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

XIAOWEI GUO

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

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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.

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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).

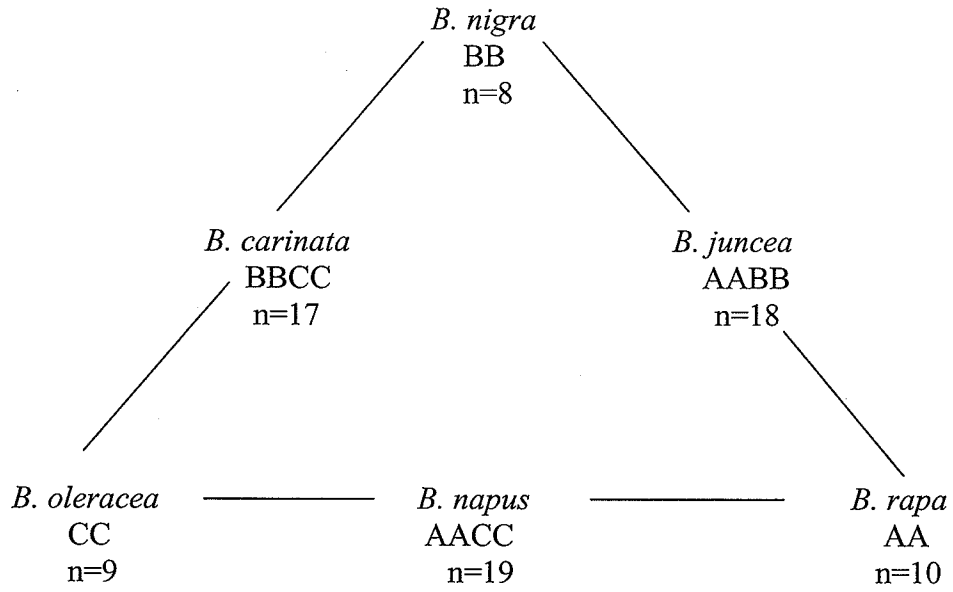

Figure 2.1. Relationship among the *Brassica* species (A, B, C = genome; n = chromosome number) (Savchuk, 2002).

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.

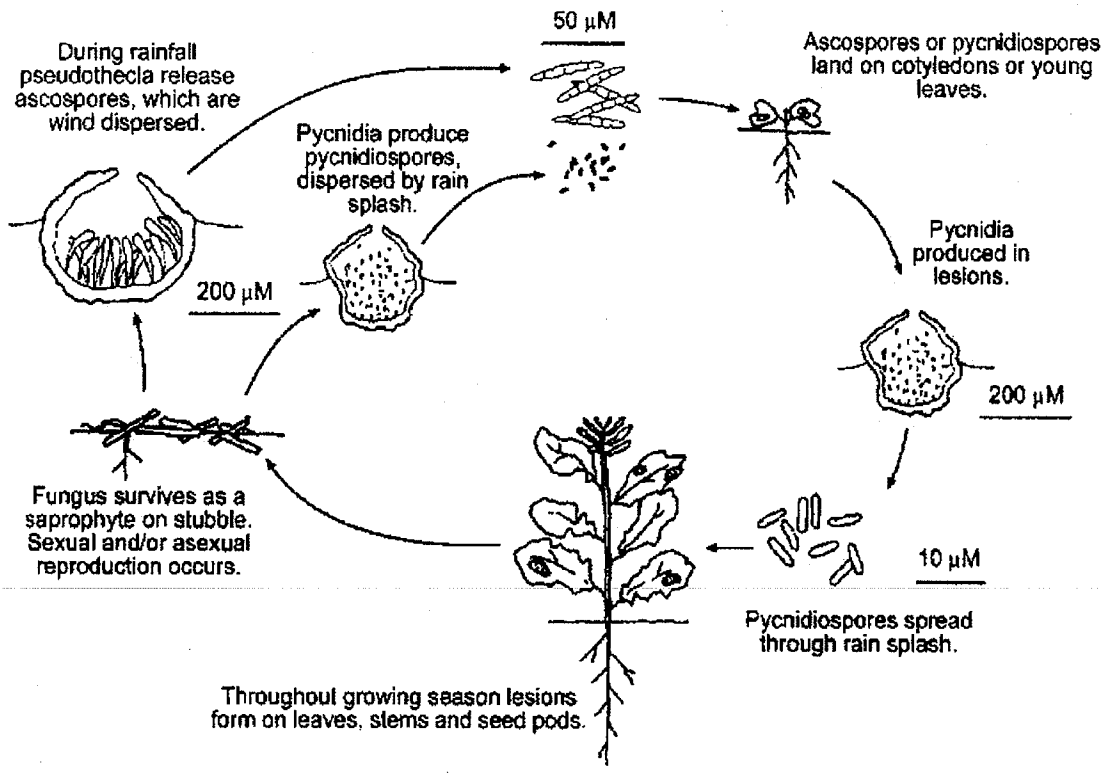

Figure 2.2. Life cycle of *Leptosphaeria maculans* on *Brassica napus* (Howlett, et al., 2001)

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