The biology of *Sclerotinia sclerotiorum* in association with carrots (*Daucus carota*) and the potential for biological control of sclerotinia rot in cold storage

by

Janet Finlayson

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THE BIOLOGY OF *SCLEROTINIA SCLEROTIORUM* IN ASSOCIATION WITH CARROTS (*DAUCUS CAROTA*) AND THE POTENTIAL FOR BIOLOGICAL CONTROL OF SCLEROTINIA ROT IN COLD STORAGE

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JANET FINLAYSON

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

MASTER OF SCIENCE

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ABSTRACT

Finlayson, Janet Elizabeth. M.Sc., The University of Manitoba, October 1987. The biology of Sclerotinia sclerotiorum in association with carrots (Daucus carota) and the potential for biological control of sclerotinia rot in cold storage. Major professors: Dr. M.K. Pritchard and Dr. S.R. Rimmer.

Field, storage and laboratory experiments were conducted to investigate and compare the infection process of Sclerotinia sclerotiorum on five cultivars of carrots. The potential for using the mycoparasites Coniothyrium minitans and Talaromyces flavus to control sclerotinia rot in storage was also studied in laboratory experiments.

Results of greenhouse trials indicated that the probable source of primary infection on growing carrots is from mycelial infection of leaf and stem tissue in contact with sclerotia in the soil. No evidence of direct mycelial infection of the carrot root from sclerotia in the soil was observed. Ascospore infection and subsequent mycelial growth on the foliage was observed only if the plants were kept under high relative humidity (RH) for 11 days. Results after two months storage indicated infection of the carrot root occurred after 9 or 11 days of high RH, but no infection occurred after 3 or 6 days high RH.

Field and storage trials demonstrated cultivar differences in susceptibility to sclerotinia rot. As well, a conductivity assay indicated cultivar differences in susceptibility of the carrot root tissue to S. sclerotiorum infection in terms of permeability changes measured by the release of electrolytes from infected tissue. Six Pak II had the highest number of rotten carrots per row at harvest, the most diseased roots after three months storage, and the most electrolyte loss from carrot discs in the conductivity assay indicating that in comparison with the other four cultivars (Paramount, Goldmine, Dess Dan, and XPH875) it was the most susceptible to sclerotinia rot. Dess Dan had the least amount of disease in the field and after three months storage, and the least amount of electrolyte loss in the conductivity assay. Thus the conductivity assay correlates well with the results in the field experiments and provides a simple measurement of relative susceptibilities of cultivars to S. sclerotiorum infection.

The growth rates (area) of S. sclerotiorum and C. minitans on carrot agar were determined at 2, 4, 6, 8, and 10°C, and best-fit regression lines of growth rate versus temperature were obtained. The growth rate of S. sclerotiorum was significantly higher than the growth rate of C. minitans at all the temperatures measured. The linear growth of T. flavus on carrot agar was measured at 4, 6, 8, 10, and 15°C. No growth was observed at 4°C, and the growth rate was slower than the other two fungi at each corresponding temperature. A best-fit regression of the log of the linear measurement of growth rate versus temperature was obtained.

The conductivity assay to compare electrolyte loss from carrot discs inoculated with S. sclerotiorum to discs inoculated with S. sclerotiorum plus the mycoparasites showed that C. minitans reduced the infectivity of S. sclerotiorum. T. flavus had no effect on reducing electrolyte loss from infected carrot discs.

Results from the growth rate experiments demonstrated that due to their comparatively slow growth rates at low temperatures, C. minitans and T. flavus would not be effective in controlling S. sclerotiorum in stored carrots. The greenhouse experiments provided information on possible target sites for application of biocontrol organisms, either in the field or in storage.

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Chapter I

INTRODUCTION

The cultivated carrot (*Daucus carota* L. var *sativa* DC.) is an important vegetable crop. Carrots are grown commercially in every province in Canada, and although the cultivated area in Manitoba has declined from 138 ha in 1982 to 80 ha in 1985, the crop ranks fifth in dollar value behind potatoes, onions, colecrops and cabbage (Man. Agric. Yearbook, 1985). Manitoba growers are usually able to supply the local market demands for the latter half of August through to the end of January or February provided the carrots are of high enough quality.

The main factors limiting the storage life of carrots are loss of turgor, sprouting of roots and shoots, and microbiological spoilage. Manipulation of the storage environment has minimized physiological changes, and currently disease is the major limiting variable in long term storage (Burton, 1977).

In Manitoba sclerotinia rot is the biggest problem in storage of carrots. Losses are sporadic and unpredictable, and some years up to one-half of the stored crop has been destroyed. The disease originates in the field, but is mainly a problem in stored carrots (Geary, 1978).

Sclerotinia sclerotiorum ((Lib.) de Bary) is an ubiquitous plant pathogen, damaging a wide range of crops, causing millions of dollars to be lost annually through the loss of yield, loss in grade and loss of production (Purdy, 1979).

There do not appear to be any carrot cultivars resistant to sclerotinia rot, and although various control methods have been used to reduce the disease levels both in the field and in storage, results have been inconsistent. Relatively little work has been conducted on the host/parasite relationship, and details of the infection process of S. sclerotiorum on carrots are not known. This could have important implications when control measures are considered.

A possible strategy for disease control is the use of microorganisms that are parasitic to S. sclerotiorum. Two such mycoparasites are Coniothyrium minitans and Talaromyces flavus, both of which can kill hyphae and sclerotia of S. sclerotiorum (Huang and Hoes, 1976; McLaren, 1983). Both fungi were reported to be effective in controlling sclerotinia wilt and reducing yield losses in sunflower in Manitoba (Huang, 1977; Mclaren, 1983). There is also a potential for biological control of postharvest plant diseases (Wilson and Pusey, 1985).

Local vegetable growers have expressed interest in new approaches to control of sclerotinia rot in carrots. Thus this work was undertaken in order to gain information on the biology of the disease in conjunction with aspects of control of S. sclerotiorum in carrots.

The objectives of this thesis are three-fold:

(1) To study the infection process of S. sclerotiorum on growing carrots in the greenhouse and the field.

(2) To compare the susceptibilities of five carrot cultivars to sclerotinia rot after a period in cold storage.
Also to measure electrolyte loss from infected carrot discs of the five cultivars using a conductivity assay to determine

the infectivity in terms of membrane permeability.

(3) To investigate the possibility of using T. flavus and C. minitans to control S. sclerotiorum in storage using the conductivity assay and growth rate comparisons.

Chapter II

LITERATURE REVIEW

2.1 BIOLOGY OF SCLEROTINIA SCLEROTIORUM

Sclerotinia sclerotiorum is one of the most non specific and successful of the plant pathogens (Purdy, 1979). It is distributed world wide, and attacks plants in over 360 species in 64 plant families, including vegetable and ornamental crops, trees and shrubs, field and forage crops and herbaceous weeds (Adams and Ayers, 1979). Millions of dollars are lost annually as a result of this fungus, either from direct loss of yield, or from indirect losses in quality. The fungus is destructive to crops in the field, as well as under greenhouse, storage and marketing conditions (Purdy, 1979).

S. sclerotiorum is a fungus of the class Ascomycetes, subclass Discomycetes. It is characterized by the production of hyaline, ellipsoidal ascospores, which have a cleft aperture at maturity. The asci develop from pedicelled apothecia which arise from a sclerotium. There is no functional conidial state (Kohn, 1979).

There is some debate on the delineation and nomenclature of Sclerotinia species, but the three main disease-causing species are S. sclerotiorum, S. minor, and S. trifoliorum (Willetts and Wong, 1980).

The growth habits of S. sclerotiorum are some of the reasons for its success as a plant pathogen. The fungus is extremely adaptable, and un-

dergoes a period of rapid growth when the host plant is invaded, but approximately 90% of the lifecycle of Sclerotinia species is spent in the soil as sclerotia (Adams and Ayers, 1979).

Sclerotia are the resting or overwintering stage of S. sclerotiorum and allow the fungus to survive for long periods of time under adverse conditions (Willetts and Wong, 1980). Viable sclerotia have been recovered in the soil after three years (Cook et al., 1975), four or five years (Adams and Ayers, 1979) and up to ten years (Brown and Butler, 1936).

Sclerotia can germinate to produce mycelia or apothecia. Myceliogenic germination of sclerotia of Sclerotinia spp. can occur in two ways. Hyphal germination occurs when individual hyphae emerge through the rind of the sclerotium. Eruptive germination, as observed in S. minor, involves the formation of a mycelial plug emerging from the medullary region of the sclerotium to rupture the rind (Adams and Tate, 1976). Myceliogenic germination of S. sclerotiorum seems to be of the hyphal type (Willetts and Wong, 1980). Carpogenic germination occurs with the development of stipes and apothecia. Ascospores are formed in the apothecia (Willetts and Wong, 1980).

Mycelial growth of S. sclerotiorum occurs over a broad range of temperatures. Tanrikut and Vaughan (1951) and Le Tourneau (1979) report that some isolates of S. sclerotiorum grow and produce sclerotia over a temperature range from near 0°C to 30°C. Minimum temperatures have been reported to be less than 0°C (van den Berg and Lentz, 1968), 4°C (Newton et al., 1973), and 5°C (Abawi and Grogan, 1975). Lauritzen (1932) ob-

served slight growth at 0.9°C and obtained infection of carrots at temperatures from 0-1°C.

The optimum temperature for growth is generally considered to be about 20°C (Tanrikut and Vaughan, 1951; Newton et al., 1973; and Abawi and Grogan, 1975). Maximum temperatures for growth have been reported at 30°C (Le Tourneau, 1979) and 32-36°C (van den Berg and Lentz, 1968; Newton et al., 1973).

2.1.1 Carpogenic germination

It is generally agreed that sclerotia require a dormancy or conditioning period in order to produce stipes. The time and optimal conditions required for preconditioning sclerotia vary for different isolates. In general, newly formed sclerotia must be held under cool and moist conditions before attaining the ability to germinate carpogenically (Abawi and Grogan, 1979).

Carpogenic germination requires prolonged exposure to saturated or near saturated conditions, and continuous moisture is required for about ten days (Abawi and Grogan, 1975,1979). The stipes are positively phototropic and light is required for differentiation of apothecial disks (Le Tourneau, 1979).

A mucilaginous material is discharged along with the ascospores and this cements the spores to the host tissue. The spore can survive for a considerable time on the leaf surface until wet conditions and exogenous energy sources are available for infection. Spores atomized onto bean leaves under field conditions survived for as long as 12 days (Grogan

and Abawi, 1975). Approximately 48-72 hours of continuous leaf wetness are required for infection by ascospores (Abawi and Grogan, 1979).

Up to 3×10^7 ascospores per apothecium have been reported to be released over a 9 day period under laboratory conditions (Schwartz and Steadman, 1978). Dispersal of the spores is by wind and insects. Asci can forcibly discharge ascospores into the air to a distance of more than one centimetre (Abawi and Grogran, 1979) and they can be carried by air currents up to at least 150 m from the source (Williams and Stelfox, 1979).

Infection can be initiated by two types of inoculum: germinated ascospores and mycelium from sclerotia or ascospores (Lumsden, 1979). Appressoria are usually formed and penetration of the host cuticle is thought to be achieved by mechanical pressure (Lumsden and Dow, 1973; Purdy, 1979; and Lumsden, 1979) and not by enzymatic dissolution. A mucilaginous material adhering the infection cushion to the host surface and the domed shape of the structure allow considerable force to be exerted on the cuticle, resulting in mechanical entry (Lumsden, 1979).

Following penetration the host tissue rapidly becomes disorganized as a result of cell wall modifying enzymes (Purdy, 1979). Pectolytic enzymes (endopolygalacturonase, pectin methyl esterase) cellulases and hemicellulases, as well as other miscellaneous enzymes have been detected in sclerotinia pathogenesis (Echandi and Walker, 1957; Calonge 1968; Lumsden, 1979). Oxalic acid and, possibly, other organic acids have also been implicated in pathogenesis (Newton et al., 1973; Lumsden, 1979).

