The use of *Talaromyces flavus* as a biological control agent for *Sclerotinia sclerotiorum*

by

Debra Leigh McLaren

A thesis
presented to the University of Manitoba
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ABSTRACT

McLaren, Debra Leigh. M.Sc., The University of Manitoba, October, 1983. The Use of Talaromyces flavus as a Biological Control Agent for Sclerotinia sclerotiorum. Major professors: Dr. H.C. Huang and Dr. S.R. Rimmer.

The relationship between Sclerotinia sclerotiorum, causal agent of sclerotinia wilt of sunflower, and its mycoparasite, Talaromyces flavus, teleomorph of Penicillium vermiculatum, was examined in laboratory, greenhouse and field experiments. Results of laboratory studies indicate that T. flavus is capable of destroying hyphae of S. sclerotiorum. The death of the host cells is due apparently to the coiling of T. flavus around host cells resulting in disintegration of host cytoplasm and collapse of cell walls. No evidence of direct penetration of the host hyphae by the mycoparasite was observed.

Results of greenhouse trials showed that T. flavus was capable of destroying sclerotia of S. sclerotiorum. When sclerotia were soaked in a spore suspension of T. flavus prior to burial in soil, fewer of the sclerotia were viable and more were infected with T. flavus as compared to the controls where no mycoparasite was applied. Similar results were obtained in the field trials when sclerotia were soaked in a spore suspension of T. flavus. When T. flavus, grown on autoclaved sclerotia, was used as inoculum and incorporated into field soil along with healthy sclerotia, fewer sclerotia were recovered in the T. flavus- treated

plots as compared to the control. Of these sclerotia, the number infected or killed by T. flavus was greater where the mycoparasite was applied.

The field trial results indicate that T. flavus is effective in controlling sclerotinia wilt of sunflower. Results from 2 locations (Winnipeg and Portage la Prairie) showed that wilt incidence was reduced significantly in the T. flavus- treated plots as compared to the control plots artificially infested with sclerotia of S. sclerotiorum. Wilt incidences in the T. flavus- treated plots and the S. sclerotiorum- treated plots were 3.8 and 47.2%, respectively, at the Winnipeg location. and 26.6 and 81.4% at the Portage la Prairie location. However, no significant differences were observed at either location between the T.flavus- treated plots and plots where sunflowers were untreated. This suggests the presence of natural inoculum of S. sclerotiorum in both fields. High disease incidence in untreated plots located at Portage la Prairie was due to a high level of natural inoculum in the soil.

The field experiments also showed that T. flavus is effective in reducing yield losses in sunflower due to sclerotinia wilt. Yields in T. flavus- treated plots and S. sclerotiorum- treated plots at the Winnipeg site were 2870 and 2350 kg/ha, respectively. Similarly, yields of the T. flavus- treated plots and S. sclerotiorum- treated plots were 2140 and 1430 kg/ha, respectively, at the Portage la Prairie location. At each site, the yield value obtained for the T. flavus- treated plots was significantly different from that of the S. sclerotiorum- treated plots. However, yield of the mycoparasite-treated plots did not differ from that obtained in plots where sunflowers alone were seeded. This indi-

cates that *T. flavus* was effective in reducing wilt incidence to a level comparable to that obtained in the untreated control.

Results obtained from the laboratory, greenhouse and field studies on the interaction between *T. flavus* and *S. sclerotiorum* indicate that: 1. *T. flavus* is a mycoparasite capable of destroying both hyphae and sclerotia of *S. sclerotiorum* and 2. *T. flavus* is effective in controlling sclerotinia wilt and reducing yield losses in sunflower.

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TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vi

LIST OF TABLES viii

LIST OF FIGURES x

LIST OF PLATES xi

LIST OF APPENDICES xii

Chapter page

I. INTRODUCTION 1

II. REVIEW OF LITERATURE 4

The host (sunflower) 4
The pathogen (Sclerotinia sclerotiorum) 5
Introduction 5
Disease cycle 5
Survival of sclerotia 11
The rind 11
Sclerotial germination 12
Production of secondary sclerotia 13
Soil moisture 14
Temperature 16
Condition of the field 19
Sclerotium size and shape 19
Depth of burial 20
Isolates of S. sclerotiorum 20
Microorganisms 21
Control of sclerotinia wilt of sunflower 25
The mycoparasite (Talaromyces flavus) 31

III. MATERIALS AND METHODS 34

Control of sclerotia of Sclerotinia sclerotiorum by Talaromyces flavus 34
Source of fungal material 34
Production of sclerotia for burial studies 34
Production of T. flavus 35

Greenhouse experiments 37
Field Experiments 38
Control of sclerotinia wilt of sunflower by *Talaromyces flavus* 40
Source of fungal material and seed 40
Inoculum production 40
Greenhouse experiments 41
Field experiments 43
Hyphal interactions between *Sclerotinia sclerotiorum* and *Talaromyces flavus* 45
Materials and methods 45
Bright field microscopy 45
Fluorescence microscopy 45
Scanning electron microscopy 46

IV. RESULTS 48

Control of sclerotia of *Sclerotinia sclerotiorum* by *Talaromyces flavus* 48
Greenhouse experiments 48
Field experiments 55
Control of sclerotinia wilt of sunflower by *Talaromyces flavus* 71
Greenhouse experiments 71
Field experiments 75
Hyphal interactions between *S. sclerotiorum* and *T. flavus* 86

V. DISCUSSION 92

LITERATURE CITED 99

APPENDIX 107

LIST OF TABLES

Table — page

1. Effect of T. flavus on the survival and viability of sclerotia of S. sclerotiorum in soil (experiments 1 and 2) 49

2. Effect of sampling date on survival and viability of sclerotia of S. sclerotiorum in soil (experiments 1 and 2) 50

3. Effect of T. flavus on survival and viability of sclerotia of S. sclerotiorum in sterile soil 53

4. Effect of sampling date on survival and viability of sclerotia of S. sclerotiorum in soil 54

5. Effect of soil sterilization on the survival and viability of sclerotia of S. sclerotiorum in soil 56

6. Effect of T. flavus on the survival and viability of sclerotia of S. sclerotiorum in soil 57

7. Effect of T. flavus on survival and viability of sclerotia of S. sclerotiorum at 2 locations 58

8. Effect of sampling date on survival and viability of sclerotia of S. sclerotiorum at 2 locations 60

9. Effect of T. flavus on survival and viability of sclerotia of S. sclerotiorum at 2 locations 63

10. Effect of sampling date on survival and viability of sclerotia of S. sclerotiorum at 2 locations 66

11. Effect of T. flavus on survival and viability of sclerotia of S. sclerotiorum 69

12. Effect of sampling date on survival and viability of sclerotia of S. sclerotiorum 70

13. Biological control of sclerotinia wilt of sunflower by Talaromyces flavus in sterile soil 72

14. Biological control of sclerotinia wilt of sunflower by *Talaromyces flavus* in unsterile soil. 73

15. Biological control of sclerotinia wilt of sunflower by *Talaromyces flavus* in sterile and unsterile soil. 74

16. The effect of *T. flavus* on the incidence of sclerotinia wilt and recoverable sclerotia in sunflowers grown in artificially infested soil. 76

17. Effect of *Talaromyces flavus* on yield and sclerotinia wilt of sunflower at two field locations (1982). 78

LIST OF FIGURES

Figure | page

1. The effect of T. flavus on survival of sclerotia of S. sclerotiorum (greenhouse experiment, Morden) 51

2. The effect of T. flavus as a spore suspension on survival and viability of sclerotia of S. sclerotiorum (field experiment, Winnipeg) 61

3. The effect of T. flavus as a spore suspension on survival and viability of sclerotia of S. sclerotiorum (field experiment, Portage la Prairie) 62

4. The effect of T. flavus produced on autoclaved sclerotia on survival and viability of sclerotia of S. sclerotiorum (field experiment, Winnipeg) 65

5. The effect of T. flavus produced on autoclaved sclerotia on survival and viability of sclerotia of S. sclerotiorum (field experiment, Portage la Prairie) 67

6. The effect of T. flavus on survival and viability of sclerotia of S. sclerotiorum (greenhouse experiment, Winnipeg) 77

7. The effect of T. flavus on the number of sclerotia recovered at 2 field locations (Winnipeg and Portage la Prairie) 79

8. The effect of T. flavus on the incidence of sclerotinia wilt of sunflower at the Winnipeg location. 81

9. The effect of T. flavus on the incidence of sclerotinia wilt of sunflower at the Portage la Prairie location. 83

10. The effect of T. flavus on survival and viability of sclerotia recovered from sunflower plots at the Winnipeg location. 84

11. The effect of T. flavus on survival and viability of sclerotia recovered from sunflower plots at the Portage la Prairie location. 85

LIST OF PLATES

PLATE		PAGE
1	Biological control of sclerotinia wilt of sunflower by *T. flavus* in plots located at the Portage la Prairie site..	80
2	Hyphal interactions between *T. flavus* and *S. sclerotiorum* observed via fluorescence and light microscopy..	87
3	Hyphal interactions between *T. flavus* and *S. sclerotiorum* observed via scanning electron microscopy..	88
4	Scanning electron micrographs of the advanced stages of the *T. flavus* - *S. sclerotiorum* hyphal interaction	90
5	Scanning electron micrograph of a healthy versus a colonized hypha of *S. sclerotiorum*..................	91

LIST OF APPENDICES

Appendix		page
1.	Analysis of variance for greenhouse burial experiment No. 1 . . .	107
2.	Analysis of variance for greenhouse burial experiment No. 2 . . .	108
3.	Analysis of variance for greenhouse burial experiment No. 3 . . .	109
4.	Analysis of variance for greenhouse burial experiment No. 4 . . .	110
5.	Analysis of variance for Winnipeg field burial experiment No. 1 .	111
6.	Analysis of variance for Portage la Prairie field burial experiment No. 1	112
7.	Analysis of variance for Winnipeg field burial experiment No. 2	113
8.	Analysis of variance for Portage la Prairie field burial experiment No. 2	114
9.	Analysis of variance for Morden field burial experiment (1981)	115
10.	Soil temperatures recorded for 3 field experiments at the Winnipeg location (1982)	116
11.	Soil moisture calculated for 3 field experiments at the Portage la Prairie location (1982)	117
12.	Analysis of variance for greenhouse experiment No. 1 (sterile soil)	118
13.	Analysis of variance for greenhouse experiment No. 2 (unsterile soil)	119
14.	Analysis of variance for greenhouse experiment No. 3 (sterile vs. unsterile soil)	120
15.	Analysis of variance for greenhouse experiment No. 4 where <u>Talaromyces</u> <u>flavus</u> was produced on wheat bran	121
16.	Analysis of variance for results of 1982 biological control field experiment (Winnipeg location)	122
17.	Analysis of variance for results of 1982 biological control field experiment (Portage la Prairie location)	123

Chapter I

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is an important plant pathogen capable of damaging crops in the field and 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). In Canada, the most serious disease of sunflower is sclerotinia wilt caused by Sclerotinia sclerotiorum (Hoes and Huang, 1976). The pathogen infects flowers and stems resulting in head and stalk rot. It also infects roots resulting in root rot and wilt. Losses in yield and seed quality of sunflower can be severe as a result of sclerotinia infections (Dorrell and Huang, 1978).

Sclerotia are the main overwintering propagules of S. sclerotiorum and they serve as the primary source of inoculum for sclerotinia wilt in sunflower (Huang, 1979, 1980b). The disease is difficult to control due to the ability of sclerotia to survive adverse environmental conditions for prolonged periods of time in the absence of a host, the rapid colonization of host tissue by Sclerotinia hyphae, and the extreme adaptability of the pathogen. While cultural practices such as crop rotation provide a measure of control, use of resistant cultivars and chemicals for effective control of this disease are still unavailable.

Due to the importance of sclerotia, a possible strategy for disease control is the use of microorganisms to reduce the sclerotial inoculum

in soil. Reduction of inoculum density has been attempted through use of mycoparasites such as Coniothyrium minitans Campbell. This fungus has been used successfully as a biological control agent of sclerotinia wilt of sunflower (Huang, 1976, 1979, 1980b). Application of C. minitans to fields naturally infested with S. sclerotiorum resulted in a significant reduction in wilt incidence. Huang (1980b) suggested that this success was based on the ability of C. minitans to control effectively the primary inoculum (sclerotia) in situ. Due to the lack of effective control procedures, Huang (1980b) proposed that biological control using a mycoparasite such as C. minitans might have great potential when used in conjunction with cultural practises recommended for control.

Talaromyces flavus is one of many microorganisms examined for possible parasitic action against S. sclerotiorum. Su and Leu (1980) found T. flavus to be parasitic on sclerotia of S. sclerotiorum. Inoculating sclerotia with a spore suspension of T. flavus resulted in sclerotia being lysed extensively. T. flavus has been used successfully as a biological control agent in reducing verticillium wilt of eggplant caused by Verticillium dahliae Kleb. (Marois et al., 1982). Field testing showed that T. flavus reduced disease by 76 and 67% in fields at two locations. Boosalis (1956) reported that Penicillium vermiculatum the conidial stage or anamorph of T. flavus, gave nearly complete control of Rhizoctonia -incited damping-off and seedling blight of peas. Boosalis also indicated that hyphae of R. solani were parasitized by P. vermiculatum. The mode of parasitism involved development of penetration pegs followed by production of internal hyphae. Penicillium vermiculatum coiled

around host hyphae resulting in deterioration of the cell protoplasm (Boosalis, 1956). Due to the ability of *T. flavus* to destroy sclerotia of *S. sclerotiorum* and its proven effectiveness as a biological control agent of other microorganisms in the field, this fungus may have potential as a future biological control agent of sclerotinia wilt of sunflower.

The objective of this thesis is to explore the potential of using *T. flavus* to control *S. sclerotiorum*. This work is divided into three sections: 1. To investigate hyphal interactions between *T. flavus* and *S. sclerotiorum* using light and scanning electron microscopy, 2. To study the effects of *T. flavus* on survival of sclerotia of *S. sclerotiorum* in soil and 3. To evaluate the efficacy of *T. flavus* as a control agent of sclerotinia wilt of sunflower in the field.

Chapter II

REVIEW OF LITERATURE

2.1 THE HOST (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 found favor 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 countries in the world include the USSR, Argentina, Rumania, South Africa and the United States (Anonymous, 1978).

In Canada, commercial production of the sunflower began in 1943 in southern Manitoba (Sackston, 1981). Canadian sunflower production is still largely confined to Manitoba (Anonymous, 1982). 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 second only to soybeans as a source of edible oils (Putt, 1978). Sunflower production has increased rapidly in Canada over the past two decades (Huang, 1979) resulting in increased attention on sunflower diseases.

There are more than thirty known diseases of sunflower. Downy mildew - *Plasmopara halstedii* (Farl.) Berl & de Toni, rust - *Puccinia helianthi* Schw., Sclerotinia white mold - *Sclerotinia sclerotiorum* (Lib.) de Bary, and Verticillium wilt - *Verticillium dahliae* Klebahn are considered to be 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).

2.2 THE PATHOGEN (SCLEROTINIA SCLEROTIORUM)

2.2.1 Introduction

Sclerotinia sclerotiorum is distributed worldwide. It attacks more than 360 species of plants in 64 families (Purdy, 1979) including 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).

2.2.2 Disease cycle

Sclerotia are the resting or overwintering stage of *S. sclerotiorum* (Coley-Smith and Cooke, 1971; Huang, 1979; Willetts and Wong, 1980). The life cycle begins with germination of the sclerotium. This can be either myceliogenic (Huang and Dueck, 1980) or carpogenic (Williams and West-

ern, 1965; Willetts and Wong, 1980). Two kinds of myceliogenic 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 sclerotia to rupture the rind (Adams and Tate, 1976). Myceliogenic germination of S. sclerotiorum appears to be of the hyphal type (Huang and Dueck, 1980; Willetts and Wong, 1980). 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; Le Tourneau, 1979), 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 (Le Tourneau, 1973; Price and Colhoun, 1975; Phipps and Porter, 1982) and between 32 C 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).

Sclerotia also germinate carpogenically by the development of stipes and apothecia. Ascospores are formed within an apothecium and these are the only infective spores produced by S. sclerotiorum (Willetts and Wong, 1980). Huang (1981) reported a new strain of S. sclerotiorum which exhibits both myceliogenic and carpogenic germination of the same sclerotium. This strain produces tan rather than the normal black sclerotia.

With sclerotinia wilt of sunflower, wilt symptoms caused by infection result from myceliogenic germination of sclerotia (Huang and Dueck, 1980; Huang and Hoes, 1980). Following myceliogenic germination of sclerotia, infection occurs at or below the soil line where the taproot-hypocotyl axis is the primary site of infection. Infection of healthy plants also occurs by mycelium spreading from neighboring infected plants (Huang and Dueck, 1980; Huang and Hoes, 1980; Willetts and Wong, 1980). Huang and Dueck (1980) found that mycelia arising from germinating sclerotia are able to infect unwounded sunflower roots and hypocotyls in the absence of exogenous nutrients. These results are similar to those obtained by Adams and Tate (1976), who observed direct infection of lettuce seedlings by germinating sclerotia of S. minor without the addition of a food base. Contrary to the two above reports, an exogenous source of energy has been suggested to be a requirement for mycelium from germinating sclerotia to infect a host plant (Tanrikut and Vaughan, 1951; Purdy, 1958; Abawi and Grogan, 1975; Abawi and Grogan, 1978).

Once infection has occurred, enzymatic processes affecting the middle lamella between cells 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 very sudden wilting. Severely diseased plants have a characteristic lesion at the base of the stem, on the taproot, and on some fibrous roots. Such le-

sions, commonly brown and water-soaked in appearance, may extend from the taproot along the hypocotyl to 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 also occurs at the stem base, on the laterally extending fibrous roots and on the outside of the taproot (Young and Morris, 1927; Hoes and Huang, 1975; Zimmer and Hoes, 1978; Huang and Hoes 1980). 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 Hoes, 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; Huang, 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 the effects of moisture levels on apothecial production, Grogan and Abawi (1974) found that continuous moisture was required for sclerotia to produce apothecia. In later work, 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 2 to 3 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 occurred in 1977 resulting in poor seed yield and reduced seed quality. This outbreak was attributed to excess rainfall occurring at the flowering and seed development stages of sunflower growth. In 1982, ascospore infection of sunflower causing stalk and head rot, was quite 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 and result 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 mycelium from germinating sclerotia. In the field, sclerotinia wilt of sunflower resulted from myceliogenic germination whereas in rapeseed fields, carpogenic germination was common.

2.2.3 Survival of sclerotia

Sclerotia enable *Sclerotinia sclerotiorum* to survive adverse conditions or the absence of a suitable host for prolonged periods of time. Ascospores and mycelia remain viable only for limited periods of time (Hungerford and Pitts, 1953; Van Den Berg and Lentz, 1968; Cook *et al*., 1975; Grogan and Abawi, 1975). Survival of sclerotia is reported to vary from one year (Davis, 1925) to 4 or 5 years (Adams and Ayers, 1979) to 10 years (Brown and Butler, 1936). Cook *et al*. (1975) reported that after 3 years of burial in soil, 78% of sclerotia buried at depths of 5 to 20 cm were recovered. McLean (1958) found that after 5 years of burial in moist soil, destruction of sclerotia was almost complete, but some were still viable and produced apothecia. Hungerford and Pitts (1953) observed that sclerotia germinated readily after being held in dry storage for 7 years. Such variability in sclerotial longevity may be associated with quantitative and qualitative differences in the soil fauna and microflora as suggested by Huang (1977).

There are a number of factors which may influence survival of sclerotia of *S. sclerotiorum* in nature. They will be discussed in the following sections.

2.2.3.1 The rind

A structural factor such as the rind may influence sclerotial survival. The rind functions as a protective layer which insulates against adverse environmental conditions as discussed by Coley-Smith and Cooke (1971) and Willetts (1971). Damage to the rind of the sclerotium may increase susceptibility to colonization by other microorganisms (Makko-

nen and Pohjakallio, 1960). Rind damage may also lead to a reduction in longevity by inducing germination of the sclerotium. Makkonen and Pohjakallio (1960) ascribed this reduction to depletion of sclerotial food reserves. Merriman (1976) demonstrated that survival of sclerotia produced in nature differs from that of sclerotia produced in culture. This difference is related to the rind. Sclerotia formed in culture survive longer in soil than those formed in nature because sclerotia produced in culture develop complete rinds. Merriman (1976) suggested that in nature, formation of sclerotia in the presence of other microorganisms results in an adverse effect on sclerotium development, and therefore, imperfect rinds are produced. Imperfections allow for colonization by microorganisms and subsequent degradation of sclerotia.

2.2.3.2 Sclerotial germination

Varying reports occur in the literature concerning sclerotial germination and its effect on the survival of the parent sclerotium. Makkonen and Pohjakallio (1960) observed that sclerotia of _S. sclerotiorum_ that produced large numbers of apothecia were more susceptible to decay. Williams and Western (1965a) observed that the production of apothecia by _Sclerotinia trifoliorum_ increased the susceptibility of the parent sclerotium to decay. It was also noted that while susceptibility to decay was increased, some parent sclerotia were able to produce two and even three crops of apothecia in successive years. The ability of a sclerotium to germinate on successive occasions has important implications for survival. Coley-Smith and Cooke (1971) state that germination results in a reduction or exhaustion of sclerotial reserves and increas-

es the susceptibility of sclerotia to decay (Coley-Smith and Cooke, 1971). Cook *et al*. (1975) conclude that carpogenic germination of sclerotia of *S. sclerotiorum* is not correlated to sclerotial deterioration. However, their results were obtained from laboratory survival trials.

