Studies on Effects of Crop Rotation and Tillage on Blackleg Disease (*Leptosphaeria maculans*) in Canola (*Brassica napus*), Dispersal Patterns of *L. maculans* Spores, and Effects of Temperature and Relative Humidity on Infection of Canola Cotyledons

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Plant Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Guo, Xiaowei. M.Sc., The University of Manitoba, January, 2004. Studies in Effects of Crop Rotation and Tillage on Blackleg Disease (*Leptosphaeria maculans*) in Canola (*Brassica napus*), Dispersal Patterns of *L. maculans* Spores, and Effects of Temperature and Relative Humidity on Infection of Canola Cotyledons. Advisor: W.G. D. Fernando.

Leptosphaeria maculans (anamorph: *Phoma lingam*), the causal agent of blackleg of canola, is an important disease affecting the crop across western Canada. Effects of cropping practices on blackleg disease of canola were studied in the Department of Plant Science, and Carman Research Station, University of Manitoba, Manitoba from 1999 to 2002. The disease could be significantly reduced when canola was rotated with wheat and flax, and was grown on tilled plots. Tillage showed a significant effect on decreasing the disease when it was performed with a single-crop rotation; however, it did not with a two-crop rotation. A non-host crop grown in the previous year before canola effectively decreased the inoculum level (amount of spores released) and disease. Survival of blackleg pathogen on canola stubble significantly decreased within nine months. With increase of soil depth, viability of the pathogen significantly decreased. The pathogen had more difficulty in surviving in clay than in loam and sand. There was a significant seasonal dispersal pattern of ascospores by *L. maculans* from the middle of June to the end of July, a dispersal pattern of pycnidiospores from the middle to end of July or beginning of August, and a diurnal dispersal pattern of ascospores and pycnidiospores from 9 pm to 4 am. The optimum temperature for ascospore and pycnidiospore release were 13-18 °C and relative humidity > 80% respectively. Peak ascospore and pycnidiospore dispersal were associated with more than 2-mm rain events. Peak pycnidiospore dispersal could

occur at the same hour as rain events. Peak ascospore dispersal could occur one or more hours after rain events; and could maintain in the next three days. More ascospores and pycnidiospores were carried by wind in its prevailing direction. Higher concentration of ascospores was observed within 10-25 m from inoculum source. Relative humidity and temperature had significant effects on development of leaf lesions. The optimum relative humidity and temperature were 70% and 18/20°C (night/day). The results of this project will benefit future disease management, and can be used in modeling and forecasting epidemics of blackleg disease.

FOREWARD

This thesis is written in manuscript style, with each manuscript including its own abstract, introduction, materials and methods, and results and discussion sections. There is a general introduction and review of the literature prior to the manuscripts, followed by the general discussion and conclusions, and the literature cited section.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

1. INTRODUCTION

Canola is one of the most important oil crops grown worldwide. The crop is well adapted to North America, Europe and Australia (Canola Council of Canada, 2002). In Canada, the crop is mainly grown in southern Manitoba, central Saskatchewan and Alberta (Sawatsky, 1989). In 2001, it was grown on 9.6 million acres in Canada, of which 4.7 million acres were in Alberta, 2.9 million acres in Saskatchewan and 1.9 million acres in Manitoba to produce a total of 5.1 million tons. From 1991 to 2001, the crop seed export reached 1.9 million tons and oil export rose from 203,000 to 715,000 tons (Savchuk, 2002).

With the significant rise in acreage, concern about canola diseases has increased. One of the most important diseases is blackleg, caused by *Leptosphaeria maculans* (Desm) Ces. & de Not (anamorph: *Phoma lingam*) (Tode ex Fr.) (Desm). Blackleg disease is widespread around the world, with severe epidemics occurring in France in 1950 (Gugel and Petrie, 1992), in Australia in 1968 (Boker et al., 1975; McGee and Emmett, 1977), and in England in1976-1977 (Sawatsky, 1989). In Canada, since the observation of the first virulent strain in 1977, blackleg disease has spread across Manitoba, Saskatchewan and Alberta, devastating many canola-based industries (Gugel et al., 1992; Evens et al., 1991; Assabgui and Hall, 1989).

A scientific approach to the control of plant diseases developed in the 1800s (Zadoks and Schein, 1979). Crop rotation and tillage practices have interested farmers as early low cost, methods to reduce risk of yield loss. Crop rotations lengthen the time between host crops so that infected crop stubble has a sufficient period to decompose, and survival of pathogen has enough time to decrease (Turkington et al., 2000). Tillage decreased

pathogen survival by burying and fracturing crop stubble, and changing the soil environment where the pathogen exists (Kharbanda, 1999). Some studies have been carried out on investigating the effects of crop rotation and tillage on stem rot of soybean (Gracia-Garza et al., 2002), fusarium head blight (Dill-Macky and Jones, 2000), and tan spot of wheat (Bockus and Claassen, 1992). There are few studies addressing the effects of crop rotation and tillage on blackleg disease of canola.

Leptosphaeria maculans over-winters on crop stubble as mycelia, pycnidia and pseudothecia. In the spring pseudothecia and pycnidia produce and release ascospores and pycnidiospores, respectively. Wind-dispersed ascospores and rain splashed-dispersed pycnidiospores land on the cotyledons and young leaves of canola plants (Howlett et al., 2001). After ascospores and pycnidiospores germinate, hyphae infect leaves through stomata and wounds, and cause lesions on the leaves, where pycnidia form and produce pycnidiospores. Pycnidiospores are dispersed through rain splash to other leaves and plants to continue their infection. The fungus reaches other leaves and stem crowns and causes black stem canker symptom (Hammond et al., 1985) that may girdle the base of the stem and cause plants to lodge (Howlett et al., 2001). Throughout the growing season, lesions form on leaves, stems and seedpods. After harvest, the pathogen living on the crop stubble over-winters and starts another life cycle. However, there are no studies done on the pattern of ascospore and pycnidiospore release by *L. maculans* under the environmental conditions in Manitoba or the effects of weather factors on infection of canola.

The objectives of this project were: (1) to study the effects of crop rotation and tillage on blackleg disease of canola; (2) to examine the viability of the blackleg pathogen over

time at different depths in different types of soil; (3) to investigate the distance spores travel, and seasonal and diurnal patterns of ascospore and pycnidiospore dispersal in relation to temperature, relative humidity, rain and wind; (4) to investigate the effects of temperature and relative humidity on infection of canola cotyledons by *L. maculans*.

2. LITERATURE REVIEW

2.1 Host

2.1.1 History

Canola, known as rapeseed, was cultivated as early as 5000 BC in China for its edible roots, stems, leaves, buds, flowers and seeds, and then was introduced into India and Japan (Background of Canola Varieties, 2002). In the 13th century the crop was introduced to Europe due to its ability to grow at lower temperatures than other oilseed crops (Background of Canola Varieties, 2002). It had been used only in foods and as cooking oil until the development of steam power, when rapeseed oil's ability to cling to water and steam and to wash metal surfaces well was discovered (Canola History, 2002).

In the early 1940's, Canadian rapeseed production was stimulated by the critical shortage of rapeseed oil following the World War II blockage of European and Asian sources of the oil (Canola History, 2002). Prior to World War II, some research had shown that the crop could be successfully grown in eastern and western Canada (Canola History, 2002). In 1936, a farmer, in Shellbrook, Saskatchewan, started growing a small amount of rapeseed he obtained from his Polish friend. With the coming of the War, the farmer increased his seed supply and sold seed to his neighbors. The species he planted was known as "Polish rapeseed" in Canada. A requirement for a considerably larger quantity of seeds was met by the purchase of 19 tons of rapeseed from U.S. seed companies in 1943. This seed, belonging to the *Brassica napus* species, had originally been obtained from Argentina, therefore, named "Argentine rapeseed". "Polish rapeseed" and "Argentine rapeseed" became Canada's two main canola crop species (Canola History, 2002).

The merits of the crop as a source of food were not fully realized until the end of World War II when the agricultural industries in Canada made essential improvements in crop processing techniques (Canola History, 2002). The first edible rapeseed oil in Canada was extracted in 1956. Fatty acids, components of rapeseed oil determine the use of oils for either edible or industrial use. Some fatty acids (linoleic) are essential for human health, but some (eicosenoic and erucic) are not. In the early 1960's, rapeseed varieties with low eicosenoic, erucic acid and glucosinolates content were quickly developed by Canadian plant breeders (Canola History, 2002). In 1974, the first "double-low" variety of *Brassica rapa*, which had low erucic and glucosinolate levels, meeting specific nutritional requirements, was developed by Baldur Stefansson at the University of Manitoba. In 1979, canola, a combination of two words - Canadian and oil - was registered by the Western Canadian Oilseed Crushers' Association, now known as the Canadian Oilseed Processors Association (Oplinger et al., 1989). The official definition of canola is: an oil that must contain less than 2% erucic acid, and the solid component of the seed must contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate, and 2-hydroxy-4-pentenyl glucosinolate per gram of air-dry, oil-free solid (Canola History, 2002). Since the 1980's, many canola varieties have been developed by plant breeders with improvements in agronomic, oil and meal quality, and disease resistance, all of which have played important roles in the rapid expansion of the Canadian canola industry (Background of Canola Varieties, 2002). Types of rapeseed, having high concentrations of erucic acid, are known as high erucic acid rapeseed (HEAR). HEAR oil used for industrial applications exceeds 46% erucic acid concentration. HEAR has been produced

for industrial oil products since the 20th century (Jacobs, 2001). Breeding work for developing high erucic acid, low glucosinolate rapeseed began in 1969 at the University of Manitoba (Jacobs, 2001). These studies culminated in recently register HEAR cultivars of *B. napus* rapeseed that contained over 50% erucic acid in the oil and less than 20 umol glucosinolates / g seed at 8.5% moisture (Scarth and McVetty, 1991).

2.1.2 Family and species

Canola belongs to the Cruciferae (mustard) family. It consists of the species *Brassica napus* L., *Brassica rapa* L., *Brassica carinata* L. and *Brassica juncea* (L.) Coss. (Sawatsky, 1989). *Brassica napus* and *B. rapa* both have spring and winter types which have morphological and physiological differences. Yield of *B. napus* is slightly more than *B. rapa*, but the earlier maturity of *B. rapa* lends itself to production in the more northern areas. The relationship among the *Brassica* species is illustrated in Figure 2.1 (Savchuk, 2002).

There are about 3,000 species of plants in the Cruciferae family found in the northern hemisphere (Background of Canola Varieties, 2002). Canola is closely related to other *Brassica* species such as cabbage, cauliflower, kale, brown and original mustard, and distantly related to the species white mustard (*Sinapis alba*) and wild mustard (*Sinapis arvesis*). Some weed species such as wormweed mustard (*Artemisia alaskana*), and flixweed (*Descurainia sophia*) are also included in the Cruciferae family (Background of Canola Varieties, 2002).

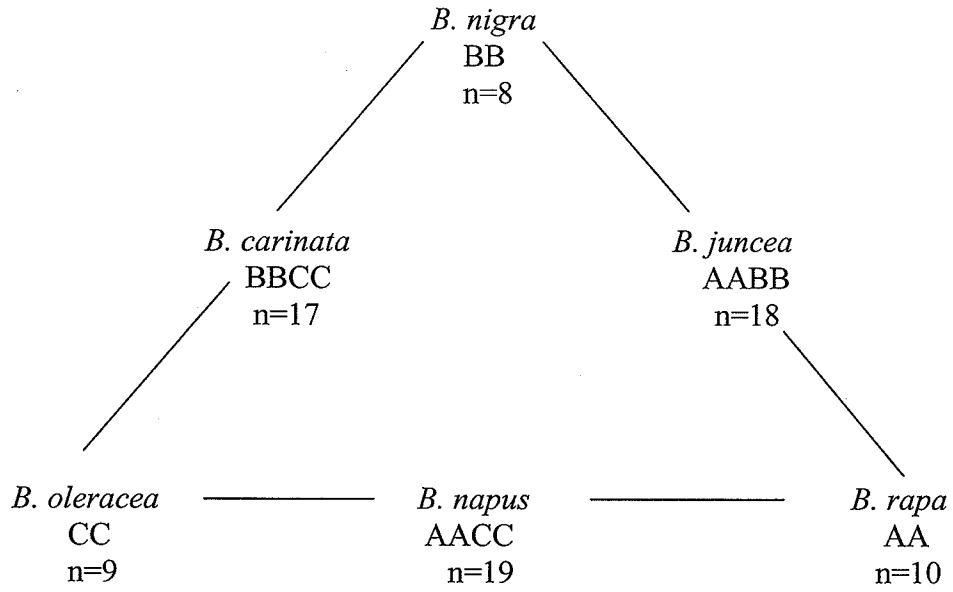


Figure 2.1. Relationship among the *Brassica* species (A, B, C = genome; n = chromosome number) (Savchuk, 2002).

2.1.3 Development of canola

The growth and development of canola are divided into six stages, the length of each of which is affected by conditions of weather, soil and crop variety (Canola Council of Canada, 2001).

Germination

After seeding, a seed starts to absorb water from the soil and swells. The seed coat splits and the root tip emerges. The root grows downward and absorbs water and nutrition while developing root hairs. The stem grows and pushes two heart shaped cotyledons up to the soil surface (Canola Council of Canada, 2001).

Seedling

Upon emergence, four to ten days after seeding, the seedling stem continues to develop. The cotyledons expand, turn green and provide nutrition for the developing plant. The root continues to develop downward (Canola Council of Canada, 2001).

Rosette

Four to eight days after emergence, the seedling forms its first true leaf. After that, the plant quickly develops a rosette with older leaves at the base increasing in size, and smaller, younger leaves developing in the center. The root system continues to develop and grow outward and downward. Leaves play a very important role in plant development because they collect sunlight for the production of dry matter necessary for plant growth and yield formation. Furthermore, rapid leaf development promotes root growth (Canola Council of Canada, 2001).

Bud

As days lengthen and temperatures increase, the buds form. A cluster of flower buds develops at the center of the rosette, and rises and enlarges as the stem elongates. Secondary branches arise from buds, developing from the axils of the upper leaves. They also develop one to four leaves and a flower bud cluster (Canola Council of Canada, 2001).

The main stem reaches 30 to 60 percent of its maximum length and produces 30-60% of the plant's total dry matter before flowering. Maximum leaf area is attained by the beginning of flowering when the upper leaves are the major source of food for the growth of stems and buds (Canola Council of Canada, 2001).

Flowering

Flowering begins from the lowest bud on the main stem, and continues upward. Approximately three to five flowers open each day. Flowering at the base of the first secondary branch begins two to three days after the first flower opens on the main stem.

Flowers are available for receiving pollen for up to three days after opening. Warm and dry weather favors pollination (Canola Council of Canada, 2001).

The productive pods are much fewer than the buds the plant initially forms. Only 40 to 55 percent of the flowers develop productive pods, which are retained until harvest. Most of the productive pods are from the flowers which open within the first 15 days of flowering on the main stem and the first three secondary branches (Canola Council of Canada, 2001).

Ripening

When the petal falls from the last-formed flower on the main stem, ripening begins. At this stage, the stem and pod walls are both major sources of food for growth. The seed coat expands until the seed reach full size. At the same time, the seed embryo grows rapidly within the seed coat, and seed weight increases. About 35 to 45 days after flowering, seed filling is complete. During this stage, the seeds in the lower pods turn green, and most of the leaves on the plant turn yellow (Canola Council of Canada, 2001).

During maturation the pod is divided into two halves by a membrane running the full length of the pod. A pod contains 15 to 40 seeds. Seeds contain about 40 percent moisture during filling. Approximately 40 to 60 days after first flower, the seeds in the lower pods ripen. When seeds are completely mature, they are bright yellow, and average seed moisture is about 30 to 35 percent (Canola Council of Canada, 2001).

2.2 Pathogen

2.2.1 Nomenclature and Taxonomy

In 1791, Tode described the saprophytic organism *Sphaeria lingam* on dead red cabbage stems (Williams, 1992). In 1849, Desmazieres obtained the same fungus from

living cauliflower, and taxonomically placed it into the genus *Phoma*. Various names *Phoma brassicae, Phoma oleracea, Phoma napobrassicae, Plenodomus lingam* were given to *Phoma lingam* (Tode ex Fr.) Desm.

The sexual stage of *Phoma lingam* was found in New Zealand and confirmed as being *Leptosphaeria maculans* (Desm.) Ces & De Not. in the order pleosporales and family loculoascomycetes (Williams, 1992). Punithalingam and Holliday (1972) described the pathogen. Pseudothecia of *L. maculans* on stems and leaves are immersed, becoming erumpent, globose, black, with protruding ostioles 300- 500 μm in diameter. Asci are cylindrical to clavate, sessile or short stipitate, 8 spores, 80 -125 × 15-22 μm and the ascus wall is bitunicate. Ascospores are biseriate, cylindrical to ellipsoidal, ending mostly rounded, yellow brown, sometimes slightly constricted at the central septum, guttulate, 35-70 × 5-8 μm. Pseudoparaphyses are filiform, hyaline and septate. There are two types of pycnidia on stems and leaves of *Brassica* spp. Type I (sclerotioid form) is immersed, becoming erumpent, gregarious, variable in shape, convex, soon becoming depressed and concave without any definite shape, with narrow ostioles 200-500 μm across. The wall is composed of several layers of thick-walled sclerenchymatous cells. Type II is globose, black, 200-600 μm in diameter. The wall is composed of several layers of sclerenchymatous cells. Pycnidiospores are hyaline, short and cylindrical, mostly straight, some curved, guttulate, with one guttule at each end of the pycnidiospore, unicellular, 3-5 × 1.5-2 μm (Punithalingam and Holliday, 1972).

2.2.2 Host Range

The pathogen can attack *B. oleracea, B. rapa, B. napus,* rutabaga, and various other genera of crucifers (Punithalingam and Holliday, 1972). Some cruciferous weeds,

including *Thlaspi* spp., *Sisymbrium* spp., *Descurainia sophia* (L.) Webb, *Lepidium* spp. and *B. kaber* (DC.) L. C. Wheder, can also be infected by the pathogen (Sawatsky, 1989).

2.2.3 Symptomology

Blackleg disease attacks leaves, stems, roots and pods of the plants (Nyvall, 1989). Young tissue is more susceptible to infection than older tissue. Symptoms first appear on cotyledons and true leaves as inconspicuous pallid areas. These later become gray to white irregularly-shaped lesions dotted with pycnidia. Stem lesions often form near the soil line as white to gray or black areas with purplish borders. With favorable moisture, pycnidia develop in lesions on lower stem portions, and exude a pinkish exudate with pycnidiospores. Severely infected stems may form cankers and girdle the plant crowns, leading to plant lodging and causing yield loss (Nyvall, 1989).

2.2.4 Disease cycle and epidemiology

Leptosphaeria maculans can over-winter on crop stubble as mycelia, pycnidia and pseudothecia. Under favorable conditions of temperature, radiation, and relative humidity, pseudothecia produce and release ascospores. Ascospores, wind dispersed, are considered to be the primary inoculum (Howlett et al., 2001). Pycnidia produce rain splash-dispersed pycnidiospores, which are regarded as the secondary inoculum (Williams, 1992). Ascospores or pycnidiospores land on cotyledons or on young leaves, and enter tissue through stomates or wounds, hyphae colonize intercellular spaces through tissues, which can be called the symptomless biotrophic phase. After that, the fungus uses resources in the necrotic leaf lesions to produce pycnidia, which are produced in lesions of recently killed tissues (Williams, 1992).

Pycnidiospores are dispersed through rain splash to other leaves and neighboring plants. The fungus enters the host cell, reaches a vascular strand and spreads down the petiole in xylem vessels or between cells of xylem parentchyma and cortex. It eventually invades and kills cells of the stem cortex and causes not only leaf lesions, but also the black stem canker symptom (Hammond, et al., 1985) that may completely girdle the base of the stem to cause plants to lodge - hence the name "blackleg" (Howlett, et al., 2001). Throughout the growing season, lesions form on leaves, stems and seedpods. After harvest, the pathogen inoculum left on the crop stubble over-winters and starts another life cycle.

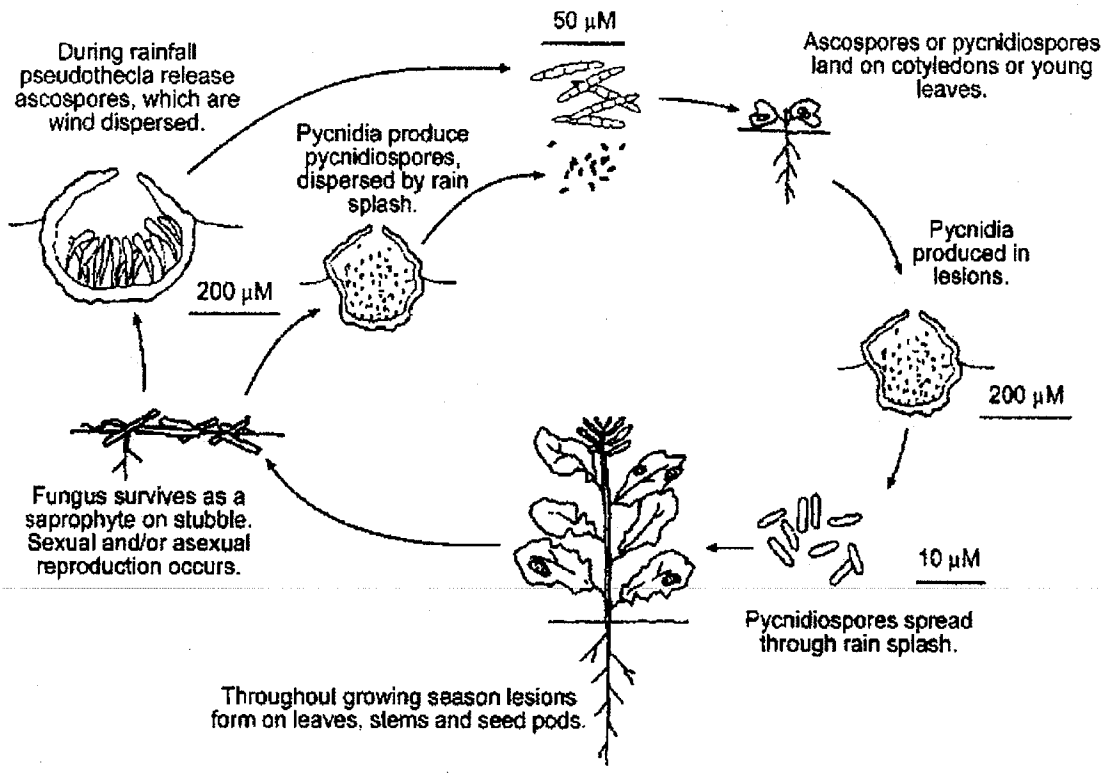


Figure 2.2. Life cycle of *Leptosphaeria maculans* on *Brassica napus* (Howlett, et al., 2001)

The disease is usually monocyclic and epidemics are generally initiated by inoculum from infected crop stubble and seeds. Pseudothecia and pycnidia may survive along with the stubble for one to five years (Hall, 1992). The primary inoculum may come from infected stubble of either the previous year (McGee and Emmett, 1977), or from three to five-year old stubble (Petrie, 1986b). There is a direct relationship between the number of ascospores dispersed from the stubble and disease severity (McGee and Emmett, 1977).

Seeds may be contaminated by the pathogen and introduced to disease free area, leading to disease epidemics (McGee and Emmett, 1977; Petrie and Vanterpool, 1974; Wood and Barbetti, 1977). The pathogen may infect seeds through the funicle (Wood and Barbetti, 1977), or by penetrating the wall of the silique (Petrie and Vanterpool, 1974).

Pseudothecia, forming on infected stubble, appear 1 to 10 months after harvest (Hall, 1992). A mean temperature of 14 °C and a high frequency of rainfall (2.5 mm every 3 - 4 days) are considered the optimum conditions for the first generation of peseudothecia in the field (Pere et al., 1999). In the laboratory, pseudothecia can be produced by incubating opposite mating types on V8 juice agar (20% V8 juice, amended with 0.1g/L streptomycin sulfate, 0.04g/L Rose Bengal and 0.75g/L CaCO3) under white light at 25 °C for 5-15 days. Temperatures of 8-12 °C generally favor ascospore release (Hall, 1992). Production of pycnidia is affected by cultivar and age of host and environmental factors such as temperature, humidity, light and duration of leaf wetness (Vanniasingham and Gilligan, 1989). Pycnidia may be produced in 14 days by inoculating wounded leaves, maintaining leaf wetness for 1-8 days, and incubating them at 10- 25°C in the laboratory. Different weather conditions in different regions lead to diverse spore release

patterns. Peak ascospore release occurs in September, October or November in Europe; and in June, July and August in Australia (Hall, 1992).

Infection of the pathogen is affected by environmental conditions. Germination of pycnidiospores starts after 12 hours at 24-28 °C. There exists a significant latent infection by *L. maculans* (Nathaniels and Taylor, 1983; Xi et al., 1991). Pycnidiospores often function as the secondary inoculum, dispersed by rain splash (Williams, 1992). Because the flight distance of splash droplets is generally shorter than 10 cm, successful deposition of spores at an infection site requires continual splashing of droplets with spores during rain events (Madden, 1997). Pycnidiospore dispersal depends on rain intensity, droplet size and kinetic energy of drop impactions (Madden, 1996; Fitt et al., 1989; Fitt et al., 1984; Walklate, 1989).

2.2.5 Leptosphaeria maculans species

Strains of *L. maculans* are classified into two pathotypes, A and B, according to the ability to cause stem canker on *B. napus* or to produce the phytotoxin sirodesmin PL, respectively (Williams and Fitt, 1999). The group A stem cankers causing strains are named aggressive, highly virulent, or virulent, whereas those in group B are nonaggressive, weakly virulent, or avirulent.

Group A is subdivided into PG-2, PG-3 and PG-4 based on differential virulence on cotyledons of *B. napus* cultivars Westar, Glacier and Quinta (Koch et al., 1991). PG-2 is virulent on Westar, avirulent on Glacier, and intermediate on Quinta; PG-3 is virulent on Westar and Glacier, and intermediate on Quinta; PG-4 is virulent on the three cultivars. Group B is PG-1, subdivided into NA1, NA2 and NA3 (Koch et al., 1991).

