# Prediction of Sclerotinia Stem Rot in Rapeseed Using Buried Sclerotia of *Sclerotinia sclerotiorum*

by

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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## GENERAL ABSTRACT

Experiments are described in which preconditioned and unconditioned axenic sclerotia of Sclerotinia sclerotiorum (Lib.) de Bary are used to simulate germination of natural populations of sclerotia. Counts of carpogenically germinated sclerotia were taken from the sclerotia that were incubated at different temperatures. Different concentrations of polyethylene glycol (PEG 20,000 MW) as a medium were used to simulate various water potentials. Various combinations of temperature and water potentials were used to study the effect of interactions of temperature and water potential on carpogenic germination. Similarly, counts of apothecia from sclerotia that had been exposed to various lengths of cold treatment and buried in the field were taken on a weekly basis. Daily temperature, rainfall and biweekly readings of soil moisture were monitored during the experiment.

The effects of temperature, water potential and their interaction on sclerotial germination were significant. Sclerotial carpogenic germination occurs over a wide range of temperatures (5-25°C) and water potentials (0 to -5 bars). The optimum temperature was 15°C and the most conducive water potentials were between 0 and -2 bars. Low temperatures (5°C) and high temperatures (25°C) alone or in combination with low water potentials (-5 to -7 bars) delayed or inhibited the rate of carpogenic germination.

The effects of cold moist treatment and time of burial on sclerotium germination were highly significant. The sclerotia that were preconditioned germinated more than those that were unconditioned. Preconditioned sclerotia that were buried in early May germinated more than those similarly treated and buried in late June. Sclerotial carpogenic germination varied from location to location due to the weather patterns that occurred at each of the locations.

Apothecia were first observed in the field during the period consisting of the last week of June and the first week of July (crop stages 3.3-4.2). This coincided with the period when there was a rainfall frequency of at least 2 in every 7 days, with a total of 5 cm for the previous 10-14 days. The mean min-max temperatures ranged from 10-24°C and water potentials were greater than -20 centibars.

Linear regression analysis indicated that apothecial numbers were neither correlated with disease incidence nor with rapeseed yield. However, there was a high negative correlation between disease incidence and rapeseed yield.

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# Chapter I

# INTRODUCTION

Since the early sixties, the area of rapeseed (*Brassica campestris* L. and *Brassica napus* L.) production has increased dramatically in Western Canada. In 1979 a total of 3.4 million hectares were sown to rapeseed compared to 2.15 million hectares in 1971 (Manitoba Department of Agriculture, 1980). Stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a major disease of rapeseed in Western Canada and other parts of the world. The disease has become more widespread since spring rapeseed was introduced into western Canada in 1942. This has been determined from the surveys carried out by Platford and Bernier (1975), Rimmer and Platford (1982) and Dueck and Sedun (1983). This fungus is responsible for serious economic losses in many agricultural crops in both storage and the field (Purdy, 1979).

Recently, general infestations of rapeseed fields have been common in some regions and have caused yield losses. Morrall *et al*. (1976) suggest that losses in rapeseed were due to reduction in thousand kernel weight and seeds per plant.

At present no Canadian cultivars of either *B. campestris* (turnip rape) or *B. napus* (summer rape) are resistant to this fungus. Control methods such as crop rotation as well as other cultural practices have been recommended against this disease but have often proved inadequate. Thomson *et al*. (1984) and Dueck *et al*. (1983) have reported successful

control of rapeseed stem rot and an increase in rapeseed yield using the fungicides benomyl and iprodione. Many farmers are now applying these fungicides to control S. sclerotiorum in rapeseed. Since fungicides must be applied prior to any expression of disease symptoms in order to be effective, farmers have a problem in deciding whether to spray or not. Usually this decision is based upon the expected yield of the farmer's crop and his past experience with sclerotinia problems in rapeseed.

The economics of fungicide use in disease control depends, in part, on the ability to predict and forecast disease outbreaks. Most workers have used the presence of apothecia of S. sclerotiorum as a criterion for the prediction of disease outbreak and as a method of selecting test sites (Williams and Stelfox, 1979; Morrall and Dueck, 1982; Dueck et al., 1983). These are areas where disease development is virtually assured. However, the work of Williams and Stelfox (1979, 1980a) shows that airborne ascospores may be quite widely dispersed. Further, abundant disease symptoms may develop in instances where apothecia are not readily found (Morrall and Dueck, 1982; Dueck et al., 1983). To improve on the current method of prediction of S. sclerotiorum in rapeseed, it appeared important to investigate the physical environmental factors which favour apothecial morphogenesis and disease development in rapeseed with the ultimate objective of developing a predictive model for this disease in rapeseed.

## 1.1 GENERAL LITERATURE REVIEW

## 1.2 HOST RANGE OF *SCLEROTINIA SCLEROTIORUM*

*Sclerotinia sclerotiorum* (Lib.) de Bary is a plant pathogenic fungus of the class Ascomycetes, subclass Discomycetes. It is characterized by the production of hyaline, ellipsoidal ascospores which arise from ascii that have a cleft aperture at maturity (Kohn, 1979). The ascii develop from pedicelled apothecia which arise from the sclerotium. There is no conidial state (Kohn, 1979). It occurs in temperate and subtropical regions of the world and has a wide host range. Purdy (1979) stated that a total of 361 species in 64 families of plants were attacked by this fungus throughout the world. In Western Canada, this pathogen attacks a total of 104 species in 28 families of plants (Morrall et al., 1976).

A survey of the literature reveals more than sixty names used to refer to diseases caused by this fungus on various crops (Purdy, 1979; Dueck, 1977). These include diseases such as stem rot of rapeseed, stem and head rot of sunflowers, stem rot of clovers, blight of peanuts, white mold of beans, etc.

## 1.3 SYMPTOMS OF STEM ROT OF RAPESEED

Symptoms of sclerotinia stem rot in rapeseed can be observed at late flowering or early maturing stages of growth. Infections are commonly found on the stems, especially near branches or stem bases. Lesions appear first as water soaked regions on the stem which rapidly spread.

A whitish cottony growth of mycelium may be observed on the surface (Williams and Stelfox, 1979) and later the affected area becomes bleached and shredded. Sclerotia form both in and outside the stem. Sclerotia are about 4-8 mm long and 3-4 mm in diameter. After flowering, patches of prematurely ripened plants become conspicuous in the field. Occasionally, siliques may be attacked.

## 1.4 ECOLOGY AND EPIDEMIOLOGY

### 1.4.1 Sclerotial Survival

Sclerotia are the structures by which the fungus survives adverse conditions. These structures may be distributed throughout the tilled levels of soil during tillage operations. Although some sclerotia are destroyed by other organisms, a substantial number remain viable near the soil surface each year regardless of the host crop being grown (Dueck, 1977).

The persistence of sclerotial populations in fields for several years in the absence of susceptible crops is intriguing. Williams and Stelfox (1980b) investigated the effects of farming practices in Alberta on the frequency of apothecia. They found more apothecia under barley in 1978 and 1979 when rapeseed had been grown on the land in both 1976 and 1977 than when rapeseed had been grown in 1976 and barley in 1977. Thus; repeated planting to rapeseed (susceptible crop) increased sclerotial populations in the soil. This could be due to: a) long-term sclerotium survival, b) regeneration, or c) production of new sclerotia in infected weeds in cereal crops (Morrall and Dueck, 1982). Morrall and Dueck state that saprophytic regeneration is probably limited by the fact that

S. sclerotiorum is not a vigorously competitive saprophyte even though it grows rapidly in pure culture. The length of time sclerotia can survive in the prairie soils and climate has not been documented.

### 1.4.2 Sclerotial Germination

Sclerotia may germinate in one of the three ways: a) carpogenic, b) myceliogenic, and c) eruptive (Huang and Dueck, 1980; Willetts and Wong, 1980; Williams and Western, 1965a). Most references to the germination of sclerotia are on carpogenic germination (Bedi, 1962a; Henson and Valleau, 1940; Purdy, 1956; Morrall, 1977). Henson and Valleau stated that constant moisture, a temperature of 14°C and sufficient time of exposure were important factors in the initiation of stipes of S. sclerotiorum. Coley-Smith and Cooke (1971) reported that light is necessary for the expansion of the discs of apothecial and that light influences the length of the apothecial stipes. In S. sclerotiorum, stipes only develop in amber light or in the dark while differentiation to form apothecia requires light for at least 8 hours in every 24 hours (Coley-Smith and Cooke, 1971; McLean, 1958). Apothecia are about 2 cm long and 5-10 mm in diameter. Sclerotia must be at or within 5 cm of the soil surface for apothecial production to occur. Thus in nature, premature spore production below the soil surface is prevented until stipes have grown sufficiently to allow the successful liberation of spores to the atmosphere.

Mycelial growth of S. sclerotiorum occurs over a broad range of temperatures. According to Tanrikut and Vaughan (1951) and Le Tourneau (1979) the hyphae will grow when the temperature is as low as 0°C. Other researchers found slightly higher temperature, 4°C (Newton and

Sequeira, 1972) or 5°C (Abawi and Grogan, 1975) to be the minimum and the maximum above 30°C. The optimum temperature for growth is generally considered to be about 20°C, with only slight amounts of variation from this temperature among specific isolates (Tanrikut and Vaughan, 1951; Imolehin *et al*., 1980).

Myceliogenic germination is not as dependent on the physical environmental conditions of the soil as is carpogenic germination. Morrall (1977) found that the percentage moisture of a heavy clay soil could vary greatly and still be sufficient to satisfy requirements of myceliogenic germination. Grogan and Abawi (1975) showed that myceliogenic germination ceased at -91 bars and the optimum for germination was at -37 bars. Waterlogging of the soil does not favour germination as it creates an anaerobic environment which promotes sclerotial decay (Moore, 1949). Mycelia can only grow for about 2 cm if a non-living organic substrate is not present (McLean, 1958; Newton and Sequira, 1972; Williams and Western, 1965b). It is not clear whether eruptive germination occurs in *S. sclerotiorum* or not. The epidemiological importance of myceliogenic and eruptive germination in rapeseed is insignificant.

The disease cycle of *S. sclerotiorum* is well known from studies of several host species (Grogan and Abawi, 1975; Purdy, 1979). The epidemic potential of an apothecium is very important in the life cycle of this fungus. Each apothecium can produce up to 2 million ascospores over a 7-10 day period. With a sclerotium averaging 4-5 apothecia the potential ascospore production is 10 million per sclerotium (Steadman, 1983). This explains why low numbers of sclerotia in the soil may produce epidemics.

### 1.4.3 Ascospore Dispersal

Wind and insects (bees) are the most important dispersal agents for ascospores of *S. sclerotiorum*. Williams and Stelfox (1979) showed that ascospores can be carried by air currents up to and beyond 50-100m from the source and that ascospores which are forcefully ejected from apothecia do escape above the canopy in rapeseed and barley. This indicates the feasibility of airborne spores being carried to rapeseed fields not infested with sclerotia.

Insects, particularly bees, are important pollinating agents in rapeseed fields. They are also, inevitably, carriers of ascospores which stick on pollen grains. Stelfox *et al*. (1978) recovered ascospores of *S. sclerotiorum* from rapeseed pollen grains carried by honey bees.

### 1.4.4 Ascospore Survival

Ascospores of *S. sclerotiorum* are the primary infective propagules of this fungus on many crops (McLean, 1958; Williams and Stelfox, 1979; Gugel and Morrall, 1984).

