INVESTIGATING PARTIAL RESISTANCE AND HOST-PATHOGEN INTERACTIONS IN THE FLAX – *SEPTORIA LINICOLA* PATHOSYSTEM

BY

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THE UNIVERSITY OF MANITOBA

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FORWARD

The Format of this thesis is presented as three manuscripts, which include Materials, Methods, Results and Discussion. A general abstract, general introduction and general review of the literature precede the manuscripts; a general discussion and bibliography follow.

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1. GENERAL ABSTRACT

Gillis, Evan Oscar. M.Sc., The University of Manitoba, February, 2009. Investigating Partial Resistance and Host-Pathogen Interactions in the Flax-*Septoria linicola* Pathosystem. Major Professors; Lakhdar Lamari and Khalid Rashid.

Septoria linicola (Speg) Garassini. (teleomorph: *Mycosphaerella linorum* Naumov), the causal agent of the pasmo disease of flax, affects production in many flax growing regions around the world. Currently, acceptable resistance to the pathogen is not available in any commercial cultivars and little is known about the characteristics of its infection of the host. A total of 208 accessions were examined for pasmo severity on the leaves and stem, and the area under disease progress curve was calculated. There was a range of susceptibility levels in the population and several promising accessions warrant further testing. However, no high level of resistance was identified. Several accessions were observed under greenhouse conditions and disease severity was estimated based on image analysis. Some accessions correlated well to previous field experiments while some did not. There was variation in the accessions tested using image analysis which was effective in accurately obtaining disease severity. Cytological observations were made, using fluorescence microscopy, on two flax cultivars and one accession from field studies. Penetration was observed by 24 hours post-inoculation and was only observed through stomata. No appressorium was required for penetration or observed at any time. Colonization of the intercellular spaces was evident by 72 hours post inoculation and although mesophyll cells would collapse no haustorium was evident and the pathogen was not observed to penetrate host cells at any time. The plant appeared to exhibit some defence responses in the form of bright fluorescing mesophyll cells and fluorescing compounds deposited at stomates or epidermal cells. Neither of these reactions have

been identified as any particular compounds, nor can they be considered to be elicited by the pathogen. Pycnidial formation occurred in the sub-stomatal chamber and pycnidial initials were observed frequently at 6 days post inoculation. Many pycnidia were mature and produced spores at 8 days post-inoculation.

2. GENERAL INTRODUCTION

Septoria linicola (Speg) Garassini. (teleomorph: *Mycosphaerella linorum* Naumov), the causal agent of pasmo of flax affects, production in many flax growing regions around the world, including North America (Rashid, 2003). The pathogen is favoured by moist temperate conditions (Brentzel, 1926; Loughnane et al., 1946) and lodging, and can cause significant defoliation, boll drop and stem breakage (Rashid, 2003; Flor, 1943).

Genetic resistance would provide the best control of this or any pathogen (Flor, 1943; Rashid, 2003). However, no such resistance exists in commercial flax cultivars at this point (Rashid, 2003). If flax continues to become a more valuable crop due to rotational benefits (Halley et al., 2004) or, perhaps more importantly, its potential health benefits (Berglund, 2002), then the management and control of *S. linicola* will also be more important.

Several studies have investigated flax accessions from many geographical locations for resistance to *S. linicola* (Sackston, 1947; Sackston, 1951; Christenson, 1952a; Michaelson, 1956; Pederson and Michaelson, 1960; Diederichsen et al., 2007). All past studies have found similar results in which there was variability within the population but no true resistance.

Some researchers have also investigated accessions of flax at a greenhouse level (Brentzel, 1926; Sackston, 1949) and results similar to those of field experiments were found. Little is known about where the variation in susceptibility originates or how the pathogen actually penetrates, colonizes, and reproduces within the host. A study by Grant (2008) suggested that populations of the pathogen from different geographical

locations were nearly genetically identical. However, there was some genetic differences observed and is did seem highly plausible that sexual reproduction was occurring in the two populations that were studied. A study by Sackston (1949) observed the pathogen on the surface and noted growth habits and stomatal penetration but did not go further. Another study by Covey (1962) documented similar findings as Sackston (1949), but also investigated some mycelium in the tissue. It is thought that the breakdown of the host tissue may be due to toxins produced by the fungus (Covey, 1962), because neither study identified any penetration of host cells. Although the two studies produced similar results, a complete investigation of the *S. linicola* infection process from penetration, to sporulation has not been completed.

Although no genetic resistance is currently available to flax growers, preliminary work to identify some potential resistance genetics is important in developing resistant cultivars in the future. Gaining an understanding of the means by which the pathogen infects, and colonizes the host will help researchers better understand the system they are dealing with.

The objectives of this study were to 1) to screen flax accessions for signs of potential or partial resistance to *S. linicola.* 2) to further investigate the resistance of several flax accessions under greenhouse conditions 3) to document the infection process of *S. linicola* of flax from germination, through penetration and colonization to sporulation.

3. REVIEW OF THE LITERATURE

3.0 The Host

Flax (*Linum usitatissimum* L.) has been cultivated since pre-historic times and knowledge of its use dates back to 5000 - 8000 BC (Berglund, 2002). It was a very important crop for many ancient civilizations including the Greeks, Romans and Egyptians. In early times, the perennial species *Linum angustifolium* (Huds.) may have been cultivated (Berglund, 2002).

It was reported by Singh (1987) that the wild ancestor of flax, *Linum bienne* (Mill.), occured in the Kurdish foothills and, therefore, it is possible that the first agricultural cultivars were domesticated in this region and later spread into temperate Europe, although the actual time and place of domestication are not well known.

Flax was originally brought into North America for its stem fibre qualities and for uses in making linen and paper. However, in the Northern Great Plains region of the USA and Canada flax has been grown as a commercial oilseed crop for over 100 years (Berglund, 2002). It was introduced into western Canada around 1875 (Lehberg and Anderson, 1941) and has historically been used for paints, varnishes and fibre among other things. Historically, flax production in Canada was below 750,000 bushels in 1933 and 1937 but surpassed 3 million bushels in 1940 (Lehberg and Anderson, 1941). Assuming no. 1 grade and a minimum pounds per Winchester bushel weight of 47.3 lbs (Canadian Grain Commission, 2006) this translates into 16,125 tonnes and 64,500 tonnes for 1933 and 1940, respectively; a four times increase over that period.

L. usitatissimum has been considered a minor crop on the prairies for the past several decades. However, production may be increasing as illustrated in North Dakota

by an increase from 32,275 hectares in 1996 to 300,000 hectares in 2002 which has been attributed to the crops rotational benefit with respect to reduced susceptibility to the pathogen *Sclerotinia sclerotiorum* (Lib.) compared to most other broadleaf crops grown in the region (Halley et al., 2004). As of September 3rd , 2008, The Canada: Grains and oilseeds outlook for the 2008-2009 season forecasts a harvested area of 605,000 hectares and total production at 765,000 tonnes for Canada, (Agriculture Canada Market Analysis Division, 2008)

Flaxseed has several characteristics that have given it appeal as a health food and food additive in North America, and may have contributed to the relatively recent increase in demand for flax in North America (Berglund, 2002). The seed has a very high content of beneficial omega-3 fatty acids (alpha linolenic acid) (Cunnane et al., 1993) as well as a high percent of both soluble and insoluble dietary fibre. The omega-3 fatty acid content can have direct benefits to humans by consuming flaxseed directly or as ground powder added to many foods (Berglund, 2002). There are also indirect benefits associated with flaxseed when it is added to animal diets increasing the omega-3 fatty acid content of products such as eggs and milk. Furthermore, flax has the highest content of plant lignans of all products used for human food (Slavin, 2003). Lignans are thought to be anti-carcinogenic compounds (Berglund, 2002). In addition to the potential dietary and health benefits from flaxseed, fibres from flax stems are being processed and used in a number of products because they have natural fibre appeal as opposed to polypropylene or other synthetic's which are normally used. Much of the recent interest in natural fibre originated in Europe and included fibre products or composite fibre/synthetic products such as automotive interior components, carpet backing, geotextiles and mats for erosion

control (Berglund, 2002). Demand for both seed and fibre products from flax have been increasing in North America and are expected to continue (Berglund, 2002).

Traditionally, rust and fusarium wilt have been the major pathogens affecting flax production worldwide (Rashid, 2003). However, many other diseases affecting flax, including pasmo, can be problematic to production but have been considered regional and of minor importance to date. For this reason, efforts of breeding programs to develop resistance to pathogens such as pasmo have historically been limited (Rashid, 2003). In areas of the world where flax is grown for fibre such as Lithuania, pasmo is thought of as one of the most important diseases along with anthracnose due to detrimental effects on the stems of the plant (Dabkevicius and Gruzdeviene, 2003). Evaluation of the susceptibility of flax to pasmo is difficult because varieties vary in their maturation dates and all varieties appear to become more susceptible as they approach maturity (Flor, 1943).

3.1 The Pathogen

Pasmo was first discribed by Spegazzini in Argentina in 1909 and documented in 1911 (Spegazzini, 1911). The disease was reported in Minnesota in 1919 (Christenson, 1952a) and identified in North Dakota in 1920 (Brentzel, 1926). Pasmo was first found in Ottawa in 1939, Manitoba in 1940 (Sackston, 1949) and its presence has been regular in the province since 1942, extending into Saskatchewan in 1946 (Sackston, 1947). It is thought to have made its way to Australia on seed imported from Canada in 1940 (Millikan, 1948). Pasmo was first found in Ireland on the perennial relatives of flax in 1944 (Lafferty and McKay, 1944). There are many other reports of the primary

identification of pasmo in certain regions documented by Sackston (1949), including Texas in 1939, California, Iowa and Montana in 1943, Siberia in 1930, New Zealand in 1931 and Jugoslavia in 1936.

3.1.1 The Anamorph (*Septoria linicola* Speg. Garassini)

The asexual state is thought to be the main means by which the pathogen propagates. *Septoria linicola* is considered to be seed, soil and stubble borne (Rashid, 2003), surviving as pycnidia. The fruiting bodies of the fungus develop on flax tissue remaining as debris over winter. The pathogen can be carried over long distances on or in the seed and is thought to have come to North America by this method, prior to 1916, likely from Argentina (Christenson, 1952b).

Several researchers documented the physical size of the asexual structures of *S. linicola* and Brentzel (1926) found that the pycnidia produced on both stems and leaves ranged from 63 μm to 126 μm in diameter and when complete had small ostioles. A later report confirmed the ostiolate pycnidia are thin-walled structures which are up to 120 μm broad and immersed in the tissue. They are sometimes flattened at the base and are globose to subglobose in nature (Sivaneson and Holliday, 1981). A third report concludes the pycnidia have a diameter of 55μm - 110μm (Millikan, 1948). Counts show that anywhere from one to seventy pycnidia may inhabit one square mm of stem or sepal (Christenson, 1952b).

Average spore length of *S. linicola* measured by six investigators was reported by Sackston (1949) and ranged from 21.7 to 26 μm with a minimum value of 13.5 μm and maximum of 36.4μm. However, there were some conflicting reports on the spore

characteristics. Spegazzini (1911) stated that the spores of *S. linicola* were hyaline, nonseptate, cylindrical, very slightly curved and approximately 20-30 μm long and 1.5-3 μm in diameter. While Brentzel (1926) reported that the spores resembled those of a *Septoria* and found them to be septate. He also found the average size to be 2.8 μm x 21.7μm which does closely resembles the results of Spegazzini (1911).

Research conducted by Brentzel (1926) found that in culture the fungus grows well between 17° C and 29° C with optimum growth at approximately 21° C, while at extremes of 5° C and 32° C there was minimal growth. These results are similar to those of Sackston (1949).

The work of Sackston (1949) found that fusions between hyphae and spores were extremely common in culture; also noted were fusions between germ tubes from different spores. It was found that when grown on culture media, the pathogen gives rise to several distinct strains. However, the differences appear to be only phenotypic as no differences in pathogenicity is noticed in greenhouse tests (Millikan, 1948; Sackston, 1949). Results for pasmo differ from those for other pathogens such as tan spot of wheat (*Pyrenophora tritici-repentis* Died.), where a group of isolates was classified into four pathotypes based on lesion type or chorosis on differential cultivars (Lamari and Bernier, 1989b).

3.1.2 The Teleomorph (*Mycosphaeralla linorum* Naumov)

Similar to the studies done on the anamorph (*S. linicola*), some research has been done on the structures of the teleomorph of the fungus. Sivanesan and Holliday (1981) reported the pseudothecia to be globose to subglobose in shape, while scattered and

immersed in the tissue. The ostiolate fruiting bodies are 75-120μm wide with a wall up to 13μm thick. The wall, composed of 3-5 layers of pseudoparenchymatous cells, is thick and dark brown towards the outside and thin and hyaline on the inside. The authors also reported the ascospore characteristics: the 13-17 x 2.5-4 μm hyaline, one-septate, ascospores are contained in rows of 8 within 30-50 x 8-9 μm bitunicate, fasciculate asci. Although there are reports describing the characteristics of the ascospores of *Mycosphaerella linorum* the sexual stage has not been identified or proven in Manitoba for decades.

However, the teleomorph is believed to have been identified in Manitoba by Sackston in December of 1944 (Sackston, 1949). In spite of this isolated case, the appearance of the perfect state is extremely rare in Manitoba and no further specimens have been found in the province to date. The size of the ascospore's found in Manitoba in 1944 by Sackston, (1949) are in agreement with those of Sivaneson and Holliday, (1981). This state of the fungus was documented as absent in North Dakota (Brentzel, 1926).

3.2 Epidemiology

The pycnidia of *S. linicola* overwinter on infested straw and are the major natural means of disease propagation. For this reason infested straw has been saved and used extensively as a source of inoculum in research plots as early as the 1900's (Brentzel, 1926). In order for the pathogen to spread and invade a field, the pycnidia need to disperse their spores. When the pycnidia are exposed to high humidity the spores are released in the form of a gelatinous mass called a cirrhus. These spore masses are not

easily dispersed by normal winds but mostly by rain splash or insects, which are believed to be the main means of dissemination (Christenson, 1952b; Sackston 1970). This is the source of primary infection with secondary symptoms resulting from the millions of spores produced in lesions from early infections (Rashid, 2003). In simulated experiments, Sackston (1949) demonstrated the possibility of dissemination by wind driven rain. This work showed that wind or rain alone may not adequately disseminate the spores but a combination of high winds and rain can be very effective.

As with most fungal pathogens, symptom development for pasmo is favoured by high moisture and warm temperatures (Brentzel, 1926; Loughnane et al., 1946) which can be increased with a dense crop canopy or lodging (Rashid, 2003). Many studies have stated that low lying or lodged areas and periods of high humidity or rain showers are known to be attributed to greater severity of pasmo (Brentzel, 1926; Flor, 1943; Sackston, 1949).

It is noted that plants between seedling and flowering stage are not usually affected by the pathogen unless there is an abundance of inoculum and favourable climatic conditions (Flor, 1943; Sackston, 1949). This may be, in part, the reason why pasmo does not usually become noticeable in fields until near maturity. Brentzel (1926) noted that under average conditions the pathogen can barely maintain itself on healthy green flax plants, but when flowering occurs and as the host nears maturity, the pathogen spreads much more rapidly.

The infection caused by *S. linicola* can appear on different plant parts at different times in the growing season as the disease progresses. A study by Perryman and Fitt (2000) at Rothamsted field station in the UK clearly shows that symptoms caused by

pasmo on the leaves, beginning July 22nd, occur earlier than symptoms on the stems, beginning August 5th, and that the stem severity may not ever reach the magnitude of the leaf severity. In some cases, disease assessments should be carried out on both the leaves and the stems in order to get accurate representation of overall disease. These findings by Perryman and Fitt (2000) may demonstrate that the pathogen attacks leaves primarily earlier in the season and moves to stems as plants mature. In a study observing both leaves and stem separately, Michaelson, (1956) noticed that scores on the leaves and stems were not always consistent, which may suggest that flax plants have different physiological characteristics on those separate plant parts creating differences in susceptibility.

In another study, temperature reduction was attributed directly to a decrease in incubation period, however, no differences in symptoms were observed (Sackston, 1949). High flax acreages or the use of winter linseed varieties in other countries may also result in increased disease pressure (Perryman and Fitt, 2000). This is caused by a constant source of infected stubble from one year to the next or even from season to season. This theory is demonstrated in the late 1940's when high prices resulted in larger acreages seeded to flax. Introduction into new areas made it relatively easy for the pathogen to build up inoculum and survive year to year by spreading from diseased stubble to plants growing in nearby fields (Sackston, 1949). Increased acres of flax can be attributed to higher prices which also result in greater appreciation for every bushel lost to disease.

3.3 Symptoms

It is well known that the characteristic symptom of this disease consists of brown bands on the stems, alternating with green healthy areas produced from coalescence of elongated brown lesions, resulting in a mottled appearance (Brentzel, 1926; Sackston, 1947; Sackston, 1949; Rashid, 2003). Brown lesions on the lower leaves, which are round to irregular in shape, are also a sign of pasmo and are generally the first distinguishable manifestation of the disease caused by primary inoculum. Stem lesions rarely develop on seedlings unless they are kept in extremely high humidity in which case the disease usually kills the seedling before the lesions reach more than ten millimetres in length (Sackston, 1949).

Lesions developing on the stems may occur from direct infection or more likely spread from infected leaves originating at the leaf scars after leaves fall off. Lesions originating from the point of leaf attachment are generally larger than those from direct infection (Sackston, 1949) and, in many cases, instances of lesions on the stem associated with leaf scars may be the only observed (Perryman and Fitt, 2000).

Sackston (1949) noted that large lesions developed on stems primarily near the soil line when infected straw is used as inoculum in plots. The lesions are the source of the pycnidia which can form on any infected plant part if sufficient humidity is present (Sackston, 1949). The primary and secondary inoculum are dispersed mainly by means of rain splash and wind to higher leaves on the host plant or to nearby plants (Flor, 1943; Rashid, 2003). The pathogen moves vertically through the growing season to cover all plant parts including the leaves, stem, branches and eventually the bolls near maturity

also causing the leaves to dry rapidly, resulting in significant defoliation (Rashid, 2003). Furthermore the pedicles may become extremely brittle due to infection (Sackston, 1949) and eventually the pathogen can weaken the host, resulting in significant boll drop and stem breakage (Flor, 1943; Rashid, 2003).

Seed from infected plants may be thinner and dull coloured. In severe cases the seed can become wrinkled and scabby in appearance and, although rare in Manitoba, on some occasions pycnidia may occur on seeds (Sackston, 1949).

At the field level, the disease appears in scattered brown patches which give the appearance of irregular ripening (Sackston, 1949). The brown areas may gradually develop and enlarge nearly covering the entire field (Brentzel, 1926; Millikan, 1948). These areas may eventually show complete necrosis and defoliation (Perryman and Fitt, 2000). Longer flowering period has been attributed to decreased expression of pasmo (Ferguson et al., 1987) which may be explained by cool conditions that are conductive to flowering and unfavourable to disease development. This may also be explained by the ability of some cultivars to stay green although approaching maturity. This is a phenomenon called stay-green stem and has been attributed to potential overall improvements in plant health, standability and overall resistance to drought and pests in sunflower (Cukadar-Olmedo and Miller, 1997). It is also suggested that this phenomenon, which contributes to longer photosynthetic periods, and possibly increased nitrogen uptake during grain filling in sorghum (Borrell et al., 2001), also has benefits related to pathogen resistance in dry bean (*Phaseolus vulgarus* L.). The work of Miklas, et al. (2007) found two quantitative trait loci conferring resistance to white mold (*Sclerotina sclerotiorum* (Lib.) de Bary) of dry bean, both of which were associated with

the stay green stem trait while one had additional disease avoidance traits. Furthermore it is thought that pasmo does not interact with flower production directly, but with seed setting and filling, which are post-flowering events (Ferguson et al., 1987). Although lesions on stems and leaves are factors associated with greatest yield losses from pasmo, the fact that the pathogen causes browning on the leaves, stems and bolls creates difficulty in quantifying the effect that damage to each tissue has on yield (Perryman and Fitt, 2000).

In an effort to distinguish reaction types to the pathogen on different cultivars of flax, Sackston (1949) studied four cultivars indoors and several hundred in field plots. Although some chlorosis was noted on two cultivars, the rapid coalescence of lesions and death of leaves present great difficulty in finding definite or consistent enough differences to distinguish any reaction types and therefore no differential lines can be confidently put into place.

3.4 Disease Control

It is well stated that genetic resistance can provide the most effective control of this or any pathogen (Flor, 1943; Rashid, 2003), however, this is likely the most difficult control method to obtain and develop. There are several sound recommendations to control the pathogen which are confirmed by many studies. These include a three year crop rotation to reduce overwintering inoculum on plant debris (Brentzel, 1926; Flor, 1943; Sackston, 1949; Rashid, 2003), using clean seed (Sackston, 1949), at the recommended rate (Rashid, 2003), and maintain control of weeds to avoid a favourable microclimate for disease development (Sackston, 1949; Rashid, 2003). Managing

fertility may also be a method of disease control with lower rates of nitrogen, possibly reducing pasmo and lodging severity although sacrificing potential yield (Rashid et al., 2002).

Although seed treatment may be an effective means of pasmo control and recommended in some cases (Flor, 1943), Sackston (1949) found little viable seed treatment options to combat *S. linicola*, especially if pycnidia or mycelium was present in the seed coat. The research did, however, find that treatment for surface-borne spores may be effective, which is in agreement with other early recommendations (Brentzel, 1926). More recent work has found planting flax with treated seed may be effective in reducing pasmo severity by 26-45.8 %, although seed treatments had little effect on disease incidence (Dabkevicius and Gruzdeviene, 2003)

Seeding date may also have an effect on pasmo development and severity. Diederichsen et al., (2007) reported that late seeding of flax approximately three weeks later than the regular seeding date supported the infection of pasmo by delaying maturity until the warm and humid conditions of August, suggesting early seeding as a method to avoid heavy pasmo infection. This is in agreement with other findings that seeding date may have an effect on pasmo severity for spring seeded (Sackston, 1951) and winter seeded varieties (Turey and Snowden, 1998). Despite some general recommendations stating otherwise, early seeding date may increase yields significantly (Rashid et al. 2002).

Fungicides can be an effective means of control, (Ferguson et al., 1987; Perryman and Fitt, 2000; Dabkevicius and Gruzdeviene, 2003; Halley et al., 2004; Grant, 2008) but different formulations come with varying effectiveness. Substantial yield

responses may be noticed when a severe attack of *S. linicola* is controlled (Dabkevicius and Gruzdeviene, 2003)

In fibre flax production in Russia and Belarus it is common practice to use fungicides added to herbicide or fertilizer applications in order to control pasmo (Dabkevicius and Gruzdeviene, 2003).

Halley et al. (2004) found that the active ingredient azoxystrobin, a quinone outside inhibitor (QoI), decreased pasmo severity and increased flax yield compared to the untreated check, while a sulphur fungicide was effective on pasmo reduction but failed to increase yield versus the control plots. It was suggested that this phenomenon may be attributed to phytotoxicity of sulphur to the host plant. On the other hand, prothioconazole, a sterol biosynthesis inhibitor and class I demethylation inhibitor, did not decrease pasmo severity but did, however, increase flax yield over the check. Data from Ferguson et al. (1987) revealed that the effect of pasmo on yield and seed weight occurs after anthesis, and furthermore that Benomyl fungicide was noted to be effective in disease control while also providing a seven percent yield increase from a single application. This suggested that protection of the flax plants during the reproductive period is important to decrease the effect of pasmo. This result suggested that there were important times in flax growth in which pasmo severity, and control of the pathogen can have the greatest detrimental and positive effects, respectively. The fact that certain growth stages are more important to flax health and yield has lead some researchers to suggest that one properly timed spray can be as effective as two or several fungicide applications (Ferguson et al., 1987; Perryman and Fitt, 2000).