2.3 Disease spread in the world

Blackleg disease is widespread around the world. In France, a severe epidemic caused disease incidence of 50%-80% in 1950 when cultivars Oleor, Titus and Tonus, all highly susceptible to blackleg, were grown (Gugel and Petrie, 1992). In 1966, a severe epidemic occurred in central France leading to a yield reduction of 40%. In 1968, blackleg was in most areas of France. In England, an increase in stem canker occurred in 1976 and 1977 (Sawatsky, 1989; Humpherson-Jones, 1983), resulting in a disease incidence of 100% and a yield loss of 50% in some areas (Gugel et al., 1992). In 1968, Canadian cultivars were introduced to Australia and grown on approximately 150,000 ha in southern and western Australia (Bokor et al., 1975; McGee and Emmett, 1977). In 1972, a severe epidemic devastated many canola industries in Australia (Wood and Barbetti, 1977; McGee and Emmett, 1977).

In Canada, the avirulent strains of *L. maculans* have been found since 1961 (Petrie, et al., 1965, 1966, 1968 and 1970; Vanterpool, 1961). The virulent strain was first discovered in Saskatchewan in 1975 (McGee and Petrie, 1978). Disease incidence and yield loss were highly variable in different provinces. In 1977, 17% of canola fields in Saskatchewan had the virulent strain, resulting in an estimated yield loss of 20%. In 1984, the development of cultivar Westar, high-yielding but highly susceptible to blackleg, led to a blackleg epidemic in Saskatchewan in 1984 and 1985 (Petrie, 1986). In 1986, 65% of canola fields were found to have the virulent strain (Gugel and Petrie, 1992). In 1983, the first virulent strain to occur in Alberta was discovered (Gugel and Petrie, 1992). By 1988, yield losses were about 10% in some areas, but much lower a fewer years later (Evans et al., 1991). The first virulent strain in Manitoba was detected in

1984 (Gugel and Petrie, 1992). Thirty six percent of canola fields were found to have blackleg disease and yield losses approached 50%. In 1987, the disease was found in 54% and 83% of Argentine and Polish canola fields, respectively; and yield loss was about 10% in Manitoba (Gugel and Petrie, 1992). The first virulent strain in Ontario was found in 1986 (Peters and Hall, 1987). In 1987, the disease incidence and yield loss were about 70% and 7.5%, respectively, in Ontario. In 1989, 60% of canola fields had blackleg disease; yield loss was 1.8% in the same region (Assabgui and Hall, 1989). In 2002, the PG-3 strain of the blackleg pathogen was isolated and characterized in Manitoba for the first time (Fernando and Chen, 2003).

2.4 Disease Control

Disease control strategies are based on the disease "tetrahedron" of epidemiology: pathogen effect, host effect, host-pathogen interactions, environmental and man effect (Fig. 2.3). Once the association between any two factors is broken, a disease epidemic will stop. Since the 20th century, many control methods of plant diseases have been developed, and different combinations have been devised for the protection of different crops (Zadoks and Schein, 1979). Methods to control crop diseases are divided into several categories: cultural, biological, physical and chemical (Agrios, 1990).

2.4.1 Cultural control

Crop rotation

Theoretically, rotation to non-hosts for a sufficient period allows for decomposition of infected crop stubble and a reduction in the viability of pathogen survival structures and the pathogen's ability to produce inoculum, thus eliminating a potential source of a

disease (Turkington, et al., 2000). Some practices of crop rotation have shown reduced incidence and severity of blackleg disease (Petrie, 1986b; Kaminski et al., 1997; Barbetti

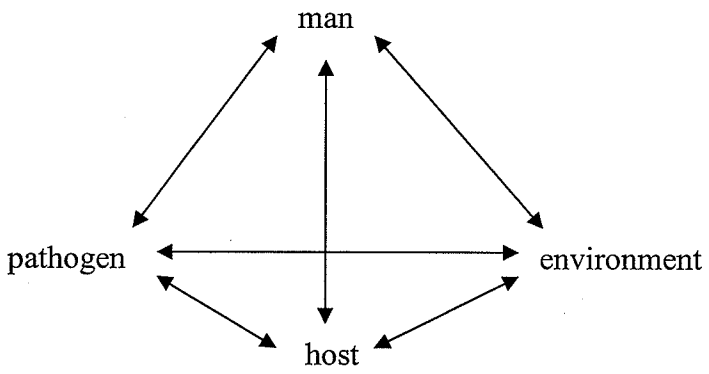


Figure 2.3. The disease tetrahedron. The base symbolizes the interaction of host, pathogen and environment. On each of these, man has various effects that are important to the development and control of epidemics (Zadoks and Schein, 1979).

and Kharbanda, 1999; Canola Council of Canada, 2002).The most damaging infections were from inoculum produced by stubble that was less than 3 year old (Kharbanda and Tewari, 1996), thus at the least, a 3-year rotation of canola with non-host crops was recommended to diminish pathogen viability in the field. However, Morrall et al. (1997 and 1999) found in their survey, that a two to five-year rotation did not effectively reduce the disease.

Tillage

Tillage systems can be grouped into three categories based on amount of stubble retained on the soil surface: conventional tillage, conservation tillage and minimum tillage. Conventional tillage systems retain less than 15% stubble on the soil surface after planting. Conservation tillage or reduced tillage systems leave 15 to 30% stubble on the soil surface, and practices that retain more than 30% stubble are considered to be minimum tillage systems, including zero tillage, mulch tillage and ridge tillage (Workneh

and Yang, 2000). It is often difficult to distinguish tillage systems between conventional and zero tillage systems.

Tillage reduces disease by burying, breaking up crop stubble and changing the physical environment where the pathogen and decomposing microorganisms exist in the soil, temperature, moisture, pH and other components (Kharbanda, 1999). In Canada, levels of blackleg disease are attributed to the accumulation of amounts and ages of infected canola stubble (Kharbanda and Tewari, 1996). Infected stubble decomposes quickly in the first 12 months (McGee, 1977). Moldboard plowing was most effective at reducing the amount of canola stubble, especially during the first two years, and stubble levels were low at the end of these years (Turkington et al., 2000). Stubble decomposition is more rapid in the soil than on the surface (Blenis et al., 1999). However, over several years of tillage, the situation may become complicated because the infected stubble already in the soil can be brought to the soil surface, and infect the current host crops. Therefore, use of different tillage systems may have both positive and negative effects at reducing blackleg disease (Turkington et al., 2000).

It is suggested that canola stubble should be buried by moldboard plowing or by deep tillage shortly after harvest or early the following spring to promote decomposition and prevent the release of ascospores in the following years. Minimum shallow tillage or zero tillage in the two following years will help reduce risk by leaving canola stubble buried in the soil (Canola Council of Canada, 2002; Gladders and Musa, 1980; Thomas, 1985).

Other methods of control

The degree of natural seed infection is usually very low, but seeds infected by blackleg are the major cause of introduction of the disease into previously uninfected areas

(Kharbanda Tewari, 1996; Wood and Barbetti, 1977). In 1989, Canada launched the first seed-testing program to control the spread of blackleg and give producers training on blackleg disease (Gugel and Petrie, 1992). The Alberta government provided free testing for all certified canola sold in the province. The spread of blackleg would have occurred at a relatively faster rate had there been no seed testing program (Kharbanda and Tewari, 1996; Harrison and Kharbanda, 1994).

Canola plants are highly susceptible to blackleg prior to the 6-leaf stage (McGee and Petrie, 1979). Changing the seeding time to avoid high levels of inoculum and/or favorable conditions for disease development can lead to reduction of disease severity (Kharbanda and Tewari, 1996). Multiple seeding times may increase the probability that at least a portion of the crop escapes serious damage (Canola Production and Management, 2002). Ascospore release by *L. maculans* is conditioned by climatic conditions, therefore strategies for adjusting the seeding time will vary from region to region.

Sanitation is useful to control blackleg disease (Bokor et al., 1975). Burning infected stubble to reduce inoculum levels has been common in Australia (Bokor et al., 1975). However, it is not recommended in Canada due to resultant air pollution and loss of organic matter.

2.4.2 Chemical control

Different combinations of fungicide seed treatments or foliar fungicide sprays are used to control blackleg disease. In Canada, seed treatment commenced in 1978 (Petrie, 1979) and is recommended even if blackleg is not found in seed lots (Thomas, 1984). Fungicide seed dressings, including Cloak, Premiere F1, Rovral ST, Vitavax RS Powder, are often

used in Canada (Canola Council of Canada, 2002). Thiram and Iprodione are used in parts of Europe and Iprodione is used in Australia (West et al., 2002). Seed treatment helps to reduce seed-borne blackleg, and avoid other problems such as seedling blight and flea beetles. However, seed treatment will not protect canola that is planted on blackleg-infested fields (Canola Council of Canada, 2002).

The application of foliar fungicides has mixed results. Ascospore release occurs continuously throughout crop growth (Hall, 1992). Thus, timing of spraying fungicides is very important to protect crops in the early period or peak period of ascospore release. There is limited potential for controlling the disease in Australia (Brown et al., 1975) and in Canada (Xi et al., 1986). It is critical to have a good disease forecasting system combined with fungicide application.

In 1994, Tilt 250 was registered for control of blackleg in canola. Application of 500 mL of Tilt 250 per hectare is recommended during the canola rosette stage. During this time, blackleg lesions develop on the leaves, but stem cankers are generally not visible. Therefore, the fungicide must be applied before there is much evidence of disease. Tilt 250 can penetrate the leaf tissue to protect against damage caused by blackleg. Application during the rosette stage protects the crop when it starts to establish yield. This protection delays the initiation of stem cankers and allows the canola plant to fill its pods more efficiently and completely. Tilt 250 will not control blackleg during the whole season. Stem cankers may be visible later in the season because the disease continuously re-infects canola plants throughout all stages of growth and development. However, this later infection usually has minimal impact on yield. Tilt 250 should be applied earlier than 60 days prior to harvest (The Growers Manual, 2002).

2.4.3 Biological control

Biological control may reduce environmental impact produced by chemicals. Partial control of virulent *L. maculans* was obtained *in vitro* and *in vivo* by using weakly virulent strains of the pathogen (Petrie, 1982; Chen and Fernando, 2003). *Paenibacillus polymyxa* (syn. *Bacillus polymyxa*) strain PKB1 produced two closely related antifungal peptides with eight amino acids and masses of 835.5 and 897.5 Da, which were able to inhibit *L. maculans* in culture, on leaves, stems and stubble (Kharbanda, 1999).

2.4.4 Cultivar resistance

The development of resistant cultivars is considered one of the most important means of blackleg control. Blackleg disease has been effectively controlled in Europe since the resistant cultivar Jet Neuf was introduced in 1977 (Rimmer et al., 1992). Most resistant cultivars now grown in Europe are derived from this cultivar. Cultivars of *B. napus* with improved resistance also were developed in Australia (Roy, 1978 and 1989; Wratten et al., 1989), and in Canada (Canola Council of Canada, 2002; Thomas, 1984; Fernando, 2001). However, the increased resistance of cultivars caused farmers to shorten rotations between canola crops, increasing inoculum level and causing serious epidemics and yield losses (West et al., 2001). In Canada, most Argentine varieties have a moderate-to-high level of resistance, but none is completely resistant. All Polish varieties are susceptible to blackleg, but the disease usually causes less damage and yield loss than in susceptible Argentine types (Canola Council of Canada, 2002). The resistance ratings of canola in Canada can be seen in Tables 2.1A and 2.1B.

Table 2.1A. Blackleg resistance rating of Argentine canola (*Brassica napus*) (Blackleg of Canola, 2002)

Resistant	44A89, 45A02, 45A03, 45A53(RR), 46A65, 46A76 (CF), Bianca 2, Conquest(RR), Foremost, Hi-Q, Impulse, Invigor 2573, Invigor 2663, LG3310, LG3366, LG3525(RR). OAC Dynamite, Q2, Quantum, Sentry
Moderately resistant	220, 500, 1134CA, 46A05, 46A52(RR), 46A73(CF), 46A74(CF), AC-H102, Agassiz, Battleford, Beacon, Bianca, Ebony, Garrison, Hero, Hudson, Hyola 428, Hyola 454(RR), Hylite 201, Bullet, Castor, Cartier BX, Challenger, Clavet, Coronet, Mercury, Neptune, PR5338(RR), Sprint, Swallow(LL), SW Legion(LL), Trailblazer, Venus, Wildcat, Zodiac BX
Moderately susceptible	1174 CA, 295 BX, 2641 LL, 1492, 2631 LL, 3640(LL), 3850(LL), 3880(LL), 45A50(RR), 45A51(RR), 45A71(CF), Exceed(LL), Frontier, Goliath, HCN14(LL), HL99, Hy-per Star 100, Impact, 46A72(CF), Alliance, Apollo, Aemor BX, Ascent, AC Invigor 2163(LL), Legacy, LG3222, LG3360, LG Dawn(RR), Magellan, Princeton, Profit, SW Arrow(RR), SW Roder(RR), Trojan
Susceptible	Bounty, Hyola 401, LG3260, OAC Springfield
Very susceptible	Allons, Westar

Table 2.1B. Resistance rating of Polish canola (*Brassica rapa*) (Blackleg of Canola, 2002)

Resistant	none
Moderately resistant	none
Moderately susceptible	none
Susceptible	100, 1007, 41P55, AC Parkland, AC Boreal, AC Sunbeam, AC Sungold, Cash, Chinook, Colt, Eldorado, Expo, Fairview, Foothills, Goldrush, Horizon Hysyn 100, Hysyn 110, Hysyn 111, Hysyn 120CS, Klondike, Maverick, Norwester, Reward, Shamrock, Spectrum, Valleyview, Westwin
Very susceptible	none

3. EFFECTS OF CROP ROTATION AND TILLAGE ON THE BLACKLEG DISEASE OF CANOLA

3.1 Abstract

The effects of a 3-year crop rotation and two tillage systems on blackleg disease of canola were determined in a field experiment carried out from 1999 through 2002. Crops in rotation were canola (*Brassica napus* L.) (C), wheat (*Triticum aestivum* L.) (W) and flax (*Linum usitatissimum*) (F). Rotations were done under conventional (T) or zero-till (Z) conditions. The number of lesions, size of lesions, and disease incidence and stem severity were assessed as disease measurements. The number and size of lesions on canola after 4-year rotation showed: CCCCT < CCCCZ and breaking continuous canola with wheat in one of the 4 year was beneficial under zero-tillage. There was no significant difference in the number of lesions between CWCCZ and CCWCZ. Disease incidence (DI) and stem severity (SS) and in 2001 and 2002 were significantly lower when canola was rotated with wheat and flax under both tillage systems. There were no significant differences in DI and SS among CCWCT, CWFCT and CWCCT, and between CWFCT and CWFCZ. Tillage significantly decreased the disease when it was performed with a simple-crop rotation, however, it did not with a two-crop rotation. In 2002, plots with rotation and conventional tillage had reduced release of ascospores and pycnidiospores relative to CCCCZ, CWCCZ and CCWCZ plots. Ascospore release occurred earlier than pycnidiospore release, from the middle of June to the beginning of July, but total trapped ascospores throughout the growth stage were much less than pycnidiospores, 38 and 584 spores/m^3, respectively. There were two peaks of pycnidiospore release at the beginning and middle of July. Ascospore and pycnidiospore release in CWFCZ plot was lower than in CWCCZ and CCWCZ plots, which were lower

than CCCCZ plot. Spore release in CWFCT, CWCCT and CCWCT plots was lower than in the CCCCT plot. Tillage significantly reduced the amount of spores released from fields. The results suggest that the appropriate combination of rotation and tillage could significantly reduce blackleg of canola in farmers' fields.

3.2 Introduction

Canola (*Brassica napus* L. and *B. rapa* L.) is one of the major oilseed crops grown worldwide. With the significant expansion of acreage sown to canola during the past two decades, concern about blackleg, the most serious disease of canola, has increased.

Blackleg disease is caused by *Leptosphaeria maculans* (Desm.) Ces. et de Not. (anamorph: *Phoma lingam* (Tode ex Fr.) Desm.). The disease can attack all parts of the plant. Symptoms first appear on cotyledons and true leaves as off-white to buff colored-lesions, round to irregular in shape, and dotted with numerous pin-head size black pycnidia. The most destructive phase of the disease is the infection of the crown. If basal infection begins early, the light brown to gray stem cankers appear from flowering onward. As the season progresses, cankers penetrate, deepen and may girdle the stem base, causing the plant to lodge. Plants slightly infected remain standing, but pod filling is seriously affected due to decreased sap flow. The fungus may also progress from the foliar lesions through the petiole to the upper area of the stem to cause lesions. Lesions can also form on siliques, which often result in the production of infected and shriveled seeds (Bokor et al., 1975; Sawatsky, 1989).

The most important source of inoculum is the stubble bearing pseudothecia from which ascospores can be released. Pseudothecia are formed on stubble in fall when moist conditions and mild temperatures prevail. Ascospores can be liberated for at least three

years after growing the original crop (McGee, 1977). Pycnidiospores, released from pycnidia as spore ooze, can be splash dispersed, but are of little importance in causing primary infection (McGee, 1977; Sawatsky, 1989). However, once infection is established by ascospores, the pycnidiospores formed in these initial lesions can contribute to secondary spread of the disease, which can result in severe epidemics providing conditions are suitable (Barbetti, 1976; Sawatsky, 1989).

Disease control methods are based on the disease "tetrahedron" of epidemiololgy, dealing with pathogen effect, host effect, host-pathogen interactions, environmental and man effect (Zadoks and Schein, 1979). When the association between any two of these factors between each other is broken, a disease epidemic will stop. Crop rotation and tillage are two methods of reducing the association between crop and pathogen, and are relatively easy for farmers to employ. Theoretically, appropriate crop rotation lengthens the time between similar host plants, so pathogen populations have time to decline. Crop rotation takes advantage of the fact that plant pathogens important on one crop may not cause problems on another crop (Kharbanda, 1999). Gracia-Garza et al. (2002) reported that 2-year crop rotations of corn (*Zea maize* L.)-soybean (*Glycine soja*), soybean-corn, winter wheat (*Triticum aestivum* L.)-soybean and soybean-winter wheat in a no-tillage system had a significant effect on reducing production of apothecia on soybean by *Sclerotinia sclerotiorum*. Germination of sclerotia was decreased in crop rotations compared with continuous soybean. Dill-Macky and Jones (2000) found that fusarium head blight (FHB) of wheat could be significantly reduced with wheat-soybean rotation and moldboard plowing. Moldboard plowing favors the decline of pathogen *F. graminearum* on wheat residue, and prevents the development of the pathogen by

burying residue. Bockus et al. (1992) showed that both wheat-sorghum (*Sorghum bicolor* L.) rotation and plowing significantly decreased severity of tan spot in winter wheat. Wrather and Kendig (1998) found disk-tillage or moldboard plowing significantly reduced the soil population density of *Macrophomina phaseolina* in a soybean field.

Individual diseases react differently to conditions induced by rotation and tillage systems. Bailey et al. (1992) reported that common root rot symptoms decreased with reduced tillage in three of four years under minimum-till or zero-till compared to conventional tillage. Another study showed that common root rot was reduced significantly in three to six years under reduced tillage (Tinline and Spurr, 1991). Disease development results from an interaction between a pathogen, and its host and environment. Each disease has unique requirements for development and it is not possible to predict the development of one disease utilizing the general knowledge of another disease. There are few publications about the effects of crop rotation and tillage on the blackleg disease of canola. Available studies are based on surveys, which reported that a long rotation (> 5 years) reduced incidence of the blackleg disease on canola crops (Kaminski et al., 1996; Khangura and Barbetti, 2001; Morrall et al., 1999). A preliminary experiment showed that a three-year rotation and tillage had little effect on the disease (Martin et al., 2001).

The objectives of this research were (1) to systematically study the effects of crop rotation and tillage on blackleg disease of canola, (2) to understand the progress of the disease under different tillage-rotation systems and the effects of crop rotation and tillage on inoculum availability and spore release, (3) to establish a reasonable rotation period

under different tillage systems and provide a foundation for future disease management strategies to be implemented.

3.3 Materials and methods

3.3.1 Experimental design and materials

The study was conducted at the University of Manitoba's Carman Research Station, Carman, Manitoba, Canada from 1999 to 2002. The soil type was a Rignold loam. A split-plot design with three replications was used with conventional till (T) and zero till (Z) as main plots, and canola (C) rotated with wheat (W) and flax (F) as subplots. The experimental field size was 155m × 81m. There were 24 plots; each plot was 10m × 4m. The distance between plots was 15m, and 10m between a plot and border of the field. The canola cultivar was Westar (susceptible to blackleg pathogen), the wheat cultivar was AC-Barrie and the flax was cultivar Norlin. Fall rye was sown as the border crop. Canola was seeded on June 02, 1999, May 17, 2000, May 30, 2001 and May 30, 2002; wheat and flax were seeded the same day in the second and third years. In 1999, canola plants were inoculated by spraying with a blackleg isolate (PG-2 isolate 86-12), pycnidiospore suspension at 1×10^7 spores/mL at the 3-leaf stage. Conventional tillage was carried out using a deep tiller with tine harrow after canola was harvested in fall and using a cultivator with tine harrow and coil packer in the following spring in 2002. There were eight treatments in total: CCCCZ, CCCCT, CWCCZ, CWCCT, CCWCZ, CCWCT, CWFCZ and CWFCT.

In 2001, disease incidence (DI) and stem severity (SS) were investigated at canola filling stage (seeds in pods turn yellow). In 2002, the number of infected plants, number of infected leaves per plant and number of lesions per plant were recorded on July 01, 05,

09, 13 and 17; percent leaf coverage with lesions was measured on July 07, 11, 15, 19 and 23. Measurements were carried out in the following ways:

Infected plants, leaves and lesion number: Sampling was done by walking through the plots in a zigzag line composed of seven segments of approximately 2.5 m in length. The angle between two neighboring segments was approximately 60°. Sampling started 0.6m into the plot from the end and 1.0m away from the side of the plot. Four canola plants were selected equidistant from each other along each segment line except the last segment line in which five plants were selected the same way. There were in total 29 plants selected for measurement.

Lesion size: In a 1m × 1m quadrat in the center of each canola plot, all plants in which the 4th leaf from the top was infected by blackleg were marked on July 07. The marked 4th leaves were photographed with a digital camera. The pictures were transferred into a computer, and the lesion size as a percentage of the total leaf was calculated using the ASSESS program (Assess, 2002).

Disease incidence and stem severity: These factors were assessed in ten 3m lines across the width of the plot. The lines were 1m apart and started 0.5m away from the edge of the plot. Ten plants were sampled at canola filling stage (seeds in pods turn yellow) along each line with approximately 0.3m between each sample for the evaluation of disease incidence and stem severity.

Spore trapping: In 2002, blackleg spores were trapped every day from June 21 to July 30. Rotorod impaction spore samplers (Aerobiology Company, Nepean, Ontario, Canada) were used for spore trapping and consisted of a sampling head, rain shelter and rebar. The sampling head was designed to support two removable clear polystyrene rods that had a

sampling surface of 1.59 mm by 32 mm. When the sampler was at rest the rods were retracted inside the sampler head. When the motor in the head spins, centrifugal force causes the rods to extend downwards at a 90° angle. Spores impact against the leading surfaces. Rods were coated with a thin layer of petroleum jelly to catch spores. Eight rotorod impaction spore samplers were set up in one replicate plot of each treatment. A Campbell Scientific CR 10 datalogger was programmed to run the rotorods for five minutes out of each hour. The rotorods were replaced every 24 hours and the spores on the rotorod were counted under a microscope (400×).

3.3.2 Analysis

a. The number of infected plants: Total number of infected plants at each sampling time.
b. The number of infected leaves per plant: (total number of infected leaves on infected plants) / (number of all infected plants).
c. The number of lesions per plant: (total number of lesions on all infected plants) / (number of all infected plants).
d. Percent leaf coverage with lesions: (lesion size / leaf area) × 100%. Determined using ASSESS program (Assess, 2002).
e. AUDPC (the area under the disease progress curve) the number of infected plants: $\Sigma \{[(Yi + Y_{i-1}) / 2] (t_i - t_{i-1})\}$. Yi is number of infected plants at each evaluation time; Y_{i-1} is number of infected plants at previous evaluation time; and $t_i - t_{i-1}$ is the time duration (Shaner and Finney, 1977).
f. Disease incidence (%): [(number of infected plants) / (total number of plants sampled)] × 100%.

g. Stem severity: using the standard 0-5 scale: 0 - no infection; 1 - lesion area is less than 25% of a cross-section area of crown; 2 – 25-50%; 3 – 51-75%; 4 – 76-100%; 5 - plant dead. Stem severity was evaluated as average stem severity over all the infected plants sampled.

h. Spore concentration (SC): total spores / (RPM × K × Min). RPM – rotations per minute; K – rod constant 0.097; Min – sampling period (minutes).

i. Statistical analyses were conducted using The SAS System 8.2 (SAS Institute Inc., Cary, NC, USA). The Fisher's LSD (least significant difference) test was selected to examine the differences among treatments ($P \leq 0.05$).

3.4 Results

3.4.1 Year 2001

Rotation and tillage had significant effects on reducing the disease (Table 3.1A and B). The continuous canola crop under zero tillage (CCCZ) had significantly higher disease incidence and stem severity caused by blackleg. The lowest disease incidence and stem severity were observed in tilled plots, when canola was rotated with wheat (CWCT). Zero-till plots always had significantly higher disease incidence and stem severity, compared to tilled plots with or without rotation. The interaction between rotation and tillage was significant (Table 3.1A and B). Effects of tillage with different rotation systems or effects of rotation with different tillage systems were different.

3.4.2 Year 2002

In 2002 there were no visible symptoms on leaves when canola was rotated with wheat and flax whether they were on zero-till or tilled plots. Also, no leaf symptoms when canola was rotated only with wheat in the second or third year and were on tilled plots.

Table 3.1A. Blackleg disease incidence (%) on canola under different rotation and tillage systems at maturity in 2001

Rotation	Tillage			Rotation × Tillage
	Conventional till (T)	Zero till (Z)	LSD	
CCC	57.0 a A	81.0 a B	17.1	-
CWC	49.0 b A	63.0 b B	8.7	-
LSD	5.1	14.2	-	-
**		**		**

C=canola; W=wheat; different small letters within a column indicate significant difference between different rotation systems; and different capital letters with a row indicate significant difference between different tillage systems according to the Fisher's least significant difference (LSD) test ($P \geq 0.05$). ** - effect of rotation, tillage or their interaction is significant at 0.01 level. Rotation × Tillage – interaction between rotation and tillage.

