Biocontrol of Sclerotinia diseases (*Sclerotinia sclerotiorum*) of sunflower and bean by *Talaromyces flavus* and *Coniothyrium minitans*.

by

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BIOCONTROL OF SCLEROTINIA DISEASES (*Sclerotinia sclerotiorum*) OF SUNFLOWER AND BEAN BY *Talaromyces flavus* and *Coniothyrium minitans*

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DEBRA LEIGH McLAREN

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

DOCTOR OF PHILOSOPHY

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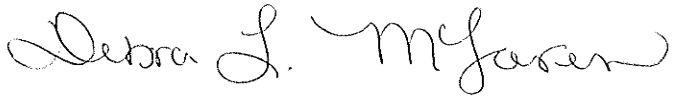

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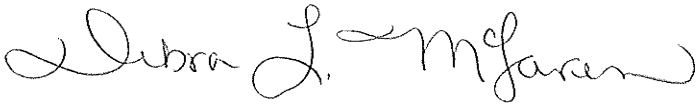

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ABSTRACT

McLaren, Debra Leigh. Ph.D. The University of Manitoba. 1989. Biocontrol of Sclerotinia diseases (Sclerotinia sclerotiorum) of sunflower and bean by Talaromyces flavus and Coniothyrium minitans.

The effect of Talaromyces flavus and Coniothyrium minitans on Sclerotinia sclerotiorum, causal agent of sclerotinia wilt of sunflower and white mold of bean was examined. Laboratory investigations, using dual culture techniques, indicate that T. flavus is destructive to hyphae and sclerotia of S. sclerotiorum. Hyphae of T. flavus grew toward and coiled around the host hyphal cells. The coiling effect intensified as the hyphae of T. flavus branched repeatedly on the host surface. Tips of the hyphal branches often invaded the host by direct penetration of the cell wall without formation of appressoria. Infection of host cells by T. flavus resulted in granulation of the cytoplasm and collapse of cell walls. Examination, using transmission electron microscopy, of sclerotia inoculated with T. flavus indicated that T. flavus is also pathogenic to sclerotia of S. sclerotiorum. Sclerotia colonized by the hyperparasite at 3,7 and 12 days after inoculation revealed that hyphae of T. flavus penetrated the cell walls directly. Etching of the cell walls at the penetration site was evident which suggests that wall-lysing enzymes may be involved in the process of infection. Hyphae of T. flavus grew both inter- and intracellularly throughout

the rind, cortical and medullary tissues. Ramification of the hyperparasite in the sclerotium resulted in destruction and collapse of sclerotial tissues.

A four-year field study (1983-86) at Lethbridge and a three-year study (1983-85) at Winnipeg indicate that the incidence of sclerotinia wilt of sunflower and yield losses are reduced significantly with the application of T. flavus and C. minitans. In 1983 at Lethbridge, the percentage of disease in the control plots was 54.9% compared with 0 and 9.8% in the plots treated with T. flavus and C. minitans, respectively. This represents a reduction in disease by 100 and 82% in the T. flavus and C. minitans treated plots, respectively. At the Winnipeg site, the percentage of disease in plots treated with T. flavus and C. minitans was 14.4 and 3.2%, respectively, compared with 65.2% in the control. In 1984, at both locations, similar results were obtained. In 1985, application of sclerotia, but no hyperparasites, to plots treated with both sclerotia and hyperparasites in the previous two years resulted in low levels of disease in the T. flavus and C. minitans -treated plots (Lethbridge) and in the C. minitans -treated plots (Winnipeg). In the control plots (sclerotia only) at Lethbridge, the percentage of disease was 38.6% compared with 4.3 and 5.1% in the T. flavus and C. minitans -treated plots, respectively. This represents a reduction in disease by 89 and 87% in the plots treated with T. flavus and C. minitans, respectively. Similar results occurred at Winnipeg in the C. minitans -treated plots only. The percentage of disease in the control was 24.4% compared with 21.1 and 1.2% in plots treated with T. flavus and

C. minitans, respectively. Talaromyces flavus was not effective in reducing significantly the incidence of wilt in the third year of the study. The one year carry-over of control did not continue at Lethbridge in 1986 with 54.1% disease in the control compared with 51.0 and 41.7% disease in the T. flavus and C. minitans -treated plots, respectively.

Seed yield of sunflower was increased with the application of hyperparasites during 1984-85 and 1984 at the Lethbridge and Winnipeg sites, respectively. At Lethbridge (1984), seed yields were 1433.0, 1326.8 and 1360.0 kg/ha in the plots treated with T. flavus, C. minitans and a combination of both hyperparasites, respectively compared with 952.7 kg/ha in the control. At Winnipeg, similar results were obtained with a yield of 664.6 kg/ha in the control compared with 1464.8 and 1862.0 kg/ha in the T. flavus and C. minitans -treated plots, respectively. In 1985 (Lethbridge), the carry-over effect of biocontrol was also reflected in the yield data with significantly greater yields in the hyperparasite-treated plots compared with the control. In 1986 at Lethbridge, the yield differences between the control and the hyperparasite-treated plots were insignificant as the carry-over effect of disease control was no longer evident.

A field study in 1984 and 1985 at Lethbridge showed that T. flavus and C. minitans reduced the carpogenic germination of sclerotia of S. sclerotiorum in a bean field. Coniothyrium minitans was more effective than T. flavus in both years. In 1984, the number of apothecia produced in the T. flavus and C. minitans -treated plots was

125.1 and 21.4, respectively compared with 188.5 in the control. This represents a reduction in apothecia production by 33.6 and 88.6% in the T. flavus and C. minitans -treated plots, respectively. In 1984, 325.4 and 25.6 apothecia were produced in the T. flavus and C. minitans -treated plots, respectively compared with 344.3 in the control. This represents a reduction in apothecia production by 5.5 and 92.6% in the plots treated with T. flavus and C. minitans, respectively. In 1984 and 1985, apothecia production was based on 1200 and 300 sclerotia buried per plot, respectively.

In both sunflower and bean fields, the application of T. flavus and C. minitans affected the survival of sclerotia of S. sclerotiorum. Fewer sclerotia were recovered from the hyperparasite-treated plots and viability of the recovered sclerotia was reduced. The percentage of recovered sclerotia infected by T. flavus or C. minitans was low but concentrated in the hyperparasite-treated plots.

In summary, the laboratory studies on the interaction between T. flavus and S. sclerotiorum indicate that T. flavus is a hyperparasite capable of destroying both hyphae and sclerotia of S. sclerotiorum. In the field, T. flavus and C. minitans are effective hyperparasites capable of reducing disease and yield losses due to sclerotinia wilt of sunflower and reducing sclerotial germination and apothecial production in field bean.

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REVIEW OF LITERATURE

1.1 THE HOSTS

1.1.1 Sunflower

The sunflower (Helianthus annuus L.) originated in temperate North America and was used by American Indians as a source of food, oil and dye (Heiser, 1978). Early in the nineteenth century, this crop was used as a source of food and edible oil in Russia. Cultivation of the sunflower expanded rapidly in Russia and breeding commenced. In North America, the present cultivated sunflower evolved from Russian material introduced into the United States during the latter part of the nineteenth century (Semelczi-Kovacs, 1975). Today, the major sunflower producing areas in the world include the USSR, Argentina, the United States and eastern Europe (USDA, 1981a, 1985).

In Canada, commercial production of the sunflower began in 1943 in southern Manitoba (Sackston, 1981). Canadian sunflower production is still largely confined to Manitoba (Statistics Canada, 1982b). Two distinct types of sunflower are produced, the oilseed and confectionary types. In Canada, most cultivars grown are of the oilseed type. On a worldwide basis, sunflowers rank fourth to soybean, palm and rapeseed as a source of edible oil (Agriculture Canada, 1987). Sunflower production increased rapidly in Canada from 1976-81 (Statistics Canada, 1982a) resulting in increased attention on sunflower diseases.

There are more than 30 known diseases of sunflower. Downy mildew [Plasmopara halstedii (Farl.) Berl & de Toni], rust (Puccinia helianthi Schw.), Sclerotinia wilt or head rot [Sclerotinia sclerotiorum (Lib.) de Bary] and Verticillium wilt (Verticillium dahliae Kleb.) are the major diseases encountered in North America (Zimmer and Hoes, 1978). These pathogens have caused significant yield losses in Canada and the United States in the past 30 years. Sclerotinia wilt has become one of the limiting factors of sunflower production in western Canada (Huang, 1979).

1.1.2 Bean

There are four main cultivated species of Phaseolus: (1) P. vulgaris L., the navy, haricot, common (field), French or snap bean, (2) P. lunatus L., the Sieva, Lima, butter or madagascar bean, (3) P. coccineus L., the scarlet runner bean and (4) P. acutifolius A. Gray, the tepary bean (Evans, 1980). All four cultivated species originated in Latin America. The two most economically important species, P. vulgaris and P. lunatus spread widely to Europe, England and the United States (Evans, 1980). In the late nineteenth century, many introductions were made into the eastern United States from Europe. Bean cultivation was also evident through California and into the western states. Today, the major dry bean producing countries in the world include India, Brazil, the United States and Mexico (USDA, 1981b).

In Canada, field or dry beans are an important commercial crop with a major portion being produced in Ontario (Martens et al., 1984). In

1985, 36,440 ha were seeded to dry white beans in Ontario (Ontario Ministry of Agriculture and Food, 1985). The area seeded to dry beans in Manitoba and Alberta in 1985 was 4860 ha (Manitoba Agriculture, 1985) and 3060 ha (Alberta Agriculture, 1985), respectively. At present, common blight [Xanthomonas pv. phaseoli (E.F.Sm.) Dows], Halo blight [Pseudomonas syringae pv. phaseolicola (Burk.) Dows], white mold (Sclerotinia sclerotiorum) and root rot (caused by Rhizoctonia solani Kuehn, Fusarium solani f. sp. phaseoli (Burk.) Snyder & Hansen, Pythium spp. and Thielaviopsis basicola (Berk and Br.) are the important diseases in the main bean producing areas of Canada (Martens et al., 1984). Quality of seed as well as yields of marketable beans are reduced annually by such diseases. White mold has become one of the major causes of crop loss in the bean production areas of the U.S.A. and Canada. In the irrigated regions of western Nebraska where Great Northern or Pinto dry edible beans are commonly grown, white mold of bean is the most important production problem (Steadman et al., 1976; Kerr et al., 1978). In Canada, white mold has been found throughout the country (Ginns, 1986) and has been reported to cause total crop destruction (Wallen and Sutton, 1967).

1.2 THE PATHOGEN SCLEROTINIA SCLEROTIORUM

1.2.1 Introduction

The fungus Sclerotinia sclerotiorum (Lib.) de Bary belongs to the Class Ascomycetes, Subclass Hymenoascomycetidae, Family Sclerotiniaceae (Alexopoulos and Mims, 1979). It has a worldwide distribution and attacks more than 360 species of plants in 64 families (Purdy, 1979). These include vegetables, ornamental crops,

trees and shrubs, field and forage crops and numerous herbaceous weeds (Adams et al., 1974; Purdy, 1979; Willetts and Wong, 1980). The pathogen is destructive to crops in the field and is also damaging under greenhouse, storage and market conditions. Millions of dollars are lost annually through loss of yield, loss in grade and loss of production (Purdy, 1979).

Besides S. sclerotiorum there are two other species, belonging to the genus Sclerotinia, that are of economic importance. These are S. minor Jagger and S. trifoliorum Erikss. (Kohn, 1979). Sclerotinia minor has a broad host range whereas that of S. trifoliorum is limited to forage legumes (Kohn, 1979). Both pathogens are destructive and the majority of the diseases that they cause have not been controlled economically and consistently (Steadman, 1979). Information concerning the history (Purdy, 1979), taxonomy (Kohn, 1979), cytology (Le Tourneau, 1979), morphology (Le Tourneau, 1979), biology (Grogan, 1979; Willetts and Wong, 1980), physiology (Le Tourneau, 1979; Lumsden, 1979), ecology (Adams and Ayers, 1979), epidemiology (Abawi and Grogan, 1979) and control (Steadman, 1979) of these two Sclerotinia species is available in the literature.

1.2.2 Disease cycle

1.2.2.1 Sunflower

Sclerotia are the resting or overwintering structures of S. sclerotiorum (Coley-Smith and Cooke, 1971; Willetts and Wong, 1980). With the sclerotinia disease of sunflower, the disease cycle begins with germination of the sclerotium. This can be either myceliogenic

(Huang and Dueck, 1980; Huang, 1985) or carpogenic (Williams and Western, 1965a; Willetts and Wong, 1980). Myceliogenic germination of the hyphal type occurs by the emergence of individual hyphae through the rind of the sclerotium (Adams and Tate, 1976; Huang and Dueck, 1980; Willetts and Wong, 1980). Carpogenic germination involves the development of stipes and apothecia. Ascospores are formed within an apothecium and are the only spores produced by S. sclerotiorum (Willetts and Wong, 1980). While carpogenic germination of sclerotia may result in the development of head rot and stalk rot of sunflower, myceliogenic germination results in the production of wilt symptoms due to root and basal stem infections (Huang and Dueck, 1980; Huang and Hoes, 1980).

For infection to occur following germination, the sclerotia must be close to host plants. Williams and Western (1965a) observed that mycelial growth of S. sclerotiorum in unsterilized soil is restricted, with the hyphae never extending more than 5 mm from the parent sclerotium. Newton and Sequeira (1972) found that infection of plants via mycelium from germinating sclerotia is unlikely to occur if sclerotia are more than 2 cm from the plants. Huang and Hoes (1980) stated that the incidence of sclerotinia wilt of sunflower is decreased significantly if sclerotia are buried 4 cm above the seed and 5 cm or 15 cm below the seed as compared to sclerotia applied adjacent to the seed. Similar results were obtained when sclerotia were buried at seed level but at a distance of 10 cm or more from the seed.

Following myceliogenic germination of sclerotia, infection occurs at or below the soil line where the taproot-hypocotyl axis is the primary site of infection. Huang and Dueck (1980) found that mycelia from germinating sclerotia are able to infect unwounded sunflower roots and hypocotyls in the absence of exogenous nutrients. Adams and Tate (1976) similarly observed direct infection of lettuce seedlings by germinating sclerotia of S. minor without the addition of a food base. Others have suggested a need for an exogenous source of energy for mycelial infection of a host plant (Tanrikut and Vaughan, 1951; Purdy, 1958; Abawi and Grogan, 1975, 1979).

Once infection has occurred, enzymatic processes affecting the middle lamella result in rapid disorganization of the plant tissues (Hancock, 1966; Lumsden, 1979; Purdy, 1979). Symptoms of such infected plants generally occur during the flowering and seed development stage but may also occur during the seedling stage (Putt, 1958; Huang and Hoes, 1980). Infected plants show wilting of the leaves which often occurs on only one side of the plant. Infection can cause sudden wilting. Severely diseased plants have a characteristic lesion at the base of the stem, on the taproot and on some fibrous roots. Such lesions, commonly brown and water-soaked in appearance, may extend from the taproot along the hypocotyl and as much as 50 cm up the stem (Jones, 1923; Young and Morris, 1927; Huang and Dueck, 1980; Huang and Hoes, 1980). If environmental conditions are favorable, white mycelium interspersed with sclerotia may develop on the surface of the lesion (Bisby, 1921; Young and Morris, 1927; Huang and Hoes, 1980). Formation of sclerotia can occur at the stem

base, on the fibrous roots and on the taproot (Bisby, 1921; Jones, 1923; Young and Morris, 1927; Hoes and Huang, 1975; Huang, 1977; Zimmer and Hoes, 1978; Huang and Hoes, 1980) as well as inside the pith cavity of the diseased stem (Bisby, 1921; Jones, 1923; Hoes and Huang, 1975; Huang 1977; Zimmer and Hoes, 1978) and taproot (Jones, 1923; Hoes and Huang, 1975; Huang, 1977). The first signs of disease are wilted plants scattered throughout the field (Bisby, 1921; Jones, 1923; Huang and Hoes, 1980). As the disease develops, neighboring plants become infected due to spread of the pathogen by root contact (Huang and Dueck, 1980; Huang and Hoes, 1980; Willetts and Wong, 1980).

The mode of sclerotial germination largely influences the manner in which hosts become infected. Head and stalk rot are initiated by airborne ascospores which are produced via carpogenic germination of sclerotia at or near the soil surface (Zimmer and Hoes, 1978; Huang and Hoes, 1980). A conspicuous white mycelial mat is produced in the head. Large black sclerotia may form around the seeds, while others develop below the seed layer. The entire head may be destroyed leaving only the vascular bundles and fibres which give the head a shredded, brush-like appearance. Stalk infections result in development of lesions similar to those produced at the base of the stem. Infected stem areas may disintegrate to leave only shredded, straw-colored fibrous tissue. Sclerotia produced from basal stem, stalk and head rot serve as the primary inoculum for sclerotinia disease from year to year (Huang, 1979, 1980b).

Information on germination behavior of sclerotia of S. sclerotiorum is still controversial. Abawi and Grogan (1979) proposed that S. sclerotiorum functioned primarily by producing apothecia and that myceliogenic germination contributed very little, if at all, to the development of epidemics. In contrast to this, Huang and Dueck (1980) reported that for sclerotinia wilt of sunflower, the sclerotia of S. sclerotiorum initiate infection chiefly via the production of mycelia. Myceliogenic germination is crucial to the development of sclerotinia wilt, which limits the production of sunflowers in Manitoba.

The incidence of head rot as compared to wilt is dependent on whether sclerotia germinate carpogenically or myceliogenically. There are many factors affecting the development of apothecia from sclerotia of S. sclerotiorum and several of these have been investigated (Coley-Smith and Cooke, 1971; Willetts and Wong, 1980). Moisture is an important factor affecting the germination of sclerotia. In studies on effects of moisture levels on apothecial production, Grogan and Abawi (1975) found that continous moisture was required for sclerotia to produce apothecia. Teo and Morrall (1985a, 1985b) studied the effect of different matric potentials on carpogenic germination of sclerotia and found that the largest number of apothecia were produced near field capacity. Abawi and Grogan (1975) concluded that moisture was the most important factor in the development of white mold epidemics in beans under New York conditions. Morrall (1977) also found moisture to be critical in the development of apothecia, and suggested that even in semi-arid regions such as western Canada, ascospores could be an important source of

inoculum if moist soil conditions persist for a minimum of two to three weeks. In some growing seasons, apothecia have become an important source of inoculum due to the development of these conditions. Huang (1979) reported that a severe outbreak of sclerotinia head rot of sunflower occurred in 1977 resulting in poor seed yield and reduced seed quality. This outbreak was attributed to rainfall occurring at the flowering and seed development stages of sunflower growth. In 1982, ascospore infection of sunflower, causing stalk and head rot, was pronounced (Hoes and Huang - pers. comm.). Heavy rains prior to sunflower bloom induced development of apothecia and airborne inoculum.

In western Canada, both sclerotinia head rot and wilt occur, but wilt is usually more prevalent than head rot (Hoes and Huang, 1976). Huang and Dueck (1980) suggested that environmental conditions in western Canada are conducive to myceliogenic germination of sclerotia which results in the predominance of sclerotinia wilt of sunflower. Wilt symptoms are prominent when conditions are dry while head and stalk rot are common when high moisture conditions prevail during late July, August and September (Huang and Hoes, 1980). Besides an effect of environmental conditions on sclerotial germination, Huang and Dueck (1980) also proposed that the host crop might influence the mode of germination. In their study, they used isolates of S. sclerotiorum from two host species, sunflower and rapeseed, and found that both had the ability to germinate myceliogenically. However, infection of rapeseed plants did not occur readily by mycelia from germinating sclerotia. In the field, sclerotinia wilt of sunflower resulted from

myceliogenic germination whereas sclerotinia stem blight of rapeseed resulted from carpogenic germination of sclerotia.

1.2.2.2 Bean

Sclerotia formed in infected bean tissue are the primary survival structures of S. sclerotiorum (Starr et al., 1953; Natti, 1971; Abawi and Grogan, 1975; Cook et al., 1975; Martens et al., 1984) and are capable of producing apothecia which are an important source of inoculum for white mold from year to year (Abawi and Grogan, 1975; Cook et al., 1975; Steadman, 1983; Martens et al., 1984; Boland and Hall, 1987). With white mold of bean, the life cycle begins with carpogenic germination of sclerotia to produce ascospores. Carpogenic germination generally begins once the soil surface is covered by the plant canopy (Steadman, 1983). Canopy coverage creates a favorable microclimate for apothecia development with temperatures seldom above 25 C and soil moisture retained at the surface between irrigations or rains (Steadman, 1983). Although long range spore dispersal is hampered by the canopy, local infection sites are often saturated, creating the possibility of high local infection. Severe white mold epidemics can result if air temperatures in the range of 15-25 C, plant surface wetness periods of greater than 39 h, a closed canopy containing petals, and apothecia occur in the bean crop at about the same time (Boland and Hall, 1987). According to Steadman (1983) the sexual stage can be produced within a few weeks if cool temperatures (4-20 C) and sustained adequate moisture occur. In a study conducted by Boland and Hall (1987) soil temperatures, determined over a two week period prior to the occurrence of apothecia, were in the range of

15-30 C. During this time, soil matric potentials were greater than -5 bars. Teo and Morrall (1985a) observed that the largest number of apothecia were produced in soil with a matric potential near field capacity (-0.11 to -0.40 bar). A supply of oxygen is critical for carpogenic germination to occur (Teo and Morrall, 1985b). Anaerobic conditions induce rotting and are the basis for pathogen control via flooding (Moore, 1949; Fry, 1982).

Following a preconditioning period, apothecia are produced if the sclerotia are within 5 cm of the soil surface. At greater depths, apothecia are often not produced as: (1) stipes are rarely longer than 5 cm and (2) formation of these asci-containing disks is dependent on light (Steadman, 1983). Stipe formation is not light-dependent as they form under the soil without light or in the light. Apothecia can develop within 6-10 days (Partyka and Mai, 1962) following the appearance of stipes. Once apothecia develop on the soil surface, ascospores are released in a "puffing" phenomenon when an abrupt change in relative humidity occurs. Each apothecium can produce more than two million ascospores over a 5-10 day functional life period (Steadman, 1983). Boland and Hall (1987) reported that apothecia which had desiccated during a period of dry weather, were revived and exhibited the puffing phenomenon following a period of rain. Once discharged, ascospores can survive on bean leaves for up to 14 days but most don't remain viable for more than six days (Caesar and Pearson, 1983).

White mold disease is commonly observed at the blossom or postbloom stage and is favored by moist conditions and a dense plant canopy

(Natti, 1971; Martens et al., 1984). The presence of senescent or injured plant tissue appears to be critical to infection of healthy tissue by ascospores. Several workers report that an exogenous energy source such as senescent bean blossoms is required for ascospore germination and subsequent mycelial infection of the host (Starr et al., 1953; Natti, 1971; Abawi and Grogan, 1975; Cook et al., 1975). Contrary to these results, direct infection of host tissues by germinated ascospores has also been reported (Dana and Vaughan, 1949; Hungerford and Pitts, 1953). Sutton and Deverall (1983) found that direct penetration by hyphae from germinated ascospores occurred only with young host tissue. Host cells exhibited a hypersensitive reaction to penetration with the fungus remaining restricted to the infected cells. Infection of host tissue by mycelia arising from myceliogenic germination of sclerotia has also been reported (Blodgett, 1946; Moore et al., 1949; Vaughan and Dana, 1949; Hungerford and Pitts, 1953; Starr et al., 1953; Cook et al., 1975). However, production of mycelia by sclerotia is not an important factor in the initiation of infection on bean (Abawi and Grogan, 1975). Secondary infections of bean are a result of mycelial growth from infected to healthy tissues (Natti, 1971). A major portion of secondary infection occurs following flowering, with senescent flowers serving as the most frequent energy base for these infections (Cook et al., 1975).

The fungal mycelia growing from colonized senescent blossoms or other senescent tissue can infect adjacent, intact host surfaces of flowers, stems, pods and leaves within two to three days (Steadman,

1983; Sutton and Deverall, 1983). Frequently the first symptoms are brown, water-soaked lesions located in the axillary tissues of the plant branches and main stems. These result from mycelia growing from senescent blossoms lodged in the branch axils. Lesions may also develop from blossoms that remain attached (Martens et al., 1984). If environmental conditions are favorable, lesions enlarge rapidly to become rotted, watery sections on plant tissue. Such lesions may become covered with extensive white, fluffy mycelial growth and within a week, black sclerotia are formed in the infected tissues. These hard, black fungal bodies are cylindrical to spherical in shape and range in size from 2-20 mm long (Martens et al., 1984). Lesions on bean tissue eventually become dry and bleached in appearance. Leaves of infected branches become yellow, wilt and are shed, leaving light brown, dry spurs on naked branches (Natti, 1971). Wilt and subsequent death of entire branches and main stems is often due to girdling of the tissue by S. sclerotiorum (Starr et al., 1953). Infected stem areas may disintegrate to leave only shredded, straw colored fibrous tissue. Infected bean pods may produce seed that is discolored (dull and chalky) and often lighter in weight than healthy seed (Hungerford and Pitts, 1953; NDSU, 1981). Although sclerotia are the primary survival structures of S. sclerotiorum , Cook et al. (1975) found that the pathogen was able to overwinter as mycelium in bean seeds. However, this was not considered to be an important source of initial inoculum.

1.2.3 Survival of sclerotia

1.2.3.1 The rind

A structural factor such as the rind may influence sclerotial survival. Many soil microorganisms produce dark-colored polymers or melanins in their cell walls (Linhares and Martin, 1978). The rind cell walls of S. sclerotiorum contain dark pigments called melanins (Jones, 1970) which are quite resistant to decay (Bloomfield and Alexander, 1967; Jones and Webley, 1968). Fungi with melanins in the cell walls are more resistant to microbial decay than are those lacking the dark-colored polymers (Martin et al., 1959; Bloomfield and Alexander, 1967; Hurst and Wagner, 1969; Wagner and Rubinska, 1971). Huang (1983b) observed that tan sclerotia were much more susceptible to infection by C. minitans than black sclerotia. Melanin deposits in the rind cell walls of tan sclerotia are almost negligible (Huang and Kokko, 1989). Huang and Kokko (1987) found that degradation of the melanized rind cells of S. sclerotiorum by C. minitans was slow compared with the nonmelanized cortical and medullary cells.

Huang (1985) reported the melanin in the rind to be a major component in controlling myceliogenic germination. In the absence of exogenous nutrients, myceliogenic germination is thought to be inhibited by the black, melanized rind of the sclerotium which appears to function in controlling dormancy. Those sclerotia having incomplete rinds or nonmelanized rinds (Huang, 1983b) were characterized by a lack of dormancy as well as susceptibility to attack by microorganisms. Makkonen and Pohjakallio (1960) postulated that sclerotia with damaged rinds might be more susceptible to

microbial colonization and more likely to germinate. This would reduce longevity of the fungal propagules.

The ability of the rind to function as a protective layer differs depending on whether the sclerotia are formed in nature or produced in culture (Merriman, 1976). Sclerotia produced in culture develop complete rinds as opposed to sclerotia produced in nature which form incomplete rinds. Imperfections of the rind allow for colonization by microorganisms and subsequent degradation of sclerotia. Trutmann et al. (1983) also found that sclerotia collected from the field did not survive as long as those formed in culture. They related decreased longevity to less intact rinds as well as reduced food reserves and an increased level of contamination by other microorganisms.

1.2.3.2 Germination

Sclerotia of S. sclerotiorum germinate myceliogenically (Huang and Dueck, 1980; Huang, 1985) or carpogenically (Williams and Western, 1965a; Willetts and Wong, 1980) to produce mycelia or apothecia, respectively. Two kinds of mycelial germination have been described. These are firstly, hyphal germination, which refers to the development of individual hyphae emerging through the rind of the sclerotium and secondly, eruptive germination which involves formation of a mycelial plug emerging from the medullary region of the sclerotium to rupture the rind (Adams and Tate, 1976). Myceliogenic germination of sclerotia of S. sclerotiorum appears to be of the hyphal type (Adams and Tate, 1976).

Sclerotia also germinate carpogenically by the development of stipes and apothecia. Ascospores are formed within an apothecium and are the only infective spores produced by S. sclerotiorum (Willetts and Wong, 1980). The production of apothecia by a sclerotium is affected by temperature (Bedi, 1962b). Sclerotia germinate freely over the temperature range of 5-20 C. Although stipes will develop, expansion to form apothecial discs occurs only at 15-20 C. Germination of sclerotia, either carpogenically or myceliogenically, has been reported to affect survival of the fungal propagules. Makkonen and Pohjakallio (1960) observed that sclerotia of S. sclerotiorum producing large numbers of apothecia were more susceptible to decay. Similar results were reported by Coley-Smith and Cooke (1971). They found germination to result in a reduction or exhaustion of sclerotial reserves which likely increased the susceptibility of sclerotia to decay. Myceliogenic germination may reduce the survival period of sclerotia more than carpogenic germination as suggested by Saito in Willetts and Wong (1980).

1.2.3.3 Production of secondary sclerotia

Sclerotia of S. sclerotiorum produce secondary sclerotia (Williams and Western, 1965b; Cook et al., 1975; Huang, 1980b) which occur as a result of myceliogenic germination. Although the presence of secondary sclerotia has been reported, information on the factors affecting their production is sparse (Adams, 1975; Le Tourneau, 1979). Cook et al. (1975) reported that secondary sclerotia develop at soil depths of 5 to 30 cm. Williams and Western (1965b) reported the formation of secondary sclerotia to be affected by soil moisture.

However, experiments conducted by Adams (1975) dealing with soil moisture, soil amendments and soil temperature failed to indicate any factors favoring formation of secondary sclerotia. While factors favoring development of secondary sclerotia are not clear, Willetts and Wong (1980) suggest that their production would enable limited multiplication and regeneration to occur. Such an increase in population of S. sclerotiorum would enhance the ability of this pathogen to survive.

1.2.3.4 Soil moisture

In the field, sclerotia are subject to variations in available moisture and these changes in soil moisture affect both survival and behavior of the sclerotia (Williams and Western, 1965b). Prolonged flooding and alternate flooding and draining have been used to promote decay of sclerotia in the field (Moore, 1949; Starr et al., 1953). More recently, Williams and Western (1965b) reported that increasing soil moisture accelerated breakdown of sclerotia. Liu and Sun (1984) found that sclerotia lysed after 20-30 days in flooded soil but survived well in dry soil. After 20 days in sterilized flooded soil, sclerotia survived and germinated. Liu and Sun (1984) suggested that sclerotial lysis may be due to the microflora in flooded soil.

Sclerotial survival is also affected by the drying and wetting of these propagules, which occurs on or just below the soil surface. Smith (1972b) found that sclerotia of S. sclerotiorum dried and rewetted, leaked more nutrients than non-dried sclerotia. Leakage favors biological breakdown of the sclerotia because the nutrients

serve as a substrate for colonization by other microorganisms. When dried and remoistened, the sclerotia did not survive longer than two to three weeks in soil. Smith attributed rapid decay of the sclerotia to increased microbial activity. His findings were confirmed by Javed and Coley-Smith (1973) and Coley-Smith et al. (1974) with Sclerotium delphinii Welch. However, results obtained by Smith (1972b) in studies on survival of sclerotia of Sclerotium cepivorum Berk. and Sclerotinia minor do not agree with those of Papavizas (1977b) and Abawi et al. (1985), respectively. Papavizas (1977b) suggested that decay of sclerotia was not due to drying sclerotia in soil even when the soil was then kept moist over a period of time. Similarly, Abawi et al. (1985) found that the survival time of sclerotia of S. minor was longer in soil subjected to a fluctuating soil moisture regime than in soil subjected to a constant water regime. Although differences in survival may be related to species differences, it is evident that additional studies on S. sclerotiorum as well as other sclerotia-producing pathogens are needed to better understand their survival under a variety of environmental conditions. Leakage of nutients from sclerotia of S. sclerotiorum has also been reported by Huang (1983a). These sclerotia were collected from sunflower heads which became desiccated in the fall. It is likely that these sclerotia were subjected to a drying effect; such an effect would cause leakage of nutrients (Smith, 1972b). Huang (1982, 1983a) examined both normal and morphologically abnormal sclerotia and found that viability and pathogenicity of abnormal sclerotia was less than that of the normal sclerotia (Huang, 1983b). The abnormal sclerotia have severely injured rinds resulting in extensive leakage of

nutrients. Such leakage may promote microbial degradation and accelerate death of the sclerotia (Huang, 1983b).

Adams (1975) reported that the inoculum density of soil containing sclerotia of S. sclerotiorum is markedly reduced when the soil is air-dried, remoistened and incubated at normal temperature. In the field, the upper soil layers tend to dry more rapidly than lower ones and therefore, sclerotia in the upper layers are subjected to more frequent periods of drying and rewetting. Williams and Western (1965b) suggested that it is this phenomenon that contributes to the more rapid disappearance of sclerotia in the upper soil layers.

Drying may affect the survival of sclerotia in yet another way. Smith (1972b) reported that a drying treatment of sclerotia of S. sclerotiorum stimulates germination in moist soil. He suggested that drying of sclerotia in the surface soil during hot, dry weather followed by rainfall or irrigation could promote sclerotia to germinate directly. Smith (1972a) found that in the absence of host plants, germinated sclerotia of Sclerotium rolfsii Sacc. decayed. Drying and remoistening of sclerotia of S. sclerotiorum may also promote decay following germination if no host plants are present.

1.2.3.5 Temperature

Temperature is also important in affecting the survival of sclerotia of S. sclerotiorum (Adams, 1975; Adams and Ayers, 1979). At temperatures up to 30 C, sclerotia survive well in soil, whereas others kept at 35 C for five to six weeks are destroyed. Singh et al. (1985) reported that good carpogenic germination occurred in sclerotia

exposed to temperature treatments of 40, 50 and 60 C for 24 h. However, as the temperature and exposure time increased, germination and apothecia formation decreased. Adams (1975) suggested that soil temperatures of 35 C are not uncommon in fields containing S. sclerotiorum, but prolonged periods at this soil temperature are rare. Consequently, high soil temperatures would not be expected to affect the survival of sclerotia under field conditions.

Sclerotia of S. sclerotiorum are able to survive at temperatures less than -18 C as indicated by the fact that in Manitoba, where winter temperatures of -18 C are not uncommon, the same areas can be affected with disease from year to year (Bisby, 1921, 1924; Zimmer and Hoes, 1978). Willetts (1971) reported that at low and subzero temperatures viability of sclerotia is maintained more effectively. Lack of competition from other organisms and dry conditions also favor survival. If food reserves of sclerotia are depleted, a loss of resistance to adverse environmental conditions results. An indirect influence of temperature on survival may be exerted by a direct influence on sclerotial germination. For example, carpogenic germination of sclerotia may be activated by low or fluctuating temperatures and may result in the production of numerous apothecia. This would consume considerable amounts of the sclerotial food reserves and therefore hasten decay (Coley-Smith and Cooke, 1971).

The ability of sclerotia to survive periods of low temperature and subsequently germinate has been related to a dormancy period (Coley-Smith and Cooke, 1971). Two kinds of dormancy occur in fungal sclerotia: (1) constitutive dormancy where germination is delayed by

a restraint on the processes that normally lead to germination and (2) exogenous dormancy where a delay in germination is imposed by unfavorable environmental conditions (Coley-Smith and Cooke, 1971). To break constitutive dormancy, application of an environmental stimulus such as low temperature treatment is required to induce sclerotial germination. Exogenous dormancy can be broken by a return to favorable environmental conditions. If environmental conditions are not favorable for germination once constitutive dormancy is broken, the sclerotium may undergo exogenous dormancy. This had been reported for S. sclerotiorum but, as pointed out by Coley-Smith and Cooke (1971), reports in the literature are contradictory. Bedi (1956) observed that sclerotia which had been freshly harvested from culture germinated carpogenically in distilled water when incubated at 15 to 20 C. Jones and Gray (1973) found that sclerotia collected from the field and incubated on soil at 20 C germinated quite readily. Only sclerotia derived from culture require a low temperature treatment (14 to 16 C) prior to incubation at 20 C in order to germinate. To obtain carpogenic germination of sclerotia harvested from culture, Huang (1981a) observed that a low temperature treatment is required. Only 5% tan and 10% black sclerotia produced apothecia when chilled for six weeks at 3.3 C followed by incubation for six weeks at 20 C. Under these conditions, myceliogenic germination also occurred, in 85% tan and 8% black sclerotia tested. This marked difference in myceliogenic germination was attributed to a lack of dormancy in tan sclerotia. Myceliogenic germination without preconditioning of tan sclerotia was also reported in further work by Huang (1983b). Adams and Tate (1976) reported that sclerotia of S.

minor exhibited dormancy following initial formation and did not immediately undergo myceliogenic germination. Although Cook et al. (1975) suggested that sclerotia do not exhibit dormancy relative to myceliogenic germination, data reported by Huang (1981a) showed that a dormancy period was involved with the black sclerotia of S. sclerotiorum. In later work, Huang (1985) reported the melanized rind of the sclerotium to be the site controlling dormancy which prevents myceliogenic germination. If the rind is incompletely formed or damaged, the ability of the sclerotium to function normally with respect to dormancy is affected.

Although reports in the literature vary, dormancy does appear to be associated with both carpogenic (Cook et al., 1975; Dueck, 1981; Huang, 1981a) and myceliogenic germination (Huang, 1985). Temperature is a factor associated with dormancy and germination of sclerotia. Continued research in this area is warranted to give a better understanding of this relationship.

Usually environmental conditions are not independent in their action but complement one another (Griffin, 1969). Interactions between environmental factors such as soil temperature and moisture affect soilborne pathogens (Papavizas, 1977a). Reductions in inoculum levels of Sclerotium oryzae Catt. (Usmani and Ghaffar, 1986), Sclerotium rolfsii, S. cepivorum, Sclerotinia minor and V. dahliae (Porter and Merriman, 1983) have been reported to be a result of a synergistic effect between soil temperature and moisture. While air-dried sclerotia of S. sclerotiorum are extremely resistant to heat treatment (Dueck et al., 1979), the combination of moisture and

temperature does favor their destruction (Ervio et al., 1964; Williams and Western, 1965b; Cook et al., 1975). Porter and Merriman (1985) observed that solarization of moist soil containing sclerotia of S. sclerotiorum reduced the number of viable propagules of the pathogen. After treatments, sclerotia were not recoverable from the 0-10 cm soil layer and the inoculum level of the pathogen was observed to decrease progressively towards the soil surface. Soil solarization in combination with a treatment such as addition of antagonists may have potential because solarization weakens propagules of the pathogen thus increasing their susceptibility to attack by biological agents (Pullman et al., 1979; Lifshitz et al., 1983; Porter and Merriman, 1983, 1985).

Thus the survival of sclerotia of S. sclerotiorum is affected by temperature alone or by a combination of temperature with other environmental factors. Temperature is also important when considering the mycelial growth of the pathogen as well as production of apothecia. Mycelial growth occurs over a broad range of temperatures with minimum temperatures reported to be slightly below 0 C (Van Den Berg and Lentz, 1968), 0 C (Tanrikut and Vaughan, 1951; Bedi, 1962a; Le Tourneau, 1979; Willetts and Wong, 1980), 4 C (Newton et al., 1973) or 5 C (Abawi and Grogan, 1975; Phipps and Porter, 1982). Maximum temperatures for growth have been reported to be 30 C (Tanrikut and Vaughan, 1951; Le Tourneau, 1979; Price and Colhoun, 1975; Phipps and Porter, 1982), 33 C (Bedi, 1962a), 35 C (Willetts and Wong, 1980) and between 32 and 36 C (Van Den Berg and Lentz, 1968; Newton et al., 1973). The optimum temperature for growth of S. sclerotiorum is

generally found to be about 20 C (Tanrikut and Vaughan, 1951; Van Den Berg and Lentz, 1968; Newton et al., 1973; Abawi and Grogan, 1975; Phipps and Porter, 1982). Tanrikut and Vaughan (1951) reported that S. sclerotiorum was able to produce sclerotia in a range of 0-30 C, with the optimum temperatures for growth being between 15 and 25 C. Similar results were obtained by Willetts and Wong (1980). They found that the optimum temperatures for growth and sclerotial formation were in the range of 15-22 C.

1.2.3.6 Condition of the field

The survival of sclerotia has been reported to be influenced by a cropped or fallowed condition of the field. However, some of the results are contradictory. Williams and Western (1965a) concluded that survival of sclerotia of both S. sclerotiorum and S. trifoliorum was enhanced under red clover because of an apparent inhibitory effect of clover on apothecial development. Willetts and Wong (1980) suggested that another reason for enhanced sclerotial survival could be the reduced drying of the surface soil layer underneath a covering crop. These results indicating that the presence of a crop can enhance survival of sclerotia of S. sclerotiorum are not in agreement with those of Gasparotto et al. (1982) and Adams (1975). Gasparotto et al. (1982) found that the survival of sclerotia in soil cultivated with grasses was reduced after a seven month period. Adams (1975) reported that the presence of a crop did not affect the survival of sclerotia. Sclerotia buried in soil maintained under fallow conditions survived as well as those buried under lettuce and bean.

Merriman et al. (1979) and Trutmann et al. (1983) reported that the degradation of sclerotia was affected by crop debris. Merriman et al. (1979) found that sclerotia buried alone were degraded more rapidly in soil than those inserted in bean straw prior to burial. Trutmann et al. (1983) reported similar results. Sclerotia buried in bean straw survived better than those formed on the outside of bean plants due to protection offered by the straw.

1.2.3.7 Soil pH and Soil Composition

Activity of S. sclerotiorum has been reported to occur over a wide range of H-ion concentration. Tanrikut and Vaughan (1951) reported the range of pH permitting growth to be 2-10. Dounine et al., (1935) and Rai and Agnihotri (1971) reported the range of pH within which growth occurred to be 2.3-7.5 and 3.5-7.5, respectively. Willetts and Wong (1980) reported that S. sclerotiorum can tolerate a wide range of pH but that fungal activity was enhanced under acidic conditions. These results support those of Dounine et al. (1935) and Rai and Agnihotri (1971) who found the optimum pH for growth to be 3.4-4.0 and 5.1, respectively.

The survival of sclerotia may differ from one location to another based on differences in soil composition. In studies with S. minor, Abawi et al. (1985) found that sclerotia buried in soil without a history of lettuce drop (soil B) exhibited greater viability than those buried in soil with a history of lettuce drop (soil A). Sclerotia recovered from soil A were often at various stages of decomposition and frequently contaminated with Trichoderma spp. Those

from soil B were firm and had essentially no fungal contamination. They suggest that the different survival rates of sclerotia in the two fields is related to differences in the biological activities of each soil. Soil B was found to have a number of ions present at high levels which may have been inhibitory to Trichoderma spp. Soil B may also have enhanced activities of microorganisms antagonistic towards Trichoderma spp. In soil A, sclerotia were more subject to attack by Trichoderma spp. whose activity may have been favored by a lower salt content of the soil. Also, the activities of organisms antagonistic to Trichoderma may have been favored in soil B.

In a previous study conducted in the same two commercial fields, Imolehin and Grogan (1980) also found differences in duration of survival and a more frequent association with Trichoderma of sclerotia recovered from soil A than those recovered from soil B. They suggested that various quantitative and qualitative differences in soil physical properties and soil microflora influence the longevity of sclerotia and hence, inoculum density of S. minor. These results concur with those of Garrabrant and Johnston (1982) who found that soil type affected significantly the incidence of lettuce drop. Disease incidence was lowest in sandy soil and increased in loam soil with the highest levels occurring in organic soil. Such differences in soil properties and microflora would be likely to influence the survival of S. sclerotiorum as well as other sclerotial-forming pathogens.

1.2.3.8 Sclerotium size and shape

The size and shape of the sclerotium may have an effect on sclerotial viability (Hoes and Huang, 1975). To some extent size determines the amount of food reserves available for use during the resting period. Shape affects the surface area exposed to drying conditions and also to attack by microorganisms. Size of the sclerotium also affects the number of apothecia formed. Bedi (1963) found that the number of apothecia produced by a sclerotium was correlated with sclerotium volume - as the volume increased, the number of apothecia produced increased.

1.2.3.9 Depth of burial

Burial depth also affects survival of sclerotia. Cook et al. (1975) indicated that survival at different depths is dependent on temperature and moisture content of the soil. Under dry, cool conditions survival of sclerotia is not affected by placement. At high soil temperatures and moisture content, deterioration of sclerotia is greater in the soil than on the soil surface. Adams (1975) stated that sclerotia of S. sclerotiorum survive well at depths of one to 12 inches but not at 24 inches. Soil samples taken at 24 inches were saturated with water. Moore (1949) found that prolonged flooding resulted in the same rate of sclerotial decay regardless of the depth of burial. Saturated anaerobic conditions induce rotting (Teo and Morrall, 1985b).

Carpogenic germination of sclerotia is also affected by depth of burial. Bedi (1961) found that sclerotia buried at depths greater

than 2.5 cm produced stipes but expansion of apothecial discs did not occur. Merriman (1976) reported that sclerotial populations declined rapidly in soil and suggested that deep ploughing to bury sclerotia for at least 30 weeks should reduce the inoculum surviving between crops. Merriman et al. (1979) confirmed the negative effect of burial on the survival of sclerotia and demonstrated that deeper burial resulted in more rapid degradation of sclerotia. Deep ploughing was effective in reducing levels of disease in lettuce due to the rapid degradation of sclerotia (Merriman et al., 1979) as well as the inability of stipes to reach the soil surface from depths greater than 8-10 cm (Williams and Western, 1965a). Garrabrant and Johnston (1982) reported a significant reduction in lettuce drop when sclerotia were buried at depths of 4,8 or 16 cm compared with 0,1 or 2 cm. Merriman et al. (1979) suggested that deep ploughing be conducted only once as ploughing a second time is likely to return viable sclerotia to the surface. Adams (1975) suggested that sclerotia produced on diseased host tissue be allowed to air-dry prior to ploughing. This would presumably hasten decay of the sclerotia due to the detrimental effects of drying and remoistening.

1.2.3.10 Microorganisms associated with sclerotia

The most significant factors affecting survival of sclerotia in soil appear to be biological (Adams and Ayers, 1979). Various workers have implicated more than 30 species of fungi and bacteria as antagonists or parasites of Sclerotinia spp. (Adams and Ayers, 1979). Rai and Saxena (1975) isolated species of Aspergillus, Penicillium and Stachybotrys from decaying sclerotia and showed that they were

antagonistic to sclerotia of S. sclerotiorum in vitro. In laboratory experiments, Bedi (1958) indicated that Bacillus subtilis Cohn emend. Prazmowski exercised a strong antibiotic effect on colonies of S. sclerotiorum. Trichoderma lignorum (Tode) Harz and a Fusarium sp. were found to suppress growth of this Sclerotinia species. Trichoderma spp. are often found to be associated with fungal plant pathogens producing sclerotia (dos Santos and Dhingra, 1982). Trichoderma viride Pers.:Fr. has been reported to parasitize sclerotia of S. sclerotiorum (Makkonen and Pohjakallio, 1960; Ervio et al., 1964; Jones and Watson, 1969; Hoes and Huang, 1975; Huang, 1980b; Lee and Wu, 1984). Lee and Wu (1984) reported that T. viride lysed hyphae of S. sclerotiorum, formed chlamydospores within sclerotia of this pathogen and produced antibiotics which inhibited mycelial growth. Other species of Trichoderma have also been reported to show antagonistic activity towards S. sclerotiorum (Artigues et al., 1984; Lee and Wu, 1984). Species of Acrostalagnus, Fusarium, Gliocladium, Verticillium and Trichoderma were observed to infect sclerotia on sterilized sand (Makkonen and Pohjakallio, 1960). Merriman (1976) isolated species of Mucor, Fusarium and Trichoderma from sclerotia. Certain species of Gliocladium are capable of destroying sclerotia of S. sclerotiorum. Gliocladium roseum (Link) Bainier caused decay of sclerotia on sand (Makkonen and Pohjakallio, 1960) and Gliocladium catenulatum Gilmon & Abbott, a hyperparasite of S. sclerotiorum (Huang, 1978), destroyed sclerotia in soil (Huang, 1980b). Su and Leu (1980) found that sclerotia inoculated with Gliocladium deliquescens Sopp. and buried in soil were lysed. Another species, Gliocladium virens Miller & Foster, was shown to parasitize mycelia and sclerotia

of S. sclerotiorum (Tu, 1980; Lee and Wu, 1984). Mueller et al. (1985) reported G. virens to reduce significantly the viability of sclerotia of S. sclerotiorum as well as the number of apothecia produced per sclerotium.

Sporidesmium sclerotivorum Uecker, Ayers and Adams has also been reported to be parasitic on sclerotia of S. sclerotiorum (Uecker et al., 1978; Ayers and Adams, 1979a; 1979b) as well as those of S. minor and Sclerotium cepivorum (Adams and Ayers, 1979; 1980). Adams and Ayers (1981) indicated that S. sclerotivorum was responsible for the decline in numbers of Sclerotinia minor, S. sclerotiorum and Sclerotium cepivorum in the field. Teratosperma oligocladum Uecker, Ayers and Adams was described by Uecker et al. (1980) as a new hyperparasite on the sclerotia of S. sclerotiorum, S. trifoliorum and S. minor. Adams and Ayers (1981) reported T. oligocladum to be a destructive parasite in unsterile soil as well as in vitro.

Watson and Miltimore (1975) observed that sclerotia of S. sclerotiorum were parasitized by Microsphaeropsis centaureae Morgan-Jones. Parasitism of sclerotia was found to be similar to parasitism by C. minitans. The genera Microsphaeropsis and Coniothyrium are closely related, and Watson and Miltimore (1975) suggested that M. centaurae and C. minitans might be synonymous.

Coniothyrium minitans is well established as a hyperparasite of S. sclerotiorum. For information regarding C. minitans, refer to the section devoted to this hyperparasite on pages 54-59.

Penicillium spp. have also been implicated as hyperparasites of S. sclerotiorum. Penicillium steckii Zaleski, Penicillium citrinum Thom and Penicillium funiculosum Thom destroyed sclerotia placed in sterilized soil previously infested with the specific parasitic fungus (Rai and Dhawan, 1978) while P. citrinum, P. steckii, P. funiculosum and Penicillium pallidum Smith affected the survival of sclerotia in field soil (Rai and Saxena, 1975). Su and Sun (1978) found three species of fungi which were able to decay sclerotia of S. sclerotiorum in one month. They suggested that these fungi were probably Penicillium spp. Su and Leu (1980) reported Penicillium vermiculatum Dangeard, the anamorph of Talaromyces flavus, to be a hyperparasite on sclerotia of S. sclerotiorum. For further information on this hyperparasite, refer to the section devoted to T. flavus on pages 47-54. Penicillium spp. reported to colonize sclerotia of S. sclerotiorum are Penicillium brevi-compactum Deirckx, Penicillium corymbiferum Westling and Penicillium cyclopium Westling var echinulatum n. var. (Merriman, 1976).

1.2.4 Control of sclerotinia disease

1.2.4.1 Sclerotinia wilt of sunflower

Sclerotinia wilt is a major disease of sunflower in Canada (Hoes and Huang, 1976) and may cause serious losses in yield, quality and production of the crop (Dorrell and Huang, 1978). Production of sclerotia and growth of Sclerotinia sclerotiorum occur at temperatures ranging from 0 C to 30 C (Tanrikut and Vaughan, 1951). With this wide range of activity, the fungus is able to maintain itself at almost any

temperature which supports crop production. The extreme adaptability of this fungus, its wide host range (Martens et al., 1984), and its ability to colonize host tissue rapidly and to produce numerous sclerotia make S. sclerotiorum very difficult to control. The sclerotia, which are the overwintering propagules of S. sclerotiorum, are of major importance in the development of sclerotinia disease of sunflower. Consequently, methods aimed at reducing or eliminating sclerotia must be an important component of control strategies for this disease.

Reducing the number of sclerotia in a field has been attempted through use of cultural methods. A prerequisite to the control of sclerotinia disease is the use of clean seed (Huang, 1983c). The danger of introducing sclerotia into clean, uncontaminated fields is minimized with the use of high quality certified seed.

Various methods have been used in attempts to destroy sclerotia in soil. Moore (1949) found that almost complete destruction of sclerotia of S. sclerotiorum occurred as a result of flooding. In most situations, this control method is impractical. Reports on the use of cultural practices to bury sclerotia and thereby reduce the inoculum density are contradictory. Zimmer and Hoes (1978) recommended that shallow harrowing of the field be practiced to retain infested residue near the soil surface. In the upper layers, sclerotia are subjected to more frequent periods of drying and rewetting which contribute to their more rapid disappearance (Williams and Western, 1965b). Adams (1975) suggested that sclerotia produced on diseased host tissue be allowed to air-dry prior to ploughing to

hasten decay. However, Steadman (1979) felt that survival of sclerotia would not be significantly affected by a drying period in the field because, under such conditions, sclerotia would not lose enough moisture to affect markedly their survival. Merriman (1976) believed that deep ploughing to bury sclerotia for at least 30 weeks should reduce the number of sclerotia surviving between crops. Poor survival of deeply buried sclerotia was attributed to high soil moisture content (Adams, 1975).

The incidence of sclerotinia wilt in sunflower can be reduced by manipulation of plant spacing (Huang and Hoes, 1980; Hoes and Huang, 1985). Young and Morris (1927) suggested that thinning rows gave good control of sclerotinia wilt because the spread from diseased to healthy plants was reduced. Huang and Hoes (1980) demonstrated that within-row spacings of 10 cm enabled the disease to spread from one initially infected plant to as many as eight neighboring plants. At spacings of 30 and 40 cm, minimal spread of the pathogen occurred confirming the observations of Young and Morris (1927). Sunflowers may be grown as a row crop or solid-seeded. Hoes and Huang (1976) found that solid-seeded fields were severely wilted. In fields that were planted in rows where the within row spacing was reduced, disease was also favored. Recommended control procedures are that plants be spaced as widely and as uniformly as possible and that overseeding be avoided (Dedio et al., 1980). Sunflower plant populations of greater than 86,450 plants per hectare are not advised (Campbell and Woods, 1979). Hoes and Huang (1985) found that yield was maximized at plant populations of 20,000 to 49,000 plants/hectare. Holley and Nelson

(1986) report that manipulation of plant population may not be important in reducing yield losses because no effect on disease incidence occurred in their studies. These results do not concur with those obtained by Hoes and Huang (1985) who found that increasing both within and between-row spacing decreased the number of nonproductive wilted plants and increased the yield.

Crop rotation has been recommended as a control measure to minimize losses due to sclerotinia wilt of sunflower (Bisby, 1924; Young and Morris, 1927; Zimmer and Hoes, 1978). However, control by crop rotation is difficult due to the wide host range and lack of specialization of the pathogen. The ability of sclerotia to survive for long periods in soil also decreases the effectiveness of control by crop rotation (Willetts and Wong, 1980; Sackston, 1981). Many crops such as rapeseed, bean, pea and mustard as well as some dicotyledonous weeds such as burdock, wild sunflower, wild mustard and Canada thistle are hosts of S. sclerotiorum (Dedio et al., 1980). It is recommended that susceptible crops be avoided in the rotation and that proper weed control be maintained. Although rotation alone may not effectively control sclerotinia wilt, the use of nonhost crops such as cereals, grasses and corn in the rotation will reduce the buildup of sclerotia in the field. A four- or five-year rotation is recommended between sclerotinia susceptible crops (Campbell and Woods, 1979; Dedio et al., 1980).

Use of chemicals aimed at reducing the population of sclerotia in soil and thereby controlling diseases caused by Sclerotinia spp. has not been promising. Alabouvette and Louvet (1973) discussed the use

of formaldehyde and methyl bromide for disinfestation of the soil. These products destroy sclerotia but the costs of these treatments and the technical difficulties involved make them impractical for use in the field. Certain chemicals such as cyanamide (Gabrielson et al., 1973), calcium cyanamide, nitrolim, hydrogen cyanamide, benlate, benzotriazole (Jones and Gray, 1973), dazomet and dicyanidiamide (Jones, 1974) have been reported to inhibit apothecial production. The chemicals found to destroy sclerotia of S. sclerotiorum were nitrolim (McLean, 1958) and dazomet (Jones, 1974). Results obtained by McLean (1958) are not in agreement with those of Jones (1974) who found that nitrolim inhibited apothecial production but did not kill all the sclerotia. Jones (1974) demonstrated that sclerotia of S. sclerotiorum were effectively destroyed by dazomet at 20 C and at temperatures as low as 5 C. Tests conducted in the laboratory showed dazomet to be an effective fungicide against S. sclerotiorum.

In the field, chemical control of S. sclerotiorum has not been that effective. Gulya (1981) tested the ability of Benlate, Botran, Mertect, Orthocide, Ronilan, Rovral and Topsin-M to control sclerotinia stalk rot of sunflower. When seeds were pelleted with these fungicides and planted in a field with a history of severe stalk rot, only Mertect inhibited stalk rot. This effect did not last the season and no significant differences in yield were observed. When these fungicides were applied as pre-plant incorporated treatments, Ronilan gave significant control but none of the treatments resulted in significant differences in yield when compared to the controls. Gulya (1981) concluded that utilizing seed or soil-applied fungicides

for control of sclerotinia stalk rot was not promising. Although results obtained using chemicals for control of sclerotinia wilt of sunflower have not been encouraging, Lee and Wu (1986) found that eight applications of DCNA, iprodione or vinclozolin reduced significantly sclerotinia disease of sunflower due to ascospore infection. Yield was improved significantly with DCNA only. Control of basal stem infection as opposed to ascospore infection involves activity of the chemicals in soil. Coley-Smith and Cooke (1971) suggested that diseases caused by sclerotial fungi are difficult to control with fungicides due to the breakdown of the chemicals in soil, inadequate contact between sclerotia and fungicide and the large amounts of fungicide required to destroy sclerotia at different soil depths.