Among the environmental factors affecting survival of ascospores or *S. sclerotiorum* in beans, the most important are temperature, relative humidity and ultra-violet light (Caesar and Pearson, 1983). Caesar and Pearson (1983) showed that temperatures of 25°C or greater combined with relative humidities in excess of 35% were most detrimental to ascospore survival. In their field experiments they found that an average of 21.5% more ascospores survived on shaded leaves approximately 12-15 cm above ground within a dense canopy than on top-most leaves.

Most rapeseed cultivars grow up to 90 cm tall and their canopy at bloom time completely shades the soil surface (Morrall and Dueck, 1982). The relative humidity under this canopy is slightly higher than the ambient while the temperatures remain close to ambient temperature. However, there are no quantitative reports on the influence of environmental factors on ascospore survival in rapeseed fields in Western Canada or elsewhere.

### 1.4.5 Infection

#### 1.4.5.1 Mycelial Infection

Huang and Hoes (1980) state that mycelial infection due to germination of sclerotia at the vicinity of sunflower roots is the most important for disease development on sunflowers in southern Manitoba. The mycelium grows towards and invades the root. The mycelial growth inside the root cuts off water and nutrient supply causing the plant to wilt (Huang and Dueck, 1980; Huang and Hoes, 1980).

Root to root spread occurs in sunflower if plants are closely spaced. In rapeseed there is no evidence that root infection or root to root spread is important epidemiologically.

#### 1.4.5.2 Ascospore Infection

McLean (1958) and Gugel and Morrall (1984) showed that foliar infection by ascospores occurs only through abrasions or senescent flower parts. Ascospores do not have enough energy to infect host plants directly unless the host has been damaged in some way (Newton and Sequeira, 1972; McLean, 1958). In both winter rapeseed in Europe and

spring rapeseed in Canada, infection has been suggested to occur primarily during the bloom period (Morrall and Dueck, 1982).

In Western Canada, the infection period is relatively brief since the flowering period lasts approximately two weeks. Leaves abscise shortly after completion of flowering. Morrall and Dueck (1982) observed the fungus producing sclerotia on fallen decaying leaves under rapeseed canopy in summer. There is little secondary spread from foliar infection via bridges of fallen, decaying leaves, particularly late in the season when the crop lodges or is swathed.

Plant phenology affects disease development not only through the influence of decaying floral parts but also by controlling inoculum production (Morrall and Dueck, 1982). Apothecia do not develop until the crop reaches growth stage 3.2 (Harper and Berkenkamp, 1975) when the plant canopy first completely shades the soil surface. Abawi and Grogan (1975) state that in white mold of irrigated beans, a dense canopy probably reduces surface evaporation such that a high water potential is maintained in the upper layers of soil long enough for sclerotia to germinate. Crop canopy also has a dampening effect on temperature fluctuations. Morrall and Dueck (1982) state that under rapeseed canopy soil surface temperatures remain close to ambient. The length of time and the physical environmental conditions required by ascospores to survive on plant surfaces when discharged before flower or other senescent tissue are available for colonisation have not been determined.

## 1.5 CONTROL

### 1.5.1 Influence of Farming Practices

Crop rotation is recommended for the control of *S. sclerotiorum* in susceptible crops. It is known that this fungus has a wide host range and a great inoculum potential that negates the effectiveness of crop rotation. Williams and Stelfox (1980b) investigated the effects of farming practices on the frequency of apothecia. They stated that crop rotation is not effective for control of *S. sclerotiorum* because carpogenic germination and ascospore production occur in fields planted to nonhost crops such as barley. Ascospores are then blown by air currents and deposited on nearby rapeseed causing infection.

They also showed that fall ploughing to a depth of 7.6-15 cm (deep or mold board ploughing) buried sclerotia and significantly reduced the numbers of apothecia which developed under a rapeseed crop the following year compared to surface (2-3 cm) cultivation. This reduction would be expected because stipes longer than 3 cm are rarely found in the field and therefore only sclerotia in the top 3-4 cm of the soil are functionalin disseminating spores.

Spring applied fertilizer increases sclerotial germination in early summer compared to fall applied and no fertilizer treatments (Williams and Stelfox, 1980b). Application of immediately available nitrogen in spring, they suggested, caused increased growth of barley initially and this flush of growth provided a canopy cover suitable for earlier germination of sclerotia than on plots not fertilized in spring. Light, soil temperature and moisture are critical factors determining carpogenic

germination and crop canopy development influences all these three factors.

1.5.2 Fungicides

Control of S. sclerotiorum with fungicides, particularly the fungicide benomyl, has been described for other Brassica spp. (Gabrielson et al., 1973). More recently, other fungicides active against sclerotium producing fungi, including S. sclerotiorum, have become available. These include vinclozolin, procymidone and iprodione (Dueck et al., 1983).

Dueck et al. (1983) evaluated all these fungicides for the control of S. sclerotiorum in rapeseed. Their results indicated that stem rot in rapeseed could be effectively controlled by a single application of one of the several fungicide treatments when plants are in early bloom stage. This growth stage precedes the period when infections are initiated and when the crop canopy becomes too dense for the fungicide application to penetrate to the lower plant parts. They concluded that benomyl applied alone at the rate of 0.5 Kg/ha or at a lower rate of 0.25 Kg/ha in combination with vinclozolin consistently controlled the disease. Vinclozolin at the rate of 0.75 Kg/ha alone or a lower rate in combination with a lower rate of benomyl was as effective as the higher rate of benomyl alone. Other researchers have found that benomyl does not effectively control S. sclerotiorum in some crops. Steadman (1983) suggested that the reason for the lack of control of S. sclerotiorum with benomyl on beans is that under high ascospore inoculum levels and favourable environment, most of the late blossoms are unprotected and become easily colonized. Benomyl acts as a contact protectant and no

evidence has been found of redistribution or systemic movement of this fungicide into newly formed blossoms which developed after spraying.

### 1.5.3 Biological Control

More than 30 species of fungi and bacteria have been implicated by various workers as antagonists or mycoparasites of *Sclerotinia* species (Adams and Ayers, 1979). Rai and Saxena (1975) isolated species of *Aspergillus*, *Penicillium* and *Stachybotrys* from decaying sclerotia and showed that they were antagonistic to sclerotia of *S. sclerotiorum in vitro*. They reported that under field conditions, sclerotia on the soil surface were extensively colonized by species of *Penicillium*.

Bedi (1958) reported that all of the sclerotia mixed into a nonsterilized soil were killed within 3 weeks of the addition of a potato dextrose broth culture of *Aspergillus flavus* Link ex Fries. The rapidity and completeness of this result indicated the effect may have been caused by toxic metabolites in the medium rather than by mycoparasitism.

Jones and Watson (1969) observed that sclerotia of *S. sclerotiorum* appear to be parasitized by certain isolates of various *Trichoderma* species but not others. One out of four single spore isolates of *Trichoderma viride* Pers. was able to decay sclerotia incubated in moist sand or buried in the soil.

*Coniothyrium minitans* Campbell, is a mycoparasite of *S. sclerotiorum* (Tribe, 1957; Jones and Watson, 1969; Ghaffar, 1972; Huang and Hoes, 1976, 1977, 1980; Huang, 1980). It infects both hyphae and sclerotia and produces pycnidia upon and within the sclerotium. Pycnidiospores

which are exuded as a black liquid mass are capable of infecting healthy sclerotia. Little is yet known of the physiology of the mycoparasitism by _C. minitans_. Jones and Watson (1969) described lysis of the pseudoparenchymatous tissue of the sclerotia of _S. sclerotiorum_ which they attributed to endo- and exoenzymes, particularly B-glucanase and chitinase produced by _C. minitans_. Ghaffar (1972) speculated that melanolytic enzymes played a role in permitting entry through the sclerotial rind.

Huang and Hoes (1976) and Huang (1980) described penetration of hyphae of _S. sclerotiorum_ by _C. minitans_ hyphae without the formation of specialized penetration structures. The host cytoplasm disintegrates and cell walls collapse as a result of infection.

The ability of _C. minitans_ to parasitize sclerotia of _S. sclerotiorum_ inside plant roots and as well as those on the root surface of infected sunflower plants was demonstrated by Huang (1977). _C. minitans_ is currently being used experimentally as a biological control agent for _S. sclerotiorum_.

### 1.5.4 Disease Prediction

At present the only effective and economical method of controlling _S. sclerotiorum_ is to use either protectant or curative fungicides. The economics of fungicide use in disease control depends in part on the ability to predict and forecast disease outbreaks. Most workers have used the presence of apothecia of _S. sclerotiorum_ as a criterion for the prediction of disease outbreaks and as a method of selecting test sites

(Williams and Stelfox, 1979; Morrall and Dueck, 1982; Dueck et al., 1983). Morrall and Dueck (1982) state that infection in rapeseed occurs primarily during the bloom period since ascospore infection requires senescent floral parts. This growth stage coincides with the period when apothecia appear on the soil surface.

Disease prediction or forecasting as developed in various crops to reduce crop loss and limit fungicide use would provide a needed management tool for the control of stem rot in rapeseed. Disease prediction methods for soil-borne pathogens, particularly sclerotium forming fungi are lacking. The information that is available in the literature deals with assay methods for measuring densities of sclerotia of Sclerotium cepivorum (Crowe et al., 1980) and the influence of temperature, moisture and host exudates on germination of sclerotia of Sclerotium rolfsii (Punja and Grogan, 1981a, 1981b; Punja and Jenkins, 1984; Shew et al,, 1984). Sclerotia of these species are known to germinate myceliogenically and eruptively and do not form apothecia (Punja and Grogan, 1981a,1981b; Crowe et al., 1980). Sclerotial myceliogenic germination is not as dependent upon the physical environmental conditions of the soil as is carpogenic germination (Morrall, 1977; Abawi and Grogan, 1975; Crowe et al., 1980). Grogan and Abawi (1975) showed that myceliogenic germination ceased at -91 bars and the optimum was at -37 bars. These water potentials are far less than the -5 bars at which most plants start to wilt. Sclerotia decay under flooding conditions (Moore, 1949; Morrall, 1977), whereas apothecial development requires continuous moisture over long periods of time (Grogan and Abawi, 1975; Purdy, 1979; Henson and Valleau, 1940).

Myceliogenic germination of the sclerotia of S. cepivorum and S. rolfsii Berks is the most important in diseases caused by these fungi, while in S. sclerotiorum apothecial development to produce ascospores is important in diseases caused by this fungus. Morrall and Dueck (1982) showed that there was no relationship between numbers of apothecia (sclerotial densities) of S. sclerotiorum and disease incidence in the field. The work of Williams and Stelfox (1979, 1980a) shows that airborne ascospores may be quite widely dispersed. Abundant disease symptoms may develop in instances where apothecia are not readily found (Morrall and Dueck, 1982; Dueck et al., 1983).

To improve on the current method of prediction of S. sclerotiorum in rapeseed, it is important to investigate the physical environmental factors which favour apothecial morphogenesis and disease development in rapeseed.

Chapter II

# THE EFFECTS OF TEMPERATURE AND WATER POTENTIAL ON CARPOGENIC GERMINATION

## 2.1 INTRODUCTION

Several factors, including temperature and water potential, are known to influence carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Abawi and Grogan (1979) state that newly formed sclerotia require various periods of exposure to cool moist conditions before attaining the capability for carpogenic germination. Duniway *et al*. (1977) state that maintaining soil moisture near field capacity (-300 mb) for long periods of time is essential for carpogenic germination. Grogan and Abawi (1975) showed that carpogenic germination does not occur below -6 bars whereas Morrall (1977) showed that carpogenic germination occurred between 0 and -7 bars.