White mold of beans (snap and dry), stem rot of rapeseed, and head rot of sunflower are caused by ascosporic infection (Huang and Hoes, 1980; Cook et al., 1975; Abawi and Grogan, 1975). These diseases are due to infection of above-ground parts by ascospores produced from apothecia on sclerotia near the soil surface. Soil moisture is important for apothecial production, and the ascospores require free moisture on the plant tissue surface as well as a food base of senescing flower parts for infection (Abawi and Grogan, 1979).

2.1.2 Myceliogenic germination

There are many reports that mycelium from germinating sclerotia requires exogenous nutrients (Purdy, 1958; Abawi and Grogan, 1975; 1979). Abawi and Grogan (1975) determined that mycelial germination was unimportant in white mold of beans, and that an exogenous source of energy must be provided for sclerotia to produce mycelial growth. They proposed that the primary function of the sclerotia is to produce ascospores, and mycelium production is a secondary attribute.

Adams and Tate (1976) observed mycelial germination in soil of sclerotia of *S. minor* but not of *S. sclerotiorum*. They also showed that *S. minor* caused infection of lettuce seedlings without first colonizing organic matter. When *S. minor* germinates by eruptive mycelial germination the dense mycelial mass is able to utilize stored food reserves in the sclerotium for growth, as opposed to the hyphal germination of *S. sclerotiorum* and *S. minor*, which is characterized by a few short hyphal strands that grow very little without an exogenous food base (Abawi and Grogan, 1979).

However, greenhouse studies by Huang and Dueck (1980) demonstrated sclerotia of S. sclerotiorum can undergo myceliogenic germination in the soil, and the mycelium produced can infect roots and hypocotyls of sunflower seedlings without the addition of nutrients or wounding of host tissue.

Huang and Hoes (1980) presented evidence that sclerotinia wilt of sunflower is from myceliogenic germination of sclerotia, and the pathogen may spread by root contact from plant to plant.

Lettuce drop can be caused by either S. sclerotiorum or S. minor although S. minor is usually the predominant species on lettuce (Newton and Sequeira, 1972; Hawthorne, 1974; Adams and Tate, 1975). Infection of lettuce by S. sclerotiorum, originating from ascosporic infection of the lower leaves, nearly always occurs at the ground level (Newton and Sequira, 1972; Abawi and Grogan, 1979). The primary inoculum from S. minor is most likely mycelium originating from sclerotia lying on the soil surface under the leaves (Hawthorne, 1974) or from mycelium from sclerotia as deep as 10 cm infecting the root and stem tissues (Abawi and Grogan, 1979).

2.1.3 Influences on mode of sclerotial germination

Whether sclerotia of S. sclerotiorum germinate carpogenically or myceliogenically is largely dependent on moisture conditions. Generally, sclerotinia wilt of sunflower is more prevalent than head rot when the crop is grown under dryland conditions in Manitoba (Huang, 1985). However, in some years ascosporic infection causing head and stalk rot has been quite serious due to infection resulting from the induction of

apothecial development by heavy rains prior to sunflower bloom (McLaren, 1983; Huang and Hoes, 1980).

Letham et al. (1976) determined that crop type has an important influence on the mode of disease development. Both aerial and basal infection were observed in trellised tomatoes, but only aerial infection occurred in cauliflower. The dense canopy of the cauliflowers prevented rapid drying of the soil surface and this inhibited mycelial germination but maintained moist environmental conditions suitable for apothecial development. In contrast, the open canopy of the trellised tomatoes allowed rapid drying of the surface soil, providing a stimulus for mycelial germination (Smith, 1972) and providing conditions unsuitable for apothecial development.

The mode of germination of sclerotia is also influenced in part by the host crop, apart from moisture effects (Huang and Dueck, 1980). Huang and Dueck (1980) isolated sclerotia from rapeseed and sunflower. These S. sclerotiorum isolates from rapeseed and sunflower had the ability to germinate either carpogenically or myceliogenically and were pathogenic on the two host crops. However, compared with sunflower, rapeseed was not as readily infected by mycelium from germinating sclerotia.

Rimmer and Menzies (1983) also found significant host x isolate interaction for mycelial germination when sclerotial germination of several S. sclerotiorum isolates was observed next to sunflower or rapeseed seedlings. It is possible host exudates play a role in the germination of sclerotia (Rimmer and Menzies, 1983).

2.2 CARROTS

The cultivated carrot (*Daucus carota* L.) has become an important vegetable crop worldwide. The dollar value for carrots in Canada is estimated at 28.5 million in 1984, ranking fifth among the vegetable crops behind potatoes, mushrooms, tomatoes, and corn (Statistics Canada, 1986).

Since the vegetable was first described in Afghanistan in the sixth century as a purple tap root (Robertson et al., 1979), selection has concentrated on the phenotypic components of yield, root shape, retardation of inflorescence development, and quality characteristics such as color to develop into the familiar orange garden vegetable. However, selection did not include resistance to disease (Lewis and Garrod, 1983).

The wild form of the carrot (*Daucus carota* ssp. *carota*) is native to cool temperate regions where the root and hypocotyl have evolved as a survival and storage organ in the biennial cycle of the plant (Lewis and Garrod, 1983). These tissues remain capable of wound repair and active defence for several months. With the onset of warmer temperatures the plant eventually becomes reactivated, producing new foliage and an inflorescence, thus completing the biennial cycle. During this period the root is highly resistant to infection (Lewis and Garrod, 1983).

Long term storage of local produce has become more important as the rising energy costs of long distance transport and local grower benefit are realized (van den Berg, 1981). Technology in environmental control has allowed the crop to be stored longer than the period of natural resistance, and this has led to the problem of disease becoming the major limiting variable in long term storage (Burton, 1977).

2.3 SCLEROTINIA ROT IN CARROTS

Sclerotinia rot is mainly a storage disease of carrots, but plants can be infected in the field where a damping-off sometimes occurs (Crete, 1980). Infection in storage is a direct consequence of infection in the field (Geary, 1978).

Early workers considered that infection arose from mycelium present in the soil although there was little experimental evidence to support this (Ramsey, 1925; Lauritzen, 1932). However Geary (1978) concluded that direct infection from sclerotia in the soil is unlikely. Tahvonen (1985) made the assumption that infection of carrot roots takes place mainly from the tops after mechanical topping during harvesting, although he states that the direct method of infection is not known. Infection by most other carrot storage pathogens rarely occurs through undamaged parts of the root but is usually confined to the foliage (Lewis and Garrod, 1983).

According to Geary (1978) ascospores produced by apothecia in the autumn are the main source of inoculum of sclerotinia rot in carrots in the United Kingdom. A long period of free moisture and a humid microclimate is necessary for penetration and lesion development and the plants are susceptible only when the older leaves are just beginning to senesce. The susceptibility of older carrot leaves compared with younger leaves was associated with increased formation of appressoria on the surface and the inability of the epidermal cells to react in a hypersensitive way to penetration. Fully senescent leaves were never invaded and the youngest leaves were seldom infected. It is not known if apothecia are formed in carrot fields in Manitoba, nor if conditions are

such that infection of carrot tops by ascospores is able to occur. However spores from apothecia formed in neighboring fields (eg. canola) are a possible source of inoculum.

Another source of infection could be sclerotia germinating myceliogenically at or very near the soil surface and infecting leaf tissue touching the soil. As leaves senesce in late summer and fall, they droop onto the ground under the crop canopy providing a ready source of nutrients for the germinating sclerotia. As well, many leaves are broken off and damaged by machinery, and hilling operations can cause foliage to become buried and covered with soil, allowing for direct contact with sclerotia.

Whether the primary infection on the foliage occurs via ascospores or sclerotia, once the mycelium is established it is able to spread rapidly amongst the foliage, becoming localized in the crown and thence the root region.

At harvest, incomplete abscission layers are present in the petiole bases allowing the fungus entry into the root region (Davies et al., 1981). Two other storage pathogens, Botrytis cinerea and Mycocentrospora acerina, also infect the leaves and petioles of the carrot plant before harvesting, and then extend into the crown and root region before and after harvest (Davies et al., 1981; Goodliffe and Heale, 1975).

2.3.1 Spread of sclerotinia rot in stored carrots

In general the carrot roots themselves are not infected until later in storage (Geary, 1978). The primary inoculum in storage is most likely actively growing mycelium present in the crown of the plant, as well as in foliage debris adhering to the roots during the harvest operation (Geary, 1978). A decreased infection potential of ascospores and sclerotia is realized at temperatures typical of commercial storage. Geary could not obtain infection at 4°C through intact periderm when either ascospores or sclerotia were used as inoculum. Goodliffe and Heale (1977) also observed lower infection potential of conidia compared with mycelium of B. cinerea on stored carrots.

Spread of S. sclerotiorum in storage occurs by mycelial growth from an infected carrot to an adjacent healthy one to form characteristic nests of infection (Lewis and Garrod, 1983; Geary, 1978). S. sclerotiorum is able to infect carrot roots through undamaged portions (Geary, 1978) although wounding facilitates entry and infection by all carrot storage pathogens (Lewis and Garrod, 1983). The initiation of primary infection appears to occur within two months of harvest (Geary, 1978).

Carrot roots seem to be highly resistant to the development of some fungal diseases early in storage (Goodliffe and Heale, 1978; Davies et al., 1981; Lewis et al., 1981). Goodliffe and Heale (1978) noted that carrots stored for short periods were resistant to invasion by B. cinerea and with increasing periods in storage, an increasing number of roots were susceptible.

This decline in resistance is associated with a fall in levels of antifungal compounds such as falcarindiol (Garrod and Lewis, 1979; Lewis et al., 1981) and 6-methoxy mellein (Goodliffe and Heale, 1978; Lewis and Garrod, 1983). However, no phytoalexin type compounds have been reported to be important in the limitation of infections caused by S. sclerotiorum on any crop (Geary, 1978) and the rapid destruction of tissues by S.sclerotiorum indicates a possible insensitivity to these antifungal compounds.

2.3.2 Storage of carrots

Controlled temperature storage is normally used to store carrots in most cool temperate countries (Lewis and Garrod, 1983). The carrots are usually stored in pallet bins or slatted crates, but if directed forced-air circulation is provided, bulk bins with roots piled up to ten feet are used (Ryall and Lipton, 1979).

The optimum keeping temperature for carrots is 0 to 1°C (International Standard ISO, 1981; Apeland and Hoftun, 1974). van den Berg and Lentz (1966) found that in long term storage tests of carrots, temperature ranges of 0-0.1 and 2.8-3.9°C did not appreciably affect decay. Decay seemed to be more dependent on variety, storage season, atmospheric composition and relative humidity than on temperature. However, at slightly higher temperatures (3.5-4.5°C) decay increased, especially at lower humidity levels (van den Berg, 1981).

Maximum relative humidity (RH) is desirable for carrot storage when the temperatures are low. van den Berg and Lentz, (1966,1973) found that in long term storage tests (up to 9 months) carrots stored at 0-1°C

and 98-100% RH resulted in less decay than in carrots stored under any other regime tested.

This was an unexpected result as traditionally it was feared that such a high humidity would lead to a drastic increase in decay, either because of the humidity level itself or because of condensation of water on the product which becomes unavoidable at this low temperature (van den Berg, 1981).

It was noted that at 98-100% RH molds grew luxuriantly on the surface of many root vegetables without causing decay or other damage (van den Berg and Lentz, 1973,1978). The surface tissues retained their integrity better under very high RH than under even slightly lower RH and thus were better able to resist invasion by microorganisms (van den Berg and Lentz, 1973,1978; Ryall and Lipton, 1979). As well, B. cinerea and S. sclerotiorum produced more extracellular pectolytic enzymes when the carrots were stored at 90-95% RH than at 98-100% RH. This higher enzyme production was caused by a lesser availablity of nutrients at the lower humidity level.

