rabiei*. Can. J. Plant. Pathol. 26: 411.

Thaher, N. 2011. Fungicide Insensitivity in *Ascochyta rabiei* in Saskatchewan. Masters thesis. University of Guelph.

Warkentin, T.D., Xue, A.G., and McAndrew, D.W. 2000. Effect of mancozeb on the control of mycosphaerella blight of field pea, Can J. Plant Sci. 80: 403−406.

Zhang, R., and Gossen, B.D. 2007. Heritability estimates and response to selection for resistance to mycosphaerella blight in pea. Crop Sci. 47: 2303–2307.

APPENDIX: SUPPLEMENTARY DATA FOR SPRAYER TECHNOLOGY CHAPTER

Table A3-1. Mycosphaerella blight severity ratings and yield under different pyraclostrobin fungicide application treatments at Morden, MB, in 2008 on pea 'Topper'.

	Severity rating*				Yield**
Treatment	**5-Aug**	**12-Aug**	**18-Aug**	**26-Aug**	**(T ha^{-1})**
Control	3.0	4.5	5.0	7.0	3.19
Single Nozzle					
- fine spray	2.8	3.2	3.5	4.8	3.55
- coarse spray	2.8	3.8	3.8	5.8	3.06
- fine spray / 100 L ha^{-1}	2.8	3.5	3.8	4.8	3.72
Double Nozzle					
- fine spray	2.8	3.8	3.8	5.5	3.03
- coarse spray	2.8	4.0	4.0	5.8	3.07
- vertical coarse, 60° fine	2.5	3.8	3.8	4.5	3.39

* Visual ratings using the Xue scale (0-9) (Xue 1996)

** No sig differences between treatments at $P \leq 0.05$ using Proc Mixed in SAS 9.2

Table A3-2. Mycosphaerella blight severity ratings and yield under different pyraclostrobin fungicide application treatments at Morden, MB, in 2009 on pea 'Topper'.

	Severity rating*				Yield
Treatment	**10-Aug**	**19-Aug**	**29-Aug**	**2-Sep**	**(T ha^{-1})**
Control	3.5	5.3	7.0	7.3	4.51
Single Nozzle					
- fine spray	3.3	4.3	5.8	6.3	4.99
- coarse spray	3.0	4.0	6.0	7.3	4.68
- fine spray / 100 L ha^{-1}	3.0	4.0	6.0	6.3	5.66
Double Nozzle					
- fine spray	3.3	3.8	5.8	6.0	5.59**
- coarse spray	3.0	4.0	6.0	6.5	5.42**#
- vertical coarse, 60° fine	3.3	4.3	5.8	6.5	5.49**

* Visual ratings using the Xue scale (0-9) (Xue 1996)

** Significantly different than untreated control at P > 0.05 using Proc Mixed in SAS 9.2

Significantly different at P ≤ 0.05 using Proc Mixed in SAS 9.2

Table A3-3. Mycosphaerella blight severity ratings and yield under various pyraclostrobin fungicide application treatments at Saskatoon, SK in 2008 on pea 'Delta'.

Treatment	Severity rating*		Yield
	6-Aug	29-Aug	(T ha^{-1})
Control	1.4	7.3	1.90
Single Nozzle			
- fine spray	1.7	5.9	2.22**
- coarse spray	1.4	6.4	2.12
- fine spray / 100 L ha^{-1}	1.7	6.5	2.13
Double Nozzle			
- fine spray	1.4	5.8	2.15**
- coarse spray	1.5	6.0	2.05
- vertical coarse, 60° fine	1.7	5.8	2.11

* Visual ratings using a scale of 0-9 (Xue 1996)

** Significantly different than untreated control at $P \leq 0.05$ using Proc Mixed in SAS 9.2

Table A3-4. Mycosphaerella blight severity ratings and yield under different pyraclostrobin fungicide application treatments at Saskatoon, SK in 2009 on pea 'Nitouche'.

Treatment	Severity rating*			Yield
	31-Jul	25-Aug	8-Sep	(T ha^{-1})
Control	2.4	5.8	7.9	3.60
Single nozzle				
- fine spray	2.3	4.7	5.8	4.01**
- coarse spray	2.4	4.9	6.2	3.79
- fine spray / 100 L ha^{-1}	2.2	4.7	6.5	3.87
Double nozzle				
- fine spray	2.2	5.3	6.6	3.53**
- coarse spray	2.3	4.5	6.2	4.06*
- vertical coarse, 60° fine	2.2	5.0	6.5	3.90**

* Visual ratings using a scale of 0-9 (Xue 1996)

* Significantly different than untreated control at P ≤ 0.05 using Proc Mixed in SAS 9.2

** Significantly different at P > 0.05 using Proc Mixed in SAS 9.2

Table A3-5. Orthoganol contrast analysis of least square means values of yield response to pyraclostrobin fungicide application treatments in field pea at Saskatoon, SK, in 2009 (pooled data).

Cultivar	Treatmeant A	Treatment B	Estimate	Std Error	t value	Pr > |t|
Nitouche/Montero	control	double coarse	-545.98	211.29	-2.58	0.0136
	single coarse	double coarse	-462.39	211.29	-2.19	0.0347

Table A3-6. Mycosphaerella blight severity ratings and yield under different pyraclostrobin fungicide application treatments at Saskatoon, SK, in 2011 on pea 'Topper'.