Bedi (1962a) claimed that germination required free water although in one of his experiments he did not obtain germination in an atmosphere of 100% relative humidity. Liquid distilled water and an atmosphere of 100% relative humidity both theoretically exert a water potential of 0 bars. Since sclerotia can absorb as much moisture in both cases, Bedi's results must be explained by other means.

Soil water content especially in the top 2-3 cm varies considerably. It is affected by weather parameters such as temperature, relative

humidity and wind velocity. There are no quantitative data on the effect of fluctuating soil water potential on sclerotial carpogenic germination.

Temperature is known to exert a significant effect on sclerotial carpogenic germination (Abawi and Grogan, 1975). However, literature on the effect of temperature on germination is inconsistent. Jones and Gray (1973) showed that sclerotia collected from the field and incubated on moist soil at 20°C germinated quite readily. They stated that only sclerotia derived from artificial media require a low temperature treatment of 14-16°C prior to incubation at 20°C in soil for germination to occur. Abawi and Grogan (1975) state that (sic) "preconditioned sclerotia incubated in water at different constant temperatures produced the most initials and mature apothecia at 11°C although production was equally good at 15°C." No apothecia were produced at 5°C and 30°C. Huang (1981) confirmed that sclerotia harvested from culture required a low temperature treatment prior to carpogenic germination.

Both temperature and soil moisture tension fluctuate in nature. It is unlikely that either factor alone would be limiting for sclerotial carpogenic germination. Most studies have been done with constant moisture or temperature and combination effects have not been determined. Since critical studies on the interactions of temperature and water potential are lacking, we undertook this work to determine the effects of interactions of temperature and water potential on apothecial germination under laboratory conditions.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Production of Sclerotia

Sclerotia of S. sclerotiorum collected from rapeseed in various crop districts of Manitoba were used. To increase the number of sclerotia, these isolates were surface sterilized with 1:1 mixture of Javex and 90% ethanol for two minutes and then rinsed several times with sterile distilled water. The sclerotia were then plated on petri plates containing potato dextrose agar (PDA) and incubated at 25°C for 4 days. Agar plugs containing mycelia were cut in an incubation chamber with a sterile cork-borer (4 mm diameter) from the periphery of the actively growing culture. These were inoculated onto new PDA plates and then incubated at 25°C for six weeks. At that time, mature, easily removable sclerotia were harvested and these were placed in plastic bags containing sand which had been autoclaved at 120°C and 103.4 Kpa for 15 minutes. Sterile distilled water was added to almost free flow. The bags were then sealed and stored for at least two months in a refrigerator maintained at 4°C. At the end of this period, the sclerotia were removed from the refrigerator, separated from sand by floating them on cold tap water in a beaker, then rinsed several times under running tap water.

### 2.2.2 Water Potential

To hold sclerotia at constant water potential, polyethylene glycol (PEG 20,000 MW) was utilized to obtain solutions of known matric potentials (Slavik, 1974; Morrall, 1977; Lowlar, 1970). The concentrations of the solutions were 0, 50, 86, 137, 220 and 260 g/L of water, and the

corresponding water potentials were 0, -1.2, -2.1, -3.3, -5.1 and -7 bars, respectively. The water potentials of these solutions were verified using Wescor L51 leaf psychrometers and Wescor HR-33T microvoltmeter.

To calibrate the psychrometers, standard solutions of sodium chloride of known water potentials were made (Brown and Havaren, 1972; Slavik, 1974). Discs of filter paper (Whatman) soaked in these solutions were placed in the chambers of the psychrometers and sealed immediately. The psychrometers were allowed a 30 minute period for the vapour pressure within the chamber to equilibrate before they were connected to the microvoltmeter. The output of the psychrometer was recorded in microvolts on a strip chart recorder (Fisher Recordall Series 500) which was moving at the rate of $0.05 . cm . s^{-1}$ (Bristow and DeJager, 1980). After calibration in a given solution the chambers were thoroughly rinsed with distilled water and then air dried before adding the next solution. A similar procedure was followed in obtaining the psychrometer output voltage for the PEG solutions.

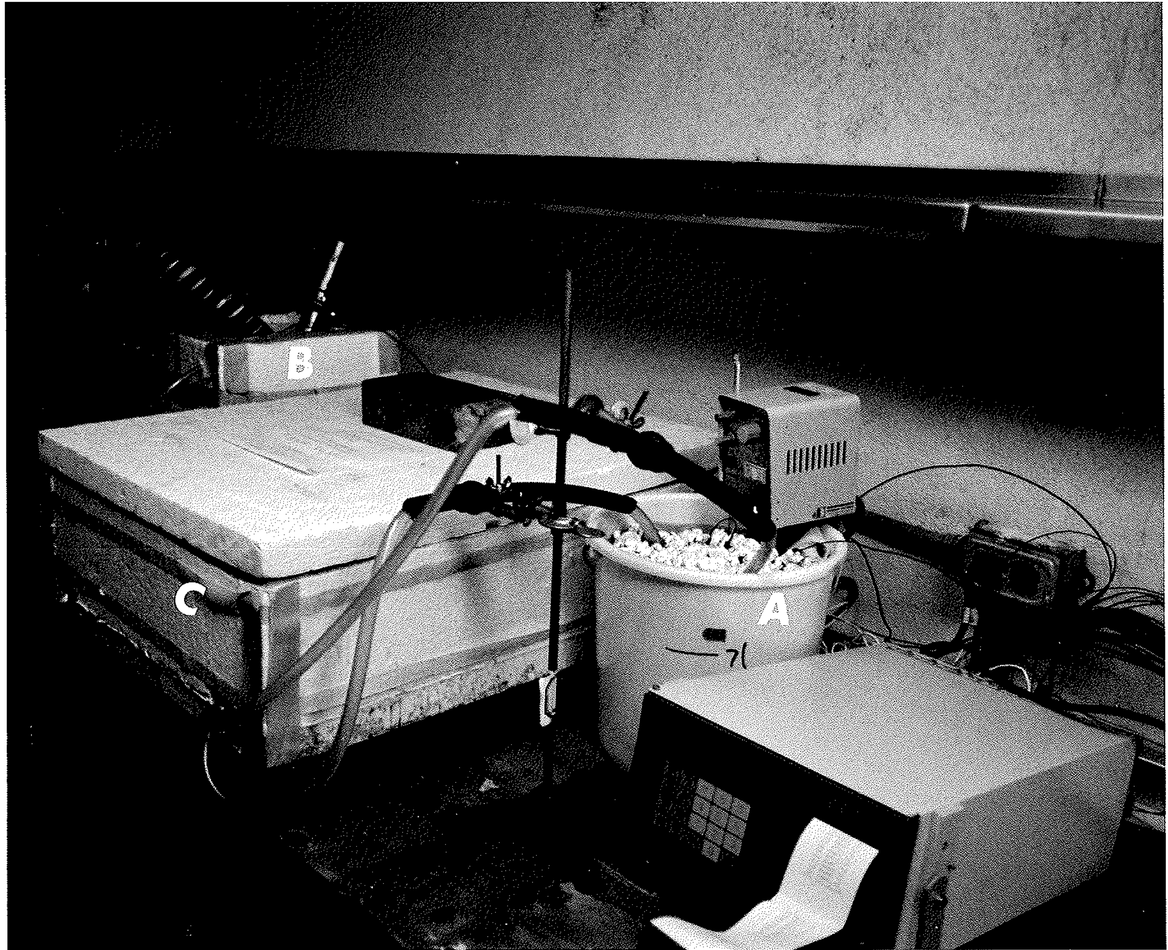
### 2.2.3 Temperature Gradient

A temperature gradient plate (Figure 1), slightly modifed from those described by Chatterton and Kadish (1969) and Clegg and Eastin (1978), was used to quantify the effects of interactions of temperature and water potential on carpogenic germination of sclerotia. The 60 X 52 cm temperature gradient plate was insulated on all sides with a 5 cm thick styrofoam. This plate was used in a laboratory in which the temperature fluctuated between 24-27°C. Two water baths were connected on either side of the long end of the plate. Heating and cooling coils were

placed in one water bath (for thermoregulation) so that a constant temperature was maintained at the hot end while only a cooling coil was connected to the other water bath. Continuously running constant speed pumps were placed in each water bath to maintain uniform heat distribution. A thermoregulating pump (Aqua-chill, Jewel)) was connected to the cold end to maintain the desired cold temperature. Temperatures across the plate were determined with thermocouples and ranged from 4°C to 30.5°C.

Figure 1: Temperature gradient plate.

A = Hot end
B = Cold end
C = Gradient plate insulated with styrofoam.


B
C
A

### 2.2.4 Sclerotial Carpogenic Germination

Very fine, acid washed silica sand, particle size 85-125 mesh (Fisher Scientific), was used as a medium for carpogenic germination studies. It was placed to a depth of 5 mm in 6x2x2 cm transparent plastic boxes. With the aid of thermocouples, five temperatures (5, 10, 15, 20, and 25°C) were determined on the plate. To determine the effect of variations in water potential and temperature, 5 ml of each PEG solution were placed in different boxes which had been labelled as to the temperature and water potential combination therein. Two replicate boxes were established for each water potential and temperature combination. Each replicate contained 16 sclerotia of uniform size. The boxes were sealed with parafilm, covered with lids to reduce moisture loss and then randomly placed along the isotherms of the plate. The temperature gradient apparatus was covered with a styrofoam lid to minimize temperature fluctuations and wind drafts. As such, light was eliminated except for the brief moments of counting germinated sclerotia. The experiment was carried out as a split-split-plot design; with 5 (levels of temperature) X 6 (levels of water potential) X 2 (replicate) factorial.

Both conditioned (exposed to cold temperature (4°C) in moist sand and then washed in running tap water) and unconditioned sclerotia were used in this experiment to study the effect of conditioning and washing on sclerotial germination. A sclerotium was considered germinated when it developed one or more stipes. The germinated sclerotia were counted and removed every week from the date of incubation. Effects of water potential and temperature on germination were expressed by onset of germination and rate of germination (percent per day).

## 2.3 RESULTS AND OBSERVATIONS

### 2.3.1 Onset of Germination

The analysis of variance indicates that the effects of temperature, water potential and their interaction on carpogenic germination are highly significant. There was no significant difference between replicates (Table 1).

Sclerotial carpogenic germination was first observed after 7 days of incubation between 15-25°C in high water potential (0 bar). Sclerotial germination was continuous and never synchronized. Fewer stipe initials were formed at 25°C than at 15 and 20°C after 7 days of incubation. The stipes that initially formed at 25°C did not develop any further nor did any more form. The sclerotia that were incubated at 25°C dried off as a result of moisture loss from the boxes by evaporation. Most of the moisture condensed on the upper lid leaving the sand at the bottom dry. As such, there was high moisture within the boxes but the sclerotia were not in direct contact with free moisture. Stipe initiation at 25°C was inhibited when sclerotia ceased to be in direct contact with free moisture. No noticeable evaporation and condensation occurred at temperatures between 5-10°C. Therefore, sclerotia were in direct contact with free moisture for a longer time than at 25°C. The most initials were formed at temperatures between 10-15°C.