Pectolytic enzymes produced by B. cinerea and S. sclerotiorum include pectin methyl esterase, endopolygalacturonase and possibly endopolymethyl galacturonase (Echandi and Walker, 1957; van den Berg and Yang, 1969). It was noted that these enzymes, when present on the surface of carrots were deactivated more rapidly during storage at 98-100% RH than at 90-95% RH (van den Berg and Lentz, 1978). Since these enzymes play an important role in the penetration of the pathogen into the host, the difference in enzyme production at different relative humidities is

probably a major factor in the effect of RH on decay (van den Berg and Lentz, 1978).

2.4 CONTROL OF SCLEROTINIA ROT IN CARROTS

2.4.1 Cultural practices

Crop rotation is generally recommended for control of Sclerotinia diseases (Steadman, 1979). A three-year rotation with resistant crops such as beets, onions, spinach, corn or cereals is recommended for carrots (Crete, 1980). However, the ability of sclerotia to survive for long periods in the soil decreases the effectiveness of rotation (Steadman, 1979).

Microclimate modification to reduce the high humidity favorable for infection under a thick crop canopy has lessened the disease severity in some crops. Susceptible lettuce cultivars create a more favorable microclimate and provide senescing leaves for infection by S. minor compared with less susceptible cultivars with upright growth habits (Hawthorne, 1974).

The canopy structure of bean cultivars has also been associated with white mold incidence, and upright indeterminate and open bush types resulted in reduced production of apothecia as compared with that under dense compact bush or vine types (Schwartz and Steadman, 1978).

Huang and Hoes (1980) suggested that manipulation of population size and plant spacing may be of some importance in minimizing yield losses in sunflowers due to sclerotinia wilt.

Ridging the soil to protect the crowns of carrots may alter the crop microclimate by preventing the susceptible foliage from accumulating in the rows and by stopping a full canopy from developing. However, late season field treatments should be kept to a minimum to avoid injury to the foliage (Geary, 1978).

2.4.2 Disease resistance

Due to the wide host range and lack of tissue specificity it was once thought that resistance to S. sclerotiorum did not exist (Steadman, 1979). Generally resistant cultivars show less disease in the field due to some form of disease escape.

However, genetic resistance was confirmed in Phaseolus coccineus (Abawi et al., 1978). The inheritance of resistance to S. sclerotiorum was studied and interspecific crosses and backcrosses made with susceptible P. vulgaris germplasm indicated that resistance was controlled by a single dominant gene. The resistance was associated with the blossom tissue although the mechanism of resistance is unknown (Abawi et al., 1978). A good strategy for breeding would be to combine germ-plasm resistance with characteristics that modify crop architecture (Willetts and Wong, 1980).

Huang (1980a) reported a significant difference in resistance among 21 inbred lines and 25 hybrid lines of sunflower. Resistance was heritable and this character was transferable from parental inbred lines to the hybrid. Huang (1980a) suggested screening inbred lines for resistance to sclerotinia wilt may be of importance to the production of commercial hybrids, although no commercial cultivars with genetic resistance to S. sclerotiorum have been released so far.

Traditionally carrots have not been bred for disease resistance (Lewis and Garrod, 1983). Lauritzen (1932) compared susceptibility to S. sclerotiorum infection and decay in 14 carrot cultivars, and although there were differences in disease levels between the cultivars, results were not repeatable, and Lauritzen concluded that any differences in susceptibility between cultivars was of no practical consequence.

Results from carrot storability trials in British Columbia indicate that site differences as well as crop management may have a greater influence than cultivar on keeping quality (Perrin, 1984).

2.4.3 Chemical control

Attacking the sclerotia with chemicals is another alternative control measure. Soil biocides such as methyl bromide or formaldehyde will destroy sclerotia, but costs and technical difficulties involved make their use impractical in the field (Steadman, 1979) and uneconomical in terms of carrot production.

Foliar protectants are effective in controlling S. sclerotiorum in some crops. Benomyl gave good protection in cabbage seed fields (Gabrielson et al., 1973) and in tomatoes (Letham et al., 1976) where good coverage was obtained. Benomyl and iprodione are currently recommended for control of S. sclerotiorum in canola and field beans in Manitoba (Field Crop Production Recommendations, 1986).

Tahvonen (1985) reduced sclerotinia rot in stored carrots from 43.8 to 2.9% and from 11.9 to 0% in 1978/79 and 1981/82 respectively, when thiophanate methyl was sprayed on the tops the day before harvesting.

Spraying just before harvest probably controls mycelium growing on the foliage that might be taken into storage with the roots as loose debris, resulting in a reduction of inoculum levels. It would not, however, prevent infections from occurring earlier in the season, which by harvest might be localized in the crown.

Foliar sprays are expensive, and they must be applied before infection occurs. Fungicide coverage of the senescing plant organs and the soil-plant interface, as well as timing are the most important factors in obtaining control (Steadman, 1979).

2.4.4 Pre-storage treatments

The use of fungicidal dips applied to the roots immediately after harvest have been effective in decreasing decay during storage.

Post-harvest treatment of washed carrots with benomyl or thiabendazole gave good control of rots caused by *S. sclerotiorum*, *B. cinerea*, and *M. acerina* (Lockhart and Delbridge, 1974; Derbyshire and Crisp, 1978). Benomyl is used commercially in Eastern Canada. However, the use of benomyl may be less effective now that some isolates of certain fungi, such as *B. cinerea*, have developed resistance to it (Siegel and Sisler, 1977).

Dipping in water alone also reduces disease. Carrots that were washed and graded before being stored had significantly less decay than carrots stored directly from the field (Lockhart and Delbridge, 1972). Grading removes sources of infection; infected, cracked and broken carrots, as well as adhering soil and debris, and this accounts for the de-

crease in decay (Lockhart and Delbridge, 1972). It also allows for more efficient use of the cold storage facilities.

However, there is still some debate as to whether vegetables should be washed before long term storage (van den Berg, 1980). The process of washing can allow propagules of infection to infect produce via hydrated or watersoaked lenticels and injuries and may possibly increase decay (Eckert, 1975). Also, washing and grading carrots immediately after harvest is expensive and time consuming, and involves labor that is usually needed elsewhere during the busy harvest season.

For this reason growers in Manitoba store their carrots unwashed in bulk storage, and the carrots are washed and graded immediately prior to marketing. Benomyl can be applied to unwashed carrots, but under Manitoba conditions benomyl treatment of unwashed carrots may result in reduced control (Veg. Crop. Prod. Guide, 1985).

2.4.5 Biological control

More than 30 species of fungi and bacteria as well as insects and other organisms have been reported to be parasites or antagonists of *Sclerotinia* spp. (Adams and Ayers, 1979). However, in most instances the parasitic activity of these organisms is based on laboratory observations or tests, and little information is available on their activity and effectiveness under natural or field situations (Steadman, 1979). *Trichoderma* spp., *Sporedesmium sclerotivorum*, *Coniothyrium minitans*, and *Talaromyces flavus* have been studied extensively. The latter two species have been studied in the field situation in Manitoba.

C. minitans is well established as a mycoparasite of S. sclerotiorum under natural conditions. Campbell (1947) first isolated this fungus from sclerotia of S. sclerotiorum and described it as a new species. In laboratory experiments sclerotia of both S. sclerotiorum and S. minor were parasitized by C. minitans, with subsequent development of pycnidia both on and within the sclerotia. Also the formation of apothecia from sclerotia of S. sclerotiorum was suppressed when pycnidiospores of C. minitans were brought into direct contact with sclerotia.

C. minitans kills hyphae as well as sclerotia of S. sclerotiorum. The hyphal tips of the parasite penetrate cell walls of the host without the formation of specialized structures, causing the host cytoplasm to disintegrate and cell walls to collapse (Tribe, 1957; Huang and Hoes, 1976). Physical forces may be involved too, as a depression occurs in the host cell walls at the point of penetration. Disintegration is a result of lysis by enzymatic action. C. minitans produces endo and exo-glucanases which break down B-glucans in the cell walls of S. sclerotiorum (Jones et al., 1974; Huang and Hoes, 1976). Huang (1977) concluded that C. minitans was a promising biological control agent for sclerotinia wilt of sunflowers. In field tests C. minitans parasitized and killed the sclerotia produced on the root surface. It continued to parasitize the pathogen inside the root and upwards into the base of the stem. The secondary infection rate was decreased by the production of pycnidia on sclerotia as well as on roots of sunflower plants killed by S. sclerotiorum. Thus, C. minitans might decrease the infection rate of subsequent crops by decreasing the survival rate of the pathogen.

Penicillium vermiculatum (conidial state or anamorph of Talaromyces flavus) was reported to be a parasite of S. sclerotiorum by Su and Leu (1980). As well, T. flavus was demonstrated to parasitize Rhizoctonia solani (Boosalis, 1956), and to overgrow colonies and suppress growth of Verticillium albo-atrum (Dutta, 1981).

T. flavus destroyed hyphae of S. sclerotiorum by coiling around host cells with consequent disintegration of host cytoplasm and collapse of cell walls. There is evidence of direct penetration of the host hyphae by the hyperparasite, and toxic substances may be involved in pathogenesis (McLaren et al., 1986). T. flavus was also destructive to sclerotia (McLaren, 1983)

In the field, T. flavus was shown to be an effective biological agent in reducing verticillium wilt of eggplant and increasing yield (Marois et al., 1982). McLaren (1983) demonstrated that T. flavus was effective in controlling sclerotinia wilt of sunflower as well as reducing yield losses due to the disease.

Chapter III

MATERIALS AND METHODS

This thesis project was divided into three sections: (1) Investigation of the infection process of S. sclerotiorum on carrots; (2) Comparison of the storability and suceptibility to sclerotinia rot of five carrot cultivars; and (3) Determination of the potential of using C. minitans and T. flavus in controlling S. sclerotiorum in stored carrots.

3.1 SOURCE OF FUNGAL MATERIAL

S. sclerotiorum was obtained from a sclerotium removed from a diseased chicory plant found in a commercial carrot storage (isolate SS13, Menzies, 1983). Infectivity of this isolate on carrots was ascertained by placing a plug of mycelium growing on potato dextrose agar (PDA) on a carrot root. S. sclerotiorum from this isolate was used in the preliminary work, the first greenhouse experiment, and the 1985 field experiments. S. sclerotiorum from a sclerotium isolated from an infected carrot in the first greenhouse experiment was used in all further work.

3.2 ASCOSPORE COLLECTION

To obtain large quantities of sclerotia, plugs of S. sclerotiorum mycelium grown on PDA were placed on autoclaved baked beans and incubated for 10 to 14 days at room temperature. The sclerotia were removed and placed in moistened sterilized sand at 2°C for 6 weeks. The sclerotia were then washed, surface sterilized for two minutes in a solution of 6%

sodium hypochlorite (Javex, Bristol-Myers Inc.) and distilled water (1:4 v/v), rinsed three times with sterile distilled water, and placed on wet sterilized silicate sand in covered glass jars at 18°C with 16 h photoperiod to promote apothecial development. After 5 or 6 weeks the apothecia were mature, and spores were collected daily for up to two weeks on 0.45 um Millipore filter membrane using a Millipore filter (Millipore Corporation, Mass., USA) inverted over the opened jar.

The spores were stored on the Millipore filters at 4°C. Inoculum was prepared by gently scraping the spores off the membrane and suspending in distilled water (1 x 10^5 spores/ml) containing one drop of Tween 20 (Allied Corporation Fisher Scientific) per 200 ml.

3.3 BIOLOGY OF SCLEROTINIA ROT IN CARROTS

In Manitoba it is not known precisely where the original infection of S. sclerotiorum on carrots occurs. The origin of mycelial inoculum was investigated in a greenhouse experiment, and a comparison of the infectivity of mycelium and ascospores as inoculum was done both in the field and greenhouse. As well, further aspects of ascospore infection related to humidity were observed on carrots grown in the greenhouse.