Treatment	Severity rating*			Yield**
	28-Jul	10-Aug	19-Aug	(T ha^{-1})
Control	1.9	3.8	5.0	2.61
Single Nozzle				
- fine spray	1.8	2.7	4.5	2.88
- coarse spray	1.7	2.4	4.2	2.73
- fine spray high angle	2.1	2.6	4.7	2.66
- coarse spray high angle	1.8	2.8	4.5	2.95
Double Nozzle				
- fine spray	1.5	2.4	4.2	2.87
- coarse spray	2.1	2.8	4.5	2.84
- fine spray high angle	2.0	2.6	4.7	2.66
- coarse spray high angle	1.8	2.7	4.1	2.82

* Visual ratings using a scale of 0-9 (Xue 1996)

** No sig differences betwen treatments at P ≤ 0.05 using Proc Mixed in SAS 9.2

Table A3-7. Mycosphaerella blight severity ratings and yield under different pyraclostrobin fungicide application treatments at Edmonton, AB, in 2009 on pea 'Topper'.

Treatment	Severity rating*		Yield**
	7-Aug	21-Aug	(T ha^{-1})
Control	4.4	6.2	1.75
Single Nozzle			
- fine spray	3.6	5.3	2.06
- coarse spray	3.7	5.1	2.04
- fine spray high angle	3.7	5.0	2.12
- coarse spray high angle	4.0	5.3	2.10
Double Nozzle			
- fine spray	4.1	4.8	2.17
- coarse spray	3.8	5.5	1.99
- fine spray high angle	3.4	4.6	2.07
- coarse spray high angle	4.1	5.0	2.20

* Visual ratings using a scale of 0-9 (Xue 1996)

** No sig differences betwen treatments at $P \leq 0.05$ using Proc Mixed in SAS 9.2

Table A3-8. Mycosphaerella blight severity ratings and yield under different pyraclostrobin fungicide application treatments at Lacombe, AB, in 2011 on pea 'Topper'.

Treatment	Severity rating*				Yield
	15-Jul	20-Jul	2-Aug	25-Aug	(T ha^{-1})
Control	4.3	5.7	5.7	5.8	1.72
Single Nozzle					
- fine spray	4.2	5.4	5.4	5.6	2.62**
- coarse spray	3.5	4.9	5.1	5.5	2.53**
- fine spray high angle	3.4	5.1	5.2	5.7	2.66**
- coarse spray high angle	4.3	5.0	5.5	5.4	2.33**
Double Nozzle					
- fine spray	4.5	5.2	5.4	5.6	2.25**
- coarse spray	3.4	5.3	4.9	5.4	2.17
- fine spray high angle	3.5	4.7	5.0	5.5	2.78**
- coarse spray high angle	4.3	5.5	5.4	5.6	2.62**

* Visual ratings using a scale of 0-9 (Xue 1996)

** Significantly different than untreated control at $P \leq 0.05$ using Proc Mixed in SAS 9.2

Table A3-9. Mycosphaerella blight severity ratings and yield under different pyraclostrobin fungicide carrier volume application treatments at Lacombe, AB, in 2011 on pea 'Cutlass'.

Treatment	Severity rating*				Yield
	18-Jul	25-Jul	5-Aug	25-Aug	(T ha^{-1})
Control	4.6	5.1	5.4	6.8	2.32
Single Nozzle					
- 50 L ha^{-1}	5.1	5.2	5.3	6.8	3.18
- 100 L ha^{-1}	5.1	5.0	5.7	6.2	2.92
- 200 L ha^{-1}	5.2	5.1	5.5	6.7	2.80
- 400 L ha^{-1}	4.9	4.8	5.7	6.6	3.04
- 800 L ha^{-1}	4.9	4.7	5.5	6.5	3.45**
Double Nozzle					
- 50 L ha^{-1}	4.9	4.8	5.4	6.6	2.93
- 100 L ha^{-1}	4.9	5.0	5.5	6.4	3.22
- 200 L ha^{-1}	5.2	5.0	5.4	6.7	3.05
- 400 L ha^{-1}	5.0	4.6	5.5	6.4	3.40
- 800 L ha^{-1}	5.2	4.6	5.8	6.7	3.01

* Visual ratings using a scale of 0-9 (Xue 1996)

** Significantly different than untreated control at $P \leq 0.05$ using Proc Mixed in SAS 9.2

Table A3-10. Mycosphaerella blight severity ratings and yield under different pyraclostrobin fungicide carrier volume application treatments at Saskatoon, SK, in 2011 on pea 'Cutlass'.

Treatment	Severity rating*			Yield**
	28-Jul	10-Aug	19-Aug	(T ha^{-1})
Control	1.3	3.0	3.8	3.19
Single Nozzle				
- 50 L ha^{-1}	1.5	2.7	3.6	3.14
- 100 L ha^{-1}	1.3	2.7	3.4	3.24
- 200 L ha^{-1}	1.1	2.8	3.1	3.51
- 400 L ha^{-1}	1.2	2.8	3.3	3.32
- 800 L ha^{-1}	1.2	2.7	3.5	3.29
Double Nozzle				
- 50 L ha^{-1}	1.2	2.7	4.0	2.81
- 100 L ha^{-1}	1.4	2.7	3.6	3.49
- 200 L ha^{-1}	1.3	2.7	3.6	3.10
- 400 L ha^{-1}	1.4	2.9	3.7	3.32
- 800 L ha^{-1}	1.2	2.7	3.5	3.28

* Visual ratings using a scale of 0-9 (Xue 1996)

** No sig differences betwen treatments at $P \leq 0.05$ using Proc Mixed in SAS 9.2