The mode of germination may affect the survival of sclerotia. Willetts and Wong (1980) discuss work by Saito who suggested that survival of sclerotia of *S. sclerotiorum* was influenced by the mode of germination. Survival was reduced more after germination by means of mycelia than following carpogenic germination.

2.2.3.3 Production of secondary sclerotia

The survival of *S. sclerotiorum* in soil may be enhanced by the ability of the sclerotia to produce secondary sclerotia. Williams and Western (1965b) concluded that sclerotia of *S. sclerotiorum* were able to regenerate and produce daughter or secondary sclerotia. They also reported that an increase in weight of sclerotial material could occur by direct absorption of food material from the soil. Cook *et al*. (1975) reported that secondary sclerotia develop at soil depths of 5 to 30 cm. Whether the formation of secondary sclerotia is related to soil moisture is unresolved. Williams and Western (1965b) reported that increasing soil moisture accelerates the breakdown of sclerotia. Soil moistures above 30% of the moisture-holding capacity of the soil allow some secondary sclerotial production, but not sufficiently to counterbalance degeneration of the original individuals. When complete saturation of the soil was maintained for 3 months, formation balanced destruction and the num-

ber of sclerotia remained constant. These results conflict with those obtained by Adams (1975). His experiments, dealing with soil moisture, soil amendments and soil temperature failed to indicate any factors that favor 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. An increase in mean dry weight of sclerotia would enhance the ability of this pathogen to survive.

2.2.3.4 Soil moisture

Environmental factors also affect the survival of sclerotia. In the field, sclerotia are subject to variations in the moisture content of the soil and these changes in soil moisture affect both survival and behavior of the sclerotia (Williams and Western, 1965b). Sclerotia of S. sclerotiorum survive better under dry as compared to wet soil conditions. Prolonged flooding and alternate flooding and draining have been used to promote decay of sclerotia in the field (Moore, 1949). More recently, Williams and Western (1965b) reported that increasing soil moisture accelerated breakdown of sclerotia.

Sclerotial survival is also affected by the drying and wetting of these propagules, which occurs on or just below the soil surface. Smith (1972c) found that sclerotia of S. sclerotiorum leak nutrients when they are dried and placed in moist soil. This 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 2 to 3 weeks in soil. Adams

(1975) reported that the inoculum density of soil containing sclerotia of _S. sclerotiorum_ is markedly reduced when the soil is air-dried, re-moistened 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 (1972a, 1972b) reported that sclerotia of _Sclerotium rolfsii_ leak nutrients when they are dried for short periods and remoistened. This treatment promotes microbial breakdown due to nutrient leakage, but also stimulated sclerotia to germinate. He suggested that drying of the sclerotia was the main mechanism for inducing germination of sclerotia of _S. rolfsii_ in nature (Smith, 1972a). Smith (1972c) reported that a drying treatment of sclerotia of _Sclerotinia sclerotiorum_ also 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 and produce mycelium to infect the host. Smith (1972a) found that in the absence of host plants, germinated sclerotia of _Sclerotium rolfsii_ decay. If hosts are present, they survive long enough to cause infection. Drying and remoistening of sclerotia of _Sclerotinia sclerotiorum_ may also promote decay following germination if no host plants are present.

For infection to occur following germination, the sclerotia must be close to host plants. Williams and Western (1965a) observed that myce-

lial 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 incidence of sclerotinia wilt of sunflower is decreased significantly if sclerotia are buried 4 cm above, 5 cm below, 15 cm below or 10 cm horizontally removed from the seed, rather than at the seed level near the seed.

2.2.3.5 Temperature

Temperature appears to be of minimal importance 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 5 to 6 weeks, are destroyed. 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, the same areas can be affected with disease from year to year (Bisby, 1921; Bisby, 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 influ-

ence 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 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 has 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 (1953) found that sclerotia collected from the field and incubated on soil at 20 C germinated quite readily. He stated that only sclerotia derived from culture require a low temperature treatment (14 to 16 C) prior to incubation, at 20 C in

soil, for germination to occur. To obtain carpogenic germination of sclerotia harvested from culture, Huang (1981) observed that a low temperature treatment is required. Only 5% tan and 10% black sclerotia produced apothecia when chilled for 6 weeks at 3.3 C followed by incubation for 6 weeks at 20 C. Under these conditions myceliogenic germination also occurred, with 85% tan and 8% black sclerotia germinating myceliogenically. This marked difference in myceliogenic germination was attributed to a lack of dormancy in tan sclerotia. Adams and Tate (1976) reported that sclerotia of _S. minor_ exhibited dormancy following initial formation and did not immediately undergo myceliogenic germination. This may also be true for black sclerotia of _S. sclerotiorum_ as suggested by Huang (1981). While Cook _et al_. (1975), Dueck (1981) and Huang (1981) feel that a dormancy period is associated with carpogenic germination, Cook _et al_. (1975) suggests that sclerotia do not exhibit dormancy relative to myceliogenic germination. It is evident that continued research in this area is required to clarify this problem.

Usually environmental conditions are not independent in their action but complement one another (Griffin, 1969). The interactions of several factors affect the viability and degradation of sclerotia. Viability of sclerotia is not reduced significantly during dry summers and winter months, but their destruction is favored during wet summers when the combination of high moisture levels and high temperatures occurs (Ervio _et al_., 1964; Williams and Western, 1965b; Cook _et al_., 1975). Cook _et al_. (1975) found that after 1 and 3 months in wet soil at 27 C, 51 and 73% of sclerotia had decayed, respectively. However, at 5 C only 7 and 15% of sclerotia had deteriorated, respectively. In dry soil, at both 5

C and 27 C, little deterioration occurred. Soil texture (Adams, 1975; Merriman, 1976) and pH (Merriman, 1976) may also affect sclerotial survival either directly, or by influencing other factors associated with the degradation of sclerotia.

2.2.3.6 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 broad 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 affect survival of sclerotia of *S. sclerotiorum* are not in agreement with those obtained by Adams (1975). His work indicated that sclerotia buried in soil maintained under fallow conditions survive as well as sclerotia buried under lettuce and beans.

2.2.3.7 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.

2.2.3.8 Depth of burial

Burial depth also affects survival of sclerotia. Adams (1975) stated that sclerotia of _S._ _sclerotiorum_ survive well at depths of 1 to 12 inches but not at 24 inches. Soil samples taken at 24 inches were saturated with water and decay was attributed to the high moisture content of the soil. 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 are not affected by placement. At high soil temperatures and moisture content, deterioration of sclerotia is greater in the soil than on the soil surface. 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. Adams (1975) suggested that sclerotia produced on diseased host tissue should be allowed to air-dry prior to ploughing. This would presumably hasten decay due to the detrimental effects of drying and remoistening of the sclerotia.

2.2.3.9 Isolates of _S._ _sclerotiorum_

Price and Colhoun (1975a) found isolates of _S._ _sclerotiorum_ to vary on the basis of length of asci, ascospores and sclerotia. Variability has also been observed in the pathogenicity of different isolates on different hosts (Bisby, 1924; Price and Colhoun, 1975b) growth rate at different temperatures (Menzies, 1983), sclerotial production (Morrall _et_ _al_., 1972) and oxalic acid production (Maxwell and Lumsden, 1970).

In the literature, two viewpoints exist concerning variability among isolates of S. sclerotiorum. These are expressed by Price and Colhoun (1975a,b) who studied many isolates to determine if they could be grouped into a number of smaller taxonomic groups of S. sclerotiorum or if they could be regarded as strains of the same species. Price and Colhoun (1975b) believe that variations among isolates are due to small genetic differences between different isolates of the fungus. Morrall et al. (1972) studied 114 isolates of S. sclerotiorum and their results supported the view that the population of Sclerotinia isolates is continuously variable. Their data indicated wide variation among isolates in morphological characteristics as well as in pathological and physiological tests. The isolates did not fall into discrete groups based on these characteristics or combinations thereof. No clear host or geographic associations occurred.

Although no study was done on the survival of sclerotia among isolates, it is probable that differences exist here as well. Morrall et al. (1972) and Price and Colhoun (1975b) feel that such differences are due to the extremely variable nature of the pathogen, with continous variation occurring among isolates.

2.2.3.10 Microorganisms

The most significant factor affecting survival of sclerotia in soil appears 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 exercised a strong antibiotic effect on colonies of S. sclerotiorum. Trichoderma lignorum 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). Sclerotia of S. sclerotiorum appear to be parasitized by certain isolates of Trichoderma spp. but not others. This was observed in work by Jones and Watson (1969) where only 1 of 4 single spore isolates of T. viride decayed sclerotia incubated in moist sand or buried in soil.

Makkonen and Pohjakallio (1960) observed the ability of many species of soil fungi to parasitize and damage the sclerotia of S. sclerotiorum. Species of Acrostalagnus, Fusarium, Gliocladium, Verticillium, and Trichoderma were observed to infect sclerotia on sterilized sand. Merriman (1976) isolated species of Mucor, Fusarium and Trichoderma from sclerotia. Ervio et al. (1964) tested T. viride and other fungi under field conditions and suggested that Trichoderma spp. played a minor role in the natural destruction of sclerotia. This was further substantiated by Huang (1980b) who observed a reduction of sclerotia numbers by 42% in soil infested with an isolate of T. viride.

Certain species of Gliocladium are capable of destroying sclerotia of S. sclerotiorum. Gliocladium roseum caused decay of sclerotia on sand (Makkonen and Pohjakallio, 1960) and G. catenulatum, a mycoparasite of S. sclerotiorum (Huang, 1978), destroyed sclerotia in soil (Huang, 1980b). Su and Leu (1980) found that sclerotia inoculated with G. deli-

quescens and buried in soil were seriously lysed. Another species, G. virens was shown to parasitize mycelia and sclerotia of S. sclerotiorum (Tu, 1980).

Coniothyrium minitans Campbell is well established as a mycoparasite 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; Huang, 1979; Huang, 1980b; Trutmann et al., 1980) and kills both hyphae and mycelium of this Sclerotinia species. This mycoparasite was first isolated from sclerotia of S. sclerotiorum by Campbell (1947) who suggested that biological control of plant diseases caused by Sclerotinia spp. might be possible with this fungus. Huang and Hoes (1976) found that sclerotia as well as hyphae of S. sclerotiorum were invaded by C. minitans. The survival of sclerotia in soil was severely affected, with a 97% reduction occurring 100 days after C. minitans was added to soil artificially infested with S. sclerotiorum (Huang, 1976). Huang (1977) showed C. minitans to be a naturally destructive agent of sclerotia. Sclerotia formed in the pith cavities and basal stems of sunflowers were parasitized and subsequently killed. C. minitans has been used successfully for biological control of S. sclerotiorum under experimental conditions (Huang, 1980b).

A new species, Sporidesmium sclerotivorum was described by Uecker et al. (1978) as being parasitic on sclerotia of Sclerotinia sclerotiorum. Proof of its ability to parasitize sclerotia of S. sclerotiorum as well as S. minor and Sclerotium cepivorum was subsequently reported (Adams and Ayers, 1979; Adams and Ayers, 1980). Evidence presented by Adams and Ayers (1981) indicated that Sporidesmium sclerotivorum was responsi-

ble for the decline in numbers of sclerotia of Sclerotinia minor, S. sclerotiorum and Sclerotium cepivorum in the field.

Teratosperma oligocladum was recently described by Uecker et al. (1980) as a new mycoparasite on the sclerotia of Sclerotinia 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 centaurae. Parasitism of sclerotia was found to be similar to parasitism by C. minitans. The genera Microsphaeropsis and Coniothyrium are closely related, and Watson and Miltmore (1975) suggested that M. centaureae and C. minitans might be synonymous.

Penicillium spp. have also been implicated as mycoparasites of S. sclerotiorum. Rai and Dhawan (1978) reported that Penicillium steckii, P. citrinum and P. funiculosum were responsible for destruction of sclerotia placed in sterilized soil previously infested with the specific parasitic fungus. Rai and Saxena (1975) found that some species of Penicillium showed strong antagonistic activity in vitro and also affected the survival of sclerotia in field soil. Sclerotia were colonized and destroyed by P. citrinum, P. steckii, P. funiculosum and P. pallidum. Rai and Saxena suggested that Penicillium spp. played an important role in sclerotial degradation. 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 to be a mycoparasite

on sclerotia of *S. sclerotiorum*. Sclerotia inoculated and buried at depths of 0, 2.5, 5, 10 and 20 cm showed 40, 75, 100, 100 and 100% infection respectively. Other *Penicillium* spp. reported to colonize sclerotia of *S. sclerotiorum* are *P. brevi-compactum*, *P. corymbiferum*, and *P. cyclopium* var *echinulatum* (Merriman, 1976).

2.2.4 Control of sclerotinia wilt of sunflower

The sclerotinia disease of sunflower is difficult to control. Growth of *S. sclerotiorum* and production of sclerotia occur in a temperature range from about 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. Tanrikut and Vaughan (1951) found that *S. sclerotiorum* was also capable of growth over a wide range of pH levels. The ability of this fungus to produce sclerotia and its extreme adaptability make *S. sclerotiorum* very difficult to control. Sclerotia are the main overwintering structures and serve as the primary source of inoculum for sclerotinia wilt, head rot and stalk rot (Huang, 1979). As a result, control of sclerotia must be an important component of control strategies for this disease. Sclerotia are well adapted to survive adverse environmental conditions and are therefore difficult to eliminate from soil (Smith, 1972c).

Various methods have been used in attempts to destroy sclerotia in soil or prevent their formation. 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. 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 large 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, beans, peas and mustard as well as some dicotyledonous weeds are hosts of S. sclerotiorum (Dedio et al., 1980). It is recommended that these crops be avoided in the rotation, that proper weed control be maintained and that sunflowers be alternated with cereals, grasses, corn and fallow. Four or five years is recommended as the rotation period between sclerotinia susceptible crops (Campbell and Woods, 1979; Dedio et al., 1980).

The removal of crop residues containing sclerotia from the field to prevent build-up of inoculum is not practical with sunflowers. Combine harvesting often crushes the stems and spreads sclerotia over the field (Alabouvette and Louvet, 1973). 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 soil layers, sclerotia are subjected to more frequent periods of drying and rewetting which contributes to their more rapid disappearance in these areas (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 be able to 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 sclerotia surviving between crops. Poor survival at deeper depths 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). 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 8 neighboring plants. At spacings of 30 and 40 cm, minimal spread of the pathogen occured. 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 with-in row spacing was reduced, disease was also favored. Recommended control procedures suggest that plants be spaced as widely and uniformly as possible and that overseeding be avoided (Dedio et al., 1980). Sunflower plant populations of greater than 35,000 plants per acre are not advised (Campbell and Woods, 1979).

Use of chemicals aimed at reducing the population of sclerotia in the soil and thereby controlling diseases caused by Sclerotinia spp. has not been promising. Alabouvette and Louvet (1973) discuss the use of formaldehyde and methyl bromide for disinfection of the soil. These products destroy sclerotia but the costs of these treatments and the technical difficulties involved make them impractial 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 decay 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. Application of these fungicides as pre-plant incorporated treatments indicated that Ronilan gave significant control but none of the treatments resulted in significant differences in yield when compared to the controls. Gulya concluded that utilizing seed or soil-applied fungicides for control of sclerotinia stalk rot was not promising. Coley-Smith and Cooke (1971) suggested that diseases caused by sclerotial fungi are difficult to control with fungicides due to 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 pathogens (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 2 year period and found a significant difference in resistance among them. In 1979 and 1980, the disease incidence varied from 6 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.

In Manitoba, cultivars used for commercial production of sunflower were found to be susceptible to diseases caused by *S. sclerotiorum* (Hoes, 1978). Resistant varieties are still unavailable (Anonymous, 1978; Huang, 1980a). Huang (1980a) suggested that a screening program, to select lines exhibiting resistance to sclerotinia wilt, is of paramount importance to the production of commercial hybrids for use in the field.

Biological control of sclerotinia wilt of sunflower also appears promising. *Coniothyrium minitans* has been used successfully for biological control of this disease (Huang, 1976; Huang, 1979; Huang, 1980b). 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 in naturally infested fields over 2 successive years. 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 mycoparasite, yield was significantly higher in both years. Huang (1980b) discussed the importance of C. minitans as a biological control agent of sclerotinia wilt of sunflower and believed it 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, Huang (1980b) concluded that biological control, using mycoparasites such as C. minitans may have great potential when used in conjunction with the cultural practices recommended for control of this disease. 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 mycoparasite is available to permit its use in a rational manner (Ayers and Adams, 1982). Use of mycoparasites as a biological control mechanism is still relatively new, and further research is required to assess the capabilities of other fungi to act as biological control agents in the field.

2.3 THE MYCOPARASITE (TALAROMYCES FLAVUS)

Talaromyces flavus (Klocker) Stolk & Sam belongs to the Class Ascomycetes, Subclass Plectomycetidae, Family Eurotiaceae. Penicillium vermiculatum Dangeard represents the conidial stage or anamorph of T. flavus.

Penicillium vermiculatum has been established as a parasite of certain fungi. 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 fungi. When tested in vitro, the fungi not parasitized by P. vermiculatum included Alternaria sp., A. solani (Ell. & G. Martin) L.R. Jones & Grout, Aspergillus fumigatus Fres., A. niger v. Tiegh., Celphalosporium sp., C. gregatum Allington & Chamberlain, Chaetomium sp., C. globosum Kuntze, Fusarium oxysporum Schlecht., F. oxysporum f. lini (Bolley) Snyd. & Hans., Gibberella zeae (Schw.) Petch, Helminthosporium sp., H. avenae Eidam, H. sativum Pam. King & Bakke, Mucor sp., 4 unidentified species of Penicillium, Pythium sp., P. debaryanum Hesse, Phytophthora infestans (Mont.) de Bary, Rhizopus sp., Sclerotinia sclerotiorum (Lib.) de Bary, Sclerotium rolfsii Sacc., Trichoderma sp., and T. lignorum (Tode) Harz.

Contrary to what Boosalis reported, Su and Leu (1980) found that Penicillium vermiculatum could parasitize sclerotia of Sclerotinia 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 5 to 20 cm were seriously lysed. Besides being parasitic on sclerotia of S. sclerotiorum, T. flavus has been reported to penetrate and overgrow mycelium of Verticillium albo-atrum Reinke & Berth. (Dutta, 1981) and to be effective against V. dahliae Kleb. (Marois et al., 1982).

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, research with P. vermiculatum and Verticillium albo-atrum (Dutta, 1981) has shown that when cultural filtrates of P. vermiculatum were added to media inoculated with V. albo-atrum the development of the pathogen was reduced. Dutta 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.

An antibiotic, vermiculine, has been isolated from the culture broth of P. vermiculatum (Fuska et al., 1972). This compound is closely related to the antifungal compound pyrenophorin (Boeckman et al., 1974). Inhibitory activities of vermiculine have been established against Gram-positive bacteria, Trypanosoma cruzi and Leishmania braziliensis, with weaker activity against mycobacteria (Fuska et. al., 1972). It is possible that vermiculine may be involved with inhibitory activities of P. vermiculatum on S. sclerotiorum, but there is no evidence of this in the literature to date.

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). P. 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 Verticillium 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 proved to be an effective biological control agent in reducing verticillium wilt of eggplant caused by V. dahliae (Marois et al., 1982). Field testing at 2 locations showed that T. flavus reduced disease by 76 and 67% in fields 1 and 2 respectively. Throughout the season, disease in the T. flavus -treated plots was always less than disease in the untreated plots. Yield was increased by 18 and 22% in fields 1 and 2 respectively. Results were taken on a weekly basis and yield was always greater from the T. flavus -treated plants than the untreated plants.

Chapter III

MATERIALS AND METHODS

3.1 CONTROL OF SCLEROTIA OF SCLEROTINIA SCLEROTIORUM BY TALAROMYCES FLAVUS

3.1.1 Source of fungal material

In 1976, H. C. Huang isolated *Talaromyces flavus* from the rhizosphere soil of a sunflower field located at Morden, Manitoba. The fungus was identified as *P. vermiculatum*, the anamorph of *T. flavus* (DAOM 172557), by the Biosystematics Research Institute, Agriculture Canada, Ottawa. *Sclerotinia sclerotiorum* was obtained from a sclerotium removed from the stalk of a diseased sunflower plant. This isolate was collected from a field located on the Agriculture Canada Research Station at Morden, Manitoba.

3.1.2 Production of sclerotia for burial studies

To produce sclerotia for burial, petri dishes containing potato dextrose agar (PDA) were inoculated with discs of PDA bearing mycelia of *S. sclerotiorum*. Following an incubation period of 3 weeks at 12 or 15 C in the dark, sclerotia were mature and were easily removed from the plates. To facilitate burial and recovery of sclerotia from soil, fiberglass screening[1] with approximately 42 squares per square centimeter was used. The mesh was cut into 28 by 8 cm sections, folded in half and soldered

[1] Fabuglass fiberglass screening, Root Wire Ltd., Brampton, Ontario and nylon screening, Beaver Lumber, Winnipeg, Manitoba.

into 10 compartments. Two sclerotia were placed in each compartment and a section of red plastic tape[2] 11.0 by 1.1 cm was attached to each bag to facilitate recovery from the soil. One mesh bag containing 20 sclerotia represented one replication of any treatment. All bags were buried at a depth of 6.5 cm in field and greenhouse experiments.

3.1.3 Production of *T. flavus*

Three types of *T. flavus* inoculum were prepared for field and greenhouse application. These were: (1) spore suspension, (2) *T. flavus* grown on autoclaved grain and (3) *T. flavus* grown on sclerotia of *S. sclerotiorum*. For production of a spore suspension, *T. flavus* was cultured in test tubes containing PDA for 12 days at room temperature. To each test tube, 15 ml of sterile distilled water were added to obtain the spore suspension. Spore concentrations were determined using a haemocytometer. Sclerotia to be soaked in a spore suspension were surface sterilized in a solution of 95% ethanol and 5.25% sodium hypochlorite (1:1, v/v) for 90 seconds, rinsed in sterile distilled water for 30 seconds and placed in the spore suspension for 15 minutes. In some experiments, sclerotia were inoculated with *T. flavus* by a dusting procedure. Surface sterilized sclerotia were added to 12 day old plates of the hyperparasite and thoroughly coated with *T. flavus* spores. Sclerotia appropriately soaked or dusted were placed in mesh bags for burial.

