Fungicide Management of Pasmo of Flax and Fungicide Sensitivity of *Septoria linicola*

A Thesis Submitted to the College of Graduate and Postdoctoral Studies

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Plant Sciences

University of Saskatchewan

Saskatoon, SK, Canada

By

Tonima Islam

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department of Plant Science or the Dean of the College of Agriculture and Bioresources. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use that may be made of any material in my thesis. Requests for permission to copy or to make other uses of materials in this thesis, in whole or part, should be addressed to:

Head, Department of Plant Sciences
University of Saskatchewan
Saskatoon, Saskatchewan
Canada, S7N 5A8
Or

Dean, College of Graduate Studies and Research
University of Saskatchewan
Saskatoon, Saskatchewan
Canada S7N 5A2

DISCLAIMER

Reference in this dissertation to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favouring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes.

ABSTRACT

Pasmo of flax (*Linum usitatissimum* L.), caused by *Septoria linicola* (Speg.) Garassini, is commonly observed in western Canada every year. Severe pasmo infection can reduce both quality and quantity of flaxseed. As no flax cultivar is pasmo resistant, flax growers depend on fungicide application to manage this disease. However, continuous use of the same fungicide or fungicides may result in the development of fungicide insensitivity in the pathogen population. The first objective of this study was to determine the impact of three fungicide products, pyraclostrobin, fluxapyroxad and fluxapyroxad+pyraclostrobin; and application timing (early-flower, mid-flower and at both stages) on pasmo severity, seed yield and quality of flaxseed. Field studies were conducted at four locations in western Canada in 2014, 2015 and 2016. The results indicated that among the three fungicide products, fluxapyroxad+pyraclostrobin was the most effective. It decreased disease severity to 15% compared with 59% for the unsprayed control, followed by pyraclostrobin (20%) and fluxapyroxad (39%). Application of fluxapyroxad+pyraclostrobin also increased the seed yield to 2562 kg ha^{-1} compared with 1874 kg ha^{-1} for the unsprayed control, followed by pyraclostrobin (2391 kg ha^{-1}) and fluxapyroxad (2340 kg ha^{-1}). However, application of these products also delayed crop maturity. Fungicide application at mid-flowering stage was the most cost effective and decreased disease severity and improved seed yield. The second objective of this study was to determine the sensitivity of *S. linicola* isolates to pyraclostrobin and fluxapyroxad fungicides by spore germination and microtiter assay methods. In the spore germination assay, the EC_{50} of pyraclostrobin for most of the isolates was between 0.0005 to 0.007 µg mL^{-1} and fluxapyroxad between 0.001 to 0.05 µg mL^{-1}. In the microtiter assay, the EC_{50} of pyraclostrobin was between 0.003 to 0.021 µg mL^{-1} and fluxapyroxad was between 1 and 4 µg

mL^{-1}. No fungicide insensitivity was observed among the isolates of *S. linicola* to these fungicide products. The findings of these studies will help to make effective pasmo management recommendations and provide a baseline against which to measure future *S. linicola* isolates suspected of insensitivity to pyraclostrobin and fluxapyroxad fungicides.

ACKNOWLEDGEMENTS

To begin with, I would like to acknowledge my supervisor, Dr. Randy Kutcher for the support and guidance he gave throughout this study.

I would also like to thank my committee members, Dr. Helen Booker, Dr. Steve Shirtliffe and Dr. Eric G. Lamb for their valuable advice, useful suggestions and feedback throughout my research project. I would thank Dr. Syama Chatterton for reviewing my thesis as the external examiner and for her time and comments to improve the quality of this thesis.

I would also like to thank my collaborators, Dr. Ramona Mohr, Dr. Jan Slaski, Dr. Khalid Y. Rashid, and Mr. Cecil L. Vera as well as their respective personnel.

This thesis work would not have been possible without the help of the Cereal and Flax Pathology crew. Thanks to Md. Mortuza Reza for helping me throughout the research project. Special thanks to the University of Saskatchewan and the Government of Saskatchewan (Agriculture Development Fund) for their financial support to carryout this research project. I would also like to thank all the friends and personnel at College of Agriculture and Bioresources for making the days at university so pleasant.

Finally, I am very thankful to all of my family members and friends for their inspiration and continued support.

DEDICATION

I dedicate this work to my father Md. Monirul Islam, my mother Farida Yeasmin and my brother Zayadul Islam for their amazing support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALA	Alpha-linoleic acid
ANOVA	Analysis of variance
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical industry
CDC	Crop Development Centre
CFIA	Canadian Food Inspection Agency
CGC	Canadian Grain Commission
EC_{50}	Effective Concentration
FRAC	Fungicide Resistant Action Group
LTN	Long-term normal
MAP	Mono-ammonium phosphate
MICs	Minimum inhibiting concentrations
ND	No data
NS	Non-significant
PDA	Potato dextrose agar
QoI	Quinone outside inhibitor
RCBD	Randomized complete block design
SDHI	Succinate dehydrogenase inhibitor
SHAM	Salicylhydroxamic acid
SOM	Soil organic matter
TBZ	Thiabendazole
PGPR	Plant growth promoting rhizobacteria
TPM	Thiophanate-methyl

CHAPTER 1

1. Introduction:

Flax (*Linum usitatissimum* L.) is an important cash crop in Canada, the world's largest producer and exporter. Western Canada produces about 40% of world flax production with an annual export value of CAD$150-180 million (Saskatchewan Flax Development Commission 2017). Flax is considered as both a fiber and an oilseed crop. Previously, flax oil was used mainly for industrial purposes, such as in paint and flooring, and flax fiber was used for linen (Berglund 2002). However, due to its high alpha-linoleic acid (ALA) content and medicinal properties, flaxseed and flax oil is now consumed by humans (Bloedon and Szapary 2004; Paschos et al. 2005). The ALA reduces the risk of cardiovascular disease, lowers blood pressure, and improves blood lipid levels and endothelial function (Oomah 2001; Bloedon and Szapary 2004). Additionally, flaxseed added to animal feed increases the ω-3 fatty acid content in animal products (Caston and Leeson 1990).

Flax is susceptible to several diseases that impact yield and quality, such as, pasmo, fusarium wilt, rust, powdery mildew, seedling blight and root rot, sclerotinia stem rot and aster yellows. Pasmo causes crop damage almost every year. The disease is caused by the pathogen *Septoria linicola* (Speg.) Garassini and when severe, it can reduce seed yield by as much as 60-70% (Rashid 2001). At present, all flax cultivars are susceptible to this disease. In 2015, up to 40% disease severity was observed in pasmo affected fields in Saskatchewan (Rashid et al. 2016). Researchers report that single or multiple applications of fungicide at the flowering stage of flax reduces yield loss (Ferguson et al. 1987). Until 2014, pyraclostrobin (Headline® EC) was the only registered fungicide to control pasmo in Canada. Fluxapyroxad (Xemium®) and the combination of

pyraclostrobin and fluxapyroxad (Priaxor®) are two new fungicide products. Little is known of the effects of these fungicide products on pasmo and flax seed yield and quality compared with pyraclostrobin. Determining the effect of these fungicides will help flax growers to choose an appropriate fungicide to manage this disease. In flax, fungicides are sprayed at the flowering stage to control pasmo. However, there is also no clear information on the flowering stage at which fungicide application is most beneficial, and whether a single application of fungicide may be as effective as two applications. This information is very important to flax growers for effective fungicide management.

Due to overuse of fungicides, insensitivity to some fungicide products are developing in many pathogen species (Avenot et al. 2008; Yin et al. 2012). Pyraclostrobin is a commonly used fungicide product to control pasmo and fluxapyroxad is a comparatively new fungicide product, which has been used in combination with pyraclostrobin in flax since 2015. Some pathogens have developed insensitivity against pyraclostrobin and fluxapyroxad (Kim and Xiao 2011; Amiri et al. 2014); however, research has not been conducted to determine if insensitivity to either pyraclostrobin or fluxapyroxad has occurred in the pathogen population of *S. linicola*. Thus, it is very important to measure the fungicide sensitivity of *S. linicola* against these fungicides. This will help to determine whether insensitivity has occurred in the pathogen population based on the EC_{50} values of the isolates. The EC_{50} values can also be used to determine the baseline sensitivity, which will facilitate identification of fungicide insensitivity in this pathogen if or when it occurs in future. Thus, the hypotheses for this study were:

1. The combination of pyraclostrobin and fluxapyroxad will improve control of pasmo over that of pyraclostrobin or fluxapyroxad alone,

2. Fungicide application at mid-flowering stage will improve pasmo control over application at the early-flowering stage, and
3. Sensitivity to each fungicide mode of action varies among isolates of *S. linicola.*

The objectives that follow from the hypotheses were:

1. To evaluate the effect of three fungicide products, pyraclostrobin (Headline EC), fluxapyroxad (Xemium) and the combination of pyraclostrobin and fluxapyroxad (Priaxor), applied at two flax flowering stages on disease severity and flax seed yield and quality, and

2. To determine the sensitivity of *S. linicola* isolates collected from western Canada in 2014 and 2015 to fluxapyroxad and pyraclostrobin.

CHAPTER 2

2. Literature Review:

2.1. History and Economic Importance of Flax

Flax (*Linum usitatissimum* L.) is in the family Linaceae and is cultivated for oil and fiber. The botanical name, *Linum usitatissimum* was given to flax by Linnaeus in his book "Species Plantarum" (Vaisey-Genser and Morris 2003). The name *Linum usitatissimum*, originated from a Latin word that means "very useful" (Singh et al. 2011). Both the Mediterranean and Southwest Asia have been proposed as the center of origin of flax (Millam et al. 2005). There are many geographical centers of genetic diversity of flax, including central Asian, Western Asian, the Mediterranean, and Abyssinia. Flax appears to be the earliest and best-documented oil and fiber crop cultivated in the early stage of agriculture, some 8,000 years ago. Over time, cultivation and trade of flax increased from the Near East to Europe, the Nile Valley and West Asia. In 1600, flax was brought to North America by French immigrants (Vaisey-Genser and Morris 2003).

Flax was domesticated for both its fiber and oil. With domestication, the weedy forms of flax (*Linum bienne* Mill) evolved some characteristic traits through random selection and consistent practices of sowing, reaping and threshing. For example, today's domestic flax bolls no longer split or dehisce to release seed, and seed size, number of seeds per boll and oil yield have increased (Vaisey-Genser and Morris 2003). Oilseed flax is grown worldwide, and fiber flax is mainly grown in Europe (Singh et al. 2011).

Historically, the principal use of flaxseed oil was as a preservative in products such as paint and flooring and use in other industries. Use of flaxseed oil makes paint and coatings durable and also improves the quality of the products (Berglund 2002). At present, however, flaxseed is used as food and animal feed due to its health benefits. Flaxseed is rich in alpha-linoleic acid (ALA), an essential omega (ω)-3 fatty acid, which constitutes about 57% of the total fatty acids in flax (Cunnane et al. 1993). Flax helps reduce cardiovascular disease by altering the ω-3 fatty acid content of cell membranes, improving blood lipids and endothelial function, and exerting antioxidant effects (Bloedon and Szapary 2004). Consumption of flax oil for 12 weeks (one tablespoon, 8 g ALA/day) in the daily diet lowers blood pressure significantly in middle-aged men with high blood cholesterol levels (Paschos et al. 2005). From animal trials, it is suggested that the nutritional components of flax reduce the risk of breast cancer, by interfering with tumor initiation and promotion by altering estrogen metabolism, which decreases cell proliferation (Hutchins et al. 2000).

Flax is added to animal feed as a means of increasing the ω-3 fatty acid content of meat and dairy products. Replacing barley with flaxseed in the diet of lactating cows increased the beneficial fatty acids in milk without decreasing nutrient quality (Soita et al. 2003). These products provide consumers with foods that have a healthy fat profile, and taste good. Feeding flax to laying hens increased the omega-3 fatty acid content five- to nine-fold in eggs (Caston and Leeson 1990). Flax improved the growth performance of rainbow trout. Flax is also a promising alternative to marine fish oil for enriching the omega-3 fatty acid content of farmed fish. The use of flax as fish meal could result in a very significant savings in feed costs without compromising fish performance and end-product quality (Thiessen 2004).

There are two primary advantages of using flax fiber in textiles. One is the strength of the yarn spun from long-line flax fibers, which is more than twice as strong as cotton. The other advantage is that the hollow flax fibers are very absorbent due to the property of wicking, the movement of moisture along the surface. This makes linen fabrics comfortable to wear in hot climates because sweat evaporates quickly, producing a cooling effect. Moreover, wicking makes cloth stronger when wet and provides durability in products such as sails, tents, rugs and heavy bags. A disadvantage of wicking is that flax fibers resist dyeing (Aranberri-Askargorta et al. 2003; Baltazar-y-Jimenez and Bismarck 2007).

2.2. Commercial Production

Canada, Russia, USA, India, and China are the main flax growing countries of the world (Singh 2011). Canada is the world's largest producer and exporter of flax, representing about 40% of world production with annual exports valued at CAN$150-180 million (M). Saskatchewan produces almost four times as much flax as Manitoba on average (Saskatchewan Flax Development Commission 2017). In 2016, flax was seeded on 378,000 ha and a total of 579,000 tonnes flaxseed was produced in western Canada (Canadian Grain Commission 2016). Canada exports 80–90% of the flaxseed produced, mainly to Europe, USA, Japan, and South Korea. More than 70% of Canada's production was exported to Europe each year prior to 2009 (Flax Council of Canada 2017a).

In the late 1980's, a genetically modified (GM) flax variety, CDC Triffid was developed by the Crop Development Centre in Saskatoon, Saskatchewan (Canada). CDC Triffid transgenic flax was genetically modified to make it tolerant to sulfonylurea based herbicides (triasulfuron and metsulfuron-methyl) (Canadian Food Inspection Agency 1998; McHughen et al. 1997). In 1998,

Triffid was approved by the Canadian Food Inspection Agency (CFIA) for full variety release and entered into the seed multiplication program. However, implementation of the program created an obstacle to the Canadian flax industry as Europe threatened to stop importing Canadian flax because they had a zero-tolerance policy for GM products (Ryan and Smyth 2012). Due to the negative effect on the export market, CDC Triffid was deregistered in 2001. However, in 2009, Europe reported that traces of GM flax were found in Canadian shipments during preprocessing, which dropped the flax bid (almost 30% in Manitoba) within a few days of publication of the report (Schmidt and Breckling 2010; Booker et al. 2014). Industry stakeholders, the Flax Council of Canada, the CFIA, the Canadian Grain Commission (CGC) and the Crop Development Centre (CDC) responded quickly to mitigate the impacts and took action to eliminate the GM contamination and reduced the rates of GM contamination in commercial flax stocks to between one GM seed per million and one seed per hundred thousand (Booker and Lamb 2012; Ryan and Smyth 2012; Booker et al. 2014). The result was a major shift in the export markets and presently China is the major export market for Canadian flaxseed (Booker et al. 2017).

2.3. Growth and Development of Flax

The cultivated form of flax (*L. usitatissimum*) is a self-pollinating diploid plant with a karyotype of 2n = 30. Morphological, genetic and molecular evidence suggest that the wild progenitor of cultivated flax is pale flax (*L. angustifolium* Huds.), which has the same chromosome number (2n = 30), strong branches, periwinkle blue flowers and dehiscent (splitting) seed bolls or capsules. This form of wild flax is widely distributed across Western Europe, the Mediterranean Basin, North Africa and the Caucasus (Vaisey-Genser and Morris 2003). Cultivated flax has long upright stems in comparison to wild forms and capsules that generally do not dehisce (Allaby et al. 2005). There are two principal types of cultivated flax, which are broadly designated ‘oil varieties’ and

‘fibre varieties’. The oil varieties tend to be shorter with large seeds that contain approximately 40% oil, while the fibre varieties are taller and more sparsely branched, with smaller and fewer seeds (Vaisey-Genser and Morris 2003). Flax is an annual crop and depending on growing conditions and morphotype the height ranges from 25-125 cm (Diederichsen 2001). The flowers have five petals surrounding a globular capsule that may contain up to ten yellow or dark brown seeds. The typical flower color of flax grown in western Canada is blue, but may also be white or pale pink (Halley 2007). The vegetative period ranges from 45-60 days, the flowering period from 15-25 days, and the maturation period 30-40 days (Saskatchewan Flax Development Commission 2017).

Flax is adapted to a wide range of environments; however, flax grows best under moderate to cool conditions, particularly during seed filling (Casa et al. 1999). Under controlled conditions high temperatures during the ripening phase reduce the number of seeds per capsule, the seed weight and decrease oil yield and quality (Canvin 1965). Flax tolerates a wide range of soils but grows best on well-drained, medium to heavy textured soils, especially silty or clay loam soil of approximately pH 6 (Alvarez 2010). In North America, flax is principally grown on the Canadian Prairies and in the North Central United States in the Black, Dark Brown, Dark Grey and Brown Chernozemic soil zones (Marchenkov et al. 2003).

Flax should be seeded in rows 15 to 20 cm apart, but can be planted at row spacing of up to 30 cm (Stevenson and Wright 1996). High plant density can sometimes result in lodging, which can reduce crop yield. The optimum plant population for maximum yield is 300 plants per square meter and seed should be sown 2.5 to 4.0 cm (1.0 to 1.5 inches) deep into moist soil (Lafond et al. 2008). In western Canada, flax is generally seeded between mid-May to early June. Yield is generally higher when the crop is seeded early (McKenzie et al. 2011). Early seeding in May rather than late

June also helps to reduce the risk of disease and the risk of damage caused by early fall frosts (McGregor 1964; Gubbels et al. 1994). Although flax is not usually irrigated, it is susceptible to water stress at the seedling stage, at flowering and during early seed development. In dry environments, irrigation at flowering and during seed filling may increase seed yield considerably (Alessi and Power 1970).

Flax, like most other crops, usually responds well to additional fertilizer, but the application of high rates of N does not always result in improved seed yield (Malhi et al. 2008). The optimum nitrogen (N) requirements for flax are 100 – 120 kg ha^{-1}. However, additional N may result in increased pasmo severity and lodging, both of which negatively affect seed yield of flax (Vera et al. 2014). A low rate of phosphate, <17 kg/ha of P_2O_5, may be placed to the side of the seed at a depth of 25 mm to improve phosphorus nutrition (Flax Council of Canada 2017b). Placement of fertilizers in bands near the seed row allows for early access of applied nutrients by plant roots, producing rapid growth in spring-seeded crops (Sadler 1980; Malhi et al. 2008).

In Manitoba, flax is mainly a stubble-sown crop. In Alberta and Saskatchewan, it is also increasingly a stubble seeded crop and recently more than 50% of flax was grown on stubble. Research has shown that flax performs poorly after canola but does well after cereals and corn (Gubbels and Kenaschuk 1989). In western Canada, a minimum crop rotation of three years with cereal crops between flax crops is recommended (Bailey et al. 1992). Flax should not be grown after legumes or potato because of the potential for infection from *Rhizoctonia* spp. (Johnston et al. 2002). A diverse crop rotation helps to reduce the incidence of soil- and stubble-borne diseases (Krupinsky et al. 2002). Sometimes weeds can also reduce flax yield and seed quality, as flax is less competitive with weeds than most other crops in western Canada. Due to limited herbicide

options, integrated weed management strategies are recommended (Friesen 1986; Stevenson and Wright 1996).

There are many diseases that can affect flax production, such as rust, caused by the pathogen *Melampsora lini* (Ehrenb.), fusarium wilt caused by *Fusarium oxysporum* Schlecht. f. sp. *lini* (Bolley), pasmo caused by *Septoria linicola* (Speg.) Garassini, powdery mildew caused by *Oidium lini* (Skoric.), stem break and browning caused by *Aureobasidium pullulan* var. *lini* (Laff.), also called *Polyspora lini*, seedling blight and root rot caused by *Rhizoctonia solani* (Kuhn.), sclerotinia stem rot caused by *Sclerotinia sclerotiorum* (Lib.) and aster yellows caused by a phytoplasma (Rashid 2003).

2.4. Pasmo disease of flax and the causal pathogen

The causal agent of the pasmo was first isolated from infected flax stems in Argentina and named *Phlyctaena linicola* (Speg.) in 1911. However, considering the spore size and septation, the fungus was transferred to the genus Septoria in 1935 (Sanderson 1963). Pasmo has been identified in all flax growing countries of the world. The disease was likely introduced into the United States when flaxseed was imported from Argentina and grown for research and other purposes (Brentzel 1926). In Canada, pasmo was first observed in Manitoba in 1940 (Sackston 1950). In New Zealand, pasmo was first discovered by Cunningham in 1931 in linseed crops (Sanderson 1963). In 1999, the fungus *Septoria linicola* was reported as a new species of Coelomycetes in Lithuania (Jovaišienė and Taluntytė 2000).

Pasmo symptoms on flax stems at crop maturity are characterized by tiny black pycnidia, which are the fruiting structures, containing the pycnidiospores of the fungus. Spores are exuded in slimy masses to form "tendrils", "spore-horns" or "cirti" (Sackston 1970). The pycnidia are lens-shaped,

about 63 to 126 µm in diameter, with small ostioles. They develop just under the epidermis of the leaves and stems, and the mycelium extends to the region of the bast-fiber cells (Brentzel 1926). The optimum temperature for fungal growth is 21°C, however, in culture, the fungus grows well from 17 to 29°C. At extreme temperatures of <5 or >32°C, there is very little fungal growth observed (Brentzel 1926). On artificial culture, the fungus grows very slowly, forming small, raised colonies circled by sparse white to dark olive gray mycelium. Thick, slimy, reddish-brown masses of conidia are produced in the center of the colony. The pycnidiospores are hyaline, sub-cylindrical, tapering slightly at the ends, straight, curved, 1.5-3 x 12-28 µm, and usually three to seven septate (Brentzel 1926). Sporulation can occur eight days after infection. Two distinct types of pycnidiospore germination were observed by Sackston (1970) in artificial culture. The terminal cells of pycnidiospores germinate and produce germ tubes that develop into hyphal threads, whereas, the intercalary cells produce secondary spores of the pathogen (Sackston 1970). In the teleomorph, perithecia develop on diseased flax stems and resemble black spherical dots. The asci are oblong, straight or hyaline. Ascospores are fusiform, generally 13-17 x 2.5-4 µm, hyaline, and one-septate (Brentzel 1926).

Pycnidiospores of *Septoria linicola* spread by rain, dew and insects (Christensen et al. 1953). Sackston (1970) observed that the spores are most often spread by humid air, rather than dry air. Pasmo can also be spread through infected flax seed (Brentzel 1926). Sanderson (1963), in New Zealand, observed that within six plots only five plants had cotyledon lesions; however, these plants were not diseased once their cotyledons senescenced. It was also determined that the origin of pasmo in this crop was from wind-blown spores from infected flax straw and stubble, rather than from a primary seed-borne source. The pathogen can persist in the seed for over a year and in crop residue for up to four years (Halley, 2007). The pycnidia overwinter on flax stubble and in

spring produce spores that cause the initial infection of both stems and leaves. Dense canopies may create a humid environment, which is favorable for disease development. *Septoria linicola* can be controlled on seed with thiram fungicide or a hot water treatment of 38°C for ten minutes (Halley 2007).

The disease appears on the above ground portions of flax plants such as the leaves, stems, flower buds and seed bolls. The symptoms mainly appear at later crop maturity stages. Flax is most resistant to pasmo between the cotyledon and the flowering stages (Brentzel 1926). The first symptoms appear and affect flax at the green boll ripening stage or at the early yellow ripening stage (Gruzdevienė et al. 2008). Symptoms of the disease first appear on leaves and produce brown to dark brown lesions. On the stem, small lesions usually first occur on the lower portions, but do not encircle the stem. Affected sections alternate with green bands of healthy tissue on the same stem. Depending on the susceptibility of the variety, these enlarge rapidly, extending both up and down the stem (Flor 1953). The disease can be identified by circular to oblong shaped lesions that change from green to yellow and eventually to dark brown in the center (Rashid 2003). The leaves dry and wither, and abundant small black pycnidia are produced in the older lesions.

As the crop ripens, the brown, infected lesions coalesce, and the stems become entirely brown (Flor 1953). Seed infected by the organism may exhibit a bluish-black tinge. Disease incidence is related to maturity of the crop (Sanderson 1963). If the pathogen attacks at maturity, the seed yield may not be materially reduced, but if the crop is attacked in early growth stages, the plant may be prematurely defoliated, the flowers blighted, and the bolls undeveloped. The stem becomes weakened and breaks readily, resulting in heavy boll-drop due to rain and wind. The seeds may be off-color and shrunken (Flor 1953). It has been reported that flax may be severely affected by

lodging, particularly when grown under above normal precipitation and high plant populations (Gubbels and Kenaschuk 1989).

Temperature and moisture conditions are very important factors for the development and spread of the disease in the field. High temperature (20 to 21°C) and high rainfall favor infection and disease spread. High disease severity was observed in low-lying areas of a pasmo infected field, where moisture was abundant, whereas, in more elevated and drier areas the disease was noticeably less severe (Brentzel 1926). Gruzdeviene et al. (2008), observed that the amount of precipitation during the growing season is one of the most important factors promoting pasmo spread; abundant rainfall in the second half of the flax growing season in particular promoted the spread of *S. linicola* in the field.

2.5. Losses and Epidemics

Pasmo has been identified in all flax growing countries of the world including Argentina, North America, Australia and New Zealand (Brentzel 1926; Sanderson 1963). In 1999, up to 40% pasmo severity was observed in flax fields surveyed in southern Manitoba and in eastern Saskatchewan (Rashid et al. 1999). When pasmo infections are severe, the disease reduces seed yield by as much as 60-70% (Rashid 2001). In 2002 and 2003, disease severity exceeded 15% and 20% of the stem tissue of each plant affected in North Dakota (Halley et al. 2004). Up to 30% disease severity was observed in Saskatchewan in 2011 (Rashid et al. 2012). In 2016, 23 flax fields in Manitoba and 58 flax fields in Saskatchewan were surveyed and 20-40% pasmo severity was observed in both provinces (Rashid et al. 2016). Severe pasmo infection can reduce seed oil content and also the iodine number of the oil (Ferguson et al. 1987). Pasmo also reduces the yield and quality of the fiber in infected stems. As a result, the market value of the straw from infested fields is reduced

(Ferguson et al. 1987). The effect of lodging was reported to be greater in flax than wheat (Vera et al. 2012), particularly when susceptible cultivars were grown under conditions of high precipitation or high plant densities (Gubbels and Kenaschuk 1989).

2.6. Host Resistance

Pasmo of flax is prevalent in western Canada and disease severity depends on many factors such as climatic conditions, variety, crop hygiene and the presence of pathogen inoculum (Mercer et al. 1991). Continuous use of fungicide may result in insensitivity in the pathogen population and poor protection against pathogens (Ma and Michailides 2005). Therefore, disease resistant cultivars are very important to reduce yield and quality losses. In North America, the majority of the early efforts for pasmo control were aimed at resistance breeding to improve flax (Fu et al. 2003). Screening of 153 flax lines, collected from 24 countries was conducted to identify pasmo-resistant flax at the All-Russian Flax Research Institute of Russia and in these lines, the average pasmo severity was 27 – 100% (Diederichsen et al. 2008). However, clear-cut pasmo resistance was not identified in North American flax lines. As all commercial fiber flax varieties lack resistance to *S. linicola,* other measures need to be taken to prevent infection of flax (Rashid 2003).

2.7. Management of pasmo

The benefit of fungicide use in crop production has long been acknowledged as it reduces crop losses due to disease. Systemic fungicide products were first used on cereal crops during the latter part of the 1960's (Russell 2005). The most common fungicides used to control Septoria diseases of cereals are triazoles, strobilurins, carboxamides, and chlorothalonil (Loughman and Thomas 1992; Fraaije et al. 2005; Vera et al. 2014).

As most flax cultivars are susceptible to pasmo, application of fungicides can reduce pasmo incidence and severity in flax, and increase yield (Halley et al. 2004; Rashid 2010). Fungicides are usually applied at full bloom, as the heaviest yield losses occur when flax plants are infected (inoculated) at this stage of plant development (Sackston 1950). In 2009, various single and multiple fungicide applications treatments were reported to reduce disease severity of pasmo by up to 50% under natural conditions in southern Manitoba and increase flax yields by 80% (Rashid 2010). Use of azoxystrobin with prothioconazole increased yield by 50% at Langdon, North Dakota (Halley et al. 2004). At Carrington, North Dakota yield increases were very high, almost 136% by applying azoxystrobin and 102% from pyraclostrobin (Halley et al. 2004). At Melfort, pasmo severity was reduced and significant yield increases were observed when pyraclostrobin fungicide was applied to flax in 2010, 2011 and 2012 (Vera et al. 2014).

There are also several cultural practices that can help to reduce pasmo. High plant density will contribute to humid conditions, which favors lodging and development of pasmo (Gubbels and Kenaschuk 1989). Therefore, recommended seeding rates should be followed to maintain appropriate plant densities. The most important control method of this stubble-borne disease is use of certified seed, early seeding and crop rotation with at least three years between flax crops (Halley et al. 2004).

2.8. Fungicide Insensitivity

In recent years, crop production has increased rapidly to supply global food demand. International trade and travel has also increased to distribute food worldwide. However, expansion of trade and travel has the potential to spread new plant pathogens around the world (Gossen et al. 2014).

Climate change may also influence plant pathogen spread (Rosenzweig et al. 2001). The use of fungicides has also increased to control these new and existing diseases (Gossen et al. 2014).

Although the need to increase agricultural productivity has led to the extensive use of pesticides, using the same fungicide repeatedly can develop fungicide tolerant or fungicide insensitive isolates (Fairchild et al. 2013). Until 1960, most agricultural fungicides were protectants and multi-site inhibitors, so despite their widespread use, development of resistance to these compounds was rare. However, since the introduction of systemic fungicides in the late 1960s, with specific modes of action, fungicide resistance in phytopathogenic fungi has become a major problem (Brent and Hollomon 2007). Fungicide insensitivity was first reported in the 1970s (Staub 1991). Thereafter fungicide insensitivity was reported in many pathogen populations to various fungicide products (Goodwin et al. 1998; Chang et al. 2007; Köycü 2012). For example, insensitivity to prochloraz was observed in *Pseudocercosporella herpotrichoides* (causal agent of eyespot in cereals) in New Zealand (King et al. 1986). In 1998, among 473 isolates of *Phytophthora capsica* (causes root, crown, and fruit rot on solanaceous and cucurbit hosts), 13% were insensitive to the fungicide mefenoxam (Lamour and Hausbeck, 2000). Fosetyl-aluminum insensitivity in *Bremia lactucae* (causes downy mildew of lettuce) was reported in California in 2004 (Brown et al. 2004).