Table 3.1B. Blackleg disease severity on canola stem under different rotation and tillage systems at maturity in 2001

Rotation	Tillage			Rotation × Tillage
	Conventional till (T)	Zero till (Z)	LSD	
CCC	3.22 a A	3.57 a B	0.24	-
CWC	2.91 b A	3.41 b B	0.38	-
LSD	0.21	0.11	-	-
**		**		**

C=canola; W=wheat; different small letters within a column indicate significant difference between different rotation systems; and different capital letters with a row indicate significant difference between different tillage systems according to the Fisher's least significant difference (LSD) test ($P \geq 0.05$). ** - effect of rotation, tillage or their interaction is significant at 0.01 level. Rotation × Tillage – interaction between rotation and tillage.

Therefore, only those rotations in which disease occurred, CCCCZ, CWCCZ, CCWCZ and CCCCT will be discussed in the following results section.

The number of infected plants: Effects of rotation, tillage and their interaction on the number of infected plants were significant from July 01 through 17 (Table 3.2). Number of infected plants increased over the growing season in all treatments in which disease occurred (Table 3.2). The increase was more evident after July 09. Among the different treatments, the number of infected plants in rotation CCCCZ was significantly higher than numbers of infected plants in rotation CWCCZ, CCWCZ and CCCCT from July 01 to July 17. There were no significant differences between treatments CWCCZ and CCWCZ except on the last sampling date (Table 3.2).

According to AUDPC values, the most infected plants were in CCCCZ plots from July 01 to 17 (Fig. 3.1). There was no significant difference in AUDPC between CWCCZ and CCWCZ from July 01 to 17.

The number of infected leaves per plant: Effects of rotation, tillage and their interaction on the number of infected leaves per plant were significant from July 01 through 17 (Table 3.2). There were no significant differences among CCCCZ, CWCCZ and CCWCZ on July 01, but CCCCZ had significantly higher number of infected leaves than other rotations from July 01 through 17 except July 13 (Table 3.2). There were no significant differences between CWCCZ and CCWCZ during the growing season.

The number of lesions per plant: Effects of rotation, tillage and their interaction on the number of lesions per plant were significant (Table 3.2). The number of lesions per plant in the CCCCZ rotation was significantly higher than in the CWCCZ and CCWCZ

Table 3.2. Numbers of infected plants, leaves and lesions of canola by blackleg disease under different rotation and tillage systems

	July 01	July 05	July 09	July 13	July 17
Number of infected plants					
Rotation	**	**	**	**	**
Tillage	**	**	**	**	**
Rotation × Tillage	**	**	**	**	**
CCCCZ	8.3 aA	9.7 aA	12.0 aA	15.3 aA	23.7 aA
CWCCZ	5.7 b	7.3 b	9.0 b	12.0 b	18.0 b
CCWCZ	4.3 b	5.7 b	6.7 c	10.7 b	16.0 c
CCCCT	2.3 B	4.0 B	5.3 B	9.3 B	14.3 B
LSD (Z-Z)	1.81	1.70	2.04	2.26	2.31
LSD (Z-T)	3.03	2.70	4.10	3.42	4.55
Number of infected leaves / plant					
Rotation	**	**	**	**	**
Tillage	**	**	**	**	**
Rotation × Tillage	**	**	**	**	**
CCCCZ	1.46 aA	1.96 aA	2.18 aA	1.46 aA	1.25 aA
CWCCZ	1.41 a	1.52 b	1.56 b	1.34 b	1.19 b
CCWCZ	1.38 a	1.46 b	1.44 b	1.42 ab	1.19 b
CCCCT	1.00 B	1.22 B	1.11 B	1.18 B	1.11 B
LSD (Z-Z)	0.25	0.19	0.18	0.09	0.03
LSD (Z-T)	0.20	0.47	0.53	0.16	0.07
Number of lesions / plant					
Rotation	**	**	**	**	**
Tillage	**	**	**	**	**
Rotation × Tillage	**	**	**	**	**
CCCCZ	1.93 aA	2.71 aA	2.90 aA	1.97 aA	1.91 aA
CWCCZ	1.63 b	1.93 b	1.98 b	1.56 b	1.45 b
CCWCZ	1.62 b	1.77 b	1.89 b	1.57 b	1.35 b
CCCCT	1.00 B	1.33 B	1.20 B	1.27 B	1.21 B
LSD (Z-Z)	0.21	0.54	0.16	0.24	0.17
LSD (Z-T)	0.39	0.88	0.91	0.35	0.29

C=canola; W=wheat; Z=zero-tilled; T=tilled; different letters within a column indicate significant differences according to the Fisher's least significant difference (LSD) test ($P \geq 0.05$); capital letters and small letters indicate differences between conventional and zero till in the same rotation systems, and differences among rotations in the same tillage systems, respectively; their corresponding LSDs are LSD (Z-T) and LSD (Z-Z). ** - effect of rotation, tillage or their interaction is significant at 0.01 level. Rotation × Tillage – interaction between rotation and tillage.

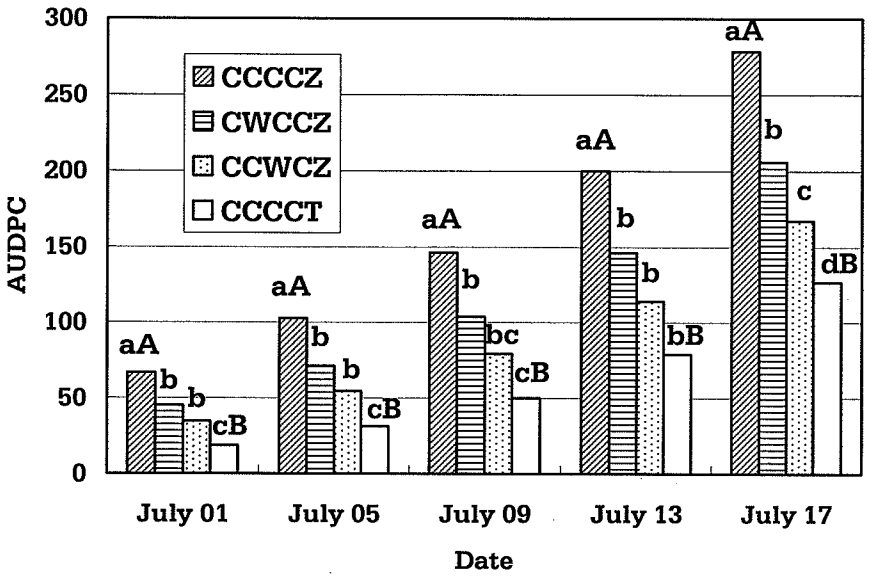


Figure 3.1. The area under the disease (number of infected plants by *Leptosphaeria maculans*) progress curve (AUDPC) under different rotation and tillage systems in 2002. C=canola; W=wheat; F=flax; Z=zero-tilled; T=tilled; same small letters on the same day indicate no significant difference between different rotation systems; and different capital letters with a row indicate significant difference between different tillage systems according to the Fisher's least significant difference (LSD) test ($P \geq 0.05$). LSD=12.3, 19.8, 28.7, 36.1 and 33.4 for difference between different rotation systems on July 01, 05, 09, 13 and 17, respectively; and 21.1, 30.5, 38.8, 51.7 and 66.4 for difference between different tillage systems.

rotations during the growing season (Table 3.2). There were no significant differences between CWCCZ and CCWCZ throughout the growing season.

Percent leaf coverage with lesions: Effects of rotation, tillage and their interaction on percent leaf coverage with lesions were significant (Table 3.3). CCCCZ rotation had significantly higher percent leaf coverage with lesions than CWCCZ, CCWCZ and CCCCT rotations from July 07 through 23; and percent leaf coverage with lesions in CWCCZ was significantly higher than CCWCZ except on July 23 (Table 3.3).

Disease incidence: Effects of rotation, tillage and their interaction on disease incidence were significant (Table 3.4A). Disease incidence scores in the CCCCZ rotation was significantly higher than in rotations CWCCZ, CCWCZ and CWFCZ (Table 3.4A). Disease incidence score in the CWCCZ rotation was significantly higher than in the CCWCZ rotation, which was significantly higher than in the CWFCZ rotation. The CCCCT rotation had a significantly higher disease incidence score than rotations CWCCT, CWFCT and CCWCT. There were no significant differences among CWCCT, CWFCT and CCWCT. Tillage showed significant effects on reducing disease incidence in all the rotations except the CWFC rotation ($P \leq 0.05$).

Stem severity: Effects of rotation, tillage and their interaction on stem severity were significant (Table 3.4B). Although leaf lesions were found in only four treatments, symptoms could still be observed on the cross sections of the stems in all eight treatments. Stem severity in plants from the CCCCZ rotation was significantly higher than all other rotations CWCCZ, CCWCZ and CWFCZ (Table 3.4B). In zero-till plots a break of one year brought a significant reduction in stem severity, and a break of two years (rotation CWFCZ) reduced stem severity to the lowest levels. There was no

Table 3.3. Percent leaf coverage with blackleg lesions on canola under different rotation and tillage systems

	July 07	July 11	July 15	July 19	July 23
Rotation	**	**	**	**	**
Tillage	**	**	**	**	**
Rotation × Tillage	**	**	**	**	**
CCCCZ	3.86 aA	6.21 aA	10.65 aA	17.96 aA	20.89 aA
CWCCZ	2.91 b	5.03 b	8.61 b	15.67 b	17.78 b
CCWCZ	2.43 c	4.34 c	7.72 c	14.54 c	16.47 b
CCCCT	1.68 B	3.41 B	6.13 B	12.45 B	14.21 B
LSD (Z-Z)	0.26	0.34	0.64	0.89	1.74
LSD (Z-T)	1.02	1.13	2.15	3.01	3.62

C=canola; W=wheat; Z=zero-tilled; T=tilled; different letters within a column indicate significant differences according to the Fisher's least significant difference (LSD) test ($P \geq 0.05$). capital letters and small letters indicate differences between conventional and zero till in the same rotation systems, and differences among rotations in the same tillage systems, respectively; their corresponding LSDs are LSD (Z-T) and LSD (Z-Z). ** - effect of rotation, tillage or their interaction is significant at 0.01 level. Rotation × Tillage – interaction between rotation and tillage.

Table 3.4A. Blackleg disease incidence (%) on canola under different rotation and tillage systems at maturity in 2002

Rotation	Tillage			Rotation × Tillage
	Conventional till (T)	Zero till (Z)	LSD	
CCCC	49.3 a A	79.3 a B	13.6	-
CWCC	21.5 b A	61.0 b B	17.5	-
CCWC	16.3 b A	56.7 c B	19.2	-
CWFC	21.0 b A	18.7 d A	8.4	-
LSD	12.5	4.0	-	-
**		**		**

C=canola; W=wheat; F=flax; different small letters within a column indicate significant difference among different rotation systems; and different capital letters with a row indicate significant difference between different tillage systems according to the Fisher's least significant difference (LSD) test ($P \geq 0.05$). ** - effect of rotation, tillage or their interaction is significant at 0.01 level. Rotation × Tillage – interaction between rotation and tillage.

Table 3.4B. Blackleg disease severity on canola stem under different rotation and tillage systems at maturity in 2002

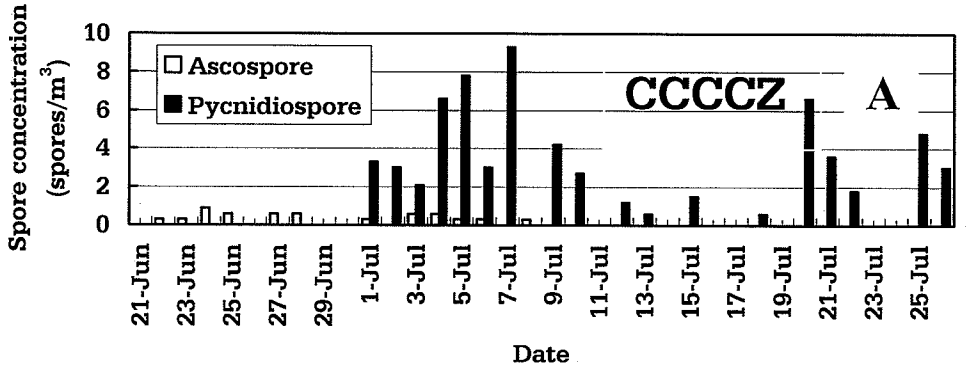
Rotation	Tillage			Rotation × Tillage
	Conventional till (T)	Zero till (Z)	LSD	
CCCC	1.34 a A	3.07 a B	0.87	-
CWCC	1.18 b A	2.01 b B	0.40	-
CCWC	1.04 b A	1.94 b B	0.39	-
CWFC	1.06 b A	1.07 c A	0.33	-
LSD	0.15	0.64	-	-
**		**		**

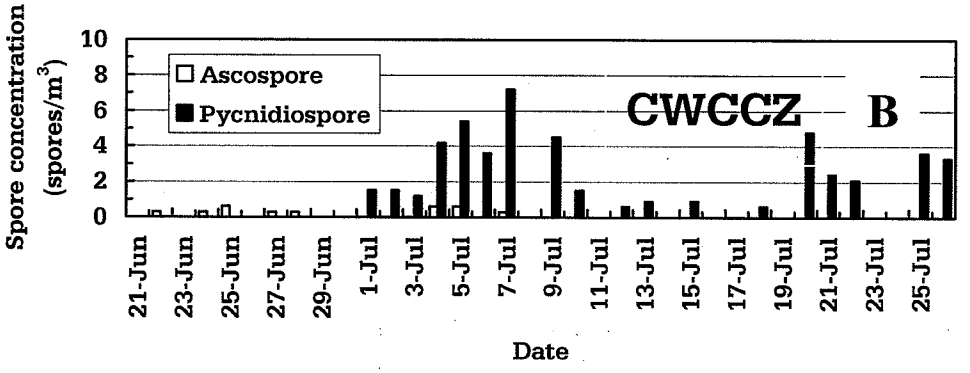
C=canola; W=wheat; F=flax; different small letters within a column indicate significant difference among different rotation systems; and different capital letters with a row indicate significant difference between different tillage systems according to the Fisher's least significant difference (LSD) test ($P \geq 0.05$). ** - effect of rotation, tillage or their interaction is significant at 0.01 level. Rotation × Tillage – interaction between rotation and tillage.

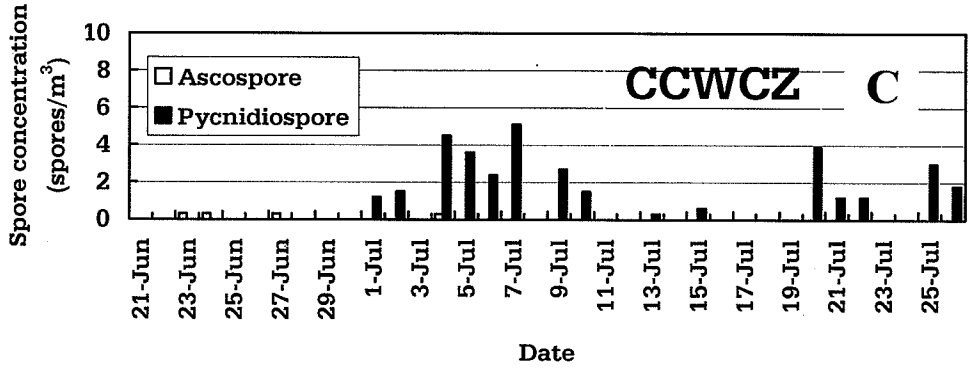
significant difference between CWCCZ and CCWCZ. Stem severity in plants from the CCCCT rotation was significantly higher than CWCCT, CWFCT and CCWCT rotations. There were no significant differences among CWCCT, CWFCT and CCWCT. Tillage showed significant effects on reducing stem severity with all rotations except the CWFC rotation ($P \leq 0.05$).

The number of spores: Numbers of ascospores and pycnidiospores trapped were higher in CCCCZ, CWCCZ and CCWCZ rotations than in CWFCZ rotation (Fig. 3.2A, B, C and D). Ascospores were trapped from the middle of June to the beginning of July, approximately eight days earlier than the first pycnidiospores trapped. The number of ascospores trapped was much lower than pynidiospores. The total number of ascospores and pycnidiospores trapped from all the plots during the whole assessing period were 38 and 584 per m^3, respectively. There were two peak periods when pycnidiospores were trapped, in the beginning and middle of July, when infection on leaves was developing (Fig. 3.2A, B, C, and 3.3A).

The numbers of spores trapped were lower in plots with rotation and tillage than with no rotation and no tillage (Fig. 3.2A, B, C, D, and 3.3A, B, C, D). In plots under crop rotations, ascospores and pycnidiospores trapped in the rotation CWFCZ plot were much lower than in plots with rotations CWCCZ and CCWCZ, which had lower spore amounts than CCCCZ rotation (Fig. 3.2A, B, C, D). Number of spores trapped in plots with rotations CWFCT, CWCCT and CCWCT was less than in the plot with rotation CCCCT. Spore release in rotations under conventional tillage was less than in rotations under zero tillage. There were ascospores trapped in plots with rotations under zero tillage except where the rotation had a two year break from canola, CWFCZ (Fig. 3.2A, B, C, D). Low


Spore concentration (spores/m3)
Ascospore
Pycnidiospore
CCCCZ
A
Date


Spore concentration (spores/m3)
Ascospore
Pycnidiospore
CWCCZ
B
Date


Spore concentration (spores/m3)
Ascospore
Pycnidiospore
CCWCZ
C
Date

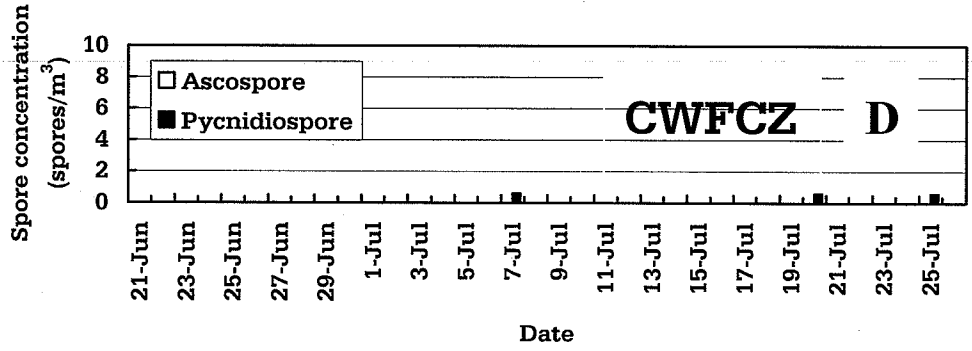
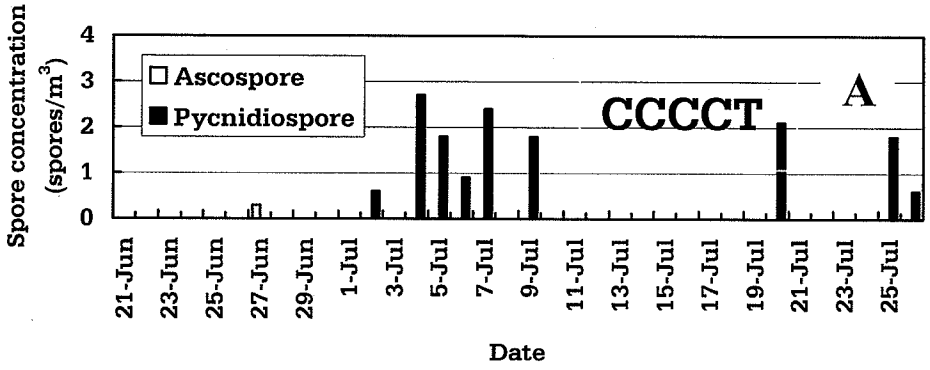
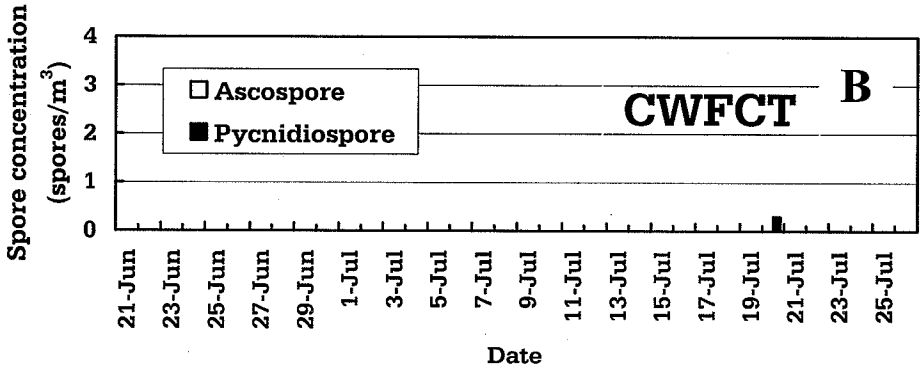
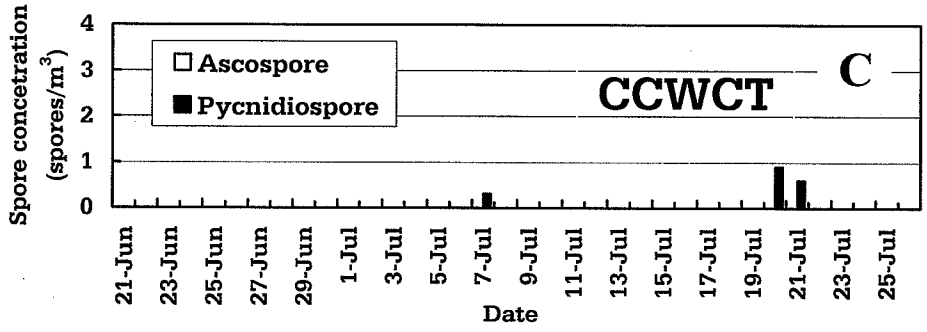

Spore concentration (spores/m3)
Ascospore
Pycnidiospore
CWFCZ
D
Date

Figure 3.2. Daily spore count of ascospores of *L. maculans* and pycnidiospores of *P. lingam* in 2002 trapped from plots under different rotations with zero tillage (Z). Pycnidiospores and ascospores were calculated as spores/m^3. C = canola; W = wheat; F = flax; Z = zero-tilled. A: CCCCZ rotation; B: CWCCZ rotation; C: CCWCZ rotation; D: CWFCZ rotation.


Spore concentration (spores/m3)
Ascospore
Pycnidiospore
CCCCT
A
Date


Spore concentration (spores/m3)
Ascospore
Pycnidiospore
CWFCT
B
Date


Spore concetration (spores/m3)
Ascospore
Pycnidiospore
CCWCT
C
Date

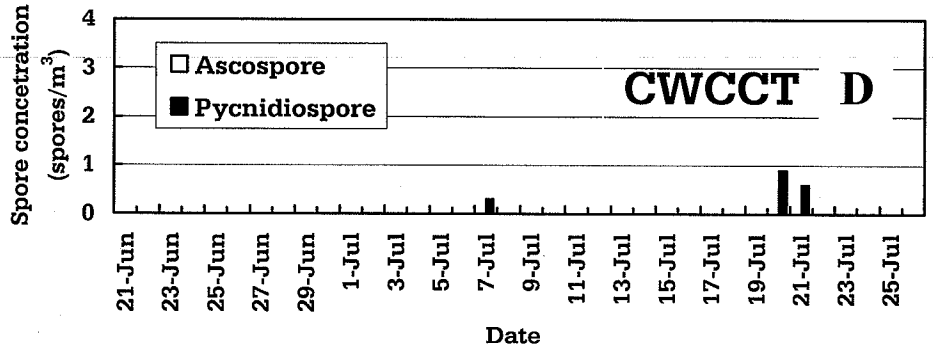

Spore concetration (spores/m3)
Ascospore
Pycnidiospore
CWCCT
D
Date

Figure 3.3. Daily spore count of ascospores of *L. maculans* and pycnidiospores of *P. lingam* in 2002 trapped from plots under different rotations with conventional tillage (T). Pycnidiospores and ascospores were calculated as spores/m^3. C = canola; W = wheat; F = flax; T = conventional till. A: CCCCT rotation; B: CWFCT rotation; C: CCWCT rotation; D: CWCCT rotation.

numbers of ascospores were also trapped from tilled plots under continuous canola. However, pycnidiospores were trapped from plots with all rotations (Fig. 3.2A, B, C, D, and 3,3A, B, C, D).

3.4.3 Comparison of disease incidence and stem severity between 2001 and 2002

Under zero till condition, disease incidence and stem severity in the rotation in which canola was grown continuously for four years were not significantly different from a rotation in which canola was grown for three years in a row (Table 3.5 and 3.6). When rotation to wheat was carried out in the second year during four years, disease incidence in canola grown in the fourth year was not significantly different from the third year, but stem severity was different.

Tillage had significant effects on reducing disease incidence and stem severity of canola (Table 3.5 and 3.6). When canola was grown for four consecutive years, disease incidence and stem severity were significantly decreased by tillage compared to canola grown for three consecutive years. When canola was rotated to wheat in the second year of four years, both disease incidence and stem severity were significantly reduced.

3.5 Discussion

This study showed that crop rotation had significant effects on reducing blackleg disease. Canola rotation with wheat significantly decreased disease incidence by 18% in the zero-tilled plots and by 8% in the tilled plots. When canola was rotated with wheat and flax in either the tilled or zero-tilled plots, blackleg disease was decreased and was not different from the canola-wheat rotation. Disease incidence scores in rotations CWFCT and CWFCZ were decreased by 28% and 44% compared to rotations CWCT and CWCZ, respectively. Results of the number of infected plants, infected leaves per

Table 3.5. Comparison of blackleg of canola disease incidence and stem severity under different rotations in zero-tilled plots in 2001 and 2002

	CCCCZ (2002)	CCCZ (2001)	LSD	CWCCZ (2002)	CWCZ (2001)	LSD
DI (%)	79.3a	80.6a	10.2	61.0a	63.7a	7.3
SS	3.1a	3.6a	0.9	2.0a	3.4b	1.1

C=canola; W=wheat; Z=zero-tilled; DI=disease incidence; SS=stem severity; different letters within a row between two treatments indicate significant differences according to the Fisher's least significant difference (LSD) test ($P \geq 0.05$).