In 1981, field studies involved the use of *T. flavus* produced on a substrate of rye and barley (1:1, v/v). The seed mixture was placed in jars, soaked in warm water for one hour, drained and autoclaved for 15

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

minutes at 121 C, three times over three successive days. The grain was inoculated with a spore suspension of T. flavus and incubated for 15 days at room temperature. The inocula was air-dried for 3 days prior to application.

In 1982, field sclerotial burial studies involved the use of T. flavus produced on sclerotia of S. sclerotiorum. These were obtained from head rot of sunflower. Sclerotia were chopped using a motorized meat grinder and screened. Those less than 4mm in size were rinsed twice in water and placed in autoclavable bags. Over two successive days, the sclerotia were autoclaved, once each day for one hour at 121 C. The sclerotia were inoculated with 20 to 40 ml of a T. flavus spore suspension and incubated for three days at room temperature. The bags were then emptied into plastic flats where T. flavus developed rapidly. The inocula were broken up and air-dried prior to application.

Talaromyces flavus produced on grain or on sclerotia was added to the soil with healthy sclerotia according to the following procedure. In the greenhouse sclerotia in mesh bags were buried at a depth of 6.5 cm in 20 cm pots. The soil layer 2.5 cm above and below the mesh bags was thoroughly mixed with 58 gm of air-dried inocula produced on grain. In the field, the inocula was applied in a similar manner with 200 gm of the air-dried T. flavus grain mixture dispersed throughout the soil above and below the five mesh bags. This involved an area of 28 cm^2. In both greenhouse and field experiments, a separate treatment utilizing uninoculated grain was incorporated. The grain was prepared in the same manner as that used for T. flavus inoculation.

When T. flavus was produced on sclerotia for use in the field, 130 gm of air-dried inocula were mixed with soil 2.5 cm above and below five mesh bags. All field experiments were set up using a split plot design, with five mesh bags per main plot. Greenhouse experiments were conducted using a randomized complete block design with one mesh bag per pot. Analyses of variance were carried out on results obtained and the means were compared using Fisher's least significant difference test. Where data was transformed for analysis, means were converted back to raw form for ease of comparison and interpretation.

3.1.4 Greenhouse experiments

The relationship between T. flavus and S. sclerotiorum was assessed by burying sclerotia, appropriately treated, in 20 cm plastic pots at a 6.5 cm depth. Four greenhouse experiments were conducted. In the first experiment, unsterilized, clay loam soil was used and treatments were sampled at one month intervals over a four month period. Treatments included: (1) sclerotia, (2) sclerotia with T. flavus and (3) sclerotia with autoclaved grain. In each pot of treatment two, 58 gm of T. flavus inoculum were added. The inoculum was produced on a rye and barley grain mixture. The third treatment involved the addition of 58 gm of autoclaved, air-dried grain to the soil. All treatments were replicated four times. Pots were watered periodically to maintain soil moisture near field capacity. The temperature range of the greenhouse was 20 to 22 C. Following the prescribed burial periods, the mesh bags were recovered and sclerotia were removed to be plated out onto PDA.

The second experiment involved the following treatments: (1) sclerotia soaked in a spore suspension of T. flavus and (2) sclerotia alone. Each treatment was replicated twice using unsterilized, clay loam soil and two sampling dates of 15 and 30 days were employed. In the third experiment, the treatments were: (1) sclerotia, (2) sclerotia soaked in water, (3) sclerotia soaked in a spore suspension of T. flavus and (4) sclerotia dusted in T. flavus spores. The soil was sterilized using a 'Lanza' soil pasteurizer[3] and the treatments were replicated four times. Mesh bags were removed at 15 and 30 days.

Treatments of the fourth experiment were: (1) sclerotia, (2) sclerotia soaked in water, (3) sclerotia soaked in a T. flavus spore suspension of 3.2×10^7 conidia/ml and (4) sclerotia soaked in a T. flavus spore suspension of 6.1×10^7 conidia/ml. All treatments were replicated four times in sterilized and unsterilized soil and sampled after 15 days. The temperature range of the greenhouse where experiments two through four were conducted ranged from 21 to 24 C.

3.1.5 Field Experiments

In 1981 and 1982, experiments were conducted to assess the ability of T. flavus to parasitize sclerotia of S. sclerotiorum in the field. In 1981, the work was conducted at the Morden Research Station in clay loam soil. The experiment consisted of five replicates of three treatments arranged in a split plot design. The treatments were: (1) sclerotia, (2) sclerotia and 200 gm of T. flavus inocula and (3) sclerotia and 200 gm of autoclaved grain. The main plots were the treatments and

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[3] Model LB, Johnson Machine Co., Ltd., Burlington, Ontario.

the subplots were the sampling dates. The grain substrate used in treatments two and three was incorporated into 3.8 kg of moist field soil and placed at a depth of 2.5 cm above and below the mesh bags. Five sampling dates were included with four occurring at monthly intervals during the season, and the last occurring the following spring in May of 1982.

Two sclerotial burial experiments were conducted in 1982 at the University of Manitoba field stations located at Portage la Prairie and Winnipeg. Each experiment was set up as a split plot design. The treatments of the first experiment were: (1) sclerotia, (2) sclerotia soaked in a spore suspension of T. flavus and (3) sclerotia soaked in water. Each treatment was replicated six times. Plots at both locations were sampled four times at three week intervals. The second experiment involved two treatments: (1) sclerotia alone and (2) sclerotia with 130 gm of T. flavus on autoclaved sclerotia incorporated into a 5.0 cm layer of soil surrounding the mesh bags. All treatments were replicated six times, with four sampling dates at three week intervals. Soil moisture readings were taken twice weekly at the Portage location using a portable tensiometer.[4] In the Winnipeg plot area, soil temperature readings were taken twice daily, two times per week. Three screen-cage psychrometers[5] were buried in the plot area at a depth of 6.5 cm. These were connected to a thermocouple thermometer[6] to read the soil temperature at the required depth. Soil types at the Portage and Winnipeg lo-

[4] Model 2900, series E, Soil Moisture Equipment Corporation, Box 30025, Santa Barbara, Calif., 93105.

[5] Model 74-13, J.R.D. Merrill Speciality Equipment, Logan, Utah.

[6] Model TH-65, Wescor Inc., Logan, Utah.

cations were silty clay loam and silty clay soil, respectively.

3.2 CONTROL OF SCLEROTINIA WILT OF SUNFLOWER BY TALAROMYCES FLAVUS

3.2.1 Source of fungal material and seed

Talaromyces flavus was isolated from the rhizosphere soil of a sunflower plant located in a field near Morden, Manitoba. *Sclerotinia sclerotiorum* was collected from a diseased sunflower plant in the same area. Sclerotia used for soil inoculation were obtained from diseased sunflower heads. They were obtained from the Agriculture Canada Research Station located in Morden, Manitoba during their seed cleaning procedures. Once sclerotia were chopped and screened, those from 4.0 to 6.0 mm in size were retained and used for field experiments. All sclerotia were stored at 4 C until required for experimental use.

Sclerotia produced in culture were used in some greenhouse experiments. Plates of PDA were inoculated with discs of agar bearing mycelia of *S. sclerotiorum*. Following a three week incubation period in the dark at 12 C, sclerotia were mature and removed for experimental use.

A sunflower hybrid, Hybrid 894, was used in all experiments. This seed was obtained from the Agric. Can. Res. Station at Morden, Manitoba.

3.2.2 Inoculum production

Three types of *T. flavus* inoculum were prepared for field and greenhouse application. These were: (1) spore suspension, (2) *T. flavus* grown on sclerotia and (3) *T. flavus* grown on wheat bran. To produce the spore suspension, *T. flavus* was cultured on PDA in test tubes for 12 days at room temperature. To each test tube, 15 ml of sterile, distilled

water was added, and spores were dislodged to obtain the spore suspension. Spore concentrations were determined using a haemocytometer. Sclerotia to be soaked in the spore suspension were surface sterilized in 95% ethanol and 5.25% sodium hypochlorite (1:1, v/v). They were soaked for 90 seconds, rinsed in sterile, distilled water for 30 seconds and placed in the spore suspension for 15 minutes. Sclerotia were used immediately, or incubated in a moist chamber for 15 days at room temperature.

Inoculum for field application was prepared by growing T. flavus on wheat bran. The bran was thoroughly soaked in water, drained and placed in glass jars. All jars were autoclaved at 121 C for one hour each day over two successive days. The bran was inoculated with T. flavus and incubated at room temperature for nine days. Prior to application, the inocula was air-dried for two days.

3.2.3 Greenhouse experiments

The ability of T. flavus to reduce sclerotinia wilt of sunflower was assessed by incorporating T. flavus and sclerotia of S. sclerotiorum into the seed region of the soil. Four greenhouse experiments were conducted. For all experiments, one 20 cm pot containing 10 plants represented one replicate. In the first experiment the treatments were: (1) sunflower, (2) sunflower with sclerotia, (3) sunflower with sclerotia soaked in a spore suspension of T. flavus and (4) sunflower, sclerotia and sclerotia supporting a 15 day growth of T. flavus. Each treatment was replicated four times. Moist clay loam soil was sterilized using a 'Lanza' soil pasteurizer. Prior to planting, sunflower seeds were sur-

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face sterilized in a solution of 5.25% sodium hypochlorite and sterile water (1:4, v/v) for five minutes, rinsed twice in sterile water and placed in moist chambers to germinate. Healthy lab-cultured sclerotia to be soaked in spore suspension were also surface sterilized prior to use.

In the second experiment, the treatments were: (1) sunflower, (2) sunflower with field sclerotia and (3) sunflower, field sclerotia and _T. flavus_ grown on lab-cultured sclerotia. The soil was not sterilized and sunflower seeds and sclerotia were not surface sterilized. Each treatment was replicated six times.

Treatments used in the second and third experiments were the same. However, in the third experiment, both unsterile and sterile soil were used and the three treatments were replicated twice. For experiments one to three, _T. flavus_ and _S. sclerotiorum_ were placed in the soil with the seed. One pregerminated seed, two healthy sclerotia and one sclerotia overgrown by _T. flavus_ were placed in each of 10 holes per pot of soil. In experiment one, treatment three, two sclerotia soaked in a _T. flavus_ spore suspension were placed with the seed. All three experiments, including the fourth experiment, were set up as randomized complete block designs. The experiments were carried out in a greenhouse having a temperature range of 21 to 22 C. Plants were watered daily to maintain normal plant growth, and disease readings were taken over a four week period.

The fourth experiment involved the use of _T. flavus_ produced on wheat bran. The treatments were: (1) sunflower, (2) sunflower and bran, (3) sunflower and _T. flavus_, (4) sunflower and sclerotia and (5) sunflower,

sclerotia and *T. flavus*. For treatments two, three and five, 16 gm of air-dried inoculum or bran were added to the soil. For treatments four and five, 250 field sclerotia were used for artificial infestation of the soil. Sclerotia, bran and/or inoculum were mixed with 0.8 kg of soil prior to placement in pots. This mixture was divided evenly and placed above and below the seed, which was planted at a depth of 6.5 cm. Pots were placed in a growth cabinet having 16 hour light periods at 20 C and 8 hr dark periods at 15 C. Each treatment was replicated four times and pots were watered daily to maintain normal plant growth. Disease readings were taken over a four week period. At the end of this period, plants were cut off at the soil level. The pots from treatments four and five remained in the growth cabinet for four weeks to dry out. Sclerotia were recovered by hand and tested for the presence of *T. flavus*.

3.2.4 Field experiments

In 1982, experiments were conducted in fields located at Winnipeg and Portage. At each location, 12 plots with four replicates of three treatments 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) sunflower (2) sunflower and sclerotia and (3) sunflower, sclerotia and *T. flavus*. At seeding time, 90 seeds of hybrid 894, 250 field sclerotia and 100 gm of air-dried inoculum 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 the length of each row and the trenches were filled with soil and packed. Plants were thinned to 0.15 m and ex-

amined each week for symptoms of sclerotinia wilt. Symptoms were recorded from the vegetative through to the seed development stages (Siddiqui et al., 1975).

Soil moisture readings were taken at the Portage location using a portable soil tensiometer. Twice weekly, four readings were taken.

In the early bloom stage, all plants in the center two rows of each four row plot were bagged with cotton bags (28 cm^2) to prevent bird damage and obtain accurate seed yields. Sunflowers at the Winnipeg and Portage locations were harvested by hand after 140 and 130 days, respectively. The heads were placed in a drying room at 30 C for 18 to 20 days, after which they were threshed and the seed cleaned and weighed.

Following harvest, 20 soil samples were taken from within the rows of each treatment at both locations. The purpose of the sampling was to screen soil for remaining sclerotia and determine whether or not they were viable or parasitized by *T. flavus*. At Winnipeg and Portage, the soil samples averaged 0.43 and 0.40 kg, respectively. To remove sclerotia from the Winnipeg samples, the soil was soaked in warm water, homogenized in a high volume Waring Blender[7] and washed through a wire screen with approximately 42 squares per square centimeter. Sclerotia remained on the screen and were retained for viability testing. The Portage soil samples were treated in a similar manner, but were not homogenized in a blender due to the lighter soil type.

Analysis of variance was carried out on field and greenhouse results and means were compared using Fisher's least significant different test. Where data was transformed for analysis, means were converted back to

[7] Canlab Division of McGaw Supply Ltd., Winnipeg, Manitoba.

raw form for ease of comparison and interpretation.

3.3 HYPHAL INTERACTIONS BETWEEN *SCLEROTINIA SCLEROTIORUM* AND *TALAROMYCES FLAVUS*

3.4 MATERIALS AND METHODS

3.4.1 Bright field microscopy

For bright field microscopy, dual cultures of *T. flavus* and *S. sclerotiorum* were established in petri dishes containing sucrose-peptone agar at one-fifth the normal concentration. The agar was inoculated with agar discs bearing mycelium of *S. sclerotiorum* and incubated at 18 C in the dark for two days. The dishes were then removed, and spores of *T. flavus* were gently dusted onto the agar surface along the edge of each *Sclerotinia* colony. Following an incubation period of two days at 18 C in the dark, agar pieces 10 x 15 x 3 mm were removed and placed on glass slides. Photomicrographs of the unstained material were taken using a Zeiss Standard Microscope.

3.4.2 Fluorescence microscopy

For fluorescence microscopy, polycarbonate membranes[8] 47 mm in diameter were placed on the surface of the agar. Each membrane was inoculated with agar bearing mycelium of *S. sclerotiorum* and incubated as above. Following the addition of *T. flavus* spores and further incubation, sections of the membranes were removed and fixed in formalin-acetic acid-alcohol (FAA) for 24 hours at room temperature. To complete the preparation of the material for fluorescence microscopy, the method of

[8] Nucleopore Corporation, Pleasanton, CA 94566.

Rohringer _et al_. (1977) was followed. The membrane sections were washed twice, for 15 minutes each time, with 50% ethanol followed by 0.05M NaOH. The material was washed three times with water over a 45 minute period and then placed in 0.1M Tris/HCl buffer, pH 8.5 for 30 minutes. A 0.1% solution of Calcofluor White M2R New[9] in the above Tris/HCl buffer was used to stain the material for 5 minutes. Following four 10 min washes with water and a 30 min wash with 25% aqueous glycerol, the specimens were mounted in glycerol containing a drop of lactophenol as a preservative. A Zeiss Standard Microscope, equipped with epifluoresence attachments, was used to examine the material.

3.4.3 Scanning electron microscopy

For scanning electron microscopy (SEM), glass slides were coated with sucrose-peptone agar (one-fifth the normal concentration) to a thickness of about 1 mm. Agar pieces bearing mycelium of the host or hyperparasite were placed 20 mm apart on the coated slides. The slides were placed in moist chambers and incubated at 18 C for 5 days in the dark. The cultures were examined briefly with a light microscope under low power for areas of interaction. Agar sections 2 x 2 x 1 mm were cut from these areas using a razor blade, and these were placed in cold phosphate buffer, 0.025M, pH 6.8, until all material was collected. The agar sections were placed in cold 5% glutaraldehyde in phosphate buffer, 0.025M, pH 6.8, for 3 hours. After 5 rinses in phosphate buffer at hourly intervals, the material remained in the buffer overnight. Agar pieces were then post-fixed in 2% phosphate-buffered osmium tetroxide

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[9] Polysciences, Inc., Warrington, Pa. 18976.

for 2 hours. Following 3 rinses with phosphate buffer at 20 minutes per rinse, the agar pieces were dehydrated using an ethanol series of 20, 40, 60, 80, 90, 95% and absolute alcohol. All previous steps were conducted on ice, while the dehydration was carried out at room temperature. The agar pieces were placed in each solution for 15 min with the exception of absolute alcohol where 3 changes of 15 min each were used. The material was stored at -12 C until required and then removed from the freezer and allowed to reach room temperature. A final change of absolute alcohol occurred and the material was critical-point dried using liquid CO_2 in a Sorvall critical-point drying Device.[10] Agar pieces were then attached to aluminum stubs and coated with gold in a Balzer Sputtering Device.[11] Total thickness of the coating was about 25 nm. Specimens were examined with a Cambridge stereoscan Mark IIA.

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[10] Ivan Sorvall Inc., Newton, Connecticut 06470.

[11] Balzers Union Ltd., P.O. Box 75, FL-9496 Balzers, Principality of Liechtenstein.

Chapter IV

RESULTS

4.1 CONTROL OF SCLEROTIA OF SCLEROTINIA SCLEROTIORUM BY TALAROMYCES FLAVUS

4.1.1 Greenhouse experiments

Greenhouse studies showed that *T. flavus* was destructive to sclerotia of *S. sclerotiorum*. Significant differences were found between the treatments in the first experiment where *T. flavus* was grown on grain (Appendix 1 and Table 1). While sclerotia infected with *T. flavus* were recovered in all treatments, the number of these sclerotia was significantly higher in the *T. flavus*- treated plots than in the untreated controls (Table 1).

Significant differences were also found between sampling dates (Table 2). Fewer sclerotia were recovered at later sampling dates for all treatments. Of these sclerotia, fewer were infected with *T. flavus* as time of burial increased.

A significant interaction between treatment and sampling date occurred for the number of viable sclerotia (Figure 1B). The number of viable sclerotia was significantly higher in the *T. flavus*- treated pots as compared to the control. However, Figure 1B suggests that at 4 and 8 weeks, there were no significant differences in number of viable sclerotia between treatments.

TABLE 1. Effect of *T. flavus* on the survival and viability of sclerotia of *S. sclerotiorum* in soil (experiments 1 and 2).

	Sclerotia/replicate[1]									
	Buried		Recovered		Viable		Infected by *T. flavus*		Infected by *Trichoderma*	
Treatment[2]	1	2	1[3]	2	1	2	1	2	1	2
Sclerotia alone	20	20	17.47a[4]	20	7.00a	11.75b	2.15a	1.25a	-	6.00a
Sclerotia + grain	20	-	18.49b	-	5.50a	-	1.70a	-	-	-
Sclerotia + *T. flavus* produced on grain	20	-	18.61b	-	8.90b	-	3.45b	-	-	-
Sclerotia soaked in spore suspension of *T. flavus*	-	20	-	20	-	1.50a	-	1.25a	-	12.50b

[1]Values averaged over 4 replicates.

[2]Comparison over all sampling dates.

[3]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 2. Effect of sampling date on survival and viability of sclerotia of *S. sclerotiorum* in soil (experiments 1 and 2).

Sampling date (wks)[2]	Sclerotia/replicate[1]									
	Buried		Recovered		Viable		Infected by *T. flavus*		Infected by *Trichoderma*	
	1	2	1[3]	2	1	2	1	2	1	2
2	-	20	-	20	-	8.00a	-	1.00a	-	8.50a
4	20	20	20	20	8.60b[4]	5.25a	4.20c	1.50a	-	10.00a
8	20	-	18.66	-	4.80a	-	2.53b	-	-	-
12	20	-	17.99	-	8.40b	-	1.93ab	-	-	-
16	20	-	15.90	-	6.73ab	-	1.07a	-	-	-

[1]Values averaged over 4 replicates.

[2]Comparison over all treatments.

[3]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).