3.3.1 Greenhouse experiments

Carrots (cv 'Paramount') were seeded into bolster bags (Bolster Pack, Horticultural Products, W.R. Grace and Co.) containing soilless mix and thinned to approximately 35 plants per bag. The plants were fertilized with 20-20-20 fertilizer (Plant Products Co. Ltd.) every two weeks. The bags were divided in the middle with a translucent plastic divider to

double the number of replications. After 2.5 to 3 months clear plastic was hung between the bags to separate the replications. After inoculation the entire system was covered with clear plastic to provide a humid environment.

3.3.2 Mycelium placement experiment

The experiment was set up in a randomized complete block design (RCBD) with two blocks and three treatments, and repeated three times. Two treatments were replicated six times in each experiment; the control was replicated four times in experiments 1 and 2 and six times in experiment 3.

Inoculum was obtained by growing S. sclerotiorum on autoclaved millet seed. Isolate SS13 of S. sclerotiorum was grown on PDA at room temperature for 2 or 3 days. The resulting colonies, covering the agar, were then cut into cubes. While these cultures were growing, millet seed was soaked overnight, drained and autoclaved in Mason jars at 121°C and 102 kPa for 20 min on two consecutive days. The mycelium-covered agar in the petri dishes was cut into cubes and one-half of the contents of a dish was mixed into each jar of millet and incubated at room temperature for five days.

The treatments consisted of placing a few inoculated seeds either 1.5 cm below the surface and beside the root, or on the soil on top of bent-over leaves. Approximately one month after inoculation the plants were visually rated for leaf damage (on a scale of 1 to 5) and disease. Leaf damage was determined by subjectively comparing the replicates with one another. A replicate containing plants with mostly upright green

foliage received a rating of 1; a rating of 5 was given to replications in which most of the plants had senesced or decayed leaves. Healthy roots were washed and placed in plastic bags at 6°C for approximately three months, when they were rated again for disease by determining the weight of diseased carrots. Leaf fresh weight was obtained from experiment 3.

3.3.3 Comparison of ascospores and mycelium as inoculum - greenhouse

This experiment was set up as an RCBD with two blocks and three treatments. Treatments were replicated 6 times each and consisted of: (1) control, (2) mycelium placed near the crown of plants, and (3) ascospore spray. The mycelial inoculum was prepared using autoclaved millet seed, as described above. Mycelial inoculum (20 ml per one-half bag) was sprinkled on the soil surface near the crown of the carrot plants. Ascospore inoculum (80 ml per one-half bag in a concentration of 10^5 spores/ml) was sprayed onto the surface of the leaves using an air pump with a DeVilbis sprayer (Braun Chemical Co.) at 69 kPa. After two weeks the plants were sprayed a second time with 50 ml inoculum and a humidifier was placed under the plastic cover to increase the humidity.

The mycelium-inoculated carrots were rated for leaf damage and disease, and harvested 11 days after inoculation. The ascospore-inoculated plants in one block were rated and harvested four days after the second spray, and the control treatments and ascospore-treated carrots in the other block were rated and harvested 23 days after the second spray. The harvested roots were placed in plastic bags and stored for 2 months at 6°C.

3.3.4 Comparison of ascospores and mycelium as inoculum - field

Carrots were planted on May 23 on the edge of a commercial carrot field of Jamor Farms, 11 km north of Portage la Prairie, Manitoba. Four rows of carrots 40 cm apart were planted in beds 180 cm in width. Seeding was done with a cone and belt push seeder with a seeding rate of 83 seeds/metre.

The carrot cultivar 'Paramount' was planted as 2 3x3 Latin squares; each plot was one bed wide and 6 meters long. The three treatments consisted of a control, and two forms of inoculum, mycelium grown on millet and an ascospore suspension. The carrots were inoculated on September 12 1986. Mycelial inoculum (250 ml) was sprinkled between the two middle rows of each mycelium-treated plot. Ascosporic inoculum in a volume of 130 ml (10^5 spores/ml) was sprayed onto a section in each ascospore-treated plot using a 4 L duralite sprayer (Hudson Manufacturing Company, ILL., USA). The carrots were hand harvested on October 10, 1986, the tops were broken off and the roots were placed in plastic bags aerated with 40 0.5 cm holes and stored at 5-6°C.

3.3.5 Effect of duration of high RH treatment on ascospore infection

This experiment was set up in a complete block design with two blocks and repeated twice. Treatments in the subplots consisted of: 1) ascospore spray and 2) control. The main plot treatments represented the length of time the carrots were under high humidity; 3, 6 or 9 days (experiment 1) and 3, 6, or 11 days (experiment 2).

Ascospore inoculum (50 ml in a concentration of 10^5 spore/ml) was sprayed onto the leaves using an air pump with a DeVilbis sprayer (Braun

Chemical Co.) at 69 kPa. A humidifier was placed under the plastic cover to maintain a high relative humidity (RH). After 3 days the plastic was removed from the first main plot; after 6 days plastic was removed from the second main plot, and the third main plot was under the plastic for 9 days (experiment 1) or 11 days (experiment 2). Two humidifiers were used in the second experiment. One month after inoculation the plants were rated for leaf damage and disease, leaf fresh weight was recorded, and the roots were placed in plastic bags at 6°C.

3.4 COMPARISON OF FIVE CARROT CULTIVARS FOR RESISTANCE TO SCLEROTINIA ROT

There have been no reports of resistance to S. sclerotiorum in any carrot cultivars, but differences in the amount of disease in storage have been noted. Five cultivars commonly grown by commercial growers in Manitoba were grown and the amount of disease in the field and after storage was compared. The five cultivars used were: Six Pak 11 (Harris Seed Company, New York, USA), Paramount (Asgrow Seed Company, Michigan, USA), and Goldmine (Sun Seed, Minnesota, USA), which are fresh market types, and two processing types: Dess Dan (Stokes Seeds Ltd., Ontario) and XPH875 (Asgrow Seed Company, Michigan).

3.4.1 Field Experiments

Cultural treatments included 36 kg per ha each of nitrogen and phosphorus fertilizer applied the previous fall, post emergence application of Lorox (1.1 kg/ha) for weed control, periodic application of Sevin (1.9 L/ha) and Belmark (62 ml/ha) for control of leafhoppers.

3.4.2 Field experiment 1985

The five carrot cultivars were planted on May 17 in a RCBD with five replications. Each plot was one bed wide and 6 m long. To obtain large quantities of sclerotia, plugs of S. sclerotiorum mycelium grown on PDA were placed on autoclaved baked beans in foil-lined cake pans. After 10-14 days at room temperature, the sclerotia were removed and placed in moistened sterilized sand at 2°C for 6 weeks.

On July 3, 20 g of sclerotia were inserted approximately 1.5 cm deep into the soil beside the carrot plants between one outer and middle row of each plot. Disease was assessed in the field in late August and September. During the period October 17-21, two 4.5 kg samples of roots were hand harvested from the uninoculated rows of each plot, and placed into mesh bags. The carrots initially were randomly placed into three pallet boxes in a commercial storage at the Jefferies Bros. Farm near Portage la Prairie. Each pallet box was placed above another box, and a humidifier and fan were located in the bottom box. The two boxes were then sealed with plastic leaving the top open, to allow humid air to circulate among the carrots. The storage temperature was extremely cold (near 0°C, 98-100 % RH) and no rot was observed. The boxes were dismantled and the carrots were brought into Winnipeg on January 10, 1986. Each bag was placed individually into a plastic bag aerated with 40 0.5 cm holes and placed into storage at 6°C in the Plant Science building. The bags were removed from storage April 30 and graded for disease.

3.4.3 Field experiment 1986

The five carrot cultivars were planted in a split-plot Latin square design with five replications consisting of five main plot treatments (the different cultivars) that were one bed wide and 6 m long, and two subplots that were each 3 m long. S. sclerotiorum was grown as for the mycelium placement experiment (3.3.2).

The subplots were the different rates of inoculum. On August 26 inoculum was sprinkled on the soil surface on either side of the two middle rows in one subplot at a rate of 500 ml, and 250 ml of inoculum was sprinkled between the two middle rows of carrots in the second subplot. The carrots were irrigated the next day.

On October 13 the numbers of diseased and healthy roots in one middle row in each plot were recorded. The carrots were harvested on October 15 and 17. The roots were hand dug with a fork, the tops broken off, and approximately 7 kg were placed in plastic bags aerated with 40 0.5 cm holes. The bags were transported to the Plant Science Building at the University of Manitoba and stored at 5-6°C until January 13, 1987 when they were removed and the number and weight of diseased roots was recorded.

3.4.4 Conductivity assay

A method was used by Newton et al. (1973) to correlate electrolyte loss (in terms of conductivity) with the virulence of Sclerotinia isolates on infected carrot tissue. This method was adapted to determine the effect of temperature on electrolyte loss and to compare the infectivity of S. sclerotiorum on five cultivars of carrots.

Carrot root discs (10 mm diameter, 4 mm thick) were placed on moist filter paper in a glass petri plate, and an 8 mm diameter inoculum plug (from the leading mycelial edge of a S. sclerotiorum colony grown on 20 ml PDA in a 9 cm diameter petri dish) was placed with the mycelial side down on top of the disc. Three replicates, each consisting of two discs, and two control replicates (incubated with a sterile PDA plug) were included in each plate. A separate plate was used for each cultivar at each temperature. The plates were wrapped with Parafilm (American Can Company) and incubated at 2, 4, 6, 8, 10, 15 and 20°C for 22 hours. After incubation two discs were placed into 10 ml deionized distilled water, and electrolyte loss was determined by the increase in conductance (electrolytes) of the bathing medium, using a dip-type conductivity electrode (Markson Science Inc.). The conductivity of the bathing solution was read at time intervals of 15, 35 and 50 min. Four to six experiments were done using each cultivar at each temperature.

3.5 BIOLOGICAL CONTROL OF SCLEROTINIA ROT IN CARROTS

3.5.1 Source of mycoparasites

An isolate of Talaromyces flavus, obtained from D. McLaren (Agriculture Canada, Lethbridge, Alberta), was cultured on PDA. Isolate CM54 of Coniothyrium minitans was also cultured on PDA.

3.5.2 Growth rates of the pathogen and mycoparasites at different temperatures

The growth rates of S. sclerotiorum and C. minitans were measured at five different temperatures (2, 4, 6, 8 and 10°C). T. flavus was grown at 6, 8, 10, 15 and 20°C. Carrot agar was prepared as follows: sliced

carrots (300 g) were boiled in 500 ml distilled water for 20 min, blended in a Waring blender, sieved through a J-cloth, made up to a 1 L solution with distilled water, and 20 g Bacto agar (Difco) was added before autoclaving.

S. sclerotiorum and C. minitans were grown on 20 ml carrot agar in 9 cm petri plates. Plugs of mycelium (6 mm) were cut from the edges of actively growing colonies of each fungus on PDA, and were used to inoculate the carrot agar plates. Ten plates per fungus per temperature were prepared and incubated at the appropriate temperature in the dark. The experiment was performed twice. The colony diameter was measured and recorded every half-day, day, or several days, depending on how fast the colonies grew.

The same procedure was followed for T. flavus except that due to excessive sporulation which spoiled measurements of the colony diameter, the fungus was grown on 10 ml carrot agar in growth tubes (75-2390, Carolina Biological Supply) and the linear growth of the fungus in the tube was measured.

3.5.3 Effect of mycoparasites on infectivity of Sclerotinia sclerotiorum on carrot discs

The ability of T. flavus and C. minitans to reduce infectivity of S. sclerotiorum on carrot roots was assessed by modifying the conductivity assay developed by Newton et al. (1972). As the growth rate of S. sclerotiorum is much faster than the growth rates of T. flavus and C. minitans, it was necessary for the mycoparasites to be established on the carrot disc before S. sclerotiorum was added.