A pathogen is fungicide resistant or insensitive when it has reduced sensitivity to a fungicide. Fungicide applications are less effective or ineffective against insensitive pathogens. Therefore, disease control failures may occur eventually (Ma and Michailides 2005). The primary factors influencing the development of fungicide insensitivity are genetic diversity of the pathogen, mutation of single or multiple genes, rapid production and dispersal of pathogen propagules, the mode of action of the fungicide, and the fungicide application rate and frequency (Ma and Michailides 2005; Brent and Hollomon 2007; Beckerman 2013). Fungicide resistance can also

occur through various mechanisms, such as an altered target site that reduces the binding of the fungicide, substitution of the target enzyme by synthesis of an alternative enzyme, metabolic breakdown of the fungicide, or reduced uptake of fungicide (Gisi et al. 2000). Fungicide insensitivity can be quantitative or qualitative. In quantitative insensitivity, the pathogens are sensitive only to high rates of fungicide, and in qualitative insensitivity, pathogen isolates become completely insensitive to the fungicide and disease control is not possible by using higher rates or more frequent fungicide applications (McGrath 2001; Gossen et al. 2014). If fungicide insensitivity is not recognized at an early stage, the sudden appearance of fungicide insensitivity in a pathogen population may result in failure of disease control, and consequently, serious crop losses (Dekker and Georgopoulos 1982).

For some 35 years the agricultural industry has faced problems arising from the development of resistance in fungal pathogens of crops, against the fungicides used to control them (Brent and Hollomon 2007). In western Canada, fungicide insensitivity was reported in many pathogen populations to different fungicide products (Daayf and Platt 2000; Gossen et al. 2001; Chang et al. 2007). For example, metalaxyl insensitive isolates of *Phytophthora infestans* were reported in British Columbia during 1992 and 1993 (Goodwin et al. 1998). Insensitivity to both thiabendazole (TBZ) and thiophanate-methyl (TPM) was observed in isolates of *Fusarium sambucinum* and *Helminthosporium solani* recovered from diseased potato tubers in Canada (Kawchuk et al. 1994). In 2007, reduced fungicide efficacy was observed in the field isolates of *Alternaria solani* to azoxystrobin in Manitoba (Peters et al. 2008). Pyraclostrobin insensitivity was observed in a population of *Didymella rabiei* obtained from Alberta in 2007 (Ahmed et al. 2014). Since the first identification of fungicide insensitivity, academic and government researchers, agrochemical

companies and crop advisers have analyzed the phenomenon and tried to find countermeasures against insensitive pathogens (Brent and Hollomon 2007).

2.9. Fluxapyroxad (Group 7 Fungicide)

Fluxapyroxad is a second generation carboxamide fungicide, developed by BASF Corporation in 2012 to control a wide spectrum of diseases (Li et al. 2014). It is a succinate dehydrogenase inhibitor (SDHI). The SDHI fungicides include seven chemical groups comprised of: furan-carboxamide, oxathiin-carboxamide, pyrazole-carboxamide, pyridine-carboxamide, and thiazole-carboxamide, and the phenyl-benzamides and the pyridinyl-ethylbenzamides. The SDHI fungicides have been assigned the Fungicide Resistant Action Group (FRAC) activity group code number 7 (McKay et al. 2011; FRAC 2017).

The mode of action of fluxapyroxad is inhibition of succinate dehydrogenase in complex II of the mitochondrial respiratory chain, which results in inhibition of spore germination, germ tube formation and mycelial growth of the target fungal species (Avenot and Michailides 2010). The first generation of SDHIs were narrow spectrum fungicides known as carboxin and oxycarboxin, introduced in 1966. These very effectively control basidiomycetes and are used as a seed treatment against smuts (Sierotzki and Scalliet 2013; Avenot and Michailides 2010). The second generation SDHIs were boscalid, fluxapyroxad, penthiopyrad, isopyrazam and fluopyram. These fungicides are effective against a wide range of fungal pathogens of various crops (Avenot and Michailides 2010; Amiri et al. 2014).

Based on single-site mutations of the gene encoding the succinate dehydrogenase enzyme, the risk is medium to high that fungal pathogens will become insensitive to SDHI fungicides. High rates of insensitivity were reported against some SDHI fungicides (Avenot and Michailides 2010), e.g,

boscalid insensitive isolates of *Didymella bryoniae* were found in gummy stem blight infected watermelon. Many isolates of *Botrytis cinerea, Corynespora cassiicola* and *Alternaria alternata* have also been reported as insensitive to boscalid (Stammler et al. 2007; Miyamoto et al. 2009; Avenot and Michailides 2010). The SDHI fungicides should be mixed with or applied in a rotation with other fungicides to prolong the effectiveness of these fungicides and to reduce the risk of development of fungicide insensitivity in the pathogens (Sierotzki and Scalliet 2013).

2.10. Pyraclostrobin (Group 11 Fungicide)

Pyraclostrobin is an important fungicide that belongs to the strobilurin group (Group 11) and is known as a Quinone outside inhibitor (QoI). The other fungicides in this group are azoxystrobin, coumoxystrobin, dimoxystrobin, enoxastrobin, famoxadone, fenamidone, fluoxastrobin, mandestrobin, picoxystrobin, pyraclostrobin, pyribencarb and trifloxystrobin (FRAC 2017). The discovery of QoI fungicides was inspired by a group of natural fungicidal derivatives of β-methoxyacrylic acid, such as strobilurin A and oudemansin A, produced by the basidiomycetes *Strobilurus* sp., *Mycena* sp., and *Oudemansiella* sp. (Kraiczy et al. 1996). Strobilurin A, the first QoI molecule, was obtained from liquid cultures of *Strobilurus tenacellus* in 1977 (Anke et al. 1977) and the mode of action of this group of compounds was elucidated in 1981 (Fernández-Ortuño et al. 2008). The strobilurin group consists of nine different chemical groups including methoxy-acrylates, methoxy-acetamide, methoxy-carbamates, oximino-acetates, oximino-acetamides, oxazolidine-diones, dihydro-dioxazines, imidazolinones and benzyl-carbamates (FRAC 2017). In 1996, the first strobilurin was sold to control diseases of cereals in temperate regions (Bartlett et al. 2001). The strobilurins (QoIs) quickly became one of the most important of agricultural fungicides, accounting for over 20% of the global fungicide market within the first ten years of their commercial offering (Fernández-Ortuño et al. 2008).

Strobilurins function as mitochondrial respiration inhibitors of fungi. They bind at the Qo site of the cytochrome bc1 enzyme complex (Complex III) and block electron transfer between cytochrome b and cytochrome c1, which results in disruption of the energy cycle within the fungus by stopping ATP production (Bartlett et al. 2001). These fungicides inhibited both spore germination and mycelial growth (Reuveni and Sheglov 2002; Balba 2007). Application of pyraclostrobin (Headline EC®) was very effective in reducing the severity of pasmo and lodging in flax (Vera et al. 2014). Strobilurin fungicides also produce greening and delayed senescence in plants, and have been reported to enhance yields of field crops in the absence of disease (Weisz et al. 2011).

Strobilurins are very effective fungicides against many pathogens due to their highly specific mode of action, which has reduced the impact of fungicides on health and the environment; however, this mode of action has also proven to be a serious weakness, since a significant number of target pathogens have developed insensitivity to QoIs (Fernández-Ortuño et al. 2008). A few years after registration, QoI fungicide insensitivity was reported in wheat pathogens such as *Blumeria graminis, Microdochium nivale, Microdochium majus,* and *Mycosphaerella graminicola* (Sierotzki et al. 2000; Amand et al. 2003; Fraaije et al. 2005; Walker et al. 2009). In addition, many other fungal pathogens such as *Botrytis cinerea, Alternaria alternata, Colletotrichum graminicola, Pyricularia grisea, Pythium aphanidermatum, Podosphaera fusca, Pyrenophora teres* and *Pseudoperonospora cubensis* are also reported to be insensitive to QoIs (Ishii et al. 2001; Gisi et al. 2002; Kim et al. 2003; Avila-Adame et al. 2003; Sierotzki et al. 2007; Banno et al. 2009).

Plant pathogens have developed insensitivity to strobilurin fungicides due to the mutations in the cytochrome b (cyt b) gene; the common genetic mutations are: (i) glycine changes to alanine at

position 143 (G143A), and (ii) phenylalanine changes to leucine at position 129 (F129L) (Kim et al. 2003; Pasche et al. 2005; Sierotzki et al. 2007). The mutation of G143A results in 1000 to 1300-fold higher EC_{50} values in *P. grisea*, whereas, the F129L mutation results in 30 to 140-fold higher EC_{50} values (Kim et al., 2003). Some isolates have been detected with high EC_{50} values without the G143A mutation (Blixt et al. 2009). This indicates that the fungus has developed an alternative respiratory pathway and mechanism to survive in presence of strobilurins *in vitro* (Ziogas et al. 1997; Wise et al. 2008). Salicylhydroxamic acid (SHAM) has been used to prevent the fungus from accessing the alternative respiratory pathway (Ziogas et al. 1997; Vincelli and Dixon 2002; Sierotzki et al. 2007). It has been also reported that SHAM has no effect on conidial germination (Wise et al., 2008).

2.11. Fungicide Sensitivity Assays

Monitoring sensitivity to systemic fungicides has become common over the past 20 years and is considered an important part of anti-resistance strategies (Brent 1992). Loss of sensitivity to a fungicide is often assessed by the reduction in mycelial growth or spore germination of the target fungus on agar medium amended with a range of concentrations of fungicide under specific growth conditions (Staub and Stozzi 1984; Köller et al. 1997). For many fungi, spore germination is the most sensitive stage to fungicides, although many fungicides strongly inhibit mycelial growth as well (Slawecki et al. 2002). Measuring spore germination and mycelial growth are typically used to determine the impact of survival of conidia during storage, to compare fungal strains, and to determine the fungicide sensitivity of many pathogens (Milner et al. 1991; El Ghaouth et al. 1992; Avenot et al. 2008; Lonergan et al. 2015). However, these techniques are also very laborious, time-consuming, and the petri plates filled with agar media occupy considerable space (Stevens 1984). Additionally, many fungi cannot be cultured on artificial media. For example, downy mildews,

powdery mildews and rusts cannot easily be cultured on artificial media (Chapman et al. 2011). In the spore germination assay, germination is considered to have occurred based on a specific minimum length of the spore germ-tube, but sometimes the rapid growth of germ tubes from germinating conidia obscure other slower germinating conidia. As a result, this assay is only applicable to the early stages of spore germination (Milner et al. 1991). Many other methods have been developed to measure fungal growth inhibition. However, most of the methods have disadvantages. For example, the disc-plate diffusion assay, which is a semi-quantitative assay measures only the degree of inhibition, and in-situ concentrations of the active ingredients cannot be measured correctly (Broekaert et al. 1990).

The microplate reader assay has several advantages over the spore germination and radial growth assays for the measurement of fungal growth inhibition. It allows fast and easy handling of large numbers of samples with the adapted multichannel pipetting equipment, as the assay is carried out in standard microplates. The assay requires minimal amounts of test substances and can be adapted to non-sporulating fungi, in which case mycelial fragments are used for inoculum. Finally, this method is very useful for rapid, accurate, non-destructive and computerized data collection (Broekaert et al. 1990). The use of the microplate reader is becoming increasingly popular for measuring the turbidity of bacterial cultures because of its ability to analyze up to 96 samples in a few seconds. This technology has been used as a reliable tool to measure the insensitivity of various fungi including; *Phycomyces blakesleeanus*, *Fusarium culmorum*, *Septoria nodorum*, *Trichoderma hamatum*, and *Rhizoctonia solani* (Broekaert et al. 1990). The microtiter method was used to evaluate sensitivity of metalaxyl, mancozeb, benomyl, and thiophanate-methyl fungicides to *Verticillium dahliae* isolates (Rampersad 2011). Sensitivity of *Botrytis squamosa* to different classes of fungicides (mancozeb and iprodione) was determined using this method (Tremblay and

Talbot 2003). Baseline sensitivity of boscalid for 137 isolates of *Botrytis cinerea* isolated from different host plants from around the world was determined using the microtiter method (Stammler and Speakman 2006). Beyer et al. (2011), measured the sensitivity of *Septoria tritici* against different types of fungicides including trifloxystrobin, folpet, chlorothalonil, propiconazole, prochloraz, tebuconazole, epoxiconazole, and prothioconazole using this method.

Sound crop production practices require a fungicide anti-insensitivity strategy (Staub 1991). To reduce the risk of fungicide insensitivity in the pathogen population and loss of efficacy of important chemical groups, fungicides with different modes of action should be rotated in sequence with one another, or used in mixtures, as this should reduce the risk that fungicide insensitivity will develop (Skylakakis 1981). Monitoring the effectiveness of the fungicide to control the disease in question and the sensitivity of pathogen populations is also essential to forewarn farmers and industry of fungicide insensitivity.

CHAPTER 3

3. Effect of fungicides and application timings on pasmo severity and flaxseed yield and quality

3.1. Introduction

Among the diseases that have the potential to cause damage to flax every year, pasmo is the most important. The disease was first identified in Argentina in 1911, and is caused by the pathogen *Septoria linicola* (Speg.) Garassini (Brentzel 1926). Pasmo is a residue-borne disease and the pathogen can persist up to four years in the crop residue (Halley 2007). It reduces both the quality and quantity of flax. The symptoms of pasmo occur on the leaves, stems and seed bolls, resulting in premature ripening and shriveled seeds (Flor 1953).

Pasmo has been identified in all flax growing countries of the world. It is prevalent in western Canada, and most flax cultivars are susceptible to this disease. Pasmo severity (proportion of the stem discolored by pasmo symptoms at maturity) was as much as 39% at North Dakota in 2002 (Halley et al. 2004). In severely affected fields, up to 30% disease severity was observed in Saskatchewan in 2011 (Rashid et al. 2012).

Though plant breeders have collected germplasm of flax from around the world and have attempted to breed pasmo resistant varieties, none have been developed (Brutch et al. 2001; Saskatchewan Flax Development Commission 2017). Thus, farmers depend on cultural practices and the application of fungicides to control pasmo. Many researchers have reported that fungicides can reduce pasmo severity and increase seed yield (Halley et al. 2004; Rashid 2010; Vera et al. 2014). However, currently only two fungicides, pyraclostrobin (Headline® EC, Group 11 fungicide) and

combination of pyraclostrobin and fluxapyroxad (Priaxor®, Groups 7 and 11) are registered for pasmo control in Canada. Fluxapyoxad (Xemium®) is a Group 7 fungicide and used to control many crop diseases. Pyraclostrobin has been used to control pasmo since 2009 and was the only product registered for pasmo in western Canada until registration of Priaxor in 2015 (BASF 2009, BASF 2015). Application of the same fungicide repeatedly may lead to development of fungicide insensitivity in the pathogen population. Thus, it is very important to evaluate the efficacy of fungicides with different mode of actions, which will help flax growers to choose appropriate fungicides and use those fungicides alternately or in tank-mixes.

Proper timing of fungicide application often is the key factor that determines success or failure of chemical control (Mueller et al. 2009). In flax, the greatest yield losses occur when the disease infects at the flowering stage (Sackston 1950). Thus, fungicide application during the flowering stage of flax may reduce pasmo severity and yield loss (Ferguson et al. 1987). However, there is very little information available on fungicide application timing for pasmo, which is needed for better management of this disease.

The objectives of this study were: 1) to evaluate the effect of different fungicide products (pyraclostrobin, fluxapyroxad and the combination of these two products) on pasmo severity and seed yield and quality, 2) to determine the appropriate plant growth stage to apply fungicide, and 3) to determine whether a single dose of fungicide would adequately control pasmo. Answering these questions will help to determine the most effective fungicide control measures for this disease and help the flax grower to make improved fungicide application decisions.

3.2. Materials and Methods:

Experimental locations and field trials:

Field experiments were conducted under a no-till seeding system from 2014 to 2016 at four locations in western Canada: Brandon, MB (49°52'04.0"N 99°58'22.2"W); Saskatoon, SK (52°09'27.5"N 106°32'02.0"W); Melfort, SK (52°49'16.3"N 104°36'29.5"W) and Vegreville, AB (53°30'16.6"N 112°06'08.0"W). Brandon and Melfort are in the black soil-climatic zone. This is the most fertile soil zone with a high level of soil organic matter (SOM) (typically 4.5 to 5.5%, up-to 10%), cool temperatures and moderate moisture conditions. The black soil zone is generally cooler than the brown and dark-brown soil zones and sub-humid. Saskatoon is in dark brown soil-climatic zone and the SOM soil content is approximately 3.5 to 4.5% with a sub-arid to semiarid climate. Vegreville is in the dark grey soil-climatic zone with an SOM level of 3.5 to 4.5% and the climate is sub-humid (Canadian Agricultural Services Coordinating Committee, 1998).

The experiments at each site-year were organized in a randomized complete block design (RCBD) with four replicates. In all years, wheat was grown previous to the experimental plots at Brandon and Melfort, canola was the previous crop at Saskatoon and barley at Vegreville. The plot sizes were 4.2 × 10 m at Brandon, 2 × 8 m at Saskatoon, 4.2 × 7.3 m at Melfort and 2 × 6 m at Vegreville. Fertilizer application rates were calculated for yield targets appropriate to each location minus soil residual determined from soil tests. Nitrogen (N) fertilizer as urea was side-banded at seeding to achieve 120 kg N ha^{-1} (applied fertilizer plus residual N); phosphorus (P) fertilizer as mono-ammonium phosphate (MAP) was applied with seed and varied among site-years, from 20–27 kg P_2O_5 ha^{-1}. The cultivar CDC Bethune was chosen for this experiment as it is the most widely-grown flax cultivar in western Canada. The seeding rate was 60 kg ha^{-1} (1000 seeds m^2) and seeding date varied from the 25th of May to the 5th of June among site-years. Plant density was

measured two weeks after seedling emergence by counting the number of seedlings in two 1-m rows length per plot. Herbicides were applied to control weeds as necessary at each site. To increase the risk of disease at each site (except at Vegreville), approximately 2 to 3 kg of pasmo infected flax residue from the previous year was spread on each plot by hand in all three years before the flowering stage (BBCH 39) (Meier, 2001).

Three commercially available fungicides, pyraclostrobin, fluxapyroxad, and the combination of these products, plus a control treatment (no fungicide) were applied to determine their effect on pasmo severity, and flax seed yield and quality (Table 1). All fungicides were manufactured by BASF Corporation.

Fungicides were applied at the early-flowering stage, 7-10 days after flower initiation when 50 - 75% of the plants had at least one flower (BBCH 61); the mid-flowering stage, 7-10 days after the first application (BBCH 65) and at both timings.

Table 3.1: Trade name, active ingredients, application rate of fungicides and the water volume used with the fungicide in the field

Trade name	Active ingredient (a.i.)	Application rate (g a.i. ha^{-1})	Water volume (L ha^{-1})
Headline® EC	pyraclostrobin	100	100
Xemium®	fluxapyroxad	50	100
Priaxor®	pyraclostrobin + fluxapyroxad	150	100

Disease assessment and data collection:

Maturity was determined as the time when 75% of the bolls in a plot had turned brown (BBCH 89); disease severity was assessed at this stage. From each plot, 25 pasmo infected flax stems were collected and disease severity was rated using the Horsfall-Barratt scale (Horsfall and Barratt, 1945). The Elanco conversion table was then used to convert Horsfall-Barratt rating numbers into estimated mean percentages (Redman et al. 1964).

Flax was harvested mechanically from each plot by a small-plot combine harvester, excluding the outside rows of each plot. Samples were air-dried for approximately 48 hours and then cleaned using an automated seed cleaner. After cleaning the seed, yield and test weight (TW) of flaxseed from each plot were determined. Subsequently, 200 seeds were weighed to calculate thousand seed weight (TSW) and a sub-sample of seed (10 g) from each plot was used to determine seed oil content.

Temperature and precipitation values were collected from Environment Canada for each site-year for the growing period from May to August.

Data analysis:

Levene's test was performed to determine the homogeneity of variance of the data among site-years. To determine the treatment effects, i.e. fungicide product and timing of application, on disease severity, yield, TSW, TW and oil content of flaxseed, analysis of variance was performed using proc MIXED in the Statistical Analysis System, SAS 9.4 (SAS Institute Inc., Cary, NC, USA) software with fungicide treatments and spray timings as fixed variables; and site-years and replicates as random variables. Contrast analysis was used to determine differences between the sprayed and unsprayed treatments and among fungicides. Fungicide and timing treatment means

were separated using Tukey's test and declared significant at $P \leq 0.05$. When the interaction effects of fungicide and application timing were determined, the unsprayed control was excluded from the analysis, as inclusion resulted in an unbalanced data set.

3.3 Results:

Climate condition:

Precipitation amounts at Saskatoon and Melfort were slightly higher than long term normal during the study period (Table 3.2). At Brandon in 2014, 2016 and at Vegreville in 2016 precipitation was higher than the long-term normal. Precipitation was very limited during the growing season of 2015 at both Brandon and Vegreville, and the 2014 started off very dry at Vegreville.

Table 3.2 Precipitation (mm) in the growing seasons (May, June, July, August and September) of 2014, 2015, and 2016, and long-term normal (LTN) from 1981 to 2010 at Saskatoon, Melfort, Brandon and Vegreville

Site	Year	May	June	July	August	September	Total
Saskatoon	2014	61.1	94.8	44.5	18.5	10.7	229.6
	2015	6.3	20.2	85.1	58.2	50.8	220.6
	2016	42.6	46.8	76.9	70.2	24.7	261.2
	LTN	36.5	63.6	44.4	38.1	18.8	201.4
Melfort	2014	24.3	167.3	38.8	57.9	9.4	297.7
	2015	7.1	54.8	150.0	57.4	70.0	339.1
	2016	16.8	53.2	129.0	80.8	41.3	320.8
	LTN	42.9	54.3	76.7	52.4	38.7	265.0
Brandon	2014	75.7	211.2	13.9	53.8	37.7	392.3
	2015	44.8	34.3	51.8	50.4	32.9	214.2
	2016	56.4	106.4	98.0	48.6	90.2	399.6
	LTN	56.5	79.6	68.2	65.5	41.9	311.7
Vegreville	2014	0.0	0.0	0.0	16.2	25.5	41.7
	2015	16.0	35.9	72.7	26.8	81.6	233.0
	2016	109.2	65.4	94.7	50.8	31.5	351.6
	LTN	37.1	60.6	76.3	51.8	40.5	266.3

At Saskatoon, mean temperatures during the growing season were generally below normal in 2014 and above normal in both 2015 and 2016 (Table 3.3). At Melfort mean temperatures were about normal in 2014 and 2015, however above normal in 2016. Mean temperatures were nearly normal at Brandon and above normal at Vegreville in all three years.

Table 3.3 Mean temperatures (°C) in the growing seasons (May, June, July, August and September) of 2014, 2015, and 2016, and long-term normal (LTN) from 1981 to 2010 at Saskatoon, Melfort, Brandon and Vegreville

Site	Year	May	Jun	Jul	Aug	Sep	Mean
Saskatoon	2014	10.1	14.1	18.3	17.9	12.4	14.6
	2015	11.3	18.1	20.1	18.6	12.9	16.2
	2016	14.7	18.5	19.3	16.9	11.8	16.2
	LTN	11.8	16.1	19.0	18.2	12.0	15.4
Melfort	2014	10.0	14.0	17.5	17.6	11.9	14.2
	2015	9.9	16.4	17.9	17.0	11.9	14.6
	2016	13.6	17.1	18.1	16.3	12.0	15.4
	LTN	10.7	15.9	17.5	16.8	10.8	14.3
Brandon	2014	11.0	15.9	18.4	18.3	12.8	15.3
	2015	10.3	17.2	19.7	18.1	14.1	15.9
	2016	13.0	17.0	18.6	17.5	12.7	15.8
	LTN	11.4	16.6	19.2	18.2	12.2	15.5
Vegreville	2014	9.2	14.7	18.4	16.9	11.0	14.0
	2015	10.1	16.3	18.0	16.6	10.3	14.3
	2016	12.0	16.8	18.1	16.8	11.0	14.9
	LTN	10.3	14.4	16.6	15.6	10.1	13.4

Disease severity:

The effect of fungicide products on pasmo severity was significant at all locations in all three years, except at Vegreville in 2014 and 2015, where disease symptoms were not observed (Table 3.4). The effect of fungicide application timing on disease severity was significant at most locations, except at Melfort in 2014 and at Saskatoon in 2014 and 2015. The fungicide and application timing

interaction were not significant at any site-year. Effects of fungicide and application timings were significant in the combined data analysis of all site-years.

Table 3.4: Probability values of fungicide, application timing and their interaction for pasmo disease severity at four locations over three years; and combined data analysis of all site-years

		***P* Value**		
		2014	**2015**	**2016**
Brandon	**Fungicide (F)**	0.0022**	0.0033**	<0.0001***
	Time (T)	0.0445*	0.0004***	<0.0001***
	F × T	NS	NS	NS
Melfort	**Fungicide (F)**	<0.0001***	<0.0001***	<0.0001***
	Time (T)	NS	0.0023**	0.0023**
	F × T	NS	NS	NS
Saskatoon	**Fungicide (F)**	0.0005***	<0.0001***	<0.0001***
	Time (T)	NS	NS	0.0060**
	F × T	NS	NS	NS
Vegreville	**Fungicide (F)**			0.0170*
	Time (T)	ND	ND	0.0025**
	F × T			NS
Combined	**Fungicide (F)**		<0.0001***	
	Time (T)		<0.0001***	
	F × T		NS	

Note: NS = non-significant, ND = no data as pasmo did not occur at Vegreville in 2014 or 2015. For the F × T interaction, the unsprayed control was excluded from the analysis; * P < 0.05, ** P < 0.01 and *** P <0.001.

At Brandon, fungicide application reduced disease severity compared to the unsprayed control in 2014, 2015, and 2016 (Fig. 3.1). In 2014, the disease severity of the unsprayed control treatment was 56% and was reduced to approximately 10% by pyraclostrobin and fluxapyroxad+pyraclostrobin, but only reduced to 30% by fluxapyroxad. In 2015, fluxapyroxad and fluxapyroxad+pyraclostrobin were more effective than pyraclostrobin, however, in 2016 fluxapyroxad+pyraclostrobin was more effective than pyraclostrobin and fluxapyroxad alone. In

2015, disease severity of the unsprayed control treatment was 56% and application of fluxapyroxad+pyraclostrobin reduced disease severity to 5%, followed by fluxapyroxad, 7% and pyraclostrobin, 16%. In 2016, disease severity was 74% in the unsprayed control and reduced to 12% by the application of fluxapyroxad+pyraclostrobin, followed by pyraclostrobin, 31% and fluxapyroxad, 37%.

At Melfort, all fungicides reduced disease severity compared to the unsprayed control in all three years. However, no difference was observed among the fungicides in 2014 and 2015 (Fig. 3.1). In 2016, disease severity of the unsprayed control treatment was 95% and fluxapyroxad+ pyraclostrobin reduced the disease severity to 12%, followed by pyraclostrobin, 30% and fluxapyroxad, 70%.

At Saskatoon, the effect of fungicides was similar in all three years (Fig. 3.1). All fungicides decreased disease severity compared to the unsprayed control. In 2014, disease severity was, approximately 37% at this site. The application of fluxapyroxad+pyraclostrobin reduced disease severity to 2%, followed by pyraclostrobin, 6% and fluxapyroxad, 19%. Contrast analysis between disease severity of the unsprayed control and fluxapyroxad indicated that the difference was not significant in 2015 or 2016. In these years, no difference was observed between the effect of pyraclostrobin and fluxapyroxad+pyraclostrobin on pasmo severity. In 2015, pasmo severity in the unsprayed control was 86%. Fluxapyroxad+pyraclostrobin reduced disease severity to 56%, followed by pyraclostrobin, 42%. In 2016, disease severity of the unsprayed control plot was also 86% and application of fluxapyroxad+pyraclostrobin reduced disease severity to 12%, followed by pyraclostrobin, 20%.

At Vegreville, pasmo was observed only in 2016 and disease severity was also much lower compared with the all other sites (Fig. 3.1). All fungicides reduced disease severity compared to

the unsprayed control in this year. The disease severity of the unsprayed control was approximately 41%. The lowest disease severity observed was that for fluxapyroxad+pyraclostrobin (15%) and fluxapyroxad (16%), followed by pyraclostrobin (21%).

The combined results of all site-years (except Vegreville 2014 and 2015 as no disease severity was observed in these site-years) indicated that all fungicides reduced disease severity compared to the unsprayed control. Among the three fungicides, the reduction in disease severity was similar for pyraclostrobin and fluxapyroxad+pyraclostrobin. Fluxapyroxad had the least effect on disease severity compared to pyraclostrobin and fluxapyroxad+pyraclostrobin.