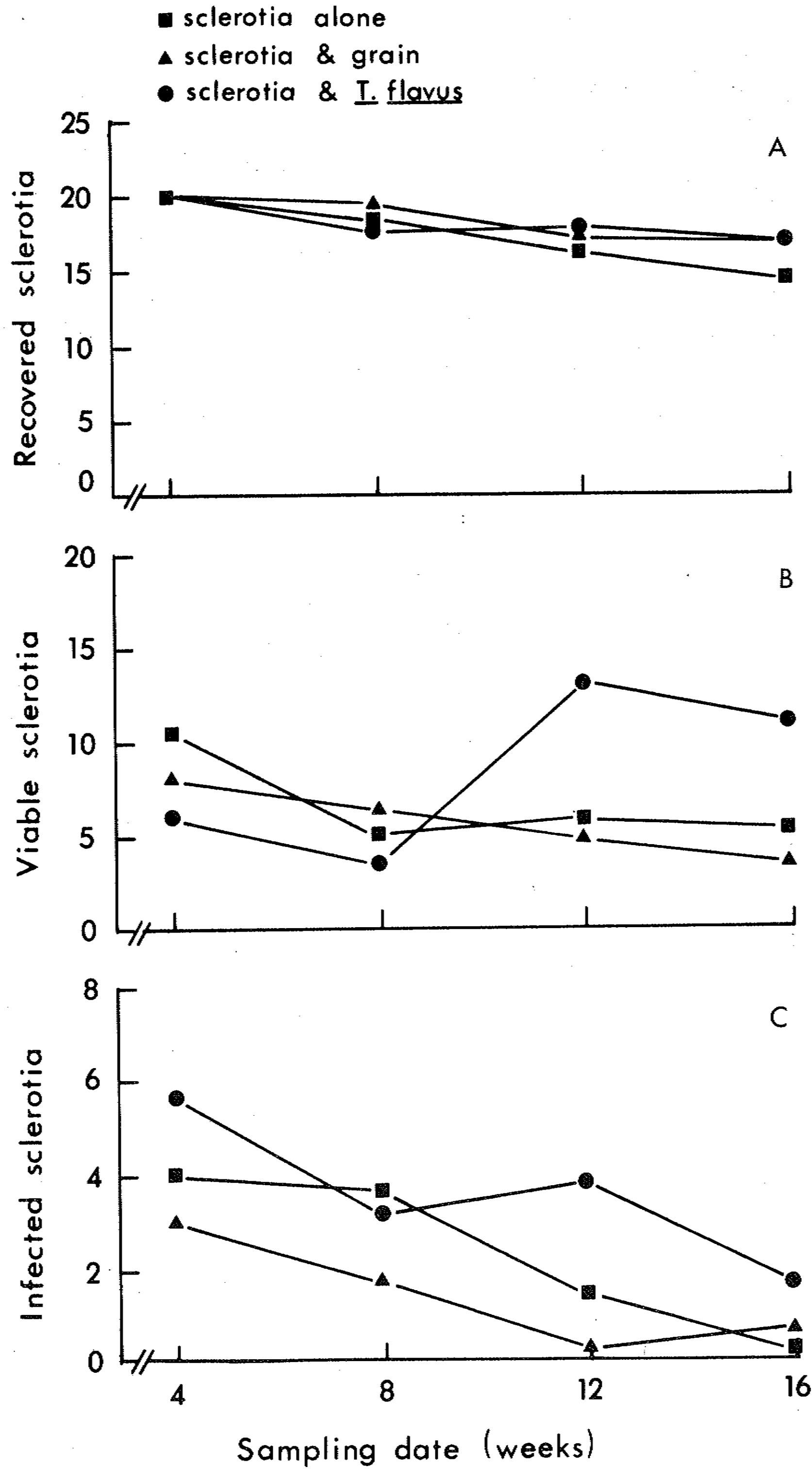


Figure 1. The effect of T. flavus on survival of sclerotia of S. sclerotiorum (greenhouse experiment, Morden).

Only at 12 and 16 weeks were there marked differences in sclerotial numbers between treatments. No significant interaction between treatments and between sampling dates were found for recovered sclerotia and infected sclerotia (Figure 1A, 1C and Appendix 1).

In the second experiment, the number of sclerotia infected with T. flavus in the T. flavus- treated pots was no different from the number found in the control (Table 1). However, the number of viable sclerotia in the T. flavus- treated pots was significantly lower than that of the control. The reduction in viability associated with the T. flavus treatment was due primarily to the presence of a Trichoderma species. Sclerotia in both the control and the T. flavus treatment were infected with Trichoderma indicating that this fungus was present as part of the soil microflora. No significant differences between sclerotial numbers occurred over time (Table 2 and Appendix 2).

The first two experiments showed a low sclerotial infection by T. flavus. Consequently, a third experiment using sterilized soil was performed. Significant differences between treatments and between sampling dates occurred (Appendix 3). The number of viable sclerotia was significantly higher in the controls as compared to the T. flavus treatments (Table 3). T. flavus was isolated at a much higher rate from the two T. flavus treatments as compared to the control. This indicates that T. flavus is effective in reducing the number of viable sclerotia of S. sclerotiorum under sterile conditions. Significant differences between numbers of viable sclerotia at 15 and 30 days occurred (Table 4 and Appendix 4). More sclerotia were infected with T. flavus at 15 days as compared to 30 days.

TABLE 3. Effect of *T. flavus* on survival and viability of sclerotia of *S. sclerotiorum* in sterile soil.

Treatment[2]	Sclerotia/replicate[1]			
	Buried	Recovered	Viable	Infected by *T. flavus*
Sclerotia	20	20	15.50c[3]	2.00a
Sclerotia soaked in water	20	20	11.60b	3.00a
Sclerotia soaked in *T. flavus* spore suspension	20	20	0.75a	12.75c
Sclerotia dusted with *T. flavus* spores	20	20	0.75a	8.38b

[1]Values averaged over 4 replicates.

[2]Comparison over all sampling dates.

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

TABLE 4. Effect of sampling date on survival and viability of sclerotia of *S. sclerotiorum* in sterile soil.

Sampling date[2] (days)	Sclerotia/replicate[1]			
	Buried	Recovered	Viable	Infected by *T. flavus*
15	20	20	6.13a[3]	9.19b
30	20	20	8.19b	3.88a

[1]Values averaged over 4 replicates.

[2]Comparison over all treatments.

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

Analysis of variance showed a significant block effect for infected sclerotia (Appendix 3). Due to a space shortage, 2 blocks were not located adjacent to the remaining blocks. Equal numbers of pots appeared to be influenced by the block effect which should minimize the importance of the effect.

The fourth experiment involved assessment of T. flavus in sterile and unsterile soil. Fewer sclerotia were infected with T. flavus under sterile soil conditions (Table 5). While soil conditions influence the number of sclerotia infected with T. flavus the numbers of viable sclerotia were not affected. Significant differences between treatments were found for both viable sclerotia and infected sclerotia (Table 6 and Appendix 4). T. flavus treatments showed fewer viable sclerotia and more infected sclerotia than the controls. Results indicate that T. flavus is capable of destroying sclerotia of S. sclerotiorum in unsterile soil.

4.1.2 Field experiments

Results of the 1982 field trials conducted at Winnipeg and Portage la Prairie indicated that T. flavus is destructive to sclerotia of S. sclerotiorum. The first experiment of 1982 showed that fewer sclerotia were recovered in the T. flavus- treated plots as compared to the plots where no mycoparasite was applied (Table 7). The numbers of viable sclerotia were also significantly less in the T. flavus- treated plots as compared to the plots where sclerotia were soaked in water prior to burial or buried dry. Although sclerotia infected with T. flavus were recovered from all plots, the number of infected sclerotia was significantly higher in the T. flavus- treated plots (Table 7).

TABLE 5. Effect of soil sterilization on the survival and viability of sclerotia of _S. sclerotiorum_ in soil.

Soil condition[2]	Sclerotia/replicate[1]			
	Buried	Recovered	Viable	Infected by _T. flavus_[3]
Unsterile	20	20	13.25a[4]	2.89b
Sterile	20	20	13.69a	1.81a

[1]Values averaged over 4 replicates.

[2]Comparison over all treatments.

[3]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 6. Effect of T. flavus on the survival and viability of sclerotia of S. sclerotiorum in unsterilized soil.

	Sclerotia/replicate[1]			
Treatment[2]	Buried	Recovered	Viable	Infected by T. flavus[3]
Sclerotia	20	20	18.50c[4]	0.00a
Sclerotia soaked in water	20	20	18.88c	0.00a
Sclerotia soaked in spore suspension of T. flavus (3.2×10^7 conidia/mL)	20	20	11.25b	5.55b
Sclerotia soaked in spore suspension of T. flavus (6.1×10^7 conidia/mL)	20	20	5.25a	7.68b

[1]Values averaged over 4 replicates.

[2]Comparison over both treatments.

[3]Analysis of variance on transformed data; means converted 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 7. Effect of *T. flavus* on survival and viability of sclerotia of *S. sclerotiorum* at 2 locations.

	Sclerotia/replicate[1]							
	Buried		Recovered[3]		Viable		Infected by *T. flavus*[3]	
Treatment[2]	W[4]	P	W	P	W	P	W	P
Sclerotia	20	20	19.59b[5]	19.35b	16.08b	13.67b	0.60a	0.71a
Sclerotia soaked in water	20	20	19.57b	19.56b	17.46c	13.96b	0.44a	0.34a
Sclerotia soaked in a spore suspension of *T. flavus*	20	20	12.78a	13.90a	2.00a	2.25a	7.12b	6.52b

[1]Values averaged over 6 replicates.

[2]Comparison over all sampling dates.

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

[4]W = Winnipeg location; P = Portage La Prairie location.

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

At both locations, significant differences between sampling dates also occurred (Table 8 and Appendices 5 & 6). In general, the numbers of recovered sclerotia, viable sclerotia and sclerotia infected with T. flavus decreased as time from burial increased. However, a significant treatment by sampling date interaction occurred for the variables recovered sclerotia and infected sclerotia (Appendices 5 & 6). This indicates that the effect of sampling date is dependent on the treatment. Results (Table 8) illustrate that over all sampling dates, the numbers of recovered sclerotia were significantly reduced for the T. flavus-treated plots as compared to the controls. However, such a significant difference was found only when sclerotia were buried for 9 weeks or longer but not at 3 weeks (Figures 2 & 3). Marked differences in the number of infected sclerotia between the T. flavus- treated plots and the 2 untreated controls occurred at 3 weeks and decreased as time from burial increased. These results suggest that T. flavus was more effective in destroying sclerotia of S. sclerotiorum as time from burial increased.

A second field experiment produced results similar to the first. T. flavus was effective in reducing the number of sclerotia of S. sclerotiorum in soil (Table 9). At both locations, fewer viable sclerotia were recovered in the T. flavus- treated plots as compared to the controls and higher numbers of sclerotia infected with T. flavus were observed in plots where the mycoparasite was applied. However, due to a significant treatment by sampling date interaction for recovered sclerotia and viable sclerotia at the Winnipeg location (Appendix 7), it is evident that at some sampling dates, results obtained from the T. flavus- treated

TABLE 8. Effect of sampling date on survival and viability of sclerotia of *S. sclerotiorum* at 2 locations.

Sampling date[2] (wks)	Sclerotia/replicate[1]							
	Buried		Recovered[3]		Viable		Infected by *T. flavus*[3]	
	W[4]	P	W	P	W	P	W	P
3	20	20	20.00c[5]	20.00c	12.61b	11.89c	3.34c	3.26b
9	20	20	17.20b	18.82b	12.28b	10.28bc	1.66ab	2.81b
12	20	20	17.07b	16.00a	11.78ab	9.72ab	2.42bc	1.29a
15	20	20	15.89a	16.01a	10.72a	7.94a	1.01a	0.75a

[1]Values averaged over 6 replicates.

[2]Comparison over all treatments.

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

[4]W = Winnipeg location; P = Portage la Prairie location.

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

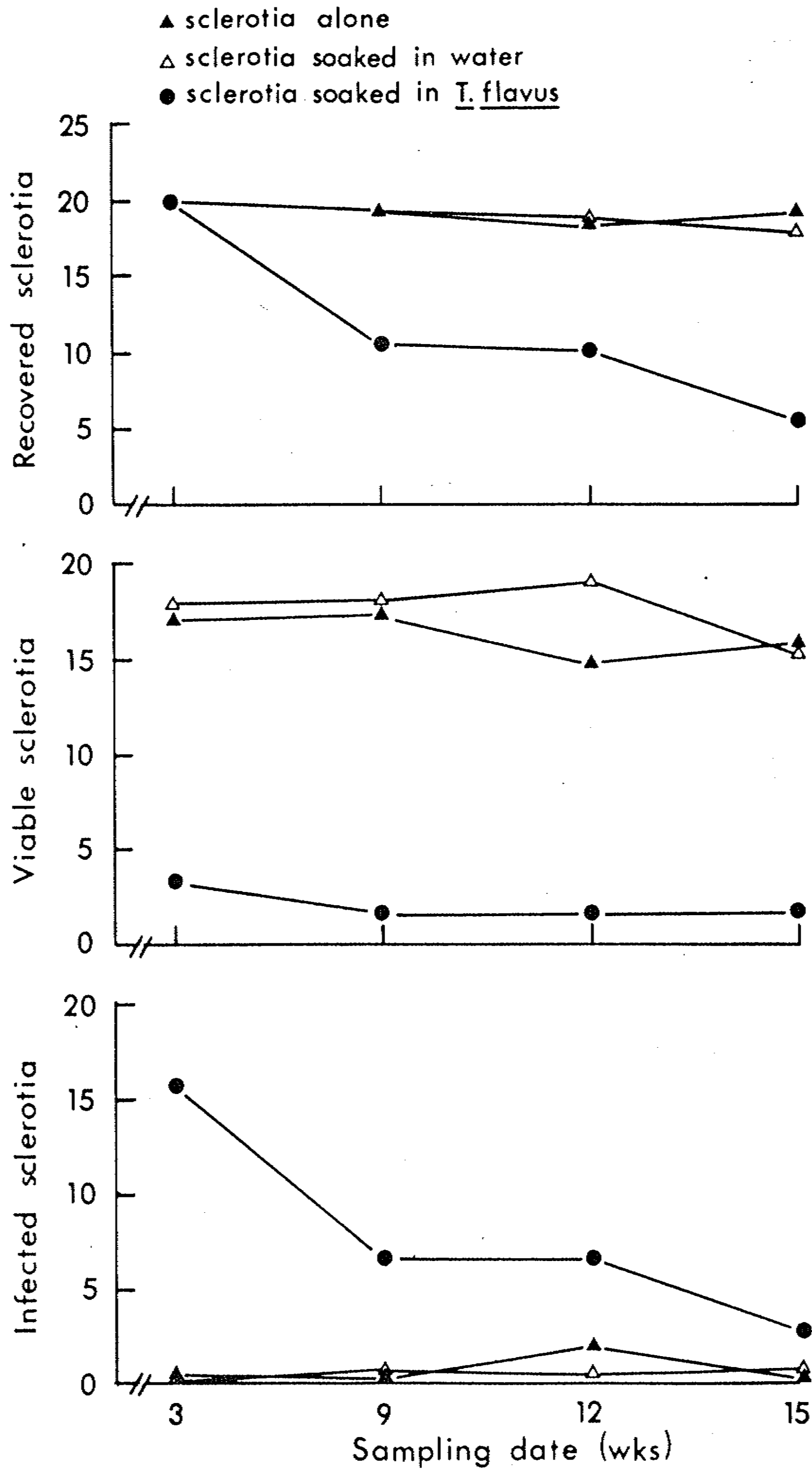


Figure 2. The effect of *T. flavus* as a spore suspension on survival and viability of sclerotia of *S. sclerotiorum* (field experiment, Winnipeg).

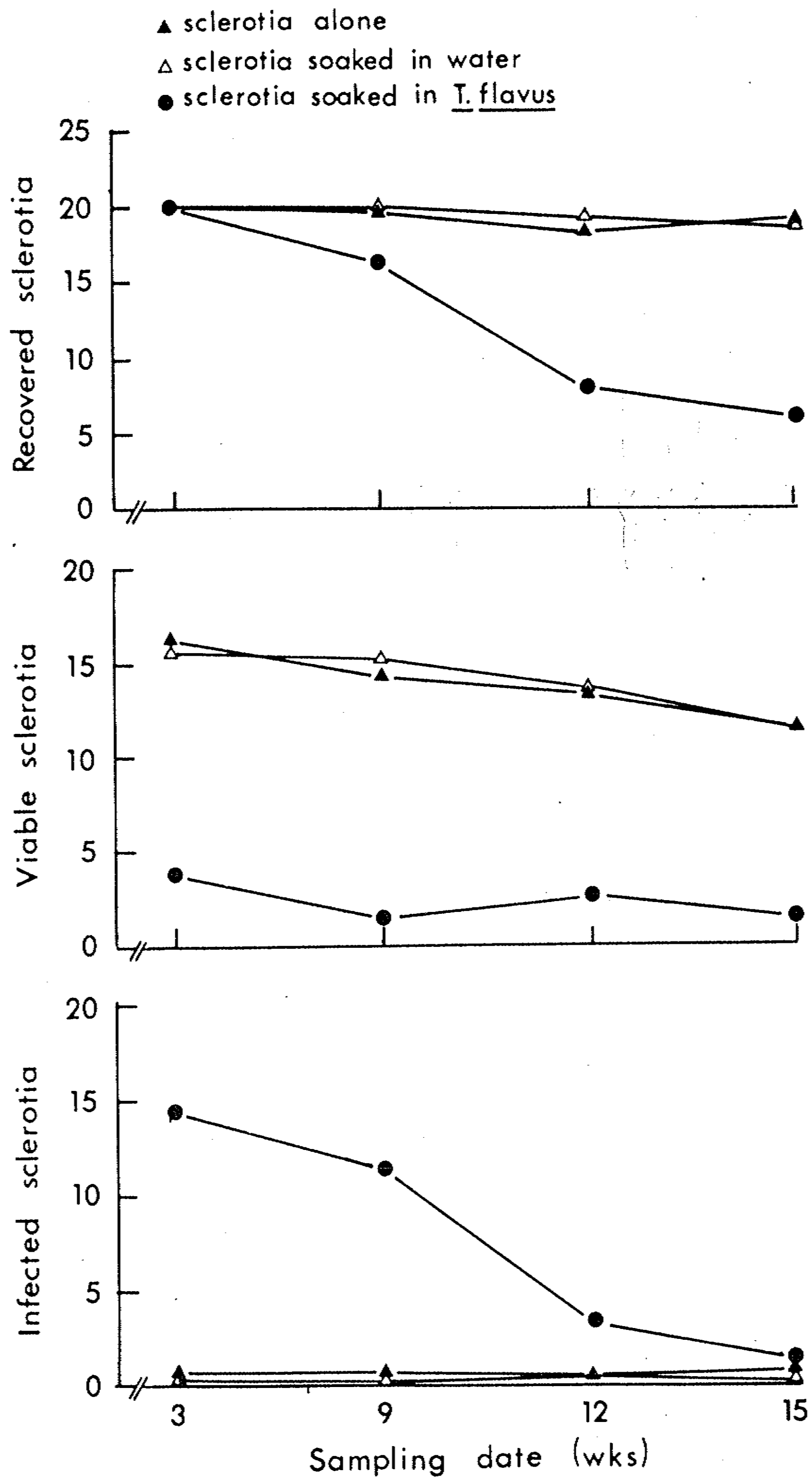


Figure 3. The effect of _T. flavus_ as a spore suspension on survival and viability of sclerotia of _S. sclerotiorum_ (field experiment, Portage la Prairie).

TABLE 9. Effect of T. flavus on survival and viability of sclerotia of S. sclerotiorum at 2 locations.

	Sclerotia/replicate [1]							
	Buried		Recovered		Viable		Infected by T. flavus[3]	
Treatment [2]	W[4]	P	W	P	W	P	W	P
Sclerotia (control)	20	20	19.79b	18.92b[5]	17.67b	13.25b	0.12a	0.58a
Sclerotia and T. flavus (grown on autoclaved sclerotia)	20	20	14.63a	15.54a	7.17a	8.71a	1.11b	2.85b

[1] Values averaged over 6 replicates.

[2] Comparison over all sampling dates.

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

[4] W = Winnipeg location; P = Portage la Prairie location.

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

plots were not significantly different from the controls (Figure 4). The numbers of recovered and viable sclerotia for the *T. flavus*- treated plots did not differ markedly from those of the controls at 3 weeks (Figure 4). However, at 9, 12 and 15 weeks, larger differences were observed. For the control plots, little difference in the numbers of recovered sclerotia occurred over time, whereas in the *T. flavus*- treated plots, a decrease in the numbers of recovered and viable sclerotia occurred as time from burial increased. When sampling dates were compared over all treatments, it was evident that the numbers of recovered sclerotia and viable sclerotia decreased over time (Table 10). However, the reduction in sclerotial numbers is much less for the control than for the *T. flavus* treatment. Similar results were obtained at the Portage location (Figure 5, Table 10 and Appendix 8).