Treatments consisted of a control, C. minitans, T. flavus, and S. sclerotiorum alone, and each mycoparasite in combination with S. sclerotiorum. Spore suspensions of T. flavus and C. minitans were obtained by rinsing 3 to 8 week old PDA cultures of the fungi with sterile distilled water. Spore suspensions of S. sclerotiorum were obtained from apothecia as described earlier. Carrot root discs (10 mm diameter, 4 mm thick) were placed on moist filter paper in a petri plate, and a drop of spore suspension of either mycoparasite (or both) was placed on top of the disc. A drop of sterile distilled water was used for the control and S. sclerotiorum treatments. After one to four weeks a drop of S. sclerotiorum spore suspension was placed on the T. flavus/S. sclerotiorum, C. minitans/S. sclerotiorum, T. flavus/C. minitans/S. sclerotiorum and S. sclerotiorum treatments; sterile distilled water was placed on the control, C. minitans/T. flavus, C. minitans, and T. flavus treatments. An 8 mm diameter plug of sterile PDA was placed on top of each carrot disc.

After an incubation period of 7 to 9 days at 10, 15 or 20°C the two discs of each treatment were placed into 10 ml deionized distilled water and the conductance of the bathing solution was read at time intervals of 15, 35 and 50 min.

Chapter IV

RESULTS AND DISCUSSION

4.1 BIOLOGY OF SCLEROTINIA ROT IN CARROTS

4.1.1 Mycelium placement experiment

The carrots treated with mycelium near the leaves had a significantly higher leaf disease rating and significantly more diseased roots than the carrots treated with mycelium near the roots or the control (Table 1). The higher leaf disease rating indicated more senescing and dead foliage, lighter color and generally less plant vigor. The leaf fresh weight at harvest (experiment 3 only) was significantly lower for the plots treated with mycelium near the leaves as compared with the other two treatments. After storage, significantly more roots treated with mycelium near the leaves were visibly infected with S. sclerotiorum compared to the control.

If the relative humidity (RH) was high enough a few days after inoculation white mold was visible on the stem and leaves of those carrots inoculated with mycelium near the leaves. The foliage then became water soaked, darkened, and senesced. The crowns of infected carrots were soft, and often decayed up to one-half the length of the root. Sclerotia were rarely observed. Infected carrots treated with mycelium near the roots were diseased near the crown and the tops were usually senesced or decayed. There was never any infection in the lower part of the root without the crown also being infected. The growing medium was

TABLE 1 Effect of inoculum placement on sclerotinia rot in greenhouse-grown carrots

Treatment	% Healthy[1] roots	Leaf rating 1-5[2]	% Roots with sclerotinia rot after storage	Leaf fresh wt per carrot[3]
Control	100.0 a[4]	2.0 a	2.1 a	10.5 a
Inoc. near roots	96.8 a	2.7 a	15.8 ab	6.7 b
Inoc. leaves	91.0 b	4.0 b	34.3 b	3.9 c

[1] Values averaged over 3 experiments, six replications/experiment.

[2] Least to most damage.

[3] Experiment 3 only.

[4] Means within columns followed by same letter are not significantly different at the 0.05 level (Duncan's Multiple Range Test).

light, and it was possible millet inoculum was washed up during watering to the surface near the crown and senescing leaves.

Only apparently healthy carrots with no visible infection were stored. In some replications although there was a high percentage of carrots with sclerotinia rot in the greenhouse, no disease developed on the healthy roots after a period in storage. In other replications all the growing plants appeared healthy, but after storage most or all of the roots were infected. This was likely due to latent infection in the crown. During the time in storage S. sclerotiorum mycelium spread from one root to another in a bag, and it was impossible to determine the number of roots carrying the original infection from the greenhouse.

In preliminary work unconditioned and conditioned sclerotia were placed 5 cm below the surface and approximately 2 cm away from roots of carrot plants growing in bolster bags. Infection was never observed, and when the sclerotia were removed there was no evidence of germination. Sclerotia were placed near the roots of sunflower plants growing in the same type of medium, and within several weeks wilting symptoms were observed.

To circumvent the possibility that conditions were unsuitable for sclerotial germination near the carrot roots, mycelium was used as the inoculum source using millet seed as a nutrient base. Under natural conditions in the soil the rhizosphere around the carrot root would be much different and sclerotia might be capable of germinating myceliogenically and infecting carrot roots. However, from these results it appears that even when mycelium was placed near the carrot roots with a

food base, either growth of the mycelium through the medium did not occur, or penetration and infection of the carrot root was inhibited or impossible. Carrot root exudates or the environmental conditions may play an inhibitory role.

The placement of mycelium near the leaves was an attempt to simulate cultural conditions in the field when machinery runs over leaves and partially buries them. The results indicated that S. sclerotiorum mycelium was able to infect leaves after growing a distance of 0.5 - 1 cm through the medium. Once the mycelium had invaded the leaf tissue it was able to move down the petiole to the crown, whence it entered the root. By entering the root via the crown the mycelium could circumvent the periderm, which may be a barrier to penetration through the exterior of the root (Lewis and Garrod, 1983).

These results tend to support the idea that mycelial infection of carrots is primarily through leaf tissue very near or in contact with sclerotia lying near the soil surface. In agreement with Geary's (1978) results, direct mycelial infection of the carrot root does not seem to occur.

4.1.2 Comparison of ascospores and mycelium as inoculum - greenhouse

The mycelial inoculum generally caused the most disease under the greenhouse conditions (Table 2). Compared with the control and spore treatments, the bags containing carrots treated with mycelium had significantly fewer healthy carrots, significantly more roots without tops when harvested, significantly lower ratio of fresh leaf weight to number of plants, and a significantly higher leaf rating, indicating more damaged

TABLE 2 Effect of ascosporic and mycelial inoculum on the level of S. sclerotiorum infection in greenhouse-grown carrots

Treatment[1]	% healthy roots before storage	% roots without tops at harvest	Leaf rating 1 - 5	Weight leaves/ number roots	% diseased roots after storage
Control	98.17 a[2]	1.83 a	2.17 a	10.48 a	2.00 a
Ascospores	84.17 a	30.33 b	2.33 a	6.68 b	78.33 b
Mycelium	24.50 b	84.00 c	4.67 b	3.87 c	-

[1] Average of six replications.

[2] Means within rows followed by same letter are not significantly different at the 0.05 level (Duncan's Multiple Range Test).

leaves and less plant vigor. The plants quickly became diseased; white mycelium was visible within a few days on plant tissue adjacent to the inoculum, and the mycelium moved rapidly amongst the foliage eventually becoming localized in the crown.

The RH was not high enough for the ascospores to infect the plants, although after two weeks on the south side of the bench there was some evidence of disease in the form of white fluffy mycelium on the stems of a few plants in each spore-treated bag. All the plants used for spore treatments were re-inoculated with spore suspension, but after four days the carrots on the south side of the bench were harvested since the original inoculum had been effective. It is possible that additional heat from the sun increased the temperature and/or the RH on the south side enough to allow ascospore germination and infection. With the addition of a humidifier the humidity was raised enough so that infection by spores could occur on the north side of the bench. As well the temperature may have been more conducive to ascospore germination after the second inoculation. The number of diseased plants among the spore-treated plots was not significantly different, which should minimize the importance of the blocking effect and variations in inoculum treatments.

Compared with the control the carrots treated with spore inoculum had significantly more roots without tops at harvest and significantly lower leaf weight per plant. The crowns of the plants without tops were likely infected with S. sclerotiorum even though the roots appeared healthy because after several months in storage 100% of the carrots were rotted in 4 out of 6 replications.

Ascospores of S. sclerotiorum were capable of germinating and infecting carrot leaf and stem tissue as long as the RH was high enough. The youngest leaves and fully senesced leaves were never initially infected. The mycelial inoculum infected roots more rapidly and frequently than the ascosporic inoculum but this seems to be due to the less stringent conditions required for infection.

4.1.3 Comparison of ascospores and mycelium as inoculum - field

The carrots in the control and ascospore treatments showed no evidence of sclerotinia disease in the field nor after three months in storage (Table 3). The millet/mycelium inoculum was effective in infecting the plants in the field, and 60 % by weight of the stored carrots had sclerotinia rot after three months in storage. No carrots were infected in the control treatment indicating that S. sclerotiorum inoculum levels were low in the field.

The ascospore application was not effective in infecting the plants. Environmental conditions are rigorous for the production, germination, and infectivity of ascospores. Under natural conditions apothecia will not develop unless there is prolonged soil moisture for around 10 days without even a slight moisture tension, and approximately 48-72 hours of continuous leaf wetness are required for infection of bean tissue by ascospores (Abawi and Grogan, 1979). These conditions are difficult to maintain artificially in the field, and the period during which the plants were sprayed in this experiment did not coincide with humid moist weather. Irrigation was of limited use since the soil was near field capacity and heavy winds and cool temperatures prevented a moist, humid microclimate from occurring within the plots.

TABLE 3 Effect of ascosporic and mycelial inoculum on the levels of S. sclerotiorum on stored carrots (field experiment)

Treatment	% diseased by number[1]	% diseased by weight[1]
Control	0 a[2]	0 a
Mycelium	57 b	60 b
Ascospores	0 a	0 a

[1] Average of six replications.

[2] Means within columns followed by same letter are not significantly different at the 0.05 level (Duncan's Multiple Range Test).

4.1.4 Effect of duration of high RH treatment on ascospore infection

Sclerotinia rot was only observed on the carrots sprayed with ascospores and placed under high RH for 9 and 11 days. Compared with the control plants and the other treatments, the inoculated plants placed under high RH for 11 days had significantly fewer healthy roots before storage, significantly more roots without tops at harvest, a significantly higher leaf rating indicating more damaged leaves and less plant vigor, and a lower leaf (fresh weight) to root ratio (Table 4). There was no significant difference between the 9 and 11--day high RH treatments with respect to the number of roots infected with S. sclerotiorum after two months storage.

Ascospores require free water and an exogenous form of energy (such as bean blossoms) in order to initiate infection (Purdy, 1958; Abawi and Grogan, 1975). However the spores will readily infect mechanically injured plants and plants with necrotic lesions incited by other plant pathogens (Abawi and Grogan, 1979). Geary (1978) obtained infection on the oldest green leaves of carrot plants (after approximately 8 weeks of growth in pots) without the addition of nutrients, using 10^5 spores/ml and placing the plants in humid conditions in a mist bench. After 3-4 days, symptoms were initially visible as watersoaked olive-green spots and after 6-7 days white aerial mycelium appeared.

The plants in this experiment were over three months old and several leaves per plant had already senesced and detached from the plant. As well, there were numerous lesions on nearly all the leaves due to insect damage (spider mites, aphids). Thus, there were older leaves as well as wound sites available for infection by the spores and it was unlikely

TABLE 4 Effect of duration of high RH on infectivity of *S. sclerotiorum* ascospores in greenhouse-grown carrots

Days under high RH	No. of replications	% healthy roots before storage	% roots without tops at harvest	Leaf rating 1 - 5[1]	Weight leaves/ number roots	% diseased roots after storage
CONTROL						
3	6[2]	100 a[3]	0 a	1.8 a	9.6	0 a
6	5	100 a	0 a	2.6 a	11.4	20.0 a
9	2	100 a	0 a	2.0 a	9.8	0 a
11	2	100 a	0 a	2.0 a	9.8	0 a
INOCULATED						
3	6	100 a	0 a	2.0 a	10.8	0 a
6	5	100 a	0 a	2.6 a	8.8	0 a
9	2	100 a	0 a	3.0 a	8.8	35.3 ab
11	2	94.5 b	13.4 b	4.5 b	5.5	66.8 b

[1] 1-5; least to most damage.