3.5 Host Resistance

Although plant resistance can be determined by a single gene as in the gene-for-gene model described by Flor, (1971), Pryor (1987) suggested that effective resistance probably involved more than one character specified by a number of different genes and that the gene-for-gene may not apply to all interactions or may be difficult to determine. There have been several reports of resistance to pasmo listed by Rashid (2003). A study conducted by Brentzel (1926) reported a few hybrids from experimental plots that appeared to be immune to the disease. Despite some early optimistic reports, no commercial cultivars presently available are resistant or show any major differences in reaction to the pathogen (Rashid, 2003). It is suggested that some of the resistance observed at some intermediate growth stages are the result of a phenomenon in which water droplets (spore solution) have less affinity for younger flax leaves and therefore drop off and do not cause infection (Sackston, 1949). This characteristic is termed "wetability" of the leaves and has also been reported between different cultivars not just growth stages (Covey, 1961). In a study conducted in North Dakota by Brentzel (1926), it was found that of six different planting dates separated by one week intervals and inoculated on the same date, the first three planting dates, or older plants had severe symptoms and the younger plants or the last three seeding dates had minor symptoms. This could possibly be attributed to this phenomenon.

Over time many researchers have attempted to screen many selections of flax varieties from numerous geographic areas for signs of resistance to *S. linicola*. Although results vary, most studies found that no true resistance was available and only some noticeable differences in susceptibility between cultivars existed.

The work of Sackston (1947) in which 130 varieties of flax were tested for resistance to pasmo revealed that none possessed any real resistance. Michaelson (1956) studied 952 varieties of flax from 31 countries and six continents for resistance to pasmo. Differences between varieties were apparent however some of the more resistant lines were later maturing and may have become more susceptible as they reached maturity. A later study also supports this conclusion (Pederson and Michaelson, 1960).

In a study by Sackston (1951) it was determined that of 800 varieties and selections observed in inoculated plots in Winnipeg, Manitoba, none were resistant to the pathogen although some plots were, to some extent, more tolerant than previous averages. In a recent study completed by Diederichsen et al. (2007), 153 flax accessions were evaluated for resistance to pasmo and it was found that disease severity fell within a range of 74.2 ± 11.8% and none of the accessions showed complete resistance. Similar results were found for anthracnose although many accessions showed high resistance to fusarium wilt. A study of six flax varieties comparing seeding and pasmo inoculation date to yield indicated that some varieties could be recommended over others depending if pasmo was likely to occur in the upcoming growing season but ultimately seeding date had the most effect on yield (Sackston, 1951). Other researchers have examined several to many varieties of flax for resistance to pasmo but report only variation in susceptibility, inferring moderate resistance but no immunity from the disease (Christenson, 1952a; Pederson and Michaelson, 1960). The overall conclusion from many studies over several decades is that no resistance has been discovered and differences noticed between varieties may be a result of varying maturity and not a sign of resistance. Research conducted by Sackston, (1949) lead him to conclude that field

data may leave a lot to be desired, and some differences in relative susceptibility noticed on certain varieties may be attributed to geographic races of the pathogen differing in virulence. Sackston (1949) further reported that in studies where plots are inoculated only by naturally occurring inoculum the distribution of infection can be quite erratic. Greenhouse investigations have also produced variable results in the past. In one greenhouse study where flax plants were inoculated, kept at high humidity for three days, and rated at eight days after inoculations, there were noticeable differences between the degree of infection on some of the 5 cultivars mentioned (Brentzel, 1926). Conversely, in another greenhouse experiment, no differences were found among more than 100 varieties inoculated with three different isolates, nor in experiments testing smaller groups (Sackston, 1949).

Christenson (1952a) suggests screening of the approximately 100 wild species of *Linum* may be potential source of genetic resistance, as this strategy has been employed with many other crops. The potential problems associated with wild relatives are that some of the wild species have different chromosome numbers and perennial growth habits. However, with modern scientific techniques introducing new genetic material should be feasible.

When breeding for particular traits, unintended and undesirable traits may be carried with the intended germplasm. This is discussed by Flor (1943) and Sackston (1949) who provided an example of a situation where new flax varieties introduced for resistance to rust in the 1940's showed greater susceptibility to pasmo.

Transferring only the R-gene to the desired cultivar can avoid carrying over unwanted and genetically linked genes however in some cases it may be difficult to separate the desired R-gene from unwanted germplasm (Rommens and Kishore, 2000). Moreover it may be possible to have an effective R-gene isolated from a non-host plant as demonstrated by Kamoun et al., (1998) where tobacco's non-host resistance to the potato pathogen *Phytopthera infestans* is correlated with hypersentivity to the elicitor INF1 of *P. infestans*. The authors stated that this indicates direct involvement of an avirulance factor and the potential to isolate R-genes from non-host germplasm as a novel source of resistance.

In some cases plants may appear resistant but in fact are not, which can be detrimental to breeding efforts. There can be several reasons that this may occur, one is apparent resistance. As described by Agrios (2005), apparent resistance occurs under certain conditions or circumstances when highly susceptible plants or varieties of crops remain free from infection or symptoms and therefore appear resistant. This phenomenon is generally the result of disease escape or tolerance. Disease escape occurs when a genetically susceptible plant does not become infected because one of the three factors required for pathogenesis is not met. For all host pathogen interactions if the host plant is resistant, the pathogen is not virulent or the environment is not favourable the three factors are not met and pathogen development ceases (Agrios, 2005). Tolerance to disease is reported as the ability to produce a good crop even when infected by a pathogen (Agrios, 2005), this is to say that the host plant has specific genetic characteristics where by the pathogen can develop and multiply within it while the host is not killed and shows little damage while producing good yield.

In the absence of resistant varieties researchers can search for partial (or quantitative) resistance in flax. Partial resistance retards the pathogen during the infection cycle and reduces the pathogens development rate (Rashid, 1991). Similarily, Parlevliet (1979) defines partial resistance as a reduced rate of disease development as a cumulative result of differences in one or more of the phases of the infection cycle. Looking for factors or plant characteristics that can limit the development and spread of pasmo should aid in developing more resistant varieties (Covey, 1961). In the case of partial resistance, there remains a susceptible reaction in all growth stages except the infection frequency, latent period, amount of spores produced and length of spore production period may vary (Parlevliet and Van Ommeren, 1975). Other factors that may be important components of partial resistance include the length of incubation period and the pustule size (Rashid, 1991). Depending upon the nature of the pathogen under observation, the equivalent of pustule size is the lesion size. Another component dependant on the host-pathogen interaction is non-successful infections due to the abortion of infections.

In a study conducted by Covey (1961) lesions and fruiting bodies were counted on flax varieties thought to differ in their susceptibility to pasmo. The ability to reduce secondary inoculum is an important aspect of reduced disease severity and practical differences were found between cultivars in, number of lesions, pycnidial production, and spore production per lesion. This indicates that some varieties have some partial resistance components.

When observing partial resistance to rust (*Melampsora lini*) on flax, Rashid (1991) observed significant differences among 11 cultivars for incubation period, latent

period, infection type, receptivity, sporulation and successful infections. This led to the conclusion that cultivars with beneficial partial resistant components may be useful in developing resistant cultivars. It is possible that resistance may not be immediately attainable but lines with high values for different resistance components could be crossed to increase the level of partial resistance (Ohm and Shaner, 1976).

Regarding the aforementioned studies involving selections of flax observed for differences in susceptibility levels, the selections were for the most part represented by percent infection scores and none with an area under disease-progress curve (AUDPC) measurement. The AUDPC may be a more accurate representation of disease severity because it is a measurement of quantitative disease resistance over repeated disease assessments and can be used to integrate all aspects of disease progress in relation to host development and growth (Jeger and Vlijanen-Rollinson, 2001). The authors also list several studies in which the AUDPC has been used effectively in the past. It was reported by Jeger and Vlijanen-Rollinson, (2001) that in cases where a well defined period for assessment existed (i.e. growth stages) then as few as two disease assessments may prove to be sufficient in providing most of the information contained in the AUDPC calculations for making many assessments. Taking fewer assessments through the growing season can be a method of managing the considerable time, space and human resources required to assess disease quantity in field plots, easing the work load when frequent assessments may not be feasible (Jeger and Vlijanen-Rollinson, 2001).

3.6 Infection of the Host Tissue

The work of Sackston (1949) found no relationship between the size of spores or number of septa and speed of germination. The study, conducted on artificial media, found that at four hours about 10% of the spores had started to germinate. Between five and six hours some spores had produced germ tubes up to six microns in length. Germination was noted from one or both terminal cells and rarely from intercalary cells. Germ tubes developing into hyphal threads were most frequent, however, secondary conidia were observed with regularity from intercalary cells. Conidia borne on spores in culture were observed individually, in pairs or even in a whorl formation (Sackston, 1949). A study conducted by Covey (1962) also mentioned secondary sporulation occurring on the leaves and found this to occur at 48 hours after inoculation without pycnidial formation. Similarly, Annone (1990) noted that secondary conidia or microconidia were produced from budding of pycnidiospores of the pathogen, causing *Septoria tritici* blotch of wheat, *Mycosphearella graminicola* (anamorph: *Septoria tritici),* on the surface of wheat leaves at 12-72 hours after inoculation.

Previous studies have identified several characteristics of the host-pathogen relationship. Brentzel (1926) stated that spores were 3-septate, and that in some cases each of the 4 cells of a spore forms what appears to be a chlamydospore. Chlamydospores occurred commonly in cultures of *S. linicola* and germinated in the same manner as the conidia (Sackston, 1949). Also, germ tube fusions between adjacent spores were observed after 7 hours on plates (Sackston, 1949). Fusions were observed between neighbouring spores and between two germ tubes of the same spore, however, no transfer of protoplasm was observed.

The pathogen was also observed on the host by Sackston, (1949). On inoculated flax cotyledons it was noted that spore germination seemed to follow the same pattern as in culture and fusions between germ tubes were once again observed. All penetrations observed on the cotyledons were via the stomata and no appressoria-like structures were observed. The stimulus to penetration was not determined as many germ tubes passed over several stomata without penetrating while others entered the first opening they came across. Furthermore, no cases were found of host epidermal cell penetration, neither directly, or by haustoria.

With respect to pycnidial formation, previous research has shown that after 80 hours, numerous dense dark staining knots of mycelium were observed at the junction of several hyphal strands. These were noted to be pycnidial primordia and by 108 hours had developed into definite pycnidia (Sackston, 1949).

In other work by Covey (1962) it was found that within six hours 60% of the spores had germinated although no penetration occurred until 36 hours after inoculation. When penetrations were observed, there was definite infiltration through the stomata although this did not occur frequently and apparent direct penetration was noted twice on one of the two cultivars observed. By 4 days after inoculation, mesophyll cells were observed to be collapsing and being replaced by hyphae. The mycelium was observed to be frequently appressed to the cells of the host, however, no intracellular mycelium or haustoria were documented. The study observed that by seven days the mycelium had completely replaced the mesophyll in the infected area with only the epidermis and vascular tissue remaining intact. The result is the appearance of a distinct lesion coupled with the formation of pycnidial initials just below the epidermis at this time. Another

study conducted by Covey (1962) attempted to identify toxins associated with pathogenicity and again concluded that the most frequent method of penetration was via the stomata. Evidence of mesophyll collapse ahead of the hyphal advance within tissue along with filtrates of *S. linicola* infected leaves causing blackening and collapse of leaves lead the authors to conclude that although the fungus does not penetrate the host cell, it apparently secretes a toxin or enzyme that causes collapse of the host cell. It was suggested that this substance may be active in pathogenesis (Covey, 1962).

In related studies the causal agent *S. tritici* which is responsible for septoria blotch of wheat has been studied more recently. The research of Cohen and Eyal (1993) discovered that at 24 hours after inoculation 85-90% of the conidia had germinated, and by 48 hours after inoculation bifurcate and unbranched hyphae were observed. Most frequent penetration was achieved via stomata, with the formation of an appressorium occurring, but not a prerequisite. Organization of pycnidia became evident at 12 days, and was restricted to substomatal spaces with the ostiole located beneath the guard cells. Autofluorescent materials were noticed in stomatal and epidermal cell walls and mesophyll cells surrounding the substomatal space of a resistant cultivar. For the *S. tritici* pathogen the absence of immunity results in resistance levels based on a limited number of symptoms (Cohen and Eyal, 1993).

4. RESULTS OF RESEARCH

4.0 Investigating potential resistance of flax to *Septoria linicola*

4.0.0 Abstract

Septoria linicola (Speg) Garassini. (teleomorph: *Mycosphaerella linorum* Naumov), the causal agent of pasmo of flax, is emerging as an important foliar pathogen affecting flax production in most flax growing regions. Historically, there have been many reports of researchers attempting to develop resistance to the pathogen, however little more than variation among cultivars has ever been reported meaning resistance to the pathogen has been difficult to find. Two hundred and eight accessions provided by Agriculture and Agri-Food Canada (AAFC) and showing signs of resistance to pasmo were collected as single plants from a pasmo nursery. They were selected from many different flax cultivars. The seed was increased and the accessions were sown in a pasmo nursery at two different seeding dates in 2006 and 2007 and assessed for leaf and stem disease severity. Plots were assessed weekly for a period of seven and five weeks for the leaves and stems, respectively, and area under disease-progress curve (AUDPC) values were generated. AUDPC values ranged from highly susceptible to moderately resistant for the 208 accessions, although most were less susceptible than the susceptible check. Leaf and stem values were positively correlated although some accessions appeared to have very different susceptibility on these separate plant tissues. Although it is apparent that genetic differences exist between the accessions observed in this study, the effects of maturation date, other pathogens, and lodging may be attributed to some of the variation. While several accessions are promising for future breeding studies, many of the accessions may not be adapted for western Canada growing conditions and further study

of the resistance, days to maturity, and genetics of the moderately resistant cultivars is needed.

4.0.1 Introduction

Pasmo, caused by the fungal pathogen *Septoria linicola* (Speg) Garassini. (teleomorph: *Mycosphaerella linorum* Naumov) is a foliar disease of flax affecting many flax growing regions around the world, including North America (Rashid, 2003). The disease was first identified in Manitoba in 1940 (Sackston, 1949). Pathogen growth and symptom development are favoured by moist conditions (Brentzel, 1926; Loughnane et al., 1946) and lodging (Rashid, 2003). However, in the absence of abundant inoculum and favourable climatic conditions disease development is generally not important between the seedling and flowering stage (Flor, 1943; Sackston, 1949). This may partially explain why some researchers suggested that the pathogen is most prevalent near maturity and that maturation date may be a factor in the variability observed when screening for resistance (Michaelson, 1956; Pederson and Michaelson, 1960).

The pathogen is seed, soil and stubble-borne and overwinters on plant debris. Although it is generally believed that pasmo occurs later in the season, early season epidemics can take place with favourable environmental conditions (Rashid, 2003). The initial inoculum is provided in the form of pycnidiospores which ooze from pycnidia on plant debris. The lesions produced from the initial infections are responsible for the secondary infections (Rashid, 2003), when spread by wind, rainsplash or other vectors (Christenson, 1952b; Sackston 1970). The teleomorph is not known to exist in Manitoba and has not been documented since 1944 (Sackston, 1949), thus reducing the potential for the pathogen to reproduce sexually and develop more genetic diversity.

Because the pathogen remains on infected stubble, crop rotation is an important management tool in control of the disease (Brentzel, 1926; Flor, 1943; Sackston, 1949;

Rashid, 2003), although other means, including fungicides, also prove to be effective in controlling pasmo (Ferguson et al., 1987; Perryman and Fitt, 2000; Dabkevicius and Gruzdeviene, 2003; Halley et al., 2004, Grant, 2008). Despite resistance being the most effective control (Flor, 1943; Rashid, 2003), there is no true resistance to pasmo among commercial cultivars (Rashid, 2003). *S. linicola* has not historically been seen as a destructive disease in Manitoba. However, when breeding for resistance to other diseases, researchers found flax rust resistant varieties that were developed were more susceptible to pasmo, demonstrating a need to study pasmo in Manitoba (Sackston, 1949). Many studies have been conducted to find genetic resistance to *S. linicola* in numerous flax selections from various geographical regions (Sackston, 1947; Sackston, 1951; Christenson, 1952a; Michaelson, 1956; Pederson and Michaelson, 1960; Diederichsen et al., 2007). Although none of the studies have been conducted in western Canada recently, they all arrived at similar conclusions. Variation in susceptibility among the selections was evident in all studies. However, true resistance was not found in any study despite some reports of its existence (Rashid, 2003). The expression of disease in a cultivar can be altered by many factors. The genetics of the host in the host-pathogen relationship is important but factors such as lodging (Brentzel, 1926; Flor, 1943; Sackston, 1949; Rashid, 2003), competition from other diseases (Al-Naimi et al., 2005), temperature and humidity (Brentzel, 1926; Loughnane et al., 1946) may result in escape from the disease.

Prior to this study, flax cultivars were selected as single plants based on low susceptibility to pasmo when grown in a disease nursery (Rashid, unpublished data). The objective of the present study was to assess 208 flax accessions for resistance or partial

resistance to *S. linicola* and to examine other agronomic factors for use in future breeding programs.

4.0.2 Materials and Methods

Flax Accessions

Prior to this experiment, 208 accessions of flax were selected as single plants in the 2005 growing season at Agriculture and Agri-Food Canada, Morden Research Station (Rashid, unpublished data). Selection was based on accessions taken as single plants from a genetically diverse population showing low levels of susceptibility to *S. linicola* on both leaves and stem as preliminary research on flax pathology to identify sources of resistance to *S. linicola*. In the 2006 and 2007 growing seasons, plots were established in a pasmo nursery and the accessions were seeded in three-meter single row plots with three grams of seed per row and 0.30 m row spacing. In 2006 the plots were seeded on May 16th into a hochfeld orthic black soil (Smith et al., 1973, Manitoba Soil Survey). This soil type consists of a fine sandy loam structure and is of a moderately coarse to coarse texture and considered a well drained soil. Permeability is moderately rapid resulting in very little runoff and this soil is quite susceptible to wind erosion due to the sandy structure. The 2007 plots were seeded into an eigenhof series soil which is a moderately well drained orthic black soil. It is a characteristically loamy soil with a moderately fine texture and agriculture capability class one (Smith et al., 1973, Manitoba Soil Survey). The 208 individual plots were replicated twice and seeded approximately 10 days apart to represent early and late seeding dates. In 2007, the early seeded plots were sewn on May 16th and the late seeded on May 28th. In 2006, however, both replications were seeded on the same date and repetition 1 and 2 corresponded with early and late seeding times in 2007. The cultivars Somme (both seasons) and AC McDuff

(2007 only) were seeded as comparative check strips because these varieties are thought to be highly susceptible and moderately resistant, respectively.

Weed control was achieved by a single herbicide application and hand weeding. Plots were harvested at maturity using a single row cutter, bundling individual plots. Single row bundles were hot air dried at 35°C and subsequently threshed individually through a stationary combine. Harvested samples were stored in envelopes until cleaned and weighed. Yield data was reported as the weight of clean seed per plot and because the seed originated from single plants, quantity was limited and seed harvested from selections in 2006 was used in 2007 to repeat the trial.

Inoculation

In order to reveal differences between accessions, disease pressure was monitored and maintained throughout the growing season. To achieve this, a combination of introduced inoculum and a misting system were used. The misting system, developed by Lamari L. and Smith R. at the University of Manitoba (Lamari and Smith, unpublished) was designed to provide moisture to the plots in the nursery area to develop a favourable microclimate for spore release (Fig. 1A). At the stem extension stage (approximately 15-25 cm tall) the inoculum was placed evenly between the single row plots in the form of straw covered with the pycnidia of S*eptoria linicola* from previous years pasmo nurseries. The infected straw was placed on the ground and pressed down to avoid direct contact with the leaves in the plots (Figure 1B). In 2006, the inoculum was applied on June 19th which was 34 days after seeding. In 2007, the inoculum was applied to the early plots on June 28th, 43 days after seeding and the late

seeded plots on July 13th 46 days after seeding. The misting system was started after the straw inoculum was spread and ran for 4-5 minutes every 30 minutes, weather permitting. In 2007, the early plots were not showing symptoms after more than two weeks and there might not have been enough inoculum applied. On July 19th, 2007, 21 days after the first inoculum on the early seeded plots, another dose of the infected straw inoculum was added to ensure sufficient amounts to cause infection. The inoculum applied to the late seeded plots six days earlier was also increased in any plots that appeared to have less than average. In 2006, the approximately weekly ratings began on June 26th, 41 days after seeding and seven days after inoculation. In 2007, the first rating was on July 12th, 55 days after seeding and 14 days after inoculation for the early seeded plots and on July 24th, 57 days after seeding and 11 days after inoculation.

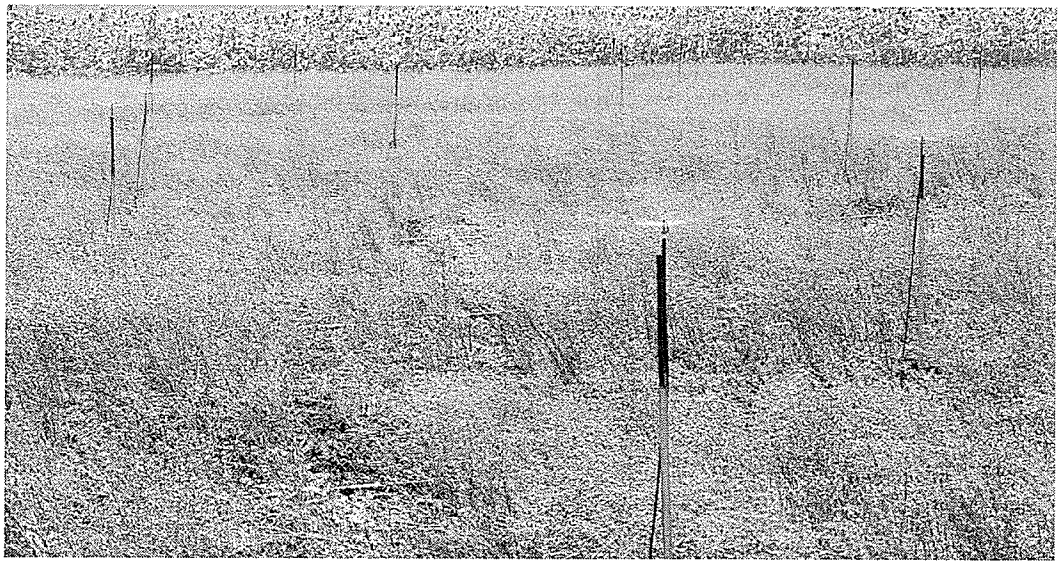
Rating System

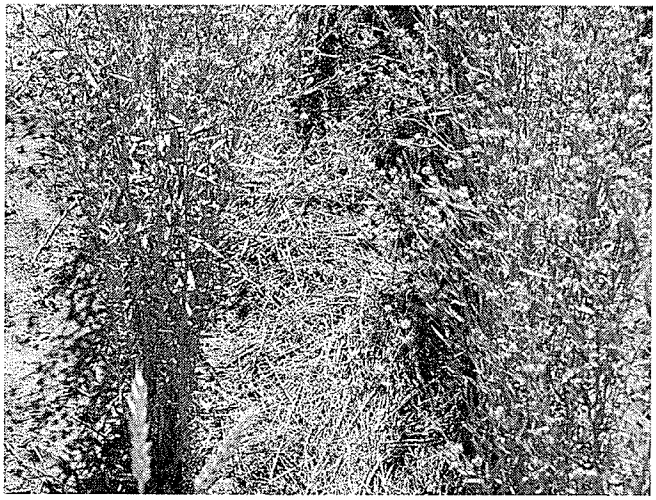
Ratings were taken separately on both leaves and stems based on a visual assessment of percentage leaf and stem area infected. Estimates were taken on both leaves and stems for each plot similar to the approach taken by Sackston (1949). Disease values were recorded on a 0 to 9 scale where 0 = no sign of infection and 9 = 90% of the plant suface area appeared infected. Observations were made at approximately weekly intervals throughout the growing season. Disease assessment began when the first signs of the disease were present. This strategy was implemented to avoid several weeks of "zero" disease scores early in the season and resulted in 7 weeks of total leaf ratings and 5 weeks of total stem ratings. The area under disease-progress curve (AUDPC) was used as a measure of quantitative disease resistance based on

(Figure 1A) Misting system providing leaf wetness to pasmo nursery

(Figure 1B) Straw inoculum containing pycnidia of *S. linicola* placed between rows of flax plants

(Figure 1C) Plots with poor germination in some of the 2007 field plots





repeated disease assessments. The equation used to calculate the AUDPC was taken from Jeger and Viljanen-Rollinson, (2001) and was used as follows;

$$AUDPC = \frac{1}{2}\sum_{i=1}^{n}(x_i + x_{i-1})(t_i - t_{i-1})$$

Where:

x_i = the visual pasmo rating on the i^{th} rating date

t_i = the date of the i^{th} rating

Plants were also rated for lodging and powdery mildew (*Oidium lini* Skoric) and were both observed, for five and four weeks, in the 2006 growing season for repetitions one and two, respectively, and recorded on a 1-3 scale representing low-medium-high severity. Flax growth stage was also recorded in 2006 only and was based on the 1-12 scale of Linseed main growth stages (Turner, 1987). The growth stage was recorded on four occasions and a mean of the four observations was used. In 2007, the plots were not noticeably affected by powdery mildew. Problems with germination of the 2007 seed (Figure 1C) made accurate lodging and stage observations difficult. The germination percent was however recorded in 2007, and was based on the observed percentage of plants emerged compared to the check rows which were set at 100%.

