POTENTIAL FOR THE BIOLOGICAL CONTROL OF ONION WHITE ROT IN THE FRASER VALLEY OF BRITISH COLUMBIA

by

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TABLE OF CONTENTS

Approval ii

Abstract v

Acknowledgements vii

List of Tables viii

List of Figures ix

A. Introduction 1

B. Protection of Host Plant Surfaces 4

I. Selection of Potential Biological Control Fungi 6

Introduction 6

Materials and Methods 9

Results 12

Discussion 28

II. Field Experiments on Biological Control of White Rot 35

Introduction 35

Materials and Methods 36

Results 39

Discussion 46

III. Conclusion 51

C. Reduction of Inoculum 54

I. Comparison of Laboratory- and Field-Produced Sclerotia 56

Introduction 56

Materials and Methods 58

Results 62

Discussion 73

II. Natural Decay of Sclerotia in Fraser Valley Soils ...81
Introduction 81
Materials and Methods 82
Results 84
Discussion 92
III. Enhanced Reduction of Sclerotia 97
Introduction 97
Materials and Methods 98
Results 101
Discussion 116
IV. Conclusions 120
D. Conclusions 123
Literature Cited 125

ABSTRACT

White rot, an onion disease caused by the fungus *Sclerotium cepivorum* Berk., is a serious problem in the Fraser Valley of British Columbia. This thesis examines the potential of controlling the disease biologically by using soil fungi to protect the plant from infection and by reducing inoculum levels in the soil.

Fungi were isolated from Fraser Valley soils and evaluated for their potential as biological control agents for onion white rot. Several of the 400 isolates obtained were *Penicillium* spp. and *Trichoderma* spp. which have been used elsewhere to control diseases caused by sclerotia-forming pathogens. Of 310 fungus isolates tested, 59% were antagonistic to *S. cepivorum in vitro.* None of the 90 isolates tested in the field caused significant reduction in white rot incidence.

The potential for controlling white rot by reducing the population of sclerotia of *S. cepivorum* in soil was evaluated by studying the survival of sclerotia under local conditions. Sclerotia produced in the laboratory decayed faster than those produced on onions in the field. Laboratory-produced sclerotia were initially sterile but after 96 h in soil they developed a mycosphere population of bacteria and fungi similar to that of field-produced sclerotia. The rind of laboratory-produced sclerotia was thicker than that of field-produced sclerotia but it was broken in many places, while that of field-produced sclerotia was continuous and underlain by a layer of ovoid

cells.

Half-life estimates for field-produced sclerotia were 25.8, 9.7, and 10.7 months for field trials conducted in 1979, 1980, and 1981, respectively. Drying sclerotia prior to burial significantly increased their rate of decay. Sclerotia left on the surface of soil decayed faster than buried ones in 1 of 2 years' field trials. The rate of decay of sclerotia was reduced by admixture of soil with the sclerotia but was not affected by soil type.

These results suggest that the potential for reducing white rot in the Fraser Valley by protecting host plant surfaces with fungal antagonists is low, but that cultural measures aimed at reducing inoculum levels may be a useful part of a disease management program.

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LIST OF TABLES

1. Comparison of dual culture interactions of soil fungi and *S. cepivorum* on PDA and SEA 22
2. Dual-Culture interactions of soil fungi and *S. cepivorum* on PDA 25
3. Known biological control agents of sclerotia -forming pathogens isolated from Fraser Valley soils................26
4. Influence of soil sterilization on mortality of onions in soil, amended or not amended with sclerotia of *S. cepivorum*. 27
5. White rot incidence in onions as affected by seed treatment with soil fungi (Experiment I)............................40
6. White rot in onions as affected by treatment with *Coniothyrium minitans*, *Bacillus subtilis*, *Trichoderma harzianum* and the fungicide Ronilan, alone or in combination...44
7. White rot in onions as affected by seed treatment with soil fungi (Experiments II and IV)...............................45
8. Comparison of the structure of laboratory-produced and field-produced sclerotia of *S. cepivorum*. 72
9. Composition of the bacterial and fungal microflora on field-produced sclerotia of *S. cepivorum* as affected by drying and incubation in soil.............................115

LIST OF FIGURES

1. The effect of fungi isolated from Fraser Valley soils on the growth of *S. cepivorum* on PDA.................................13

2. The effect of *S. cepivorum* on the growth on PDA of fungi isolated from Fraser Valley soils...........................15

3. Non antagonistic interactions of soil fungi and *S. cepivorum* on PDA..18

4. Antagonistic interactions of soil fungi and *S. cepivorum* on PDA..20

5. Incidence of white rot in onion treated with antagonistic and non antagonistic soil fungi (Experiment II)...........42

6. Decline in populations of laboratory-produced and field-produced sclerotia of *S. cepivorum* in muck soil.....63

7. Effect of residue from infected onions on the decline in the population of laboratory-produced sclerotia of *S. cepivorum* in muck soil...65

8. Bacterial populations of laboratory-produced and field-produced sclerotia of *S. cepivorum* as affected by incubation in soil..68

9. Fungal populations of laboratory-produced and field-produced sclerotia of *S. cepivorum* as affected by incubation in soil. 70

10. Cross sections of *S. cepivorum* *sclerotia.* a) laboratory-produced b) field-produced.....................74

11. Cross sections of rind of *S. cepivorum* sclerotia. a) laboratory-produced b) field-produced....................76

12. The effect of admixture of muck soil with sclerotia on the decline in the population of sclerotia of _S. cepivorum_ exposed on the surfaace pf muck soil.....................85

13. The effect of soil source on the proportion of sclerotia of _S. cepivorum_ surviving 8 and 12 months of field exposure .88

14. Patterns of decline in the population of field-produced sclerotia of _S. cepivorum_ in muck soilin the Fraser Valley for three successive years...............................90

15. Sclerotia of _S. cepivorum_ infected by _C. minitans_........102

16. The effect of _C. minitans_ on the proportion of sclerotia of _S. cepivorum_ surviving 8 and 12 months field exposure in muck soil...104

17. The effect of preburial desiccation on the decline in the population of field-produced sclerotia of _S. cepivorum_ in muck soil...106

18. The effect ofthe position of the sclerotia in soil (surface or buried 15 cm deep) on the decline in the population of field-produced sclerotia of _S. cepivorum_ in muck soil....108

19. Bacterial populations on sclerotia of _S. cepivorum_ as affected by drying and incubation in soil...............111

20. Fungal populations on sclerotia of _S. cepivorum_ as affected by drying and incubation in soil........................113

A. Introduction

White rot, an onion disease caused by the fungus *Sclerotium cepivorum* Berk., was first discovered in the Fraser Valley in 1970 (88). The disease, which can cause severe yield losses (5, 40, 41, 82) has continued to spread (115) and now threatens the local onion industry. It is therefore essential to identify measures which can be used to control white rot in this area. The purpose of this thesis is to examine the potential of biological control as a means of limiting losses due to white rot in the Fraser Valley.

The pathogen, *S. cepivorum*, is an imperfect fungus which attacks only *Allium spp.* (27, 120). The fungus produces micro-conidia in nature (28) and in culture (13, 39) but these spores have not been observed to germinate and their function is unknown. The only known means by which the pathogen survives in the absence of the host is as small (0.2 to 0.5 mm diam) black sclerotia (27, 40, 120). These sclerotia remain dormant in non-sterile soil until stimulated to germinate by exudates from the roots of *Allium spp.* (32, 33, 35, 46, 70, 71). Infection of the host is either from germinated sclerotia, (primary infection) (27, 38, 39, 120) or from mycelial spread from adjacent infected plants (secondary infection) (38, 98, 99). Hyphae of *S. cepivorum* invade the roots and colonize both inter- and intracellularly, eventually destroying the parenchymatous tissue (4). New sclerotia are formed within and on the host

tissue and are deposited in soil where they may persist for many years in the absence of the host (27, 41, 79).

Complete control of white rot has not been achieved, and in some cases where infestation of soil is severe, onion production has had to be abandoned (9, 42, 82). Chemical control has reduced white rot in some studies. Effective chemicals include calomel (23, 42), PCNB (97), dichloran (49) iprodione (47, 48, 116) and vinclozolin (116). Of these materials only dichloran is registered for use on onions in Canada. Iprodione and vinclozolin have significantly reduced white rot in field trials in the Fraser Valley (116) and may provide a short term solution to the disease problem. They cannot be relied upon for long, however, as there is already evidence that the pathogen can develop resistance to them (76). Cultural methods such as the use of organic amendments (97), altering planting dates (97), and the use of onions as a trap crop (81) have been tried but are of little use in controlling the disease. One method which is giving promising results in field trials is the use of onion oil applications to stimulate germination of sclerotia in the absence of the host (82, 84, 119). This measure is effective because sclerotia of S. cepivorum that germinate in the absence of the host decay quickly and do not readily form secondary sclerotia (27, 39, 119). This method gave significant control of white rot in field trials in the Fraser Valley, but at prohibitive cost (119). Alternative control procedures for white rot in the Fraser Valley are still needed.

Biological control may ultimately provide the answer to the white rot problem. Cook (37) identified three mechanisms by which biological controls can act to prevent disease:

> " 1. through the management of incompatability between host and pathogen through plant breeding or inoculation with a mild or avirulent strain of the pathogen, 2. through protection of host plant surfaces with antagonists of the target pathogen and 3. through reduction of inoculum by antagonists of the target pathogen."

Biological control of white rot by each of these mechanisms is possible. Recent work at Simon Fraser University has identified sources of resistance to white rot and a breeding program is currently underway in the Fraser Valley (114). It will be several years before a commercially acceptable cultivar can be developed, however, and it is unlikely that the level of resistance that can be achieved will provide complete control.

This thesis examines potential for biological control of white rot in the Fraser Valley via protection of host plant surfaces with fungal antagonists and via reduction in the inoculum potential of the pathogen.

B. Protection of Host Plant Surfaces

Biological control by protection of host plant surfaces involves (37)

> " the establishment of antagonists in or near the infection court of the host, thereby providing a biological barrier against the target pathogen."

Use of antagonists in this manner essentially treats them as biological pesticides and they are often produced and marketed in a manner similar to chemical pesticides. Commercial preparations of _Agrobacterium radiobacter_ (68), _Peniophora gigantea_ (54, 96), _Trichoderma viride_ (43) and ectomycorrhizae for the control of _Phytopthora cinnamoni_ on pine (78) are currently available. Several other diseases have been controlled biologically in field trials but the agents have not yet been developed for commercial use (16, 55, 62, 72, 109).

Organisms used as biological control agents for plant diseases may be bacteria, fungi, viruses or nematodes (17). Bacteria and fungi are most commonly used. The successful use of these organisms involves extensive tests of many organisms before one can be chosen for further development. These tests may be both laboratory and field studies. Organisms are usually screened in the laboratory to identify ones that are antagonistic to the pathogen and these are tested in the field for their ability to control the disease.

Control of white rot by protection of the host plant surfaces has been achieved using both bacterial and fungal

antagonists (3, 11, 51, 118). Field trials in the Fraser Valley have shown that a bacterium, *Bacillus subtilis*, can reduce the levels of white rot significantly in some cases (117).

This study on the protection of host plant surfaces was designed to determine if fungi could be used as biological control agents for white rot in the Fraser Valley. Section I describes the isolation of fungi from local soil and the selection of potential antagonists. Section II describes the subsequent testing of these organisms in the field.

I. Selection of Potential Biological Control Fungi

Introduction

The first step in developing a biological control program for any disease is to select organisms which have potential to protect the host. Biological control agents to be used against soil-borne pathogens may be isolated from soil, from the rhizosphere of the host, or from the surface of the pathogen (17). Any isolation procedure will likely yield more organisms than can be practically tested in field trials. An initial screening procedure must therefore be used to select a smaller subset of potentially useful organisms which can then be tested in the field. This process involves both laboratory studies and field observations. Organisms which may have potential as biological control agents and which should be retained for further testing may be grouped into three categories:

1. organisms which have demonstrated antagonistic activity towards the pathogen in dual culture tests,
2. organisms which have controlled the disease being studied or similar diseases in other areas,
3. organisms which are thought to be responsible for naturally occurring biological control.

Dual culture tests are a common means of selecting organisms for use in biological control experiments (24, 50, 73,

93, 124). The pathogen and the test isolate are plated on opposite sides of a culture plate and the two colonies are allowed to grow together. The formation of a clear zone between the two colonies is regarded as an indication that the isolate is antagonistic to the pathogen. These tests, which are usually carried out on a rich medium, do not necessarily indicate that an organism will be a good biological control agent. Kommedahl and Windels (73) did not find any consistent relationship between the size of the inhibition zone and disease control in the field. Wood and Tveit (124) suggested that measurements of inhibition of growth of the pathogen or the use of a more natural medium such as soil extract agar (SEA) may give more meaningful results. Despite their limitations, these tests still provide us with one method of selecting potential biological control agents.

Organisms which have controlled the disease being studied or similar diseases are good candidates for field testing., Several bacteria and fungi, notably _Bacillus spp._ (72, 74, 107, 118), _Pseudomonas spp._ (37, 74, 87), _Coniothyrium spp._ (11, 62, 110, 111) and _Trichoderma spp._ (2, 16, 45, 55, 56), have been used successfully as biological control agents on several occasions. Their performance is not guaranteed, however, as different isolates may have different abilities to control disease (2 21, 22). Local environmental factors may also have profound effects on the efficacy of a given agent (56, 65). Diseases caused by sclerotium-forming pathogens other than _S._

cepivorum have been controlled by applications of Coniothyrium minitans (61, 109, 110), Gliocladium catenulatum (61, 62), Gliocladium roseum (85), Sporidesmium sclerotivorum (7, 8, 15), Trichoderma harzianum (45, 57, 109) and Trichoderma hamatum (55, 57, 65). White rot has been controlled with C. minitans (11), Penicillium nigricans (51) and Trichoderma spp. (2, 3). Any one of these fungi is a potential biological control agent for white rot in the Fraser Valley.

The best place to look for biological control agents is in soils where, despite conditions favorable to disease development, the disease does not occur (17). Such soils are known as suppressive soils. Suppressive soils often have microfloral populations which are significantly different from those of conducive soils (12, 26, 44). Disease control in conducive soils may be achieved by introduction of a proportion of the microfloral populations from suppressive soils (26, 37). In some situations one organism can be identified as the primary cause of the suppressiveness of a soil. The introduction of large amounts of this organism to a conducive soil may be sufficient to control the disease. Chet and Baker (26) identified T. hamatum as the cause of the suppressiveness of a Columbian soil to Rhizoctonia solani and were able to use this fungus to control the diseases caused by R. solani in Colorado. Soil in some fields in Burnaby, an area of small market gardens in the Fraser Valley of British Columbia, may be suppressive to white rot. In a survey conducted in 1977, these fields were

found to contain viable sclerotia of S. *cepivorum*, but white rot was not found in onions planted in these fields (115). If this effect is due to a biological factor, these fields may represent good source of biological control agents.

This section describes the selection of potential biological control agents for onion white rot. The organisms were selected from fungi isolated from soil from fields in Burnaby (possibly suppressive to white rot) and from soil from fields in Cloverdale (conducive to white rot). The fungi were evaluated as biological control agents on the basis of the criteria discussed above.

Materials and Methods

Isolation of Fungi

Soil samples were collected in September 1978 from eight fields in the Fraser Valley: four from fields in the Cloverdale area that had white rot in the 1977 survey and four from fields in Burnaby that did not have white rot but were infested with *S. cepivorum*. Soil from 30 to 50 randomly located sites in each field was bulked in polyethylene bags and stored at 5° C until it could be processed. Dilution and soil plates were prepared within 48 h of field sampling.

Dilution plates were used to estimate the population of fungi in each soil. The soil from each bag was first mixed in a rotary mixer. Twenty-five g of soil (dry weight basis) was added

to 250 mL of a 0.1 % agar solution. The suspension was shaken on a mechanical shaker for 20 min, and serial dilutions were prepared. One mL aliquots of the $1x10^5$ dilution were spread over the surface of each of 10 plates of OHIO agar (67) and 10 plates of Dextrose Peptone Yeast Extract Agar (DPYEA) (67). The plates were incubated at 22° C for seven days. The number of colonies on each plate was recorded and subcultures were made of presumed species.

Soil plates were prepared to isolate fungi which are not normally obtained on dilution plates. One g of soil was mixed with 99 g of sterile sand. Approximately 0.01 g of this mixture was placed in the bottom of each of 20 petri dishes. Twenty mL of either OHIO agar or DPYEA; cooled to 45° C, was added to each of 10 replicate dishes per soil sample. The dishes were swirled to suspend the soil in the agar and incubated for 14 days at 22° C. Subcultures were made of presumed species.

Identification

One hundred and fifty isolates were identified at least to genus. Cultures for identification were grown on Czapeks Dox Agar (CDA); Potato Dextrose Agar (PDA); and Malt Extract Agar (MEA). Some of the isolates were sent to the Biosystematics Research Institute, Agriculture Canada, Ottawa, for further identification.

Dual Culture Tests

Three hundred and ten isolates were evaluated for antagonism to *S. cepivorum* on PDA. Thirty-four isolates were also tested on SEA. The tests were done on either 60 x 15 mm or 150 x 15 mm plates. The test isolates were inoculated on one edge of the plate and allowed to grow for 24 h before a 5 mm diam core of *S. cepivorum* was placed at the opposite edge. The core was taken from the edge of an actively growing colony of *S. cepivorum* with a number 2 cork borer. The plates were incubated at 22° C and examined for the type of interaction between the test fungus and *S. cepivorum* after five days for the smaller plates and seven days for the larger plates. All tests were conducted at least twice. Thirty isolates were tested further to quantify inhibition of growth of *S. cepivorum* and the test fungus. The plates (60 x 15 mm) were prepared as described above and the colony diameter of each isolate and *S. cepivorum* in pure and in dual culture was recorded at 24, 48 and 72 h. There were four replicates of each test.

Evaluation of Suppressiveness of Burnaby Soil

Muck soil was collected from a vegetable farm in Burnaby British Columbia and brought into the laboratory where half of it was sterilized by autoclaving twice (103.5 KPa pressure; 15 min). The soil was placed in 42 pots, 10 cm in diameter. There were seven replicate pots for each of the following six treatments: sterilized and non-sterilized soil, each with

field-produced sclerotia, laboratory-produced sclerotia or no sclerotia added. Onion seeds (cv. Autumn Spice) were planted in the soil and the pots were kept in a growth chamber for 12 weeks. The number of healthy plants was recorded weekly. Percent mortality was calculated as the number of dead plants/number of onions emerged x 100. The data were subjected to an arc-sine transformation and analyzed using analysis of variance and Duncan's Multiple Range Test.

Results

Dual Culture Tests

Qualitative evaluation of the interactions of 310 fungus isolates and S. cepivorum showed that 184 (59.2 %) were antagonistic to the pathogen. Quantitative evaluation of 30 isolates showed that 97 % inhibited the growth of S. cepivorum by at least 20% (Fig. 1). Two colonies of S. cepivorum plated on opposite sides of the plate continued growth through each other with no apparent inhibition of growth although measurement of their colony diameters was impossible as the mycelium of the two colonies could not be distinguished from each other. S. cepivorum inhibited the growth of 56.6% of the test isolates by at least 20% (Fig. 2).

Fig.1. The effect of fungi isolated from Fraser Valley soils on the growth of *S. cepivorum* on PDA.

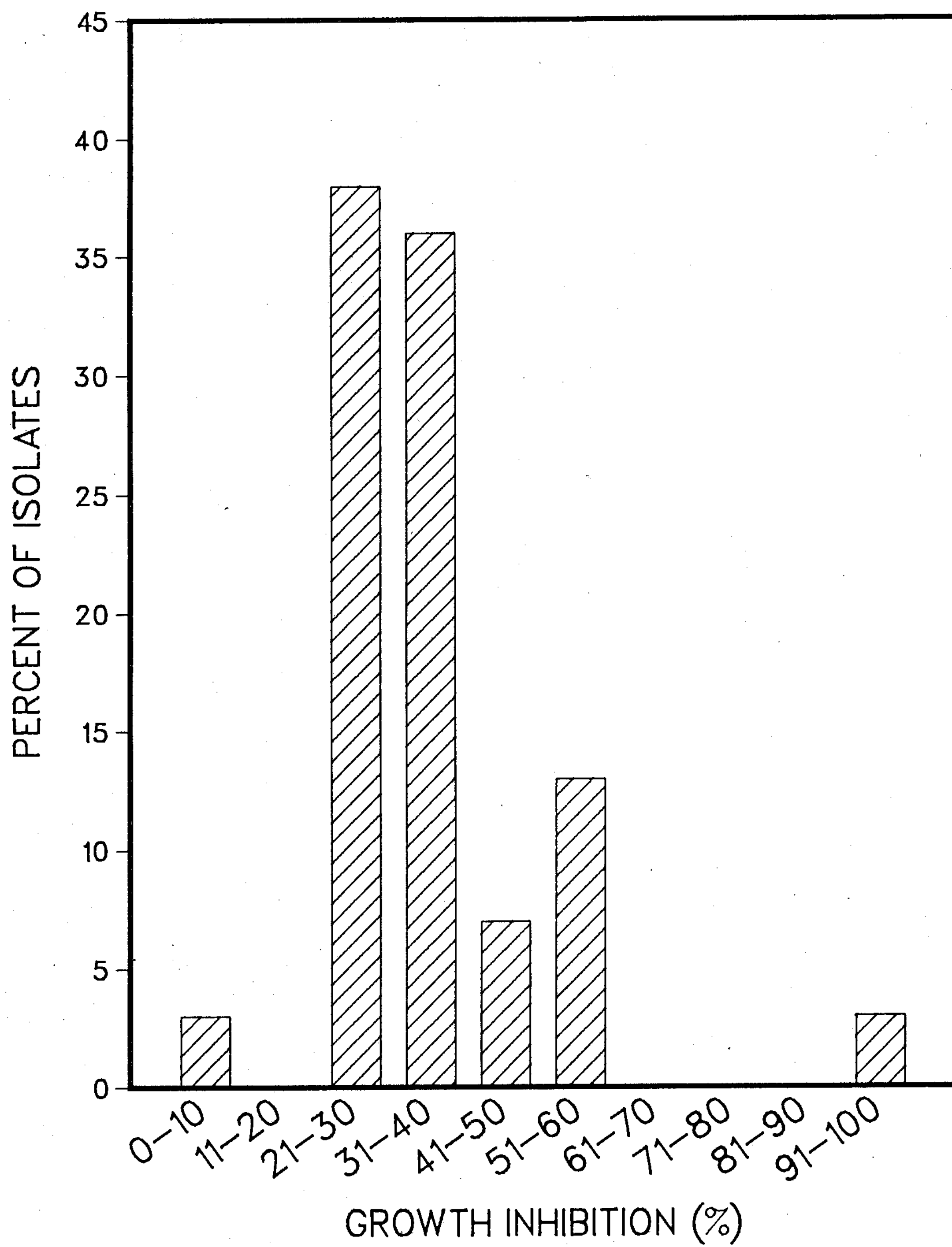
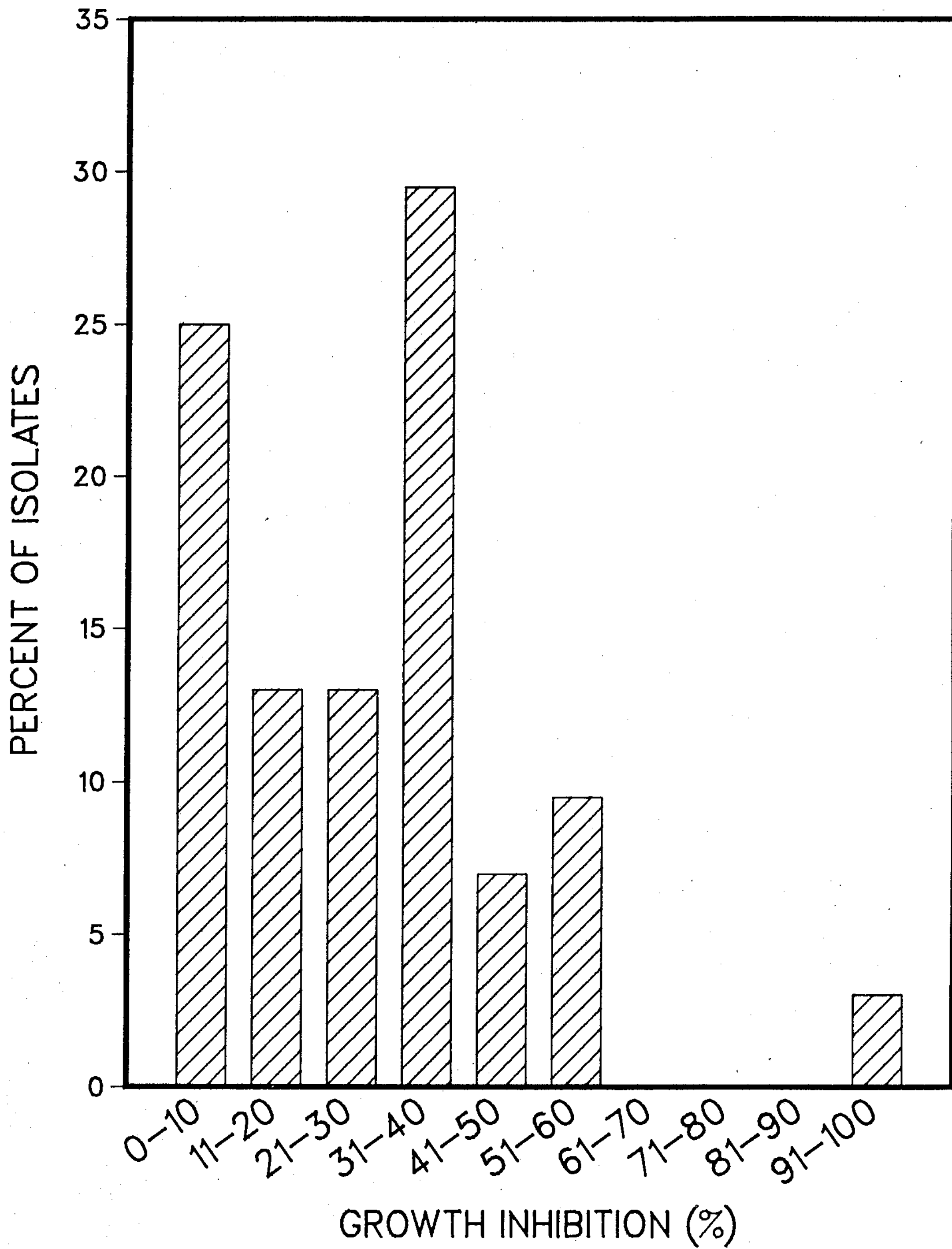