[2] Average over 2 experiments.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Duncan's Multiple Range Test).

that infection was inhibited due to lack of nutrients. It was impossible to detect the early symptoms of infection as observed by Geary (1978) (watersoaked olive green spots) since the leaves were so covered with lesions from other pests. However the white aerial mycelium was readily observed on the inoculated plants after 11 days at high RH. Geary (1978) observed white mycelium after 6 or 7 days, but this shorter incubation and infection period is likely due to variations in temperature, humidity, amount of free water, fungal isolate, and plant vigor. The results from the number of diseased roots after storage indicate that the fungus was able to infect carrots after 9 days high RH even though the white fluffy mycelial growth was not visible on the foliage of the growing plants.

A comparison of the results from inoculated carrots in the 9 and 11 day high RH treatments (Table 4) demonstrates the sensitivity of ascospores to an additional two days of high RH for disease development. This experiment demonstrates the importance of duration of leaf wetness for infection by ascospores of S. sclerotiorum and is in general agreement with published data (Abawi and Grogan, 1975).

4.2 COMPARISON OF FIVE CARROT CULTIVARS FOR RESISTANCE TO SCLEROTINIA ROT

4.2.1 Field experiment 1985

The summer of 1985 was unusually wet and cool, and the carrot plots were situated in a depression in the field. Consequently the soil was saturated for lengthy periods and soft rot (Erwinia spp.) was prevalent. S. sclerotiorum was found in only a few plots in the field. Mycelium was first observed in August on senescing leaves touching the ground,

and by September it had gradually progressed down the petioles to the crown of the plants. However, by harvest many roots were completely destroyed by soft rot and it was impossible to determine the primary infection organism. In some plots it was difficult to find enough healthy roots to make up the two 4.5 kg samples for storage.

The carrots were stored in pallet boxes with humid air circulated through the bottom of the pile to mimic commercial bulk storage. When after three months there was no sign of any disease the bags were removed to warmer conditions to determine whether the low temperature was inhibiting disease development. However, by April 1986 only one bag (cv Paramount) had S. sclerotiorum rot. Although the original storage temperature was near 0°C, growth of S. sclerotiorum has been observed at less than 0°C (van den Berg and Lentz, 1968) and it is unlikely incipient infections would be prevented from progressing. However since the growth rate is very slow at such low temperatures (Lauritzen, 1932) spread of the mycelium from root to root would be diminished. In addition, survival of the mycelium is short for this pathogen (Geary, 1978; van den Berg and Lentz, 1968; Cook et al., 1975). van den Berg and Lentz (1968) could not isolate S. sclerotiorum from healthy carrots even though they had been inoculated before storage. Their results indicated that the fungus survived less than three months on healthy carrots during storage. Geary (1978) observed that the initiation of primary infection appeared to occur within four weeks of harvest, and if healthy roots were not infected by mycelium on the foliage debris or poorly trimmed tops within the first 2 months, it was unlikely that they would be infected at all, unless by contact from already infected roots (Geary, 1978).

It is also possible that the sclerotia were destroyed due to flooding, causing limited primary infection to occur in the field. Drying and remoistening of sclerotia is detrimental to the survival of the pathogen (Adams, 1975) although moisture fluctuations may be the primary germination stimulus (Cook et al, 1975).

4.2.2 Field experiment 1986

The fungal inoculum was applied in the form of mycelium to ensure the presence of disease. The mycelium quickly colonized any adjacent plant material (usually leaves) and in cases where millet seed was sprinkled or washed into the crown region of the plant, colonization of the crown and root occurred within one week.

The numbers of infected carrots in a row were significantly different between the two inoculum levels (Table 5). The higher rate of inoculum proved too much, and there was complete destruction of the plants in some sections of the rows. When the carrots were harvested one and one-half months after inoculation the carrots from the subplots treated with the higher rate of millet inoculum could not be harvested, and the effect of inoculum level on storability could not be evaluated.

There were no differences among cultivars for the number of infected carrots in a row when they were inoculated at the lower rate. In the higher inoculum level subplots, Six Pak II had significantly more diseased plants than Goldmine and Dess Dan, and Dess Dan had significantly fewer diseased plants than Six Pak II, XPH875 and Paramount (Table 5).

TABLE 5 Effect of cultivar and two levels of inoculum on the number of carrot plants per row infected with S. sclerotiorum

Cultivar	Number infected plants/row (%)[1] Inoculum level 500 ml/plot	Inoculum level 250 ml/plot
Six Pak II	87.4 a[2]	62.8 a
XPH875	80.1 ab	54.8 a
Paramount	72.9 ab	53.2 a
Goldmine	68.0 bc	49.1 a
Dess Dan	53.8 c	50.5 a

[1] Average of 5 replications

[2] Means within columns followed by same letter are not significantly different at the 0.05 level (Duncan's Multiple Range Test).

After three months in storage there were significant differences in disease levels among cultivars, with respect to the number of diseased roots (Figure 1). Six Pak II had significantly more rotted roots than Paramount and Dess Dan. In terms of weight of diseased roots there was a row effect, and there were no significant differences among cultivars. This could be due to a nutrient and/or moisture gradient across the experiment.

4.2.3 Conductivity Assay

In order to correlate electrolyte loss with the virulence of S. sclerotiorum on infected carrot tissue, the conductivity of the carrot discs in the bathing solution was measured. The conductivity of each treatment was compared to the conductivity of the control, and a ratio was determined for each treatment. A higher ratio indicates more electrolyte loss due to S. sclerotiorum and thus more infectivity. There were significant differences among temperatures and among replications when all five cultivars were considered together (Table 6). At higher temperatures much more electrolyte loss occurred, indicating more tissue damage due to S. sclerotiorum. As the temperature increased from 2°C to 6°C there was no significant increase in the infectivity of S. sclerotiorum. There was significantly more infection at 8°C and 10°C and infectivity at 20°C was significantly greater than at 15°C. Comparisons were made between the conductivity ratio at each temperature for each cultivar separately. Above 10°C there was significant increase in electrolyte loss due to infection by S. sclerotiorum for all 5 cultivars (Table 6).

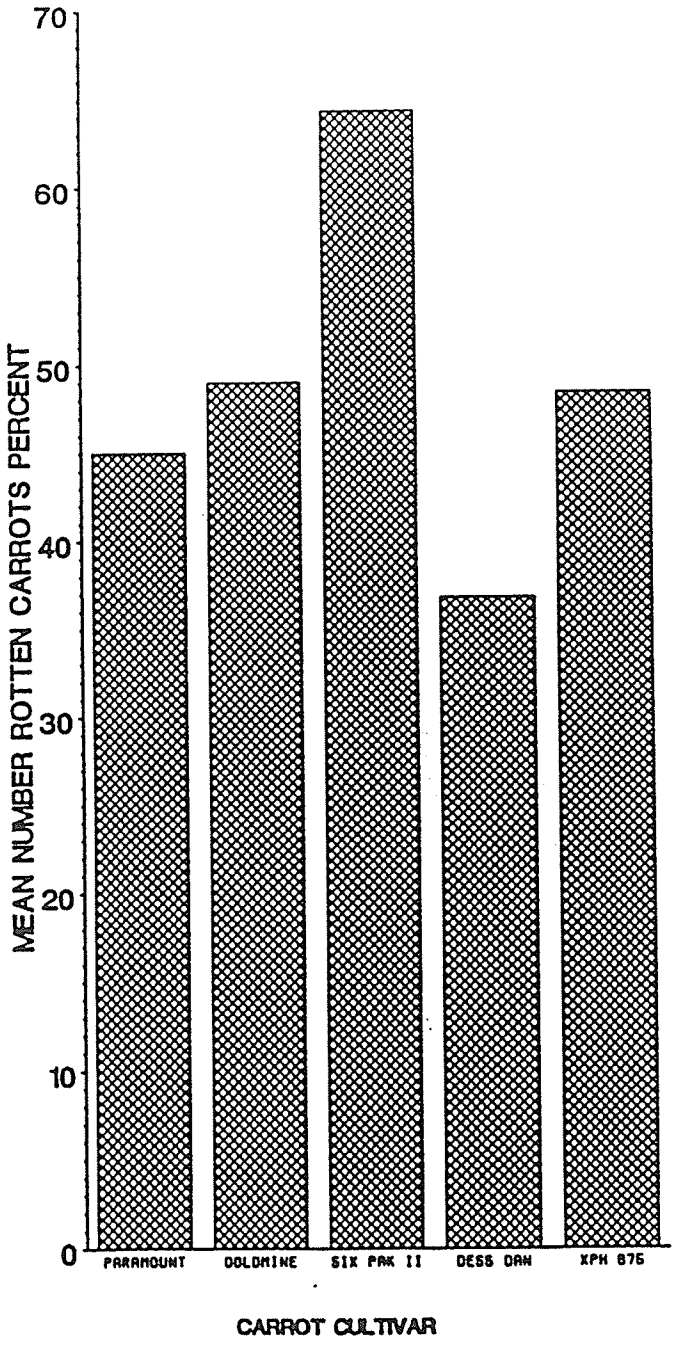

Figure 1. Amount of sclerotinia rot on five carrot cultivars after three months storage.

TABLE 6 Effect of temperature on electrolyte loss induced by S. sclerotiorum on five carrot cultivars

	Conductivity Ratio[1]					
			Cultivar			
Temp.°C	Dess Dan	Goldmine	Paramount	SixPak II	XPH875	Average
2	1.48[2]a[3]	1.44 a	1.56 a	1.53 a	1.46 a	1.49 a
4	1.46 a	1.69 a	1.63 a	1.70 a	1.61 a	1.62 a
6	1.69 a	1.83 a	1.77 a	1.87 a	1.74 a	1.78 a
8	2.69 a	2.82 a	2.70 a	2.90 a	2.73 a	2.77 b
10	3.07 ab	3.49 a	3.43 ab	3.48 a	3.02 a	3.30 b
15	5.30 b	6.02 b	5.12 b	5.68 b	5.78 b	5.58 c
20	7.93 c	7.38 b	7.43 c	8.23 c	7.36 b	7.67 d

[1] Ratio of electrolyte loss of carrot discs inoculated with fungus to control.

[2] Average of 4 to 6 replications.

[3] Means within column followed by same letters are not significantly different at the 0.05 level (Duncan's Multiple Range Test).

Results among experiments were extremely variable due to factors such as the individual carrot root, age and virulence of the mycelium, and temperature fluctuations. The conductivity ratios of the five cultivars were ranked from lowest to highest for each replication at each temperature, and an overall mean ranking value was determined for each cultivar (Table 7). A higher ranking (numerically) indicates more infectivity due to S. sclerotiorum in comparison to the other cultivars. There were significant differences among cultivars. Six Pak II and Goldmine were ranked significantly higher than XPH875 and Dess Dan. Thus in terms of electrolyte loss of inoculated carrot discs Six Pak II and Goldmine are significantly more susceptible to infection by S. sclerotiorum than XPH875 and Dess Dan.

S. sclerotiorum induces permeability changes in host cells due to the action of several cell wall and cell membrane modifying enzymes, oxalic acid and, perhaps, other as yet unknown factors (Newton et al., 1973). The water-soaking of host tissues generally associated with infection by S. sclerotiorum is a result of this process. The change in permeability of the host cells results in increased leakage of materials which can be utilized by the fungus for growth (Newton et al., 1973). Newton et al. (1973) related the ability to induce membrane permeability changes and consequent electrolyte loss of damaged tissue to the virulence of the fungus. Conversely, the ability of the host tissue to resist fungal invasion and retain its cell integrity can also be related to electrolyte loss after infection.