TABLE 1

Analysis of variance for the effect of interaction of temperature and water potential on carpogenic germination.

| Source Variation | df | SS | F | PR>F |
|---|---|---|---|---|
| Replicates | 1 | 7.00 | 3.10 | 0.07 |
| Time | 1 | 34.50 | 15.30 | 0.0001 |
| Rep X Time | 1 | 0.33 | 0.15 | 0.69 |
| Temperature | 4 | 678.80 | 75.23 | 0.0001 |
| Temp X Time | 4 | 209.14 | 23.18 | 0.0001 |
| Temp X Rep | 4 | 6.55 | 0.73 | 0.57 |
| Moisture | 5 | 524.82 | 46.53 | 0.0001 |
| Moist X Rep | 5 | 12.12 | 1.07 | 0.37 |
| Moist X Time | 5 | 54.82 | 4.86 | 0.0004 |
| Moist X Temp | 20 | 353.49 | 7.84 | 0.0001 |
| MoistXTimeXTemp | 20 | 238.65 | 5.29 | 0.0001 |
| Error | 169 | 381.27 | | |
| Total | 239 | 2501.49 | | |

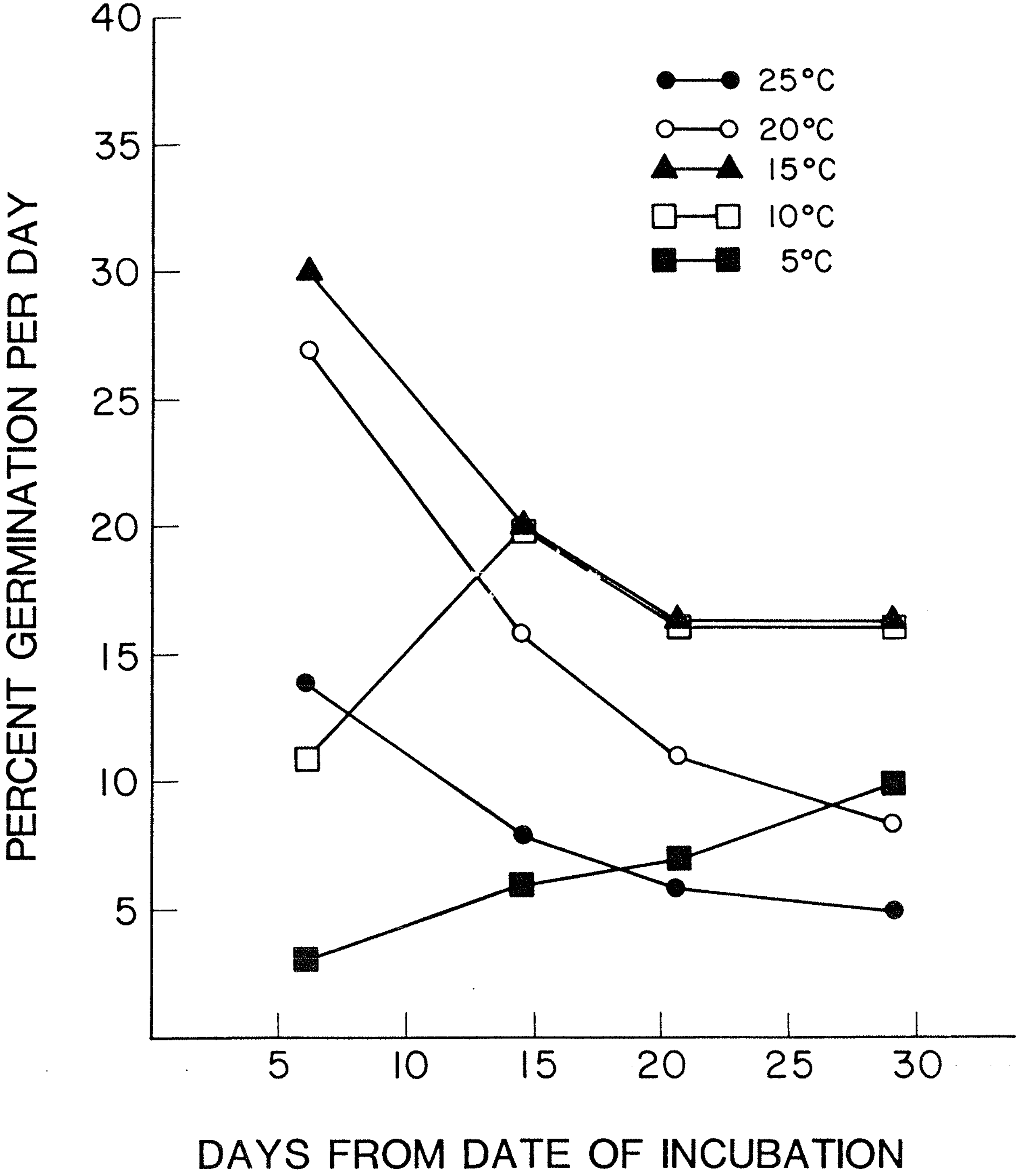
### 2.3.2 The Rate of Carpogenic Germination

The percent rate of carpogenic germination per day,

$$RCG = \frac{\text{\# germinated}}{\text{Total \#}} \times \frac{100}{\text{\# of days from date of incubation}}$$

was faster at temperatures between 15-20°C and decreased with time as more and more sclerotia germinated. Germination was completed within 28 days from the date of incubation at these temperatures in high water potential (0 bar) (Figure 2). At low temperatures (5-10°C), the rate of germination was initially lower than at high temperatures (15-20°C) but later on increased with time. The rate was faster at 10°C than at 5°C. After 14 days of incubation, the rate at 10°C caught up with the rate at 15°C. Although the rate of germination at low temperatures showed an increase after 7 days, germination was never complete within 28 days of incubation. The rate of germination was initially higher at 25°C than at 10 and 5°C but decreased rapidly with time due to moisture loss from the sand.

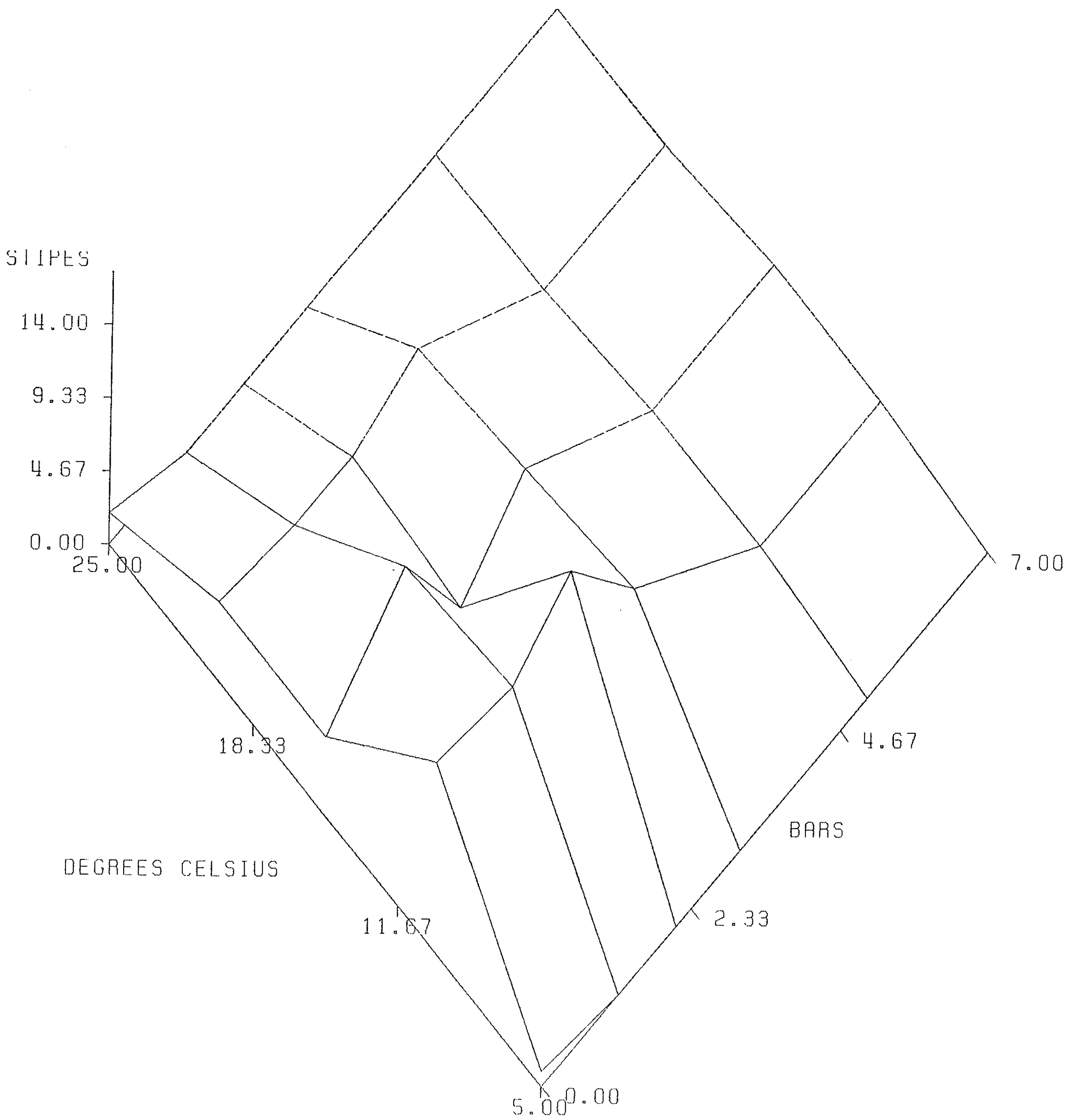
Figure 2: The effect of temperature on the rate of carpogenic germination of *Sclerotinia sclerotiorum* at 0 bars.


Effect of temperature on the rate of carpogenic germination at O bars
PERCENT GERMINATION PER DAY
40
35
30
25
20
15
10
5
5
10
15
20
25
30
DAYS FROM DATE OF INCUBATION
25°C
20°C
15°C
10°C
5°C

### 2.3.3 The Effect of Interaction of Temperature and Water Potential on Sclerotial Carpogenic Germination

Sclerotial carpogenic germination occurs over a wide range of temperatures (5-25°C) in combination with water potentials ranging from 0 to -5 bars (Figure 3). At low temperatures (5-10°C) germination was delayed for various periods of time depending on the water potential. Generally, the delay increased with a decrease in water potential and germination never occurred in water potentials between -3 to -7 bars. At higher temperatures (25°C) sclerotial carpogenic germination was inhibited after a few days in high water potentials (0 to -2 bars) due to moisture loss and never occurred in water potentials between -3 to -7 bars. The optimum temperature for carpogenic germination was at 15°C in combination with water potentials between 0 and -3 bars while the optimum water potential was at -2 bars in combination with temperatures between 10-20°C (Figure 3). Carpogenic germination increased with increase in temperature up to the optimum (15°C) then it decreased with further increase in temperature from the optimum. Similarly, the most conducive water potentials were between 0 to -3 bars and carpogenic germination decreased rapidly with a decrease in water potential from -3 bars at all temperatures.

Figure 3: Response surface of carpogenic germination of sclerotia to temperature and water potential.


STIPES
14.00
9.33
4.67
0.00
25.00
18.33
11.67
5.00
0.00
2.33
4.67
7.00
DEGREES CELSIUS
BARS

### 2.3.4 Effect of Cold Moist Conditioning and Washing on Carpogenic Germination

The sclerotia that were not exposed to a cold (4°C) moist treatment did not germinate whether washed for twenty minutes or not. They leaked substances that turned distilled water and PEG solutions dirty-yellow-brown. The media then acquired a characteristic foul smell similar to spoiled mushrooms.