Table 3.6. Blackleg disease incidence and stem severity of canola under different rotations in tilled plots in 2001 and 2002

	CCCCT (2002)	CCCT (2001)	LSD	CWCCT (2002)	CWCT (2001)	LSD
DI (%)	49.3a	57.2b	5.4	21.5a	48.9b	4.2
SS	1.3a	3.2b	0.6	1.2a	2.9b	0.5

C=canola; W=wheat; T=tilled; DI=disease incidence; SS=stem severity; different letters within a row between two treatments indicate significant differences according to the Fisher's least significant difference (LSD) test ($P \geq 0.05$).

plant, lesions per plant, percent leaf coverage with lesions, and stem severity suggested that canola rotation with wheat and flax could significantly reduce the pathogen levels on the canola stubble infected by *L. maculans* both under conventional and zero tillage practices. Disease incidence scores in the CWCCZ rotation were significantly higher than in plots with the CCWCZ rotation, which indicates infected stubble from the previous crop may be a major contributor to the disease spread of the current year. However, disease incidence scores in the CCCCZ rotation were also significantly higher than in the CWCCZ rotation, indicating that just one extra year with canola infected stubble may be important for inoculum build-up. In a study to investigate pathogen persistence we observed that pathogen levels decreased in 12 months when stubble was left on the soil surface (Guo and Fernando, unpublished). Few articles have been published concerning the effects of crop rotation on blackleg disease (Martin et al., 2001), but some reports of disease surveys and preliminary experiments have addressed this (Kharbanda, 1999; McGee, 1977; Morrall et al., 1999; Petrie, 1986a; Turkington and Clayton, 2002; West et al., 2001). Petrie (1986a) showed that a 3-year rotation between canola crops reduced the severity of blackleg disease. Turkington and Clayton (2002) reported that, under conventional tillage condition, a 4-year-rotation helped to limit the level of blackleg inoculum. Morrall et al. (1999) found in their survey that, when canola was rotated to non-host crops for more than five years, blackleg disease showed a decreasing trend, but it did not when rotation was performed for two or three years. Other studies, however, showed no significant effect of crop rotation at reducing the disease (Martin et al., 2001). The fields they investigated were considered to be infected by air-borne ascospores of *L. maculans* from neighboring fields.

A preliminary survey indicated that there was no significant difference in the amount of disease between the long rotations (no canola in the previous four years) and short rotations (canola, wheat and canola) (McGee, 1977). The physical amount of canola stubble was a key factor in spore density. The volume of stubble from the previous crop was more than ten times that of older stubble. The stubble decomposed quickly during the first 12 months, leaving the woody root section, which remained intact for at least four years (McGee, 1977). This observation is consistent with the results of our study. We found that the number of spores trapped from plots in which canola was grown in the previous year, was much higher than from plots in which flax or wheat was grown in the previous year.

Survival of stubble-borne pathogens has been closely correlated with stubble decomposition (Blenis et al., 1999; Kharbanda, 1999; Wrather and Kending, 1998). Tillage affected pathogens in three ways: 1) breaking up infected stubble to promote its decomposition, 2) improving the environment for stubble-decomposing microorganisms to survive in the soil, e.g., by burying them to protect them from drying out at the soil surface, and 3) changing (for better or worse) the conditions in which pathogens exist in the soil (Kharbanda, 1999). Turkington and Clayton (2002) and Blenis et al. (1999) showed that canola stubble decomposition was more rapid in the soil than on the surface. Guo and Fernando (unpublished) described the persistence of *L. maculans* on canola stubble at the surface and at depths of 5 and 10 cm in the soil. Eight, nine and ten months after stubble was buried, pathogen survival at a depth of 10 cm in the soil was significantly lower than pathogen viability at a depth of 5 cm, and the latter was significantly lower than on the soil surface. Turkington and Clayton (2002) reported that

with tillage practice over several years, the problem could become complicated because the infected stubble already in the soil could be brought to the soil surface and spread and infect the current host crops. A preliminary study showed that disease incidence did not significantly vary among conventional, minimum and zero tillage treatments in three-year rotation plots (Martin et al., 2001).

In our investigation of infected leaves, leaf lesions were only found in plots with rotations CCCCZ, CWCCZ, CCWCZ and CCCCT. However, symptoms were observed on the cross sections of the stems in all the treatments. Hammond et al. (1985) reported that when colonizing the lamina and petiole, the pathogen hyphae were predominantly limited to intercellular spaces in the parenchyma and the adjacent cell did not appear obviously necrotic. Therefore, stem cankers can also be produced through a systemic pathway of infection.

The analyses of infected plants and leaves showed there was no significant difference between rotations CWCCZ and CCWCZ. However, lesion size was significantly higher in rotation CWCCZ than in rotation CCWCZ. It is speculated that there was more infected stubble from which ascospores could be produced, offering more opportunities for early infection on healthy plants in plots with rotation CWCCZ than in plots with rotation CCWCZ. Thus, larger lesions were formed on the leaves in rotation CWCCZ.

This study showed that tillage had significant effects on reducing blackleg disease. Disease incidence in zero till plots with three years of canola was 24% higher than in tilled plots. The difference was 14% when canola was broken with a one year rotation. Disease incidence in the tilled plots with continuous canola in four years decreased by 30% compared to the same rotation under zero tillage. Disease incidence decreased by

39.5% and 40.4%, respectively, on the tilled plots with wheat rotated in the second year and third year in four years compared to the same rotation on zero tillage. In the same rotations, daily ascospores and pycnidiospores released from tilled plots were lower than from no-tilled plots. Tillage reduced the amount of infected stubble available for releasing spores. Further study is needed to prove whether the significant differences among the numbers of lesions, infected plants, leaves, and lesion size among the two tillage systems resulted from the differences in amount of infected canola residue available to release ascospores. The effects of tillage on blackleg disease will be clarified with a further understanding of the following aspects: a. Effects of different types of tillage systems on movement and distribution of canola residue at different depths in different soils; b. Decomposition rate of canola residue at different depths in different soils over time and the effects of soil moisture and temperature; c. Persistence of the blackleg pathogen at different depths in different soils over time.

Our study showed that rotation and tillage had significant effects on reducing blackleg disease. With the interaction between rotation and tillage, tillage showed different effects combined with different rotation systems, and rotation had different effects with different tillage systems. Canola rotation with wheat and flax can largely reduce blackleg disease and gain the same effects on both tilled and zero-tilled plots. Conventional tillage had a significant effect on reducing the disease when it was performed with simple rotations. Zero tillage could be applied in complicated rotations. Crop rotation and tillage can effectively reduce amount of spores released, and decrease the possibility of crop being infected. This suggests that appropriate rotation to non-host crops with tillage could significantly reduce blackleg disease of canola in farmers' fields.

4. PERSISTENCE OF *LEPTOSPHAERIA MACULANS* IN SOIL

4.1 Abstract

A study on the persistence of *L. maculans* in the soil was conducted at the Carman Research Station from 2000 to 2002. Survival of the pathogen was assessed at 0, 5 and 10-cm depths in three types of soil: loam (in 2001 and 2002), sand and clay (in 2002), with three replicates. In 2001, survival of the pathogen was significantly lower nine months after stubble was left on the soil surface or buried at 5-cm depth than at seven and eight months. There were no differences in the frequency of isolations from stubble on the surface and at 5-cm depth between seven and eight months, and at 10-cm depth at seven, eight and nine months. With soil depth from 0 to 10 cm, survival of the pathogen significantly decreased during the seventh and eighth months after stubble burial. The pathogen was not isolated from stubble buried at 10 cm eight and nine months after burial. In 2002, under all conditions of soil type and burial depth, pathogen survival was significantly lower at nine months after stubble burial than at seven or eight months. On the three types of soil, the pathogen survival significantly decreased with soil depth from 0 to 10 cm seven, eight and nine months after stubble burial. Of the three soil depths, the viability in clay was significantly lower than in sand seven months after burial, and in loam and sand eight and nine months after burial. The experiment helped explain the positive effects of conventional tillage to reduce pathogen inoculum, by assessing persistence of the pathogen on stubble on the soil surface or buried at 5 or 10 cm in the soil.

4.2 Introduction

Tillage may shorten the time pathogens can survive on infected host material by burying and breaking up stubble in the soil, reducing the potential for infection by stubble-borne pathogens that release air-borne spores (Kharbanda, 1999). Tillage is being experimentally used to reduce various crop diseases: tan spot of winter wheat caused by *Pyrenophora tritici-repentis* (Bockus and Claassen, 1992; Sutton and Vyn, 1990; Wright and Sutton, 1990); fusarium head blight primarily caused by *Fusarium graminearum* (Dill-Macky and Jones, 2000; Miller et al., 1998; Seaman, 1982; Sutton and Vyn, 1990; Teich and Nelson, 1984); and stem rot of soybean caused by *Sclerotinia sclerotiorum* (Gracia-Garza et al., 2002; Grau and Radke, 1984; Subbarao, et al., 1996; Williams and Stelfox, 1980; Workneh and Yang, 2000). However, few studies have been conducted on the effects of tillage on blackleg disease of canola (Kharbanda, 1999; Martin et al., 2001). Turkington et al. (2000) reported that blackleg infected canola stubble released fewer pycnidiospores when incorporated into the soil. Fall mold-board plowing effectively reduced the amount of canola residue in the first two years, although stubble amounts were very low under all tillage systems after three years. Blenis et al. (1999) suggested that buried canola straw underwent 1.6 to 2 times as much decomposition as straw on the soil surface. Baird et al. (1999) reported that isolation frequencies of *L. maculans* on the stubble buried in loam soil decreased over time from 85% before burial to 15% two months, 3% five months, and no isolation of the pathogen eleven months after burial.

The objectives of this study were to examine survival of the blackleg pathogen at different depths in different types of soil in western Canada in an effort to further understand the effects of tillage on blackleg of canola.

4.3 Materials and methods

4.3.1 Field

The experiment was conducted at the University of Manitoba's Carman Research Station, Carman, Manitoba, from 2000 to 2002. The experiment included three sub-experiments which were performed in three types of soils: sand, loam and clay. A completely random block design was used in each sub-experiment. The treatment included three depths (0, 5 and 10 cm) in the soil.

In November 2000, infected stubble with typical blackleg lesions (black-dot structures identified as pseudothecia by observation and identification of *L. maculans* its ascospores under a microscope) was collected from canola plots under zero till. The stubble was cut into 1-cm piece sections containing pseudothecia. Stubble pieces were mixed up well, and pieces were randomly selected and put into one of ten cells of a mesh bag (Fig. 4.1). Bags were buried at 0, 5 and 10-cm depths in loam soil. There were three replicates. On May 30, June 30 and July 30 of 2001, the buried stubble was pulled out from the field and examined to determine fungal survival. The experiment from 2000 to 2001 was performed in a completely random block design.

In November 2001, the same methods were repeated and stubble buried at three depths (0, 5, 10 cm) in three different types of soil: Plum Coulee clay (poorly-drained; particles are less than 0.002 mm in diameter), Rignold loam (moderately well-drained; particles are 0.002-0.005 mm in diameter) and Hochfeld sand (well-drained; particles are 0.005-

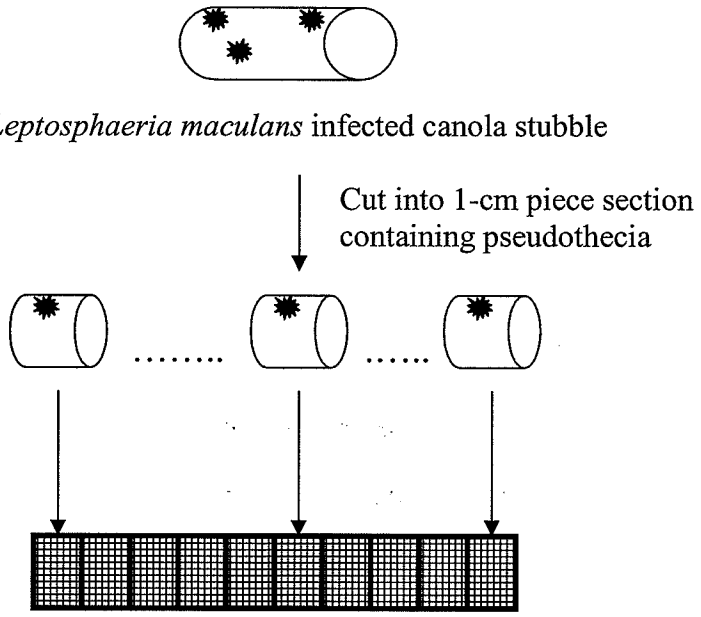


Figure 4.1. Treatment of infested canola stubble before burial in the soil

2.0 mm in diameter). There were three replicates. On May 31, June 30 and July 28 of 2002, the stubble was examined to determine fungal survival. The experiment from 2001 to 2002 was performed in a completely random block design in each type of soil.

4.3.2 Retrieval of pathogen

The buried stubble from the mesh bags was washed under running cold water, and cut into 1-cm pieces. Pieces with lesions were soaked in 1% bleach solution in Petri dishes for 4 minutes, then taken out and rinsed in sterile distilled water in a Petri dish three times. After drying on filter paper, pieces of stubble were placed on V-8 agar media amended with 0.1g/L streptomycin sulfate (Sigma-Aldrich Co., Louis, Mo., USA), 0.04g/L Rose Bengal (Fisher Scientific Co., New Jersey, USA) and 0.75g/L $CaCO_3$. Ten pieces of stubble from ten cells of a mesh bag (one piece of stubble in one cell) at the same depth of soil were placed on ten plates. The plates were incubated under fluorescent light at 24°C for 10 days, and the pathogen survival was assessed.

4.3.3 Assessment of the pathogen survival

Percent pathogen survival at a depth of soil = [(number of stubble pieces from which the pathogen growth occurred) / (total number of stubble pieces)] × 100%.

4.4 Results

4.4.1 Year 2001

Survival of the pathogen on infected stubble significantly decreased with increase in soil depth, seven and eight months after stubble was buried (Table 4.1). Viability of the pathogen on the soil surface was significantly higher than at 10-cm depth, and there was no difference between the surface and at 5-cm depth, and 5-cm and 10-cm depths nine

Table 4.1. Percent recovery of the pathogen, *Leptosphaeria maculans,* seven, eight and nine months after infested stubble was buried at 0, 5 and 10-cm depths in loam soil in 2001

Soil depth	Months after stubble burial			LSD
	7 months	8 months	9 months	
0 cm	40 a A	33 a A	20 a B	7.9
5 cm	27 b A	20 b A	10 ab B	17.0
10 cm	10 c A	0 c A	0 b A	18.6
LSD	12.4	12.0	13.0	

Different capital letters in rows indicate significant differences among seven, eight and nine months, and small letters in columns indicate significant differences among 0, 5, and 10-cm depths according to Fisher's Least Significant Difference (LSD) test ($P \geq 0.05$).

months after stubble was buried. There was no recovery of the pathogen from buried stubble at 10 cm for eight and nine months.

Pathogen survival significantly decreased on the soil surface and at 5-cm depth nine months after stubble was buried compared to seven and eight months; and there was no difference in viability between seven and eight months (Table 4.1). Survival of the pathogen decreased to a low level at 10 cm after seven months and no pathogen isolates were recovered at eight and nine months.

4.4.2 Year 2002

There were significant effects of depth on pathogen survival (Table 4.2). Seven, eight and nine months after stubble was buried it was consistently observed that pathogen survival was significantly lower at 10 cm than at 5 cm, and the survival was significantly lower at 5 cm than on the soil surface, except in sand eight months and in loam seven months after burial.

Pathogen survival was significantly lower nine months after stubble was buried than seven months under all conditions of soil type and soil depth (Table 4.2).

4.5 Discussion

The physical and chemical alterations of soil during different tillage systems have a critical effect on pathogens. Microorganisms responsible for decomposing organic materials, have a direct impact on stubble decomposition due to changes in soil temperature and moisture (Lafond and Derksen, 1996). The major factors in the soil, including moisture, temperature, pH, aeration and oxygen supply, available nutrients, chemical compositions of crop stubble, carbon/nitrogen component ratio of stubble and other factors, involving stubble position in the soil and soil types, especially soil moisture

Table 4.2. Percent recovery of the pathogen, *Leptosphaeria maculans,* seven, eight and nine months after infested stubble was buried at 0, 5 and 10-cm depths in sand, loam and clay soils in 2002

Soil type and depth	Months after stubble burial			LSD
	7 months	8 months	9 months	
Sand				
0 cm	77 aA	60 aB	57 aB	5.7
5 cm	57 bA	47 aB	37 bC	6.3
10 cm	37 cA	27 bB	23 cB	5.8
LSD	8.7	13.0	9.2	
Loam				
0 cm	73 aA	67 aA	50 aB	7.7
5 cm	43 bA	40 bA	33 bB	5.0
10 cm	33 bA	27 cB	23 cB	4.2
LSD	10.5	7.6	6.9	
Clay				
0 cm	67 aA	50 aB	47 aB	4.9
5 cm	43 bA	33 bB	27 bC	3.8
10 cm	27 cA	20 cB	10 cC	5.5
LSD	9.5	7.7	9.4	

Capital letters in rows indicate significant differences among seven, eight and nine months. Small letters in columns in the same type of soil indicate significant differences among 0, 5, and 10-cm depths, according to Fisher's Least Significant Difference (LSD) test ($P \geq 0.05$). Percent recovery of the pathogen at different depths derived from three types of soils.

and temperature, may affect activities of microorganisms and stubble decomposition (Lafond and Derksen, 1996). The physical and chemical characters are different among different depths in the soil. Temperature on the surface of soil is higher than in the soil, and moisture increases with increase of soil depth within one meter in the summer (Guo, unpublished). There were differences in organic matter, soil minerals and amount of pore space among different soil depths, which could impact soil pH, water movement, moisture-holding capacity, oxygen and nutrient availability to pathogens and decomposing microorganisms (Agrios, 1997). In the soil of pH >7.5, high concentrations of $CaCO_2$ could tied up essential microelements $Fe^{3+}$and Zn^{2+}, making them unavailable to soil microbes. These factors in the soil are inextricably combined to affect the stubble and pathogen survival. There are no studies done on effects of soil depth on pathogen survival.

The changes in moisture level, temperature and activities of decomposition by microorganisms in soil through tillage may lead to an increase, decrease, or maintenance of disease (Bailey and Duczek, 1996). Sumner (1981) reviewed effects of tillage on plant diseases and suggested that several foliar pathogens of corn, *Helminthosporium turcium, H. maydis, Phyllosticta maydis*, were known to survive in corn stubble in minimal tillage or zero tillage systems. Most of these diseases were more severe in minimal or zero tillage than when the stubble was buried by plowing. Minimal or zero tillage decreased soil temperatures in spring and early summer, which could be conducive to root diseases induced by soil or stubble-borne pathogens favored by low temperatures. Bailey and Duczek (1996) reported that wheat stubble infected by *Pyrenophora tritici-repentis* produced fewer ascocarps on stubble in disked soil than on stubble in zero-tilled soil. It

was found that stubble completely buried by tillage decomposed more rapidly than when left on the soil surface (Summerell and Burgess, 1989). Tillage favors an increase in soil temperature (Bockus, et al., 1994) and promotes the decomposition of crop stubble (Summerell and Burgess, 1989). Doran (1980) reported that in the upper layer of soil with zero-tillage, populations of microorganisms were double those in conventional tillage. However, Gracia-Garza et al. (2002) observed a reduction in numbers of apothecia and apothecial clumps by *Sclerotinia sclerotiorum* on soybean stubble in zero tilled soil and suggested that zero till after harvest might prevent apothecia in the soil from reaching the surface and therefore reduced the spread of ascospores and ultimately reduced disease severity.

In canola-wheat-canola rotations under zero tillage in the present study, survival of pathogen from infected stubble left on the soil surface significantly decreased during the period of wheat growth so that canola grown following wheat had less infection than canola grown for three years in a row. In canola-wheat-canola rotation under conventional tillage, there were possibly two situations co-existing: infected stubble was buried and stayed in soil; and infected stubble in the soil was brought back to the soil surface. In either situation, viability of pathogen would decreased over time, especially the pathogen staying buried in the soil, which showed significantly weaker survival than the pathogen on the soil surface. Therefore, this rotation and tillage system obtained less infection than canola-canola-canola with tillage. The burial of stubble reduced viability of pathogen which was observed in canola-canola-canola, and canola-wheat-canola rotations under conventional tillage. The crop infection in these two systems was significantly lower than canola-canola-canola and canola-wheat-canola rotations under

zero-tillage. The results from year 2001 and 2002 consistently suggested that two- or three-year conventional tillage had significant effects at reducing the disease by burying infected stubble to decrease the possibility of pathogen survival. Canola plants in canola-wheat-canola-canola and canola-canola-wheat-canola rotations under zero-tillage exhibited lower infection than in the system of canola grown for four years in a row. Although the viability of the pathogen on the soil surface significantly decreased within twelve months, canola grown in each year might help the pathogen maintain its life cycle on canola plants and keep some level of inoculum alive. Thus, in canola-canola-canola-canola under zero-tillage crop infection was still at a higher level than canola-wheat-canola-canola and canola-canola-wheat-canola rotations under zero tillage. Between the two latter systems, there were significant differences in disease incidence and severity. In canola-wheat-canola-canola rotation in which wheat was grown in the second year, pathogen survival decreased in approximately twenty months, but canola grown following wheat helped increase the levels of inoculum in the third and fourth year of the rotation. In the rotation in which wheat was grown in the third year, the level of inoculum from the first two years significantly decreased in the third year when wheat was grown. Therefore, crop infection in the fourth year after wheat was grown in the third year showed a lower level than the system in which wheat was grown in the second year.

The results from loam soil in these two years showed a similar trend, however, there were some differences. These differences may be due to changes of weather conditions (Table 4.3), which affect soil temperature and moisture. How soil temperature and moisture impact stubble decomposition and pathogen survival is yet clear.

Table 4.3. Temperature, relative humidity and precipitation during consecutive period from August 2000 to August 2002

Month	January	February	March	April	May	June	July	August	September	October	November	December
Year 2000												
T_{max} (°C)								26.2	18.0	14.0	-0.7	-15.2
T_{min} (°C)								12.2	5.7	-1.3	-8.4	-25.8
RH_{max} (%)								101.9	100.9	95.8	102.5	92.5
RH_{min} (%)								50.8	54.9	48.1	90.4	78.8
Precipitation (mm)								75.4	55.8	5.6	54.8	30.7
Year 2001												
T_{max} (°C)	-5.9	-12.5	-1.0	8.8	19.1	22.6	25.7	26.5	20.4	15.3	-1.2	-16.7
T_{min} (°C)	-16.8	-22.3	-10.9	-10.9	-0.5	10.2	13.4	13.1	6.0	-0.8	-9.6	-26.4
RH_{max} (%)	99.2	92.5	99.4	100.5	99.9	99.7	102.0	101.9	101.5	96.6	101.3	92.8
RH_{min} (%)	83.8	73.9	76.7	67.9	48.9	53.1	60.4	52.7	49.8	50.7	91.0	77.9
Precipitation (mm)	215.6	10.8	13.8	69.2	162.9	30.0	160.4	19.0	29.8	10.7	48.7	23.2
Year 2002												
T_{max} (°C)	-7.8	-2.7	-5.7	7.9	15.2	23.6	27.1	23.9				
T_{min} (°C)	-17.0	-14.3	-15.6	-4.0	0.1	11.8	13.5	11.5				
RH_{max} (%)	95.3	97.3	91.5	99.5	95.3	99.1	102.0	103.8				
RH_{min} (%)	78.2	70.5	60.9	47.6	36.7	52.1	53.8	59.3				
Precipitation (mm)	11.9	5.7	20.0	30.2	103.6	141.0	30.0	106.4				

T_{max} - average maximum temperature, T_{min} - average minimum temperature, RH_{max} - average maximum relative humidity, RH_{min} - average minimum relative humidity.

Our study in persistence of blackleg pathogen in the soil was the first one that was involved in different depths of soil and different types of soil. The results confirmed that lower amounts of the blackleg pathogen survived in deeper soil than in the upper layer of soil or on the soil surface, and less pathogen inoculum survives in clay soil than in sand. Survival of the pathogen significantly decreased within nine months. However, studies were not carried out to understand the environmental conditions of soil where stubble was buried.

5. SEASONAL AND DIURNAL PATTERNS OF SPORE DISPERSAL BY *LEPTOSPHAERIA MACULANS* FROM CANOLA STUBBLE IN RELATION TO ENVIRONMENTAL CONDITIONS

5.1 Abstract

Seasonal and diurnal patterns of spore dispersal by *Leptosphaeria maculans*, which causes blackleg disease of canola, were studied in two consecutive field seasons, using a 7-day Burkard spore sampler and rotorod impaction spore samplers. Ascospores of *L. maculans* were trapped from the middle of June to the end of July, whereas pycnidiospores were trapped from the middle until the end of July or beginning of August. Ascospores and pycnidiospores were trapped between 9 pm to 4 am when air temperatures were 13-18 °C and relative humidity of over 80 %. Peak ascospore and pycnidiospore dispersal was associated with rain events. Peak ascospore dispersal was found to occur several hours after rainfall ≥ 2 mm with ascospore dispersal being maintained for approximately 3 days after such events. Peak pycnidiospore dispersal occurred around the same hours as rain events. More ascospores and pycnidiospores were carried in the direction of the prevailing winds than in other directions. To the south of the inoculated area, the gradients of disease incidence and stem severity were -19.2 m^{-1} and -0.8 m^{-1}, respectively. Disease incidence and stem severity declined by 50 % 12.5 and 5.5 m from the inoculated area, respectively. To the north of the inoculated area, the gradients of disease incidence and stem severity were -21.5 and -0.7 m^{-1}, respectively. Disease incidence and stem severity declined by 50 % 14.0 and 5.2 m from the inoculated area. In 2001, ascospores and pycnidiospores were trapped within 25 m of the inoculated area, whereas pycnidiospores were trapped up to 45 m from the inoculated area.

5.2 Introduction

Canola (*Brassica napus* L. *and B. rapa* L.), is one of the major oilseed crops grown worldwide. With significant expansion of acreage sown to canola during the past two decades, concern about blackleg, the most serious disease of canola, has increased.