Analysis

Data was analysed separately for 2006 and 2007 field seasons. Data from seven disease assessments was transformed into area under disease-progress curve (AUDPC) scores for each plot and analysed individually as two separate variables, namely leaf and stem. A combined AUDPC score variable was also created as the mean of the leaf and

stem for each plot. SAS software version 9.1, (Copyright, 2002-2003, The SAS Institute inc., Cary, NC, USA) was used for analysis and the "proc mixed" function was employed for these data. Estimates for each accession were compared to the sample mean for each variable. This estimate and comparison was determined with the Best Linear Unbiased Prediction-Variance Structure otherwise known as BLUP analysis on the mixed procedure. Subsequently, the sort procedure was used to rank the accessions from lowest to highest disease severity as calculated by AUDPC values. The BLUP function compared a particular accession's AUDPC value to the mean of all 208 accessions plus check cultivars to determine if the accessions AUDPC value is significantly different from the mean. This is effective in determining accessions with values significantly lower and higher than the mean (Table 1).

4.0.3 Results

The results of the statistical analysis are shown in Appendix 1. The accessions themselves were considered a random factor and had a highly significant ($P \leq 0.01$) effect with respect to differences among them for the leaves, stem and combined AUDPC scores (Table 1, 2). Variability was apparent among the accessions for leaves and stems and subsequently the combined score. The effect of field season was found to be highly significant ($P \leq 0.01$) between the 2006 and 2007 growing seasons. The effect of seeding time was found to be significant ($P \leq 0.05$) in 2007 based on average AUDPC scores. The plots in 2006 were seeded on the same date so there was no seeding date discrepancy on combined data, although the two replications were found to be significantly different ($P \leq 0.05$) with respect to leaf scores. It was also found that early seeded plots were significantly higher yielding ($P \leq 0.05$) than later seeded plots in 2007 based on a t-test assuming unequal variances. Furthermore, one replication in 2006 was surprisingly higher yielding than the other although both were seeded at the same time.

Combined Observations

Statistical BLUP estimation of differences ($P \leq 0.05$) for the combined scores for each accession found that there were 36 and 14 accessions which are significantly lower than the mean of the observed population in 2006 and 2007, respectively. Three accessions appear to be significantly better than the population observed both field seasons (Table 1). Accessions 79, 170 and 178 (Table 1) were significantly better than the population means for the combined score but they also are ranked highly for both leaves and stems individually. Conversely, with respect to average AUDPC values, there

were 32 and 25 accessions that were significantly greater than the population mean for 2006 and 2007, respectively (Table 1). The checks were also found to have significantly ($P \leq 0.05$) higher AUDPC values than the population mean for combined scores.

Leaf and Stem Observations

When analysing the leaf and stem AUDPC values separately, leaf AUDPC analysis identified three accessions which had significantly lower values than the mean for both field seasons (170, 164, 63), while the AUDPC analysis in stems identified six accessions (79, 53, 35, 29, 80, 82) (Table 1). Although many accessions were ranked highly on the individual variables, leaves and stems, the average AUDPC value for some may not be. Many accessions which were significantly better than the mean for stem AUDPC scores may rank relatively low for leaf AUDPC scores and vice versa. Despite some exceptions, leaf and stem AUDPC values were positively correlated in both 2006 and 2007 field seasons (Table 2).

Other Factors

Correlation coefficients were determined for several of the variables (Table 2). Due to poor germination in 2007, the growth stage, powdery mildew and lodging for that season were not included in the correlation because accurate observations were difficult to determine in situations with very few plants in a plot. It was found, however, that in 2006 the growth stage was positively correlated with AUDPC values for leaves and stems (Table 2). A higher growth stage value equates to earlier maturing varieties. It was also observed that lodging had a positive correlation with all AUDPC variables calculated

(Table 2). The presence of powdery mildew was found to be negatively correlated with AUDPC for all variables.

In 2007, poor germination resulted in four accessions being omitted from disease scoring (77, 90, 91, 84). These accessions had plots with either zero germination or very few plants which died early in the growing season and, therefore, were not evaluated. Accessions were included in the analysis only if scores were available for both seeding dates.

Table 1. Mean area under disease progress curve (AUDPC) values for pasmo and yields in 2006 and 2007 and stage, powdery mildew severity and lodging in 2006 for 208 flax selections. Selections are sorted in ascending order by the overall combined score of leaf and stem AUDPC values for both field seasons observed.

Cultivar	Plot # early	Plot # late	Accession	2006 AUDPC scores			2007 AUDPC scores			Overall combined[d]
				leaf[a]	stem[b]	combined[c]	leaf	stem	combined	
Somme	C	C	Check 1	263.4	188.8	226.1	199.0	122.0	160.5	193.3
AC McDuff	C	C	Check 2	-	-	-	254.9	154.5	204.7	204.7
Diva	7570	7870	170	135.5*	23.8*	79.6*	90.8*	24.1	57.4*	68.5
Ilona	7566	7866	166	116.0*	23.5*	69.8*	123.1	38.6	80.9	75.3
Argos	7564	7864	164	122.6*	28.8*	75.7*	112.1*	43.1	77.6	76.7
SOMxLINDA	7578	7878	178	175.0	9.5*	92.3*	100.0*	23.6	61.8*	77.0
CDC Bethune/Double Low	7479	7779	79	171.8*	1.8*	86.8*	129.6	9.1*	69.4*	78.1
Elektra	7567	7867	167	113.3*	32.0*	72.6*	137.9	30.8	84.3	78.5
Argos	7563	7863	163	120.8*	23.8*	72.3*	136.0	36.4	86.2	79.2
(VERNExACLIN)-1xCH10-1	7463	7763	63	154.8*	18.3*	86.5*	112.9*	54.3	83.6	85.0
Marina	7428	7728	28	162.5*	13.8*	88.1*	123.9	41.4	82.6	85.4
Hermes	7561	7861	161	132.4*	17.0*	74.7*	143.5	51.3	97.4	86.0
Hermes	7562	7862	162	138.5*	45.8	92.1*	121.3	41.3	81.3	86.7
Marina	7427	7727	27	165.5*	13.5*	89.5*	140.4	29.3	84.8	87.2
M7406	7453	7753	53	172.0*	13.8*	92.9*	155.4	8.6*	82.0	87.4
N2014	7572	7872	172	200.8	19.5*	110.1	101.9*	28.5	65.2*	87.7
SOMxLINDA	7577	7877	177	187.0	13.5*	100.3*	119.1	35.5	77.3	88.8
Astelle	7435	7735	35	158.5*	31.8*	95.1*	148.3	21.1*	84.7	89.9
Astelle	7436	7736	36	151.8*	43.5	97.6*	137.6	27.0	82.3	90.0
Astelle	7417	7717	17	140.3*	11.8*	76.0*	161.0	49.0	105.0	90.5
SOMxLINDA	7468	7768	68	190.3	11.9*	101.1*	115.6*	44.8	80.2	90.6
Natasja(Can)	7429	7729	29	197.9	27.1*	112.5	122.1	17.3*	69.7*	91.1
Escalina	7440	7740	40	146.9*	63.8	105.3*	123.6	33.5	78.6	91.9
(VERNExACLIN)-1xCH10-1	7470	7770	70	179.6	37.0	108.3*	126.8	31.9	79.3	93.8
M7418	7458	7758	58	180.6	16.1*	98.4*	133.4	46.5	89.9	94.2
Liflax	7414	7714	14	160.9*	17.9*	89.4*	145.3	53.0	99.1	94.3
99L01	7416	7716	16	164.8*	48.8	106.8*	145.6	24.0	84.8	95.8
M7406	7454	7754	54	178.1	39.8	108.9*	149.6	23.8	86.7	97.8
FP 2070/Double Low	7481	7781	81	226.9	41.8	134.3	109.8*	14.8*	62.3*	98.3
Torzhokij	7452	7752	52	172.6*	38.8	105.7*	165.6	22.0*	93.8	99.8

Cultivar	Plot # early	Plot # late	Accession	2006 AUDPC scores			2007 AUDPC scores			Overall combined[d]
				leaf[a]	stem[b]	combined[c]	leaf	stem	combined	
Natasja(Can)	7441	7741	41	172.5*	21.8*	97.1*	152.3	55.0	103.6	100.4
Torzhokij	7451	7751	51	170.5*	37.0	103.8*	171.6	22.6*	97.1	100.4
FP 2070/Double Low	7482	7782	82	215.3	31.8*	123.5	144.5	10.3*	77.4	100.4
(VERNExACLIN)-1xCH10-1	7469	7769	69	202.6	55.3	128.9	109.4*	34.8	72.1*	100.5
SOMxLINDA	7589	7889	189	213.6	51.8	132.7	111.0*	32.4	71.7*	102.2
(VERNExACLIN)-1xCH10-1	7464	7764	64	180.1	30.9*	105.5*	153.6	45.4	99.5	102.5
SOMxLINDA	7471	7771	71	203.1	42.1	122.6	137.5	28.9	83.2	102.9
M7418	7457	7757	57	184.0	20.3*	102.1*	150.9	61.6	106.3	104.2
Ariane	7437	7737	37	172.3*	87.5	129.9	135.3	22.6*	78.9	104.4
Arriane	7560	7860	160	145.0*	26.1*	85.6*	157.5	89.3	123.4	104.5
810/5//Flanders	7476	7776	76	223.9	25.5*	124.7	138.3	30.4	84.3	104.5
Natasja(Can)	7442	7742	42	173.0*	21.8*	97.4*	160.8	68.5	114.6	106.0
(VERNExACLIN)-1xCH10-1	7587	7887	187	212.3	58.4	135.3	105.5*	48.1	76.8	106.1
CDC Bethune/Double Low	7480	7780	80	210.5	15.1*	112.8	179.9	20.5*	100.2	106.5
Natasja sel	7448	7748	48	179.9	26.8*	103.3*	164.5	60.6	112.6	107.9
(VERNExACLIN)-1xCH10-1	7588	7888	188	217.4	81.8	149.6	96.3*	38.5	67.4*	108.5
M7407	7456	7756	56	202.4	43.5	122.9	152.1	36.0	94.1	108.5
N2014	7571	7871	171	222.9	64.1	143.5	121.4	26.1	73.8*	108.6
Liflax	7413	7713	13	183.1	50.3	116.7	144.1	63.1	103.6	110.2
Laura	7422	7722	22	175.3	17.0*	96.1*	172.3	79.0	125.6	110.9
Natasja sel	7447	7747	47	175.9	26.8*	101.3*	163.8	77.9	120.8	111.1
AC Emerson/Double Low	7478	7778	78	213.6	55.4	134.5	138.9	36.5	87.7	111.1
ACLIN-3-1xCH17-3-1	7583	7883	183	226.0	65.3	145.6	108.9*	44.3	76.6	111.1
(VERNExACLIN)-1xCH10-1	7550	7850	150	220.4	56.6	138.5	127.1	42.6	84.9	111.7
Double Low/SP 2047	7487	7787	87	231.8	38.5	135.1	140.1	36.5	88.3	111.7
SOMxLINDA	7472	7772	72	218.0	21.8*	119.9	167.5	40.1	103.8	111.8
FP 2142	7535	7835	135	224.4	72.0	148.2	128.1	23.1*	75.6*	111.9
Escalina	7439	7739	39	159.1*	63.8	111.4	170.0	56.9	113.4	112.4
810/5//Flanders	7475	7775	75	241.0	33.8	137.4	139.0	36.1	87.6	112.5
(VERNExACLIN)-1xCH10-1	7539	7839	139	234.8	53.8	144.3	114.8*	46.9	80.8	112.5
SOMxLINDA	7590	7890	190	225.4	97.0	161.2	91.4*	36.4	63.9*	112.5
Laura	7421	7721	21	174.3	20.3*	97.3*	192.5	64.4	128.4	112.8
Ariane	7426	7726	26	205.0	42.3	123.6	159.8	46.5	103.1	113.4

Cultivar	Plot # early	Plot # late	Accession	2006 AUDPC scores			2007 AUDPC scores			Overall combined[d]
				leaf[a]	stem[b]	combined[c]	leaf	stem	combined	
AG2-BLK	7466	7766	66	189.0	35.1	112.1	147.9	84.9	116.4	114.2
Astelle	7418	7718	18	189.0	65.8	127.4	166.0	37.4	101.7	114.5
K-6	7543	7843	143	221.1	75.0	148.1	122.8	40.9	81.8	114.9
Natasja sel	7433	7733	33	197.1	63.9	130.5	160.6	40.9	100.8	115.6
M7407	7455	7755	55	194.3	51.8	123.0	180.4	38.4	109.4	116.2
99L01	7415	7715	15	195.4	60.5	127.9	165.1	44.6	104.9	116.4
Natasja sel	7434	7734	34	195.9	59.5	127.7	156.5	58.6	107.6	117.6
AC Carnduff/Double Low	7495	7795	95	232.8	41.8	137.3	164.5	32.5	98.5	117.9
VERNE-1xNT16-1	7504	7804	104	227.5	50.3	138.9	166.0	30.9	98.4	118.7
Ariane	7438	7738	38	161.5*	108.8	135.1	171.0	38.1	104.6	119.8
(VERNExACLIN)-1xCH10-1	7549	7849	149	219.8	82.0	150.9	134.4	46.4	90.4	120.6
CRYSTAL x ISTRU LINE 36-	7545	7845	145	229.4	87.3	158.3	126.8	41.0	83.9	121.1
Webster	7412	7712	12	202.1	41.8	121.9	184.5	56.8	120.6	121.3
(VERNExACLIN)-1xCH10-1	7538	7838	138	236.3	82.0	159.1	126.3	42.8	84.5	121.8
Webster	7530	7830	130	222.0	63.6	142.8	147.1	58.0	102.6	122.7
N0010	7537	7837	137	241.5	76.8	159.1	137.8	35.4	86.6	122.8
AG2-BLK	7465	7765	65	201.3	22.6*	111.9	183.9	84.8	134.3	123.1
Evelin	7419	7719	19	211.3	82.5	146.9	165.3	34.8	100.0	123.4
SOMxLINDA	7467	7767	67	218.9	65.5	142.2	140.5	68.9	104.7	123.4
ZARIJA-87	7556	7856	156	229.4	64.8	147.1	139.8	61.5	100.6	123.8
AC Carnduff/Double Low	7494	7794	94	243.0	67.3	155.1	150.9	35.0	92.9	124.0
(VERNExACLIN)-1xCH10-1	7503	7803	103	238.1	68.8	153.4	145.3	44.5	94.9	124.2
M6850	7459	7759	59	241.8	67.0	154.4	156.0	33.8	94.9	124.6
LINDAxATAL	7582	7882	182	219.8	80.4	150.1	127.6	71.3	99.4	124.8
BILTON	7527	7827	127	227.5	88.4	157.9	142.8	41.0	91.9	124.9
N0010	7536	7836	136	256.9	87.0	171.9	125.0	31.4	78.2	125.1
(VERNExACLIN)-1xCH10-1	7502	7802	102	248.5	77.0	162.8	139.9	35.5	87.7	125.2
FP 2142	7534	7834	134	237.5	85.1	161.3	149.4	30.9	90.1	125.7
CRYSTAL x ISTRU LINE 36-	7544	7844	144	238.6	118.8	178.7	122.0	26.6	74.3*	126.5
M6850	7460	7760	60	220.4	97.3	158.8	145.0	43.8	94.4	126.6
Double Low/SP 2047	7488	7788	88	253.6	41.9	147.8	182.8	29.6	106.2	127.0
Ariane	7425	7725	25	203.0	42.0	122.5	204.6**	58.4	131.5	127.0
(VERNExACLIN)-1xCH10-1	7548	7848	148	243.0	91.8	167.4	135.4	39.0	87.2	127.3

Cultivar	Plot # early	Plot # late	Accession	2006 AUDPC scores			2007 AUDPC scores			Overall combined[d]
				leaf[a]	stem[b]	combined[c]	leaf	stem	combined	
Linola/Flanders	7473	7773	73	229.3	77.0	153.1	150.8	53.9	102.3	127.7
Linola/Flanders	7474	7774	74	238.0	91.9	164.9	140.6	44.1	92.4	128.7
ACLIN-1-1xCH10-1	7515	7815	115	236.1	91.9	164.0	143.9	44.5	94.2	129.1
AC McDuff/Double Low	7497	7797	97	248.0	60.5	154.3	142.9	65.8	104.3	129.3
MCDFxLINDA	7579	7879	179	228.9	95.5	162.2	141.9	53.9	97.9	130.0
VERNE-1xNT16-1	7540	7840	140	247.5	79.6	163.6	145.4	52.3	98.8	131.2
Double Low/99HN8141	7489	7789	89	264.0	58.8	161.4	174.6	27.5	101.1	131.2
Adoptiv	7551	7851	151	239.6	88.8	164.2	147.1	49.4	98.3	131.2
Linola/Flanders	7492	7792	92	270.3	107.3	188.8	119.4	28.3	73.8*	131.3
Linola 947	7405	7705	5	222.0	70.5	146.3	182.8	50.9	116.8	131.5
MCDFxLINDA	7580	7880	180	248.4	88.8	168.6	140.4	48.6	94.5	131.5
M6869	7444	7744	44	240.5	70.5	155.5	175.3	40.9	108.1	131.8
Ilona	7565	7865	165	208.5	115.1	161.8	125.8	78.0	101.9	131.8
FP 2070/Double Low	7483	7783	83	245.5	62.1	153.8	183.9	36.9	110.4	132.1
NORMxATAL	7575	7875	175	254.4	98.5	176.4	140.4	37.5	88.9	132.7
NORMxLINDA	7574	7874	174	254.5	75.0	164.8	148.8	53.9	101.3	133.0
SP 2047/AC Emerson	7485	7785	85	242.9	77.0	159.9	173.3	39.9	106.6	133.3
AG2-BLK	7586	7886	186	259.8	92.3	176.0	116.6	64.9	90.8	133.4
ACLIN-3-1xCH17-3-1	7462	7762	62	241.4	84.0	162.7	150.9	59.0	104.9	133.8
Hermes	7424	7724	24	212.0	115.8	163.9	166.9	45.3	106.1	135.0
N0003	7532	7832	132	251.9	103.3	177.6	132.4	52.8	92.6	135.1
VERNE-1xNT16-1	7541	7841	141	247.5	89.5	168.5	160.3	43.1	101.7	135.1
ACLIN-3-1xCH17-3-1	7584	7884	184	252.8	100.3	176.5	122.8	64.6	93.7	135.1
AG2-BLK	7585	7885	185	271.8	100.5	186.1	119.3	49.1	84.2	135.2
Webster	7446	7746	46	221.8	51.8	136.8	174.5	93.8	134.1	135.4
Hermes	7423	7723	23	213.3	107.5	160.4	163.6	59.0	111.3	135.8
Astelle	7449	7749	49	259.6	115.5	187.6	133.5	35.6	84.6	136.1
ACLIN-1-1xCH10-1	7514	7814	114	239.1	85.0	162.1	151.5	71.0	111.3	136.7
Natasja(Can)	7430	7730	30	188.1	30.5*	109.3*	218.6**	110.6**	164.6**	137.0
NorMan	7596	7896	196	247.3	120.3	183.8	132.6	48.0	90.3	137.0
NORMxATAL	7576	7876	176	257.9	100.3	179.1	148.1	42.3	95.2	137.1
ACLIN-3-1xCH17-3-1	7461	7761	61	251.6	89.0	170.3	155.1	54.8	104.9	137.6
N0003	7533	7833	133	261.0	107.0	184.0	140.6	42.4	91.5	137.8

Cultivar	Plot # early	Plot # late	Accession	2006 AUDPC scores			2007 AUDPC scores			Overall combined[d]
				leaf[a]	stem[b]	combined[c]	leaf	stem	combined	
NORMxLINDA	7573	7873	173	247.5	97.0	172.3	141.5	65.3	103.4	137.8
LINDAxATAL	7608	7908	208	206.5	107.0	156.8	179.8	59.9	119.8	138.3
AC McDuff/Double Low	7496	7796	96	245.0	75.6	160.3	172.9	62.1	117.5	138.9
Webster	7445	7745	45	239.8	68.8	154.3	185.3	70.4	127.8	141.0
Evelin	7420	7720	20	196.0	111.0	153.5	195.3	62.9	129.1	141.3
Linola/Flanders	7493	7793	93	264.8	125.5	195.1	136.5	38.6	87.6	141.3
VERNE-1xNT16-1	7505	7805	105	205.9	61.8	133.8	228.4**	74.5	151.4**	142.6
ZARIJA-87	7555	7855	155	224.0	120.3	172.1	163.6	63.8	113.7	142.9
Webster	7411	7711	11	225.4	72.1	148.8	186.0	88.3	137.1	142.9
Rio	7557	7857	157	154.3*	89.0	121.6	207.3**	122.5**	164.9**	143.3
M6869	7443	7743	43	264.1	74.0	169.1	182.5	53.6	118.1	143.6
Rio	7558	7858	158	185.1	107.0	146.1	194.5	90.4	142.4**	144.3
Astelle	7450	7750	50	235.3	127.0	181.1	149.3	66.3	107.8	144.4
LINDAxATAL	7607	7907	207	203.0	102.1	152.6	188.4	87.3	137.8	145.2
FP 1082	7599	7899	199	224.0	121.5	172.8	140.5	98.9**	119.7	146.2
BILTON	7526	7826	126	255.0	92.0	173.5	169.8	74.4	122.1	147.8
Webster	7531	7831	131	274.4	73.4	173.9	155.8	90.9	123.3	148.6
SP 2090	7601	7901	201	246.8	153.5**	200.1	139.8	55.6	97.7	148.9
LINDAxATAL	7581	7881	181	252.4	105.4	178.9	164.9	73.3	119.1	149.0
SP 2090	7602	7902	202	244.9	142.3**	193.6	148.5	60.9	104.7	149.1
Elektra	7568	7868	168	228.0	121.6	174.8	156.0	92.3	124.1	149.5
Linola 947	7406	7706	6	232.5	90.5	161.5	201.1**	74.8	137.9	149.7
AC McDuff	7593	7893	193	244.3	130.5	187.4	157.3	72.4	114.8	151.1
ACLIN-3-1xVERNE-1	7528	7828	128	244.1	131.5	187.8	164.3	64.6	114.4	151.1
Arriane	7559	7859	159	196.0	135.3	165.6	174.9	99.4**	137.1	151.4
CEB0211	7524	7824	124	292.4**	108.5	200.4	155.9	49.9	102.9	151.7
K-6	7510	7810	110	255.4	84.0	169.7	184.0	83.8	133.9	151.8
Webster	7546	7846	146	278.3**	109.0	193.6	159.3	62.4	110.8	152.2
ACLIN-3-1xCH10-1	7501	7801	101	283.9**	149.0**	216.4**	142.6	36.9	89.8	153.1
FP 897	7598	7898	198	235.0	152.3**	193.6	143.9	82.1	113.0	153.3
FP 1082	7600	7900	200	255.7	150.3**	203.0**	124.3	85.4	104.8	153.9
Linola 2047	7409	7709	9	216.9	125.8	171.3	204.1**	77.4	140.8**	156.0
FP935-1x(VERNExACLIN)-1	7517	7817	117	268.5	135.3	201.9**	153.8	67.3	110.5	156.2