PERCENT OF ISOLATES
45
40
35
30
25
20
15
10
5
0
0–10
11–20
21–30
31–40
41–50
51–60
61–70
71–80
81–90
91–100
GROWTH INHIBITION (%)

Fig.2. The effect of *S. cepivorum* on the growth on PDA of fungi isolated from Fraser Valley soils.



PERCENT OF ISOLATES
35
30
25
20
15
10
5
0
0–10
11–20
21–30
31–40
41–50
51–60
61–70
71–80
81–90
91–100
GROWTH INHIBITION (%)

The interactions between the test fungi and *S. cepivorum* in dual culture tests were grouped into five major categories:

A Two colonies grow through each other

B The pathogen overgrows the test isolate

- a grows around
- b grows over

C The two colonies grow until they meet and growth stops

D The growth of both colonies stops at a distance from each other leaving a clear zone

- a zone less than 5 mm
- b zone between 5 and 10 mm
- c zone greater than 10 mm

E The test isolate overgrows *S. cepivorum*

- a grows around
- b grows over

The first two categories (A and B) describe interactions in which the pathogen is not severely limited by the test isolate, the third category (C) describes an interaction in which both organisms are equally affected. The isolates in these categories (Fig. 3) are classified as non antagonistic to *S. cepivorum* . The last two categories (D and E) describe interactions where the pathogen is significantly affected by the test organism. The organisms in these categories (Fig. 4) are classified as antagonistic to *S. cepivorum* . In these interactions the mycelium of *S. cepivorum* often becomes darkened.

Fig.3. Non antagonistic interactions of soil fungi and *S. cepivorum* on PDA. (Arrows represent the direction and extent of fungus growth, asterisks show the location of *S. cepivorum*)

A *S. cepivorum* and the test isolate grow through each other.

B *S. cepivorum* overgrows the test isolate .

C *S. cepivorum* and the test isolate grow until they meet and growth stops.

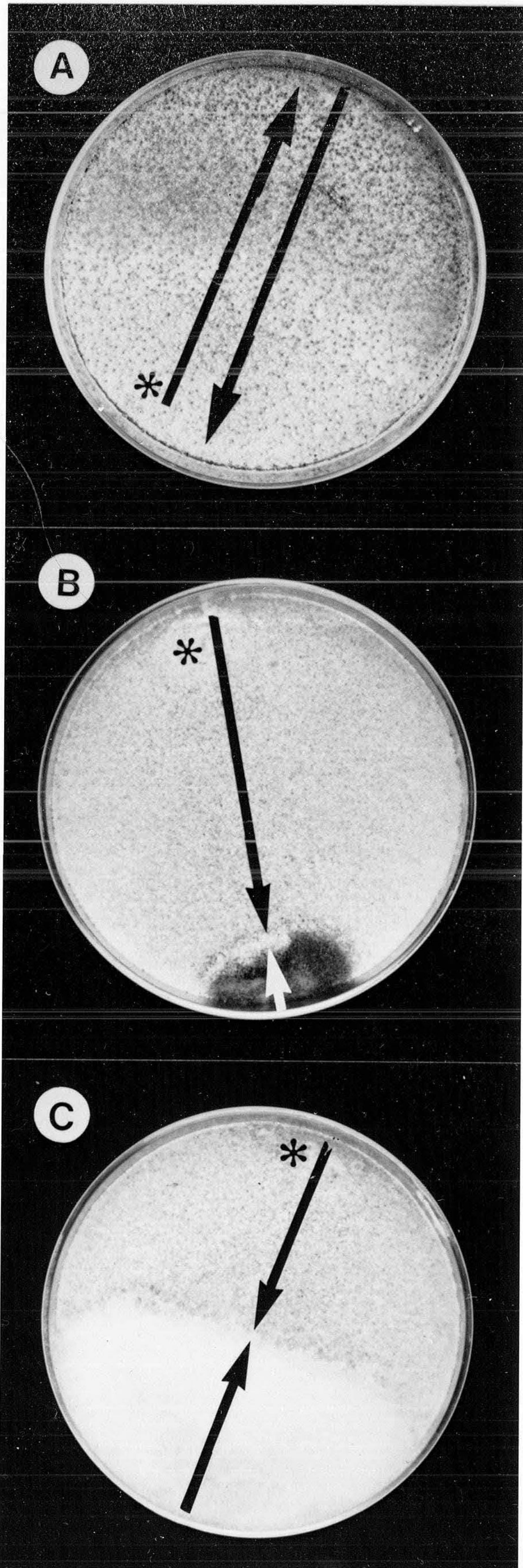

A
*
B
*
C
*

Fig.4. Antagonistic interactions of soil fungi and _S. cepivorum_ on PDA. (Arrows represent the direction and extent of fungus growth, asterisks show the location of _S. cepivorum_)

A The growth of _S. cepivorum_ and the test isolate stop at a distance from each other leaving a clear zone (5 to 10 mm) .

B The growth of _S. cepivorum_ and the test isolate stop at a distance from each other leaving a clear zone (>10 mm).

C The test isolate overgrows _S. cepivorum_ .

D The test isolate causes a browning of the _S. cepivorum_ mycelium .

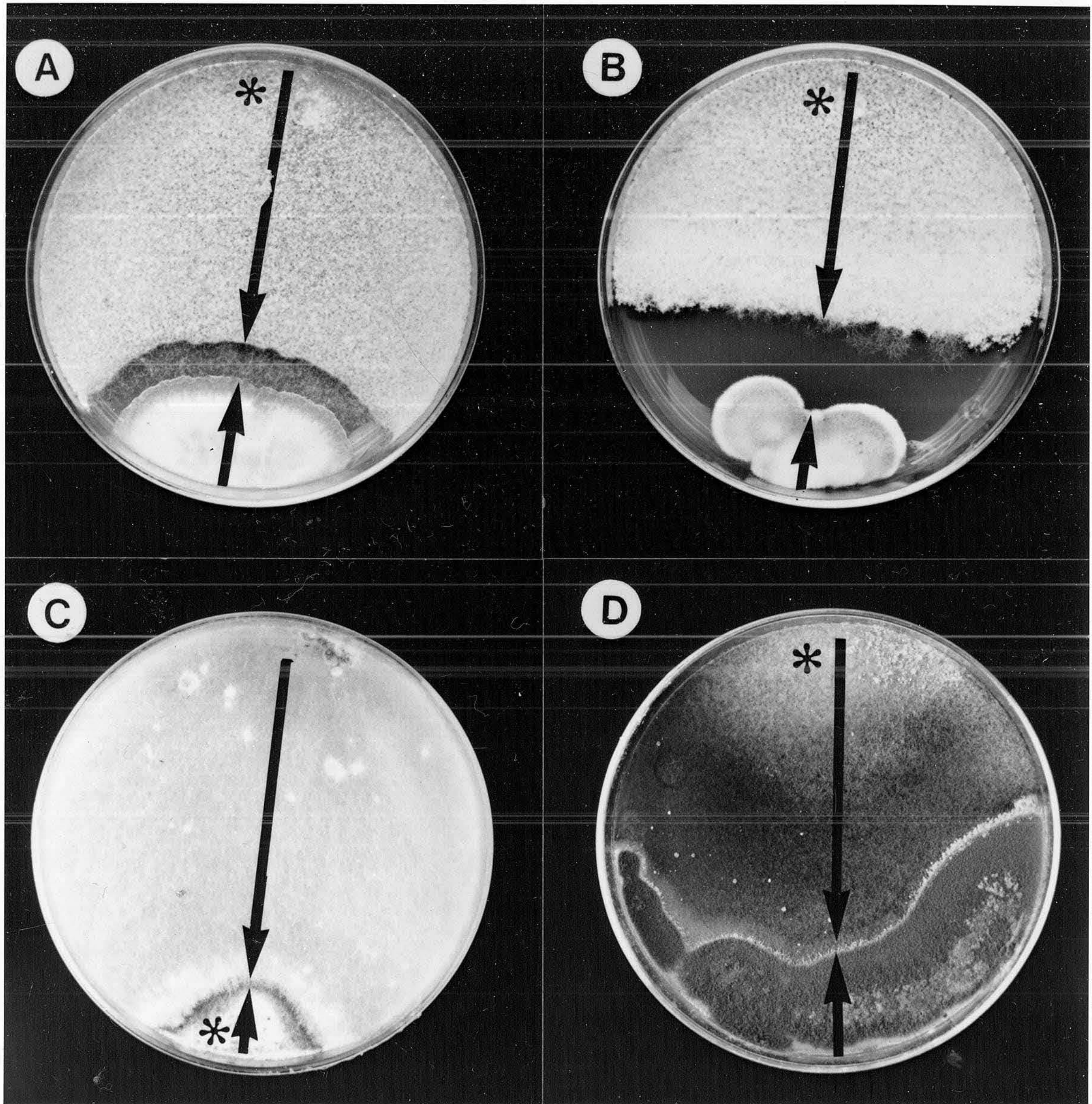

A
B
C
D

Table 1. Comparison of dual-culture interactions of soil fungi and *S. cepivorum* on PDA and SEA.

Interaction	Percent of Isolates	
	PDA	SEA
A. Two colonies grow through each other	5.8	2.9
B. *S.cepivorum* overgrows the test isolate	14.7	17.6
C. Two colonies grow until they meet and stop growing	5.8	2.9
D. Two colonies stop growing at a distance from each other	61.8	64.7
E. Test isolate overgrows *S.cepivorum*	11.7	8.8

In category D this browning may occur from 0 to 1 cm back from the edge of the contact zone. In category E the mycelium often becomes totally darkened and formation of sclerotia is reduced or eliminated.

The growth of the pathogen and the test isolates was less on SEA than on PDA. There were few differences in the interactions observed on the two media (Table 1). Twenty-five of the isolates tested (73.5%) were antagonistic to *S. cepivorum* on both media. *S. cepivorum* was antagonistic to 33.9% of the fungi tested. Forty-nine percent of the isolates tested caused the formation of an inhibition zone, and a further 9.5% grew over the pathogen (Table 2). Wide inhibition zones were produced by *Penicillium spp.*, notably *P. nigricans* and *P. jenseni*, and *Acremonium furcatum*. Both of the *Trichoderma spp.* overgrew the pathogen and caused a darkening of the mycelium and reduction in sclerotia. *Gliocladium catenulatum*, a *Penicillium spp.* and an ascomycete(likely *Emercellopsis*) also overgrew the pathogen.

Occurrence of Known Biocontrol Agents in Local Soils

All of the species which have controlled sclerotia-forming pathogens in other areas with the exception of *C. minitans* and *S. sclerotivorum* were found in local soils. All of these isolates were antagonistic to *S. cepivorum* in dual culture tests (Table 3). *C. minitans* was occasionally isolated from sclerotia of *S. cepivorum* from local soil.

Suppressiveness of Burnaby Soil

There was significantly ($p \leq 0.05$) more mortality of the onions in soil with sclerotia of S. cepivorum than in the control soil. There was no significant difference in the mortality of onions in pots with sclerotia produced in the laboratory or the field. Onion mortality was significantly greater ($p \leq 0.05$) in sterilized soil than in non-sterilized soil when laboratory-produced sclerotia were added. Although there was no significant difference ($p > 0.05$) in mortality in sterilized and non-sterilized soil amended with sclerotia from onions the mortality was twice as great in the latter (Table 4).

There were no significant differences ($p \leq 0.05$) in the fungal populations of Burnaby and Cloverdale soils, which contained populations of soil fungi of 6.53×10^5 and 8.66×10^5 colony forming units per g, respectively. The proportion of antagonistic isolates was over 50% in both soils. There were no obvious differences in the proportions of the most common genera in the two soils or any indication that any species was predominant in Burnaby soil and not in Cloverdale soil.

Table 2. Dual-culture interactions of soil fungi and *S. cepivorum* on PDA.

Interaction	% of 310 Isolates	Species
A. Two colonies grow through each other	2.0	*Rhizopus* spp., *Mucor* spp.
B. *S.cepivorum* overgrows the test isolate	33.9	*Cladosporium cladosporiodes*, *Fusarium* spp., *Gliocladium viride*, *Humicola grisea*, *Monocillium mucidum*, *Paecilomyces inflatus*, *Penicillium* sp., *Trichocladium asperum*, *Verticillium* sp.
C. Two colonies grow until they meet and growth stops	4.0	*Fusarium* sp.
D. Two colonies stop growing at a distance from each other		
distance<5mm.	25.0	*Acremonium atrogriseum*, *Eupenicillium* sp., *Fusarium proliferatum*, *Fusarium moniliforme*, *Gliocladium roseum*, *Gliocladium virens*, *Mortiarella alpina*, *Mortiarella vinacea*, *Nectria inventa*, *Penicillium* sp., *Verticillium* sp.
distance 5mm to 10mm	15.1	*Aspergillus* sp., *Cladosporium herbarum*, *Penicillium* sp., *Pseudoeurotium zonatum*, *Ulocladium* sp., *Verticillium* sp.
distance >10mm	9.6	*Acremonium furcatum*, *Fusarium* sp., *Penicillium nigricans*, *Penicillium jenseni*, *Penicillium* sp., *Myrothecium roridum*
E. Test isolate grows over *S. cepivorum*	9.5	*Gliocladium catenulatum*, *Penicillium* sp., *Trichoderma hamatum* , *Trichoderma harzianum*

Table 3. Known biological control agents of sclerotia-forming pathogens isolated from Fraser Valley Soils

Species	Pathogen Controlled	Local Isolation	Dual-Culture Interaction
Coniothyrium minitans	*Sclerotium cepivorum* *Sclerotinia sclerotiorum*	from sclerotia	D a
Gliocladium catenulatum	*Sclerotinia sclerotiorum*	B(.1)C(.1)*	E
Gliocladium roseum	*Phomopsis sclerotiodes*	B(.1)C(.1)	D a
Penicillium nigricans	*Sclerotium cepivorum*	B(.22)C(.15)	D c
Sporidesmium sclerotivorum	*Sclerotinia minor*	not isolated	
Trichoderma hamatum	*Rhizoctonia solani*	B(.1)C(.1)	E
Trichoderma harzianum	*Rhizoctonia solani* *Sclerotinia rolfsii*	B(.2)C(.15)	E

*B=Burnaby, C=Cloverdale ,numbers in brackets are average populations of species in soil x10^5

Table 4. Influence of soil sterilization on mortality of onions in soil amended or not amended with sclerotia of *Sclerotium cepivorum*

Treatment	Average % Mortality
Sterilized Soil	
Laboratory Sclerotia	74.9a*
Field Sclerotia	65.0ab
No Sclerotia	5.7d
Non-Sterilized Soil	
Laboratory Sclerotia	26.8cd
Field Sclerotia	32.3bc
No Sclerotia	3.1d

*numbers followed by the same letter are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

Discussion

Too many (310) fungi were isolated from Fraser Valley soils to test them all in the field for their ability to control white rot. Additional studies were, therefore, conducted to select promising organisms for use in further tests.

Dual Culture Tests

Wood and Tveit (124) suggested that dual culture tests should be carried out on a medium which has nutrient conditions similar to those of the natural environment of the pathogen: SEA for soil-borne pathogens. Ideally each organism should be tested using conditions of nutrient composition, water potential, and temperature specific to each antagonist, pathogen and soil combination (17). As knowledge of the specific environmental conditions present in Fraser Valley soils is minimal and as it would be impractical to develop specific conditions for each dual culture test, the interactions of *S. cepivorum* and soil fungi were compared only on PDA and SEA. The interactions of *S. cepivorum* and the soil fungi were similar on the two media. This result agrees with the observations of Broadbent *et al.* (24) and Porter (93) who found that the media influenced the intensity but not the type of interaction. The remaining dual culture tests were all conducted on PDA as it is readily available and easy to prepare.

Wood and Tveit (124) also suggested that quantitative measurements on the inhibition of growth of the pathogen may give more meaningful results than the standard qualitative evaluations used in most studies. The growth of S. cepivorum was inhibited by at least 20% by the majority of the isolates tested (Fig. 1). Those isolates which reduced the growth of the pathogen by at least 50% were also classified as antagonistic on the basis of qualitative evaluation. These measurements alone, therefore, do not significantly improve one's ability to distinguish between potential antagonists. The measurements, however, also showed that S. cepivorum could inhibit the growth of a large proportion (56.6%) of the test isolates. If similar inhibition occurs in soil it may help to explain in part why some organisms, antagonistic in culture, are not as successful in biological control experiments as might be hoped. Measurements of the inhibition of growth of both the pathogen and the test isolate may be a useful means of selecting potential biological control agents. They are time-consuming and laborious, however, and are not a practical means of screening large numbers of organisms. It may be possible to use quantitative measurements such as these to distinguish between promising isolates selected on the basis of qualitative evaluation.

The observation that S. cepivorum was antagonistic to 33.9% of the isolates tested is contrary to the reports of Papavizas (89) and Scott (98) who cite S. cepivorum as a poor competitor.

Ghaffar (50), in his study of the interactions of soil fungi and *S. cepivorum* , did not mention any instances of the pathogen inhibiting the test fungi. The observation that a pathogen can antagonize a soil saprophyte is not unique as Bell and Wells (21) found that *Rhizoctonia solani* grew over some isolates of *Trichoderma spp.* in dual culture tests.

Wood and Tveit (124) define antagonism as

> " any action of one organism which in some way adversely affects another growing in association with it."

Most soils contain a large number of organisms antagonistic to pathogens. Broadbent *et al.* (24) found that 40% of the bacteria and actinomycetes tested were antagonistic to at least one of nine pathogens. Kommedahl and Windels (73) reported that 59% of the bacteria and fungi they tested were antagonistic to *Fusarium solani* or *R. solani.*

The larger proportion (>50%) of isolates antagonistic to *S. cepivorum* in this study than the 25% reported by Ghaffar (50) is a consequence of the predominance of *Penicillium spp.* and *Trichoderma spp.* isolated from local soils. These genera are commonly over-represented on dilution plates due to their ability to produce large numbers of spores (121). The large proportion of antagonists reported here is representative of the fungi isolated but not necessarily representative of the *in situ* population of soil fungi.

The majority of the isolates which produced a wide inhibition zone on agar (category D-3) were *Penicillium spp.* Many *Penicillium spp.* are noted for their ability to produce

broad spectrum antibiotics and are often reported to produce wide inhibition zones when plated in dual culture tests against many pathogens (50, 73, 86, 93).

Both of the *Trichoderma spp.* isolated overgrew the pathogen and many caused a browning of the mycelium and inhibited sclerotia formation. Similar observations were made by Ghaffar (50) and Moubasher *et al.* (86). If this is characteristic of a reaction that occurs in soil, these organisms could be very effective biological control agents.

If all organisms which are classified as antagonistic to *S. cepivorum* were selected for further tests there would still be too many to test in field trials. While the chances of success will likely be improved by testing as wide a variety of fungal species as possible, further selection procedures must be used.

Known Biocontrol Agents

Almost all of the species which have been used in other areas to control diseases caused by sclerotia-forming pathogens were isolated from local soils. *C. minitans* and *S. sclerotivorum* are specialized mycoparasites and are not isolated by the procedures used here (6, 14, 15). Known biocontrol agents were all isolated with similar frequencies from soils from both Burnaby and Cloverdale. The presence of these fungi in soil from Cloverdale, an area with a severe white rot problem, indicates that at present they are not actively controlling the disease. It may, however, be possible to increase their populations

sufficiently to achieve control of white rot in local soils. The populations of selected antagonists can be increased by applying inoculum to the seed of the host plant (74). As these fungus species have all given disease control in other situations it is likely worthwhile to test them in the field in the Fraser Valley.

Analysis of Suppressive Soils

There was significantly more onion mortality in sterilized soil from Burnaby fields than in non-sterilized soils (Table 4) when both of these were amended with sclerotia of *S. cepivorum*. This indicates that the soil is naturally suppressive to white rot and that the suppressiveness is a biological rather than a physical or chemical phenomenon. The suppressiveness is not total as disease can be induced in Burnaby soil by the addition of large numbers of sclerotia.