The sclerotia that were stored in cold moist sand for at least two months and then washed in running tap water for twenty minutes germinated after seven days in distilled water at temperatures between 10-25°C. More stipes were formed between 15 - 20°C than at 25°C or 10°C. Germination was complete within 28 days at 15°C. At 10°C there was an initial delay in the number of sclerotia that germinated, but later, germination increased with time, although it was not completed within 28 days. Germination was slightly higher at 25°C than at 10°C, but it decreased with time.

## 2.4 DISCUSSION

Polyethylene glycol (PEG 20,000 MW) was chosen for this *in vitro* study because its large molecules cannot penetrate the plasmalemma and, in some cases, cell walls (Hoch and Mitchell, 1973; Steuter, 1981). PEG has been used in physiological experiments because of its relative stability and inertness, solubility in water and nontoxicity even at high concentrations (Jackson, 1962).

Water movement occurs as the result of water potential gradients and moves from high potentials to low potentials. When the water potential of the system (solutions or soil matrix) decreases below that of the sclerotia, water molecules move from the sclerotia to the surrounding medium. When moisture levels within the sclerotia fall below the level required for carpogenic germination, the sclerotia become dormant (Trevethick and Cooke, 1973).

Results of this study indicate that temperatures above 25°C decrease sclerotial carpogenic germination by the direct effect of temperature on the process of germination as well as its effect on the movement of water molecules. Sclerotia were exposed to moisture for a shorter period at 25°C than at lower temperatures (10-15°C) due to the rapid moisture loss at 25°C. Low temperatures (5°C) delay sclerotial carpogenic germination by inactivation of the sclerotia and the reduction in the movement of water molecules. This reduces moisture availability to the sclerotia. The results indicate that the optimum temperature for carpogenic germination is at 15°C. The minimum temperature has not been precisely determined. Sclerotia that were stored in moist sand in a refrigerator at 4°C for more than 3 months were observed to form stipes although they did not form apothecia. This may be due to the lack of light in the closed refrigerator. No germination of sclerotia incubated at 5°C occurred within the limited time of 28 days. Similar results were obtained by Abawi and Grogan (1975) and Grogan and Abawi (1975). The maximum temperature for carpogenic germination in this experiment was at 25°C. Abawi and Grogan (1975) showed that no apothecia were produced at 30°C, but they do not indicate whether they obtained apothecia at temperatures between 25-30°C.

The results of this study indicate that the minimum water potential for carpogenic germination is -5 bars at temperatures between 10-20°C while germination occurs well in water potentials between -3 to 0 bars in combination with temperatures between 10-20°C. The optimum water potential is -2 bars at temperatures up to 25°C. Germination is equally good at 0 bars. Grogan and Abawi (1975) found the minimum water potential to be -6 bars and Morrall (1977) found -7 bars to be the minimum. The above authors did not study the effect of interaction of temperature and water potential on carpogenic germination. The differences in results between these experiments and Morrall's (1977) with respect to the minimum water potential for carpogenic germination may be due to the experimental procedures used. Morrall used sclerotia that were placed in moist soil in permeable pig skin membranes which he immersed in various concentrations of PEG. He had to change the solution several times in order to equilibrate the soil moisture and the PEG solutions. As such, sclerotia must have been exposed to varying water potentials longer than necessary. During this study, sclerotia were immersed directly in PEG solution. The sclerotia were at constant water potential during the experiment except at 25°C where there was rapid moisture loss.

The sclerotia that were preconditioned in moist sand at 4°C and incubated at various temperatures in high water potential (0 bar) germinated well while the unconditioned sclerotia did not germinate. These results support the findings of (Huang, 1981; Grogan and Abawi, 1975; Steadman, 1983) that sclerotia of _S._ _sclerotiorum_ grown in culture require a cold moist treatment as stimulus for carpogenic germination. Bedi (1962a) failed to obtain germination at 100% relative humidity when he suspended sclerotia above distilled water in air tight jars. The results of this

experiment indicate that sclerotia must be in direct contact with free moisture for at least 7 days in order to germinate carpogenically. This may explain why Bedi (1962a) did not obtain carpogenic germination in an atmosphere of 100% relative humidity and the poor germination at 25°C in this experiment.

Free moisture may leach out from the sclerotia substances potentially inhibitory to carpogenic germination. Smith (1972) showed that sclerotia that were dried for various periods of time leaked nutrients (sugars and amino acids) when returned to moist conditions. Casale and Hart (1983) showed that preleached sclerotia germinated more than unleached sclerotia and they suggested that the leachates contain inhibitors of germination. At present there is no evidence with respect to the nature of potential inhibitor(s).

The stipes that formed under these experimental conditions did not differentiate into apothecia. This may be due to insufficient time of exposure to light. Coley-Smith and Cooke (1971) and McLean (1958) state that stipe initiation in S.Sclerotiorum can occur in both light and darkness, but stipe differentiation to form apothecia requires at least 8 hours of light in every 24 hours. Morrall (1977) and this study both utilized restricted systems with limited gaseous exchange and this fact may also contribute to the lack of stipe differentiation.

Chapter III

# PREDICTION OF SCLEROTINIA STEM ROT IN RAPESEED USING BURIED SCLEROTIA

## 3.1 INTRODUCTION

Disease prediction is the science of monitoring the physical conditions of the environment and declaring after "disease weather" but before symptoms are visible that infection has occurred (Jones, 1983, Jones and Fisher, 1984; Krause and Massie, 1975; MacKenzie, 1981); while forecasting is based on anticipated weather (Scarpa and Raniere, 1964).

The key to prediction is to quantify the relationship between some biological or environmental variable and disease. Ideally, we would like to use information from all the components of the disease tetrahedron. Practically, most predictive systems are based on only one component of the disease tetrahedron and assumptions are made about the others (MacKenzie, 1981; Zadoks, 1984; Scarpa and Raniere, 1964). For practical purposes, only those elements that are relevent to disease prediction at a particular time, place and under specific technical constraints are needed.

For the past twenty-five years, several predictive systems have been advanced based on one, two or three components of the disease tetrahedron (Zadoks, 1984; Royle, 1972; Thomson *et al*., 1982; Scarpa and Raniere, 1964). Each system was developed in its own context and none of them is universally accepted.

Environment (weather), is the only basic component frequently used *per se*. A few workers (Thomson *et al*., 1982; Eversmeyer *et al*., 1973; Burleigh *et al*., 1972) have integrated the host growth stage, the pathogen and the physical environmental conditions in their predictive models.

Disease prediction methods for soilborne plant pathogens, particularly sclerotial forming fungi, are lacking. Disease prediction or forecasting as developed in various crops to reduce crop loss and limit fungicide use would provide a needed management tool for control of stem rot in rapeseed.

This work was undertaken in order first to study the possibility of using samples of buried sclerotia as indicators of germination in natural sclerotial populations. Secondly, to study disease incidence in rapeseed in the locality of buried sclerotia. And, lastly, to relate sclerotial germination and disease development to crop canopy temperature, moisture (relative humidity and rainfall) and to crop loss (or yield loss) with the ultimate objective of developing a predictive model for stem rot in rapeseed.

## 3.2 MATERIALS AND METHODS

Sclerotial germination and stem rot development were observed at rapeseed field plots in three locations (Winnipeg, Glenlea and Portage) in 1983 and two locations (Winnipeg and Portage) in 1984. The rapeseed variety Regent used in this study was sown in early May in rows, 3.6 m long and 30 cm betwen rows at a rate of 7 Kg/ha. The experiment was designed as a randomized complete block. Block sizes for 1983 were 12 X

12 m with 4 replicates for each treatment and 28 X 36 m with 12 replicates for each treatment in 1984.

3.2.1 Inoculum

Sclerotia of S. sclerotiorum collected from rapeseed in various crop districts of Manitoba were used. To increase the inoculum, these isolates were surface sterilized with 1:1 mixture of Javex and 90% ethanol for two minutes and then rinsed several times with sterile distilled water. The sclerotia were then plated in petri plates containing potato dextrose agar (PDA) and these were then incubated at 25°C for 4 days. Agar plugs containing mycelia were cut from within 2 cm of the periphery of the actively growing colony using a sterile cork borer (4 mm diameter). Such plugs were then inoculated onto new PDA plates and then incubated at 25°C for six weeks.

The sclerotia that were used in Portage La Prairie in 1984 were grown on baked beans (Heinz Canada Ltd.) which had been homogenized in a waring blender. The blended beans were then poured into aluminum cake pans to a depth of 2 cm and covered with aluminum foil and sterilized in an autoclave at 120°C, 103.4 Kpa for 15 minutes. After cooling, the aluminum plates were inoculated aseptically with mycelial plugs derived as above, and then left at room temperature for about six weeks. Mature, easily removable sclerotia from both PDA plates and homogenized bean preparations were then harvested. Mature sclerotia were bulked and divided into three batches in 1983 and four batches in 1984. In 1983 the first three treatments were employed; in 1984, the fourth was added:

1. Left at room temperature until required. (Treatment One).
2. Placed in a plastic bag which contained autoclaved sand. Distilled water was added to almost free flow. The bag was then sealed and kept for one month in a refrigerator maintained at 4°C. (Treatment Two).
3. Treated as in (2) but left in the refrigerator for two months until required for the experiment. (Treatment Three).
4. Buried in nylon mesh bags at Winnipeg and Portage la Prairie, in Nov. 1983, in the field at a depth of 3 cm overwinter. Sclerotia were recovered in May 1984 for experimental purposes. (Treatment Four).

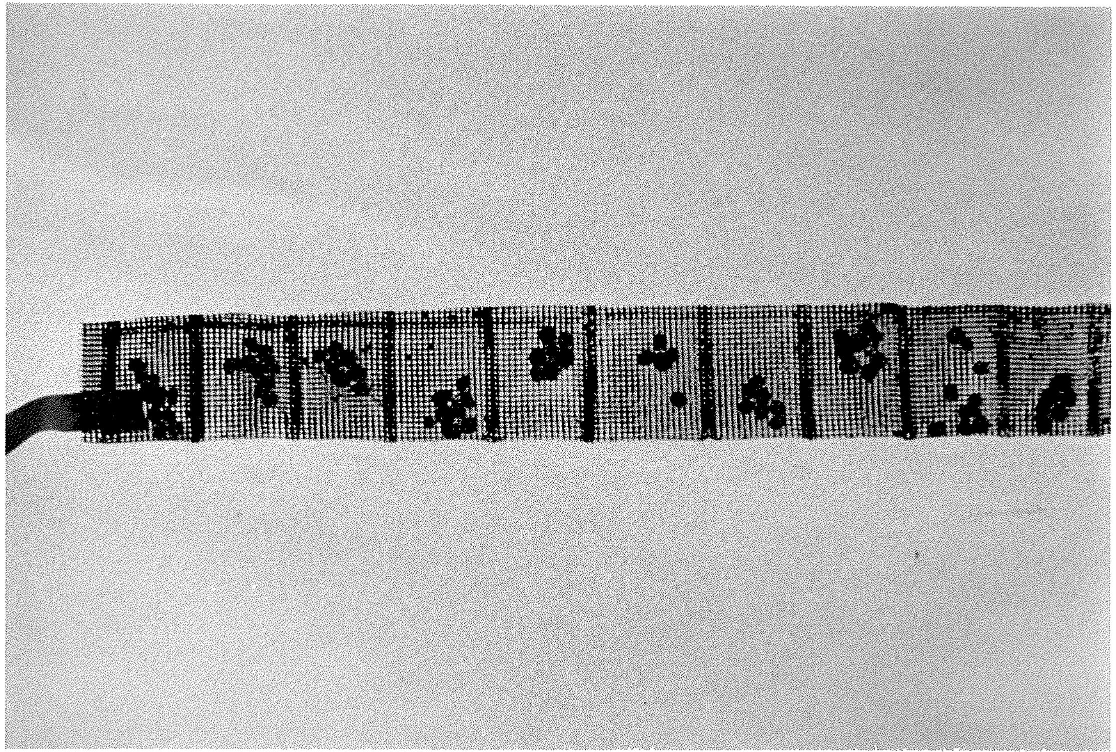
### 3.2.2 Burial of Sclerotia

Sclerotia subjected to each of the four treatments described above were later buried in the field on two separate dates. The first burial date was within a week of rapeseed planting in the experimental area, while the second burial (June 20th) date was one month after first burial date. To facilitate burial and recovery of sclerotia from the soil, a screen of nylon netting, approximately forty-two squares per centimetre was used to create storage pouches (Figure 4). The mesh was cut into 28 X 8 cm sections, folded in half and glued into ten compartments. Five sclerotia were placed in each compartment and a section of red plastic tape 11 X 1 cm was attached to each bag to facilitate recovery from the soil. One mesh bag containing fifty sclerotia represented a replicate. All bags were buried to a depth of 2 cm and approximately 2 cm from the stem bases of the cover crop. In 1984, treatment number four at Portage had less than 50 sclerotia per bag because most

of the sclerotia were rotted or heavily colonized by other microorganisms when they were recovered in early May of that year.