Cultivar	Plot # early	Plot # late	Accession	2006 AUDPC scores			2007 AUDPC scores			Overall combined[d]
				leaf[a]	stem[b]	combined[c]	leaf	stem	combined	
Linola 2047	7410	7710	10	220.0	131.0	175.5	205.8**	70.4	138.1	156.8
Adoptiv	7552	7852	152	255.5	105.8	180.6	184.1	81.9	133.0	156.8
ACLIN-3-1xCH10-1	7500	7800	100	278.3**	153.1**	215.7**	145.3	51.0	98.1	156.9
Webster	7547	7847	147	259.4	122.0	190.7	157.6	89.5	123.6	157.1
CRYSTAL x ISTRU LINE 36-	7512	7812	112	254.0	114.0	184.0	182.1	83.8	132.9	158.5
N2004	7522	7822	122	275.9**	133.5	204.7**	159.0	68.3	113.6	159.2
CEB0211	7525	7825	125	290.5**	110.3	200.4	178.8	58.4	118.6	159.5
(ACLINxFP935-8)-1xNT16-1	7499	7799	99	271.5	137.0	204.3**	165.0	65.0	115.0	159.6
K-6	7511	7811	111	253.4	118.8	186.1	187.0	79.4	133.2	159.6
NorMan	7595	7895	195	272.0	155.3**	213.6**	140.3	72.6	106.4	160.0
Diva	7569	7869	169	240.3	170.5**	205.4**	144.3	85.3	114.8	160.1
FP 897	7597	7897	197	238.1	150.0**	194.1	168.6	84.1	126.4	160.2
SP 2047/AC Emerson	7486	7786	86	264.3	143.8**	204.0**	151.4	81.8	116.6	160.3
Linola 1084	7408	7708	8	228.6	142.3**	185.4	197.9	73.8	135.8	160.6
FP935-1x(VERNExACLIN)-1	7516	7816	116	262.0	155.8**	208.9**	165.5	66.1	115.8	162.3
AC Hanley	7404	7704	4	241.6	112.3	176.9	213.0**	90.8	151.9**	164.4
AC McDuff	7594	7894	194	257.1	147.0**	202.1**	178.9	79.9	129.4	165.7
N2004	7523	7823	123	282.4**	130.0	206.2**	179.1	76.0	127.6	166.9
(ACLINxFP935-8)-1xNT16-1	7498	7798	98	272.5	150.3**	211.4**	170.5	74.4	122.4	166.9
Linola 1084	7407	7707	7	250.3	125.8	188.0	201.6**	92.9	147.3**	167.6
MINERVA	7507	7807	107	246.1	130.3	188.2	200.1**	100.4**	150.3**	169.2
Atalante	7591	7891	191	287.8**	149.3**	218.5**	165.8	76.5	121.1	169.8
MINERVA	7506	7806	106	240.3	125.5	182.9	211.3**	102.4**	156.8**	169.8
Atalante	7592	7892	192	284.6**	145.9**	215.3**	173.8	76.3	125.0	170.1
Norland	7432	7732	32	255.5	144.1**	199.8	209.0**	72.5	140.8**	170.3
Norland	7431	7731	31	260.1	144.0**	202.1**	200.6**	83.1	141.9**	172.0
ACLIN-3-1xVERNE-1	7529	7829	129	263.1	146.8**	204.9**	188.5	91.4	139.9**	172.4
AC Hanley	7403	7703	3	241.4	120.5	180.9	227.8**	102.0**	164.9**	172.9
N2005	7520	7820	120	274.4	179.0**	226.7**	169.5	71.5	120.5	173.6
FP935-1xNT16-1	7519	7819	119	260.5	143.5**	202.0**	197.3	94.8**	146.0**	174.0
AC Carnduff	7401	7701	1	204.6	127.3	165.9	237.3**	127.4**	182.3**	174.1
CREE	7508	7808	108	261.8	145.8**	203.8**	197.9	95.9**	146.9**	175.3
FP935-1xNT16-1	7518	7818	118	263.5	145.3**	204.4**	200.0**	93.6	146.8**	175.6

Cultivar	Plot # early	Plot # late	Accession	2006 AUDPC scores			2007 AUDPC scores			Overall combined[d]
				leaf[a]	stem[b]	combined[c]	leaf	stem	combined	
N2005	7521	7821	121	276.0**	180.8**	228.4**	179.8	68.1	123.9	176.2
NORMxLINDA	7604	7904	204	256.3	182.5**	219.4**	174.1	96.0**	135.1	177.2
CREE	7509	7809	109	264.4	204.5**	234.4**	181.0	78.8	129.9	182.2
AC Carnduff	7402	7702	2	226.8	127.5	177.1	236.9**	141.1**	189.0**	183.1
NORMxLINDA	7603	7903	203	268.1	184.3**	226.2**	176.3	107.3**	141.8**	184.0
MCDFxLINDA	7553	7853	153	263.8	167.0**	215.4**	171.8	139.1**	155.4**	185.4
EMERSxLINDA	7606	7906	206	249.4	160.3**	204.8**	216.4**	120.4**	168.4**	186.6
MCDFxLINDA	7554	7854	154	267.0	168.5**	217.8**	183.6	129.6**	156.6**	187.2
EMERSxLINDA	7605	7905	205	259.3	175.8**	217.5**	214.1**	116.6**	165.4**	191.4
CRYSTAL x ISTRU LINE 36-	7513	7813	113	264.8	208.0**	236.4**	194.3	120.3**	157.3**	196.8
K-6	7542	7842	142	275.3**	196.0**	235.6**	199.4**	138.0**	168.7**	202.2
AC Emerson/Double Low	7477	7777	77	208.5	17.0*	112.8	-	-	-	-
FP 2070/Double Low	7484	7784	84	286.5**	155.8**	221.1**	-	-	-	-
SP 2047/M6155	7490	7790	90	257.3	108.5	182.9	-	-	-	-
SP 2047/M6155	7491	7791	91	244.1	110.3	177.2	-	-	-	-
Mean				224.1	86.2	155.1	157.1	58.7	1.7.9	131.3

[a] Area under disease progress curve values for leaves based on seven assessments
[b] Area under disease progress curve values for stems based on five assessments
[c] Mean area under disease progress curve of leaf and stem values for the select season
[d] The overall mean area under disease progress curve value for the accession based on two years of observations on the leaves and stems. Accessions are ranked in table based on this overall value
[e] Mean growth stage based on four assessments following the 1-12 growth stage scale (Turner, 1987) lower stage values equal later maturing varieties
* accession area under disease progress curve value is significantly lower than the population mean for the particular variable and year
** accession area under disease progress curve value is significantly higher than the population mean for the particular variable and year
- value is missing due to low germination, plant death or the variable was not assessed for the particular accession.

Table 2. Pearson correlation coefficients for seven variables observed in 2006 and four variables in 2007 field seasons.

2006	Leaf AUDPC[a]	Stem AUDPC[b]	Combined AUDPC[c]	Stage[d]	PM[e]	Lodge[f]
Leaf AUDPC	1.000					
Stem AUDPC	0.679 **	1.000				
Combined AUDPC	0.901 **	0.931 **	1.000			
Stage	0.553 **	0.623 **	0.645 **	1.000		
PM	-0.482 **	-0.207 **	-0.362 **	-0.205 **	1.000	
Lodging	0.164 *	0.316 **	0.269 **	0.126 *	0.081 ns	1.000

2007	Leaf AUDPC	Stem AUDPC	Combined AUDPC
Leaf AUDPC	1.000		
Stem AUDPC	0.780 **	1.000	
Combined AUDPC	0.957 ns	0.928 ns	1.000

[a] Area under disease progress curve values for seven leaf assessments; [b] and five stem assessments; [c] Mean area under disease progress curve values for leaf and stem assessments; [d]Mean growth stage based on four assessments following the 1-12 growth stage scale (Turner, 1987) ; [e] Powdery mildew (*Oidium lini*) observed as a low-medium-high (1-3) scale for severity; [f] lodging observed as a low-medium-high (1-3) scale for severity; * Correlation is significant at ($P < 0.05$); ** Correlation is significant ($P < .0001$); [ns] not significant correlation

4.0.4 Discussion

The results of this experiment were similar to several past studies, which found noticeable differences in flax accessions susceptibility levels to pasmo, but no high levels of resistance in either the field (Sackston, 1947; Sackston, 1951; Christenson, 1952a; Michaelson, 1956; Pederson and Michaelson, 1960; Rashid, 2003; Diederichsen et al., 2007) or in the greenhouse (Brentzel, 1926; Sackston, 1949). The 208 accessions assessed in this study originated from single plants selected for low susceptibility and subsequently revealed differences with respect to pasmo severity on both the leaves and stems. However, no high level of resistance was identified for any accession. The results of this study suggested that some of the accessions observed were partially resistant to *S. linicola,* while some appeared highly susceptible and yet yielded relatively well. This suggests that some plants may be more tolerant to the pathogen. However, with only two years of testing, the authenticity of the partial resistance found for some accessions may be questioned due to variable abiotic factors such as weather conditions.

The germination issues encountered in 2007 could partially explain the 2006 field seasons significantly ($P \leq 0.05$) higher AUDPC scores and also be responsible for some of the relatively small yields and missing values in the 2007 accessions. The results from the early and late seeding dates were combined for correlations and for presentation in Table 1. In the current study, accessions would ideally maintain low AUDPC scores under varying conditions including different seeding dates. In addition to genetic differences between accessions, there are many other reasons why the differences in AUDPC values shown in Table 1 occurred. Aust and Hoyningen-Huene (1986) stated that an epidemic is a result of complex interactions between the host, the pathogen, the

environment and man. Therefore, one would expect disease severity to be affected by factors such as the misting systems coverage of the plot area, where some of the single row plots may have escaped disease pressure and yielded an un-representative disease rating. This is called apparent resistance or escape and may be responsible for some cultivars appearing resistant even though they genetically are not resistant (Agrios, 2005). One example of potential escape is Natashja(Can) (30) (Table 1) which was ranked significantly ($P \leq 0.05$) better than the population mean in 2006 but significantly worse ($P \leq 0.05$) in 2007 and therefore may have only escaped disease pressure in 2006. Over a growing season the weather is unfavourable for disease development for some periods of time and if some plots did not receive sufficient misting in these periods, they may have developed symptoms slower or possibly not at all.

Leaf AUDPC values were significantly different ($P \leq 0.05$) in 2006 between the two replications although the plots were seeded on the same date. The combined AUDPC values were not significantly different, therefore, it is surprising that the leaf values were, because all factors were the same and the two blocks were seeded adjacent to each other. Although not likely a large factor, this difference could be attributed to human error or one repetition block may have been located in a slightly more suitable place. The fact that differences appeared between repetitions proves that the environment and microclimate has an effect on the disease severity even at the small plot level. Some accessions had very different AUDPC scores for leaves and stems, which brought the combined AUDPC value down and could have caused the cultivars to appear relatively susceptible overall. An example of such a cultivar is Natashja (Can) (29) (Table 1) which had very low stem AUDPC values but only moderate leaf AUDPC values, resulting in a

rank that was not as high as some others with low scores on both tissues. If a cultivar had low AUDPC values for one tissue (leaves or stems) then it might have benefited from partial resistance for that one type of tissue even if the other tissue was heavily infected. Leaf and stem scores were not always consistent and it is likely that different physiological characteristics of these separate plant parts created different levels of susceptibility (Michaelson, 1956). Perhaps the reactions on the leaves and stems of individual plants were controlled by independent resistance genes in certain cultivars. One characteristic that may be responsible for resistance observed on the stems of some accessions is the "stay-green phenomenon". Although no true resistance was found on the leaves of any accession, there were some stems that appeared to be near immune to *S. linicola*. Accessions CDC Bethune/Double low (79) and M7406 (53) had almost no visible symptoms on the stems (Table 1). The stems of these and other accessions were green for an extended period of time and appeared to remain green even when the plots were harvested. These cultivars may have been very late maturing varieties, however, the "stay-green phenomenon" has been attributed to disease resistance (Cukadar-Olmedo and Miller, 1997; Miklas et al., 2007) and could have potential for resistance to stem diseases of flax.

It was observed through the growing season that disease developed on the lower leaves first and moved upward. Eventually, symptoms developed on the stems. Lesions developing first on the leaves, then stems was documented by Perryman and Fitt (2000), and it was thought that the lesions on the stems tended to develop at the point of leaf attachment after diseased leaves fall off (Sackston, 1949). This delay in development of symptoms on the stems versus the leaves is why the AUDPC scores on the leaves and

stems were not of similar values. The observation of the 208 accessions was carried out two more times on the leaves than on the stems, and the evaluation periods were overlapping, subsequently affecting the AUDPC values. Although there are cases where accessions had relatively high AUDPC values on leaves and low on stems or vice versa, it would still seem logical that the amount of disease present on the leaves early in the season would have a strong effect on the disease development on the stems later on. There was a significant correlation between leaf and stem AUDPC values to support this assumption (Table 2).

The location of the pasmo nursery in 2006 and 2007 differed and, therefore, could be a factor in pasmo severity observed in each year. Although the misting system was expected to keep the leaf wetness similar throughout the nursery, disease development could have been affected by soil pH and structure, temperature, soil nutrient status (Colhoun, 1973), crop rotation (Brentzel, 1926; Flor, 1943; Sackston, 1949; Rashid, 2003), proximity to field edges and other plots or "edge effects". In 2006, the nursery was located in a hochfeld fine sandy loam soil and many plant leaves were damaged at a young age due to wind erosion from the sandy soil blowing in dry windy conditions. Any abiotic stress such as wind damage could potentially induce "resistance" pathways and elicit enzymes responsible for cell wall modification or secondary metabolism as it does in *Arabidopsis* when it is wounded (Cheong et al., 2002).

It is known that pasmo is not often noticed on young flax but becomes more prevalent near maturity of the flax plant (Brentzel, 1926; Flor, 1943; Sackston, 1949). Variability in maturation dates may have played a role in observed disease severity as implied by other researchers in the past (Michaelson, 1956; Pederson and Michaelson,

1960). This leads to the assumption that differences in AUDPC values in the current study may have been a result of differences in maturation dates inherent to cultivars originating from different geographical regions. The maturation dates were noted to differ among the 208 accessions, and there was a strong positive correlation between growth stage and the AUDPC variables (Table 2). Higher growth stage equates to earlier maturity and, therefore, the correlation suggests that the earlier maturing varieties were most diseased and maturity may play an important role in pasmo severity.

Due to the seeding date discrepancy, it was found that seeding date was significant in 2007 only ($P \leq 0.05$), which would be expected due to the two replications different seeding dates in 2007. This result is consistent with the suggestions in several reports that seeding date has an effect on pasmo severity (Sackston, 1951; Turey and Snowden, 1998; Diederichsen et al., 2007). There are, however, conflicting reports on how seeding date effects pasmo severity. The later seeding date in 2007 had significantly less disease based on AUDPC values which agreed with the suggestion of Turey and Snowden, (1998) that later seeding has lower infection.

Poor germination of some accessions in 2007 (Figure 1C) is another reason the field seasons were analysed separately. Some plots had only a few plants which does not favour crop canopy and subsequently provides a poor microclimate for disease development. A favourable microclimate is important because epidemics are influenced by the special microclimate inside the crop canopy (Aust and Hoyningen-Huene, 1986). The poor germination likely occurred because the seed originated from a single plant and was, therefore, limited requiring the potentially diseased seed harvested from the 2006 pasmo nursery to be used in the 2007 nursery. Furthermore, the 2007 plots were seeded

into an eigenhoff soil, which is characteristically fine textured, versus a more coarse textured soil in the 2006 location. A finer textured or clay soil creates difficulty for germination when compared to more coarse textured sandy soils (Benvenuti, 2003).

In 2006, powdery mildew (*O. lini*) was prevalent on many accessions, covering the surface of the host leaves with superficial whitish colonies of mycelium and has been reported to reduce yield of susceptible cultivars (Rashid, 2003). The presence of this pathogen may have impeded the ability of the pasmo pycnidiospores to reach the leaf surface and penetrate, or, if it arrived on the host first, may out-compete *S. linicola* and deny access to nutrients from the plant. The incidence of powdery mildew was negatively correlated with AUDPC values in 2006 (Table 2), suggesting that *O. lini* infected accessions had reduced pasmo severity. Lodging was to some extent positively correlated with AUDPC values for pasmo (Table 2) which would be expected because low lying or lodged areas are partially attributed to greater pasmo severity (Brentzel, 1926; Flor, 1943; Sackston, 1949; Rashid, 2003).

Besides the many abiotic and non-host biotic factors discussed that may explain the observed differences in disease, each individual accession's genetics providing partial resistance will also play a factor. Although only partial resistance was observed in this study, if a high level of resistance to pasmo was ever found, the possibility exists that single genes could perhaps be identified and introgressed or inserted into plants directly, reducing the time-consuming process of breeding for resistance by selection (Rommens and Kishore, 2000).

In the future, researchers could look at AUDPC values for pasmo assessment and determine at which growth stage measurements correlate best with the total AUDPC

value, similar to the work of Jeger and Viljanen-Rollinson (2001), reducing the amount of assessments required while maintaining similar accuracy. Selection for future breeding programs and in depth genetic investigations of accessions should take into account the contradictory scores observed on leaves and stems. It is possible that the different tissues may have different disease responses and this phenomenon may be especially important for future flax cultivars that are destined for alternate or scrupulous markets, such as fibre markets. For oil crops, which are the focus of Manitoba and Saskatchewan production, there is no evidence that one tissue is more important than the other, however, the most important portion of the plant is perhaps the pedicels and bolls. In the future, it could be beneficial to assess accessions for the amount of disease present on the upper plant parts although this quantity is likely a function of the amount of disease present on the leaves and stem. Conceivably, disease levels on the leaves or stem at certain growth stages could be correlated with the amount observed on the bolls and subsequently the effect of pasmo related boll drop or any disease aided yield loss. Accessions strong for particular components of partial resistance, such as leaf or stem resistance, may be crossed in the future to develop varieties which have increased levels of partial resistance (Ohm and Shaner, 1976). The five accessions, CDC Bethune/Double Low (79), Agros (164), Ilona (166), Diva (170) and SOMxLINDA (178) had the lowest overall AUDPC values and may be useful in flax breeding programs.

4.1 Cytological investigation of the infection process of *Septoria linicola*

4.1.0 Abstract

Septoria linicola (Speg) Garassini. (teleomorph: *Mycosphaerella linorum* Naumov), the causal fungus of pasmo of flax causes round circular lesions on the leaves and is characterized by brown lesions encircling the stem. The pathogen can be detrimental to flax yields and seed quality in Canada and elsewhere. To date, no high level of resistance was reported in any commercial cultivar and only partial resistance has been identified as a reduction in symptoms. There is no documented knowledge of what causes the arrested development of the disease in certain cultivars. In this study, three flax cultivars were inoculated with a single-spore culture from an isolate collected in Saskatchewan and designated 06-F-97. Two commercial cultivars AC Emerson and AC McDuff, and one partially resistant accession (accession # 178), were chosen based on their respective decreased susceptibility. An aniline blue clearing and staining fluorescence technique was used to study the infection and colonization processes and the host response to the pathogen at a histological level. Germination, stomatal penetration, colonization and pycnidial formation were similar for all three cultivars. However, minor differences were apparent. Penetration events were observed beginning at 24 hours post-inoculation, with notable absence of an appressorium. Intercellular hyphae could be noticed in the mesophyll 3 days post-inoculation, with host cell collapse and necrosis beginning by 4 days post-inoculation. Pycnidial initials were evident in lesions by 6 days post-inoculation and at this point, necrosis and chlorosis were visible macroscopically on leaves. Fully mature pycnidia were evident by 8 days post-inoculation in all cultivars. There was no evidence of intracellular hyphal growth or haustorium formation. Some

mesophyll cells around penetration sites fluoresced brightly upon pathogen penetration of the mesophyll intercellular space. This phenomenon was occasionally noticed on control leaves also and may have been more of a general response. The host also deposited an unidentified substance at stomatal openings and surrounding cells, which may be lignin or papillae formation elicited by the pathogen. This phenomenon was observed more frequently on accession 178.

4.1.1 Introduction

The pathogen, *Septoria linicola* (Speg) Garassini. (teleomorph: *Mycosphaerella linorum* Naumov), the causal agent of pasmo infects flax crops in many regions around the world (Rashid, 2003). It was first discovered in Argentina in 1909 (Spegazzini, 1911) and was found in Manitoba in 1940 (Sackston, 1949). The disease can affect all above-ground plant parts resulting in significant defoliation and eventually considerable boll drop and stem breakage (Flor, 1943; Rashid, 2003), causing decreased yield and seed quality.

There are many recommended control measures (Rashid, 2003). Although genetic resistance would be ideal, there are no resistant commercial cultivars available. Discovering and understanding the genetic basis of available resistance, and incorporating traits into cultivars suitable for prairie growing conditions is an important goal in disease management practices. Several researchers have previously searched for resistance based on visual observations of host symptoms (Sackston, 1947; Sackston, 1951; Christenson, 1952a; Michaelson, 1956; Pederson and Michaelson, 1960; Diederichsen et al., 2007). All past studies, including the one in Chapter 4.0 of this thesis, have found a range of susceptibility to the pathogen. Therefore, some genetic differences among cultivars must promote some level of resistance reactions. Effective quantitative resistance probably involves more than one character controlled by a number of different genes (Pryor, 1987). There are likely several genetic factors involved in the partial resistance observed over and above whatever differences occur due to abiotic factors such as plot microclimate. It is known that plants may have many defence responses at the cellular level including hypersensitive responses, reactive oxygen

species, cell wall fortification, pathogenesis related proteins and phytoalexins (Hammond-Kosack and Jones, 1996) acting independently or together to provide resistance.

Very few studies have investigated this host-pathogen system using microscopy (Sackston, 1949; Covey, 1962). Related pathogens such as *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), the causal agent of septoria blotch of wheat, have been studied more extensively in the past and the infection process and timeline are well documented (Cohen and Eyal, 1993; Duncan and Howard, 2000). For *S. linicola*, Sackston (1949) found that the pathogen only penetrated stomates and there did not appear to be any stimulus to penetration as germ tubes would frequently pass over, or by, stomates without penetrating. Covey (1962) investigated further, finding that stomates were again the main penetration site and that mycelium was observed around mesophyll cells but there were no intracellular hyphae or haustoria noticed. No previous work investigated the infection process thoroughly from start to finish and therefore a documentation of the entire infection process would be of benefit. The primary objective of this study was to observe the *S. linicola* – flax relationship at a microscopic level using fluorescence microscopy, examining the host response and fungal colonization. This was carried out on three cultivars that have shown differential responses macroscopically in field plots, developing a timeline for the infection process while potentially identifying differences at the cellular level between the three different flax varieties.

4.1.2 Materials and Methods

Isolates of *Septoria linicola*

Fungal Isolates of *Septoria linicola* were collected in the 2006 and 2007 field seasons from various flax growing locations in Manitoba and Saskatchewan. Single-spore isolates were derived from cultures obtained from infected flax tissue collected near maturity from randomly selected fields. The plant samples were dried and kept in cool storage at 4°C until needed. Infected stems containing pycnidia were cut into 2-4 cm pieces and placed in a Petri dish with a moist filter paper to hydrate until pycnidiospores could be seen oozing from the pycnidia. If an abundance of saphrophytic fungi appeared on the stem surface, the tissue was surface sterilized by soaking in 3% sodium hypochloride (bleach solution) for 30 seconds prior to rehydration. At this point, a single cirrhus of conidia was transferred to yeast malt agar, containing 0.25% chloramphenicol, to prevent bacterial contamination. The plates were kept under light on a laboratory bench at room temperature for two to three weeks until pycnidiospores were visible. These pure cultures were harvested and either used for inoculation, transferred to another yeast malt agar plate, or stored for future use. To achieve long term storage of cultures, the pycnidiospores were harvested, suspended in sterilized deionized water, combined (1:4 v/v) with glycerol and frozen at -70 °C (Appendix 2). Inoculation of host material was conducted using cultures from isolate 06-F-97 that were stored at -70 °C

Host Material and Inoculation

The experiment was set up in a split plot design with three randomized complete blocks, consisting of three cultivars grown in four replicates and a control. All flax pots,

except the control, were treated with the same isolate (06-F-97). The three flax cultivars selected for this experiment were considered to have variable susceptibility to the *S. linicola* pathogen (K.Y. Rashid, unpublished). AC McDuff and AC Emerson were, chosen as moderately resistant and highly susceptible commercial cultivars, respectively, and accession 178 (SOMxLINDA) was chosen because of low apparent susceptibility in field nurseries, observed as low area under disease progress curve (AUDPC) values on the leaves and stem for two growing seasons (Table 1, Chapter 4.0). The cultivars were grown under growth chamber conditions at the University of Manitoba and subjected to 22 °C / 18 °C (day / night) temperature, approximately 65% relative humidity and a 16 hour photoperiod. The four replications, and control, were grown in 15.25-cm-diameter clay pots in a soil: sand: peat (2:1:1 v/v) mixture amended with 16-20-0-14 (N-P-K-S) fertilizer. Plant stand was thinned to ten seedlings per pot at the cotyledon stage to correspond with the number of required sampling times. Plants were inoculated at 18-20 days after planting.