Breeding resistant varieties to control diseases caused by Sclerotinia spp. was once thought to be impractical due to the lack of tissue specificity and the wide host range of the pathogen (Willetts and Wong, 1980). However, some cultivars of susceptible crops have exhibited differences in their response to Sclerotinia spp. Putt (1958), Kolte et al. (1976) and Huang (1980a) indicated that various inbred lines of sunflower exhibited differences in resistance to sclerotinia wilt. Huang (1980a) evaluated 21 inbred lines over a two year period and found a significant difference in resistance among them. In 1979 and 1980, the disease incidence varied from six to 50% and from 12 to 73% respectively. When 25 hybrid lines produced from screened inbreds were tested, they also showed a significant difference in their response to S. sclerotiorum. Huang (1980a)

suggested that resistance to sclerotinia wilt was heritable and was passed from inbred lines to hybrid progeny. Pirvu et al. (1985) reported that the resistance of hybrids could not be predicted based on the response of the parental inbred lines. In a study on the susceptibility of sunflower to capitulum infection by ascospores, Tourvieille de Labrouhe and Vear (1984) found that hybrids could be classified according to resistance and that the resistance was partial and polygenic. Clearly, more research is needed in determining the genetic mechanisms of sunflower resistance. In Manitoba, cultivars used for commercial production of sunflower were found to be susceptible to diseases caused by S. sclerotiorum (Hoes, 1978). Resistant cultivars are still unavailable (Huang, 1980a; NDSU, 1985).

Biological control of sclerotinia wilt of sunflower also appears promising. Two strains of Trichoderma viride applied as leaf sprays and soil applications were found to suppress sclerotinia disease of sunflower due to ascospore infection (Lee and Wu, 1986). Control of sclerotinia wilt of sunflower using such microorganisms may be feasible as suggested by application of these antagonists to soil. Coniothyrium minitans has been used successfully for biological control of this disease (Huang, 1976, 1979, 1980b; Bogdanova et al., 1986). Huang (1980b) reported that addition of C. minitans to soil artificially infested with S. sclerotiorum resulted in a 97% reduction of sclerotia following an incubation period of 100 days. In the field, C. minitans was added to the soil with the seed at planting time. Experiments were conducted over two successive years in fields naturally infested with S. sclerotiorum. In 1976 field trials, the

incidence of sclerotinia wilt in C. minitans -treated plots was 25% as compared with 43% in the control. Similar results were obtained in 1977, where C. minitans plots showed 24% disease as compared to 40% in the untreated control. In the plots treated with the hyperparasite, yield was significantly higher in both years. Huang (1980b) discussed C. minitans as a biological control agent of sclerotinia wilt of sunflower and believed its effectiveness to be based on the ability of C. minitans to control effectively the primary inoculum or sclerotia. His data indicated that C. minitans is ineffective in controlling the actively growing state of S. sclerotiorum in soil, and therefore, while primary sources of inoculum (sclerotia) were reduced, the spread of the fungus was not. While resistant cultivars or effective chemicals are not yet available, biological control, using hyperparasites such as C. minitans may have great potential when used in conjunction with the cultural practices recommended for control of this disease (Huang, 1980b). The success of C. minitans as a biological control agent of S. sclerotiorum is due in part to the fact that C. minitans is active under natural conditions and enough basic information on the ecology and behavior of this hyperparasite is available to permit its use in a rational manner (Ayers and Adams, 1981). Use of hyperparasites as biological control agents is still relatively new, and continued research is required to assess further the potential of C. minitans as well as the capabilities of other fungi to act as biological control agents in the field.

1.2.4.2 White mold of bean

White mold of bean is an important cause of crop loss wherever beans are produced (Martens et al., 1984). The disease is difficult to control due to the wide host range of the pathogen (Adams et al., 1974; Martens et al., 1984; Purdy, 1979; Willets and Wong, 1980) , the extreme adaptability of the fungus and the ability of the fungus to produce sclerotia and ascospores. Ascospores are of major importance in the development of epidemics of white mold disease of bean (Abawi and Grogan, 1975). Consequently control measures aimed at: (1) reducing the number of ascospores produced and (2) preventing infection of host plants by ascospores are of primary importance.

Methods used to reduce or eliminate the production of ascospores are often aimed at reduction of the sclerotia population in soil. In the southern states, bean fields infested with white mold were often flooded for a three week period during the summer to destroy sclerotia (Starr et al., 1953). However, in most areas, this control method would not be practical.

Chemicals have also been used to reduce the population of sclerotia in soil. Calcium cyanamid was applied to S. sclerotiorum -infested bean fields in northern Wyoming during the 1947-1949 field seasons (Starr et al., 1953). Results were variable and the procedure costly but Starr et al. (1953) concluded that the cost may be justified if the soil was heavily infested with sclerotia and if large amounts of nitrogen were needed. In most experiments the excess nitrogen supplied by calcium cyanamid delayed maturation of the bean crop. Other chemicals affecting the survival of sclerotia are discussed under sclerotinia wilt of sunflower on pages 34-36.

Certain herbicides have been reported to affect carpogenic germination and apothecium development of sclerotia of S. sclerotiorum. In the presence of atrazine or simazine, sclerotia either did not produce apothecia from stipes or produced abnormal apothecia that did not develop ascospores (Liu, 1977; Liu and Sun, 1984; Casale and Hart, 1986; Radke and Grau, 1986). Other herbicides have also been found to affect sclerotia of S. sclerotiorum (Cerkauskas et al., 1986; Radke and Grau, 1986). Although carpogenic germination was not affected, sclerotial viability was observed to decline consistently with increasing triallate concentrations. Several fungicides have also been reported to have an effect on carpogenic germination of sclerotia. Patterson and Grogan (1985) reported that certain rates of DCNA, iprodione and vinclozolin inhibited apothecial production by sclerotia of S. sclerotiorum when tested under greenhouse conditions.

Crop rotation as a control measure to minimize losses due to white mold is recommended (Starr et al., 1953; NDSU, 1981). In the New York area, Natti (1971) found that repeated culture of bean crop in the same fields contributed to the serious white mold disease problem. Although control by crop rotation is difficult to achieve due to the wide host range of S. sclerotiorum, it is recommended that beans be alternated with nonhost crops such as cereals, grasses and corn (Starr et al., 1953). In the North Platte valley of western Nebraska, the dry bean crop is commonly rotated with corn and sugar beets every third year (Cook et al., 1975). Control of white mold has not been satisfactory and this is related to the ability of the pathogen to

survive in soil for extended periods of time in the absence of a host crop. The recommended rotation period between sclerotinia susceptible crops is a minimum of four to five years (Campbell and Woods, 1979; Dedio et al.; 1980). It is also important to avoid planting next to a field which was cropped to beans in the previous season (NDSU, 1981). Severe disease may occur as a result of windblown ascospores produced in the adjacent field.

Sclerotia added to soil with seed increase the inoculum potential of the pathogen. Under the appropriate conditions each sclerotium may produce numerous apothecia, each capable of discharging a cloud of ascospores on more than one occasion (Newton and Sequeira, 1972; Saur, 1983). Sclerotinia sclerotiorum has also been reported to be spread by seed that is infected with mycelia of the fungus (Starr et al., 1953; Tu, 1988). Cook et al. (1975) found that the pathogen was able to overwinter as mycelium in bean seeds located on the soil surface. However they were doubtful that infected seed played an important role in disease initiation. This contradicts a report by Tu (1988) who found that infected seeds were important both in the spread and the epidemiology of S. sclerotiorum in bean.

Sclerotia may also be introduced into a non-infested locality via infected bean straw or by movement of infested soil. Spread of white mold disease from one area to another through combine operations has also been reported (Starr et al., 1953; NDSU, 1981). Reuse of surface irrigation runoff water may also spread S. sclerotiorum from one field to another (Steadman, 1983). Steadman et al. (1979) reported that specific treatments of this water could eliminate ascospores but

sclerotia were not killed. To minimize dissemination of S. sclerotiorum in irrigation water, application of contaminated reuse water to nonsusceptible crops was recommended. Steadman (1983) also reported that spores of S. sclerotiorum can be efficiently distributed to infection sites by honeybees.

Inhibiting production of or reducing the numbers of apothecia produced by sclerotia will also affect the incidence of white mold disease. This can be achieved through modifications of bean cultural practices. High moisture and humidity favor the development of white mold (Starr et al., 1953; Abawi and Grogan, 1975; Weiss et al., 1980a). For example, the development of apothecia occurs in saturated or near-saturated soil (Abawi and Grogan, 1975). Also, infection of tissue by S. sclerotiorum will not occur unless a film of water is maintained at the tissue surface (Abawi and Grogan, 1975; Grogan and Abawi, 1975). Consequently cultural practices that reduce the humidity, leaf wetness or soil surface moisture will reduce the incidence of white mold. Such practices include increasing row spacing (Starr et al., 1953; NDSU, 1981) and planting with the prevailing wind (NDSU, 1981). Although narrow rows can increase yield, a wider spacing decreases the drying time of plants. The drying time can also be decreased if beans are planted in rows parallel with the prevailing wind. This increases the airflow within the canopy (NDSU, 1981). Avoiding excessive irrigation is also important (Starr et al., 1953; Steadman et al., 1976; Weiss et al., 1980a; Steadman, 1983). A reduced irrigation frequency can result in fewer apothecia being produced and a lowered disease severity

(Steadman, 1983). It is also important to follow the recommendations for planting rates (Starr et al., 1953; NDSU, 1981). With a high plant population, canopy development is more rapid and is increased, resulting in higher moisture and humidity levels within the canopy. These conditions are favorable for the development of white mold.

Developing resistant varieties for control of white mold disease has not been very successful. Genetic resistance of most beans to S. sclerotiorum is minimal (NDSU, 1981). Controlled environment and inoculated greenhouse tests have shown the common bean (Phaseolus vulgaris) to be susceptible or semiresistant at best. Although differences in levels of resistance to S. sclerotiorum have been reported (Coyne et al., 1976; Hunter et al., 1982), it has not been established in all cases whether the reduced levels of disease are due to the host crop resisting pathogen attack or to a disease avoidance mechanism (Steadman, 1983). A low level of disease has often been attributed to avoidance or escape due to an open plant canopy and upright growth habit (Coyne et al., 1976; Hunter et al., 1982; Campbell and Steadman, 1985). In open bean canopies, the microclimate temperature can exceed 27 C which has a significant effect on ascospore germination. A reduced germination of ascospores results in a lower incidence of disease (Campbell and Steadman, 1985). Blad et al. (1978) and Weiss et al. (1980b) found disease severity to be associated with irrigation treatments and canopy structure. Disease severity is often highest in fields of viny cultivars which produce dense canopies and, when irrigated heavily, are the wettest and coolest (Steadman, 1983). The canopy effect is also seen in other

crops such as potato where plants with a heavy dense foliage showed more hills infected with S. sclerotiorum than plants with upright smaller foliage (Partyka and Mai, 1962).

The identification of resistance to S. sclerotiorum has been difficult and time consuming, but attempts to incorporate resistance into Phaseolus vulgaris cultivars continue (Coyne et al., 1981; Dickson et al., 1981; Fuller et al., 1984). The best approach to producing high-yielding bean cultivars which minimize the incidence of white mold involves the combination of disease avoidance due to plant morphology and breeding for resistance (Hunter et al., 1982; Steadman, 1983; Casciano and Schwartz, 1985).

Starr et al. (1953) reported that chemicals were not yet considered practical for control of white mold disease of bean. More recent reports indicate that fungicides such as Ronilan, Rovral and benomyl, which are registered for control of white mold, have provided varying degrees of control. Studies using benomyl an aerial application for control of white mold have produced variable results. In New York, Natti (1971) demonstrated that two spray applications of benomyl, one at prebloom and the other at full bloom, gave maximum control of white mold of bean. Conversely, sprays applied after full bloom did not give effective control. Morton (1986) reported the greatest reduction in disease to occur with either one application of benomyl at full bloom or with two applications, one at full bloom and one at late bloom. Two sprays could be reduced to one if the one spray was well-timed. Hunter et al. (1978) found that effective control was obtained when the whole plant was sprayed with benomyl whereas, when

all plant parts excluding the blossoms were sprayed, no control was observed. These results indicate the importance of blossom coverage and proper timing of spray application.

Other chemicals used for control of S. sclerotiorum on bean include vinclozolin, the active ingredient of Ronilan (Baraer, 1979; Lartaud and Duchon-Doris, 1980; Vulsteke and Meeus, 1982), and procymidone (Vulsteke and Meeus, 1982). Baraer (1979) reported that good results were obtained using two treatments of vinclozolin. In 1982, Vulsteke and Meeus found that two spray applications of vinclozolin gave the best control of S. sclerotiorum. The first was applied at early flowering and the second two weeks later when pod set was almost complete. Treatments with these two fungicides resulted in increased seed yield and did not affect quality and color of the pods. Iprodione, the active ingredient of Rovral, is also reported to be effective against Sclerotinia species (Rowe, 1983).

Chemical control of the disease has been erratic (Coyne et al., 1976; Steadman and Kerr, 1976). Failure to obtain good control can be related to factors such as incorrect timing of spray applications (Natti, 1971), inefficient application procedures (Steadman, 1983), severe disease pressure (Abawi, 1976; Steadman, 1983) and inadequate coverage of bean blossoms due to growth habit and indeterminate flowering (Abawi, 1976; Steadman, 1983).

Biological control of white mold of bean is a possibility. Biocontrol of S. sclerotiorum on the phylloplane of bean has been reported. Fungi isolated from bean or rapeseed flowers have been

found to suppress white mold lesion development on bean seedlings (Boland and Inglis, 1989). The fungi most effective in suppressing disease development were isolates of Epicoccum purpurascens Ehrenb. & Schlecht., Fusarium graminearum Schwabe, F. heterosporum Nees ex Fr. [= F. roseum (Lk.) Snyd. and Hans.] and Drechsclera sp.. In the field, white mold of bean was suppressed significantly by foliar applications of E. purpurascens (Zhou and Reeleder, 1987). Coniothyrium minitans has been evaluated as a soil treatment (Howard and Dueck, 1980; Huang, 1976, 1979, 1980b) and as an aerial application (Trutmann et al., 1982) for control of S. sclerotiorum on bean. Coniothyrium minitans is hyperparasitic to both sclerotia and hyphae of S. sclerotiorum (Hoes and Huang, 1975; Trutmann et al., 1980, 1982; Huang and Kokko, 1987; Huang, 1988) and has been used successfully for control of sclerotinia wilt of sunflower (Huang, 1976, 1979, 1980b). Howard and Dueck (1980) reported that the use of C. minitans resulted in only a slight reduction of sclerotia numbers in soil and concluded that C. minitans does not hold much promise as a biocontrol agent against S. sclerotiorum on dry bean. Results obtained by Trutmann et al. (1982) indicate that aerial application of C. minitans onto bean plants reduced significantly the number and weight of sclerotia produced on diseased plants. Apothecial production by such sclerotia was reduced significantly as compared to the control where no C. minitans was applied. Although C. minitans did not protect the bean crop against the pathogen, Trutmann et al. (1982) concluded that the hyperparasite was effective in reducing the inoculum potential of S. sclerotiorum. They suggested that it might be feasible to use C. minitans as a postharvest treatment applied to

crop residues containing sclerotia. A reduction in the level of disease in subsequent crops would be expected.

Unfortunately the development of resistant cultivars is slow and difficult and control through the use of chemicals can often be erratic (Steadman, 1983). Consequently, use of a hyperparasite such as C. minitans may have potential as a biological control agent for white mold of bean. Coniothyrium minitans has many attributes of an ideal biological control agent, some of which are: (1) the ability to grow well and sporulate in pure culture, (2) the production of pigmented and thick-walled conidia (3) the common occurrence of the fungus in nature, (4) the production of melanized pycnidia which can be harvested and as such provide further protection to conidia and (5) the ability to be readily prepared as an aqueous suspension for application through irrigation or spraying equipment (Trutmann et al., 1980). With so many attributes, further research on the use of C. minitans as a biological control agent is warranted.

1.3 THE HYPERPARASITES

1.3.1 Talaromyces flavus

Talaromyces flavus (Klocker) Stolk and Sampson belongs to the Class Ascomycetes, Subclass Plectomycetidae, Family Eurotiaceae (Alexopoulos and Mims, 1979). The most common species of Talaromyces is T. flavus, the sexual stage or teleomorph of Penicillium vermiculatum (Stolk and Sampson, 1972). This ascomycete has been found in soil in numerous locations including Spain (Guarro and Calvo, 1981), Japan (Horie et al., 1977; Ueda, 1980a; 1980b), Korea (Min et al., 1982), Poland

(Rataj-Guranowska, 1981), Greece (Tjamos and Paplomatas, 1986; Tjamos et al., 1986), the Netherlands (Bollen and van der Pol-luiten, 1975), Nepal (Minoura et al., 1975), Czechoslovakia (Fuska et al., 1972), North America (Widden and Parkinson, 1973; Dutta, 1981; Zlattner and Gochenaur, 1984) and Great Britain (Warcup, 1951). Raper and Thom (1968) reported P. vermiculatum to be abundant in nature and to have a worldwide distribution. Stolk and Sampson (1972) found similar results, reporting T. flavus to be isolated frequently from soil and to be worldwide in distribution. In a recent study by Fravel and Adams (1986), distribution of T. flavus throughout the United States and the world has been estimated.

Talaromyces flavus has been isolated from the rhizosphere of tomato (Dutta, 1981), sunflower (Huang, pers. comm.), groundnut (Horie et al., 1977), cotton and potato (Marois et al., 1984), eggplant (Marois et al., 1982; 1984) and olive (Tjamos et al., 1986).

Penicillium vermiculatum has been established as a parasite of a number of fungi. Husain and McKeen (1962) reported P. vermiculatum to be parasitic on an undescribed species of Rhizoctonia. Boosalis (1956) showed that hyphae of Rhizoctonia solani were parasitized by P. vermiculatum in unsterilized field soil. The mode of parasitism involved production of penetration pegs which developed from mycelium contacting the host hyphae. Coiling of Penicillium hyphae around host hyphae occurred and parasitic hyphae were observed within host hyphae. Boosalis (1956) tested the ability of P. vermiculatum to parasitize 28 species of fungi belonging to 16 genera . Of the 28 species including S. sclerotiorum that were tested in vitro, only hyphae of R. solani

were reported to be parasitized by P. vermiculatum. McLaren et al. (1982; 1983; 1985; 1986) found that hyphae of S. sclerotiorum were parasitized by T. flavus, the teleomorph of P. vermiculatum. Host hyphal cells were invaded by direct penetration of the cell walls without the formation of appressoria (McLaren et al., 1986). Infection of host cells resulted in granulation of the cytoplasm and collapse of cell walls.

Su and Leu (1980) found that P. vermiculatum could parasitize sclerotia of S. sclerotiorum. Inoculating sclerotia with a spore suspension of P. vermiculatum resulted in more than 70% of the sclerotia being infected. Sclerotia inoculated and buried in soil at depths of five to 20 cm were lysed. McLaren et al. (1983) reported that sclerotia of S. sclerotiorum were parasitized by T. flavus in both field and greenhouse trials. Fewer sclerotia were recovered in treatments where the hyperparasite was applied. An ultrastructural study of the infection of sclerotia of S. sclerotiorum by T. flavus indicates that host cells are degraded and destroyed due to ramification of T. flavus within the sclerotium tissue (McLaren et al., 1989). Besides being parasitic on sclerotia of S. sclerotiorum, T. flavus has been reported to inhibit growth of Rhizoctonia fragariae sp. nov. (Husain and McKeen, 1963), to penetrate and overgrow mycelia of Verticillium albo-atrum Reinke and Berthold (Dutta, 1981), to inhibit spore germination of V. albo-atrum (Feinstein and Morehart, 1984) and to be effective against V. dahliae Kleb. (Marois et al., 1982, 1984).

Boosalis (1956) suggested that P. vermiculatum does not produce toxic substances resulting in injury to hyphae of Rhizoctonia solani. Filtered culture extracts of the parasite had no effect on the host fungus. However, Bollen and van der Pol-luiten (1975) reported T. flavus to show a marked antibiotic activity against Streptomyces and bacteria. Mycelial growth of a number of fungi was also inhibited. Research with P. vermiculatum and V. albo-atrum (Dutta, 1981) has shown that when culture filtrates of P. vermiculatum were added to media inoculated with V. albo-atrum the development of the pathogen was reduced. Dutta (1981) suggested that an inhibitory substance was left in the potato dextrose solution. Spore germination and germ tube growth of V. albo-atrum were reduced by culture filtrates of P. vermiculatum. Husain and McKeen (1963) reported toxic substances to be involved in the parasitism of R. fragariae by P. vermiculatum. Colonies of R. fragariae were stunted and flattened due to media-diffusible toxic substances excreted from P. vermiculatum.

Talaromyces flavus has been reported to produce a metabolite which reduced the growth of V. albo-atrum in culture (Dutta, 1981) and the viability of microsclerotia of V. dahliae (Fravel et al., 1987a; 1987b). This metabolite is thought to be different from the antibiotics produced by T. flavus due to its smaller molecular weight (Kim et al., 1986). The antibiotics produced by T. flavus are talaron (Mizuno et al., 1974), vermiculine (Fuska et al., 1972; Sedmera et al., 1973; Boeckman et al., 1974; Burri et al., 1978; Horakova et al., 1980), vermistatin (Fuska et al., 1979a) and vermicillin (Fuska et al., 1979b). Of these antibiotics, talaron exhibits activity against

fungi including Pyricularia oryzae Cav. and Hormodendrum pedrosoi Brumpt (= Cladosporium pedrosoi) (Mizuno et al., 1974) and vermiculine is closely related to the antifungal agent pyrenophorin (Fuska et al., 1972).

Use of Talaromyces flavus as a biological control agent has been attempted. When sterilized field soil was infested with both Rhizoctonia solani and P. vermiculatum, nearly complete control of Rhizoctonia-incited damping-off and seedling blight of peas resulted (Boosalis, 1956). Penicillium vermiculatum gave little or no control in a comparable treatment in unsterilized soil. In work conducted by Dutta (1981), tomato seedlings dipped in culture filtrates of P. vermiculatum and then inoculated in a V. albo-atrum spore suspension prior to planting, showed fewer symptoms than the infected control. Similar results were obtained when plants were dipped in a spore suspension of P. vermiculatum and then planted into soil infested with V. albo-atrum grown on wheat grain. Talaromyces flavus has been found to be effective in reducing the incidence of verticillium wilt in the field (Davis et al., 1986; Fravel et al., 1986, 1987a). Fravel et al., (1986, 1987a) reported that a single application of the antagonist which was broadcast throughout a naturally infested field was able to suppress wilt incidence for two consecutive seasons. Spink and Rowe (1989) reported that T. flavus has no significant effect on the development of verticillium wilt of potato in the field. They suggested that T. flavus may need to be present in soil for some time to be effective as a biological control agent.

Tjamos and Paplomatas (1986) reported that T. flavus may be involved in the control of verticillium wilt of artichokes. A three year study in naturally infested fields showed effective control of the disease through solarization. They found that T. flavus and Aspergillus terreus Thom survived solarization and suggest that control may be explained in part by the activity of these microorganisms. Tjamos et al. (1986) suggest that T. flavus and A. terreus may also be involved in the control of verticillium wilt of olive trees which have been subjected to individual soil solarization treatments. The ability of T. flavus to withstand heat treatment has been reported by Bollen and van der Pol-luiten (1975), Van der Spuy et al. (1975) and Katan (1985). The latter two authors found that ascospores of T. flavus tolerated high temperatures well, whereas mycelia and conidia were very heat sensitive. Ascospores enclosed within cleistothecia showed a marked increase in heat resistance as compared to those removed from their fruiting bodies (Van der Spuy et al., 1975).

Biological control of white mold of bean on the phylloplane using T. flavus has been attempted (Boland and Inglis, 1989). The mean lesion diameter on bean seedlings inoculated with T. flavus and S. sclerotiorum was reduced compared with the mean lesion diameter on plants inoculated with S. sclerotiorum alone.

Talaromyces flavus has been used for biological control of sclerotinia wilt of sunflower (McLaren, 1983; McLaren et al., 1983; 1985). In a study conducted at two locations, sclerotinia wilt was reduced and yields were greater in plots treated with the

hyperparasite (McLaren et al., 1983). In a three-year study, T. flavus was found to reduce the incidence of sclerotinia wilt in sunflower. Wilt incidence for T. flavus -treated plots was 4, 0 and 2% compared with 47, 54 and 50% in control plots (McLaren et al., 1985).

Studies have also been conducted on the ability of T. flavus to establish in soils having different biological, chemical and physical properties (Fravel et al., 1984). Of 23 parameters measured in 25 soil types, six were found to relate to proliferation and survival of T. flavus. These were total soil bacteria, potassium, zinc, sodium, soluble salts and cation exchange capacity. Marois and Fravel (1983) found that when spores of T. flavus were added to natural soil, soil respiration increased from 5-20% depending on soil type and condition. Of the seven fungal species tested, only T. flavus increased in population density when added to natural soil. Similar results concerning population density of T. flavus in soil were reported by Lewis and Papavizas (1984b). The population density of T. flavus when applied to natural soil as hyphae in a sand-bran mixture, increased up to tenfold during the first three weeks following incorporation. As the period of incubation in soil increased, the propagule numbers declined and stabilized.

The production of biological control agents for incorporation into soil has involved the use of different types of growth media. Husain and McKeen (1963) used a mixture of cornmeal and sand (1:1 w/w) for growth of P. vermiculatum whereas Marois et al. (1982) used cornmeal alone. Papavizas et al. (1984) studied a fermentation process using molasses and brewer's yeast for the production of T. flavus and other

biological control fungi. Once produced, mats of T. flavus were air-dried and mixed with a suitable carrier for application. Wheat bran has also been used as a food base for biocontrol agents. Hadar et al. (1979) used bran for growth of Trichoderma harzianum Rifai. This antagonist was applied to Rhizoctonia solani -infested soil and gave effective control of damping-off of bean, tomato and eggplant seedlings. A combination of wheat bran, quartz sand and water (1:1:2 w/w/v) has been used to produce inocula of T. flavus as well as Trichoderma viride, T. harzianum, Gliocladium virens, G. roseum, G. catenulatum and Aspergillus ochraceus Wilhelm (Lewis and Papavizas, 1984b). Fravel et al. (1985b) studied the use of an alginate-clay matrix to encapsulate T. flavus as well as other test organisms. Talaromyces flavus remained viable following the pellet formation process and generally survived longer than other microorganisms pelleted in a similar manner. Fravel et al. (1985b) report this technique to be inexpensive and versatile and to provide a product compatible with modern production practices. Further testing of formulation procedures on the proliferation and survival of T. flavus in alginate-encapsulated pellets has shown that bran is a good bulking agent and enhances the metabolic activity of T. flavus (Papavizas et al., 1987). Use of alginate-pyrophyllite granules containing ascospores of T. flavus for the control of Verticillium dahliae in the field has been reported (Fravel et al., 1987a). Application of T. flavus in this form resulted in a suppression of wilt incidence for two consecutive years.

1.3.2 Coniothyrium minitans

The soilborne fungus Coniothyrium minitans Campbell lacks a perfect state and reproduces only by means of conidia. Such fungi are commonly referred to as "Fungi Imperfecti" and comprise the form-class Deuteromycetes (Alexopoulos and Mims, 1979). This fungus has been found in soil in many locations throughout the world including the United States (Campbell, 1947), England (Tribe, 1957), Poland (Zub, 1960), Finland (Ervio, 1965), Hungary (Voros, 1969), Scotland (Jones and Watson, 1969), East Germany (Schmidt, 1970), New Zealand (Jarvis and Hawthorne, 1971), Italy (Zazzerini and Tosi, 1985), Canada (Hoes and Huang, 1975; Huang, 1981b), U.S.S.R. (Fedulova, 1983) and Australia (Trutmann et al., 1980, 1983; McCredie and Sivasithamparam, 1985).

Coniothyrium minitans has been tested for pathogenicity to a number of plant species. Turner and Tribe (1976) tested 17 species, mainly hosts to S. trifoliorum and S. sclerotiorum, and found that no infection by C. minitans occurred. The plants tested included Antirrhinum majus L., Apium graveolens L., Beta vulgaris L., Brassica oleracea L., Cheiranthus cheiri L., Cichorium intybus L., Coleus blumei Benth., Cucumis sativus L., Daucus carota L., Helianthus annuus L., Lactuca sativa L., Lupinus regalis Bergermans, Lycopersicon esculentum Mill., Solanum melongena L., Solanum tuberosum L., Trifoliorum repens L. and Vicia faba L. Coniothyrium minitans has been reported to be a parasite of a number of fungi. Tribe (1957) and Turner and Tribe (1975) found that C. minitans parasitized sclerotia of Sclerotinia trifoliorum in the field. Under laboratory conditions,

Turner and Tribe (1976) reported C. minitans to parasitize sclerotia of Sclerotinia minor as well as some strains of Botrytis cinerea Persoon ex Fries, B. fabae Sardina and Sclerotium cepivorum. Ahmed and Tribe (1977) found C. minitans to be pathogenic to Sclerotium cepivorum when tested in a glasshouse experiment resulting in effective control of white rot of onion seedlings. Coniothyrium minitans was also reported to reduce the severity of white rot of garlic caused by Sclerotium cepivorum (De Oliveira et al., 1984).

Coniothyrium minitans is well established as a hyperparasite of S. sclerotiorum (Campbell, 1947; Jones and Watson, 1969; Voros, 1969; Ghaffar, 1972; Hoes and Huang, 1975; Huang, 1976; Huang and Hoes, 1976; Turner and Tribe, 1976; Huang, 1977, 1979, 1980b; Trutmann et al., 1980, 1982; Fedulova, 1983; Phillips and Price, 1983; Tu, 1984; McCredie and Sivithamparam, 1985; Zazzerini and Tosi, 1985; Huang and Kokko, 1987) and kills hyphae, and sclerotia of this Sclerotinia species. The mode of parasitism of C. minitans on hyphae of S. sclerotiorum involves penetration of host cell walls from both the inside and the outside. Tu (1984) reported that appressorium-like swellings are produced on hyphae of C. minitans when they contact host hyphae. These results are not in agreement with those obtained by Huang and Hoes (1976), Trutmann et al. (1982) and Huang (1988) whose work indicates that host cell walls are penetrated directly by hyphae of C. minitans without formation of appressoria. Phillips and Price (1983) reported that physical pressure was critical to the penetration of rind cells by C. minitans and that wall-lysing enzymes were of little significance. However, one of the principal components of the

cell walls of sclerotia (Jones, 1970; Saito, 1977) and hyphae (Jones and Watson, 1969) of S. sclerotiorum is ß-glucan. Jones and Watson (1969) reported that pseudoparenchymatous and hyphal walls of S. sclerotiorum were lysed by C. minitans. Culture filtrates of C. minitans contained ß-glucanase and chitinase. Jones et al. (1974) attributed the degradation of ß-glucan to the presence of ß-(1->3)-glucanases produced by C. minitans. Huang and Kokko (1987) reported that ß-(1->3)-glucanases may be critical to the degradation of cell walls of sclerotia of S. sclerotiorum by C. minitans.

Coniothyrium minitans was first isolated from sclerotia of S. sclerotiorum by Campbell (1947) who suggested that biological control of plant diseases caused by Sclerotinia species might be possible with this fungus. The survival of sclerotia in soil has been reported to be severely affected by C. minitans. Huang (1976) found a 97% reduction in sclerotial survival occurring 100 days after the hyperparasite was added to soil artificially infested with S. sclerotiorum. McCredie and Sivasithamparam (1985) reported that survival of sclerotia in soil was also reduced by C. minitans. Huang (1977) showed C. minitans attacking sclerotia under natural conditions. Sclerotia formed in the pith cavities and basal stems of sunflowers were parasitized and subsequently destroyed by C. minitans.

Coniothyrium minitans has been used successfully for biological control of Sclerotinia trifoliorum (Turner and Tribe, 1975) and S. sclerotiorum (Huang, 1976; 1979; 1980b; Bogdanova et al., 1986) in the field and Sclerotium cepivorum under controlled environmental conditions (De Oliveira et al., 1984). De Oliveira et al. (1984)

found that the severity of white rot of onion was reduced when seedling roots were immersed in a conidial suspension of C. minitans at transplanting time. Treated plants were then placed in soil artificially infested with sclerotia of S. cepivorum. Turner and Tribe (1975) placed sclerotia of S. trifoliorum on the surface of field plots and treated them with three levels of a C. minitans pycnidial dust preparation. As the level of applied inoculum increased, 88, 93 and 98 per cent infection of sclerotia occurred. Huang (1980b) conducted experiments in fields naturally infested with S. sclerotiorum. Coniothyrium minitans was added to the soil with the seed at planting time and reduced significantly the incidence of sclerotinia wilt of sunflower. These results have been discussed in the section on sclerotinia wilt of sunflower on pages 37-38. Coniothyrium minitans has also been examined as a biological control agent of S. sclerotiorum in bean (Howard and Dueck, 1980; Trutmann et al., 1982). This has been discussed in a previous section under white mold of bean on pages 45-46.

Environmental factors play a vital role in hyperparasitism of S. sclerotiorum by C. minitans. Trutmann et al. (1983) reported parasitism by C. minitans to be limited by high temperatures. Few sclerotia collected from bean crops were found to be parasitized during the summer months when ambient temperatures often exceeded 30 C. Turner and Tribe (1976) reported the optimum temperature for parasitism of sclerotia of S. sclerotiorum by C. minitans to be 20 C.

Use of C. minitans as a biological control agent has involved the use of different types of growth media. Tribe and Ahmed (1975) used

coarse milled rice soaked in water and autoclaved prior to inoculation with C. minitans. Various other types of media used for production of C. minitans are milled rice (Turner and Tribe, 1975, 1976; Ahmed and Tribe, 1977) and a barley, rye and sunflower seed mixture (1:1:1, v/v/v) (Huang, 1980b). Coniothyrium minitans has also been grown on autoclaved crushed sclerotia of S. sclerotiorum for biochemical studies (Jones et al., 1974; Webley, 1974) and Ahmed and Tribe (1977) indicated that C. minitans grows well on bran.

In some studies on biocontrol using C. minitans, the fungus has been prepared as a spore suspension. Under laboratory conditions, soaking sclerotia in a spore suspension of C. minitans yielded high levels of infection (Turner and Tribe, 1976). When sclerotia were placed on soil or sand and sprayed with a spore suspension of C. minitans, no infection occurred (Turner and Tribe, 1976). A similar experiment using pycnidial dust prepared from C. minitans grown on rice proved very successful (Turner and Tribe, 1976). Coniothyrium minitans prepared on rice has also been used successfully against white rot of onion (Sclerotium cepivorum) in greenhouse experiments (Ahmed and Tribe, 1977) and against Sclerotinia trifoliorum in field plots (Turner and Tribe, 1975). Clearly, the preparation of C. minitans for experimental use varies. For practical application, Turner and Tribe (1976) found pycnidial dust containing spores protected within broken or whole pycnidia to give good results. Addition to soil of propagules of a microorganism not embedded in a substrate generally results in a significant reduction in the propagule numbers (Baker, 1981). Propagules embedded in a substrate

have an increased inoculum potential over single propagules without substrate (Baker et al., 1984). The literature indicates that a number of substrates have been utilized for the growth of C. minitans. Providing the appropriate substrate is an important factor affecting the efficacy and longevity of the biocontrol agent in soil.

1.4 ANTAGONISM AMONG MICROORGANISMS

In terms of biological control, antagonists can be defined as "biological agents with the potential to interfere in the life processes of plant pathogens" (Cook and Baker, 1983). All classes of organisms include antagonists which may be viruses, fungi, nematodes, bacteria, actinomycetes, protozoa, nematodes, collembola and rotifers (Baker and Cook, 1982). The influence of such antagonists on plant pathogens is exerted through competition, antibiosis or exploitation (Baker, 1968).

Competition can be defined as active demand surpassing supply of condition or material (Clark, 1965). Conditions or material(s) involved may be water, oxygen, nutrients and space. Although Clark (1965) felt that microorganisms do not compete for water or space and rarely for oxygen, Baker and Cook (1982) felt that competition for oxygen and space as well as for nutrients in terms of biocontrol could be very important.

Competition for space may be involved in the prior colonization of a substrate by microorganisms (Leach, 1939; Barton, 1957; Bruehl and Lai, 1968). Earlier occupation generally leads to ultimate possession (Barton, 1961; Bruehl and Lai, 1966; Nash and Snyder,

1967). Possession of a substrate by a microorganism is generally retained even when that organism is confronted by vigorous saprophytes such as Trichoderma viride (Baker and Cook, 1982). Biological control through substrate occupation as a type of antagonism may also be effective against pathogens which possess high competitive saprophytic ability eg. Pythium spp. and Rhizoctonia solani. Cook (1970) reported that wheat straw allowed to decay on the soil surface prior to incorporation into soil was thoroughly colonized by airborne saprophytes. These fungi prevented colonization by Fusarium roseum f. sp. cerealis (Cooke) Snyder & Hansen 'Culmorum' following tillage. Fresh residue ploughed under immediately is more favorable to Fusarium as well as Rhizoctonia and Pythium because tissues are often not yet occupied and therefore available to colonization by facultative-type pathogens (Baker and Cook, 1982). Prior colonization of the ecological niche required by the pathogen has been suggested to explain, in part, biocontrol due to wilt-resistant soils in which crops susceptible to Fusarium wilt have been grown successfully. Alabouvette et al. (1979) reported that Fusarium solani (Mart.) Sacc. and F. oxysporum Schlecht. play an important role in certain soils in France. These soils are rich in montmorillonite clays and are suppressive to fusarium wilt. The montmorillonite clays reportedly allow the suppressive microflora to develop but do not inhibit the pathogen. Alabouvette et al. (1979) suggested that nonpathogenic Fusarium are favored in suppressive soils and the ecological niche required by the pathogen is previously occupied by these fungi.

Aside from competition for space, competition for nutrients may also occur. For competition in biological control to be effective, one or more essential elements must become limiting to inhibit the activity of the pathogen. The most common limiting factors to germination and penetration appear to be nitrogen and carbon (Baker, 1968). Competition between two fungi in the presence of limiting nitrogen has been demonstrated (Lindsey, 1965). Biological control where disease has been suppressed due to limiting levels of nitrogen has also been demonstrated. For example, Snyder et al. (1959) reported that additions of barley amendments to soil reduce crop losses due to bean rot caused by Fusarium solani. The indigenous soil microflora decomposed the barley amendments and in doing so utilized the soil nitrogen. This element is required by F. solani for germination (Griffin, 1964; Cook and Schroth, 1965) and penetration (Toussoun et al., 1960). Consequently, infection of the host did not occur. Nitrogen also appears to be a limiting factor in other biocontrol systems (Blair, 1943; Papavizas and Davey, 1961).

Competition for carbon can also be an important factor in biological control systems. Cook and Schroth (1965) found that germination of chlamydospores of F. solani f. sp. phaseoli in soil was limited due to reduced availability of carbon. Powelson (1966) found carbon to be the major factor limiting germination of Verticillium dahliae in soil. Maurer and Baker (1964) worked with biological control of bean root rot and also found carbon to be a limiting factor. In this system, chitin and lignin amendments were utilized and together reduced disease severity. Similar findings were reported

by Khalifa (1965). Addition of chitin to soil reduced significantly the incidence of pea wilt caused by Fusarium oxysporum f. sp. pisi (Jones) Snyder & Hansen. Microorganisms that antagonize the pathogen were thought to be stimulated by the addition of chitin to soil. Generally the addition of organic materials is responsible for the increase in population of the soil saprophytes. These microorganisms in turn are responsible for suppression of the plant pathogen.

A relatively new concept in biological control is competition for iron occurring in suppression. Kloepper et al. (1980) attributed suppression to Pseudomonas spp.. These bacteria produce extracellular siderophores which complex with iron and limit its availability to the pathogen. Soils can be altered from the conducive to the suppressive state with the addition of these bacteria. Conversely, suppressive soils can become conducive with the addition of ferric iron. The development of suppression through the addition of fluorescent Pseudomonas strain 10 or its siderophore pseudobactin, has been reported with both Fusarium and Gauemannomyces (Kloepper et al., 1980).

The second category of antagonism to be discussed is antibiosis which refers to the inhibition of one organism by a metabolite produced by another (Baker and Cook, 1982). Such metabolites include antibiotics as well as compounds such as lactic acid, alcohols, enzymes and other similar substances. This form of antagonism has certain advantages over competition and the third form of antagonism, hyperparasitism. For antibiosis to be effective, contact between the antagonist and subject is not necessary. The toxic substances may

diffuse in water-filled pores or water films through the soil or, in the case of a volatile, through air-filled pores (Baker and Cook, 1982). The zone of influence of an antibiotic is therefore greater than the volume of the microorganism. The distance of antagonism via antibiosis encompasses a larger area than that of hyperparasites and competitors and is therefore more effective and rapid. The end result of antibiosis is generally endolysis. The effects may continue for some time following cessation of colony growth because antibiotic release still continues briefly after cell death. With antibiotic release during and following active growth of the colony, antibiosis tends to provide more of a constant state of antagonism than does competition or hyperparasitism (Baker and Cook, 1982).

An abundance of nutrients provides the best conditions under which antibiosis will be effective. Very little antibiotics will be produced except where leakage from plant parts occurs or where organic debris is found. Consequently, to enhance antibiosis by increasing production of antibiotics and/or other toxic metabolites, an adequate supply of organic amendments is required. Biological control of common scab of potato using soybean refuse is such an example (Weinhold and Bowman, 1968). The bacterium Bacillus subtilis colonizes soybean refuse and maintains possession of the substrate through production of a metabolite. Annual additions of soybean substrate to the tillage layer increased the number of zones of antibiosis so that activities of Streptomyces became restricted over time. When application of soybean was discontinued, scab severity increased, strengthening the conviction that it is Streptomyces that

is inhibited by antibiotics produced by B. subtilis on soybean refuse. Weinhold and Bowman (1968) reported no increase in scab severity occurring with the application of soybean refuse. However, when barley amendments were used, the population of Streptomyces increased. They postulated that, in extracts of soybean tissue, B. subtilis was able to produce two to three times as much antibiotic as in similar barley extracts.

Crown gall, caused by Agrobacterium tumefaciens (Smith & Townsend) Conn. has been controlled biologically with an antibiotic-producing suppressive agent closely related to the pathogen (Moore and Guylyn, 1979; Kerr, 1980). The suppressive agent, A. radiobacter (Beijerinck and van Delden) Conn. strain 84 controls the pathogenic strain. New and Kerr (1972) found that when they treated tomato seeds and roots with the nonpathogenic strain, they recorded 78 and 95% control of crown gall, respectively. Following root or seed inoculation, the nonpathogenic strain was found to establish in the rhizosphere and provide the host with protection against the pathogenic strain at specific points such as wounds. Subsequent research, discussed by Rovira (1982) indicates that an antibiotic known as agrocin 84 is produced by the nonpathogenic suppressive Agrobacterium strain. This antibiotic inhibits the common pathogenic biotype which causes crown gall on peaches and almonds.

Another example of biological control associated with production of toxic metabolites is the resistance of shortleaf pine seedlings with naturally occurring ectomycorrhizae to infection by zoospores of Phytophthora cinnamomi Rands (Marx, 1972; 1973). Resistance to P.

cinnamomi was very effective with the fungus Leuopaxillus cerealis var. piceina (Peck) ined. [syn. C. piceina Peck, L. albissimus (Pk.) Sing. var piceinus (Peck) Singer & Smith and L. cerealis (Lasch) Sing.] (Marx 1969; 1972). When grown in association with shortleaf pine, this fungus produces diatretyne nitrile which totally inhibits zoospore germination of the pathogen at 2 ppm. Many root-infecting fungi and bacteria were also inhibited. Krupa and Fries (1971) reported increased production of volatiles, such as sesquiterpenes and terpenes, in association with the ectomycorrhizal fungus Boletus variegatus Fr. as compared to the noninoculated controls. By producing antibiotics, the antagonists were suppressed sufficiently for the mycorrhizal fungi to gain dominance in the rhizosphere and to enter roots. The mycorrhizal roots then exhibited an increased production of volatiles which further inhibited the root pathogens.

Examples of antagonism where antibiosis may possibly be responsible for reduction in disease include Penicillium notatum Westling and two species of Aspergillus against Pythium debaryanum Hesse (Joshi and Keshwal, 1969), Penicillium patulum Bainier against Phytophthora cryptogea Pethybr. & Laff. (El-Goorani et al., 1976), Streptomyces griseus (Krain.) Waks. & Henrici against Phomopsis sclerotioides Kesteren (Ebben and Spencer, 1978), Bacillus subtilis against Sclerotium rolfsii (Agrawal et al., 1977), Bacillus subtilis against Sclerotium cepivorum (Utkhede and Rahe, 1980), Corticum sp. against Pythium ultimum Trow (Hoch and Abawi, 1979) and Fusarium oxysporum and Penicillium patulum against Gaeumannomyces graminis (Sacc.) Arx & Olivier var tritici Walker (Sivasithamparam and Parker, 1980).

The third form of antagonism is hyperparasitism. There are a number of means by which hyperparasites attack other organisms. The components of the host organism may be penetrated directly by a hyperparasitic fungus. Such is the case with Gliocladium virens and its host Rhizoctonia solani (Tu and Vaartaja, 1981). Once contact has been made between G. virens and R. solani, appressoria are formed and host cells are penetrated. Intracellular hyphae develop followed by collapse and death of host cells. Another hyperparasite, Coniothyrium minitans also penetrates its host Sclerotinia sclerotiorum but does so without the formation of appressoria (Huang and Hoes, 1976; McLaren et al., 1986). Hyphal walls of the host are penetrated by C. minitans from both the inside and the outside. As a result of infection, disintegration of host cytoplasm and collapse of cell walls occurs. Huang and Hoes (1976) suggest that enzymatic action as well as physical forces may be involved in penetration of Sclerotinia hyphae by C. minitans. The hyperparasitism of R. solani by Penicillium vermiculatum also exhibits direct penetration without the formation of appressoria (Boosalis, 1956). However, cell walls of the host are penetrated from the outside only and, although cell walls often collapse, they do not disintegrate. Parasitism by penetration is also evident between Alternaria brassicae (Berk.) Sacc. and Nectria inventa Pethybr. (Tsuneda et al., 1976).

Another means by which fungal structures are attacked by other microorganisms involves parasitism by means of contact without penetration (Tsuneda et al., 1976). This mode of parasitism also occurs in the N. inventa-A. brassicae system. Hyphae of N. inventa

coil around the host hyphae and commonly produce appressorium-like bodies that attach to the Alternaria hyphae. At these points of contact, an abnormal response of the host cells usually occurs. Eventually disruption of the cellular organelles follows leaving only remnants of membranous elements and ribosomes.

A similar relationship occurs between Gliocladium roseum and Rhizoctonia solani (Jager et al., 1979) as well as between G. catenulatum and S. sclerotiorum (Huang, 1978). In the first system, hyphae of G. roseum commonly coil around Rhizoctonia hyphae but parasitism of the host is rarely observed. Death of Rhizoctonia hyphae by coiling and clamping was also reported by Barnett and Lilly (1962). With the G. catenulatum-S. sclerotiorum system, Huang (1978) observed direct hyphal contact but no penetration and no intracellular hyphae. Host hyphal cells eventually disintegrated or collapsed as a result of infection.

In summary, the influence of antagonistic microorganisms on plant pathogens can therefore be categorized as competitive, hyperparasitic or operative through antibiosis. However, establishing which mechanism is involved in each case is difficult. In some cases, mechanisms may not be distinct. For example, hyperparasitism and antibiosis may be involved simultaneously or act at different times within the same system. Once knowledge of the mechanism is obtained, it is critical to assess the capabilities of the antagonist to function in natural soils. In many cases, the mechanism has been established in vitro but may not be operative in vivo. Knowledge of the mode of action of an antagonist and its ability to function in

vivo are critical to establishing an effective management system under natural conditions (Baker and Cook, 1982).

Chapter II

HYPHAL INTERACTIONS BETWEEN SCLEROTINIA SCLEROTIORUM AND TALAROMYCES FLAVUS

2.1 INTRODUCTION

Talaromyces flavus (Klocker) Stolk and Sampson (conidial state Penicillium vermiculatum Dangeard) was reported to be antagonistic to the plant pathogens Sclerotinia sclerotiorum (Lib.) de Bary, Rhizoctonia solani Kuehn, Verticillium albo-atrum Reinke & Berth. and V. dahliae Kleb. (Boosalis, 1956; Su and Leu, 1980; Dutta, 1981; Marois et al., 1982). Boosalis (1956) observed that T. flavus invaded hyphae of R. solani directly by producing penetration pegs. These pegs developed either from a mycelium coiling around the host hyphae or from a hypha in direct contact with the host. Dutta (1981) suggested that the mode of parasitism of V. albo-atrum by T. flavus involved antibiosis and competition. Marois et al. (1982) reported the fungus to be antagonistic towards V. dahliae under greenhouse and field conditions but did not determine the mode of action. Su and Leu (1980) demonstrated that more than 70% of the sclerotia of S. sclerotiorum became infected when inoculated with a conidial suspension of the hyperparasite and incubated at 24-26 C for 3-6 weeks.

In Canada, sclerotinia wilt is a serious disease of sunflower (Hoes and Huang, 1976) caused by infection of roots by hyphae from

myceliogenic germination of sclerotia (Huang and Dueck, 1980). Huang (1980b) demonstrated effective control of this disease in the field with the use of the hyperparasite Coniothyrium minitans. The success of C. minitans is based on its ability to parasitize sclerotia of S. sclerotiorum, the primary source of inoculum for wilt in sunflower. Hyperparasitic invasion of sclerotia by T. flavus has been reported (Su and Leu, 1980) but no information on hyphal interaction between the two fungi is available. Therefore, a study[1] was conducted to investigate, using light and scanning electron microscopy, the mode of hyperparasitism of S. sclerotiorum by T. flavus.

2.2 MATERIALS AND METHODS

One isolate of Sclerotinia sclerotiorum (SS3) obtained from diseased sunflower plants near Morden, Manitoba, and one isolate of Talaromyces flavus (DAOM 172557) from the rhizosphere of sunflower at the Morden Research Station of Agriculture Canada were used. Two methods were used to establish dual cultures on slides coated with modified Wilbrink Agar (Koike, 1965). The agar was comprised of 5.0 g of peptone, 1.0 g of KH_2PO_4, 0.5 g of $MgSO_4 7H_2O$, 10.0 g of sucrose, 15.0 g of agar and 1 L of distilled water. In method one, the medium-coated slides were inoculated with agar pieces bearing mycelia of S. sclerotiorum. These slides were placed in moist chambers and incubated at 18 or 20 C. After 2 days, spores of T. flavus were placed, with the tip of an inoculating needle, approximately 10 mm from the edge of the colony of S. sclerotiorum. After a further incubation of 2 days at 18 or 20 C in the moist chamber, agar blocks

[1] This chapter has been published as a paper in Can. J. Plant Pathol. 8: 43-48.

from the contact areas of the two fungi were removed for study. For method two, medium-coated slides were simultaneously inoculated with mycelia-bearing agar pieces of the host and of the hyperparasite at the same time. The slides were incubated at 18 or 20 C in moist chambers for 5 days. Areas of hyphal interaction between the two fungi were then removed for study using light and scanning electron microscopy (SEM).

For light microscopy, the fungal material was stained with cotton blue in lactophenol (1% cotton blue dissolved in equal parts of lactic acid, phenol, glycerine and water) and photographed on a Zeiss standard microscope. For SEM, agar blocks bearing mycelia of T. flavus and S. sclerotiorum were processed in two ways. In the first method, agar blocks were fixed in 5% glutaraldehyde in 0.025 M phosphate buffer (pH 6.8) at 0 C for 3 h. The specimens were rinsed in buffer, post-fixed in 2% osmium tetroxide in 0.025 M phosphate buffer (pH 6.8) at 0 C for 2 h, rinsed again in buffer, warmed to room temperature, and dehydrated in an ethanol series. Following ethanol dehydration, the material was critical-point dried using carbon dioxide, mounted on SEM stubs, sputter coated with gold and examined on a Cambridge Stereoscan Mark 11A scanning electron microscope. For the second method, agar blocks were fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.0) at room temperature for 1 h. Following buffer-rinsing, the specimens were post-fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer at room temperature for 1 h. The material was rinsed in buffer, dehydrated through an ethanol series and prepared as before for examination on a Hitachi S-500 scanning electron microscope.

2.3 RESULTS

In dual culture, the hyperparasite T. flavus was readily distinguishable from its host S. sclerotiorum by the fine hyphae it produced as opposed to the coarse hyphae of the host. Initially, hyphae of T. flavus established an intimate contact with the host by coiling around the host hyphal cell (Fig. 1) and by producing small hyphal branches that penetrated host cell walls without forming appressoria (Figs. 2, 3, 4, 6 and 7). Most of the side branches produced by hyphae of T. flavus situated near the host grew unilaterally toward the hyphae of S. sclerotiorum (Figs. 2, 3, 4, and 10). Penetration of the host was frequently observed (Figs 3, 6 and 7). Hyphae of T. flavus often grew through host hyphal cells at an angle perpendicular to the host hypha (Fig. 4). A hyperparasitic hypha within a host cell rarely ran parallel to the host hypha.

Invasion by T. flavus, caused the host cytoplasm to aggregate and form numerous fine granules. These granulated hyphal cells (Figs. 2,3 and 4) were lightly stained in contrast to the healthy cells (Fig. 5) which were densely stained by cotton blue in lactophenol. Some host cells adjacent to the infected cell also showed granulation of the cytoplasm even though they were not in direct contact with the hyperparasite (Fig. 4). Frequently, disintegration of the cytoplasm occurred following penetration even though extensive growth of the hyperparasite within the host cell was not apparent (Figs. 3 and 10). Plasmolysis was also evident in some of the host cells infected by the hyperparasite (Figs. 4 and 10).

As the host-hyperparasite interaction progressed, the coiling effect intensified as the hyphae of T. flavus branched repeatedly on the host surface (Figs. 6,7,8,9 and 10). Cytoplasmic granulation became more pronounced, cell contents disintegrated and host hyphal cells appeared empty (Fig. 10). Host cell walls remained intact even after the cell contents were completely disintegrated. Finally, the host hyphae collapsed, their cell walls appearing rough and wrinkled (Fig. 11). Coiling of the hyperparasite around hyphae of S. sclerotiorum was so profuse in the late stages of infection, that the host hypha was almost totally covered by hyphae of the hyperparasite (Fig. 12).

2.4 DISCUSSION

This study shows that T. flavus is destructive to hyphae of S. sclerotiorum, with hyphae of the hyperparasite invading the host hyphae without the aid of appressoria. This mode of penetration and the coiling of the hyperparasite around the host hyphae are similar to the behavior of T. flavus on R. solani, described by Boosalis (1956).

Of the two methods used to examine hyphal interactions between S. sclerotiorum and T. flavus, method one was preferred because the number of hyperparasitic hyphae coming in contact with the edge of the host colony was reduced. This facilitated microscopic observation of sites of interaction between the two fungi.

The parasitic activity of T. flavus on S. sclerotiorum resulted in deterioration of the host hypha and granulation of the cytoplasm in hyphal cells. Observations that the granulation may extend from the

cells infected by T. flavus to the neighboring hyphal cells which were not in direct contact with the hyperparasite suggest the possibility that toxic substances may be involved in pathogenesis. Talaromyces flavus has been reported to produce an inhibitor which reduced the growth of V. albo-atrum in culture (Dutta, 1981) and the viability of microsclerotia of V. dahliae (Fravel et al., 1985a). The antibiotics talaron (Mizuno et al., 1974), vermiculine (Fuska et al., 1972), vermistatin (Fuska et al., 1979a), and vermicillin (Fuska et al., 1979b) are produced by P. vermiculatum. Metabolites may be involved in the deterioration of Sclerotinia hyphae in the present study as talaron exhibits antifungal activity (Mizuno et al., 1974) and vermiculine is closely related to the antifungal agent pyrenophorin (Boeckman et al., 1974). Although Boosalis (1956) reported that P. vermiculatum did not produce toxic substances capable of injuring hyphae of R. solani, T. flavus may produce compounds destructive to hyphae of S. sclerotiorum.

Hyphal cells of S. sclerotiorum eventually collapsed as a result of infection by T. flavus but host cell walls remained intact even after cell contents were completely destroyed. This suggests that cell wall degrading enzymes may not play a major role in the hyperparasitism of S. sclerotiorum by T. flavus. A similar phenomenon was seen in the hyperparasitism of R. solani by P. vermiculatum where cell walls often collapsed but did not disintegrate (Boosalis, 1956). However, the mode of hyperparasitism of T. flavus observed in this study differs from that of C. minitans, another important hyperparasite of S. sclerotiorum (Huang, 1980b). Coniothyrium minitans does not coil

intensively around hyphae of S. sclerotiorum (Huang and Hoes, 1976) and the host cell walls are often disintegrated by the enzymes produced from the hyperparasite (Jones et al., 1974; Jones and Watson, 1969).