The two experiments conducted at the Winnipeg location show that similar numbers of sclerotia were recovered (Figures 2 & 4). Fewer sclerotia were recovered in the *T. flavus*- treated plots as compared to the remaining plots as time from burial increased. The numbers of viable sclerotia were less in the *T. flavus*- treated plots as compared to the control plots in both experiments (Figures 2 & 4). However, a greater number of viable sclerotia occurred at 3 weeks in the first experiment as compared to the second experiment. This is probably related to the application of *T. flavus* directly to the sclerotia in the first experiment as compared to dispersal of the mycoparasite throughout the soil in the second experiment. When *T. flavus* was applied directly to sclerotia, more were infected with the mycoparasite than when *T. flavus* was added to soil surrounding the sclerotia (Figures 2 & 4). Again, this is

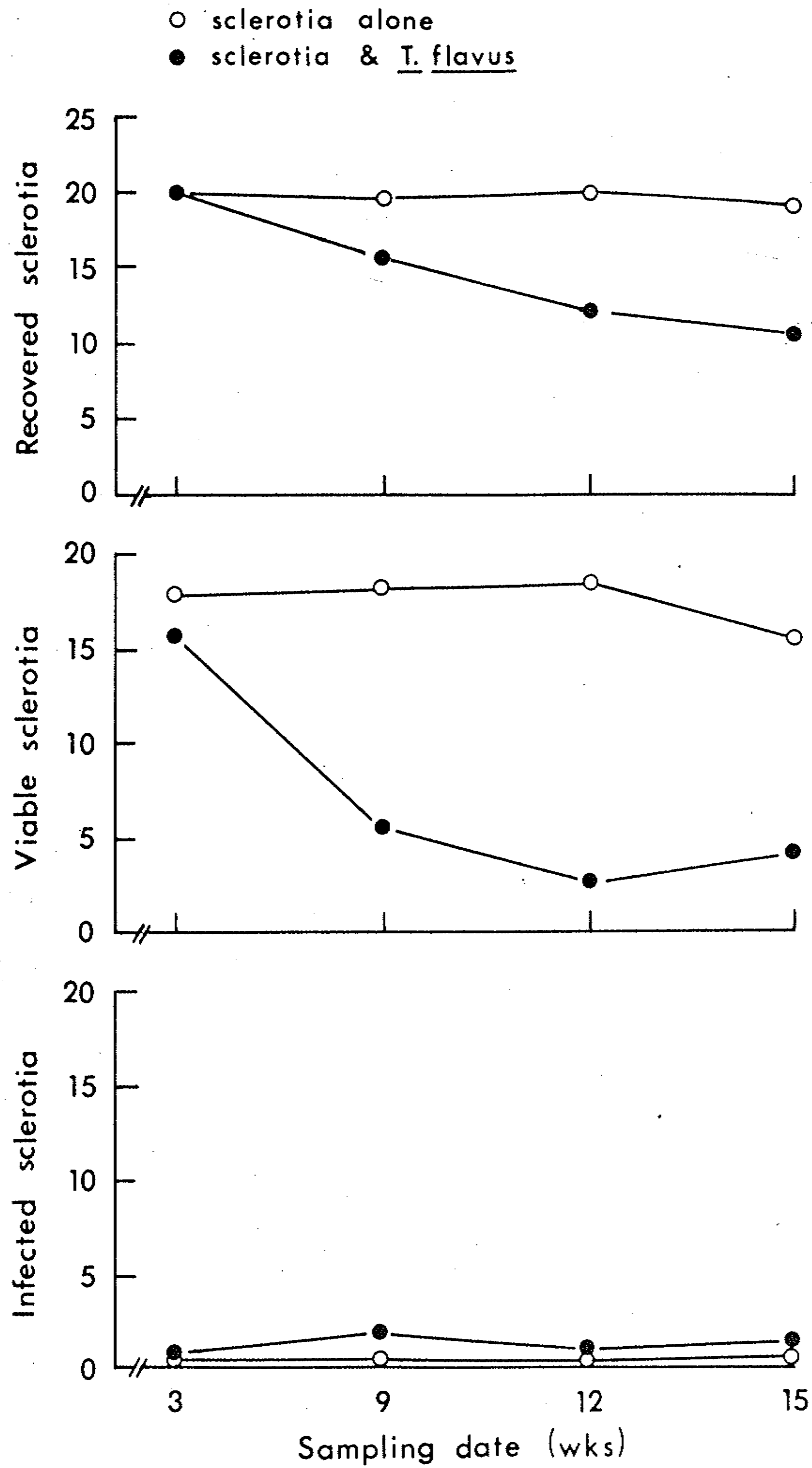


Figure 4. The effect of T. flavus produced on autoclaved sclerotia on survival and viability of sclerotia of S. sclerotiorum (field experiment, Winnipeg).

TABLE 10. Effect of sampling date on survival and viability of sclerotia of *S. sclerotiorum* at 2 locations.

Sampling date[2] (wks)	Sclerotia/replicate[1]							
	Buried		Recovered		Viable		Infected by *T. flavus*[3]	
	W[4]	P	W	P	W	P	W	P
3	20	20	20.00c	20.00c[5]	16.75c	15.00b	0.36a	1.49a
9	20	20	17.92b	19.33c	12.00b	13.00b	0.73a	1.93a
12	20	20	16.08a	15.83b	10.75ab	7.25a	0.52a	1.96a
15	20	20	14.83a	13.75a	10.17a	8.67a	0.64a	0.92a

[1]Values averaged over 6 replicates.

[2]Comparison over all treatments.

[3]Transformed data converted back to raw form for presentation.

[4]W = Winnipeg location; P = Portage la Prairie location.

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

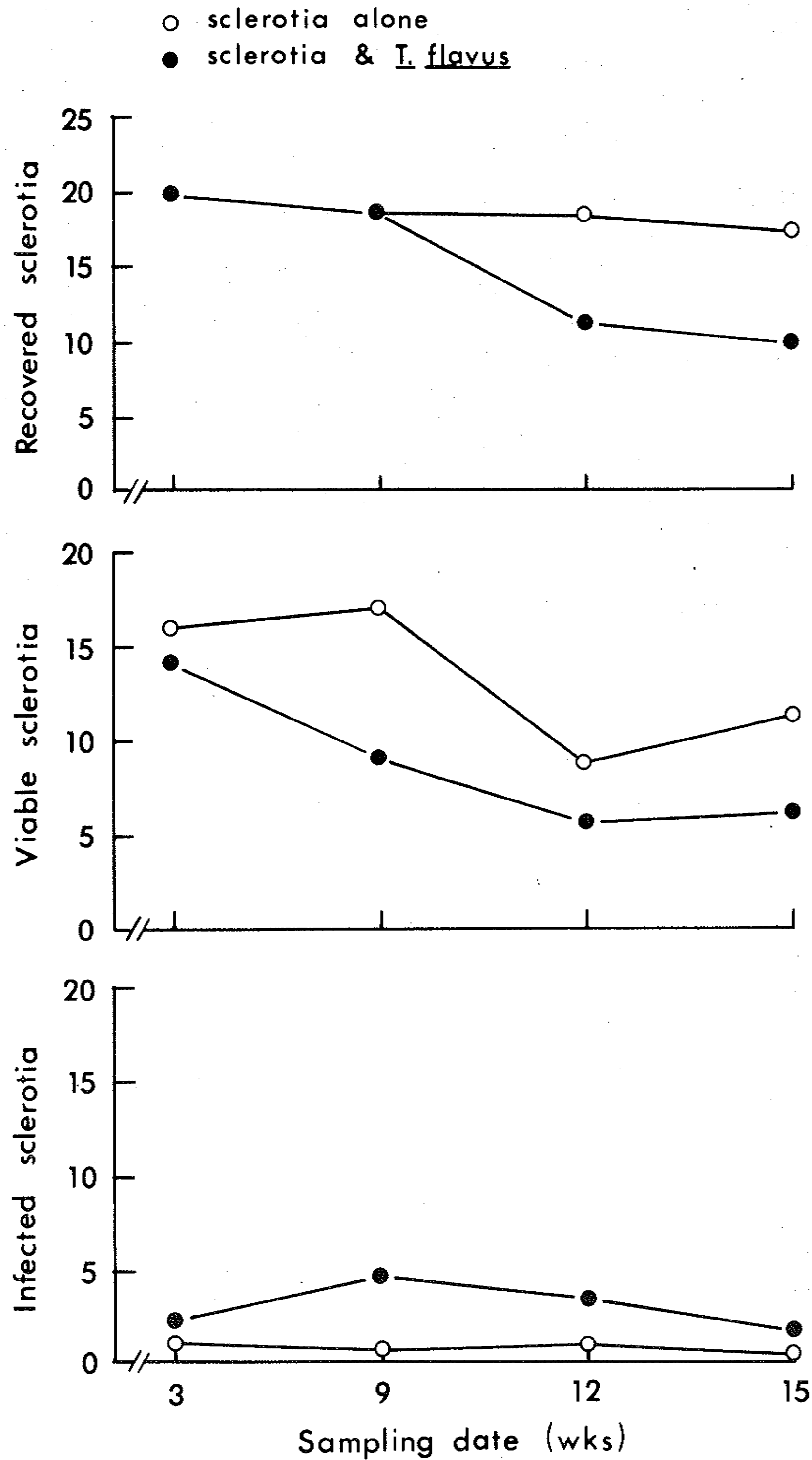


Figure 5. The effect of T. flavus produced on autoclaved sclerotia on survival and viability of sclerotia of S. sclerotiorum (field experiment, Portage la Prairie).

probably due to differences in the method of application of *T. flavus* between experiments.

Results of a field test conducted at Morden (1981) showed no significant differences between the *T. flavus*- treated plots and the plots where sclerotia or sclerotia and autoclaved grain were buried (Appendix 9). Sclerotia infected with *T. flavus* were recovered from all treatments and no treatment showed a significantly higher level of infected sclerotia (Table 11). Although no significant differences occurred between treatments, significant differences between sampling dates for each variable were found (Table 12). The numbers of recovered sclerotia, viable sclerotia and sclerotia infected with *T. flavus* decreased over time, with more sclerotia recovered early in the season. No significant interactions between treatments and sampling dates for all three variables were found, indicating that the effect of sampling date was not dependent on the treatment (Appendix 9).

Soil temperature and moisture influence the survival of sclerotia and the activity of soil microorganisms. Soil temperatures recorded throughout the summer in the soil burial experiments located at Winnipeg ranged from 11.6 to 22.6 C at 8:30 a.m. and from 14.8 to 33.6 C at 4:30 p.m. (Appendix 10). Tensiometer readings taken at the Portage la Prairie site revealed that soil moisture was quite variable at a depth of 6.5 cm. Soil moisture percentages ranged from 11.3 to 44.8% (Appendix 11). Experimental results indicate that *T. flavus* was capable of destroying sclerotia under environmental conditions encountered at both locations. It is probable however that some combinations of soil moisture and temperature are more favorable than others for the destruction of sclerotia by *T. flavus*.

TABLE 11. Effect of *T. flavus* on survival and viability of sclerotia of *S. sclerotiorum*.

	Sclerotia/replicate[1]			
Treatment[2]	Buried	Recovered[3]	Viable[3]	Infected by *T. flavus*
Sclerotia	20	15.27a[4]	7.47a	0.64a
Sclerotia and grain	20	15.55a	8.41a	0.56a
Sclerotia and *T. flavus* produced on grain	20	13.21a	7.42a	0.52a

[1]Values averaged over 5 replicates.

[2]Comparison over all sampling dates.

[3]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 12. Effect of sampling date on survival and viability of sclerotia of *S. sclerotiorum*.

Sampling date[2] (mos)	Sclerotia/replicate[1]			
	Buried	Recovered[3]	Viable[3]	Infected
1	20	18.25c[4]	12.40b	1.33b
2	20	13.45ab	7.47a	0.47a
3	20	15.01b	7.53a	0.53a
4	20	13.97ab	5.77a	0.20a
12	20	12.18a	5.70a	0.33a

[1]Values averaged over 5 replicates.

[2]Comparison over all treatments.

[3]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).

4.2 CONTROL OF SCLEROTINIA WILT OF SUNFLOWER BY TALAROMYCES FLAVUS

4.2.1 Greenhouse experiments

The results in Tables 13 and 14 show that *T. flavus* is effective in controlling sclerotinia wilt of sunflower under greenhouse conditions (Appendices 12 & 13). When the first experiment was set up in sterile soil, sclerotia soaked in a spore suspension of *T. flavus* did not incite sclerotinia wilt on sunflower, and results were not significantly different from the treatment of sunflower alone (Table 13). Where *T. flavus* was grown on sclerotia and added to the soil with healthy sclerotia, wilt of sunflower did occur, but was significantly less than the treatment of soil with sunflower and *S. sclerotiorum* alone.

Where soil was not sterilized, similar results occurred (Table 14). Sclerotinia wilt was evident in the *T. flavus* treatment, but the level of disease was significantly different from treatments lacking the mycoparasite. Results indicate that *T. flavus* was also effective in unsterile soil.

Another experiment was conducted to compare the effectiveness of *T. flavus* in unsterile and sterile soil. Although there were significant differences between treatments, no differences were found between unsterile and sterile soil (Appendix 14). *T. flavus* reduced wilt to a level that did not differ significantly from the treatment of sunflower alone (Table 15). Where no *T. flavus* was applied, incidence of sclerotinia wilt was high.

The effectiveness of *T. flavus* as a biological control agent when grown on wheat bran and dispersed in soil was evaluated in the next experiment. Significant differences between treatments occurred (Appendix

TABLE 13. Biological control of sclerotinia wilt of sunflower by *Talaromyces flavus* in sterile soil.

Treatment	No. of seeds/pot[1]	No. of diseased plants/pot[1]	% disease	Arcsin trans-formation
Sunflower	10	0	0	11.54a[2]
Sunflower & sclerotia soaked in *T. flavus* spore suspension	10	0	0	11.54a
Sunflower & sclerotia and *T. flavus* grown on surface sterilized sclerotia	10	1.75	17.50	24.16b
Sunflower & Sclerotia	10	5.25	52.50	46.51c

[1] Values averaged over 4 replicates.

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

TABLE 14. Biological control of sclerotinia wilt of sunflower by *Talaromyces flavus* in unsterile soil.

Treatment	No. of Seeds/pot[1]	No. of diseased plants/pot[1]	% disease	Arcsin trans-formation
Sunflower	10	0	0	12.25a[2]
Sunflower, sclerotia and *T. flavus* grown on surface sterlized sclerotia	10	1.5	15.0	22.58b
Sunflower & sclerotia	10	3.3	33.3	34.72c

[1] Values averaged over 6 replicates.

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

TABLE 15. Biological control of sclerotinia wilt of sunflower by *Talaromyces flavus* in sterile and unsterile soil.

Treatment	No. of seeds/pot[1]		No. of diseased plants/pot[1]		% diseased		Arcsin transformation[2]	
	Sterile	US	Sterile	US	Sterile	US	Sterile	US
Sunflower	10	10	0	0	0	0	9.98a	9.98a
Sunflower, sclerotia, and *T. flavus* grown on surface sterilized sclerotia	10	10	1.0	0.5	10	5	18.3a	14.2a
Sunflower & sclerotia	10	10	7.5	5.0	75	50	61.2b	45.0b

[1] Values averaged over 2 replicates.

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

15) with less disease and fewer sclerotia recovered in the T. flavus treatment (Table 16). Though less sclerotia from the T. flavus treatment were viable as compared to the control, few of these sclerotia were infected with T. flavus (Figure 6). The number of Talaromyces- infected sclerotia from the T. flavus treatment did not differ significantly from the control. The predominant fungus isolated from nonviable sclerotia was a Trichoderma species which was found in both treatments, but primarily in the control.

4.2.2 Field experiments

Results of the 1982 field trials at Winnipeg and Portage la Prairie show that T. flavus significantly reduced the incidence of sclerotinia wilt and increased yield in sunflower (Table 17, Appendices 16 and 17). Natural inoculum of S. sclerotiorum was present in both fields, averaging 2.9 sclerotia/kg soil at the Portage site and 1.1 sclerotia/kg soil at the Winnipeg site (Figure 7). The incidence of sclerotinia wilt of sunflower incited by the natural inoculum was 30.1 and 3.4% in the control plots of the Portage and Winnipeg sites, respectively (Table 17). The incidence of sclerotinia wilt of sunflower was significantly higher in the S. sclerotiorum- treated plots than in either the control or the mycoparasite-treated plots (Table 17 and Plate 1). However, results from the T. flavus- treated plots did not differ significantly from the control in both fields.

At both locations, the wilt incidence in the S. sclerotiorum- treated plots increased slowly during the vegetative and budding stages of growth (Figures 8 and 9). Throughout the anthesis and seed development

TABLE 16. The effect of *T. flavus* on the incidence of sclerotinia wilt and recoverable sclerotia in sunflowers grown in artificially infested soil.

Treatment	% disease[1]	Arcsin trans-formation	Recovered sclerotia[2,3]
T. flavus and *S. sclerotiorum*	2.5	14.3a	21.50a
S. sclerotiorum	38.2	37.9b	165.00b

[1]Average values from 4 replicates of 10 plants each.

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

[3]Average values from 4 pots of 250 sclerotia each.

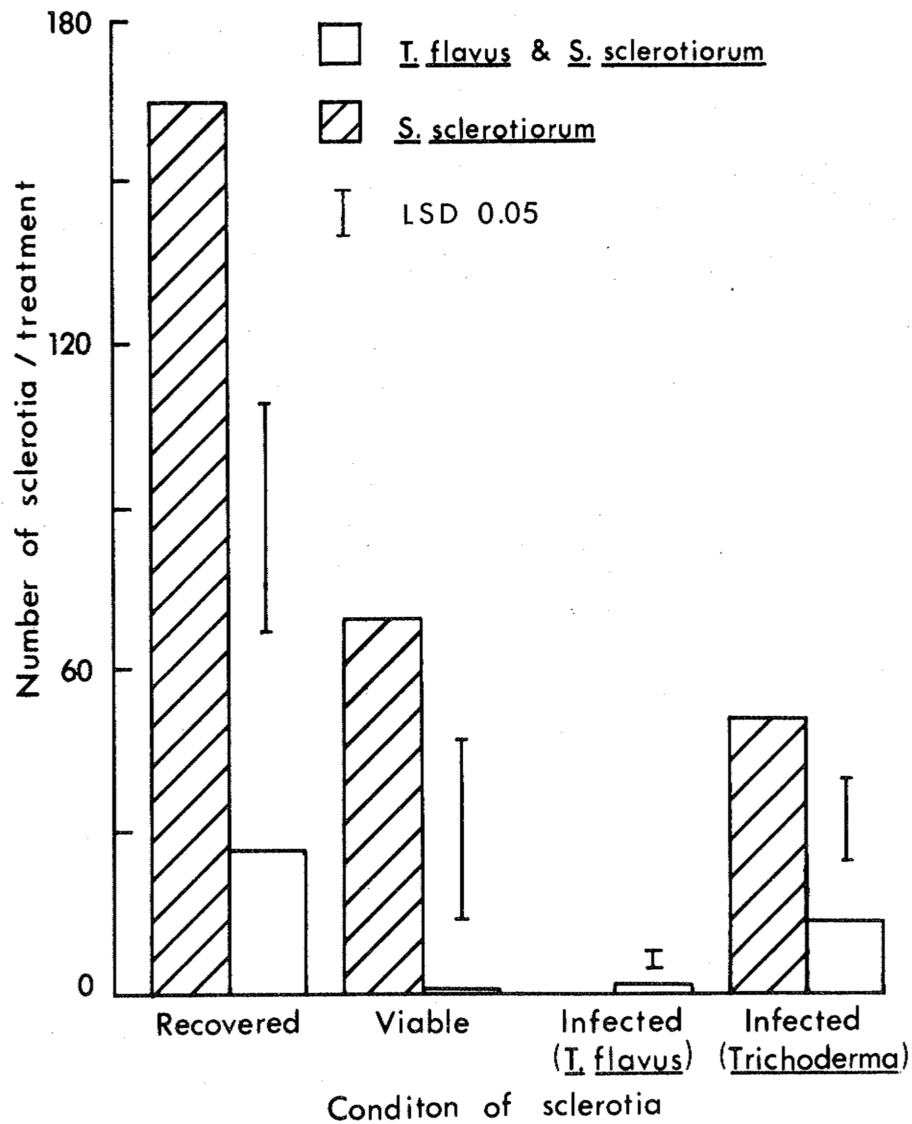


Figure 6. The effect of T. flavus on survival and viability of sclerotia of S. sclerotiorum (greenhouse experiment, Winnipeg).

TABLE 17. Effect of *Talaromyces flavus* on yield and sclerotinia wilt of sunflower at two field locations (1982).

Treatment	Winnipeg		Portage la Prairie	
	Yield[1] (kg/ha)	Diseased plants[1] (%)	Yield[1] (kg/ha)	Diseased plants[1] (%)
Sunflower alone (control)	2,897b[2]	3.4a	1,926b	30.1a
S. sclerotiorum	2,350a	47.2b	1,430a	81.4b
S. sclerotiorum and *T. flavus*	2,870b	3.8a	2,140b	26.6a

[1]Average values from 4 replicates of 4-row plots.

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

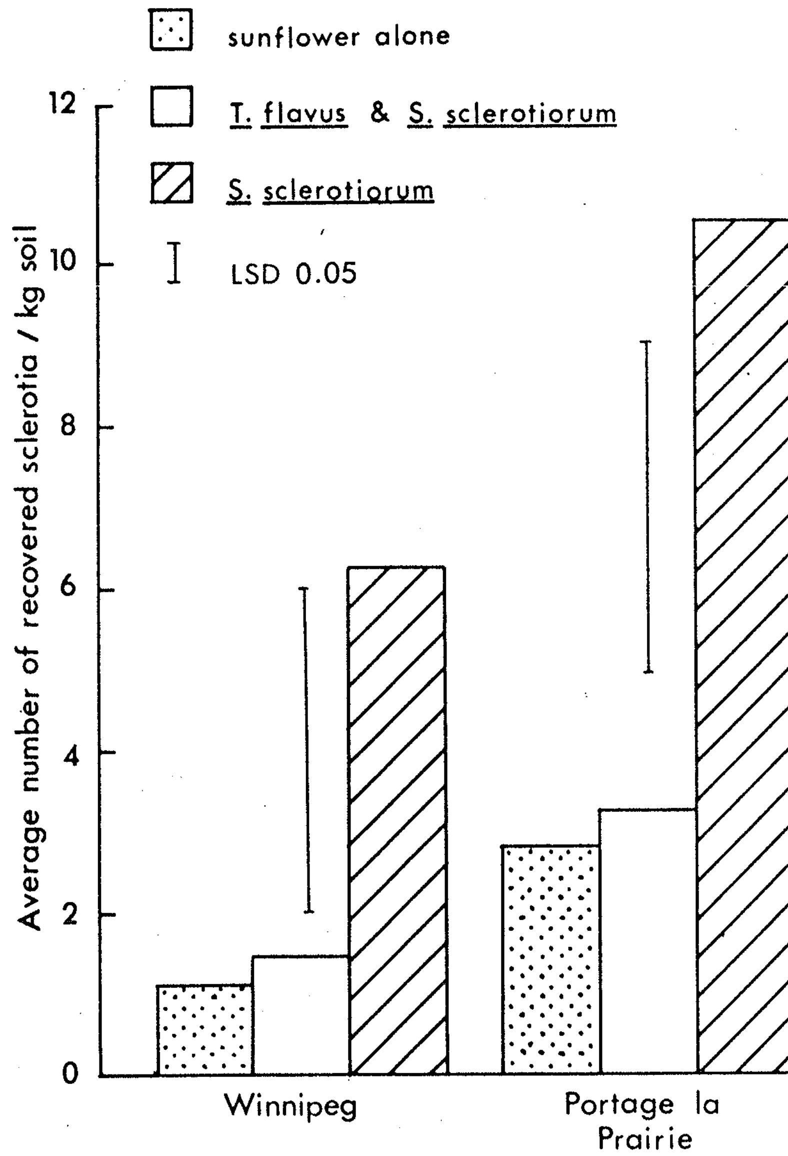


Figure 7. The effect of T. flavus on the number of sclerotia recovered at 2 field locations (Winnipeg and Portage la Prairie).