Figure 4: A nylon netting screen with sclerotia of *Sclerotium sclerotiorum* before burial.

Figure 5: Expanded apothecia of *Sclerotinia sclerotiorum* growing from within the screen.



### 3.2.3 Environmental Data

At Winnipeg, a hygrothermograph (Serdex, Bacharach, Ambac, Pittsburg, U. S. A.) was placed within the crop to monitor air temperature and relative humidity. At Portage La Prairie, a hygrothermograph equipped with a leaf wetness recorder, digital rainfall gauge, and temperature and relative humidity sensors was used (MacHardy and Sondej, 1981). This was located in an adjacent potato plot. In 1984, soil moisture was recorded using a quick draw tensiometer (Soil Moisture Equipment Corp., Santa Barbara, CA 93105) at a soil depth of 3 cm. Mean soil moisture readings for two separate days per week were taken. Data obtained from these instruments were compared to the data from local weather stations. Data obtained from these stations were recorded at standard meteorological conditions in louvered screens at a height of 1.14 m whereas our crop canopy data were obtained at about 15 cm above the soil surface.

### 3.2.4 Apothecial Germination and Stem Rot Counts

A sclerotium was considered to have germinated when the stipe appeared on the soil surface and later developed into an apothecium (Figure 5). Counts of germinated sclerotia were taken on a weekly basis. Plants were recorded as diseased during the season if leaves were wilted and yellow and the characteristic white mycelia and later sclerotia developed in association with the water soaked lesions on stems or branches. At maturity, about 90-105 days from the date of planting, 5.5 m long double rows were trimmed to 4.5 m length by deleting 0.5 m from each end. The total number of plants (healthy and diseased) per row were counted, cut using sickles, and then threshed.

Chaff was removed from the seeds by passing them through a seed blower. Plot yields (in g) were obtained from weights of clean seed. The data were analysed using analysis of variance and regression procedures (SAS) and by Duncan's multiple range test for comparison of means with an alpha level of either 0.01 or 0.05.

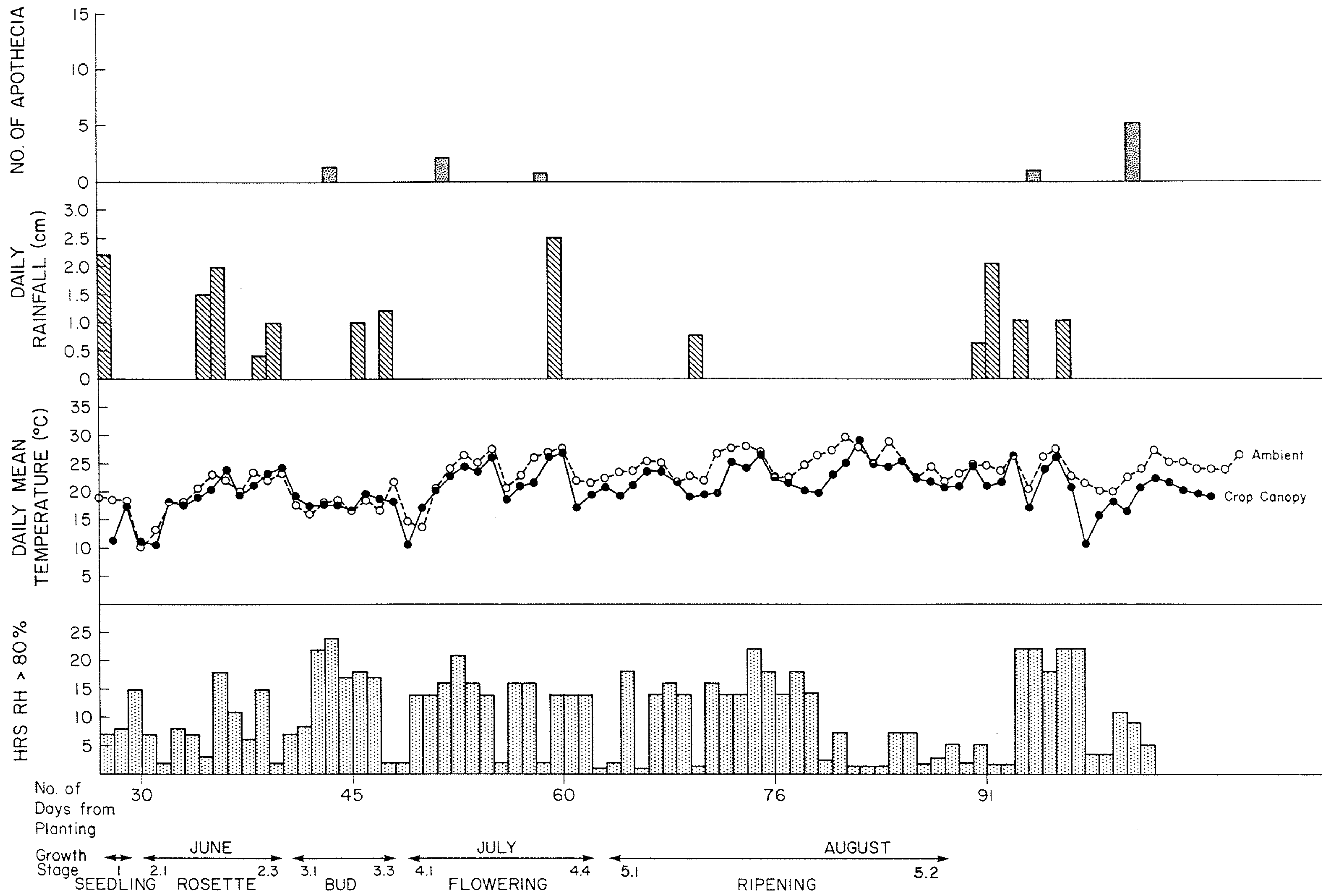
## 3.3 RESULTS AND OBSERVATIONS

### 3.3.1 Meteorological Data and Carpogenic Germination of Sclerotia

In both 1983 and 1984, stipes and apothecia were first observed within the experimental plots during the last week of June - first week of July (about 40 to 55 days from the date of planting). This coincided with the period when the crop was in growth stages 3.2-4.1 (Harper and Berkenkamp, 1975). Rainfall within this period occurred on at least 2 out of every 7 days with a total of at least 5cm in the 10-14 days prior to germination (ie the 3rd and 4th weeks of June). Soil moisture readings ranged from -5 to -20 centibars (Figures 6 and 7). The mean min-max temperatures within this period ranged from 10 to 25°C. Crop canopy temperatures for the most part of 1984 season were below 20°C until August when temperatures rose and were close to 25°C. In 1983, ambient and crop canopy temperatures were above 25°C early in the season but dropped to about 20°C during bud stage. After about 10 days, temperatures rose again to above 25°C (Figure 6). In both years, crop canopy temperatures were usually lower than ambient.

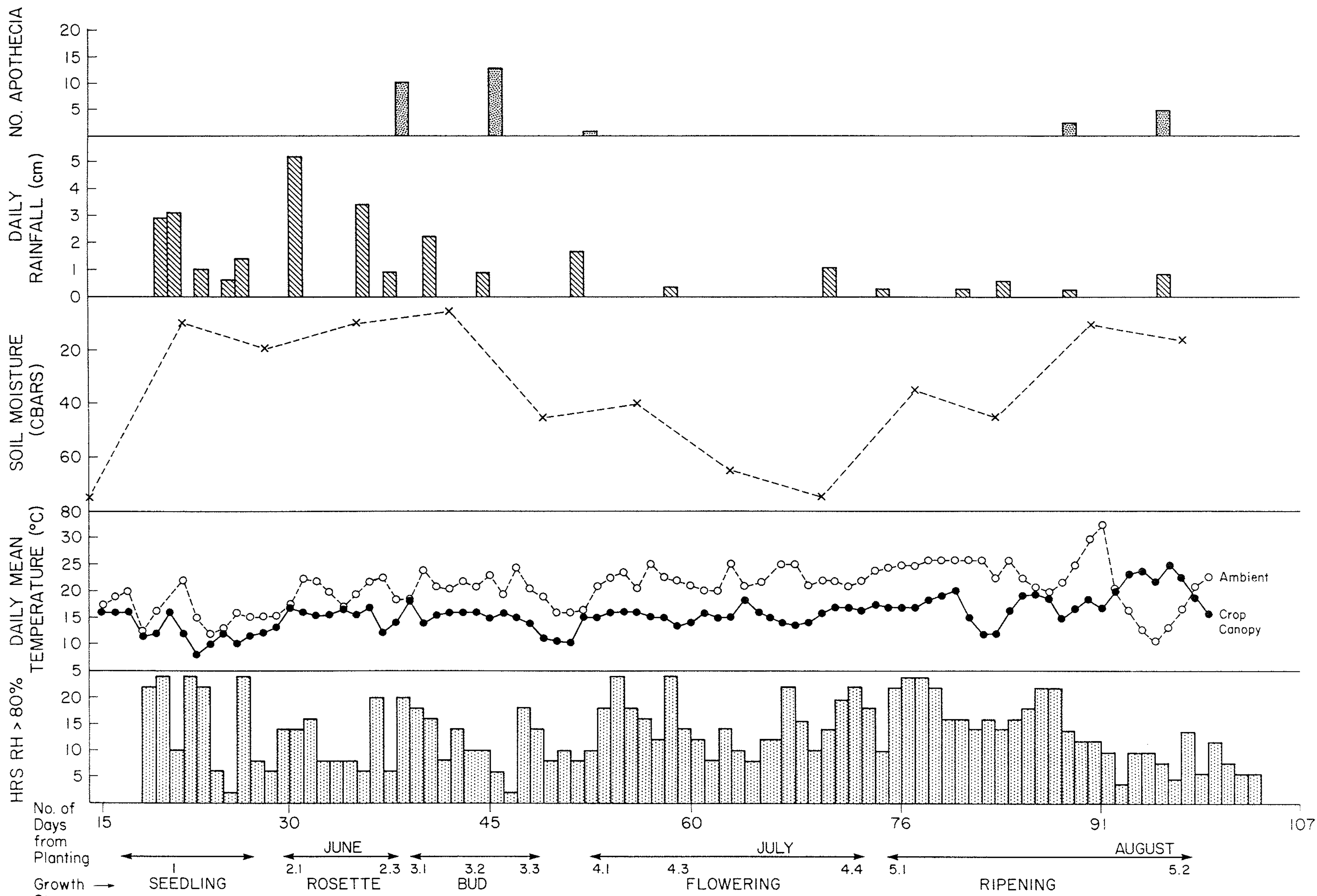
In both 1983 and 1984, there was little or no rainfall from the beginning of the second week of July to the second week of August (Table 2); for the greater part of this period temperatures were above 25°C.