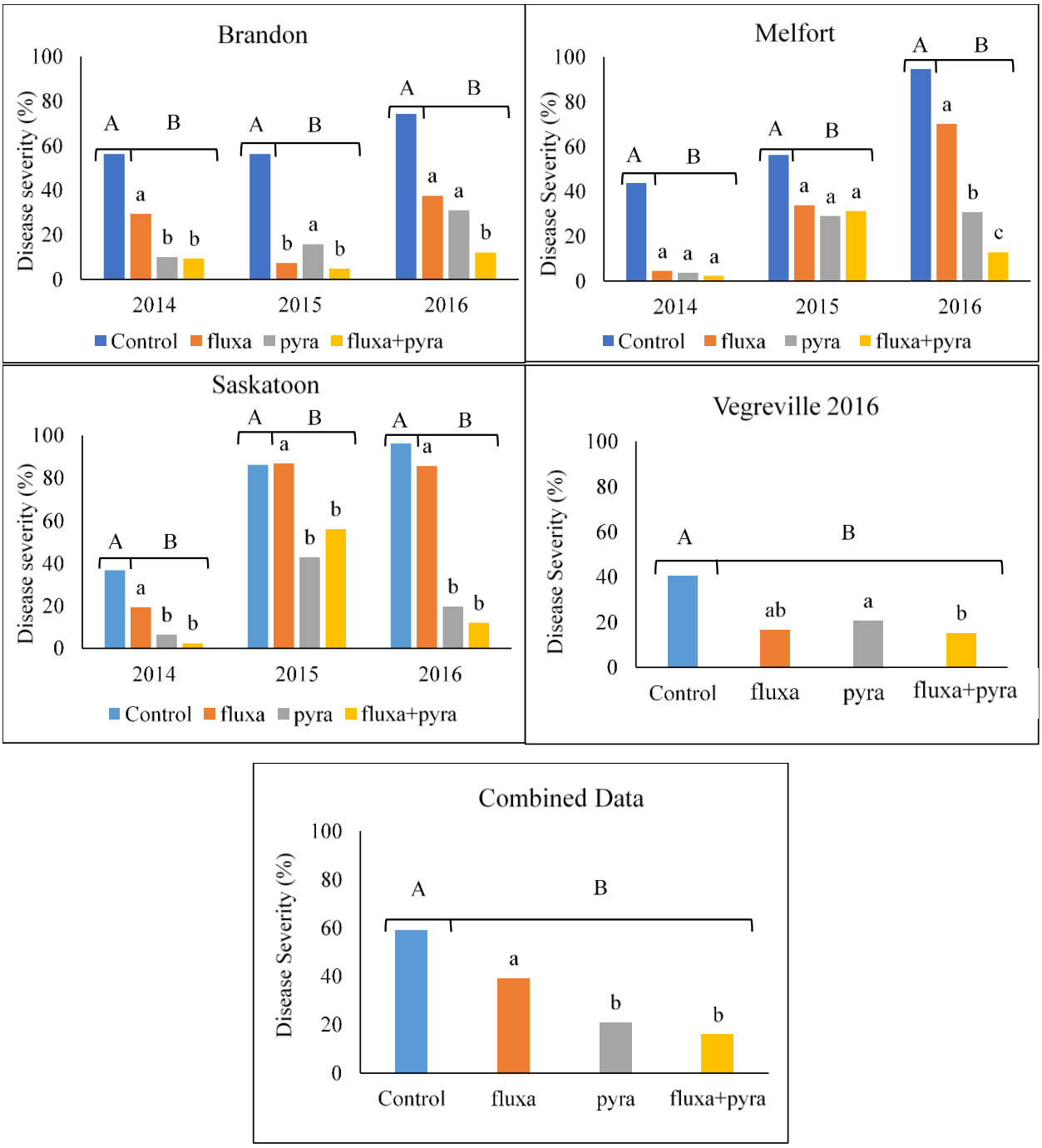

Fig 3.1. Effect of fluxapyroxad (fluxa), pyraclostrobin (pyra) and fluxapyroxad+pyraclostrobin (fluxa+pyra) on pasmo severity of flax compared with the unsprayed control at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015 and 2016, and all site-years combined. The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide products at the 5% level of significance, using Tukey's post hoc test

Fungicide application timing had an effect on disease severity at Brandon in all three years (Fig. 3.2). In 2014, no difference in disease severity was observed between early- and mid-flowering stage fungicide applications. However, fungicide application at both flowering stages reduced disease severity more than the fungicide applied at mid-flowering stage. In 2015, disease severity of 17% was observed when fungicide was applied at the early-flowering stage. The application of fungicide at both flowering stages (early + mid-flowering) reduced disease severity to approximately 3%, followed by the application at the mid-flowering stage, 8%. In 2016, application of fungicide at both flowering stages reduced disease severity more than a single application at either early- or mid-flowering stage. No difference was observed between the effect of fungicide applied at early- and mid-flowering stages.

At Melfort, fungicide application reduced pasmo severity compared to the unsprayed control in all years; however, the effect was greatest in 2014, although disease severity of the unsprayed check was not as great in 2014 as it was in 2015 or 2016. No difference in disease severity was observed among the fungicide application timings treatments in 2014 (Fig. 3.2). In 2015, disease severity of the unsprayed control was 56%. The application of fungicide at both flowering stages reduced the disease severity to 23%, followed by a single application at mid-flowering stage (30%), which was less than at early-flowering stage (40%). In 2016, disease severity of 40 to 45% was observed when fungicide was applied at the early- or mid-flowering stages and 28% by fungicide application fungicide at both stages.

Timing of fungicide application had an effect on disease severity at Saskatoon in 2014 and 2015 compared with the unsprayed control, however, no difference was observed among the timings in these years (Fig. 3.2). In 2016, fungicide application at mid and both flowering stages had a greater reduction in disease severity than application at the early-flowering stage.

At Vegreville in 2016, fungicide application timing had an effect on disease severity compared with the unsprayed control (Fig. 3.2). Fungicide application at both flowering stages reduced disease severity to a lower level than single applications at early- or mid-flower.

Combined data analysis of all site-years (except Vegreville 2014 and 2015 as no disease severity was observed in these site-years) indicated that there was no difference between the effect of fungicide application at early- and mid-flowering stage for disease severity (Fig. 3.2). Disease severities of approximately 27% and 30% were observed when fungicide was applied at mid-flowering stage and early flowering stage, respectively. Fungicide application at both flowering stages reduced the disease severity to 18%.

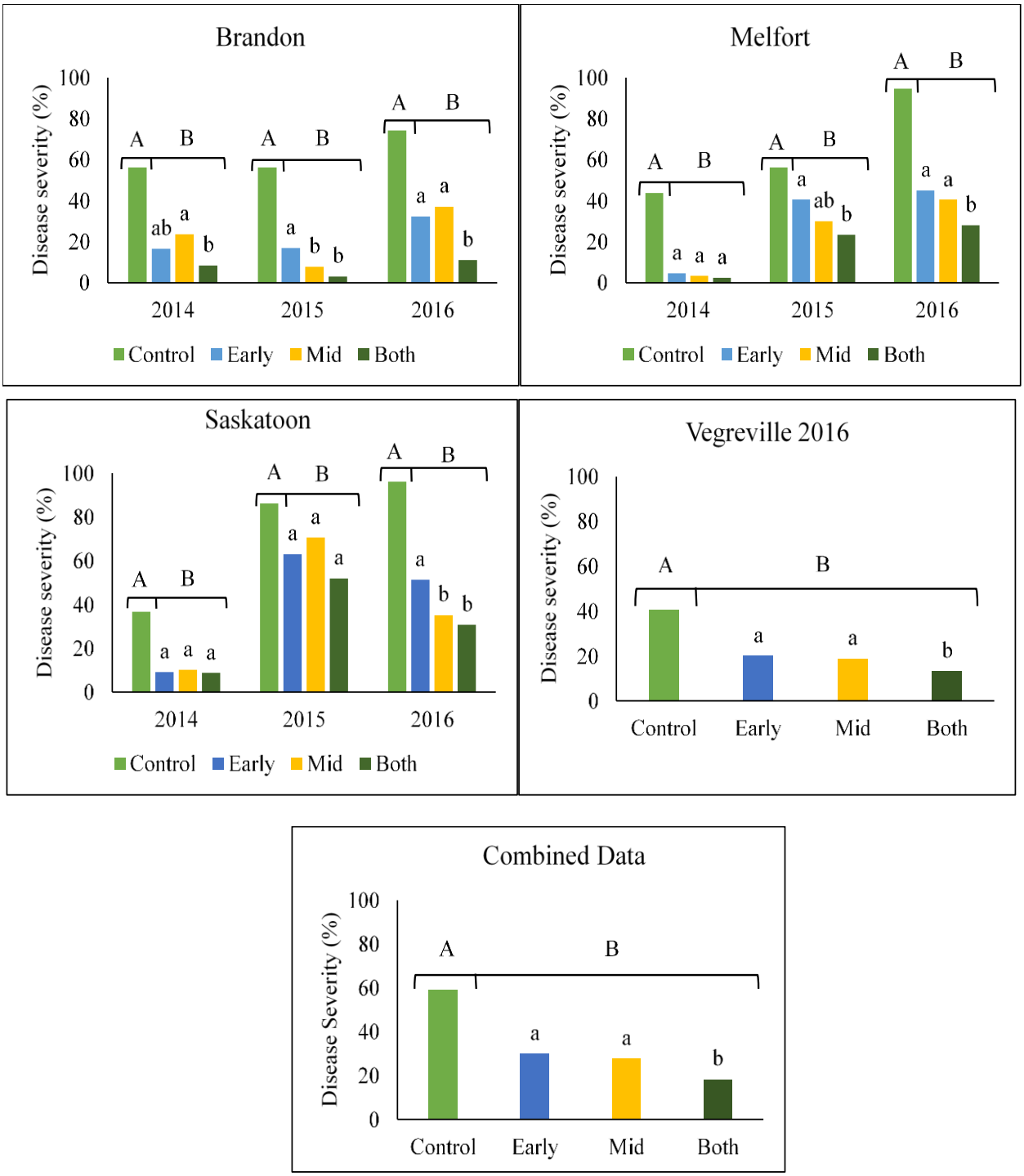

Fig 3.2. Effect of fungicide application timings on pasmo disease severity of flax at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015 and 2016, and all site-years combined. The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide products at the 5% level of significance using Tukey's post hoc test

Crop maturity:

In 2014, application of fungicide affected crop maturity at Melfort, Saskatoon and Vegreville, but not at Brandon (Table 3.5). In 2015, fungicide application had an effect on crop maturity only at Melfort, and in 2016, only at Brandon. Fungicide product was significant in the combined analysis of all site-years.

Fungicide application timing had an effect on maturity only at Brandon in 2016 and at Melfort in 2014 and 2015 and in the combined analysis of all site-years. The interaction of fungicide product and application timing was not significant at any site-year, or in the combined analysis.

Table 3.5: Probability values of fungicide product, application timing and their interaction for flax maturity at four locations and three years; and combined data analysis of all site-years

		P Value		
		2014	**2015**	**2016**
Brandon	**Fungicide**	NS	NS	0.0009***
	Time	NS	NS	<0.0001***
	F × T	NS	NS	NS
Melfort	**Fungicide**	<0.0001***	0.0015**	NS
	Time	<0.0001***	<0.0001***	NS
	F × T	NS	NS	NS
Saskatoon	**Fungicide**	0.0003***	NS	NS
	Time	NS	NS	NS
	F × T	NS	NS	NS
Vegreville	**Fungicide**	<0.0001***	NS	NS
	Time	NS	NS	NS
	F × T	NS	NS	NS
Combined	**Fungicide**		<0.0001***	
	Time		<0.0001***	
	F × T		NS	

Note: NS = non-significant. For F × T effect, unsprayed control data was excluded from the analysis; * P < 0.05, ** P < 0.01 and *** P <0.001.

At Brandon, the combined effect of fungicides on crop maturity was not different from the unsprayed check in any year (Fig. 3.3). In 2016, there was an effect of fungicide product, fluxapyroxad and fluxapyroxad+pyraclostrobin had similar effects on crop maturity, and delayed crop maturity approximately 3 to 4 days longer than pyraclostrobin, and by approximately 10 - 11 days compared with the unsprayed control.

At Melfort, application of fungicides delayed crop maturity in 2014 (Fig. 3.3); fluxapyroxad and fluxapyroxad+pyraclostrobin delayed crop maturity almost five days, and pyraclostrobin by three days, compared with the unsprayed control. In 2015, application of fungicides delayed crop maturity approximately 4 to 6 days compared with the unsprayed control. Maturity was delayed almost 6 days by the application of fluxapyroxad+pyraclostrobin compared with the unsprayed control, followed by fluxapyroxad, 5 days and pyraclostrobin, 4 days. In 2016, application of fungicide delayed maturity compared to the unsprayed control. Application of pyraclostrobin delayed the crop maturity almost 7 days, followed by fluxapyroxad, 5 days and fluxapyroxad+pyraclostrobin, 2 days compared to the unsprayed control.

At Saskatoon and Vegreville, fungicide delayed crop maturity compared with the unsprayed control only in 2014 (Fig. 3.3). At Saskatoon, fluxapyroxad+pyraclostrobin delayed maturity by 14 days compared with the unsprayed control. Pyraclostrobin and fluxapyroxad alone delayed crop maturity by approximately 5 to 6 days compared with the unsprayed control. At Vegreville, the difference in crop maturity between the unsprayed control and fluxapyroxad+pyraclostrobin was only 3 days. Contrast analysis indicated that there was no difference between the effect of pyraclostrobin and fluxapyroxad and the unsprayed control in crop maturity.

Combined data analysis of all site-years indicated that application of fungicide products delayed crop maturity compared with the unsprayed control (Fig. 3.3). Among the three fungicides,

fluxapyroxad+pyraclostrobin delayed crop maturity by 5 days compared with the unsprayed control, followed by fluxapyroxad, 4 days and pyraclostrobin, 3 days.

At Brandon, fungicide application timing had no effect on crop maturity in 2014 or 2015 (Fig. 3.4). In 2016, fungicide application at early-flowering stage delayed the crop maturity by 5 days. There was no difference between fungicide application at mid-flowering and both flowering stages; application at both stages delayed crop maturity by approximately 11 – 12 days compared to the unsprayed control.

At Melfort, fungicide delayed maturity compared to the unsprayed check in 2014 and 2015, but not in 2016 (Fig. 3.4). In 2014, there was no difference between the effect of fungicide application at mid-flowering and both flowering stages, however, when fungicides were applied at these stages, maturity was delayed by almost 6 days compared to the unsprayed control, followed by the early stage, 3 days. In 2015, fungicide application at both flowering stages delayed maturity by 6 days compared to the unsprayed control, followed by mid application, 5 days and early application, 3 days.

In most years, fungicide application timing had no effect on crop maturity at Saskatoon and Vegreville, except at Saskatoon in 2014 (Fig. 3.4). In that year, application of fungicides delayed crop maturity compared with the unsprayed control.

The combined analysis of all site years indicated that fungicide application at mid-flowering and both flowering stages delayed maturity by 3 days compared to the unsprayed control, followed by the early-flowering stage, 2 days (Fig. 3.4).

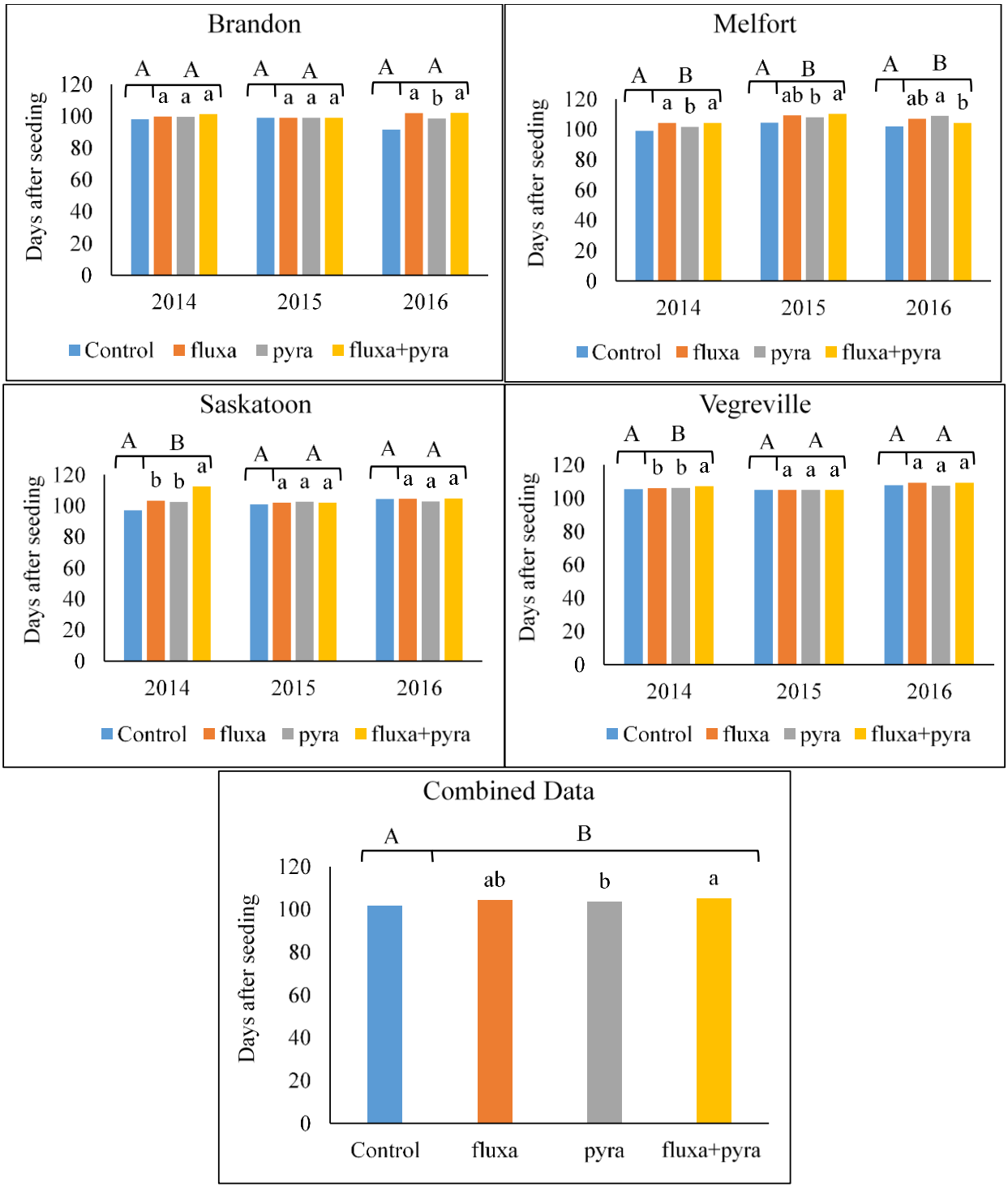

Fig 3.3. Effect of fluxapyroxad (fluxa), pyraclostrobin (pyra) and fluxapyroxad+pyraclostrobin (fluxa+pyra) on flax maturity compared with the unsprayed control at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015 and 2016, and all site-years combined. The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide products at the 5% level of significance, using Tukey's post hoc test

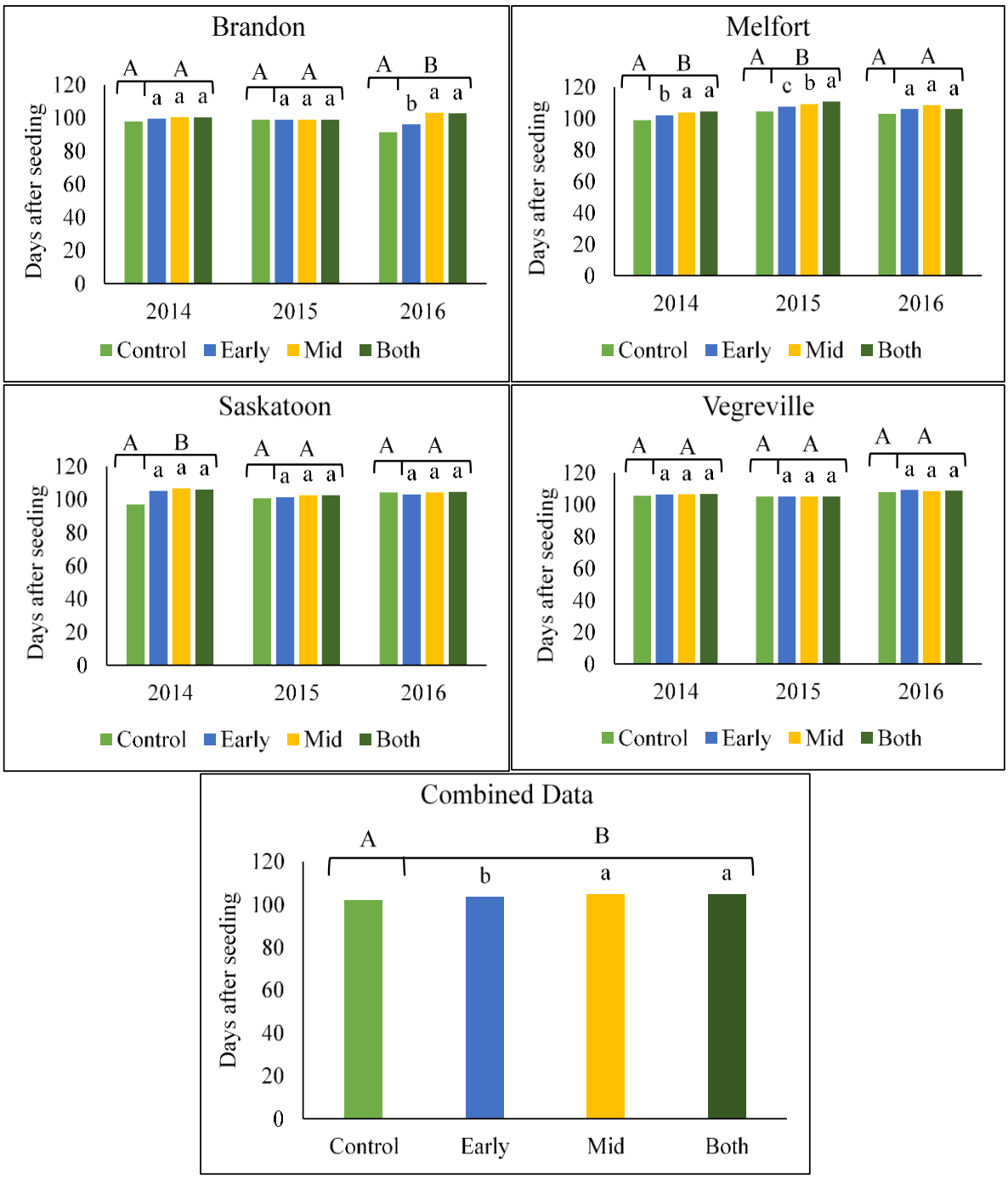

Fig 3.4. Effect of fungicide application timing on flax maturity at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015 and 2016, and all site-years combined. The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide application timings at the 5% level of significance, using Tukey's post hoc test

Seed yield:

The effects of fungicide products and application timings were observed on seed yield at Brandon in 2016 and at Melfort and Saskatoon in all three years (Table 3.6). There was no effect of fungicide products and application timings on seed yield at Vegreville in any year. The effects of fungicides were also observed in the combined data analysis of all site-years. No interaction effect of fungicide product and application timing was observed at any site-year.

Table 3.6: Probability values of fungicide product, application timing and their interaction for seed yield of flax at four locations and three years; and combined data analysis of all site-years

		***P* Value**		
		2014	**2015**	**2016**
Brandon	**Fungicide (F)**	NS	NS	0.0208*
	Time (T)	NS	NS	0.0353*
	F × T	NS	NS	NS
Melfort	**Fungicide (F)**	0.0118*	0.0024**	0.0169*
	Time (T)	0.0357*	0.0003***	0.0153*
	F × T	NS	NS	NS
Saskatoon	**Fungicide (F)**	0.0004***	0.0055**	<0.0001***
	Time (T)	0.0012**	0.0116*	0.0047**
	F × T	NS	NS	NS
Vegreville	**Fungicide (F)**	NS	NS	NS
	Time (T)	NS	NS	NS
	F × T	NS	NS	NS
Combined	**Fungicide (F)**		0.0328*	
	Time (T)		0.0368*	
	F × T		NS	

Note: NS = Non-significant. For F × T effect, unsprayed control data was excluded from the analysis; * P < 0.05, ** P < 0.01 and *** P <0.001.

Application of fungicides had no effect on seed yield at Brandon in 2014 or 2015 (Fig. 3.5). In 2016, yield was improved by the application of fungicide compared with the unsprayed control. However, no difference was observed among fungicide products. The unsprayed control had a mean yield of 1504 kg ha^{-1}. Application of fluxapyroxad increased the seed yield to 1857 kg ha^{-1}, followed by fluxapyroxad+pyraclostrobin, 1818 kg ha^{-1} and pyraclostrobin, 1720 kg ha^{-1}.

At Melfort, application of fungicides increased seed yield compared with the unsprayed control in all three years, however, no difference was observed among the fungicides in 2014 or 2015. In 2014, the unsprayed control had a mean yield of 2848 kg ha^{-1} and the fungicide products increased the seed yield to between 3145 and 3219 kg ha^{-1}. In 2015, the seed yield of the unsprayed control was 2374 kg ha^{-1}, whereas the seed yield for the fungicide products ranged from 2712 to 2777 kg ha^{-1}. In 2016, the unsprayed control had the seed yield of 1930 kg ha^{-1}. Application of fluxapyroxad+pyraclostrobin increased the seed yield to 2969 kg ha^{-1}, followed by pyraclostrobin, 2641 kg ha^{-1} and fluxapyroxad, 2484 kg ha^{-1}.

At Saskatoon, effects of fungicide products were observed in all three years compared with the unsprayed control. In 2014, the unsprayed control had a seed yield of 1383 kg ha^{-1}. Fluxapyroxad+pyraclostrobin increased yield to 2200 kg ha^{-1}. No difference in yield was observed between pyraclostrobin and fluxapyroxad. Application of fungicide products increased seed yield by an average of 1857 kg ha^{-1}. In 2015, no difference in yield was observed among fungicide products at this site. In 2016, the unsprayed control had a very low seed yield of 1412 kg ha^{-1}, and fluxapyroxad+pyraclostrobin (2726 kg ha^{-1}) and pyraclostrobin (2574 kg ha^{-1}) had higher seed yields than fluxapyroxad (2123 kg ha^{-1}).

Pasmo was not observed at Vegreville in 2014 or 2015, and no difference was observed between the unsprayed control and the fungicide treatments. In these years, the average seed yield at this

location was very low compared with other locations. Although, pasmo was observed in 2016, application of fungicides had no effect on the seed yield compared with the unsprayed control.

Effects of fungicide products on seed yield were observed at seven site-years and combine data analysis of these seven site-years indicated that application of fungicides increased seed yield compared with the unsprayed control. Fluxapyroxad+pyraclostrobin improved the seed yield more than fluxapyroxad alone. However, no difference in seed yield was observed between pyraclostrobin and fluxapyroxad. The seed yield of the unsprayed control was 1822 kg ha^{-1} and fluxapyroxad+pyraclostrobin increased the yield by 26% (473 kg ha^{-1}) compared with the unsprayed control, followed by pyraclostrobin, 19% (350 kg ha^{-1}) and fluxapyroxad, 18% (337 kg ha^{-1}).

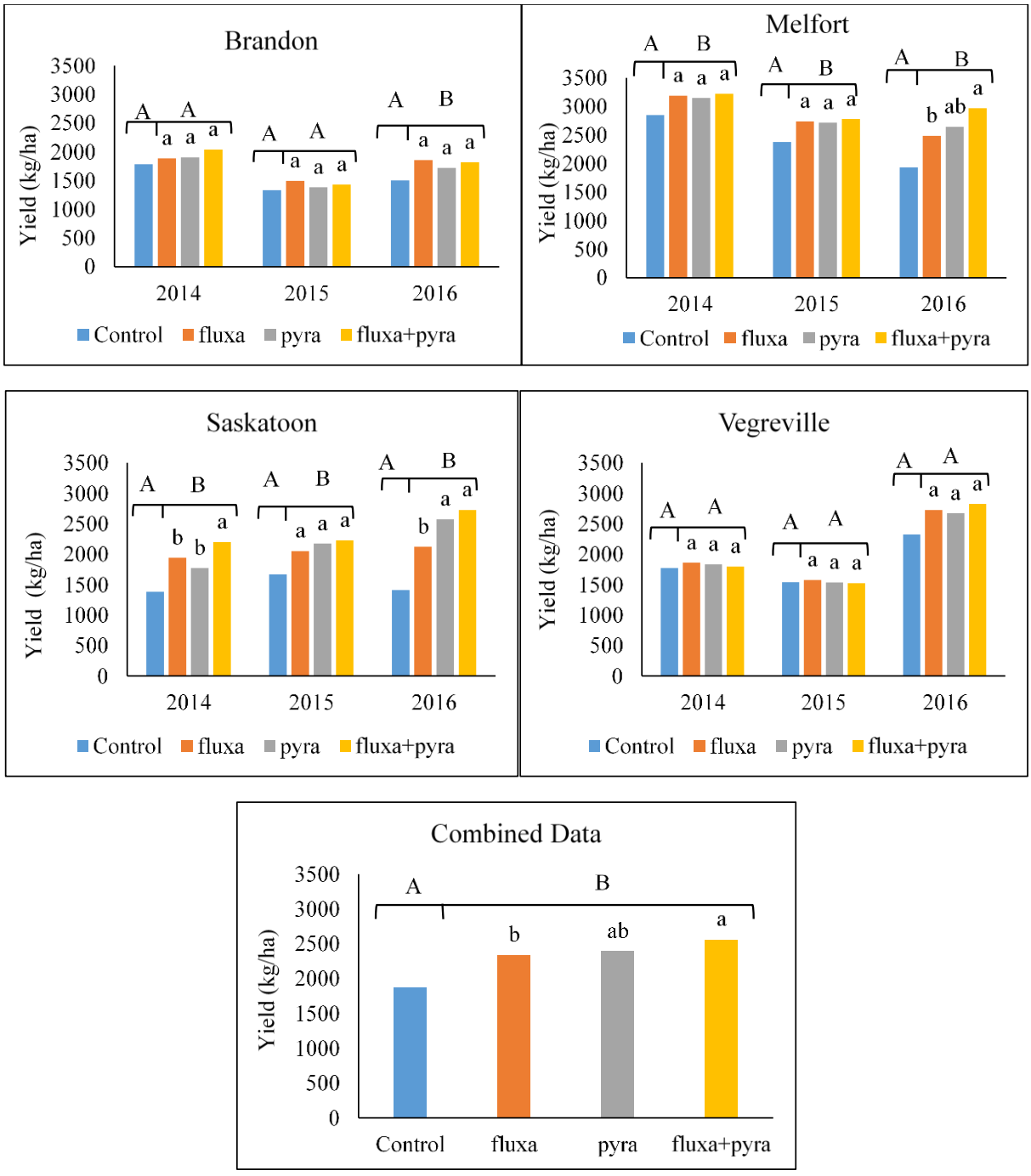

Fig 3.5. Effect of fluxapyroxad (fluxa), pyraclostrobin (pyra) and fluxapyroxad+pyraclostrobin (fluxa+pyra) on seed yield of flax compared with the unsprayed control at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015, 2016 and combined result of all site-years where effect of fungicides was significant. The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide products at the 5% level of significance, using Tukey's post hoc test

At Brandon, application timing had no effect on seed yield in 2014 or 2015 (Fig. 3.6). In 2016, seed yield was improved to 1798 kg ha^{-1} on average by the application of fungicide at any flowering stage compared with the unsprayed control for which the yield was 1504 kg ha^{-1}.

At Melfort, in 2014, fungicide application timing had an effect on seed yield compared with the unsprayed control. No difference was observed between the effect of fungicide application at mid-flowering and both flowering stages. However, there was a difference between the effects of application at early- and mid-flowering stages. Application of fungicides at early-flowering stage yielded 3079 kg ha^{-1}, whereas application at mid-flowering stage had an average yield of 3271 kg ha^{-1} (Fig. 3.6). In 2015, no difference was observed among application timings on seed yield, however, there was in 2016. Yield of the unsprayed control was 1930 kg ha^{-1}, however, seed yield was higher when fungicide was applied at the mid-flowering stage, 2804 kg ha^{-1} and both stages, 2881 kg ha^{-1}, compared with the early-flowering stage, 2410 kg ha^{-1}.

At Saskatoon, in 2014 and 2015, application of fungicide at any flowering stage increased the seed yield more than the unsprayed control. In 2016, the unsprayed control had a seed yield of 1412 kg ha^{-1}. Application of fungicide at both stages increased the seed yield to 2679 kg ha^{-1}, followed by mid-flowering stage, 2459 kg ha^{-1} and early-flowering stage, 2290 kg ha^{-1}.