The growth of hyphal branches of T. flavus toward hyphae of S. sclerotiorum was readily apparent, suggesting that positive tropism may be involved in the host-hyperparasite relationship. A similar phenomenon is seen with Gliocladium catenulatum Gilman & Abbott and its host S. sclerotiorum where growth of hyphal branches of the hyperparasite toward S. sclerotiorum frequently occurs (Huang, 1978).

Our study indicates that, in addition to the infection of sclerotia by T. flavus (Su and Leu, 1980), the hyperparasite is capable of invading actively growing hyphae of the host. Destruction of sclerotia and hyphae of the host by T. flavus will influence the ability of S. sclerotiorum to survive.

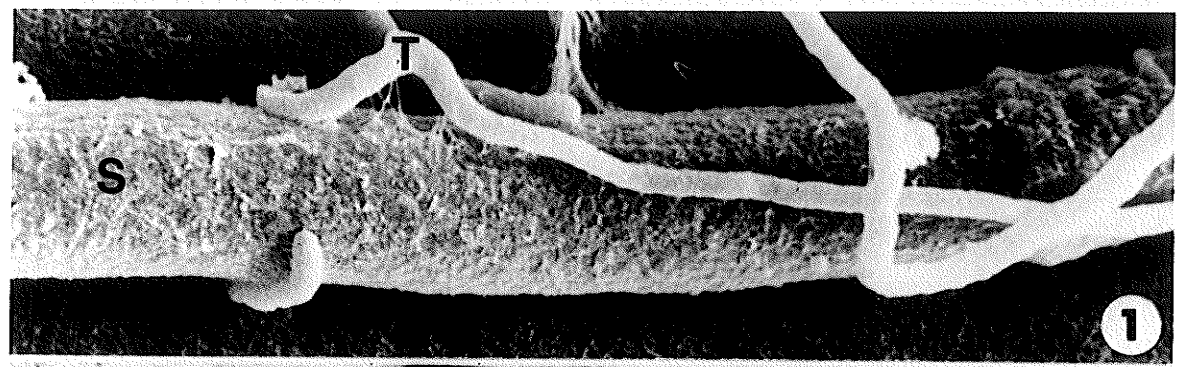
Talaromyces flavus was reported to be an effective biological control agent for verticillium wilt of eggplant in the greenhouse as well as in the field (Marois et al., 1982). Similar results have been found in a three-year study using T. flavus for control of sclerotinia wilt of sunflower in the field (D.L. McLaren, H.C. Huang and S.R. Rimmer, unpublished data). Thus, in addition to the control of verticillium wilt of eggplant (Marois et al., 1982), T. flavus may also have potential for the control of sclerotinia wilt of sunflower.

Plate 1. Hyphal interactions between T. flavus and S. sclerotiorum during the early stages of interaction (McLaren et al., 1986).

Figure 1. A SEM micrograph showing a hypha of T. flavus (T) coiling around the host hypha S. sclerotiorum (S). x 3125. (McLaren, 1983).

Figures 2, 3 and 4. Light micrographs showing invasion of a host hypha (S) by short hyphal branches of T. flavus (arrows). Note granulation of the cytoplasm in the infected cells (Figs. 2 and 3) and the neighboring non-infected cells (Fig. 4). Material stained with lactophenol cotton blue. Fig. 2, x 910; Fig. 3, x 1375; Fig. 4, x 430.

Figure 5. A light micrograph showing young and old healthy hyphae of S. sclerotiorum stained darkly with lactophenol cotton blue. x 240.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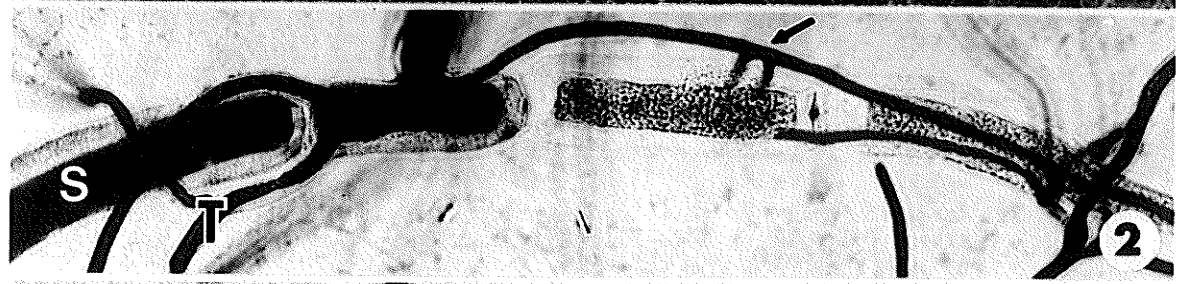
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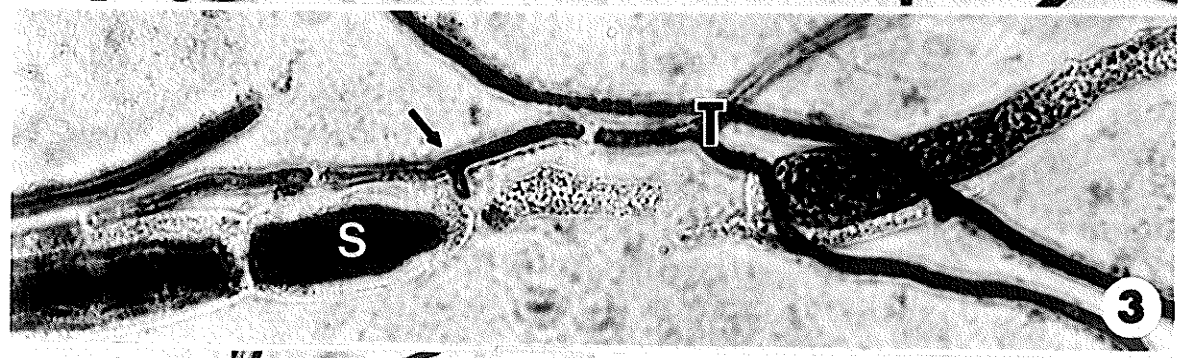
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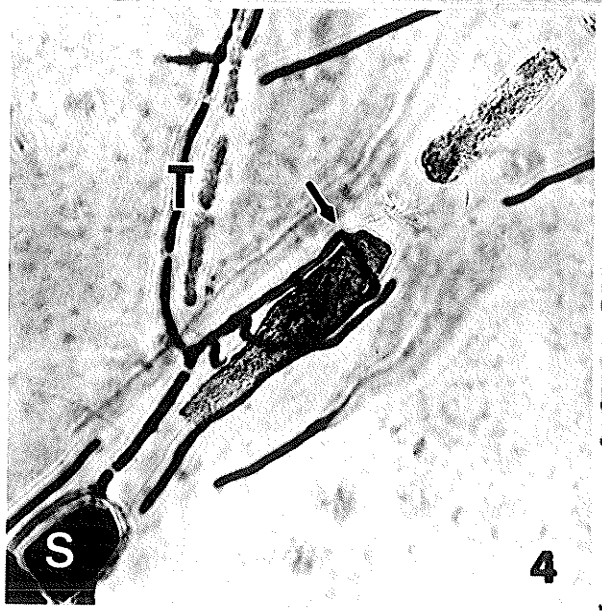
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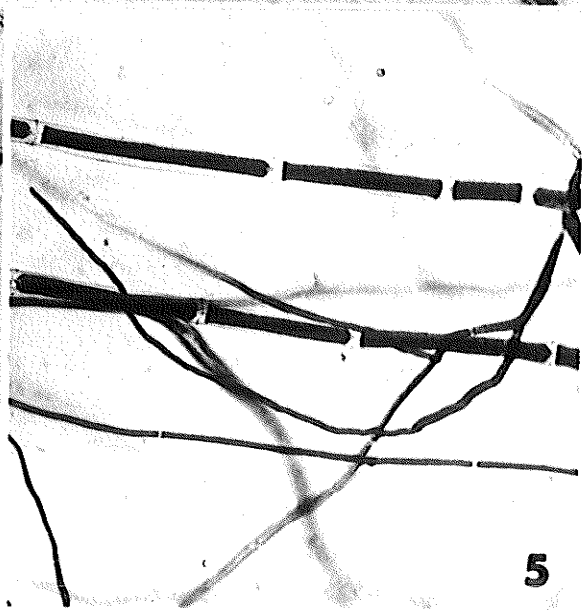
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Plate 2. Hyphal interactions between T. flavus and S. sclerotiorum as the infection process progressed (McLaren et al., 1986).

Figures 6, 7, 8, 9 and 10. SEM (Figs. 6, 7 and 8) and light (Figs. 9 and 10) micrographs showing hyphal interactions between T. flavus and S. sclerotiorum as the infection process progressed. Note the repeated branching of the T. flavus hyphae on the host surface (Figs. 6, 7 and 8) and the penetration of the host hyphae by short, lateral branches of T. flavus (T) (arrows, Figs. 6 and 7). The breakage of the host cell wall is discernible (Fig. 6). Note also the hyphal coiling of T. flavus around the host hypha (Figs. 9 and 10), the growth of many hyphal branches of T. flavus toward the host (Fig. 10), and the disintegration of host cell contents (arrows, Fig. 10). Figs. 9 and 10 stained with lactophenol cotton blue. Fig. 6, x 6080; Fig. 7, x 6100; Fig. 8, x 2250; Fig. 9, x 460; Fig. 10, x 460.

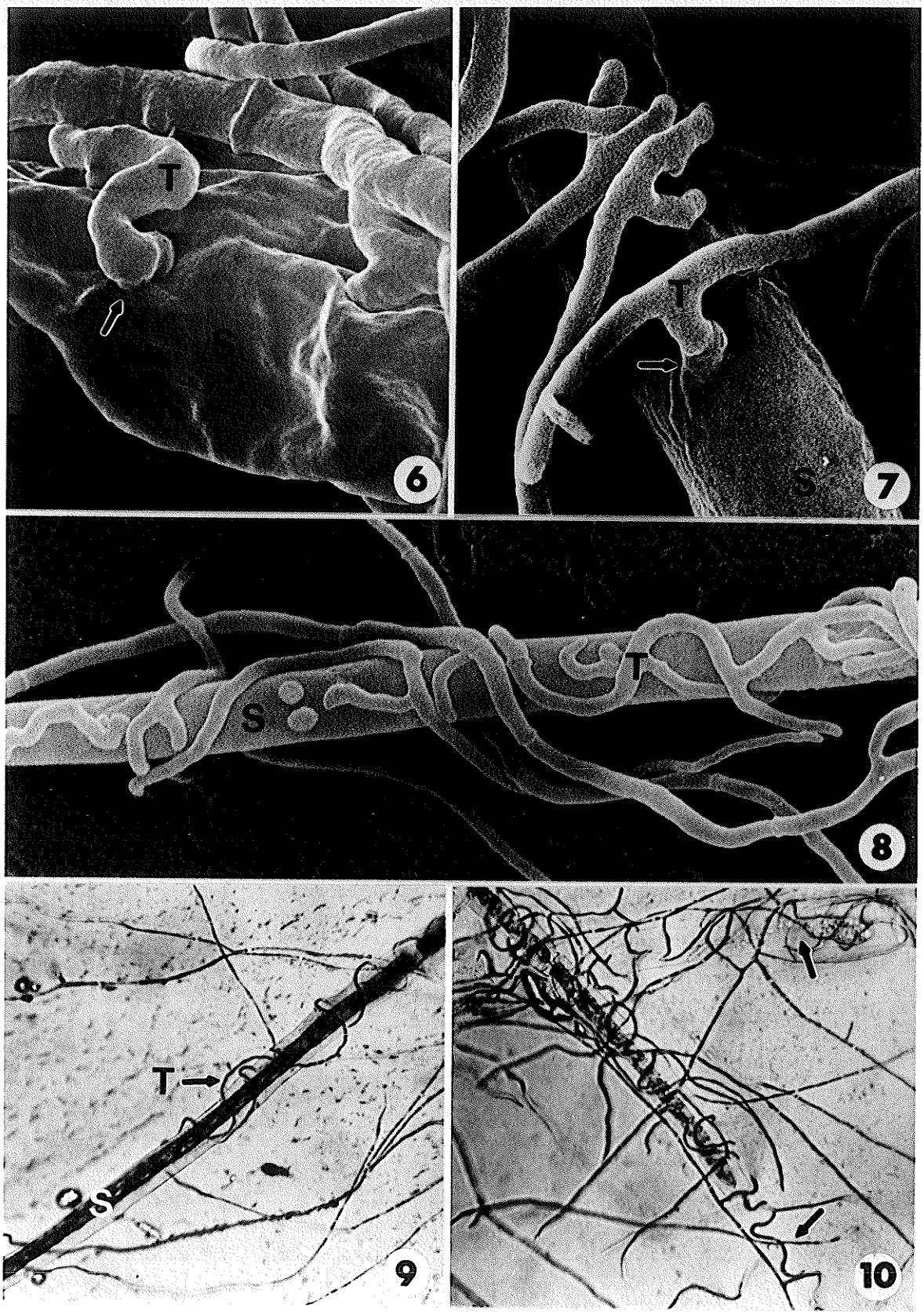
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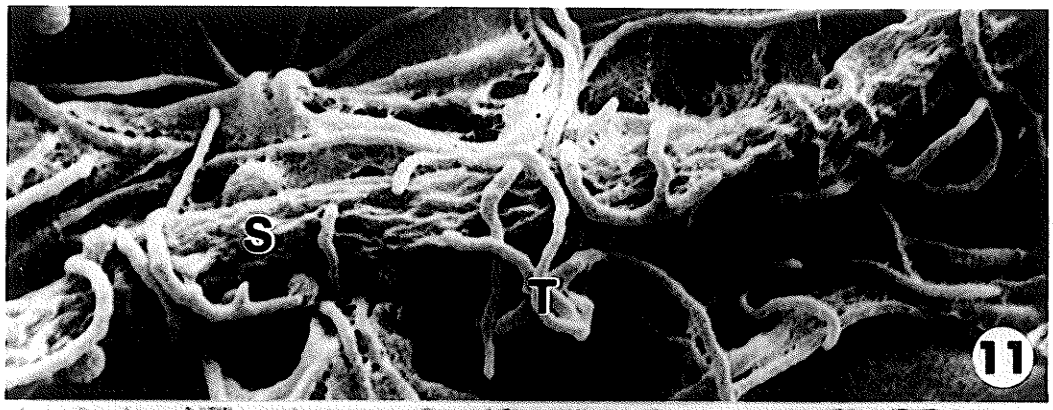
Plate 3. Hyphal interactions between T. flavus and S. sclerotiorum during the late stages of infection (McLaren et al., 1986).

Figures 11 and 12. SEM (Fig. 11; McLaren, 1983) and light (Fig. 12) micrographs showing hyphal interactions during the late stages of infection. Note the intensive coiling of the hyperparasitic hyphae around the host (Figs. 11 and 12) and the distorted, wrinkled appearance of the host hypha (Fig. 11). Fig. 11, x 3240; Fig. 12, x 550.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Chapter III

ULTRASTRUCTURAL STUDIES ON INFECTION OF SCLEROTIA OF SCLEROTINIA SCLEROTIORUM BY TALAROMYCES FLAVUS

3.1 INTRODUCTION

Talaromyces flavus (Klocker) Stolk and Sampson (anamorph Penicillium vermiculatum (Dangeard) is a hyperparasite of Sclerotinia sclerotiorum (Lib.) de Bary (Su and Leu, 1980; McLaren et al. 1986) and Verticillium dahliae (Kleb.) (Marois et al., 1984; Fravel et al., 1987a; 1987b). This fungus has been reported to reduce the germinability and viability of microsclerotia of V. dahliae (Marois et al., 1984; Fravel et al., 1987a; 1987b). Fravel et al. (1987b) suggested that a metabolite from T. flavus may be involved in the control of V. dahliae. Kim et al. (1988) further identified the metabolite as glucose oxidase. Other toxic metabolites produced by T. flavus are talaron (Mizuno et al., 1974), vermiculine (Fuska et al., 1972), vermistatin (Fuska et al., 1979a) and vermicillin (Fuska et al., 1979b).

Talaromyces flavus attacks both mycelia (McLaren et al., 1986) and sclerotia (Su and Leu, 1980) of S. sclerotiorum. McLaren et al. (1986) found that T. flavus invaded hyphae of S. sclerotiorum by direct penetration of the cell wall without the formation of appressoria. This mode of penetration is similar to that of T. flavus on Rhizoctonia solani, described by Boosalis (1956). The coiling

effect of T. flavus on its host R. solani (Boosalis, 1956) was also observed on the host S. sclerotiorum by McLaren et al. (1986). Su and Leu (1980) reported that more than 70% of the sclerotia of S. sclerotiorum were infected when inoculated with a conidial suspension of the hyperparasite and incubated at 24-26 C for 3-6 weeks. They also reported that inoculated sclerotia, buried in soil at depths of 5-20 cm, were lysed after a 15 or 30-day burial period.

In Canada, sclerotinia wilt is a serious disease of sunflower (Hoes and Huang, 1976) that results from infection of roots by hyphae from myceliogenic germination of sclerotia (Huang and Dueck, 1980). Huang (1980b) reported effective control of this disease under field conditions with the use of the hyperparasite Coniothyrium minitans Campbell. The success of C. minitans is based on its ability to parasitize and destroy the sclerotia of S. sclerotiorum, the primary source of inoculum for wilt in sunflower. Although T. flavus is parasitic to sclerotia of S. sclerotiorum (Su and Leu, 1980), no information is available on the host-hyperparasite relationship at the ultrastructural level. This chapter[2] reports results from a study, using transmission electron microscopy, on the mode of hyperparasitism of T. flavus on sclerotia of S. sclerotiorum.

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[2] This chapter has been accepted for publication in Can. J. Bot. (in press).

3.2 MATERIALS AND METHODS

The isolate of T. flavus (DAOM 172557) used in this study was isolated from the rhizosphere of sunflower at the Morden Research Station in Manitoba. The isolate of S. sclerotiorum (SS3) was isolated from diseased sunflower plants near Morden, Manitoba. Talaromyces flavus was grown on potato dextrose agar (PDA) for 15 days at room temperature. The cultures were washed with sterile, distilled water and the spore suspension was poured through two layers of sterile cheesecloth and adjusted to a concentration of 1.4×10^7 spores ml^{-1} Sclerotia of S. sclerotiorum obtained from 17-day old PDA cultures were surface sterilized in 95% ethanol for 90 seconds and soaked in the spore suspension of T. flavus for 5 minutes. The sclerotia were placed on moist filter paper in Petri dishes and incubated at 20 C in the dark. After incubation for 3, 7 and 12 days, the sclerotia were removed and processed for study by transmission electron microscopy (TEM).

For TEM, sclerotia were fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.0, at room temperature for 4 h, rinsed in buffer and postfixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.0, at room temperature for 4 h. Following buffer rinsing, the sclerotia were dehydrated in an ethanol series, embedded in Spurr's resin (Spurr, 1969) and placed in a polymerization oven for 8 h at 70 C. Silver to gold sections (approximately 80 nm) were cut using a Reichert OM U3 ultramicrotome, stained with uranyl acetate and lead citrate and examined in an H-500 Hitachi transmission electron microscope.

3.3 RESULTS

A sclerotium of S. sclerotiorum is composed of three layers of tissues, namely the rind, cortex and medulla. The rind consisted of a layer of two to three cells that were characterized by thick, melanized cell walls (Fig. 1a). The cortex, located beneath the rind, was two to four cells thick and distinguished from the rind by thin, nonmelanized cell walls (Fig. 1b). The medullary tissue comprised the large central portion of the sclerotium and had cells with thin, nonmelanized cell walls surrounded by a thick fibrous layer (Figs. 1b and 1c). In a sclerotium infected by T. flavus, hyphal cells of the hyperparasite were present in all three layers of tissues (Fig. 2).

Infection of the rind tissue of S. sclerotiorum by T. flavus occurred as early as three days following inoculation. Direct penetration of the rind cell walls by hyphae of the hyperparasite was observed (Figs. 3,4 and 5). Etching of the cell walls at each penetration site was evident (Figs. 4 and 5). The progress of hyperparasitism resulted in severe cell-wall degradation on numerous rind cells of the outermost layer (Fig. 6). Extensive growth and ramification of the hyperparasite in the rind caused disruption of the host cytoplasm and the breakdown of cell walls (Figs. 2 and 7).

Within the cortex, extensive proliferation of T. flavus, both intercellularly and intracellularly, was observed after 7-12 days of interaction with the host (Figs. 2 and 8). Degradation of cell walls and disintegration of cytoplasm were evident in the infected cortical tissue (Fig. 8).

A few hyphal cells of the hyperparasite were observed in the medullary tissue as early as three days after inoculation (Fig. 9). Although disintegration of the cytoplasm occurred, the structure of cell walls and fibrous layers remained intact in most of the affected cells. As the interaction progressed to 7 and 12 days, the frequency of hyphae of the hyperparasite in the medullary tissue increased (Figs. 2 and 10). They were observed in the intercellular and intracellular spaces as well as within the fibrous layer (Figs. 2 and 10). Hyphae of T. flavus grew from one host cell to another (Fig. 10). Most of the infected cells, with signs of disintegration of cytoplasm and collapse of the cell, showed intensive colonization by the hyperparasite (Fig. 2). Eventually the fibrous layers deteriorated and cell walls were destroyed leading to collapse of the medullary tissue.

3.4 DISCUSSION

This study confirms a previous report (Su and Leu, 1980) that T. flavus is a destructive hyperparasite capable of invading sclerotia of S. sclerotiorum. Sclerotia inoculated with T. flavus were parasitized after 3, 7 and 12 days of incubation. Etching of the cell walls of sclerotia of S. sclerotiorum by T. flavus was evident in the TEM micrographs. This suggests that wall-lysing enzymes of T. flavus may play a significant role in cell-wall dissolution of the melanized rind and the nonmelanized cortex and medulla. This phenomenon is similar to the infection of sclerotia of S. sclerotiorum by C. minitans reported by Huang and Kokko (1987). One of the principal components of the cell walls of sclerotia (Jones, 1970; Saito, 1977) and hyphae

(Jones and Watson, 1969) of S. sclerotiorum is ß-glucan. Jones and Watson (1969) reported that pseudoparenchymatous and hyphal walls of C. minitans contained ß-glucanase and chitinase. Jones et al. (1974) attributed the degradation of ß-glucan to the presence of ß-(1->3)-glucanases produced by C. minitans. Huang and Kokko (1987) suggested that enzymes such as ß-(1->3)-glucanases may be critical to the degradation of cell walls of sclerotia of S. sclerotiorum by C. minitans. Whether or not T. flavus produces glucan-lysing enzymes similar to those of C. minitans warrants further investigation.

Dark pigments, melanins, are found in the rind cell walls of sclerotia of S. sclerotiorum (Jones, 1970) and are known to be resistant to decay (Bloomfield and Alexander, 1967; Jones and Webley, 1968). Fungi with melanins in their cell walls are more resistant to decay than are those lacking these dark-colored polymers (Martin et al., 1959; Bloomfield and Alexander, 1967; Hurst and Wagner, 1969; Wagner and Rubinska, 1971). Huang and Kokko (1987) found that the degradation of the melanized rind cells of S. sclerotiorum by C. minitans was slow compared with the nonmelanized cortical and medullary cells. Huang (1983b) observed that tan sclerotia of S. sclerotiorum were much more susceptible to infection by C. minitans than black sclerotia. Melanin deposits in the rind cell walls of tan sclerotia are almost negligible (Huang and Kokko, 1989). In our study, although degradation of cell walls occurred throughout the sclerotium, rind cell walls may be more resistant to decay by T. flavus than the walls of the cortical and medullary cells.

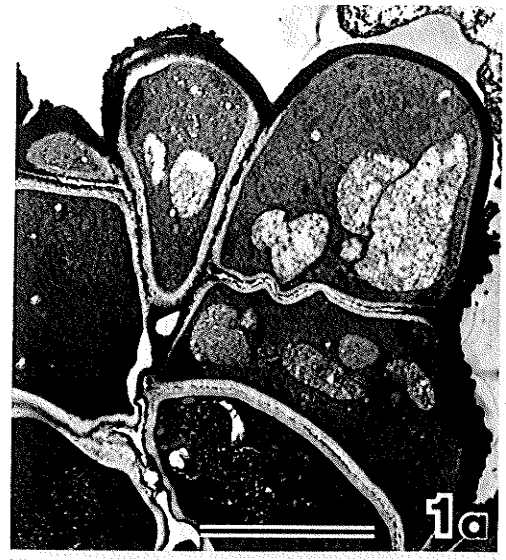
Talaromyces flavus affects the survival of sclerotia of V. dahliae (Fravel et al., 1987a; 1987b) and hyphae (McLaren et al., 1986) as well as sclerotia (Su and Leu, 1980) of S. sclerotiorum. Our study indicates that the survival of sclerotia of S. sclerotiorum is affected by the physical presence of hyphae of T. flavus within the sclerotium. Wall-lysing enzymes may also be involved in the destruction of sclerotia. Previous studies indicated that T. flavus produces a metabolite (Kim et al., 1986, 1988; Fravel et al., 1987a; 1987b) and four antibiotics (Fuska et al., 1972, 1979a, 1979b; Mizuno et al., 1974). Whether or not any of these substances are involved in the hyperparasitism of sclerotia of S. sclerotiorum by T. flavus remains unknown.

This study indicates that T. flavus is a hyperparasite capable of parasitizing and destroying sclerotia, the primary source of inoculum of S. sclerotiorum. Results of field studies with T. flavus showed that the hyperparasite was effective in reducing the inoculum potential of S. sclerotiorum in field bean (D.L. McLaren, H.C. Huang and S.R. Rimmer, unpublished data) and reducing the incidence of sclerotinia wilt of sunflower (McLaren et al., 1985). Talaromyces flavus was also reported to reduce the germinability and viability of microsclerotia of V. dahliae (Marois et al., 1984; Fravel et al., 1985a; 1987a; 1987b) and to be an effective biological control agent for verticillium wilt of eggplant (Marois et al., 1982). Talaromyces flavus appears to be nonhost specific and may have potential as a biological control agent for important soilborne pathogens such as S. sclerotiorum and V. dahliae.

Plate 4. Comparison of a healthy sclerotium of *S. sclerotiorum* to one infected by *T. flavus*.

Figure 1. A healthy sclerotium of *S. sclerotiorum* showing the rind (Fig. 1a), cortex (C) (Fig. 1b) and medulla (M) (Figs. 1b and 1c). Scale bars = 5 µm.

Figure 2. A sclerotium infected by *T. flavus* (Tf) with numerous hyphal cells of the hyperparasite (Tf) in all of the tissue zones. Note the signs of dissolution of some of the cortical and medullary cells (asterisks). Scale bar = 5 µm.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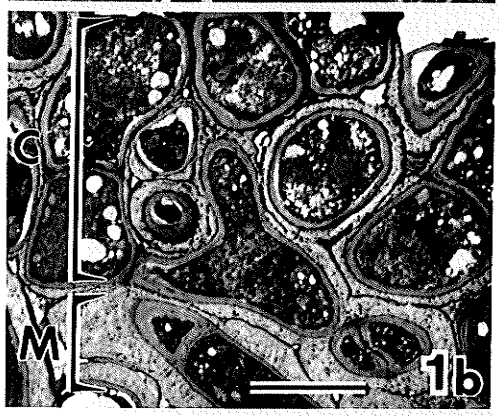
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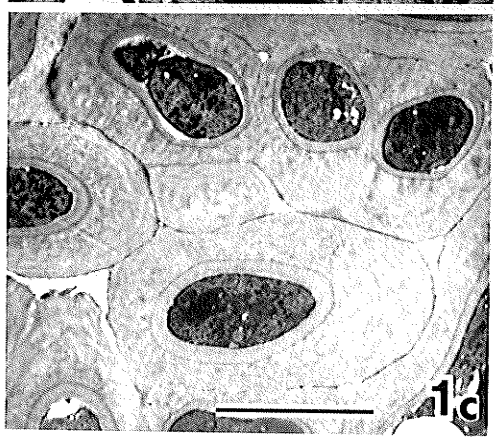
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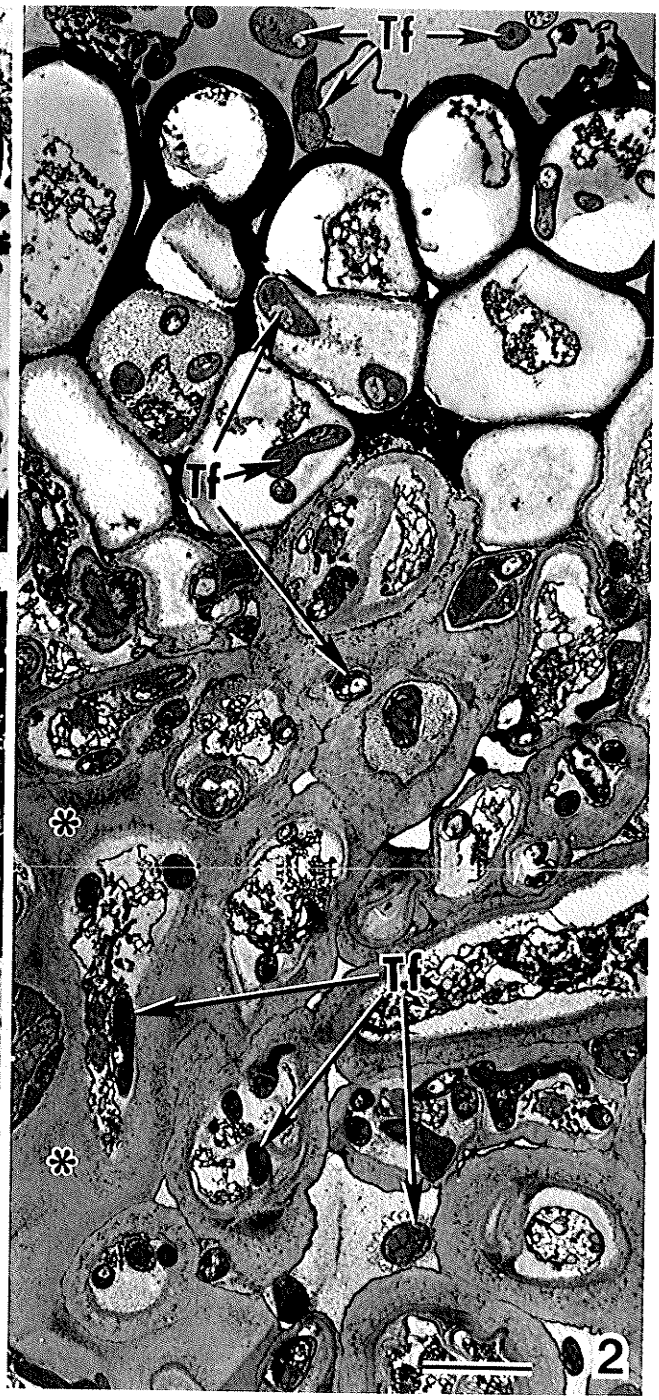
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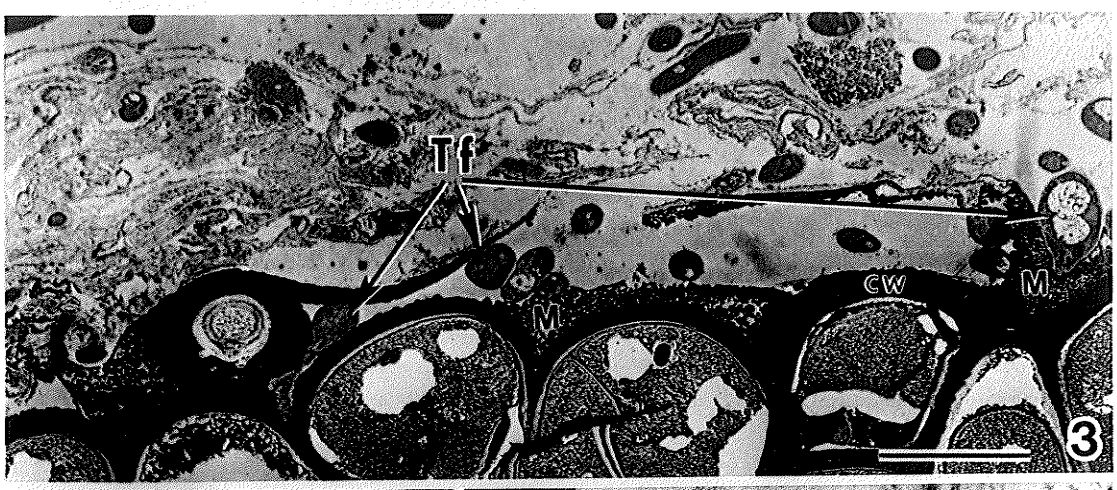
Plate 5. Infection of the rind cells of a sclerotium of *S. sclerotiorum* by *T. flavus*.

Figures 3, 4, 5 and 6. Penetration of the cell walls (Figs. 3, 4 and 5) and destruction of rind cells (Fig. 6). Note etching of the cell wall (CW) at the penetration site (asterisk) (Fig. 5) and the loose melanin (M) associated with the hyphae of *T. flavus* (Tf) (Figs. 3, 4 and 5). Note also the disintegration of the affected rind walls (arrows) (Fig. 6). Figs. 3, 4 and 6, scale bars = 5 µm. Fig. 5, scale bar = 1 µm.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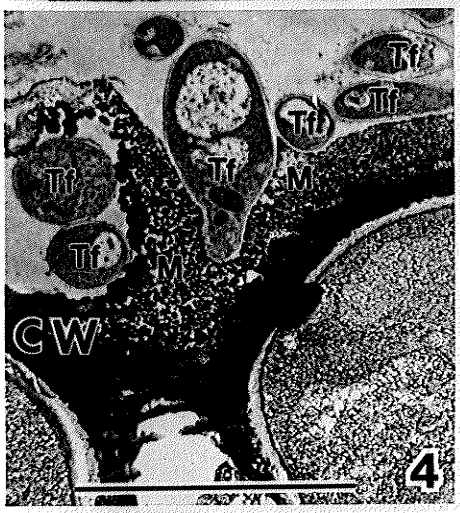
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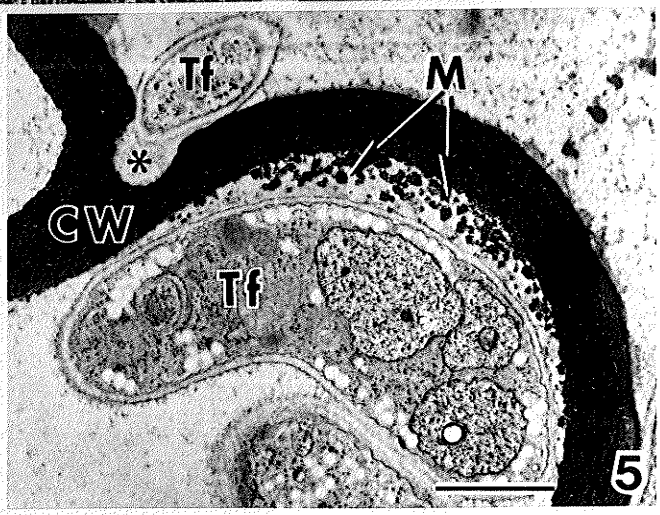
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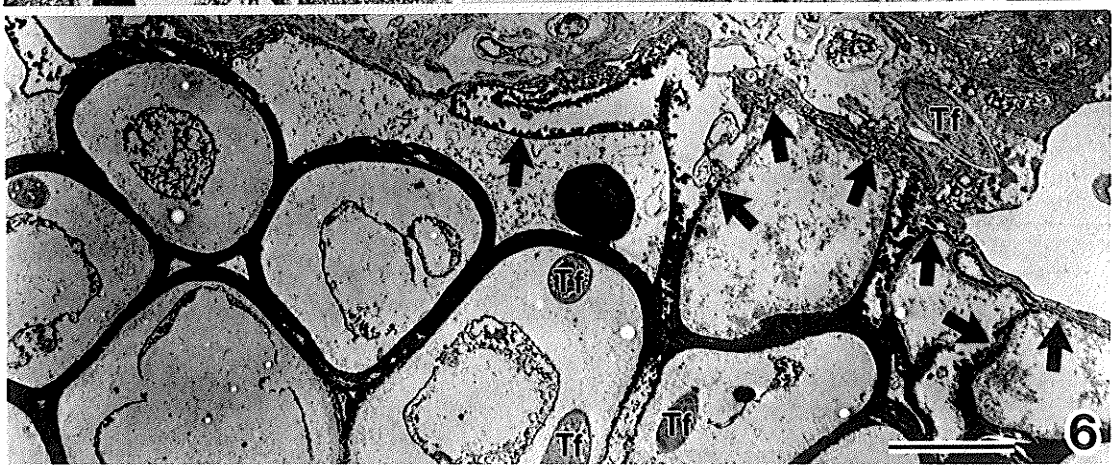
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Plate 6. Infection of the rind, cortical and medullary tissues of a sclerotium of *S. sclerotiorum* by *T. flavus*.

Figures 7 and 8. *T. flavus* (Tf) in the infected rind (Fig. 7), cortex and medulla (Fig. 8). Note vacuolation of the host cytoplasm and the funnel-shaped opening (arrows) of the host cell wall at the site of penetration (Fig. 7). Note also the hyphae of *T. flavus* in the inter- (asterisk) and intra- (square) cellular spaces of the infected cortical and medullary tissues (Fig. 8). Scale bars = 5 μm.

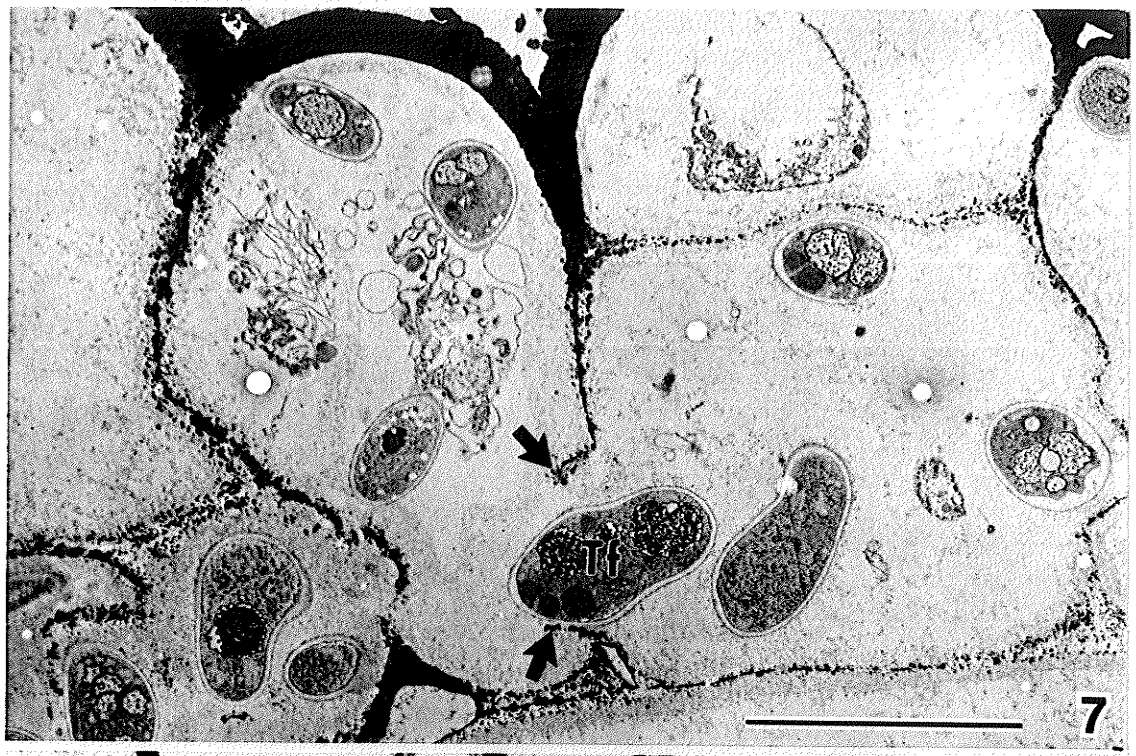
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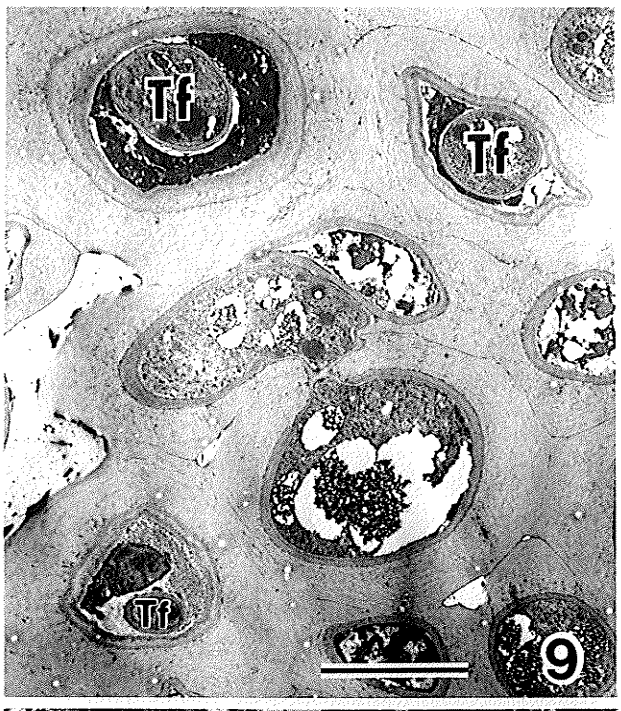
Plate 7. Infection of the medullary tissue of a sclerotium of *S. sclerotiorum* by *T. flavus*.

Figures 9 and 10. Infection of the medullary tissue of sclerotia by *T. flavus* (Tf) at 3 days (Fig. 9) and 7 days (Fig. 10) after inoculation. Note the difference in distribution of hyphae of *T. flavus* between early (Fig. 9) and late (Fig. 10) stages of infection. Scale bars = 5 μm.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Chapter IV

BIOLOGICAL CONTROL OF SCLEROTINIA WILT OF SUNFLOWER BY TALAROMYCES FLAVUS AND CONIOTHYRIUM MINITANS

4.1 INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary, the causal agent of sclerotinia wilt of sunflower (Helianthus annuus L.), is a destructive pathogen and can cause serious losses in yield, quality and production of the crop (Dorrell and Huang, 1978). The sclerotia are the overwintering structures and serve as the primary source of inoculum for sclerotinia wilt (Huang, 1977). These structures are soil-borne and therefore subject to attack by soil microorganisms such as Coniothyrium minitans Campbell (Campbell, 1947; Tribe, 1957; Jones and Watson, 1969; Ghaffar, 1972; Hoes and Huang, 1975; Turner and Tribe, 1976; Trutman et al., 1980; 1982; Fedulova, 1983) and Penicillium vermiculatum, Dangeard, the anamorph of Talaromyces flavus (Su and Leu, 1980). Coniothyrium minitans is effective as a biological control agent of Sclerotinia sclerotiorum in the field (Huang, 1976; 1979; 1980b; Bogdanova et al., 1986). Talaromyces flavus has also shown promise as a biological control agent for Rhizoctonia solani (Boosalis, 1956) and Verticillium albo-atrum (Dutta, 1981; Fravel et al., 1986). This chapter reports the effects of Talaromyces flavus and Coniothyrium minitans on disease development and yield of sunflower as well as on survival of sclerotia of Sclerotinia sclerotiorum.

4.2 MATERIALS AND METHODS

4.2.1 Source of fungal cultures and sunflower seed

Talaromyces flavus (DOAM 172557) was isolated from the rhizosphere soil of a sunflower field near Morden, Manitoba in 1976 by H.C. Huang. Coniothyrium minitans (DAOM 149432) was isolated from a sclerotium of Sclerotinia sclerotiorum (SS3) obtained from a diseased sunflower field near Morden, Manitoba (Hoes and Huang, 1975). Sclerotia of S. sclerotiorum used for artificial infestation of field soil were obtained from a number of sources. Sclerotia formed on diseased sunflower heads, on diseased pods and stems of bean and on diseased rapeseed tissue were obtained from commercial companies[3] during their cleaning processes and stored at 5 C until use in the field experiments.

Sclerotia from pure culture of the isolate SS3 of S. sclerotiorum were used in some field experiments. Canned red kidney beans (Heinz and Steak House brands) or baked beans (Heinz brand) were homogenized in a Waring blender and approximately 200 ml were poured into each of several 18 x 18 cm foil containers.[4] The foil containers were covered with aluminum foil, autoclaved 40 minutes at 121 C and cooled to room temperature. Each container was inoculated with 13 plugs of SS3, each 10 mm in size, cut from 10 day old plates of S. sclerotiorum produced on potato dextrose agar (PDA). All foil containers were then incubated at 20 C for 12 days at which time the sclerotia were

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[3] Alberta Sunflower Seeds, Ltd., Box 767, Bow Island, Alberta T0K 0G0 and Alberta Wheat Pool (Bean Plant), Box 96, Bow Island, Alberta T0K 0G0.

[4] Echo foil containers, Daxion Inc., 1016 1st Ave. S., Lethbridge, Alberta T1J 0B1.

removed, air-dried overnight and stored at 5 C until use.

The source(s) of sclerotia used each year for artificial infestation of soil were: (1) diseased sunflower heads - Experiment A (1983 and 1986), (2) diseased bean plants - Experiment A (1984 and 1985), (3) diseased rapeseed plants - Experiment B (1984) and (4) pure culture - Experiment B (1985). The source of sclerotia used for mesh bags buried at seeding time and in the fall (where applicable) was the same as that used for soil infestation at seeding time in all years, with the exception of Experiment B (1984) when sclerotia from diseased bean plants were used for fall burial. In 1983, sclerotia from sunflower were chopped and screened and those from 4 to 6 mm in size were used for soil inoculation. In other experiments, sclerotia obtained from sunflower, rapeseed or bean were not chopped but were used whole. Sclerotia from sunflower ranged in size from 8 to 10 mm in diameter. Rapeseed sclerotia were oblong and ranged from 4 to 6 mm in length, while the irregularly shaped bean sclerotia ranged in size from 5 to 10 mm.

Each year, excluding 1983, the viability of approximately 400 sclerotia was determined on agar media prior to spring seeding or fall burial in the soil. The percentage viability of the samples was as follows: (1) 1984 - sclerotia from bean (spring burial) 96%; sclerotia from bean (fall burial) 86%; sclerotia from rapeseed 94%; (2) 1985 - sclerotia from bean 94%; sclerotia produced artificially 98% and (3) 1986 - sclerotia from sunflower 97%. When determined in 1982, the percentage viability of sclerotia used for the 1983 field studies was greater than 90%. All sclerotia were surface sterilized and plated

onto potato dextrose agar (PDA) and PDA + streptomycin sulphate in 1983 and 1984-86, respectively. The viability of sclerotia was confirmed with the development of daughter sclerotia.

The sunflower Hybrid 894, obtained from the Agriculture Canada Research Station, Morden, Manitoba was used in all experiments during 1983-85. In 1986, the sunflower hybrid Cargill 204 was used due to a shortage of untreated Hybrid 894 seed. This was obtained from the Alberta Department of Agriculture, Lethbridge, Alberta. No seed treatment was used in any year.

4.2.2 Production of Talaromyces flavus and Coniothyrium minitans

Talaromyces flavus and C. minitans were grown on wheat bran to produce inocula for field application . For the 1983 test, bran was thoroughly soaked in water, drained and placed in glass jars. All jars were autoclaved at 121 C for 1 hr each day over two consecutive days. The bran was inoculated with C. minitans or T. flavus, which had been grown on PDA at room temperature for 10 days. The inoculated bran was incubated at room temperature for 28 days and air-dried for 3 to 4 days. In 1984, 1985 and 1986, dry bran was moistened with water (850 ml/kg bran), placed in 18 x 18 cm foil containers, autoclaved at 121 C for a total of 1 hr, cooled to room temperature and inoculated with a spore suspension of T. flavus or C. minitans. To produce a spore suspension, 25-35 ml of sterile distilled water were added to each 9.0 cm diameter Petri dish containing a 10 day old culture of T. flavus or C. minitans. The spores were dislodged, and the spore suspension from two washings was used to inoculate wheat bran in two

foil containers. Following inoculation, the bran was incubated at room temperature for 21 days, air-dried at room temperature for 3 to 4 days and stored at 5 C until use. Non-inoculated wheat bran was required for some experiments. It was prepared in the same manner as the inoculated bran, but was air-dried immediately following autoclaving.

4.2.3 Field experiments

Two experiments were conducted in fields during 1983-86. Experiment A was established at two locations, the University of Manitoba, Winnipeg for three years (1983-85) and the Lethbridge Research Station for four years (1983-86). Experiment B was established at the Lethbridge Research Station over a two-year period from 1984-85.

4.2.3.1 Experiment A

The experiments in Winnipeg and Lethbridge were designed to evaluate the ability of T. flavus and/or C. minitans to reduce the incidence of sclerotinia wilt of sunflower in the field. At the Lethbridge location, 28 plots of seven treatments with four replicates were arranged in a randomized complete block design. Each plot consisted of four rows, 6.0 m long and 0.9 m apart. The treatments were (1) control (sunflower alone), (2) bran, (3) S. sclerotiorum (SS), (4) SS and bran, (5) SS and T. flavus, (6) SS and C. minitans, (7) SS and a combination of T. flavus and C. minitans (1:1 w/w). In 1983 (May 23) and 1984 (May 22), 90 seeds of hybrid 894, 250 sclerotia and 45.0 g (50.0 g in 1984) of air-dried inoculum (

C. minitans or T. flavus) or wheat bran were applied to each row according to treatment. In 1985 (May 14-15) and 1986 (May 12), 250 sclerotia were applied to each row without fresh inoculum of the hyperparasites. Each year sclerotia, seed and/or inoculum were distributed uniformly in a 6.5 cm-deep, 8.0 cm-wide trench in each row. Trenches were filled with soil and packed. Plant spacing was adjusted by thinning to 0.13 m. Plants were examined weekly for symptoms of sclerotinia wilt from the vegetative through to the seed development stages. (Siddiqui et al., 1975; Appendix 1).

At the Winnipeg location, a similar experiment of 24 plots of six treatments with four replicates was arranged in a randomized complete block design. The treatments were (1) control (sunflower only), (2) bran, (3) S. sclerotiorum (SS), (4) SS and bran, (5) SS and T. flavus, and, (6) SS and C. minitans. Row length, row spacing, number of seeds of Hybrid 894, number and source of sclerotia and method of seed, sclerotia and inoculum application were the same as those used at the Lethbridge site. The dates of seeding in 1983, 1984 and 1985 were May 16, May 31 to June 1 and May 23-24, respectively. In 1983 and 1984, the amount of air-dried inocula of the hyperparasites or bran applied to each row of the appropriate treatment was 22.5 and 100 g, respectively. In 1985, 250 sclerotia were applied without adding fresh inocula of the hyperparasites. The Winnipeg experiment differed from the Lethbridge experiment as follows: (1) The amounts of air-dried inocula of the hyperparasites or bran applied to each row of the appropriate treatment in 1983 and 1984 differed, (2) plant spacing was adjusted by thinning to 0.15 m, (3) no irrigation was required and (4) the experiment was terminated after the 1985 season.

The natural inoculum density of S. sclerotiorum at each location was determined in 1984. Twenty-four (6 treatments x 4 reps) and twenty-eight (seven treatments x 4 reps) soil samples were collected from the Winnipeg and Lethbridge locations, respectively, shortly after seeding. Each sample was comprised of five smaller subsamples collected from within each plot. The samples, approximately 8000 cc each, were air-dried, weighed and then processed by wet sieving (Hoes and Huang, 1975) to separate the sclerotia. The inoculum density was not determined in 1985 and 1986 because all experiments were sown into the same sites which were not tilled from year to year.

Sunflower seed yield was determined at the Lethbridge location (1984-86) and at the Winnipeg location (1984-85). Severe bird damage prevented the determination of yield in 1983. Consequently, in later years, seed yield was estimated using a method involving the measurement of individual heads (Dr. W.O. Chubb, pers. comm.). Ten heads per plot were bagged with cotton sacs for individual yield determination. The diameter of all heads in the center two rows of each plot was measured late in the season when seeds were filled but heads were still green. The largest and smallest diameter measurements were recorded for each head. Once mature, the ten bagged heads/plot (40 heads/treatment) were harvested and individual head yields were determined. These values were used to calculate the regression of yield on head diameter. The intercept and slope, determined by regression, were calculated for each treatment and applied to data collected from plots of the same treatment to determine yield.

The important question is how well the yields, estimated from a regression equation derived from an independent set of data, agree with the actual yields. Data including both observed diameters and yields have been collected over a number of years and locations. Regression equations were obtained and estimates using these equations were compared with the observed yields to judge their value. A measure of the agreement (V) is given by the mean of the squares of the deviations between the actual (YA) and estimated (YE) values. The value of V can be partitioned into components which explain the nature of the agreement between the actual and observed values. This involves testing if the linear regression of the actual yields on estimated yields has a slope of 1 and if the mean of the observed and estimated yields are not different. The smaller the V value, the better. A perfect agreement would result in a V=0. Similar means and a slope not significantly different from 1 suggest that the agreement is good. Observed diameter and yield data obtained from the groups Winnipeg (1983-84) and Lethbridge (1984-86) were used to compare the estimated and actual values. Regressions were determined for subsets of the five location-years data and used to compare estimated and observed yields for independent sets of data.

Efficacy of the hyperparasites against S. sclerotiorum over time was assessed by burying additional sclerotia in each plot of the Winnipeg (1983-85), and Lethbridge (1983-86) experiments. To facilitate burial and recovery of sclerotia from soil, fiberglass (mesh) screen[5] (1.0 mm pore size) was used. In 1983 and 1984, the

[5] Fiberglass screen, Spalding Hardware Ltd., Whlse 1616, 10th Ave. S.W., Calgary, Alberta T3C OJ5.

screen was cut into 12 x8 cm sections, folded in half and heat sealed into five compartments to form a mesh bag. Each compartment had one sclerotium. Four mesh bags, each containing five sclerotia, represented one replication (20 sclerotia) of any treatment. A section of red plastic tape,[6] 11.0 x 1.1 cm, was attached to each bag for identification and to aid in location in the field. In May of 1983 and 1984, 240 mesh bags representing 12 bags per treatment with four replicates (sclerotia were applied in only five of the seven treatments; the two remaining treatments were sunflower + bran and sunflower alone) were buried at a depth of 6.5 cm at the Lethbridge location. With one less treatment at the Winnipeg location, 192 mesh bags (12 bags per treatment x four treatments x four replicates) were buried at a depth of 6.5 cm. The 12 mesh bags per treatment represented three sampling dates with four bags removed from each plot at five week intervals.

In the fall of 1984, 20 mesh bags were buried in plots at the Lethbridge location to assess the overwintering activity of the hyperparasites. One mesh bag containing 20 sclerotia was buried per plot. From this time onward, each mesh bag measured 25 x 5 cm in size and was divided into 10 compartments with two sclerotia per compartment. Use of larger bags containing 20 sclerotia reduced the loss of sclerotia occurring with the four smaller bags containing a total of 20 sclerotia. In May, 1985, the twenty bags buried the previous fall were removed prior to seeding. At seeding time, 60 mesh bags, representing three sampling dates for each of the five

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[6] Max Tape for Max Tapener Model HT-B, Westcan Horticultural Specialists Ltd., Calgary, Alberta.

treatments replicated four times, were buried at Lethbridge. At the Winnipeg location, where one less treatment occurred, 48 bags were buried. These were sampled three times during the season at five week intervals. In 1986, 60 mesh bags (five treatments x three sampling dates x four replicates) were buried in the experimental site at Lethbridge. The bags were removed at intervals of five weeks. The split-plot design was used when several sampling dates were involved, with hyperparasite treatments assigned to main plots and sampling dates assigned to subplots within each main plot.

For the 1984 and 1985 field seasons at Lethbridge, soil temperatures were recorded using a Tel-Tru thermometer.[7] twice daily, two times per week at a depth of approximately 6.5 cm. No soil temperatures were recorded in 1986. In 1984 and 1985, a portable tensiometer[8] was used to take soil moisture readings two and one times per week, respectively at the Lethbridge location. No readings were available for the Winnipeg location. In 1986, soil moistures were determined weekly at the Lethbridge site by collecting, drying and weighing soil samples.

4.2.3.2 Experiment B

The experiment was conducted in 1984 and 1985 in a field located at the Lethbridge Research Station. The objectives were: (1) to determine the effect of *T. flavus*, *C. minitans* and various combinations of these hyperparasites on the survival of sclerotia of

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[7] Model GT100, Tel-Tru Manufacturing Co., Rochester, N.Y., U.S.A.

[8] Model 2900F Quick Draw Soilmoisture Probe, Hoskin Scientific (Western) Ltd., 239 East 6th Ave., Vancouver, B.C. V5T 1J7.