Figure 6: The relationship between some weather factors and germination of sclerotia of *Sclerotinia sclerotiorum* 1983 at Winnipeg.



NO. OF APOTHECIA
DAILY RAINFALL (cm)
DAILY MEAN TEMPERATURE (°C)
Ambient
Crop Canopy
HRS RH > 80%
No. of Days from Planting
30
45
60
76
91
Growth Stage
JUNE
JULY
AUGUST
1
2.1
2.3
3.1
3.3
4.1
4.4
5.1
5.2
SEEDLING
ROSETTE
BUD
FLOWERING
RIPENING

Figure 7: The relationship between some weather factors and germination of sclerotia of *Sclerotinia sclerotiorum* 1984 at Winnipeg.



NO. APOTHECIA
DAILY RAINFALL (cm)
SOIL MOISTURE (CBARS)
DAILY MEAN TEMPERATURE (°C)
Ambient
Crop Canopy
HRS RH > 80%
No. of Days from Planting
15
30
45
60
76
91
107
JUNE
JULY
AUGUST
1
SEEDLING
2.1
2.3
ROSETTE
3.1
3.2
3.3
BUD
4.1
4.3
4.4
FLOWERING
5.1
5.2
RIPENING
Growth Stage

Weekly mean hours of relative humidity greater than 80% for the two months were less than 12 h. Tensiometer readings for soil moisture ranged from -45 to -75 Cbars (Figure 7). The few apothecia that had developed withered rapidly.

TABLE 2

Rainfall and temperature patterns at Winnipeg and Portage 1982 - 1984.

| LOCATION | | | | WINNIPEG | | | | PORTAGE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Rainfall | | Temperature (°C) | | Rainfall | | Temperature (°C) | |
| MONTH | Week | Days From Planting | Year | Total (cm) | Freq Days | Min | Max | Total (cm) | Freq Days | Min | Max |
| JUNE | 2 | 22 to 30 | 1982 | .5 | 3 | 5 | 20 | .68 | 3 | 5.9 | 19.9 |
| | | | 1983 | 2.2 | 2 | 13 | 26 | 1 | 4 | 15 | 25 |
| | | | 1984 | 3 | 3 | 5 | 15 | 4.3 | 4 | 8.5 | 22 |
| | 3 | 31 to 38 | 1982 | .8 | 1 | 7 | 19 | 2.84 | 3 | 8.3 | 18 |
| | | | 1983 | 3.6 | 2 | 12 | 23 | 2.5 | 4 | 10 | 20 |
| | | | 1984 | 8.7 | 2 | 10 | 16 | 1.98 | 3 | 13 | 26 |
| | 4 | 39 to 46 | 1982 | 0 | 0 | 7 | 23 | .18 | 3 | 7.7 | 22.5 |
| | | | 1983 | 1.4 | 2 | 15 | 26 | 2.5 | 5 | 16 | 24 |
| | | | 1984 | 4.1 | 3 | 10 | 20 | 1.2 | 5 | 13 | 23 |
| JULY | 1 | 47 to 54 | 1982 | 5.8 | 2 | 15 | 25 | 2.1 | 3 | 13 | 26.7 |
| | | | 1983 | 2.3 | 2 | 13 | 22 | 2.4 | 4 | 13 | 25 |
| | | | 1984 | 1.7 | 1 | 10 | 19 | .75 | 3 | 10.4 | 23.3 |
| | 2 | 55 to 62 | 1982 | 0 | 0 | 12 | 26 | 7.36 | 3 | 11 | 25 |
| | | | 1983 | 0 | 0 | 19 | 31 | 0.24 | 2 | 20 | 28 |
| | | | 1984 | .38 | 1 | 14 | 20 | .48 | 3 | 14 | 28 |
| | 3 | 63 to 70 | 1982 | 4.3 | 2 | 15 | 25 | 5.53 | 3 | 13 | 25 |
| | | | 1983 | 2.54 | 1 | 18 | 30 | 0 | 0 | 21 | 28 |
| | | | 1984 | 0 | 0 | 10 | 20 | 1.46 | 3 | 13 | 27 |
| | 4 | 71 to 78 | 1982 | 1.9 | 1 | 14 | 27 | 8.9 | 5 | 13 | 25.8 |
| | | | 1983 | 0.43 | 1 | 18 | 29 | 1.6 | 2 | 14 | 26 |
| | | | 1984 | 1.4 | 2 | 10 | 20 | 1 | 2 | 14 | 25 |
| AUGUST | 1 | 79 to 87 | 1982 | 0 | 0 | 15 | 26 | 0.14 | 3 | 14 | 25 |
| | | | 1983 | 0 | 0 | 20 | 33 | 0.06 | 2 | 18 | 33 |
| | | | 1984 | .25 | 1 | 17 | 32 | 2.13 | 2 | 15 | 26 |
| | 2 | 88 to 95 | 1982 | 1.3 | 1 | 10.5 | 23 | 2.4 | 4 | 11 | 22 |
| | | | 1983 | 0 | 0 | 16 | 30 | 0 | 0 | 13 | 29 |
| | | | 1984 | .9 | 1 | 18 | 29 | 3.8 | 2 | 16 | 27 |

### 3.3.2 Germination of Sclerotia at Different Locations

To account for the possible effect of location on carpogenic germination, data from the three locations in 1983 were combined and analyzed. Analysis of variance indicated that the effect of location was highly significant (Table 3). The mean number of germinated sclerotia at Portage differed significantly from the mean number of germinated sclerotia at both Winnipeg and Glenlea (Duncan's multiple rage test P = 0.01). The mean number of germinated sclerotia at Winnipeg did not differ significantly from the number of germinated sclerotia at Glenlea (Table 4).

TABLE 3

Effect of burial date, sclerotial pretreatment and location on carpogenic germination

| Source of Variation | df | SS | F | PR>F |
|---|---|---|---|---|
| Location | 2 | 1124.11 | 8.78 | 0.0007 |
| Block | 3 | 631.82 | 3.29 | 0.0305 |
| Loc X Block | 6 | 720.88 | 1.88 | 0.1093 |
| Trt | 2 | 1121.36 | 8.76 | 0.0007 |
| Block X Trt | 6 | 390.64 | 1.02 | 0.4283 |
| Loc X Trt | 4 | 43.38 | 0.17 | 0.9526 |
| Date | 1 | 3514.01 | 54.92 | 0.0001 |
| Loc X Date | 2 | 1032.11 | 8.07 | 0.0012 |
| Date X Trt | 2 | 944.53 | 7.38 | 0.0019 |
| Loc X Trt X Date | 4 | 90.72 | 0.35 | 0.8393 |
| Error | 39 | 2495.40 | | |
| Total | 71 | 12108.96 | | |

TABLE 4

Sclerotium germination at different locations in 1983.

| Location | N | Mean* |
| --- | --- | --- |
| Portage | 24 | 39.5 a |
| Winnipeg | 24 | 12.6 b |
| Glenlea | 24 | 11.2 b |

* Mean germination for the 3 treatments. Means with the same letter are not significantly different. Duncan's multiple range test P = .01.

### 3.3.3 The Effect of Low Temperature Treatment on Carpogenic Germination

The analysis of variance using the number of carpogenically germinated sclerotia as the dependent variable (Tables 3 and 5) indicates that the effect of cold moist treatment on sclerotial carpogenic germination is highly significant. In 1983, the mean number of carpogenically germinated sclerotia for two months conditioning differed significantly from one month and unconditioned treatments (Table 6). One month conditioning did not differ signficantly from unconditioned sclerotia (Duncan's multiple range test P = 0.01).

TABLE 5

Analysis of variance using apothecia as the dependent variable. 1984 data.

| Source of Variation | df | SS | F | PR>F |
|---|---|---|---|---|
| Block | 11 | 488.75 | 1.72 | 0.08 |
| Date | 1 | 408.37 | 15.81 | 0.0002 |
| Trt | 3 | 936.33 | 12.08 | 0.0001 |
| Date X Trt | 3 | 215.13 | 2.78 | 0.046 |
| Error | 77 | 1989.42 | | |
| Total | 95 | 4038.00 | | |

TABLE 6

Effect of cold treatment on sclerotium germination 1983.

| | Treatment | N | Mean* |
|---|---|---|---|
| Two Month Conditioning | (2) | 24 | 13.58 a |
| One Month Conditioning | (1) | 24 | 5.54 b |
| No Conditioning | (0) | 24 | 4.91 b |

* Mean germination per replicate.
Means with the same letter are not significantly different. Duncan's multiple range test. P = 0.01.

Data from the two locations in 1984 were analyzed separately. In both locations, all treatments differed significantly from each other. At Winnipeg, overwintered sclerotia (treatment 4) had more germinated sclerotia than treatment 3, 2 and 1. The order of magnitude of the number of germinated sclerotia were treatment 4 >3 >2 >1 (Table 7). While the order of magnitude of germinated sclerotia at Portage was treatment 2 >3 >4 >1 (Table 8).

TABLE 7

Effect of cold treatment on sclerotium germination at Winnipeg 1984.

| | Treatment | N | Mean* |
|---|---|---|---|
| Overwintered | (4) | 24 | 9.0 a |
| Two Month Conditioning | (3) | 24 | 6.6 b |
| One Month Conditioning | (2) | 24 | 2.7 c |
| No Conditioning | (1) | 24 | 0.5 d |

* Means per replicate for both burial dates. Means with the same letter are not significantly different. Duncan's multiple range test P = .01.

TABLE 8

Effect of cold treatment on sclerotium germination at Portage 1984.

| | Treatment | N | Mean* |
|---|---|---|---|
| One Month Conditioning | (2) | 24 | 5 a |
| Two Month Conditioning | (3) | 24 | 3.8 b |
| Overwintered | (4) | 24 | 2.4 c |
| No Conditioning | 1 | 24 | 0.5 d |

* Means per replicate for both burial dates. Means with the same letter are not significantly different. Duncan's multiple range test P = .01.

### 3.3.4 The Effect of Time of Burial (Length of Burial) on Sclerotial Carpogenic Germination

The results for 1983 combined data indicate that sclerotia from all treatments which were buried in early May (early burial) produced approximately ten times more apothecia than the same treatments buried in late June (second burial). The ratio of germinated sclerotia for the early and late burial dates in 1983 were: no conditioning - 9.1 ; one month conditioning - 10.1; two month conditioning - 13.1 (Table 9). There were four treatments in each of the two locations in 1984. Data from each location were analyzed separately. All sclerotial treatments buried in early May had more germinated sclerotia than similar treatments buried in late June at both locations (Tables 10 and 11). The ratios of germinated sclerotia for the early and late burial dates at Winnipeg were: no conditioning - 0:1 ; one month conditioning - 7:3; two month conditioning - 5:2; overwintered - 13:4 (Table 10).