Isolate 06-F-97 was grown on yeast malt agar (Appendix 3) under constant light at room temperature and spores were harvested from 12-14 day old cultures. Spores were suspended in sterilized, distilled and deionized water. The concentration was measured using a hemocytometer and adjusted to 1.0×10^6 spores per ml by addition of sterile distilled water. One drop of Polyoxyethylene (20) sorbitan monolaurate (Tween 20) per 100 ml of spore suspension was added to act as a surfactant. Control samples were inoculated with sterile distilled water and one drop of Polyoxyethylene (20) sorbitan monolaurate (Tween 20) per 100 ml of spore suspension. The plants were sprayed until run-off using a Delvibis sprayer with air pressure of approximately 67 kPa. Inoculated

plants were kept in a misting chamber for 48 hours and continuous leaf wetness was provided by two commercial ultrasonic (familycare HM480) humidifiers. After 48 hours the plants were returned to the growth room bench at 22°C / 18°C, (day / night) temperature and 16 hour photoperiod.

Sampling and Staining Procedure

The cytological study was conducted using a KOH-aniline blue fluorescence technique described by Hood and Shew (1996). The 6-7 lowermost leaves, not including the cotyledons, were excised from one plant of each replication at 6, 12, 24, 36 hours post-inoculation and at 2, 3, 4, 6 and 8 days post-inoculation. Control samples were also taken at 6 and 24 hours post-inoculation and at 2, 4 and 8 days post-inoculation. The leaves were placed in tissue capsules, submerged in 1M potassium hydroxide (KOH) for 24 hours and subsequently autoclaved for 15 minutes at 121°C. Samples remained viable for a period of at least 3 weeks at room temperature. For viewing, the leaves were triple rinsed in sterile deionized distilled water, removed from tissue capsules, and mounted in a staining solution consisting of 0.01% aniline blue (C.I. 42755 BDH Chemicals ltd.) in 67 mM dipotassium hydrogen phosphate (K_2HPO_4).

Whole leaf mounts were examined with a Vanguard microscope (model 1486 FL, Kirkland, WA, USA) equipped with custom 425 nm excitation and 460 nm emission filter set. This technique provided ample distinction between host and fungal tissue. Quantitative data was collected for percent germination, spore length, germ tube length, percent penetration, percent colonization and the number of lesions and pycnidia. Observations were based on three pots for each cultivar and five leaves per pot for each

sampling time with the exception of germination percent and germ tube length in which leaves from three pots were used. Digital photographs were taken with a Nikon Coolpix 8800 Digital Camera (Nikon Corporation, Japan). Photo scales were achieved by comparison with a micrometer slide ruled to 0.01 mm and calibrated with imagine analysis software, ASSESS version 2.0 (Lamari, 2008, APS press, St. Paul, MN, USA).

Spore germination percentage was assessed at 6, 12, 24, and 36 hours post-inoculation from approximately 100 spores observed per leaf and 20 leaves per cultivar. Germ tube and spore length were measured on 100 spores per cultivar using the "digital ruler" feature of ASSESS 2.0 (Lamari, 2008). The penetration percentage was calculated at 6, 12, 24, 36, 48, 72 and 96 hours post-inoculation and is a measure of the number of penetration events observed for every 100 spores counted on the leaf surface. Fifteen leaves were observed from each cultivar. The sub-stomatal hyphae, observed extending into the mesophyll tissue, were quantified as a percentage of penetration sites that had hyphal development below the leaf surface originating from the original penetration site. This was assessed at 48, 72 and 96 hours post-inoculation on 15 leaves per cultivar. The number of lesions observed per leaf was quantified at 6 and 8 days post-inoculation, and the number of pycnidia per leaf, were counted at 8 days post-inoculation on 15 leaves per cultivar.

Phase Contrast Microscopy

The KOH-aniline blue fluorescence technique was effective at staining the fungal tissue within the host tissue. In order to confirm the results and rule out the presence of fluorescent artifacts or other forms of error, the aniline blue/lactophenol clearing/staining

technique described by Bruzzese and Hasen (1983) was used. Clearing and staining was achieved by submerging whole leaf samples into the clearing/staining solution consisting of 95% ethanol (300 ml), chloroform (150 ml), lactophenol (660 ml) (Note: Lactophenol was used in place of lactic acid and phenol), chloral hydrate (450 g) and aniline blue (0.6 g) (C.I. #42755 BDH Chemicals ltd.) for a minimum of 96 hours. Leaves were stored in lactophenol until mounted in 0.01% aniline blue in lactophenol and observed with a Vanguard microscope (model 1486 FL). The staining procedure did not always adequately provide discrimination between fungal and plant tissues in the mesophyll. However, the clearing effect was achieved and leaves were observed with phase contrast objectives which provided enough distinction between the fungus and the host to confirm the results obtained with fluorescence.

Analysis

The variables in which quantitative data were taken were analysed with the "mixed" procedure for analysis of variance from SAS: Statistical Analysis Software, version 9.1.3 (The SAS Institute inc., Cary, NC, USA). Sampling time was considered as a repeated measure in the analyses. All data sets with the exception of lesion and pycnidia counts were transformed with arcsine square-root (ARSIN(sqrt)) function to achieve normality prior to analyses. Differences in cultivars and sampling times were determined based on least square means and were deemed significant at a level of $P \leq 0.05$.

4.1.3 Results

Germination and Host Penetration

Overall there were no major differences between the three cultivars observed with respect to germination, penetration and colonization or the time frame for disease development; however, some minor differences were observed.

Spore length was measured on the surface of the three host cultivars, had a mean of 20.36 μm ranging from 6.24 μm to 38.0 μm with a standard deviation of 4.39 μm.

On the host surface, germination began at or before 6 hours post-inoculation at a low percentage and continued until at least 36 hours post-inoculation (Fig. 2B and 3A). The sampling times were found to be a significant factor ($P \leq 0.05$) with respect to percent germination. However, no significant differences were found between any of the cultivars. Germ tubes were observed elongating across the surface of the leaf from 6 hours post-inoculation until at least 36 hours post-inoculation at which point spore germination was near 100% (Fig. 3A). The germ tube length increased significantly over time but did not significantly differ ($P \leq 0.05$) between cultivars for any of the three sampling times measured (Fig. 2A). The longest germ tube recorded was 144.04 μm, however, there may have been longer germ tubes at later sampling times, which were not recorded. In rare cases, germ tubes were observed to be following guard cell boundaries on the surface of the leaf (Fig. 4A) but in most circumstances appeared to grow randomly with no specific direction or affinity for any cells. In some instances the pathogen appeared to grow directly towards some penetration sites, elongating away or directly in between in other cases, or growing directly over some stomatal openings without penetrating (Fig. 3A). Overall the pathogen showed no consistent attraction to the

penetration sites. Hyphal anastomosis was observed many times on all cultivars. The germ tubes were observed to fuse to other germ tubes or to conidia on the leaf surface (Fig. 3A).

Penetration occurred via stomatal openings (Fig. 3B and 3C) beginning at approximately 24 hours post-inoculation, at which time rare penetration events could be observed. In one case, the pathogen was seen to penetrate at the meeting of epidermal and guard cells. However, this was only noticed once and it was not obvious that penetration had occurred. In almost all cases, only one germ tube was observed penetrating a stomate. However, it is not unlikely that more than one germ tube could penetrate a single opening. Penetration percent was not equal for each cultivar at every sampling time (Fig. 2C). Cultivars were not significantly different ($P \leq 0.05$) at any sampling time. However, the sampling times were significant ($P \leq 0.05$) with the exception of 6-12 hours post-inoculation and 72-96 hours post-inoculation.

Appressoria were not observed at any point in the experiment. In many cases there was fluorescence at the site of penetration, suggesting some plant reaction to the pathogen. In some rare cases prior to penetration or afterwards, the stomates appeared to be sealed with a bright red-orange fluorescing substance (Fig. 4A and 4B). Any fungal tissue on, or near the stomatal opening also appeared to develop the same bright red-orange fluorescence (Fig. 4B). This phenomenon was noticed most often on accession 178 but was also observed on cultivars AC McDuff and AC Emerson.

Penetration continues for several days after the initial observations becoming more frequent with time (Fig. 2C), and can be observed on leaves even after earlier

penetrations have advanced and hyphal development was occurring below the surface in other areas.

Host Colonization

In most cases, after the pathogen had entered and colonized the sub-stomatal chamber, intercellular hyphae could be seen branching between the parenchyma cells of the healthy mesophyll tissue by 72 hours post-inoculation (Fig. 3D and 4C). The amount of penetration sites with hyphal branching observed below the stomate was quantified at 48, 72 and 96 hours post-inoculation and there was no significant ($P \leq 0.05$) effect of cultivar, sampling time or any interaction between them. Although not significant due to high variability, the occurrence of sub-stomatal hyphae appears to remain lower for accession 178 until 72 hours post-inoculation (Fig. 2D).

In some cases, the parenchyma cells immediately below the stomata, where the pathogen penetrated, would fluoresce brightly, but did not noticeably impede the pathogen development (Fig. 4C). This was also noticed on leaf areas without any spores and on the control leaves (Fig 4D). After the pathogen penetrates and begins to develop in the mesophyll, the hyphal network continues to expand away from the initial infection site with apparently no preferred direction. The hyphae were much larger than the initial germ tubes and grew in an intercellular fashion between the mesophyll cells (Fig. 5A). Although the hyphae do not visibly penetrate any cells, they often completely surround the mesophyll cells creating a "honeycomb" appearance (Fig. 6A and 6B). Intercellular hyphae were also examined using phase contrast microscopy with no evidence of appressorium formation, cell penetration or haustorium formation as the hyphae were

Figure 2. Germ tube length and germination, penetration, and colonization percentages for cultivars AC McDuff, AC Emerson, and accession 178. A) Germ tube length measured in micrometers (μm) and formatted with exponential trend lines. B) Germination percent on host leaf surface based on approximately 100 spores per leaf and formatted with quadratic trend lines. C) Penetration percent formatted with sigmoidal trend lines. D) Percent of penetration sites where hyphal colonization could be observed sub-surface formatted with linear trend lines. Legend on bottom of figure.

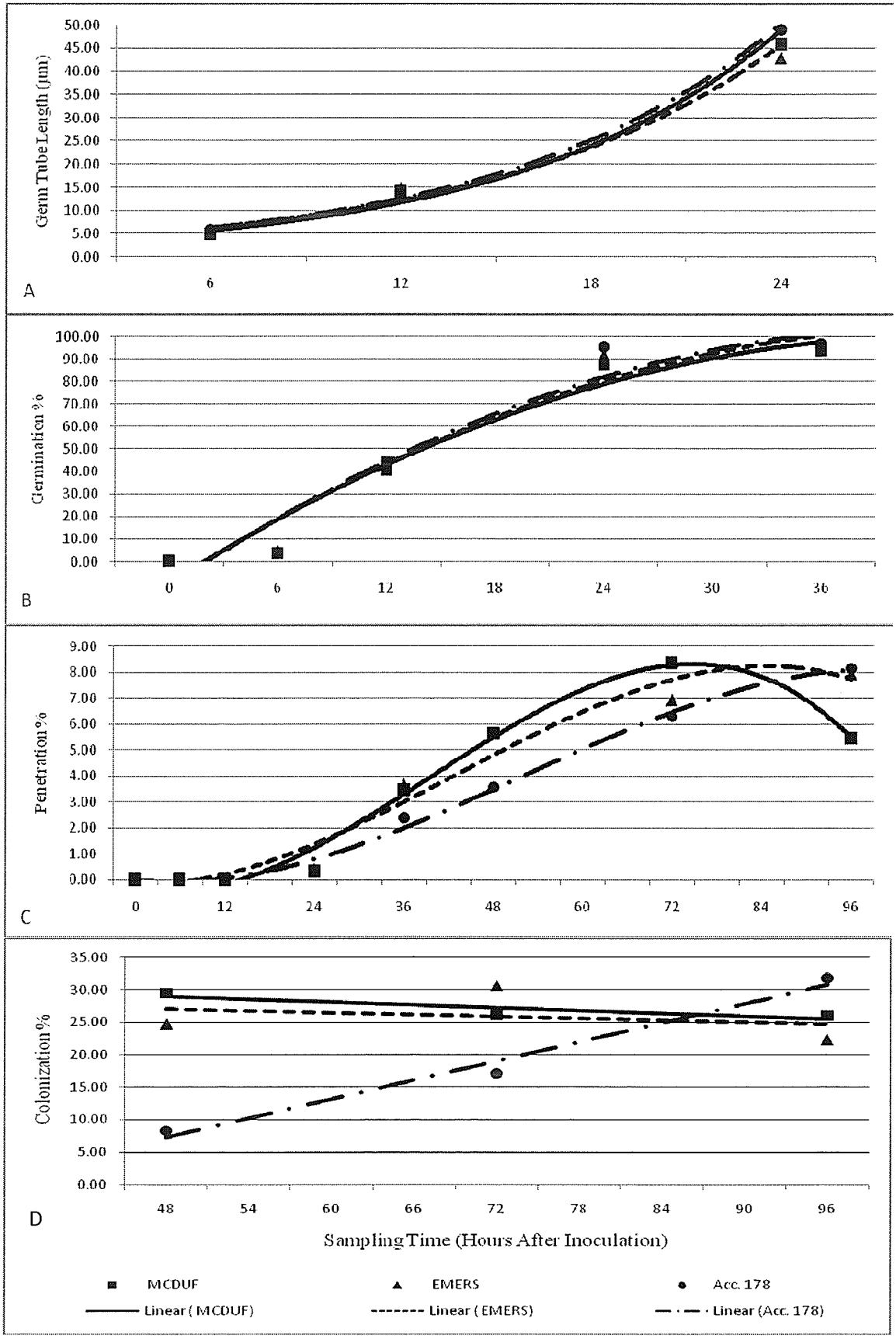

Germ Tube Length (µm)
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
6
12
18
24
A
Germination %
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
6
12
18
24
30
36
B
Penetration %
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
12
24
36
48
60
72
84
96
C
Colonization %
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
48
54
60
66
72
78
84
90
96
D
Sampling Time (Hours After Inoculation)
MCDUF
EMERS
Acc. 178
Linear (MCDUF)
Linear (EMERS)
Linear (Acc. 178)

Figure 3. Spore germination, surface phenomenon and host penetration processes of *S. linicola*. A) Pycnidiospores germinated on the surface of accession 178 at 24 hours post-inoculation. Germ tubes pass directly by stomates without penetrating, germ tube fusion is evident (arrow) B) Two, germinated, three septate pycnidiospores. One germ tube is about to penetrate a stomate on the surface of accession 178 at 24 hours post-inoculation. C) Stomatal penetration visible from the surface of cultivar AC McDuff 72 hours post-inoculation (arrow). Intercellular hyphae is faintly visible below the surface in the mesophyll tissue. D) View below the leaf epidermis of leaf in figure 3C, intercellular hyphae is developing underneath the penetration site and the pycnidiospores are faintly visible on the surface.

Abbreviations: GT = germ tube, GTF = germ tube fusion IH= intercellular hyphae, MC = mesophyll cell, P = penetration site, PS = pycnidiospore, ST = stomate,

Bar = 50μm

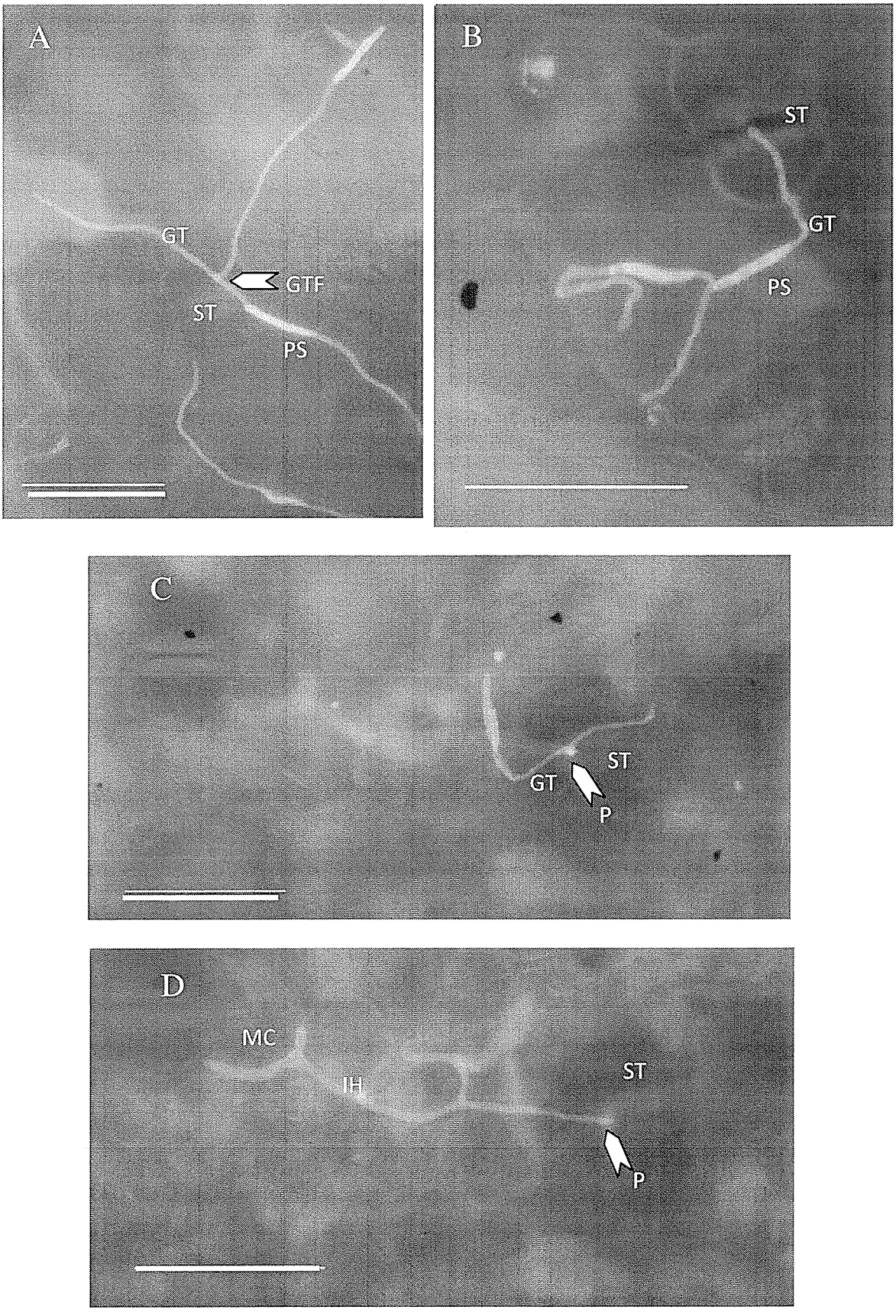

A
GT
GTF
ST
PS
B
ST
GT
PS
C
ST
GT
P
D
MC
IH
ST
P

Figure 4. Resistance related phenomenon from the host, and surface actions of the pathogen. A) Fungal germ tube appears to be following the boundary between a guard cell and epidermal cell while the stomate is exhibiting red-orange fluorescence (arrow) on cultivar AC McDuff at 48 hours post-inoculation. B) Stomate is expressing deposition of red fluorescing substance and fungal tissue is also observed to fluoresce red-orange (arrow) instead of the normal green color on cultivar AC Emerson at 36 hours post-inoculation. C) Below the leaf surface of cultivar AC McDuff at 48 hours post-inoculation, mesophyll cells fluoresce brightly around the penetration area. Intercellular hyphae is visible and growing in the same area as the affected mesophyll cells. D) Similar bright green fluorescence (arrow) as seen in figure 4C was observed on control leaves at 4 days post-inoculation.
Abbreviations: EC = epidermal cell, GC = guard cell, GF = green fluorescence GT = germ tube, IH= intercellular hyphae, MC = mesophyll cell, PS = pycnidiospore, ROF = red-orange fluorescence, ST = stomate,

Bar = 50μm

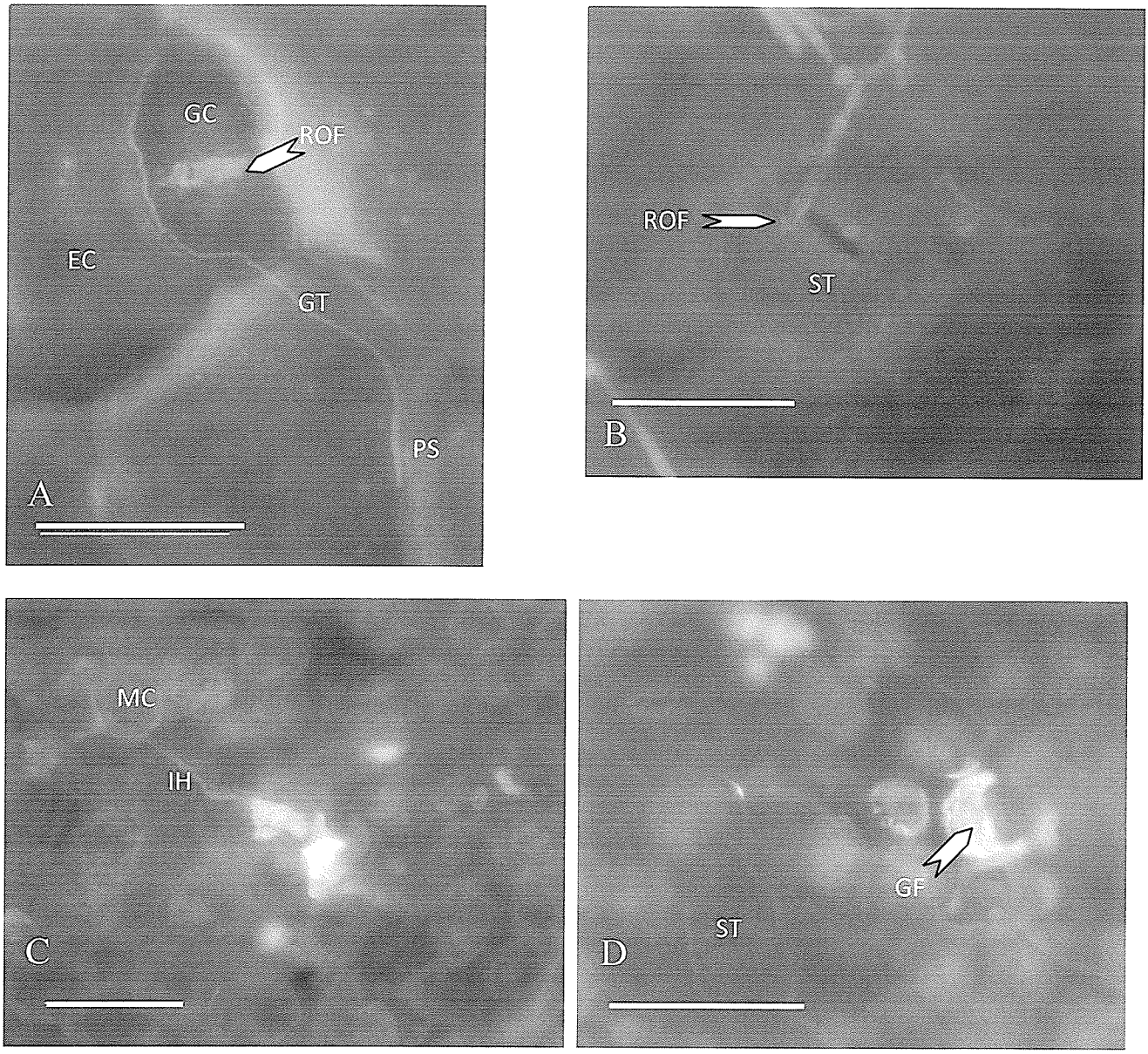

GC
ROF
EC
GT
PS
A
ROF
ST
B
MC
IH
C
GF
ST
D

Figure 5. Intercellular hyphae, and mycelium development at vascular tissue. A) Intercellular hyphae in the leaf tissue of cultivar AC McDuff at 6 days post-inoculation (Bar = 50μm). B) Intercellular hyphae viewed with phase contrast microscopy on cultivar AC Emerson at 6 days post-inoculation (Bar = 50μm). C) Mycelium within the host tissue of accession 178 at 8 days post inoculation. The mycelium is observed meeting the vascular tissue and growth is halted in that direction. Two pycnidial initials can also be observed (arrow) (Bar = 100μm).