S. sclerotiorum and on the incidence of sclerotinia wilt of sunflower, (2) to determine whether T. flavus and C. minitans alone or in combination were able to overwinter and control sclerotinia wilt of sunflower in the next season and (3) to compare the ability of overwintered T. flavus and C. minitans to control sclerotinia wilt of sunflower with that of spring-applied hyperparasites. The treatments in 1984 (spring) were: (1) bran, (2) S. sclerotiorum (SS) (3) SS and bran, (4) SS and T. flavus, (5) SS and C. minitans, (6) SS and a combination of T. flavus and C. minitans (1:1 w/w), (7) SS and a combination of T. flavus and C. minitans (2:1 w/w) and (8) SS and a combination of T. flavus and C. minitans (1:2 w/w). The experiment was designed as a randomized complete block design with 16 plots in each of four replicates. In the spring of 1984, each of the eight treatments listed was assigned to two plots per replicate. In the fall of 1984, one of the two plots of each treatment per replicate received an application of hyperparasites or bran. The remaining plots received a spring application of hyperparasites or bran. Each plot consisted of three rows of sunflower, 6 m long and 0.6 m apart.

On May 25, 1984, 90 seeds of sunflower Hybrid 894, 250 sclerotia and 100 g of air-dried C. minitans, T. flavus or bran were applied to each row of the appropriate treatment. The material was distributed uniformly in a 6.5 cm-deep, 8.0 cm-wide trench in each row. Trenches were filled with soil and packed. Plots were irrigated immediately after seeding to insure good germination and emergence. Upon emergence, plant spacing was adjusted by thinning to 0.13 m. Plants were examined weekly for symptoms of sclerotinia wilt by the same method described for Experiment A.

In the fall of 1984, the duplicate plots of each treatment received hyperparasite inocula or bran at the rate of 100 g of air-dried material per row. The same proportions of T. flavus and C. minitans, applied in the spring of 1984 (2:1, 1:1 and 1:2), were used for the fall application. No sclerotia, aside from those buried in mesh bags, were applied to plots at this time. Those plots which did not receive inocula or bran in the fall received the same rate of application of this material during spring seeding (May 15, 1985). Ninety seeds of Hybrid 894 and 250 sclerotia were also applied to each row of the appropriate treatment in spring of 1985.

Soil samples to determine natural sclerotial levels in the experimental site were collected and processed as described previously for Experiment A.

Mesh bags containing sclerotia were buried in field plots during both seasons to assess the activity of the hyperparasites against S. sclerotiorum. In the spring of 1984, 112 mesh bags representing four bags per treatment with four replicates were buried (seven treatments x four sampling dates x four replicates). Each bag contained 20 sclerotia and represented one sampling date; four bags per plot allowed for four sampling dates. The first three sampling dates occurred at five week intervals from the date of seeding with the fourth bag recovered prior to seeding in 1985. Mesh bags containing sclerotia were buried in only seven of eight treatments as the eighth treatment was bran alone. On October 16, 1984, 52 mesh bags were buried to compare the effect on sclerotia of a fall application of inocula with no fall application of inocula. The 52 bags represented

one bag/treatment (13 treatments) with four replicates. The two bran-treated plots and one of the plots treated with sclerotia alone did not receive a mesh bag. These bags were removed prior to seeding in 1985. During seeding (1985), 168 mesh bags representing three bags per treatment (three sampling dates) with four replicates were buried. Of the 16 plots per replicate, 14 received mesh bags; the two remaining plots had bran only and no application of sclerotia. Sampling of sclerotia occurred at five week intervals from the date of seeding. The split-plot design was used when several sampling dates were involved, with hyperparasite treatments assigned to main plots and sampling dates assigned to subplots within each main plot.

4.3 RESULTS

4.3.1 Control of sclerotinia wilt of sunflower

The application of T. flavus and C. minitans reduced significantly the incidence of sclerotinia wilt of sunflower at both the Lethbridge and Winnipeg locations over a three year period (Tables 1,2,3,4,5 and 6; Appendices 2-11). At Lethbridge, treatment differences became noticeable during approximately the third (growth stage approximately 2.3; Appendix 1) to fifth (growth stage approximately 3.2) week of rating with fewer plants becoming infected in the hyperparasite-treated plots compared with the control (SS) and the SS+bran-treated plots (Tables 3,4 and 5; Figure 1). Similar results occurred at the Winnipeg site during 1983-84 with the exception of the T. flavus -treated plots (Appendices 2 and 3). In 1984 (Winnipeg), a significant reduction in disease in the T. flavus -treated plots

compared with the control did not occur until the eighth week of rating (Appendix 3). In 1985, there was no significant difference in levels of disease between the T. flavus -treated plots and the SS or SS+bran-treated plots at any week of rating (Appendix 4). At the Lethbridge site, the experiment was continued into 1986. During this season, the level of disease in the hyperparasite-treated plots compared with the control (SS) and the SS+bran-treated plots did not differ at any week of rating (Table 6). A trace of head rot occurred in the sunflower plots at Lethbridge (1983-86) and Winnipeg (1983-85).

In 1983 at the Lethbridge location, the final percentages of disease in the T. flavus -treated and C. minitans -treated plots were 0 and 9.8%, respectively compared with 54.9% in the plots treated with sclerotia only (Table 1, Appendix 5). This represents a reduction in disease by 100% (T) and 82.1% (C) in the hyperparasite-treated plots. In 1984, similar results were obtained with 49.9% disease in the plots treated with sclerotia only compared with 0.1 and 5.3% disease in the plots treated with T. flavus and C. minitans, respectively (Table 1 and Appendix 6). In 1985, application of sclerotia, but no hyperparasites, to plots treated with both sclerotia and hyperparasites in the previous two years resulted in low levels of disease (Figure 1). In the plots treated with sclerotia only, the mean percentage of disease was 38.6% compared with 4.3, 5.1 and 0.3% in the T. flavus (T), C. minitans (C) and C/T-treated plots, respectively (Table 1 and Appendix 7). This represents a reduction in disease by 88.9% (T), 86.9% (C) and 99% (C/T) in the hyperparasite-treated plots. In 1986, sclerotia, but no

hyperparasites, were applied as in 1985. However, the level of control achieved in previous years did not reoccur. The incidence of disease in the hyperparasite-treated plots did not differ significantly from that of the control (SS) plots and the SS+bran-treated plots (Table 1 and Appendix 8).

At the Winnipeg location (Experiment A), the results obtained during 1983-1984 were similar to those which occurred at the Lethbridge site (Table 2, Appendices 9 and 10). The application of sclerotia and the hyperparasites T. flavus and C. minitans reduced significantly the incidence of sclerotinia wilt of sunflower (Table 2) in 1983 with 14.4 and 3.1% disease in the T. flavus and C. minitans -treated plots, respectively compared with 65.2% disease in the plots treated with sclerotia only. Similar results occurred in 1984 (Table 2). In 1985, application of sclerotia, but no hyperparasites, to plots treated with both hyperparasites and sclerotia in the previous two years resulted in 21.1 and 1.2% disease in plots treated with T. flavus and C. minitans, respectively, compared with 24.4% in the plots treated with SS only. The incidence of disease was reduced significantly in the C. minitans -treated plots but not in the T. flavus -treated plots (Table 2 and Appendix 11).

The application of hyperparasites to field soil for two consecutive seasons (1983-1984) was effective in reducing the incidence of disease during these seasons as well as during a third field season. Sclerotia had been applied with hyperparasites during 1983-84 but were applied alone in 1985. The one-year carry-over effect of biocontrol was evident with C. minitans at the Winnipeg site (Table 2) and with

T. flavus and C. minitans at the Lethbridge site (Table 1, Figure 1 and Plate 8, Figure A) in 1985. This phenomenon did not continue at Lethbridge during 1986, the second season of sclerotia application without further additions of hyperparasites (Plate 8, Figure B). In the plots treated with sclerotia only, the percentage of disease was 54.1% compared with 51.0 and 41.7% in the plots treated with T. flavus and C. minitans, respectively (Table 1). The lack of a carry-over effect of biocontrol was evident early in the season and continued throughout the summer (Table 6 and Figure 2).

The use of C. minitans as a biocontrol agent was as effective in reducing disease incidence as the use of T. flavus at the Lethbridge location (Table 1). At the Winnipeg site, application of C. minitans resulted in levels of disease that were significantly less than those occurring in the plots treated with T. flavus in any year (Table 2). During 1983-85, the percentage of disease in the C. minitans -treated plots was 3.1, 1.0 and 1.2% compared with 14.4, 33.4 and 21.1% in the T. flavus -treated plots, respectively. Use of a combination of T. flavus and C. minitans during 1983-1985 resulted in a level of disease which was as low as, or lower than that obtained with the use of each hyperparasite alone (Table 1 and Figure 2). This effect was no longer apparent in 1986 at the Lethbridge location. No results on this interaction are available from the Winnipeg site as the supply of inocula was limited and therefore prevented the inclusion of this treatment in the experiment.

In 1983, and during 1983 and 1984 at the Winnipeg and Lethbridge sites, respectively, application of bran reduced significantly the

incidence of sclerotinia wilt of sunflower (Tables 1 and 2). At Winnipeg, the incidence of disease was 54.6% in the SS+bran-treated plots compared with 65.2% in the plots treated with sclerotia only. At Lethbridge, percentages of disease in the SS+bran-treated plots during 1983-84 were 25.2 and 27.1%, respectively, compared with 54.9 and 49.9% in the plots treated with sclerotia alone. Although bran has affected disease incidence, the greatest reductions in the level of disease at both locations were due to the application of hyperparasites.

Experiment B was designed to test various combinations of T. flavus and C. minitans for control of sclerotinia wilt of sunflower and to study the effect of a fall (1984) and a spring (1985) application of hyperparasites on the incidence of disease. In 1984, application of sclerotia and T. flavus or C. minitans or combinations of T. flavus and C. minitans produced little difference between the percentage of diseased plants per treatment (Table 7 and Appendix 12). The incidence of disease in all plots was quite low with 0.12 and 0.34% disease in the T. flavus and C. minitans -treated plots, respectively, compared with 1.32% disease in the plots where sclerotia, but no hyperparasites, were applied.

In 1985, the levels of disease were greater than those seen in 1984. In plots treated with sclerotia and hyperparasites, a significantly lower level of disease occurred in comparison to plots where sclerotia only were applied (Table 7 and Appendix 13). The greatest reduction in disease was due to the application of C. minitans. Use of a combination of T. flavus and C. minitans reduced

significantly the incidence of wilt compared with the use of T. flavus alone. The most effective combination of hyperparasites was the 2:1 ratio of C. minitans to T. flavus. However, the control achieved in this treatment appeared to be due to C. minitans as this mixture was as effective as the use of C. minitans alone.

The time of application of hyperparasites had no effect on the incidence of sclerotinia wilt of sunflower (Table 7). The incidence of disease occurring in plots where hyperparasites were applied in the fall did not differ significantly from the disease incidence in plots where hyperparasites were applied in the spring.

The natural inoculum density of S. sclerotiorum in soil samples collected shortly after seeding averaged 0.01, 0.01 and 0.02 sclerotia per kilogram of soil at the Lethbridge (Experiment A), Lethbridge (Experiment B) and Winnipeg field sites, respectively (Table 8). No significant difference in inoculum density occurred between the control and the hyperparasite-treated plots in all three fields (Appendix 14). The low incidence of disease found in the control (none) and bran-treated plots reflects the low inoculum density of S. sclerotiorum (Tables 1 and 2).

4.3.2 Seed yield

Seed yield of sunflower was increased with the application of hyperparasites to the soil during 1984-85 and 1984 at the Lethbridge and Winnipeg sites, respectively. The 1984 field trials at Lethbridge showed that seed yield was greater in plots treated with sclerotia and

hyperparasites compared with plots treated with sclerotia only (Table 1). Seed yields were 1433.0, 1326.8 and 1360.0 kg/ha in the plots treated with T. flavus (T), C. minitans (C), and a combination of T. flavus and C. minitans, respectively. This represents an increase in yield by 50% (T), 39% (C) and 43% (C/T) in the hyperparasite-treated plots. No significant differences in seed yield occurred between the untreated plots (none) or plots treated with bran alone and those plots treated with sclerotia and T. flavus, C. minitans or a combination of T. flavus and C. minitans. In 1985, similar results were obtained with a seed yield of 866.5 kg/ha in plots treated with sclerotia only compared with 1905.6, 1732.8 and 2095.5 kg/ha in the T. flavus, C. minitans and C/T-treated plots (Table 1). This represents an increase in yield by 119% (T), 100% (C) and 142% (C/T) in the hyperparasite-treated plots. In this season, no hyperparasites were applied indicating that the carry-over effect of biocontrol was also reflected in the yield data. In 1986, the carry-over effect of biocontrol was no longer noticeable. No significant differences in seed yield occurred between the hyperparasite-treated plots and the plots treated with sclerotia but no hyperparasites. Seed yields in the untreated plots and in the plots with bran alone were significantly higher than in any plots to which sclerotia were applied (Table 1).

In 1984 at the Winnipeg location, an increase in yield also occurred as a result of reduction in disease due to application of C. minitans or T. flavus (Table 2). Yields of 1464.3 and 1862.0 kg/ha were obtained in the T. flavus and C. minitans -treated plots,

respectively, compared with 664.6 kg/ha in the plots treated with sclerotia only. During the next season no hyperparasites were applied and yields of 1209.5 and 1215.3 kg/ha, obtained from the T. flavus and C. minitans -treated plots, respectively, did not differ significantly from a yield of 992.2 kg/ha obtained from the plots treated with sclerotia alone. Differences in disease incidence may not have been large enough to create significant yield differences.

The application of bran did not result in significant increases in yield at the Winnipeg (Table 2) and Lethbridge (Table 1) sites during 1984-86 and 1984-85, respectively. The greatest increases in yield were due to the application of hyperparasites.

Plot yields were estimated with the estimated values based on a previously determined regression equation. The results presented below are representative of the range of agreement obtained. When the Lethbridge (1984) yields were estimated from a regression equation derived from other location-year combinations, the observed and estimated values agreed fairly closely with a V value of 20.7 (Appendix 15). There appears to be no bias as the means are equal and the slope is not different from 1. The largest difference between an actual and estimated value was 12 g. When 1985 (Lethbridge) yields were estimated, the agreement was not as good with a higher V value and a lower r value (Appendix 15). The difference between the observed and estimated means was significant, but the slope was not significantly different from 1. The lower r value reflected a larger scatter of points about the fitted regression as compared to the Lethbridge (1984) data. The differing means suggested that the actual

yield values were underestimated consistently throughout the range of head diameters. Similar results were obtained when the 1986 Lethbridge yields were estimated; the means differed for the observed and estimated values and the slope was equal to 1 (Appendix 16).

When the 1984 Winnipeg yields were estimated, the observed and estimated values agreed fairly closely with equal means (YA=YE) and a slope not different from 1 (Appendix 17). The largest difference between an observed and estimated value was 23 g. In 1985 (Winnipeg), the means were different and the slope was significantly less than 1 (Appendix 17). The estimates from the regression equation were biased upwards and deviated more from the actual yield values as the size of the head increased. Deviations as large as 30 g between the observed and estimated yields occurred.

4.3.3 Survival of sclerotia

In 1983 (Lethbridge, Experiment A), fewer sclerotia were recovered from the hyperparasite-treated plots compared with the control (SS) plots at 10 and 15 weeks after burial (Table 9). At 10 weeks after burial, 14.8 sclerotia were recovered from the control plots compared with 8.0 and 12.2 sclerotia recovered from the T. flavus and C. minitans treated plots, respectively. Similar results occurred at 15 weeks after burial. No significant treatment differences occurred at five weeks after burial (Appendix 18). Fewer sclerotia recovered from the hyperparasite-treated plots were viable compared with sclerotia recovered from the control plots. At five weeks after burial, 22.5% of the sclerotia recovered from the control plots were viable compared

with only 1.9 and 0.8% in the T. flavus and C. minitans -treated plots, respectively. At 10 weeks after burial, the percentage of viable sclerotia was reduced with 3.9% in the control plots compared with 0 and 0.9% in the T. flavus and C. minitans -treated plots, respectively. Viability testing of sclerotia recovered at 15 weeks after burial showed a similar trend with 4.6% viability in the control plots compared with 0% viability in the T. flavus and C. minitans -treated plots.

The length of burial time affected both the recovery and viability of sclerotia of S. sclerotiorum. The number of sclerotia recovered decreased from the first to the last sampling with significant reductions occurring most often from the first to the second sampling date. A significant interaction between sampling date and hyperparasites indicates that the number of sclerotia recovered over time was affected by treatment. Fewer sclerotia were recovered from the hyperparasite-treated plots compared with the untreated control as time from burial increased. The percentage of recovered sclerotia that were viable tended to be the highest at the first sampling date and decreased as time from burial increased. However, the interaction between sampling date and treatment was not significant indicating that the viability was not affected by treatment.

In 1984 (Lethbridge, Experiment A) significant reductions in the number of sclerotia recovered from the T. flavus and C. minitans -treated plots compared with the control plots occurred at 15 weeks after burial (Table 10, Appendix 21). There were 18.6 sclerotia recovered from the control plots compared with 15.8 and 16.3 sclerotia

recovered from the T. flavus and C. minitans -treated plots, respectively. Sclerotia buried in May of 1984 and recovered after 50 weeks of burial also showed a significant reduction in numbers. There were 10.5 sclerotia recovered from the control compared with 5.5 and 4.3 sclerotia recovered from the T. flavus and C. minitans -treated plots, respectively. As time from burial progressed, significantly fewer sclerotia were recovered. The interaction between sampling date and treatment is significant indicating that the number of sclerotia recovered over time was affected by the treatment (Appendix 21). As time of burial progressed, fewer sclerotia were recovered from the hyperparasite-treated plots compared with the control (SS). The greatest reduction occurred in the C. minitans -treated plots. The viability of sclerotia recovered from the hyperparasite- treated plots was significantly less than that of sclerotia recovered from the control plots at five weeks after burial. Only 0.8 and 0% of sclerotia recovered from the T. flavus and C. minitans -treated plots were viable compared with 12.9% from the control. At 10, 15 and 50 weeks after burial, the viability of sclerotia recovered from any treatment was low. No significant differences occurred between treatments at any of these sampling dates.

In 1985 (Lethbridge, Experiment A), there was little difference between the number of sclerotia recovered from the hyperparasite-treated plots compared with the control plots (Table 11, Appendix 24). At five weeks after burial, 20 sclerotia were recovered from the control plots compared with 19.5 sclerotia recovered from the T. flavus and the C. minitans -treated plots. Similar results

occurred at 10 and 15 weeks after burial. These results indicate that the recovery of sclerotia was not affected by treatment. Sclerotia recovery was also not affected by sampling date as the numbers recovered did not change significantly from one sampling date to another. The viability of sclerotia recovered from the hyperparasite-treated plots was lower than that of sclerotia recovered from the control but the differences were not significant. Thirty-one percent of the sclerotia recovered from the control plots were viable compared with 23.3 and 24.9% in the T. flavus and C. minitans -treated plots. At 10 weeks after burial, there were no treatment differences in the percent viability of recovered sclerotia. At 15 weeks after burial, fewer sclerotia recovered from the hyperparasite-treated plots, compared with the control plots were viable.

In 1986 (Lethbridge, Experiment A), no significant reductions in the number of sclerotia recovered from the hyperparasite-treated plots compared with the control plots occurred at 5, 10 or 15 weeks after burial (Table 12, Appendix 27). The percent viability of sclerotia was low regardless of treatment and sampling date. However, the length of burial time affected the recovery of sclerotia with fewer sclerotia being recovered as time from burial increased. Recovery over time was not affected by treatment as indicated by a nonsignificant interaction between sampling date and hyperparasites.

Sclerotia infected by T. flavus or C. minitans were found in all experiments but the percentages of infection were low. In 1983, (Lethbridge, Experiment A) the percent of recovered sclerotia infected by T. flavus was not significantly greater in the T. flavus -treated

plots compared with the control (Table 9, Appendix 18). At five weeks after burial, 16.4% of sclerotia recovered from the T. flavus -treated plots were infected with T. flavus compared with 9.2% in the control. Similar results occurred after 15 weeks of burial. At 10 weeks after burial, a significantly lower percent of sclerotia recovered from the T. flavus -treated plots were infected with this hyperparasite compared with the sclerotia recovered from the control. Although the infection of sclerotia by T. flavus was low, more sclerotia were recovered from the control plots compared with the T. flavus -treated plots.

In 1984 (Table 10, Appendix 21) and 1986 (Table 12, Appendix 27), sclerotia infected by T. flavus were recovered primarily from the plots treated with this hyperparasite. However, differences between treatments were often not significant. In 1985, T. flavus -infected sclerotia were found in each treatment at 10 weeks after burial (Table 11). The remaining sampling dates showed little infection by T. flavus with no significant treatment differences (Appendix 24). Other microorganisms such as Trichoderma and Fusarium spp. were associated with sclerotia recovered from soil during 1983-84 (Appendix 39). The frequency of sclerotia infected by microorganisms other than T. flavus or C. minitans was high in all treatments (Appendix 39).

During 1983-86 (Lethbridge, Experiment A), higher percentages of sclerotia recovered from the T. flavus -treated plots were infected with T. flavus at the first sampling date than at subsequent dates (Tables 9-12). As time from burial increased, the percent of sclerotia infected by T. flavus decreased with the exception of 1985

when a greater percentage of T. flavus -infected sclerotia were found at the second sampling date compared with both the first and last sampling dates.

Although the number of sclerotia recovered from the C. minitans treated plots was consistently lower than in the control, the percentages of sclerotia infected with C. minitans were quite low and variable. During 1983-86, the percentages of recovered sclerotia infected with C. minitans tended to be higher in the C. minitans -treated plots.

During 1983-85 at the Winnipeg location (Experiment A), the number of sclerotia recovered and the viability of these sclerotia varied from year to year (Tables 13,14 and 15; Appendices 30-38). In 1983, fewer sclerotia were recovered from the hyperparasite-treated plots compared with the control at 10 and 15 weeks after burial (Table 13, Appendix 30). At 10 weeks after burial, 18.8 sclerotia were recovered from the control compared with 10.6 and 8.4 sclerotia from the T. flavus and C. minitans -treated plots, respectively. Similar results occurred at 15 weeks after burial but no treatment differences were evident. The percentages of recovered sclerotia that were viable did not differ significantly between treatments at five weeks after burial. At 10 weeks after burial, 9.6% of sclerotia recovered from the control plots were viable compared with 0% viability of sclerotia recovered from the T. flavus and the C. minitans -treated plots. Similar results occurred after 15 weeks of burial. The length of burial time affected the recovery of sclerotia. Fewer sclerotia were recovered as time from burial progressed with the greatest reductions

occurring in the hyperparasite-treated plots. However, viability did not differ significantly from one sampling date to another.

In 1984 (Winnipeg, Experiment A), fewer sclerotia were recovered from the hyperparasite-treated plots at 15 but not at 5 and 10 weeks after burial (Table 14). Viability was significantly less in sclerotia recovered from the hyperparasite-treated plots compared with the control at all sampling dates (Appendix 33). Sampling date was significant for recovered sclerotia only with fewer sclerotia recovered as time from burial increased.

In 1985 (Winnipeg, Experiment A), the results are similar to those obtained in previous years with fewer sclerotia recovered from the C. minitans -treated plots compared with the control (Table 15, Appendix 36). Although the recovery of sclerotia was reduced with the application of C. minitans, a significant reduction in the number of sclerotia recovered from the T. flavus -treated plots did not occur. At 10 weeks after burial, 18.2 sclerotia and 16.8 sclerotia were recovered from the control and T. flavus -treated plots, respectively compared with 12.5 sclerotia from the C. minitans -treated plots. Similar results occurred at 15 weeks after burial. The length of burial time affected the recovery of sclerotia. A significant reduction in the number of sclerotia recovered occurred from one sampling date to another with means of 18.9, 16.6 and 14.3 sclerotia recovered at 5, 10 and 15 weeks after burial, respectively.

Sclerotia infected by T. flavus or C. minitans were recovered from the hyperparasite-treated plots in 1983, 1984 and 1985 (Winnipeg,

Experiment A). In 1983, the percentage of recovered sclerotia infected by T. flavus was significantly greater in the T. flavus -treated plots compared with the control at 5, 10 and 15 weeks after burial (Table 13). No sclerotia recovered from the control plots were infected with T. flavus compared with 27.9% in the T. flavus -treated plots at five weeks after burial. Similar results occurred at 10 and 15 weeks after burial. Although T. flavus infected sclerotia were recovered from the T. flavus -treated plots at all sampling dates in 1984, the percentages were not significantly different from the control (Table 14). In 1985, a significantly greater percentage of sclerotia recovered from the T. flavus -treated plots were infected by T. flavus compared with the control at 5 and 10 weeks after burial (Table 15). No significant treatment differences occurred at 15 weeks following burial. Other microorganisms associated with sclerotia recovered from field plots during 1983-84 at Winnipeg included Trichoderma and Fusarium spp. (Appendix 39).

During 1983-85 (Tables 13-15), the percentage of recovered sclerotia infected by C. minitans was not significantly higher in the C. minitans -treated plots compared with the controls at all sampling dates with the exception of 15 weeks after burial (1984). At this sampling date, 3.4% of sclerotia recovered from the C. minitans -treated plots were infected with C. minitans compared with 0% in all other treatments. Although the recovery of C. minitans -infected sclerotia was low, recovery of sclerotia from the C. minitans -treated plots was often less than from the untreated control. The length of burial time had no effect on the percentage of sclerotia infected by C. minitans or T. flavus during 1983-85 at the Winnipeg site.

In Experiment B (Lethbridge), S. sclerotiorum, T. flavus, C. minitans and bran were applied in May, 1984. Three combination treatments of C. minitans and T. flavus were included (1:2, 1:1 and 2:1). Sclerotia, in mesh bags, were buried and recovered at 5, 10 15 and 50 weeks after burial. In 1984 (Table 16, Appendix 40), the greatest reduction in sclerotial recovery occurred after 15 weeks of burial with 7.6, 8.9, 8.7 and 8.0 sclerotia recovered from the T. flavus, C/T 1:2, C/T 1:1 and C/T 2:1-treated plots, respectively compared with 14.3 in the control. After 50 weeks of burial, similar results were obtained with the exception of the C. minitans -treated plots. Although a significant reduction in sclerotial numbers recovered from the C. minitans -treated plots did not occur at 15 weeks following burial, only 6.4 sclerotia were recovered at 50 weeks of burial compared with 11.0 in the control. The viability of sclerotia did not differ significantly between treatments at any sampling date. However, the lowest viability ratings occurred in the hyperparasite-treated plots.

Although sclerotia infected by T. flavus were recovered from plots to which T. flavus was applied, no significant differences between treatments for sclerotia infected by T. flavus at five, 10 and 15 weeks after burial occurred (Table 16, Appendix 40). No sclerotia recovered from the control or C. minitans -treated plots were infected by T. flavus. Similar results occurred with sclerotia infected by C. minitans.

The length of burial time affected the recovery of sclerotia but not the viability or infection of sclerotia by T. flavus or C.

minitans (Table 16, Appendix 40). Fewer sclerotia were recovered as time from burial increased with 16.5, 14.1, 11.3 and 7.2 sclerotia recovered after 5, 10, 15 and 50 weeks of burial, respectively. A significant interaction between treatment and sampling date indicates that the number of sclerotia recovered over time was affected by treatment. Fewer sclerotia were recovered from the hyperparasite-treated plots compared with the untreated control as time from burial increased.

Fourteen of the 16 plots per replicate of Experiment B had been treated with either bran or hyperparasites in the spring of 1984. The remaining two plots received sclerotia only. In October of 1984, T. flavus, C. minitans, bran and combinations of T. flavus and C. minitans (2:1, 1:1, 1:2) were applied to seven of 14 plots per replicate that had received the same treatment in May, 1984. The remaining seven plots per replicate did not receive a fall application of bran or hyperparasites. Sclerotia were buried in 13 of the 16 plots per replicate (two plots/rep= bran alone; one plot of SS omitted) during the fall application of bran or hyperparasites. These sclerotia were recovered in May, 1985. The number of sclerotia recovered from these plots did not differ significantly between treatments (Table 17, Appendix 43). No less than 19 sclerotia were recovered from each of the hyperparasite-treated plots compared with 20 from the control. The viability of sclerotia recovered from the hyperparasite-treated plots was significantly less than that of sclerotia recovered from the control. In the control plots, the percent viability of sclerotia was 22.3% compared with 11.9, 3.4, 5.7

6.2, 4.2 and 10.3% in the plots treated with bran, T. flavus, C/T 1:2, C/T 1:1, C/T 2:1 and C. minitans, respectively. Viability of sclerotia recovered from plots which received a fall application of bran or hyperparasites was even less. A fall application of C. minitans compared with no application resulted in 0 and 10.3% viability of recovered sclerotia, respectively.

Infection of sclerotia by hyperparasites was low. However, the majority of sclerotia infected by T. flavus or C. minitans was recovered from the hyperparasite-treated plots as opposed to the control and the SS+bran-treated plots (Table 17, Appendix 43). A fall application of hyperparasites did not result in a significant difference in the percentage of sclerotia infected by T. flavus or C. minitans in each treatment.

In May of 1985 (Experiment B), T. flavus, C. minitans, bran and combinations of T. flavus and C. minitans (1:2, 1:1, 2:1) were applied to the seven plots per replicate that had received the same treatment in May, 1984 but not in October, 1984. Sclerotia (250/row) were applied to 14 plots per replicate (two plots/rep.= bran alone). Sclerotia in mesh bags were also buried in these plots and recovered at 5, 10 and 15 weeks after burial. The number of sclerotia recovered after five weeks of burial ranged from 18.1 to 20 and did not differ significantly between treatments (Table 18, Appendix 44). At 10 weeks after burial, differences in the recovery of sclerotia began to appear. In the untreated control (SP) 19.3 sclerotia were recoverd compared with 17.4 and 11.9 in the T. flavus and C. minitans -treated plots, respectively. At 15 weeks after burial, similar results were

obtained. A significant interaction between sampling date and treatment occurred indicating that the number of sclerotia recovered over time was affected by treatment. As time from burial increased, fewer sclerotia were recovered from plots receiving a spring compared with a fall application of hyperparasites. The greatest reductions in the recovery of sclerotia occurred in the C/T 1:1, C/T 2:1 and the C. minitans -treated plots.

The viability of recovered sclerotia was significantly less in the hyperparasite-treated plots compared with the control (SS) and the SS+bran-treated plots (Table 18, Appendix 44). After five weeks of burial, 24.3% of sclerotia recovered from the control (SP) were viable compared with 0.8 and 0.8% in the T. flavus and C. minitans -treated plots, respectively. At 10 and 15 weeks after burial, viability was also less in most hyperparasite-treated plots compared with the untreated control. The viability of sclerotia recovered from the control as time from burial increased was affected by the treatment. Viability of sclerotia recovered from the control (SS) and SS+bran-treated plots decreased significantly from the first to the last sampling date. This trend was not apparent in most of the hyperparasite-treated plots. Application of hyperparasites in the fall compared with a spring application had no effect on the viability of the sclerotia that were recovered.

The percent of recovered sclerotia infected by T. flavus was low (Table 18). At five weeks after burial, the percentage of sclerotia infected with T. flavus ranged from 0 - 4.2% (Table 18). Similar results occurred at 10 and 15 weeks after burial. Application of T.

flavus in the fall compared with a spring application had little effect on the infection of sclerotia by T. flavus. The percentage of sclerotia infected by T. flavus did not differ significantly from one sampling date to another (Appendix 44).

Sclerotia infected by C. minitans were found in all plots to which C. minitans had been applied (Table 18). At five weeks after burial, infection ranged from 0.8 - 22.4% in the C/T and C. minitans -treated plots, respectively. As the proportion of C. minitans in the hyperparasite mixture increased, the number of sclerotia infected by C. minitans tended to increase. In the plots treated with C/T 1:2, C/T 1:1, C/T 2:1 and C. minitans, the percentages of sclerotia infected by C. minitans were 1.8, 0.8, 9.5 and 22.4, respectively. At 10 weeks after burial infection of recovered sclerotia by C. minitans was low and did not differ significantly between treatments (Appendix 39). At 15 weeks after burial, higher percentages of sclerotia infected with C. minitans were recovered from the C. minitans -treated plots compared with the control. In the C/T 1:1, C/T 2:1 and C. minitans -treated plots (SP) 11.3, 7.9 and 3.3% of sclerotia were infected with the hyperparasite compared with 0% in the control. Application of C. minitans in the fall compared with a spring application produced variable results. At five weeks after burial, higher percentages of C. minitans -infected sclerotia were recovered from the fall treated plots. At 10 weeks after burial, although treatment differences were not significant, sclerotia infected by C. minitans were recovered mainly from the plots where the hyperparasite had been applied in the fall. The reverse is true at 15 weeks after

burial where many of the C. minitans -infected sclerotia were recovered from the plots treated with the hyperparasite in the spring. Sampling date is significant indicating that the percentage of recovered sclerotia infected with C. minitans differed significantly from one sampling date to another.

4.3.4 Environmental factors

Soil temperature and moisture influence the survival of sclerotia and the activity of soil microorganisms. Soil temperatures, recorded at a depth of 6.5 cm throughout the 1983, 1984 and 1985 growing seasons at Lethbridge, ranged from 7.6 to 28.9, 5.8 to 31.5 and 2.3 to 29.3 C, respectively (Figure 3). During the main period of wilt development (from the onset of budding to maturity), the mean soil temperatures were 18.1, 17.8 and 18.4 in 1983, 1984 and 1985, respectively (Appendix 48 and 49). Soil temperatures were not recorded in 1986 at Lethbridge or during 1983-1985 at the Winnipeg location. Total moisture (precipitation and irrigation) received per week at the Lethbridge location during the growing season (Experiment A) ranged from 0 to 45.3, 0 to 43.3, 0 to 60.6 and 0 to 49.2 mm in 1983, 1984, 1985 and 1986, respectively (Figures 4 and 5). From the onset of budding to the completion of anthesis, the experimental site received 104.5, 104.0, 133.3 and 130.5 mm in 1983, 1984, 1985 and 1986, respectively (Appendix 49). The percentage soil moisture ranged from 9.8 to 25.5, 8.4 to 22.3 and 6.0 to 23.4% during the 1984, 1985 and 1986 growing seasons, respectively (Appendix 50). Soil moisture averaged 15.4, 16.3 and 13.4% from the onset of budding to maturity in

1984, 1985 and 1986, respectively (Appendices 49 and 50). Similar results occurred in Experiment B (Appendix 50). Soil moisture was not taken at the Winnipeg field site.

An analysis of soil samples collected from the Lethbridge and Winnipeg field sites (1984) indicates that the pH, available phosphorous and level of nitrate (NO3-N) are similar (Appendix 47). The greatest difference in nutrient level of the soil occurs with NH4-N with 3.0 and 16.7 ppm in the Lethbridge and Winnipeg soil samples, respectively.

4.4 DISCUSSION

Data from four years of studies in three fields showed that application of the hyperparasites T. flavus and/or C. minitans to soil at seeding time reduced the incidence of sclerotinia wilt in sunflower. These findings support previous reports on the success of C. minitans (Bogdanova et al., 1986; Huang, 1980b) and T. flavus (McLaren, 1983) as biological control agents for sclerotinia wilt of sunflower in the field.

The percentage of disease in the hyperparasite-treated plots was significantly less than in the control plots. Compared to the control plots, the hyperparasite-treated plots had a high frequency of infection of sclerotia by C. minitans and T. flavus and fewer numbers of sclerotia recovered. These results support previous findings on the ability of T. flavus (Su and Leu, 1980; McLaren et al., 1989) and C. minitans (Huang and Hoes, 1976; Huang and Kokko, 1987) to kill

actively growing hyphae and sclerotia of S. sclerotiorum and suggest that the hyperparasites were responsible for the destruction of sclerotia and consequently the reduction in disease incidence and yield losses in sunflower.

The incidence of disease in the control plots (Experiment B, 1984-85, Lethbridge) was much higher in 1985 than 1984. This may have been due to the different source of sclerotia used for each year. In 1984 and 1985, the sources of sclerotia were infested canola plants and pure culture, respectively.

The variability in the plating out results obtained during 1984-1986 may have been related to the media used. Although PDA was enhanced with streptomycin sulphate, the addition of antifungal compounds such as pentachloronitrobenzene (PCNB) (Tuite, 1969) may have reduced the variability of the results. Although the results do vary, the percentage of disease in the hyperparasite-treated plots was significantly less than in the control plots. Also, fewer sclerotia were recovered from the hyperparasite-treated plots compared with the control plots at Lethbridge (Experiment A, 1983-84; Experiment B, 1984-85) and Winnipeg (1983-85). These results indicate that hyperparasite treatments result in the destruction of sclerotia and consequently the reduction in disease incidence and yield losses in sunflower.

Sunflower seed yield was estimated using previously determined regression equations. To judge how well estimated yields predicted the actual yields, data from a number of groups (years and locations)

were studied. From the five sets of data examined (Appendices 15-17), it is evident that the nature of the agreement varies. Using a regression equation to predict yield can provide fairly accurate estimates as seen with L84 and W84. The observed and estimated values agree fairly closely and there appears to be no bias. For L85 and L86, the regression equations overestimated consistently the actual yields throughout the range of head diameters. Although the estimates are biased, they are off by a constant amount over the range of head diameters which suggests that the method of yield estimation is still useable but that caution should be used when interpreting the results. For the W85 example, the estimates from the regression were biased upwards with the greatest deviations from the actual yield occurring as the head diameter increased. The reasons for this are not known but may be related to some biological factor associated with the different location-years being used to obtain the regression equation, or may be an isolated case.

These examples indicate that the r values can be quite large and using a regression equation to predict yield can provide fairly accurate estimates. However, even though r values may be large, using a regression equation to predict yield can also produce estimates that are biased. Although one example suggested that use of a regression equation derived using data from independent locations and years may not give accurate estimates, the majority suggest that using a regression equation to predict yield may be useful.

Yield losses due to sclerotinia wilt of sunflower were reduced with the application of hyperparasites. At both the Lethbridge and

Winnipeg locations, yield losses were reduced significantly in 1984, the second year of hyperparasite application. In 1985, when sclerotia but no hyperparasites were applied, yield losses were still significantly less at the Lethbridge site. This indicates that a carry-over effect of control occurred with sclerotia of S. sclerotiorum, applied in the spring, being destroyed without a further application of hyperparasites. Destruction of new sclerotia produced by S. sclerotiorum is important as infected sunflower stalks harbor many new sclerotia which contribute to the source of inocula for the following crop season. A reduction in the number of sclerotia would reduce the inoculum potential of S. sclerotiorum in the next season.

At the Winnipeg site, seed yields were lower but not significantly so in the hyperparasite-treated plots compared with the control plots. This may have been due to reduced differences between disease incidence in the control plots compared with the hyperparasite-treated plots. The differences in disease incidence may not have been large enough to create significant differences in yield.

A carry-over effect of biocontrol has important economic considerations. Production of a biofungicidal product is costly and the marketability of such a product would be enhanced if it was effective not only during the year of application but for another year at no extra cost to the landowner. Although the carry-over effect did not last for a second season (1986), a reduction in yield loss and disease incidence for a period of one year is significant.

Seed yield in the control plots was quite different from one year to the next at each location. At the Lethbridge site, yield was greater in 1985 that in 1984. Such difference may be related to variations in soil moisture. The amount of moisture received each week at the Lethbridge site in 1984 compared with 1985 varied with large differences occurring from the budding to the anthesis growth stages (Appendix 50), a period during which the crop is most sensitive to moisture stress (NDSU, 1985). The total precipitation and irrigation received in 1984 and 1985 during this growth period was 104 and 134 mm, respectively. This represents an increase in moisture of 29% (Appendix 49) and corresponds with an increase in yield of 56% (Table 1). These findings support previous results on the effects of moisture reduction on yield potential of sunflower. A 50% reduction in yield has been reported with a 20% reduction in application of irrigation moisture from the onset of budding to the completion of anthesis (NDSU, 1985).

The application of hyperparasites in the fall compared with a spring application had little effect on the viability and infection of sclerotia by T. flavus and C. minitans. Significant differences between times of application occurred within the hyperparasite treatments for recovered sclerotia with fewer sclerotia being removed from plots that received a spring compared with a fall application of hyperparasites. The recovery of sclerotia was less in the hyperparasite-treated plots compared with the control regardless of the application time indicating that the introduction of hyperparasites into soil may have altered the biological balance to

favor the destruction of sclerotia of S. sclerotiorum. The effect of the addition of hyperparasites to soil was evident during the same season of application as well as after an overwintering period.

Sclerotia recovered from the experimental plots showed a low percentage of infection by T. flavus and C. minitans. A significant reduction in the number of sclerotia recovered from the C. minitans -treated plots indicates that a treatment effect occurred. Non-recovered sclerotia may have been affected by the presence of C. minitans and destroyed prior to the recovery dates. The presence of a variety of microorganisms recovered from the buried sclerotia suggests that while C. minitans may have been involved in the parasitism of the sclerotia, secondary microorganisms may have invaded the diseased tissue making the recovery of C. minitans on agar media difficult.

Sclerotia are subject to variations in moisture content of the soil and these changes affect their survival (Williams and Western, 1965b). In the present sclerotial studies, survival of sclerotia was frequently reduced as time from burial progressed. During 1984-86, the largest reductions in the number of sclerotia recovered after 15 weeks of burial occurred in 1984 (Lethbridge, Experiment A). Soil moisture was more variable in 1984 than in 1985 or 1986 at Lethbridge (Appendix 50). Smith (1972b) found that sclerotia which are dried and rewetted leak nutrients which may promote microbial degradation and accelerate death of the sclerotia. Variations in soil moisture during the 1984 study may have contributed to the reduction in the recovery of sclerotia from all treatments.

Usually environmental conditions are not independent in their actions but complement each other (Griffin, 1969). The combination of soil temperature and moisture favors the destruction of sclerotia of S. sclerotiorum (Ervio et al., 1964; Williams and Western, 1965b; Cook et al., 1975). In the present study, the recovery of sclerotia was reduced from the control and hyperparasite-treated plots as time from burial increased. Both hyperparasites were effective in reducing sclerotia inocula over the range of soil temperature and moisture encountered during 1983-86. As well, both hyperparasites were effective in reducing the incidence of disease at the Lethbridge location. However, C. minitans was more effective against S. sclerotiorum than T. flavus at the Winnipeg site. The environmental activity of a biocontrol agent can depend on specific environmental requirements (Phillips, 1986). The environmental conditions required to induce biocontrol using T. flavus may be more specific than those required for optimal activity of C. minitans. Baker (1987) cites examples of fungi such as Sporidesmium sclerotivorum and G. virens which have specific environmental requirements for optimal biological control activity. For the best results, these fungi should be applied to environments conforming to or adjusted to their optimal activity. This may be the case with T. flavus. Information on the factors affecting the activity and survival of both T. flavus and C. minitans, produced in various formulations, is meager. Further investigations on the ecology of both hyperparasites are warranted.

Synergism - a state in which two organisms work better together than alone - is not apparent in the combination of T. flavus and C.

minitans. The organisms do not appear to be antagonistic to each other as such activity would likely nullify the biocontrol effect and this is not apparent in the data (Table 1). Competition for one or two resources (eg. space, nutrients) may be involved, but not to such a degree that the biocontrol activity of either hyperparasite is affected seriously.

Sclerotia used in the biocontrol experiments were whole (1984-1986) or chopped (1983). Sclerotia having incomplete or non-melanized rinds are characterized by a lack of dormancy as well as susceptibility to attack by other microorganisms (Huang, 1980b). At both the Winnipeg and Lethbridge locations, the incidence of sclerotinia wilt in the control plots is greater in the early part of the 1983 season compared with the same period in 1984. This may be related to a lack of dormancy in the chopped sclerotia used in 1983 compared with whole sclerotia used in 1984. During the latter portion of the season in 1983 and 1984, the development of sclerotinia wilt is similar suggesting that some sclerotia may have regenerated complete rinds rendering them less susceptible to destruction by hyperparasites. Plant to plant spread (Huang and Hoes, 1980) would also account for the increased levels of disease during the latter portion of each season.

The density of natural inocula of S. sclerotiorum at the Lethbridge (Experiment A and B) and Winnipeg sites averaged 0.01, 0.01 and 0.02 sclerotia per kilogram of soil, respectively. These levels of inocula are quite low compared with the concentrated application of sclerotia within the rows at seeding time. Such low levels of natural inocula

of S. sclerotiorum indicate that introduced sclerotia were responsible for the high levels of disease in the plots treated with sclerotia only compared with the plots receiving no treatment (sunflower alone).

The substrate used for the growth of hyperparasites is important. Wheat bran has been used successfully for the production of T. flavus (McLaren et al., 1983) and C. minitans (Ahmed and Tribe, 1977). Application of a hyperparasite to small plot trials in this form is feasible. However, on a large scale basis, a formulation suitable for application with conventional farm equipment is necessary. Talaromyces flavus has been produced in alginate pellets by a process which is inexpensive and versatile (Fravel et al., 1985). Such a procedure may prove to be suitable for C. minitans as well. Addition of wheat bran to pellets has been used to enhance the proliferation of biological control agents (Lewis and Papavizas, 1984a). The addition of a food base to T. flavus inocula has enhanced significantly the colonization of soil by this fungus (Spink and Rowe, 1989). Various compounds have been incorporated into soil along with biocontrol agents selectively to enhance their activity (Baker, 1986). Further investigations on the addition of compounds such as food bases, antibiotics, etc. to pellets or to soil in order to enhance the establishment of hyperparasites such as T. flavus and C. minitans within a new environment are warranted.

The addition of undigested organic matter to soil can result in biological control (Baker, 1981; 1983). In the present study, bran was applied with the sclerotia to determine whether a food base could induce biological control. Although the most significant reductions

in disease incidence occurred with the application of hyperparasites, a reduced incidence of disease in the bran-treated plots compared with the control (SS) plots indicates that a degree of biological control has occurred. Much of the nutrients in the bran would be utilized by the resident population of microorganisms some of which may be antagonistic to sclerotia of S. sclerotiorum. Any increase in activity of such microorganisms would be deleterious to the survival of sclerotia of S. sclerotiorum.

Use of sterile wheat bran as a treatment is fine but as a control it is not recommended (Baker et al., 1984) because the bran itself has its own 'interference'. Although Baker et al. (1984) suggest that using autoclaved inocula (hyperparasite grown on sterile bran and autoclaved) is a not an appropriate control, because the physical, chemical and ecological properties are not identical to those of the live inocula, use of such a control may be more appropriate than use of bran alone due to the likelihood of more similarities in properties of the live inocula and the autoclaved live inocula compared with the bran and the live inocula.

A hyperparasite such as Sporidesmium sclerotivorum reduces significantly the survival of sclerotia of S. sclerotiorum (Adams and Ayers, 1981). The success of this hyperparasite is partly due to the fact that it is effective under natural conditions. Huang (1977) demonstrated that hyperparasitism by C. minitans was an important factor affecting the survival of S. sclerotiorum in a sunflower field. Hyperparasitism can have a substantial impact on the inoculum potential of plant pathogenic microorganisms as indicated from

evidence accumulated from a number of systems (Baker, 1987). Effective control of sclerotinia wilt of sunflower has not been available through the use of chemicals or resistant cultivars. Consequently, biological control using the hyperparasites T. flavus and C. minitans may have good potential when used in conjunction with cultural practises recommended for the control of this disease.

Table 1. Effect of *T. flavus* and *C. minitans* on the incidence of sclerotinia wilt and seed yield of sunflower (Experiment A, Lethbridge, 1983-86).

	% disease[1]			
Hyperparasites	1983	1984	1985	1986
none	0[2]d[3]	0[2]d	0[2]c	0.1[2]b
bran	0.6 d	0.2 cd	0.1 bc	0 b
S. sclerotiorum (SS)[4]	54.9 a	49.9 a	38.6 a	54.1 a
SS + bran	25.2 b	27.1 b	25.6 a	51.8 a
SS + *T. flavus*[5] (T)	0.0 d	0.1 d	4.3 b	51.0 a
SS + *C. minitans* (C)	9.8 c	5.3 c	5.1 b	41.7 a
SS + C/T	2.0 d	0.1 d	0.3 bc	45.9 a

	Yield (kg/ha)[6]		
Hyperparasites	1984	1985	1986
none	1260.3 abc	1962.6 ab	1489.0 a
bran	1393.0 a	2370.5 a	1487.5 a
S. sclerotiorum	952.7 c	866.5 c	296.5 b
SS + bran	988.7 bc	1194.5 bc	329.8 b
SS + *T. flavus*	1433.0 a	1905.6 ab	340.2 b
SS + *C. minitans*	1326.8 abc	1732.8 ab	589.1 b
SS + C/T	1360.0 ab	2095.5 a	327.8 b

1 Values averaged over 4 replicates; based on disease incidence from 4-row plots.

2 Analysis of variance on transformed data; means converted back to raw form for presentation.

3 Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

4 Sources of sclerotia were diseased sunflower heads (1983, 1986) and diseased bean plants (1984); sclerotia were produced in pure culture on a bean substrate (1985).

5 Rates of application for bran, T, C, and C/T were 45 (1983) and 50 (1984) grams per 6.1m row; sclerotia and hyperparasites were applied in 1983 and 1984; only sclerotia were applied in 1985 and 1986.

6 Yield taken on the center two rows of each 4-row plot; no yield data were available in 1983.

Table 2. Effect of *T. flavus* and *C. minitans* on the incidence of sclerotinia wilt and seed yield of sunflower (Experiment A, Winnipeg, 1983-85).

	% disease[1]			Yield[1] (kg/ha)	
Hyperparasites	1983	1984	1985	1984	1985
none	0.6[2]d[3]	1.8[2]c	1.7[2]b	1934.7 a	1234.7 a
bran	0.6 d	2.1 c	2.6 b	2038.7 a	1314.5 a
SS[4]	65.2 a	77.2 a	24.4 a	664.6 d	992.2 a
SS + bran	54.6 b	59.9 a	26.5 a	1123.7 dc	1085.6 a
SS + T[5]	14.4 c	33.4 b	21.1 a	1464.3 bc	1209.5 a
SS + C	3.1 d	1.0 c	1.2 b	1862.0 ab	1215.3 a

[1] Values averaged over 4 replicates; based on disease incidence from 4-row plots; no yield data available in 1983; yield was
[2] taken from the 2 center rows of each 4-row plot.
Analysis of variance on transformed data; means converted back
[3] to raw form for presentation.
Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least
[4] significant difference test).
SS = *S. sclerotiorum*; sources of sclerotia were diseased sunflower heads (1983, 1986) and diseased bean plants (1984); sclerotia were produced in pure culture on a bean substrate
[5] (1985).
T = *T. flavus*; C = *C. minitans*; rates of application for bran, C, T, and C/T were 22.5 g (1983) and 100 g (1984) per 6.1 m row.

Table 3. The effect of *T. flavus* and *C. minitans* on the development of sclerotinia wilt of sunflower over time at the Lethbridge field site (Experiment A, 1983).

	Disease incidence[1] (%)							
Hyperpar.	20/6[2]	4/7	18/7	1/8	15/8	29/8	12/9	26/9
none	0 de[3]	0 de	0 d	0 d	0 e	0 d	0 d	0 d
bran	0 e	0 e	0 d	0 d	0.2 de	0.4 cd	0.5 d	0.6 d
SS[4]	5.7 a	7.5 a	9.1 a	13.6 a	23.7 a	32.0 a	42.9 a	54.9 a
SS + bran	3.5 b	4.0 b	4.3 b	5.0 b	9.1 b	12.9 b	17.7 b	25.2 b
SS + C[5]	0.7 c	0.8 c	1.1 c	1.4 c	2.8 c	4.7 c	6.3 c	9.8 c
SS + T[5]	0 e	0 e	0 d	0 d	0 e	0 d	0 d	0 d
SS + C/T	0.6 cd	0.6 cd	0.6 e	0.8 c	1.1 cd	1.1 c	1.5 cd	2.0 d

[1] Values represent percent disease averaged over 4 replicates; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Disease rated weekly over a 15-week period; data presented from alternate weeks only.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] SS = *S. sclerotiorum*.

[5] C = *C. minitans*; T = *T. flavus*; C/T = 1:1 (w/w).

Table 4. The effect of *T. flavus* and *C. minitans* on the development of sclerotinia wilt of sunflower over time at the Lethbridge field site (Experiment A, 1984).

	Disease incidence[1] (%)							
Hyperpar.	25/6[2]	9/7	23/7	6/8	20/8	3/9	17/9	1/10
none	0 b[3]	0 b	0 b	0 b	0 b	0 c	0 c	0 c
bran	0 b	0 b	0 b	0 b	0.1 b	0.1 c	0.1 c	0.1 c
SS[4]	0.3 a	1.1 a	6.1 a	9.9 a	21.6 a	34.6 a	43.9 a	49.9 a
SS + bran	0.3 a	0.8 a	4.7 a	7.7 a	12.2 a	18.9 b	24.3 b	27.1 b
SS + C[5]	0.1 ab	0.1 a	0.9 b	1.3 b	2.4 b	4.0 c	4.8 c	5.3 c
SS + T[5]	0 b	0 a	0 b	0 b	0 b	0 c	0.0 c	0.1 c
SS + C/T	0 b	0 a	0 b	0 b	0 b	0 c	0 c	0 c

[1] Values represent percent disease averaged over 4 replicates; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Disease rated weekly over a 15-week period; data presented from alternate weeks only.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] SS = *S. sclerotiorum*.

[5] C = *C. minitans*; T = *T. flavus*; C/T = 1:1 (w/w).

Table 5. The effect of *T. flavus* and *C. minitans* on the development of sclerotinia wilt of sunflower over time at the Lethbridge field site (Experiment A, 1985).

	Disease incidence[1] (%)							
Hyperpar.	17/6[2]	1/7	15/7	29/7	12/8	26/8	9/9	23/9
none	0 b[3]	0 b	0 c	0 d	0 c	0 c	0 c	0 d
bran	0 b	0 b	0 c	0 cd	0 c	0 c	0 c	0.1 cd
SS[4]	0.3 a	1.8 a	5.1 a	13.0 a	24.1 a	30.8 a	35.2 a	38.6 a
SS + bran	0.2 ab	1.4 a	3.2 ab	6.1 ab	14.3 a	19.5 a	23.5 a	25.6 a
SS + C[5]	0 b	0.1 b	0.3 c	0.9 cd	2.4 b	3.6 b	4.3 b	5.1 bc
SS + T[5]	0 b	0.3 b	1.0 bc	2.2 bc	2.4 b	2.8 b	3.9 b	4.3 bc
SS + C/T	0 b	0 b	0 c	0 d	0.2 bc	0.3 bc	0.3 bc	0.3 cd

[1] Values represent percent disease averaged over 4 replicates; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Disease rated weekly over a 15-week period; data presented from alternate weeks only.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] SS = *S. sclerotiorum*.

[5] C = *C. minitans*; T = *T. flavus*; C/T = 1:1 (w/w).

Table 6. The effect of *T. flavus* and *C. minitans* on the development of sclerotinia wilt of sunflower over time at the Lethbridge field site (Experiment A, 1986).

	Disease incidence[1] (%)								
Hyperpar.	9/6[2]	16/6	30/6	14/7	28/7	11/8	25/8	8/9	22/9
none	0 a[3]	0 a	0 b	0 b	0 b	0 b	0 b	0.1 b	0.1 b
bran	0 a	0 a	0 b	0 b	0 b	0 b	0 b	0 b	0 b
SS[4]	0 a	0.2 a	2.0 a	4.5 a	16.3 a	30.5 a	41.8 a	46.0 a	54.1 a
SS + bran	0.2 a	0.3 a	1.9 a	5.6 a	18.1 a	33.6 a	40.9 a	46.2 a	51.8 a
SS + C[5]	0 a	0.2 a	1.0 a	2.7 a	13.9 a	23.1 a	30.3 a	34.8 a	41.7 a
SS + T[5]	0.2 a	0.3 a	2.2 a	6.9 a	16.5 a	31.1 a	40.4 a	44.7 a	51.0 a
SS + C/T	0.1 a	0.3 a	0.9 a	3.8 a	11.4 a	28.6 a	37.5 a	41.6 a	45.9 a

[1] Values represent percent disease averaged over 4 replicates; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Disease rated weekly over a 16-week period; data presented from alternate weeks only.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] SS = *S. sclerotiorum*.

[5] T = *T. flavus*; C = *C. minitans*; C/T = 1:1 (w/w).