Many researchers have found differences in the microbial populations of suppressive and conducive soils (12, 26, 44). In some cases they have been able to identify the active agent in the suppressive soils and use it to control the disease in conducive soils (26). There were no discernible differences in the fungal populations of soil from Burnaby and Cloverdale. The comparison of the fungi in these soils therefore did not provide a useful means of selecting potential biological control agents for white rot.

The differences in disease incidence in Burnaby and Cloverdale soils reported by Utkhede *et al.* (115) may have been due to differences in agricultural practices in the two areas rather than biological differences in the soil. In Burnaby, green bunching onions are produced in small (0.1 to 0.4 ha) plots using considerable hand labour. In Cloverdale, on the other hand, dry bulb storage onions are produced on larger (8 to 16 ha) fields with modern equipment and agricultural methods.

There may also be differences in the microbial populations of soil from the two areas which would not have been detected by the methods used in this study. The suppressiveness of Burnaby soil may be due to bacteria rather than fungi or may be due to organisms which are not readily isolated on dilution or soil plates. The suppressiveness may also be the result of a complex interaction of many organisms rather than the action of any one antagonist. None of these differences would have been detected by the methods used here and a potential biological control agent may have been overlooked.

Conclusions

None of the methods described in this paper were very useful as a means of selecting organisms for further tests. The dual culture tests eliminated only 50% of the isolates. All of the known biocontrol agents were present in local soils and are not presently keeping white rot incidence below an economically acceptable threshold level. They may, however, be of value as

biological control agents if their populations can be increased sufficiently. The comparison of supposedly suppressive and conducive soils did not reveal any organism which was evidently controlling white rot and which could be used as a biocontrol agent in conducive soils. The methods described above are all based on indirect evidence and at best can only indicate potential. It is important to test the isolates as soon as possible in the field.

II. Field Experiments on Biological Control of White Rot

Introduction

The only reliable means of evaluating the biological control potential of an organism is to test its ability to reduce disease incidence in the field. Only a few organisms which are antagonistic in the laboratory will be effective in the field. Of the 3500 bacteria and actinomycetes tested by Broadbent *et al.* (24) 40% were antagonistic on agar plates and only 4% were effective in soil.

To be a good candidate for commercial development an organism should be easy to grow on standard media, able to withstand storage, not be detrimental to the host, and of course be able to control the disease under normal environmental conditions.

There have been several reports of successful attempts to control white rot biologically. *P. nigricans* (51) and *C. minitans* (11) controlled white rot in greenhouse trials and *B. subtilis* (118) and *T. harzianum* (2, 3) were effective in the field.

This chapter describes field experiments in the Fraser Valley designed to test the effectiveness of fungi isolated from local soils as biological control agents for white rot.

Materials and Methods

Four experiments were conducted to test the ability of soil fungi to control white rot. All experiments were conducted in muck soil on a commercial vegetable farm in Burnaby. Onion seeds were planted by hand in raised beds in holes made in the soil with a peg board. The holes were spaced 7 cm apart, and two seeds were planted in each hole. The number of seedlings was counted after three weeks to determine emergence and observations were made at biweekly intervals thereafter to determine the number of plants killed. The cause of plant death was determined by plating diseased tissue and by visual examination of the onion. At harvest, the number of healthy plants and the number showing signs of white rot were recorded. Weed and insect control followed the recommendations in the Vegetable Production Guide produced by the B.C. Ministry of Agriculture and Food (1).

Seed Treatment

All of the fungi used in the field experiments, with the exception of *C. minitans*, were isolated from Fraser Valley soils and tested for antagonism to *S. cepivorum* as described in the previous chapter. *C. minitans* was obtained from the Biosystematics Research Institute of Agriculture Canada.

The fungi were grown on PDA for two to three weeks at 25° C. In experiment I methyl cellulose sticker (2%) was added to the plate and a spore suspension was prepared by rubbing the surface

of the culture with a flamed glass rod. The onion seeds (surface sterilized in 0.5% NaOCl) were coated with this suspension and dried overnight on sterile filter paper. In the other experiments, the mycelium and spores of each fungus isolate were scraped from the surface of the agar and placed in sterile petri dishes to air dry for three to ten days. The dried material was ground with a mortar and pestle, to pass through a 1 mm mesh sieve. Carborundum (60 grade) was added to the fungus to aid the grinding. Onion seeds (surface sterilized in 0.5% NaOCl) were covered with 2% methyl cellulose sticker, added to test tubes containing the powdered fungus and shaken vigorously until the seeds were evenly coated. The seeds were dried overnight in sterile petri dishes, and transferred to glass vials for storage at 5° C. Seeds were treated with the bacterium, B. subtilis and the fungicide Ronilan as described by Utkhede and Rahe (118). The seeds were plated after one, two and three months storage to test for the viability of the organisms on the seeds.

Preparation of Granular Material

Granular preparations of C. minitans and T. harzianum were made as described by Backman and Rodriquez-Kabana (16) using florex as the carrier. The granular preparation of the bacterium, B. subtilis, was made by soaking florex in a broth culture of the bacteria (2:1 v/v). The granular preparations were stored at 5° C and the viability of the material was determined by plating it on PDA after one, two and three months

storage.

The Experiments

The onion seeds for experiment I were planted on May 5, 1980, in a four by four simple lattice design. Fifteen soil fungi were applied to onion seeds (cv. Autumn Spice) as described above. Control seeds were treated only with methyl cellulose sticker. Each treatment consisted of two rows of onions (26 seeds per row) spaced 20 cm apart. The onions were harvested on September 11.

The onion seeds for experiment II were planted on June 1 and 2, 1981, in a ten by ten simple lattice design. Ninety-five soil fungi were selected randomly and applied to onion seeds (cv. Autumn Spice), as described above. The _Bacillus_ isolate, EBW6, and the fungicide Ronilan were also applied to seed. Control seeds were treated with carborundum and a methyl cellulose sticker, with methyl cellulose alone, or were left untreated. Each treatment consisted of one row of onions (50 seeds per row). The onions were harvested on September 8, 1981.

The onions for experiment III were planted on June 3, 1981, in a five by five triple lattice design. Two fungi, _C. minitans_ and _T. harzianum_, a bacterium, _B. subtilis_, and the fungicide Ronilan were prepared as seed and granular treatments as described above. The treatments were applied alone or in combination. The combinations consisted of seed treatments and granular applications. There were a total of 25 treatments. The

onions (cv. Autumn Spice) were harvested on September 8 and 9, 1981.

Experiment IV was planted on May 26, 1982, in a randomized complete block design with four replications. Thirteen fungi were applied to seeds (cv. Canada Maple). Seeds were also treated with the fungicide Ronilan or left untreated. Each treatment was planted in one bed, with three data rows per bed and 40 seeds per row. The plots were harvested on September 9, 1982. Percentage data were subjected to arc-sine transformation and analyzed using analysis of variance and Duncan's Multiple Range Tests.

Results

Experiment I

All of the fungi used as seed treatments were antagonistic to *S. cepivorum* on agar (Table 5). Onion emergence ranged from 48.2% to 74.3% and was unaffected by treatment. Disease incidence was high ranging from 78.0% to 98.1%. No treatment significantly ($p \leq 0.05$) affected disease.

Experiment II

Sixty-four % of the fungal isolates used in this experiment were antagonistic to *S. cepivorum* on PDA. Eighty-seven % of the isolates applied to seed survived three months storage at 5° C.

Table 5. Ranked White rot incidence in onions as affected by seed treatment with soil fungi (Experiment I).

Treatment	Dual-Culture Interaction Type *	% Emergence (no significant difference)	% Disease (no significant difference)
<u>Penicillium sp.</u>	D c	62.6	78.0
<u>Trichoderma sp.</u>	E	64.5	79.3
Control		66.3	83.8
<u>Fusarium sp.</u>	D c	48.2	84.0
<u>Penicillium sp.</u>	D c	68.9	85.4
<u>Gliocladium sp.</u>	E	65.5	87.1
<u>Cladosporium sp.</u>	D b	57.3	89.5
<u>T. harzianum</u>	E	51.6	89.8
<u>Ulocladium sp.</u>	D c	59.0	91.1
<u>T. hamatum</u>	E	59.8	93.3
<u>Penicillium sp.</u>	E	66.3	93.5
<u>P. nigricans</u>	D c	67.4	93.6
<u>Ulocladium sp.</u>	D c	63.5	94.6
<u>Penicillium sp.</u>	D b	50.6	95.5
<u>Penicillium sp.</u>	D b	69.9	97.2
<u>Trichoderma sp.</u>	E	74.3	98.1

* see page 17 for explanation of symbols

The emergence of the onions was low ranging from 0 to 40%. One treatment, Myrothecium roridum significantly ($p \leq 0.05$) reduced emergence. Disease incidence ranged from 0 to 52% and no treatment significantly ($p \leq 0.05$) affected disease. There was no relationship between the interaction of the fungi with S. cepivorum on PDA and disease incidence (Fig. 5).

Experiment III

Emergence ranged from 8 to 46.7% (Table 6). Eight treatments significantly reduced emergence, five in which Ronilan was added as a granular treatment and three where seed was treated with B. subtilis either alone or in combination with granular applications of C. minitans or T. harzianum . Disease incidence ranged from 0.0 to 63.9% and was unaffected by treatment.

Experiment IV

Fungi used in this experiment were selected on the basis of their performance in experiment II in which five had no diseased onions and the remaining had disease ranging from 2.8 to 52.2% (Table 7). Ten of the 13 isolates were antagonistic to S. cepivorum on PDA. In Experiment IV emergence ranged from 12.5 to 45.3%. Two treatments, Acremonium furcatum and Fusarium spp., had significantly lower emergence ($p \leq 0.05$) than the control.

Fig.5. Incidence of white rot in onions treated with antagonistic and non antagonistic soil fungi(Experiment II).

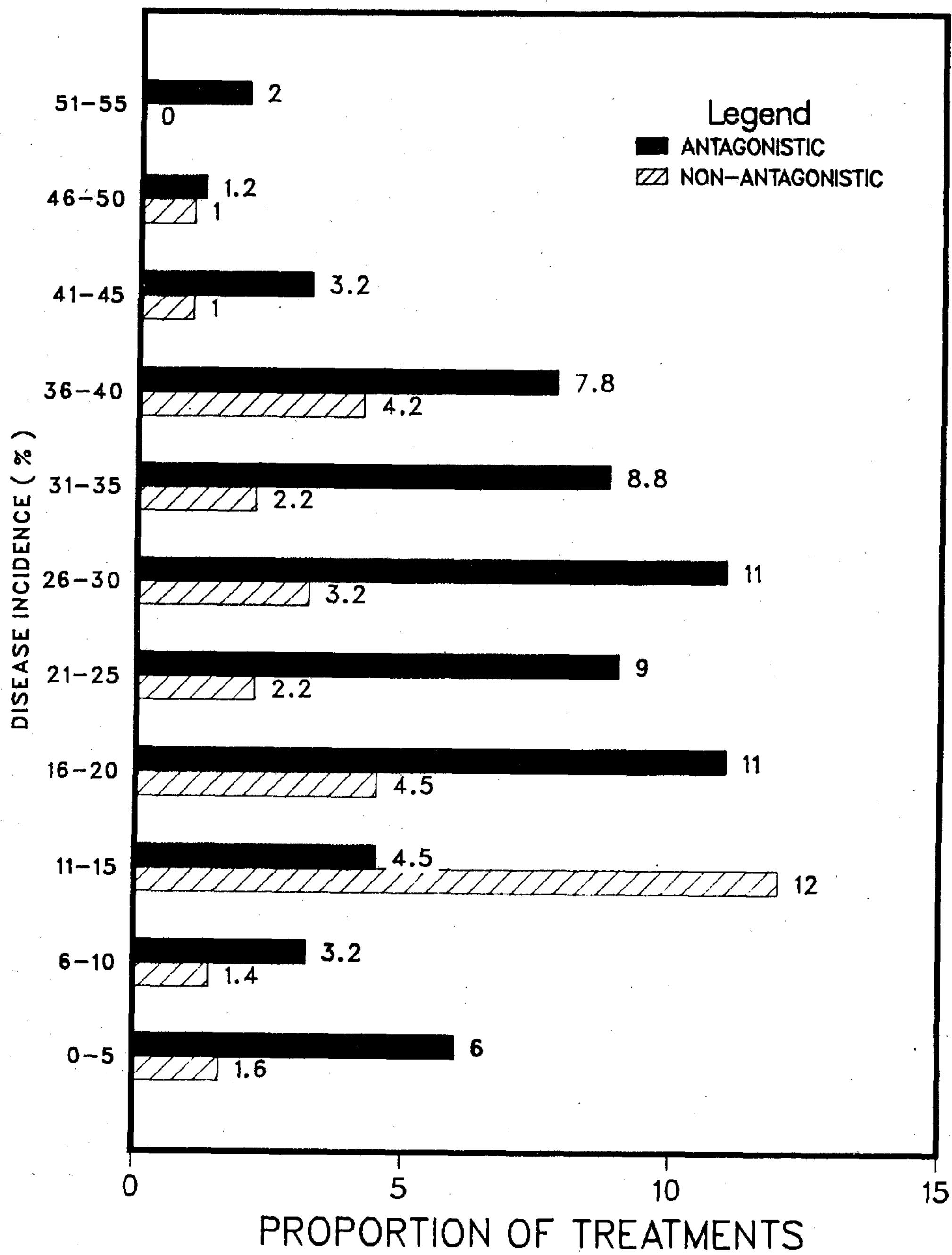

Legend
ANTAGONISTIC
NON-ANTAGONISTIC
DISEASE INCIDENCE (%)
51-55
46-50
41-45
36-40
31-35
26-30
21-25
16-20
11-15
6-10
0-5
2
0
1.2
1
3.2
1
7.8
4.2
8.8
2.2
11
3.2
9
2.2
11
4.5
4.5
12
3.2
1.4
6
1.6
0
5
10
15
PROPORTION OF TREATMENTS

Table 6. White rot in onions as affected by treatment with <u>B.subtilis</u>, <u>C.minitans</u>, <u>T.harzianum</u>, and the fungicide Ronilan, alone or in combination.

Treatment*		% Emergence	% Disease
seed	granular		N.S.
R	R	22.0 bcd**	0.0
R	B	44.7 ij	10.2
B	B	19.3abc	11.7
T	T	35.3 defghij	17.4
X	B	35.3 defghij	19.8
C	C	32.7 cdefghij	20.2
X	T	29.3 cdefg	20.6
T	B	32.7 cdefghij	21.4
R	X	39.3 cdefghij	21.8
R	C	42.0 fghij	22.2
R	T	42.7 ghij	22.4
T	X	40.0 fghij	22.9
C	T	37.3 efdhij	23.6
X	X	46.7 j	27.4
B	T	30.0 cdefgh	33.3
B	R	22.0 bcd	33.3
C	R	11.3ab	33.3
T	R	8.0a	33.3
B	C	24.6 bcd	34.0
C	B	29.3 cdefg	39.5
T	C	28.0 cdef	41.2
C	X	30.6 cdefghi	41.7
B	X	20.7abc	46.0
X	C	30.0 cdefgh	47.1
X	R	25.3 bcde	63.9

*R=Ronilan; C=<u>C.minitans</u>; B=<u>B.subtilis</u>; T=<u>T.harzianum</u> X=Control
**Means followed by the same letter are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test
N.S. not significant ($p \leq 0.05$)

Table 7. White rot in onions as affected by seed treatment with soil fungi (Experiments II & IV).

Treatment	Dual culture Interaction Type**	% Emergence II	% Emergence IV	% Disease Total II	% Disease Total IV	% Disease Primary IV
Fusarium sp.	D a	17.0	25.0*	23.7	25.3*	22.2
Ronilan		40.0	36.7	19.3	26.3*	16.2
Acremonium furcatum	D c	6.0	12.5*	52.2	33.2	27.3
Trichoderma harzianum	E	15.0	40.0	28.8	40.0	18.4
Ulocladium sp.	D b	15.0	31.7	0.0	42.9	22.6
Fusarium sp.	B	24.0	35.0	12.0	43.2	17.7
Acremonium sp.	D a	16.0	34.0	0.0	46.4	19.5
Gliocladium roseum	D a	19.0	30.0	43.7	46.9	24.5
Nectria inventa	D a	25.0	32.8	0.0	47.2	20.1
Penicillium nigricans	D c	13.0	30.0	2.8	47.6	26.7
Control		27.0	41.1	27.7	47.7	21.1
Monocillium mucidum	B 1	14.0	40.8	0.0	56.4	24.6
Gliocladium viride	B 1	15.0	45.3	13.3	58.8	24.6
Verticillium sp.	D a	13.0	35.0	0.0	58.9	25.9
Coniothyrium minitans	D a	30.6	39.8	41.7	60.4	25.7

** see page 17 for explanation of symbols
* differs significantly ($p \leq 0.05$) from the other values in the column

Disease incidence ranged from 25.3 to 60.4%. Two treatments, Ronilan and the _Fusarium spp._, resulted in significantly lower disease incidence than the control, with 25.3 and 26.3% white rot, respectively. The percentage of primary infections estimated from counts of disease foci ranged from 16.2 to 27.3% and was unaffected by treatment.

Discussion

All of the fungi used in this study grew well on PDA and thus would be easy to produce commercially if they proved to be effective biocontrol agents. The ability of the organisms to survive a period of storage is also an important characteristic. In the Fraser Valley it is often difficult to predict when it will be possible to plant and it is important to have seed which is ready when it is needed.

Emergence varied depending on the year of the experiment. Experiments II and III were conducted in 1981 which was an extremely wet and cold year. Emergence was reduced by _Myrothecium roridum_ in experiment II, Ronilan added as a granular material and _B. subtilis_ as a seed treatment in experiment III, and _A. furcatum_ and a _Fusarium spp._ in experiment IV. Ronilan added as a granular material was toxic, but as the material was added to the holes directly on top of the seeds the phytotoxicity of the chemical was likely greater than would be the case with normal use. The cause of the reduced emergence of seeds treated with the bacteria and fungi is

unknown. Kommedahl and Windels (74) suggest that seed treatment with organisms may reduce emergence via depletion in oxygen around the seed. Alternatively, the organisms may produce toxic metabolites or may be weak parasites and contribute to preemergence damping-off. Whatever the cause, these organisms would not be suitable as biocontrol agents unless a means can be found to overcome their detrimental effect on emergence (74).

None of the organisms which have successfully controlled white rot in other studies reduced the disease in experiments in the Fraser Valley. *P. nigricans* which successfully reduced white rot infection in Ghaffar's experiments (51) was ineffective in the Fraser Valley. Ghaffar's experiments were carried out in sterile soil using large amounts of *P. nigricans* inoculum. He states that the protection was due to the build up of griseofulvin in the soil. This antibiotic however loses its effectiveness in nonsterile soil (125) and so the fungus may not be effective under natural conditions. Treatment of seed with *C. minitans* was also ineffective in reducing disease incidence. *C. minitans* was tested against white rot by Ahmed and Tribe only in greenhouse experiments (11), but it has been used successfully in the field to control sclerotinia wilt of sunflower (62). The control of sclerotinia wilt was due to a reduction in the number of primary infections, presumably caused by parasitization of the sclerotia of the pathogen. In this study *C. minitans* did not reduce either primary or secondary infection (Table 7). *C. minitans* may have been unable to compete with other organisms

present in Fraser Valley soils. T. harzianum was used successfully in the field to control white rot by Abd-El Moity et al. in Egypt (3). They applied the Trichoderma inoculum as a dry material and transplanted 60 day-old-onions into the treated soil, and were able to reduce disease from 86 to 18%. In this study neither granular application nor seed treatment with T. harzianum significantly reduced disease (Table 5, 6, 7). Different strains of T. harzianum have different antagonistic properties (2, 22) and it is possible that the strain used was a less effective biocontrol agent than that used by Abd-El-Moity et al. The fungus may have been unable to compete with other soil organisms and would, therefore, not be able to protect the plant throughout the growing season. The medium used by Abd-El-Moity et al. contained onion extract which may have contributed to the biological control ability of the fungus by stimulating germination of the sclerotia and increasing the susceptibility of the pathogen to T. harzianum . However, they did not have any treatment where T. harzianum was added without the onion extract and so we cannot tell if it affected the results.

There was no apparent relationship between the ability of an isolate to inhibit S. cepivorum on agar and the proportion of diseased onions in the field (Fig. 5). This result is unlike the findings of Ghaffar (50) who found that all fungi which were antagonistic on agar reduced white rot to some degree in sterile soil. Sterilizing soil removes micro-organisms which under

normal conditions might compete with and prevent the establishment of a biological control agent.

The inability of antagonistic fungi to control white rot in the field may be due to factors which affect either their ability to colonize the rhizosphere or their antagonistic activity.

The organisms may have been unable to establish in the rhizosphere. Generally seed treatment organisms are not able to colonize the rhizosphere of the host. Kommedahl and Windels (74) state that

> " antagonists applied to seeds generally protect seeds and not roots, although they can be recovered from the rhizospheres for several weeks after planting."

As _S. cepivorum_ can infect onions throughout the growing season, an organism should be present in the rhizosphere for as long as four months if the host is to be protected from the pathogen. There is some evidence that onions leak chemicals which inhibit the growth of some organisms. Moubasher _et al._ (86) reported that _Allium spp._ extracts were inhibitory to the growth of most fungi. However, they used higher concentrations than would normally be present in onion rhizospheres. Coley-Smith _et al._ (30) report that there is no evidence that at normal concentrations exudates from _Allium spp._ will reduce the rhizosphere microflora. The chances that an organism can establish in the rhizosphere of a plant are greater if it was isolated from that region, and the chances of successful biological control through seed treatments will likely be

greater if antagonists are selected from rhizosphere micro-organisms (17).

The antagonistic activities of any organism may be reduced when it is placed into nonsterile soil. As noted before for griseofulvin, the antibiotic produced by _P. nigricans_, some compounds are only effective in sterile soil (125). An antibiotic may also be adsorbed onto soil particles and quickly inactivated. Keyworth and Milne (69) found that _S. cepivorum_ was more tolerant of antibiotics in the presence of onion exudates which could also affect the ability of organisms to inhibit the pathogen _in vivo_ .

In experiment IV one isolate, a _Fusarium spp._, did significantly reduce the proportion of disease. This isolate, however, also caused a reduction in emergence, and this is most likely the cause of the reduction in disease rather than a protective effect of the fungus. The proportion of primary infections was similar to the other treatments but the secondary spread was lower as the onions were spaced farther apart. It is important to consider plant spacing when the results of a field experiment on white rot are being analyzed as it is easy to misinterpret the results. There is no evidence that onion white rot in the Fraser Valley can be reduced by treatment with fungal antagonists. It may be possible, however, to improve the chances of success by improving application methods and by selecting organisms from the rhizosphere of onions.