Combined data analysis indicated that application timing had effect on seed yield compared with the unsprayed control (Fig. 3.6). Overall, there was no difference between fungicide applications at early- and mid-flowering stages. However, a difference was observed between the effect of application at early-flowering stage, 2144 kg ha^{-1} and both stages, 2274 kg ha^{-1}.

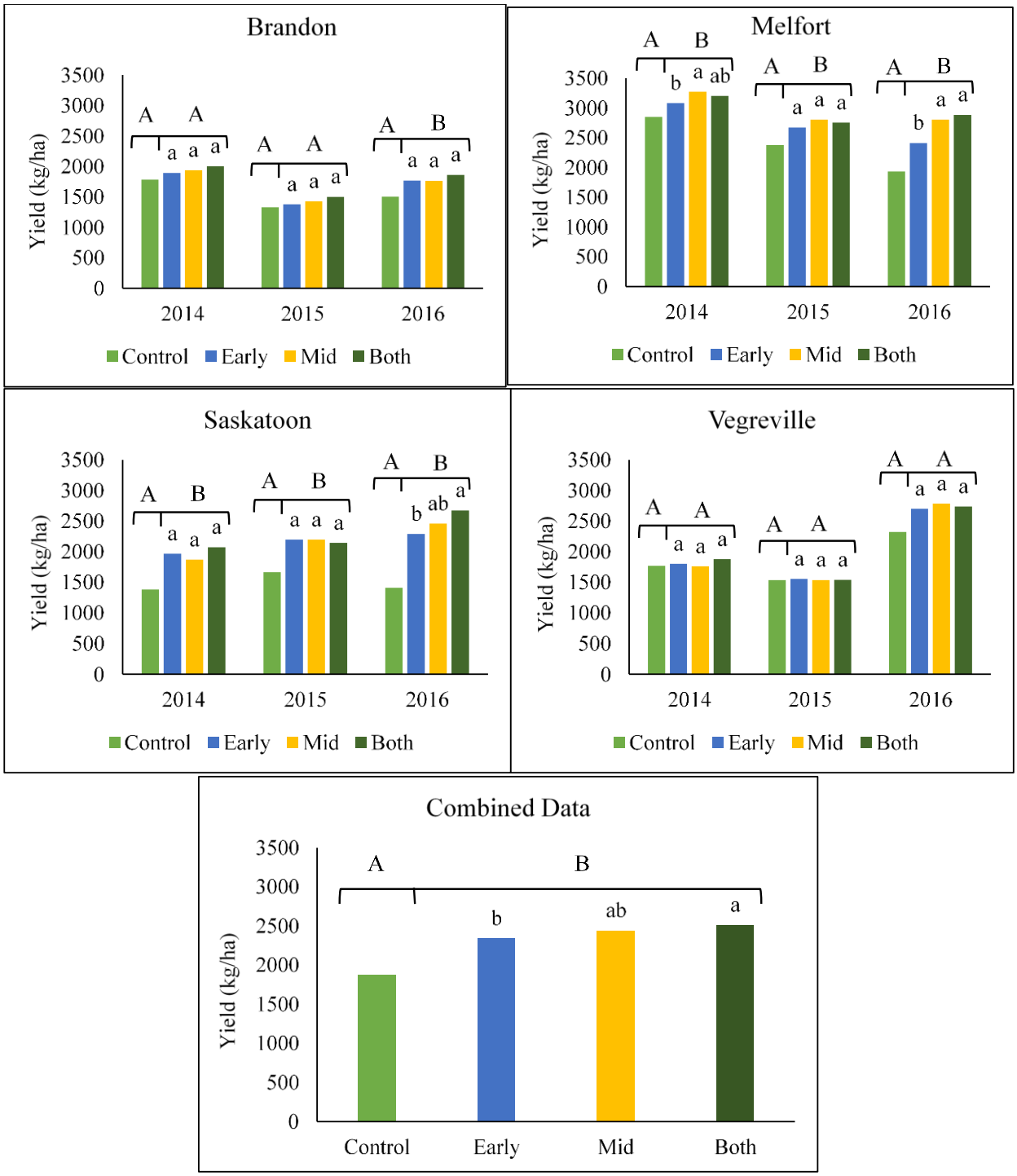

Fig 3.6. Effects of fungicide application timings (early, mid and both stages) on seed yield of flax at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015, 2016 and combined result of all site-years where effect of fungicides were significant. The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide application timings at the 5% level of significance, using Tukey's post hoc test

Thousand seed weight (TSW):

The interaction of fungicide products and application timings was significant only at Saskatoon in 2016 (Table 3.7). Fungicide products increased TSW at all sites, except Vegreville in 2014 and 2015. The increase in TSW varied among application timings at all sites, except Brandon in 2015 and Vegreville in 2014 and 2015. Effects of both fungicide products and application timings were also significant in the combined analysis of all site-years (TSW data of Saskatoon 2016 was excluded in the combined analysis).

Table 3.7: Probability values of fungicide product, application timing and their interaction effect for thousand seed weight (TSW) of flax at four locations and three years; and combined data analysis of all site-years (except Saskatoon 2016)

		***P* Value**		
		2014	**2015**	**2016**
Brandon	**Fungicide (F)**	0.0174*	0.0355*	<0.0001***
	Time (T)	0.0010**	NS	<0.0001***
	F × T	NS	NS	NS
Melfort	**Fungicide (F)**	0.0002***	<0.0001***	<0.0001***
	Time (T)	<0.0001***	<0.0001***	0.0003***
	F × T	NS	NS	NS
Saskatoon	**Fungicide (F)**	<0.0001***	0.0108*	<0.0001***
	Time (T)	0.0032**	0.0007***	<0.0001***
	F × T	NS	NS	0.0021**
Vegreville	**Fungicide (F)**	NS	NS	0.0335*
	Time (T)	NS	NS	0.0007***
	F × T	NS	NS	NS
Combined	**Fungicide (F)**		<0.0001***	
	Time (T)		<0.0001***	
	F × T		NS	

Note: NS = non-significant. For F × T effect, unsprayed control data was excluded from the analysis; * P < 0.05, ** P < 0.01 and *** P <0.001.

In 2016, at Saskatoon, when fluxapyroxad+pyraclostrobin was applied at mid- and both flowering stages, the TSW increased compared with the early application (Fig. 3.7). Application of pyraclostrobin at mid- and both flowering stages also improved TSW more than the early-flowering application. The effect of fluxapyroxad was similar at all stages and lower than pyraclostrobin at mid-flowering and both application stages.

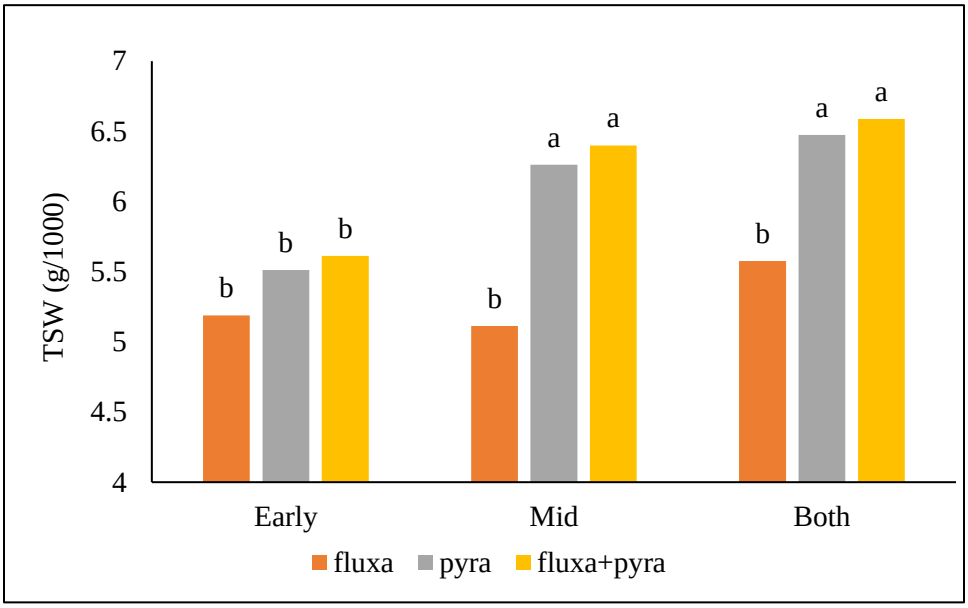

Fig 3.7. The interaction effect of fungicides and application timings on TSW of flax at Saskatoon in 2016. Lower case letters indicate significant differences among fungicide products at the 5% level of significance, using Tukey's post hoc test

At most of the site-years, the average TSW was 5 to 6 grams (Fig. 3.8). At Brandon, application of fungicide products improved the TSW compared with the unsprayed control in all three years. In 2014, application of fluxapyroxad+pyraclostrobin increased TSW by 0.5 g compared with the unsprayed control (4.7 g), followed by fluxapyroxad, 0.4 g and pyraclostrobin, 0.3 g. In 2015, the TSW of the unsprayed control was 5.1 g and the TSW of the pyraclostrobin treatment was 5.2 g; contrast analysis indicated that there was no difference between the TSW of pyraclostrobin and the unsprayed control. Application of fluxapyroxad+pyraclostrobin increased the TSW to 5.5 g, followed by fluxapyroxad, 5.4 g. In 2016, the unsprayed control had a TSW of 5.3 g. No difference

was observed between the TSW of fluxapyroxad+pyraclostrobin and fluxapyroxad in 2016, however, the application of these combined products increased the TSW to 6.1 g, compared with pyraclostrobin, 5.8 g.

At Melfort, fungicide products increased the TSW in all three years compared with the unsprayed control (Fig. 3.8). In 2014, the TSW of the unsprayed control was 5.9 g. Fluxapyroxad and fluxapyroxad+pyraclostrobin were equally effective and increased TSW to 6.3 g. Pyraclostrobin was less effective than fluxapyroxad or fluxapyroxad+pyraclostrobin, but still increased the TSW to 6.1 g. In 2015, no difference was observed among the fungicide products for TSW. Application of fungicide products increased the TSW by approximately 0.5 g compared with the unsprayed control (5.8 g). In 2016, fluxapyroxad+pyraclostrobin had higher TSW, 5.7 g than pyraclostrobin, 5.4 g or fluxapyroxad, 5.3 g, whereas the unsprayed control was 4.8 g.

At Saskatoon, application of fungicide increased TSW compared with the unsprayed control in 2014 and 2015 (Fig. 3.8). In 2014, the unsprayed control had the lowest TSW, 4.8 g. The highest TSW was observed by the application of fluxapyroxad+pyraclostrobin, 6 g. Fluxapyroxad increased TSW to 5.6 g, followed by pyraclostrobin, 5.3 g. In 2015, the TSW of the fluxapyroxad treatment was 5.9 g, which was not statistically different from the unsprayed control, 5.7 g. Both pyraclostrobin and fluxapyroxad+pyraclostrobin increased TSW to 6 g.

At Vegreville, fungicide application had no effect on TSW in 2014 and 2015 (Fig. 3.8). In 2016, application of fungicide product increased TSW compared with the unsprayed control, however, there was no difference among fungicides. The unsprayed control had a TSW of 6.4 g and application of fungicide products increased the TSW to 6.8 g.

The combined TSW from of all locations and years (except Saskatoon 2016, as there was an interaction effect of fungicide and application timing in this site-year) indicated that application of fungicide products increased TSW compared with the unsprayed control. Fluxapyroxad+pyraclostrobin resulted in higher TSW (approximately 6 g) than fluxapyroxad (5.8 g) or pyraclostrobin (5.7 g), whereas the unsprayed control had the lowest TSW of 5.3 g.

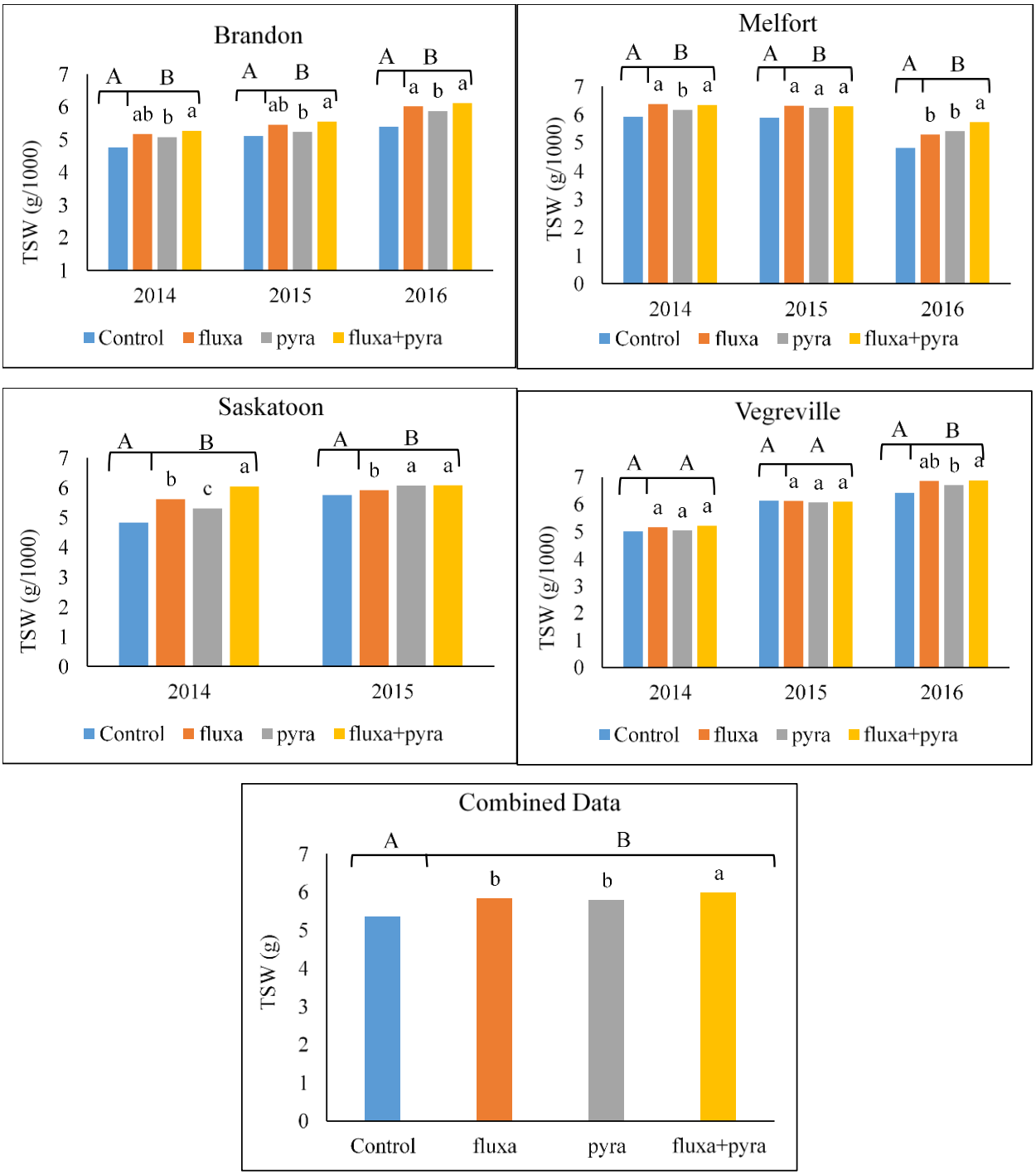

Fig 3.8. Effect of fluxapyroxad (fluxa), pyraclostrobin (pyra) and fluxapyroxad+pyraclostrobin (fluxa+pyra) on thousand seed weight (TSW) of flax compared with the unsprayed control at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015 and 2016, and all site-years combined (except Saskatoon 2014). The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide products at the 5% level of significance, using Tukey's post hoc test

At Brandon, in 2014, the unsprayed control had a TSW of 4.6 g and fungicide application at both flowering stages had a similar increase in TSW of approximately by 0.4 g compared with the unsprayed control (Fig. 3.9). In 2015, application timings had no effect on TSW at this site. In 2016, the TSW of the unsprayed control was 5.3 g. Fungicide application at both flowering stages resulted in greater TSW (6.1 g) than at early-flowering stage (5.8 g), but neither differed from the TSW of the mid-flowering stage (6 g).

At Melfort, similar results were observed in 2014 and 2015 (Fig. 3.9). In these years, no difference was observed among the application timings for TSW. Application of fungicide product at any flowering stage had similar increases of TSW compared with the unsprayed control. In 2016, the TSW of the unsprayed control was 4.8 g. Fungicide application at mid- and both flowering stages resulted in greater TSW (5.7 and 5.4 g, respectively) than application at the early-flowering stage (5.2 g).

At Saskatoon, in 2014, application of fungicide products at either flowering stage, or at both flowering stages increased the TSW by approximately 0.8 g compared with the unsprayed control (Fig. 3.9). In 2015, an effect of fungicide application timing was observed on TSW compared with the unsprayed control. The TSW of the unsprayed control was 5.7 g. Fungicide application at both flowering stages increased the TSW to 6.1 g, followed by early- and mid-flowering applications, 5.9 g.

At Vegreville, fungicide application timing had no effect on TSW in 2014 or 2015. In 2016, fungicide application at either flowering stage or at both flowering stages improved TSW similarly (approximately by 0.4 g) compared with the unsprayed control (6.4 g). No difference in TSW was observed among fungicide application timings.

Combined data analysis from all site-years (except Saskatoon 2016) indicated that application timing had an effect on TSW compared with the unsprayed control. Fungicide application at both flowering stages had higher TSW (5.9 g) than the early application (5.7 g) (Fig. 3.9). However, TSW of these treatments was not different from the TSW of mid-flowering application timing (5.8 g).

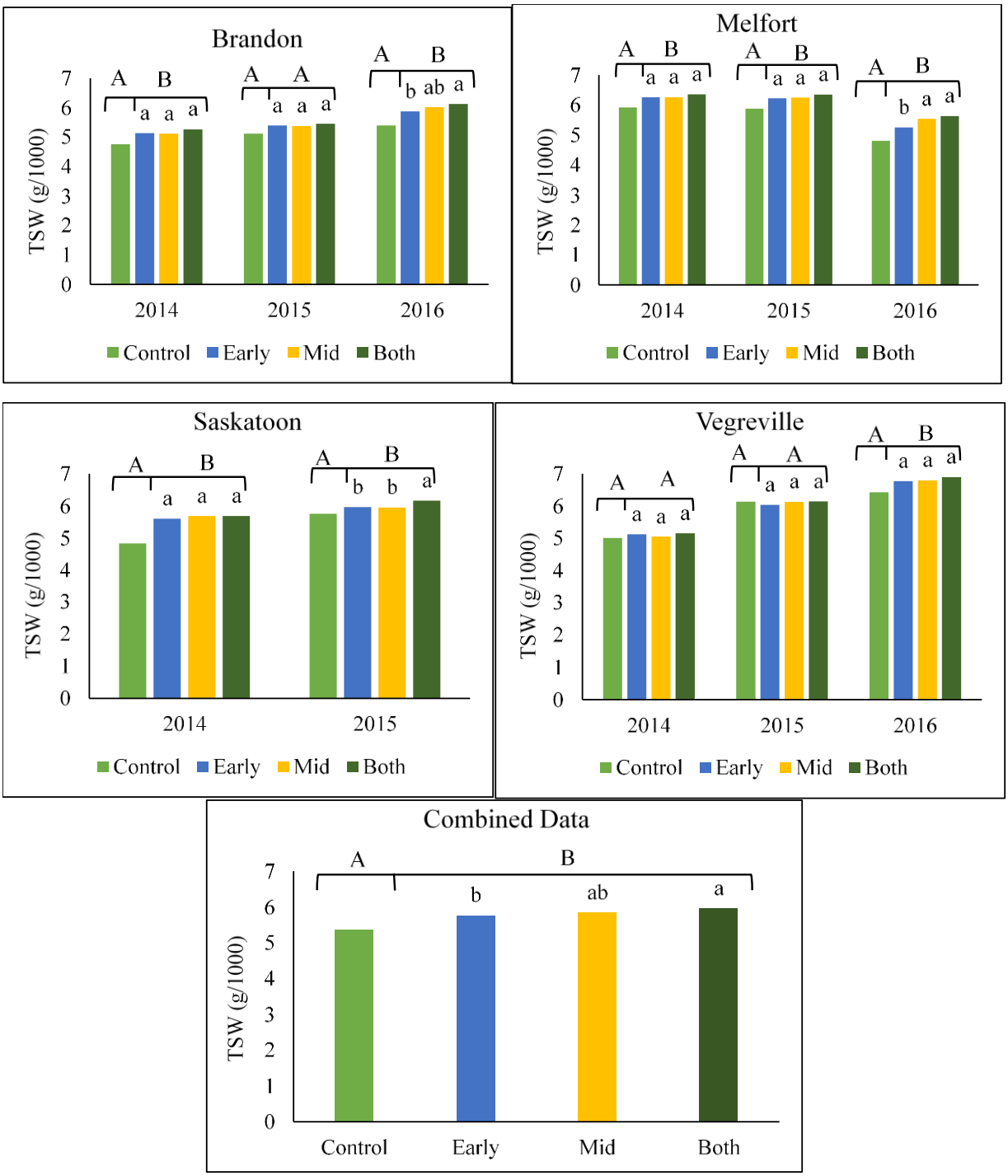

Fig 3.9. Effect of fungicide application timing on thousand seed weight (TSW) of flax at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015 and 2016, and all site-years combined (except Saskatoon 2016). The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide application timings at the 5% level of significance, using Tukey's post hoc test

Test weight (TW):

Application of fungicides had an effect on TW at Melfort in 2016 and at Saskatoon in all three years (Table 3.8). Fungicide application timing had an effect only at Melfort in 2015 and 2016, and Saskatoon in 2016. The effect of fungicide product or application timing was not significant in the combined data analysis of all site-years. There was no interaction of fungicide product and application timing at any site-year.

Table 3.8: Probability values of fungicide, application time and treatment effect for test weight of flax at four locations and three years; and combined data analysis of all site-years

		***P* Value**		
		2014	**2015**	**2016**
Brandon	**Fungicide (F)**	NS	NS	NS
	Time (T)	NS	NS	NS
	F × T	NS	NS	NS
Melfort	**Fungicide (F)**	NS	NS	0.0001***
	Time (T)	NS	0.0338*	0.0003***
	F × T	NS	NS	NS
Saskatoon	**Fungicide (F)**	0.0054**	0.0063**	0.0421*
	Time (T)	NS	NS	0.0001***
	F × T	NS	NS	NS
Vegreville	**Fungicide (F)**	NS	NS	NS
	Time (T)	NS	NS	NS
	F × T	NS	NS	NS
Combined	**Fungicide (F)**		NS	
	Time (T)		NS	
	F × T		NS	

Note: NS = non-significant. For F × T effects, unsprayed control data was excluded from the analysis; * P < 0.05, ** P < 0.01 and *** P <0.001.

At most of the locations, the average TW of flaxseed was approximately 70 kg hL^{-1} (Fig. 3.10). At Mefort, in 2016, application of fungicides increased TW by an average of 2 kg hL^{-1} compared with the unsprayed control, 66 kg hL^{-1}. However, no difference was observed among the fungicide products. At Saskatoon, application of fungicides increased TW in all three years (Fig. 3.10). In 2014, the TW of the fluxapyroxad+pyraclostrobin treatment was greater than for the pyraclostrobin treatment, but neither differed from the fluxapyroxad treatment. The TW of the unsprayed control was 67.3 kg hL^{-1} and application of fluxapyroxad+pyraclostrobin increased TW by 2 kg hL^{-1} compared with the unsprayed control, followed by fluxapyroxad, 1.5 kg hL^{-1} and pyraclostrobin, 1 kg hL^{-1}. In 2015, the TW of the pyraclostrobin and fluxapyroxad+pyraclostrobin treatments were similar, and both increased TW to 67.8 kg hL^{-1} compared with the fluxapyroxad treatment, 67.3 kg hL^{-1}. In 2016, there were no differences among the fungicide products for TW, however, application of fungicide increased TW to 69.2 kg hL^{-1} on average compared with the unsprayed control, 67.7 kg hL^{-1}.

The combined analysis of all site-years indicated that fungicide application had no effect on TW compared with the unsprayed control and neither was there a difference among fungicide products.

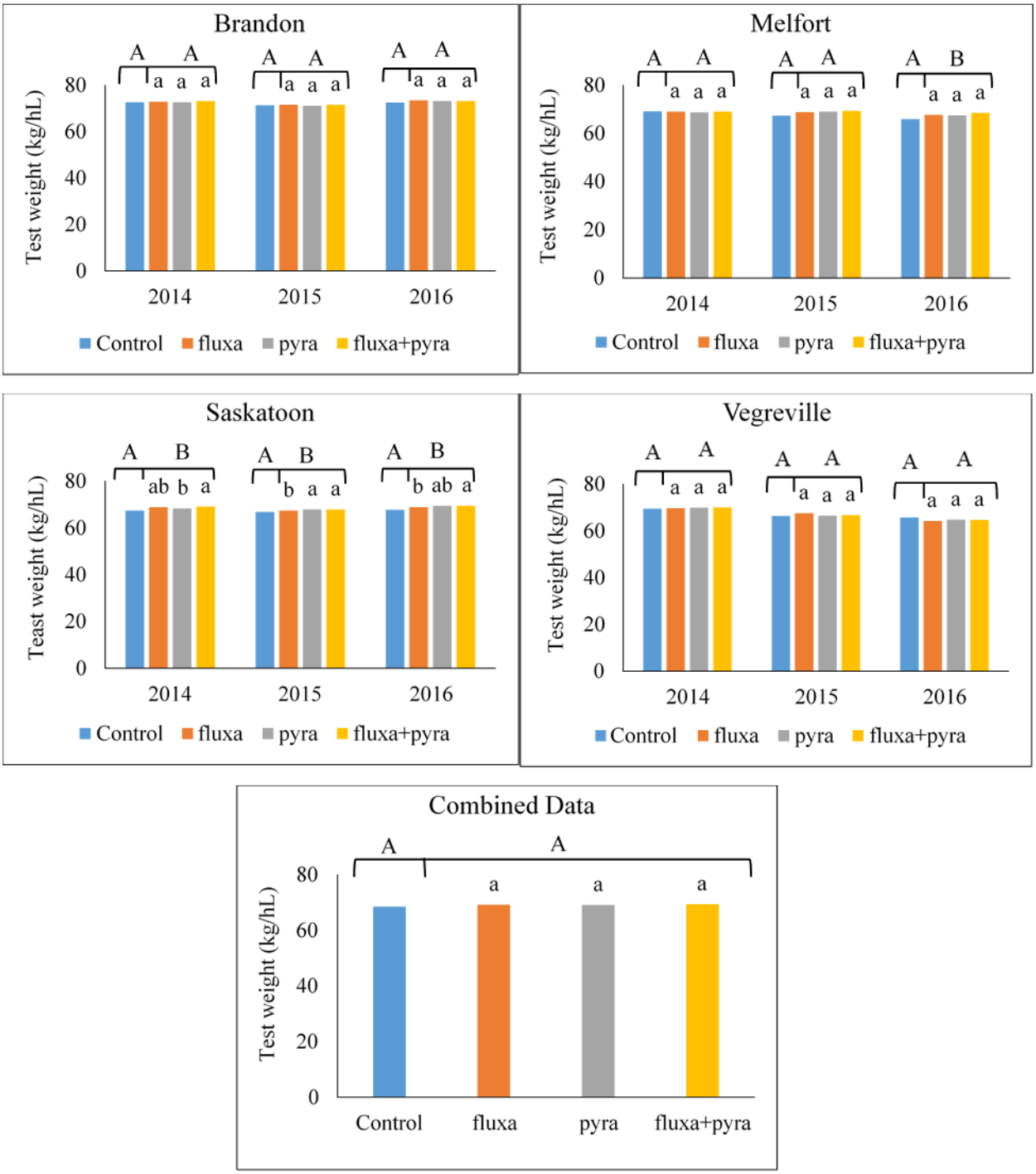

Fig 3.10. Effect of fluxapyroxad (fluxa), pyraclostrobin (pyra) and fluxapyroxad+pyraclostrobin (fluxa+pyra) on TW of flax compared with the unsprayed control at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015 and 2016, and all site-years combined. The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide products at the 5% level of significance, using Tukey's post hoc test

At Melfort, fungicide application timing had no effect on TW of flaxseed in 2014 (Fig. 3.11). In 2015, fungicide application improved TW over the unsprayed control at this site. The TW of the unsprayed control was 67.4 kg hL^{-1}. When fungicide was applied at both flowering stages and at the mid-flowering stage, it increased the TW to 69.3 and 69.1 kg hL^{-1}, respectively. Application of fungicides at the early-flowering stage increased the TW to 68.7 kg hL^{-1}. In 2016, fungicide application at the mid-flowering stage and at both stages increased TW similarly and both resulted in higher TW (68.4 kg hL^{-1}) than the early-flowering application (66.8 kg hL^{-1}).

Fungicide application timing had no effect on TW at Saskatoon in 2014 and 2015. However, in 2016, application of fungicide product at either flowering stage and at both flowering stages resulted in a similar increase in TW (69.2 kg hL^{-1}) over the unsprayed control (67.7 kg hL^{-1}).

Results from the combined data analysis of all site-years indicated that application timing had no effect on TW of flaxseed.