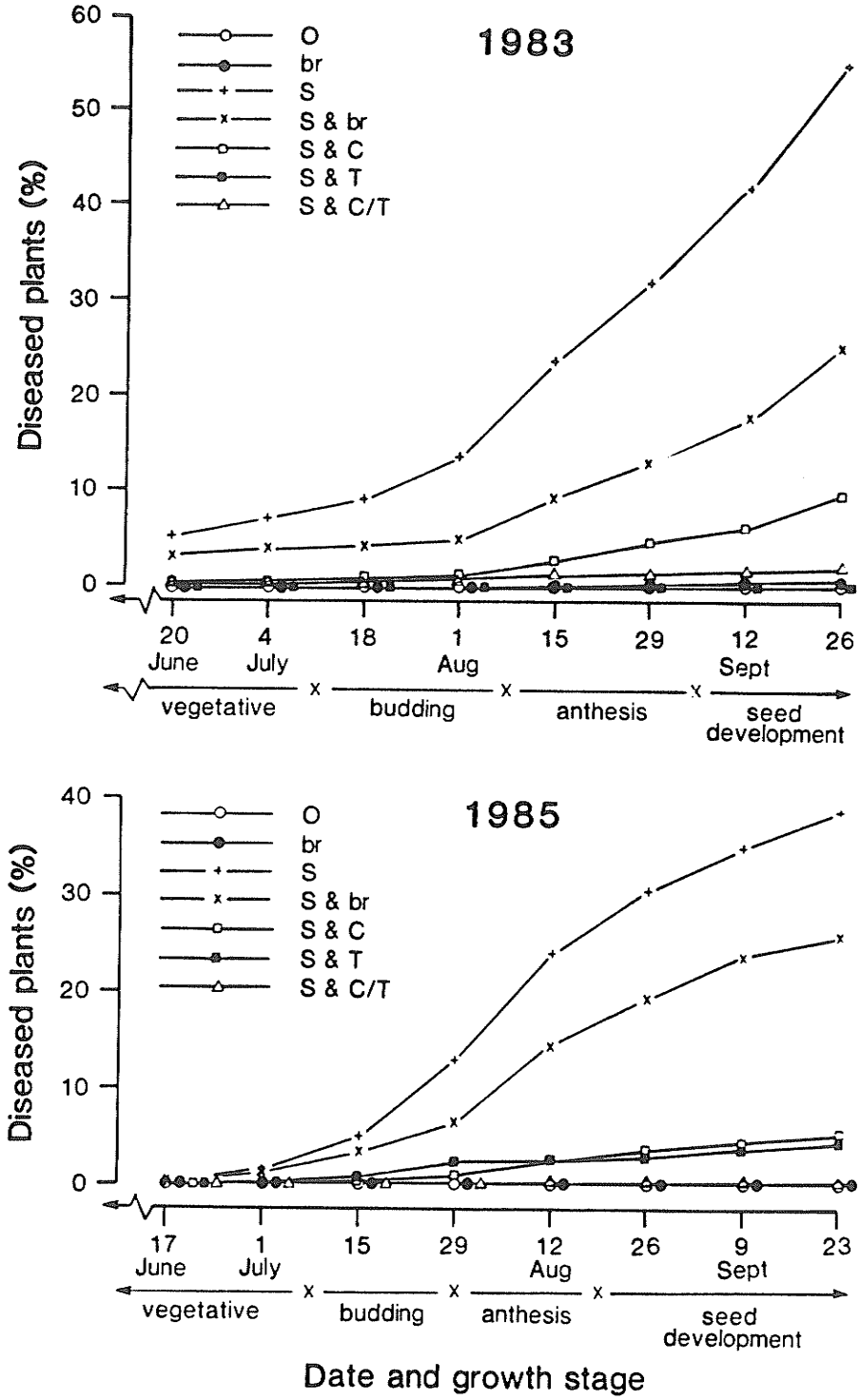
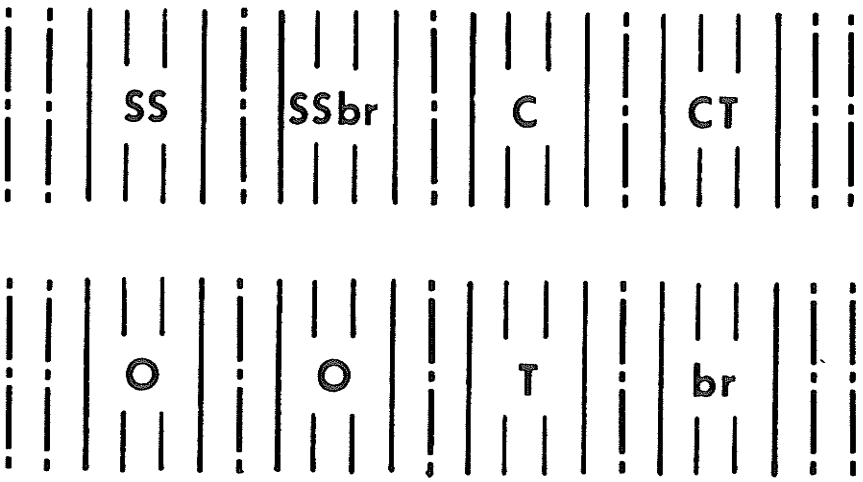


Figure 1. The effect of T. flavus (T) and C. minitans (C) on the development of sclerotinia wilt of sunflower in 1983 and 1985 (Lethbridge). Hyperparasites were applied in 1983 and 1984, but not in 1985. Note the carry-over effect of biocontrol (1985) in the T, C and C/T-treated plots.

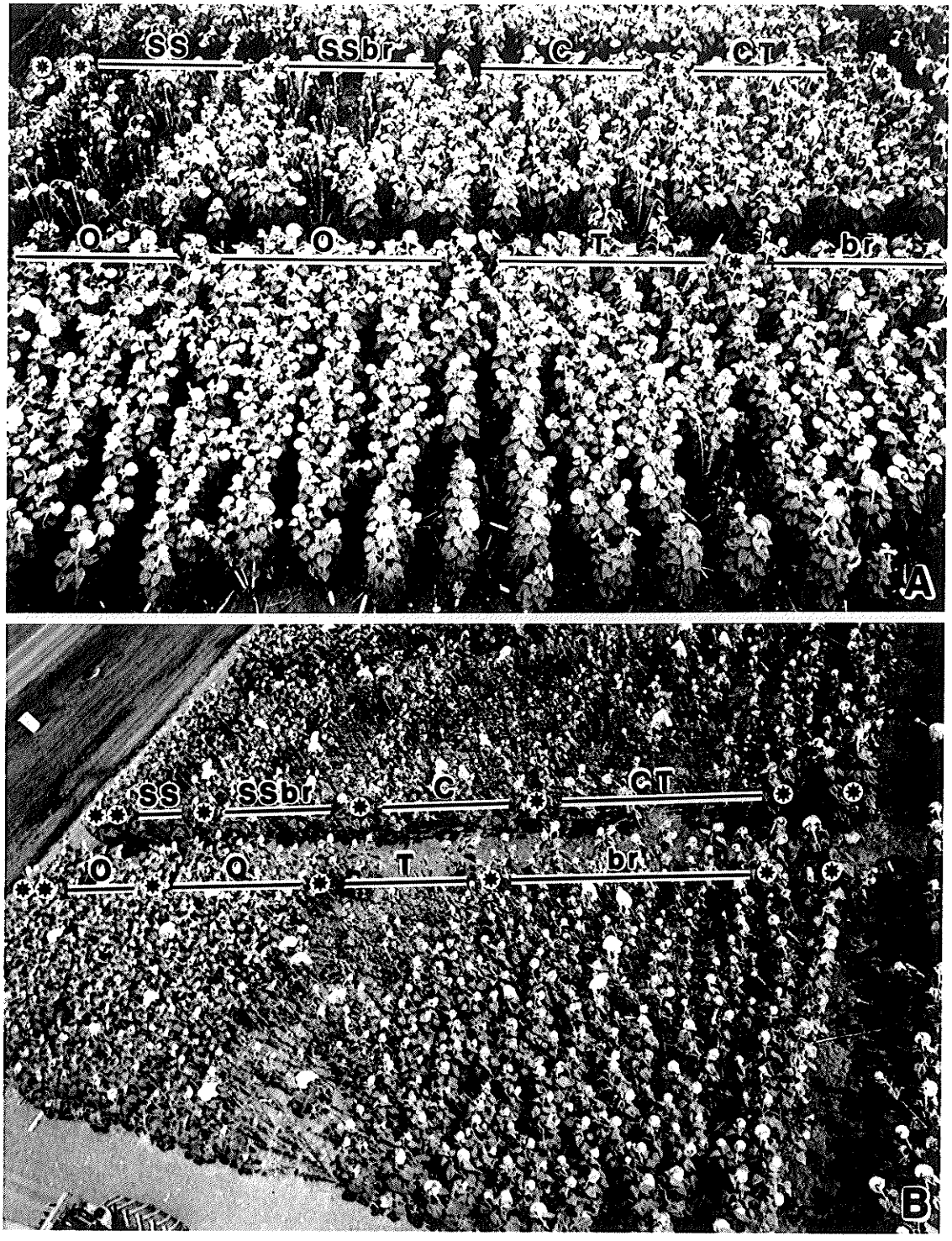
Plate 8. Carry-over effect of *T. flavus* and *C. minitans* on sclerotinia wilt of sunflower (Lethbridge, 1985-86).

Figure A is the fourth replicate of the 1985 test; Figure B is the same fourth replicate of the 1986 test. Sunflower has been sown to the same rows of this field since 1983.

In 1983 and 1984, *T. flavus* and/or *C. minitans* were applied to field plots along with sclerotia of *S. sclerotiorum*. Sclerotia were applied in 1985 and 1986 without the addition of the biocontrol agents. While disease remained severe in 1985 in the control plot, where only sclerotia were applied, a carry-over effect of biocontrol ws evident in the plots treated with *C. minitans* (CM), *T. flavus* (TF), and a combination of *C. minitans* and *T. flavus* (CT). In 1986, the carry-over effect was no longer noticeable in the CM, TF and CT-treated plots.



O - sunflower alone
br - bran
SS - *S. sclerotiorum* (SS)
SSbr - SS and bran
T - SS and *T. flavus*
C - SS and *C. minitans*
CT - SS and *T. flavus* and *C. minitans*
guard rows


SS
SSbr
C
CT
O
O
T
br
A
SS
SSbr
C
CT
O
O
T
br
B

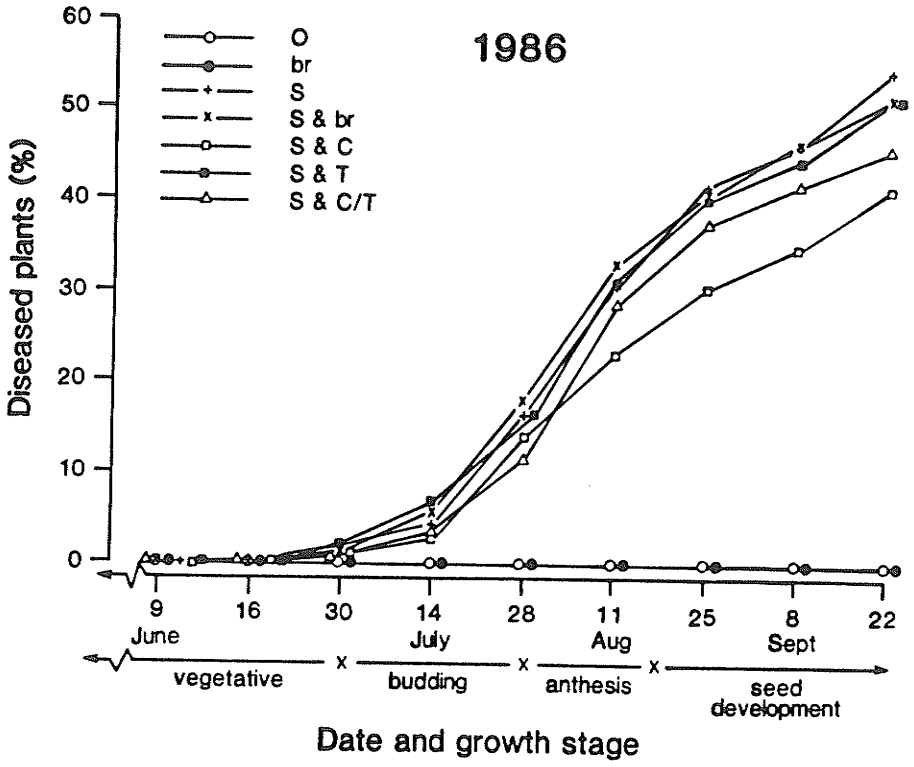


Figure 2. The effect of T. flavus (T) and C. minitans (C), applied in 1983 and 1984, on the development of sclerotinia wilt of sunflower in 1986 (Lethbridge). The carry-over effect of biocontrol is no longer evident as illustrated by the high levels of disease in the C, T and C/T-treated plots.

Table 7. Effect of hyperparasites and time of application (season) on the incidence of sclerotinia wilt of sunflower (Experiment B, Lethbridge, 1984-85).

Hyperparasites	1984		1985	
	Appl.[1]	Disease (%)[2]	Appl.[3]	Disease (%)[2]
bran	SP	0[4]b[5]	F	0[4]f[5]
			SP	0 f
S. sclerotiorum (SS)	SP	1.3 a	F	17.2 a
			SP	17.1 a
SS[6] + bran	SP	1.4 a	F	12.4 abc
			SP	13.1 ab
SS + *T. flavus* (T)	SP	0.1 ab	F	5.2 cd
			SP	5.9 bcd
SS + C/T 1:2	SP	0.2 ab	F	0.2 ef
			SP	0.6 ef
SS + C/T 1:1	SP	0 b	F	1.9 de
			SP	0.3 ef
SS + C/T 2:1	SP	0 b	F	0 f
			SP	0 f
SS + *C. minitans* (C)	SP	0.3 ab	F	0 f
			SP	0 f

1 Rate of application of bran, C, T, and C/T was 100 g/6.1m row in the spring of 1984. Sclerotia (250) were also added to each row (excluding bran plots).

2 Values averaged over 4 replicates; based on the incidence of disease from 3-row plots.

3 F = fall (1984) application of 100 g of bran, C, T, and C/T. SP = spring (1985) application of 100 g of bran, C, T and C/T. All sclerotia (250/6.1m row) were applied in the spring of 1985.

4 Analysis of variance on transformed data; means converted back to raw form for presentation.

5 Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

6 Sclerotia were produced in pure culture on a bean substrate (1985).

Table 8. Inoculum density of *S. sclerotiorum* in three fields (Winnipeg and Lethbridge, 1984).

Treatment	Avg. inoculum density[1] (no. scler./kg soil)		
	Exp. A		Exp. B
	Leth.	Wpg.	Leth.
none	0.03 a[2]	0.02 a	-[3]
bran	0 a	0 a	-
S. sclerotiorum (SS)	0 a	0.02 a	0 a
SS + bran	0.02 a	0.04 a	0.02 a
SS + *T. flavus* (T)	0 a	0.02 a	0.02 a
SS + *C. minitans* (C)	0 a	0.02 a	0 a
SS + C/T 1:1	-	-	0.02 a
SS + C/T 1:2	-	-	0.03 a
SS + C/T 2:1	-	-	0 a

[1] Analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[3] Treatments not included in the experiment are designated as -.

Table 9. The effect of hyperparasites and sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field in May, 1983 (Lethbridge, Experiment A).

Hyperparasites	Weeks after burial			Treatment means
	5	10	15	
	A. Recovered sclerotia[1]			
Control (SS)	20.0 a[2]	14.8 a[2]	15.0 a[2]	16.8 a[2]
SS + bran	20.0 a	13.7 a	13.7 a	16.1 a
SS + T. flavus (T)	19.5 a	8.0 b	8.3 b	13.1 b
SS + C. minitans (C)	20.0 a	12.2 ab	14.6 a	15.9 a
SS + C/T 1:1	20.0 a	7.3 b	7.2 b	13.0 b
Date means[3]	19.9 a	11.6 b	12.2 b	
	B. Percent of recovered sclerotia that were viable[1]			
Control (SS)	22.5 a[2]	3.9 a	4.6 a	8.8 a
SS + bran	6.3 b	0 b	0 b	1.3 b
SS + T. flavus (T)	1.9 b	0 b	0 b	0.5 b
SS + C. minitans (C)	0.8 c	0.9 ab	0 b	0.5 b
SS + C/T 1:1	4.2 b	0 b	0 b	0.9 b
Date means[3]	5.5 a	0.7 b	0.5 b	
	C. Percent of recovered sclerotia infected by T. flavus[1]			
Control (SS)	9.2 ab[2]	32.1 a	0.9 ab	10.6 a
SS + bran	12.2 ab	21.6 a	1.1 ab	9.6 a
SS + T. flavus (T)	16.4 a	10.6 b	10.4 a	12.3 a
SS + C. minitans (C)	3.4 b	2.9 b	0 b	1.8 a
SS + C/T 1:1	19.2 a	35.2 a	0 b	13.3a
Date means[3]	11.3 a	18.1 a	1.6 b	
	D. Percent of recovered sclerotia infected by C. minitans[1]			
Control (SS)	0 b[2]	0 a	0 a	0 a
SS + bran	0 b	0 a	0 a	0 a
SS + T. flavus (T)	2.8 a	0 a	0 a	0.7 a
SS + C. minitans (C)	2.6 a	0 a	0 a	0.6 a
SS + C/T 1:1	0 b	0 a	0 a	0 a
Date means[3]	0.8 a	0 a	0 a	

[1] Values averaged over 4 replicates with 20 sclerotia per replicate; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Means (for A,B,C or D) within columns (excluding overall date means) followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test); the hyperparasite x sampling date interaction is significant for recovered sclerotia and sclerotia infected by T. flavus. Sampling date is significant for recovered sclerotia, viable sclerotia and sclerotia infected by T. flavus.

[3] For tables of treatment and date means, see appendices 19 and 20.

Table 10. The effect of hyperparasites and sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field in May, 1984 (Lethbridge, Experiment A).

Hyperparasites	Weeks after burial				Treatment means
	5	10	15	50	
	A. Recovered sclerotia[1]				
Control (SS)	19.8 a[2]	20.0 a[2]	18.6 ab[2]	10.5 a[2]	17.7 a[2]
SS + bran	20.0 a	17.7 b	20.0 a	9.9 a	17.4 ab
SS + T. flavus (T)	20.0 a	19.8 a	15.8 c	5.5 b	16.4 bc
SS + C. minitans (C)	20.0 a	19.5 a	16.3 c	4.3 b	16.3 c
SS + C/T 1:1	20.0 a	17.3 b	16.8 bc	8.4 ab	16.2 c
Date means[3]	20.0 a	19.1 ab	17.6 b	8.1 c	
	B. Percent of recovered sclerotia that were viable[1]				
Control (SS)	12.9 a[2]	0.8 a	3.3 a	4.0 a	4.0 a
SS + bran	3.3 b	0 a	1.8 a	0 a	1.0 b
SS + T. flavus (T)	0.8 b	0 a	0 a	0 a	0.2 b
SS + C. minitans (C)	0 b	0 a	0 a	3.4 a	0.6 b
SS + C/T 1:1	0 b	0.9 a	0 a	0 a	0.2 b
Date means[3]	2.2 a	0.3 a	0.8 a	1.1 a	
	C. Percent of recovered sclerotia infected by T. flavus[1]				
Control (SS)	0 b[2]	0 a	0.8 a	0 a	0.2 a
SS + bran	0 b	0 a	0 a	1.4 a	0.3 a
SS + T. flavus (T)	4.9 a	1.8 a	2.4 a	0 a	2.0 a
SS + C. minitans (C)	0 b	0 a	0.9 a	0 a	0.2 a
SS + C/T 1:1	0 b	0 a	0.9 a	0 a	0.2 a
Date means[3]	0.6 a	0.3 a	0.9 a	0.2 a	
	D. Percent of recovered sclerotia infected by C. minitans[1]				
Control (SS)	0 a[2]	0 a	0 a	1.7 ab	0.3 a
SS + bran	0 a	0 a	0 a	0 b	0 a
SS + T. flavus (T)	0 a	0 a	0 a	0 b	0 a
SS + C. minitans (C)	0 a	0 a	0.8 a	3.4 a	0.8 a
SS + C/T 1:1	0 a	0 a	1.5 a	0 b	0.3 a
Date means[3]	0 a	0 a	0.4 a	0.8 a	

[1] Values averaged over 4 replicates per treatment; based on 20 sclerotia per replicate; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Means (for A,B,C or D) within columns (excluding overall date means) followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test); the hyperparasite x sampling date interaction is significant at the 5% level for recovered sclerotia only; sampling date is significant for recovered sclerotia only.

[3] For tables of treatment and date means, see appendices 22 and 23.

Table 11. The effect of hyperparasites and sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field in May, 1985 (Lethbridge, Experiment A).

Hyperparasites	Weeks after burial 5	10	15	Treatment means
	A. Recovered sclerotia[1]			
Control (SS)	20.0 a[2]	20.0 a[2]	19.3 b[2]	19.8 a[2]
SS + bran	20.0 a	20.0 a	20.0 a	20.0 a
SS + T. flavus (T)	19.5 a	19.8 a	20.0 a	19.8 a
SS + C. minitans (C)	19.5 a	20.0 a	20.0 a	19.8 a
SS + C/T 1:1	19.8 a	20.0 a	20.0 a	19.9 a
Date means[3]	19.8 a	19.9 a	19.9 a	
	B. Percent of recovered sclerotia that were viable[1]			
Control (SS)	31.5 ab[2]	0.8 a	9.0 a	10.3 a
SS + bran	4.1 a	0 a	3.3 ab	8.6 a
SS + T. flavus (T)	23.3 bc	0 a	0.8 b	4.5 a
SS + C. minitans (C)	24.9 bc	1.8 a	0.8 b	6.1 a
SS + C/T 1:1	16.1 c	1.8 a	0 b	3.9 a
Date means[3]	26.7 a	0.8 b	2.0 b	
	C. Percent of recovered sclerotia infected by T. flavus[1]			
Control (SS)	0 a[2]	20.5 a	0 a	3.5 a
SS + bran	1.8 a	20.6 a	0 a	4.7 a
SS + T. flavus (T)	0 a	17.0 a	1.8 a	4.1 a
SS + C. minitans (C)	0 a	16.2 a	0 a	2.9 a
SS + C/T 1:1	0 a	3.4 b	0 a	0.8 b
Date means[3]	0.3 b	14.6 a	0.3 b	
	D. Percent of recovered sclerotia infected by C. minitans[1]			
Control (SS)	0.8 ab[2]	1.8 a	0 a	0.8 a
SS + bran	0.8 ab	0 a	0.8 a	0.5 a
SS + T. flavus (T)	0.8 ab	1.9 a	0 a	0.8 a
SS + C. minitans (C)	3.4 a	0.8 a	3.3 a	2.3 a
SS + C/T 1:1	0 b	1.3 a	1.8 a	0.9 a
Date means[3]	1.0 a	1.1 a	0.9 a	

[1] Values averaged over 4 replicates per treatment with 20 sclerotia per replicate; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Means (for A,B,C or D) within columns (excluding overall date means) followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test); the hyperparasite x sampling date interaction is significant at the 5% level for viable sclerotia only; sampling date is significant for viable sclerotia and sclerotia infected by T. flavus.

[3] For tables of treatment and date means, see appendices 25 and 26.

Table 12. The effect of hyperparasites and sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field in May of 1986 (Lethbridge, Experiment A).

Hyperparasites	Weeks after burial			Treatment means
	5	10	15	
	A. Recovered sclerotia[1]			
Control (SS)	19.8 a[2]	16.4 a[2]	12.6 b[2]	16.5 b[2]
SS + bran	19.5 a	16.7 a	16.3 a	17.6 ab
SS + T. flavus (T)	19.8 a	16.5 a	15.9 ab	17.5 ab
SS + C. minitans (C)	19.0 a	18.6 a	13.1 b	17.1 b
SS + C/T 1:1	19.1 a	18.6 a	18.8 a	18.8 a
Date means[3]	19.4 a	17.4 ab	15.5 b	
	B. Percent of recovered sclerotia[1] that were viable			
Control (SS)	0 a[2]	3.1 a	1.1 a	1.2 a
SS + bran	0 a	0.8 ab	0 a	0.2 a
SS + T. flavus (T)	0.8 a	0 b	0 a	0.2 a
SS + C. minitans (C)	0 a	0 b	0 a	0 a
SS + C/T 1:1	0 a	0.8 ab	0 a	0.2 a
Date means[3]	0.1 a	0.7 a	0.2 a	
	C. Percent of recovered sclerotia[1] infected by T. flavus			
Control (SS)	0.8 ab[2]	0 a	0 a	0.2 a
SS + bran	0 b	4.5 a	0 a	1.0 a
SS + T. flavus (T) a	4.9 a	2.2 a	1.3 a	2.6 a
SS + C. minitans (C)	0 b	0 a	0 a	0 a
SS + C/T 1:1	0.8 ab	3.7 a	1.9 a	2.0 a
Date means[3]	0.9 a	1.6 a	0.5 a	
	D. Percent of recovered sclerotia[1] infected by C. minitans			
Control (SS)	0 a[2]	0 a	0 b	0 a
SS + bran	0 a	0 a	0 b	0 a
SS + T. flavus (T)	0 a	0 a	0.8 ab	0.2 a
SS + C. minitans (C)	0 a	1.4 a	5.5 a	1.7 a
SS + C/T 1:1	0 a	2.1 a	4.6 a	1.8 a
Date means[3]	0 b	0.5 ab	1.6 a	

[1] Values averaged over 4 replicates per treatment with 20 sclerotia per replicate; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Means (for A,B,C or D) within columns (excluding overall date means) followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test); the hyperparasite x sampling date interaction is nonsignificant for all variables; sampling date is significant for recovered sclerotia and sclerotia infected by C. minitans.

[3] For tables of treatment and date means see appendices 28 and 29.

Table 13. The effect of hyperparasites and sampling date (weeks after burial) on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field in May, 1983 (Winnipeg).

Hyperparasites	Weeks after burial			Treatment means
	5	10	15	
	A. Recovered sclerotia[1]			
Control (SS)	20.0 a[2]	18.8 a[2]	16.5 a[2]	18.5a[2]
SS + bran	20.0 a	17.3 b	14.8 b	17.5 b
SS + *T. flavus*	20.0 a	10.6 c	5.6 c	13.5 c
SS + *C. minitans*	20.0 a	8.4 c	4.4 c	12.8 c
Date means[3]	20.0 a	14.5 b	11.7 b	
	B. Percent of recovered sclerotia that were viable[1]			
Control (SS)	4.9 a[2]	9.6 a	7.5 a	7.2 a
SS + bran	11.2 a	3.8 ab	4.6 a	6.2 a
SS + *T. flavus*	5.6 a	0 b	2.7 ab	2.2 ab
SS + *C. minitans*	2.6 a	0 b	0 b	0.6 b
Date means[3]	5.7 a	2.3 a	3.1 a	
	C. Percent of recovered sclerotia infected by *T. flavus*[1]			
Control (SS)	0 b[2]	0.8 b	0.8 b	0.5 b
SS + bran	1.8 b	0.9 b	0 b	0.8 b
SS + *T. flavus*	27.9 a	17.0 a	12.4 a	18.5 b
SS + *C. minitans*	0 b	2.3 b	0 b	0.6 b
Date means[3]	3.8 a	3.7 a	1.9 a	
	D. Percent of recovered sclerotia infected by *C. minitans*[1]			
Control (SS)	0 a[2]	0 a	0 a	0 a
SS + bran	0 a	0 a	0 a	0 a
SS + *T. flavus*	0 a	0 a	0 a	0 a
SS + *C. minitans*	1.8 a	1.6 a	1.7 a	1.7 a
Date means[3]	0.3 a	0.3 a	0.3 a	

[1] Values averaged over 4 replicates per treatment with 20 sclerotia per replicate; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Means (for A,B,C or D) within columns (excluding the overall date means) followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test); the hyperparasite x sampling date interaction is significant for recovered sclerotia only as is sampling date.

[3] For tables of treatment and date means, see appendices 31 and 32.

Table 14. The effect of hyperparasites and sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field in May, 1984 (Winnipeg).

Hyperparasites	Weeks after burial			Treatment means
	5	10	15	
	A. Recovered sclerotia[1]			
Control (SS)	20.0 a[2]	19.3 a[2]	19.3 a[2]	19.5 a[2]
SS + bran	20.0 a	18.3 a	19.5 a	19.3 a
SS + T. flavus	20.0 a	18.0 a	14.9 b	17.8 b
SS + C. minitans	20.0 a	17.8 a	14.2 b	17.5 b
Date means[3]	20.0 a	18.4 ab	17.1 b	
	B. Percent of recovered sclerotia that were viable[1]			
Control (SS)	10.3 a[2]	9.1 a	6.3 a	8.5 a
SS + bran	11.8 a	4.2 a	8.0 a	7.7 a
SS + T. flavus	0.8 b	3.0 ab	0 b	1.0 a
SS + C. minitans	0 b	0 b	1.0 ab	0.3 b
Date means[3]	4.2 a	3.3 a	3.0 a	
	C. Percent of recovered sclerotia infected by T. flavus[1]			
Control (SS)	0 a[2]	0 a	0 a	0 a
SS + bran	1.8 a	0.8 a	0.8 a	1.1 a
SS + T. flavus	2.6 a	2.1 a	0.9 a	1.8 a
SS + C. minitans	0 a	0 a	0 a	0 a
Date means[3]	0.9 a	0.6 a	0.4 a	
	D. Percent of recovered sclerotia infected by C. minitans[1]			
Control (SS)	0 a[2]	0 a	0 b	0 a
SS + bran	0 a	0.8 a	0 b	0.2 a
SS + T. flavus	0 a	0 a	0 b	0 a
SS + C. minitans	2.6 a	1.3 a	3.4 a	2.3 a
Date means[3]	0.4 a	0.4 a	0.5 a	

[1] Values averaged over 4 replicates per treatment with 20 sclerotia per replicate; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Means (for A,B,C or D) within columns (excluding overall date means) followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test); the hyperparasite x sampling date interaction is significant for recovered sclerotia only as is sampling date.

[3] For tables of treatment and date means, see appendices 34 and 35.

Table 15. The effect of hyperparasites and sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field in May, 1985 (Winnipeg).

Hyperparasites	Weeks after burial 5	10	15	Treatment means
	A. Recovered sclerotia[1]			
Control (SS)	19.8 a[2]	18.2 a[2]	14.8 a[2]	17.7 a[2]
SS + bran	19.5 a	18.3 a	15.2 a	17.8 a
SS + T. flavus	19.5 a	16.8 a	16.8 a	17.8 a
SS + C. minitans	16.7 a	12.5 b	9.5 b	13.2 b
Date means[3]	18.9 a	16.6 b	14.3 c	
	B. Percent of recovered sclerotia that were viable[1]			
Control (SS)	12.5 a[2]	8.8 a	4.0 a	8.0 a
SS + bran	7.9 a	6.7 a	4.8 a	6.4 a
SS + T. flavus	5.4 a	3.1 ab	2.2 a	3.5 a
SS + C. minitans	4.6 a	0 b	2.7 a	2.0 a
Date means[3]	7.3 a	3.8 a	3.4 a	
	C. Percent of recovered sclerotia infected by T. flavus[1]			
Control (SS)	0 b[2]	0 b	1.2 a	0.3 b
SS + bran	0.8 b	1.8 ab	0.8 a	1.1 b
SS + T. flavus	7.5 a	7.0 a	2.2 a	5.3 a
SS + C. minitans	0 b	2.1 ab	0 a	0.5 b
Date means[3]	1.3 a	2.2 a	1.0 a	
	D. Percent of recovered sclerotia infected by C. minitans[1]			
Control (SS)	0 a[2]	0.8 a	0 a	0.2 a
SS + bran	0 a	0 a	0 a	0 a
SS + T. flavus	0.8 a	0.9 a	1.0 a	0.5 a
SS + C. minitans	0.8 a	0 a	1.6 a	0.7 a
Date means[3]	0.3 a	0.4 a	0.3 a	

[1] Values averaged over 4 replicates per treatment with 20 sclerotia per replicate; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Means (for A,B,C or D) within columns (excluding the overall date means) followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test); the hyperparasite x sampling date interaction is not significant at the 5% level for any of the variables; sampling date is significant for recovered sclerotia only.

[3] For tables of treatment and date means, see appendices 37 and 38.

Table 16. The effect of hyperparasites and sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field in May, 1984 (Lethbridge, Experiment B).

Hyperparasites	Weeks after burial				Treatment means
	5	10	15	50	
	A. Recovered sclerotia[1]				
Control (SS)	17.8 a[2]	16.6 a[2]	14.3 a[2]	11.0 a[2]	15.6 a[2]
SS + bran	17.0 ab	12.4 b	13.8 a	11.1 a	13.8 ab
SS + T. flavus (T)	11.9 c	16.1 ab	7.6 b	5.6 ab	11.0 c
SS + C/T 1:2	17.5 a	12.4 b	8.9 b	3.7 b	11.8 bc
SS + C/T 1:1	17.5 a	13.2 ab	8.7 b	2.6 b	11.9 bc
SS + C/T 2:1	13.6 bc	13.5 ab	8.0 b	4.6 ab	10.6 c
SS + C. minitans (C)	16.2 a	13.7 ab	14.9 a	6.4 ab	14.2 a
Date means[3]	16.5 a	14.1 b	11.3 c	7.2 d	
	B. Percent of recovered sclerotia that were viable[1]				
Control (SS)	3.3 a[2]	4.1 a	3.1 a	5.1 a	3.8 a
SS + bran	0.8 a	3.4 a	0 a	6.9 a	2.2 ab
SS + T. flavus (T)	0 a	0 a	0 a	0 a	0 b
SS + C/T 1:2	0 a	0 a	2.7 a	2.7 ab	1.1 ab
SS + C/T 1:1	0 a	0 a	0 a	0 a	0 b
SS + C/T 2:1	0 a	0 a	0 a	0 a	0 b
SS + C. minitans (C)	0 a	0 a	0 a	0 a	0 b
Date means[3]	0.4 a	0.7 a	0.6 a	1.4 a	
	C. Percent of recovered sclerotia infected by T. flavus[1]				
Control (SS)	0 a[2]	0 a	0 a	0 a	0 a
SS + bran	0 a	0 a	0 a	1.1 a	0.2 a
SS + T. flavus (T)	0.9 a	0.9 a	1.6 ab	0 a	0.8 a
SS + C/T 1:2	0 a	0 a	0 a	2.0 a	0.4 a
SS + C/T 1:1	0 a	0 a	0 a	0 a	0 a
SS + C/T 2:1	0 a	0 a	3.0 a	2.0 a	1.0 a
SS + C. minitans (C)	0 a	0 a	0 a	0 a	0 a
Date means[3]	0.1 a	0.1 a	0.5 a	0.6 a	
	D. Percent of recovered sclerotia infected by C. minitans[1]				
Control (SS)	0 a[2]	0 a	0 a	1.1 ab	0.2 a
SS + bran	0 a	0 a	0 a	0 b	0 a
SS + T. flavus (T)	0 a	0 a	0 a	0 b	0 a
SS + C/T 1:2	0 a	0 a	1.3 a	0 b	0.3 a
SS + C/T 1:1	0 a	0 a	0 a	0 b	0 a
SS + C/T 2:1	0 a	0.9 a	0 a	2.0 a	0.6 a
SS + C. minitans (C)	0 a	0.9 a	0 a	1.2 ab	0.5 a
Date means[3]	0 a	0.2 a	0.1 a	0.5 a	

[1] Values averaged over 4 replicates per treatment with 20 sclerotia per replicate; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Means within columns (for A,B,C or D) followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test); the hyperparasite x sampling date interaction is significant for recovered sclerotia only; sampling date is significant for recovered sclerotia only.

[3] For tables of treatment and date means, see Appendices 41 and 42.

Table 17. Effect of hyperparasites on the recovery and viability of sclerotia of S. sclerotiorum buried in a sunflower field in October, 1984 and recovered prior to seeding in May, 1985 (Lethbridge, Experiment B).

Hyperparasites	F[2]	Number of sclerotia[1] Recovered	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
Control (SS)	-	20.0 a[3]	22.3 a	0 d	0 e
SS + bran	-	19.3 a	11.9 b	0 d	0 e
SS + bran	F	20.0 a	7.0 cd	0 d	0.8 de
SS + T. flavus	-	19.3 a	3.4 efg	0.8 d	5.5 a
SS + T. flavus	F	20.0 a	1.8 g	0 d	2.6 bc
SS + C/T41:2	-	20.0 a	5.7 de	3.2 c	3.3 b
SS + C/T 1:2	F	20.0 a	0 h	4.2 bc	2.6 bc
SS + C/T 1:1	-	19.8 a	6.2 de	6.2 ab	3.3 b
SS + C/T 1:1	F	19.5 a	4.9 de	4.2 bc	0.8 de
SS + C/T 2:1	-	20.0 a	4.2 def	2.6 c	0.8 de
SS + C/T 2:1	F	20.0 a	2.3 fg	6.3 a	0.8 de
SS + C. minitans	-	19.0 a	10.3 bc	0 d	0.9 d
SS + C. minitans	F	19.5 a	0 h	0 d	1.3 cd

[1] Values averaged over 4 replicates per treatment; based on 20 sclerotia per replicate; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] F = plots to which hyperparasites were applied in the fall (1984); - = plots which did not receive an application of hyperparasites in the fall of 1984.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Table 18*. The effect of hyperparasites, time of application (season) and sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field in May, 1985 (Lethbridge, Experiment B).

Hyperparasites	Season[1]	Weeks after burial 5	10	15	Treatment means
		A. Recovered sclerotia[2]			
Control (SS)	SP	20.0 a[3]	19.3 ab[3]	19.8 a[3]	19.7 a[3]
	F	20.0 a	19.8 a	19.5 a	19.8 a
SS + bran	SP	20.0 a	19.3 ab	19.3 a	19.5 ab
	F	20.0 a	20.0 a	19.0 ab	19.7 a
SS + T. flavus (T)	SP	19.8 a	17.4 bcd	15.7 cd	17.7 de
	F	19.8 a	19.8 a	19.3 a	19.6 a
SS + C/T 1:2	SP	20.0 a	18.3 abc	17.0 bcd	18.5 bcd
	F	19.3 a	20.0 a	17.0 bcd	18.8 abc
SS + C/T 1:1	SP	19.1 a	16.4 cde	3.9 e	14.7 f
	F	20.0 a	20.0 a	17.9 abc	19.3 ab
SS + C/T 2:1	SP	18.1 a	14.5 ef	6.5 e	13.9 f
	F	19.5 a	16.0 de	14.6 d	16.8 e
SS + C. minitans (C)	SP	20.0 a	11.9 f	4.3 e	13.7 f
	F	19.8 a	18.8 ab	14.6 d	17.9 cde
Date means[4]		19.7 a	18.1 b	15.9 c	
		B. Percent of recovered sclerotia that were viable[2]			
Control (SS)	SP	24.3 a[3]	9.0 a	5.3 a	11.7 a
	F	15.1 ab	1.8 b	4.3 ab	6.0 c
SS + bran	SP	15.7 ab	1.8 b	3.4 ab	5.8 c
	F	23.9 a	12.8 a	1.9 ab	10.9 a
SS + T. flavus (T)	SP	0.8 cd	0 b	0 b	0.2 e
	F	1.9 cd	0 b	0 b	0.5 e
SS + C/T 1:2	SP	0 d	0 b	2.0 ab	0.5 e
	F	0.9 cd	0 b	2.0 ab	0.2 e
SS + C/T 1:1	SP	1.6 cd	0 b	0 b	0.4 e
	F	0.8 cd	1.3 b	0 b	0.6 e
SS + C/T 2:1	SP	0.9 cd	0 b	0 b	0.2 e
	F	6.3 bc	0 b	0 b	1.3 e
SS + C. minitans (C)	SP	0.8 d	0 b	0 b	0.2 e
	F	21.4 a	0 b	0 b	3.6 c
Date means[4]		5.6 a	1.1 b	0.8 b	

* Table 18 is continued on the next page.

Table 18 (continued).

Hyperparasites	Season[1]	Weeks after burial 5	10	15	Treatment means
		C. Percent of recovered sclerotia infected by T. flavus[2]			
Control (SS)	SP	0 b[3]	0 b	0 b	0 c
	F	0 b	1.3 ab	0 b	0.3 bc
SS + bran	SP	0.8 ab	1.4 ab	0 b	0.6 bc
	F	0 b	0.8 ab	0 b	0.2 c
SS + T. flavus (T)	SP	1.9 ab	1.9 ab	4.4 a	2.6 a
	F	1.3 ab	3.4 a	0.8 ab	1.7 ab
SS + C/T 1:2	SP	4.2 a	2.6 ab	2.1 ab	2.9 a
	F	1.8 ab	0 b	2.0 ab	1.1 abc
SS + C/T 1:1	SP	0 b	0 b	3.4 a	0.8 bc
	F	0.8 ab	0 b	0 b	0.2 c
SS + C/T 2:1	SP	0.8 ab	1.6 ab	0 b	0.7 bc
	F	0 b	0 b	0 b	0 c
SS + C. minitans (C)	SP	0 b	0 b	0 b	0 c
	F	0 b	0.8 ab	0 b	0.2 c
Date means[4]		0.6 a	0.8 a	0.6 a	
		D. Percent of recovered sclerotia infected by C. minitans[2]			
Control (SS)	SP	1.8 bcd[3]	0.8 a	0 d	0.8 def
	F	0 d	0 a	0.8 d	0.2 f
SS + bran	SP	0.8 cd	0 a	0 d	0.2 f
	F	0 d	0 a	0 d	0 f
SS + T. flavus (T)	SP	0 d	0 a	1.3 cd	0.3 ef
	F	0 d	0.8 a	0.8 d	0.5 ef
SS + C/T 1:2	SP	1.8 bcd	0 a	0 d	0.5 ef
	F	1.8 bcd	0.8 a	0.8 d	1.1 cde
SS + C/T 1:1	SP	2.6 bcd	0 a	11.3 b	3.4 bcd
	F	0.8 cd	4.8 a	4.0 bcd	2.9 cde
SS + C/T 2:1	SP	6.9 bc	0 a	7.9 bc	4.0 bc
	F	9.5 ab	1.7 a	1.3 cd	3.5 bcd
SS + C. minitans (C)	SP	5.2 bc	3.9 a	3.3 a	11.2 a
	F	22.4 a	4.1 a	2.3 cd	7.7 ab
Date means[4]		2.6 a	0.9 b	2.7 a	

[1] F = fall = application of hyperparasites in October, 1984; SP = spring = application of hyperparasites in May, 1985; all sclerotia in mesh bags were buried in the spring (May, 1985).

[2] Values averaged over 4 replicates with 20 sclerotia per replicate; analysis of variance on transformed data; means converted back to raw form for presentation; the hyperparasite x sampling date interaction is significant at the 5% level for recovered sclerotia, viable sclerotia and sclerotia infected with C. minitans.

[3] Means within columns (for A,B,C or D) followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] For tables of treatment and date means, see appendices 45 and 46.

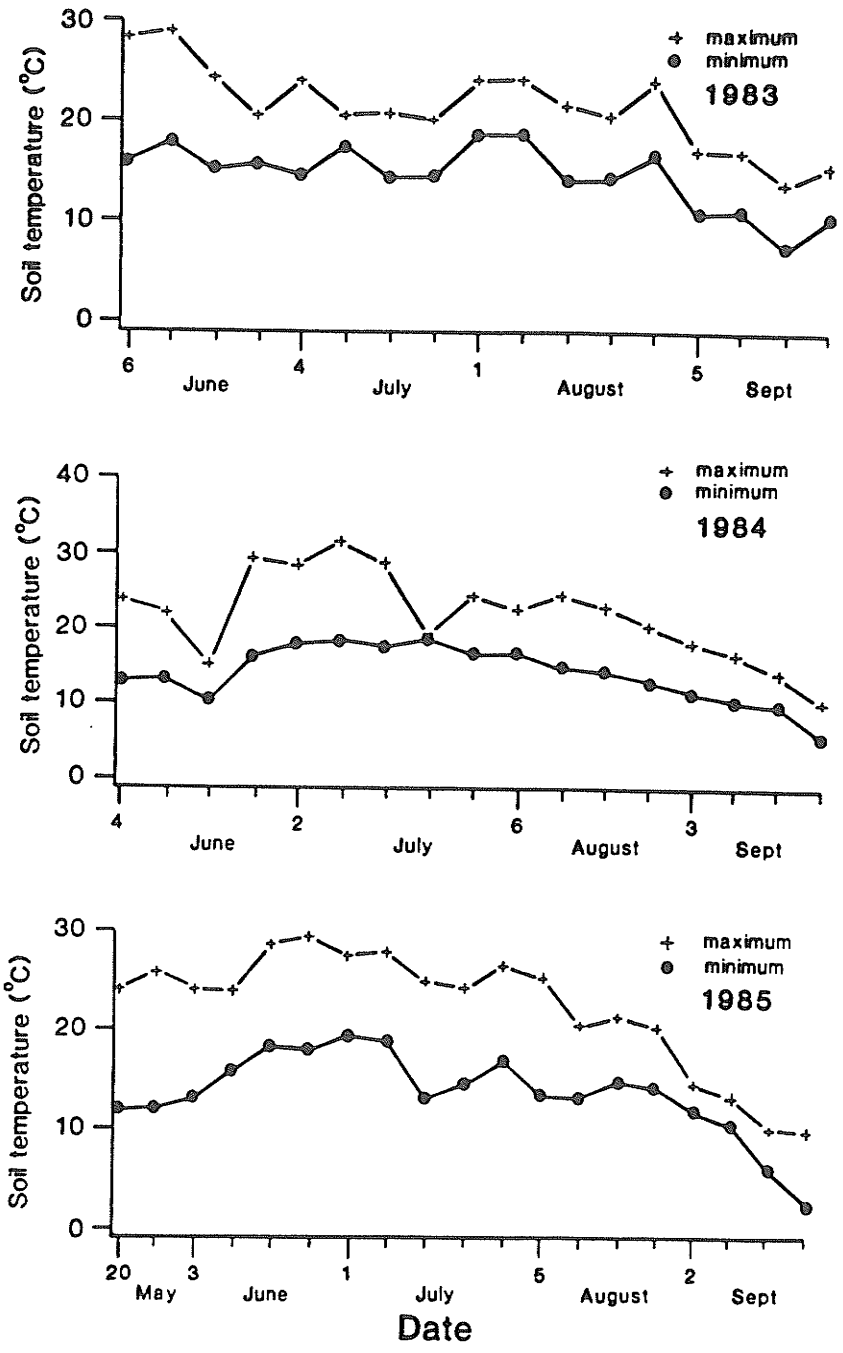


Figure 3. Soil temperatures recorded in a sunflower field during the 1983-1985 field seasons (Lethbridge).

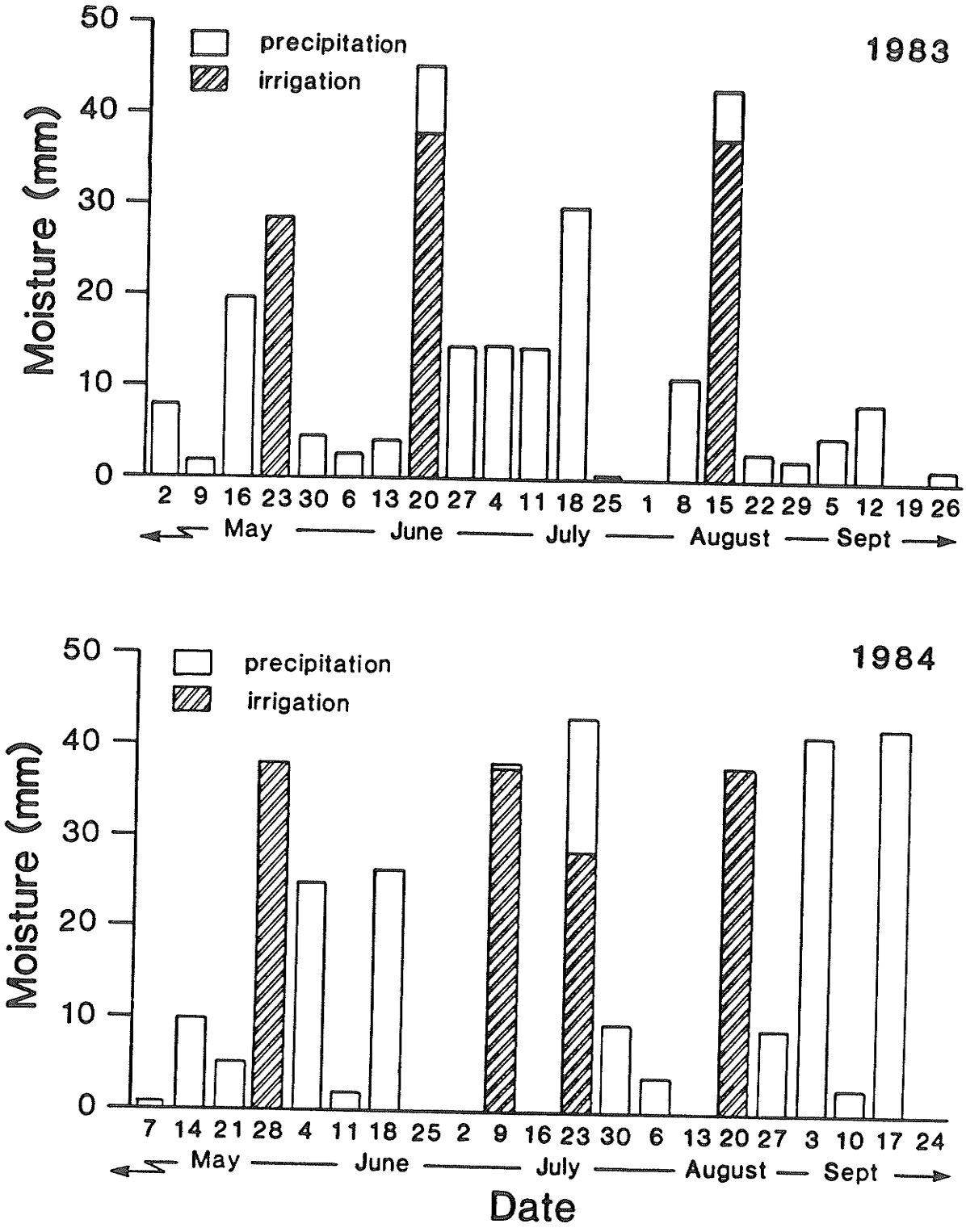


Figure 4. Precipitation and irrigation recorded in a sunflower field during the 1983 and 1984 field seasons (Lethbridge).

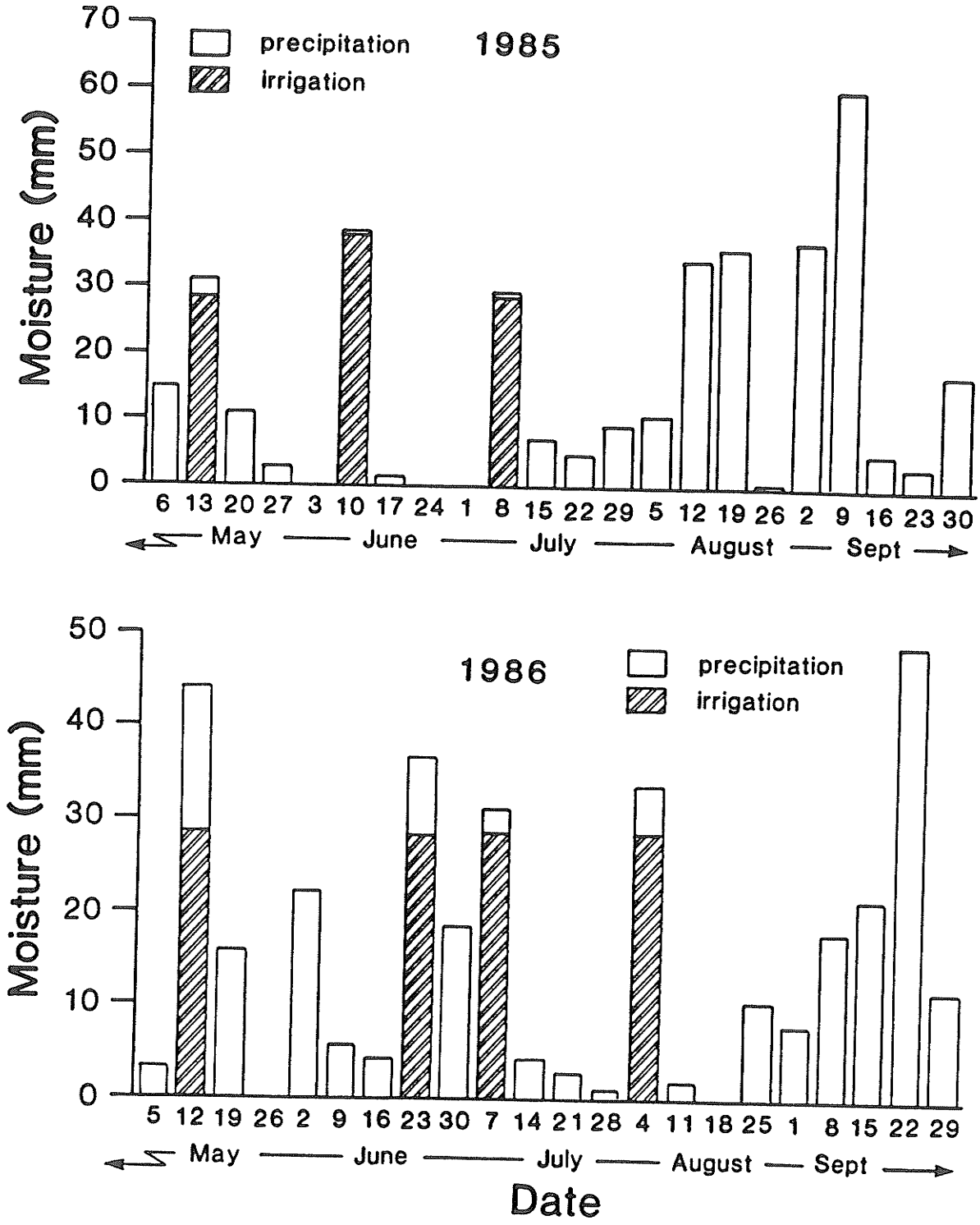


Figure 5. Precipitation and irrigation recorded in a sunflower field during the 1985 and 1986 field seasons (Lethbridge).

Chapter V

CONTROL OF APOTHECIAL PRODUCTION OF SCLEROTINIA SCLEROTIORUM BY TALAROMYCES FLAVUS AND CONIOTHYRIUM MINITANS IN FIELD BEAN

5.1 INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is found in many countries throughout the world and has an extensive host range. Purdy (1979) reported 361 species of plants from 64 families to be the hosts of this pathogen. The ability of S. sclerotiorum to colonize rapidly host tissue, to produce sclerotia as survival structures and to grow at temperatures that support optimal crop growth make it difficult to control (Martens et al., 1984). Some of the economically important crop plants commonly affected by S. sclerotiorum are soybean [Glycine max (L.) Merr], lettuce (Lactuca sativa L.), peanut (Arachis hypogaea L.), cucumber (Cucumis sativus L.), bean (Phaseolus vulgaris L.), sunflower (Helianthus annuus L.) (Fuller et al., 1984) and oilseed rape (Brassica napus L.) (Martens et al., 1984). In Canada, white mold of bean, caused by S. sclerotiorum, is one of the most important diseases occurring in the main bean producing regions and can cause significant economic losses (Martens et al., 1984; Huang et al., 1988).

The lack of adequate control of white mold disease through cultural (Fuller et al., 1984) or chemical means (Steadman, 1979; 1983) as well as the lack of resistant cultivars which maximize yield potential

(Coyne et al., 1977) emphasizes the need to investigate other means of control. Use of hyperparasites to control S. sclerotiorum in the field has met with some success. Coniothyrium minitans has been used successfully as a biological control agent for Sclerotinia trifoliorum (Turner and Tribe, 1975) and S. sclerotiorum (Huang, 1980b; Fedulova, 1983; Bogdanova et al., 1986). Huang attributed the success of C. minitans to its ability to destroy the primary inoculum or sclerotia of S. sclerotiorum in the field. Another hyperparasite, Talaromyces flavus has shown promise as a biological control agent for Rhizoctonia solani (Boosalis, 1956) and Verticillium dahliae (Dutta, 1981; Fravel et al., 1986). Talaromyces flavus has also been reported to be destructive to hyphae and sclerotia of S. sclerotiorum (McLaren et al., 1986; 1989). Apothecia of S. sclerotiorum are the important source of inoculum for white mold disease of bean (Abawi and Grogan, 1975). Wherever beans are produced, white mold is an important cause of crop loss (Martens et al., 1984). Consequently, studies were conducted to evaluate the ability of the hyperparasites T. flavus and C. minitans, applied in the spring or in the fall, to prevent the development of apothecia from sclerotia of S. sclerotiorum in a field of dry bean.

5.2 MATERIALS AND METHODS

5.2.1 Source of fungal cultures and bean seed

In 1976, H.C. Huang isolated Talaromyces flavus from the rhizosphere of sunflower plants located in a field at Morden, Manitoba. This fungus was identified as Penicillium vermiculatum the anamorph of T. flavus (DAOM 172557). Coniothyrium minitans (DAOM 149432) was

isolated from a sclerotium of S. sclerotiorum obtained from a sunflower field near Morden, Manitoba (Hoes and Huang, 1975). Sclerotinia sclerotiorum was isolated from a diseased sunflower plant collected from a field located on the Agriculture Canada Research Station at Morden, Manitoba. Sclerotia from naturally infected bean plants were obtained during seed cleaning procedures from the Alberta Wheat Pool Bean Plant.[9] All sclerotia were stored at 5 C until used for the 1984 and 1985 field experiments. The viability of sclerotia used in the spring of 1984 and 1985 was 96 and 94%, respectively. Sclerotia buried in the fall of 1984 had a viability rating of 86%. The numbers of sclerotia buried in the field were adjusted for viability. A Great Northern cultivar, US-1140, was used in all field experiments. This seed was obtained from Hill's Pantry,[10] Lethbridge, Alberta.