III. Conclusion

The results reported in this section illustrate the importance of testing biological control agents in the field. Although over 50% of fungi isolated from Fraser Valley soils were antagonistic on PDA, none significantly reduced disease in field tests. This result supports the findings of many other researchers who have found that only a small portion of isolates which are antagonistic *in vitro* can control a disease under natural conditions (24, 73, 83). The high proportion of isolates which were antagonistic on PDA is not unusual. Broadbent *et al.* report that many soils, especially those high in organic matter contain a high proportion of antagonists (24). The results of this study also indicate that there is very little relationship between the results of dual culture tests and disease incidence in the field. This is in agreement with the findings of Kommedahl and Windels (73) and Sivasithamparam and Parker (102). These results indicate that, in searching for biological control agents, a large number of organisms should be screened and these should be chosen to represent as wide a variety of organisms as possible.

The inability of any of the fungi tested to reduce white rot in the field may be due to the unsuitability of the disease and local environmental conditions to biological control by protection of host plant surfaces. The Fraser Valley is subject to highly variable weather conditions. Rainfall varies greatly

from year to year. In 1979 the area received 192 mm of rain between May and September, while in 1980, 520 mm fell within the same period. Local variations in temperature and moisture increase the difficulty of finding a biological control agent which would be suitable for commercial development as it would have to be effective under a variety of conditions. Fraser Valley onion soils are muck soils, high in organic matter. These soils represent well buffered systems that resist any attempt to increase the population of any one organism, even temporarily. In addition, the onions themselves may resist attempts to establish an organism in their rhizosphere by producing antibiotics which inhibit the growth of soil organisms within this region. Onions can be infected by S. cepivorum at any time during the growing season. Any biocontrol agent must, therefore, be able to protect the host for as long as five months. When all of these factors are considered it is not surprising that it will be difficult to find an effective biocontrol agent for use against white rot in the Fraser Valley.

There are ways of increasing the probability of obtaining biological control of a plant disease and using these measures it may be possible to find an organism which could be used in the Fraser Valley. Isolation of organisms from the rhizosphere of onions rather than from soil should yield organisms which have a higher probability of being able to colonize the root surface of the host and thus provide a longer lasting control (37). A large number of organisms should be tested over several

years in field tests so that biocontrol agents which are effective under different conditions can be identified. It should then be possible to use combinations of the organisms which will be effective under a variety of conditions. Recent work by Abd-El-Moity *et al.* (3) has indicated that it is possible to produce isolates of given organisms which have increased antagonistic potential, and it is, therefore, possible to improve the biological control ability of a given isolate. Isolates which are tolerant of low doses of fungicide can also be produced and the combination of chemical and biological treatments may be used to control disease (3). Improving the technology of applying the organisms will also improve the effectiveness of a given agent (74). At the moment there appears to be little hope of controlling white rot with applications of soil fungi, but as our knowledge of the ecology of the soil increases we may eventually be able to prevent disease by using fungi to protect onions from infection by *S. cepivorum.*

C. Reduction of Inoculum

Biological control measures will never completely eliminate the inoculum of a pathogen from any area. The goal is rather to reduce inoculum to a level that causes economically acceptable levels of disease (37). Even if this level cannot be achieved, reduction in inoculum levels may increase the effectiveness of other control procedures by reducing inoculum stress (92).

Reduction in inoculum levels is, at present, achieved primarily by crop rotation (37). Crop rotation takes advantage of the natural decay of inoculum. The rate of decay may be increased by cultural manipulation such as the use of organic manures (18) or by the addition of mycoparasites to the host reservoir (15).

The development of a program designed to cause a reduction in inoculum requires accurate information on the behavior of the pathogen in the local area.

In this section I have evaluated the potential for controlling white rot by reducing the numbers of sclerotia in soil. Most of the information in the literature on the behavior of sclerotia of *S. cepivorum* in soil is based on results obtained using laboratory-produced rather than field-produced (natural) sclerotia. The structure and behavior of these two types of sclerotia were compared in order to determine if the results obtained with laboratory-produced sclerotia can be used to predict occurrences with natural sclerotia. Survival of

sclerotia can vary depending on environmental conditions and the species and isolate of the pathogen (29). The natural decline of sclerotia in Fraser Valley soils using a local isolate of *S. cepivorum* was studied to determine if crop rotation could be used to control white rot. In addition, as it is often desirable to shorten the rotation period, measures which could be used to increase decay were investigated.

I. Comparison of Laboratory- and Field-Produced Sclerotia

Introduction

In order to design an effective biological control program, it is important to know that experimental results adequately reflect occurrences in the natural system. Results of experiments using sclerotia can vary depending on whether the sclerotia are produced in the laboratory or the field. Sclerotia produced in the laboratory are formed under different conditions than those produced in the field, and this may result in structural and /or physiological differences. Takashi and Tadao (108) found that bigger sclerotia of *Rhizoctonia solani* were produced in culture than in soil. Laboratory-produced sclerotia of *S. rolfsii* were less uniform in size and appearance than sclerotia produced in soil (75). In addition, the laboratory-produced sclerotia were more susceptible to bleaching than field-produced sclerotia, a condition which may be due to differences in the thickness or integrity of the rind (75). Sclerotia produced in the laboratory are normally grown aseptically and therefore do not become associated with soil organisms as is common with those produced in the field. Sclerotia of *S. sclerotiorum* produced and incubated in soil had more fungal colonists than those produced in the laboratory and incubated in soil(80, 110).

Differences in sclerotia produced in the laboratory and the field may result in differences in the ability of sclerotia to germinate or survive in soil, but there is no consistent relationship. Sclerotia produced in the laboratory have been found to have lower survival (*Macrophomina phaseolina*) (101), the same survival (*Sclerotinia minor*) (66) or longer survival (*S. sclerotiorum*) (110) than those produced in the field.

Studies on survival of sclerotia commonly use sclerotia separated from the substrate they were formed on, mixed with soil and placed back into the field. Sclerotia in the field, however, are mixed into soil along with decaying and healthy host tissue. The presence of this host tissue may also affect survival.

Sclerotia of *S. cepivorum* are easily produced in the laboratory on artifical media. Sclerotia produced in this manner are easily handled and as a result most studies on survival of sclerotia of *S. cepivorum* have used laboratory produced sclerotia (27, 31, 39, 91, 105). This section compares sclerotia of *S. cepivorum* produced in the laboratory on sand-maize meal medium with those produced on onions in the field, to determine if they can be used interchangeably in field studies. The sclerotia are compared with regard to germination and ability to survive in soil, presence of associated micro-organisms, and thickness and integrity of the rind. The effect of residue from decaying onions and healthy onion tissue on survival is also examined.

Materials and Methods

The sclerotia used in this study were of local origin and were either produced in the laboratory (laboratory-produced) or were harvested directly from infected onions (field-produced). Laboratory-produced sclerotia were grown on sterilized sand-maize meal medium (99:1 v/v) (27) in 500 mL flasks. Each flask was inoculated with a 1 cm diam agar disc containing mycelium and sclerotia of *S. cepivorum* originally obtained from a commercial onion field in Burnaby. The cultures were incubated in the dark at 22° C for three weeks.

Field-produced sclerotia were collected from infected onions from field plots in the same field in Burnaby. White rot infected onion bulbs were rubbed on a .595 mm (28 mesh) brass sieve and dislodged sclerotia and decaying onion tissue were washed through and trapped on a lower .210 mm (70 mesh) sieve. The material in the top sieve was discarded and the material in the bottom sieve was collected in a 250 mL beaker. After two h, the residue from the decaying onions was decanted off and the sclerotia were picked from the material at the bottom of the beaker.

Survival of Sclerotia Under Field Conditions

Experiments were conducted in 1980 and 1981 to compare the survival of laboratory-produced and field-produced sclerotia under local field conditions, and to evaluate the effects of

healthy onion tissue and residue material from *S. cepivorum*-infected onion bulbs on their survival. Six treatments were evaluated in the 1980 experiment: field-produced sclerotia with and without addition of a small (0.05 g) piece of healthy onion tissue, laboratory-produced sclerotia soaked for two h in residue material from *S. cepivorum*-infected onions with or without healthy onion tissue, and laboratory produced sclerotia soaked in tap water for two h, with or without healthy onion tissue. The residue was the debris (less sclerotia) retained on the bottom sieve during the collection of field-produced sclerotia from infected onions. This debris consists of soil particles, rotting onion tissue, mites, nematodes, and a complex and undefined mixture of fungal, actinomycete and bacterial propagules.

Four treatments were evaluated in the 1981 experiment: field-produced sclerotia, laboratory-produced sclerotia, laboratory-produced sclerotia treated with residue (as above), and laboratory-produced sclerotia treated with sterilized residue.

For both experiments, replicate samples of 40 sclerotia were placed in nylon mesh bags. The bags were placed on the surface of field plots (muck soil) in Cloverdale B.C. in 1980, and on the surface of soil from the Cloverdale test site in clay pots kept on the S.F.U. campus in Burnaby B.C. in 1981. Randomized complete block designs with six replications were used for each experiment. The bags were arranged in a randomized

complete block design with six replications. The bags were recovered after 2, 9 and 12 months field exposure in the 1980 experiment, and 2 and 6 months in the 1981 experiment. At each recovery time the bags were brought into the laboratory where they were stored at 5° C until the contents could be examined. The sclerotia that remained intact after being touched with a dissecting needle were recorded as recovered. Intact sclerotia were plated on PDA as described by Utkhede and Rahe (117) to determine viability. The data were analyzed by analysis of covariance with time as the covariate. Duncan's Multiple Range Test was used for comparing treatments.

Mycosphere Micro-organisms

Micro-organisms associated with sclerotia were isolated using a dilution plate technique and two selective media. Bacteria were isolated on Thornton's Agar (T.A.) (67) and fungi on Rose Bengal Agar (R.B.A.) (67). Ten sclerotia were rinsed in distilled water and placed into 1 mL test tubes with three to five drops of sterile distilled water. The sclerotia were crushed with a sterile glass rod and the resulting suspension was added to ten mL of sterile distilled water. Serial dilutions were then prepared. The R.B.A. plates were inoculated with dilutions of 1:1, 1:10 and 1:100 sclerotia per mL, while T.A. plates were inoculated with dilutions of 1:10, 1:100 and 1:1000 sclerotia per mL. Half a mL of each dilution was spread over the surface of each of three plates of each medium. Each treatment

was replicated five times. The plates were incubated at 25° C. Bacterial and fungal colonies were counted after four and seven days incubation, respectively. The mycoflora of both laboratory-produced sclerotia and field-produced sclerotia was assessed directly after isolation from their respective sources, and after they had been buried in moist soil for 96 h.

Sclerotial Morphology

Comparison of morphological features of laboratory-produced and field-produced sclerotia was made on sections of sclerotia.

Sections of 15 sclerotia of each type were prepared. Sclerotia were fixed in 3% glutaraldehyde for two h, washed in sodium phosphate buffer, pH 7.0, transferred to osmium tetroxide for a further two h and washed again in the buffer. The sclerotia were dehydrated, and infiltrated with polyethelene glycol. Sections were prepared from each sclerotium and stained with crystal-violet. Five sections of each sclerotium were measured for diameter and rind width using a light microscope fitted with a calibrated ocular scale. The rind was measured at three points on each section.

Results

Survival of Sclerotia Under Field Conditions

Sclerotia produced in the laboratory decayed faster ($p \leq 0.05$) than those produced in the field in both experiments (Fig. 6). In the 1980 experiment 32.9% of the laboratory-produced and 44.8% of the field-produced sclerotia were recovered after 12 months in soil. In the 1981 experiment 30% of the laboratory-produced sclerotia and 77.5% of the field-produced sclerotia survived 6 months in soil. The decline in numbers of the laboratory-produced sclerotia was greatest in the first two months in soil in both experiments (Fig. 6).

Healthy onion tissue did not significantly ($p > 0.05$) affect the survival of either laboratory-produced or field-produced sclerotia. The residue from infected onions had opposite effects on the survival of the sclerotia in the two experiments (Fig. 7). In the 1980 experiment the survival of the sclerotia was greater ($p \leq 0.05$) when they had been soaked in residue; 32% of the untreated sclerotia and 44.6% of the treated sclerotia survived 12 months in soil. In the 1981 experiment the proportion of surviving sclerotia was reduced ($p \leq 0.05$) when they were treated with unsterilized residue.

Fig.6. Decline in populations of laboratory-produced and field-produced sclerotia of *S. cepivorum* in muck soil.

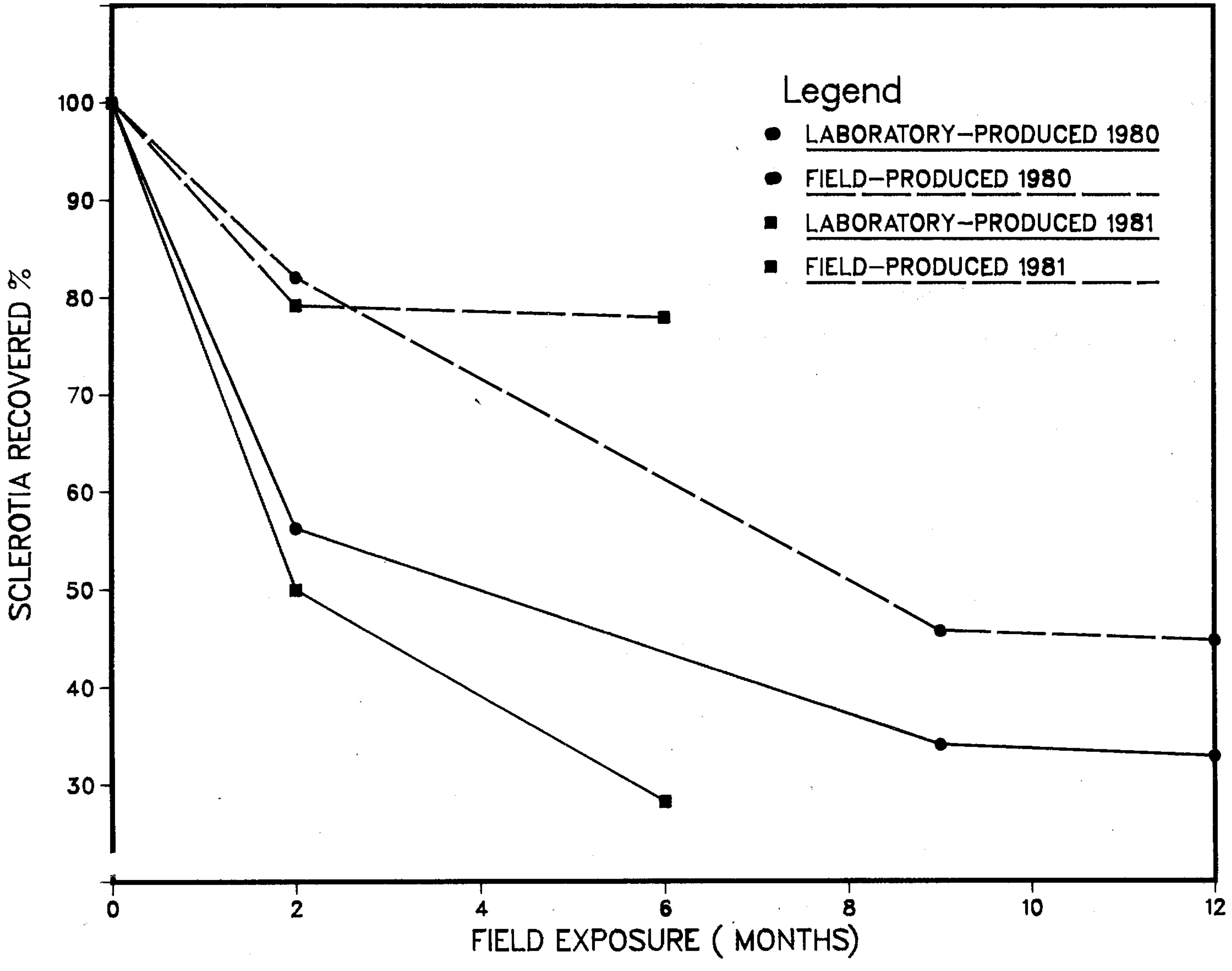
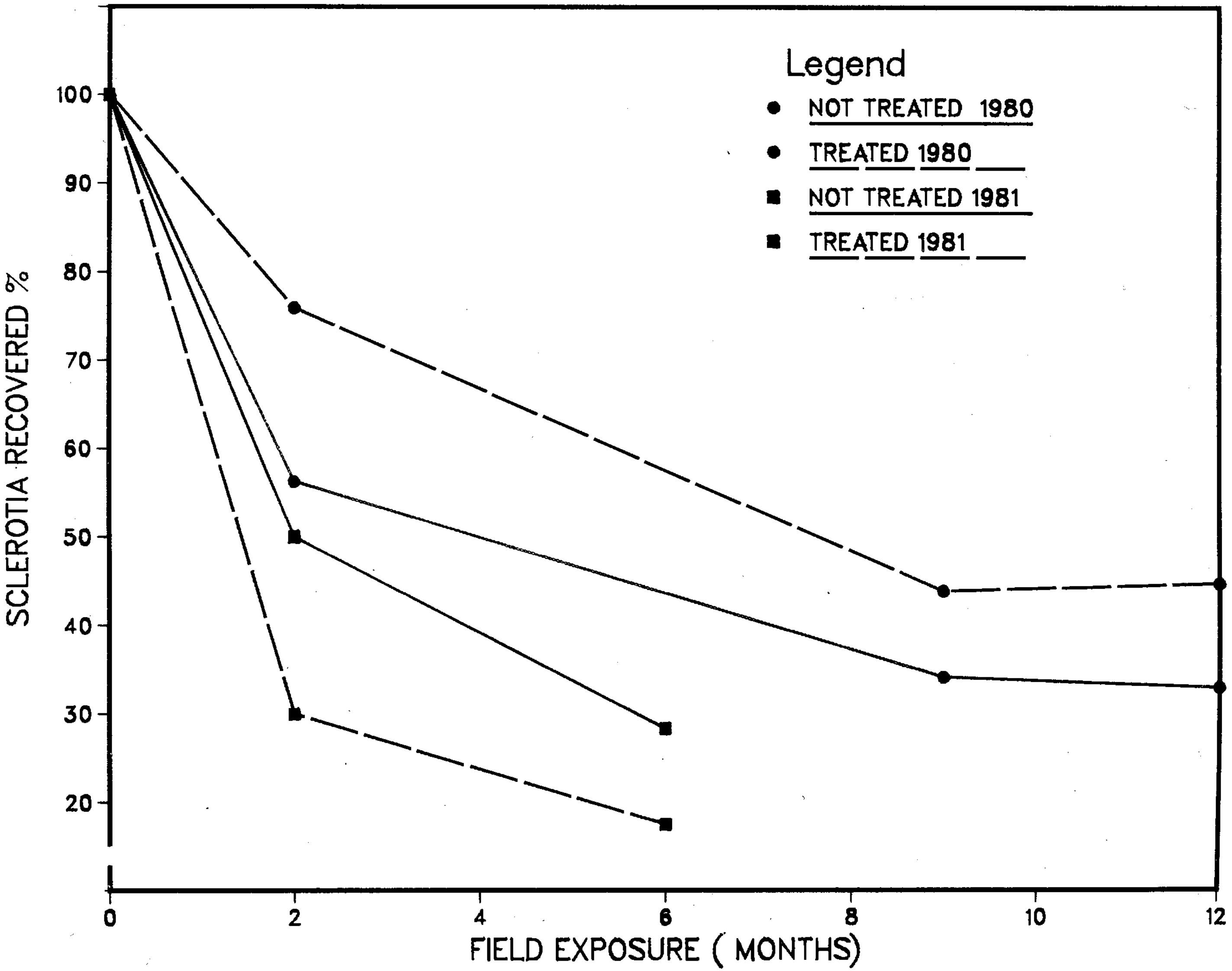

Legend
LABORATORY–PRODUCED 1980
FIELD–PRODUCED 1980
LABORATORY–PRODUCED 1981
FIELD–PRODUCED 1981
SCLEROTIA RECOVERED %
100
90
80
70
60
50
40
30
FIELD EXPOSURE (MONTHS)
0
2
4
6
8
10
12

Fig. 7. Effect of residue from infected onions on the decline in the population of laboratory-produced sclerotia of *S. cepivorum* in muck soil.



Legend
NOT TREATED 1980
TREATED 1980
NOT TREATED 1981
TREATED 1981
SCLEROTIA RECOVERED %
100
90
80
70
60
50
40
30
20
FIELD EXPOSURE (MONTHS)
0
2
4
6
8
10
12

The sterilized residue did not affect survival. After six months in soil, 17.5% 29.5% and 28.3% of the sclerotia which were treated with unsterilized residue, treated with sterilized residue, or untreated, respectively remained.

Germination of the recovered sclerotia was high for all treatments in both experiments varying from 94% to 98%.

Large numbers of bacteria and fungi were associated with field-produced sclerotia (Fig. 8, 9). Sclerotia plated directly after isolation from onions yielded an average of 21,853 cfu (colony forming units) of bacteria and 50.2 cfu of fungi per sclerotium. After 96 h in soil these numbers were reduced to 1686 cfu of bacteria and 5.3 cfu of fungi per sclerotium. Laboratory-produced sclerotia were initially sterile but after 96 h in soil had acquired an average of 2122 cfu of bacteria and 7.1 cfu of fungi per sclerotium. After 96 h in soil there was no significant difference ($p \leq 0.05$) in the numbers of either bacteria or fungi isolated from laboratory-produced or field-produced sclerotia. The fungi isolated from laboratory-produced and field-produced sclerotia were primarily _Penicillium spp._, _Fusarium spp._, _Verticillium spp._ and _Trichoderma spp._.

Microscopic examination revealed considerable differences between the two types of sclerotia. Laboratory-produced sclerotia were larger and had a thicker rind than did field-produced sclerotia (Table 8).

Fig 8. Bacterial populations of laboratory-produced and field-produced sclerotia of _S. cepivorum_ as affected by incubation in soil.