The conductivity assay demonstrated that there are differences in electrolyte loss among carrot cultivars, indicating variations in root

TABLE 7 The relative ranking of five cultivars in terms of electrolyte loss from carrot discs induced by S. sclerotiorum

Temp.°C	No. of Reps.	Rank[1] Cultivar: Six Pak II	Goldmine	Paramount	XPH875	Dess Dan
2	5	3.1	2.3	4.0	2.8	2.8
4	4	3.6	4.0	3.2	2.5	1.6
6	5	3.4	3.4	3.2	2.6	2.4
8	4	3.4	3.0	3.4	2.5	2.8
10	6	3.5	3.3	3.5	2.3	2.3
15	4	3.8	3.8	2.2	2.2	3.0
20	4	4.2	3.5	2.2	2.5	2.5
Average		3.55 a[2]	3.30 a	3.17 ab	2.50 b	2.48 b

[1] Comparative ranking of 5 cultivars (1 to 5); average of replications at each temperature.

[2] Means within row followed by same letter are not significantly different at the 0.05 level (Duncan's Multiple Range Test).

cell membrane integrity and permeability when exposed to S. sclerotiorum. The susceptibility of a plant to S. sclerotiorum is influenced by the susceptibility of the leaf tissue as well as the crop architecture such as canopy cover and uprightness of the foliage. The susceptibility of the root tissue to S. sclerotiorum mycelium is not necessarily indicative of the susceptibility of the rest of the plant. Also compared with intact plants there are differences in the permeability changes induced in excised plant parts and results must be interpreted with caution (Hancock 1972).

However results from the field experiments showed that Six Pak II had the highest number of rotten carrots per row, significantly more than Dess Dan and Goldmine, and after three months in storage Six Pak II again had significantly more diseased roots than Dess Dan and Paramount. Thus the permeability changes of the root tissues induced by S. sclerotiorum may play an important role in the relative susceptibility and resistance of Six Pak II and Dess Dan, or at least be indicative of cultivar differences for susceptibility to the fungus.

4.3 BIOLOGICAL CONTROL OF SCLEROTINIA ROT IN CARROTS

Since fungicide dips are not used on unwashed carrots in Manitoba, it would be useful to have some other method of controlling S. sclerotiorum on the harvested product. Wilson and Pusey (1985) reported some successful attempts at postharvest biocontrol using an introduced antagonist. Since T. flavus and C. minitans have been established as mycoparasites of S. sclerotiorum in Manitoba (Huang, 1977; McLaren, 1983) their potential as postharvest biocontrol agents of S. sclerotiorum on carrots was investigated.

The cardinal temperatures for growth and reproduction of the antagonists should correspond to those of the target pathogen (Cook and Baker, 1983). Thus the growth rates of all three fungi were compared over a range of temperatures.

4.3.1 Growth rates of the pathogen and mycoparasites at different temperatures

The growth rate (area mm^2/day) was determined for C. minitans and S. sclerotiorum at each temperature. The growth rate at each temperature was not significantly different between the two repetitions for each fungus, and the values from both experiments were averaged (Table 8). The growth rate of C. minitans was significantly lower than the growth rate of S. sclerotiorum at each temperature. A regression analysis was carried out on the pooled growth rate for each fungus, and R^2 values of 0.974 and 0.961 were obtained for S. sclerotiorum and C. minitans, respectively (Figure 2).

The growth rate (length mm/day) was determined for T. flavus (Table 9). Slight growth occurred at 6°C and no growth was observed at temperatures less than 6°C. A natural log transformation was performed on the data, and a good fit was obtained (R^2=0.96) (Figure 3). Direct comparisons of the growth rate of T. flavus cannot be made with the other two fungi due to the different measurements of growth rate obtained. However, from a comparison of the growth rates of S. sclerotiorum and T. flavus at 10°C (135 mm^2/day and 0.049 mm/day, respectively) it can be seen that growth of S. sclerotiorum is obviously much more vigorous.

TABLE 8 Effect of temperature on the growth rate of *C. minitans* and *S. sclerotiorum* on carrot agar

Temp °C	Growth rate (mm^2/day) *C. minitans* Exp 1	*C. minitans* Exp 2	*C. minitans* Average	*S. sclerotiorum* Exp 1	*S. sclerotiorum* Exp 2	*S. sclerotiorum* Average
2	8.68[1]	11.50[1]	10.90	195.14	109.35	152.24
4	25.28	-	25.28	228.98	264.56	246.77
6	70.07	84.19	77.13	700.12	632.42	666.27
8	110.82	119.86	115.34	1129.96	902.93	1016.44
10	189.31	177.83	183.57	1520.37	1194.78	1357.58

[1] Means of replications.

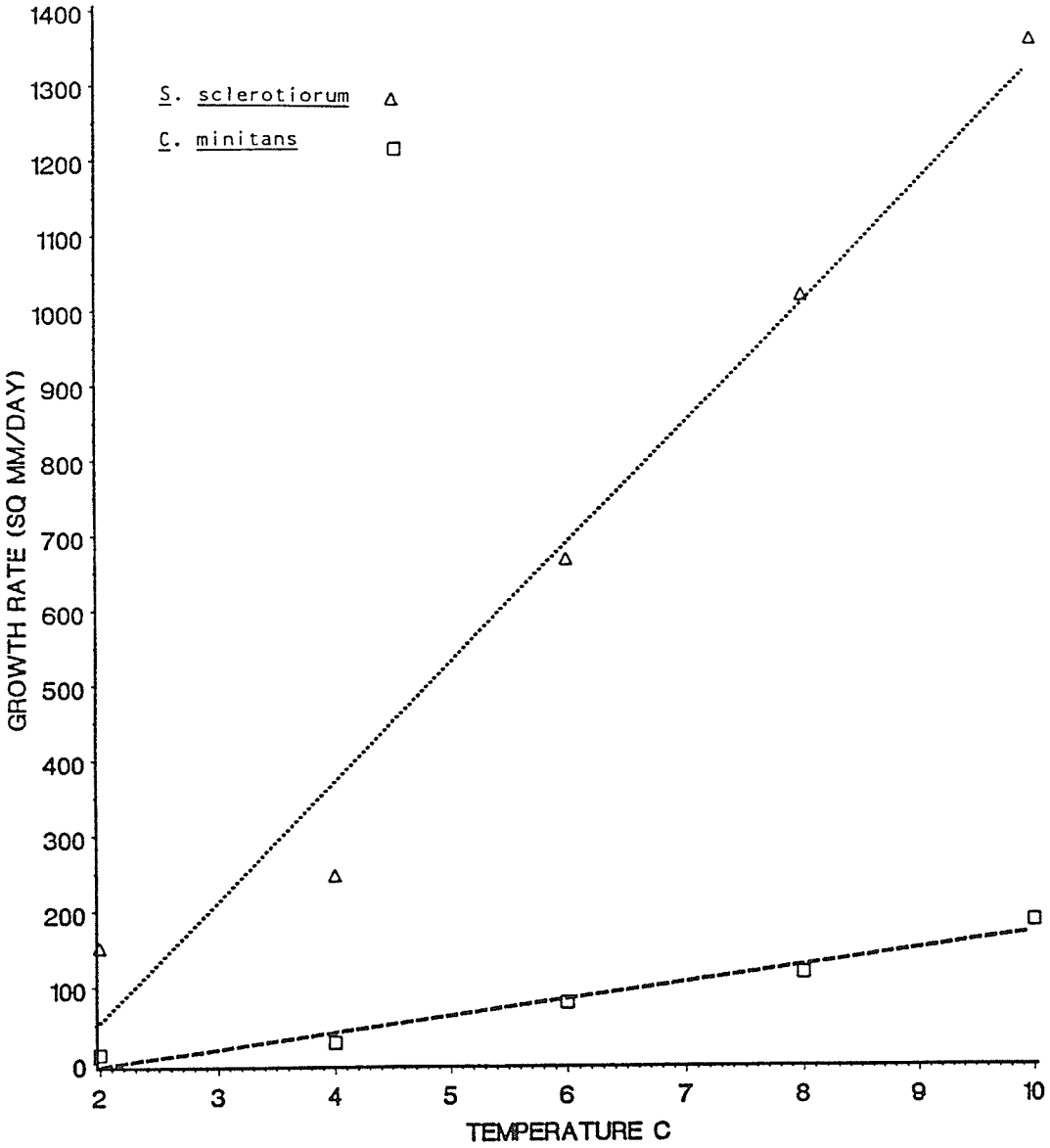

Figure 2: Growth rates of S. sclerotiorum and C. minitans on carrot agar.

TABLE 9 Effect of temperature on the growth rate of *T. flavus* on carrot agar

Temp°C	Growth rate (mm/day)		
	Expt 1	Expt 2	Average
4	0	0	0
6	0.034	0.017	0.025
8	0.049	0.047	0.048
10	0.076	0.061	0.049
15	0.87	0.81	0.84
20	1.68	1.91	1.80

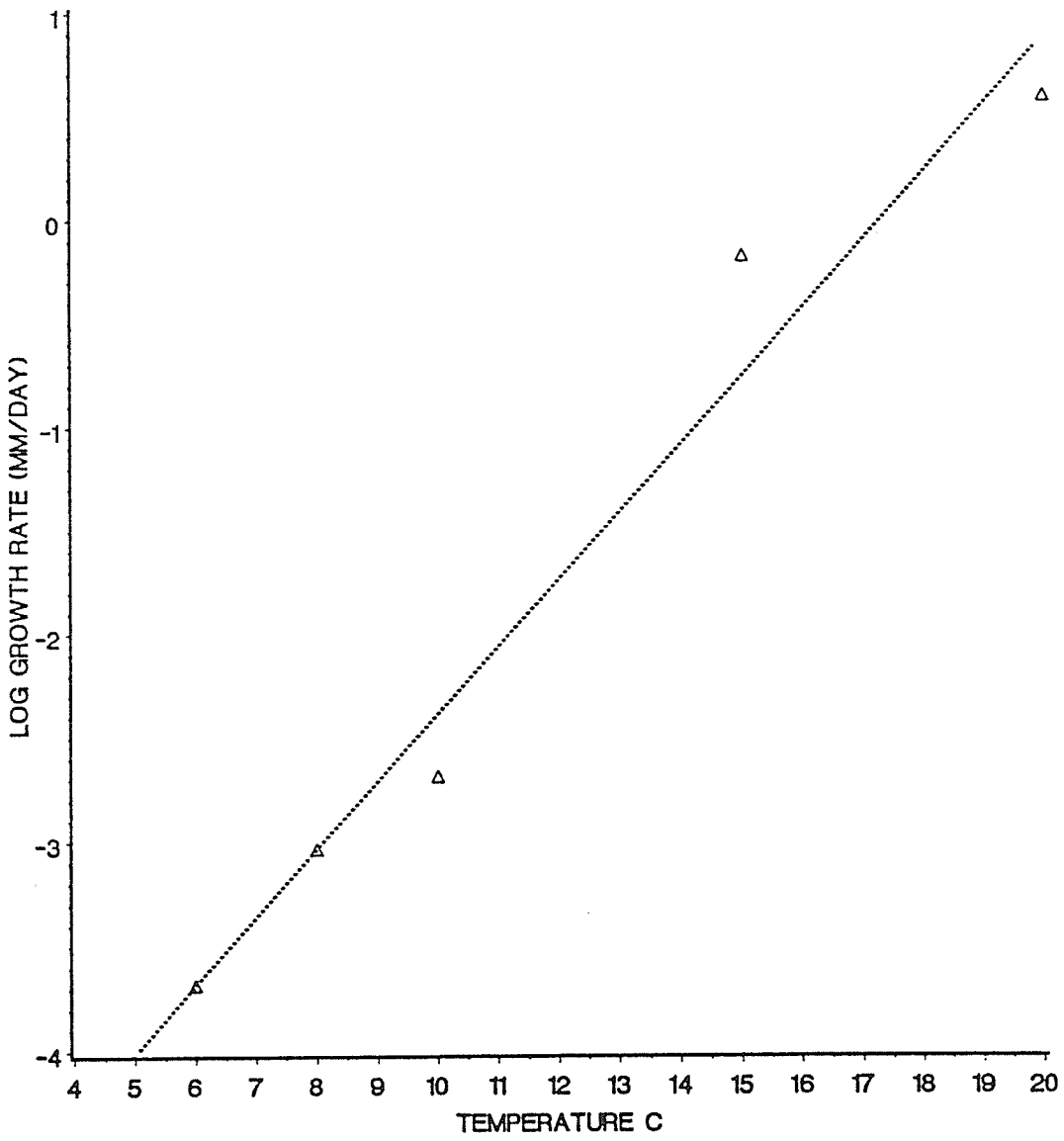

Figure 3. Log growth rate of T. flavus on carrot agar.