5.2.2 Production of T. flavus and C. minitans

Talaromyces flavus and C. minitans were grown on wheat bran to produce inocula for field application. The wheat bran was moistened with water (850 ml/kg dry bran), placed in 18 x 18 cm aluminium foil containers[11] and autoclaved at 121 C for two one-half hour sessions. The bran was cooled to room temperature (approximately 22 C) between autoclavings and before being inoculated with cultures of T. flavus or C. minitans which had grown on potato dextrose agar (PDA) at room

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[9] Alberta Wheat Pool (Bean Plant), Box 96, Bow Island, Alberta, T0K 0G0.

[10] Hill's Pantry Ltd., 1269 2nd Ave. S., Lethbridge, Alberta T1J OE7.

[11] Echo foil containers, Daxion Inc., 1st Ave. S., Lethbridge, Alberta T1J OB1.

temperature for 10 days. A spore suspension, produced by washing a 9.0 cm petri dish containing either T. flavus or C. minitans with approximately 50-70 ml of sterile distilled water, was used to inoculate wheat bran in two foil containers. The inoculated bran was incubated at room temperature for 21 days, air-dried at the same temperature for three to four days and stored at 5 C until use. The bran inocula had a spore concentration of 1 x 10^7 colony-forming units (cfu)/gm. Non-inoculated wheat bran was used in some treatments. It was prepared in the same manner as the inoculated bran, but was air-dried immediately following autoclaving.

5.2.3 Field Experiments

Experiments were conducted, in 1984 and 1985, in a bean field at the Lethbridge Research Station. In 1984, twenty-eight plots of seven treatments with four replicates were arranged in a randomized complete block design (RCBD). Each plot consisted of eight rows, 2.0 m long and 0.45 m apart. Twelve burial areas (BA's) were located in each plot and were subdivided into four groups of three BA's in the fall of 1984. Two BA's, each 0.61 x 0.30 m in size, were located in one inter-row space and were separated by a distance of approximately one foot (Plate 9, Figure A). The BA's were placed in a total of six inter-row spaces per plot with one empty inter- row space located between the two groups of six BA's (Plates 10 and 11). The treatments for spring of 1984 were: (1) S. sclerotiorum (SS), (2) SS and bran, (3) SS and C. minitans, (4) SS and T. flavus, (5) SS and a combination of C. minitans and T. flavus (2:1 w/w), (6) SS and a combination of C. minitans and T. flavus (1:1 w/w) and (7) SS and a combination of C.

minitans and T. flavus (1:2 w/w). For treatment one, 100 viable sclerotia from infected bean plants, were distributed evenly within each BA at a depth of approximately 4 cm. For treatments two to seven, 30 g of air-dried inoculum or bran were mixed with 1000 cc of field soil. Prior to application of sclerotia (100 viable/BA), this mixture was divided evenly and one-half was applied to the BA. The sclerotia were then distributed in the BA at a depth of 4 cm and covered with the remaining half. A thin layer of soil was placed over the soil-hyperparasite mixture before packing. The plots were seeded on May 18 using a 4-row seeder.[12] Some reseeding was necessary due to cutworm damage and was done by hand on June 15 and 18. The sclerotia and/or inoculum of the hyperparasites were buried during June 25-29, 1984.

To assess the number of apothecia produced in each BA, wooden sticks,[13] 15.0 cm in length, were used (Plate 9, Figure B). Groups of sticks were painted one of 10 colors to represent one to 10 apothecia arising from a single sclerotium. Combinations of sticks were used where greater than 10 apothecia were produced per sclerotium. In 1984, the first apothecia were sighted on August 22 (bean growth stage=R8; Appendices 51 and 53) and three counts were taken ending on August 28 (R9), September 24 (R9+) and October 3 (R9+). Similarly, in 1985 the first apothecia were observed on July 24 (R4-R5) and two counts were taken ending on August 8 (R5) and September 24 (R9+).

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[12] Custom made 4-row seeder manufactured by Fabro Enterprises Ltd., Swift Current, Saskatchewan S9H 3W3 (1977).

[13] Applicator sticks, American Hospital Supply Can. Inc., 11620-181 St., Edmonton, Alberta, T5S 1M6.

The natural inoculum density of S. sclerotiorum was determined in 1984 by collecting 28 samples (seven treatments x 4 reps) shortly after seeding. Each sample was comprised of 5 smaller samples collected at random from within the inter-row spaces of each plot. The samples, which were approximately 8000 cc in size, were air-dried, weighed and processed to separate the sclerotia. The inoculum density was not determined in 1985 because the experiment was sown into the same site which was not tilled following the 1984 season.

Efficacy of the hyperparasites against S. sclerotiorum over time was assessed by burying additional sclerotia in each plot. To facilitate burial and recovery of sclerotia from soil, fiberglass screen[14] (1.0 mm pore size) was used. The screen was cut into 28 x 8 cm sections, folded in half and soldered into ten compartments to form a mesh bag. Two sclerotia were placed in each compartment and a section of red plastic tape[15] 11.0 x 1.1 cm in size, was attached to each bag for identification and to aid in location of mesh bags in the field. During June 25-29, 1984, four mesh bags representing four sampling dates were buried per treatment. Sampling dates were at four week intervals after burial except for sampling date four which was recovered on May 7, 1985 (52 weeks after burial). The split-plot design was used, with hyperparasite treatments assigned to the main plots and sampling dates assigned to the subplots within each main plot.

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[14] Fiberglass screen, Spalding Hardware Ltd., Whlse 1616, 10th Ave. S.W., Calgary, Alberta T3C OJ5.

[15] Max Tape for Max Tapener Model HT-B, Westcan Horticultural Specialists Ltd., Calgary, Alberta.

Soil temperature was taken twice daily, two times per week, using a Tel-Tru thermometer.[16] Readings were taken at a depth of 4 cm. At the beginning of each season, the plots were irrigated to insure germination and emergence. Growth stages (NDSU, 1981) and canopy development of bean were recorded on a weekly basis. Canopy development was based on the following criteria: 0 = no coverage of inter-row space (IRSP), 1 = 25% of IRSP covered, 2 = 50% of IRSP covered, 3 = 75% of IRSP covered and 4 = 100% of IRSP covered. Irrigation continued periodically until the inter-row space for all plots was 75-100% covered. At this time, all plots were irrigated twice weekly to maintain moist soil surface conditions. A portable tensiometer[17] was used to measure soil moisture twice weekly, at a depth of approximately 4 cm, within the experimental area.

In the fall of 1984, the experiment which was set up as a RCBD in the spring was converted to a split-plot design. This design was used in order to evaluate the ability of the overwintered T. flavus and/or C. minitans to control the population of apothecia as compared to a new addition of the hyperparasite(s). Each treatment assigned in the spring of 1984 became a main plot and was subdivided into four subplots of three BA's each. The subplots were: (1) spring (1985) application of hyperparasites or bran and spring (1985) application of sclerotia, (2) fall (1984) application of hyperparasites or bran and spring (1985) application of sclerotia, (3) spring (1985) application of hyperparasites or bran and fall (1984) application of sclerotia and

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[16] Model GT100, Tel-Tru Manufacturing Co., Rochester, N.Y., U.S.A.

[17] Model 2900F Quick Draw Soilmoisture Probe, Hoskin Scientific (Western) Ltd., 239 East 6th Ave., Vancouver, B.C. V5T 1J7.

(4) fall (1984) application of hyperparasites or bran and fall (1984) application of sclerotia. The main plots treated with S. sclerotiorum alone were divided into subplots of fall versus spring application of sclerotia. The fall application of hyperparasites/bran/sclerotia and the burial of mesh bags containing sclerotia were done during November 23-25, 1984.

Mesh bags, with 20 sclerotia per bag, were prepared in the same manner as those used in the spring of 1984. Two hundred and twenty-four mesh bags representing 8 bags for each of the 7 main plot treatments replicated 4 times were buried. The 8 bags per plot represented four bags (= four sampling dates) in each of two subplots (subplots 3 & 4). The time of burial of mesh bags containing sclerotia corresponded to the time of application of the 100 viable sclerotia per burial area. After the fall burial of material, straw bales were placed around the experimental plot area to reduce soil erosion throughout the winter. On May 7, 1985 the first set of bags was recovered with the remaining sets of bags being recovered at three week intervals thereafter. The data collected from the sclerotia buried in mesh bags were analyzed as a split-split plot with seven main plots (hyperparasite), two subplots (season) and four sub-subplots (sampling dates).

In 1985, application of material to the subplots was carried out in the same manner as the 1984 fall application except that sclerotia buried in subplots one, two and three were placed at a depth of approximately 2 cm. Application of material to a subplot both in the fall and spring (subplots 2 & 3) was facilitated by using a 0.61 x

0.30 m section of fiberglass screen (1.0 mm pore size) which was placed in each BA. The screens were buried at a depth of approximately 2 cm with the sclerotia located immediately below or with a soil-hyperparasite/bran mixture located both above and below the screen. On May 7-8, 1985, the screens were lifted carefully and the sclerotia were applied (subplot 2) or the inoculum was mixed into the soil surrounding the sclerotia (subplot 3). Each BA was covered with a thin layer of soil and packed. The plots were seeded by hand to the Great Northern cultivar US-1140 on May 18-19, 1985. Apothecia were counted using the same method as described in the 1984 experiment. Data collected per subplot represented a sum of the data collected from the three BA's. The data were analyzed as a split-plot design with seven main plots (hyperparasite) and four subplots (season of application).

One hundred and sixty-eight mesh bags representing six bags for each of the seven main plot treatments replicated four times were buried. The six bags per main plot represented three bags (= three sampling dates) in each of two subplots (subplots 1 & 2). The time of burial of mesh bags containing sclerotia corresponded to the time of application of the 100 viable sclerotia per burial area. Sampling of mesh bags occurred at three week intervals starting from the date of burial (May 7, 1985). The data were analyzed as a split-split plot with seven main plots (hyperparasite), two subplots (season) and three sub-sub plots (sampling date).

In September 1985, sclerotia were also collected from the above ground portion of infected bean plants to determine their viability

and parasitization by T. flavus or C. minitans. Twenty sclerotia were collected from each main plot at a distance of no greater than 10 cm above the soil surface. All sclerotia were plated onto PDA amended with streptomycin sulphate (0.2 g/L media) and examined for viability and/or infection by the hyperparasites.

5.3 RESULTS

5.3.1 Carpogenic germination

In 1984 and 1985, apothecia were first observed on August 22 and July 24, respectively (Figure 6). In both cases, the canopy was well developed with an average of 95% (1984) and 70% (1985) of the inter-row space covered during the two weeks prior to the appearance of apothecia. During the two weeks before apothecia were observed in the field, irrigation and/or precipitation provided moist soil surface conditions (Figure 7 and Appendix 53) with an average of 18.1 and 21.7% soil moisture in 1984 and 1985, respectively. Soil temperatures ranged from 13 to 23 C and 13 to 33 C during this period in 1984 and 1985, respectively (Figure 8). Once apothecia were observed, three and two counts were taken in 1984 and 1985, respectively. In 1984, the counts ended on August 28, September 24 and October 3 and corresponded with bean growth stages of R9, R9+ and R9. During each count, a full canopy (100% of inter-row space covered) was present. In 1985, two counts ending August 8 and September 24 were taken. The bean growth stages at the first and second count were R5 and R9, respectively. Greater than 95% of the inter-row space was covered during this period.

In 1984, application of T. flavus, C. minitans or combinations of both reduced the number of germinable sclerotia of S. sclerotiorum and the number of apothecia produced (Table 19 and Appendix 54). In the T. flavus and C. minitans -treated plots, 37.9 and 6.9 sclerotia germinated, respectively compared with 57.6 in the control. The percentage of buried sclerotia that germinated was significantly less in the hyperparasite-treated plots compared with the control. The number of apothecia observed in the hyperparasite-treated plots was also significantly less than in the control with 125.1 and 21.4 apothecia in the T. flavus and C. minitans -treated plots, respectively compared with 188.5 in the control. Although application of T. flavus reduced both the number of apothecia and germinable sclerotia compared with the untreated control plots (Table 19 and Plate 10), the greatest reductions were seen in plots treated with C. minitans alone (Plate 11). As the proportion of C. minitans in the hyperparasite mixture increased, the numbers of germinable sclerotia and apothecia decreased. No significant differences occurred between treatments for the average number of apothecia produced from each germinated sclerotium (Table 19). When bran alone was applied, the greatest numbers of apothecia and germinable sclerotia were observed.

In 1985, similar results were obtained (Table 20 and Appendix 55). Coniothyrium minitans was the more effective hyperparasite in reducing the number of germinated sclerotia and apothecia produced. Only 8.6 of 300 sclerotia germinated in the plots treated with C. minitans compared with 83.9 in the control. The numbers of apothecia produced in the C. minitans -treated plots and the control plots were 25.6 and

344.3, respectively. Application of T. flavus alone was ineffective with 82.6 germinated sclerotia and 325.4 apothecia produced in plots treated with this hyperparasite. Use of a combination of T. flavus and C. minitans significantly reduced the germination of sclerotia and the production of apothecia compared with the use of T. flavus alone. The numbers of sclerotia that germinated in the plots treated with T. flavus, C/T 1:2, C/T 1:1, C/T 2:1 and C. minitans were 82.6, 17.0, 13.2, 8.6 and 8.6, respectively. Similarly, the numbers of apothecia produced in the plots treated with T. flavus, C/T 1:2, C/T 1:1, C/T 2:1 and C. minitans were 325.4, 62.0, 41.6, 24.8 and 25.6, respectively. As the proportion of C. minitans in the hyperparasite mixture increased, the effectiveness of the treatment improved. Use of C. minitans alone or in combination with T. flavus resulted in the lowest number of apothecia produced per germinable sclerotia.

The time of application of hyperparasites and sclerotia had an effect on the carpogenic germination of sclerotia of S. sclerotiorum (Table 21 and Appendix 55). Although time of application was significant, the interaction between time of application and hyperparasite treatment was not, indicating that there was an effect for time of application. This effect is not related to any specific hyperparasite treatment (Note: hyperparasite treatment refers to the seven main plot treatments of control, bran, T. flavus, C/T 1:2, C/T 1:1, C/T 2:1 and C. minitans). Application of hyperparasite treatments in the fall prior to application of sclerotia in the spring resulted in a significantly lower number of apothecia being produced compared with application of hyperparasites and sclerotia

simultaneously in the spring. Application of hyperparasites in the spring to sclerotia buried the previous fall was not as effective in reducing carpogenic germination as a fall application of hyperparasites to sclerotia. The most significant reductions in apothecia production occurred with the application of hyperparasites in the fall regardless of the time of application of sclerotia. No significant differences in the average number of apothecia produced from each sclerotium occurred with respect to time of application of hyperparasites or sclerotia.

5.3.2 Survival of sclerotia

Sclerotia of S. sclerotiorum placed in mesh bags and buried in the field plots were affected by the application of T. flavus and C. minitans. In 1984, fewer sclerotia were recovered from the hyperparasite-treated plots compared with the control at eight, 12 and 52 weeks after burial (Table 22, Appendix 56). At 12 weeks after burial, 18.5 sclerotia were recovered from the control plots compared with 16.1, 9.7, 12.0 11.3 and 10.8 sclerotia recovered from the T. flavus, C/T 1:2, C/T 1:1, C/T 2:1 and C. minitans -treated plots, respectively. Similar results occurred at eight and 52 weeks after burial. No treatment differences occurred at four weeks after burial. Fewer sclerotia recovered from the hyperparasite-treated plots were viable compared with sclerotia recovered from the control plots at 4 weeks after burial. Thirty-five percent of the sclerotia recovered from the control plots were viable compared with 5.2% or less viability of sclerotia recovered from the plots treated with T.

flavus, C. minitans or combinations of both. Similar results occurred at 12 and 52 weeks after burial. No treatment differences occurred at eight weeks following burial.

The length of burial affected both the recovery and viability of sclerotia buried in the spring of 1984. The number of sclerotia recovered decreased significantly from the first to the last sampling date. A significant interaction between sampling date and hyperparasite indicates that the number of sclerotia recovered over time was affected by treatment. Fewer sclerotia were recovered from the hyperparasite-treated compared with the untreated control as time from burial increased.

The viability of sclerotia was greater in the SS and SS+bran-treated plots than in plots treated with hyperparasites at 4 weeks after burial. Viability of sclerotia recovered from the hyperparasite-treated plots was low at all sampling dates. A significant interaction between sampling date and treatment indicates that viability of sclerotia recovered over time was affected by treatment as time from burial increased. A significant reduction in the viability of sclerotia recovered from the control, SS+bran, C/T 2:1 and C. minitans -treated plots occurred from the first to the second sampling date.

Infection of sclerotia by T. flavus and C. minitans was low. However, the majority of sclerotia infected by T. flavus or C. minitans were recovered from the hyperparasite-treated plots compared with the control plots. At five weeks after burial, 8.6% of sclerotia

recovered from the T. flavus -treated plots were infected with this hyperparasite compared with 0% in the control and SS+bran-treated plots. Similar results occurred at 12 weeks after burial. At eight and 52 weeks after burial, although treatment differences were not significant, T. flavus -infected sclerotia were recovered from the T. flavus -treated plots but not from the controls. Sclerotia infected with C. minitans were recovered from the C/T 1:1, C/T 2:1 and C. minitans -treated plots. At eight weeks after burial, 0.9, 1.7 and 2.9% of sclerotia recovered from the C/T 1:1, C/T 2:1 and C. minitans -treated plots were infected with C. minitans respectively. Although treatment differences were not significant at most sampling dates, sclerotia infected with C. minitans were recovered from the C. minitans -treated plots but not from the controls or the T. flavus -treated plots. Sampling date was not significant for the percent of recovered sclerotia infected by T. flavus or C. minitans, indicating that as time from burial increased, no significant differences in the percent of infection of sclerotia by either hyperparasite occurred.

In the fall of 1984, sclerotia were buried in subplots to which additional hyperparasites or bran were also applied (subplot 4) and in subplots which did not receive a fall application of bran or hyperparasites (subplot 3). These sclerotia remained in the field over winter and were recovered after 30, 33, 36 and 39 weeks of burial (Tables 23-26). In the spring of 1985, sclerotia were buried in subplots along with a spring application of hyperparasites or bran (subplot 1) and in subplots which received a fall (1984) but not a spring (1985) application of hyperparasites or bran (subplot 2).

These sclerotia were recovered after three, six and nine weeks of burial (Tables 27-30).

Sclerotia buried in the fall and recovered in the spring were affected by the application of hyperparasites at 36 and 39 weeks after burial (Table 23). No significant reduction in the recovery of sclerotia occurred following 30 and 33 weeks of burial. After 36 weeks of burial, 307.5, 171.5, 264.8, 150.8 and 184.5 sclerotia were recovered from the T. flavus, C/T 1:2, C/T 1:1, C/T 2:1 and C. minitans -treated plots, respectively compared with 333.8 sclerotia recovered from the control plots. This represents a significant reduction in recovery of sclerotia from the C. minitans and C/T-treated plots compared with the controls. Similar results occurred after 39 weeks of burial. Sclerotia buried in the spring and recovered during the same season were also affected by the application of hyperparasites (Table 27). At six weeks after burial, 267.0, 221.3, 281.8, 111.0 and 152.8 sclerotia were recovered from the plots treated with T. flavus, C/T 1:2, C/T 1:1, C/T 2:1 and C. minitans, respectively, compared with 370.8 sclerotia recovered from the control plot. This represents a significant reduction in the recovery of sclerotia from the hyperparasite-treated plots compared with the control. At nine weeks after burial, fewer sclerotia were recovered with the greatest reductions occurring in the plots to which C. minitans alone or in combination with T. flavus was applied.

Recovery of sclerotia buried in the fall was not affected significantly by the time of application of hyperparasites (Table 23). Little difference in the recovery of sclerotia between seasons (SP =

application of hyperparasites and bran in spring 1984; F = application of hyperparasites and bran in fall 1984) for the same treatment occurred. Recovery of sclerotia buried in the spring was affected by the time of application of hyperparasites and bran (Table 27). At six weeks after burial, fewer sclerotia were recovered from plots which received a fall (1984) compared with a spring (1985) application of hyperparasites or bran. Similar results occurred at nine weeks after burial with the exception of the C/T 2:1 and C. minitans -treated plots where recovery of sclerotia was reduced significantly with a spring as opposed to a fall application of C. minitans.

Sampling date was significant for the recovery of sclerotia buried in the fall (Table 23; Appendix 59) and in the spring (Table 27; Appendix 63). In the spring, a significant interaction occurred between treatment and sampling date indicating that the number of sclerotia recovered over time was affected by treatment. Fewer sclerotia were recovered from the hyperparasite-treated plots compared with the untreated control as time from burial increased.

The viability of sclerotia buried in the fall and recovered after 30-39 weeks (Table 24; Appendix 59) and buried in the spring and recovered after three to nine (Table 28; Appendix 63) weeks of burial was low. For sclerotia buried in the fall, and in the spring, the percentages of recovered sclerotia that were viable tended to be lowest in the hyperparasite-treated plots compared with the controls. Viability of sclerotia was not affected by the time of application of hyperparasites and bran (Appendices 59 and 63). Sampling date affected the viability of sclerotia buried in the fall but not in the

spring. A significant reduction in the viability of sclerotia buried in November occurred at the first sampling date and from the first to the last sampling date (Table 24; Appendix 59).

The infection of sclerotia by T. flavus was low for both sclerotia buried in the fall (Table 25) and in the spring (Table 29). However, in both cases, the percentage of recovered sclerotia infected by T. flavus was greater in the plots to which this hyperparasite was applied than in the control plots. The infection of sclerotia, buried in the fall, by T. flavus was affected by sampling date. Fewer of the recovered sclerotia were infected by this hyperparasite as time from burial increased. The time of application of hyperparasites had little effect on the infection of sclerotia by T. flavus whether sclerotia were buried in the fall (Table 25) or in the spring (Table 29).

Although the number of sclerotia recovered from the C. minitans -treated plots was frequently lower than from the controls (Tables 23 and 27), the percentages of sclerotia infected with C. minitans were low (Tables 26 and 30). For sclerotia buried in the fall and in the spring, infection of sclerotia by C. minitans was greater in plots to which this hyperparasite was applied. Sampling date was significant for sclerotia buried in the fall and spring. A significant treatment by sampling date interaction indicates that the percentage of sclerotia infected by C. minitans over time was affected by treatment. The percentages of sclerotia infected by C. minitans tended to be higher in the C. minitans -treated plots at 33 weeks after burial and decreased thereafter. The time of application of hyperparasites did

not affect significantly the percentages of recovered sclerotia infected by C. minitans for sclerotia buried in the spring (Table 30; Appendix 63) but affected the infection rate of sclerotia buried in the fall (Table 26; Appendix 59). Many microorganisms were isolated from sclerotia recovered during 1984-85 from the bean plots. Fungi belonging to genera such as Trichoderma and Fusarium were often associated with sclerotia of S. sclerotiorum (Appendix 39). The frequency of infection of sclerotia by microorganisms other than T. flavus or C. minitans was high in all treatments (Appendix 39).

Examination of sclerotia collected from bean plants in the fall of 1985 indicated that relatively few sclerotia were viable and that the viability of sclerotia did not differ between treatments (Table 31 and Appendix 67). The average numbers of viable sclerotia collected from the control, bran, C. minitans and T. flavus -treated plots were 2.6, 4.5, 3.0 and 5.2, respectively. Upon examination of the sclerotia for infection by hyperaparasites, C. minitans was found to be the most frequent colonizer of sclerotia. Coniothyrium minitans was associated with sclerotia collected from all treatments with 5.8, 7.0, 7.7 and 7.9 C. minitans -infected sclerotia collected from the control, bran, T. flavus and C. minitans -treated plots, respectively. The percent of recovered sclerotia infected with T. flavus was very low and found only in the C/T 1:2 treatment.

The inoculum density of S. sclerotiorum in soil samples collected from the field site averaged 0.006 sclerotia per kilogram of soil (Table 32). No significant differences in the inoculum density occurred between the control plots and the hyperparasite-treated plots (Appendix 68).

5.4 DISCUSSION

Data from two years of field studies at Lethbridge showed that the application of the hyperparasites T. flavus and C. minitans to soil reduced the carpogenic germination of sclerotia of S. sclerotiorum in a bean field. Both the number of germinable sclerotia and the number of apothecia produced were reduced. These findings support previous reports on the ability of T. flavus (Su and Leu, 1980; McLaren et al., 1983; 1989) and C. minitans (Huang, 1976; 1977; McCredie and Sivasithamparam, 1985) to affect the survival of sclerotia of S. sclerotiorum.

Coniothyrium minitans was more effective than T. flavus in controlling apothecial production of S. sclerotiorum in field bean during 1984-85. Combinations of C. minitans and T. flavus were effective in reducing the germination of sclerotia and apothecial production. As the proportion of C. minitans in the mixture of the two hyperparasites increased, the effectiveness of the treatment increased. However, the greatest reductions in carpogenic germination were seen with the application of C. minitans alone indicating that a synergistic effect did not occur with the combination of C. minitans and T. flavus. Also, T. flavus did not affect the efficacy of C. minitans.

Many enviromnental factors can have a profound effect on biological control agents (Ayers and Adams, 1979b). The optimal activity can depend on specific environmental requirements (Phillips, 1986). The mean daily soil temperature at the Lethbridge field site ranged from 6

to 25 C and from 8 to 26 C in 1984 and 1985, respectively. The optimum temperature at which C. minitans grows on agar, germinates and infects sclerotia of S. sclerotiorum is 20 C (Turner and Tribe, 1976: Trutmann et al., 1980). Good parasitism has also been reported to occur at 10 C (Turner and Tribe, 1976). At higher temperatures such as 30 C, parasitism is reduced (Turner and Tribe, 1976; Trutmann et al., 1983). The mean soil temperatures occuring in the present study are in the range in which C. minitans is active as an effective hyperparasite of sclerotia of S. sclerotiorum. In a study of the Penicillium spp., Raper and Thom (1986) used incubation temperatures of 23-25 C and found this range to be suitable for most species, including P. vermiculatum (teliomorph T. flavus). At 30 C, they found that growth of many species was more rapid and sporulation was heavier. Talaromyces flavus is able to withstand heat treatment (Bollen and van der Pol-luiten, 1975; Van der Spuy et al., 1975; Katan, 1985) and may be involved in the control of verticillium wilt of artichokes (Tjamos and Paplomatos, 1986) and olive trees (Tjamos et al., 1986) after soil solarization treatments. These results suggest that T. flavus may be more effective at higher temperatures. The soil temperatures encountered at the Lethbridge site during 1984-85 may not have favored the optimal activity of T. flavus resulting in the reduced effectiveness of this hyperparasite against sclerotia of S. sclerotiorum.

Soil moisture is critical to the development of apothecia of S. sclerotiorum (Teo and Morrall, 1985a; 1985b). The duration of wetness required for the development of apothecia may not provide optimal soil

moisture conditions required for the hyperparasitism of sclerotia of S. sclerotiorum by specific hyperparasites. Trutmann et al. (1980) found that infection of sclerotia by C. minitans was greatly reduced at relative humidities below 95%. If moderate temperatures and moist conditions prevailed, many sclerotia were killed. At the Lethbridge site, moderate temperatures prevailed and the average percentage of soil moisture was 17.6%. These conditions appeared to promote the activity of C. minitans as indicated by the effect of the hyperparasite treatment on carpogenic germination of sclerotia. As well, the infection of sclerotia recovered from above-ground parts of bean plants indicates that the introduced hyperparasite was able to survive and proliferate under the environmental conditions encountered at the Lethbridge site. Although moist soil conditions seem to favor the activity of C. minitans, they may not be conducive to the activity of T. flavus as indicated by the lack of reduction in carpogenic germination of sclerotia in the T. flavus -treated plots compared with the control plots.

Coniothyrium minitans, produced on bran and applied to the soil in the spring, is capable of destroying new sclerotia produced on the above-ground tissues of infected bean plants thereby reducing the inoculum potential of S. sclerotiorum in the next season. These findings support previous reports on the ability of C. minitans to reduce the number of sclerotia produced on diseased bean plants (Trutmann et al., 1982). However, in Trutmann's study, C. minitans was produced as a spore suspension and sprayed on plants at flowering time. The spray was not effective as a crop protectant but fewer

sclerotia were produced by the C. minitans -treated plants. The sclerotia were often infected with C. minitans compared with sclerotia recovered from other treatments. Trutmann et al. (1982) suggest treatment with a fungicidal spray (Benlate) followed by a post-harvest treatment of crop residue with C. minitans to reduce the population of sclerotia. The present study indicates that it may be feasible to apply C. minitans at seeding time for the control of sclerotia present in the soil at the time of application and for the control of new sclerotia produced on infested plants during the season.

Coniothyrium minitans was able to infect sclerotia on above-ground parts of infected bean plants, regardless of the plot treatment. This indicated that the hyperparasite is capable of spreading from one area to another. Spread of a pathogen through irrigation practices may occur (Steadman, 1983). It is probable that spores of C. minitans were spread from plot to plot by irrigation splash. The ability of C. minitans to spread and destroy sclerotia in areas removed from the site of application improves its attractiveness as a biological control agent.

The density of natural inocula of S. sclerotiorum at Lethbridge is low (<0.02 sclerotia/kg soil) compared with the concentrated application of sclerotia within the burial areas. This suggests that apothecial production in each BA resulted from introduced sclerotia and not natural inocula of S. sclerotiorum.

The substrate used for the growth of the biocontrol agent is important. Bran has been used successfully for the production of T.

flavus (McLaren et al., 1983) and C. minitans (Ahmed and Tribe, 1977). Application of a hyperparasite to small plot trials in this form is feasible. However, on a large scale basis, a formulation suitable for application with conventional farm equipment is necessary. Talaromyces flavus has been produced in alginate pellets by a process which is inexpensive and versatile (Fravel et al., 1985b). Such a procedure may prove to be suitable for C. minitans as well.

In the fall of 1984, sclerotia were buried in the bean field with and without a fall application of hyperparasites. Time of application was significant only for the percentage of sclerotia infected with C. minitans. In the spring of 1985, sclerotia were buried in the bean field in plots that received a fall application of hyperparasites and in plots that received an application of fresh hyperparasite inocula (spring, 1985). Time of application was significant only for recovered sclerotia. This suggests that introduction of hyperparasites into the soil prior to addition of sclerotia affected the recovery, viability and infection of sclerotia by T. flavus and C. minitans in the same way as did the application of hyperparasites and sclerotia to soil at the same time. The number of sclerotia recovered from the C. minitans -treated plots was consistently less than in the control regardless of the time of application indicating that the introduced hyperparasite may have altered the biological balance to favor the destruction of sclerotia of S. sclerotiorum. This effect was evident during the same season of application as well as after an overwintering period.

Although the application of C. minitans reduced the carpogenic germination of sclerotia, the percentage of buried sclerotia that were infected with C. minitans was low. A significant reduction in the number of sclerotia recovered from the C. minitans -treated plots indicates that a treatment effect occurred. Non-recovered sclerotia may have been affected by the presence of C. minitans and destroyed prior to the recovery dates. The presence of a variety of microorganisms recovered from buried sclerotia suggests that while C. minitans may have been involved in the initial parasitism of the sclerotia, secondary microorganisms may have invaded the diseased tissue making the recovery of C. minitans on agar media difficult.

The lack of adequate control of white mold disease through cultural or chemical means as well as the lack of resistant cultivars which maximize yield potential emphasizes the need to investigate other means of control. Biological control through the use of hyperparasites can have a substantial impact on the inoculum potential of a plant pathogen as indicated from evidence collected from a number of systems (Baker, 1987). Hyperparasitism by C. minitans is an important factor affecting the survival of sclerotia of S. sclerotiorum in the field (Huang, 1977; Trutmann et al., 1982). Talaromyces flavus has also been reported to be destructive to sclerotia of S. sclerotiorum (McLaren et al., 1989) and has shown promise as a biological control agent for Rhizoctonia solani (Boosalis, 1956) and Verticillium dahliae (Dutta, 1981; Fravel et al., 1986). The present study indicates that both T. flavus and C. minitans are effective in reducing the carpogenic germination of

sclerotia of S. sclerotiorum in a bean field. Consequently, biological control using the hyperparasites T. flavus and C. minitans may have potential when used in conjunction with cultural and/or chemical practices recommended for control of this disease.

Plate 9. Design of field plots for studying the control of apothecial production of *S. sclerotiorum* by *T. flavus* and *C. minitans* (Lethbridge, 1984).

Figure A. Two burial areas (BA's), each 0.61m x 0.31m in size, located between two 2-m long rows of beans. Twelve BA's located within 6 inter-row spaces comprised one plot.

Figure B. Applicator sticks of different colors (arrow) were used to label the clusters of apothecia originating from germinated sclerotia. Each color of the sticks represents a defined number of apothecia in the cluster. As more apothecia emerged within each cluster, the sticks were replaced with ones corresponding to the total number of apothecia produced/cluster.


BA
BA
A
B

Plate 10. Control of apothecial production of *S. sclerotiorum* in a bean field with (Figure B) and without (Figure A) *T. flavus* (Lethbridge, 1984).

Sclerotia were buried in 12 burial areas (BA's). Each germinated sclerotium with apothecia is marked by an applicator stick. Note that the number of sticks is high in the untreated plot (Figure A) and in the *T. flavus*-treated plot (Figure B).

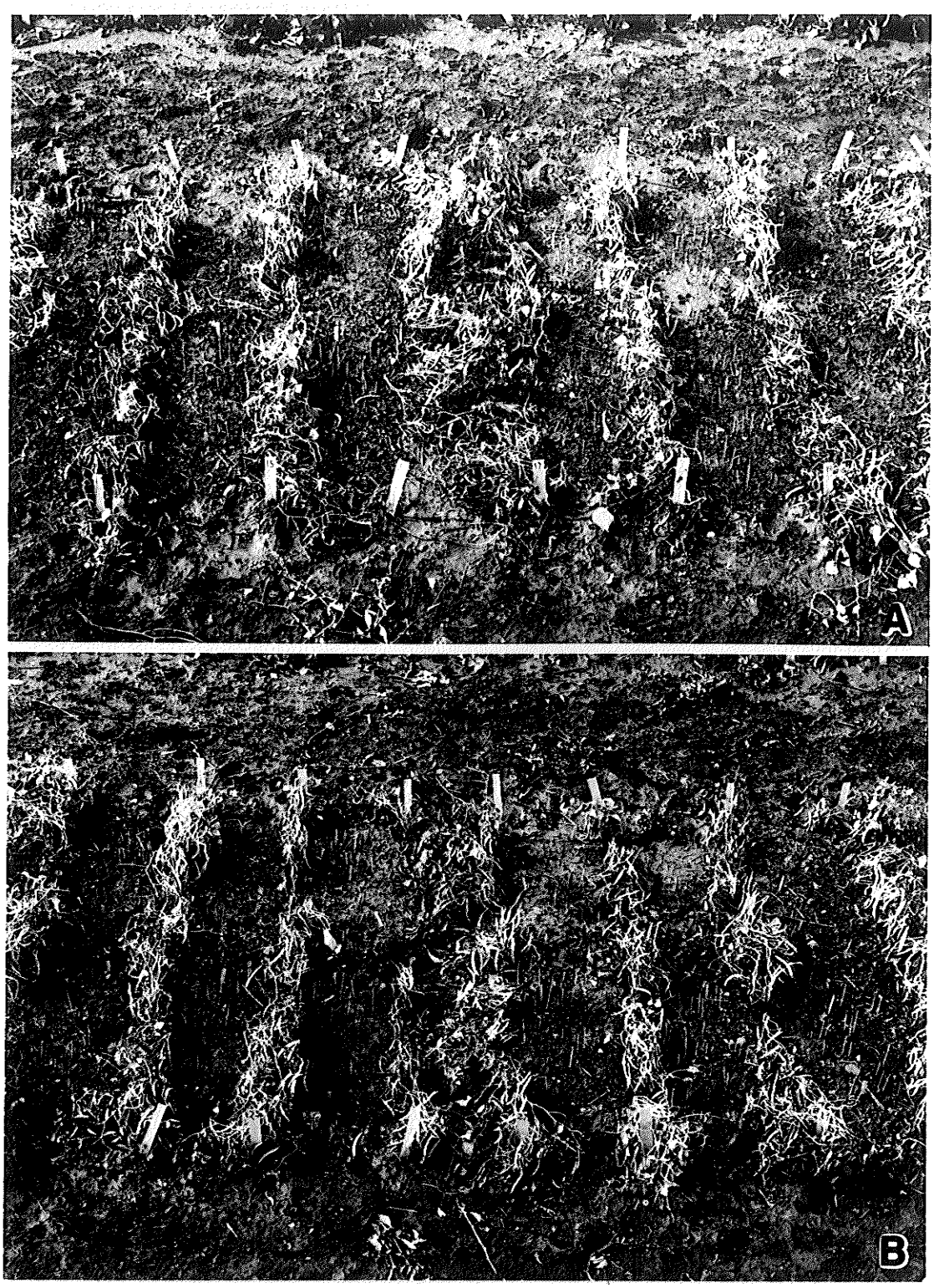
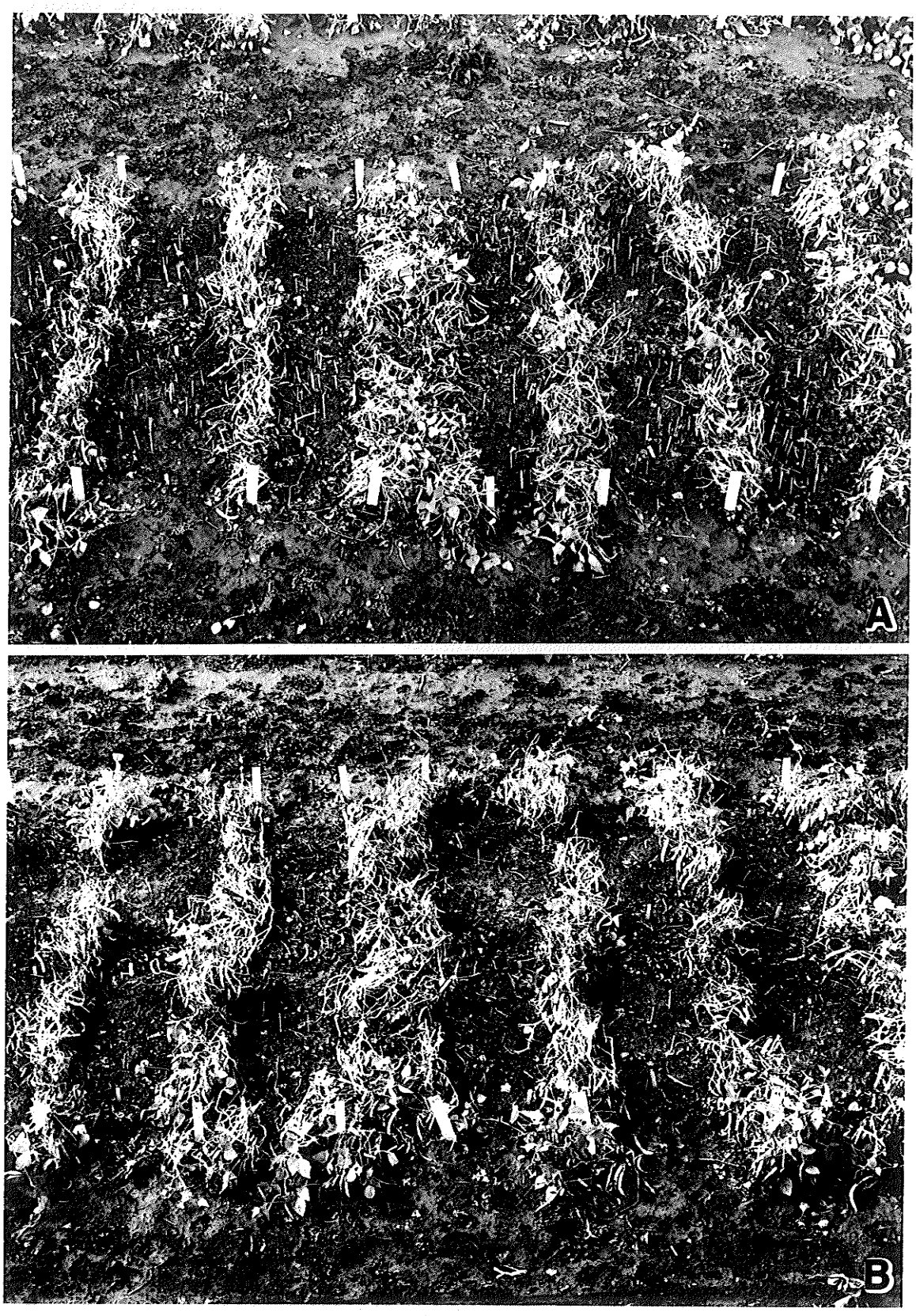

A
B

Plate 11. Control of apothecial production of *S. sclerotiorum* in a bean field with (Figure B) and without (Figure A) *C. minitans* (Lethbridge, 1984).

Sclerotia were buried in 12 BA's. An applicator stick marks the location of each germinated sclerotium having apothecia. Note that the number of sticks is high in the control (Figure A) but low in the *C. minitans*-treated plot (Figure B).


A
B

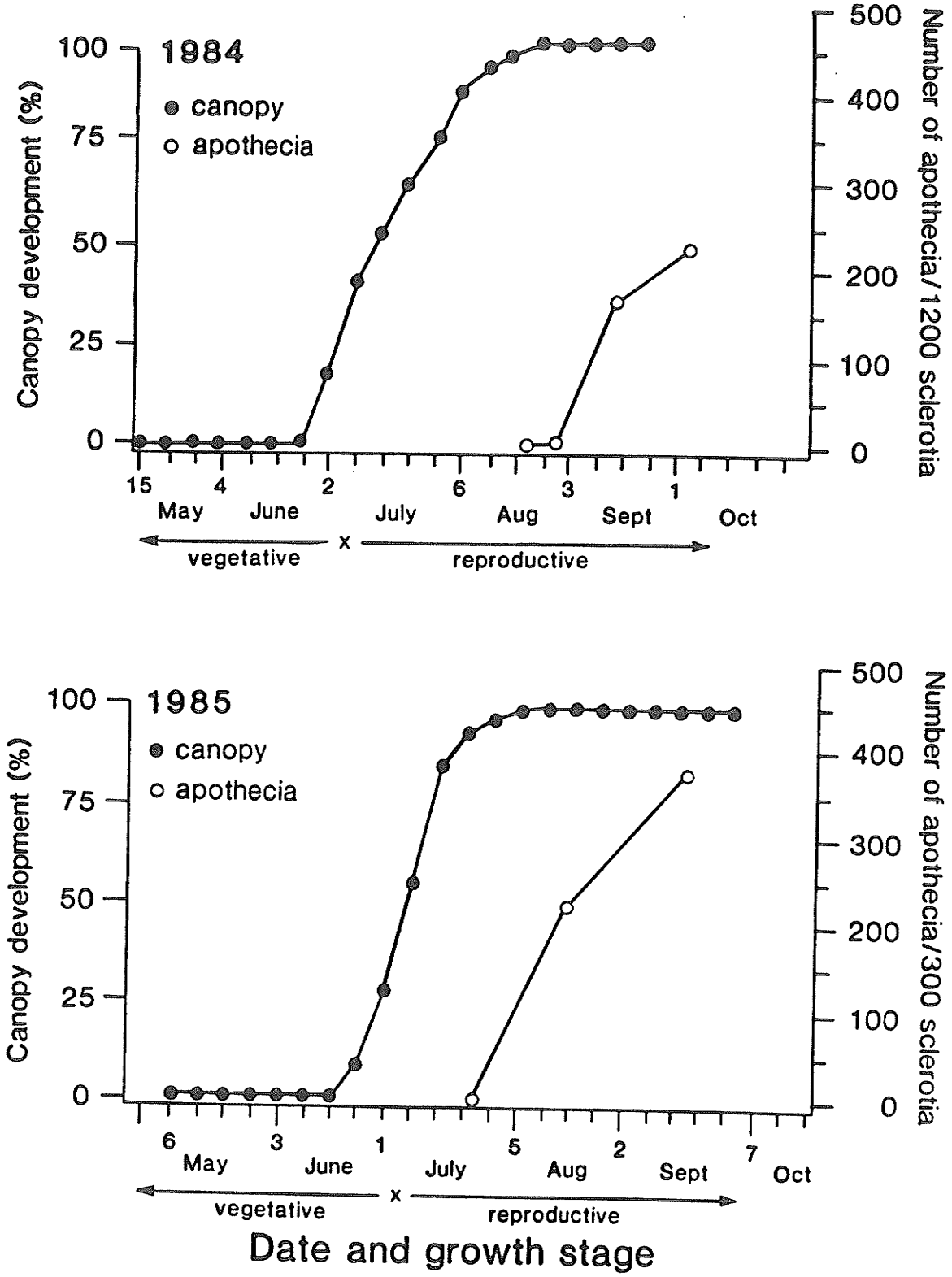


Figure 6. Canopy development of dry bean and production of apothecia in a field during 1984 and 1985 (Lethbridge). Canopy development is based on coverage of inter-row space.

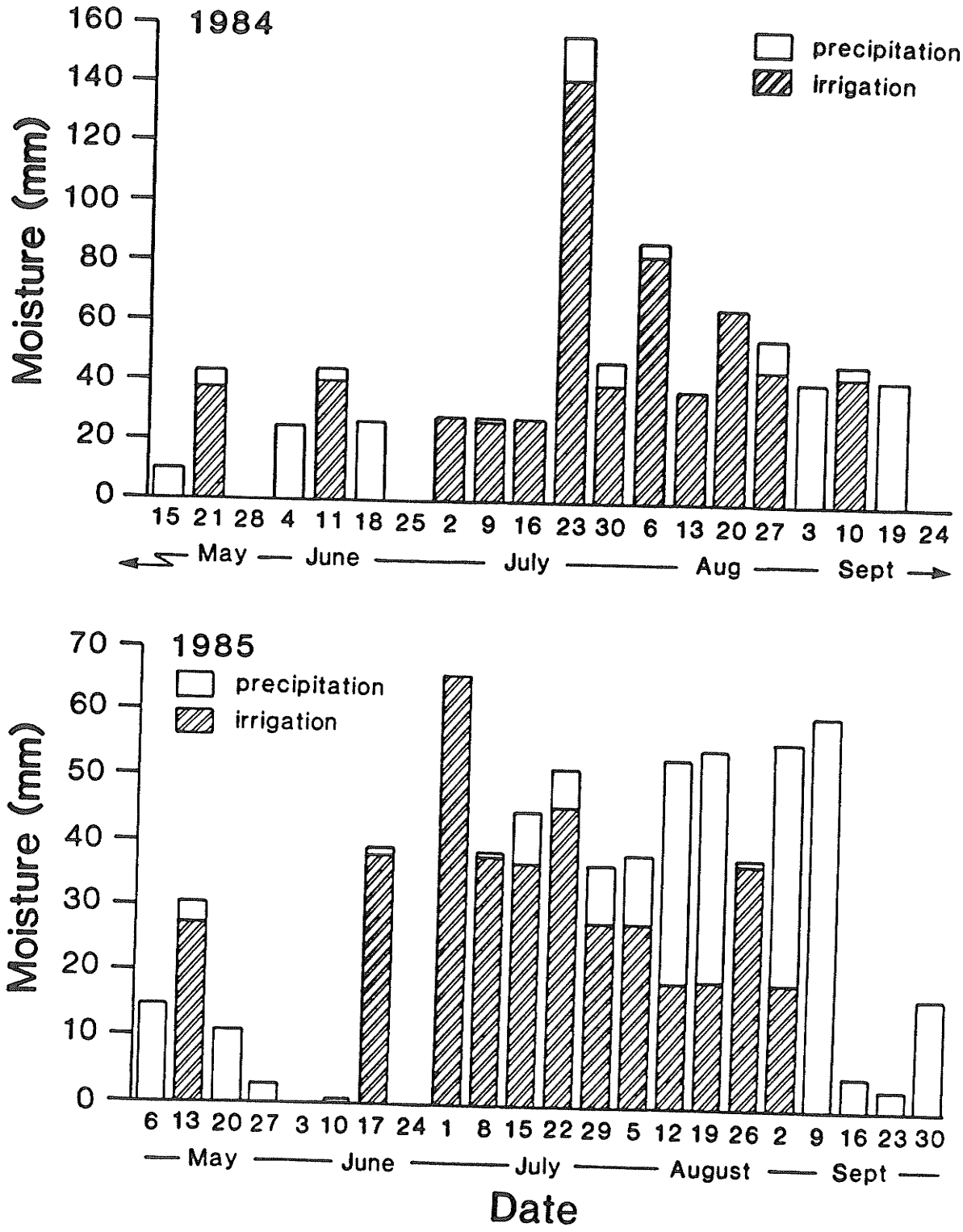


Figure 7. Precipitation and irrigation recorded in a bean field during the 1984 and 1985 field seasons (Lethbridge)

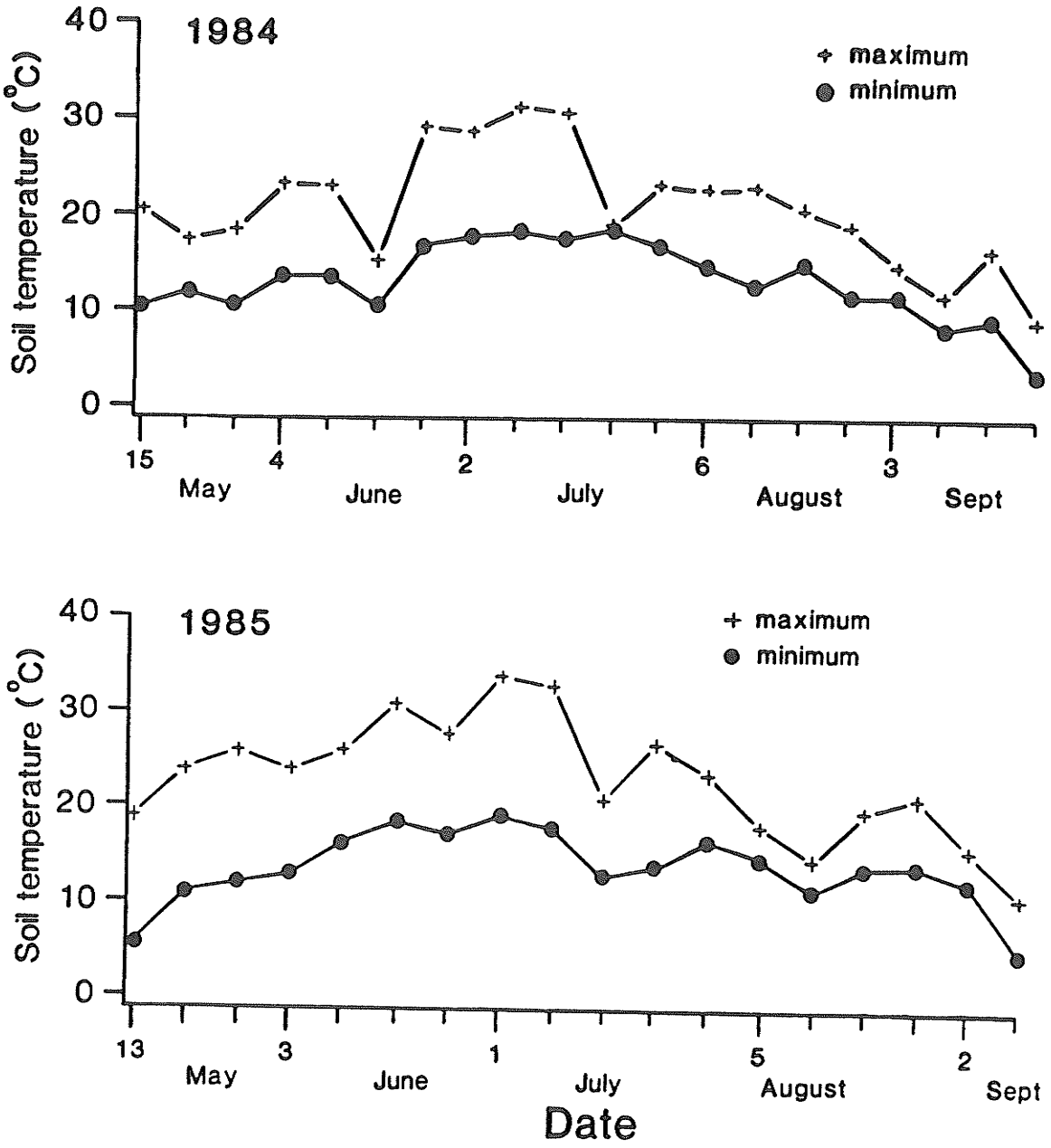


Figure 8. Soil temperatures recorded in a bean field in 1984 and 1985 (Lethbridge).

Table 19. The effect of *T. flavus* and *C. minitans* on the carpogenic germination of sclerotia of *S. sclerotiorum* buried in a bean field in June, 1984 at Lethbridge.

Hyperparasites	No. of apothecia[1]	No. of germinable sclerotia[1]	% germ.[1,2]	Avg. no. of apoth./germ. sclerotia[1]
Control (SS)	188.5ab[4]	57.6[3]a	5.0[3]ab	3.3[3]a
SS + bran	287.1 a	78.6 a	6.4 a	3.7 a
SS + *T. flavus* (T)	125.1 abc	37.9 ab	3.3 bc	3.4 a
SS + C/T 1:2	74.8 bcd	19.2 bc	1.6 cd	4.1 a
SS + C/T 1:1	46.7 cd	15.1 c	1.3 d	3.7 a
SS + C/T 2:1	27.0 d	9.1 c	0.8 d	3.0 a
SS + *C. minitans* (C)	21.4 d	6.9 c	0.5 d	3.1 a

[1] Values averaged over 4 replicates with 1200 sclerotia per replicate (12 BA's/replicate with 100 viable sclerotia/BA); sclerotia obtained from an infected bean crop.

[2] % germ. = the percentage of buried sclerotia that germinated.

[3] Analysis of variance on transformed data; means converted back to raw form for presentation.

[4] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Table 20. The effect of T. flavus and C. minitans on the carpogenic germination of sclerotia of S. sclerotiorum (SS) buried in a bean field in November, 1984 and in May, 1985.

Hyperparasites	No. of apothecia[1]	No. of germinable sclerotia[1]	% germ.[1,2]	Avg. no. of apoth./germ. sclerotia[1]
Control (SS)	344.3[3]a[4]	83.9[3]a	26.1[3]a	4.2[3]ab
SS + bran	433.2 a	88.7 a	27.2 a	5.0 a
SS + T. flavus (T)	325.4 a	82.6 a	25.7 a	4.0 b
SS + C/T 1:2	62.0 b	17.0 b	6.0 b	4.1 ab
SS + C/T 1:1	41.6 c	13.2 b	4.4 bc	3.5 bc
SS + C/T 2:1	24.8 d	8.6 c	2.9 c	3.0 c
SS + C. minitans (C)	25.6 d	8.6 c	2.8 c	3.4 bc

[1] Design is split-plot with 7 main plots (hyperparasites) and 4 subplots (time of application); values averaged over 4 replicates with 300 sclerotia per replicate. Each subplot contained 3 burial areas with 100 viable sclerotia per burial area. Sclerotia were obtained from an infected bean crop.

[2] The hyperparasite treatment X time of application interaction is not significant at the 5% level.

[3] % germ. = percentage of buried sclerotia that germinated. Analysis of variance on transformed data; means converted back to raw form for presentation.

[4] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Table 21. The effect of time of application of hyperparasites and S. sclerotiorum on the carpogenic germination of sclerotia in the bean field.

Time of application[1]					
Hyper.	Sclerotia	No. of apoth.[2]	No. of germ. sclerotia[2]	% germ[2]	Avg. no. of apoth./germ. sclerotia[2]
SP85	SP85	111.0[3]b[4]	33.4[3]b	12.5[3]b	3.8[3]a
F84	SP85	74.4 c	22.3 c	8.2 c	4.0 a
SP85	F84	168.1 a	47.6 a	16.8 a	3.8 a
F84	F84	81.3 c	24.1 c	9.0 c	4.0 a

[1] SP85 = spring application (May, 1985) of hyperparasites and/or sclerotia; F84 = fall application (November, 1984) of hyperparasites and/or sclerotia.

[2] Design is split-plot with 7 main plots (hyperparasites) and 4 subplots (time of application); values based on 4 replicates with 300 sclerotia per replicate. Sclerotia were obtained from an infected bean crop.

[3] The hyperparasite treatment X time of application interaction is not significant at the 5% level.

[4] Analysis of variance on transformed data; means converted back to raw form for presentation.

Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Table 22. The effect of hyperparasites and sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a bean field in May, 1984.