Blackleg disease is caused by *Leptosphaeria maculans* (Desm.) Ces. et de Not. (anamorph: *Phoma lingam* (Tode ex Fr.) Desm.). The pathogen can attack all parts of the plant. Symptoms first appear on cotyledons and true leaves as dirty white to buff-colored lesions, round to irregular in shape, and dotted with numerous black pin-head-size pycnidia (Sawatsky, 1989).

The most important primary inoculum is ascospores released from pseudothecia (Howlett, 2001). In Australia, pseudothecia are formed on infested crop stubble 1 to 10 months after harvest when moist conditions and mild temperatures prevail. Ascospores can be liberated from infested stubble for at least 3 years (McGee, 1974, 1977; McGee and Petrie, 1978). Pycnidiospores act as secondary inoculum after infection is established by ascospores, and are believed to spread by rain splash to other leaves and neighboring plants (Barbetti, 1976; McGee, 1977; Sawatsky, 1989).

Although the life cycle of the pathogen is well known, understanding of the epidemiology and weather factors affecting release and dispersal of spores is limited. Canola is most susceptible to infection by *L. maculans* prior to the 6-leaf stage of growth (Petrie, 1995; Rempel and Hall, 1993). In western Canada, neither timing of spore release and dispersal nor impact of temperature, humidity, rainfall, and wind on spore dispersal are well understood. Answers to such questions have important epidemiological implications for management of the blackleg disease.

Pycnidiospores, released from pycnidia as spore ooze, constituted secondary inoculum and were dispersed by rain splash to neighboring plants (Barbetti, 1976; Howlett, 2001; Williams, 1992). Fitt et al. (1986; 1989) and Madden (1997) described many of the characteristics of rain-splash dispersed fungi. Madden (1997), studying the splash dispersal of *Phytophthora cactorum* and *Colletotrichum acutatum*, found that number of splash droplets produced, number of spores disseminated, and flight distance of the splash droplets all increased with increasing size, velocity and kinetic energy of impacting raindrops. Transport distance was generally shorter than 15 cm in each splash event, but even rain durations shorter than 2 min resulted in substantial dispersal.

There is a lack of field scale studies on dispersal patterns of spores by *L. maculans* in western Canada. The objectives of this study were to investigate 1) seasonal and diurnal ascospore and pycnidiospore dispersal patterns, 2) relationship of ascospore and pycnidiospore dispersal to temperature, relative humidity and rain events, 3) the effects of wind on ascospore and pycnidiospore dispersal, and 4) disease gradients in relation to spore dissemination distance.

5.3 Materials and methods

5.3.1 Inoculum preparation

Leptosphaeria maculans PG-2 isolate 86-12 was collected from a field in Swan Lake, Manitoba, Canada in 1986, and identified using morphological technique. The isolate was stored on PDA medium under sterilized mineral oil at 20 °C in the dark. Fifteen days before use, mycelia were transferred onto V-8 agar media amended with 0.1g/L streptomycin sulfate (Sigma-Aldrich Co., Louis, Mo., USA) and 0.75g/L $CaCO_3$, and incubated in light at 24 °C. After 12 days pycnidia were produced on the media,

pycnidiospores were harvested, filtered, counted and made into a spore suspension at 1×10^7 spores/ml using a Hemacytometer (Hausser Scientific Company, Horsham, PA).

5.3.2 Field plots

The experiments were carried out in a 126 × 45 m plot from 1999 to 2001 (plot 1) and in a 120 × 50 m plot from 2001 to 2002 (plot 2), at Carman Research Station, Carman, Manitoba, Canada.

In plot 1, wheat was planted in 1998. In 1999, the whole plot was planted to canola variety Westar (susceptible to blackleg pathogen) at seeding rate of 5 kg/ha on May 30. The central point of plot 1 was determined as the point of intersection of 2 diagonals of the plot before seeding (Fig. 5.1 A). A 25 m line was determined in the middle of the short side of the plot by measuring out 2 10-m lines from 2 ends of the short side. Another 25 m line was determined in the middle of the long side of the plot by measuring out 2 10-m lines from 2 ends of the long side. The same measurements were done on the other short and long sides of the plot. Therefore, 4 lines A, B, C and D were drawn using ropes, and 4 points of intersection p, q, r and s brought out a square area of 25 × 25 m, which was use as the inoculated area in the center of the plot. At 3-leaf stage, the 25 × 25 m area was uniformly inoculated using a backpack sprayer with a pycnidiospore suspension of PG-2 isolate 86-12 at 1×10^7 spores/ml at 6:00 pm on June 10. In the fall of 1999, canola in the 25 × 25 m area was harvested, and the plot left un-tilled; canola around this area was harvested, then the area around the inoculated area was tilled using a tiller with a tine harrow (reach 10 cm depth in soil). In 2000, the whole plot was planted to Westar at the same seeding rate on May 30 as in 1999. In the fall of 2000, the plot was

harvested, and canola in the 25 × 25 m area was left un-tilled, and canola in the other area was tilled the

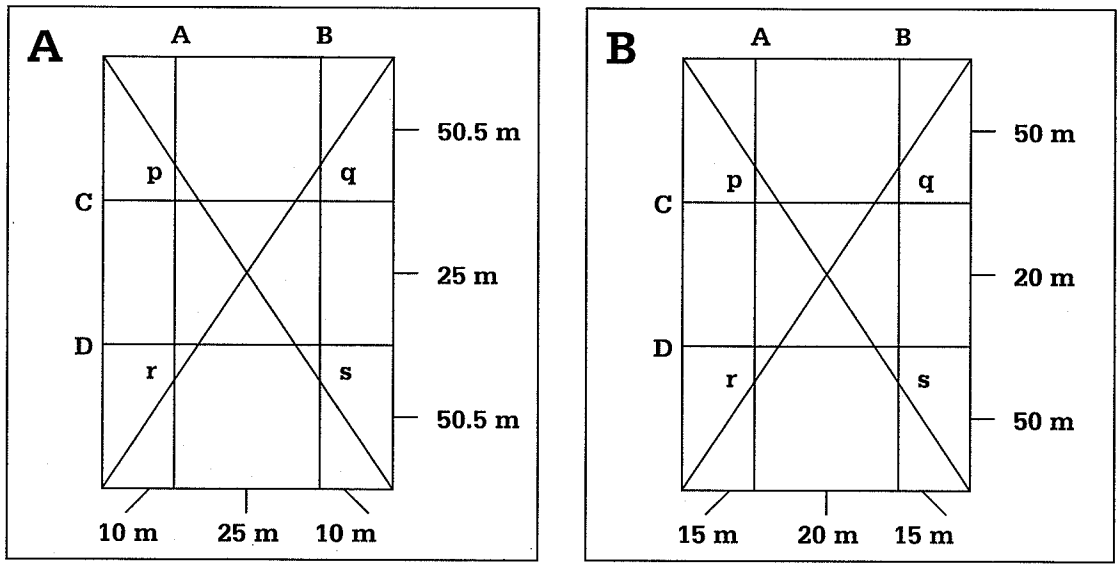


Figure 5.1. Determination of the centre and inoculated areas of plot 1 (A) and plot 2 (B). The central point of plot 1 was determined as the point of intersection of 2 diagonals of the plot. A 25 m line was determined in the middle of the short side of the plot by measuring out 2 10 m lines from 2 ends of the short side. Another 25 m line was determined in the middle of the long side of the plot by measuring out 2 10 m lines from 2 ends of the long side. The same work was done on the other short and long sides of the plot. Four lines A, B, C and D were drawn using ropes, and 4 points of intersection p, q, r and s brought out the inoculated area of 25 × 25 m. The similar work was done in plot 2.

same way as in 1999. In 2001, the plot was again planted to Westar on June 1. In plot 2, hemp and wheat were planted in 1999 and 2000, respectively. In 2001, the whole plot was planted to Liberty Link variety 2153 (moderately susceptible to blackleg pathogen) on May 30. The inoculum source of 20 × 20 m was determined the same way as in plot 1 (Fig. 5.1 B). At 3-leaf stage, the 20 × 20 m area was inoculated using the same backpack sprayer with a pycnidiospore suspension of PG-2 isolate 86-12 at 1×10^7 spores/ml at 6:00 pm on June 10. After harvest, Liberty Link in the 20 × 20 m area was left un-tilled, and Liberty Link in the other area was tilled. In 2002, the whole plot was planted to Liberty Link variety 2153 on June 1.

5.3.3 Spore sampling and weather data collecting

In 2001, rotorod impaction spore samplers (Aerobiology Company, Nepean, Ontario, Canada) were set up in the plot 1 (Fig. 5.2 A). Based on the historical weather data at Carman, prevailing wind often came from NE and SW. Therefore, four spore samplers were placed 1, 5, 25 and 45 m to the northwest (NW) and southeast (SE) of edge of the inoculated area on the diagonals of the plot. Three spore samplers were placed 1, 5 and 25 m to the northeast (NE) and southwest (SW) of edge of the inoculated area, and 2 spore samplers were placed 5 m east (E) and west (W) from the side of the inoculated area, to trap spores. A CR10 datalogger (Campbell Scientific, Logan, Utah, US) was programmed to run the rotorods for 5 minutes/h. A thin coat of petroleum jelly was brushed on the rotorod to catch spores. Each rotorod was replaced every 24 hours. In addition, a 7-day Burkard spore trap (Burkard Scientific Ltd., Uxbridge, Middlesex, UK) was set up in the center of the inoculated area. The tape in the Burkard was coated with a

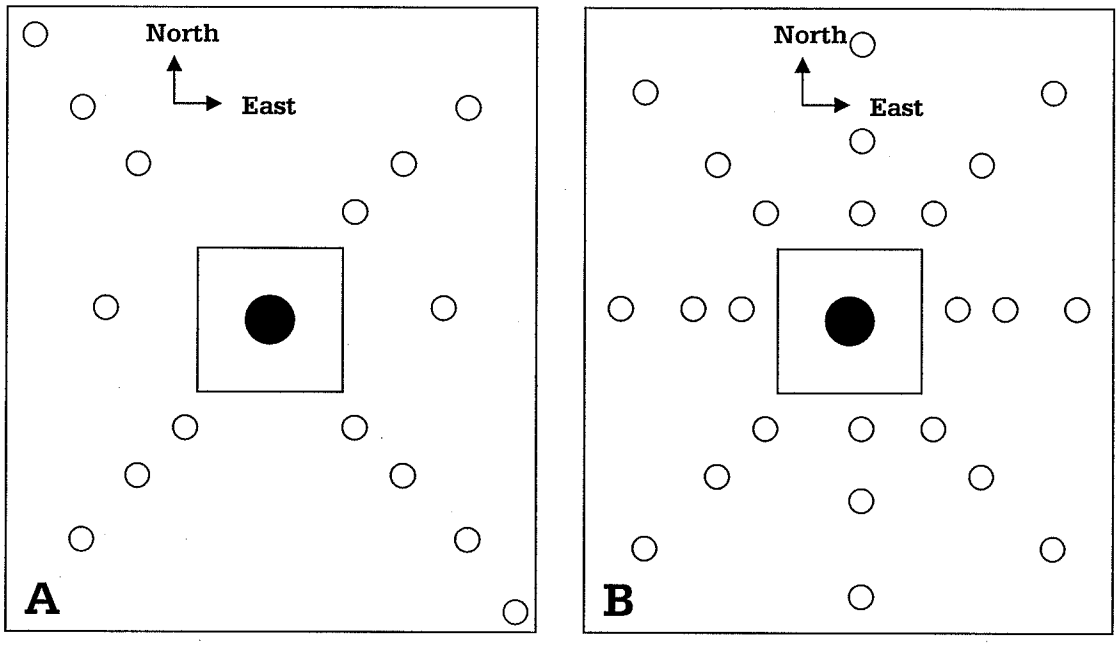


Figure 5.2. Location of rotorod impaction spore samplers and a 7-day Burkard spore trap in plot 1 (A) and plot 2 (B). The small square areas in the centre of the plot 1 and plot 2 were the inoculated areas. The big close circles in the plot centres represented the 7-day Burkard spore traps, and small open circles in different directions represented rotorod impaction spore samplers.

thin layer of petroleum jelly the same way as the rotorods were done. The tape was replaced weekly.

In 2002, rotorod impaction spore samplers were set up in the plot 2 in N, NE, E, SE, S, SW, W and NW directions, 1, 5 and 10 m from the perimeter of the 20 × 20 m inoculated area (Fig. 5.2 B). A Burkard spore trap was set up in the centre of the inoculated area as in 2001.

Hourly rainfall, hourly and mean daily temperature and relative humidity, and wind direction were collected from the weather station at the Carman Research Station, which was located approximately 100 m to the east of the experimental plots.

In 2001 and 2002, cotyledon stage of the crop was on June 10, 1st-3rd leaf stages were between June 14 and 18, 3rd-6th leaf stages were between June 18 and 30, and 6th leaf-flowering stages were between June 30 and July 10.

5.3.4 Measurement and analysis

Spore concentration based on the Burkard spore trap was used for the analysis of daily and hourly spore counts in the air. Each Burkard tape was divided into 7 equal sections, each section representing 1 day. Each section was then divided into 24 divisions, each representing 1 h. The area of 1 section (24 hours) was 20 × 48 mm; the area of 1 division (1 hour) was 2 × 20 mm. Five frames were determined and well distributed within each 1 hour division with approximately 3.5 mm between 2 neighbor frames. The area of each frame was 0.20 mm^2. Spores from the 5 frames were counted under a microscope at 400 ×. The number of spores in the 5 frames was transferred into the numbers of spores in 1 division and section. Spore concentration at a certain hour was calculated as the number of spores in 1 division divided by throughput at orifice of the Burkard 0.6 m^3/h. Mean

concentration of spores on a certain day was calculated as total number of spores on 1 section divided by the throughput 0.6 m^3/h and 24 h.

Spore concentration based on a rotorod spore sampler was used for an analysis of spores trappped at different distances from the inoculated area and in different directions. Length of the rod for collecting spores was 20 mm. The collecting face of a rod was divided into 15 fields. The spores in the 15 fields were counted under a microscope at 400 ×. Mean concentration of spores per day was calculated as total number of spores on 1 rod divided by rotations per minute of rod (2400), rotation constant 0.097, sampling period in minutes per day (120 minutes) and 24 h.

Stem disease severity and disease incidence were assessed at the pod filling stage of canola (seeds in a pod turn yellow) in plot 2 in 2002. The 120 × 50 m plot was divided into 120 10 × 5 m units. Because row spacing was 0.20 m, 24 plants in each line diagonal of a unit were equidistantly sampled, approximately 0.45 m from each other. Sampling started 0.45 m from the corner of a unit. Stem disease severity was rated using the standard 0-5 scale, 0 – no infection, 1 – lesion area is less than 25% of cross-section area of crown, 2 – 25-50%, 3 – 51-75%, 4 – 76-100%, 5 – plant dead (Fernando's lab, unpublished). Disease incidence was assessed as the number of the infected plants divided by total number of the plants sampled.

D_{50} was assessed as the distance from edge of the inoculated area to where the disease (incidence or severity) declined by 50%.

Gradient of disease spread was estimated as reduction of disease (incidence or severity) per unit distance (m^{-1}).

Mean daily concentration of spores in a certain direction was calculated as average concentration of spores released from all rotorod spore samplers in this direction over all the sampling days. North wind was defined as 0°, northeast wind was 0-89°, east wind 90°, southeast wind 91-179°, south wind 180°, southwest wind 181-269°, west wind 270° and northwest wind 271-360°. Prevailing wind direction on each day was determined as the direction in which a wind took the most hours among all winds occurring on this day.

5.4 Results

5.4.1 Seasonal spore dispersal in relation to rain events

In 2001, ascospores were trapped daily from June 19 to July 31 except on July 30 (Fig. 5.3). Pycnidiospores were trapped every day from June 19 to August 10. A greater number of ascospores were trapped prior to June 23 than pycnidiospores. After June 23 a greater number of pycnidiospores were trapped than ascospores. Peak numbers of ascospore were observed on June 21, 25 and 28, July 06, 16, 22, 26 and 27, and peak pycnidiospores were observed on June 27, July 06, 16, 26 when rain events occurred.

Higher numbers of ascospores and pycnidiospores were associated with rainfall $\geq$2 mm (Fig. 5.3). Peak ascospores were observed on the same day as rain or one day after rain events. Peak pycnidiospores occurred on the same days as rain events.

In 2002, ascospores and pycnidiospores were first trapped on June 21 (3-leaf stage) and June 29 (6-leaf stage), respectively (Fig. 5.4).

Rain events favored spore release (Fig. 5.4). More ascospores were trapped by July 11 (flowering stage), and no ascospores were trapped after July 22, 9 days earlier than in 2001. The highest number of pycnidiospores were trapped on July 04 (budding

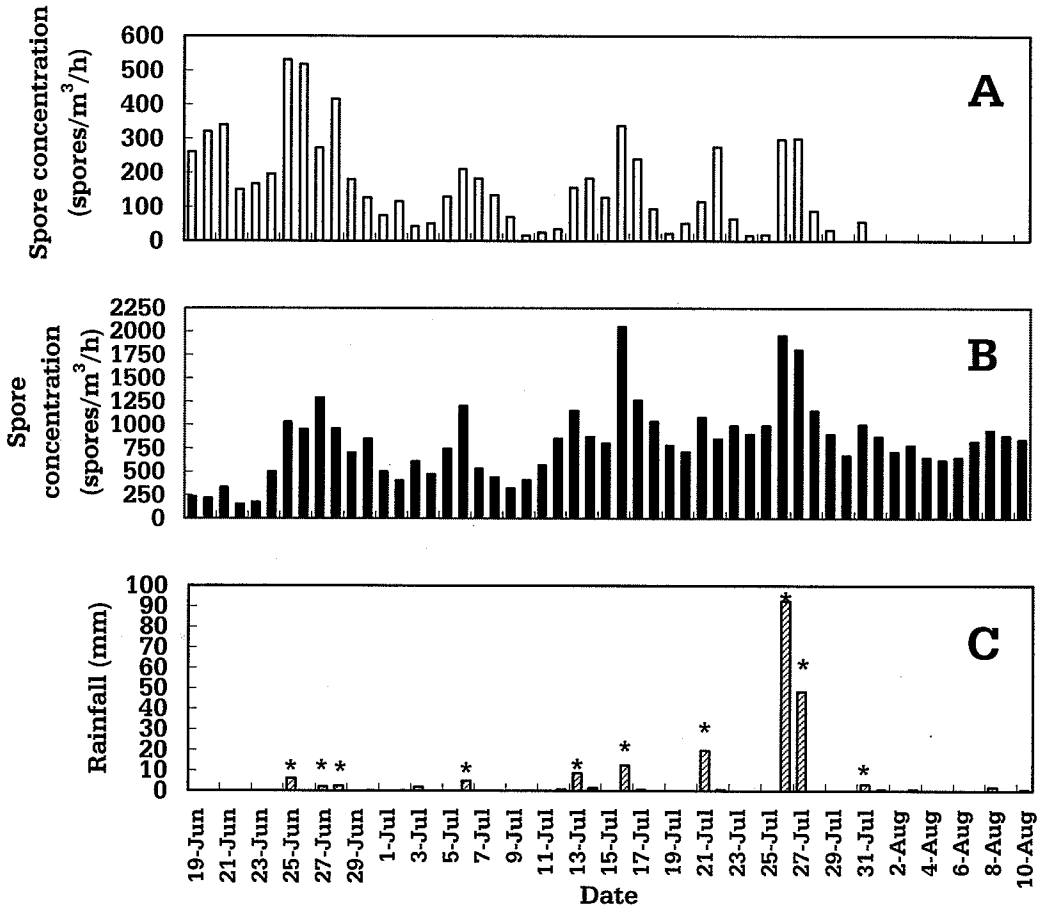


Figure 5.3. Seasonal patterns of ascospore (A) and pycnidiospore (B) dispersal by *Leptosphaeria maculans* from a point source inoculum trapped by a Burkard 7-day spore trap, and rain events (C) in 2001. * on the rain bars represents rainfall of ≥2 mm.

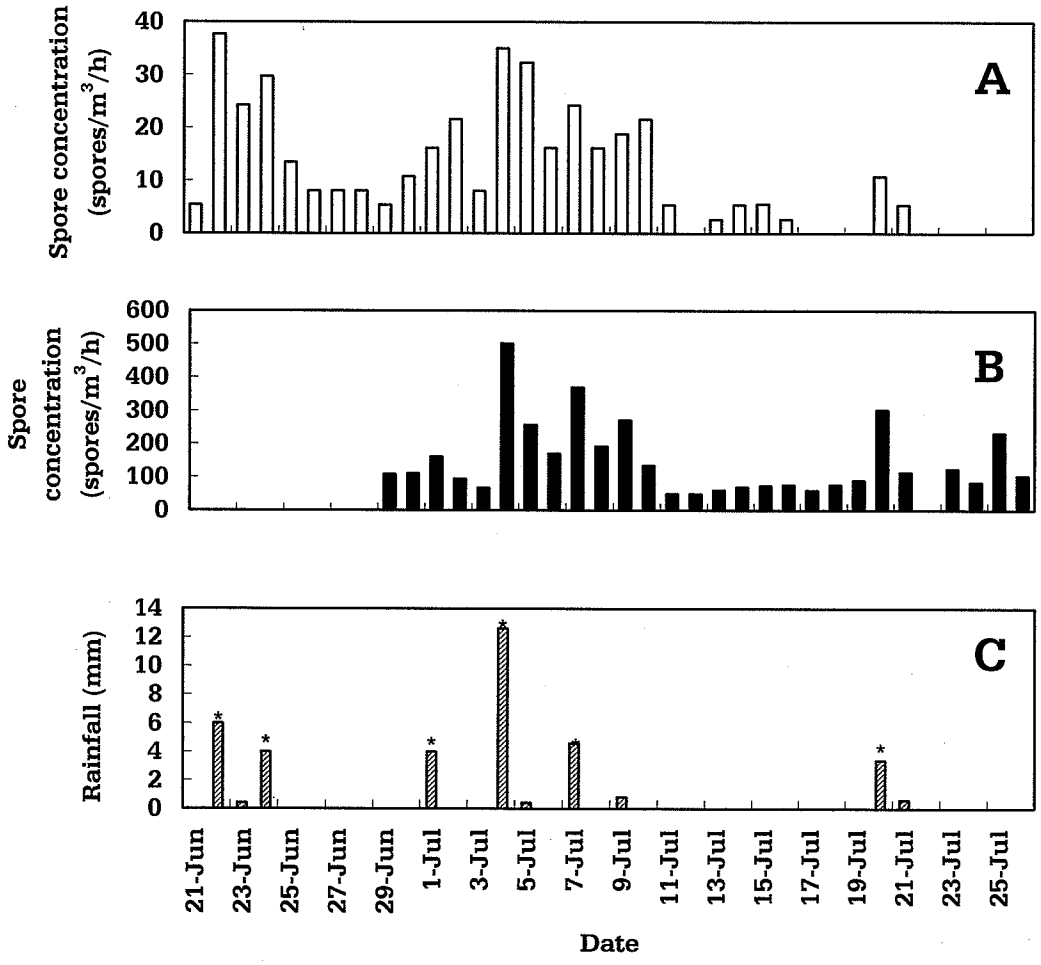


Figure 5.4. Seasonal patterns of ascospore (A) and pycnidiospore (B) dispersal by *Leptosphaeria maculans* from a point source inoculum trapped by a Burkard 7-day spore trap, and rain events (C) in 2002. * on the rain bars represents rainfall of ≥2 mm.

stage) when the highest amount of rain fell during the season. Effects of rainfall ≥2 mm in 2002 showed the same trend as in 2001 (Fig. 5.3 and Fig. 5.4). Peak amounts of ascospores were observed on the same day as rain or one day after rainfall events, and peak pycnidiospore release occurred on the same days as rain events (Fig. 5.4).

5.4.2 Diurnal spore dispersal in relation to air temperature and relative humidity on days without rain

On days without rain, more ascospores and pycnidiospores were trapped between 9 pm and 4 am, when the temperatures were 13-18°C and relative humidity > 80%, than at other times of day in 2001 (Fig. 5.5). A similar trend was observed in 2002 (Fig. 5.6). More ascospores and pycnidiospores were trapped between 9 pm and 4 am, when the temperatures were 15-18°C and relative humidity > 80%.

5.4.3 Relationship between time of rain and spores trapped

In 2001, the first peak of ascospore dispersal was observed 5 h after rain on July 6; then peak number of ascospores were trapped 10, 13, 17 and 18 h after rain on the same day (Fig. 5.7A and B). Peaks of ascospores decreased in magnitude during the three following days. The highest peak of pycnidiospores were trapped at the same hour as the rainfall on June 6; following this peak, spore release dispersal was trapped 3, 7, 12, 16 and 19 h after rain on the same day (Fig. 5.7B). Only a few pycnidiospores were trapped between 8 am and 5 pm on July 7, 8 and 9.