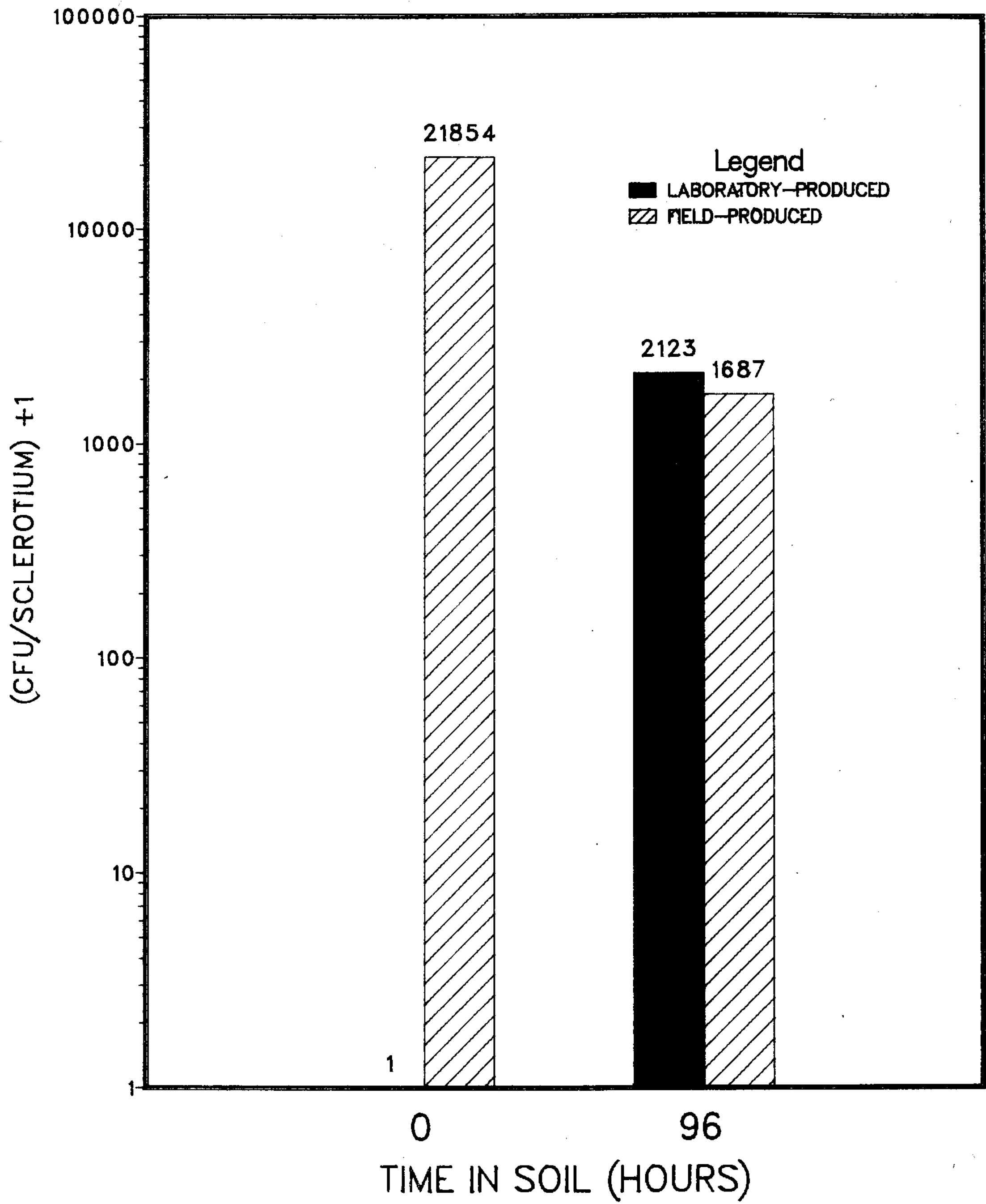

100000
10000
1000
100
10
1
(CFU/SCLEROTIUM) +1
21854
2123
1687
1
Legend
LABORATORY–PRODUCED
FIELD–PRODUCED
0
96
TIME IN SOIL (HOURS)

Fig 9. Fungal populations of laboratory-produced and field-produced sclerotia of S. cepivorum as affected by incubation in soil.

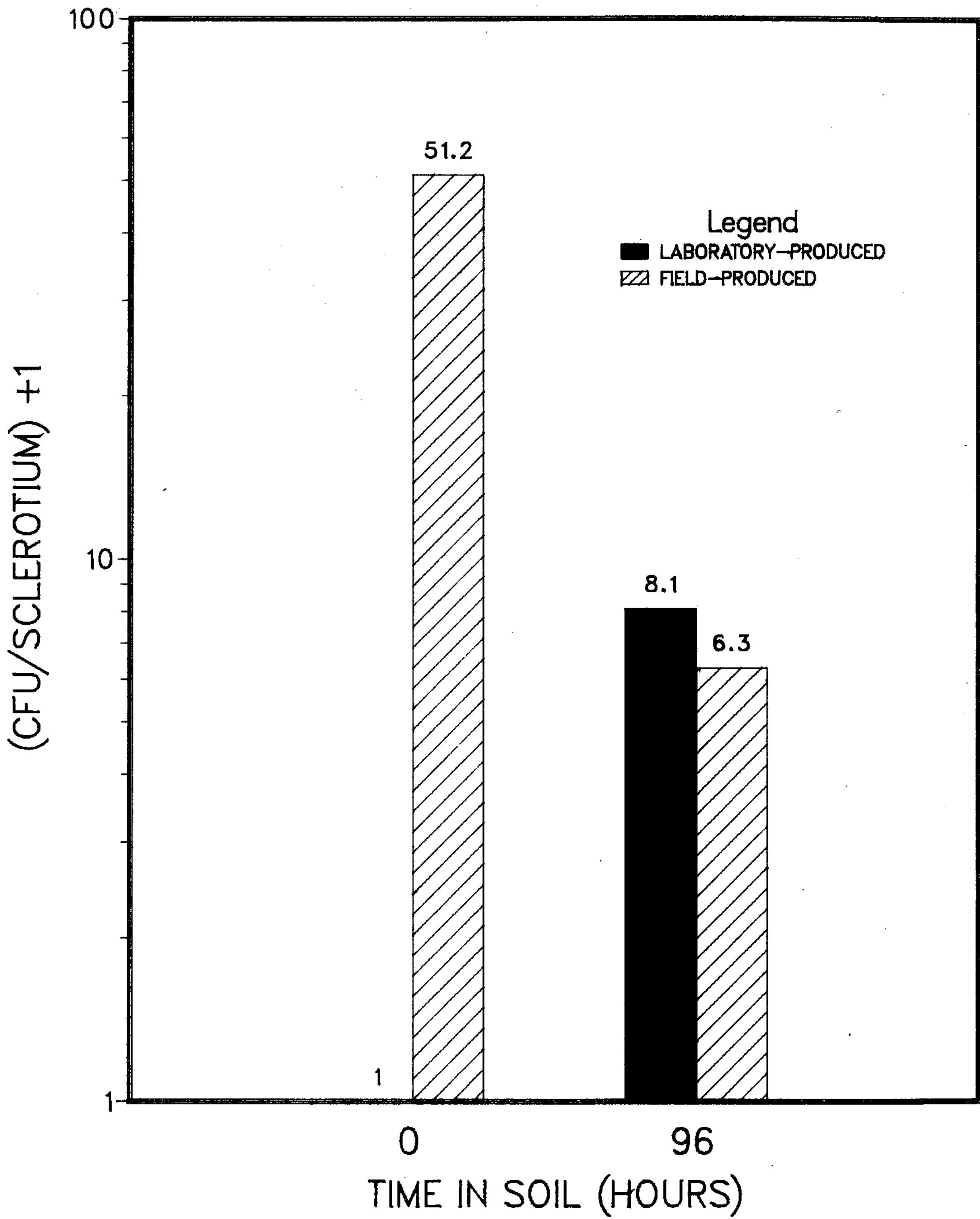

100
10
1
(CFU/SCLEROTIUM) +1
51.2
8.1
6.3
1
Legend
LABORATORY-PRODUCED
FIELD-PRODUCED
0
96
TIME IN SOIL (HOURS)

Table 8. Comparison of the structure of laboratory-produced and field-produced sclerotia of *S. cepivorum.*

Characteristics	Field-Produced Sclerotia	Laboratory-Produced Sclerotia
Diameter(mean ±SD)	0.25±0.01 mm	0.29±0.03 mm
Number of Layers	3	2
Rind Thickness (mean ±SD)	.015±.001 mm	.025±.005 mm
Rind Integrity	Continuous	Broken

The field-produced sclerotia were more uniform in size and shape than those produced on sand-maize meal. There were three distinct layers in the sclerotia produced in the field, an outer dark covering (rind), a middle layer of ovoid cells filled with many large vacuoles and an inner layer or medulla of interwoven hyphae with many densely staining nucleii (Fig. 10, 11). The laboratory-produced sclerotia had only two visible layers, a thick dark outer layer or rind which was broken in many places and an inner layer or medulla of interwoven hyphae (Fig. 10, 11).

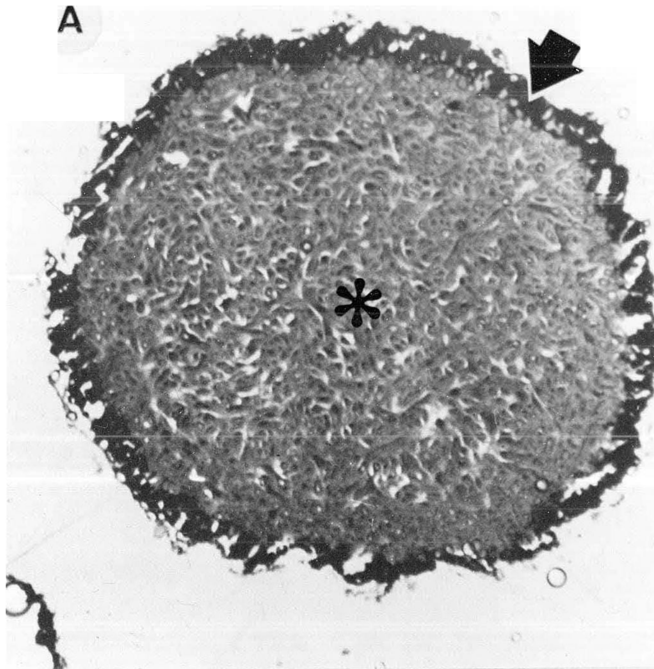
Discussion

Sclerotia of *S. cepivorum* produced in the laboratory decayed more rapidly than did those produced on onions in the field. Similar results were obtained by Short et al. (101) for *Macrophomina phaseolina*. Merriman found an opposite relationship for *S. sclerotiorum*: sclerotia produced in the field decayed while those produced in artificial culture did not (80). He attributed the decay of the field-produced sclerotia to the presence of micro-organisms within the sclerotia. Sclerotia produced in the field may incorporate foreign organisms within their mass during their formation (80). Sclerotia placed in soil also acquire a characteristic flora over their surface as leakage of nutrients creates a mycosphere effect analogous to the rhizosphere effect of plants (52). The determinations of the bacteria and fungi associated with the sclerotia

Fig.10. Cross sections of *S. cepivorum* sclerotia showing distinct layers: outer rind (wide arrow); middle layer of ovoid cells (narrow arrow); and inner medulla (asterisk)

A laboratory-produced, with two distinct layers

B field-produced with three layers


A
*

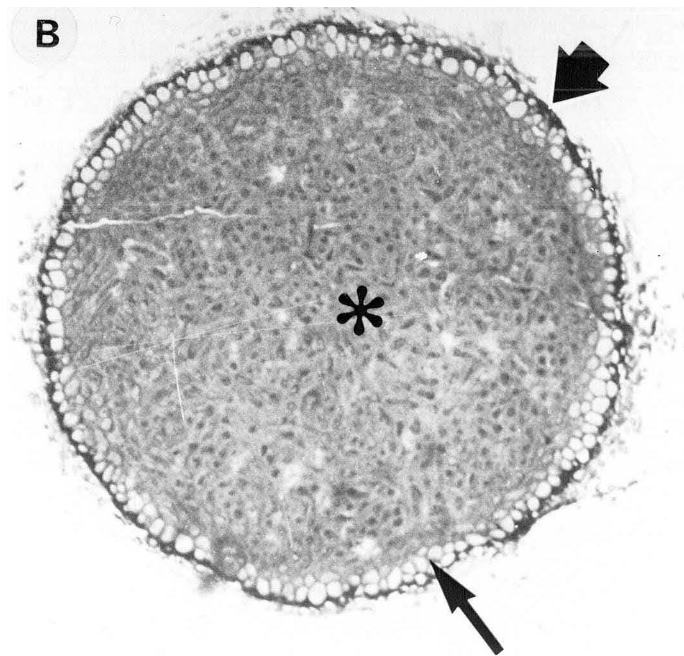
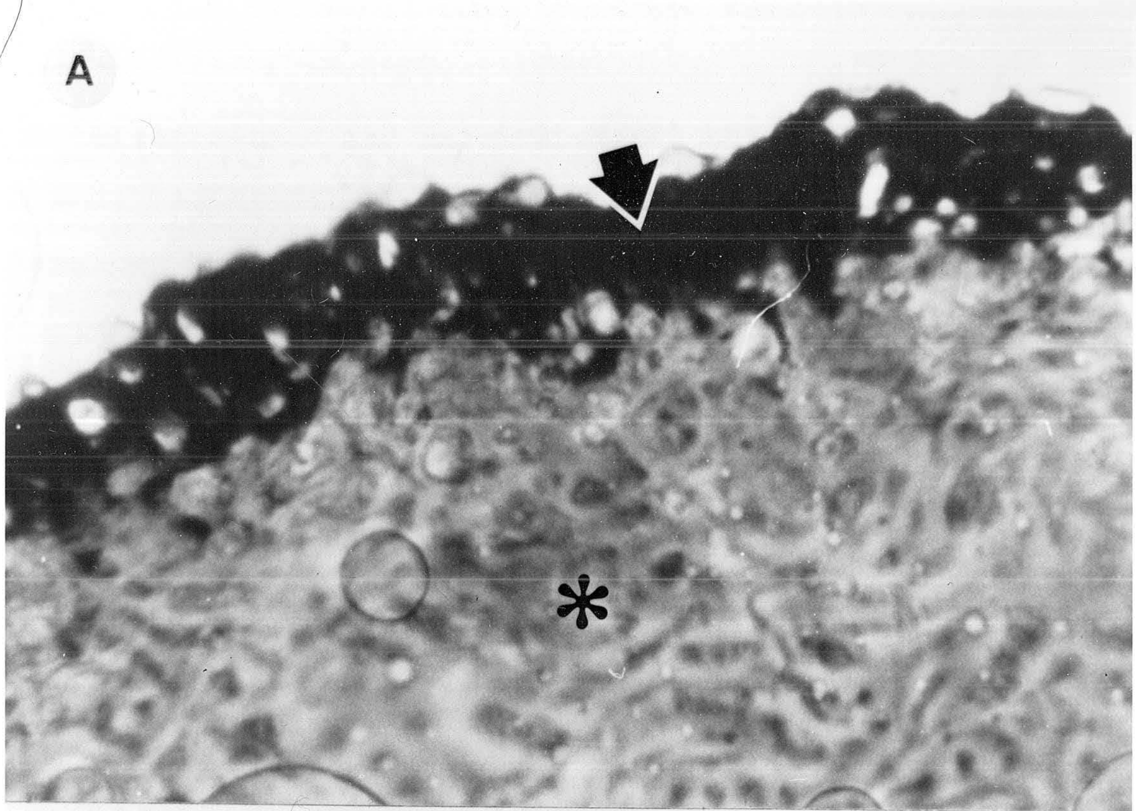
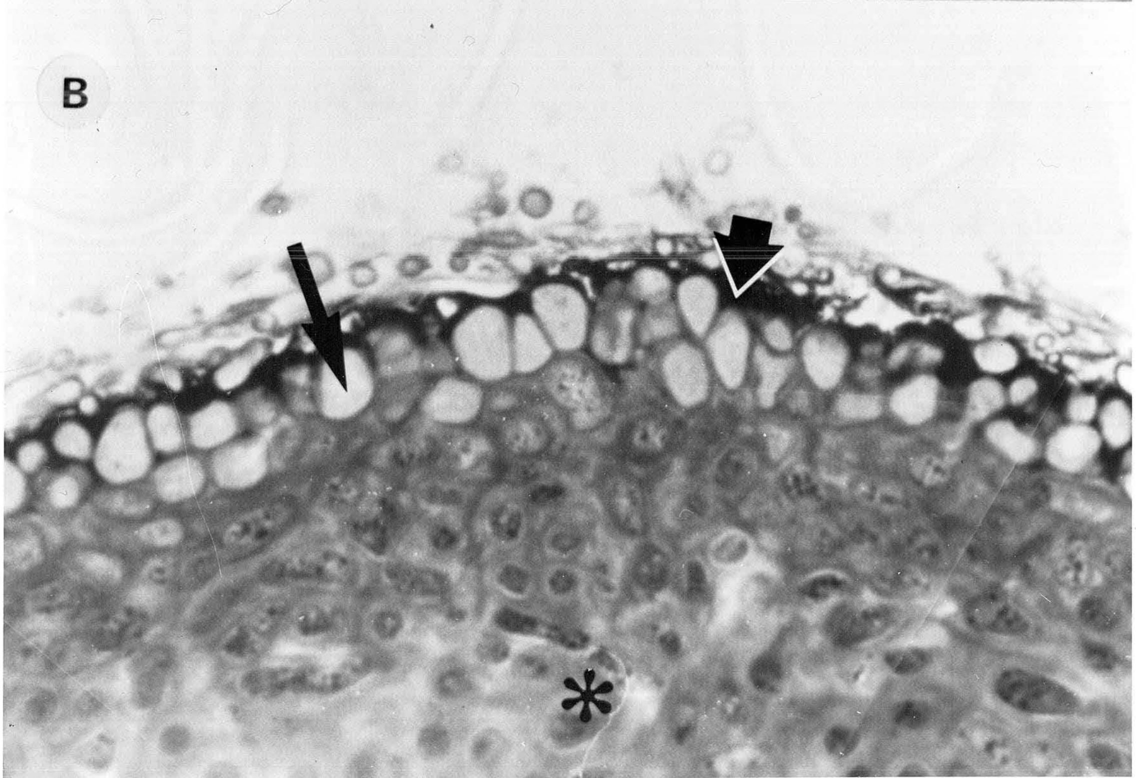

B
*

Fig.11. Cross sections of the rind of S.cepivorum sclerotia

A laboratory-produced , note the thickness of the rind (wide arrow) and the absence of the ovoid cells beneath it.

B field-produced, note the thin rind(wide arrow) and the second layer of ovoid cells(narrow arrow).


A
*


B
*

in this study would measure both internal and external mycoflora. The nature of this mycoflora does not seem to be responsible for the observed differences in the survival of laboratory-produced and field-produced sclerotia of S. cepivorum in Fraser Valley soils. While there are initially large differences in the number of micro-organisms associated with field-produced and laboratory-produced sclerotia, differences in populations of bacteria and fungi on the two types of sclerotia were non-apparent after 96 h in soil. The mycosphere colonization of laboratory sclerotia may have occurred even before 96 h, as Gilbert and Linderman found that it was detectable on sclerotia of S. rolfsii within 24 h. It is unlikely that such a short-lived difference in mycoflora of sclerotia would contribute to the differences in survival of the sclerotia.

The observed differences in survival may instead be due to differences in the structure of the sclerotia. Many workers have pointed out that the integrity and structure of the rind may have a significant effect on the persistence of sclerotia in soil (25, 29, 36, 76). A rind which is broken allows the entrance of decomposing micro-organisms into sclerotia and may also increase their germination, which in the absence of a host leads to their eventual decay (25, 28). The rinds of the laboratory-produced sclerotia of S. cepivorum used in this study were often broken (Fig. 10, 11) and contained many cracks which could have allowed micro-organisms to enter the sclerotia. The

rinds of the field-produced sclerotia, on the other hand, were continuous and would presumably provide an effective barrier. In addition, the field-produced sclerotia had an extra layer of cells beneath the dark outer layer. Coley-Smith and Cooke (29) described sclerotia of S. cepivorum as having two layers, the dark rind and an inner medulla, but it is likely that they only examined laboratory-produced sclerotia. The function of the extra layer observed in the field-produced sclerotia is unknown but it is conceivable that it could play a role in protecting the sclerotia from decay organisms.

The addition of host tissue along with sclerotia in soil may cause either an increase or decrease in inoculum levels. Inoculum levels will increase if the pathogen is able to occupy the tissue and form new inoculum (18, 103). The inoculum levels may decline rapidly if the pathogen germinates but is unable to form new propagules on the substrate, or if the tissue acts as a substrate for the increase of decay organisms which will then attack the pathogen.

In the 1980 experiment the addition of healthy onion tissue to bags containing sclerotia of S. cepivorum did not significantly affect ($p \leq 0.05$) the survival of the pathogen. Sclerotia of S. cepivorum undergo a period of constitutive dormancy which prevents them from germinating until they have undergone a period in soil (28, 39). Constitutive dormancy may have prevented the pathogen from colonizing the onion tissue and forming new sclerotia.

The fact that sterilized residue had no effect on the survival of the sclerotia suggests that the effect of residue from infected onion bulbs was a biological rather than a chemical phenomenon. The composition of the residue would likely vary depending on the environmental conditions. The residue used in the 1980 experiment could have contained a high proportion of organisms antagonistic to local mycoparasites and, if so, would likely have had a protective effect. The residue used in the 1981 experiment may have contained a high proportion of mycoparasites which could have caused an increase in the decay of the sclerotia.

As sclerotia produced in the laboratory and in the field were structurally different and decayed at different rates, it is concluded that field studies on the behavior of the sclerotia in soil should be done using sclerotia produced in the field.

II. Natural Decay of Sclerotia in Fraser Valley Soils

Introduction

Inoculum reduction may be achieved by simply taking advantage of naturally occurring biological control. Crop rotation removes the host from an area for long enough for resident organisms to reduce the inoculum potential of the pathogen in question to acceptable levels. The time required for this to occur varies depending on the pathogen and on local environmental conditions.

Survival of sclerotia in soil can range from four months for *Mycosphaerella pinoides* to 13 years for *Verticillium dahliae* (29). There can also be large variations in longevity of sclerotia within a species, as different isolates have different susceptibilities to parasitism (77). The survival of sclerotia is also markedly influenced by environmental factors such as soil moisture and temperature (29, 39, 90) and soil type (53, 66, 80). Every situation must be examined separately before crop rotation recommendations can be made.

Crop rotation has not been seriously considered as a means of controlling white rot as there is considerable evidence that sclerotia of *S. cepivorum* are capable of surviving for long periods in the absence of the host. White rot is often found in fields where onions have not been grown for many years (27, 40,

79). Crowe (40) isolated viable sclerotia of *S. cepivorum* where no onions had been grown for 10 to 15 years. The most intensive study on survival of sclerotia of *S. cepivorum* was conducted by Coley-Smith (27) who found that the sclerotia could survive for over four years in soil with little loss in viability. These studies were conducted under soil and moisture conditions which are very different from those in the Fraser Valley. As these conditions can affect sclerotial survival, this study was initiated to determine survival of sclerotia in the Fraser Valley. The effect of different local soils on survival was examined. The half-life of field-produced sclerotia was determined for sclerotia placed in local soil in 1979, 1980, and 1981.

Materials and Methods

Effect of Soil and Soil Source

Two experiments were conducted to examine the effect of soil source on survival of field-produced sclerotia. The first experiment compared the survival of sclerotia mixed with two cc of muck soil with that of sclerotia alone placed in nylon mesh bags. The second experiment compared the survival of sclerotia in three Fraser Valley soils. The three soils were collected from vegetable fields in the Fraser Valley. Soil A was a muck soil collected from a small commercial vegetable farm in Burnaby. Soil B, another muck soil, was from a commercial

vegetable farm in Cloverdale, and soil C, a mineral soil, was from a field in Abbotsford.

The sclerotia were isolated from infected onions as described in the previous section. Forty sclerotia mixed with two cc of soil A, B, or C were placed into nylon mesh bags . The bags were placed on the surface of the corresponding soil contained in large clay pots which were kept outside on the Simon Fraser University campus for the duration of the study. Each experiment was conducted with six replications.

Natural Decay

Experiments were conducted in 1979, 1980, and 1981 (Experiments 1, 2, and 3 respectively) to evaluate the rate of decay of field-produced sclerotia of _S. cepivorum_ , and the effect of drying, location in soil, and other environmental factors on this decay. The effects of associated factors are presented and discussed in the next section (C-III Enhanced Reduction of Sclerotia). The rate of natural decay of sclerotia is considered in this section and was estimated from the numbers of untreated field-produced sclerotia recovered after various times of exposure of sclerotia on the surface of soil under field conditions.