Plate 1. Biological control of sclerotinia wilt of sunflower by *T. flavus* in plots located at the Portage la Prairie site. Note the healthy appearance of plants in the *T. flavus*-treated plots as compared to the plots where *S. sclerotiorum*, but no mycoparasite, was applied.

S. sclerotiorum
T. flavus &
S. sclerotiorum
sunflower alone

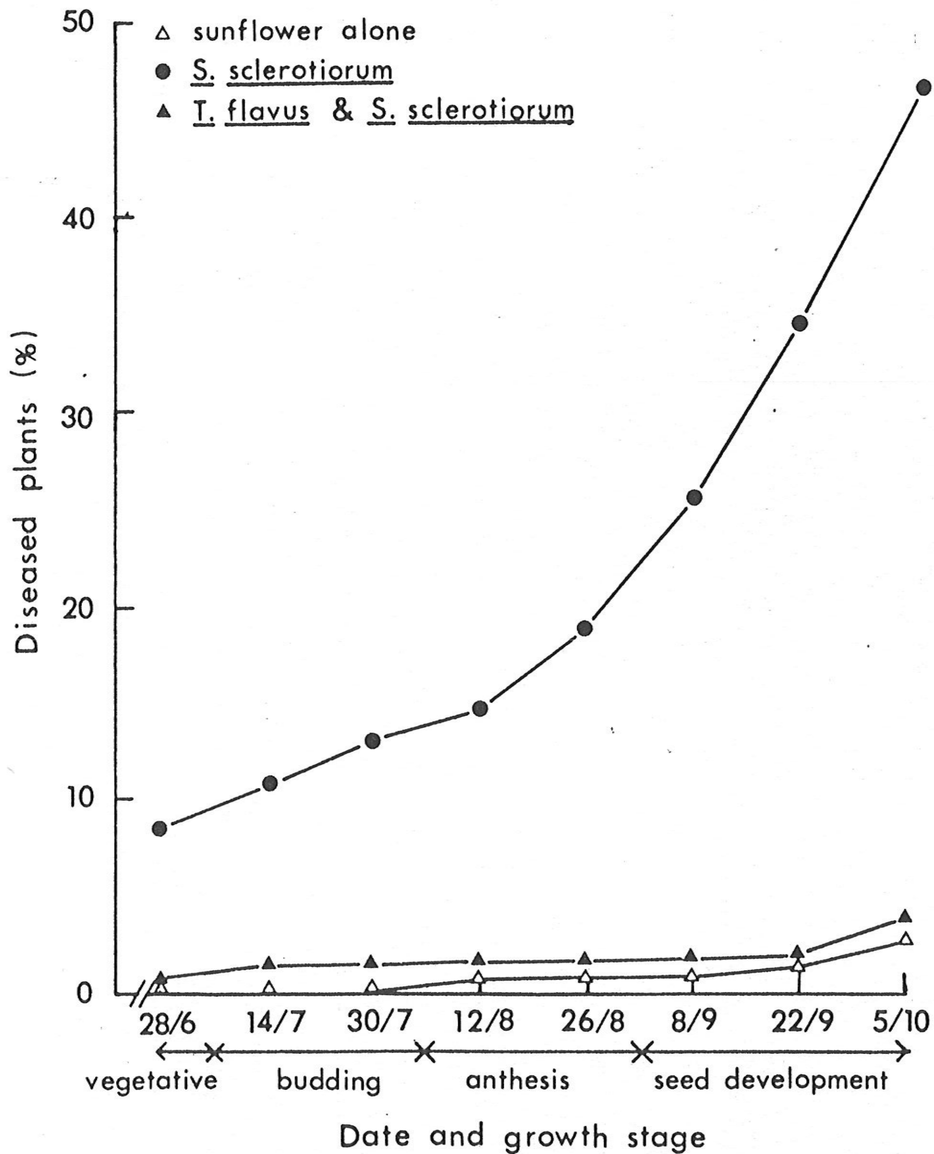


Figure 8. The effect of T. flavus on the incidence of sclerotinia wilt of sunflower at the Winnipeg location.

stages, wilt incidence increased rapidly (Figure 7). In the T. flavus-treated plots and the control, disease increased slowly but steadily, with the number of wilted plants at each growth stage much less than those of the S. sclerotiorum- treated plots. By the late seed development stage, disease incidence in the T. flavus- treated plots at Winnipeg was 3.8% as compared to 47.2% for the S. sclerotiorum- treated plots. At the Portage location, wilt incidences for the T. flavus-treated and S. sclerotiorum- treated plots were 26.6 and 81.4%, respectively. This represents a reduction of wilt incidence in T. flavus-treated plots by 91.9 and 67.3% at the Winnipeg and Portage la Prairie sites, respectively.

Significant differences in yield between T. flavus- treated plots and S. sclerotiorum- treated plots also occurred (Table 17). No significant differences between yield of the T. flavus- treated plots and the control were found. The T. flavus- treated plots at Winnipeg and Portage yielded 2870 and 2140 kg/ha, respectively while the S. sclerotiorum-treated plots gave yields of only 2350 and 1430 kg/ha, respectively. This represents an increase of sunflower seed yield of 22.1 and 49.7% at the Winnipeg and Portage la Prairie sites due to the application of T. flavus.

The survival of sclerotia of S. sclerotiorum was affected by T. flavus. The number of sclerotia recovered and the number of viable sclerotia in the T. flavus- treated plots were significantly lower than in the S. sclerotiorum- treated plots (Figures 10 and 11). No significant differences were observed in the number of recovered sclerotia and the number of viable sclerotia between T. flavus- treated plots and the control (Appendices 16 and 17).

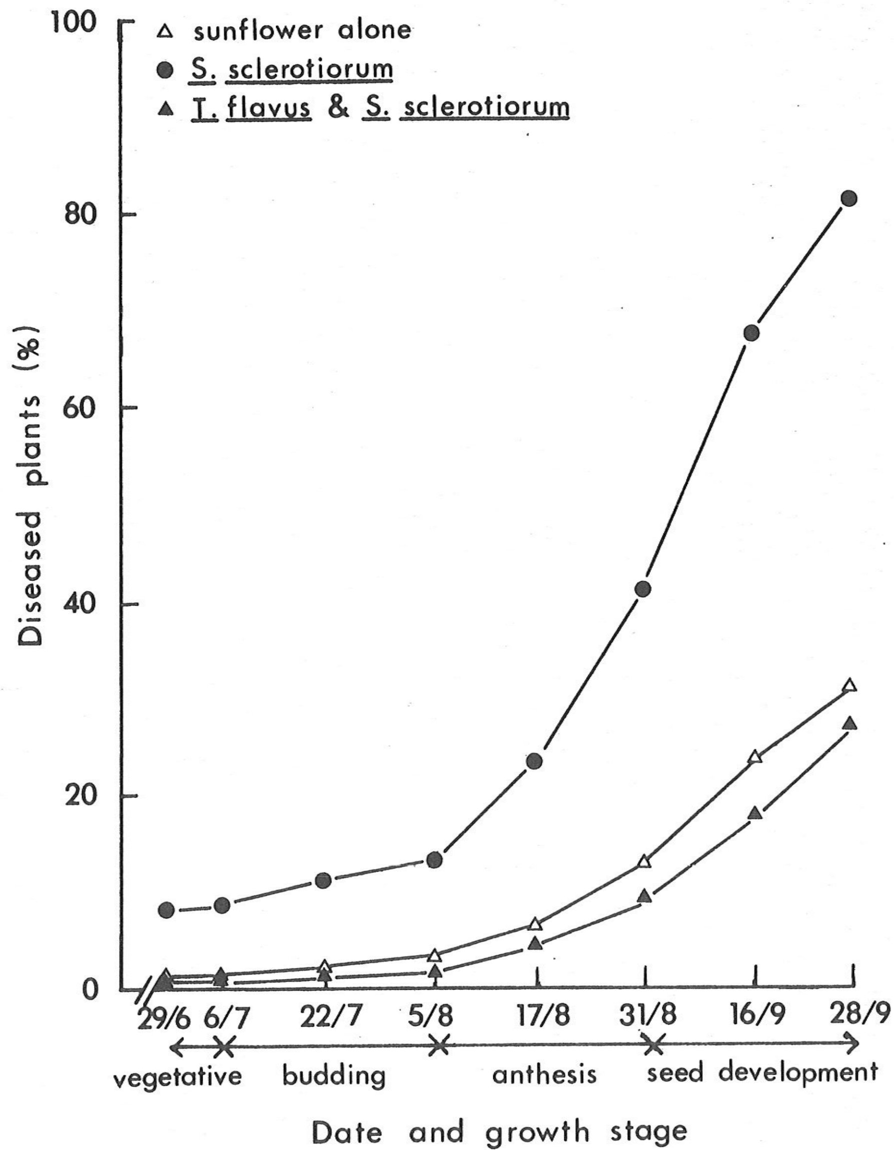


Figure 9. The effect of T. flavus on the incidence of sclerotinia wilt of sunflower at the Portage la Prairie location.

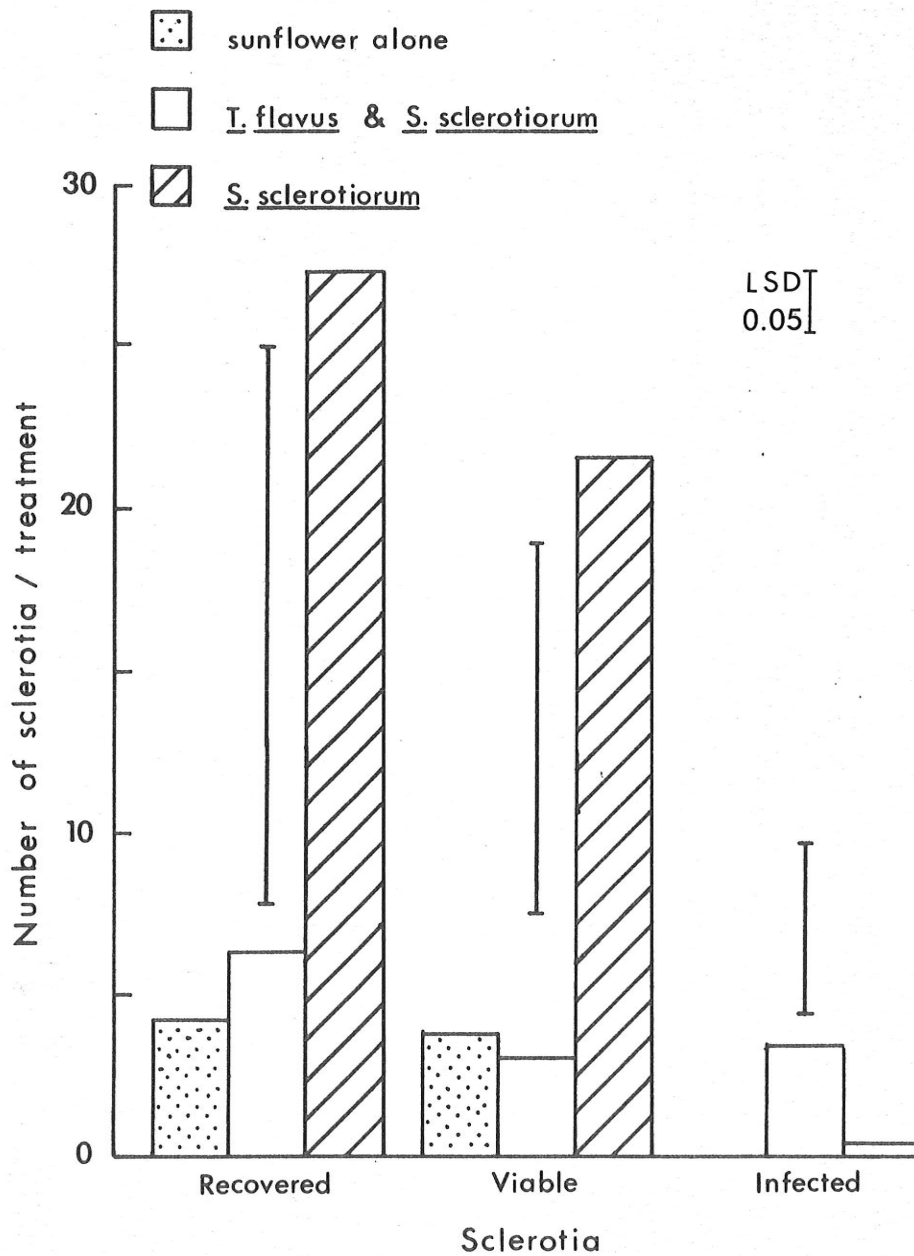


Figure 10. The effect of T. flavus on survival and viability of sclerotia recovered from sunflower plots at the Winnipeg location.

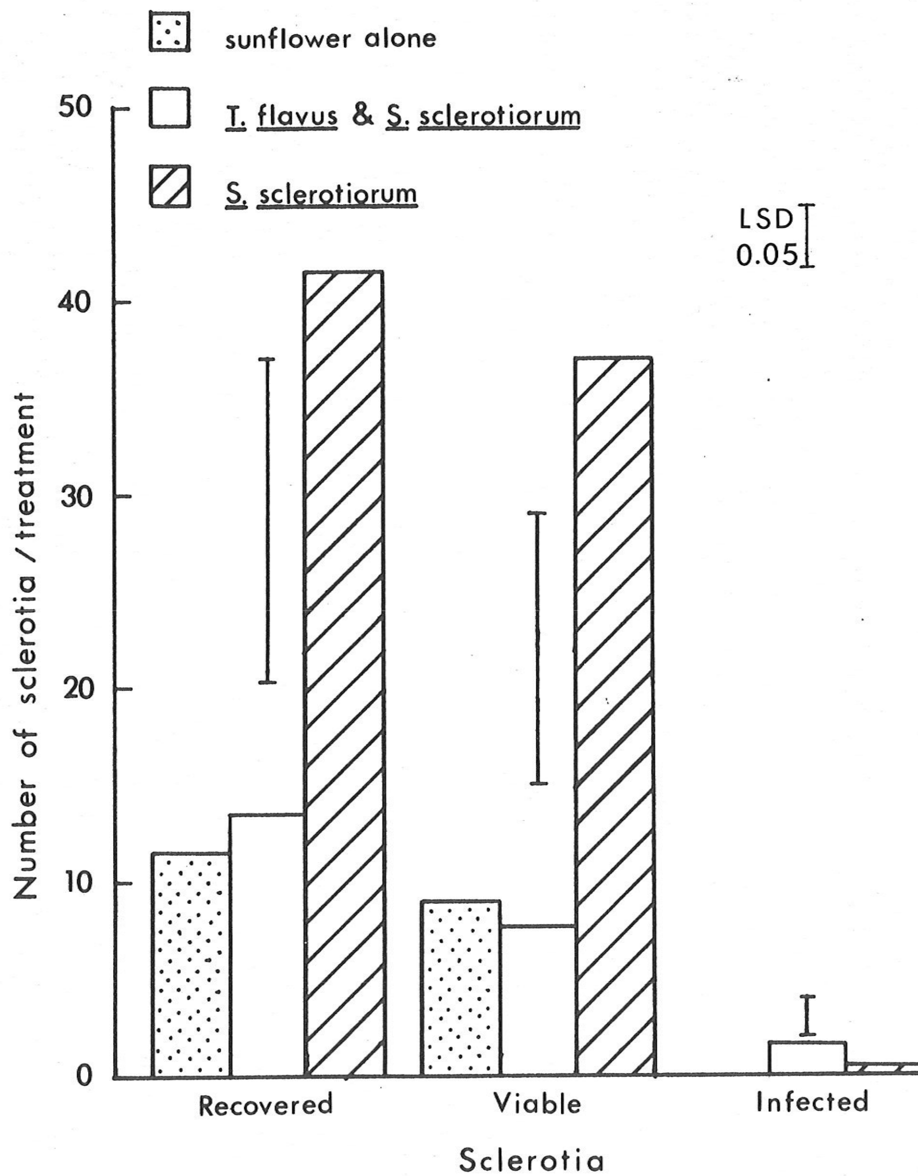


Figure 11. The effect of T. flavus on survival and viability of sclerotia recovered from sunflower plots at the Portage la Prairie location.

At the Winnipeg location, fewer sclerotia from the T. flavus- treated plots were viable compared to the control and the S. sclerotiorum-treated plots (Figure 10). The viability of sclerotia was affected by T. flavus as the number of sclerotia infected with the mycoparasite was significantly higher in the T. flavus- treated plots as compared to the S. sclerotiorum- treated plots and the control. Similar results were obtained at the Portage site (Figure 11).

Sclerotia were found in control plots at both locations. The level of natural inoculum was much higher at the Portage la Prairie location than at the Winnipeg site (Figure 7) and this is reflected in the higher incidence of wilt in the Portage la Prairie plots.

Soil temperatures recorded in the sclerotinia wilt experiment located at the Winnipeg site ranged from 8.2 to 25.6 C at 8:30 a.m. and from 11.3 to 35.9 C at 4:30 p.m. (Appendix 10). Tensiometer readings taken at the Portage la Prairie site revealed that soil moisture was quite variable at a depth of 6.5 cm, with moisture percentages ranging from 4.5 to 44.8% (Appendix 11).

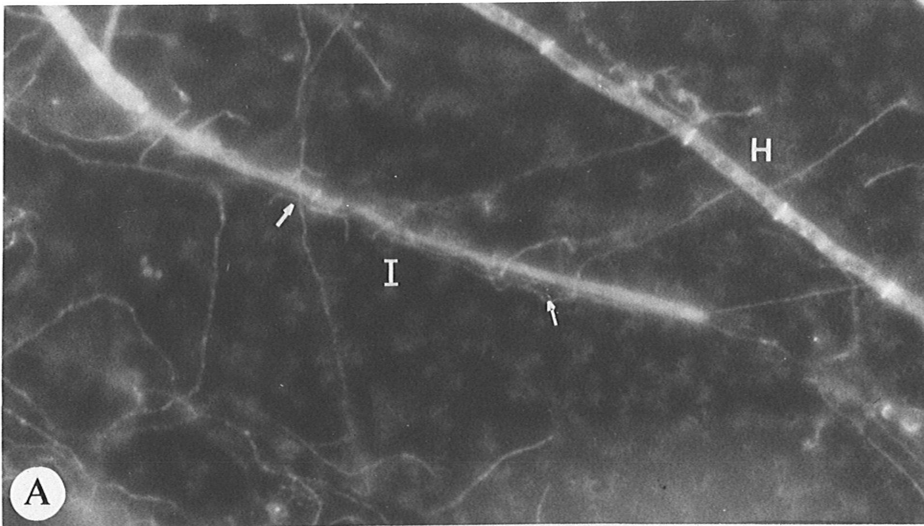
4.3 HYPHAL INTERACTIONS BETWEEN S. SCLEROTIORUM AND T. FLAVUS

Laboratory studies showed that T. flavus is destructive to growing hyphae of S. sclerotiorum (Plates 2-5). Hyphal interactions between S. sclerotiorum (the host) and T. flavus (the mycoparasite) were evident after 4 or 5 days of incubation. Hyphae of T. flavus commonly coil around Sclerotinia hyphae (Plates 2B, 3B). The early stages of this host-mycoparasite interaction involve trophic growth of T. flavus toward the host hyphae (Plates 2A, 3A) and initiation of coiling (Plate 3A).

Plate 2. Hyphal interactions between *T. flavus* and *S. sclerotiorum* observed via fluorescence and light microscopy.

Figure A. Healthy hypha of *S. sclerotiorum* (H) compared to infected hypha (I) surrounded by *T. flavus* (arrows). Approximate magnification: x275.

Figure B. Coiling of *T. flavus* around hypha of *S. sclerotiorum*. Note granular host cytoplasm and empty cell areas. Approximate magnification: x400.