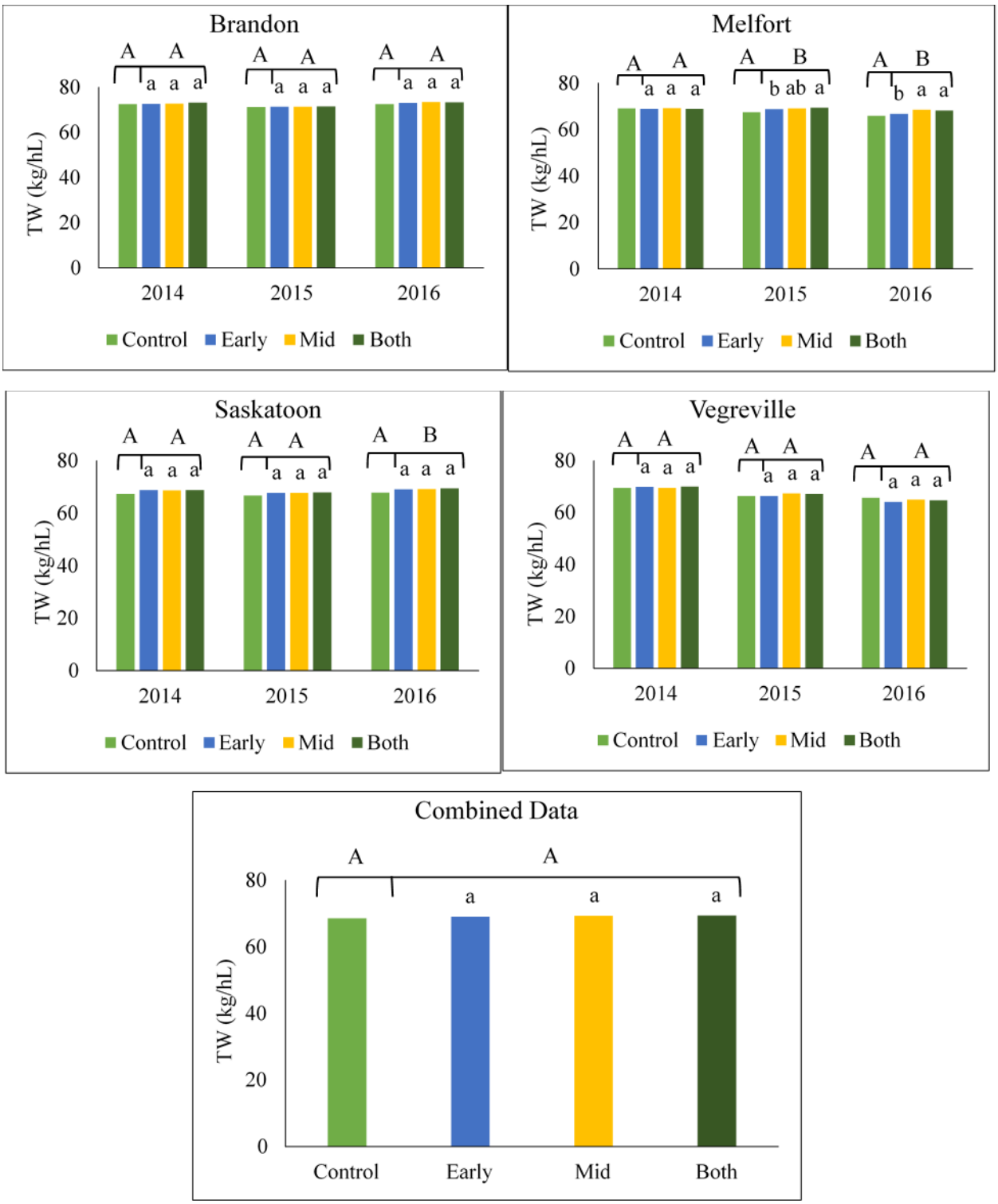

Fig 3.11. Effect of fungicide application timing on TW of flax at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015 and 2016, and all site-years combined. The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide application timings at the 5% level of significance, using Tukey's post hoc test

Oil content:

Fungicides and application timing had an effect on oil content at Melfort and Saskatoon only in 2014. (Table 3.9). At Brandon and Vegreville, there was no effect of fungicide application on oil content, except at Brandon in 2014. Effects of fungicide products and application timings on oil content were not significant in the combined data analysis of all site-years. The interaction effect of fungicide product and application timing was not significant at any site-year or in the combined analysis.

Table 3.9: Probability values of fungicide, application timing and treatment effect for oil content of flax at four locations and three years; and combined data analysis of all site-years

		***P* Value**		
		2014	**2015**	**2016**
Brandon	**Fungicide (F)**	<0.0001***	NS	NS
	Time (T)	NS	NS	NS
	F × T	NS	NS	NS
Melfort	**Fungicide (F)**	0.0050*	NS	NS
	Time (T)	0.0068*	NS	NS
	F × T	NS	NS	NS
Saskatoon	**Fungicide (F)**	<0.0001***	NS	NS
	Time (T)	0.0007***	NS	NS
	F × T	NS	NS	NS
Vegreville	**Fungicide (F)**	NS	NS	NS
	Time (T)	NS	NS	NS
	F × T	NS	NS	NS
Combined	**Fungicide (F)**		NS	
	Time (T)		NS	
	F × T		NS	

Note: NS = non-significant. For F × T effect, unsprayed control data was excluded from the analysis; * P < 0.05, ** P < 0.01 and *** P <0.001.

At Brandon in 2014, the application of fungicide improved oil content over the unsprayed control (Fig. 3.12). The unsprayed control had an oil content of 42.1%. Fluxapyroxad and fluxapyroxad+pyraclostrobin treatments had similar effects on the oil content of flax and increased oil content to 43.5% on average. The pyraclostrobin treatment had an oil content of 42.4%, which was not statistically different from the unsprayed control. Fungicide products had no effect on oil content in 2015 or 2016 at this site.

At Melfort in 2014, a slightly higher oil content (43.7%) was observed when fungicides were applied compared with the unsprayed control (42.9%); however, no difference was observed among the fungicide products (Fig. 3.12). Application of fungicide products had no effect in 2015 or 2016.

At Saskatoon, fungicide products increased oil content compared with the unsprayed control only in 2014 (Fig. 3.12). In this year, the oil content of the unsprayed control was 40.5% and fluxapyroxad+pyraclostrobin increased oil to 44.1%, followed by fluxapyroxad, 43.4% and pyraclostrobin, 42.6%.

In the combined analysis of all site-years, no benefit of fungicide application was detected on the oil content of flaxseed (Fig. 3.12).

At most of the site-years fungicide application timing had no effect on oil content (Fig. 3.13); however, at Melfort and Saskatoon in 2014, oil content was improved when fungicide was applied at any flowering stage of flax compared with the unsprayed control.

The combined analysis from all site-years did not indicate that fungicide application timing had an effect on the oil content of flaxseed (Fig. 3.13).

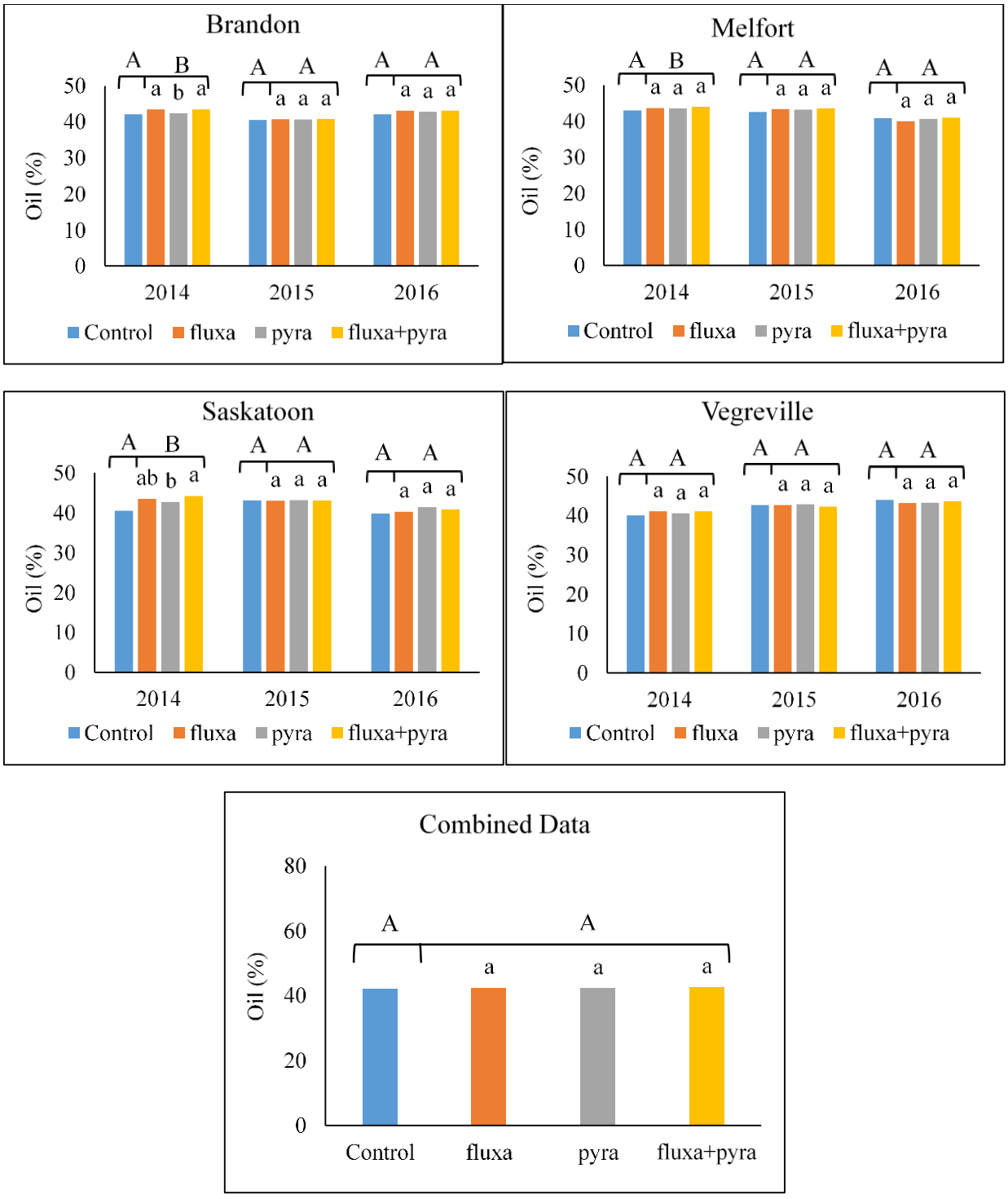

Fig 3.12. Effect of fluxapyroxad (fluxa), pyraclostrobin (pyra) and fluxapyroxad+pyraclostrobin (fluxa+pyra) on oil content of flax compared with the unsprayed control seed at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015 and 2016, and all site-years combined. The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide products at the 5% level of significance, using Tukey's post hoc test

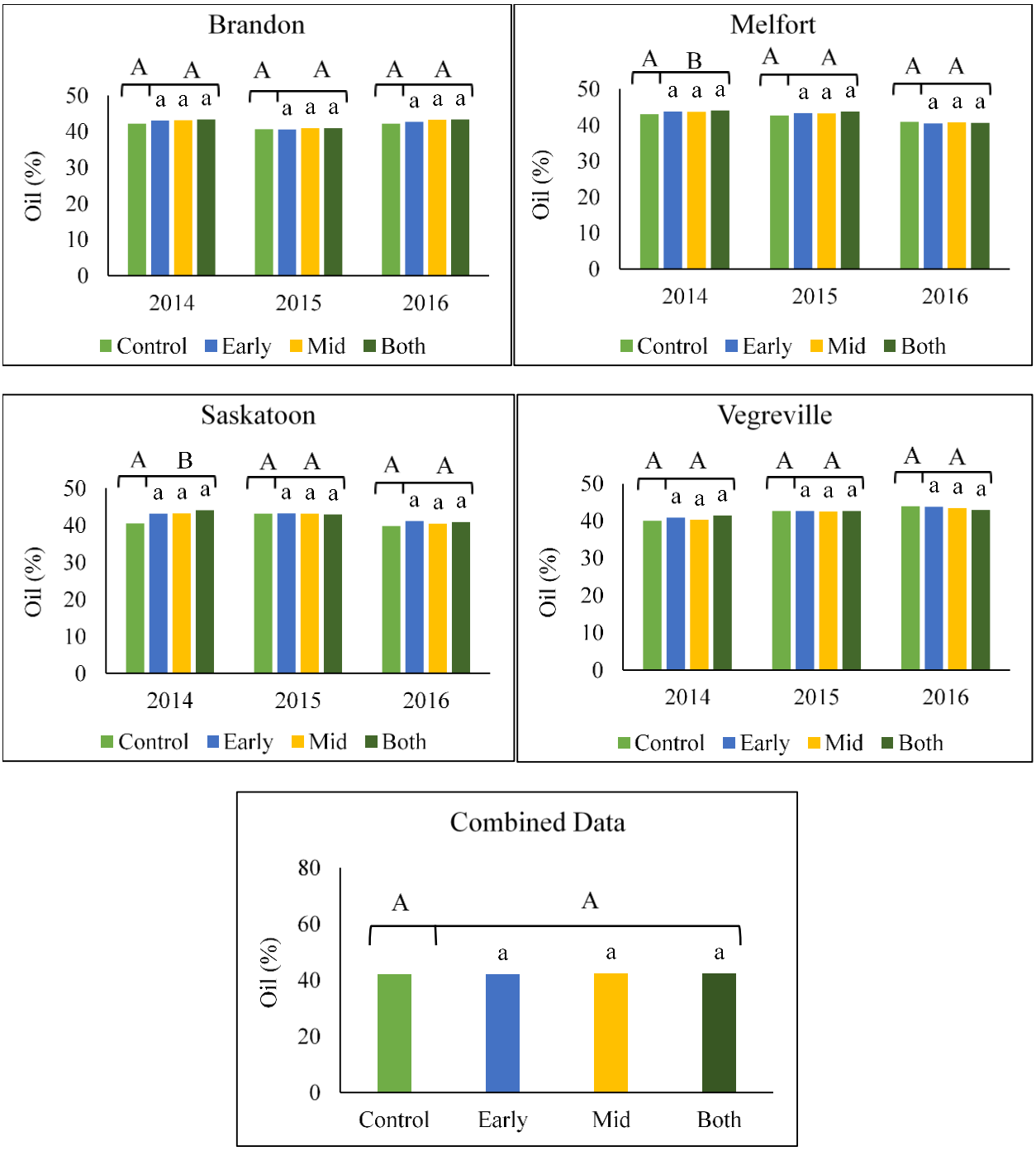

Fig 3.13. Effect of fungicide application timing on oil of flax at Brandon, Melfort, Saskatoon and Vegreville in 2014, 2015 and 2016, and all site-years combined. The upper-case letters indicate significant differences between the treated and untreated plots, and the lower-case letters indicate significant differences among fungicide application timing treatments at the 5% level of significance, using Tukey's post hoc test

3.4. Discussion:

Pasmo is an important disease of flax that can be managed through effective fungicide application. However, very little research has been done to determine the effect of various fungicides on pasmo severity, seed yield and quality of flax. In the 1980's, application of benomyl was shown to reduce pasmo severity, which resulted in a 7% yield increase (Ferguson et al. 1987). Pyraclostrobin effectively reduced disease in various crops, including soybean rust, leaf spot diseases of winter wheat, and blackleg of canola (Ransom and McMullen 2008; Nelson et al. 2010; Hwang et al. 2016). In North Dakota, application of azoxystrobin (a quinone outside inhibitor [QoI] fungicide) was reported to reduce pasmo severity to 65% from 77% and increased seed yield by 50% (Halley et al. 2004). Application of pyraclostrobin decreased pasmo severity by 50-60% and increased seed yield by 80% at Morden, Manitoba in 2009 (Rashid 2010). In Saskatchewan, pasmo was reduced up to 59% and seed yield was increased up to 35% with the application of pyraclostrobin fungicide (Vera et al. 2014). In our field trials, both pyraclostrobin and fluxapyroxad+pyraclostrobin reduced pasmo severity and improved flax yield compared with the unsprayed control. Fluxapyroxad has also been used to reduce disease in many crops (Smith et al. 2013; Amiri et al. 2014; Chen et al. 2014). This fungicide product was also used in our study to determine its effect on pasmo severity and seed yield of flax. At most of the locations, fluxapyroxad decreased disease severity compared with the unsprayed control, however, at Saskatoon in 2015 and 2016, application of fluxapyroxad did not reduce the pasmo severity compared with the unsprayed control. There are many factors that affect fungicide activity including volatilization, plant uptake, biotic degradation (microbial metabolism), and abiotic degradation (temperature, precipitation) (Gao 2012). Thus, to identify the reason why fluxapyroxad did not have an effect on pasmo at this site, more research needs to be conducted.

Fluxapyroxad was less effective than pyraclostrobin and fluxapyroxad+pyraclostrobin for pasmo control and resulted in lower seed yield than fluxapyroxad+pyraclostrobin. Fluxapyroxad+pyraclostrobin, trade name Priaxor, is a comparatively new fungicide and the combination of the two active ingredients were more effective in reducing pasmo severity than fluxapyroxad alone. Application of fungicides reduced disease severity and protected upper plant parts during the seed development stage contributing to seed fill and improved seed weight, which resulted the seed yield improvement.

In our study, no pasmo was observed at Vegreville in 2014 or 2015. There was no precipitation from May to July in 2014 at this location and in 2015, the average precipitation during the growing season was also low compared to the long-term normal. The below normal precipitation was likely the most important factor limiting pasmo in 2014 and 2015 at this site. The amount of precipitation during the growing season is one of the main factors affecting pasmo development (Gruzdevienė et al. 2008). Positive correlations were found between pasmo severity and total accumulated rainfall from June to August (Halley et al. 2004).

In this experiment, disease pressure was established by spreading pasmo infected flax stubbles in the experimental plots. Among the 10 site-years where disease was present, application of fungicide decreased disease severity at all site-years; however, seed yield was increased at only seven of these 10 site-years. Although application of fungicides decreased disease severity at Brandon in 2014 and 2015 and Vegreville in 2016 compared with the unsprayed control, no effect was detected on seed yield at these locations. The reason for this inconsistency may be because of late disease development, which may have occurred after boll formation or at the green boll ripening stage of flax. Yield loss depends on the time (host growth stage) of pasmo infection; infection at the flowering stage may reduce yield by 20% to 50% (Sackston et al. 1950).

Flax is purchased on the basis of several quality factors, among these factors, TSW is one of the most important. High TSW results in high seed yield (Copur et al. 2006). Thus, it is very important to determine the effects of fungicide on TSW of pasmo infected flax. Severe pasmo causes premature ripening, which reduces the seed weight of flax (Sackston 1950). In our study, application of fungicide improved the TSW compared with the unsprayed control. Among the three fungicides examined in our study, fluxapyroxad+pyraclostrobin increased the TSW more than either pyraclostrobin or fluxapyroxad alone. At most of the sites fluxapyroxad and pyraclostrobin caused a similar increase in TSW. At Saskatoon, fluxapyroxad was less effective than pyraclostrobin in increasing TSW. The reason may be due to high disease severity. At this site, high disease severity was observed after the application of fluxapyroxad compared with pyraclostrobin, which may also have affected the TSW of the flaxseed.

Test weight is an important flax grading factor, and is defined as the weight of grain that fills a given volume (Ghaderi et al. 1971). High test weight values are desirable because they positively influence market grade and price. In our study, however, neither fungicide products nor application timing had any effect on the TW of flaxseed.

In Canada, flax is grown as an oilseed crop. Thus, the oil content of flax is a very important factor for the flax industry (Jhala and Hall 2010). In our study, no difference was observed between fungicide products and the unsprayed control in terms of oil content of flaxseed; neither did application timing have any effect on the oil content of flaxseed. The reason may be due to the late appearance of disease. The oil content of flaxseed is greatly affected by the early occurrence of the disease at the flowering stage of flax and very little or no effect is observed when the disease appears at seed ripening stage (Sackston and Carson 1951). However, the growth and development of flaxseed and oil content can also be affected by environmental conditions, including temperature

and soil moisture (Kraft et al. 1963). Inadequate rainfall and high temperatures during the growing period result in early maturity, which reduce yield, TSW and oil content (Casa et al. 1999).

It is very important to apply fungicide at the appropriate crop growth stage for maximum benefit. For example, applications of pyraclostrobin at the early- and late-flowering stages of bean (*Phaseolus vulgaris*) provided the most consistent control of anthracnose and reduced yield and quality losses (Conner et al. 2004). When soybean rust infection occurs before the beginning of pod development, the plants treated with fungicide at the beginning of the flowering stage have the lowest disease severity and highest yields (Mueller et al. 2009). In our study, fungicides applied at both flowering stages decreased disease severity by 10 to 12% compared to a single application at the early- or mid-flowering stage. However, at most sites, application of fungicide at both flowering stages gave approximately the same increase in seed yield and TSW as a single application at mid-flowering stage. A difference was observed between the early-flowering application and application at both flowering stages on seed yield and TSW, however, no difference was observed between early-flowering application and mid-flowering application on seed yield and TSW. Therefore, our results suggest that, a single application of fungicide at early- or mid-flowering stage will be as effective as fungicide application at both flowering stage. Moreover, use of multiple applications of the same fungicides may increase the risk of selecting for fungicide insensitive isolates of *S. linicola*.

Pyraclostrobin, which is a strobilurin fungicide, has been reported to have some physiological effects on crops that delay crop maturity and extend the grain filling period leading to increased grain yield due to a longer period of dry matter accumulation (Smith et al. 2013; Hill et al. 2013; Joshi et al. 2014). Delayed maturity is also known as the “greening effect” (Venancio et al. 2003). In our study, pyraclostrobin delayed maturity by 7 days at Brandon in 2016 and by 2 to 3 days

compared to the unsprayed control in a few site-years. Fluxapyroxad+pyraclostrobin delayed maturity by 14 days at Saskatoon in 2014, and by 10 days at Brandon in 2016. However, at most site-years, the maturity difference between fluxapyroxad+pyraclostrobin and the unsprayed control was 3 to 5 days. In most of the flax growing areas the typical frost-free days are between 105 to 115 days (Agricultural Land Resource Atlas of Alberta, Manitoba Agriculture and Food, Saskatchewan Crop Insurance Corporation). Thus, flax growers should consider maturity before applying fungicides, because the application of fungicides may delay crop maturity and the crop could be adversely affected by frost. Early autumn frosts can cause serious damage to flax. It can reduce the seed weight and density and cause dark seed and oil color, and lower oil and protein contents, as well as reduce seed germination rate (Gubbels et al. 1994).

The application of pyraclostrobin has been reported to increase crop yield in the absence of disease or at minimal disease pressure (Nelson and Meinhardt 2011; Hill et al. 2013; Kanungo and Joshi 2014). For example, the application of pyraclostrobin increased the seed yield of soybean by 100 kg ha^{-1} under minimal disease pressure (Henry et al. 2011). In contrast, many researchers have not observed any benefit of pyraclostrobin application in the absence of any disease. For example, pyraclostrobin application had no effect on winter wheat in the absence of disease (Weisz et al. 2011). No benefit was observed when pyraclostrobin was applied in sugar beet in the absence of disease (Khan and Carlson 2009). In our study, no benefit of fungicide application was observed for seed yield, TSW, TW or oil content of flax seed in absence of pasmo as occurred at Vegreville in 2014 and 2015.

Our results will help flax growers to make appropriate fungicide application decisions to control pasmo. Fungicide application always decreased pasmo in our study, but did not always improve seed yield; the results varied depending on the fungicide product applied, weather conditions,

disease severity and timing. Based on these results, it appeared that both pyraclostrobin and fluxapyroxad+pyraclostrobin provided protection against pasmo and resulted in similar yield increases. However, fluxapyroxad+pyraclostrobin should be the fungicide product of choice to manage pasmo rather than pyraclostrobin alone, because of the risk of fungicide insensitivity. It will be more difficult for the pathogen to develop insensitivity against a fungicide with two active ingredients than a fungicide with one active ingredient. Flax growers should be aware that application of fluxapyroxad+pyraclostrobin may also delay crop maturity. This study also indicated that the mid-flowering stage of flax was a better fungicide application timing than early-flowering, and that no benefit was observed when fungicide was applied in the absence of disease. Therefore, it is very important for flax growers to consider the climatic zone in which they grow flax, the weather conditions, their crop rotation, and the maturity considerations of the crop, and their experience with pasmo before making the decision to apply fungicide.

CHAPTER 4

4. Sensitivity of *Septoria linicola* to pyraclostrobin and fluxapyroxad fungicides

4.1. Introduction:

Fungicides have been used to protect plants against disease for over 200 years (Brent and Hollomon 2007). However, due to long-term and continuous use of fungicides many pathogens have become insensitive to some fungicides (Sierotzki et al. 2000; Ishii et al. 2001; Gisi et al. 2002; Amand et al. 2003; Kim et al. 2003). Monitoring of pathogens for fungicide sensitivity has become common over the past 20 years and is an essential part of anti-resistance approaches (Brent 1992). Without proper monitoring and effective product management, resistance may arise quickly (Brent and Hollomon 2007). Many studies have been conducted to determine the fungicide sensitivity of many pathogens against various fungicides (Stammler et al. 2007; Wise et al. 2009; Banno et al. 2009; Miyamoto et al. 2009; Avenot and Michailides 2010).

The standard or traditional technique for measuring fungicide sensitivity is to determine the minimum inhibiting concentration (MIC) using the spore germination assay or the radial growth assay (Pasche et al. 2005; Mondal et al. 2005; Blixt et al. 2009; Avenot et al. 2014). However, many researchers have reported that determination of fungal growth inhibition using a microtiter assay method has several advantages compared with traditional methods (Beyer et al. 2011; Rampersad 2011; Akhavan et al. 2017). In the microtiter method, the pathogen is grown in a multi-well plate containing liquid medium amended with a range of fungicide concentrations (Brent and Hollomon 2007). The microtiter assay allows handling of a large number of samples in a short

period of time. This technique has been used as a reliable tool to monitor biomass of various fungi including *Fusarium culmorum, Septoria nodorum* and *Rhizoctonia solani* (Broekaert et al. 1990).

Pasmo is one of the most important diseases of flax, which is caused by the pathogen *Septoria linicola* (Speg.) Garassini. Pyraclostrobin is a strobilurin fungicide, registered in Canada for use in flax to control pasmo since 2009 (Health Canada 2011; BASF 2009). Fluxapyroxad is a succinate dehydrogenase inhibitor (SDHI) fungicide, applied in flax along with pyraclostrobin since 2015 (BASF 2015). A number of pathogens have already developed insensitivity to these active ingredients (Avenot et al. 2008; Kim and Xiao 2011; Yin et al. 2012; Amiri et al. 2014). Therefore, it is very important to determine the sensitivity of *S. linicola* to these fungicides, and thus, the objective of the current study was to determine the sensitivity of *S. linicola* isolates *in vitro* against pyraclostrobin and fluxapyroxad using the spore germination assay and the microtiter assay method.

4.2. Materials and Methods:

Media preparation:

Isolates of *S. linicola* were grown on potato dextrose agar (PDA) media, which was prepared by thoroughly mixing 19.5 g of media in 500 mL of distilled water and autoclaving for approximately 20 min at 121°C. After autoclaving, 500 µL of streptomycin sulphate (100 µg mL^{-1}) antibiotic was added in the media to prevent bacterial growth. Fifteen mL of media was poured into each petri-dish (Fisher Scientific Inc.); after cooling, media was stored at 4°C.

Isolation of the pathogen:

Pasmo infected flax stems were collected from the fungicide field trials at the maturity stage (see previous chapter), which were at three locations in western Canada (Brandon, Melfort and

Saskatoon). The stems were collected from the plots where pyraclostrobin or fluxapyroxad were applied at both early- and mid-flowering stages. Stems were washed with distilled water and cut into three to four cm pieces. The pieces were incubated on wet filter paper in a petri dish under light for 24 hours. Stems were then observed under the dissecting microscope and the ooze containing spores from a single pycnidium (Fig. 4.1a) transferred with a sterilized needle to the PDA media. The petri-dishes were sealed with parafilm and cultured for 15 days in the dark at room temperature (Fig. 4.1b). After 15 days, spore masses formed in the middle of the fungal growth, and were harvested by adding 2 mL of autoclaved water to the culture plate and gently rubbing with a sterilized loop. To increase the culture, 1 mL of spore solution was spread on solid PDA media and incubated for 2 days at room temperature in the dark. After 2 days, 2 mL of glycerol was added to the plate and the concentrated spores collected with a sterilized pipette. The spore solution was stored in microfuge tubes at -80°C as a concentrated stock.

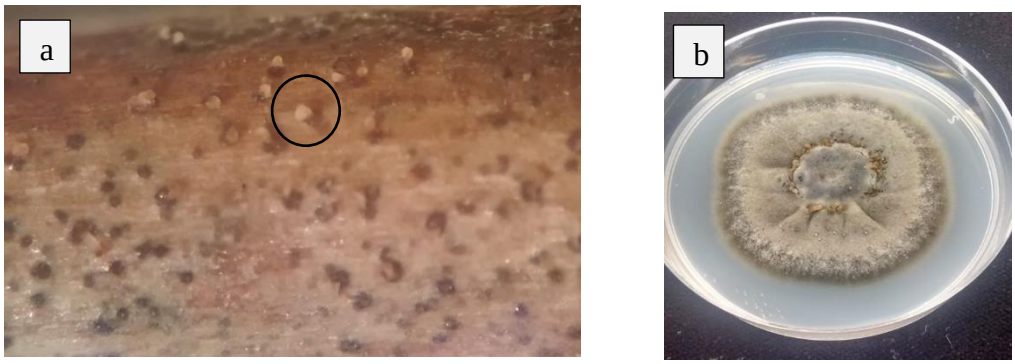

Fig 4.1: a) pycnidiospore containing ooze produced by pycnidia of *Septoria linicola*, and b) growth of a *Septoria linicola* isolate on PDA media

Preparation of fungicides:

Technical grade pyraclostrobin and fluxapyroxad (BASF Corporation) were used in these *in vitro* experiments. Both fungicides were diluted with methanol in a dilution series of 100, 10, 1 and 0.1 µg mL^{-1}, for use as stock fungicide solutions for the experiments. Salicylhydroxamic acid (SHAM) was also used alone and with each concentration of pyraclostrobin in both experiments to block the use of alternative respiration pathway by the pathogen. The SHAM was dissolved in methanol and used at a concentration of 100 µg mL^{-1}. All fungicides were stored at room temperature in dark bottles. In the spore germination assay, fungicide concentrations of 0.1, 0.01, 0.001 and 0.0001 µg mL^{-1} were used with a no-fungicide control for both pyraclostrobin and fluxapyroxad. In the microtiter assay, fungicide concentrations of 0.1, 0.01, 0.001 and 0.0001 µg mL^{-1} and a no-fungicide control were used for pyraclostrobin, and 0.5, 1.0, 2.5 and 5.0 µg mL^{-1} and a no-fungicide control for fluxapyroxad. The dosage ranges were selected based on preliminary experiments.

Spore germination assay:

Water agar was used as the media for the spore germination assay. It was prepared by thoroughly mixing 7.5 g of the agar in 500 mL of distilled water and autoclaving for 20 min at 121°C. The autoclaved agar was cooled to 50 °C. In a sterile Falcon tube, 45 mL of media and 45 µL of each fungicide concentration were added and used to pour three Petri plates (9 cm) of each treatment. The fungicides were diluted with methanol; therefore, a fungicide-free methanol control was included.

For this experiment, the stock *S. linicola* isolates were grown on PDA media for 2 days to obtain fresh spores. After 2 days, the spores were harvested by pipetting autoclaved water onto the media

and suspending them by dislodging with a sterile loop. Spores were counted under the microscope using a hemocytometer and diluted to a spore concentration of 1×10^6 spores per mL. Ten µL of spore solution was pipetted onto the water agar as drops and incubated for 6 hours in the dark. Three replications were used for the test. At this point, 15 µL of lacto phenol-cotton blue (stain) was pipetted onto the spores to stop spore germination. Fifty spores were then observed under a compound microscope to determine germination rate. Spores with germ tube lengths equal to the length of the spore were considered germinated and spores with germ tubes smaller than the length of the spore were considered non-germinated (Fig. 4.2).