In 2002, similar trends were observed in 2002 as in 2001. The first peak of ascospores were observed 1 h after rain ended (Fig. 5.7C and D). Additional ascospores were trapped occasionally (on July 2); and fewer spores were trapped on July 3 (Fig. 5.7C). The highest peak of pycnidiospores were observed between 8 and 9 am when the rain

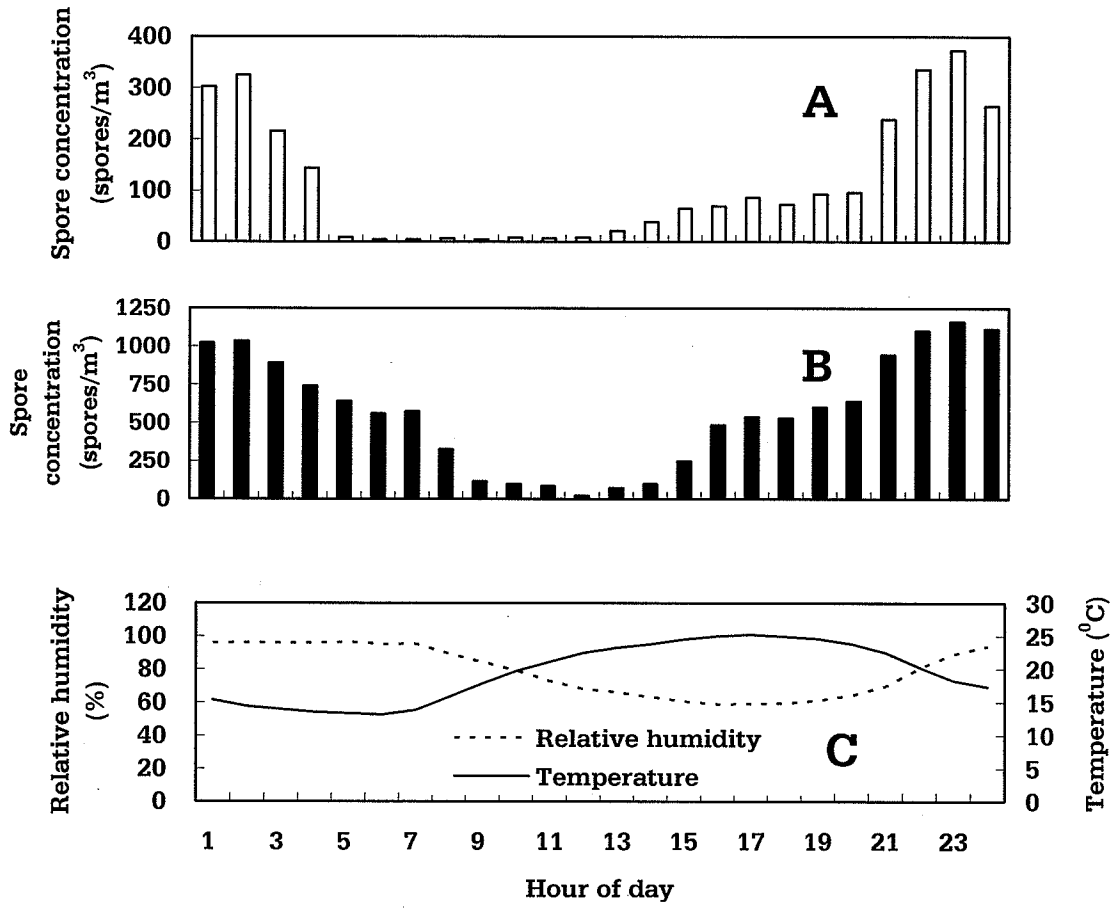


Figure 5.5. Diurnal dispersal of ascospores (A) and pycnidiospores (B) by *Leptosphaeria maculans* in relation to air temperature and relative humidity (C) averaged over 34 days without rain during the spore sampling period in 2001.

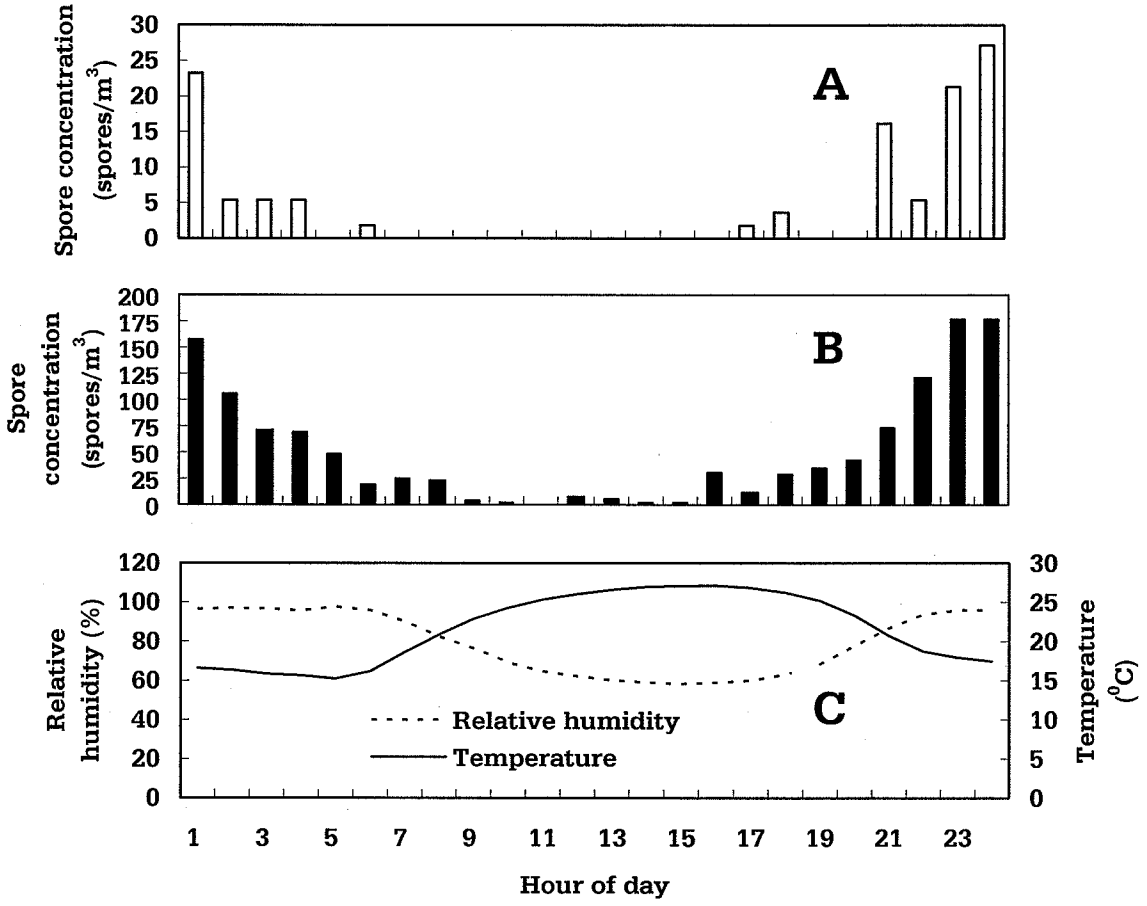


Figure 5.6. Diurnal dispersal of ascospores (A) and pycnidiospores (B) by *Leptosphaeria maculans* in relation to air temperature and relative humidity (C) averaged over 26 days without rain during the spore sampling period in 2002.

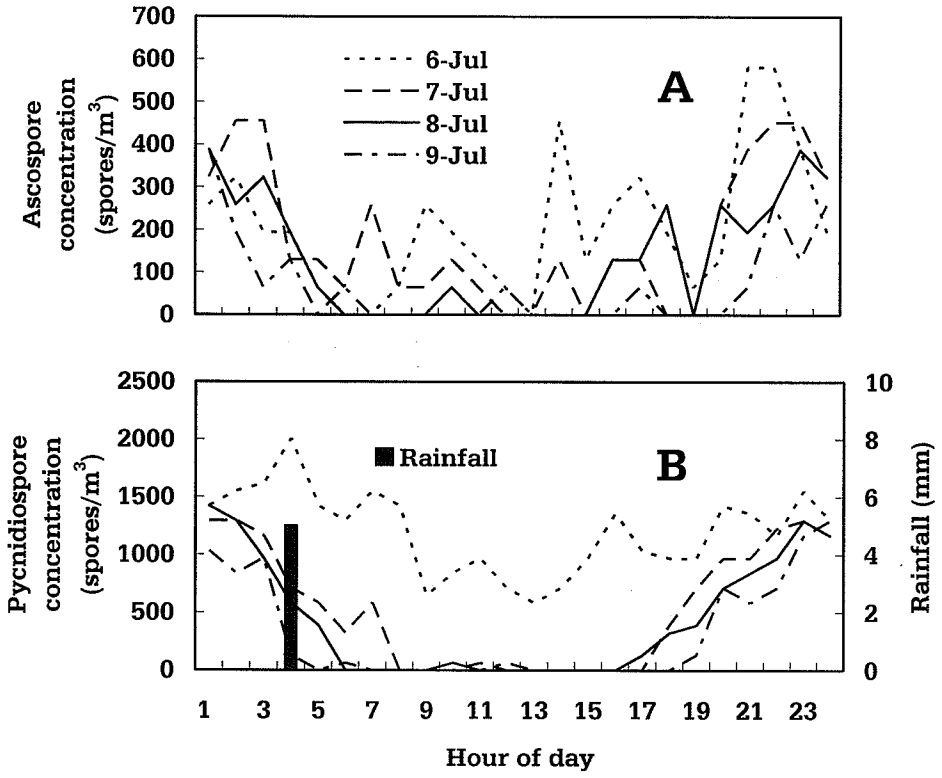


Figure 5.7 A and B. Relationship between time of rain and ascospore (A) and pycnidiospore (B) dispersal on 6-9 July 2001. A rain event of 5 mm occurred at 4 am on June 6; and there was no rain on June 7, 8 and 9.

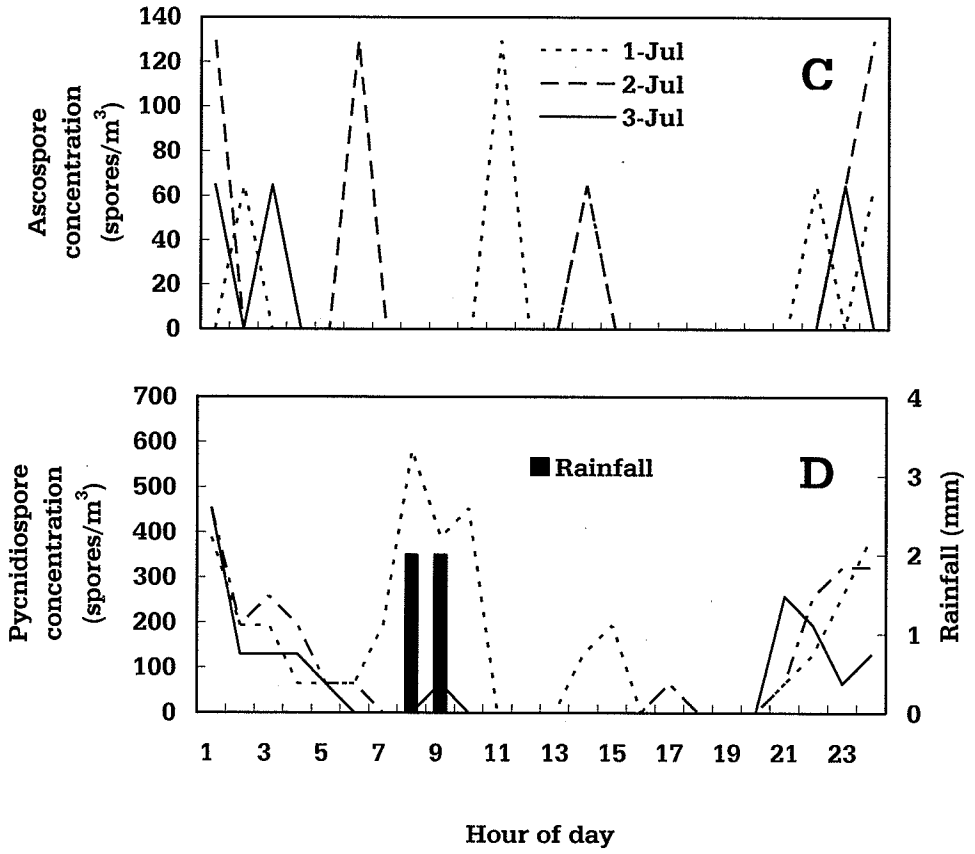


Figure 5.7 C and D. Relationship between time of rain and ascospore (C) and pycnidiospore (D) dispersal on 1-3 July 2002. Two rain events occurred between 8 and 9 am on July 1, rainfall for each hour was 2 mm. There was no rain on July 2 and 3.

event occurred on July 1, however, the rain event with the same amount continuing between 9 and 10 am did not trigger similar spore numbers (Fig. 5.7D). Instead, a higher peak of spores was observed 1 h after the second rain event. There were fewer pycnidiospores trapped on July 02 and 03.

The 2001 and 2002 results indicated that peak ascospore and pycnidiospore dispersal was associated with rain events. Pycnidiospore dispersal tended to occur at the same time as rain events, and ascospore dispersal tended to occur 1 h or more after rain events.

5.4.4 Relationship between spore concentration and distance from the inoculum source

A higher number of ascospores and pycnidiospores were trapped within 25 m from the inoculated area than farther distances in 2001 (Fig. 5.8A and B), and within 10 m from the inoculated area in 2002 (Fig. 5.8C and D). The gradients of ascospore and pycnidiospore concentrations were -2.8 and -7.0 m^{-1} in 2001, and -0.4 and -2.3 m^{-1} in 2002.

Ascospore and pycnidiospore concentrations were negatively correlated with the distance from the inoculum source in 2001 (ascospore: $r = -0.92$; pycnidiospore: $r = -0.93$) (Fig. 5.8A and B); and in 2002 (ascospore: $r = -0.99$; pycnidiospore: $r = -0.98$) (Fig. 5.8C and D).

5.4.5 Effect of wind direction on spore dispersal

In 2001, there were 2 directions of the prevailing winds, SE and SW. Higher ascospore and pycnidiospore concentrations were observed downwind from the direction of prevailing winds (Fig. 5.9A and B). Higher ascospore and pycnidiospore concentrations were observed in traps in the NW and NE corners of the experimental plot when winds come from the SE, SW, and both SE and SW during a day.

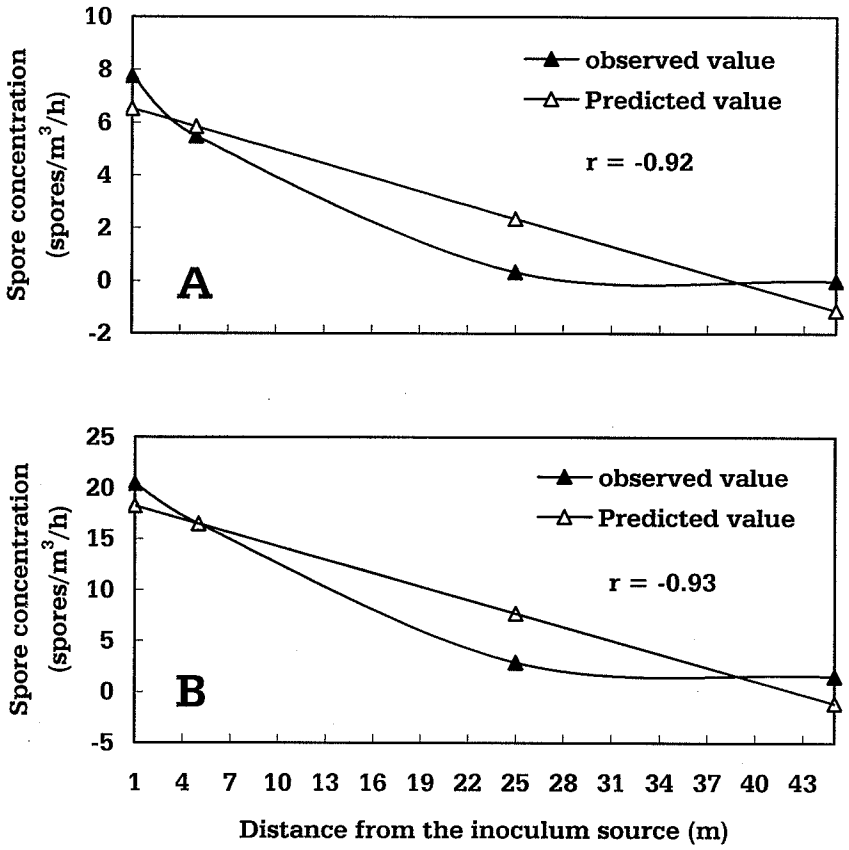


Figure 5.8 A and B. Relationship between ascospore (A) and pycnidiospore (B) concentrations, and distance from the inoculum source in 2001. The rate equation for ascospore concentration: $y = -0.1741x + 6.7055$; and for pycnidiospore concentration: $y = -0.4394x + 18.6710$, where y is spore concentration (spores/m^3/h), and x is distance (m) from the inoculum source. All lines and curves were drawn using Trendline in the Excel of Microsoft 2000.

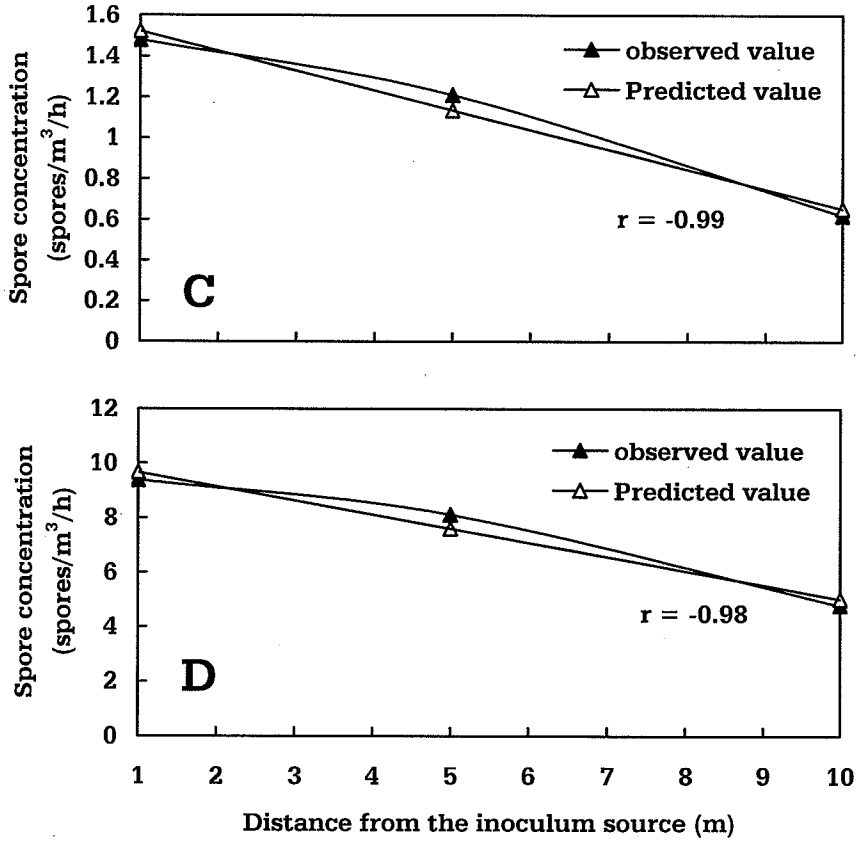


Figure 5.8 C and D. Relationship between ascospore (C) and pycnidiospore (D) concentrations, and distance from the inoculum source in 2002. The rate equation for ascospore concentration: $y = -0.0965x + 1.6179$; and for pycnidiospore concentration: $y = -0.5163x + 10.1800$, where y is spore concentration (spores/m^3/hr), and x is distance (m) from the inoculum source. All lines and curves were drawn using Trendline in the Excel of Microsoft 2000.

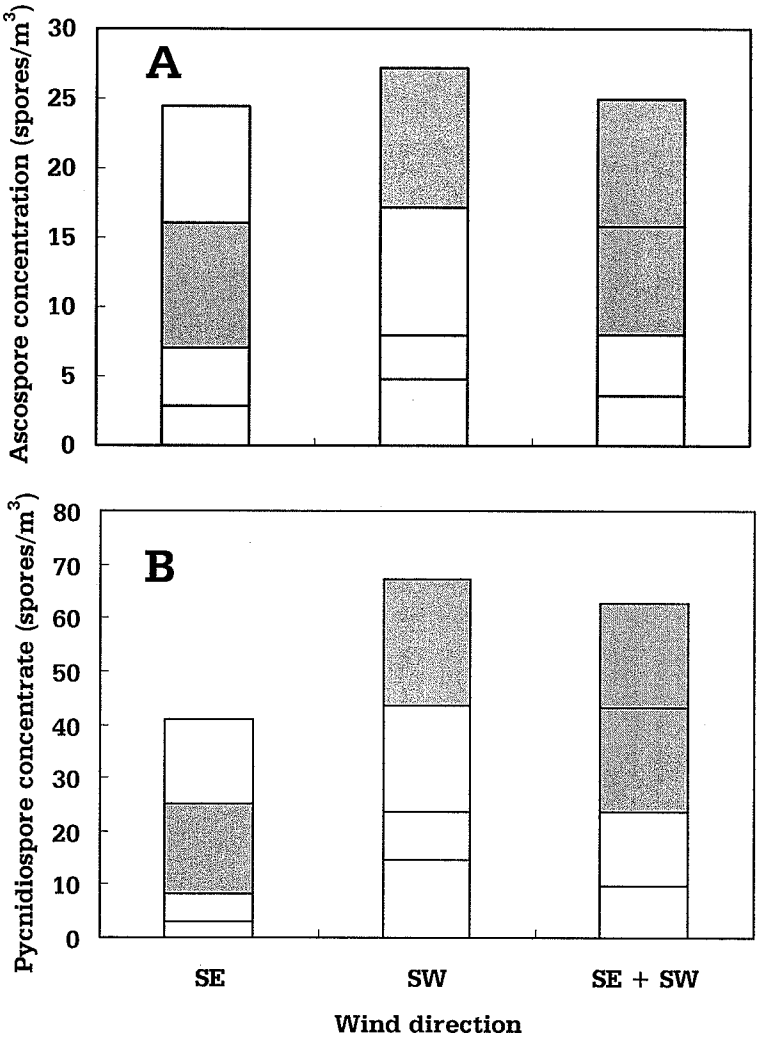


Figure 5.9 A and B. Mean daily concentration of ascospores (A) and pycnidiospores (B) trapped in the northeast (NE), northwest (NW), southwest (SW) and southeast (SE) of the inoculated area in a 126 m × 45 m plot with the wind coming from SE, SW, SW+SE over 53 days in 2001. Gray boxes in different bars represented mean daily concentration of spores downwind from the directions of winds.

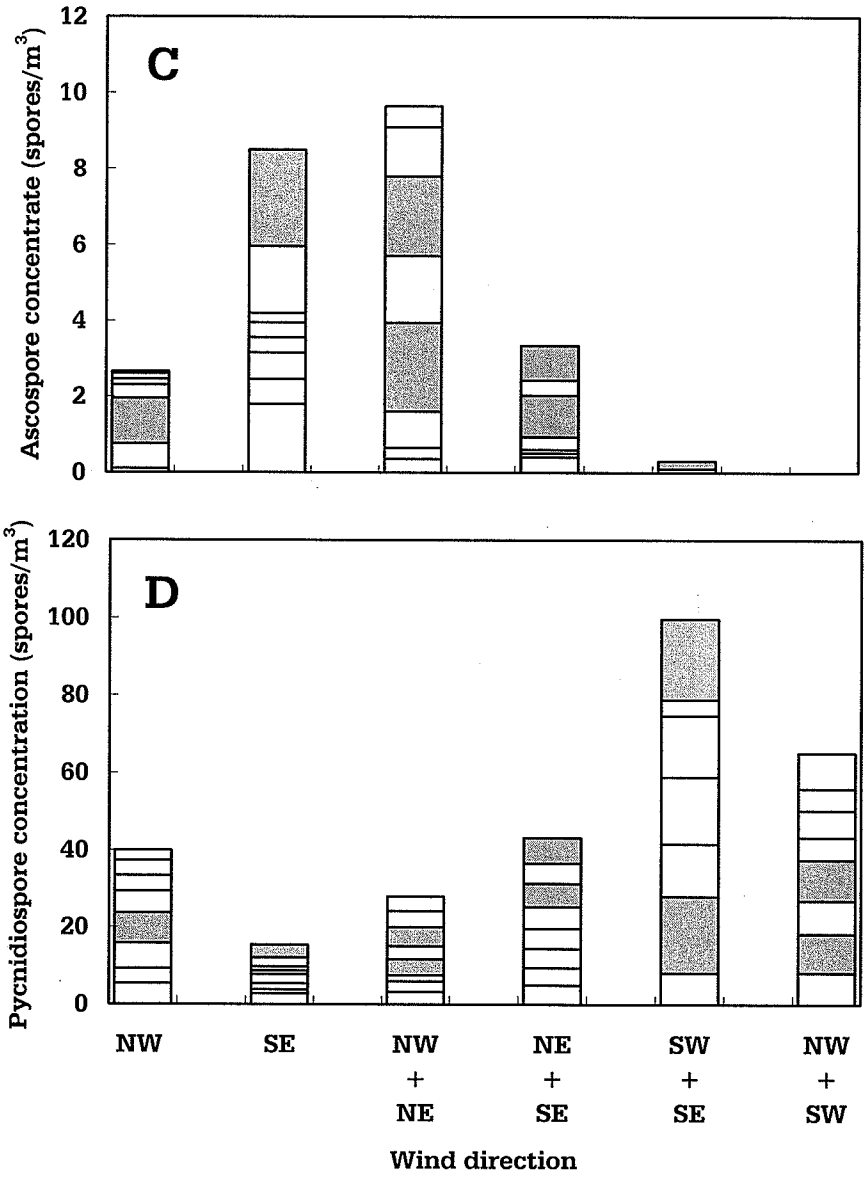


Figure 5.9 C and D. Mean daily concentration of ascospores (C) and pycnidiospores (D) trapped in the northwest (NW), southeast (SE), northwest and northeast (NW+NE), northeast and southeast (NE+SE), southwest and southeast (SW+SE), and northeast and southeast (NW+SW) of the inoculum source in a 120 m × 50 m plot with the wind coming from NW, W, SW, S, SE, E, NE and N over 36 days in 2002. Gray boxes in different bars represented mean daily concentration of spores downwind from the directions of winds.

In 2002, the directions of the prevailing winds were SE and SW+SE. The same effect of wind direction on ascospore and pycnidiospore dispersal was observed in 2002 as in 2001 (Fig. 5.9C and D). The highest ascospore and pycnidiospore concentration was in the direction SE when wind was from NW, in NW with a SE wind, in SE and SW with a NW+NE wind, in SW and NW with the NE+SE wind, and NW and NE with the SW+SE wind (Fig. 5.9C and D).

5.4.6 Disease spread in the field

Disease incidence and stem disease severity decreased with distance from the inoculum source (Table 5.1 and Fig. 5.10). The gradients of disease incidence and stem severity were steeper to the south of the inoculum source than to the north (Table 5.1).