Abbreviations: IH= intercellular hyphae, MC = mesophyll cell, PI = pycnidial initials, VT = vascular tissue

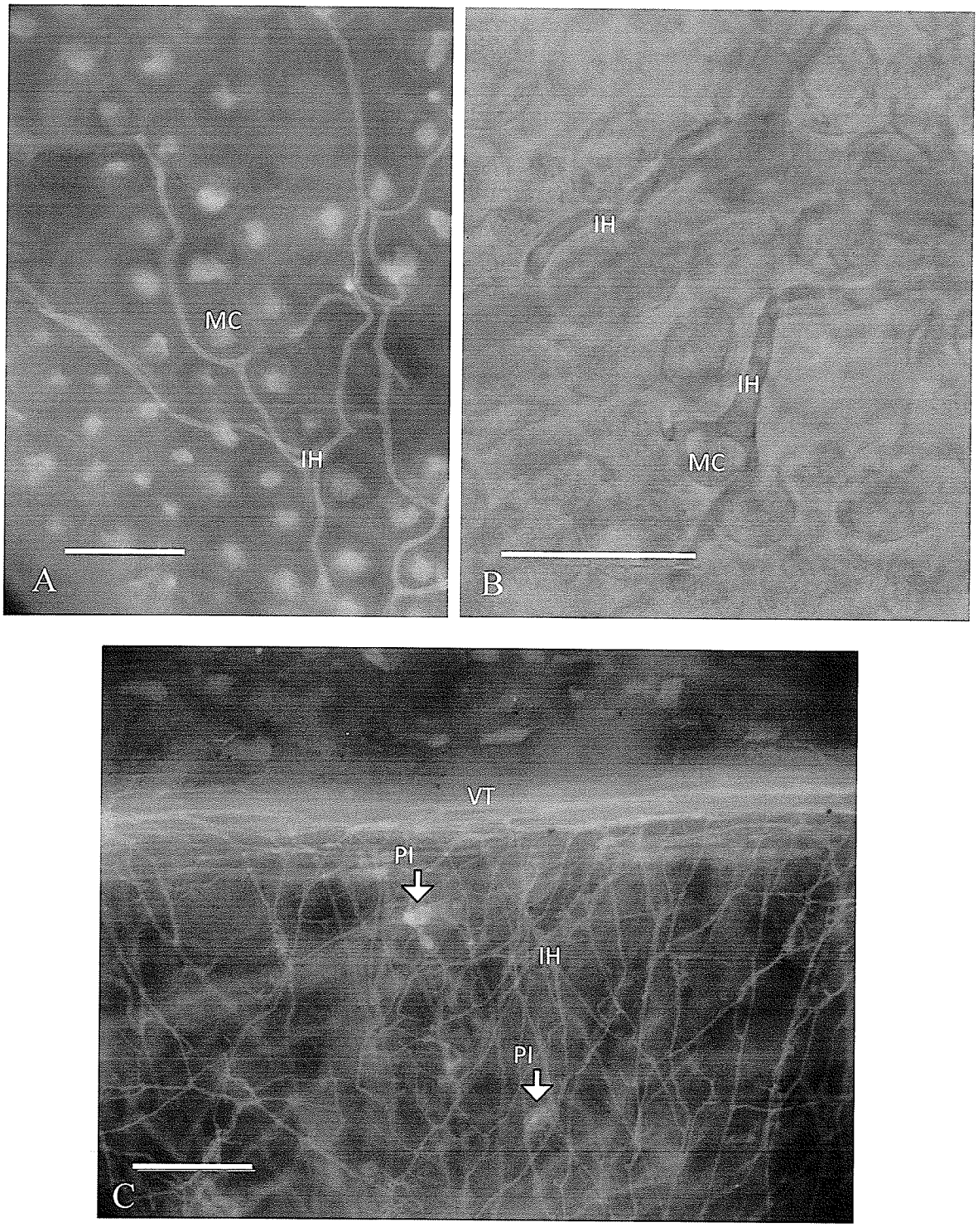
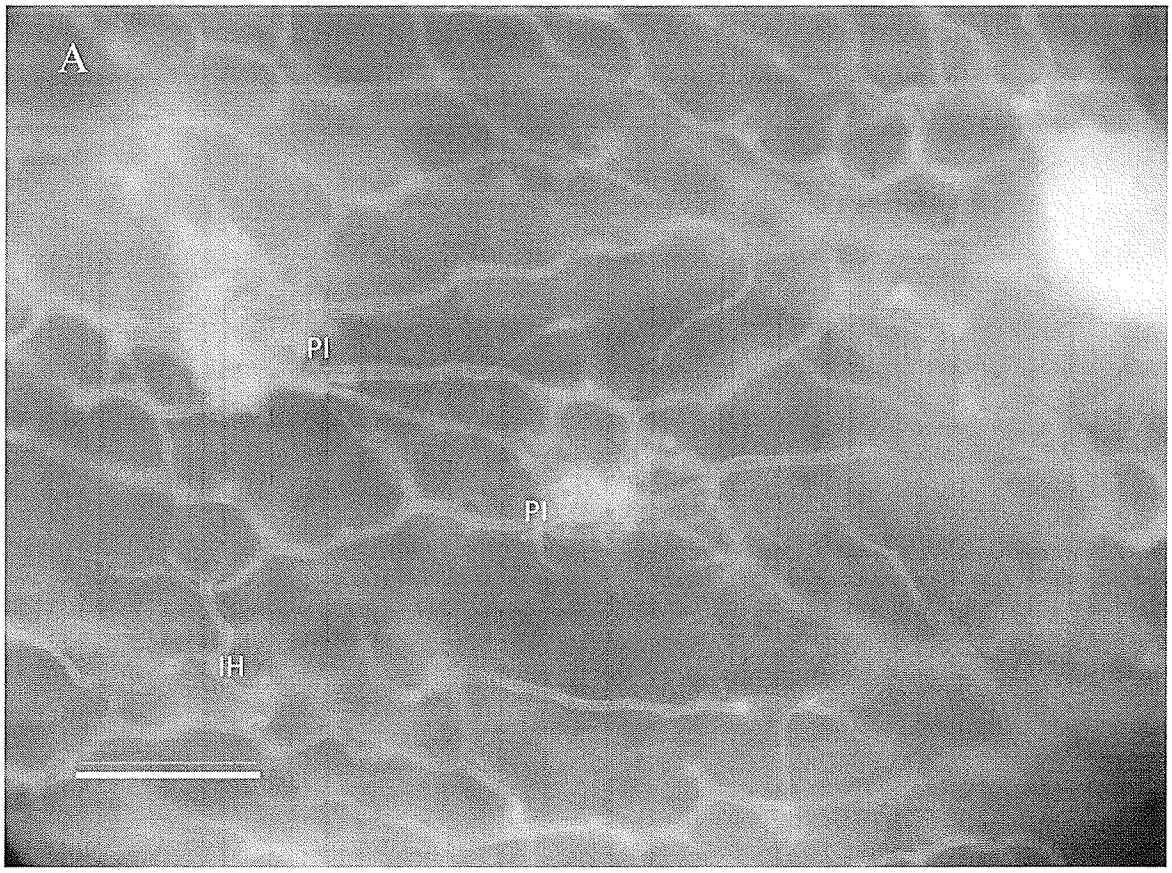
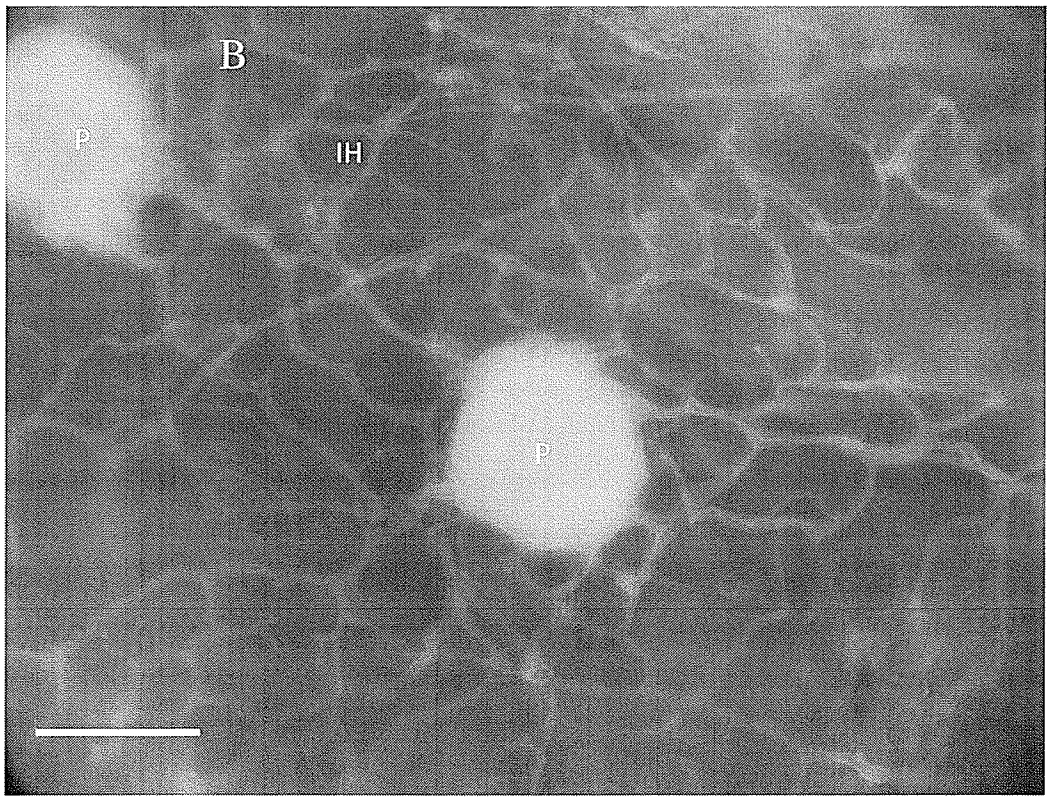

MC
IH
A
IH
IH
MC
B
VT
PI
IH
PI
C

Figure 6. Pycnidial development of *S. linicola* in host leaves. A) Pycnidial development with pycnidial initials shown at three different development stages on cultivar AC Emerson at 8 days post-inocualtion. (Bar = 50 μm) B) Mature pycnidia surrounded by mycelium on cultivar AC McDuff at 8 days post-inoculation. Most host cells do not fluoresce and are necrotic at this point. (Bar = 50 μm)

Abbreviations: IH= intercellular hyphae, MC = mesophyll cell, PI = pycnidial initial, P = pycnidium.


A
PI
PI
IH


B
P
IH
P

observed to be growing and expanding into the intercellular space (Fig. 5B). At 4-6 days post-inoculation, lesions began to form, and necrotic lesions would be seen. macroscopically in the greenhouse by 5-6 days post-inoculation. Round circular lesions develop on the leaves due to radial hyphal growth away from the initial penetration site. In some cases it appeared that the hyphae became stalled at large vascular bundles of the leaf, where normally a circular expanding lesion, became flattened at the vascular tissue and did not expand as rapidly (Fig. 5C). Furthermore, it was not evident that the pathogen entered the vascular system of the leaf at any point. The vascular tissue appeared to be the last tissue to be broken down by the pathogen. This phenomenon is most evident at the mid-vein of the leaf.

The number of lesions developing on each leaf was counted at 144 and 192 hours post-inoculation (Appendix 4). It was found that time was a significant ($P \leq 0.05$) factor on lesion number, however, the cultivar and interaction effects were not.

Pycnidial Formation and Sporulation

By 6 days post-inoculation, visible lesions were evident on the leaf both macro- (as necrotic brown tissue), and microscopically (as non autofluorescing cells). Pycnidial initials were forming at this time and observed as visible masses of mycelium congregating together (Fig. 5C and 6A). The masses of mycelium fluoresced quite brightly and it was difficult to distinguish their location relative to leaf cells. When this stage was observed with phase contrast microscopy, it was discovered that, although difficult to distinguish, the pycnidium form either directly beneath the stomatal opening or slightly off to one side but always appeared to be within the substomatal chamber.

The pycnidia were observed to form earliest in the center of the lesion near the presumed initial penetration site and their development moved outward similar to the expansion of the intercellular hyphae in the lesion. By 8 days post-inoculation, leaves were beginning to senesce and some were nearly 100% infected with no healthy green tissue remaining. The mycelium in the tissue was extensive and many pycnidia were nearly or entirely mature. Leaves at this stage, were placed in high humidity, and the pycnidia oozed a pycnidiospore mass in the form of a cirrhus producing secondary inoculum. The pycnidia are circular in appearance. However, some are slightly oblong in shape (Fig. 6B).

4.1.4 Discussion

In order to develop effective resistance to a pathogen, a clear understanding of the host-pathogen interactions must be achieved. A step towards this understanding includes a description of how the pathogen penetrates and develops within the host tissue. Previous histological studies of the flax – rust pathosystem revealed quantative resistance that differed from the partial resistance to the barley leaf rust fungus (*Puccinia hordei*) (Kowalska and Niks, 1999).

Of the three cultivars selected, accession 178 (plot 7878) was selected because of signs of moderate resistance in field studies. AC McDuff and AC Emerson were commercial cultivars.

The spore length should not be influenced by sampling time or any particular cultivar although some spores have been noted to adhere more strongly at different times to dissimilar surfaces via extracellular matrix materials (Braun and Howard, 1994). Because the leaves in which the spores were counted were autoclaved, which creates a relatively harsh environment, some of the spores which did not adhere to the leaf surface may have been removed. However, it is possible that a larger sample size would have revealed more similar spore sizes. If a more detailed microscopy procedure was utilized, such as laser scanning or electron microscopy, it might be possible to determine if the conidium produces an extracellular matrix for adhesion to the leaf surface as is observed in some other host- pathogen systems.

Overall, the mean spore length of 20.36 μm coincides with previous researcher's measurements (Sackston, 1949) and the spores were visibly septate, which is in

agreement with Brentzel (1926) and further disproves the suggestion of non-septate spores (Spegazzini, 1911).

Spore germination was similar on all cultivars, and reached nearly 100% by 36 hours post inoculation. The germ tubes emerged relatively quickly and followed a similar growth pattern as noted by Sackston (1949). Overall, the fungus showed no consistent or obvious attraction to the main penetration sites. Some germ tubes seemed to exhibit thigmotropic responses and appeared to follow the boundaries of guard cells but this response was rare in occurrence and could not be considered a consistent behaviour. It did not appear that the length of germ tube had any influence on the ability to penetrate as some spores penetrated almost as soon as the germ tube emerged while others appeared to pass by several stomates before entering the sub-stomatal chamber or failed to penetrate all together.

The germ tube length was not different between cultivars but as anticipated, it increased drastically within the first 24 hours post-inoculation. The fact that germ tube length did not differ between the three cultivars indicates that there are no differences on the surface of the leaves that would cause reduced or increased fungal growth. Using ASSESS 2.0 (Lamari, 2008) was an efficient method of determining length even on randomly curved germ tubes. This method may, therefore, be efficient in testing the effect of fungicides on pasmo at a more in depth level than visual symptoms. A similar method of germ tube measurement using ASBA, from Wild and Leitz Ltd., Switzerland has been used in the past to examine the effect of various fungicide concentrations (Hilber and Schuepp, 1992). Germ tube fusions were observed on the surface and were

expected to occur as they have been observed previously in a similar fashion (Sackston, 1949)

The penetration process clearly required high leaf wetness as preliminary studies found that minimal symptoms developed when a 24 hour leaf wetness period was used versus a 48 hour period. The differences noticed with respect to penetration are outlined in figure 2C. This shows that there were differences in the amount of successful penetrations, and that the time of penetration may be a point of plant defence. There was only one observation of the pathogen penetrating at any other venue besides stomates. It may be possible that the pathogen can penetrate at alternative sites, however, it is apparently extremely rare and the one observation was not conclusive. Appressorium formation was not observed and therefore not mandatory for penetration. Appressora are not required for other similar pathogens such as septoria leaf blotch (*Mycosphaerella graminicola*) of wheat (Cohen and Eyal, 1993; Kema et al. 1996; Grieger, 2001).

The bright red-orange fluorescence of stomates that was observed on many occasions appears to be a plant defence response. The fact that the normally blue green fluorescing fungal germ tubes and intercellular hyphae were observed fluorescing red-orange when they came into contact with the affected area suggests that the pathogen may elicit the production of compounds from the plant which perhaps affects the fungal tissue. Mauch-mani and Slusarenko (1996) have noted this phenomenon where lignification takes a more active defence role and lignifies not only the host cell but the fungal tissue as well.

The fact that some of these fluorescing stomates have been observed essentially unchanged later in disease development while all tissue around appear necrotic, suggests

that this substance must be strong in preventing fungal induced break down of the cell. Cohen and Eyal (1993) reported that callose depositions and lignification play a limited role in resistance to *S. tritici*. It is possible that this unidentified substance is a lignified papillae forming in response to elicitation from the pathogen at the penetration site. Papilla formation on plant cell walls has been linked to partially resistant varieties of barley to leaf rust (Kowalska and Niks, 1999), and is thought to be an important defence mechanism.

Although the red fluorescing deposition was observed on all cultivars it was much more frequent on accession 178 and, therefore, may be considered partially responsible for the differences in penetration and hyphal development and the lower susceptibility observed in field conditions. It is possible that some of the autofluorescing areas observed in this study could be correlated to some compounds produced under pathogen elicitation.

Host colonization should increase with time because one would expect that more penetrations would result in increased hyphal density. All mycelium in the mesophyll was intercellular in nature and no penetration of the host cells was observed which agrees with a previous study by Covey (1962). The work of Covey (1962) also discussed the possibility of the fungus releasing a pathogenesis related toxin, which would be responsible for degrading host cells in order to acquire nutrients. This can be explained, in part, by the fact that the host cells appeared to become necrotic slightly before the mycelium actually engulfed them and filtrates of mycelium cause blackening of healthy leaves, suggesting that the pathogen may produce a toxin. However, it is possible that something in the pathogen simply elicits a kind of a programmed cell death or partial

hypersentitive response in the plant which caused the cells to collapse prior to hyphal advance and that the response was not quick enough to fully arrest pathogen development. The mycelium clearly has a method of extracting nutrients from, and destroying the mesophyll cells without direct penetration, because necrotic plant tissue could be observed macroscopically by 4-6 days post-inoculation.

Host cells below and around the stomata were sometimes observed to autofluoresce bright green, which may be indicative of a resistance response involving some form of secondary metabolites, reactive oxygen species, or pathogenesis related proteins. Duncan and Howard, (2000) observed autofluorescence of host cell walls of wheat infected with *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), but described no apparent elicitation from fungal contact. The observed autofluorescence of flax cells may be a response to wounding of the plant when leaves were removed for the staining procedure. Certain compounds produced in response to a wound or environmental stress may be the same as those elicited due to pathogen related stress. The affected cells were found not only near the penetration sites, but also in other locations on the same leaf and on control leaves. There were also many instances where there was no autofluorescence and penetration appeared to be entirely uncontested. Therefore, similar to *S. tritici,* there appears to be no consistent correlation between the fluorescing host cells and fungal elicitation.

As the hyphal network was expanding below the leaf surface, the pathogen did not appear to penetrate the vascular system. It is therefore confined to surface lesions on leaves and forced to move to other plant parts through rain splash or other vectors instead of systemically making its way through the leaves and stem of the plant. This may also

explain why lesions seem to develop on edges of the leaf more frequently as there is less major vascular tissue. Furthermore, there are more small wounds observed on the edges which may provide easier entry for the pathogen and in many cases, in greenhouse inoculated plants, the water droplets would pool at the leaf edge or tip and lead to a very large concentration of spores in that area.

The expanding mycelium was observed to either change direction and grow parallel to the vascular tissue or even stall growth compared to seemingly uninhibited growth through the mesophyll tissue. The work of Heller and Gierth (2001) found that the degradation process of different tissues of sunflower by *Phomopsis helianthi* (Munt.-Cvet) was not uniform and suggested that differences in degradation for certain tissues were the result of differences in enzyme activities of the fungus or differences in cell wall composition of different host tissues. It is possible that for the flax-pasmo pathosystem the differences in host tissue between vascular bundles and mesophyll tissue are the reason for which the vascular system does not appear to be penetrated by the pathogen when observed with our microscopy technique. Covey (1962) also noted that the epidermis and vascular tissues were the only tissues remaining intact when lesions were present. In the current study, when all other host tissues ceased to fluorescence, the vascular tissue remains fluorescing red-orange in colour which may suggest that the same material that resists pathogen penetration of stomata, exists in vascular tissue as well. A potential example of such a material is lignin which is known to abound in the xylem.

It was observed that lesion number decreased over time (appendix 4). This was likely due to lesions coalescing resulting in two or more lesions appearing to be one large lesion.

The pycnidial initials begin forming between 4 and 6 days post-inoculation because in rare cases hyphae could be observed beginning to congregate at 4 days post-inoculation. This is similar to previous work of Sackston (1949) who noticed pycnidial primordia at 80 hours and definite pycnidia forming at 108 hours post-inoculation. The timeline for pycnidial formation and sporulation suggests that the pathogen has a relatively short cycle to produce secondary inoculum and explains why *S. linicola* can cause detrimental epidemics if infections occur early in the season and favourable conditions persist. Other studies of Septoria pathogens on wheat have noted pycnidial initials forming at 9 days post-inoculation and pycnidia visible at 11 days post-inoculation. The pycnidia of *S. linicola* were noted to form under the stomates in the sub stomatal chamber when observed with phase contrast microscopy. This observation is consistant with reports on *Septoria tritici* blotch of wheat where pycnidial formation was restricted to sub stomatal spaces and the ostiole was located beneath the guard cells. Pycnidia formation of *S. tritici* on wheat was also found to occur later than *S. linicola* in flax at approximately 12 days post-inoculation (Cohen and Eyal, 1993). The one day discrepancy on pycnidial formation between Cohen and Eyal (1993) and Duncan and Howard (2000) shows that with slightly different experimental conditions the results can change slightly and certain processes in fungal development may be either promoted or delayed

It must be noted that the present study of the *S. linicola* – flax relationship was conducted under near ideal conditions for pathogen development and it is probable that the conditions may not be realized under field conditions every growing season.

In this study, penetration was observed only through stomata, which may be a reflection of this host-pathogen relationship in general but also may be a function of the particular isolate used and subsequently other isolates may yield slightly different results with respect to penetration and the entire infection process. It is also very likely that the particular growth room conditions influenced the pathogen development and that in variable conditions such as field environments some differences could be expected.

All three cultivars observed were very similar in that there was no evidence of appressorium or haustorium formation. Furthermore, all penetration events were via stomates and there was no cell penetration observed at any point. Although there were no major differences in the pathogen development in terms of time frame for penetration, lesion development, and pycnidial production, any small differences could lead to a reduction in the secondary inoculum required for field scale epidemics and therefore a more pasmo resistant cultivar.