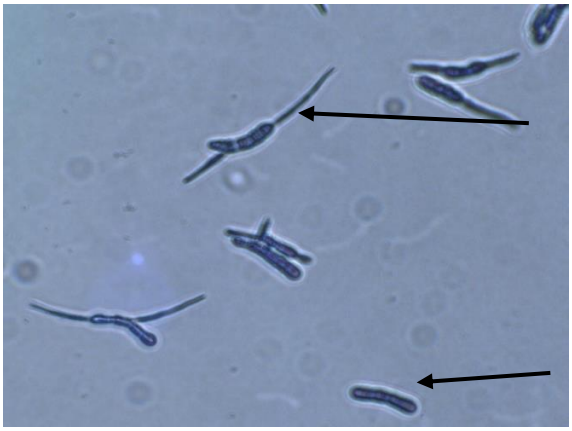

Fig 4.2. Germinated and non-germinated spores of *Septoria linicola* on PDA media observed under the microscope with magnification of 40× after 15 hours of incubation in dark at room temperature

Microtiter assay method:

Flat-bottomed, 96-well microtiter plates (Fig. 4.3a) were used in this experiment. The growth media was PDA broth prepared by thoroughly mixing 19.5 g of the media in 500 mL of distilled water and autoclaving for 20 min at 121°C. When the media cooled, 500 µL of streptomycin antibiotic was added to prevent bacterial growth. The spore solutions were prepared following the

same procedure as the spore germination assay, however, the concentration of the spore solution was 2×10^6 spore mL^{-1}.

The spore solution was vortexed vigorously. Then 60 µL of spore solution was pipetted into each well of a microtiter plate, and 140 µL of media amended with each fungicide concentration added for a total volume of 200 µL in each well. An initial absorbance reading was recorded using the FLUOstar Omega microtiter plate reader (BMG Labtech) (Fig. 4.3b) at 405 nm wavelength because the highest absorbance was observed at this wavelength. The plates were then sealed with parafilm and incubated in a growth chamber in the dark at 20°C. After seven days, the final reading was taken at the same wavelength. The fungal growth was measured by subtracting the initial reading from the final reading.

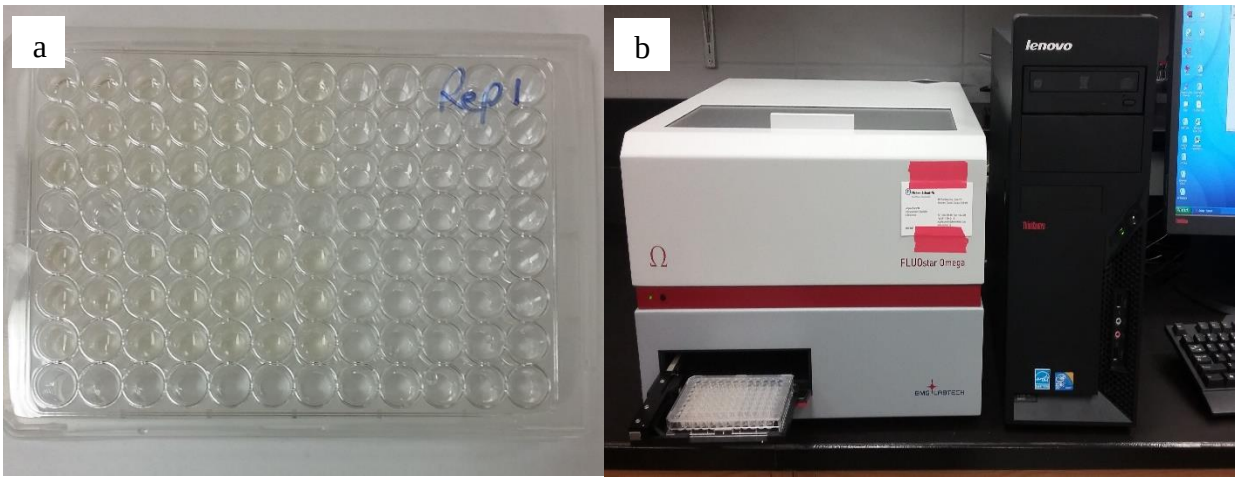

Fig 4.3. a) Flat-bottomed microtiter plate, and b) microtiter plate reader (FLUOstar Omega, BMG Labtech)

Data analysis:

For all experiments, isolates were arranged in a completely randomized design with three replicates for each isolate–fungicide concentration. The EC_{50} value and standard error of each isolate were calculated using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and plotted for each fungicide. The Anderson-Darling normality test was used to determine the normality of EC_{50}

values for each fungicide and Levene's test was performed to ensure the homogeneity of variances of EC_{50} data. Data was subjected to analysis of variance (ANOVA) and treatment differences separated using Tukey's post-hoc analysis. All analyses were performed in R (R Foundation for Statistical Computing, Vienna, Austria), version 3.4.1.

4.3. Results:

Spore germination assay:

A total of 73 isolates were assessed in this experiment: 51 isolates from diseased flax stem samples collected in 2014 (21 from Melfort, 16 from Saskatoon, and 14 from Brandon) and 22 isolates in 2015 (nine from Melfort, seven from Saskatoon and six from Brandon).

In the spore germination assay, the EC_{50} of most of the isolates tested for pyraclostrobin sensitivity were between 0.0005 to 0.007 µg mL^{-1}, mean of 0.003 µg mL^{-1} (Fig 4.4). The highest EC_{50} was 0.009 µg mL^{-1}, for Isolate 15sl29, and the lowest EC_{50} was 0.0004 µg mL^{-1} for Isolate 14sl10.

The effect of fluxapyroxad on spore germination differed among isolates (Fig 4.5). For most isolates, the EC_{50} for fluxapyroxad was between 0.001 to 0.05 µg mL^{-1}, mean 0.014 µg mL^{-1}. The highest EC_{50} for fluxapyroxad was 0.0371 µg mL^{-1} for Isolate 14sl36. The lowest EC_{50} was 0.0004 µg mL^{-1} for Isolate 14sl81.

The spore germination rate for SHAM alone did not differ from the un-amended water agar control (Fig 4.6). When SHAM was added to the media with pyraclostrobin, the spore germination results were similar to pyraclostrobin without SHAM.

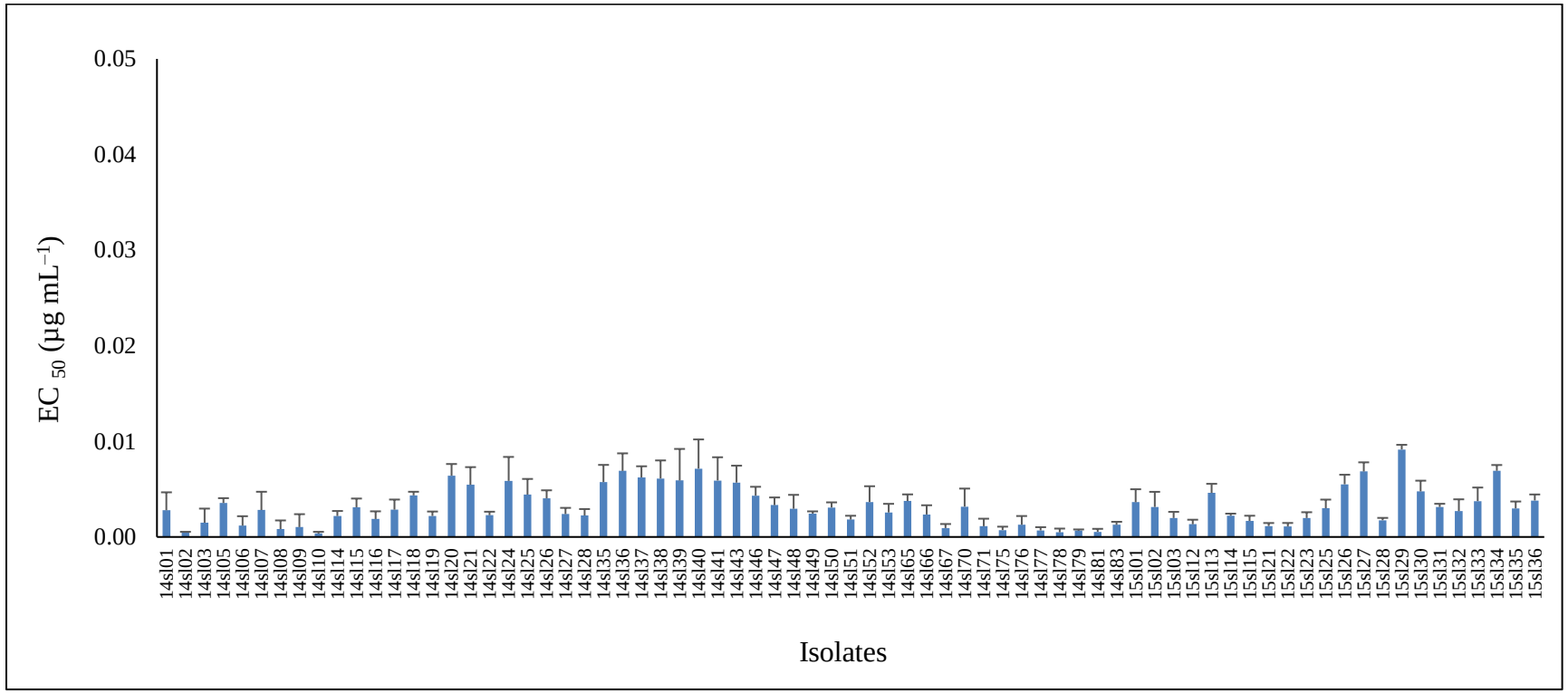

Fig 4.4: Effective concentrations (EC_{50}) of pyraclostrobin required to reduce spore germination by 50% for 73 isolates of *Septoria linicola* from flax fields in Brandon, Melfort and Saskatoon in 2014 and 2015

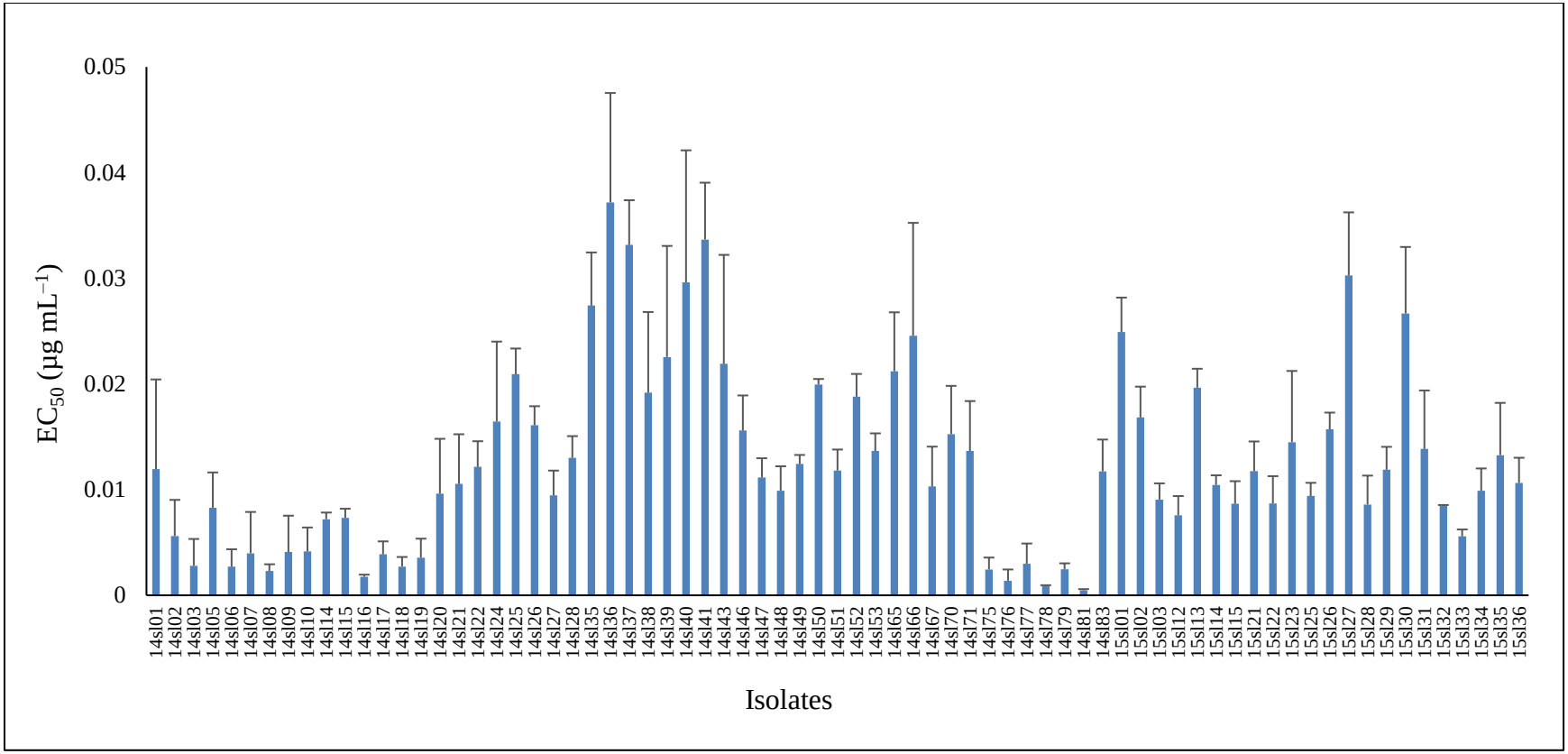

Fig 4.5: Effective concentrations (EC_{50}) of fluxapyroxad required to reduce the spore germination by 50% for 73 isolates of *Septoria linicola* from flax fields in Brandon, Melfort and Saskatoon in 2014 and 2015

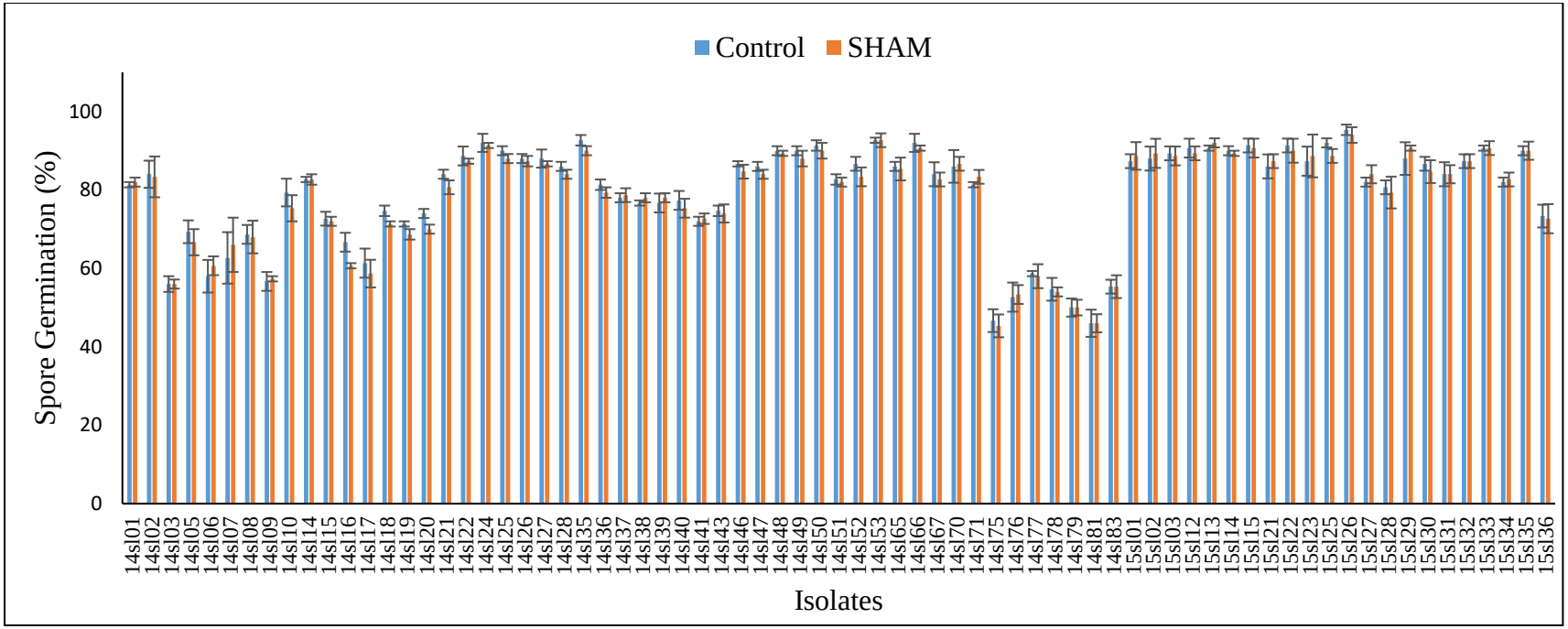

Fig 4.6: Spore germination (%) of 73 isolates of *Septoria linicola* (obtained from flax fields in Brandon, Melfort, and Saskatoon in 2014 and 2015). Spore germination values were obtained from the spore germination assay on water agar media amended with salicyl hydroxamic acid (SHAM) and control (media only)

Microtiter assay:

Of the 73 isolates used in the spore germination assay, 42 isolates were used in the microtiter assay. Thirty-one isolates were excluded because they did not grow in the liquid media. In the microtiter assay, the EC_{50} of most of the isolates on media amended with pyraclostrobin was between 0.003 to 0.021 µg mL^{-1}, mean of 0.009 µg mL^{-1} (Fig 4.7). The highest EC_{50} (0.021 µg mL^{-1}) was that of Isolate 14sl39. The lowest EC_{50} (0.003 µg mL^{-1}) was for Isolate 14sl43. The EC_{50} of most of the isolates for fluxapyroxad was between 1 and 4 µg mL^{-1} and the mean EC_{50} was 2.40 µg mL^{-1} (Fig 4.8). The highest effective concentration for fluxapyroxad was 3.89 µg mL^{-1} and the lowest 1.14 µg mL^{-1}, which was observed for Isolates 14sl46 and 14sl03, respectively.

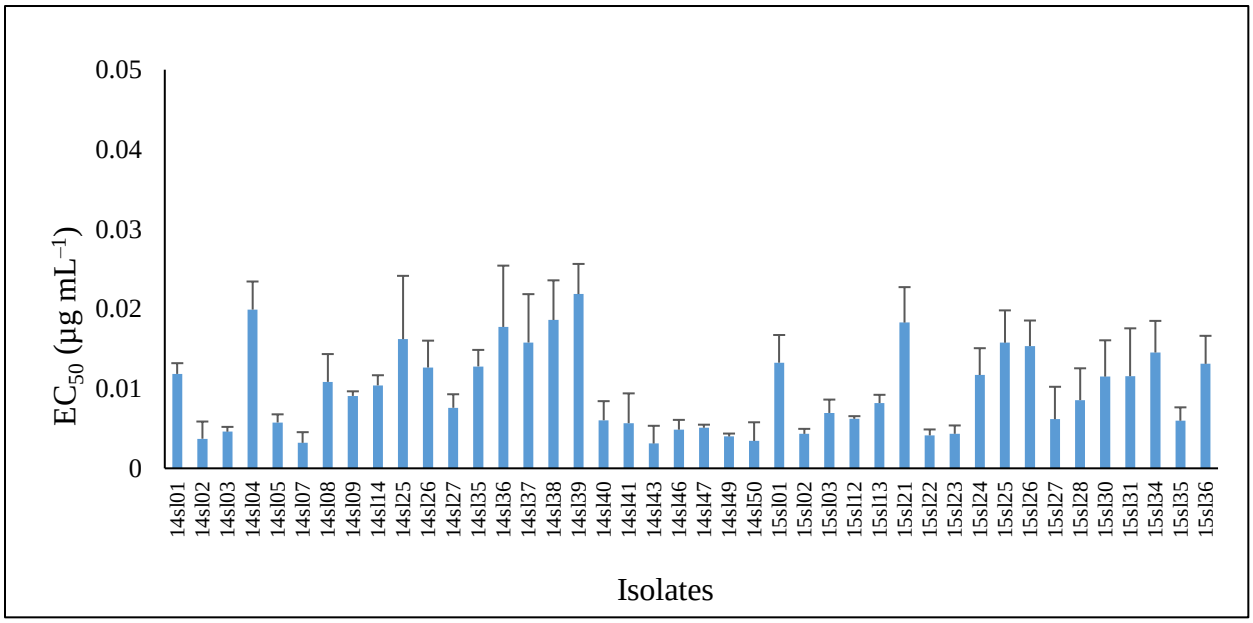

Fig 4.7: Effective concentrations (EC_{50}) of pyraclostrobin required to reduce the mycelial growth by 50% for 42 isolates of *Septoria linicola* from flax fields in Brandon, Melfort, and Saskatoon in 2014 and 2015.

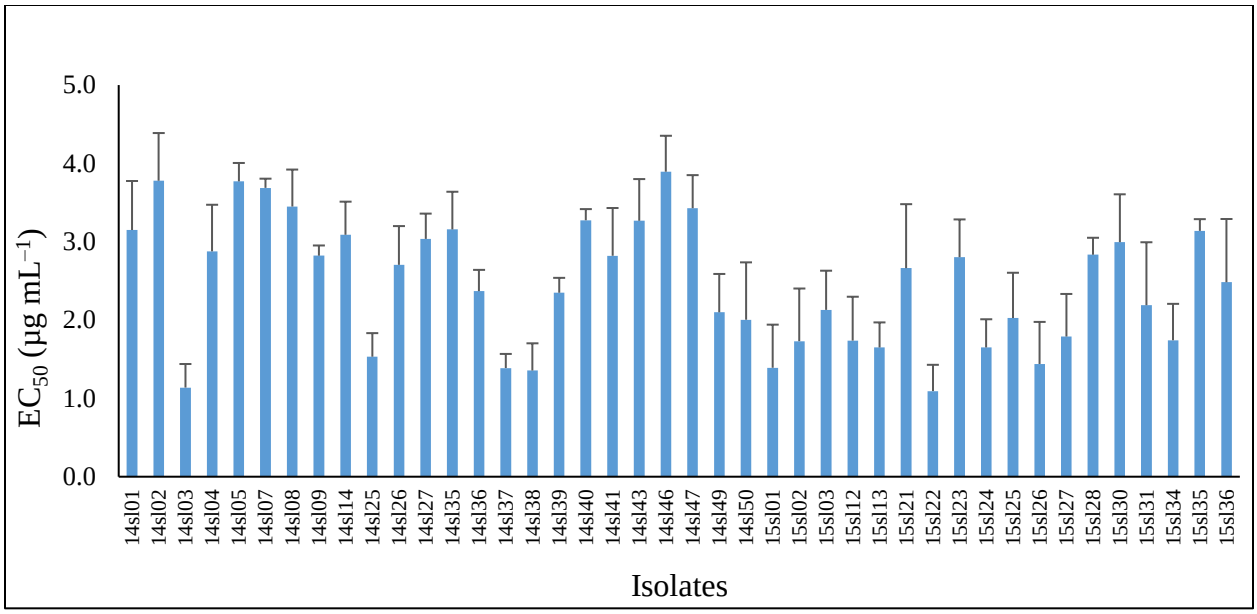

Fig 4.8: Effective concentrations (EC_{50}) of fluxapyroxad required to reduce the mycelial growth by 50% for 42 isolates of *Septoria linicola* from flax fields in Brandon, Melfort, and Saskatoon in 2014 and 2015

There was no effect of SHAM on spore germination of *S. linicola* isolates; however, there was an effect of SHAM in the microtiter assay. When SHAM without fungicide was added to the media in the microtiter assay it reduced fungal growth compared to the control (Fig 4.9). When SHAM was added to pyraclostrobin amended media it completely inhibited fungal growth. The fungal growth of six *S. linicola* isolates after incubation for 7 days clearly demonstrated the effect of SHAM on fungal growth with and without pyraclostrobin (Fig 4.10).

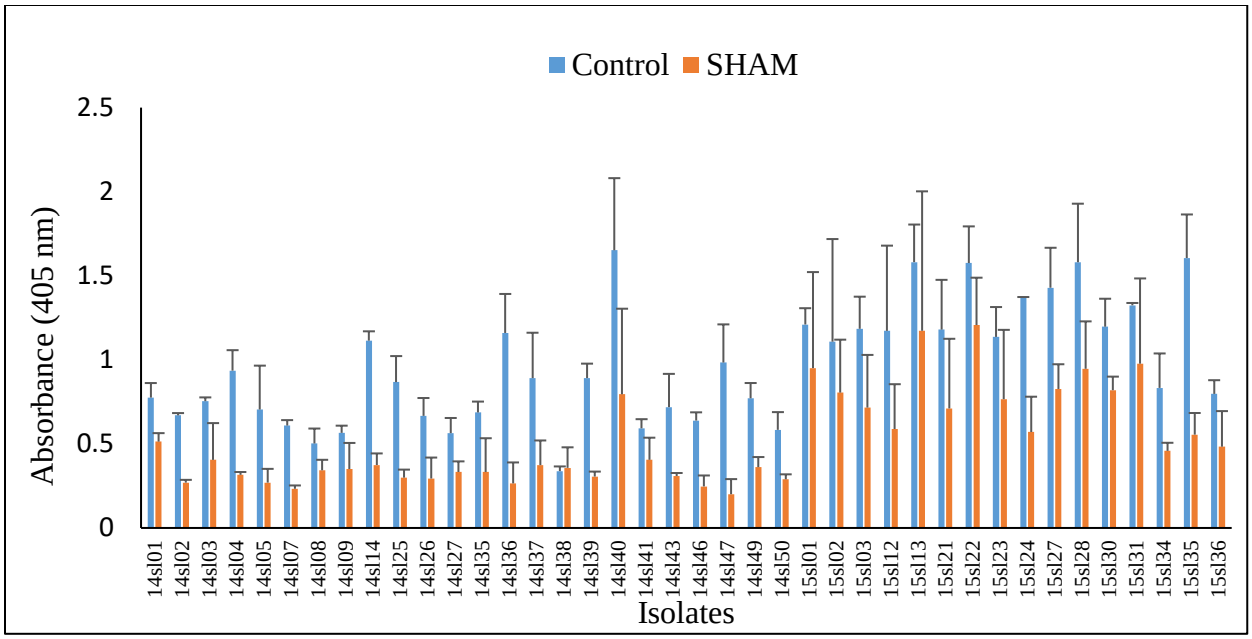

Fig 4.9: Absorbance of 42 isolates of *Septoria linicola* (obtained from flax fields in Brandon, Melfort, and Saskatoon in 2014 and 2015). The absorbance values were obtained from the microtiter assay in PDA broth media amended with salicyl hydroxamic acid (SHAM) and control (media only)

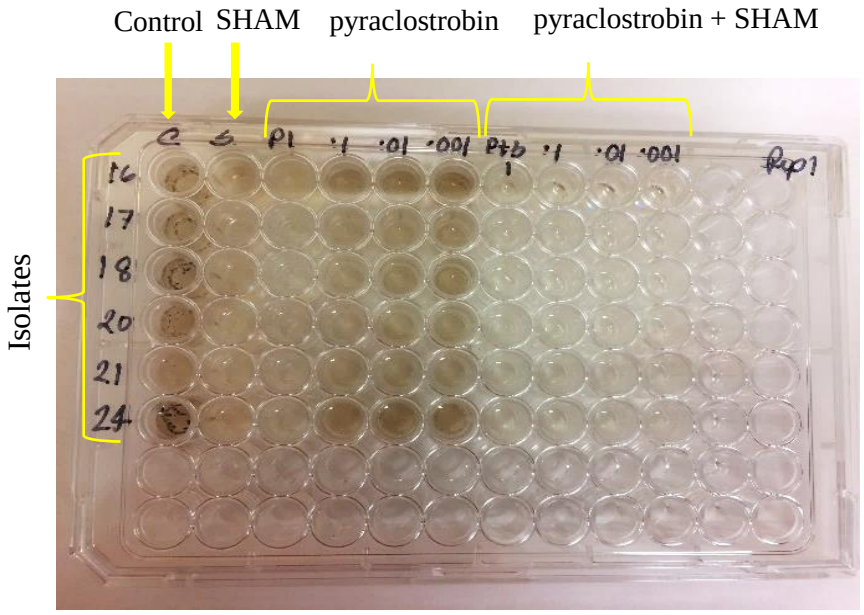

Fig 4.10: Microtiter assay of six *Septoria linicola* isolates (16 – 24) with media amended with fungicide concentrations of 1.0, 0.1, 0.01, 0.001 µg mL^{-1} on a microtiter plate (Column 1: Un-amended control, Column 2: SHAM control, Columns 3 – 6: pyraclostrobin and Columns 7 – 10: pyraclostrobin + SHAM)

4.4. Discussion:

Pyraclostrobin and fluxapyroxad are two common fungicides used to control pasmo disease of flax in Canada; however, resistance to these fungicides has been reported in many pathogens (Avenot et al. 2008; Kim and Xiao 2011; Yin et al. 2012; Amiri et al. 2014). For example, of 216 isolates of *Botrytis cinereal* (causal agent of gray mold disease of strawberry), collected from 11 fields in North and South Carolina in 2011, 144 isolates (66.7%) were insensitive to pyraclostrobin (Fernández-Ortuño et al. 2012). Among 324 isolates of *Mycosphaerella pinodes* (cause Mycosphaerella blight of field pea) which were collected from the commercial fields of Alberta, Saskatchewan, North Dakota and Washington State in 2010 and 2011, 19 isolates were highly insensitive to pyraclostrobin with EC_{50} values of 80 to 216 mg L^{-1} (Bowness et al. 2016). However, there are no reports on the sensitivity of *S. linicola* to pyraclostrobin or fluxapyroxad. Fungicide sensitivity is usually measured by the effective concentration (EC_{50} value) of the fungicide at which spore germination or fungal growth is inhibited by 50% compared with the fungicide free control (LaMondia and Douglas 1997; Pasche et al. 2004). In the current study, the mean EC_{50} for pyraclostrobin was 0.003 µg mL^{-1} in the spore germination assay of *S. linicola*. In general, *S. linicola* was more sensitive than other fungi tested against pyraclostrobin fungicide. For example, among 34 isolates of *Alternaria alternate* (causal agent of alternaria late blight of pistachio), 27 isolates were sensitive to pyraclostrobin with an EC_{50} <0·01 µg mL^{-1}, six isolates had low insensitivity with a mean EC_{50} value 4.71 µg mL^{-1} and one isolate had an EC_{50} >100 µg mL^{-1}, which was considered pyraclostrobin insensitive (Avenot et al. 2008). Spore germination of *Ascochyta rabiei*, cause of ascochyta blight of chickpea, was inhibited by 50% at a pyraclostrobin concentration of 0.01 µg mL^{-1} (Chang et al. 2007). Baseline sensitivity of 55 *Botrytis cinerea*

isolates, cause of gray mold of various vegetables (e.g. tomato, cucumber and eggplant), was determined against pyraclostrobin and the EC_{50} was 0.033 µg mL^{-1} (Myresiotis et al. 2008).