H
I
A

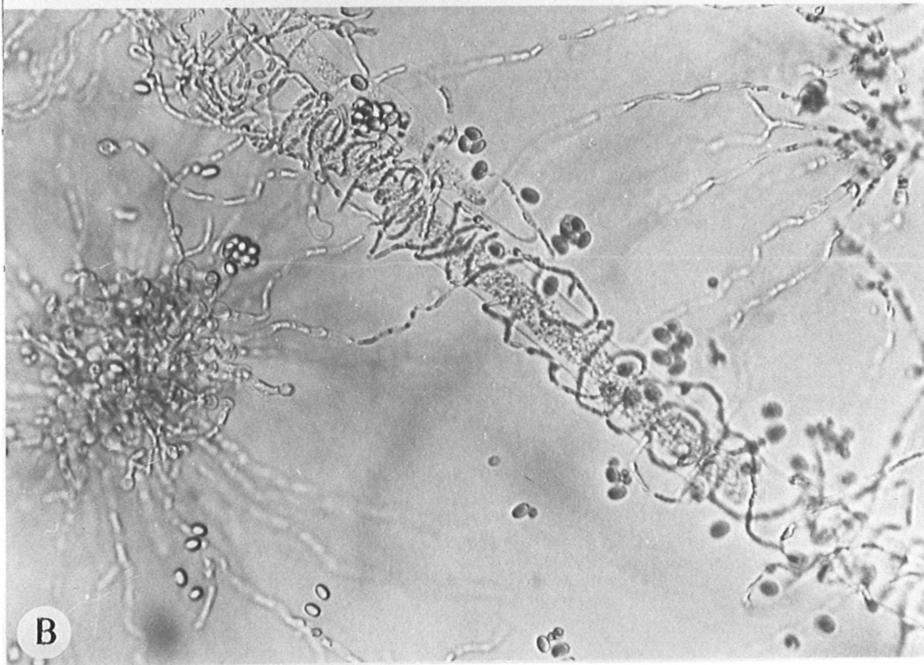
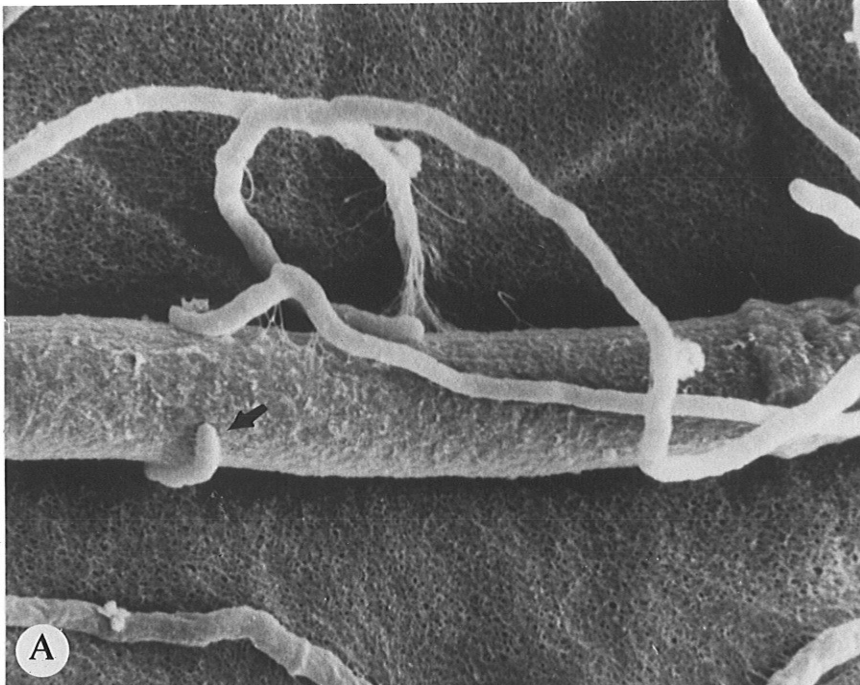
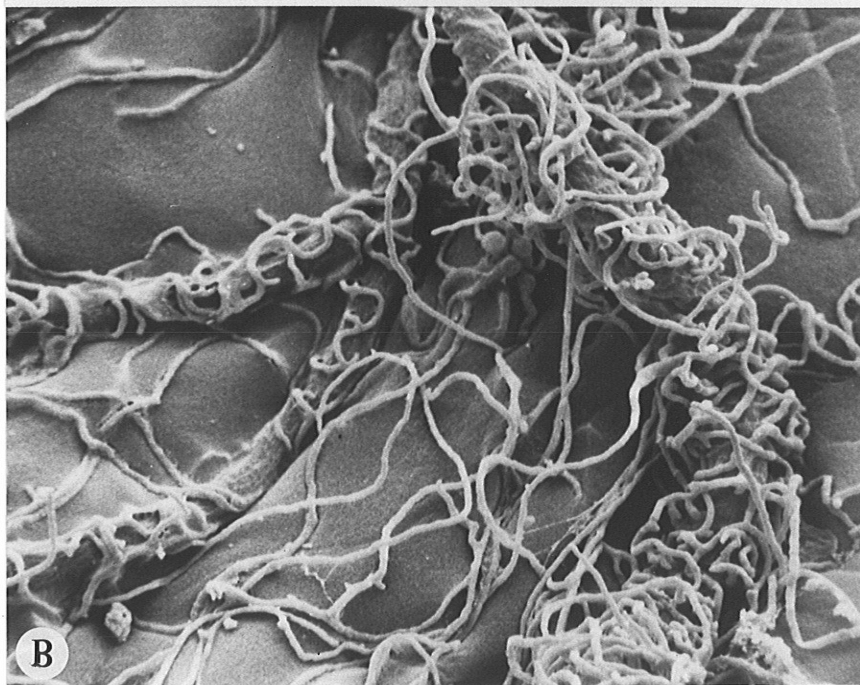

B

Plate 3. Hyphal interactions between *T. flavus* and *S. sclerotiorum* observed via scanning electron microscopy.

Figure A. Early stage of the host-mycoparasite interaction. Note initial coiling of *T. flavus* hypha around host hypha (arrow). Approximate magnification: x2500.

Figure B. Advanced stage of the host-mycoparasite interaction. Intense coiling and frequent branching of *T. flavus* occur. Approximate magnification: x625.


A


B

The first visible signs of the host cell affected by _T_. _flavus_ are plasmolysis and granulation of the cytoplasm (Plate 2B). The cell walls appear to be intact and not affected by _T_. _flavus_.

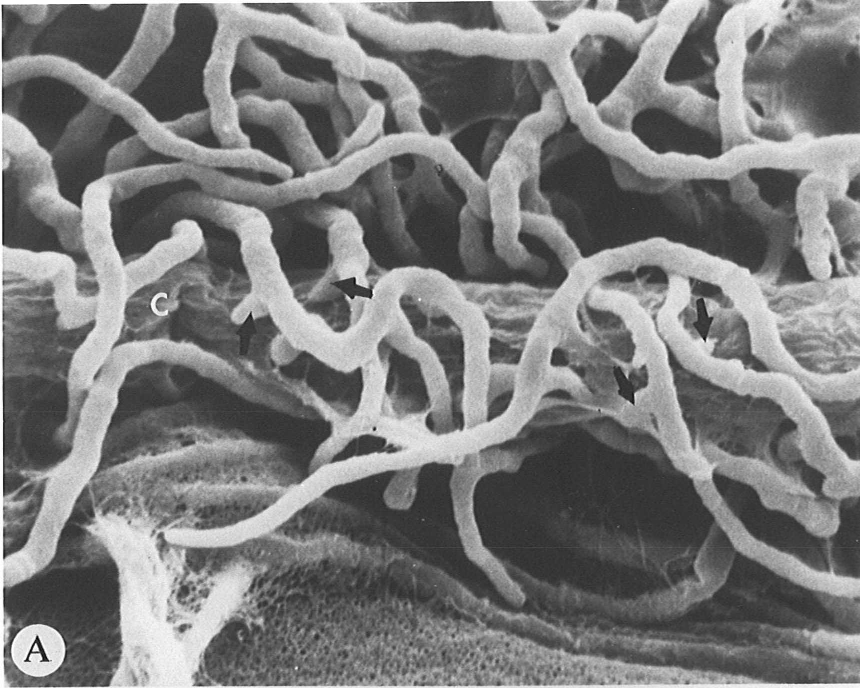
In later stages, coiling can become quite intense with hyphal branching of _T_. _flavus_ frequently occurring toward the host hyphae (Plates 3B, 4A). Sections of the _Sclerotinia_ hypha may collapse as a result of attack by _T_. _flavus_ (Plate 4B). _S_. _sclerotiorum_ hyphae become rough and pitted in appearance (Plates 3B, 4A, 5). In contrast, the unaffected, healthy hyphae of _S_. _sclerotiorum_ remain smooth and rigid (Plate 5).

No evidence was obtained that specialized infection structures were formed or that penetration of the host hyphae occurred. Due to their interaction with _T_. _flavus_, hyphal cells of _S_. _sclerotiorum_ appear to collapse with the cell walls remaining intact.

Plate 4. Scanning electron micrographs of the advanced stages of the *T. flavus* - *S. sclerotiorum* hyphal interaction.

Figure A. Hyphal branches of *T. flavus* (arrows) growing toward the host hypha. Note wrinkled appearance of the *Sclerotinia* hypha and initiation of hyphal collapse (C). Approximate magnification: x2500.

Figure B. Collapse of host hypha (C). Trophism is quite evident. Approximate magnification: x2000.


C
A

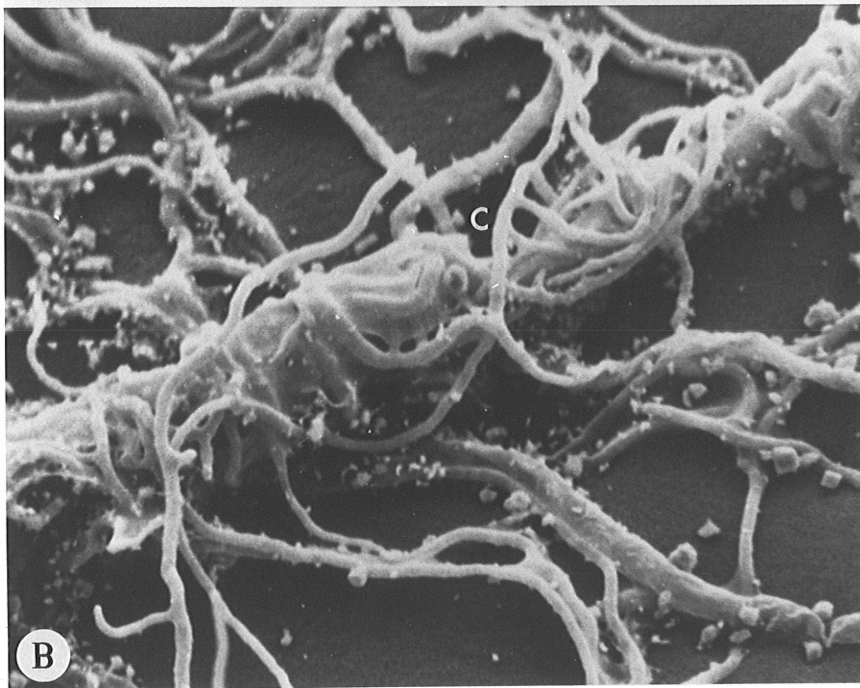
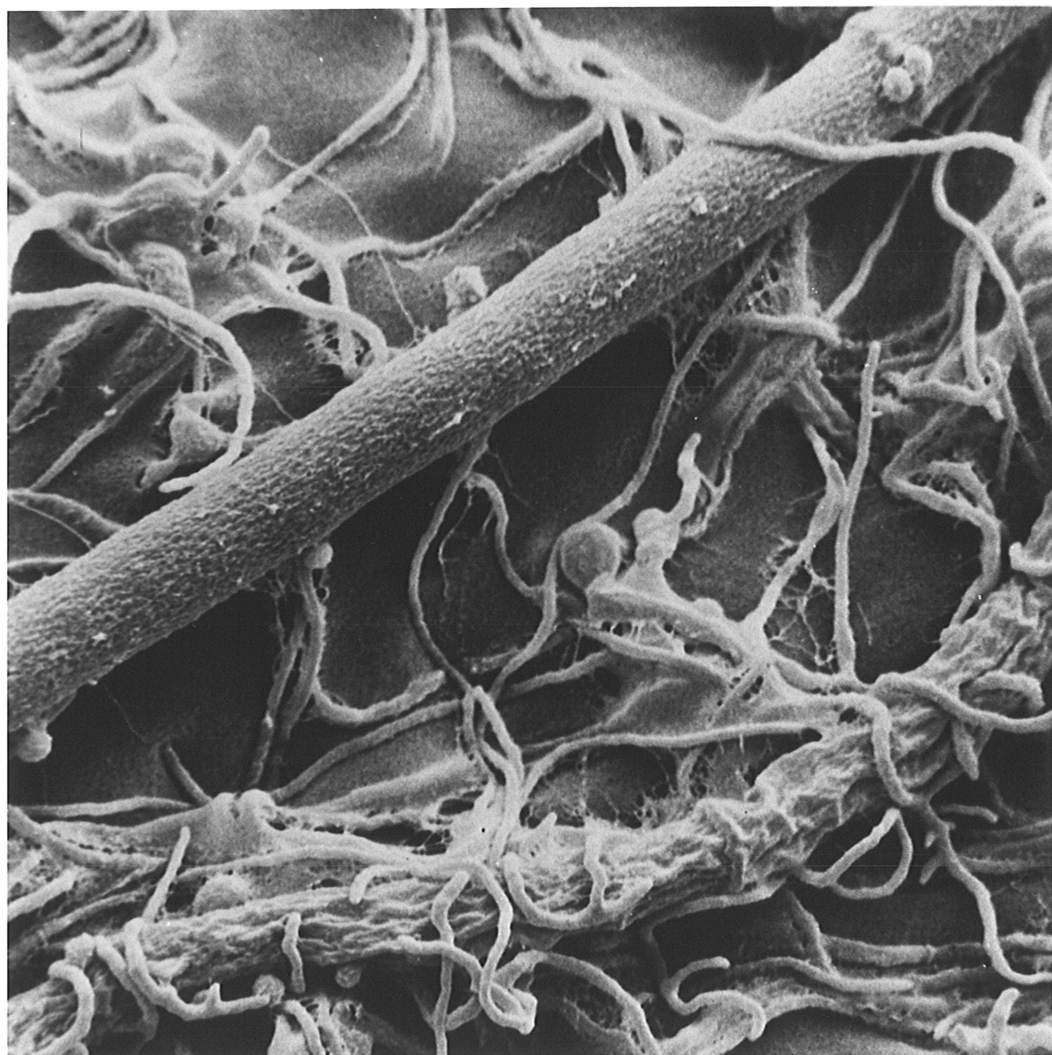

C
B

Plate 5. Scanning electron micrograph of a healthy versus a colonized hypha of *S. sclerotiorum*. Note the difference in texture and diameter of the two hyphae. Approximate magnification: x3000.



Chapter V

DISCUSSION

This study shows that *Talaromyces flavus* is a mycoparasite destructive to both hyphae and sclerotia of *S. sclerotiorum*. It is effective in controlling the sclerotial population of *S. sclerotiorum*, thereby reducing the incidence of sclerotinia wilt under greenhouse and field conditions. It appears that *Talaromyces flavus* is one of the potentially effective control agents for sclerotinia wilt of sunflower.

Although *T. flavus* is parasitic to the plant pathogens *Rhizoctonia solani* (Boosalis, 1956), *Verticillium dahliae* (Marois *et al*., 1982) and *S. sclerotiorum* (Su and Leu, 1980) the mode of parasitism may vary among these hosts. Boosalis (1956) reported that *Penicillium vermiculatum* invaded the hyphae of *Rhizoctonia solani* by penetration pegs. Parasitic hyphae were established within the host hyphae. Cell walls of *R. solani* were penetrated from the outside only and eventually collapsed, but did not disintegrate. However, the present study of the *T. flavus*-*S. sclerotiorum* interaction shows no evidence of direct penetration of host hyphae by the mycoparasite. The death of the host hyphal cells is apparently due to the coiling of *T. flavus* around host cells resulting in disintegration of host cytoplasm and collapse of cell walls. Ultrastructural studies are necessary to clarify our understanding of the mode of parasitism of *T. flavus* on *S. sclerotiorum*.

Some mycoparasites may kill the host by the production of antibiotics. Dennis and Webster (1971) described the vacuolation, granulation and disintegration of hyphal cell walls in Fusarium annosus and Rhizoctonia solani by the antibiotic-producing strains of Trichoderma. Dutta (1981) suggested that P. vermiculatum produced an inhibitory substance which reduced the growth of Verticillium albo-atrum. P. vermiculatum is capable of producing an antibiotic named vermiculine, as reported by Fuska et al. (1972). This compound is closely related to the antifungal agent pyrenophorin (Boeckman et al., 1974) and may be involved in the destruction of Sclerotinia hyphae and inhibition of V. albo-atrum. Enzymatic action may also be a factor involved in the T. flavus-S. sclerotiorum system. Although Boosalis (1956) reported that P. vermiculatum did not produce toxic substances capable of causing injury to hyphae of Rhizoctonia solani, the possibility exists that P. vermiculatum may produce enzymes destructive to hyphae of S. sclerotiorum.

Talaromyces flavus, applied directly to sclerotia or mixed into the surrounding soil, destroyed sclerotia of S. sclerotiorum in both field and greenhouse burial experiments. In general, a marked decrease in the number of viable sclerotia and a corresponding increase in the number of sclerotia infected or killed by T. flavus occurred. Results from the 1981 and 1982 field experiments indicate that the medium used for production of T. flavus is important. In 1981, T. flavus produced on grain resulted in a low level of sclerotial decay by this mycoparasite. Though T. flavus grows well on this medium, the grain mixture does not appear to be the optimal medium for establishment of T. flavus in soil. A variety of fungal colonizers was isolated from recovered sclerotia in-

dicating that this nutrient-rich food base might have attracted other soil microorganisms and thereby suppressed or inhibited growth and establishment of T. flavus. Talaromyces flavus produced on sclerotia and dispersed throughout the soil exhibited an increase in mycoparasitic activity. Therefore, growth substrate appears to be an important factor affecting the efficacy of T. flavus as a biological control agent for S. sclerotiorum.

Soil moisture and soil temperature are important factors affecting the survival of T. flavus and sclerotia of S. sclerotiorum. As time from sclerotial burial increased, the numbers of recovered sclerotia decreased for the controls and the T. flavus treatments. In general, results indicate that T. flavus enhanced this reduction. This mycoparasite was effective in reducing sclerotial inoculum over the range of soil moistures and temperatures encountered during the summer of 1982.

Sclerotia have been reported to survive from one year (Davis, 1925) to 4 or 5 years (Adams and Ayers, 1979) to 10 years (Brown and Butler,1936). Present studies show that sclerotia in contact with, or close to T. flavus in soil, rapidly lose their viability as a result of deterioration via the mycoparasite. Such differences in sclerotial longevity may be related to dissimilarities in soil microflora which affect the survival of sclerotia.

Greenhouse and field trials indicate that introduction of T. flavus into soil along with S. sclerotiorum at seeding time reduces the amount of sclerotinia wilt of sunflower. The importance of mycoparasites as biological control agents of S. sclerotiorum is dependent primarily on their ability to destroy sclerotia in the field (Huang, 1980b). At both

the Winnipeg and Portage la Prairie locations, results indicate that _T. flavus_ is effective in reducing the introduced population of sclerotia. However, the natural population of sclerotia did not appear to be affected, as indicated by similar levels of disease in the _T. flavus_-treated plots as compared to the control plots. Huang and Hoes (1980) reported that the primary site of infection of sunflower by _S. sclerotiorum_ was always within the zone in which lateral roots were found ie. 5-12 cm. Lateral roots spread widely within the upper 30 cm of soil to a distance of 60-150 cm (Knowles, 1978). Infection of lateral roots extending outside the seed zone likely occurred as a result of the natural population of sclerotia. This would account for similar levels of disease in the _T. flavus_- treated plots and the control plots, with _T. flavus_ effectively controlling the introduced population of sclerotia within the seed zone of the _T. flavus_- treated plots.

Huang and Hoes (1980) reported that incidence of sclerotinia wilt of sunflower was highest when sclerotia were buried next to the seed, and decreased as the distance between sclerotia and seed increased. In terms of disease reduction, control of the sclerotial inoculum next to the seed appears to be more important than control of sclerotia located away from the seed, as _T. flavus_ produced dramatic reductions in wilt incidence when applied to the seed zone at 2 field locations. It is probable that natural sclerotia located where the mycoparasite was applied, ie. within rows, were effectively controlled. Between rows and at deeper depths, infection of sunflower roots likely occurred as a result of mycelia arising from the natural population of sclerotia. Failure of the mycoparasite to destroy these sclerotia may be due to an in-

ability of the mycoparasite to spread within soil or a limited viability of the inoculum under soil conditions encountered. Application of _T. flavus_ into the seed zone rather than throughout the field is likely to result in a higher level of crop protection. However, further research is required for this to be established.

Seed yield of sunflower was significantly increased when _T. flavus_ was applied to soil. Though both locations illustrated this effect, plot yields at Winnipeg were higher than those at the Portage site. Lower levels of natural inoculum at the Winnipeg location resulted in fewer diseased plants, and therefore contributed to an increase in yield. Environmental conditions may also have influenced yield differences between locations by affecting head size. At Winnipeg, the head diameters of plants were much larger than at the Portage la Prairie site and resulted in an increase in yield per plant.

Greenhouse trials also indicate that _T. flavus_ is able to destroy sclerotia of _S. sclerotiorum_ and reduce the level of disease. However, in a few cases, _T. flavus_ was not effective and the majority of sclerotia were parasitized by a _Trichoderma_ species, most likely _Trichoderma viride_ Pers. ex Fr. as this species was reported to be a common mycoparasite of _S. sclerotiorum_ (Jones and Watson, 1969; Huang, 1977). The condition of the soil (sterile versus unsterile) and the presence of other soil microorganisms will affect the ability of _T. flavus_ to perform. In unsterile soil, interactions such as competition for nutrients, parasitism or competition may have occurred between _T. flavus_ and _T. viride_. This would reduce the effectiveness of _T. flavus_. When soil was sterilized, _T. flavus_ reduced the number of sclerotia of _S._

sclerotiorum in soil. Sterilization eliminates many soil microorganisms and provides a favorable environment for introduced pathogens, as competition and other microbial interactions are reduced or eliminated. In the first experiments conducted, the soil was not sterilized and T. flavus did not appear to be effective in reducing the sclerotial population of S. sclerotiorum. However, in later experiments where unsterile soil was also used, T. flavus was effective against sclerotia of S. sclerotiorum. Differences in results obtained using unsterile soil were likely due to variations in type of soil microorganisms present and environmental conditions, which might favor certain microorganisms and inhibit others. The numbers and types of soil microorganisms ie. fungi, bacteria, algae, actinomycetes, etc. will vary with soil pH, moisture, temperature, organic matter content, presence of other microorganisms, etc. Different batches of soil are likely to contain different proportions of microorganisms which will be influenced by a number of environmental factors. In some experiments where unsterile soil was used, viability of sclerotia was reduced but not all of the nonviable sclerotia were infected by T. flavus. It is likely that some resident soil microorganisms were capable of parasitizing sclerotia of S. sclerotiorum, but were not inhibitory to T. flavus. Information on factors affecting the survival of T. flavus in soil are meager. Further investigations on the ecology of this mycoparasite are warranted.