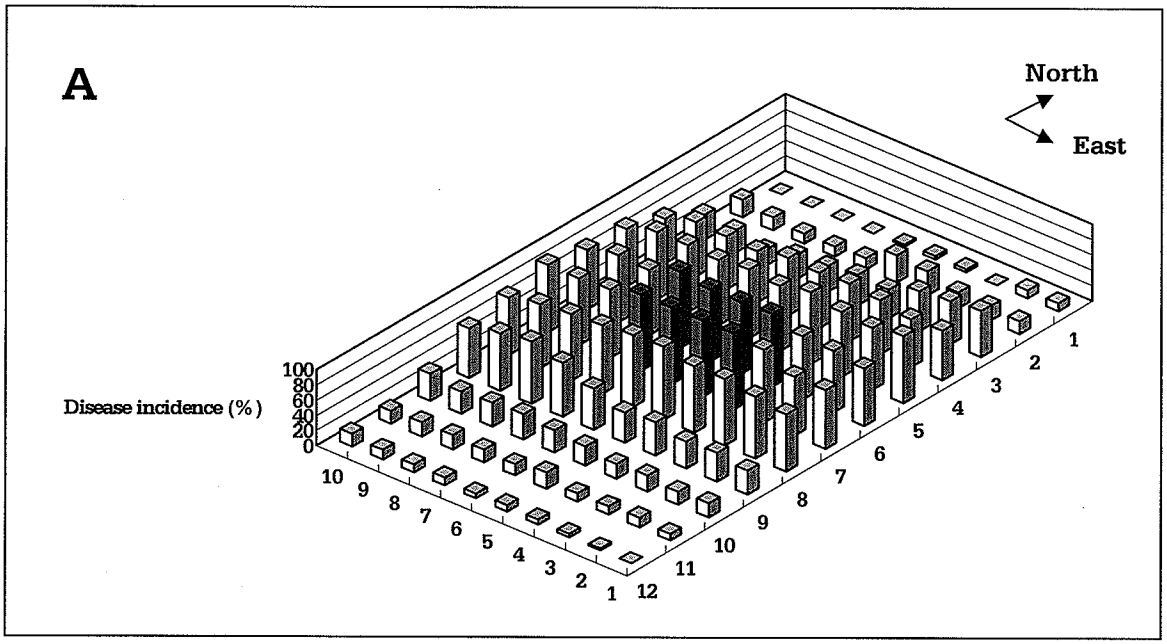
5.5 Discussion

This is the first study in seasonal and diurnal dispersal patterns of ascospores and pycnidiospores by the blackleg pathogen from canola stubble in a field under weather conditions of western Canada. Our study showed seasonal and diurnal dispersal patterns of spores by *L. maculans* in canola plots. Consistent results were obtained in the seasonal release of ascospores in 2001 and 2002. Ascospores release were trapped from the middle of June to the middle or end of July. Petrie (1995) reported in a survey that ascospores often began to be produced on canola stubble in June in Saskatchewan, but few ascospores usually were released before the end of July. Hershman and Perkins (1995) showed that infected stubble released ascospores one week after harvest, and released significant quantities of ascospores in the fall and winter months following canola crop harvest in Kentucky. Ascospores could be released during several months of a year, but peak ascospore release often occurred in September, October, or November in Europe

Table 5.1. Blackleg disease spread in the field in 2002

	Gradient of disease incidence (m^{-1})	Gradient of stem severity (m^{-1})	D_{50}-disease incidence (m)	D_{50}-stem severity (m)
South	-21.45	-0.79	12.5	5.5
North	-19.22	-0.70	14.0	5.2

Gradient of disease incidence - Reduction of disease incidence per unit distance (m^{-1}). Gradient of stem severity - Reduction of stem disease severity per unit distance (m^{-1}); D_{50}-disease incidence: Distance from inoculum center to where the disease incidence declines by 50%; D_{50}-stem severity: Distance from inoculum center to where the stem disease severity declines by 50%; South: To the south of the inoculated area; North: to the north of the inoculated area.


A
North
East
Disease incidence (%)
100
80
60
40
20
0
10 9 8 7 6 5 4 3 2 1
1 2 3 4 5 6 7 8 9 10 11 12

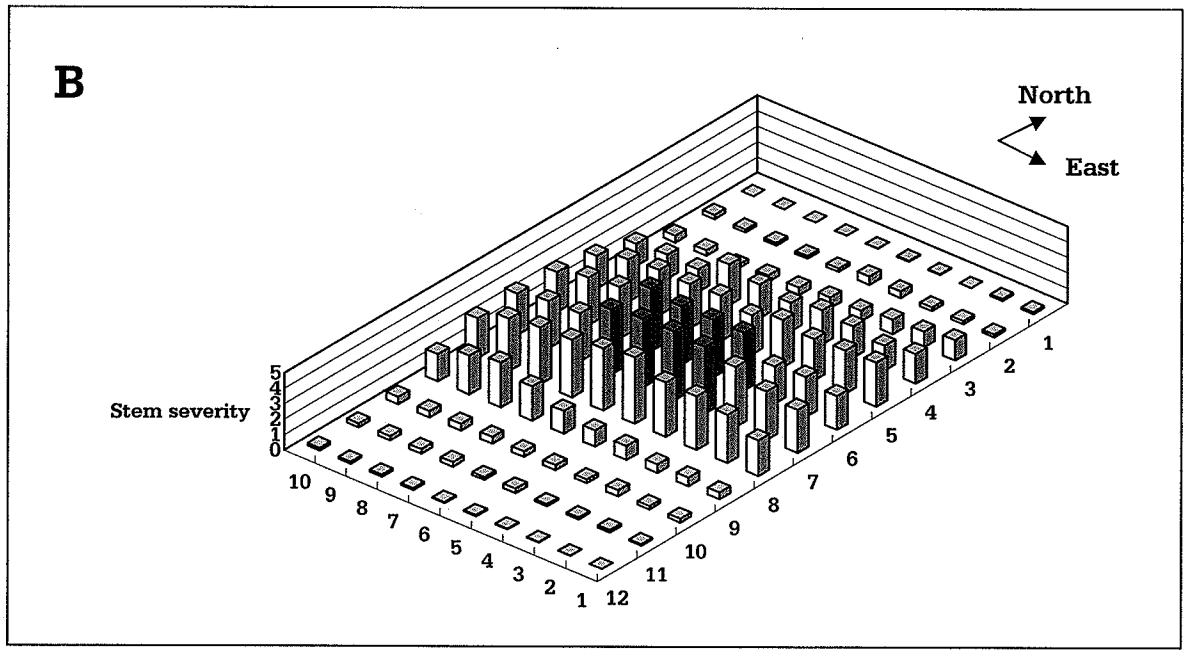

B
North
East
Stem severity
5
4
3
2
1
0
10 9 8 7 6 5 4 3 2 1
1 2 3 4 5 6 7 8 9 10 11 12

Figure 5.10. Disease incidence (A) and stem severity (B) at maturity in the field in 2002. The deep gray bars in the center of the figure represent the inoculum area. Stem severity: using 0-5 scale where by 0 _ no infection; 1 _ lesion area is less than 25% of cross-section area of crown, 2 _ 25-50%, 3 _ 51-75%, 4 _ 76-100%, 5 _ plant dead. Disease incidence = [(number of stem-infected plants) / (total number of plants sampled)] × 100%.

(Gladders and Musa, 1980), and from June through August in Australia (Bokor, 1975; McGee, 1974a, 1974b, 1977). The patterns of ascospore release in different regions were dependent on different weather conditions and crop planting systems. In Australia, summer was hot and dry, and winter canola was grown (McGee, 1974a). In Europe, wet and mild climate was predominant, and winter canola was grown in most areas (Gladders and Musa, 1980). However, spring canola is grown in Canada during hot, dry summers and cold winters. Maturation of pseudothecia and decomposition of canola stubble, relative to weather conditions (Petrie, 1994), could be different.

The starting times of ascospore and pycnidiospore release were not detected in 2001, however, there was a large difference in the amount of spores trapped and time of spores trapped between year 2001 and 2002. This may be contributed to the difference in weather conditions between these two years (Table 5.2). The precipitation from January through May in 2001 was greater than in 2002, causing earlier spore release in 2001. The precipitation in August in 2001 was also greater than in 2002, leading to an extended period of spores in the air in 2001.

Petrie (1994) speculated that the optimum temperature for ascospore production on naturally infected canola stubble was 15 °C. Ascospore sporulation occurred earlier under favorable moisture conditions from April through June. Petrie (1995) also noted that ascospore numbers were negatively correlated with number of days from April through June having a maximum temperature 30 °C. McGee (1977) found that temperatures between 8 and 12 °C generally occurred during the period of maximum ascospore release, but no relative humidity was described. Our study showed that the air temperatures between 13 and 18 °C and relative humidity > 80 % during the growth stage of canola

Table 5.2. Temperature, relative humidity and precipitation from January to August in 2001 and 2002

Month	January	February	March	April	May	June	July	August
Year 2001								
T_{max} (°C)	-5.9	-12.5	-1.0	8.8	19.1	22.6	25.7	26.5
T_{min} (°C)	-16.8	-22.3	-10.9	-10.9	-0.5	10.2	13.4	13.1
RH_{max} (%)	99.2	92.5	99.4	100.5	99.9	99.7	102.0	101.9
RH_{min} (%)	83.8	73.9	76.7	67.9	48.9	53.1	60.4	52.7
Precipitation (mm)	215.6	10.8	13.8	69.2	162.9	30.0	160.4	19.0
Year 2002								
T_{max} (°C)	-7.8	-2.7	-5.7	7.9	15.2	23.6	27.1	23.9
T_{min} (°C)	-17.0	-14.3	-15.6	-4.0	0.1	11.8	13.5	11.5
RH_{max} (%)	95.3	97.3	91.5	99.5	95.3	99.1	102.0	103.8
RH_{min} (%)	78.2	70.5	60.9	47.6	36.7	52.1	53.8	59.3
Precipitation (mm)	11.9	5.7	20.0	30.2	103.6	141.0	30.0	106.4

T_{max} - average maximum temperature, T_{min} - average minimum temperature, RH_{max} - average maximum relative humidity, RH_{min} - average minimum relative humidity.

were associated with periods of ascospore release. Peak ascospores in the air was also associated with rainfall ≥2 mm.

Our two-year study found that more pycnidiospores were trapped at temperatures between 13 and 18 °C and relative humidity of > 80 % under field conditions, which occurred from 4th leaf stage through maturity of canola when infection of leaves developed quickly. Furthermore, rainfall of ≥ 2 mm played an important role in pycnidiospore release. Hammond and Lewis (1987) reported high relative humidity could favor pycnidia to development and production of numerous quantities of pycnidiospores. Vanniasingham and Gilligan (1989) characterized pycnidiospore production counting sporulating pycnidia and the maximum number of pycnidia produced per lesion. Their results indicated that high moisture could increase pycnidiospore production. However, they did not describe the relationship between moisture and pycnidiospore release and effects of temperature on pycnidiospore release.

There are no published articles on the diurnal patterns of ascospore and pycnidiospore release by *L. maculans*. However, Paulitz (1996) and Fernando et al. (2000) investigated the diurnal release pattern of ascospores by *Fusarium* species. Paulitz (1996) reported peak release of ascospores trapped in the evening between 4 and 12 pm. Fernando et al. (2000) trapped both ascospores and macroconidia. Ascospore release showed a daily periodicity but macroconidia did not. Ascospores were recovered mostly at night. Paulitz (1996) speculated that the increase in relative humidity following drying in the evening could increase the turgor pressure of asci.

This is the first report on the diurnal patterns of ascospore and pycnidiospore dispersal by *L. maculans*. Our study revealed that ascospore dispersal by *L. maculans* occurred in

the hours around midnight when RH was > 80% and temperatures were 13-18 °C. Few ascospores were trapped during the daytime when lower RH and higher temperatures prevailed. There may be similar mechanisms as high RH triggered ascospore release in *L. maculans* and *F. graminearum*. Conditions for the production of pseudothecia by *L. maculans* have been described (Pere, 1999; Venn, 1979; Xu, 1987). In these studies, the temperature of 14 °C and relative humidity of 100 % were the best conditions for the production of pseudothecia. However, few reports have been published on the effects of temperature and relative humidity on the formation of asci and ascospore release. An interaction between temperature and relative humidity was observed in our study. There were few ascospores trapped under conditions of higher relative humidity and higher temperature, or lower temperature and lower relative humidity.

A periodicity of pycnidiospore dispersal on days without rain was also found in our study. This indicated that pycnidiospore release was affected not only by rain events, but also by higher relative humidity (Hammond and Lewis, 1987) often occurring at night.

Our study demonstrated that peak pycnidiospore release occurred at the same time as rain events; rain events increased trapped pycnidiospore concentration on the current day. Peak ascospores in the air occurred one or more hours after rain events on the current day, and maintained greater levels of spores during the next three days. The differences in the hours of ascospores trapped may hence result from differences in pseudothecia and asci maturity on stubble in the field. Maturity is largely dependant on temperature and moisture (Petrie, 1994).

No study has reported on the distance of ascospore and pycnidiospore dispersal by *L. maculans* in the field in different wind directions. Our study showed that ascospores

could be dispersed to a distance of 25 m away from the inoculum source. Higher ascospore concentration was observed within 5 m from the inoculum source than further distances. However, pycnidiospores were still trapped 45 m from the inoculum source in 2001. This may point to the fact that ascospores may be responsible for the primary infection and pycnidiospores contributing to the secondary infection. In our study, both ascospores and pycnidiospores were detected more in the prevailing wind direction than from other directions. That suggested that pycnidiospore dispersal was affected not only by rain events but also carried thereafter by wind.

Canola stem canker is caused by infection of cotyledons, and the 1st and 2nd true leaves through a systemic pathway (Hammond and Lewis, 1987). The amount of spores released early and carried to canola young leaves by wind may contribute to levels of disease incidence and stem severity at maturity. In our 2002 study, the gradients of disease incidence and stem severity were steeper to the south of the inoculum source than to the north. It is believed that the prevailing wind from the end of June to beginning of July when canola plants were growing cotyledons, 1st and 2nd leaves came from the south of the inoculum source. The results of disease incidence gradients and stem severity gradients can help predict the spread of blackleg inoculum from infected fields.

There was a large difference between slopes of reduction of disease incidence and stem severity. Disease incidence declined by 50% approximately 12-14 m from the inoculum source. However, stem severity decreased by 50% approximately 5 m from the inoculum source. This indicated that plants with higher levels of stem severity were mainly distributed in the area approximately 5 m from the inoculum source. This is consistent with the distribution of higher ascospore concentration in the field.

Our two-year study showed the seasonal and diurnal patterns of spore dispersal by *L. maculans*, but it did not address the effects of temperature, RH and other weather factors on the development of pseudothecia and asci maturity. Results of the present study will help to clarify the process of spore release and spread. An understanding of all these factors affecting spore release will be useful in modeling and forecasting epidemics of blackleg disease.

6. EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON INFECTION OF CANOLA COTYLEDONS BY *LEPTOSPHAERIA MACULANS*

6.1 Abstract

This study was conducted in growth cabinets to investigate the effects of temperature and relative humidity on blackleg disease development at the seedling stage. The experiment was arranged in a split plot design with temperature as the main plots, relative humidity as the subplots. Relative humidity and temperature had significant effects on development of lesion size on canola cotyledons. There was significant interaction between relative humidity and temperature. At 50% and 70% relative humidity, lesion size significantly increased with decrease in temperature from eight through fourteen days after cotyledons were inoculated. The optimum temperature for disease was 18/20°C (night/day). At relative humidity 30%, there were no significant differences among temperature 18/20°C, 23/25°C and 27/30°C. At temperature 18/20°C and 23/25°C, lesion size significantly increased with increase in relative humidity. At temperature 27/30°C, there was no significant difference between RH 30% and 50%. The optimum relative humidity was 70% at the three temperature regimes. Therefore, both temperature 18/20°C and relative humidity 70% were the optimum conditions for development of blackleg leaf lesions of canola cotyledons.

6.2 Introduction

Blackleg, caused by *Leptosphaeria maculans* (Desm.) Ces. et de Not. (anamorph: *Phoma lingam* (Tode ex Fr.) Desm.), is a serious and economically disease of canola (*Brassica napus* and *B. rapa*) worldwide. Severe epidemics have occurred on canola in

France, Britain and Australia since 1950 (Gugel and Petrie, 1992; Sawatsky, 1989; Bokor et al., 1975). The disease virtually wiped out the canola industry in Australia in 1972, two years after canola cultivars were introduced into Canada (Wood and Barbetti, 1977). In Canada, a virulent strain of the fungus was detected in Saskatchewan in 1975, and then occurred in most provinces that produced canola, causing significant economic losses (McGee and Petrie, 1978; Gugel and Petrie, 1992).

Blackleg disease is monocyclic (Howlett et al., 2001). Primary inoculum comes from ascospores from canola stubble, mycelium from seed and pycnidiospores from canola stubble and seeds. Secondary inoculum is generated from pycnidiospores produced on infected plants, but the secondary infections are not considered to cause serious yield losses. The pathogen, *L. maculans* can attack all parts of the canola plant: cotyledons, leaves, stems, roots and pods. It may kill seedlings soon after emergence or cause lesions varying in size and shape on cotyledons or leaves. A number of pycnidia are often present in the dead tissue. Mucilage with a mass of spores from pycnidia can be observed when favorable conditions of temperature and moisture occur (Howlett et al., 2001).

The differences in severity of epidemics between seasons and between regions may be attributed to differences in the favorable conditions, especially to differences in temperature and moisture, for infection of canola by *L. maculans* (Gladders and Musa, 1980). Our study in effects of crop rotation and tillage on blackleg disease of canola in Manitoba showed that leaf lesions were evident from the beginning of June (cotyledon stage) to the middle of July (No. 6-leaf stage) in inoculated plots. Our study in patterns of spore release by *L. maculans* found that pycnidiospore release occurred during this period. Vanniasingham and Gilligan (1989) found in the study of pycnidia production,

that pycnidia could be produced within two weeks on wounded leaves in the laboratory when canola plants were inoculated with 5×10^4 pycnidiospores/mL and exposed to surface wetness at 10-25°C for 1-8 days. Pycnidia were produced in the highest number on leaves kept wet at 25°C and under 500μmol·m^{-2}·s^{-1} light intensity for more than 8 days. However, there has been no study on the effects of temperature and moisture on leaf lesion development.

The objectives of this study were: (1) to investigate the effects of temperature and relative humidity on the infection of canola leaves by *L. maculans*; (2) to use the results of the growth cabinet study to explain the effects of weather conditions on development of leaf lesions in canola grown with different crop rotation and tillage systems (Chapter 2).

6.3 Materials and Methods

6.3.1 Experimental design

The study was conducted in two growth cabinets (Model: GC15; Enconaire Systems Ltd., Winnipeg, Manitoba, Canada) in the Department of Plant Science and one (Model: GC15; Enconaire Systems Ltd., Winnipeg, Manitoba, Canada) in the Department of Entomology, University of Manitoba. The experiment was arranged in a split plot design with temperature as the main plots and relative humidity as the subplots. There were three replicates. Temperature treatments included three levels: 18/20°C (night/day), 23/25°C and 27/30°C; and relative humidity (RH) treatments included three levels: 30%, 50% and 70%. In each replicate, there were twelve plants; ten of them were inoculated; and the other two were non-inoculated (control treatment). A Bacharach sling psychrometer (Model 12-7011, Bacharach Instrument Company, Pittsburgh, Pa., US) was

used to measure true relative humidity and temperature in the cabinets, after temperature and relative humidity were set up.

6.3.2 Inoculation

Canola cultivar was Westar, a highly susceptible cultivar to blackleg. PG-2 isolate 86-12 was selected as the blackleg pathogen. After canola cotyledons expanded, each cotyledon was punctured with a single prick using a needle. A 10μL drop of pycnidiospore suspension at 4×10^7 spores/mL was placed on the wound. At the same time, non-inoculated cotyledons were pricked and the wound was covered with 10μL drop of sterile distilled water. All plants were left at 24°C for 12 hours, then put in the growth cabinets.

6.3.3 Data analysis

Statistical analyses were conducted using The SAS System 8.2 (SAS Institute Inc., Cary, NC, USA). The Fisher's LSD (least significant difference) test was selected to examine the differences among treatments ($P \leq 0.05$).

6.3.4 Disease assessment

Lesion size was assessed as sum of the horizontal and vertical length (mm). Assessment of lesion size was performed 8, 10, 12 and 14 days after plants were inoculated.

6.4 Results

Relative humidity and temperature had significant effects on development of lesion size on canola cotyledons. There was significant interaction between relative humidity and temperature (Table 6.1).

Table 6.1. Size of lesions on cotyledons of canola variety Westar caused by *Leptosphaeria maculans* in relation to relative humidity (RH) and temperature (T)

Treatment	Days after cotyledons were inoculated			
	8	10	12	14
Relative humidity (RH)	**	**	**	**
Temperature (T)	**	**	**	**
RH × T	**	**	**	**
70%-18/20°C	4.10aA	6.19aA	10.11aA	13.48aA
70%-23/25°C	3.18bA	5.53bA	9.23bA	11.81bA
70%-27/30°C	2.75cA	4.70cA	8.22cA	10.56cA
50%-18/20°C	3.23aB	5.51aB	9.29aB	11.75aB
50%-23/25°C	2.74bB	4.77bB	8.29bB	10.46bB
50%-27/30°C	2.20cB	3.50cB	6.95cB	8.64cB
30%-18/20°C	2.24aC	3.54aC	7.04aC	8.75aC
30%-23/25°C	2.38aC	3.85aC	7.02aC	9.00aC
30%-27/30°C	2.28aB	3.52aB	7.01aB	8.84aB
LSD (70%)	0.33	0.41	0.71	1.04
LSD (50%)	0.40	0.60	0.54	0.90
LSD (30%)	0.29	0.45	0.37	0.36
LSD (18/20°C)	0.59	0.52	0.48	1.12
LSD (23/25°C)	0.28	0.70	0.63	0.78
LSD (27/30°C)	0.39	0.83	0.77	1.25

Lesion size was assessed as sum of the horizontal and vertical length (mm). Different small letters within a column indicate significant differences in lesion size among different temperature regimes at the same relative humidity; and different capital letters within a column indicate significant differences in lesion size among different relative humidity at the same temperature according to the Fisher's least significant difference (LSD) test ($P \geq 0.05$). LSD (RH) – LSD value for same RH with different temperature; LSD (Temperature) – LSD value for same temperature with different relative humidity. ** – effects of relative humidity, temperature or their interaction were significant at 0.01 level.

At temperature 18/20°C, lesion size significantly increased with increase of RH during eight to fourteen days after plants were inoculated (Table 6.1). Seventy percent was the optimum RH for lesion development. At temperature 23/25°C a similar trend was observed during the same period after inoculation, and the largest lesion size also occurred at RH 70%. At temperature 27/30°C, there was no significant difference between RH 30% and 50%.

At 30% RH, change of temperature had no significant effect on lesion size during each period after inoculation (Table 6.1). At RH 50% and 70%, lesion size significantly increased with decrease of temperature during each period. Temperature 18/20°C was the optimum for lesion development at RH 50% and 70%.

6.5 Discussion

There have been limited studies in effects of temperature and RH on infection of canola by *L. maculans*. Hall (1992) reported that stem lesions were produced after 4-8 weeks when temperature increased from <10°C to 15°C after being inoculated with pycnidiospores. Leaf and stem lesions appeared earlier at 14-16°C than at <10°C. Number of infected cotyledons and stem severity increased when temperature increased from 8°C to 15°C after inoculation. Disease incidence on cotyledons increases with increase of duration of high RH. Relative humidity of lower than 100% can alleviate disease severity (Hall, 1992). Our study showed that both RH 70% and temperature 18/20°C were the optimum conditions for lesion development on canola cotyledons. Lesion development would be suppressed when RH was lower than 70% and temperature was higher than 18/20°C. Our study did not address effects of relative humidity >70% and temperature <18/20°C on lesion development. When combined with Hall's study

(1992), we can speculate that relative humidity >70% and temperature 15-19°C (18/20°C) may be the optimum conditions for lesion development.

Weather factors showed different effects on lesion development on different parts of the crop. Nathaniels and Taylor (1983) found that there was no symptom accompanied by infection on canola stems at 1-10°C after inoculation but stem cankers developed quickly when transferred to higher temperature. The pathogen of *L. maculans* infects canola in various ways: symptomless colonization of the leaf, necrosis of the leaf, systemic symptomless colonization of the petiole, symptomless colonization of the stem, and necrosis of the stem (Hammond et al., 1985).

Relative humidity and temperature exhibited different effects on lesion development in different host-pathogen systems. Optimum conditions in various systems included 25°C and 72-hour wetness for infection of celery by *Septoria apiicola* (Mathieu and Kushalappa, 1993), 15/22°C and 72-hour wetness for development of Septoria tritici blotch on wheat (Chung et al., 2001), combinations of temperatures between 10 and 25°C and leaf-wetness periods between 6 and 24 hours for infection of saskatoon leaves by *Entomosporium mespili* (Holtslag et al., 2003).

Grove (2002) found that an increase in relative humidity could compensate for the negative effect of an increase in temperature on infection of cherry and peach foliage by *Wilsonomyces carpophilus* causing shot holes of stone fruit. A similar result was observed in our study. When RH was 50% and temperature was 18/20°C-23/25°C, or RH was 30% and RH was 18/20°C, the reduction of lesion size due to rise of 5°C could be compensated by rise of 20% RH. For example, when RH and temperature were changed from 50%-18/20°C to 50%-23/25°C, the reduction of lesion size could be compensated

by rise of 20% RH to 70%-23/25°C. When RH was 30% and temperature was 27/30°C, the reduction of lesion size due to rise of 10°C could be compensated by rise of 40% RH.

The study on effects of crop rotation and tillage on blackleg disease showed that the temperature and RH from June to August were approximately 18-20°C and 70%-80%, respectively, which coincided with the best weather conditions for lesion development. This was consistent with the observation of the crop leaf lesions in the field.

7. GENERAL DISCUSSION AND CONCLUSIONS

This is the first report that systematically attempts to elucidate effects of cropping practices on the progress, incidence and severity of blackleg disease of canola. The viability of the blackleg pathogen at different soil depths in different soil types, seasonal and diurnal patterns of ascospore and pycnidiospore dispersal by *L. maculans* from canola stubble in the field in relation to weather conditions in Manitoba, the distance of spore dispersal, and the effects of temperature and relative humidity on infection of canola leaves by *L. maculans* were also studied.