TABLE 9

Effect of time of burial on sclerotium germination 1983.

| | Date of Burial | | | |
|---|---|---|---|---|
| | 1st Burial Date | | 2nd Burial Date | |
| Treatment | N | Mean* Germination | N | Mean* Germination |
| Zero conditioning | 12 | 8.9 | 12 | 0.9 |
| One month conditioning | 12 | 10.4 | 12 | 0.6 |
| Two month conditioning | 12 | 25.6 | 12 | 1.5 |

* Mean for the three locations.

TABLE 10

Effect of time of burial on sclerotium germination in Winnipeg 1984.

| | Date of Burial | | | |
|---|---|---|---|---|
| | 1st Burial Date | | 2nd Burial Date | |
| Treatment | N | Mean* Germination | N | Mean* Germination |
| Zero conditioning | 12 | 1.6 | 12 | 0.0 |
| One month conditioning | 12 | 4.8 | 12 | 0.6 |
| Two month conditioning | 12 | 7.5 | 12 | 5.8 |
| Overwintered | 12 | 13.25 | 12 | 4.25 |

* Mean apothecial germination per replicate.

TABLE 11

Effect of time of burial on sclerotium germination at Portage 1984.

| Treatment | Date of Burial<br>1st Burial Date<br>N | <br><br>Mean* Germination | <br>2nd Burial Date<br>N | <br><br>Mean* Germination |
|---|---|---|---|---|
| Zero conditioning | 12 | 0 | 12 | 1.2 |
| One month conditioning | 12 | 6.8 | 12 | 3.2 |
| Two month conditioning | 12 | 5.4 | 12 | 2.3 |
| Overwintered | 12 | 4.3 | 12 | 0.5 |

* Mean apothecial germination per replicate.

### 3.3.5 Correlations of Apothecia, Disease Incidence and Rapeseed Yield

Little or no foliar infection was observed within the experimental plots in 1983. The mean disease incidence per plot was about 0.5% and this consisted mainly of plants with basal infections. In 1984, the mean disease incidence was 1.2% per plot and consisted of plants showing various degrees of damage (basal infections, stem and branch rots and, bleached and shredded plants). There was a higher disease incidence in 1984 than there was in 1983.

There was no correlation between the number of germinated apothecia with the number of diseased plants ($r = 0.14$) nor with rapeseed yield per plot ($r = 0.17$). However, there was a high negative correlation between disease incidence and rapeseed yield per plot ($r = -0.81$) (Table 12).

TABLE 12

Correlations of apothecia, disease incidence and rapeseed yield.

| | APOTHECIA | INCIDENCE | YIELD |
|---|---|---|---|
| APOTHECIA | 1 | 0.14 | 0.17 |
| INCIDENCE | 0.14 | 1 | -0.81 |
| YIELD | 0.17 | -0.81 | 1 |

## 3.4 DISCUSSIONS

Except for the sclerotia that were overwintered at Portage, all preconditioned sclerotia germinated more than the unconditioned sclerotia (Tables 6, 7, 8). Almost 20% of the sclerotia that overwintered in Portage were rotted when they were recovered in early May. This effectively reduced the number of sclerotia per replicate to thirty. Species of Fusarium, Penicillium and Trichoderma were isolated from rotted sclerotia. The remaining sclerotia were not tested for viability and many of them could have been colonized by mycoparasites even though they appeared normal. Several researchers (Jones and Watson, 1969; Bedi, 1958), also isolated species of these fungi from sclerotia of S. sclerotiorum.

The results of this study support the findings of Huang (1981), Henson and Valleau (1940) and Steadman (1983) that sclerotia obtained from culture require a cold temperature (3°C) treatment as a stimulus for carpogenic germination. The minimal length of time required for this preconditioning has not been determined. Isolates of S. sclerotiorum are well adapted to survival under the subzero temperatures that occur in Manitoba (Bisby, 1921). The sclerotia that overwintered at Winnipeg had a greater degree of germination than the sclerotia that were preconditioned in the refrigerator (Table 7). This may be due to the fact that when they were recovered in early May, only those which could withstand parasitism and had a high germination capability were unconsciously selected.

Apothecia were first observed in the field during the period comprising the last week of June and the first week of July (40 to 55 days from the date of planting) (Figures 6 and 7). Abawi and Grogan (1975), Grogan and Abawi (1975), Steadman (1983), Bedi (1962a) and Henson and Valleau (1940) state that sclerotia of _S_. _sclerotiorum_ require continuous moisture and temperatures of between 10-20°C for 7-21 days for carpogenic germination to occur. During the period ending with the first week of July, all locations had rainfall on at least 2 out of every 7 days, with a total of at least 5 cm during the 10 to 14 day period just prior to observation of the first apothecia (Table 2). Soil water potential ranged from -5 to -20 centibars and temperatures were between 10-24°C. Although hours of relative humidity of greater than 80% were more than 12 h per day, relative humidity alone does not contribute to sclerotial carpogenic germination. Bedi (1962a) failed to obtain carpogenic germination at 100% relative humidity.

Forty to fifty-five days from the date of planting coincided with rapeseed growth stages 3.1 to 4.2. This is the period, for the first time, when the crop canopy completely shades the soil surface. This reduces surface evaporation and dampens temperature fluctuations within the crop canopy (Morrall and Dueck, 1982). In 1983 and 1984, crop canopy temperatures were usually lower than ambient and the soil thereunder remained wetter between rains (Figures 6 and 7). These conditions created the cool moist environment necessary for carpogenic germination and apothecial development.

In 1983, high temperatures of above 25°C in combination with lack of moisture occurred beginning the second week of July (Figure 6). This interrupted sclerotial germination. Although temperatures were cool

enough (10-24°C) in 1984, lack of moisture alone interrupted sclerotial carpogenic germination beginning the second week of July (Figure 7).

The variously treated sclerotia that were buried in early May all showed a greater degree of germination than similarly treated sclerotia that were buried in late June (Tables 9, 10, and 11). Sclerotia that were buried in early May must have been exposed to a longer cool, moist environmental period than those that were buried in late June. The length of time that sclerotial treatments were exposed to these conditions contributed significantly to the differences in germination (Table 3 and 5). There were differences in mean numbers of germinated sclerotia between the populations at Winnipeg and Glenlea and that at Portage (Table 4) and this could be due to the differences in weather patterns that occurred at these locations. Temperatures were relatively uniform, but the locations differed in the amount and frequency of rainfall. The total amount of rainfall received between the second week of June to the first week of July were: Portage 8.4 cm, Winnipeg 9.4 and Glenlea 5 cm while the total rainy days were 17, 8 and 10, respectively.

Although Portage had almost a similar amount of rainfall as Winnipeg, the locations differed in respect to the frequency of rainy days (Table 2). It seems that it is not only the amount of rain received in a given area that influences sclerotial carpogenic germination but also how frequently that amount is received or sustained. This provides the necessary long periods of moisture required for carpogenic germination. These differences were consistent in 1982, 1983 and 1984 (Table 2). Therefore, a region with frequent periods of favourable weather and soil

moisture would be expected to have more sclerotia germinating than a region with unfavourable conditions.

## Chapter IV

# GENERAL DISCUSSION

In stem rot disease of rapeseed, sclerotial carpogenic germination is the key event needed for infection to occur. The results of our studies indicate that continuous moisture (near -2 bars) for about 7-10 days and cool temperatures (10-20°C) promote carpogenic germination (Figure 3). The results also indicate that carpogenic germination does not occur below water potentials of -5 bars. Sclerotial germination is not synchronized. It is continuous for up to 28 days in favourable environmental conditions (Figure 2). The results support the findings of Abawi and Grogan (1975), Purdy (1956), Henson and Valleau (1940), Grogan and Abawi (1975), and Morrall (1977).

The sclerotia that were pre-conditioned in moist sand at 4°C germinated more than the unconditioned sclerotia under similar conditions (Tables 6, 7 and 8). Sclerotia of *S. sclerotiorum* require preconditioning in cool moist environment as stimulus for carpogenic germination. This preconditioning or physiological maturation occurs during the winter or non-cropped season (Steadman, 1983; Huang, 1981). Freezing is not necessary as evidenced by the distribution of *S. sclerotiorum* in most parts of the world (Kohn, 1979; Purdy, 1979; Bedi, 1962b).

Sclerotia may be conditioned anytime from fall harvest to rapeseed flowering the next year (growth stage 4.1-4.3). This is the growth stage when crop canopy first completely shades the soil surface. This

creates micrometeorological conditions required for apothecial development (Morrall and Dueck, 1982). The field studies indicate that crop canopy temperatures were usually lower than the ambient between rains and the soil remained wetter than the uncropped area (Figures 6 and 7). Under dry conditions crop canopy temperatures are almost similar to ambient because the crop canopy opens up as plants wilt. This happened in 1983 which was generally hotter and drier than 1984 for the greater part of the growing season (Figures 6 and 7). Apothecia rapidly withered on exposure to extreme drying but quickly absorbed moisture and resumed development when cool moist conditions returned. Trevethick and Cooke (1973) obtained similar results in their laboratory experiments.

The critical factors for carpogenic germination of sclerotia of *S. sclerotiorum* in rapeseed are a total of at least 5 cm of rainfall over the last 10-14 days with temperatures between 10-25°C beginning crop stages 3.1-4.3. Apothecia were first observed in the field in both 1983 and 1984 during the last week of June to first week of July when these weather periods occurred. In 1982 these conditions occurred during the 1st and 3rd week of July (Table 2). While our work supports the findings of Morrall and Dueck (1982) that apothecia first appear on the soil surface under rapeseed canopy during the last week of June, it differs from theirs in that they worked on natural populations of sclerotia in the field and that they did not quantify the environmental factors necessary for carpogenic germination.

In order to predict carpogenic germination in rapeseed we consider a week as favourable when the 7 day mean temperature is between 10-25°C and a rainfall total of at least 2 cm for at least 2 in every 7 days

occurs. These minimal conditions were met in 1982, 1983 and 1984 between the 2nd week of June and 2nd week of July (Table 2). These conditions supply the necessary cool moist conditions for carpogenic germination. The initial appearance of apothecia can be forecast 1-2 weeks after the first occurrence of 1-2 weeks of favourable conditions.

S. sclerotiorum requires an exogenous energy source for the ascospores to infect healthy plant tissue. In rapeseed and other Brassica species the most frequent site for infection are senescent floral parts (McLean, 1958; Gugel and Morrall, 1984). After colonization of the flower, the fungal mycelium can infect adjacent plant parts within 2 or 3 days. The length of time and the environmental conditions required by ascospores to survive on plant surfaces when discharged before flower or other senescent tissue is available has not been demonstrated. This information is potentially useful for developing fungicide application schedules to control stem rot in rapeseed.

Occurence of sclerotinia favourable weather factors earlier on in the season before the crop is in bud stage (cropstages 3.1 to 3.3) poses no danger. While continued occurrence of these factors from beginning of flowering (stage 4.1) may lead to apothecial development and subsequent infection, our results indicate that apothecial numbers are correlated neither with disease incidence nor with rapeseed yield (Table 12). There were as many diseased plants at the locality of buried sclerotia as were in guard rows. This suggests that sclerotia were present in the soil providing endogenous inoculum or that exogenous sources of ascospores were responsible. Future research should be directed in this area, first to determine the relationship between sclerotial levels in

the soil, ascospores and disease incidence in the field. Secondly to verify this preliminary prediction-forecasting model for stem rot in rapeseed.

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