In experiment 1, 200 sclerotia along with a small (0.5 g) piece of healthy onion bulb tissue were placed in each bag. In experiment 2, the number of sclerotia in each bag was estimated by volume and varied from 100 to 200 per bag. Forty sclerotia

were placed in each bag in experiment 3. Sclerotia were mixed with two cc of muck soil in experiments 2 and 3. Randomized complete block designs were used for all experiments, with three, four and six replications for experiments 1, 2 and 3 respectively. The test plots were located on a commercial vegetable farm in Burnaby in experiments 1 and 2 and on a vegetable farm in Cloverdale in experiment 3. The pattern of decline over a calendar year was examined. Half-lives were determined by using a log conversion (18). As there appeared to be an overriding seasonal effect on sclerotial decay, the half-life was calculated for results representative of one calendar year for experiment 1 and 3 and nine months for experiment 2.

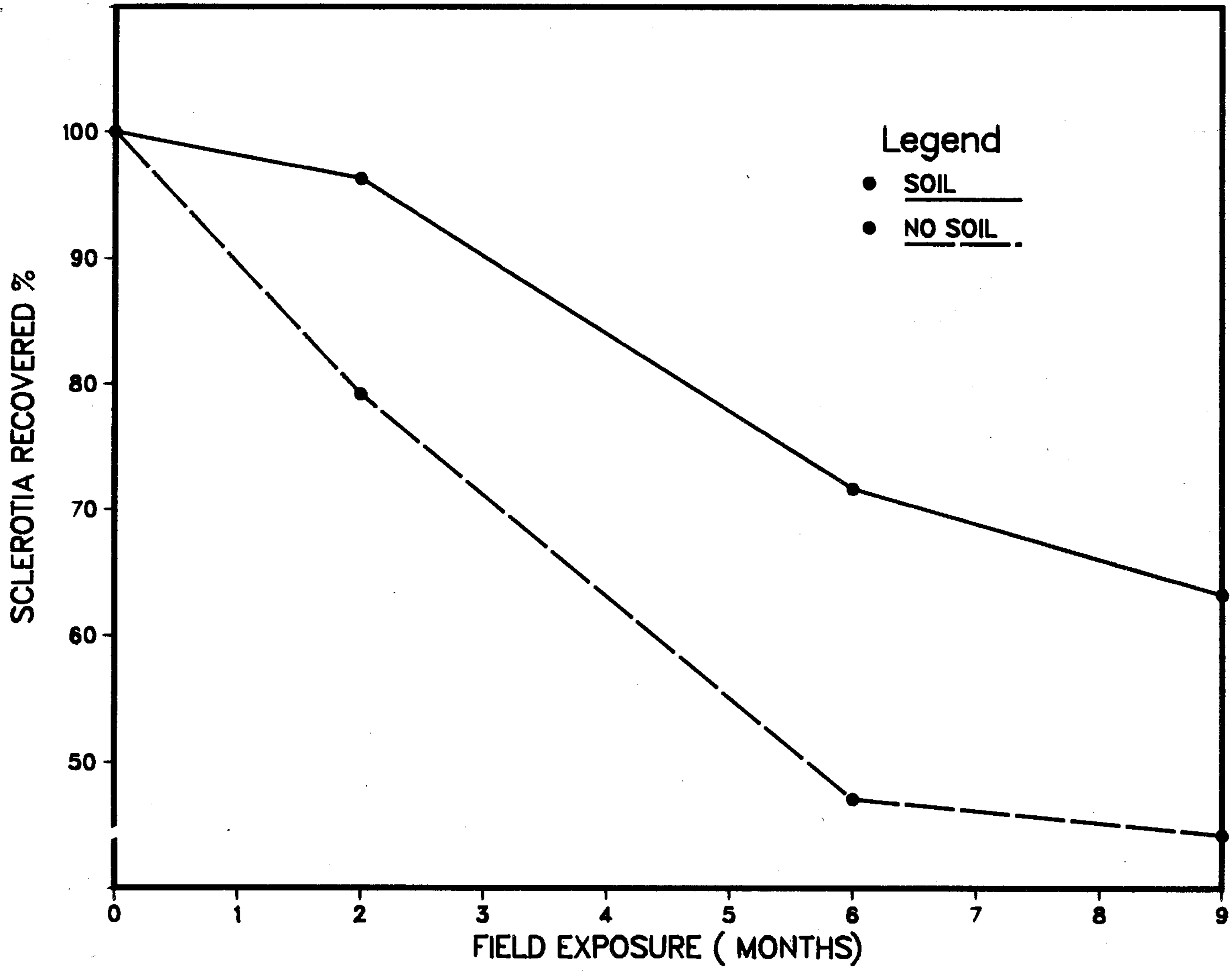
Results

Effect of Soil and Soil Source

In the first experiment, survival of sclerotia was lower ($p \leq 0.05$) for those not mixed with soil than for those mixed with muck soil (Fig. 12). After nine months in the field, 44.2% of sclerotia which were not mixed with soil, and 63.8% of sclerotia mixed with soil remained intact. Germination was high in both experiments and was not affected by treatment.

In the second experiment, which compared the effect of different soil types on survival of sclerotia, there was no significant difference in the rate of decay of sclerotia

Fig.12. The effect of admixture of muck soil with sclerotia on the decline in the population of sclerotia of *S. cepivorum* exposed on the surface of muck soil.


Legend
SOIL
NO SOIL
SCLEROTIA RECOVERED %
100
90
80
70
60
50
0
1
2
3
4
5
6
7
8
9
FIELD EXPOSURE (MONTHS)

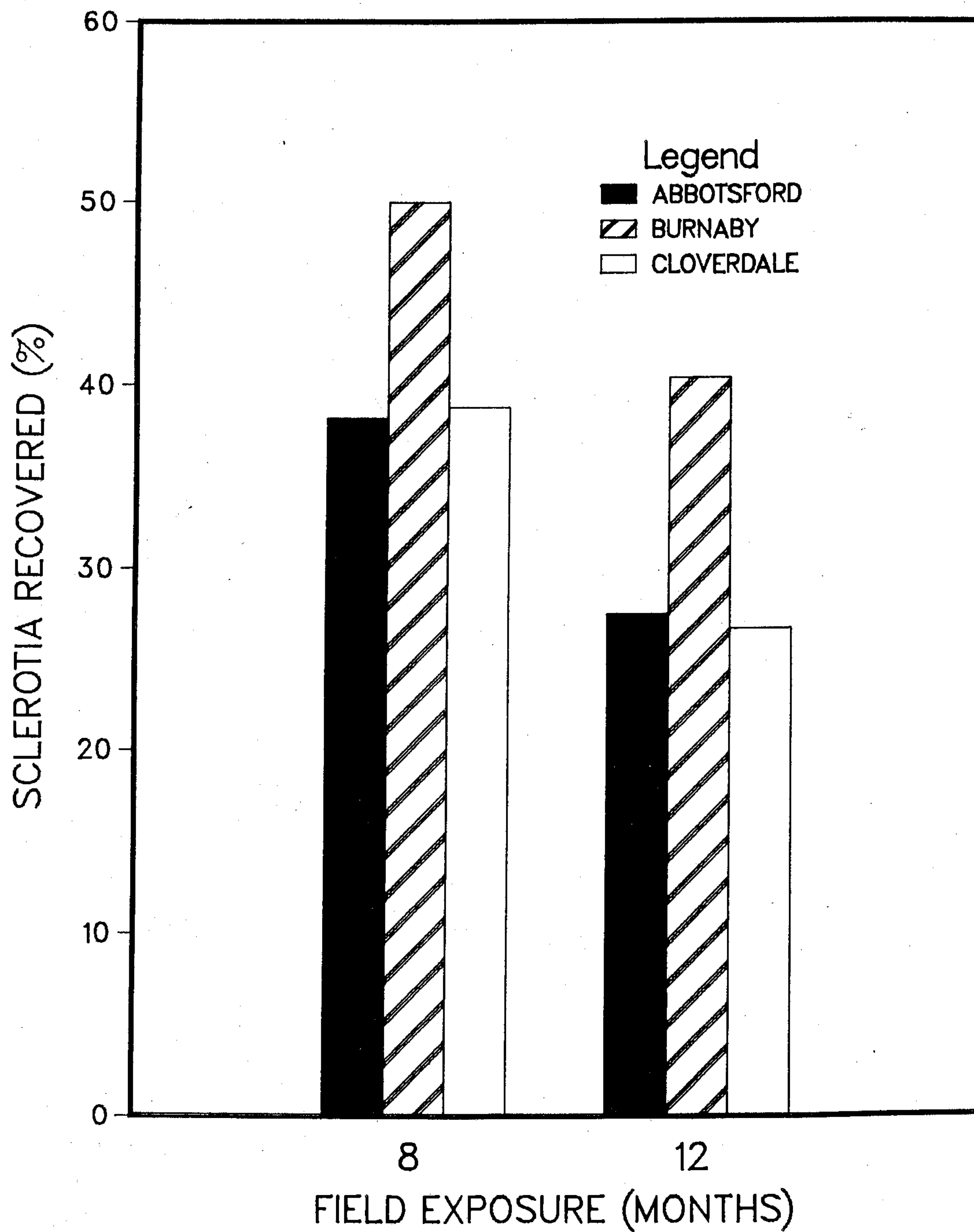
in the three soils tested (Fig. 13). Numbers of sclerotia decreased significantly with time ($p \leq 0.05$) in all soils and after 12 months, 27.5%, 40.1% and 26.7% of sclerotia remained intact in the soils from Abbotsford, Burnaby, and Cloverdale, respectively.

Natural Decline of Sclerotia

In each of the survival experiments the number of sclerotia recovered decreased with time. Examination of the pattern of sclerotial decay on a yearly basis (Fig. 14) shows that most of the decay occurred during the winter months when the fields were flooded.

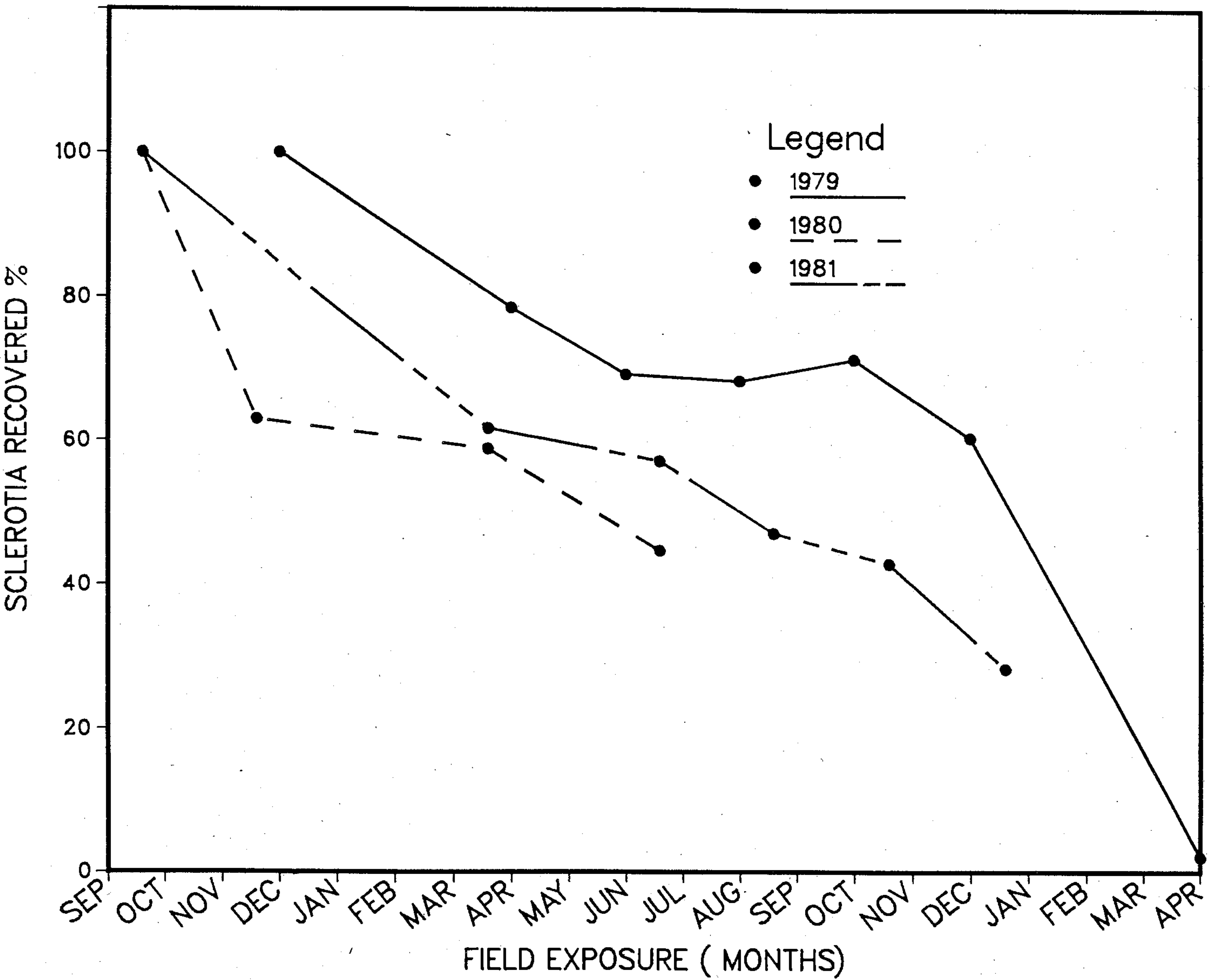
Half-lives for the sclerotia in each experiment, determined by calculating regression lines for the numbers of sclerotia surviving in soil in experiments 1, 2, 3, were 26.8, 9.7 and 10.7 months respectively.

Fig.13. The effect of soil source on the proportion of sclerotia of *S. cepivorum* surviving 8 and 12 months of field exposure.



Legend
ABBOTSFORD
BURNABY
CLOVERDALE
SCLEROTIA RECOVERED (%)
0
10
20
30
40
50
60
8
12
FIELD EXPOSURE (MONTHS)

Fig. 14. Patterns of decline in the populations of field-produced sclerotia of *S. cepivorum* in muck soil in the Fraser Valley for three successive years.


Legend
1979
1980
1981
SCLEROTIA RECOVERED %
100
80
60
40
20
0
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
FIELD EXPOSURE (MONTHS)

Discussion

The addition of soil to sclerotia of *S. cepivorum* may reduce their rate of decay in the field by protecting the sclerotia from temperature or moisture fluctuations or by absorbing compounds which could cause germination and therefore decay. Soil may also prevent the spread of mycoparasites from one sclerotium to another. This latter effect is in fact indicated in that in the first year of field tests when no soil was mixed with sclerotia there were several bags in which all of the sclerotia had disappeared, suggesting that mycoparasitic epidemics may have occurred within the bags. These results suggest that it may be useful to avoid fall cultivation on fields which are severely affected by white rot. This would leave the sclerotia formed on infected onions in clumps that would be susceptible to epidemics caused by mycoparasites.

The similar rates of decay for sclerotia of *S. cepivorum* in the three soils tested (Fig.13) was unexpected as there are many instances where soil type has influenced the rate of decay of sclerotia (53, 66, 80). Different soils have different physical and biological characteristics which can influence survival of sclerotia (29). Adams and Ayers (6) found that some soils had high populations of *S. sclerotivorum* that could be correlated with the decline in numbers of sclerotia of *S. minor* and *S. cepivorum* . Green (53) found that the extent of differences in survival of sclerotia of *V. dahliae* in two different soils

varied depending on the soil moisture. The survival of the sclerotia of _S. cepivorum_ in the three Fraser Valley soils was studied under natural uncontrolled environmental conditions. It is possible that these conditions may have contributed to the lack of detectable differences in the survival of the sclerotia in the soils tested.

The rate of decay of sclerotia of _S. cepivorum_ in the Fraser Valley was greatest during the winter when the soils were flooded. Soil flooding has been used to control diseases caused by _S. sclerotiorum_ (29) and _V. dahliae_ (94). Crowe (39) found that decay of sclerotia of _S. cepivorum_ was greatest at soil saturation but this occurred only at high temperatures. The decay observed here, however, occurred at temperatures predominately in the range 0° C to 10° C and at times below 0° C. These conditions are similar to those cited by Papavizas (90) as being optimal for the decay of _S. rolfsii_. Decay of sclerotia in waterlogged soils may result from effects on the physical structure of the sclerotia or changes in the microbial composition of the soil.

Immersion of sclerotia in water may cause the rind to be disrupted through imbibition of water (123), while freezing may cause the rind to rupture through ice crystal formation (90). Any disruption in the integrity of the rind will lead to decay of sclerotia either by increasing germination or by allowing parasites to invade the sclerotia. The anaerobic conditions created by the flooding may also affect the physiology of the

sclerotia and may thereby increase their susceptibility to parasitism. Makkonnen and Pohjakallio (77) found that normally resistant sclerotia were attacked when they were boiled, indicating that some vital process was involved in the resistance. In contrast, Ayers and Adams(15), found that sclerotia of *S. minor* killed by autoclaving were only poorly colonized by the mycoparasite *S. sclerotivorum*. There is a need for research on the effect of waterlogging on the structure and physiology of sclerotia and the subsequent effect on the susceptibility of the pathogen to parasitism.

Flooding can cause drastic changes in the chemical and biological characteristics of a soil and these changes may be responsible for sclerotial decay under these conditions. Waterlogged soils, particularly those high in organic matter produce volatile sulphides, including methyl mercaptan, dimethyl sulphide, and dimethyl disulphide (19, 20). These compounds are only moderately effective in causing germination (34, 70) of *S. cepivorum*, but concurrent changes in the microbial composition of the soil could conceivably increase the sensitivity of sclerotia to these compounds. Intensive irrigation of onion fields can cause increased infection by white rot (97). It is unknown if this is due to an increase in the germination of sclerotia or some other factor. Coley-Smith (28) found that sclerotial germination increased with increasing soil moisture ~~but that~~ infection was greatest at moderate levels. Crowe (39), however, found that germination decreased at moisture levels

above or below the -300 mb. These latter two studies examine the effect of soil moisture on stimulated germination. To my knowledge there is no published information on the effect of soil moisture on spontaneous germination which is important in this study. The factors affecting the proportion of sclerotia germinating in the absence of a stimulant, which can be as high as 17%, should be studied more intensively as they may be important in determining the longevity of sclerotia in soil. Survival of sclerotia in waterlogged soil would also be decreased if there was a shift in the microflora population in favor of organisms capable of parasitizing the sclerotia. The cause of the increased decay of sclerotia in the flooded soils is unknown, but this phenomenon may provide a practical means of controlling white rot.

Sclerotial survival in the Fraser Valley appears to be much lower than in other areas. Coley-Smith (27) found that almost 100% of the sclerotia persisted after four years in soil. In this study a significant reduction in viable sclerotia after one year was observed in all of the field trials. The half-lives of the sclerotia in the three experiments specifically evaluating this phenomenon ranged from 9.7 to 26.8 months. The reduced survival of sclerotia observed in this study, relative to that reported by other authors, is likely due to the flooding which is common in the winter in the muck soil areas of the Fraser Valley. Under these conditions crop rotation may be a practical means of controlling white rot. Before recommendations can be

made, however, we need information on the relationship between inoculum density and disease incidence. Adams (5) has developed a method of predicting disease incidence in New England but he is dealing with different soil and farming practices than are present here. It is important to derive a formula applicable to the Fraser Valley and to use it to determine the length of rotation needed to keep disease levels below an economically acceptable threshold.

III. Enhanced Reduction of Sclerotia

Introduction

The time required for crop rotation to be effective for control of white rot may be too long for it to be a practical means of disease control. It is therefore advisable to look for measures which will increase the rate of decay of the sclerotia and shorten the rotation period.

Decay of sclerotia can be increased by increasing microbiological activity on or around their surface. This activity can be achieved by the application of mycoparasites such as *C. minitans* and *S. sclerotivorum* to the reservoir of the pathogen (15). Creating conditions which cause sclerotia to leak nutrients also increases microbiological activity on their surface.

Many fungi are able to parasitize sclerotia *in vitro* but only two, *C. minitans* and *S. sclerotivorum* have demonstrated ability to destroy sclerotia in the field (14). *C. minitans* has been used in the field to reduce numbers of sclerotia of *S. sclerotiorum* (62, 110) and *S. trifoliorum* (113) and has been used in greenhouse trials to control *S. cepivorum* (11).

Smith (103, 104, 105) reported that the survival of sclerotia of *S. cepivorum* and other sclerotia-forming pathogens was reduced if they were air-dried before burial. He observed

that nutrients leaked from dried sclerotia, and promoted microbial decay (103, 104). He proposed that biological control of diseases could be obtained by producing conditions that would lead to drying of sclerotia (105). Coley-Smith et al. (31) and Papavizas (91), however, found that drying did not increase the decay of *S. cepivorum*. Papavizas (91) did observe that colonization of sclerotia by fungi and *Streptomyces spp.* was three times higher on sclerotia kept at 0% relative humidity for four and seven days than on those kept at higher humidities for the same periods.

This study examines the potential for increasing the rate of decay of sclerotia of *S. cepivorum* in the Fraser Valley by various methods: 1. by the application of the mycoparasite *C. minitans* to soil containing sclerotia of *S. cepivorum*, 2. by drying the sclerotia prior to placing them in soil, and 3. by leaving sclerotia on the surface of soil, where they would be exposed to variable drying conditions depending on environmental factors. The relationship between drying sclerotia and changes in microbial populations on the surface of sclerotia was also studied.

Materials and Methods

Parasitism by *C. minitans*

The *C. minitans* isolate (DAOM 149432) used in this study was obtained from the Biosystematics Research Institute,

Agriculture Canada. The ability of the isolate to parasitize sclerotia of S. cepivorum in vitro was tested in the laboratory. C. minitans was grown on PDA for two weeks and a spore suspension was prepared. Sclerotia of S. cepivorum were soaked in this suspension for 24 h and then incubated in moist sand for six weeks at 25° C. The sclerotia were examined after three and six weeks for signs of parasitism.

The ability of C. minitans to parasitize sclerotia in vivo was studied by mixing parasite inoculum with sclerotia of S. cepivorum and soil in nylon mesh bags and subjecting the bags to field exposure. The C. minitans inoculum was prepared by growing the fungus on a rye:barley:sunflower medium (62), which was ground to produce a powder, and added to the bags containing sclerotia at a rate of 0.1 g per bag. Half of the bags received inoculum which had been autoclaved to kill the parasite (control). Forty sclerotia of S. cepivorum were added to each bag with two cc of muck soil. The sclerotia were either dried by placing them in a desiccator over Drierite for 24 h or were not dried. The bags of sclerotia were placed on the surface of field plots in Cloverdale. They were arranged in a randomized complete block design with six replications. The bags were recovered after 8 and 12 months of field exposure and the contents were analyzed as described in section I.

The Effect of Drying on Sclerotial Survival

The effect of drying sclerotia was studied in experiments 1, 2, and 3 referred to in the previous chapter (C-II). In experiment 1 the sclerotia were air-dried by leaving them in an open dish at 25° C for 48 to 72 h. In experiments 2 and 3 the sclerotia were dried by placing them in a desiccator over Drierite ($CaSO_4$) for 24 h. The effect of natural drying was evaluated by comparing survival of sclerotia which were buried 15 cm below the soil surface with that of sclerotia left on the surface in experiments 1 and 3. The bags were arranged in field plots in a randomized complete block design and analyzed as described in section C-II.