4.2 Assessment of partial resistance of flax to pasmo using image analysis

4.2.0 Abstract

Testing accessions under more controlled conditions than field plots is important for breeding programs. The accurate determination of disease severity is also an important factor. Four accessions selected for their reaction to pasmo in previous field experiments, along with two commercial cultivars, were inoculated with four exponentially increasing spore concentrations and a water control under growth room conditions. The incubation period was recorded and, after symptoms developed, all leaves were visually scored for disease, removed from each plant and scanned. The percent leaf area infected was measured using ASSESS 2.0, image analysis software for plant disease quantification. The results of this study showed that the spore concentration had the largest effect on incubation period, percent leaf area infected and host receptivity. The accessions did show significant ($P \leq 0.5$) differences from each other although they differed in disease severity and/or host receptivity at various spore concentrations. Accessions 30, 172 and 178 appeared to best correlate to field ratings. ASSESS 2.0 was effective in accurately quantifying the disease whereas visual assessment differed by up to +50% and -20% leaf infection in rare cases. Overall the differences were approximately 5-8% above the computer analysis.

4.2.1 Introduction

Historically, scientists relied on visual assessment to quantify disease and components of disease. This method is laborious, time-consuming, imprecise and inaccurate. Furthermore, it is subject to the assessor's bias and subjectivity. (Diaz-Lago, et al. 2003). Image analysis has become an important tool for plant pathologists since the early 1990's (Holmes et al., 2000) where it was used for transferring knowledge allowing scientists to identify diseases as digital photographs sent to them. Determining the percent area infected by disease is a more recent application of image analysis in plant pathology and has not been used extensively, likely because of high labour requirements compared to visual assessments.

There have been many studies investigating partial resistance components in crops such as barley infected with *Pyrenophora teres* (Nutter and Pederson, 1985), which looked at receptivity, incubation period and lesion size to identify resistance. Parlevliet and Van Ommeren (1975) also examined partial resistance in barley to leaf rust (*Puccinia hordei*). In the case of rust's, partial resistance is sometimes quantified as slow leaf-rusting. This has been studied in wheat on *Puccinia recondita tritici* by Ohm and Shaner (1976) and on flax rust (*Melampsora lini*) (Rashid, 1991; Rashid, 1997). In cases where no high level of resistance was found, there have historically been partially resistant accessions or cultivars identified based on the components of partial resistance.

Studies aiming to identify resistance to pasmo have consisted of both field and growth room research in the past (Brentzel, 1926; Sackston, 1947; Sackston, 1949; Sackston, 1951; Christenson, 1952a; Michaelson, 1956; Pederson and Michaelson, 1960;

Diederichsen et al., 2007). However, they have all relied on visual rating techniques and growth room investigations have not been much more detailed than field studies.

The objectives of this study were to examine several cultivars and accessions based on percent infection, receptivity and incubation period for pasmo susceptibility under growth room conditions and to determine if the differences in susceptibility, assessed visually in the field, could be reproduced under growth room conditions when assessed in detail, by computer image analysis.

4.2.2 Materials and Methods

Flax Cultivars and Accessions

Four flax accessions were chosen based on susceptibility to *S. linicola* in field plots over two seasons (chapter 4.0). Accessions 172 (N2014) and 178 (Somme x Linda) were found to have low severity of pasmo in field plots while accession # 30 (Natasja (Can)) and 1 (AC Carnduff) (Table 1) had relatively high severity based on AUDPC calculations from leaf scores. The commercial cultivars AC McDuff and AC Emerson were also selected based on their respective moderately resistant and highly susceptible reactions to the pathogen in previous experimental field trials (K. Y. Rashid, Unpublished).

A greenhouse experiment was conducted to evaluate the susceptibility of the selected flax cultivars to the pathogen *S. linicola*. The flax plants were grown in 4-inch pots on a growth room bench at the Department of Plant Science, University of Manitoba. The growth room conditions were the same as described in Chapter 4.1 of this thesis for the microscopy study. After the seedlings emerged and became established, the plants were thinned to three or four seedlings per pot.

A misting chamber was used to provide continuous leaf wetness. However, due to restricted space, the trial was limited to the six cultivars described above, inoculated with five spore concentrations and replicated four times. The experiment was repeated once.

Inoculation and Sampling

The plants were inoculated at approximately 18-20 days after seeding with four different spore concentrations and a water control, consisting of distilled deionised water amended with l drop of Polyoxyethylene (20) sorbitan monolaurate (Tween 20) per 100 ml of water. The spore suspensions contained a mixture of *S. linicola* pycnidiospores harvested from 12-14 day old cultures in distilled deionised water and 1 drop of Polyoxyethylene (20) sorbitan monolaurate (Tween 20) per 100 ml suspension. The suspensions were adjusted to approximately $1x10^3$, $1x10^4$, $1x10^5$, and $1x10^6$ spores/ml using a hemocytometer. The plants were inoculated as described in chapter 4.1, beginning with the lowest concentration. The pots were then placed in the misting chamber for a period of 48 hours and subsequently returned to the growth room bench and subject to 22 °C / 18 °C day / night temperature, approximately 65% relative humidity and a 16 hour photoperiod.

The incubation period is defined as the number of days from inoculation until the first symptoms are noticed. The symptoms were monitored visually in each pot and the number of days recorded when plants in each pot began to show symptoms. Plants in each pot were also given a common visual rating based on the zero to nine scale described in chapter 4.0. This rating was carried out by two different people.

At 8 days after inoculation, all leaves from each plant were removed with tweezers and scanned in order to obtain images for later use with image analysis software to determine the percent leaf area infected of each plant.

Image Acquisition and Analysis: The ASSESS Software

Each plant that was scanned was analysed using the automatic and/or manual features of ASSESS 2.0 (Lamari, 2008). All images were scanned with an Epson Perfection 2580 photo scanner at 300 dots-per-inch (dpi) and 24-bit color depth and saved as TIFF images. Scanned images containing all leaves from individual plants were opened using the ASSESS program. If the automatic disease assessment panel was not accurate enough in separating lesion from leaf tissue, the manual panel was employed and adjusted until proper distinction was achieved between lesion and leaf tissue. Percent leaf area infected was calculated for each plant per pot by the program. The software requires a difference such as colour or brightness to effectively distinguish between the background and the leaf or lesion. First, the leaf is separated from the background. To aid in this process a blue background is scanned behind the leaves, making discrimination between the two easy to achieve. It is important to have a background that is a different in colour from both the leaf and lesions because once the leaves are separated from the background the lesions must be separated from the leaves in the same manner. The "hue" component of Hue-Saturation-Intensity (HSI) color space was the one used in these analyses. This colour space is believed to be intuitive and corresponds more so to human understanding of colour (Foley and Van Dam, 1990). The zero to nine scale, is an attempt to visually quantify the percent leaf area covered with disease. The software identifies the exact amount of discoloured tissue (lesions) and returns a percent of leaf area infected. The number of leaves and the number of lesions were counted for each plant and, subsequently, the host-receptivity was calculated using the following formula.

$$Host\ Receptivity = \frac{Number\ of\ lesions\ per\ plant}{Number\ of\ leaves\ per\ plant}$$

Analyses

Data were analysed using the "Mixed" procedure in SAS: Statistical Analysis Software, version 9.1.3 (Copyright, 2002-2003, The SAS Institute Inc., Cary, NC, USA). The two repetitions of the experiment were combined and analysis was carried out by grouping the cultivars by concentration and using the "Satterthwaite" method for degrees of freedom because there was heterogeneity of variance between the concentrations analysed. The individual accessions and concentrations were separated into letter groups based on Fisher's least significant difference (LSD) method ($P \leq 0.05$) using the "pdmix800" macro (Saxton, 1998).

4.2.3 Results

The concentration of $1x10^3$ spores /ml was clearly not high enough to provide many disease symptoms and therefore the data was extremely variable with many observations equal to zero. There were symptoms appearing as one or two lesions per pot, however, they were very rare. For these reasons analysis was only carried out on concentrations of $1x10^4$, $1x10^5$ and $1x10^6$ spores per ml. The control treatment was all zero's because no leaves showed any symptoms of disease. Overall analysis of the cultivars was only carried out for the three highest concentrations

Percent Leaf Infection

The six different cultivars used were selected because they were thought to be genetically diverse with respect to pasmo susceptibility. The cultivars were found to have a highly significant effect ($P \leq .01$) on variability within each spore concentration (Fig. 7) on percent leaf infection. The spore concentration x cultivar interaction effect was also found to be highly significant on both variables observed. Individual cultivars and concentrations showed significant differences in percentage intefection at all three spore concentrations. Percent infection of accession # 30 appeared to be relatively high for all three concentrations analyzed and accessions # 178 and 172 had significantly ($P \leq 0.05$) lower leaf area infected than several cultivars at higher spore concentrations but the only consistent difference was that accession 172 was significantly ($P \leq 0.05$) lower than accession 30 (Fig 7A). The two commercial cultivars observed in this study were not significantly different from each other with respect to percent leaf infection for any of the concentrations.

Host Receptivity

The number of lesions per leaf increased as spore concentration increased but was quite variable between cultivars at each spore concentration. It was not possible to distinguish any particular cultivar for significantly higher or lower receptivity at all three concentrations. However, there were noticeable differences such as selection 178 which appeared to have a relatively low receptivity for all three concentrations and was significantly lower than several cultivars at particular concentrations but none consistently (Fig 7B). The effect of concentration, cultivar and the concentration x cultivar interaction effect were all found to be highly significant ($P \leq 0.01$) with respect to host receptivity. The effect of concentration can be clearly seen in (Fig 7B).

Spore Concentration

Overall, the effect of spore concentration was a highly significant factor on each individual cultivar ($P \leq 0.01$). This effect can be observed in figure 7A and 7B. $1x10^6$ spores per ml caused the highest symptoms, host receptivity, percent infection and shortest incubation periods.

Incubation Period

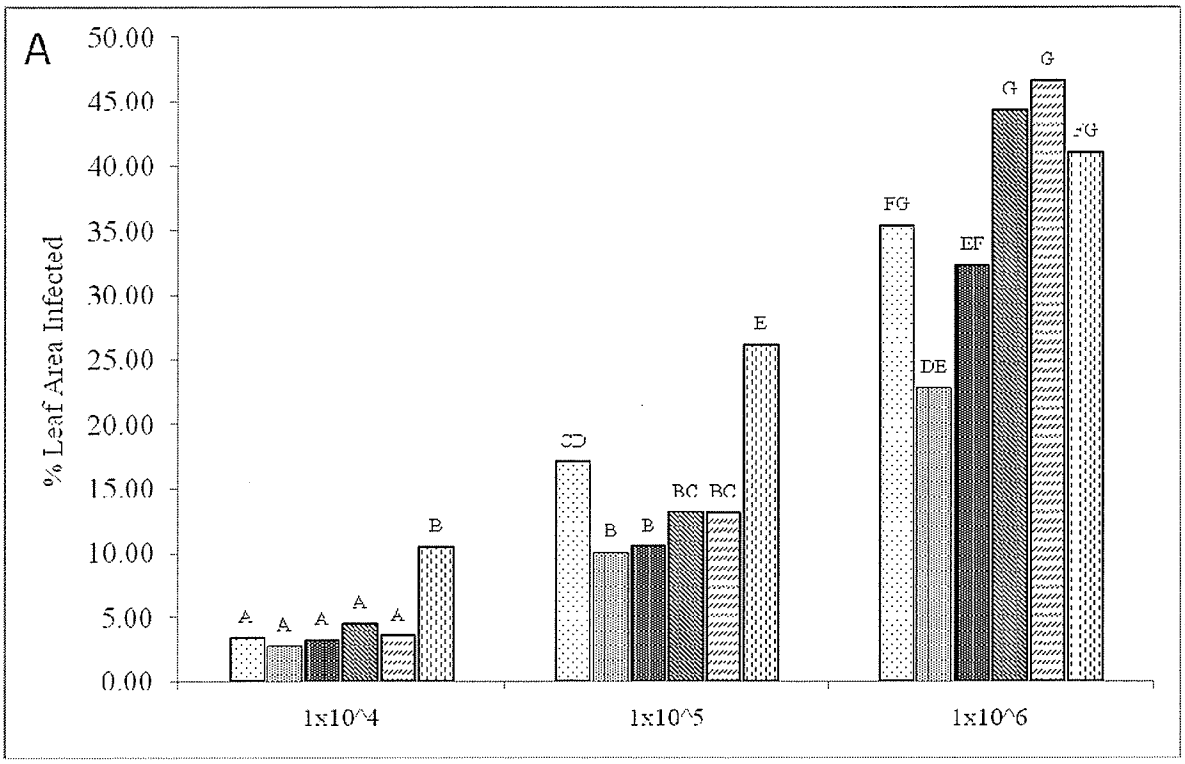
All pots that did not show symptoms after nine days were given an incubation period of 10 days. Some pots inoculated with the $1x10^3$ spore/ml concentration may not have shown symptoms by 10 days, however, because all leaves were removed, the incubation period could not be assessed after nine days. Overall the incubation period was different between higher and lower concentrations, and there were minor differences

found among the six cultivars. Accession # 30 was notably lower than other cultivars at each concentration and was the only cultivar to be consistently in a relatively low position (Appendix 5). No accessions appeared to show consistently longer incubation periods across the four spore concentrations. The incubation period was negatively correlated with percent leaf area infected and host receptivity (Table 3)

Visual Assessments

Visual assessments were taken by two separate assessors with the same instructions and rating scale used to rate leaves for pasmo symptoms in field trials (Chapter 4.0). Assessor 1 had experience rating pasmo on leaves in the field whereas assessor 2 had minimal experience. Both assessor's scores were highly correlated to each other and highly correlated to the percent leaf area infected established using image analysis (Table 3). The visual assessments of percent disease were on average 5.86% and 8.17% above the ASSESS values for assessors 1 one and 2 respectively, showing that human perception may tend to over-estimate the leaf area that is showing infection. The visual percent leaf area ratings were almost always above those from the ASSESS program and were up to 50% higher for some plants. The visual assessments that were below those from ASSESS were not as frequent and only were at most 20% below the values obtained by image analysis.

Figure 7. Results from image analysis with the ASSESS program for the six accessions and three concentrations. A) The percent leaf area infected as determined with the ASSESS program based on all leaves from each plant per pot. B) Host receptivity measured as the number of lesions divided by the number of leaves. Letter groups represent significance at $P \leq 0.05$


A
% Leaf Area Infected
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
A A A A A B
CD B B BC BC E
FG DE EF G G FG
1x10^4
1x10^5
1x10^6

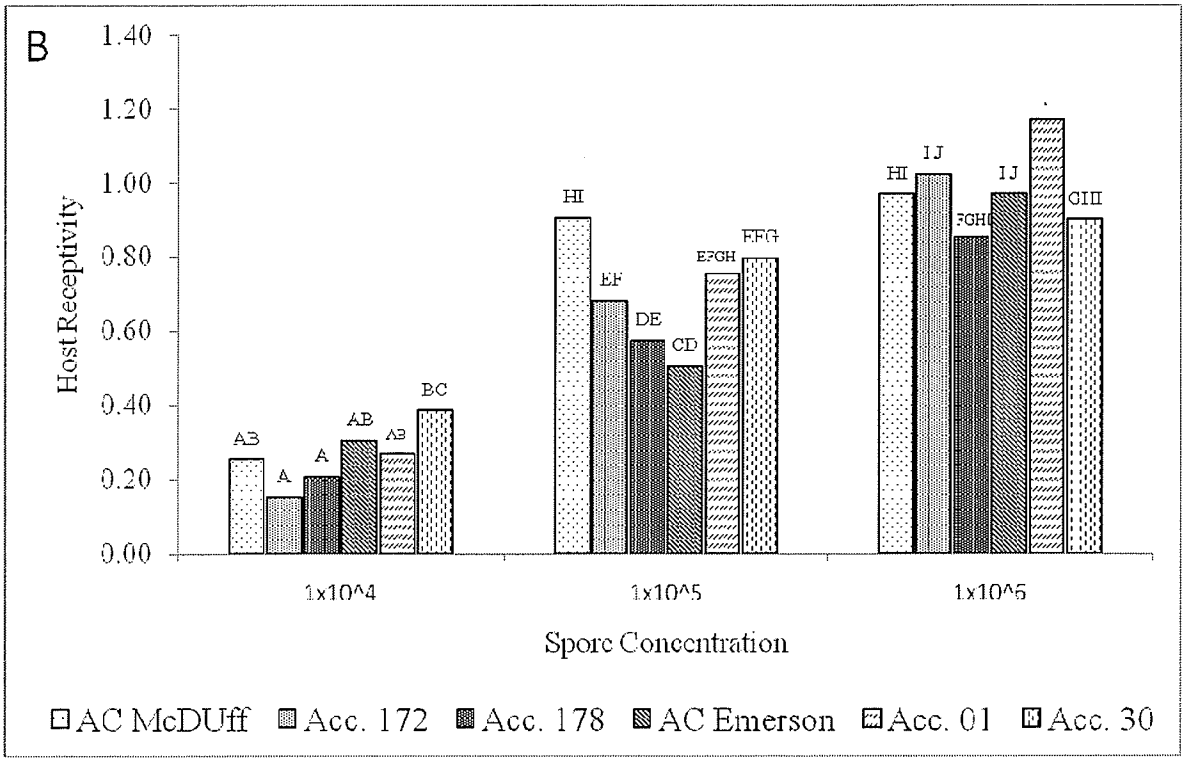

B
Host Receptivity
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
AB A A AB AB BC
HI EF DE CD EFGH EFG
HI IJ FGH IJ GHI
1x10^4
1x10^5
1x10^6
Spore Concentration
AC McDUff
Acc. 172
Acc. 178
AC Emerson
Acc. 01
Acc. 30

Table 3. Correlation matrix for the three parameters observed including the image analysis ratings and human ratings of percent leaf area infected.

			Percent Leaf Area		
	Incubation Period	Host Receptivity	Assess software	Assessor 1	Assessor 2
Incubation Period	1.00				
Host Receptivity	-0.72	1.00			
Assess software	-0.59	0.79	1.00		
Assessor 1	-0.66	0.84	0.89	1.00	
Assessor 2	-0.66	0.76	0.85	0.89	1.00

4.2.4 Discussion

The results of the present study were similar to other studies performed in the greenhouse and aiming at quantifying pasmo severity on several cultivars (Brentzel, 1926; Sackston, 1949). It was not expected that the accessions would behave exactly as observed in the field. Factors such as amount of inoculum, misting pressure and microclimate in field plots could have lead to misleading results as to how resistant some accessions truly were. Therefore it is not surprising that the results obtained in growth room conditions were different from those obtained in field studies. However, positive results were obtained, where accessions showed similar disease severity trends as observed in field studies. Selections # 30, 172 and 178 exhibited the clearest and most consistent differences with respect to leaf area infected and reflected what was observed in the field. Through both field and green house studies, selection 30 was notably more susceptible than selections 172 and 178 based on leaf assessments (Table 1). The commercial cultivars AC McDuff and AC Emerson were thought to differ under field conditions but were not different in the current study. The observed lack of differences from what would have been expected from field plots is perhaps the result of only looking at one growth stage for this study compared to seven different assessments in the field. Results may vary if observed over several growth stages because it is noted that the plants are actually less susceptible between seedling and flowering (Flor, 1943; Sackston, 1949). Although plants are more susceptible near maturity, the leaves senesce too easily when diseased and a study of this nature would be nearly impossible.

It seems logical that host receptivity would decrease with an increase in spore concentration because at higher concentrations the leaves can become heavily or almost

completely infected because lesiosns may coalesce and what may have been several small lesions becomes one larger one. This was not the case in this experiment. Perhaps if spore concentrations increased further the host receptivity would likely plateau or it may decrease although the lower concentrations had many leaves with no lesions at all. Host receptivity and lesion size are suggested to increase as the duration of leaf wetness increases in barley infected with *Pyrenophora teres* (Nutter and Pederson, 1985), especially after approximately 40 hours. Therefore, if constant wetness occurs for several days in the field, or if the plants were left in the misting chamber longer, the number of lesions may increase drastically. If a cultivar was able to withstand prolonged wetness more than others it may have a significant advantage. A future study should take into account different leaf wetness periods if space permits. The lower host receptivity noticed for accession 178 may partially explain why that selection had less leaf area infected.

Longer incubation period undoubtedly plays a role in providing reduced disease symptoms and subsequently provides some partial resistance. There were no major differences between the six accessions with respect to incubation period. Overall, and most notably in the case of accession 30, the more consistently shorter incubation periods were correlated to higher infection. In field conditions, where several cycles can be achieved in one season, a short incubation period would likely promote epidemics. Slow-rusting partial resistance in flax plants was thought to perhaps be linked to shorter incubation periods and host receptivity (Rashid, 1997).

The visual ratings were overall quite close to the computer analysed images but did prove to be higher in most cases. There were some extreme cases where the margin

was 50% from the computer to the visual rating. As symptoms increased it appeared that the humans participating in this study tended to over estimate the actual amount of lesions present because the differences noticed were much more drastic at higher concentrations. Although human observation may not be as accurate and digital analysis would be ideal, at this point digital analysis may still be too laborious to be considered feasible for larger field studies.

The results of this study show that there were consistent differences among cultivars with respect to reaction to *S. linicola* under a controlled environment and at different spore concentrations. This provides sufficient evidence to suggest that if differences in reaction to the pathogen are found in the greenhouse then the differences found under field conditions were in some part a factor of the plant's genetics and not entirely a product of the environment in the field nursery.

The use of image analysis proved to be an effective method of accurately quantifying pasmo on flax leaves. Although digital analysis is not yet developed for use at a large scale field level, further testing of accessions from field experiments with high precision may be very effective to mitigate the potential effects of human error and variable environments when trying to identify potential genetics for future breeding.

5. GENERAL DISCUSSION

Identifying resistance to the *Septoria linicola* pathogen has many limitations in western Canada and around the world. Not unlike the current study, many researchers have tested accessions of flax with little more than variation in the susceptibility levels as a result (Christenson, 1952a; Sackston, 1947; Sackston, 1951; Michaelson, 1956; Pederson and Michaelson, 1960; Diederichsen et al., 2007). The fact that pasmo is not a particularly devastating disease in many growing seasons makes screening accessions difficult. Furthermore, effective chemical control is available (Dabkevicius and Gruzdeviene, 2003), in some cases increasing flax yields irrespective of the disease control. This may make developing resistant varieties a lower priority to producers of flax.

There are some promising accessions identified in the current study, especially with respect to pasmo severity on the stems, where some appeared almost clear of symptoms. Although there was differential response between leaf and stem tissues in many cases, any sign of resistance warrants further investigation. The apparently resistant stems observed may be attributed to the "stay green phenomenon" as in other plants (Cukadar-Olmedo and Miller, 1997) and the differential response suggests the leaves and stem reaction to *S. linicola* may be controlled by separate genetic factors. Different physiological characteristics may also play a role (Michaelson, 1956). The 0-9 rating scale uses to assess disease severity was somewhat limited as the leaves and stem severity must be assessed separately. It is also and subject to the assessor's potential bias (Diaz-Lago et al. 2003). With a disease affecting two separate plant parts there is an issue as to how to determine the overall severity of the disease. In some geographical

regions one plant part, such as stems, may be more important, with respect to disease and, therefore, selected for over time (Kowalaska and Niks, 1998). This would result in a higher weight placed on that particular plant part when evaluating severity. For the current study equal weight was placed on both plant parts when determining overall severity.

It is likely that the population of *S. linicola* that appeared in the field over the two seasons did not vary significantly because the sexual state of the pathogen, which is primarily responsible for genetic variability, has not been documented in Manitoba since 1944 (Sackston, 1949). Therefore, genetic recombination is not likely to have occurred. However, fungi can also have a parasexual phase which will result in sexual recombination. Further investigations to document the perfect state of the fungus (*M. linorum*) in Manitoba would be of benefit.

Observing accessions at the greenhouse level should have provided more accurate information as to how to compare them based mostly on plant traits. If disease development can be affected by soil pH and structure, temperature, soil nutrients (Colhoun, 1973), crop rotation (Brentzel, 1926; Flor, 1943; Sackston, 1949; Rashid, 2003) and variable weather in the field, then the controlled experiment on several accessions should have mitigated these factors. The inoculum concentration was un-questionably the most significant factor affecting disease severity. Inoculum concentration affects disease severity in many host-pathogen systems including *Septoria tritici* blotch on wheat (Chungu et al., 2000) and ascochyta blight of chickpea (Trapero-Casas and Kaiser, 1992). Some accessions were effective in reducing the leaf area infected and the number of lesions relative to the other accessions examined. Three of

the accessions followed similar trends of disease severity as was observed in field experiments. However, the other cultivars/accessions did not follow the same pattern. Although the field data was taken over a longer assessment period, image analysis of cultivars in a controlled environment proved to be an effective way to confirm results from field experiments. The work of Diaz-Lago et al. (2003) also successfully used image analysis to analyse partial resistance against rust.