A three-year rotation with non-host crops effectively reduced blackleg disease, and decreased the level of inoculum (amount of spores released) and the potential for crop infection. Survival of the blackleg pathogen in the soil significantly decreased within nine months, consistent with the study by McGee (1977). Morrall et al. (1990) found in their survey that a rotation with non-host crops for less than four years did not reduce disease levels. The investigated fields were considered to be infected by blackleg pathogen from the neighboring fields by air-borne ascospores of *L. maculans*. The amount of stubble and the level of inoculum in the field were not investigated in our first study; they may impact the effects of rotation and tillage on reducing the disease (McGee, 1977).

Conventional tillage had a significant effect on reducing the disease and amount of spores released in simple rotations. Zero tillage is proposed to be used in complicated rotations. Survival of the pathogen rapidly decreased at 5 and 10-cm depths in different types of soil within nine months. Turkington et al. (2002) reported that amount of canola stubble left on the soil surface was less for conventional tillage than for zero tillage. They found, however, that several years of tillage practice might bring infected stubble already

buried in the soil, to the soil surface and increase the level of inoculum in the field. Tillage effect on the disease will be clarified with further understanding of the following aspects: a. Effects of different types of tillage systems on canola residue movement and distribution at different depths in different soils; b. Decomposition rate of canola residue at different depths in different soil over time and effects of environmental conditions; c. Persistence of blackleg pathogen at different depths in different soils over time.

The seasonal and diurnal patterns of spore dispersal by *L. maculans* under the weather conditions in Manitoba, and the conditions of spore dispersal by *L. maculans* and the effect of wind on pycnidiospore dispersal were described for the first time. Pycnidiospores cannot be only dispersed by rain splash (Howlett et al., 2001; Williams, 1992), but also released at relative humidity of >80% and the optimum temperatures of 13-18 °C. This study did not address the effects of temperature, relative humidity and other weather factors on the development of pseudothecia and asci maturation. The individual effects of temperature and relative humidity on spore release will further aid in understanding the process of spore dispersal and spread.

There were significant effects of relative humidity and temperature on development of leaf lesions. The optimum relative humidity and temperature for development of leaf lesion coincided with the weather conditions during the period canola plants were sensitive to the disease, explaining the effects of weather conditions on development leaf lesion in the field. In future, a further study should be carried out to examine differences in infection of canola leaves by ascospores and pycnidiospores of the blackleg pathogen which shed light to the importance of these two kinds of spores in initial infection and their ability to infect the canola crop.

In summary, the results of this study elucidated the role of crop rotation and tillage in reducing blackleg disease; knowledge of epidemiology of blackleg disease will allow future disease management strategies to be implemented.

8. RECOMMENDATION AND FUTURE STUDIES

1. Effects of crop rotation and tillage on blackleg disease should be estimated in other regions with different weather conditions which may affect decomposition of crop stubble and *L. maculans* survival, and further impact the effects of rotation and tillage on blackleg disease.

2. It is recommended that study on crop rotation and tillage should be performed in different plots with different levels of inoculum, which could impact effects of rotation and tillage on survival of the blackleg pathogen and subsequent disease.

3. To further understand the effect of tillage on reducing the disease, some studies could be performed: movement of crop stubble in the soil of different tillage systems, tillage-caused changes of soil texture and environmental conditions (temperature, relative humidity, pH etc.) at different depths in different types of soils, and their effects on the pathogen survival.

4. In the study of spore dispersal patterns, it is important to understand the effects of temperature and relative humidity on pseudotheical and pycnidial development, and mechanisms of spore release from pseudothecia and pycnidia.

5. Based on the study of spore dispersal patterns, a predict model of blackleg disease can be developed by integrating indexes of crop development and weather factors.

9. LITERATURE CITED

Agrios, G. N. 1997. Plant Pathology, 4th Edition (Academic Press Ltd., UK). P: 143-225.

Assabgui, R., and Hall, R. 1989. Incidence and severity of blackleg in spring and winter rapeseed in Ontario, 1988. Can. Plant Dis. Surv. 69: 68.

ASSESS, Image Analysis Software for Plant Disease Quantification. 2002. The American Phytopathological Society. St. Paul, Minnesota.

Background of Canola Varieties. 2002. The Grower Manual. Canola Council of Canada. Website: http://www.canola-council.org./production/canvarys.html.

Bailey, K. L., and Duczek, L. J. 1996. Managing cereal diseases under reduced tillage. Can. J. Plant Pathol. 18: 159-167.

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

Baird, R. E., Phillips, D. V., Mullinix, B. G., and Alt, P. J. 1999. Relative longevity of *Leptosphaeria maculans* and associated mycobiota on canola debris. Phytoprotection. 80(1): 1-11.

Barbetti, M.J. 1976. The role of pycnidiospores of *Leptosphaeria maculans* in the spread of blackleg disease in rape. Aust. J. Exp. Agric. Anim. Husb. 16: 911-914.

Barbetti, M. J., and Khangura, R. K. 1999. Managing blackleg in the disease-prone environment of Western Australia. Proceedings of the 10th International Rapeseed Congress, 1999. Canberra, Australia. http://www.regional.org.au/papers/index.htm.

Baudoin, A. B. A. M. 1986. Environmental conditions required for infection of photinia leaves by *Entomosporium mespili*. Plant Dis. 70: 519-521.

Blenis, P. V., Chow, P. S., and Stringam, G. R. 1999. Effects of burial, stem portion and cultivar on the decomposition of canola straw. Can. J. Plant Sci. 79(1): 97-100.

Bockus, W. W., and Claassen, M. M. 1992. Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. Plant Dis. 76: 633-636.

Bockus, W. W., Davis, M. A., and Norman, B. L. 1994. Effects of soil shading by surface residues during summer fallow on take-all of winter wheat. Plant Dis. 78: 50-54.

Bokor, A., Barbetti, M.J., Brown, A.G.P., and MacNish, G. C. 1975. Blackleg of rapeseed. J. Agri. West. Aust. 16: 7-10.

Brown, A. G. P., Barbetti, M. J., and Wood, P. M. 1975. Effect of benomyl on "blackleg" disease of rapeseed in Western Australia. Aust. J. Exp. Agric. Anim. Husb. 16: 276 – 279.

Canola Council of Canada. 2002. The Growers Manual. Canola-Connection website: http://www.canola-council.org.

Canola Council of Canada. 2001. Growth Stages of the Canola Plant. Website: http://www.canola-council.org/manula/growstag.htm.

Canola History. 2002. Website: http://www.canola-council.org/pubs/originhistory5.pdf.

Canola Production and Management. 2002. *Diseases* by Hershman, D. E. Website: http://www.ca.uky.edu/agc/pubs/id/id114/id114.htm.

Chen, Y. and Fernando, W.G.D. 2003. Interactions between weakly and highly virulent strains of *Leptosphaeria maculans* leading to induced resistance to blackleg disease in canola. Can. J. Plant Pathol. 25:421.

Chungu, C., Gilbert, J., and 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.

Crop Profiles. Crop Profile for Canola in Minnesota. 2000. Website: http://nestdata.ncsu.edu/cropprofiles/decs/mncanola.htm1.

Dill-Macky, R., Jones, R.K. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. 84: 71-76.

Doran, J. W. 1980. Soil microbial and biochemical changes associated with reduced tillage. Soil Sci. Soc. Am. J. 44: 765-771.

Evens, I. R., Kharbanda, P. D., Harrison, L., and Kaminski, D. 1991. Blackleg of canola survey in Alberta – 1989. Can. Plant Dis. Surv. 71: 98-99.

Fernando, W. G. D. and Chen, Y. 2003. First report on the presence of *Leptosphaeria maculans* pathogenicity group-3, the causal agent of blackleg of canola in Manitoba. Plant Dis. 87: 1268.

Fernando, W. G. D., Miller, J. D., Seaman, W. L., Seifert, K. and Paulitz, T. C. 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other Fusarium species sampled over wheat plots. Can. J. Bot. 78: 497 - 505.

Fitt, B. D. L., Creighton, N. F., Lacey, M. E. and McCartney, H. A. 1986. Effects of rainfall intensity and duration on dispersal of *Rhynchosporium secalis* conidia from infected barley leaves. Trans. Brit. Mycol. Soc. 86: 611 - 618.

Fitt, B. D. L., Lysandrou, M. 1984. Studies on mechanisms of splashed dispersal of spores, using *Pseudocercosporella herpotrichoides spores*. Phytopathology. 111:323-331.

Fitt, B. D. L. McCartney, H. A. and Walklate, P. J. 1989. The role of rain in dispersal of pathogen inoculum. Ann. Rev. Phytopathol. 27: 241 - 270.

Fitt, B. D. L., Walklate, P. J., McCartney, H. A., Bainbridge, H. A., Creighton, N. F., Hirst, J. M., Lacey, B. J. and Legg, B. J. 1986. A rain tower and wind tunnel for studying the dispersal of plant pathogens by rain and wind. Ann. Appl. Biol. 109: 661 - 671.

Gladders, P. and Musa, T. M. 1980. Observations on the epidemiology of *Leptosphaeria maculans* stem canker in winter oilseed rape. Plant Pathol. 29: 28 - 37.

Gracia-Garza, J.A., Neumann, S., Vyn, T.J., and Boland, G.J. 2002. Influence of crop rotation and tillage on production of apothecia by *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 24: 137-143.

Grau, C. R., and Radke, V. L. 1984. Effects of cultivars and cultural practices on Sclerotinia stem rot of soybean. Plant Dis. 68: 56-58.

Gregory, S., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology. 67: 1051 – 1056.

Grove, G. G. 2002. Influence of temperature and wetness period on infection of cherry and peach foliage by *Wilsonomyces carpophilus*. Can. J. Plant Pathol. 24: 40-45.

Gugel, R. K., and Petrie, G. A. 1992. History, occurrence, impact, and control of blackleg of rapeseed. Can. J. Plant Pathol. 14: 36 – 45.

Hall, R. 1992. Epidemiology of blackleg of oilseed rape. Can. J. Plant Pathol. 14: 46 – 55.

Hammond, K.E. and Lewis, B.G. 1986. The timing and sequence of events leading to stem canker disease in populations of *Brassica napus* var. *oleifera* in the field. Plant Pathol. 35: 551 – 564.

Hammond, K.E. and Lewis, B.G. 1987. The establishment of systemic infection in leaves of oilseed rape by *Leptosphaeria maculans*. Plant Pathol. 36: 135 - 147.

Hammond, K., Lewis, B. G., and Musa, T. M. 1985. A systemic pathway in the infection of oilseed rape plants by *Leptosphaeria maculans*. Plant Pathol. 34: 557-565.

Harrison, L. M., and Kharbanda, P. D. 1994. The first report on extensive spread and distribution of virulent blackleg of canola in the Peace River region of Alberta. Can. J. Plant Dis. Surv. 74: 92 – 93.

Hershman, D.E. and Perkins, D.M. 1995. Etiology of canola blackleg in Kentucky and seasonal discharge patterns of *Leptosphaeria maculans* ascospores from infected canola stubble. Plant Dis. 79: 1225 - 1229.

Holtslag, Q. A., Remphrey, W. R., and Fernando, W. G. D. 2003. Leaf-wetness duration and temperature required for infection of saskatoon leaves by *Entomosporium mespili* under controlled conditions. Can, J. Plant Pathol. 25: 168-173.

Howlett, B.J., Idnurm, A. and Pedras, M.S.C. 2001. *Leptosphaeria maculans*, the causal agent of blackleg disease of Brassicas. Fungal Genet. Biol. 33: 1 - 14.

Jacobs, J. L. 2001. A comparison of pedigree selection and doubled haploid line breeding methods for cultivar development in high erucic acid rapeseed. Masters thesis. P: 159.

Kaminski, D.A., Rapp, B., and Celetti, M. 1997. Diseases of cereals, oilseeds and pulses. Principles and Practices of Crop Rotation.
Website: http://www.agr.gov.sk.ca/DOCS/crops/integrated_pest_management/soil_fertility_fertilizers.

Kaminski, D.A., Morrall, R.A.A. and Duczek, L.J. 1996. Survey of canola diseases in Saskatchewan, 1995. Can. Plant Dis. Surv. 76: 99-102.

Khangura, R.K. and Barbetti, M.J. 2001. Prevalence of blackleg (*Leptosphaeria maculans*) on canola (*Brassica napus*) in Western Australia. Aust. J. Exp. Agric. 41(1): 71-80.

Kharbanda, P. 1989. Effectiveness of fungicides to control blackleg of canola/rapeseed. Can. J. Plant Pathol. 11: 193.

Kharbanda, P. 1999. Influence of tillage on blackleg and other diseases of canola in rotation with barley. Website: http://www.scdc.sk.ca/php-bin/articles/a038.html.

Kharbanda, P., Yang, J., Beatty, P., Jensen, S., and Tewari, P. J. 1999. Biocontrol of *Leptosphaeria maculans* and other pathogens of canola with *Paenibacillus polymyxa* PKB1. Website: http://www.regional.org.au/au/gcirc/3/448.htm.

Kharbanda, P. D., and Tewari, J. P. 1996. Integrated management of canola diseases using cultural methods. Can. J. Plant Pathol. 18: 168-175.

Koch. E., Song, K., Osborn, T. C., and Williams, P. H. 1991. Relationship between pathgenicity based on restriction fragment length polymorphism in *Leptosphaeria maculans*. Mol. Plant Microbe Interact. 4: 341 – 349.

Lafond, G. P., and Derksen, D. A. 1996. Long-term potential of conservation tillage on the Canadian prairies. Can. J. Plant Pathol. 18: 151-158.

Madden, L.V. 1977. Effects of rain on splash dispersal of fungal pathogens. Can. J. Plant Pathol. 19: 225 - 230.

Martin, K., Kutcher, H.R. and Johnston, A.M. 2001. Impact of tillage system, rotation, and fungicide application on field crop production. Abstracts, Saskatchewan Regional Meeting, The Canadian Phytopathological Society, 1999. Can. J. Plant Pathol. 23: 187.

Mathieu, D., and Kushalappa, A. C. 1993. Effects of temperature and leaf wetness duration on the infection of celery by *Septoria apiicola*. Phytopathology. 83: 1036-1040.

McGee, D.C. 1974a. The seasonal pattern of ascospore discharge of *Leptosphaeria maculans*. Aust. Plant Pathol. Soc. Newsl. 3: 27.

McGee, D.C. 1974b. The application of findings from surveys of the incidence of blackleg of rapeseed in Victoria. Aust. Plant Pathol. Soc. Newsl. 3: 27.

McGee, D.C. 1977. Blackleg (*Leptosphaeria maculans* (Dsem.) Ces. Et de Not.) of rapeseed in Victoria: Sources of infection and relationship between inoculum, environmental factors and disease severity. Aust. J. Agri. Res. 28: 53 - 62.

McGee, D.C., and Emmett, R. W. 1977. Blackleg (*Leptosphaeria maculans* (Desm.) Ces. Et de Not.) of rapeseed in Victoria: Crop losses and factors which affect disease severity. Aust. J. Agri. Res. 28: 47 - 51.

McGee, D.C. and Petrie, G.A. 1978. Variability of *Leptosphaeria maculans* in relation to blackleg of oilseed rape. Phytopathology. 68:625 - 630.

McGee, D.C. and Petrie, G.A. 1979. Seasonal patterns of ascospore discharge by *Leptosphaeria maculans* in relation to blackleg of oilseed rape. Phytopathology. 69: 586 - 589.

Miller, J. D., Culley, J., Fraser, K., Hubbard, S., Meloche, F., Ouellet, T., Seaman, W. L., Seifert, K. A., Turkington, K., and Voldeng, H. 1998. Effect of tillage practice on fusarium head blight of wheat. Can. J. Plant Pathol. 20: 95-103.

Morrall, R.A.A., Kaminski, D.A. and Kaminski, L-A. 1999. The 1996-98 western Canada disease survey; Do agronomic practices affect disease or vice-versa? Proceedings of the 10th international rapeseed congress, Canberra, Australia.

Morrall, R.A.A. 1997. The evolution of lentil disease over 25 years in western Canada. Can. J. Plant Pathol. 19: 197-207.

Nathaniels, N. Q. R., and Taylor, G. S. 1983. Latent infection of winter oilseed rape by *Leptosphaeria maculans*. Plant Pathol. 32: 23 – 31.

Nyvall, R. F. 1989. Field Crop Diseases Handbook. P: 353 – 354.

Oplinger, E. S., Hardman, L. L., Gritton, E. T., Doll, J. D., and Kelling, K. A. 1989. Canola (rapeseed) history. Website: http://newcrop.hort.purdue.edu/newcrop/afcm.

Paulitz, T.C. 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Dis. 80: 674 - 678.

Pere, A., Poisson, B., Le Sourne, V. and Maisonneuve, C. 1999. *Leptosphaeria maculans*: Effect of temperature, rainfall and humidity on the formation of pseudothecia. Proceedings of the 10th International Rapeseed Congress, Canberra, Australia.
Website: http://www.regional.org.au/au/gcirc/3/106.htm#p1_103.

Peters, R., and Hall, R. 1987. Incidence and severity of blackleg in Ontario winter rapeseed. Phytopathology. 77: 1618.

Petrie, G.A. 1978. Ocurrence of a highly virulent strain of blackelg (*Leptosphaeria maculans*) on rape in Saskatchewan (1975 - 1977). Can.Plant Dis.Surv. 58:21 - 25.

Petrie, G.A. 1979. Prevalence of a highly virulent strain of *Leptosphaeria maculans* (blackleg) in seed sample of rape and turnip rape produced in western Canada in 1976 and 1977. Can. J. Plant Sci. 59: 899 – 901.

Petrie, G. A. 1982. Blackleg of rapeseed (canola) caused by *Leptosphaeria maculans*: interactions of virulent and weakly virulent strains and implications for biological control. Can. J. Plant Pathol. 4: 309.

Petrie, G. A. 1986a. Blackleg and other diseases of canola in Saskatchewan in 1984 and 1985. Can. Plant Dis. Surv. 66: 51 – 53.

Petrie, G. A. 1986b. Consequences of survival of *Leptosphaeria maculans* (blackleg) in canola stubble residue through an entire crop rotation sequence. Can. J. Plant Pathol. 8: 353.

Petrie, G.A. 1994. Effects of temperature and moisture on the number, size and septation of ascsopores produced by *Leptosphaeria maculans* (blackleg) on rapeseed stubble. Can. Plant Dis. Surv. 74 (2): 141 - 151.

Petrie, G.A. 1995. Patterns of ascospore discharge by *Leptosphaeria maculans* (blackleg) from 9- to 13-month-old naturally-infected rapeseed/canola stubble from 1977 to 1993 in Saskatchewan. Can. Plant Dis. Surv. 75 (1): 35 - 43.

Petrie, G.A., and Vanterpool, T. C. 1965. Diseases of rape and cruciferous weeds in Saskatchewan in 1965. Can. Plant Dis. Surv. 45: 111-112.

Petrie, G.A., and Vanterpool, T. C. 1966. Diseases of rape, mustard and cruciferous weeds in the prairie provinces. Can. Plant Dis. Surv. 46: 117-120.

Petrie, G.A., and Vanterpool, T. C. 1968. Diseases of crucifers in in 1967. Can. Plant Dis. Surv. 48: 25-27.

Petrie, G.A., and Vanterpool, T. C. 1970. Diseases of rape and other crucifers in Saskatchewan in 1969. Can. Plant Dis. Surv. 50: 106-107.

Petrie, G.A., and Vanterpool, T. C. 1974. Infestation of crucifer seed in western Canada by the blackleg fungus *Leptosphaeria maculans*. Can. Plant Dis. Surv. 54: 119-123.

Ploetz, R. C., Mitchell, D. J., and Gallaher, R. N. 1985. Population dynamic of soilborne fungi in a field multicropped to rye and soybeans under reduced tillage in Florida. Phytopathology. 75: 1447 – 1451.

Punithalingam, E., and Holliday, P. 1972. CMI Descriptions of Pathogenic Fungi and Bacteria. P: 331.

Rees, R. G., Platz, G.J., and Mayer, R. J. 1982. Yield loesses in wheat from yellow spot: Comparison of estimates derived from single tiller and plots. Aust. J. Agric. Res. 33: 899 – 908.

Rempel, C.B. and Hall, R. 1993. Dynamics of production of ascospores of *Leptosphaeria maculans* in autumn on stubble of the current year's crop of spring rapeseed. Can.J.Plant Pathol. 15:182 - 184.

Rimmer, S. R., and van den Berg, C. G. J. 1992. Resistance of oilseed Brassica spp. to blackleg caused by *Leptosphaeria maculans*. Can. J. Plant Pathol. 14: 56 – 66.

Roy, N. 1979. Register of Australia oilseed cultivars: *Brassica napus* (L) var. *napus* (rapeseed) cv. Wesbarker. Aust. J. Exp. Agric. 29: 151.

Savchuk, S. C. 2002. Evaluation of biololgical control of *Sclerotinia sclerotiorum* on canola (*Brassica napus*) in the laboratory, in the greenhouse, and in the field. Masters thesis. P: 170.

Sawatsky, W.M. 1989. Evaluation of screening techniques for resistance to *Leptosphaeria maculans* and genetic studies of resistance to the disease in *Brassica napus*. Master's thesis. University of Manitoba. P: 85.

Scarth, R., and McVetty, P. B. E. 1991. Hero summer rape. Can. J. Plant Sci. 71: 865-866.

Seaman, W. L. 1982. Epidemiology and control of mycotoxigenic fusaria on cereal grains. Can. J. Plant Pathol. 4: 187-190.

Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology. 67: 1051-1056.

Subbarao, K. V., Koike, S. T., and Hubbard, J. C. 1996. Effect of deep plowing on the distribution and density of *Sclerotinia minor* sclerotia and lettuce drop incidence. Plant Dis. 80: 28-33.

Summerell, B. A., and Burgess, L. W. 1989. Decomposition and chemical composition of cereal straw. Soil Bio. Biochem. 21: 551-559.

Sumner, D. R., Ben Doupnik, J., and Boosalis, M. G. 1981. Effects of reduced tillage and multiple cropping on plant diseases. Ann Rev. Phytopathol. 19: 167-187.

Sutton, J. C., and Vyn, T. J. 1990. Crop sequences and tillage practices in relation to diseases of winter wheat in Ontario. Can. J. Plant Pathol. 12: 358-368.

Teich, A. H., and Nelson, K. 1984. Survey of fusarium head blight and possible effects of cultural practices in wheat field in Lambton County in 1983. Can. Dis. Surv. 64: 11-13.

Tinline, R. D., and Spurr, D. T. 1991. Agronomic practices and common root rot in spring wheat: Effect of tillage on disease and inoculum density of *Cochliobolus sativus* in soil. Can. J. Pant Pathol. 13: 258-266.

Thomas, P. M. 1984. Weeds, insects, diseases. Chapter 10 in Canola Grower Manual. Canola Council of Canada, Winnipeg.

Thomas, P. M. 1985. Canola Growers Manual. Canola Council of Canada, Winnipeg, Canada. P: 1424.

Turkington, T.K., and Clayton, G.W. 2002. Crop rotation and plant disease management. Website: http://ssca.usak.ca/2002proceedings/Turkington.htm.

Turkington, T.K., Clayton, G.W., Klein-Gebbinck, H., and Woods, D. L. 2000. Residue decomposition and blackleg of canola: influence of tillage practices. Can. J. Plant Pathol. 22(2): 150-154.

Vanniasingham, V.M. and Gilligan, C.A. 1989. Effects of host, pathogen and environmental factors on latent period and production of pycnidia of *Leptosphaeria maculans* on oilseed rape leaves in controlled environments. Mycol. Res. 93(2): 167 - 174.

Vanterpool, T. C. 1961. rape diseases in Saskatchewan in 1961. Can. Plant Dis. Surv. 41: 372-373.

Venn, L. 1979. The genetic control of sexual compatibility in *Leptosphaeria maculans*. Aust. Plant Pathol. 8: 5 - 6.

Walklate, P. J., McCartney, H. A., and Fitt, B. D. L. 1989. Vertical dispersal of plant pathogens by splashing. Part II: Experimental study of the relationship between raindrop size and the maximum splash height. Plant Pathol. 38: 64-70.

West, J.S., Kharbanda, P.D., Barbetti, M.J. and Fitt, B.D.L. 2001. Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. Plant Pathol. 50: 10-27.

Williams, P.H. 1992. Biology of *Leptosphaeria maculans*. Can. J. Plant Pathol. 14: 30 - 35.

Williams, P.H., and Fitt, B. D. L. 1999. Differentiating A and B groups of *Leptosphaeria maculans*, causal agent of stem canker (blackleg) of oilseed rape. Plant Pathol. 48: 161 – 175.

Williams, J. R., and Stelfox, D. 1980. Influence of farming practices in Alberta on germination and apothecium production of sclerotia of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 2: 169-172.

Wood, P. M., and Barbetti, M. J. 1977. The role of seed infection in the spread of blackleg of rape in Western Australia. Aust. J. Exp. Agri. Anim. Husb. 17: 1040 – 1044.

Workneh, F., and Yang, X. 2000. Prevalence of sclerotinia stem rot of soybeans in the north-central United States in relation to tillage, climate, and latitudinal positions. Phytopathology. 90: 1375-1382.

Wrather, J.A. and Kending, S.R. 1998. Tillage effects on *Macrophomina phaseolina* population density and soybean yield. Plant Dis. 82: 247-250.

Wratten, N., and Mailer, R. J. 1989. Register of Australian oilseed cultivars: *Brassica napus* L. var. *napus* (rapeseed) cv. Eureka. Aust. J. Exp. Agric. 29: 602.

Wright, K. H., and Sutton, J. C. 1990. Inoculum of *Pyrenophora tritici-repentis* in relation to epidemics of tan spot of winter wheat in Ontario. Can. J. Plant Pathol. 12: 149-157.

Xi, K., Kutcher, H. R., Westcott, N. D., Morrall, R. A. A., and Rimmer, S. R. 1991. Efficacy of foliar-applied fungicides against blackleg of rapeseed. Can. J. Plant Pathol. 8: 356.

Xu, X.H., Hill, C.B. and Williams, P.H. 1987. Environmental conditions for the production of pseudothecia of *Leptosphaeria maculans*. Acta Mycologica Sinica. 6: 236 - 241.

Zadoks, J. C., Schein, R.D. 1979. Epidemiology and plant disease management. Oxford University Press, New York. P: 340.