Hyperparasites	Weeks after burial 4	8	12	52	Treatment means
	A. Recovered sclerotia[1]				
Control (SS)	20.0 a[2]	17.6 ab[2]	18.5 a[2]	16.5 a[2]	18.2 a
SS + bran	20.0 a	19.3 a	17.8 a	15.3 ab	18.2 a
SS + T. flavus (T)	20.0 a	18.2 ab	16.1 a	12.8 b	17.0 a
SS + C/T 1:2	20.0 a	16.4 b	9.7 b	8.4 c	14.4 b
SS + C/T 1:1	20.0 a	12.7 c	12.0 b	10.8 bc	14.3 b
SS + C/T 2:1	19.8 a	8.4 d	11.3 b	6.3 c	12.5 b
SS + C. minitans (C)	20.0 a	11.8 cd	10.8 b	9.0 b	13.6 b
Date means[3]	20.0 a	15.4 b	14.2 bc	11.8 c	
	B. Percent of recovered sclerotia that were viable[1]				
Control (SS)	34.7 a[2]	1.9 a	6.4 a	2.9 ab	8.4 a
SS + bran	14.8 b	1.3 a	2.0 ab	5.5 a	4.9 a
SS + T. flavus (T)	0 d	0.8 a	0 b	0 b	0.2 b
SS + C/T 1:2	1.8 cd	3.3 a	0 b	1.2 ab	1.4 b
SS + C/T 1:1	0.8 cd	2.9 a	0 b	1.0 ab	1.0 b
SS + C/T 2:1	5.0 c	2.0 a	1.6 ab	0 b	1.8 b
SS + C. minitans (C)	5.2 bc	0 a	1.4 ab	0 b	1.2 b
Date means[3]	6.1 a	1.6 b	1.2 b	1.1 b	
	C. Percent of recovered sclerotia infected by T. flavus[1]				
Control (SS)	0 c[2]	0 a	0 c	0 a	0 c
SS + bran	0 c	0 a	0 c	0 a	0 c
SS + T. flavus (T)	8.6 a	0.8 a	8.7 a	1.3 a	4.0 a
SS + C/T 1:2	6.0 ab	2.3 a	0 c	3.4 a	2.5 ab
SS + C/T 1:1	1.3 bc	0 a	2.5 bc	1.3 a	1.2 abc
SS + C/T 2:1	0 c	0 a	0 c	0 a	0 c
SS + C. minitans (C)	0 c	0 a	1.1 bc	0 a	0.2 bc
Date means[3]	1.4 a	0.3 a	1.1 a	0.6 a	
	D. Percent of recovered sclerotia infected by C. minitans[1]				
Control (SS)	0 a[2]	0 a	0 a	0 c	0 b
SS + bran	0 a	0 a	0 a	0 c	0 b
SS + T. flavus (T)	0 a	0 a	0 a	0 c	0 b
SS + C/T 1:2	0 a	0 a	0 a	0 a	0 b
SS + C/T 1:1	0 a	0.9 a	0 a	1.3 bc	0.5 b
SS + C/T 2:1	2.6 a	1.7 a	3.6 a	9.5 a	3.9 a
SS + C. minitans (C)	2.6 a	2.9 a	3.3 a	3.7 ab	3.1 a
Date means[3]	0.5 a	0.6 a	0.7 a	1.3 a	

[1] Design is split-plot with 7 main plots (hyperparasites) and 4 subplots (sampling date); values averaged over 4 replicates with 20 sclerotia per replicate. Analysis of variance performed on transformed data; means converted back to raw form for presentation.

[2] Means within columns, excluding date means (for A,B,C or D) followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test); the hyperparasite treatment X sampling date interaction is significant at the 5% level for sclerotia that were recovered, viable and infected by T. flavus. Sampling date is significant for recovered and viable sclerotia only.

[3] For tables of treatment and date means, see Appendices 57 and 58.

Table 23. Effect of hyperparasites, time of burial of hyperparasites (season) and sampling date (weeks after burial) on the recovery of sclerotia of S. sclerotiorum (SS) buried in a bean field in November, 1984 (Lethbridge).

Hyperparasites	Season (S)	Weeks after burial (D) 30	33	36	39	Treatment means[1]
		Number of recovered sclerotia[2] (T x S x D means[3])				
Control (SS)	SP	400.0	400.0	333.8	282.3	353.1
	F	400.0	400.0	342.5	266.5	(18.8 a)
SS + bran	SP	400.0	400.0	282.3	250.8	339.0
	F	400.0	400.0	307.5	271.3	(18.4 ab)
SS + T. flavus	SP	400.0	400.0	307.5	271.3	318.9
	F	390.3	400.0	236.3	187.5	(17.9 b)
SS + C/T[4] 1:2	SP	400.0	400.0	171.5	83.5	269.8
	F	400.0	400.0	169.5	133.5	(16.4 d)
SS + C/T 1:1	SP	400.0	400.0	264.8	171.0	294.0
	F	390.3	390.3	171.3	164.3	(17.2 c)
SS + C/T 2:1	SP	400.0	380.5	150.8	49.3	251.3
	F	400.0	400.0	143.3	86.3	(15.9 d)
SS + C. minitans	SP	400.0	400.0	184.5	75.5	258.0
	F	400.0	400.0	139.3	64.5	(16.1 d)
		Season x Date means[5]				
Spring (SP)[6]		400.0	397.2	245.9	159.4	(17.3 a)
Fall		397.2	398.6	215.6	167.7	(17.2 a)
Date means		(20.0 a)	(20.0 a)	(15.2 b)	(12.8 c)	

[1] LSD, 5%: 23.40.

[2] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (season) and 4 sub-subplots (sampling date). Values averaged over 4 replicates with 20 sclerotia per replicate. Analysis of variance on transformed data which is presented here; data in parentheses represents transformed data converted back to raw form (see Appendices 60, 61 and 62).

[3] LSD, 5% between sampling dates for same treatment and season: 52.38; between seasons for the same treatment and the same or different sampling date: 53.46; between treatments for the same or different season or sampling date: 56.96. The T x S x D interaction is not significant at the 5% level.

[4] T = T. flavus; C = C. minitans.

[5] LSD, 5% between sampling dates for the same season: 19.80; between seasons for the same or different sampling dates: 20.20. The S x D interaction is significant at the 5% level.

[6] Spring = application of hyperparasites in May, 1985; fall = application of hyperparasites in November, 1984; all sclerotia were buried in the fall.

Table 24. Effect of hyperparasites, time of burial of hyperparasites (season) and sampling date (weeks after burial) on the viability of sclerotia of S. sclerotiorum (SS) buried in a bean field in November, 1984 (Lethbridge).

Hyperparasites	Season (S)	Weeks after burial (D) 30	33	36	39	Treatment means[1]
		Percent of recovered sclerotia that were viable[2] (T x S x D means[3])				
Control (SS)	SP	2.2	2.6	3.2	1.8	2.3
	F	2.6	1.5	2.0	2.3	(4.6 a)
SS + bran	SP	2.7	0.7	2.3	2.2	2.3
	F	4.5	1.8	1.4	2.5	(4.6 a)
SS + T. flavus	SP	0.7	0.7	0.7	0.7	1.1
	F	2.4	1.1	1.2	1.3	(0.7 b)
SS + C/T[4] 1:2	SP	1.1	0.7	0.7	0.7	1.0
	F	1.8	0.7	1.3	1.3	(0.6 b)
SS + C/T 1:1	SP	1.1	1.1	0.7	1.5	1.0
	F	1.1	0.7	0.7	0.7	(0.4 b)
SS + C/T 2:1	SP	1.5	0.7	1.3	3.3	1.6
	F	1.3	1.5	1.3	1.4	(1.9 b)
SS + C. minitans	SP	2.8	1.1	0.7	1.8	1.3
	F	1.7	0.7	0.7	0.7	(1.1 b)
		Season x Date means[5]				
Spring (SP)[6]		1.8	1.1	1.4	1.7	(1.7 a)
Fall (F)		2.2	1.2	1.2	1.5	(1.8 a)
Date means		(3.4 a)	(0.8 c)	(1.2 bc)	(2.0 b)	

[1] LSD, 5%: 0.75.

[2] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (season) and 4 sub-subplots (sampling date); values averaged over 4 replicates with 20 sclerotia per replicate. Analysis of variance on transformed data which is presented here; data in parentheses represents transformed data converted back to raw form (see Appendices 60, 61 and 62).

[3] LSD, 5% between sampling dates for same treatment and season: 1.65; between seasons for the same treatment and the same or different sampling date: 1.75; between treatments for the same or different season or sampling date: 1.74. The T x S x D interaction is not significant at the 5% level.

[4] T = T. flavus; C = C. minitans.

[5] LSD, 5% between sampling dates for the same season: 1.90; between seasons for the same or different sampling dates: 0.66. The S x D interaction is significant at the 5% level.

[6] Spring = application of hyperparasites in May, 1985; fall = application of hyperparasites in November, 1984; all sclerotia were buried in the fall.

Table 25. Effect of hyperparasites, time of burial of hyperparasites (season) and sampling date (weeks after burial) on the infection of sclerotia of S. sclerotiorum (SS) by T. flavus (sclerotia buried in November, 1984 at Lethbridge).

Hyperparasites	Season (S)	Weeks after burial (D) 30	33	36	39	Treatment means[1]
		Percent of recovered sclerotia that were infected by T. flavus[2] (T x S x D means[3])				
Control (SS)	SP	1.3	0.7	0.7	0.7	1.1
	F	1.5	1.8	0.7	0.7	(0.5 cd)
SS + bran	SP	0.7	1.1	0.7	1.7	0.9
	F	0.7	0.7	0.7	0.7	(0.3 d)
SS + T. flavus	SP	2.0	3.0	1.1	2.3	2.2
	F	4.0	3.7	0.7	1.3	(4.5 a)
SS + C/T[4] 1:2	SP	3.7	2.7	2.4	0.7	2.0
	F	2.9	1.8	1.3	0.7	(3.6 ab)
SS + C/T 1:1	SP	2.4	2.9	1.2	1.2	1.7
	F	2.4	1.1	0.7	1.8	(2.4 abc)
SS + C/T 2:1	SP	0.7	1.8	0.7	1.4	1.5
	F	1.5	2.1	1.2	2.5	(1.8 bcd)
SS + C. minitans	SP	0.7	1.5	0.7	0.7	0.9
	F	1.1	1.1	0.7	0.7	(0.3 d)
		Season x Date means[5]				
Spring (SP)[6]		1.7	2.0	1.1	1.3	(1.7 a)
Fall (F)		2.0	1.8	0.9	1.2	(1.6 a)
Date means		(2.9 a)	(3.0 a)	(0.4 b)	(1.0 b)	

[1] LSD, 5%: 0.70.

[2] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (season) and 4 sub-subplots (sampling date). Values averaged over 4 replicates with 20 sclerotia per replicate. Analysis of variance on transformed data which is presented here; data in parentheses represents transformed date converted back to raw form (see Appendices 60, 61 and 62).

[3] LSD, 5% between sampling dates for same treatment and season: 1.65; between seasons for the same treatment and the same or different sampling date: 1.65; between treatments for the same or different season or sampling date: 1.70. The T x S x D interaction is not significant at the 5% level.

[4] T = T. flavus; C = C. minitans.

[5] LSD, 5% between sampling dates for the same season: 0.62; between seasons for the same or different sampling dates: 0.62. The S x D interaction is not significant at the 5% level.

[6] Spring = application of hyperparasites in May, 1985; fall = application of hyperparasites in November, 1984; all sclerotia were buried in the fall.

Table 26. Effect of hyperparasites, time of burial of hyperparasites (season) and sampling date (weeks after burial) on the infection of sclerotia of S. sclerotiorum (SS) by C. minitans (sclerotia buried in November, 1984 at Lethbridge).

Hyperparasites	Season (S)	Weeks after burial (D) 30	33	36	39	Treatment means[1]
		Percent of recovered sclerotia that were infected by C. minitans[2] (T x S x D means[3])				
Control (SS)	SP	1.1	1.5	1.2	0.7	1.1
	F	1.5	0.7	0.7	1.3	(0.7 cd)
SS + bran	SP	0.7	1.3	1.1	0.7	1.0
	F	0.7	1.1	1.6	0.7	(0.5 cd)
SS + T. flavus	SP	0.7	0.7	1.1	0.7	0.9
	F	0.7	1.3	0.7	1.3	(0.3 cd)
SS + C/T[4] 1:2	SP	0.7	3.1	1.2	2.7	2.1
	F	1.7	3.8	2.1	1.6	(4.0 b)
SS + C/T 1:1	SP	0.7	2.7	1.2	1.2	1.7
	F	1.9	3.2	1.8	1.3	(2.5 bc)
SS + C/T 2:1	SP	1.5	4.3	3.5	2.2	3.1
	F	3.1	5.2	4.2	0.7	(9.1 a)
SS + C. minitans	SP	2.2	3.5	3.4	4.4	3.7
	F	2.7	6.0	4.8	2.6	(13.2 a)
		Season x Date means[5]				
Spring (SP)[6]		1.1	2.5	1.8	1.8	(2.7 b)
Fall (F)		1.8	3.1	2.3	1.4	(4.0 a)
Date means		(1.6 c)	(7.1 a)	(4.0 b)	(2.0 c)	

[1] LSD, 5%: 0.74.

[2] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (season) and 4 sub-subplots (sampling dates); values averages over 4 replicates with 20 sclerotia per replicate. Analysis of variance on transformed data which is presented here; data in parentheses represents transformed data converted back to raw form (see Appendices 60, 61, and 62).

[3] LSD, 5% between sampling dates for same treatment and season: 1.70; between seasons for the same treatment and the same or different sampling date: 1.60; between treatments for the same or different season or sampling date: 1.71. The T x S x D interaction is not significant at the 5% level.

[4] T = T. flavus; C = C. minitans.

[5] LSD, 5% between sampling dates for the same season: 0.64; between seasons for the same or different sampling dates: 0.61. The S x D interaction is significant at the 5% level.

[6] Spring = application of hyperparasites in May, 1985; fall = application of hyperparasites in November, 1984; all sclerotia were buried in the fall.

Table 27. Effect of hyperparasites, time of burial of hyperparasites (season) and sampling date (weeks after burial) on recovery of sclerotia of S. sclerotiorum (SS) buried in a bean field in May, 1985 at Lethbridge.

Hyperparasites	Season (S)	Weeks after burial (D) 3	6	9	Treatment means[1]
		Number of recovered sclerotia[2] (T x S x D means[3])			
Control (SS)	SP	390.3	370.8	272.5	338.4
	F	380.5	325.5	291.0	(18.4 a)
SS + bran	SP	400.0	318.3	352.8	354.2
	F	400.0	380.5	273.5	(18.8 a)
SS + T. flavus	SP	400.0	267.0	196.5	267.0
	F	400.0	192.8	145.8	(16.3 b)
SS + C/T[4] 1:2	SP	400.0	221.3	126.8	241.2
	F	390.3	212.5	96.3	(15.5 b)
SS + C/T 1:1	SP	400.0	281.8	210.5	265.0
	F	400.0	204.3	93.3	(16.3 b)
SS + C/T 2:1	SP	400.0	111.0	31.5	191.0
	F	390.3	111.5	101.5	(13.8 c)
SS + C. minitans	SP	400.0	152.8	49.5	203.2
	F	380.5	124.8	111.5	(14.3 c)
			Season x Date means[5]		
Spring (SP)[6]		398.6	246.1	177.1	(16.6 a)
Fall (F)		391.6	221.7	159.0	(16.0 b)
Date means		(19.9 a)	(15.3 b)	(13.0 c)	

[1] LSD, 5%: 26.06.

[2] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (season) and 3 sub-subplots (sampling date); values averaged over 4 replicates with 20 sclerotia per replicate. Analysis of variance on transformed data which is presented here; data in parentheses represents transformed data converted back to raw form (see Appendices 64, 65 and 66).

[3] LSD, 5% between sampling dates for same treatment and season: 63.18; between seasons for the same treatment and the same or different sampling date: 63.12; between treatments for the same or different season or sampling date: 65.47. The T x S x D interaction is significant at the 5% level.

[4] T = T. flavus; C = C. minitans.

[5] LSD, 5% between sampling dates for the same season: 23.88; between seasons for the same or different sampling dates: 23.86. The S x D interaction is not significant at the 5% level.

[6] Spring = application of hyperparasites in May, 1985; fall = application of hyperparasites in November, 1984; all sclerotia were buried in the spring.

Table 28. Effect of hyperparasites, time of burial of hyperparasites (season) and sampling date (weeks after burial) on the viability of sclerotia of S. sclerotiorum (SS) buried in a bean field in May, 1985 at Lethbridge.

Hyperparasites	Season (S)	Weeks after burial (D) 3	6	9	Treatment means[1]
		Percent of recovered sclerotia that were viable[2] (T x S x D means[3])			
Control (SS)	SP	2.0	3.2	2.1	2.0
	F	1.1	2.3	1.1	(3.3 a)
SS + bran	SP	1.3	1.3	1.6	1.5
	F	1.7	1.7	1.2	(1.7 a)
SS + T. flavus	SP	0.7	0.7	1.2	1.2
	F	1.5	1.2	1.8	(0.9 a)
SS + C/T[4] 1:2	SP	0.7	1.5	1.9	1.1
	F	0.7	0.7	1.3	(0.8 a)
SS + C/T 1:1	SP	0.7	1.2	1.7	1.3
	F	2.2	1.4	0.7	(1.2 a)
SS + C/T 2:1	SP	0.7	0.7	3.0	1.3
	F	1.3	0.7	1.4	(1.2 a)
SS + C. minitans	SP	0.7	0.7	0.7	0.9
	F	1.1	1.2	0.7	(0.2 a)
		Season x Date means[5]			
Spring (SP)[6]		1.0	1.3	1.7	(1.3 a)
Fall (F)		1.4	1.3	1.2	(1.2 a)
Date means		(0.9 a)	(1.3 a)	(1.6 a)	

[1] LSD, 5%: 0.79.

[2] Design is split plot with 7 main plots (hyperparasite), 2 subplots (season) and 3 sub-subplots (sampling date); values averaged over 4 replicates with 20 sclerotia per replicate. Analysis of variance on transformed data which is presented here; data in parentheses represents transformed data converted back to raw form (see Appendices 64, 65 and 66).

[3] LSD, 5% between sampling dates for same treatment and season: 1.47; between seasons for the same treatment and the same or different sampling date: 1.43; between treatments for the same or different season or sampling date: 1.54. The T x S x D interaction is not significant at the 5% level.

[4] T = T. flavus; C = C. minitans.

[5] LSD, 5% between sampling dates for the same season: 0.55; between seasons for the same or different sampling dates: 0.54. The S x D interaction is not significant at the 5% level.

[6] Spring = application of hyperparasites in May, 1985; fall = application of hyperparasites in November, 1984; all sclerotia were buried in the spring.

Table 29. Effect of hyperparasites, time of burial of hyperparasites (season) and sampling date (weeks after burial) on the infection of sclerotia of S. sclerotiorum (SS) by T. flavus (sclerotia buried in May, 1985 at Lethbridge).

Hyperparasites	Season (S)	Weeks after burial (D) 3	6	9	Treatment means[1]
		Percent of recovered sclerotia infected by T. flavus[2] (T x S x D means[3])			
Control (SS)	SP	0.7	0.7	0.7	0.9
	F	1.1	0.7	1.2	(0.2 b)
SS + bran	SP	0.7	0.7	1.1	0.9
	F	1.3	0.7	0.7	(0.3 b)
SS + T. flavus	SP	2.1	2.6	0.7	1.8
	F	2.8	1.6	1.3	(2.9 a)
SS + C/T[4] 1:2	SP	3.5	2.7	0.7	2.1
	F	2.1	1.3	2.1	(3.7 a)
SS + C/T 1:1	SP	1.9	0.7	0.7	1.5
	F	2.2	1.8	2.1	(1.6 ab)
SS + C/T 2:1	SP	1.3	0.7	3.4	1.6
	F	1.8	0.7	1.3	(1.9 ab)
SS + C. minitans	SP	0.7	0.7	0.7	0.7
	F	0.7	0.7	0.7	(0 b)
		Season x Date means[5]			
Spring (SP)[6]		1.6	1.3	1.2	(1.3 a)
Fall (F)		1.7	1.0	1.3	(1.3 a)
Date means		(2.2 a)	(0.8 a)	(1.0 a)	

[1] LSD, 5%: 0.90.

[2] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (season) and 3 sub-subplots (sampling date); values averaged over 4 replicates with 20 sclerotia per replicate. Analysis of variance on transformed data which is presented here; data in parentheses represents transformed data converted back to raw form (see Appendices 64, 65 and 66).

[3] LSD, 5% between sampling dates for same treatment and season: 1.74; between seasons for the same treatment and the same or different sampling date: 1.75; between treatments for the same or different season or sampling date: 1.84. The T x S x D interaction is not significant at the 5% level.

[4] T = T. flavus; C = C. minitans.

[5] LSD, 5% between sampling dates for the same season: 0.66; between seasons for the same or different sampling dates: 0.66. The S x D interaction is not significant at the 5% level.

[6] Spring = application of hyperparasites in May, 1985; fall = application of hyperparasites in November, 1984; all sclerotia were buried in the spring.

Table 30. Effect of hyperparasites, time of burial of hyperparasites (season) and sampling date (weeks after burial) on the infection of sclerotia of S. sclerotiorum (SS) by C. minitans (sclerotia buried in May, 1985 at Lethbridge).

Hyperparasites	Season (S)	Weeks after burial (D) 3	6	9	Treatment means[1]
		Percent of recovered sclerotia infected by C. minitans[2] (T x S x D means[3])			
Control (SS)	SP	1.9	1.1	0.7	1.3
	F	1.5	1.6	1.1	(1.3 c)
SS + bran	SP	0.7	1.1	0.7	0.9
	F	1.5	0.7	0.7	(0.3 c)
SS + T. flavus	SP	1.1	0.7	0.7	1.0
	F	0.7	1.3	1.2	(0.4 c)
SS + C/T[4] 1:2	SP	1.1	0.7	1.3	1.2
	F	2.4	1.2	0.7	(1.0 c)
SS + C/T 1:1	SP	1.1	2.3	2.2	2.2
	F	4.8	1.9	0.7	(4.3 b)
SS + C/T 2:1	SP	3.2	4.9	0.7	3.1
	F	4.3	2.6	2.6	(8.7 a)
SS + C. minitans	SP	3.8	3.8	2.4	3.2
	F	3.8	3.7	1.4	(9.6 a)
		Season x Date means[5]			
Spring (SP)[6]		1.9	2.1	1.3	(2.5 a)
Fall (F)		2.7	1.9	1.2	(3.2 a)
Date means		(4.8 a)	(3.4 a)	(1.0 b)	

[1] LSD, 5%: 0.75.

[2] Design is split plot with 7 main plots (hyperparasites), 2 subplots (season) and 3 sub-subplots (sampling date); values averaged over 4 replicates with 20 sclerotia per replicate. Analysis of variance on transformed data which is presented here; data in parentheses represents transformed data converted back to raw form (see Appendices 64, 65 and 66).

[3] LSD, 5% between sampling dates for same treatment and season: 1.65; between seasons for the same treatment and the same or different sampling date: 1.75; between treatments for the same or different season or sampling date: 1.74. The T x S x D interaction is not significant at the 5% level.

[4] T = T. flavus; C = C. minitans.

[5] LSD, 5% between sampling dates for the same season: 1.90; between seasons for the same or different sampling dates: 0.66. The S x D interaction is significant at the 5% level.

[6] Spring = application of hyperparasites in May, 1985; fall = application of hyperparsites in November, 1984; all sclerotia were buried in the spring.

Table 31. Effect of *T. flavus* and *C. minitans* on the viability of sclerotia collected from above-ground parts of bean plants (sclerotia collected in September, 1985).

Hyperparasites	No. of sclerotia[1]		
	Viable	Infected (C)	Infected (T)
Control (SS)	2.6[2]a[3]	5.8[2]a	0.0[2]a
SS + bran	4.5 a	7.0 a	0.0 a
SS + *T. flavus* (T)	5.2 a	7.7 a	0.0 a
SS + C/T 1:2	3.2 a	7.0 a	0.2 a
SS + C/T 1:1	4.7 a	4.7 a	0.0 a
SS + C/T 2:1	5.2 a	5.6 a	0.0 a
SS + *C. minitans* (C)	3.0 a	7.9 a	0.0 a

[1] Values averaged over 4 replicates; 20 sclerotia were collected from each main plot per replicate.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Table 32. Inoculum density of *S. sclerotiorum* in a field at Lethbridge (dry bean site, 1984).

Treatment	Avg. inoculum density[1] (no. scler./kg soil)
S. sclerotiorum (SS)	0 a[2]
SS + bran	0 a
SS + *T. flavus* (T)	0 a
SS + *C. minitans* (C)	0 a
SS + C/T 1:1	0.02 a
SS + C/T 1:2	0 a
SS + C/T 2:1	0.02 a

[1] Analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Chapter VI

GENERAL DISCUSSION AND SUMMARY

The effect of T. flavus and C. minitans on S. sclerotiorum, causal agent of sclerotinia wilt of sunflower and white mold of bean was examined. Laboratory investigations indicate that T. flavus is destructive to hyphae and sclerotia of S. sclerotiorum. Hyphae of T. flavus grew toward and coiled around the host hyphal cells. Tips of the hyphal branches often invaded the host by direct penetration of the cell wall without formation of appressoria. Infection of host cells by T. flavus resulted in granulation of the cytoplasm and collapse of cell walls. Talaromyces flavus is also destructive to sclerotia of S. sclerotiorum as revealed by transmission electron microscopy. Hyphae of T. flavus penetrated rind cell walls directly. Ramification of the hyperparasite in the sclerotium resulted in destruction and collapse of sclerotial tissues. Destruction of sclerotia and hyphae of S. sclerotiorum will affect the ability of S. sclerotiorum to survive.

A four-year field study at Lethbridge (1983-86) and a three-year field study at Winnipeg (1983-85) indicate that the incidence of sclerotinia wilt of sunflower and seed yield are reduced significantly with the application of T. flavus and C. minitans. In 1983 at Lethbridge, the percentage disease in the control plots was 54.9% compared with 0 and 9.8% in the plots treated with T. flavus and C.

minitans, respectively. This represents a reduction in disease by 100 and 82% in the T. flavus and C. minitans -treated plots, respectively. Similar results were obtained at the Winnipeg location and at both sites in 1984. These findings support previous reports on the success of C. minitans (Bogdanova et al., 1986; Huang, 1980b) and T. flavus as biological control agents for sclerotinia wilt of sunflower in the field.

In 1985, application of sclerotia, but no hyperparasites, to Lethbridge plots treated with both sclerotia and hyperparasites in the previous two years resulted in low levels of disease. In the control plots (sclerotia only), the percentage of disease was 38.6% compared with 4.3 and 5.1% in the T. flavus and C. minitans -treated plots, respectively. This represents a reduction in disease by 89 and 87% in the plots treated with T. flavus and C. minitans, respectively. Similar results occurred at the Winnipeg location for the C. minitans -treated plots. Talaromyces flavus was not effective in reducing significantly the incidence of wilt in 1985 at the Winnipeg site. In 1986, at Lethbridge, the one-year carry-over of control exhibited in 1985 did not reoccur. In the control, T. flavus and C. minitans -treated plots, the percentage of disease were 54.1, 51.0 and 41.7%, respectively.

Seed yield of sunflower was increased significantly with the application of hyperparasites during 1984-85 and 1984 at the Lethbridge and Winnipeg locations, respectively. In 1984 at Lethbridge, seed yields were 1433.0, 1326.8 and 1360.0 kg/ha in the plots treated with T. flavus, C. minitans and a combination of both

hyperparasites, respectively compared with 952.7 kg/ha in the control. A similar trend was evident at the Winnipeg location in 1984. In 1985, the carry-over effect of disease control was also reflected in the yield data at both locations. In 1986 at Lethbridge, the carry-over effect of biocontrol was no longer evident.

A carry-over of biocontrol has important economic considerations. Production of a biofungicidal product is costly and the marketability of such a product would be enhanced if it was effective not only during the year of application but for another year at no extra cost to the landowner. Although the carry-over effect did not last for a second season (1986) a reduction in yield loss and disease incidence for a period of one year is significant. Further research on this phenomenon with sunflower as well as with other susceptible hosts such as bean and canola is warranted.

In a two-year study at Lethbridge (1984-85), T. flavus and C. minitans reduced the carpogenic germination of sclerotia of S. sclerotiorum in a bean field. Coniothyrium minitans was more effective than T. flavus in both years. In 1984, apothecia production was reduced by 88.6 and 33.6% with the application of C. minitans and T. flavus, respectively. Similar results were obtained in 1985.

In both sunflower and bean fields, the application of T. flavus and C. minitans affected the survival of sclerotia of S. sclerotiorum. Fewer sclerotia were recovered from the hyperparasite-treated plots and the viability of the recovered sclerotia was reduced. Although the percentage of recovered sclerotia infected by hyperparasites was

low, the recovery of sclerotia from these plots was consistently lower than from the control indicating that a treatment effect occurred. The reduced recovery of the introduced hyperparasites from sclerotia may have been due to sclerotial colonization by other soil microorganisms whose activity may have been promoted by the addition of T. flavus and C. minitans, produced on bran, to soil.

In the sunflower fields (Experiment A), a reduced incidence of disease in the bran-treated plots compared with the control (SS) plots indicates that some biological control has occurred with the application of bran. In the bean fields, application of bran increased (but not significantly) carpogenic germination of sclerotia in the bran-treated plots compared with the control plots. The different effects of bran may be related to the burial depth of sclerotia. In the bean and sunflower fields, sclerotia were buried at depths of two or four and 6.5 cm, respectively. The activity of microorganisms may be reduced at shallow depths due to greater fluctuations in environmental conditions. At deeper depths, where the activity of microorganisms is more intense, the survival of sclerotia is affected to a greater degree by resident microorganisms.

Synergism, a state in which two organisms work better together than alone, is not apparent in the combination of T. flavus and C. minitans in sunflower fields. The organisms do not appear to be antagonistic to each other as such activity would likely nullify the biocontrol effect and this is not apparent. In a bean field, C. minitans was more effective than T. flavus in controlling apothecial production of sclerotia of S. sclerotiorum. Combinations of both hyperparasites

were effective in reducing germination and apothecial production. As the proportion of C. minitans in the mixture of the two hyperparasites increased, the effectiveness of the treatment increased. However, the greatest reductions occurred with the application of C. minitans alone. This indicates that, although a synergistic effect did not occur, the presence of T. flavus in the hyperparasitic mixture did not affect the efficacy of C. minitans.

The laboratory studies on the interaction between T. flavus and S. sclerotiorum indicate that T. flavus is a hyperparasite capable of destroying sclerotia and hyphae of S. sclerotiorum. In the field, T. flavus and C. minitans are effective hyperparasites capable of reducing the incidence of disease and yield losses due to sclerotinia wilt of sunflower. Coniothyrium minitans is more effective than T. flavus in reducing sclerotial germination and apothecial production in field bean.

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Appendix 1. A descriptive growth stage key for cultivated sunflower (Helianthus annuus var. macrocarpus) (Siddiqui et al., 1975).

1. Establishment Stage: From emergence of the cotyledons to the formation of the last pair of leaves showing opposite phyllotaxy.
   - 1.1 Cotyledons emerged.
   - 1.2 First pair of opposite leaves formed[a].
   - 1.3 Second pair of opposite leaves formed.
   - 1.4[b]

2. Vegetative Stage: From the formation of the first leaf showing spiral phyllotaxy to the appearance of the inflorescence head.
   - 2.1 First alternate leaf formed.
   - 2.2 Second alternate leaf formed.
   - 2.3 Third alternate leaf formed.
   - 2.4[b]

3. Budding Stage: From the appearance of the inflorescence head[c] to emergence of the first anther.
   - 3.1 Inflorescence head visible but tightly surrounded by young leaves.
   - 3.2 Inflorescence head pushed above the crown or plate of leaves. A few young leaves indistinguishable from inflorescence bracts.
   - 3.3 Inflorescence head fully separated from leaves. Last vegetative leaf distinguishable from bracts.
   - 3.4 Inflorescence begins to open. Ray florets visible.

4. Anthesis Stage: From emergence of the first to emergence of the last anther.
   - 4.1 Anthesis begins.
   - 4.2 Anthesis in outer quarter of inflorescence radius complete.
   - 4.3 Anthesis in half the inflorescence radius complete. Seed filling in outer florets commences.
   - 4.4 Anthesis in three-quarters of the inflorescence radius complete. Seed filling in outer florets continues.
   - 4.5 Anthesis complete. Seed filling continues.

5. Seed Development Stage: From emergence of the last anther to commercial maturity of the plant.
   - 5.1 Seed filling continues. Head inverted. Senescence of lower leaves obvious. Outer seeds soft.
   - 5.2 Inflorescence cup and bracts yellow. Younger leaves begin to senesce.
   - 5.3 Seed hard, stem and leaves dry and commercial maturity complete.

[a] Leaf formed refers to the stage of leaf development when the petiole of the leaf is just visible through the crown.

[b] Extra leaves or leaf pairs may be added if necessary.

[c] Appearance of inflorescence head refers to when close examination without dissection revealed that the terminal bud was a head rather than a cluster of leaves.

Appendix 2. The effect of *T. flavus* and *C. minitans* on the development of sclerotinia wilt of sunflower over time at the Winnipeg field site (Experiment A, 1983).

	Disease incidence[1] (%)								
Hyperpar.	13/6[2]	20/6	4/7	18/7	1/8	15/8	29/8	12/9	26/9
none	0c[3]	0d	0.1e	0.1c	0.1d	0.1e	0.6de	0.6c	0.6d
bran	0c	0d	0e	0c	0d	0e	0.4e	0.6c	0.6d
SS[4]	7.8a	9.9a	10.9a	12.0a	16.1a	26.7a	43.2a	57.1a	65.2a
SS + bran	5.5a	6.3b	7.6b	8.3a	10.8b	18.7b	33.4b	46.8a	54.5b
SS + C[5]	1.0b	1.0c	1.1d	1.2b	1.7c	1.8d	2.6d	3.0c	3.1d
SS + T[5]	1.7b	1.8c	2.9c	2.6b	2.9c	4.7c	7.8c	11.0b	14.4c

[1] Values represent percent disease averaged over 4 replicates; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Disease rated weekly over a 16-week period; data presented from weeks 1, 2 and all alternate weeks thereafter.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] SS = *S. sclerotiorum*.

[5] T = *T. flavus*; C = *C. minitans*.

Appendix 3. The effect of T. flavus and C. minitans on the development of sclerotinia wilt of sunflower over time at the Winnipeg field site (Experiment A, 1984).

	Disease incidence[1](%)								
Hyperpar.	25/6[2]	2/7	16/7	30/7	13/8	27/8	10/9	24/9	8/10
none	0.5a[3]	0.6a	0.6ab	0.6c	0.9bc	1.2c	1.2c	1.6c	1.8c
bran	0.3a	0.3a	0.3ab	0.3c	0.3c	0.3c	0.3c	1.1c	2.1c
SS[4]	0a	0.2a	2.8a	15.9a	33.8a	41.9a	53.3a	71.8a	77.2a
SS + bran	0.1a	0.2a	1.1ab	6.2b	19.9a	29.5a	37.2a	53.1a	59.9a
SS + C[5]	0a	0a	0b	0c	0c	0.1c	0.3c	0.5c	1.0c
SS + T[5]	0.3a	0.3a	0.6ab	1.9bc	6.4b	11.0b	15.7b	27.9b	33.4b

[1] Values represent percent disease averaged over 4 replicates; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Disease rated weekly over a 16-week period; data presented from weeks 1,2 and alternate weeks thereafter.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] SS = S. sclerotiorum.

[5] C = C. minitans; T = T. flavus.

Appendix 4. The effect of T. flavus and C. minitans on the development of sclerotinia wilt of sunflower over time at the Winnipeg field site (Experiment A, 1985).

	Disease incidence[1] (%)								
Treatment	1/7[2]	8/7	22/7	5/8	19/8	2/9	16/9	30/9	14/10
none	0a[3]	0a	0b	0.3b	0.5b	1.1b	1.3b	1.6b	1.7b
bran	0a	0a	0.1ab	0.1b	0.2b	0.9b	1.3b	2.2b	2.6b
SS[4]	0a	0.1a	1.6ab	6.4a	11.7a	14.7a	18.9a	23.6a	24.4a
SS + bran	0a	0.1a	2.0a	5.6a	14.4a	18.2a	21.2a	24.4a	26.5a
SS + C[5]	0a	0a	0b	0b	0.1b	0.6b	0.7b	0.8b	1.2b
SS + T[5]	0a	0a	1.2ab	5.3a	10.5a	14.2a	18.0a	20.1a	21.1a

[1] Values represent percent disease averaged over 4 replicates; analysis of variance on transformed data; means converted back to raw form for presentation.

[2] Disease rated weekly over a 16-week period; data presented from weeks 1,2 and alternate weeks thereafter.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] SS = S. sclerotiorum.

[5] C = C. minitans; T = T. flavus.

Appendix 5. Analysis of variance on the incidence of sclerotinia wilt of sunflower (Lethbridge, 1983; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		% disease (arcsin)		
Replication	3	35.3198	11.7733	0.25
Treatment	6	7142.7428	1190.4571	25.46**
Dummy	1	488.8834	488.8834	10.46**
Error	17	794.8425	46.7554	
Total	27	8129.3246		

** Significant at the 1% level.

Appendix 6. Analysis of variance on the incidence of sclerotinia wilt and seed yield of sunflower (Lethbridge, 1984; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		% disease (arcsin)		
Replication	3	123.1782	41.0594	0.72
Treatment	6	7017.1454	1169.5242	20.61**
Dummy	1	583.2830	583.2830	10.28**
Error	17	964.8864	56.7580	
Total	27	8686.1407		
		Yield		
Replication	3	2637427.5943	879142.5313	10.42**
Treatment	6	1136262.7454	189377.1242	2.25
Error	18	1517964.5291	84331.3627	
Total	27	5291654.8688		

** Significant at the 1% level.

Appendix 7. Analysis of variance on the incidence of sclerotinia wilt and seed yield of sunflower (Lethbridge, 1985; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		% disease (arcsin)		
Replication	3	224.0801	74.6934	1.31
Treatment	6	4984.6013	830.7669	14.62**
Dummy	1	400.2144	400.2144	7.04*
Error	17	966.2257	56.8368	
Total	27	7046.1305		
		Yield		
Replication	3	2737322.8162	912440.9387	2.64
Treatment	6	8259024.7253	1376504.1210	3.99*
Error	18	6214711.2091	345261.7338	
Total	27	17211058.7507		

*Significant at the 5% level.
**Significant at the 1% level.

Appendix 8. Analysis of variance on the incidence of sclerotinia wilt and seed yield of sunflower (Lethbridge, 1986; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		% disease (arcsin)		
Replication	3	384.7604	128.2535	2.34
Treatment	6	9474.8287	1579.1381	28.76**
Dummy	1	1487.3451	1487.3451	27.09**
Error	17	933.4050	54.9062	
Total	27	12123.9165		
		Yield		
Replication	3	24498.2068	8166.0689	0.07
Treatment	6	9060785.2496	1510130.8750	12.58**
Error	18	2160104.7574	120005.8199	
Total	27	11245388.2138		

** Significant at the 1% level.

Appendix 9. Analysis of variance on the incidence of sclerotinia wilt of sunflower (Winnipeg, 1983; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		% disease (arcsin)		
Replication	3	113.11048	37.70349	2.39
Treatment	5	9671.21313	1934.24263	122.54**
Error	15	236.75937	15.78396	
Total	23	10021.08298		

** Significant at the 1% level.

Appendix 10. Analysis of variance on the incidence of sclerotinia wilt and seed yield of sunflower (Winnipeg, 1984; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		% disease (arcsin)		
Replication	3	258.75711	86.25237	1.47
Treatment	5	11977.72914	2395.54583	40.93**
Error	15	877.88341	58.52556	
Total	23	13114.36966		
		Yield		
Replication	3	280413.53962	93471.17700	0.79
Treatment	5	7206953.69130	1441390.70000	12.12**
Error	15	1783303.78073	118886.91872	
Total	23	9270671.01165		

** Significant at the 1% level.

Appendix 11. Analysis of variance on the incidence of sclerotinia wilt and seed yield of sunflower (Winnipeg, 1985; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		% disease (arcsin)		
Replication	3	835.07775	278.35926	3.63*
Treatment	5	2854.43592	570.88718	7.45**
Error	15	1148.70613	76.58041	
Total	23	4838.21979		
		Yield		
Replication	3	606527.98264	202175.99000	3.04
Treatment	5	333112.04187	66622.40800	1.00
Error	15	997495.95858	66499.73057	
Total	23	1937135.98309		

*Significant at the 5% level.
**Significant at the 1% level.

Appendix 12. Analysis of variance on the incidence of sclerotinia wilt of sunflower (Lethbridge, 1984; Experiment B).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		% disease (arcsin)		
Replication	3	164.37698	54.79233	3.71*
Treatment	7	186.75899	26.67985	1.81
Error	21	309.98984	14.76142	
Total	31	661.12582		

*Significant at the 5% level.

Appendix 13. Analysis of variance on the incidence of sclerotinia wilt of sunflower (Lethbridge, 1985; Experiment B).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		% disease (arcsin)		
Replication	3	182.90094	60.96698	2.14
Treatment	15	5539.27389	369.28493	12.94**
Error	45	1284.12213	28.53605	
Total	63	7006.29697		

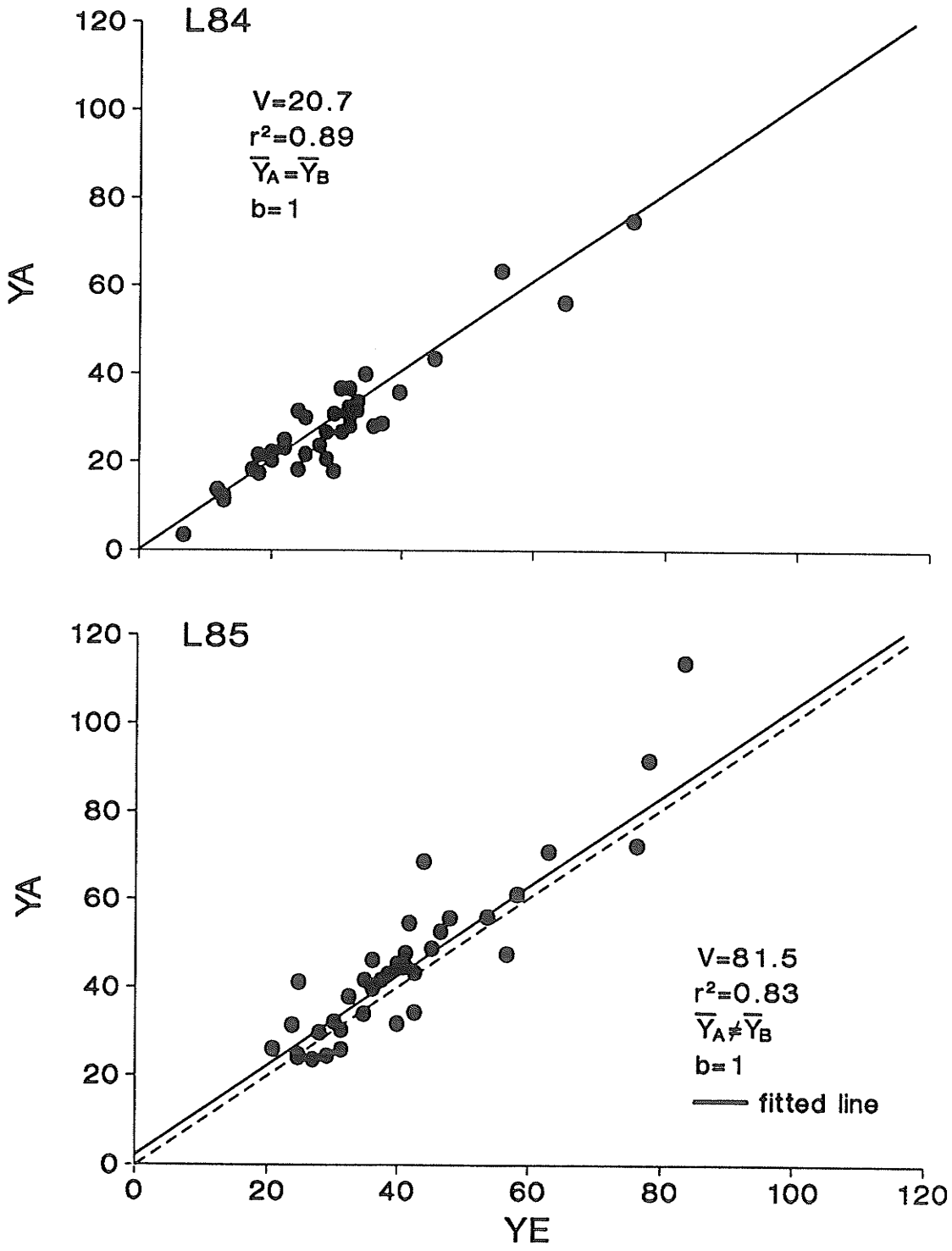
**Significant at the 1% level.

Appendix 14. Analysis of variance on the inoculum density[1] of S. sclerotiorum at the Lethbridge (Experiment A and B) and Winnipeg field sites in 1984.

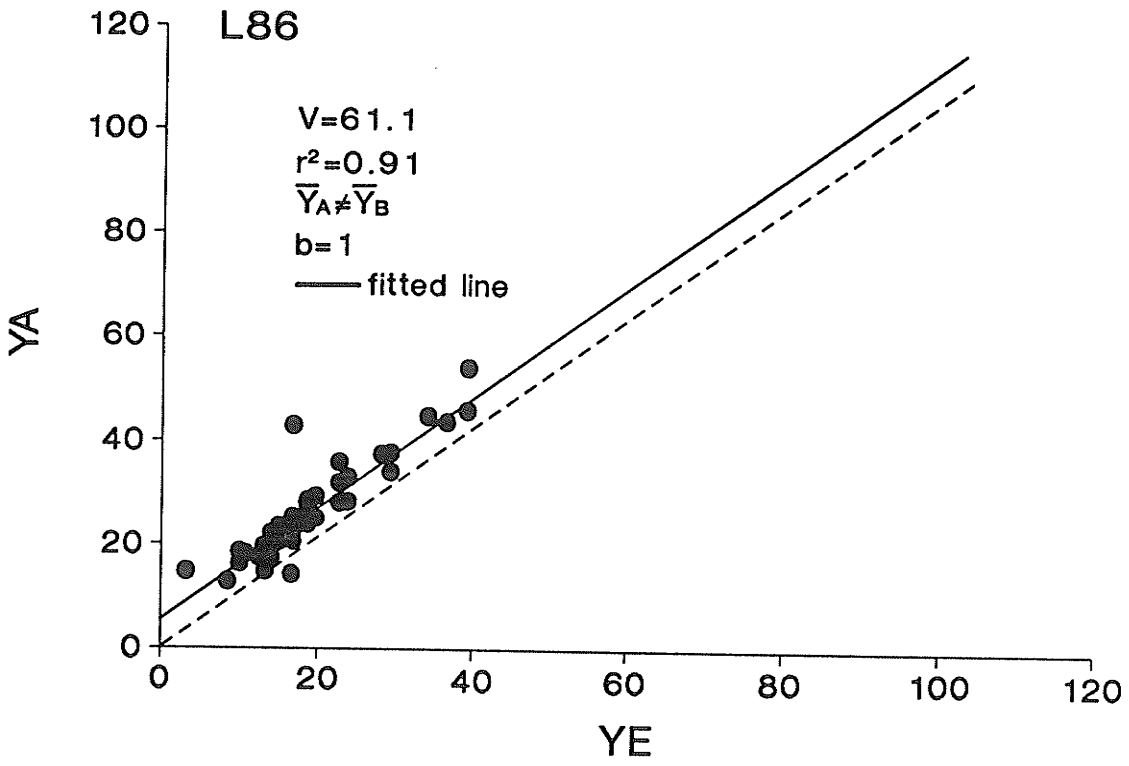
Source of variation	D.F.	Sum of squares	Mean squares	F value
Lethbridge (Experiment A)				
Replication	3	0.00047	0.00016	0.31
Treatment	6	0.00185	0.00031	0.60
Error	18	0.00921	0.00051	
Total	27	0.01154		
Lethbridge (Experiment B)				
Replication	3	0.00119	0.00040	0.60
Treatment	6	0.00189	0.00032	0.48
Error	18	0.01190	0.00066	
Total	27	0.01498		
Winnipeg (Experiment A)				
Replication	3	0.00165	0.00055	0.45
Treatment	5	0.00161	0.00032	0.26
Error	15	0.01838	0.00123	
Total	23	0.02164		

[1] Square root transformation used [SQRT(X+0.5)].

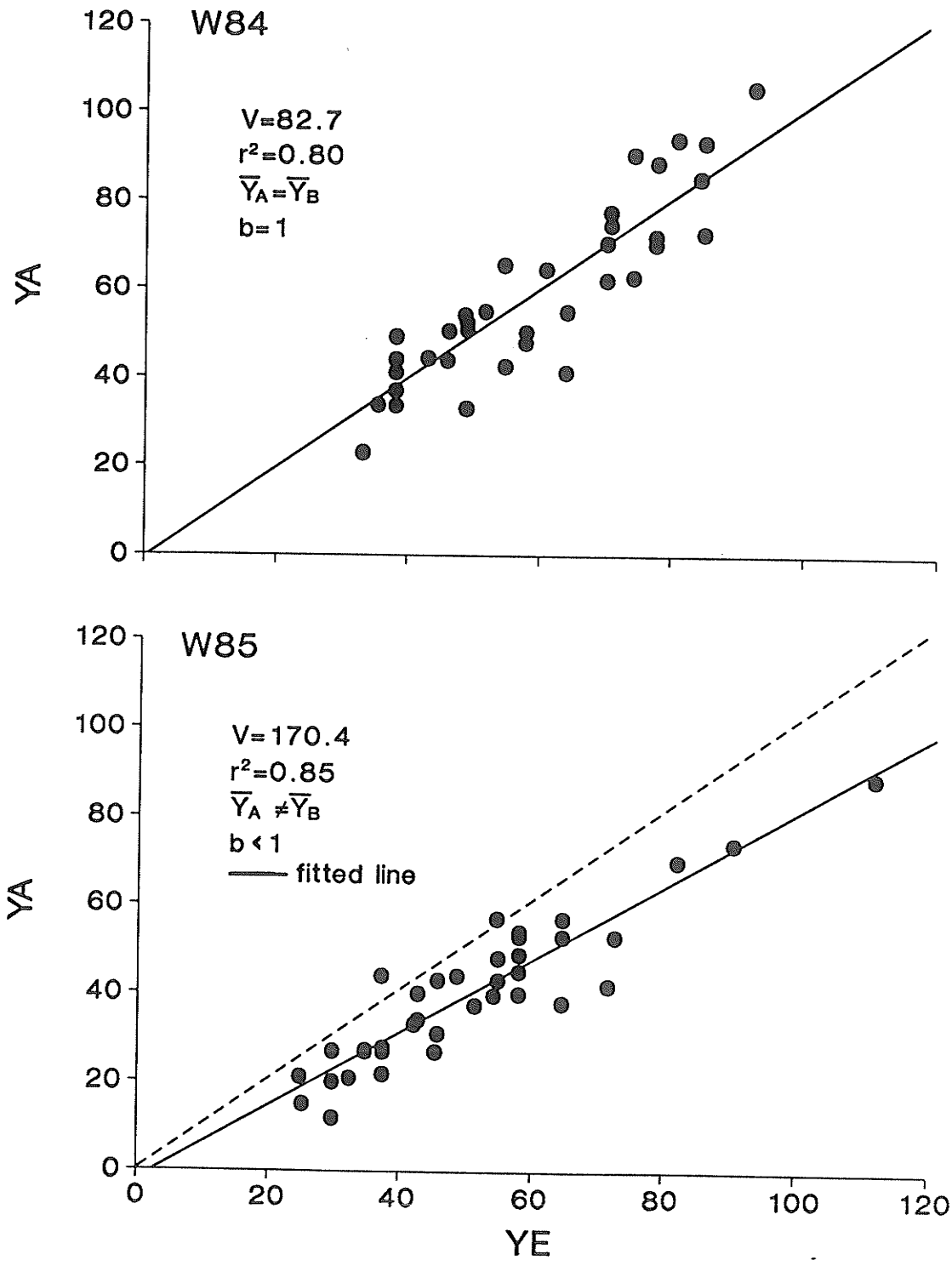
Appendix 15 Regression of actual yield (YA) on expected yield (YE) for Lethbridge 1984 (L84) and 1985 (L85).



Appendix 16 Regression of actual yield (YA) on expected yield (YE) for Lethbridge 1986 (L86).



Appendix 17 Regression of actual yield (YA) on expected yield (YE) for Winnipeg 1984 (W84) and 1985 (W85).



Appendix 18. Analysis of variance on the effect of hyperparasites and sampling date on the sclerotia of S. sclerotiorum buried in a sunflower field (Lethbridge, 1983; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	34751.80000	11583.93333	1.85
Hyperparasite (T)	4	135128.43333	33782.10833	5.40*
Error a (R x T)	12	75075.70000	6256.30833	1.85
Sampling date (S)	2	864033.43333	432016.71670	127.68**
T x S	8	62487.56667	6810.94583	2.31*
Error b	30	101505.00000	3383.50000	
Total	59	1272981.93333		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	3.48742	1.16247	2.93
Hyperparasite (T)	4	36.71888	9.17972	23.14**
Error a (R x T)	12	4.76137	0.39678	0.34
Sampling date (S)	2	26.03209	13.01605	11.20**
T x S	8	9.82932	1.22867	1.06
Error b	30	34.86636	1.16221	
Total	59	115.69545		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	1.67121	0.55707	1.36
Hyperparasite (T)	4	1.87113	0.46778	1.14
Error a (R x T)	12	4.91047	0.40921	0.93
Sampling date (S)	2	2.48985	1.24493	2.84
T x S	8	3.74227	0.46778	1.07
Error b	30	13.16337	0.43878	
Total	59	27.84831		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	14.09974	4.69991	1.34
Hyperparasite (T)	4	38.16919	9.54229	2.71
Error a (R x T)	12	42.21340	3.51778	2.16*
Sampling date (S)	2	87.29357	43.64679	26.80**
T x S	8	43.08886	5.38611	3.71**
Error b	30	48.85891	1.62863	
Total	59	273.72367		

*Significant at the 5% level.
**Significant at the 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 19. Effect of T. flavus and C. minitans on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field (Lethbridge, 1983; Experiment A).

Hyperparasite	Number of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
Control (SS)	16.8[2]a[3]	8.8[2]a	10.6[2]a	0.0[2]a
SS + bran	16.1 a	1.3 b	9.6 a	0.0 a
SS + T. flavus	13.1 b	0.5 b	12.3 a	0.7 a
SS + C/T[4] 1:1	13.0 b	0.9 b	13.3 a	0.0 a
SS + C. minitans	15.9 a	0.5 b	1.8 a	0.6 a

[1] Design is split-plot with 5 main plots (hyperparasites) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected sunflower crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] T = T. flavus; C = C. minitans.

Appendix 20. Effect of sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum buried in a sunflower field (Lethbridge, 1983; Experiment A).

Weeks after burial	No. of sclerotia[1]			
	Recovered	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
4	19.9[2]a[3]	5.5[2]a	11.3[2]a	0.8[2]a
8	11.6 b	0.7 b	18.1 a	0.0 a
12	12.2 b	0.5 b	1.6 b	0.0 a

[1] Design is split-plot with 5 main plots (hyperparasites) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected sunflower crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 21. Analysis of variance on the effect of hyperparasites and sampling date on the sclerotia of S. sclerotiorum buried in a sunflower field (Lethbridge, 1984; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	8993.23750	2997.74583	1.36
Hyperparasite (T)	4	34589.42500	8647.35625	3.93*
Error a (R x T)	12	26411.07500	2200.92292	1.07
Sampling date (S)	3	1333877.03750	444625.67900	215.65**
T x S	12	91101.77500	7591.81458	3.68**
Error b	45	92779.43750	2061.76528	
Total	79	1587751.98750		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	0.51194	0.17065	0.11
Hyperparasite (T)	4	21.50794	5.37699	3.45*
Error a (R x T)	12	18.72241	1.56020	1.67
Sampling date (S)	3	5.79164	1.93055	2.07
T x S	12	18.38318	1.53193	1.64
Error b	45	42.05556	0.95679	
Total	79	106.97268		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	1.74062	0.58021	1.18
Hyperparasite (T)	4	1.93938	0.48485	0.98
Error a (R x T)	12	5.92260	0.49355	0.82
Sampling date (S)	3	2.37379	0.79126	1.31
T x S	12	5.28943	0.44079	0.73
Error b	45	27.17463	0.60388	
Total	79	44.44046		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	2.80599	0.93533	1.54
Hyperparasite (T)	4	6.90325	1.72581	2.84
Error a (R x T)	12	7.27982	0.60665	1.36
Sampling date (S)	3	1.48924	0.49641	1.11
T x S	12	7.09516	0.59126	1.32
Error b	45	20.10161	0.44670	
Total	79	45.67506		

*, **Significant at the 5% and 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 22. Effect of T. flavus and C. minitans on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field (Lethbridge, 1984; Experiment A).