4.3.2 Effect of mycoparasites on the infectivity of S. sclerotiorum on carrot discs

Overall the conductivity ratio increased in the order of mycoparasite, mycoparasite plus S. sclerotiorum, and S. sclerotiorum. Nearly always the conductivity of the solution containing carrot discs incubated with the mycoparasite alone was less than the conductivity of the control. It is possible that placement of fungi on carrot discs caused the production of antifungal compounds, thus altering the conductivity of the solution.

The data were analyzed to compare the overall effects of mycoparasites on the infectivity of S. sclerotiorum in terms of electrolyte loss from inoculated carrot discs. Results from all the experiments combined (both mycoparasites at 10, 15 and 20°C) indicate significant differences among electrolyte loss from carrots inoculated with S. sclerotiorum compared with the electrolyte loss from carrots inoculated with either mycoparasite (Table 10). When each mycoparasite was considered separately (average of results from experiments at 10, 15 and 20°C) S. sclerotiorum caused significantly more electrolyte loss than C. minitans alone; there were no significant differences among treatments when T. flavus was added. The incubation temperature did not significantly affect the results among treatments (Table 10).

The incubation period of the mycoparasite alone ranged from 7 to 15 days for C. minitans, and 8 to 27 days for T. flavus. The germination of these fungi, especially T. flavus was slower than the germination of S. sclerotiorum spores, and it was important that the colony was established on the carrot disc before the pathogen was added. However, dete-

TABLE 10 Effect of mycoparasites (*C. minitans* and *T. flavus*) on electrolyte loss from carrot discs induced by *S. sclerotiorum*

Temperature °C	Mycoparasite	Conductivity ratio[1]		
		Scler.	Myco. + Scler.	Myco.
All[2]	Both	1.16 a[3]	1.05 ab	0.98 b
All	C. minitans	1.12 a	1.03 ab	0.98 b
All	T. flavus	1.17 a	1.15 a	1.04 a
10	Both	1.09 a	1.08 a	1.02 a
15	Both	1.00 a	0.97 a	0.97 a
20	Both	1.21 a	1.06 a	0.98 a

[1] Ratio of electrolyte loss of carrot disc inoculated with fungus to control.

[2] Data combined from results at 10, 15 and 20°C.

[3] Means within rows followed by same letter are not significantly different at the 0.05 level (Duncan's Multiple Range Test).

rioration of the carrot disc by other organisms during the incubation period was a problem, and it is possible that this incubation period was not long enough. Colonies of C. minitans were visible on the filter paper around the edge of the carrot discs at the time of the assay, but no evidence of T. flavus was observed. Preliminary work was carried out using drops of spore suspension on very thin pieces of carrot (1 to 3 cells) and observing the spores under a microscope (40x). Both mycoparasites could germinate within one or two days at 20°C and growth of individual hyphae was observed for up to 6 days later. Growth of S. sclerotiorum was very rapid and within two days the carrot tissue was degraded.

When plugs of S. sclerotiorum from the edge of a growing colony were placed on carrot discs (as in the cultivar/conductivity assay) the discs were soft and mushy after 22 hrs at 20°C. However after 8 days of incubation with S. sclerotiorum spores on the carrot disc, the discs were firm, and there was no visible evidence of tissue degradation, although the conductivity ratio of the S. sclerotiorum treated discs was always greater than 1.00, indicating some electrolyte loss and hence some infectivity of the fungus. Germination and growth was less vigorous when the spores were in contact with carrot tissue and PDA than with PDA alone. It is possible that antifungal compounds which have been observed in carrot tissue (Davies et al., 1981; Lewis and Garrod, 1983) affect the growth of the germinating S. sclerotiorum spores; fewer enzymes would be produced causing less change in membrane permeability and hence less electrolyte loss. A mycelial plug taken from the edge of an established growing colony on PDA may not be affected by the antifungal

compounds, and degradation enzymes may be already present in the PDA plug, allowing cell wall modification of the carrot tissue to occur immediately.

Overall there was significantly more electrolyte loss from carrot discs treated with S. sclerotiorum than with either T. flavus or C. minitans, indicating some infectivity from germinating S. sclerotiorum spores. Conductivity from discs treated with C. minitans and the pathogen was less than discs treated with S. sclerotiorum, and thus there was some evidence of biological control.

Chapter V

GENERAL DISCUSSION

Results and observations made in this thesis are an attempt to describe some of the aspects involved in the infection and control of S. sclerotiorum on carrot. Results from greenhouse experiments indicate that direct mycelial infection of the carrot root from sclerotia in the soil is unlikely. Primary infection probably results from mycelial colonization of leaf and stem tissue in contact with sclerotia in the soil. There are few reports of this infection process in other crops. The mycelial infection of sunflowers by S. sclerotiorum originates in the root and hypocotyl tissue (Huang and Dueck, 1980), and Letham et al. (1976) reported basal infection of tomato plants due to mycelial germination of sclerotia, but they did not indicate whether leaf tissue in contact with the soil was important. Most of the crops susceptible to S. sclerotiorum are infected via ascospores (eg. stem rot of rapeseed, cottony rot of lemons, wilt of tomatoes, white mold of beans, drop of lettuce, and head rot of sunflower) (Purdy, 1979; Newton and Sequeira, 1972; Abawi and Grogan, 1975).

The importance of ascosporic infection in sclerotinia disease in carrots in Manitoba was not established. A more appropriate method for determining the importance of ascospores in the disease cycle would have been to place conditioned sclerotia in the carrot plots to allow apothecial development and spore release to occur naturally at the most oppor-

tune time for infection. As well, a study of the association between the natural occurrence of ascospores (including those originating outside the host field) on leaves and development of sclerotinia rot would be important in determining whether ascosporic infection does play a role in the disease cycle.

Greenhouse experiments demonstrated the importance of free water and high relative humidity (RH) for ascospore infection, and it is possible that a suitable microclimate exists in carrot fields simultaneously with ascospore production. It is also possible that the mode of infection is dependent on the moisture conditions, similar to the relationship between rainfall and whether sclerotinia wilt or head rot develops in sunflowers (McLaren, 1983; Huang and Hoes, 1980).

The fact that direct mycelial infection of the root is unlikely suggests that only sclerotia near the soil surface are important in the disease cycle. The distance in the soil through which mycelium from germinating sclerotia can grow is likely dependent on the isolate and soil microclimate. Williams and Western (1965) observed that growth of S. sclerotiorum in unsterilized soil was restricted to 5 mm from the parent sclerotium, and Newton and Sequeira (1972) stated that mycelium from germinating sclerotia was unlikely to cause infection of plants located more than 2 cm from the sclerotia.

Air-drying the sclerotia near the soil surface has been proposed as a method of control (Adams, 1975). Adams (1987) suggested that in areas with low RH and rainfall, a field could be prepared for planting in the off season, and after waiting an interval of 1-2 months for the sclero-

tia in the upper 2 cm to die, the crop could then be planted. However, drying has also been found to stimulate mycelial germination (Smith, 1972). Cultural practices to prevent contact between foliage and the soil are possible control methods to prevent mycelial infection. Chemically burning off the foliage would not be feasible since the carrot tops are required for the mechanical harvesting operation.

There were definite differences among cultivars in terms of disease levels after storage, in agreement with data presented by Lauritzen (1932). However, the experiment should be repeated in different locations over a period of several growing seasons to determine if site and cultural practices are influential.

The conductivity assay demonstrated cultivar differences in susceptibility of the carrot root tissue to S. sclerotiorum infection, in terms of permeability changes measured by the release of electrolytes from infected tissue. The permeability changes result from the action of cell wall and cell membrane modifying enzymes (Newton et al., 1973). The nutrient levels of the substrate have been found to have an important effect on the germination of spores and growth of S. sclerotiorum mycelium (Purdy, 1958; Geary, 1978; Abawi et al, 1975). In a study comparing the storability of carrots at 98-100% RH and 90-95% RH van den Berg and Lentz (1978) discovered that more pectolytic enzymes were produced by S. sclerotiorum on carrot roots stored at the lower humidity level due to a lesser availability of nutrients. The differences in electrolyte loss among cultivars could be due to different levels of nutrients in the root tissue, possibly affecting enzyme production by the pathogen.

The cultivar (Six Pak II) with the larger number of diseased roots both in the field and after three months storage also had the most electrolyte loss in the conductivity assay indicating more susceptibility to S. sclerotiorum compared with the other four cultivars. As well, the cultivar (Dess Dan) which had the least amount of disease in the field and in storage had the least amount of electrolyte loss. The number of diseased roots in the field may be related to crop architecture, which influences the microclimate and conditions for germination and growth of S. sclerotiorum. This in turn would affect the original number of diseased roots brought into storage. There may be, however, a correlation between the susceptibility of the root tissue to S. sclerotiorum and the spread of mycelium from one root to another in storage. Thus the conductivity assay may provide a simple measurement to determine the relative susceptibilities of root tissue to S. sclerotiorum infection among cultivars.

The growth rate experiments and the conductivity assay demonstrated that S. sclerotiorum can grow and infect carrot tissue at low temperatures. Even at the optimum storage temperature of 0°C (International Standard ISO, 1981), S. sclerotiorum inoculum brought into storage is able to grow and infect carrot roots. However, the growth rate is very slow at low temperatures, and rapid removal of field heat and maintenance of the optimum storage temperature are extremely important in controlling the spread of S. sclerotiorum in storage.

The growth rates of C. minitans and T. flavus are such that these mycoparasites are not well enough adapted to low temperatures to control S. sclerotiorum in storage. However, several factors indicate post har-

vest biocontrol as being a potential area to explore (Wilson and Pusey, 1985). First, one of the main reasons for the failure of biocontrol procedures has been the inability to control environmental conditions. Under storage conditions for harvested food, exact environmental conditions can be established and maintained. Second, targeting biocontrol agents to the effective site is often difficult. Harvested food does not present that problem because the areas for application are much more limited than those on whole plants. Third, elaborate control procedures that may not be economically feasible under field conditions are cost-effective for harvested food when the cumulative costs of soil preparation, planting, fertilizing, watering, pest control, and harvesting are considered (Wilson and Pusey, 1985).

Biocontrol of mycelium already established in the crown of the root would be difficult, but mycelium growing on poorly trimmed tops and on foliar debris could possibly be destroyed by antagonists. It would be necessary to use an antagonist well adapted to low temperatures in order to compete with the hardy S. sclerotiorum.

There is also the potential for using mycoparasites to destroy sclerotia in the field. The introduction of C. minitans and T. flavus into S. sclerotiorum-infested soil at seeding time decreased sclerotinia wilt of sunflower and reduced yield losses (Huang, 1980b; McLaren, 1983) due mainly to the control of the primary inoculum or sclerotia. C. minitans was ineffective in controlling S. sclerotiorum in an actively growing state and it failed to reduce plant-to-plant spread of the pathogen. The spread of S. sclerotiorum in infected tissue may be faster than the ability of the mycoparasite to parasitize it (Huang, 1980b). Thus the

slow growth rates of C. minitans and T. flavus compared with S. sclerotiorum render it unlikely that they would be effective in parasitizing growing mycelium of the pathogen in carrot foliage. However, the possibility remains of using C. minitans and/or T. flavus to destroy sclerotia in the soil, thus reducing the number of primary infection propagules. Perhaps other faster growing antagonists would be more effective in controlling the actively growing mycelium, thereby reducing the amount of inoculum brought into storage.

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