Sclerotia to be used in biological control experiments were chopped prior to incorporation into the soil. This action damages the rind which is the protective layer insulating against adverse environmental conditions. Damage to the rind may increase susceptibility of the scle-

rotia to colonization by microorganisms. However, the low level of disease in the *T. flavus*- treated plots as compared to the control treatment indicates that sclerotia of *S. sclerotiorum* are effectively destroyed by this mycoparasite. It may be that many sclerotia in the *T. flavus*- treated plots were destroyed early in the season by the mycoparasite before they were able to regenerate complete rinds. A comparative study on the susceptibility of injured and non-injured sclerotia of *S. sclerotiorum* to infection by *T. flavus* would therefore be useful in understanding the role of this mycoparsite in the sunflower field.

Other mycoparasites, *Coniothyrium minitans* (Huang, 1976, 1977) and *Sporidesmium sclerotivorum* (Adams and Ayers, 1981) significantly reduce the survival of sclerotia of *S. sclerotiorum*. The success of these mycoparasites is partly due to the fact that both fungi are true mycoparasites and effective under natural conditions. As well, basic information is available concerning their use in a biological control program. Further research in the ecology and biology of *T. flavus* is essential to determine the potential usefulness of this mycoparasite under a variety of field conditions. Soils containing inoculum of *S. sclerotiorum* in the presence of a susceptible crop may be induced to become suppressive to disease development. The effect of crop plant, soil moisture, temperature, pH, texture and microflora on the activity and survival of *T. flavus* will determine the ability of the mycoparasite to control *S. sclerotiorum* and induce suppressiveness in the field. An understanding of the biology and ecology of *T. flavus*, particularly the survival of this mycoparasite in soil, is critical to the success of using *T. flavus* as a biological control agent of *S. sclerotiorum* in the future.

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APPENDIX 1. Analysis of variance for greenhouse burial experiment No. 1.

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia $(x)^2$		
Replication	4	16,722.0000	4,180.5000	1.43
Treatment	2	20,205.7333	10,102.8670	3.46*
Sampling date	3	168,363.5167	56,121.1700	19.21**
T. x S.D.	6	22,174.5333	3,695.7555	1.27
Error	44	128,544.8000	2,921.4727	
Total	59	356,010.5833		
		c.v. = 16.32%		
		Viable sclerotia		
Replication	4	10.1000	5.0500	0.37
Treatment	2	116.1333	58.0667	8.41**
Sampling date	3	140.4000	46.8000	6.78**
T. x S.D.	6	396.4000	66.0667	9.57**
Error	44	303.9000	6.9068	
Total	59	966.9333		
		c.v. = 36.84%		
		Infected sclerotia		
Replication	4	11.5667	2.8917	0.82
Treatment	2	33.0333	16.5167	4.66*
Sampling date	3	78.7333	26.2444	7.40**
T. x S.D.	6	21.3667	3.5611	1.00
Error	44	156.0333	3.5462	
Total	59			
		c.v. = 77.39%		

*Significant at the 5% level.

**Significant at the 1% level.

APPENDIX 2. Analysis of variance for greenhouse burial experiment No. 2.

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Viable sclerotia		
Replication	1	6.1250	6.1250	0.82
Treatment	1	210.1250	210.1250	28.17*
Sampling date	1	15.1250	15.1250	2.03
T. x S.D.	1	6.1250	6.1250	0.82
Error	3	22.3750	7.4583	
Total	7			
		c.v. = 41.22%		
		Infected sclerotia (*T. flavus*)		
Replication	1	0.0000	0	0
Treatment	1	0.0000	0	0
Sampling date	1	0.5000	.5000	0.50
T. x S.D.	1	0.0000	0	0
Error	3	3.0000	1.0000	
Total	7			
		c.v. = 80.00%		
		Infected sclerotia (*Trichoderma* sp.)		
Replication	1	0.5000	0.5000	0.07
Treatment	1	84.5000	84.5000	11.79*
Sampling date	1	4.5000	4.5000	0.63
T. x S.D.	1	4.5000	4.5000	0.63
Error	3	21.5000	7.1667	
Total	7			
		c.v. = 28.94%		

*Significant at the 5% level.

APPENDIX 3. Analysis of variance for greenhouse burial experiment No. 3.

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Viable sclerotia		
Replication	3	96.8438	32.2813	4.81
Treatment	3	1,373.3438	457.7813	68.23**
Sampling date	1	34.0313	34.0313	5.07*
T. x S.D.	3	13.0938	4.3646	0.65
Error	21	140.9063	6.7098	
Total	31	1,658.2188		
		c.v. = 36.20%		
		Infected sclerotia		
Replication	3	72.8438	24.2813	6.59**
Treatment	3	600.5938	200.1980	54.31**
Sampling date	1	225.7813	225.7813	61.25**
T. x S.D.	3	23.3438	7.7813	2.11
Error	21	77.4063	3.6860	
Total	31	999.9688		
		c.v. = 29.39%		

*Significant at the 5% level.

**Significant at the 1% level.

APPENDIX 4. Analysis of variance for greenhouse burial experiment No. 4.

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Viable sclerotia		
Replication	3	7.5938	2.5313	0.59
Treatment	3	1,016.0938	338.6980	78.89**
Soil	1	1.5313	1.5313	0.36
Treatment x soil	3	28.5938	9.5313	2.22
Error	21	90.1563	4.2932	
Total	31	1,143.9688		
		c.v. = 15.38%		
		Infected sclerotia (x+0.5)		
Replication	3	0.3599	0.1200	0.71
Treatment	3	31.1715	10.3905	61.14**
Soil	1	0.8187	0.8187	4.82*
Treatment x soil	3	1.3911	0.4637	2.73
Error	21	3.5688	0.1699	
Total	31	37.3101		
		c.v. = 24.49%		

*Significant at the 5% level.

**Significant at the 1% level.

APPENDIX 5. Analysis of variance for Winnipeg field burial experiment No. 1.

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia $(x)^2$		
Replication	5	9,002.8333	1,800.5667	1.23
Treatment	2	775,729.7500	387,864.8800	264.15**
Error a (RxT)	10	14,683.4167	1,468.3416	1.48
Sampling date	3	214,836.3333	71,612.1100	72.39**
T. x S.D.	6	264,579.9167	44,096.6520	44.58**
Error b	45	44,515.7500	989.2389	
Total	71	1,323,348.0000		
		c.v. = 10.14%		
		Viable sclerotia		
Replication	3	21.2361	4.2472	1.20
Treatment	2	3,513.5278	1,756.7639	497.59**
Error a (RxT)	10	35.3056	3.5306	0.66
Sampling date	3	36.7083	12.2361	2.30
T. x S.D.	6	46.9167	7.8195	1.47
Error b	45	239.6250	5.3250	
Total	71	3,893.3194		
		c.v. = 19.48%		
		Infected sclerotia (x+0.5)		
Replication	5	0.8943	0.1789	0.89
Treatment	2	49.4867	24.7434	122.53**
Error a (RxT)	10	2.0193	0.2019	1.38
Sampling date	3	5.3308	1.7770	12.16**
T. x S.D.	6	11.9227	1.9871	13.60**
Error b	45	6.5749	0.1461	
Total	71	76.2287		
		c.v. = 23.99%		

**Significant at the 1% level.

APPENDIX 6. Analysis of variance for Portage la Prairie field burial experiment No. 1.

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia $(x)^2$		
Replication	5	9,690.5694	1,938.1139	1.34
Treatment	2	549,618.1111	274,809.0600	190.39**
Error a (RxT)	10	14,433.7222	1,443.3722	1.07
Sampling date	3	281,598.9306	93,866.3110	69.34**
T. x S.D.	6	273,720.4444	45,620.0731	33.70**
Error b	45	60,913.8750	1,353.6417	
Total	71	1,189,975.6528		
		c.v. = 11.62%		
		Viable sclerotia		
Replication	5	3.6250	0.7250	0.06
Treatment	2	2,140.0833	1,070.0417	81.42**
Error a (RxT)	10	131.4167	13.1417	1.50
Sampling date	3	142.9306	47.6435	5.43**
T. x S.D.	6	33.6944	5.6157	0.64
Error b	45	395.1250	8.7806	
Total	71	2,846.8750		
		c.v. = 29.76%		
		Infected sclerotia (x+0.5)		
Replication	5	1.2687	0.2537	1.67
Treatment	2	43.5699	21.7850	143.32**
Error a (RxT)	10	1.5200	0.1520	0.76
Sampling date	3	8.0814	2.6938	13.43**
T. x S.D.	6	16.8884	2.8147	14.03**
Error b	45	9.0285	0.2006	
Total	71	80.3570		
		c.v. = 28.84%		

**Significant at the 1% level.

APPENDIX 7. Analysis of variance for Winnipeg field burial Experiment No. 2.

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia		
Replication	5	29.9167	5.9833	1.29
Treatment	1	320.3333	320.3333	69.01**
Error a (RxT)	5	31.1667	6.2333	1.34
Sampling date	3	182.4167	60.8056	13.10**
T. x S.D.	3	150.8333	50.2778	10.83**
Error b	30	139.2500	4.6417	
Total	47	853.9167		
		c.v. = 12.52%		
		Viable sclerotia		
Replication	5	9.6667	1.9333	0.67
Treatment	1	1,323.0000	1,323.0000	456.21**
Error a (RxT)	5	14.5000	2.9000	0.62
Sampling date	3	321.5000	107.1667	22.77**
T. x S.D.	3	309.8333	103.2778	21.95**
Error b	30	141.1667	4.7056	
Total	47	2,119.6667		
		c.v. = 17.47%		
		Infected sclerotia (x+0.5)		
Replication	5	0.5275	0.1055	0.97
Treatment	1	2.7339	2.7339	25.11**
Error a (RxT)	5	0.5444	0.1089	0.45
Sampling date	3	0.2307	0.0769	0.31
T. x S.D.	3	0.5079	0.1693	0.69
Error b	30	7.3308	0.2444	
Total	47	11.8753		
		c.v. = 47.89%		

**Significant at the 1% level.

APPENDIX 8. Analysis of variance for Portage la Prairie field burial experiment No. 2.

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia		
Replication	5	16.8542	3.3708	1.74
Treatment	1	136.6875	136.6875	70.55**
Error a (RxT)	5	9.6875	1.9375	0.67
Sampling date	3	313.8958	104.6319	35.96**
T. x S.D.	3	140.0625	46.6875	16.05**
Error b	30	87.2917	2.9097	
Total	47	704.4792		
		c.v. = 9.90%		
		Viable sclerotia		
Replication	5	46.1042	9.2208	1.15
Treatment	1	247.5208	247.5208	30.86**
Error a (RxT)	5	40.1042	8.0208	0.79
Sampling date	3	474.0625	158.0208	15.51**
T. x S.D.	3	61.5625	20.5208	2.01
Error b	30	305.6250	10.1875	
Total	47	1,174.9792		
		c.v. = 29.07%		
		Infected sclerotia (x+0.5)		
Replication	5	0.3688	0.0738	0.18
Treatment	1	7.5316	7.5316	17.99**
Error a (RxT)	5	2.0931	0.4186	1.62
Sampling date	3	1.1423	0.3808	1.47
T. x S.D.	3	2.5018	0.8340	3.22*
Error b	30	7.7605	0.2587	
Total	47	21.3980		
		c.v. = 35.49%		

*Significant at the 5% level.

**Significant at the 1% level.

APPENDIX 9. Analysis of variance for Morden field burial experiment (1981).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Recovered sclerotia $(x)^2$		
Replication	4	118,620.6133	29,655.1530	0.95
Treatment	2	66,981.9467	33,490.973	1.07
Error a (RxT)	8	249,392.5867	31,174.0730	4.53
Sampling date	4	300,093.5467	75,023.3850	10.89**
T. x S.D.	8	60,460.4533	7,557.5566	1.10
Error b	48	330,645.2000	6,888.4417	
Total	74	1,126,194.3467		
		c.v. = 38.32%		
		Viable sclerotia (log x+10)		
Replication	4	0.6549	0.1637	1.49
Treatment	2	0.0289	0.0145	0.13
Error a (RxT)	8	0.8796	0.1010	2.80
Sampling date	4	1.2682	0.3171	8.06**
T. x S.D.	8	0.3341	0.0418	1.06
Error b	48	1.8873	0.0393	
Total	74	5.0530		
		c.v. = 6.92%		
		Infected sclerotia		
Replication	4	1.5467	0.3867	0.50
Treatment	2	0.1867	0.0933	0.12
Error a (RxT)	8	6.2133	0.7767	1.28
Sampling date	4	11.8133	2.9533	4.88**
T. x S.D.	8	3.5467	0.4433	0.73
Error b	48	29.0400	0.6050	
Total	74	52.3467		
		c.v. = 135.67%		

**Significant at the 1% level.

APPENDIX 10. Soil temperatures[1] recorded for 3 field experiments at the Winnipeg location (1982).

Week starting	Temperature (°C)			
	Sclerotinia wilt experiment (sunflower)		Sclerotial survival experiment[2] (no sunflower)	
	8:30 a.m.	4:30 p.m.	8:30 a.m.	4:30 p.m.
May 31	16.7	24.5	17.3	26.3
June 6	17.9	22.3	17.9	23.3
14	19.9	25.8	19.8	27.2
21	22.5	26.3	21.9	26.3
28	21.2	23.7	21.4	24.1
July 5	22.1	28.8	21.6	27.7
12	25.6	35.9	22.6	33.6
19	22.9	34.0	21.9	29.3
26	23.0	33.4	22.4	28.4
Aug 2	24.4	33.1	22.0	31.7
9	18.2	26.7	15.0	24.5
16	21.3	29.6	19.7	27.2
23	11.4	17.4	11.6	14.8
30	15.2	27.8	12.5	25.1
Sept 6	21.5	26.0	-	-
13	16.7	22.5	-	-
20	11.9	25.9	-	-
27	8.2	11.3	-	-

[1]Readings taken twice weekly at 4 and 3 sites within sunflower and no sunflower experiments, respectively. Values for each experiment were averaged over 2 days to obtain temperature means for each week.

[2]Two sclerotial survival experiments included in this category.

APPENDIX 11. Soil moisture[1] calculated for 3 field experiments at the Portage la Prairie field location (1982).

Date	Soil moisture (%)	
	Sclerotinia wilt experiment (sunflower)	Sclerotial survival experiment[2] (no sunflower)
May 25	44.8	42.2
26	44.0	42.0
June 3	22.0	21.6
8	43.0	44.0
10	44.2	42.2
15	37.0	31.7
17	35.3	32.4
22	36.8	33.7
24	23.0	25.6
29	27.8	31.9
July 2	33.7	29.1
6	25.4	27.0
8	22.6	24.0
13	26.0	35.7
15		
20	31.7	31.5
22	22.0	19.0
27	39.0	44.8
29	42.7	44.0
Aug 3	13.8	31.9
5	4.9	28.4
10	9.4	15.0
12	11.0	11.3
17	13.8	23.6
19	22.0	27.8
24	35.3	43.0
26	28.0	38.0
31	6.2	35.7
Sept 2	8.5	21.4
7	11.7	20.4
9	4.5	15.0

[1]Readings taken twice weekly at 4 and 2 sites within sunflower and no sunflower experiments, respectively. Values for each date were averaged over the number of sites.

[2]Two sclerotial survival experiments included in this category.

APPENDIX 12. Analysis of variance for greenhouse experiment No. 1 (sterile soil).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Disease (arcsin)		
Replication	3	92.1596	30.7199	0.55
Treatment	3	3,262.9448	1,087.6483	19.49**
Error	9	502.1848	55.7983	
Total	15	3,857.2891		
		c.v. = 31.87%		

**Significant at the 1% level.

APPENDIX 13. Analysis of variance for greenhouse experiment No. 2 (unsterile soil).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Disease (arcsin)		
Replication	5	418.5202	83.7040	2.96
Treatment	2	1,518.0030	759.0015	26.81**
Error	10	283.1028	28.3103	
Total	17	2,219.6259		
		c.v. = 22.95%		

**Significant at the 1% level.

APPENDIX 14. Analysis of variance for greenhouse experiment No. 3 (sterile vs. unsterile soil).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Disease (arcsin)		
Replication	1	48.8437	48.8437	0.60
Treatment	2	4,339.1772	2,169.5886	26.65**
Soil	1	136.3502	136.3502	1.67
Treatment x soil	2	141.4406	70.7203	0.87
Error	5	407.0882	81.4176	
Total	11	5,072.8999		
		c.v. = 34.13%		

**Significant at the 1% level.

APPENDIX 15. Analysis of variance for greenhouse experiment No. 4 where *T. flavus* was produced on wheat bran.

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Disease (arcsin)		
Replication	3	42.2109	14.0703	1.24
Treatment	4	1,955.2358	488.8090	43.12**
Error	12	136.0270	11.3356	
Total	19	2,133.4737		
		c.v. = 18.49%		
		Recovered sclerotia		
Replication	3	157.8000	52.6000	0.53
Treatment	4	82,280.0000	20,570.0000	208.27**
Error	12	1,185.2000	98.7667	
Total	19	83,623.0000		
		c.v. = 25.81%		
		Viable sclerotia		
Replication	3	471.0000	157.0000	0.92
Treatment	1	9,112.5000	9,112.5000	53.55**
Error	3	510.5000	170.1667	
Total	7	10,094.0000		
		c.v. = 36.75%		
		Infected sclerotia (*T. flavus*)		
Replication	3	5.0000	1.6667	1.0
Treatment	1	8.0000	8.0000	4.80
Error	3	5.0000	1.6667	
Total	7	18.0000		
		c.v. = 129.09%		
		Infected sclerotia (*Trichoderma* sp.)		
Replication	3	435.3750	145.1250	2.99
Treatment	1	3,828.1250	3,828.1250	79.00**
Error	3	145.3750	48.4583	
Total	7	4,408.8750		
		c.v. = 21.67%		

**Significant at the 1% level.

APPENDIX 16. Analysis of variance for results of 1982 biological control field experiment (Winnipeg location).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Yield		
Replication	3	76,671.5833	25,557.1940	1.67
Treatment	2	921,741.1667	460,870.58	30.09**
Error	6	91,886.1667	15,314.3611	
Total	11	1,090,298.9167		
		c.v. = 4.52%		
		% disease		
Replication	3	185.3259	61.7753	0.99
Treatment	2	5,069.1342	2,534.5671	40.66**
Error	6	374.0262	62.3377	
Total	11	5,628.4864		
		c.v. = 43.47%		
		Recovered sclerotia/kg soil		
Replication	3	21.6867	7.2289	1.37
Treatment	2	69.0882	34.5441	6.55*
Error	6	31.6558	5.2759	
Total	11	122.4307		
		c.v. = 78.07%		
		Recovered sclerotia		
Replication	3	402.0000	134.0000	1.37
Treatment	2	1,282.1667	641.0833	6.56*
Error	6	586.5000	97.75	
Total	11	2,270.6667		
		c.v. = 78.05%		
		Viable sclerotia		
Replication	3	204.2500	68.0833	1.76
Treatment	2	890.1667	445.0834	11.49**
Error	6	232.5000	38.7500	
Total	11	1,326.9167		
		c.v. = 64.95%		
		Infected sclerotia		
Replication	3	38.2500	12.7500	1.17
Treatment	2	24.5000	12.2500	1.12
Error	6	65.5000	10.9167	
Total	11	128.2500		
		c.v. = 264.32%		

*Significant at the 5% level.

**Significant at the 1% level.

APPENDIX 17. Analysis of variance for results of 1982 biological control field experiment (Portage la Prairie location).

Source of variation	D.F.	Sum of squares	Mean squares	F value
		Yield		
Replication	3	206,847.0163	68,949.0030	2.43
Treatment	2	1,051,669.6331	525,834.8000	18.55**
Error	6	170,045.6487	28,340.9414	
Total	11	1,428,562.2981		
		c.v. = 9.19%		
		Disease (%)		
Replication	3	492.6889	164.2296	2.13
Treatment	2	7,529.5555	3,764.7778	48.91**
Error	6	461.8305	76.9717	
Total	11	8,484.0748		
		c.v. = 19.07%		
		Recovered sclerotia/kg soil		
Replication	2	18.8561	6.2854	1.04
Treatment	2	143.6643	71.8321	11.84**
Error	6	36.3868	6.0645	
Total	11	198.9072		
		c.v. = 44.30%		
		Recovered sclerotia		
Replication	3	297.5833	99.1944	1.03
Treatment	2	2,268.1667	1,134.0834	11.83**
Error	6	575.1667	95.8611	
Total	11	3,140.9167		
		c.v. = 44.33%		
		Viable sclerotia		
Replication	3	282.2500	94.0833	1.48
Treatment	2	2,188.1667	1,094.0834	17.25**
Error	6	380.5000	63.4167	
Total	11	2,850.9167		
		c.v. = 44.44%		
		Infected sclerotia		
Replication	3	6.0000	2.0000	1.60
Treatment	2	7.1667	3.5834	2.87
Error	6	7.5000	1.2500	
Total	11	20.6667		
		c.v. = 167.70%		

*Significant at the 5% level.

**Significant at the 1% level.