Hyperparasite	Number of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
Control (SS)	17.7[2]a[3]	4.0[2]a	0.2[2]a	0.3[2]a
SS + bran	17.4 ab	1.0 b	0.3 a	0.0 a
SS + T. flavus	16.4 bc	0.2 b	2.0 a	0.0 a
SS + C/T[4] 1:1	16.2 c	0.2 b	0.2 a	0.3 a
SS + C. minitans	16.3 c	0.6 b	0.2 a	0.8 a

[1] Design is split-plot with 5 main plots (hyperparasites) and 4 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected bean crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] T = T. flavus; C = C. minitans.

Appendix 23. Effect of sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field (Lethbridge, 1984; Experiment A).

Weeks after burial	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
5	20.0[2]a[3]	2.2[2]a	0.6[2]a	0.0[2]a
10	19.1 ab	0.3 a	0.3 a	0.0 a
15	17.6 b	0.8 a	0.9 a	0.4 a
50	8.1 c	1.1 a	0.2 a	0.8 a

[1] Design is split-plot with 5 main plots (hyperparasites) and 4 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected bean crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 24. Analysis of variance on the effect of hyperparasites and sampling date on the sclerotia of S. sclerotiorum buried in a sunflower field (Lethbridge, 1985; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	864.05000	288.01667	0.95
Hyperparasite (T)	4	828.50000	207.12500	0.69
Error a (R x T)	12	3620.70000	301.72500	0.87
Sampling date (S)	2	577.60000	288.80000	0.83
T x S	8	3564.90000	445.61250	1.28
Error b	30	10451.50000	348.38333	
Total	59	19907.25000		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	3.31516	1.10505	0.93
Hyperparasite (T)	4	12.22680	3.05670	2.57
Error a (R x T)	12	14.28344	1.19029	2.38*
Sampling date (S)	2	200.88033	100.44017	200.53**
T x S	8	17.03919	2.12989	4.25**
Error b	30	15.02654	0.50088	
Total	9	262.77145		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	3.40597	1.13532	1.20
Hyperparasite (T)	4	3.41199	0.85299	0.90
Error a (R x T)	12	11.37169	0.94764	1.68
Sampling date (S)	2	0.02403	0.01202	0.02
T x S	8	6.50480	0.81310	1.44
Error b	30	16.96535	0.56551	
Total	9	41.68383		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	4.79458	1.59819	4.22*
Hyperparasite (T)	4	9.63865	2.40966	6.36**
Error a (R x T)	12	4.54629	0.37886	0.37
Sampling date (S)	2	121.00154	60.50077	58.34**
T x S	8	13.73899	1.71737	1.66
Error b	30	31.11363	1.03712	
Total	59	184.83368		

*Significant at the 5% level.
**Significant at the 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 25. Effect of *T. flavus* and *C. minitans* on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field (Lethbridge, 1985; Experiment A).

Hyperparasites	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
Control (SS)	19.8[2]a[3]	10.3[2]a	3.5[2]a	0.8[2]a
SS + bran	20.0 a	8.6 a	4.7	0.5 a
SS + *T. flavus*	19.8 a	4.5 a	4.1 a	0.8 a
SS + C/T[4] 1:1	19.9 a	3.9 a	0.8 b	0.9 a
SS + *C. minitans*	19.8 a	6.1 a	2.9 a	2.3 a

[1] Design is split-plot with 5 main plots (hyperparasites) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were produced on homogenized beans.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] T = *T. flavus*; C = *C. minitans*.

Appendix 26. Effect of sampling date (weeks after burial) on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field (Lethbridge, 1985; Experiment A).

Weeks after burial	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
4	19.8[2]a[3]	26.7[2]a	0.3[2]b	1.0[2]a
8	19.9 a	0.8 b	14.6 a	1.1 a
12	19.9 a	2.0 b	0.3 b	0.9 a

[1] Design is split-plot with 5 main plots (hyperparasites) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were produced on homogenized beans.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 27. Analysis of variance on the effect of hyperparasites and sampling date on the sclerotia of S. sclerotiorum buried in a sunflower field (Lethbridge, 1986; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	27656.98333	9218.99444	2.10
Hyperparasite (T)	4	42402.83333	10600.70833	2.42
Error a (R x T)	12	52667.43333	4388.95278	0.79
Sampling date (S)	2	187962.23333	93981.11665	16.92**
T x S	8	86382.26667	10797.78333	1.94
Error b	30	166630.83333	5554.36111	
Total	59	563702.58333		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	1.38562	0.46187	2.38
Hyperparasite (T)	4	2.31482	0.57871	2.98
Error a (R x T)	12	2.33101	0.19425	0.46
Sampling date (S)	2	1.31339	0.65669	1.57
T x S	8	2.94153	0.36769	0.88
Error b	30	12.56881	0.41896	
Total	59	22.85519		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	2.51989	0.83996	1.00
Hyperparasite (T)	4	8.38718	2.09679	2.50
Error a (R x T)	12	10.04873	0.83739	1.11
Sampling date (S)	2	5.35628	2.67814	3.56*
T x S	8	5.70023	0.71253	0.95
Error b	30	22.57608	0.75254	
Total	59	54.58839		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	1.68887	0.56296	0.57
Hyperparasite (T)	4	9.94473	2.48618	2.54
Error a (R x T)	12	11.76188	0198016	0.84
Sampling date (S)	2	2.15716	1.07858	0.92
T x S	8	8.11984	1.01498	0.87
Error b	30	35.14535	1.17151	
Total	59	68.81783		

*Significant at the 5% level.
**Significant at the 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 28. Effect of *T. flavus* and *C. minitans* on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field (Lethbridge, 1986; Experiment A).

Hyperparasite	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
Control (SS)	16.5[2]b[3]	1.2[2]a	0.2[2]a	0.0[2]a
SS + bran	17.6 ab	0.2 a	1.0 a	0.0 a
SS + *T. flavus*	17.5 ab	0.2 a	2.6 a	0.2 a
SS + C/T[4] 1:1	18.8 a	0.2 a	2.0 a	1.8 a
SS + *C. minitans*	17.1 b	0.0 a	0.0 a	1.7 a

[1] Design is split-plot with 5 main plots (hyperparasites) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected sunflower crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] T = *T. flavus*; C = *C. minitans*.

Appendix 29. Effect of sampling date (weeks after burial) on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field (Lethbridge, 1986; Experiment A).

Weeks after burial	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
5	19.4[2]a[3]	0.1[2]a	0.9[2]a	0.0[2]b
10	17.4 ab	0.7 a	1.6 a	0.5 ab
15	15.5 b	0.2 a	0.5 a	1.6 a

[1] Design is split-plot with 5 main plots (hyperparasite) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate.

[2] Sclerotia obtained from an infected sunflower crop.

[3] Analysis of variance on transformed data; means converted back to raw form for presentation. Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 30. Analysis of variance on the effect of hyperparasites and sampling date on the sclerotia of S. sclerotiorum buried in a sunflower field (Winnipeg, 1983; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	14030.56250	4676.85417	6.38*
Hyperparasite (T)	3	286744.56250	95581.52083	130.46**
Error a (R x T)	9	6593.68750	732.63194	0.47
Sampling date (S)	2	594463.04167	297231.52080	189.90**
T x S	6	144691.12500	24115.18750	15.41**
Error b	24	37564.50000	1565.18752	
Total	47	1084087.47916		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	9.22866	3.07622	1.53
Hyperparasite (T)	3	23.41386	7.80462	3.87*
Error a (R x T)	9	18.15268	2.01696	1.22
Sampling date (S)	2	5.80698	2.90349	1.75
T x S	6	9.23384	1.53897	0.93
Error b	24	39.74324	1.65597	
Total	47	105.57927		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	1.80922	0.60307	1.00
Hyperparasite (T)	3	5.42047	1.80682	3.00
Error a (R x T)	9	5.42765	0.60307	1.58
Sampling date (S)	2	0.00457	0.00229	0.01
T x S	6	0.01370	0.00228	0.01
Error b	24	9.13306	0.38054	
Total	47	21.80866		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	5.60084	1.86695	0.98
Hyperparasite (T)	3	98.77132	32.92377	17.26**
Error a (R x T)	9	17.16954	1.90773	0.81
Sampling date (S)	2	2.85785	1.42892	0.61
T x S	6	7.69665	1.28278	0.55
Error b	24	56.36927	2.34872	
Total	47	188.46547		

*Significant at the 5% level.
**Significant at the 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 31. Effect of *T. flavus* and *C. minitans* on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field (Winnipeg, 1983; Experiment A).

Hyperparasite	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
Control (SS)	18.5[2]a[3]	7.2[2]a	0.5[2]b	0.0[2]a
SS + bran	17.5 b	6.2 a	0.8 b	0.0 a
SS + *T. flavus*	13.5 c	2.2 ab	18.5 a	0.0 a
SS + *C. minitans*	12.8 c	0.6 b	0.6 b	1.7 a

[1] Values averaged over 4 replicates and 3 sampling dates; based on 20 sclerotia per replicate; sclerotia obtained from an infected bean crop.
[2] Analysis of variance on transformed data; means converted back to raw form for presentation.
[3] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 32. Effect of sampling date (weeks after burial) on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field (Winnipeg, 1983; Experiment A).

Weeks after burial	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
4	20.0[2]a[3]	5.7[2]a	3.8[2]a	0.3[2]a
8	14.5 b	2.3 a	3.7 a	0.3 a
12	11.7 b	3.1 a	1.9 a	0.3 a

[1] Design is split-plot with 4 main plots (hyperparasite) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected sunflower crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 33. Analysis of variance on the effect of hyperparasites and sampling date on the sclerotia of S. sclerotiorum buried in a sunflower field (Winnipeg, 1984; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	18967.08333	6322.36111	2.85
Hyperparasite (T)	3	52403.08333	17467.69444	7.86**
Error a (R x T)	9	19991.08333	2221.23148	0.84
Sampling date (S)	2	91088.37500	45544.18750	17.19**
T x S	6	63747.79167	10624.63195	4.01**
Error b	24	63587.83333	2649.49306	
Total	47	309785.25000		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	0.98039	0.32679	0.20
Hyperparasite (T)	3	43.17892	14.39297	8.75**
Error a (R x T)	9	14.80589	1.64510	0.82
Sampling date (S)	2	0.72315	0.36158	0.18
T x S	6	7.31509	1.21918	0.61
Error b	24	48.06039	2.00252	
Total	47	115.06384		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	1.93961	0.64654	0.80
Hyperparasite (T)	3	7.97611	2.65870	3.31
Error a (R x T)	9	7.23426	0.80380	1.95
Sampling date (S)	2	0.03087	0.01544	0.04
T x S	6	1.22208	0.20368	0.49
Error b	24	9.89549	0.41231	
Total	47	28.29843		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	2.15498	0.71833	0.85
Hyperparasite (T)	3	5.93967	1.97989	2.33
Error a (R x T)	9	7.64208	0.84912	2.62*
Sampling date (S)	2	0.48085	0.24043	0.74
T x S	6	0.66324	0.11054	0.34
Error b	24	7.79207	0.32467	
Total	47	24.67289		

*Significant at the 5% level.
**Significant at the 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 34. Effect of *T. flavus* and *C. minitans* on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field (Winnipeg, 1984; Experiment A).

Hyperparasite	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
Control (SS)	19.5[2]a[3]	8.5[2]a	0.0[2]a	0.0[2]a
SS + bran	19.3 a	7.7 a	1.1 a	0.2 a
SS + *T. flavus*	17.8 b	1.0 b	1.8 a	0.0 a
SS + *C. minitans*	17.5 b	0.3 b	0.0 a	2.3 a

[1] Design is split-plot with 4 main plots (hyperparasite) and 3 subplots (sampling dates). Values were averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected bean crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 35. Effect of sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field (Winnipeg, 1984; Experiment A).

Weeks after burial	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
4	20.0[2]a[3]	4.2[2]a	0.9[2]a	0.4[2]a
8	18.4 ab	3.3 a	0.6 a	0.4 a
12	17.1 b	3.0 a	0.4 a	0.5 a

[1] Design is split-plot with 4 main plots (hyperparasite) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected bean crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 36. Analysis of variance on the effect of hyperparasites and sampling date on the sclerotia of S. sclerotiorum buried in a sunflower field (Winnipeg, 1985; Experiment A).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	14185.06250	4728.35417	0.91
Hyperparasite (T)	3	174409.22917	58136.40970	11.14**
Error a (R x T)	9	46983.35417	5220.37269	0.73
Sampling date (S)	2	186968.66667	93484.33330	13.07**
T x S	6	21701.33333	3616.88889	0.51
Error b	24	171717.33333	7154.88889	
Total	47	615964.97917		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	3.42630	1.14210	0.56
Hyperparasite (T)	3	13.18978	4.39659	2.17
Error a (R x T)	9	18.24939	2.02771	0.99
Sampling date (S)	2	6.51559	3.25779	1.59
T x S	6	5.09228	0.84871	0.42
Error b	24	49.03501	2.04313	
Total	47	95.50886		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	2.27543	0.75848	4.66*
Hyperparasite (T)	3	1.05628	0.35209	2.16
Error a (R x T)	9	1.46588	0.16288	0.34
Sampling date (S)	2	0.01375	0.00688	0.01
T x S	6	2.02598	0.33766	0.70
Error b	24	11.57291	0.48220	
Total	47	18.41022		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	2.76419	0.92139	1.02
Hyperparasite (T)	3	17.09672	5.69891	6.32*
Error a (R x T)	9	8.11956	0.90217	0.99
Sampling date (S)	2	1.68266	0.84133	0.92
T x S	6	5.30487	0.88415	0.97
Error b	24	21.95121	0.91463	
Total	47	56.91921		

*Significant at the 5% level.
**Significant at the 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 37. Effect of *T. flavus* and *C. minitans* on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field (Winnipeg, 1985; Experiment A).

Hyperparasite	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
Control (SS)	17.7[2]a[3]	8.0[2]a	0.3[2]b	0.2[2]a
SS + bran	17.8 a	6.4 a	1.1 b	0.0 a
SS + *T. flavus*	17.8 a	3.5 a	5.3 a	0.5 a
SS + *C. minitans*	13.2 b	2.0 a	0.5 b	0.7 a

[1] Design is split-plot with 4 main plots (hyperparasite) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were produced on homogenized beans.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 38. Effect of sampling date (weeks after burial) on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field (Winnipeg, 1985; Experiment A).

Weeks after burial	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
4	18.9[2]a[3]	7.3[2]a	1.3[2]a	0.3[2]a
8	16.6 b	3.8 a	2.2 a	0.4 a
12	14.3 c	3.4 a	1.0 a	0.3 a

[1] Design is split-plot with 4 main treatments (hyperparasites) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were produced on homogenized beans.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 39. Microorganisms associated with sclerotia of S. sclerotiorum (SS) recovered from a bean (Lethbridge, 1984-85) and two sunflower fields (Lethbridge and Winnipeg 1983-84, Experiment A).

	% of recovered sclerotia infected[1,2]					
			Sunflower[3]			
	Bean[3]		Lethbridge		Winnipeg	
	84	85	83	84	83	84
Treatment						
Control (SS)	90.6	89.5	77.1	93.7	82.6	89.2
SS + bran	95.0	95.4	87.3	96.3	75.5	90.6
SS + T. flavus (T)	95.3	85.9	76.2	90.2	69.3	84.9
SS + C. minitans (C)	94.7	90.7	79.0	96.7	78.2	87.8
SS + C/T 1:1	96.5	83.1	75.5	95.2	-	-
SS + C/T 1:2	95.6	81.9	-[4]	-	-	-
SS + C/T 2:1	93.2	84.6	-	-	-	-
Microorganism						
Trichoderma spp.	18.1	26.9	20.4	17.9	9.5	13.5
Fusarium spp.	12.6	8.3	15.6	14.1	21.7	5.6
Aspergillus spp.	38.4	34.6	32.2	27.1	26.1	36.3
Other	26.4	27.5	10.8	35.3	19.2	32.7

[1] Microorganisms include species of Trichoderma, Fusarium, Aspergillus Mucor etc.
[2] Values represent percentage of total number of recovered sclerotia infected by microorganisms. All sclerotia were buried in the spring of each year.
[3] Values averaged over three sampling dates.
[4] - = treatments not included in experiment.

Appendix 40. Analysis of variance on the effect of hyperparasites, alone or in various combinations, and sampling date on the sclerotia of S. sclerotiorum buried in a sunflower field in the spring of 1984 (Lethbridge, Experiment B).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	92970.39286	30990.13095	4.96*
Hyperparasite (T)	6	192842.46429	32140.41070	5.14**
Error a (R x T)	18	112492.10714	6249.56151	1.19
Sampling date (S)	3	753717.60714	251239.20240	47.77**
T x S	18	206364.39286	11464.68849	2.18*
Error b	63	331365.00000	5259.76191	
Total	111	1689751.964286		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	2.53903	0.84634	0.45
Hyperparasite (T)	6	29.79583	4.96597	2.61*
Error a (R x T)	18	34.20336	1.90019	2.48**
Sampling date (S)	3	3.11125	1.03708	1.36
T x S	18	11.89793	0.66099	0.86
Error b	63	48.17598	0.76469	
Total	111	129.72338		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	0.31774	0.10591	0.37
Hyperparasite (T)	6	1.78101	0.29684	1.05
Error a (R x T)	18	5.11044	0.28391	0.82
Sampling date (S)	3	1.22509	0.40836	1.18
T x S	18	4.20309	0.23351	0.68
Error b	63	21.76404	0.34546	
Total	111	34.40142		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	1.55672	0.51891	0.96
Hyperparasite (T)	6	4.26376	0.71063	1.32
Error a (R x T)	18	9.69382	0.53855	0.89
Sampling date (S)	3	1.48873	0.49624	0.82
T x S	18	7.02138	0.39008	0.64
Error b	63	38.29646	0.60788	
Total	111	62.32087		

*Significant at the 5% level.
**Significant at the 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 41. Effect of *T. flavus* and *C. minitans* on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a sunflower field in May, 1984 (Lethbridge, Experiment B).

Hyperparasites	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
Control (SS)	15.6[2]a[3]	3.8[2]a	0.0[2]a	0.2[2]a
SS + bran	13.8 ab	2.2 ab	0.2 a	0.0 a
SS + *T. flavus*	11.0 c	0.0 b	0.8 a	0.0 a
SS + C/T[4] 1:2	11.8 bc	1.1 ab	0.4 a	0.3 a
SS + C/T 1:1	11.9 bc	0.0 b	0.0 a	0.0 a
SS + C/T 2:1	10.6 c	0.0 b	1.0 a	0.6 a
SS + *C. minitans*	14.2 a	0.0 b	0.0 a	0.5 a

[1] Design is split-plot with 7 main plots (hyperparasites) and 4 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected rapeseed crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] T = *T. flavus*; C = *C. minitans*.

Appendix 42. Effect of sampling date (weeks after burial) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field in May, 1984 (Lethbridge, Experiment B).

Weeks after burial	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
5	16.5[2]a[3]	0.4[2]a	0.1[2]a	0.0[2]a
10	14.1 b	0.7 a	0.1 a	0.2 a
15	11.3 c	0.6 a	0.5 a	0.1 a
50	7.2 d	1.4 a	0.6 a	0.5 a

[1] Design is split-plot with 7 main plots (hyperparasite) and 4 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected rapeseed crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 43. Analysis of variance on the effect of hyperparasites, alone or in various combinations, on the sclerotia of S. sclerotiorum buried in a sunflower field in the fall of 1984 (Lethbridge, Experiment B).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication	3	4683.28846	1561.09615	2.37
Hyperparasite	12	9130.57692	760.88141	1.15
Error	36	23758.96154	659.97115	
Total	51	37572.82692		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication	3	6.04129	2.01376	1.21
Hyperparasite	12	60.33197	5.02766	3.02**
Error	36	59.94783	1.66522	
Total	51	126.32109		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication	3	1.64804	0.54935	0.62
Hyperparasite	12	13.10100	1.09175	1.23
Error	36	32.05183	0.89033	
Total	51	46.80088		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication	3	1.23051	0.41017	0.50
Hyperparasite	12	29.56221	2.46352	2.98**
Error	36	29.73239	0.82589	
Total	51	60.52512		

** Significant at the 1% level.
[1] Represents percent of recovered sclerotia.

Appendix 44. Analysis of variance on the effect of hyperparasites, alone or in various combinations, and sampling date on the sclerotia of S. sclerotiorum buried in a sunflower field in the spring of 1985 (Lethbridge, Experiment B).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	17733.63690	5911.21230	2.43
Hyperparasite (T)	13	867785.07738	66752.69825	27.40**
Error a (R x T)	39	95019.61309	2436.40033	0.71
Sampling date (S)	2	512704.75000	256352.37500	75.11**
T x S	26	464062.08333	17848.54167	5.23**
Error b	84	286684.50000	3412.91071	
Total	167	2243989.66071		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	13.69347	4.56449	4.20*
Hyperparasite (T)	13	148.62663	11.43281	10.52**
Error a (R x T)	39	42.37168	1.08645	0.87
Sampling date (S)	2	60.59879	30.29940	24.13**
T x S	26	63.97384	2.46053	1.96*
Error b	84	105.49092	1.25584	
Total	167	434.75533		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	19.35929	6.45310	4.83**
Hyperparasite (T)	13	108.18279	8.32175	6.22**
Error a (R x T)	39	52.14500	1.33705	0.95
Sampling date (S)	2	13.08637	6.54319	4.67*
T x S	26	87.53297	3.36665	2.40**
Error b	84	117.69919	1.40118	
Total	167	398.00563		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	1.26512	0.42171	0.82
Hyperparasite (T)	13	22.33505	1.71808	3.33**
Error a (R x T)	39	20.12240	0.51596	0.67
Sampling date (S)	2	0.22452	0.11226	0.15
T x S	26	13.87073	0.53349	0.69
Error b	84	64.92129	0.77287	
Total	167	122.73912		

*Significant at the 5% level.
**Significant at the 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 45. Effect of hyperparasites and time of application (season) on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a sunflower field in 1985 and recovered during the same season (Lethbridge, Experiment B).

Hyperpara.	S	No. of sclerotia[1] Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
Control (SS)	SP	19.7[2]a[3]	11.7[2]a	0.0[2]c	0.8[2]def
	F	19.8 a	6.0 c	0.3 bc	0.2 f
SS + bran	SP	19.5 ab	5.8 c	0.6 bc	0.2 f
	F	19.7 a	10.9 a	0.2 c	0.0 f
SS + T	SP	17.7 de	0.2 e	2.6 a	0.3 ef
	F	19.6 a	0.5 e	1.7 ab	0.5 ef
SS + C/T[4] 1:2	SP	18.5 bcd	0.5 e	2.9 a	0.5 ef
	F	18.8 abc	0.2 e	1.1 abc	1.1 cde
SS + C/T 1:1	SP	14.7 f	0.4 e	0.8 bc	3.4 bcd
	F	19.3 ab	0.6 e	0.2 c	2.9 cde
SS + C/T 2:1	SP	13.9 f	0.2 e	0.7 bc	4.0 bc
	F	16.8 e	1.3 e	0.0 c	3.5 bcd
SS + C	SP	13.7 f	0.2 e	0.0 c	11.2 a
	F	17.9 cde	3.6 c	0.2 c	7.7 ab

1 Design is split-plot with 14 main plots (hyperparasite) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were produced on homogenized beans.

2 Analysis of variance on transformed data; means converted back to raw form for presentation.

3 Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

4 T = T. flavus; C = C. minitans.

Appendix 46. Effect of sampling date (weeks after burial) on the recovery and viability of sclerotia of *S. sclerotiorum* buried in a sunflower field in May, 1985 (Lethbridge, Experiment B).

Weeks after burial	No. of sclerotia[1]			
	Recov.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
4	19.7[2]a[3]	5.6[2]a	0.6[2]a	2.6[2]a
8	18.1 b	1.1 b	0.8 a	0.9 b
12	15.9 c	0.8 b	0.6 a	2.7 a

[1] Design is split-plot with 14 main plots (hyperparasite) and 3 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were produced on homogenized beans.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

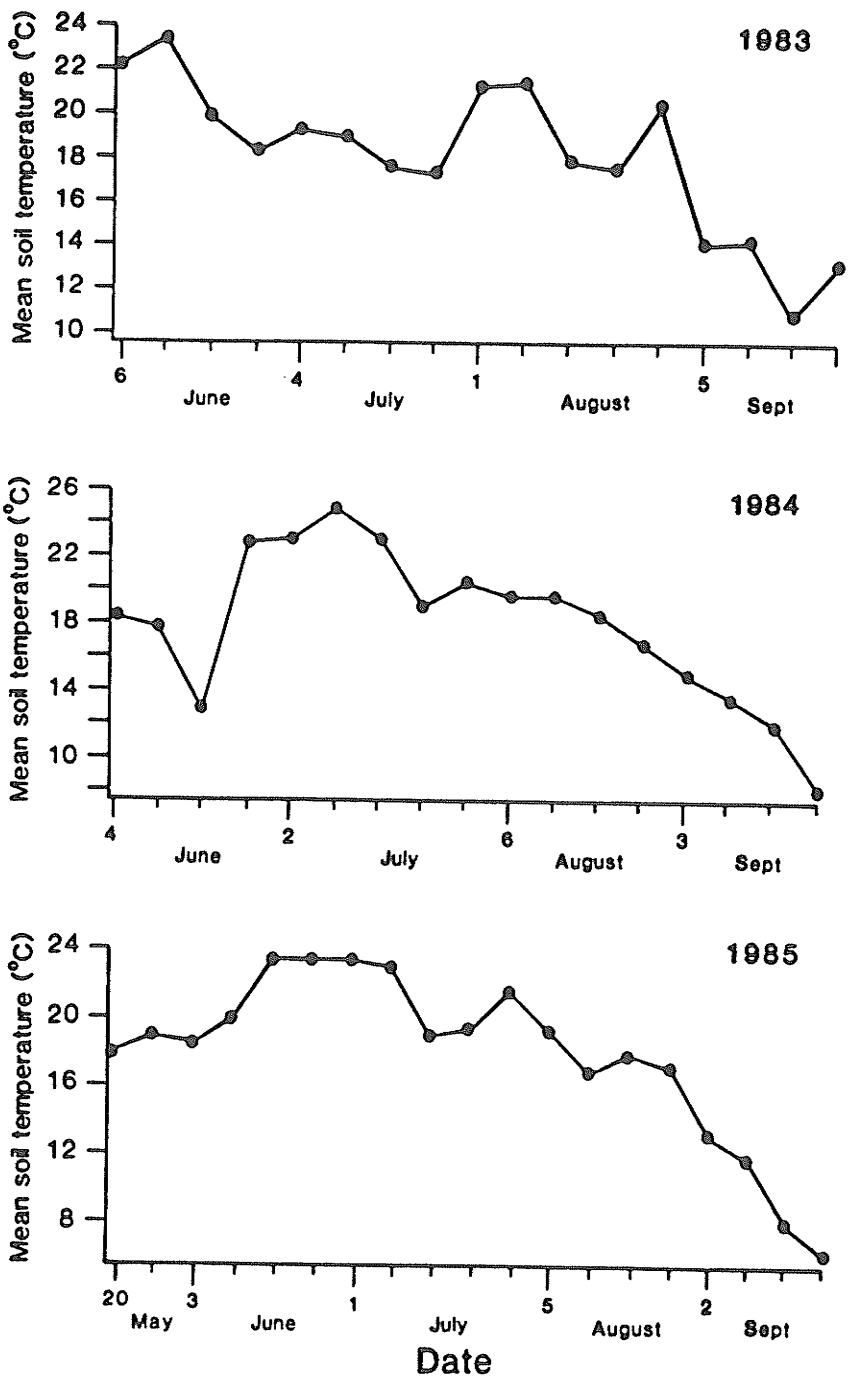
[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 47. Analysis of soil collected from the Lethbridge and Winnipeg field sites (1984).

Site[2]	pH	Cond.[1] mmhos/cm sat.	Avail. P (ppm)	NO_3-N (ppm)	NH_4-N (ppm)	Extractable K (meq./100 gm)
LRS	7.8	0.5	61.4	5.5	3.0	1.8
WPG	7.7	-	56.0	3.4	16.7	-

[1] Cond. = conductivity
[2] The two sites were located at the Lethbridge Research Station (LRS) Lethbridge, Alberta and the University of Manitoba, Winnipeg (WPG), Manitoba.

Appendix 48. Mean soil temperatures occurring in a sunflower field during the 1983-85 field seasons (Lethbridge).



Appendix 49. Growth stages of sunflower and moisture received at Lethbridge (Experiment A) during 1983-86.[1]

1983			1984		
Week of	Growth stage[2]	Moisture (mm)	Week of	Growth stage	Moisture (mm)
May 16	-	19.7	May 21	-	5.2
23	-	28.5	28	-	38.0
30	-	4.5			
June 6	-	2.6	June 4	-	24.8
13	-	4.1	11	-	1.9
20	1.3-2.1	45.3	18	1.3-2.1	26.3
27	2.2	14.6	25	2.1	0
July 4	2.3	14.7	July 2	2.2	0
11	3.1	14.4	9	2.3	38.2
18	3.2	30.0	16	3.1	0
25	3.3	0.4	23	3.2	43.3
			30	3.3	9.6
Aug. 1	3.4	0	Aug. 6	3.4	3.9
8	4.1-4.2	11.3	13	4.1-4.2	0
15	4.3	43.0	20	4.3	38.0
22	4.3	3.0	27	4.4-4.5	9.2
29	4.4	2.4			
Sept. 5	5.1	5.0	Sept. 3	5.1	41.6
12	5.2	8.6	10	5.1	2.8
19	5.3	0	17	5.2	42.5
			-	-	-

1985			1986		
Week of	Growth stage	Moisture (mm)	Week of	Growth stage	Moisture (mm)
May 20	-	11.1	May 19	-	15.8
27	-	2.8	26	-	0
June 3	-	0	June 2	-	22.2
10	1.3	38.6	9	2.1	5.6
17	2.1	1.4	16	2.4	4.2
24	2.3	0	23	2.4-3.1	36.7
			30	3.1	18.6
July 1	2.3-3.1	0	July 7	3.1	31.3
8	3.1	29.6	14	3.2-3.3	4.2
15	3.2	7.3	21	3.3	2.8
22	3.3	5.0	28	3.4	1.0
29	3.4-4.1	9.4			
Aug. 5	4.2-4.3	11.0	Aug. 4	4.2-4.3	33.9
12	4.4	34.7	11	4.5	2.0
19	4.5	36.3	18	5.1	0
26	4.5-5.1	0.5	25	5.1	10.6
Sept. 2	5.1-5.2	37.5	Sept. 1	5.2	8.0
9	5.2	60.6	8	5.2	18.1
16	5.2	5.4	15	5.2	21.7

[1] In 1983, 1984, 1985 and 1986 the crop was sown on May 23, 22, 15 and 12, respectively.

[2] Growth stages: 1 = establishment, 2 = vegetative, 3 = budding, 4 = anthesis and 5 = seed development. For detailed information on growth stages, please refer to Appendix 1.

Appendix 50. Soil moisture[1] (%) recorded in sunflower fields in 1984, 1985 and 1986 at Lethbridge.

1984				1985				1986	
Week of		A[2]	B[2]	Week of		A[2]	B[2]	Week of	A[2]
May	21	10.3	9.8	May	20	14.1	13.9	May 19	15.2
	28	19.4	18.5		27	9.8	11.1	26	10.1
June	4	15.4	14.7	June	3	9.8	8.9	June 2	14.0
	11	10.7	9.5		10	17.5	18.2	9	13.8
	18	13.7	16.2		17	13.2	12.9	16	13.5
	25	9.8	11.1		24	12.1	10.0	23	22.3
								30	13.8
July	2	8.2	8.9	July	1	10.4	9.3	July 7	16.4
	9	22.1	24.3		8	17.6	18.1	14	10.9
	16	7.4	7.3		15	14.0	15.1	21	9.3
	23	6.5	6.7		22	10.7	8.5	28	8.4
	30	14.4	14.5		29	11.9	8.7		
Aug.	6	12.8	11.5	Aug.	5	15.7	12.0	Aug. 4	20.2
	13	6.0	8.0		12	13.8	14.8	11	14.2
	20	23.4	8.5		19	22.6	17.0	18	9.3
	27	16.0	14.7		26	12.1	11.0	25	12.5
Sept.	3	22.9	21.7	Sept.	2	21.2	20.5	Sept. 1	11.9
	10	14.5	13.2		9	25.5	24.3	8	16.5
	17	23.1	24.1		16	15.9	14.7	15	16.9

[1] Readings were taken twice weekly at four sites within each experimental area; values for each date were averaged over the number of sites; a portable tensiometer was used to obtain the readings during 1984-85; in 1986, soil moisture was determined from soil samples collected from each site.

[2] A represents Experiment A (1983-86) and B represents Experiment B (1984-85); no soil moisture data is available from the 1983 season.

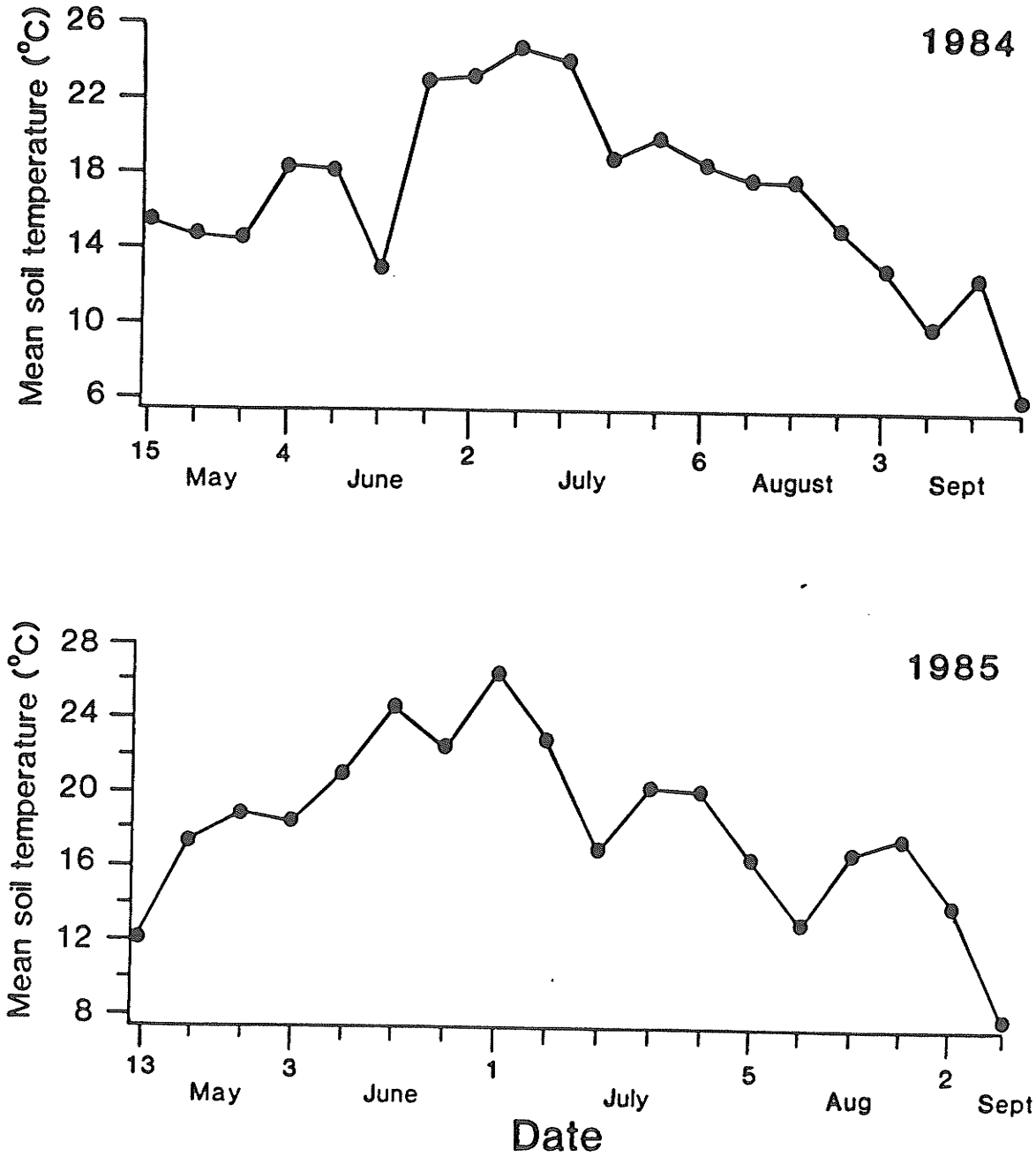
Appendix 51. Stages of vegetative and reproductive development in indeterminate (vining or trailing) dry beans (adapted from NDSU, 1981).

Stage no.	General description*	Days** from planting
	Vegetative stages	
V1	Completely unfolded leaves at the primary (unifoliate) leaf node.	10
V2	First node above primary leaf node. Count when leaf edges no longer touch.	19
V3	Three nodes on the main stem including the primary leaf node. Secondary branching begins to show from branch of V1.	29
V(n)	n nodes on the main stem, but with blossom clusters still not visibly opened.	A new node each 3 days
V8	Vine (indeterminate) plants may begin to exhibit blossom and become stage R1.	40
	Reproductive stages	
R1	One blossom open at any node. Tendril will begin to show.	40
R1	Pods 1/2 inch long at first blossom position. Node 2 to 5 most plants. Blossom would have just sluffed.	43
R3	Pods 1 inch long at first blossom position. Pods are showing at higher nodes when blossom sluffs. 1/2 bloom.	46
R4	Pods 2 inches long at first blossom position.	50
R5	Pods 3 plus inches long, seeds discernible by feel.	56
R6	Pods 4-5 inches long with spurs (maximum length). Seeds at least 1/4 inch in long axis.	60
R7	Oldest pods have fully developed green seeds. Other parts of plant will have full length pods with seeds near same size. Pods to the top and blossom on tendril, nodes 10-13.	70
R8	Leaves yellowing over half of plant, very few small new pods/blossom developing, small pods may be drying. Point of maximum production has been reached.	82
R9	Mature, at least 80% of the pods showing yellow and mostly ripe. Only 30% of leaves are still green.	94

* Growth stages according to Marshall J. Lebaron (University of Idaho, College of Agriculture, Current Information Series No. 228, April 1974).

** Approximate number of days. This will vary from season to season and variety to variety.

Appendix 52. Mean soil temperatures occurring in a bean field in 1984 and 1985 (Lethbridge).



Appendix 53. Growth stage of bean and soil moisture recorded in a bean field in 1984 and 1985 at Lethbridge.

1984				1985			
Week of		Growth stage[1]	Moisture (%)[2]	Week of		Growth stage	Moisture (%)
May	21	V1	16.1	May	20	-	13.6
	28	V1-V2	10.2		27	V1	10.9
June	4	V2	11.1	June	3	V1	9.4
	11	V3	13.2		10	V2	10.7
	18	V4	10.7		17	V3	17.2
	25	V5	9.1		24	V5	11.4
July	2	V6	12.6	July	1	V6	20.1
	9	R1-R3	12.1		8	R1	18.9
	16	R4	11.7		15	R3	24.5
	23	R5	9.7		22	R4	21.3
	30	R6	24.6		29	R5	24.6
Aug.	6	R6	17.5	Aug.	5	R6	23.2
	13	R7	18.7		12	R7	21.9
	20	R8	18.9		19	R8	23.6
	27	R9	23.6		26	R8	28.0
Sept.	3	-	19.2	Sept.	2	R9	24.1
	10	-	20.4		9	-	23.7
	17	-	19.8		16	-	25.4

[1] Growth stages: V = vegetation, R - reproductive. For detailed information on growth stages, please refer to Appendix 44a.

[2] Readings were taken twice weekly at four sites within each experimental area; values for each date were averaged over the number of sites; a portable tensiometer was used to obtain the readings.

Appendix 54. Analysis of variance on the effect of *T. flavus* and *C. minitans* on the carpogenic germination of sclerotia of *S. sclerotiorum* buried in a bean field in June, 1984 (Lethbridge).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Number of apothecia log(x + 10)		
Replication	3	0.70083	0.23361	0.49
Hyperparasite	6	17.33185	2.88864	6.10**
Error	18	8.52049	0.47336	
Total	27	26.55316		
		Number of germinable sclerotia log(x + 10)		
Replication	3	0.21568	0.07189	0.41
Hyperparasite	6	9.73954	1.62326	9.21**
Error	18	3.17269	0.17626	
Total	27	13.12791		
		% germination (arcsin)		
Replication	3	8.71586	2.90529	0.41
Hyperparasite	6	388.72989	64.78832	9.13**
Error	18	127.73057	7.09614	
Total	27	525.17631		
		Number of apothecia/sclerotia log(x + 10)		
Replication	3	0.00423	0.00141	0.23
Hyperparasite	6	0.01809	0.00302	0.49
Error	18	0.11117	0.00618	
Total	27	0.13348		

** Significant at the 1% level.

Appendix 55. Analysis of variance on the effect of hyperparasites and time of burial on the carpogenic germination of sclerotia of S. sclerotiorum buried in a bean field in November, 1984 and in May, 1985 (Lethbridge).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Number of apothecia log(x +10)		
Replication	3	3.27107	1.09035	7.39**
Hyperparasite (T)	6	126.27962	21.04660	142.73**
Error a (R x T)	18	2.65424	0.14746	0.92
Subplot	3	9.57443	3.19148	19.97**
T x sub	18	3.83201	0.21289	1.33
Error b	63	10.06740	0.15980	
Total	111	155.67878		
		Number of germinable sclerotia log(x + 10)		
Replication	3	1.24952	0.41651	6.06**
Hyperparasite (T)	6	61.86269	10.31045	149.93**
Error a (R x T)	18	1.23783	0.06877	0.79
Subplot	3	5.86576	1.95525	22.47**
T x sub	18	2.66747	0.14819	1.70
Error b	63	5.48195	0.08702	
Total	111	78.36523		
		% germination (arcsin)		
Replication	3	235.43320	78.47773	6.01**
Hyperparasite (T)	6	10650.07170	1775.01180	135.86**
Error a (R x T)	18	235.16252	13.06458	0.79
Subplot	3	990.24215	330.08072	19.87**
T x sub	18	415.05050	23.05836	1.39
Error b	63	1046.38896	16.60935	
Total	111	13572.34899		
		Number of apothecia/sclerotia log(x + 10)		
Replication	3	0.01778	0.00593	0.76
Hyperparasite (T)	6	0.20966	0.03494	4.50**
Error a (R x T)	18	0.13992	0.00777	1.44
Subplot	3	0.00580	0.00193	0.36
T x sub	18	0.18162	0.01009	1.86
Error b	63	0.34088	0.00541	
Total	111	0.89567		

*Significant at the 5% level.
**Significant at the 1% level.

Appendix 56. Analysis of variance on the effect of hyperparasites and sampling date on the sclerotia of S. sclerotiorum buried in a bean field in June, 1984 (Lethbridge).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication	3	26961.8214	8987.2738	1.58
Hyperparasite (T)	6	496219.3571	82703.2262	14.53**
Error a (R x T)	18	102435.9286	5690.8849	1.87*
Sampling date	3	1029950.6786	343316.8927	113.06**
T x S.D.	18	250435.5714	13913.0873	4.58**
Error b	63	191299.7500	3036.5039	
Total	111	2097303.1071		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication	3	3.5927	1.1975	1.12
Hyperparasite (T)	6	53.4901	8.9150	8.36**
Error a (R x T)	18	19.1887	1.0660	0.91
Sampling date	3	31.6322	10.5441	9.00**
T x S.D.	18	52.6699	2.9261	2.50**
Error b	63	73.7812	1.1711	
Total	111	234.3548		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication	3	2.6151	0.8717	1.27
Hyperparasite (T)	6	36.4901	6.0817	8.84**
Error a (R x T)	18	12.3893	0.6883	0.73
Sampling date	3	1.9323	0.6441	0.68
T X S.D.	18	6.0039	0.3336	0.35
Error b	63	59.6387	0.9466	
Total	111	119.0696		
		Sclerotia infected by T. flavus SQRT (x^1 + 0.5)		
Replication	3	4.0467	1.3489	0.87
Hyperparasite (T)	6	31.7471	5.2912	3.42*
Error a (R x T)	18	27.8747	1.5486	2.71**
Sampling date	3	3.9267	1.3089	2.29
T x S.D.	18	19.4575	1.0809	1.89*
Error b	63	35.9825	0.5712	
Total	111	123.0352		

*Significant at the 5% level.
**Significant at the 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 57. Effect of T. flavus and C. minitans on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a bean field in June, 1984 (Lethbridge).

Hyperparasites	Number of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
Control (SS)	18.2[2]a[3]	8.4[2]a	0.0[2]c	0.0[2]b
SS + bran	18.2 a	4.9 a	0.0 c	0.0 b
SS + T. flavus	17.0 a	0.2 b	4.0 a	0.0 b
SS + C/T[4] 1:2	14.4 b	1.4 b	2.5 ab	0.0 b
SS + C/T 1:1	14.3 b	1.0 b	1.2 abc	0.5 b
SS + C/T 2:1	12.5 b	1.8 b	0.0 c	3.9 a
SS + C. minitans	13.6 b	1.2 b	0.2 bc	3.1 a

[1] Design is split-plot with 7 main plots (hyperparaaites) and 4 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Rec. = recovered sclerotia.

[2] Sclerotia were obtained from an infected bean crop.

[3] Analysis of variance on transformed data; means converted back to raw form for presentation.

[4] Means within columns followed by the same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

T = T. flavus; C = C. minitans.

Appendix 58. Effect of sampling date on the recovery and viability of sclerotia of S. sclerotiorum buried in a bean field in June, 1984 (Lethbridge).

Weeks after burial	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
4	20.0[2]a[3]	6.1[2]a	1.4[2]a	0.5[2]a
8	15.4 b	1.6 b	0.3 a	0.6 a
12	14.2 bc	1.2 b	1.1 a	0.7 a
52	11.8 c	1.1 b	0.6 a	1.3 a

[1] Design is split-plot with 5 main plots (hyperparasites) and 4 subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate; sclerotia obtained from an infected bean crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 59. Analysis of variance on the effect of hyperparasites, time of burial (season) and sampling date on sclerotia of S. sclerotiorum buried in a bean field in the fall of 1984 (Lethbridge).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	22478.728	7492.909	3.775*
Hyperparasite (T)	6	312235.854	52039.309	26.218**
Error	18	35727.183	1984.844	1.340
Season (S)	1	1915.290	1915.290	1.293
T x S	6	17886.435	2981.072	2.012
Error	21	31113.900	1481.614	1.059
Date (D)	3	2391178.328	797059.443	569.475**
T x D	18	312092.072	17338.448	12.388**
S x D	3	11984.272	3994.757	2.854*
T x S x D	18	21621.657	1201.203	0.858
Error	126	176354.389	1399.638	
Total	223	3334588.108		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	4.06339	1.35446	0.887
Hyperparasite (T)	6	60.16005	10.02667	6.568**
Error	18	27.47883	1.52660	1.435
Season (S)	1	0.02930	0.02930	0.028
T x S	6	15.3064	2.55107	2.398
Error	21	22.34298	1.06395	1.011
Date (D)	3	23.43799	7.81266	7.421**
T x D	18	27.48495	1.52694	1.450
S x D	3	4.09705	1.36568	1.297
T x S x D	18	21.56403	1.19800	1.138
Error	126	132.65032	1.05278	
Total	223	338.61538		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	17.69846	5.89948	2.976
Hyperparasite (T)	6	229.58096	38.26349	19.300**
Error	18	35.68698	1.98261	2.639
Season (S)	1	5.80903	5.80903	7.732*
T x S	6	3.65314	0.60885	0.810
Error	21	15.77735	0.75130	0.510
Date (D)	3	58.98367	19.66122	13.336**
T x D	18	57.91182	3.21732	2.182
S x D	3	11.57588	3.85862	2.617
T x S x D	18	30.87581	1.71532	1.163
Error	126	185.76577	1.47433	
Total	223	653.31884		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	22.69588	7.56532	4.248*
Hyperparasite (T)	6	57.86728	9.64454	5.416**
Error	18	32.05297	1.78072	1.388
Season (S)	1	0.03183	0.03183	0.025
T x S	6	11.59920	1.93320	1.506
Error	21	26.95111	1.28338	0.929
Date (D)	3	33.33882	11.11294	8.048**
T x D	18	46.42531	2.57918	1.868*
S x D	3	3.26356	1.08785	0.788
T x S x D	18	19.24764	1.06931	0.774
Error	126	173.98424	1.35082	
Total	223	427.45799		

*, **Significant at the 5% and 1% level.

[1]Represents percent of recovered sclerotia.

Appendix 60. Effect of T. flavus and C. minitans on the recovery and viability of sclerotia of S. sclerotiorum (SS) buried in a bean field in the fall of 1984 and recovered in 1985.

Hyperparasites	Number of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
Control (SS)	18.8[2]a[3]	4.6[2]a	0.5[2]cd	0.7[2]cd
SS + bran	18.4 ab	4.6 a	0.3 d	0.5 cd
SS + T. flavus	17.9 b	0.7 b	4.5 a	0.3 cd
SS + C/T[4] 1:2	16.4 d	0.6 b	3.6 ab	4.0 b
SS + C/T 1:1	17.2 c	0.4 b	2.4 abc	2.5 bc
SS + C/T 2:1	15.9 d	1.9 b	1.8 bcd	9.1 a
SS + C. minitans	16.1 d	1.1 b	0.3 d	13.2 a

[1] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (time of application) and 4 sub-subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected bean crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] T = T. flavus; C = C. minitans.

Appendix 61. Effect of time of application of T. flavus and C. minitans on the recovery and viability of sclerotia of S. sclerotiorum buried in a bean field in the fall of 1984 and recovered in 1985.

Time of application	Number of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
November ('84)	17.2[2]a[3]	1.8[2]a	1.6[2]a	4.0[2]a
May ('85)	17.3a	1.7[2]a	1.7[2]a	2.7[2]b

[1] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (time of application) and 4 sub-subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected bean crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 62. The effect of sampling date on the recovery and viability of sclerotia of S. sclerotiorum buried in a bean field in the fall of 1984 and recovered in the spring of 1985.

Weeks after burial	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
30	20.0[2]a[3]	3.4[2]a	2.9[2]a	1.6[2]c
33	20.0 a	0.8 c	3.0 a	7.1 a
36	15.2 b	1.2 bc	0.4 b	3.7 b
39	12.8 c	2.0 b	1.0 b	2.0 c

[1] Design is split-plot with 7 main plots (hyperparasites), 2 subplots (time of application) and 4 sub-subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate; sclerotia obtained from an infected bean crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 63. Analysis of variance on the effect of hyperparasites, time of burial (season) and sampling date on the sclerotia of S. sclerotiorum buried in a bean field in May, 1985 (Lethbridge).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia (x^2)		
Replication (R)	3	11236.286	3745.429	2.030**
Hyperparasite (T)	6	557158.201	92859.700	50.333**
Error	18	33208.452	1844.914	1.004
Season (S)	1	11467.523	11467.523	6.242*
T x S	6	29516.203	4919.367	2.678*
Error	21	38582.586	1837.266	0.909
Date (D)	2	1528651.641	764325.820	377.963**
T x D	12	294667.354	24555.613	12.143**
S x D	2	2192.472	1096.236	0.542*
T x S x D	12	60000.556	5000.046	2.473**
Error	84	169866.663	2022.222	
Total	167	2736547.936		
		Viable sclerotia SQRT(x^1 + 0.5)		
Replication (R)	3	2.50853	0.83617	0.499
Hyperparasite (T)	6	16.95075	2.82512	1.686
Error	18	30.16395	1.67577	2.022
Season (S)	1	0.13005	0.13005	0.157
T x S	6	9.62450	1.60408	1.935
Error	21	17.40768	0.82893	0.760
Date (D)	2	2.10290	1.05145	0.964
T x D	12	19.72035	1.64336	1.506*
S x D	2	6.88117	3.44058	3.153**
T x S x D	12	6.34875	0.52906	0.485
Error	84	91.66322	1.09122	
Total	167	203.50189		
		Sclerotia infected by C. minitans SQRT(x^1 + 0.5)		
Replication (R)	3	11.62105	3.87368	2.540**
Hyperparasite (T)	6	134.28975	22.38162	14.677**
Error	18	27.44914	1.52495	0.888
Season (S)	1	1.57429	1.57429	0.917
T x S	6	3.13441	0.52240	0.304
Error	21	36.06463	1.71736	1.244
Date (D)	2	33.56640	16.78320	12.162**
T x D	12	24.91829	2.07652	1.505*
S x D	2	9.82552	4.91276	3.560**
T x S x D	12	49.19313	4.09942	2.971**
Error	84	115.91901	1.37998	
Total	167	447.55565		
		Sclerotia infected by T. flavus SQRT(x^1 + 0.5)		
Replication (R)	3	20.44563	6.81521	3.125*
Hyperparasite (T)	6	40.00199	6.66699	3.057*
Error	18	39.25068	2.18059	1.517
Season (S)	1	0.00977	0.00977	0.007
T x S	6	6.62800	1.10466	0.769
Error	21	30.18274	1.43727	0.933
Date (D)	2	8.13552	4.06776	2.640
T x D	12	25.96419	2.16368	1.404
S x D	2	1.90153	0.95076	0.617
T x S x D	12	21.81141	1.81761	1.179
Error	84	129.45005	1.54107	
Total	167	323.78154		

*Significant at the 5% level.
**Significant at the 1% level.
[1]Represents percent of recovered sclerotia.

Appendix 64. Effect of *T. flavus* and *C. minitans* on the recovery and viability of sclerotia of *S. sclerotiorum* (SS) buried in a bean field (sclerotia buried in May, 1985).

Hyperparasites	Number of sclerotia[1]			
	Rec.	Viable (%)	Infected by *T. flavus* (%)	Infected by *C. minitans* (%)
Control (SS)	18.4[2]a[3]	3.3[2]a	0.2[2]b	1.3[2]c
SS + bran	18.8 a	1.7 a	0.3 b	0.3 c
SS + *T. flavus*	16.3 b	0.9 a	2.9 a	0.4 c
SS + C/T[4] 1:2	15.5 b	0.8 a	3.7 a	1.0 c
SS + C/T 1:1	16.3 b	1.2 a	1.6 ab	4.3 b
SS + C/T 2:1	13.8 c	1.2 a	1.9 ab	8.7 a
SS + *C. minitans*	14.3 c	0.2 a	0.0 b	9.6 a

[1] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (time of application) and 3 sub-subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate.

[2] Sclerotia were obtained from an infected bean crop.

[3] Analysis of variance on transformed data; means converted back to raw form for presentation.
Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

[4] T = *T. flavus*; C = *C. minitans*.

Appendix 65. Effect of time of application of T. flavus and C. minitans on the recovery and viability of sclerotia of S. sclerotiorum buried in and recovered from a bean field in the 1985 season.

Time of application	Number of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
November ('84)	16.0[2]b[3]	1.2[2]a	1.3[2]a	3.2[2]a
May ('85)	16.6 a	1.3 a	1.3 a	2.5 a

[1] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (time of application) and 3 sub-subplots (sampling dates). Values are averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected bean crop.

[2] Rec. = recovered sclerotia.

[3] Analysis of variance on transformed data; means converted back to raw form for presentation.

Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 66. The effect of sampling date on the recovery and viability of sclerotia of S. sclerotiorum buried in a bean field in May, 1985.

Weeks after burial	No. of sclerotia[1]			
	Rec.	Viable (%)	Infected by T. flavus (%)	Infected by C. minitans (%)
3	19.9[2]a[3]	0.9[2]a	2.2[2]a	4.8[2]a
6	15.3 b	1.3 a	0.8 a	3.4 a
9	13.0 c	1.6 a	1.0 a	1.0 b

[1] Design is split-split plot with 7 main plots (hyperparasites), 2 subplots (time of application) and 3 sub-subplots (sampling dates). Values averaged over 4 replicates with 20 sclerotia per replicate. Sclerotia were obtained from an infected bean crop.

[2] Analysis of variance on transformed data; means converted back to raw form for presentation.

[3] Means within columns followed by same letter are not significantly different at the 0.05 level (Fisher's least significant difference test).

Appendix 67. Analysis of variance on the effect of T. flavus and C. minitans on sclerotia of S. sclerotiorum collected from above-ground portions of bean plants (Lethbridge, 1985).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Viable sclerotia log(x + 10)		
Replication	3	0.11242	0.03747	2.18
Hyperparasite	6	0.14840	0.02473	1.44
Error	18	0.31011	0.01722	
Total	27	0.57094		
		Sclerotia infected by C. minitans log(x + 10)		
Replication	3	0.10885	0.03628	1.68
Hyperparasite	6	0.13115	0.02186	1.01
Error	18	0.38969	0.02165	
Total	27	0.62969		
		Sclerotia infected by T. flavus log(x + 10)		
Replication	3	0.00097	0.00032	1.00
Hyperparasite	6	0.00195	0.00032	1.00
Error	18	0.00584	0.00032	
Total	27	0.00876		

Appendix 68. Analysis of variance on the inoculum density[1] of *S. sclerotiorum* in a field at Lethbridge (dry bean site, 1984).

Source of variation	D.F.	Sum of squares	Mean squares	F value
Replication	3	0.00059	0.00020	0.63
Treatment	6	0.00148	0.00025	0.79
Error	18	0.00561	0.00031	
Total	27	0.00769		

[1] Square root transformation used [SQRT(x+0.5)].