It is well known from the many studies investigating resistance to *S. linicola,* including the current studies, that genetic differences in flax plants produced varying levels of moderate resistance to *S. linicola*. Until the current study, only two researchers have attempted to look at the flax pasmo relationship from a cytological view (Sackston 1949; Covey, 1962). They found very similar results with respect to the characteristics of surface phenomenon and penetration as this study. With the current methodology to differentiate between host and pathogen tissue this study provided a more in depth look at the colonization of the host tissue. Unlike Septoria pathogens of wheat, which produce appressorium, or appressorium like structures (Cohen and Eyal, 1993; Duncan and Howard, 2000; Grieger, 2001), *S. linicola* did not produce anything resembling an appressorium at any point. However, *S. linicola* did penetrate only the stomates, and produced its pycnidium in a similar manner. Covey (1962), suggested that the pathogen produces a toxin to break down host cells. This would be a logical scenario because the mesophyll cells of the flax leaf break down without any direct penetration of the cells or haustoria formation. Other fungi produce toxins such as *Pyrenophora tritici-repentis* (Lamari and Bernier, 1989a).

The fluorescing mesophyll cells of the plant could not be consistently linked to fungal elicitation similar to the findings of Duncan and Howard, (2000) in *S. tritici*. There is a possibility that they are defence mechanisms such as reactive oxygen species or accumulation of pathogenesis related proteins or secondary metabolites. The changing of colour of the fungal tissue in some instances to that of the host tissue was an interesting phenomenon. Whatever the plant did to transform the host cell was working on the fungal cells also. Mauch-mani and Slusarenko (1996), working with downy mildew (*Perenospora parasitica*) on Arabidopsis, discuss the possibility that the deposition of a substance such as lignin or callose by the plant may affect the fungal tissue also.

Although some of the differences in severity observed between the accessions examined in the field and greenhouse are significant, there is still no resistant check in this system with which to work. Working based on relative comparisons has its limitations. Perhaps the moderate resistance expressed by some of the cultivars is a result of some of the phenomona noticed from the cultivars, both at the cellular level, with fluorescing mesophyll cells and depositions around stomata, and at the macroscopic level, with respect to slight variation in incubation period observed.

The purpose of this study was to investigate potential resistance at a macroscopic level and document the pathogen infection process. More in depth study of the interactions and the fluorescing substances produced would be valuable to determine if they are in fact related to fungal elicitation or, perhaps, a response to wounding. Identifying these unknown substances may be important to better understanding the system.

It may be possible to develop an integrated resistance plan for pasmo in the future with cultivars that are physiologically adapted to avoid or delay infection and have defence mechanisms at a cellular level. Slightly more resistant cultivars coupled with avoidance techniques such as lodging resistance and more open canopy have been used effectively in dry bean if disease pressure is not extreme (Miklas et al. 2001). Furthermore, the pathogen is harboured in the soil and on above ground plant stubble (Rashid, 2003), therefore, crop rotation and stubble reduction would also play a role in reducing disease. There are still several accessions that appeared moderately resistant in field experiments that warrant further study in greenhouse and possibly microscopic evaluation.

6. LITERATURE CITED

[Anonymous]. 2008. Canada: Grains and oilseeds outlook. Website: http://www.agr.gc.ca/pol/mad-dam/index_e.php?s1=pubs&s2=go-co. Accessed September 3rd 2008.

[Anonymous]. 2006. Minimum test weights for grains under the canadian grain act.

Agrios, G. N. 2005. Plant Pathology. 5th ed. Elsevier Academic Press, Burlington, MA, USA. 922 pp.

Al-Naimi, F. A., Garrett, K. A., Bockus, W. W. 2005. Competition facilitation and niche differentiation in two foliar pathogens. Oecologia 143(3): 449-457.

Annone, J. G. 1990. Observation de conidios secundarios de S*eptoria tritici* Rob. ex desm por medio de impressiones foliares. Conferencia regional sobre la septoriosis del trigo, Uruguay. CIMMYT, Mexico.

Aust, H. and Hoyningen-Huene, J. V. 1986. Microclimate in relation to epidemics of powdery mildew. Annu. Rev. Phypathol 24: 491-510.

Benvenuti, S. 2003. Soil texture involvement in germination and emergence of buried weed seeds. Agron. J. 95: 191-198.

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

Borrell, A., Hammer, G., Van Oosterom, E. 2001. Stay-green: a consequence of the balance between supply and demand for nitrogen during grain filling? Ann. Appl. Biol. 138: 91-95.

Braun, E. J. and Howard, R. J. 1994. Adhesion of fungal spores and germlings to host plant surfaces. Protoplasma 181: 202-212.

Brentzel. 1926. The pasmo disease of flax. Journal of Agriculture Research 32(1): 25-37.

Bruzzese, E. and Hasan, S. 1983. A whole leaf clearing and staining technique for host specificity studies of rust fungi. Plant Pathology 32: 335-338.

Cheong, Y. H., Chang, H., Gupta, R., Wang, X., Zhu, T., Laun, S. 2002. Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in arabidopsis. Plant Physiology 129: 661-677.

Christenson, J. J. 1952a. Report of pasmo of flax in Minnesota. Proceedings of the 33rd Flax Institute of the Untied States. 1952, Flax institute of the United States, Fargo North Dakota. USA.

Christenson, J. J. 1952b. Dissemination of *Septoria linicola* in relation to epidemics of pasmo. Proceedings of the 33rd Flax Institute of the United States. 1952, Flax Institute of the United States, Fargo North Dakota. USA.

Chungu, C., Gilbert, J., Townley-Smith, F. 2001. Septoria tritici blotch development as affected by temperature, duration of leaf wetness, inoculum concentration, and host. Plant Dis. 85: 430-435.

Cohen, L. and Eyal, Z. 1993. The histology of processes associated with the infection of resistant and susceptible wheat cultivars with *Septoria tritici*. Plant Pathology 42: 737-743.

Colhoun, J. 1973. Effects of environmental factors on plant disease. Annu. Rev. Phypathol 11: 343-364.

Covey, R. P. 1962. Mode of actoin of *Septoria linicola*. Phytopathology 52(11): 1229-1230.

Covey, R. P. 1961. Field resistance of flax to pasmo. Proceedings of the 38th Flax Institute of the United States. 1962, Flax Institute of the United States, Minneapolis Minnesota. USA.

Cukadar-Olmedo, B. and Miller, J. F. 1997. Inheritance of the stay green trait in sunflower. Crop Science 37: 150-153.

Cunnane, S. C., Ganuli, S., Menard, C., Liede, A. C., Chen, Z., Wolever, T. M. S., Jenkins, D. J. A. 1993. High a-linolenic acid flaxseed (*Linum usitatissimum*): some nutritional properties in humans. British Journal of Nutrition 69: 443-453.

Dabkevicius, Z. and Gruzdeviene, E. 2003. Investigation and use of plant protection products against flax diseases. DJF Rapport. Markbrug 96: 220-226.

Diaz-Lago, J. E., Stuthman, D. D., Leonard, K. J. 2003. Evaluation of components of partial resistance to oat crown rust using digital image analysis. Plant Dis. 87(6): 667-674.

Diederichsen, A., Rozhmina, T. A., Kudrjavceva, P. 2007. Variation patterns within 153 flax (*Linum usitatissimum* L.) genebank accessions based on evaluation for resistance to fusarium wilt, anthracnose, and pasmo. Plant Genetic Resources: Characterization and Utilization 6(1): 22-32.

Duncan, K. E. and Howard, R. J. 2000. Cytological analysis of wheat infection by the leaf blotch pathogen *Mycosphaerella graminicola*. Mycol. Res 104(9): 1074-1082.

Ferguson, M. W., Lay, C. L., Evenson, P. D. 1987. Effect of pasmo disease on flower production and yield components of flax. Phytopathology 77(6): 805-808.

Flor, H. H. 1943. Pasmo disease of flax. North Dakota State University, Bi-Monthly Bulletin 6: 31-33.

Flor, H. H. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phypathol 9: 275-296.

Foley, J. D., Van Dam, A. 1990. Fundamentals of Interactive Computer Graphics, 2nd edition, Addison Wesley. Reading Mass. USA.

Grieger, A. P. 2001. Host-pathogen interactions in the wheat-*Mycosphaerella graminicola* pathosystem. Masters Thesis at the University of Manitoba: 93pp.

Grant, L. 2008. Effect of Pasmo on Flax in Manitoba and Inference of the Sexual State of the Fungus by Molecular Polymorphism. Masters Thesis at the University of Manitoba: 207pp.

Halley, S., Bradley, C. A., Lukach, J. R., McMullen, M., Knodel, J. J., Endres, G. J., 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.

Hammond-Kosack, K. E. and Jones, Jonathan. D. G. 1996. Resistance gene-dependent plant defence responses. The Plant Cell 8: 1773-1791.

Heller, A. and Gierth, K. 2001. Cytological observations of the infection process by *Phomopsis helianthi* (munt.-cvet) in leaves of sunflower. J. Phytopathology 149(6): 347-357.

Hilber, U. W. and Schuepp, H. 1992. Accurate and rapid measurement of lengths of fungal germ tubes by image analysis. Can. J. Plant Pathol. 14: 185-186.

Holmes, G. J., Brown, E. A., Ruhl, G. 2000. Whats a picture worth? The use of modern telecommunications in diagnosing plant diseases. Plant Dis. 84(12): 1256-1265.

Hood, M. E. and Shew, H. D. 1996. Applications of KOH-aniline blue fluorescence in the study of plant-fungal interactions. Phytopathology 86: 704-708.

Jeger, M. J. and Viljanen-Rollinson, S. L. H. 2001. The use of the area under disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theor Appl Genet 102(1): 32-40.

Kamoun, S., van West, P., Vleeshouwers, V. G., de Groot, K. E., Govers, F. 1998. Resistance of *Nicotiana benthamiana* to *Phytopthora infestans* is mediated by the recognition of the elicitor protien INF1. Plant Cell 10: 1413-1426.

Kema, G. H. J., Yu, D., Rijkenberg, F. H. J., Shaw, M. W., Baayen, R. P. 1996. Histology of the pathogenesis of *Mycosphaerella graminicola* in wheat. Phytopathology 86: 777-786

Kowalska, A. and Niks, R. E. 1999. Histology of quantative resistance in flax to the flax rust fungus (*Melampsora lini*). Can. J. Plant Pathol. 21: 354-360.

Kowalska, A. and Niks, R. E. 1998. Quantitative resistance of flax to flax rust (*Melampsora lini*). Can. J. Plant Pathol. 20: 182-188.

Lafferty, H. A. and McKay, R. 1944. "Pasmo" disease on wild flax, *Linum angustifolium*. Nature 154(3918): 709.

Lamari, L. 2008. Assess 2.0 image analysis software for disease quantification. The American Phytopathological Society. St. Paul, MN, U.S.A.

Lamari, L. and Bernier, C. C. 1989a. Toxin of *Pyrenophora tritici-repentis*: host-specificity, significance in disease, and inheritance of host reaction. Phytopathology 79: 740-744.

Lamari, L. and Bernier, C. C. 1989b. Virulance of isolates of *Pyrenophora tritici-repentis* on 11 wheat cultivars and cytology of the differential host reactions . Can. J. Plant Pathol. 11: 284-290.

Lehberg, F. H. and Anderson J.A. 1941. Western Canadian flaxseed. Scientific Agriculture 21(12): 727-745.

Loughnane, J. B., McKay, R., Lafferty, H. A. 1946. Observations on the pasmo disease of flax and the causal fungus *Sphaerella linorum*. Scient. Proc., R. D. S. 24(10): 89-98.

Mauch-Mani, B. and Slusarenko, A. J. 1996. Production of salicylic acid precursers is a major function or phenylalanine ammonia-lyase in the resistance of arabidopsis to *Perenospora parasitica*. The Plant Cell 8: 203-212.

Michaelson, M. E. 1956. Evaluation of the world collection of flax for resistance to pasmo. Proceedings of the 35th Flax Institute of the United States. 1956, Flax institute of the United States, Fargo, North Dakota. USA.

Miklas, P. N., Jonhson, W. C., Delorme, R., Gepts, P. 2001. QTL conditioning physiological resistance and avoidance to white mold in dry bean. Crop Sci 41(309): 315.

Miklas, P. N., Larsen, K. M., Terpstra, K. A., Hauf, D. C., Grafton, K. F., Kelly, J. D. 2007. QTL analysis of ICA bunsi-derived resistance to white mold in a pinto x navy bean cross. Crop Sci 47: 174-179.

Millikan, C. R. 1948. "Pasmo" disease of flax. Journal of the Department of Agriculture, Victoria, Australia 46(2): 90-92.

Nutter, F. W., Jr. and Pederson, V. D. 1985. Receptivity, incubation period, and lesion size as criteria for screening barley genotypes for resistance to *pyrenophora teres*. Phytopathology (75): 603-606.

Ohm, H. W. and Shaner, G. E. 1976. Three components of slow leaf-rusting [*Puccinia recondita tritici*] at different growth stages in wheat. Phytopathology 66(11): 1356-1360.

Parlevliet, J. E. 1979. Components of resistance that reduce the rate of epidemic development. Annu. Rev. Phytopathol. 17: 203-222.

Parlevliet, J. E. and Van Ommeren, A. 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*, II. relationship between field trials, micro plot tests and latent period. Euphytica 24: 293-303.

Pederson, V. and Michaelson, M. 1960. Resistance of flax to pasmo. Proceedings of the 37th Flax Institute of the United States. 1960, Flax institute of the United States, Fargo, North Dakota. USA.

Perryman, S. A. M. and Fitt, B. D. L. 2000. Effects of diseases on the growth and yield of spring linseed (*Linum usitatissimum*), 1988-1998. Ann. Appl. Biol. 136: 197-207.

Pryor, T. 1987. The origin and structure of fungal disease resistance genes in plants. TIG 3(6): 157-161.

Rashid, K. Y. 1997. Slow-rusting in flax cultivars. Can. J. Plant Pathol. 19: 19-24.

Rashid, K. Y. 2003. Principal diseases of flax. Pages 92-124 *in:* Muir, A. and Westcott, N., eds. Flax, the genus linum. Taylor and Francis, London.

Rashid, K. Y. 1991. Evaluation of components of partial resistance to rust in flax. Can. J. Plant Pathol. 13: 212-217.

Rashid, K. Y., McAndrew, D. and Irvine, B. 2002. Effects of nitrogen level, cultivar, seeding rates and seeding dates on diseases in flax. Proceedings of the 59th flax institute of the United States, 2002, Flax Institute of the United States. Fargo ND, USA.

Rommens, C. M. and Kishore, G. M. 2000. Exploiting the full potential of disease-resistance genes for agriculture use. Current Opinion in Biotechnology 11: 120-125.

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

Sackston, W. E. 1951. Flax diseases, flax varieties, and cultural practices. Proceedings of the 33rd Flax Institute of the United States. 1952, Flax Institute of the United States Fargo, North Dakota. USA.

Sackston, W. E. 1949. Studies on the pasmo disease of flax. Masters thesis at the University of Minnesota. USA. 194pp.

Sackston, W. S. 1947. The effect of pasmo on yield of flax. Canadian Phytopathological Society 15: 16-17.

SAS Institute Inc. 2003. SAS: Statistical analysis software. 9.1.3.

Saxton, A. M. 1998. A macro for converting mean seperation output to letter groupings in proc mixed. SAS Institute, Cary, NC, USA,. .

Singh, G. K. 1987. History and origin. Pages 12 *in:* Jaiswal, P. L. and Wadhwani, A. M., eds. Linseed. Kapoor Art Press ed. Indian Council of Agricultural Research, New Delhi, India.

Sivaneson, A. and Holliday, P. 1981. *Mycosphaerella linicola. in:* Descriptions of fungi and bacteria. Commonwealth Mycological Institute, Great Britan.

Slavin, J. 2003. Why whole grains are protective: Biological mechanisms. Proceedings of the Nutrition Society 62(129): 134.

Smith, R. E., Michalyna, W., Wilson, G. 1973. Soils of the Morden-Winkler area. Manitoba Soil Survey number 18.

Spegazzini, C. 1911. *Phlyctena ? linicola* speg. mycetes argentinenses. Anales Del Museo Nacional De Buenos Aires 3(13): 329-468.

Trapero-Casas, A. and Kaiser, W. J. 1992. Influence of temperature, wetness period, plant age, and inoculum concentration on infection and development of ascochyta blight of chickpea. Phytopathology 82(589): 596.

Turey, D. B. and Snowden, K. L. 1998. Observation on the effect of winterlinseed cultivar and sowing date on pasmo (*Mycosphaerella linicola*) disease. Tests of Agrochemicals and Cultivars 19: 44-45.

Turner, J. A. 1987. Linseed law: A handbook for growers and advisors. Alderman Printing & Bookbinding Co Ltd, Russell Road, Ipswich. 356 pp.

7. APPENDICES

Appendix 1

Analysis of variance for the leaf, stem and combined area under disease progress curve (AUDPC) values. Values are shown for the source of variation, and each sources respective degrees of freedom, sum of squares, mean square and F value. The probability > F is also shown.

2006 Season

Variable	Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Combined AUDPC	Seed time	1	79.28	79.28	0.10	0.7520
	Accession	207	663729.00	3206.42	4.05	0.0001
	Residual	207	163899.00	791.78		
Leaf AUDPC	Seed time	1	4511.12	4511.12	4.94	0.0273
	Accession	207	629820.00	3042.61	3.33	0.0001
	Residual	207	189084.00	913.45		
Stem AUDPC	Seed time	1	2436.17	2436.17	2.23	0.1368
	Accession	207	931257.00	4498.82	4.12	0.0001
	Residual	207	226093.00	1092.24		

2007 Season

Variable	Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Combined AUDPC	Seed time	1	411016.00	411016.00	1131.21	0.0001
	Accession	203	262735.00	1294.26	3.56	0.0001
	Residual	203	73759.00	363.34		
Leaf AUDPC	Seed time	1	592287.00	592287.00	1030.56	0.0001
	Accession	203	345367.00	1701.31	2.96	0.0001
	Residual	203	116669.00	574.73		
Stem AUDPC	Seed time	1	262768.00	262768.00	598.75	0.0001
	Accession	203	295475.00	1455.54	3.32	0.0001
	Residual	203	89089.00	438.86		

Appendix 2

Sampling locations for *Septoria linicola* isolates stored in -70°C

Isolate	Town	Province
06[a]-F-71[b]	Weyburn	Saskatchewan
06-F-92	Liberty	Saskatchewan
06-F-134	Somerset	Manitoba
06-F-NB	Grand Falls	New Brunswick
06-F-99	Jansen	Saskatchewan
06-F-97	Manitou Beach	Saskatchewan
06-F-55	Boissevain	Manitoba
07-F-54	Darlingford	Manitoba
07-F-52	Glenora	Manitoba
07-F-46	Agquest Minto	Manitoba
07-F-41	St. Alphonse	Manitoba
07-F-48	Boissevain	Manitoba

[a] The year in which the sample was taken
[b] The field number of the isolate

Appendix 3

Yeast Malt Agar

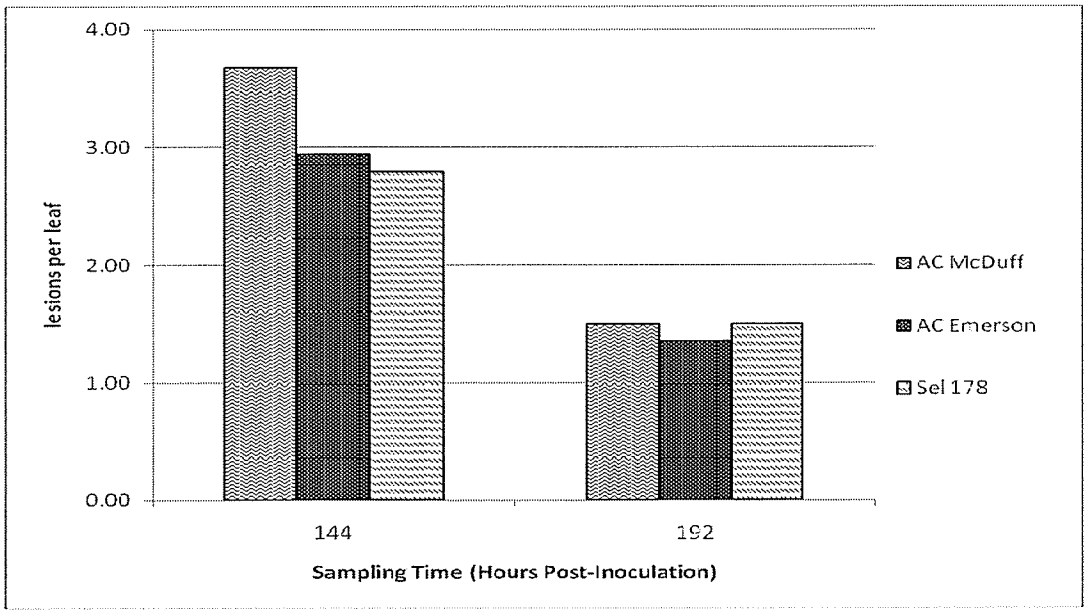
Yeast Extract 4 grams
Malt Extract 4 grams
Sucrose 4 grams
Agar 15 grams
Distilled water 1000 ml

To prevent bacterial contamination: add 0.25 g/L of chloramphenicol (equiv. To 250 ppm)

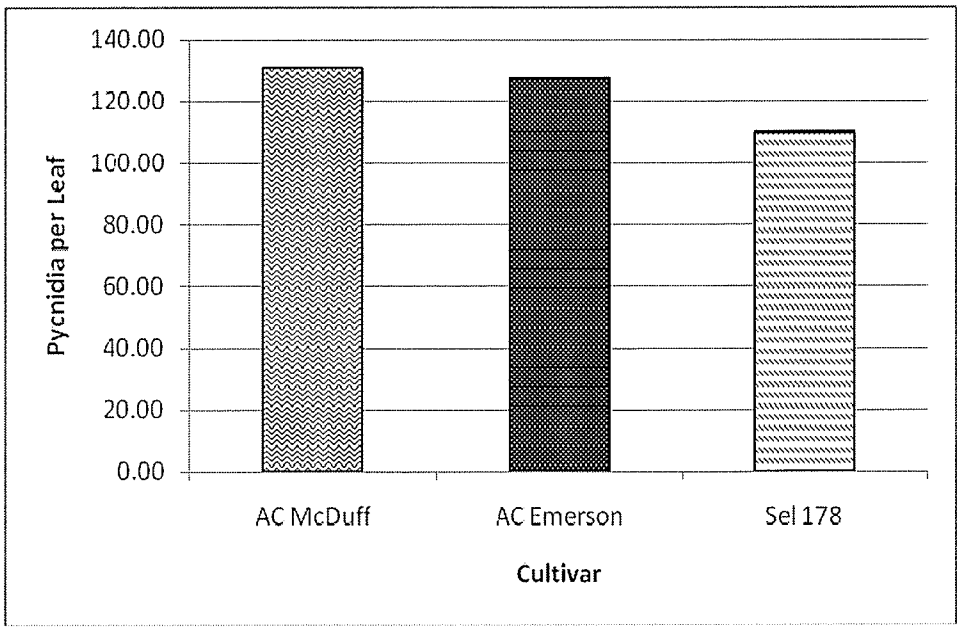
Appendix 4

Lesion and pycnidial development for cultivars AC McDuff, AC Emerson and accession 178. A) The number of lesions per leaf for each cultivar at 6 and 8 days post-inocualtion. B) The number of developed pycnidia per leaf counted at 8 days post-inoculation

A)



B)



Appendix 5

Incubation period measured in days for six accessions observed at four concentrations. Accessions were inoculated and stored in misting chamber for a period of 48 hours prior to being placed on a growth room bench, all accessions received the same treatment.