Mycoflora Determinations

The effect of drying sclerotia on their surface mycoflora was determined by the dilution plate method described in section C-I. The proportions of gram negative and positive bacteria were estimated by testing 20 to 50 colonies selected at random for each replication for their behavior in KOH (3%) as described by Suslow *et al.* (106). The number of fungal colonies were counted and the proportion of *Trichoderma spp.*, *Penicillium spp.*, *Verticillium spp.* and *Fusarium spp.* was estimated. The mycoflora populations were determined for sclerotia which had been 1. taken directly from onions, 2. dried over Drierite for 24 h, 3. placed into soil for 96 h and, 4. dried and placed into soil for 96 h.

Results

Parasitism by *C. minitans*

C. minitans was able to parasitize sclerotia of *S. cepivorum in vitro.* Almost all of the treated sclerotia were infected by *C. minitans* within 6 weeks. Infected sclerotia were filled with pycnidia of the parasite, and dark *C. minitans* conidia were released when the sclerotia were broken open (Fig. 15). In the field the parasite did not have any significant ($p>0.05$) effect on the survival of sclerotia of *S. cepivorum* that had not been dried (Fig. 16). After 12 months in soil none of the dried sclerotia, 18.8% of the sclerotia mixed with autoclaved medium (control), and 16.3% of the sclerotia mixed with viable *C. minitans* inoculum remained intact.

Effect of Drying on Survival of Sclerotia

Drying sclerotia prior to burial significantly ($p\leq0.05$) reduced the survival of sclerotia of *S. cepivorum* (Fig. 17). The half-lives of the sclerotia were reduced to less than five months in all three experiments.

Sclerotia on the soil surface decayed more than buried ones in 1979 (experiment 1) (Fig. 18). After 16 months in the field 23.6% of the not-dried sclerotia which were buried and only 2.1% of those on the surface survived.

Fig.15. Sclerotia of *S. cepivorum* infected by *C. minitans*

Top Photograph : Arrow shows conidia of *C. minitans* being released from sclerotium of *S. cepivorum*.

Bottom Photograph : Arrow shows deformation of the wall of sclerotium of *S. cepivorum* by pycnidia of *C. minitans*

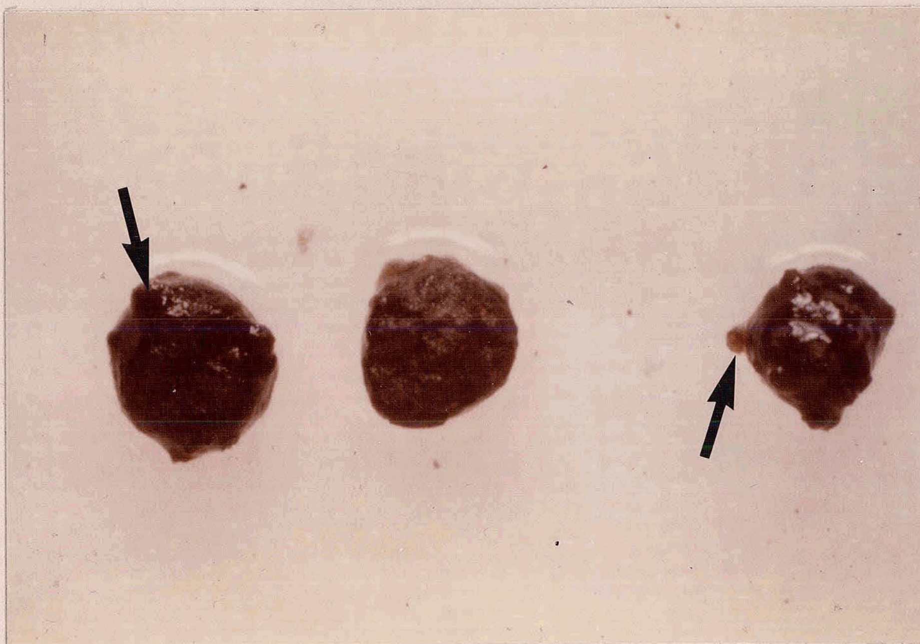
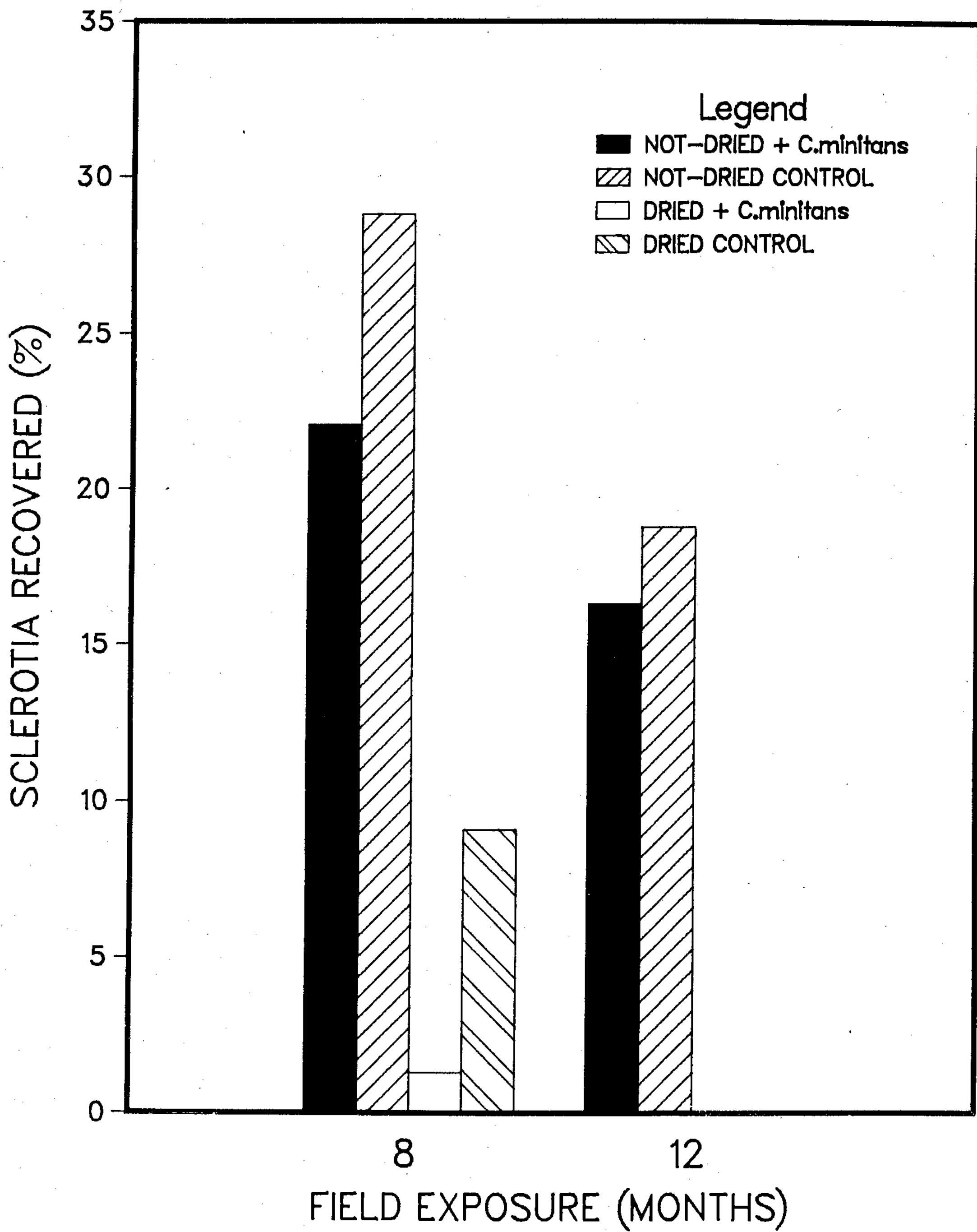
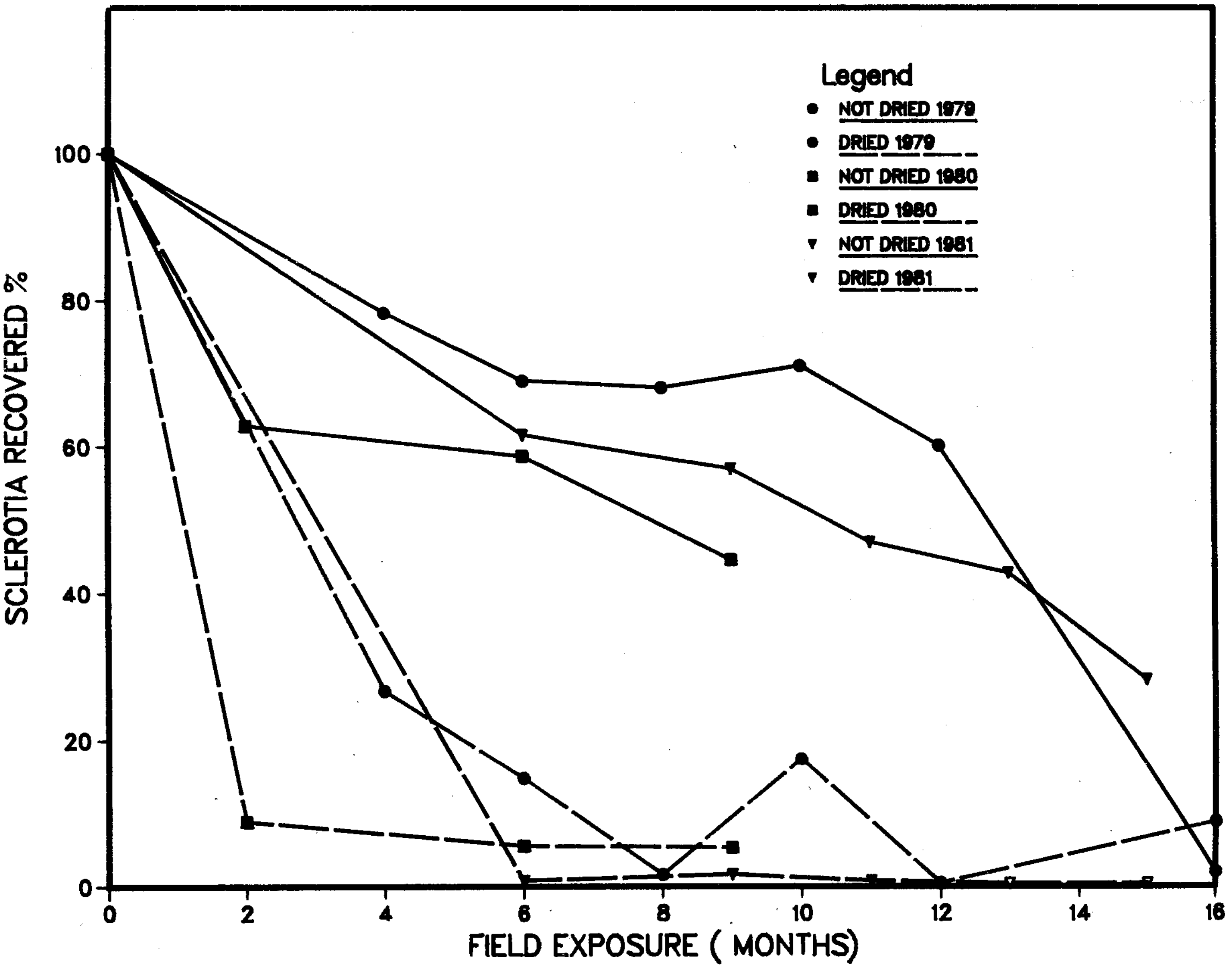


Fig.16. The effect of *C. minitans* on the proportion of sclerotia (dried; not-dried) of *S. cepivorum* surviving 8 and 12 months field exposure in muck soil.



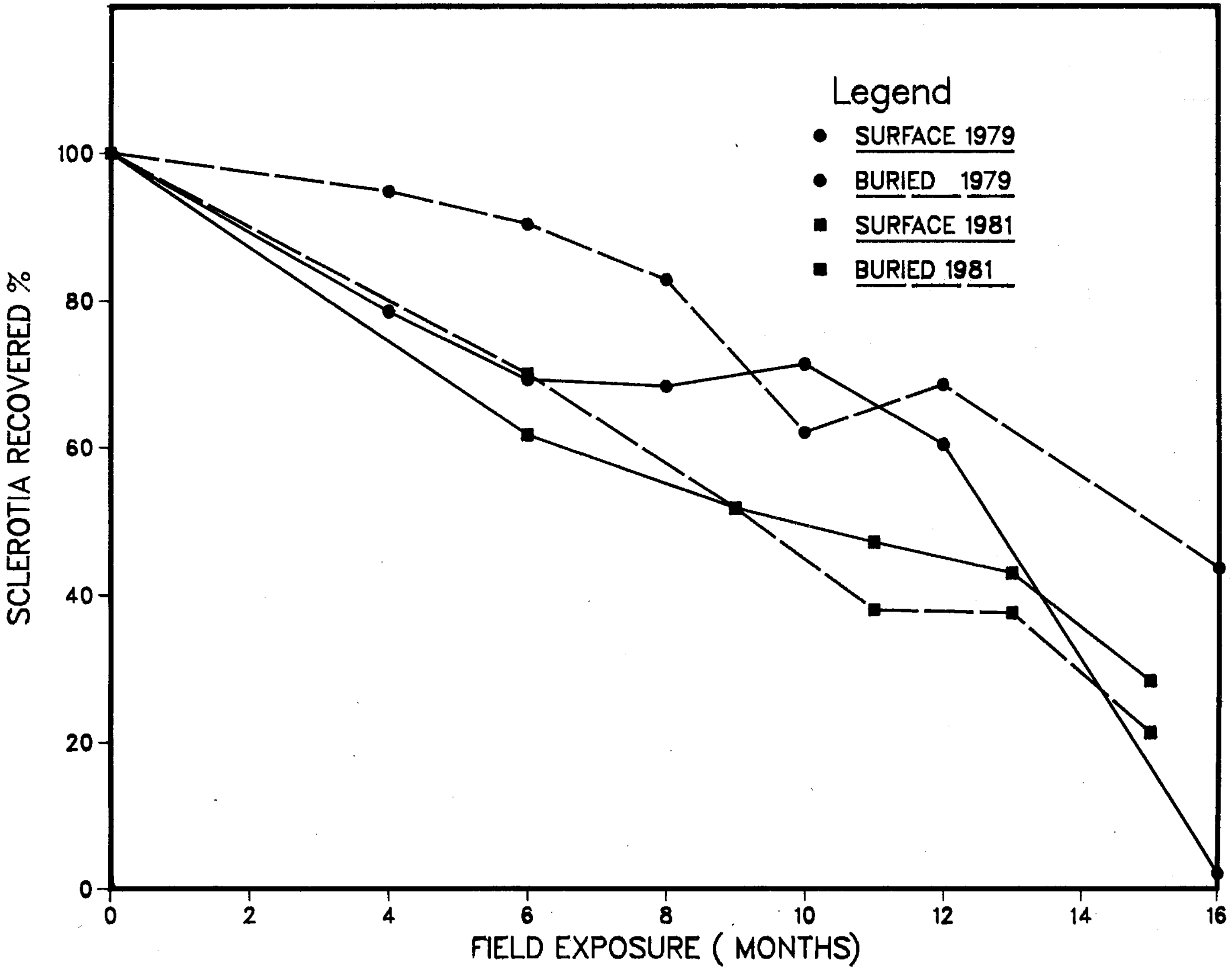
Legend
NOT–DRIED + C.minitans
NOT–DRIED CONTROL
DRIED + C.minitans
DRIED CONTROL
SCLEROTIA RECOVERED (%)
0
5
10
15
20
25
30
35
8
12
FIELD EXPOSURE (MONTHS)

Fig. 17. The effect of pre-burial desiccation on the decline of the population of field-produced sclerotia of *S. cepivorum* in muck soil



Legend
NOT DRIED 1979
DRIED 1979
NOT DRIED 1980
DRIED 1980
NOT DRIED 1981
DRIED 1981
SCLEROTIA RECOVERED %
100
80
60
40
20
0
FIELD EXPOSURE (MONTHS)
0
2
4
6
8
10
12
14
16

Fig. 18. The effect of the position of the sclerotia in soil (surface or buried 15 cm deep) on the decline in the population of field-produced sclerotia of *S. cepivorum* in muck soil.


Legend
SURFACE 1979
BURIED 1979
SURFACE 1981
BURIED 1981
SCLEROTIA RECOVERED %
100
80
60
40
20
0
FIELD EXPOSURE (MONTHS)
0
2
4
6
8
10
12
14
16

Mycoflora Populations of Sclerotia

The population of bacteria on the sclerotia was significantly ($p \leq 0.05$) affected by drying (Fig. 19). The proportion of gram negative bacteria decreased from 44.8% to 12.4% when the sclerotia were dried (Table 9).

The fungal populations of the sclerotia also changed significantly with the different treatments (Fig. 20). The dramatic increase in fungal populations observed on dried sclerotia after 96 h in soil was primarily the result of an increase in *Trichoderma* spp. which accounted for 3.8% of the fungal isolates from not-dried sclerotia in soil and 77.9% of the isolates from dried sclerotia placed in soil (Table 9). The remaining fungi isolated from sclerotia were *Penicillium* spp., *Verticillium* spp. and *Fusarium* spp..

Fig.19. Bacterial populations on sclerotia of _S. cepivorum_ as affected by drying and incubation in soil.

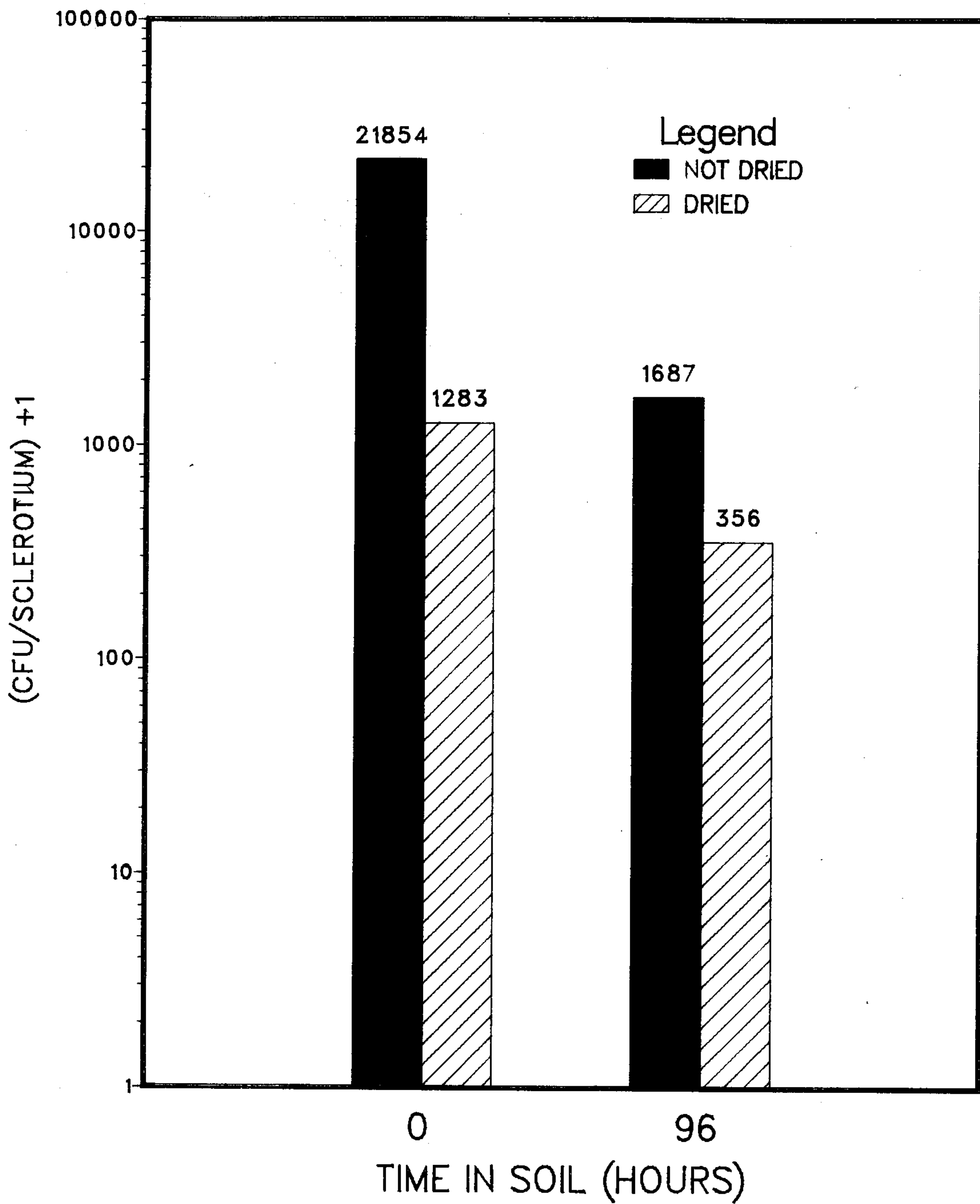

100000
10000
1000
100
10
1
(CFU/SCLEROTIUM) +1
21854
1283
1687
356
Legend
NOT DRIED
DRIED
0
96
TIME IN SOIL (HOURS)

Fig 20. Fungal populations on sclerotia of *S. cepivorum* as affected by drying and incubation in soil.