Fluxapyroxad is a relatively new fungicide in the SDHI group; therefore, the baseline sensitivity to this fungicide has been measured for only a limited number of pathogens. The baseline sensitivity of *Ascochyta lentis, A. pinode* and *A. rabiei* was determine against fluxapyroxad using the radial growth assay and the mean was 0.05, 0.76 and 0.06 µg mL^{-1}, respectively (Lonergan et al. 2015). Among 113 isolates of *A. alternate* (cause of alternaria late blight of pistachio), collected from commercial pistachio orchards in California in 1999 and 2000, 73 isolates were sensitive to fluxapyroxad with an EC_{50} value of 0.001 to 0.14 µg mL^{-1} (Avenot et al. 2014). The baseline EC_{50} value of *B. cinerea* was between 0.08 and 1.34 µg mL^{-1} (Amiri et al. 2014). In our study, the mean EC_{50} of fluxapyroxad for *S. linicola* was 0.012 µg mL^{-1} in the spore germination assay, which was in the sensitive range of other pathogens.

Although the spore germination assay is the standard method of testing fungicide sensitivity, many researchers have used the microtiter assay method as a rapid, reproducible, and efficient method for testing fungal growth inhibition (Broekaert et al. 1990; Beyer et al. 2011). The EC_{50} of *Pyrenophora teres* f. *teres*, the cause of the net form of net blotch of barley, for pyraclostrobin sensitivity was 0.015 mg L^{-1} and for *P. teres* f. *maculate*, the spot form of net blotch, 0.024 to 0.015 mg L^{-1} as determined using the microtiter method (Akhavan et al. 2017). In the current study, the mean EC_{50} for pyraclostrobin was 0.009 µg mL^{-1} and for fluxapyroxad was 2.5 µg mL^{-1}.

Many researchers consider an EC_{50} of more than 100-fold higher than the mean EC_{50} of the isolates as an indication of fungicide insensitivity. For example, insensitivity to azoxystrobin was reported in isolates of *Pyricularia grisea*, causing gray leaf spot of perennial ryegrass turf, which had EC_{50}

values 1300 fold higher than the mean (Kim et al. 2003). Sensitivity of *A. rabiei* to pyraclostrobin was evaluated by the spore germination assay and EC_{50} values of two isolates were calculated as 704-fold higher than the average of all other isolates, and were considered fungicide insensitive (Wise et al. 2009). The spore germination assay of *Cercospora beticola*, cause of cercospora leaf spot of sugar beet, determined the sensitivity to pyraclostrobin and an EC_{50} 400-fold higher than average was detected (Secor et al. 2010). Almost 690- and 827-fold higher EC_{50} were reported when sensitivity of *Pyricularia grisea* isolates, causal agent of gray leaf spot of perennial ryegrass, were determined against azoxystrobin and trifloxystrobin, respectively (Vincelli and Dixon 2002).

The isolates used in this experiment were isolated from pasmo infected flax stems, which were exposed to pyraclostrobin and fluxapyroxad in field experiments. No baseline sensitivity reports of *S. linicola* to any fungicide are available and this is the first study of sensitivity of *S. linicola* to these fungicides; thus, it is difficult to establish the concentration of pyraclostrobin and fluxapyroxad at which an isolate of the pathogen should be considered insensitive. Therefore, the EC_{50} value of individual isolates was compared with the mean EC_{50} of all isolates. In the spore germination assay, Isolate 15sl29 exhibited a three-fold higher EC_{50} to pyraclostrobin than the mean and Isolate 14sl41 exhibited a four-fold higher EC_{50} than the mean to fluxapyroxad. In the microtiter assay, the highest EC_{50} was observed in Isolates 14sl39 and 14sl46, which were only two-fold higher than the mean EC_{50} against pyraclostrobin and fluxapyroxad, respectively. Therefore, the results from both spore germination and microtiter assays suggested that all the isolates of *S. linicola* were sensitive to pyraclostrobin and fluxapyroxad. Since, we did not find an isolate highly sensitive to these fungicides, we conclude that fungicide insensitivity has not occurred in the population tested.

When pyraclostrobin is used to control any pathogen *in vitro*, many pathogens can use a secondary respiration mechanism to avoid the effect of pyraclostrobin (QoI fungicide) (Wise et al. 2008). This alternative respiration mechanism can be inhibited by using SHAM in addition to pyraclostrobin in fungicide sensitivity assays (Mondal et al. 2005; Wise et al. 2008; Patel et al. 2012). For example, SHAM did not inhibit spore germination of *Guignardia citricarpa*, causal agent of citrus black spot, but affected mycelium growth slightly when exposed to pyraclostrobin (Hincapie et al. 2014). A similar result was observed for the baseline sensitivity of *Penicillium digitatum*, cause of citrus green mold, against azoxystrobin in the presence and absence of SHAM (Kanetis et al. 2008). In the current study, the EC_{50} values obtained with and without SHAM for the 73 isolates indicated that spore germination was not affected by SHAM. Similar EC_{50} values were observed when SHAM was included with pyraclostrobin or SHAM alone. However, an effect of SHAM was detected in the microtiter assay. When media was amended with SHAM only, it reduced fungal growth compared to the control. When pyraclostrobin was amended with SHAM, the fungal growth was completely inhibited in the microtiter assay, which indicated that there is likely to be an alternative respiratory pathway present in *S. linicola.*

This study has provided up-to-date information on the sensitivity status of *S. linicola* against pyraclostrobin and fluxapyroxad in western Canada. These results provide valuable information on which to establish sensitivity profiles of *S. linicola* to pyraclostrobin and fluxapyroxad. Pyraclostrobin and fluxapyroxad insensitive isolates of *S. linicola* were not identified in this study. This information will facilitate the monitoring of pathogen sensitivity for shifts in fungicide sensitivity and help to identify disease management strategies with the aim of extending use of the available fungicides for better management of pasmo in flax.

CHAPTER 5

5. General discussion and future perspectives

5.1 General discussion

Among flax growing countries, Canada is the world's largest producer and exporter. Production can be limited due to pasmo in many years. Many plant breeders have tried to develop a pasmo resistant flax cultivar, however, there are no current pasmo resistant commercial flax cultivars (Flor 1953; Varieties of Grain Crops 2017). Therefore, fungicides are required to control pasmo when the environment is favorable for disease development.

The focus of this project was to determine the effect of pyraclostrobin, fluxapyroxad and the combination of pyraclostrobin and fluxapyroxad, applied at early- and mid-flowering stages on pasmo severity and flax seed yield and quality. In our study, pasmo was best controlled by fluxapyroxad+pyraclostrobin and pyraclostrobin, compared with fluxapyroxad. No difference was observed between the seed yield of fluxapyroxad+pyraclostrobin and pyraclostrobin. However, application of fluxapyroxad+pyraclostrobin increased the seed yield compared with fluxapyroxad. Application of pyraclostrobin+fluxapyroxad also improved TSW over that of either pyraclostrobin or fluxapyroxad. However, application of pyraclostrobin+fluxapyroxad may delay crop maturity by up to 14 days. Thus, flax growers should also consider seeding date and the time to crop maturity before fungicide application.

Fungicide application at the optimal time is one of the major factors necessary for successful disease management. Fungicide application at the appropriate flowering stage can effectively

control pasmo (Ferguson et al. 1987). In the current study, although no statistical difference was observed in disease severity or yield between early-flower application and mid-flower application, the mid-flower application resulted in 100 kg ha^{-1} higher yield than the early-flower application. The application of fungicide at the mid-flowering stage increased the seed yield similar to two applications. Applying fungicide at both stages increased the seed yield by 173 kg ha^{-1} compared with a single application at early flowering stage, and by 73 kg ha^{-1} compared with a single application at mid-flowering stage. The cost of pyraclostrobin fungicide (Headline) is \$37.6 ha^{-1} and fluxapyroxad+pyraclostrobin (Priaxor) is \$34.4 ha^{-1}, with an additional \$37.00 ha^{-1} to apply the fungicide. With a flax price of \$491.12 per MT, applying fungicide at mid-flowering stage resulted in additional income of \$49.11 ha^{-1} compared with a single application at early flowering stage. Applying fungicide at both stages resulted in additional income of \$84.96 ha^{-1} compared with a single application at early flowering stage and \$35.85 ha^{-1} compared with a single application at mid-flowering stage, however the two applications of fungicide cost approximately \$150 ha^{-1}. Therefore, these increases in revenue were not profitable and a single application of fungicide would be the most cost effective.

Our study also suggested that application of fungicide is cost effective only when pasmo is severe. No yield benefit was observed from fungicide application in the absence of disease. At the same time unnecessary and/or continuous use of fungicide application in the absence of disease may increase the risk of development of fungicide insensitive pathogens. Most of the systemic fungicides are single-site inhibitors and due to continuous application of the same fungicide, genetic changes can occur in the pathogen population that may result in fungicide insensitivity of pathogenic fungi. Thus, flax growers should use fungicides in rotation with multiple modes of action when possible, or in mixtures, and should not spray when the environmental conditions are

unfavorable for pasmo development. For example, hot and dry weather is unfavorable for pasmo development (Brentzel 1926; Gruzdevienė et al. 2008), therefore, growers might avoid fungicide application in this environment.

Pasmo may not reduce seed yield if pathogen infection occurs after flax maturity; however, it may be very destructive when the environmental conditions are favorable and pathogen infection occurs during the flowering stages of flax (Sackston 1950). Long, warm humid periods, especially when storms cause the flax to lodge, favor severe pasmo development and reduced seed yield (Flor 1953; Ferguson et al. 1987). The weather conditions for pasmo development differ from year to year and from location to location. In this study, fungicide treatment did not always decrease disease severity or improve yield; the results varied with location, as a result of varying weather conditions and disease pressure. As pasmo usually appears when the crop is approaching maturity, it is very difficult to make the decision to apply fungicide. However, it is very important to consider the location, weather conditions, previous cropping history, and information from previous disease surveys before making the decision to apply fungicide. As pasmo is a residue borne disease and can persist in the flax residue for up to four years, flax should not be grown in the same field every year. A crop rotation with three other crops should be considered. High precipitation or moist conditions influence pasmo development. Thus, flax growers should check the weather conditions and apply fungicide at the mid-flowering stage if high precipitation or moist conditions are observed. Moreover, it is very important to scout flax fields for presence of pasmo on a regular basis.

At present, only pyraclostrobin (Headline) and fluxapyroxad+pyraclostrobin (Priaxor) are registered for pasmo. However, in flax, pyraclostrobin has been applied since 2009 and many pathogens have already developed insensitivity against this product. Therefore, application of

pyraclostrobin alone may result in the development of fungicide insensitivity in the *S. linicola* population. For this reason, a single application of fluxapyroxad+pyraclostrobin at mid-flowering stage appears to be the best option for flax growers to manage pasmo at this time. However, it would be prudent for the crop protection industry to register new fungicide products with different modes of action to increase management options available for growers to manage this disease and minimize the risk of fungicide insensitivity due to continuous use of the same fungicides and/or combined fungicide products (Kable and Jeffery 1980; Dekker and Georgopoulos 1982).

Currently, there have been no reports of sensitivity of *S. linicola* to any fungicide; however, the second study of this thesis focused on sensitivity of *S. linicola* to pyraclostrobin and fluxapyroxad in anticipation that this will occur in future. This study included isolates from a limited number of sites in western Canada (Brandon, Melfort and Saskatoon in 2014-2015), however, this research is the first assessment of the sensitivity of the *S. linicola* population in western Canada to pyraclostrobin and fluxapyroxad. None of the isolates tested displayed reduced sensitivity to either pyraclostrobin or fluxapyroxad based on the EC_{50} values calculated from the spore germination assay or the microtiter assay. The variation in EC_{50} values we observed in either assay was within a range that did not suggest insensitivity of *S. linicola* to either fungicide. It appears that pyraclostrobin and fluxapyroxad are both effective to manage pasmo of flax in western Canada at present. However, continued fungicide insensitivity monitoring programs for *S. linicola* are necessary as a precautionary measure.

The effect of SHAM was also measured in the presence and absence of pyraclostrobin, as many plant pathogens are known to have an alternative respiration pathway *in vitro* when in contact with pyraclostrobin. The use of SHAM prevents the pathogen from accessing the alternative pathway (Olaya et al. 1998; Wise et al. 2008). Although no effect of SHAM was observed in the spore

germination assay, results from the microtiter assay indicated that in the absence of SHAM, *S. linicola* was able to access the alternative respiration pathway.

5.2 Future perspectives

In the future, fungicide studies of pasmo of flax could include more fungicides with different active ingredients and different modes of action than used in this study to determine their effect on pasmo severity, seed yield and quality of flax. More research is recommended to determine the factors affecting delayed crop maturity due to fungicide application. Bioproducts and biocontrol agents, including beneficial fungi or bacteria are used to control various plant diseases (Elad and Freeman 2002; Compant et al. 2005). For example, use of two yeasts species (*Candida saitoana* and *C. oleophila*), chitosan and harpin effectively reduced the severity of blue mold of apple caused by *Penicillium expansum* (De Capdeville et al. 2002). Thus, various bio-control agents may have the ability to control pasmo as an alternative to fungicides.

With regards to the fungicide sensitivity study, more isolates from other locations and countries could be collected and tested to determine sensitivity against pyraclostrobin and fluxapyroxad. The effect of SHAM was observed in this study; therefore, further research should be conducted to understand the effect of the alternative respiratory pathway of *S. linicola*.

REFERENCES

Agricultural Land Resource Atlas of Alberta. [Online]. Available: http://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/agdex10304/$FILE/pg_8_frost-free_period.pdf [14 Oct. 2017].

Ahmed, H.U., Chang, K.-F., Hwang, S.-F., Gossen, B.D., Strelkov, S.E., and Turnbull, G.D. 2014. Baseline sensitivity and the population shifts of *Didymella rabiei* in chickpea to the QoI fungicide pyraclostrobin in Alberta, Canada. J. Plant Dis. Prot. **121**: 164–170.

Akhavan, A., Strelkov, S.E., Askarian, H., Kher, S. V., Fraser, M., Kutcher, H.R., and Turkington, T.K. 2017. Sensitivity of western Canadian *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata* isolates to propiconazole and pyraclostrobin. Can. J. Plant Pathol. **39**: 11–24.

Alessi, J., and Power, J.F. 1970. Influence of row spacing, irrigation, and weeds on dryland flax yield, quality, and water use. Agron. J. **62**: 635.

Allaby, R.G., Peterson, G.W., Merriwether, D.A., and Fu, Y.B. 2005. Evidence of the domestication history of flax (*Linum usitatissimum* L.) from genetic diversity of the sad2 locus. Theor. Appl. Genet. **112**: 58–65.

Alvarez, J.M. 2010. Influence of soil type and natural Zn chelates on flax response, tensile properties and soil Zn availability. Plant Soil **328**: 217–233.

Amand, O., Calay, F., Coquillart, L., Legat, T., Bodson, B., Moreau, J.M., and Maraite, H. 2003. First detection of resistance to QoI fungicides in Mycosphaerella graminicola on winter wheat in Belgium. Commun. Agric. Appl. Biol. Sci. **68**: 519–31.

Amiri, A., Heath, S.M., and Peres, N.A. 2014. Resistance to Fluopyram, Fluxapyroxad, and Penthiopyrad in *Botrytis cinerea* from Strawberry. Plant Dis. **98**: 532–539.

Anke, T., Oberwinkler, F., Steglich, W., and Schramm, G. 1977. The strobilurins - new antifungal antibiotics from the basidiomycete *Strobilurus tenacellus*. J. Antibiot. (Tokyo). **30**: 806–810.

Aranberri-Askargorta, I., Lampke, T., and Bismarck, A. 2003. Wetting behavior of flax fibers as reinforcement for polypropylene. J. Colloid Interface Sci. **263**: 580–589.

Avenot, H., Morgan, D.P., and Michailides, T.J. 2008. Resistance to pyraclostrobin, boscalid and multiple resistance to Pristine® (pyraclostrobin + boscalid) fungicide in *Alternaria alternata* causing alternaria late blight of pistachios in California. Plant Pathol. **57**: 135–140.

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 2010. Crop Prot. **29**: 643–651.

Avenot, H.F., van den Biggelaar, H., Morgan, D.P., Moral, J., Joosten, M., and Michailides, T.J. 2014. Sensitivities of Baseline Isolates and Boscalid-Resistant Mutants of *Alternaria alternata* from Pistachio to Fluopyram, Penthiopyrad, and Fluxapyroxad. Plant Dis. **98:** 197–205.

Avila-Adame, C., Olaya, G., and Köller, W. 2003. Characterization of *Colletotrichum graminicola* Isolates Resistant to Strobilurin-Related QoI Fungicides. Plant Dis. **87**: 1426–1432.

Bailey, K.L., Mortensen, K., and Lafond, G.P. 1992. Effects of tillage systems and crop rotations on root and foliar diseases of wheat, flax, and peas in Saskatchewan. Can. J. Plant Sci. **72**: 583–591.

Balba, H. 2007. Review of strobilurin fungicide chemicals. J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes **42**: 441–451.

Baltazar-y-Jimenez, A., and Bismarck, A. 2007. Wetting behaviour, moisture up-take and electrokinetic properties of lignocellulosic fibres. Cellulose **14**: 115–127.

Banno, S., Yamashita, K., Fukumori, F., Okada, K., Uekusa, H., Takagaki, M., Kimura, M., and Fujimura, M. 2009. Characterization of QoI resistance in *Botrytis cinerea* and identification of two types of mitochondrial cytochrome *b* gene. Plant Pathol. **58**: 120–129.

Bartett, D.W., Clough, J.M., Godfrey, C.R.A., Godwin, J.R., Hall, A.A., Heaney, S.P., and Maund, S.J. 2001. Understanding the strobilurin fungicides. Pestic. Outlook **12**: 143–148.

BASF. 2009. Headline® label amended to include flax. [Online]. Available: https://agro.basf.ca/West/News/item1103.html [14 Oct. 2017].

BASF. 2015. BASF Canada receives registration of Priaxor® fungicide in Western Canada. [Online]. Available. https://www.basf.com/us/en/company/news-and-media/news-releases/2014/06/p-13-601.html [14 Oct. 2017].

Beckerman, J.L. 2013. Detection of Fungicide Resistance. Pages 281–310 in M. Nita, ed. Fungicides - Showcases of integrated plant disease management from around the world. InTech.

Berglund, D.R. 2002. Flax: New uses and demands. Pages 358–360 in J. Janick and A. Whipkey, eds. Trends in new crops and new uses. ASHS Press, Alexandria, VA, USA.

Beyer, M., Kiesner, F., Verreet, J.A., and Klink, H. 2011. Fungicide sensitivity of *Septoria tritici* field isolates is affected by an interaction between fungicidal mode of action and time. J. Plant Pathol. **93**: 7–14.

Blixt, E., Djurle, A., Yuen, J., and Olson, Å. 2009. Fungicide sensitivity in Swedish isolates of *Phaeosphaeria nodorum*. Plant Pathol. **58**: 655–664.

Bloedon, L.T., and Szapary, P.O. 2004. Flaxseed and cardiovascular risk. Nutr. Rev. **62**: 18–27.

Booker, H.M., and Lamb, E.G. 2012. Quantification of low-level GM seed presence in Canadian commercial flax stocks. AgBioForum **15**: 31–35.

Booker, H.M., Mischkolz, J.M., St.Louis, M., and Lamb, E.G. 2014. Analysis of the prevalence of CDC triffid transgenic flax in Canadian grain stocks. AgBioForum **17**: 75–83.

Booker, H.M., Lamb, E.G., and Smyth, S.J. 2017. Ex-post assessment of genetically modified, low level presence in Canadian flax. Transgenic Research **26:** 399-409.

Bowness, R., Gossen, B.D., Chang, K.F., Goswani, R., Willenborg, C.J., Holtz, M., and Strelkov, S.E. 2016. Sensitivity of *Mycosphaerella pinodes* to pyraclostrobin fungicide. Plant Dis. **100**: 192–199.

Brent, K. 1992. Monitoring fungicide resistance: purposes, procedures and progress. Pages 1–18 in I. Denholm, A.L. Devonshire and D.W. Hollomon, eds. Resistance '91: Achievements and developments in combating pesticide resistance. Springer Netherlands, Dordrecht.

Brent, K.J., and Hollomon, D.W. 2007. Fungicide resistance in crop pathogens: how can it be managed?. Pages 5–33 in K.J. Brent and D.W. Hollomon, eds. Fungicide resistance in crop pathogens: how can it be managed?. FRAC, Brussels, Belgium.

Brentzel, W.E. 1926. The pasmo disease of flax. **32**: 25–37.

Broekaert, W.F., Terras, F.R.G., Cammue, B.P.A., and Vanderleyden, J. 1990. An automated quantitative assay for fungal growth inhibition. FEMS Microbiol. Lett. **69**: 55–59.

Brown, S., Koike, S.T., Ochoa, O.E., Laemmlen, F., and Michelmore, R.W. 2004. Insensitivity to the fungicide fosetyl-aluminum in California isolates of the lettuce downy mildew pathogen, Bremia lactucae. Plant Dis. **88**: 502–508.

Brutch N.B., Kutuzova S.N., Porohovinova E.A. 2001. The exposure of intra-specific diversity of *linum usitatissimum* as a basis of the development of particular flax genetics and breeding. Pages: 94-104. Proceedings of the second global workshop. Bust plants in the new millennium. 3-6 June, Borovets, Bulgaria.

Canadian Agricultural Services Coordinating Committee. 1998. The Canadian system of soil classification (No. 1646). NRC Research Press, Ontario.

Canadian Food Inspection Agency. 1998. Determination of the safety of the crop development centre's 'CDC Triffid', a flax (*Linum usitatissimum* L.) Variety tolerant to soil residues of triasulfuron and metsulfuron-methyl. [Online]. Available: http://www.inspection.gc.ca/plants/plants-with-novel-traits/approved-under-review/decision-documents/dd1998-24/eng/1312578625754/1312578859063 [14 Oct. 2017].

Canadian Grain Commission. 2016. Quality of western Canadian flaxseed 2016 - Weather and production. [Online]. Available: http://www.grainscanada.gc.ca/flax-lin/harvest-recolte/2016/hqf16-qrl16-2-en.htm [14 Oct. 2017].

Canvin, D.T. 1965. The effect of temperature on the oil content and fatty acid composition of the oils from several oil seed crops. Can. J. Bot. **43**: 63–69.

Casa, R., Russell, G., Lo Cascio, B., and Rossini, F. 1999. Environmental effects on linseed (*Linum usitatissimum* L.) yield and growth of flax at different stand densities. Eur. J. Agron. **11**: 267–278.

Caston, L., and Leeson, S. 1990. Research note: Dietary flax and egg composition. Poult. Sci. **69**: 1617–1620.

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 effect of strobilurin fungicides on the progress of ascochyta blight of chickpea. Can. J. Plant Sci **87**: 937–944.

Chapman, K.S., Pathology, P., Lafayette, W., and Sundin, G.W. 2011. Identification of resistance to multiple fungicides in field populations of *Venturia inaequalis*. Plant Dis. **95**: 921–926.

Chen, Y., Yao, J., Yang, X., Zhang, A.-F., and Gao, T.-C. 2014. Sensitivity of *Rhizoctonia solani* causing rice sheath blight to fluxapyroxad in China. Eur. J. Plant Pathol. **140**: 419–428.

Christensen, J.J., Henderson, L. and Aragaki, M., 1953. Dissemination of *Septoria linicola*. Phytopathology, 43.

Compant, S., Duffy, B., Nowak, J., Clément, C., and Barka, E.A. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. **71**: 4951–9.

Conner, R.L., Mcandrew, D.W., Kiehn, F.A., Chapman, S.R., and Froese, N.T. 2004. Effect of foliar fungicide application timing on the control of bean anthracnose in the navy bean "Navigator" 1. Can. J. Plant Pathol **26**: 299–303.

Çopur, O., Gür, M.A., Karakuş, M., and Demirel, U. 2006. Determination of correlation and path analysis among yield components and seed yield in oil flax varieties (*Linum usitatissimum* L.). J. Biol. Sci. **6**: 738-743.

Cunnane, S.C., Ganguli, S., Menard, C., Liede, a C., Hamadeh, M.J., Chen, Z.Y., Wolever, T.M., and Jenkins, D.J. 1993. High alpha-linolenic acid flaxseed (Linum usitatissimum): some nutritional properties in humans. Br. J. Nutr. **69**: 443–453.

Daayf, F., and Platt, H.W. 2000. Changes in metalaxyl resistance among glucose phosphate isomerase genotypes of Phytophthora infestans in Canada during 1997 and 1998. Am. J. Potato Res. **77**: 311–318.

De Capdeville, G., Wilson, C.L., Beer, S. V, and Aist, J.R. 2002. Alternative disease control agents induce resistance to blue mold in harvested "red delicious" apple fruit. Phytopathology **92**: 900–908.

Dekker, E.J. 1982. Countermeasures for avoiding fungicide resistance. Pages 177-186 in E.J. Dekker and S.G. Georgopoulos, eds. Fungicide resistance in crop protection. PUDOC, Wageningen, Netherlands.

Diederichsen, A. 2001. Comparison of genetic diversity of flax (*Linum usitatissimum* L.) between Canadian cultivars and a world collection. Plant Breed. **120**: 360–362.

Diederichsen, A., Rozhmina, T.A., and Kudrjavceva, L.P. 2008. Variation patterns within 153 flax (*Linum usitatissimum* L.) genebank accessions based on evaluation for resistance to fusarium wilt, anthracnose and pasmo. Plant Genet. Resour. **6**: 22–32.

Elad, Y., and Freeman, S. 2002. Biological control of fungal plant pathogens. Agric. Appl.: 1–16.

El Ghaouth, A., Arul, J., Asselin, A., and Benhamou, N. 1992. Antifungal activity of chitosan on post-harvest pathogens: induction of morphological and cytological alterations in *Rhizopus stolonifer*. Mycol. Res. **96**: 769–779.

Fairchild, K.L., Miles, T.D., and Wharton, P.S. 2013. Assessing fungicide resistance in populations of Alternaria in Idaho potato fields. Crop Prot. **49**: 31–39.

Ferguson, M.W., Lay, C.L., and Evenson, P.D. 1987. Effect of pasmo disease on flower production and yield components of flax. Phytopathology **77**: 805.

Fernández-Ortuño, D., Chen, F., and Schnabel, G. 2012. Resistance to Pyraclostrobin and Boscalid in Botrytis cinerea Isolates from Strawberry Fields in the Carolinas. Plant Dis. **96:** 1198–1203.

Fernández-Ortuño, D., Torés, J.A., De Vicente, A., and Pérez-García, A. 2008. Mechanisms of resistance to QoI fungicides in phytopathogenic fungi. Int. Microbiol. **11**: 1–9.

Flax Council of Canada. 2017a. Canada – A Flax Leader. [Online]. Available: https://flaxcouncil.ca/resources/about-flax/canada-a-flax-leader/ [14 Oct. 2017].

Flax Council of Canada. 2017b. Chapter 3: Fertilizer Practices. [Online]. Available: https://flaxcouncil.ca/growing-flax/chapters/fertilizer-practices/ [14 Oct. 2017].

Flor, H.H. 1953. Wilt, rust, and pasmo of flax. [Online] Available: https://naldc.nal.usda.gov/download/IND43894445/ [2017 Oct. 12].

Fraaije, B.A., Cools, H.J., Fountaine, J., Lovell, D.J., Motteram, J., West, J.S., and Lucas, J.A. 2005. Role of ascospores in further spread of QoI-resistant cytochrome b Alleles (G143A) in field populations of *Mycosphaerella graminicola*. Phytopathology **95**: 933–941.

FRAC. 2017. Fungicides sorted by mode of action (including FRAC Code numbering). [Online]. Available: http://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2017-final.pdf?sfvrsn=2 [14 Oct. 2017].

Friesen, G.H. 1986. Effect of weed interference on yield and quality of flax seed oil. Can. J. Plant Sci. **66**: 1037–1040.

Fu, Y.B., Rowland, G.G., Duguid, S.D., and Richards, K.W. 2003. RAPD analysis of 54 North American flax cultivars. Crop Sci. **43**: 1510–1515.

Ghaderi, A., Everson, E.H., and Yamazaki, W.T. 1971. Test weight in relation to the physical and quality characteristics of soft winter wheat (*Triticum aestivum* L. em Thell)1. Crop Sci. **11**: 515.

Gao, J., Wang, Y., Gao, B., Wu, L. and Chen, H., 2012. Environmental fate and transport of pesticides. Pages 29-41 in H. S. Rathore and L.M.L. Nollet, eds. Pesticides—evaluation of environmental pollution. CRC Press Taylor and Francis Group, Boca Raton.

Gisi, U., Chin, K.M., Knapova, G., Küng Färber, 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 Manag. Sci. **58**: 859–867.

Goodwin, S.B., Smart, C.D., Sandrock, R.W., Deahl, K.L., Punja, Z.K., and Fry, W.E. 1998. Genetic change within populations of *Phytophthora infestans* in the United States and Canada during 1994 to 1996: Role of migration and recombination. Phytopathology **88**: 939–949.

Gossen, B.D., Carisse, O., Kawchuk, L.M., Van Der Heyden, H., and McDonald, M.R. 2014. Recent changes in fungicide use and the fungicide insensitivity of plant pathogens in Canada. Can. J. Plant Pathol. **36**: 327–340.

Gossen, B.D., Rimmer, S.R., and Holley, J.D. 2001. First Report of Resistance to Benomyl Fungicide in *Sclerotinia sclerotiorum*. Plant Dis. **85**: 1206–1206.

Gruzdevienė, E., Brazauskienė, I., Repečkienė, J., and Lugauskas, A. 2008. The occurrence of pathogenic fungi during flax growing season in central Lithuania. J. Plant Prot. Res. **48**: 255–266.

Gubbels, G.H., Bonner, D.M., and Kenaschuk, E.O. 1994. Effect of frost injury on quality of flax seed. Can. J. Plant Sci. **74**: 331–333.

Gubbels, G.H., and Kenaschuk, E.O. 1989. Effect of seeding rate on plant and seed characteristics of new flax cultivars. Can. J. Plant Sci. **69**: 791–795.

Halley, S. 2007. Pasmo Disease on Flax. [Online] Available: https://www.ag.ndsu.edu/agnic/flax/doc_images/Scott Haley Pasmo Disease on Flax.pdf [2017 Oct. 14].

Halley, S., Bradley, C.A., McMullen, M., Lukach, J.R., Knodel, J.J., Endres, G.J., and Gregoire, T. 2004. Distribution and severity of pasmo on flax in North Dakota and evaluation of fungicides and cultivars for management. Plant Dis. **88**: 1123–1126.

Health Canada. 2011. Proposed registration decision PRD2011-15, pyraclostrobin seed treatment. [Online]. Available: https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/public/consultations/pyraclostrobin-seed-treatment-proposed-registration-decision-prd2011-15-health-canada-consultation-document.html [14 Oct. 2017].

Henry, R.S., Johnson, W.G., and Wise, K.A. 2011. The impact of a fungicide and an insecticide on soybean growth, yield, and profitability. Crop Prot. **30**: 1629–1634.

Hill, C.B., Bowen, C.R., Hartman, G.L., Sciences, C., Bowen, C.R., and States, U. 2013. Effect of fungicide application and cultivar on soybean green stem disorder. Plant Dis. **97**: 1212–1220.

Hincapie, M., Wang, N.-Y., Peres, N.A., and Dewdney, M.M. 2014. Baseline Sensitivity of *Guignardia citricarpa* Isolates from Florida to Azoxystrobin and Pyraclostrobin. Plant Dis. **98**: 780–789.

Horsfall, J.G. and Barratt, R.W., 1945. An improved grading system for measuring plant diseases. Phytopathology **35:** 655.

Hutchins, A.M., Martini, M.C., Olson, B.A., Thomas, W., and Slavin, J.L. 2000. Flaxseed influences urinary lignan excretion in a dose-dependent manner in postmenopausal women. Cancer Epidemiol. Biomarkers Prev. **9**: 1113–1118.

Hwang, S.-F., Strelkov, S., Peng, G., Ahmed, H., Zhou, Q., and Turnbull, G. 2016. Blackleg (*Leptosphaeria maculans*) severity and yield loss in canola in Alberta, Canada. Plants **5**: 31.

Ishii, H., Fraaije, B.A., Sugiyama, T., Noguchi, K., Nishimura, K., Takeda, T., Amano, T., and Hollomon, D.W. 2001. Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. Phytopathology **91**: 1166–1171.

Jhala, A.J., and Hall, L.M. 2010. Flax (*Linum usitatissimum* L.): Current uses and future applications. Aust. J. Basic Appl. Sci. **4**: 4304–4312.

Johnston, A.M., Tanaka, D.L., Miller, P.R., Brandt, S.A., Nielsen, D.C., Lafond, G.P., and Riveland, N.R. 2002. Oilseed Crops for Semiarid Cropping Systems in the Northern Great Plains. Agron. J. **94**: 231–240.

Joshi, J., Sharma, S., and Guruprasad, K.N. 2014. Foliar application of pyraclostrobin fungicide enhances the growth, rhizobial-nodule formation and nitrogenase activity in soybean (var. JS-335). Pestic. Biochem. Physiol. **114**: 61–66.

Jovaišienė, Z. and Taluntytė, L. 2000. *Septoria linicola* in Lithuania. Bot. Lith. **6**: 97–100.

Kable, P.F., and Jeffery, H. 1980. Selection for tolerance in organisms exposed to sprays of biocide mixtures: a theoretical model. Phytopathology **70**: 8-12.

Kanetis, L., Förster, H., and Adaskaveg, J.E. 2008. Baseline sensitivities for new postharvest fungicides against *Penicillium* spp. on citrus and multiple resistance evaluations in *P. digitatum*. Plant Dis. **92**: 301–310.

Kanungo, M., and Joshi, J. 2014. Impact of pyraclostrobin (F-500) on crop plants. Plant Sci. Today **1**: 174–178.

Kawchuk, L.M., Holley, J.D., Lynch, D.R., and Clear, R.M. 1994. Resistance to thiabendazole and thiophanate-methyl in canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani*. Am. Potato J. **71**: 185–192.

Khan, M.F.R., and Carlson, A.L. 2009. Effect of fungicides on sugar beet yield, quality, and postharvest respiration rates in the absence of disease. Plant Heal. Prog. doi:10.1094/PHP-2009-1019-01-RS.

Kim, Y., Dixon, E.W., Vincelli, P., and Farman, M.L. 2003. Field resistance to strobilurin (QoI) fungicides in *Pyricularia grisea* caused by mutations in the mitochondrial cytochrome b Gene. **93**: 891–900.

Kim, Y.K., and Xiao, C.L. 2011. Stability and fitness of pyraclostrobin- and boscalid-resistant phenotypes in field isolates of *Botrytis cinerea* from apple. **101**: 1385–1391.

King, A., Cole, A., and Sanderson, F. 1986. Prochloraz insensitivity in isolates of the cereal Eyespot Fungus, Pseudocercosporella Herpotrichoides, in New Zealand. Australas. Plant Pathol. **15**: 22.

Köller, W., Wilcox, W.F., Barnard, J., Jones, A.L., and Braun, P.G. 1997. Detection and quantification of resistance of *Venturia inaequalis* populations to sterol demethylation inhibitors. Phytopathology **87**: 184–190.

Köycü, N.D. 2012. Sensitivity of *Botrytis cinerea* isolates against some fungicides used in vineyards. African J. Biotechnol. **11**: 1892–1899.

Kraft, J.M., Kommedahl, T., and Linck, A.J. 1963. Histological study of malformation in flax seed after exposure to 31 °C. Source Bot. Gaz. **124**: 367–371.

Kraiczy, P., Haase, U., Gencic, S., Flindt, S., Anke, T., Brandt, U., and Jagow, G. 1996. The molecular basis for the natural resistance of the cytochrome bc1 complex from strobilurin-producing basidiomycetes to center QP inhibitors. Eur. J. Biochem. **235**: 54–63.

Krupinsky, J.M., Bailey, K.L., McMullen, M.P., Gossen, B.D., and Turkington, T.K. 2002. Managing plant disease risk in diversified cropping systems. Agron. J. **94**: 198–209.

Lafond, G.P., Irvine, B., Johnston, a. M., May, W.E., McAndrew, D.W., Shirtliffe, S.J., and Stevenson, F.C. 2008. Impact of agronomic factors on seed yield formation and quality in flax. Can. J. Plant Sci. **88**: 485–500.

Lamondia, J.A., and Douglas, S.M. 1997. Sensitivity of *Botrytis cinerea* from connecticut greenhouses to benzimidazole and dicarboximide fungicides. Plant Dis. **81:** 729–732.

Lamour, K.H., and Hausbeck, M.K. 2000. Mefenoxam Insensitivity and the Sexual Stage of Phytophthora capsici in Michigan Cucurbit Fields. Phytopathology **90:** 396–400.

Li, S., Liu, X., Zhu, Y., Dong, F., Xu, J., Li, M., and Zheng, Y. 2014. A statistical approach to determine fluxapyroxad and its three metabolites in soils, sediment and sludge based on a combination of chemometric tools and a modified quick, easy, cheap, effective, rugged and safe method. J. Chromatogr. A **1358**: 46–51.

Lonergan, E., Pasche, J., Skoglund, L., and Burrows, M. 2015. sensitivity of ascochyta species infecting pea, lentil, and chickpea to boscalid, fluxapyroxad, and prothioconazole. Plant Dis. **99**: 1254–1260.

Loughman, R., and Thomas, G.J. 1992. Fungicide and cultivar control of Septoria diseases of wheat. Crop Prot. **11**: 349–354.

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

Malhi, S.S., Lemke, R., Mooleki, S.P., Schoenau, J.J., Brandt, S., Lafond, G., Wang, H., Hultgreen, G.E., and May, W.E. 2008. Fertilizer N management and P placement effects on yield, seed protein content and N uptake of flax under varied conditions in Saskatchewan. Can. J. Plant Sci. **88**: 11–33.

Manitoba Agriculture and Food. [Online] Available: https://www.gov.mb.ca/agriculture/weather/images/figure8.gif [14 Oct. 2017]

Marchenkov, A., Rozhmina, T., Uschapovsky, I., and Muir, A.D. 2003. Cultivation of flax. Flax, the genus Linum **85**: 74–91.

McGrath, M.T. 2001. Fungicide Resistance in Cucurbit Powdery Mildew: Experiences and Challenges. Plant Dis. **85**: 236–245.

McGregor, W.G. 1964. Influence of date of seeding on yield and yield components in flaxseed. Can. J. Plant Sci. **44**: 145–148.

McHughen, A., Rowland, G.G., Holm, F.A., Bhatty, R.S., and Kenaschuk, E.O. 1997. CDC Triffid transgenic flax. Can. J. Plant Sci. **77**: 641–643.

McKay, A.H., Hagerty, G.C., Follas, G.B., Moore, M.S., Christie, M.S., and Beresford, R.M. 2011. Succinate dehydrogenase inhibitor(SDHI) fungicide resistance prevention strategy. New Zeal. Plant Prot. **64**: 119–124.

McKenzie, R.H., Bremer, E., Middleton, A.B., Pfiffner, P.G., and Woods, S.A. 2011. Optimum seeding date and rate for irrigated cereal and oilseed crops in southern Alberta. Can. J. Plant Sci. **91**: 293–303.

Meier, U. 2001. Growth stages of mono- and dicotyledonous plants. BBCH Monograph. Blackwell Wissenschafts-Verlag.

Mercer, P.C., Hardwick, N. V., Fitt, B.D.L., and Sweet, J.B. 1991. Status of diseases of linseed in the UK. Status Dis. linseed UK. Home-Grown Cereals Authority (Oilseeds). [Online] Available: https://www.cabdirect.org/cabdirect/abstract/19922322366 [2017 Oct. 12].

Millam, S., Obert, B., and Pret'ová, A. 2005. Plant cell and biotechnology studies in *Linum usitatissimum* – a review. Plant Cell. Tissue Organ Cult. **82**: 93–103.

Milner, R.J., Huppatz, R.J., and Swaris, S.C. 1991. A new method for assessment of germination of *Metarhizium conidia*. J. Invertebr. Pathol. **57**: 121–123.

Miyamoto, T., Ishii, H., Seko, T., Kobori, S., and Tomita, Y. 2009. Occurrence of Corynespora cassiicola isolates resistant to boscalid on cucumber in Ibaraki Prefecture, Japan. Plant Pathol. **58**: 1144–1151.

Mondal, 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.

Mueller, T.A., Miles, M.R., Morel, W., Marois, J.J., Wright, D.L., Kemerait, R.C., Levy, C., and Hartman, G.L. 2009. Effect of fungicide and timing of application on soybean rust severity and yield. Plant Dis. **93**: 243–248.

Myresiotis, C.K., Bardas, G.A., Karaoglanidis, G.S., and Agri-, F. 2008. Baseline sensitivity of *Botrytis cinerea* to pyraclostrobin and boscalid and control of anilinopyrimidine- and benzimidazole-resistant strains by these fungicides. Plant Dis. **92**: 1427–1431.

Nelson, K.A., and Meinhardt, C.G. 2011. Foliar boron and pyraclostrobin effects on corn yield. Agron. J. **103**: 1352–1358.

Nelson, K.A., Motavalli, P.P., Stevens, W.E., Dunn, D., and Meinhardt, C.G. 2010. Soybean response to preplant and foliar-applied potassium chloride with strobilurin fungicides. Agron. J. **102**: 1657.

Olaya, G., Zheng, D., and Köller, W. 1998. Differential responses of germinating *Venturia inaequalis* conidia to kresoxim-methyl. Pestic. Sci. **54**: 230–236.

Oomah, B.D. 2001. Flaxseed as a functional food source. J. Sci. Food Agric. **81**: 889–894.

Pasche, J.S., Wharam, C.M., and Gudmestad, N.C. 2004. Shift in sensitivity of *Alternaria solani* in response to QoI fungicides. Plant Dis. **88**: 181–187.

Paschos, G.K., Yiannakouris, N., Rallidis, L.S., Davies, I., Griffin, B.A., Panagiotakos, D.B., Skopouli, F.N., Votteas, V., and Zampelas, A. 2005. Apolipoprotein E genotype in dyslipidemic patients and response of blood lipids and inflammatory markers to alpha-linolenic acid. Angiology **56**: 49–60.

Peters, R.D., Drake, K.A., Gudmestad, N.C., Pasche, J.S., and Shinners-Carnelley, T. 2008. First report of reduced sensitivity to a QoI fungicide in isolates of *Alternaria solani* causing early blight of potato in Canada. Plant Dis. **92**: 1707–1707.

Rampersad, S.N. 2011. A rapid colorimetric microtiter bioassay to evaluate fungicide sensitivity among *Verticillium dahliae* isolates. Plant Dis. **95**: 248–255.

Ransom, J.K., and McMullen, M. V. 2008. Yield and disease control on hard winter wheat cultivars with foliar fungicides. Agron. J. **100**: 1130.

Rashid, K. Y., Kutcher, H.R., Islam, T., Stephens, D., Dokken-Bouchard, F., Pradhan, M.P., and Duguid, S. 2016. Diseases of flax in Manitoba and Saskatchewan in 2015. Can. Plant Dis. Surv. **96**:180-181.

Rashid, K.Y. 2001. Pasmo disease in flax: impact on yield and potential control methods. Can. J. Plant pathol. **23**:204. A poster presented at the CPS meeting 2000.

Rashid, K.Y., 2003. Principal diseases of flax. Pages 124-49 in A.D. Muir and N.D. Westcott, eds. Flax: the genus Linum. CRC Press, London, UK.

Rashid, K.Y. 2010. Efficacy of fungicides in reducing pasmo and yield loss in flax. Pages 73-77 in: Proceedings of the 63rd Flax Institute of the United States. March 25-26, 2010, Fargo, North Dakota, USA.

Rashid, K.Y. 2012. Screening for resistance to pasmo (Septoria linicola) in flax. Can. J. Plant Pathol. **34**:343. A poster presented at the CPS 2011 meeting.

Rashid, K.Y., Kenaschuk, E.O. and Platford, R.G., 1999. Diseases of flax in Manitoba and eastern Saskatchewan in 1998. Can. Plant Dis. Surv. **79**:122-123.

Redman, C.E., King, E.P., and Brown, J.F. 1969. Elanco conversion tables for Barratt-Horsfall rating numbers. Eli Lilly and Co. Research Labs, Indianapolis, IN.

Reuveni, M., and Sheglov, D. 2002. Effects of azoxystrobin, difenoconazole, polyoxin B (polar) and trifloxystrobin on germination and growth of *Alternaria alternata* and decay in red delicious apple fruit. Crop Prot. **21**: 951–955.

Rosenzweig, C., Iglesius, A., Yang, X.B., Epstein, P.R., and Chivian, E. 2001. Climate change and extreme weather events - Implications for food production, plant diseases, and pests. Glob. Chang. Hum. Heal. **2**: 90–104.

Russell, P.E. 2005. A century of fungicide evolution. J. Agric. Sci. **143**: 11–25.

Ryan, C.D., and Smyth, S.J. 2012. Economic implications of low-level presence in a zero-tolerance European import market: The case of Canadian triffid flax. AgBioForum **15**: 21–30.

Sackston, W.E. 1950. Effect of pasmo disease on seed yield and thousand kernel weight of flax. Can. J. Res. **28c**: 493–512.

Sackston, W.E. 1970. A possible mechanism of dispersal of septoria spores. Can. J. Plant Sci. **50**: 155–157.

Sackston, W.E., and Carson, R.B. 1951. Effect of pasmo disease of flax on the yield and quality of linseed oil. Can. J. Bot. **29**: 339–351.

Sadler, J.M. 1980. Effects of placement location for phosphorus banded away from the seed on growth and uptake of soil and fertilizer phosphorus by flax. Can. J. Soil Sci. **60**: 251–262.

Sanderson, F.R. 1963. An ecological study of pasmo disease (*Mycosphaerella linorum*) On linseed in Canterbury and Otago. New Zeal. J. Agric. Res. **6**: 432–439.

Saskatchewan Crop Insurance Corporation. [Online]. Available: http://www.saskcropinsurance.com/resources/maps/first-fall-frost-map [14 Oct. 2017].

Saskatchewan Flax Development Commission. 2017. Growing Flax. [Online]. Available: https://saskflax.com/industry/index.php [14 Oct. 2017].

Schmidt, G. and Breckling, B., 2010. The Triffid case: A short résumé on the rediscovery of a de-registered GMO. Implications of GMO-crop cultivation at large spatial scales. Theorie in der Ökologie, pp.79-81.

Secor, G.A., Rivera, V. V., Khan, M.F.R., and Gudmestad, N.C. 2010. Monitoring Fungicide Sensitivity of Cercospora beticola of Sugar Beet for Disease Management Decisions. Plant Dis. **94**: 1272–1282.

Sierotzki, H., Frey, R., Wullschleger, J., Palermo, S., Karlin, S., Godwin, J., and Gisi, U. 2007. Cytochromeb gene sequence and structure of *Pyrenophora teres* and *P. tritici*-repentis and implications for QoI resistance. Pest Manag. Sci. **63**: 225–233.

Sierotzki, H., and Scalliet, G. 2013. A review of current knowledge of resistance aspects for the next-generation succinate dehydrogenase inhibitor fungicides. Phytopathology **103**: 880–887.

Sierotzki, H., Wullschleger, J., and Gisi, U. 2000. Point Mutation in cytochrome b Gene conferring resistance to strobilurin fungicides in *Erysiphe graminis* f. sp. tritici field isolates. Pestic. Biochem. Physiol. **68**: 107–112.

Singh, K.K., Mridula, D., Rehal, J., and Barnwal, P. 2011. Critical reviews in food science and nutrition flaxseed: a potential source of food, feed and fiber. Crit. Rev. Food Sci. Nutr. **51**: 210–222.

Skylakakis, G. 1981. Effects of alternating and mixing pesticides on the buildup of fungal resistance. Phytopathology. **71**: 1119-1121.

Slawecki, R.A., Ryan, E.P., and Young, D.H. 2002. Novel fungitoxicity assays for inhibition of germination-associated adhesion of *Botrytis cinerea* and *Puccinia recondita* spores. Appl. Environ. Microbiol. **68**: 597–601.

Smith, J., Grimmer, M., Waterhouse, S., and Paveley, N. 2013. Quantifying the non-fungicidal effects of foliar applications of fluxapyroxad (Xemium) on stomatal conductance, water use efficiency and yield in winter wheat. Commun. Agric. Appl. Biol. Sci. **78**: 523–35.

Soita, H.W., Meier, J.A., Fehr, M., Yu, P., Christensen, D.A., Mckinon, J.J., and Mustafa, A.F. 2003. Effects of flaxseed supplementation on milk production, milk fatty acid composition and nutrient utilization by lactating dairy cows. Arch. Anim. Nutr. **57**: 107–116.

Stammler, G., Benzinger, G., and Speakman, J. 2007. A rapid and reliable method for monitoring the sensitivity of *Sclerotinia sclerotiorum* to boscalid. J. Phytopathol. **155**: 746–748.

Stammler, G., and Speakman, J. 2006. Microtiter method to test the sensitivity of *Botrytis cinerea* to boscalid. J. Phytopathol. **154**: 508–510.

Staub, T. 1991. Fungicide resistance: Practical experience with antiresistance strategies and the role of integrated use. Annu. Rev. Phytopathol **29**: 421–42.

Staub, T. and Sozzi, D., 1984. Fungicide resistance. Plant Dis, **68**: 1026-1031.

Stevens, D.A. 1984. Antifungal drug susceptibility testing. Mycopathologia **87**: 135–140.

Stevenson, F.C., and Wright, A.T. 1996. Seeding rate and row spacing affect flax yields and weed interference. Can. J. Plant Sci. **76**: 537–544.

Thiessen, D.L. 2004. Optimization of feed peas, canola and flaxseed for aqua feeds: the Canadian prairie perspective. Av. en Nutr. Acuicola **7**: 259–277.

Tremblay, D.M., and Talbot, B.G. 2003. Sensitivity of *Botrytis squamosa* to different classes of fungicides. Plant Dis. **87**: 573–578.

Vaisey-Genser, M. and Morris, D.H., 2003. History of the cultivation and uses of flaxseed. Pages 1-21 in A.D. Muir and N.D. Westcott, eds. Flax: the genus Linum. CRC Press, London, UK.

Varieties of Grain Crops. 2017. [Online]. Available: http://publications.gov.sk.ca/documents/20/96889-Varieties%20of%20Grain%20Crops%202017.pdf [14 Oct. 2017]

Venancio, W S; Rodrigues, M A T; Begliomini, E; Souza, N.L. 2003. Physiological effects of strobilurin fungicides on plants. Publ. UEPG Ci. Exatas Terra, Ci. Agr. Eng. **9**: 59–68.

Vera, C.L., Duguid, S.D., Fox, S.L., Rashid, K.Y., Dribnenki, J.C.P., and Clarke, F.R. 2012. Short communication: Comparative effect of lodging on seed yield of flax and wheat. Can. J. Plant Sci. **92**: 39–43.

Vera, C.L., Irvine, R.B., Duguid, S.D., Rashid, K.Y., Clarke, F.R., and Slaski, J.J. 2014. Pasmo disease and lodging in flax as affected by pyraclostrobin fungicide, N fertility and year. Can. J. Plant Sci. **94**: 119–126.

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.

Walker, A.-S., Auclair, C., Gredt, M., and Leroux, P. 2009. First occurrence of resistance to strobilurin fungicides in *Microdochium nivale* and *Microdochium majus* from French naturally infected wheat grains. Pest Manag. Sci. **65**: 906–915.

Weisz, R., Cowger, C., Ambrose, G., and Gardner, A. 2011. Multiple mid-Atlantic field experiments show no economic benefit to fungicide application when fungal disease is absent in winter wheat. Phytopathology **101**: 323–333.

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.

Yin, Y.N., Kim, Y.K., and Xiao, C.L. 2012. Molecular characterization of pyraclostrobin resistance and structural diversity of the cytochrome b gene in *Botrytis cinerea* from apple. **102**: 315–322.

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.

APPENDIX

Table A1: Pearson correlation coefficients between factors: crop maturity, disease severity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Brandon 2014

	Maturity	Disease	Yield	TSW	TW	Oil
Maturity	1.00					
Disease	-0.41	1.00				
Yield	0.48	-0.30	1.00			
TSW	0.58	-0.44	0.39	1.00		
TW	0.44	-0.38	0.39	0.39	1.00	
Oil	0.17	-0.06	-0.06	0.07	-0.13	1.00

Table A2: Pearson correlation coefficients between factors: crop maturity, disease severity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Brandon 2015

	Maturity	Disease	Yield	TSW	TW	Oil
Maturity	1.00					
Disease	NA	1.00				
Yield	NA	-0.13	1.00			
TSW	NA	-0.31	0.35	1.00		
TW	NA	-0.06	0.10	0.52	1.00	
Oil	NA	-0.32	0.41	0.27	0.22	1.00

NA=Not applicable as there was no difference in crop maturity

Table A3: Pearson correlation coefficients between factors: crop maturity, disease severity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Brandon 2016

	Maturity	Disease	Yield	TSW	TW	Oil
Maturity	1.00					
Disease	-0.44	1.00				
Yield	0.37	-0.29	1.00			
TSW	0.82	-0.67	0.44	1.00		
TW	0.38	-0.15	0.44	0.42	1.00	
Oil	0.38	-0.31	0.06	0.42	-0.35	1.00

Table A4: Pearson correlation coefficients between factors: among crop maturity, disease severity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Melfort 2014

	Maturity	Disease	Yield	TSW	TW	Oil
Maturity	1.00					
Disease	-0.59	1.00				
Yield	0.49	-0.41	1.00			
TSW	0.74	-0.59	0.48	1.00		
TW	0.21	0.09	0.26	0.04	1.00	
Oil	0.45	-0.48	0.26	0.46	-0.02	1.00

Table A5: Pearson correlation coefficients between factors: among crop maturity, disease severity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Melfort 2015

	Maturity	Disease	Yield	TSW	TW	Oil
Maturity	1.00					
Disease	-0.56	1.00				
Yield	0.36	-0.27	1.00			
TSW	0.59	-0.42	0.38	1.00		
TW	0.62	-0.62	0.56	0.48	1.00	
Oil	0.29	-0.09	0.22	0.27	0.36	1.00

Table A6: Pearson correlation coefficients between factors: crop maturity, disease severity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Melfort 2016

	Maturity	Disease	Yield	TSW	TW	Oil
Maturity	1.00					
Disease	0.34	1.00				
Yield	-0.10	-0.55	1.00			
TSW	-0.29	-0.75	0.73	1.00		
TW	-0.20	-0.48	0.72	0.73	1.00	
Oil	-0.11	-0.19	0.06	0.21	0.12	1.00

Table A7: Pearson correlation coefficients between factors: crop maturity, disease severity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Saskatoon 2014

	Maturity	Disease	Yield	TSW	TW	Oil
Maturity	1.00					
Disease	-0.60	1.00				
Yield	0.68	-0.54	1.00			
TSW	0.66	-0.49	0.85	1.00		
TW	0.49	-0.37	0.77	0.74	1.00	
Oil	0.63	-0.49	0.65	0.69	0.37	1.00

Table A8: Pearson correlation coefficients between factors: crop maturity, disease severity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Saskatoon 2015

	Maturity	Disease	Yield	TSW	TW	Oil
Maturity	1.00					
Disease	-0.12	1.00				
Yield	-0.01	-0.40	1.00			
TSW	0.15	-0.43	0.33	1.00		
TW	0.24	-0.60	0.53	0.70	1.00	
Oil	-0.30	0.03	0.20	-0.09	-0.24	1.00

Table A9: Pearson correlation coefficients between factors: crop maturity, disease severity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Saskatoon 2016

	Maturity	Disease	Yield	TSW	TW	Oil
Maturity	1.00					
Disease	0.02	1.00				
Yield	0.01	-0.75	1.00			
TSW	-0.01	-0.86	0.89	1.00		
TW	-0.02	-0.58	0.80	0.62	1.00	
Oil	0.04	-0.59	0.83	0.84	0.59	1.00

Table A10: Pearson correlation coefficients between factors: crop maturity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Vegreville 2014

	Maturity	Yield	TSW	TW	Oil
Maturity	1.00				
Yield	0.15	1.00			
TSW	0.29	0.36	1.00		
TW	0.01	-0.21	0.02	1.00	
Oil	0.14	0.54	0.74	-0.16	1.00

Table A11: Pearson correlation coefficients between factors: crop maturity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Vegreville 2015

	Maturity	Yield	TSW	TW	Oil
Maturity	1.00				
Yield	NA	1.00			
TSW	NA	0.61	1.00		
TW	NA	-0.36	-0.41	1.00	
Oil	NA	0.37	0.28	-0.31	1.00

NA=Not applicable as there was no difference in crop maturity

Table A12: Pearson correlation coefficients between factors: crop maturity, disease severity, seed yield, thousand seed weight (TSW), test weight (TW) and oil content (Oil) in Vegreville 2016

	Maturity	Disease	Yield	TSW	TW	Oil
Maturity	1.00					
Disease	-0.04	1.00				
Yield	0.03	-0.46	1.00			
TSW	0.18	-0.64	0.33	1.00		
TW	-0.23	0.17	0.13	-0.58	1.00	
Oil	-0.04	0.04	0.05	0.07	0.44	1.00

Table A13: Isolates of *Septoria linicola*, the locations from where the isolates were collected, the year of collection and the assay used to determine fungicide sensitivity

Isolate	Location	Year	Assay
14sl01	Melfort	2014	Spore germination and microtiter
14sl02	Melfort	2014	Spore germination and microtiter
14sl03	Melfort	2014	Spore germination and microtiter
14sl05	Melfort	2014	Spore germination and microtiter
14sl06	Melfort	2014	Spore germination
14sl07	Melfort	2014	Spore germination and microtiter
14sl08	Melfort	2014	Spore germination and microtiter
14sl09	Melfort	2014	Spore germination and microtiter
14sl10	Melfort	2014	Spore germination and microtiter
14sl14	Brandon	2014	Spore germination
14sl15	Brandon	2014	Spore germination
14sl16	Brandon	2014	Spore germination
14sl17	Brandon	2014	Spore germination
14sl18	Brandon	2014	Spore germination
14sl19	Brandon	2014	Spore germination
14sl20	Brandon	2014	Spore germination
14sl21	Brandon	2014	Spore germination
14sl22	Brandon	2014	Spore germination
14sl24	Brandon	2014	Spore germination
14sl25	Brandon	2014	Spore germination and microtiter
14sl26	Brandon	2014	Spore germination and microtiter
14sl27	Brandon	2014	Spore germination and microtiter
14sl28	Brandon	2014	Spore germination
14sl35	Brandon	2014	Spore germination and microtiter
14sl36	Saskatoon	2014	Spore germination and microtiter
14sl37	Saskatoon	2014	Spore germination and microtiter
14sl38	Saskatoon	2014	Spore germination and microtiter
14sl39	Saskatoon	2014	Spore germination and microtiter
14sl40	Saskatoon	2014	Spore germination and microtiter
14sl41	Saskatoon	2014	Spore germination and microtiter
14sl43	Saskatoon	2014	Spore germination and microtiter
14sl46	Saskatoon	2014	Spore germination and microtiter
14sl47	Saskatoon	2014	Spore germination and microtiter
14sl48	Saskatoon	2014	Spore germination
14sl49	Saskatoon	2014	Spore germination and microtiter
14sl50	Saskatoon	2014	Spore germination and microtiter
14sl51	Saskatoon	2014	Spore germination

Isolate	Location	Year	Assay
14sl52	Saskatoon	2014	Spore germination
14sl53	Saskatoon	2014	Spore germination
14sl65	Melfort	2014	Spore germination
14sl66	Melfort	2014	Spore germination
14sl67	Melfort	2014	Spore germination
14sl70	Melfort	2014	Spore germination
14sl71	Melfort	2014	Spore germination
14sl75	Melfort	2014	Spore germination
14sl76	Melfort	2014	Spore germination
14sl77	Melfort	2014	Spore germination
14sl78	Melfort	2014	Spore germination
14sl79	Melfort	2014	Spore germination
14sl81	Melfort	2014	Spore germination
14sl83	Melfort	2014	Spore germination
15sl01	Saskatoon	2015	Spore germination and microtiter
15sl02	Saskatoon	2015	Spore germination and microtiter
15sl03	Saskatoon	2015	Spore germination and microtiter
15sl12	Saskatoon	2015	Spore germination and microtiter
15sl13	Saskatoon	2015	Spore germination and microtiter
15sl14	Saskatoon	2015	Spore germination
15sl15	Saskatoon	2015	Spore germination
15sl21	Brandon	2015	Spore germination and microtiter
15sl22	Brandon	2015	Spore germination and microtiter
15sl23	Brandon	2015	Spore germination and microtiter
15sl25	Brandon	2015	Spore germination and microtiter
15sl26	Brandon	2015	Spore germination and microtiter
15sl27	Brandon	2015	Spore germination and microtiter
15sl28	Brandon	2015	Spore germination and microtiter
15sl29	Brandon	2015	Spore germination
15sl30	Melfort	2015	Spore germination and microtiter
15sl31	Melfort	2015	Spore germination and microtiter
15sl32	Melfort	2015	Spore germination
15sl33	Melfort	2015	Spore germination
15sl34	Melfort	2015	Spore germination and microtiter
15sl35	Melfort	2015	Spore germination and microtiter
15sl36	Melfort	2015	Spore germination and microtiter