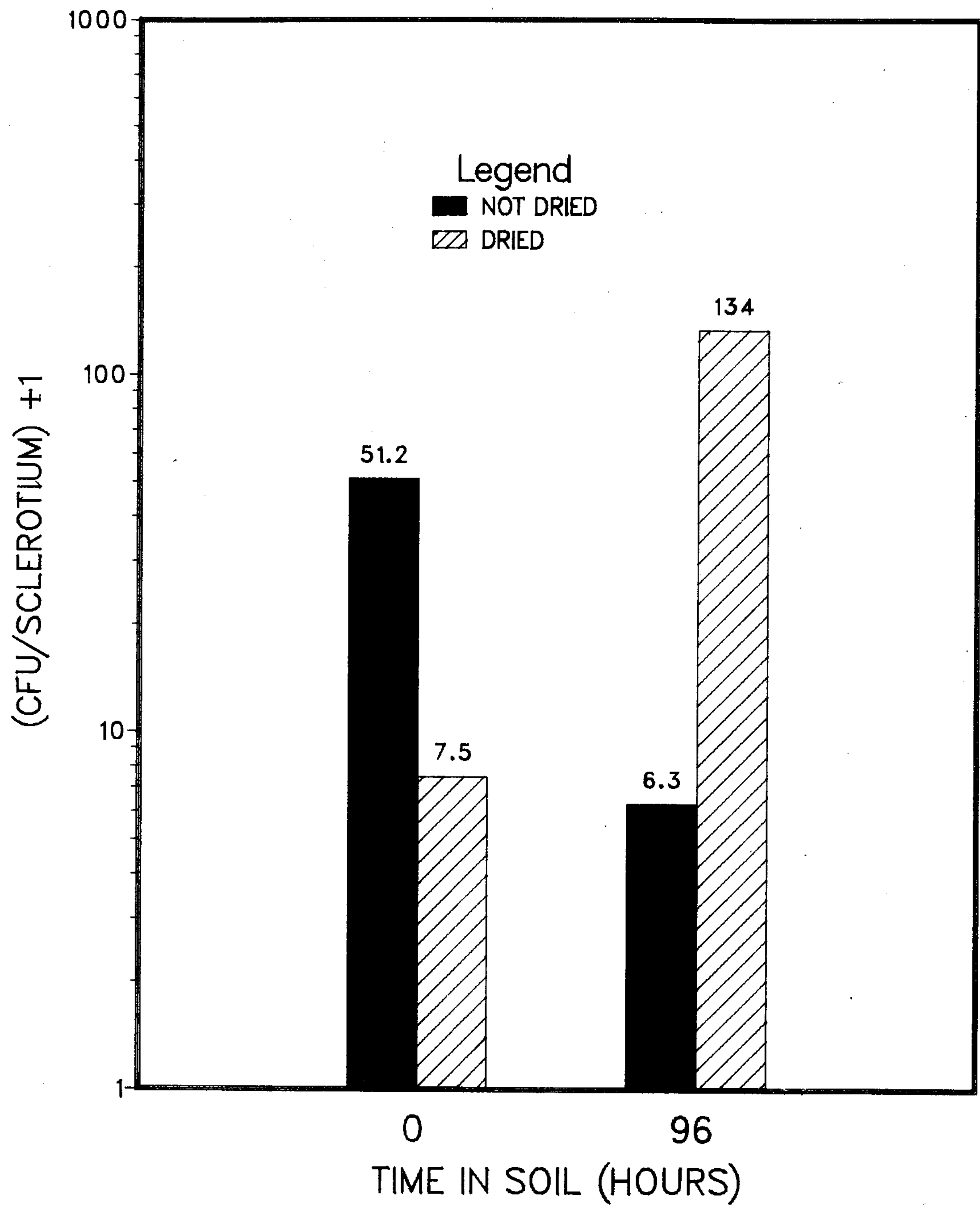

1000
100
10
1
(CFU/SCLEROTIUM) +1
Legend
NOT DRIED
DRIED
134
51.2
7.5
6.3
0
96
TIME IN SOIL (HOURS)

Table 9. Composition of the bacterial and fungal microflora on field-produced sclerotia of *S. cepivorum* as affected by drying and incubation in soil.

TREATMENT	Not Dried from Onions	Not Dried in Soil	Dried from Onions	Dried in Soil
	****	BACTERIA	****	
* CFU/SCLEROTIUM	2.2 $x10^5$	1.7 $x10^3$	1.3 $x10^3$	3.6 $x10^2$
Proportion by Type				
gram +	.243	.551	.939	.875
gram -	.746	.448	.061	.124
	****	Fungi	****	
CFU/SCLEROTIUM	50.2	6.3	7.5	134.0
Proportion by Genus				
Penicillium spp.	.245	.259	.415	.035
Verticillium spp.	.358	.435	.254	.013
Fusarium spp.	.246	.237	.154	.154
Trichoderma spp.	.059	.038	.128	.779
Other spp.	.092	.031	.049	.019

Discussion

C. minitans was able to parasitize sclerotia in the laboratory. The ability of the parasite to infect the sclerotia and form pycnidia within the body of the sclerotia was also noted by Ghaffar (50). *C. minitans*, however, did not significantly reduce the numbers of sclerotia of *S. cepivorum* in the field. This result is contrary to the reports of many researchers who have found that *C. minitans* is an effective mycoparasite under field as well as laboratory conditions (59, 62, 111, 113). The inability of the parasite to reduce sclerotial numbers in this study may be due to inhospitable conditions in the Fraser Valley soils at the time of the experiment. *C. minitans* may be unable to compete with other micro-organisms present in local soils or the temperature and moisture conditions may have been unfavorable. Most researchers have applied *C. minitans* to soil during warm weather but in this study the fungus was added to bags of sclerotia in the fall. The temperature during most of this study was rarely above 15° C and the soil was often flooded. Trutman *et al.* (111) reported that *C. minitans* was able to infect and decay sclerotia of *S. sclerotiorum* over a wide temperature range but decay was substantial only between 15 and 24° C. If this is also true for sclerotia of *S. cepivorum* it follows that *C. minitans* might be more effective if added to soil in the spring or summer.

Dried sclerotia decayed significantly faster than those which were not dried in all experiments, thereby supporting the

findings of Smith (103, 104, 105). The decay of the dried sclerotia presumably results from increased microbial activity on the surface of sclerotia. Dried sclerotia leak nutrients, which promote the growth of micro-organisms. Gilbert and Linderman (52) found that the numbers of bacteria increased while the number of fungi did not. Smith (105) and Papavizas (91), however, noticed increases in fungi on the sclerotial surface. In this study the dried sclerotia incubated in soil for 96 h had lower numbers of bacteria and higher numbers of fungi than the sclerotia which had not been dried. The observation that the sclerotia which had been dried and placed in soil had bacterial populations lower than those which had been dried but not placed in soil (Fig. 19, Table 9) indicates that the decline in numbers of bacteria on the sclerotia was not the result of death of bacteria due to desiccation but was due instead to competition, presumably from fungi which increased dramatically on the dried sclerotia in soil.

The treatment of sclerotia alters the types of bacteria and fungi isolated from their surface as well as the total populations (Table 9). The proportion of gram positive bacteria increased on the dried sclerotia. The gram positive bacteria on the sclerotial surface may, like _B. subtilis_, be spore formers and therefore more resistant to desiccation. The major fungal genera isolated from the sclerotia were _Penicillium_ _spp._, _Verticillium_ _spp._, _Fusarium_ _spp._ and _Trichoderma_ _spp._ (Table 9). Similar observations have been made by other workers who have

studied the microflora associated with sclerotia (42, 80). The proportion of these genera, however, changed with treatment. Drying of itself did not significantly alter the relative proportions of the genera predominant on sclerotia (Table 9). The increase of *Trichoderma spp.* occurred only on the sclerotia which had been dried and incubated in soil. This result indicates that dried sclerotia placed in soil are actively colonized by *Trichoderma spp.* at the expense of other sclerotial mycoflora. This change in the composition of the microflora associated with the sclerotia could account for the increased decay of these bodies. Merriman (80) reported that decay of sclerotia of *S. sclerotiorum* in soil was related to their associated microflora. The decrease in bacterial populations may have removed protective organisms from the surface of the sclerotia. This effect, combined with the increase in *Trichoderma spp.* which are known for their mycoparasitic activity (59, 62, 77), may lead to the decay of the dried sclerotia.

While the effectiveness of preburial desiccation on decay of sclerotia is biologically interesting, it is of no practical benefit unless the effect can be reproduced in the field. Leaving sclerotia on the surface of the soil should expose them to a natural drying. Sclerotia of *Mycospaerella pinoides* (100) decayed more if they were placed on the surface but sclerotia of *R. solani* (58) and *S. sclerotiorum* decayed more if they were buried. Rai and Saxena (95), however, found that sclerotia of *S.*

sclerotiorum on the surface of soil were more heavily colonized than buried ones. Sclerotia of *S. cepivorum* left on the soil surface decayed more than those which were buried in experiment 1 but not experiment 3. Experiment 3 was conducted in 1981 which was an abnormally wet year in the Fraser Valley, and the fields were flooded for much of the time these sclerotia were in the field. It is likely that the sclerotia on the soil surface in 1979, which was a relatively dry year, probably dried during the warm summer months, to a greater extent than did those sclerotia on the soil surface in 1981.

These results indicate that given favorable environmental conditions, the survival of sclerotia of *S. cepivorum* can be reduced by cultural manipulation. If there is a period of hot dry weather following harvest, leaving decaying onions covered with sclerotia on the soil surface may help to reduce inoculum levels. The results of the mycofloral determinations indicate that *Trichoderma spp.* may be effective mycoparasites. The effectiveness of applications of these parasites may be increased by applying them with low doses of pesticides to reduce the numbers of competing organisms. Any combination of these measures may, by causing decay of sclerotia, help to bring about economically useful reductions in the population levels of *S. cepivorum* .

IV. Conclusions

The results reported in this section illustrate the importance of studying the biology of an organism under natural conditions . There were structural differences in sclerotia of *S. cepivorum* produced in the laboratory and the field. These sclerotia also had different abilities to survive in soil. In most areas sclerotia of *S. cepivorum* can persist for years in soil, but this study showed that in the Fraser Valley there was substantial decay of the sclerotia within one year. The decay of the sclerotia was presumably due to the local environmental conditions. It is therefore possible that control measures which are ineffective in other areas of the world may be practical means of reducing white rot in the Fraser Valley.

While the characteristics of the disease and of local environmental conditions were detrimental to biological control measures aimed at the protection of host plant surfaces, they appear to be ideally suited to disease control through inoculum reduction. The sclerotia of *S. cepivorum* are the only known means by which the pathogen overwinters. It does not increase in the absence of the host, and it has a low capacity to produce secondary sclerotia. Thus any control measure which causes the sclerotia to decay will lower the inoculum levels in soil and reduce disease incidence. Sclerotia are relatively large bodies and therefore provide large targets for mycoparasites (17). The sclerotia of *S. cepivorum* decay more rapidly in the winter

months when soils are saturated. The effect of flooding on sclerotia survival is an important factor that should be evaluated. Fraser Valley fields are often flooded in the winter and if this is the factor responsible for enhanced decay of sclerotia, intentional flooding combined with crop rotation could be a feasible means of limiting white rot in the Fraser Valley.

The results of this study indicate that there is potential for controlling white rot in the Fraser Valley by reducing the numbers of sclerotia in soil, but there is a need for more information before concrete recommendations can be made. Crop rotation studies, which include experiments on the effectiveness of winter flooding are needed to determine how long a rotation is required to reduce the disease below threshold levels. It may be possible to predict the decline of sclerotia based on soil moisture data, and to use this information to estimate expected disease levels. Before this is practical, however, we need data on the relationship of the inoculum density of S. cepivorum to disease incidence in onions in the Fraser Valley. There should be more studies on methods of increasing the rate of decay of sclerotia. These will include experiments on techniques of manipulating the environment to cause the sclerotia to dry, and the continued search for effective mycoparasites and ways of applying them.

Biological control of white rot by reducing inoculum levels in soil should provide a long term means of preventing the

disease from destroying the onion industry in the Fraser Valley.

D. Conclusions

> " The art and science of plant disease control must continue to move in the direction of the biological control of plant pathogens including the use of introduced antagonists. Chemical controls are necessary with many diseases at present; but are undesirable and even inadequate as long term solutions to plant health" (37).

The search for effective biocontrol procedures must involve extensive investigations of many different methods, and should consider the ecology of the system being studied. Two types of biological control of plant diseases were investigated in this thesis. The first, protecting plant surfaces with antagonistic fungi did not lead to any reduction in the incidence of white rot in the Fraser Valley. The second, that of reducing inoculum levels in soil, appears to have substantial potential as a practical means of lowering disease levels.

The first technique involves causing a drastic change in the microbiological composition of the soil and maintaining this change for up to five months. Given our lack of knowledge of the interactions which occur in soil, and the known resistance of the soil to biological changes, (17) this method is not at present a feasible means of control. The second technique, on the other hand, takes advantage of the prevailing conditions in Fraser Valley soils, and so is at the moment a more promising method of reducing white rot.

Biological control, by itself may not solve the white rot problem in the Fraser Valley but it can be a useful part of an integrated disease management program.

Literature Cited

1. Anonymous.1980. Vegetable Production Guide B.C. Ministry of Agriculture and Food.

2. Abd-El-Moity, T.H. and M.N. Shatla. 1981. Biological control of white rot disease of onion (Sclerotium cepivorum) by Trichoderma harzianum. Phytopathol. Z. 100: 29-35.

3. Abd-El-Moity, T.H., G.C. Papavizas and M.N. Shatla. 1982. Induction of new isolates of Trichoderma harzianum tolerant to fungicides and experimental use for control of white rot of onion. Phytopathology 72: 396-400.

4. Abd-El-Razik, M.N. Shatla and M. Rushdi. 1973. Studies on the infection of onion plants by Sclerotium cepivorum Berk. Phytopathol. Z. 76: 108-116.

5. Adams, P.B. 1981. Forecasting onion white rot disease. Phytopathology 71: 1178-1181.

6. Adams, P.B. and W.A. Ayers. 1981. Sporidesmium sclerotivorum : distribution and function in natural biological control of sclerotial fungi. Phytopathology 71: 90-93.

7. Adams, P.B. and W.A. Ayers. 1982. Factors affecting the parasitic activity of Sporidesmium sclerotivorum on sclerotia of Sclerotinia minor in soil. Phytopathology 72: 366-368.

8. Adams, P.B. and W.A. Ayers. 1982. Biological control of sclerotinia lettuce drop in the field by Sporidesmium sclerotivorum. Phytopathology 72: 485-488.

9. Adams, P.B. and G.C. Papavizas. 1971. Effect of inoculum density of Sclerotium cepivorum and some soil environmental factors on disease severity. Phytopathology 61: 1253-1256.

10. Agrawal, S.C., M.N. Khare and P.S. Agrawal. 1977. Biological control of Sclerotium rolfsii causing collar rot of lentil. Indian Phytopathology 3: 176-179.

11. Ahmed, A.H.M. and H.T. Tribe. 1977. Biological control of white rot of onion (Sclerotium cepivorum) by Coniothyrium minitans. Plant Pathol. 26: 75-78.

12. Arjunarao, V. 1971. Biological control of cotton wilt. I. Soil fungistasis and antibiosis in cotton fields. Proc. Indian Acad. of Sci. Sect. B. 73: 265-272.

13. Asthana, R.P. 1947. Studies on sclerotium forming fungi. I. Sclerotium cepivorum Berk. and S. tuliparum Klebahn. Part 1. Cultural studies. Part 2. Symptoms, mode of infection and host range. Part 3. Pectinase activity and preparation. Proc. Indian Acad. Sci. Sect. B. 26: 93-124 (Abst).

14. Ayers, W.A. and P.B. Adams. 1979. Mycoparasitism of sclerotia of Sclerotinia and Sclerotium species by Sporidesmium sclerotivorum. Can. J. Microbiol. 25: 17-23.

15. Ayers, W.A. and P.B. Adams. 1981. Mycoparasitism and its application to biological control of plant diseases. Pages 91-103 in G.C. Papavizas ed. Biological Control in Crop Production. Allanheld, Osmun and Co. New Jersey. 450 pp.

16. Backman, P.A. and R. Rodrigues-Kabana. 1975. A system for the growth and delivery of biological control agents to the soil. Phytopathology 65: 819-821.

17. Baker, K.F. and R.J. Cook. 1974. Biological Control of Plant Pathogens. W.H. Freeman and Sons, San Francisco, 433 pp.

18. Baker, R. 1981. Biological control: eradication of plant pathogens by adding organic amendments to soil. p. 317-327 in D. Pimentel ed. Handbook of Pest Management in Agriculture. Vol. II C.R.C. Press, Florida, 501 pp.

19. Banwart, W.L. and J.M. Bremner. 1976. Volatilization of sulphur from unamended and sulphate treated soils. Soil Biol. Biochem. 8: 19-22.

20. Banwart, W.L. and J.M. Bremner. 1976. Evolution of volatile sulfur compounds from soils treated with sulfur containing organic materials. Soil Biol. Biochem. 8: 439-443.

21. Bell, D.K. and H.D. Wells. 1977. Reactions of Rhizoctonia solani and Ceratobasidium sp. to Trichoderma spp. in vitro. Proc. Amer. Phytopathol. Soc. 4: 167.

22. Bell, D.K., H.D. Wells and C.R. Markham. 1982. In vitro antagonism of Trichoderma species against six fungal plant pathogens. Phytopathology 72: 379-382.

23. Booer, J.R. 1945. Control of white rot in onions. Nature 155: 241.

24. Broadbent, P., K.F. Baker and Y. Waterworth. 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. Aust. J. Biol. Sci. 24: 925-944.

25. Chet, I. 1969. The role of sclerotial rind in the germinability of sclerotia of Sclerotium rolfsii. Can. J.

Bot. 47: 593-595.

26. Chet, I. and R. Baker. 1981. Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive to *Rhizoctonia solani*. *Phytopathology* 71: 286-290.

27. Coley-Smith, J.R. 1959. Studies of the biology of *Sclerotium cepivorum* Berk. III. Host Range; persistence and viability of sclerotia. *Ann. Appl. Biol.* 47: 511-518.

28. Coley-Smith, J.R. 1960. Studies on the biology of *Sclerotium cepivorum* Berk. IV. Germination of sclerotia. *Ann. Appl. Biol.* 48: 8-18.

29. Coley-Smith, J.R. and R.C. Cooke. 1971. Survival and germination of fungal sclerotia. *Annu. Rev. Phytopathol.* 9: 65-92.

30. Coley-Smith, J.R., D.J. Dickinson, J.E. King and R.W. Holt. 1968. The effect of species of *Allium* on soil bacteria in relation to germination of sclerotia of *Sclerotium cepivorum* Berk. *Ann. Appl. Biol.* 62: 103-111.

31. Coley-Smith, J.R., A. Ghaffar and Z.U.R. Javed. 1974. The effect of dry conditions on subsequent leakage and rotting of fungal sclerotia. *Soil Biol. Biochem.* 6: 307-312.

32. Coley-Smith, J.R. and C.J. Hickman. 1957. Stimulation of sclerotium germination in *Sclerotium cepivorum* Berk. *Nature* 180: 445.

33. Coley-Smith, J.R. and R.W. Holt. 1966. The effect of species of *Allium* on germination in soil of sclerotia of *Sclerotium cepivorum* Berk. *Ann. Appl. Biol.* 58: 273-78.

34. Coley-Smith, J.R. and J.E. King. 1969. The production by species of *Allium* of alkyl sulphides and their effect on germination of sclerotia of *Sclerotium cepivorum* Berk. *Ann. Appl. Biol.* 64: 289-301.

35. Coley-Smith, J.R., J.E. King, D.J. Dickinson and R.W. Holt. 1967. Germination of sclerotia of *Sclerotium cepivorum* Berk. under aseptic conditions. *Ann. Appl. Biol.* 60: 109-115.

36. Colotelo, N. 1973. A scanning electron microscope study of developing sclerotia of *Sclerotinia sclerotiorum*. *Can. J. Bot.* 52: 1127-1130.

37. Cook, R.J. 1981. Biological control of plant pathogens: Overview. Pages 23-44 in G.C. Papavizas ed. *Biological Control in Crop Production.* Allanheld, Osmun and Co., New

Jersey, 450 pp.

38. Crowe, F.J. and D.H. Hall. 1980. Vertical distribution of sclerotia of Sclerotium cepivorum and host root systems relative to white rot of onion and garlic. Phytopathology 70: 70-73.

39. Crowe, F.J. and D.H. Hall. 1980. Soil temperature and moisture effects on sclerotium germination and infection by onion seedlings by Sclerotium cepivorum. Phytopathology 70: 74-78.

40. Crowe, F.J., D.H. Hall, A.S. Greathead and K.G. Baghott. 1980. Inoculum density of Sclerotium cepivorum and the incidence of white rot of onion and garlic. Phytopathology 70: 64-69.

41. Croxall, H.E., R.W. Sidwell and J.E. Jenkins. 1953. White rot (Sclerotium cepivorum) of onions in Worcestershire with special reference to control by seed treatment with calomel. Ann. Appl. Biol. 40: 166-175.

42. Curl, E.A. and J.D. Hansen. 1964. The microflora of natural sclerotia of Sclerotium rolfsii and some effects on the pathogen. Plant Disease Reptr. 48: 446-450.

43. De Trogoff, H. and J.L. Ricard. 1976. Biological control of Verticillium malthousei by Trichoderma viride spray on casing soil in commercial mushroom production. Plant Disease Reptr. 60: 677-680.

44. Dhyani, S.C. and S.K. Chauhan. 1976. Survival of Sclerotium cepivorum Berk. as competitive saphrophyte on agar plate. J. Indian Bot. Soc. 55: 197-200.

45. Elad, Y., I. Chet and J. Katan. 1980. Trichoderma harzianum: A biocontrol agent effective against Sclerotium rolfsii and Rhizoctonia solani. Phytopathology 7: 119-121.

46. Elnaghy, M.A., A.H. Moubasher and S.E. Meyala. 1971. Stimulation of sclerotial germination of Sclerotium cepivorum by host plant extract. Plant and Soil 34: 109-119.

47. Entwistle, A.R. and M.L. Munasinghe. 1980. The effect of seed, furrow, and stem base spray treatment with iprodione on white rot disease (Sclerotium cepivorum) in spring sown salad onions. Ann. Appl. Biol. 95: 215-225.

48. Entwistle, A.R. and H.L. Munasinghe. 1980. The effect of iprodione on sclerotium germination, root infection and mycelial spread of Sclerotium cepivorum in salad onions. Ann. Appl. Biol. 95: 329-339.

49. Fletcher, J.T., B.C. Knight, E. Bate and I.A. Cragg. 1971. The control of white rot (*Sclerotium cepivorum*) in salad onions with dichloran. *Plant Pathol.* 20: 88-92.

50. Ghaffar, A. 1969. Biological control of white rot of onion. I. Interactions of soil micro-organisms with *Sclerotium cepivorum* Berk. *Mycopathol. et Mycol. appl.* 38: 38-48.

51. Ghaffar, A. 1969. Biological control of white rot of onion. II. Effectiveness of *Penicillium nigricans* (Bain) Thom. *Mycopathol et Mycol appl.* 38: 114-127.

52. Gilbert, R.G. and R.G. Linderman. 1971. Increased activity of soil micro-organisms near sclerotia of *Sclerotium rolfsii* in soil. *Can. J. Microbiol.* 17: 557-562.

53. Green, R.J., Jr. 1980. Soil factors affecting survival of sclerotia of *Verticillium dahliae. Phytopathology* 70: 353-355.

54. Greig, B.J.W. 1975. Biological control of *Fomes annosum* by *Peniophora gigantea. Eur. J. For. Pathol.* 6: 65-71.

55. Harman, G.E., I. Chet and R. Baker. 1980. *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *Pythium spp.* or *Rhizoctonia solani. Phytopathology* 70: 1167-1172.

56. Harman, G.E., I. Chet and R. Baker. 1981. Factors affecting *Trichoderma hamatum* as a biocontrol agent. *Phytopathology* 71: 569-572.

57. Henis, Y., A. Ghaffar and R. Baker. 1978. Integrated control of *Rhizoctonia solani* damping off of radish: Effect of successive plantings, PCNB and *Trichoderma harzianum* on the pathogen and disease. *Phytopathology* 68: 900-907.

58. Herr, L.J. 1976. In field survival of *Rhizoctonia solani* in soil and in diseased sugar beets. *Can. J. Microbiol.* 22: 983-988.

59. Hoes, J.A. and H.C. Huang. 1975. *Sclerotinia sclerotiorum:* Viability and separation of sclerotia from soil. *Phytopathology* 65: 1431-1432.

60. Howell, C.R. 1982. Effect of *Gliocladium virens* on *Pythium ultimum, Rhizoctonia solani* and damping off of cotton seedlings. *Phytopathology* 72: 496-498.

61. Huang, H.C. 1978. *Gliocladium catenulatum:* hyperparasite of *Sclerotinia sclerotiorum* and *Fusarium* species. *Can. J. Bot.* 56: 2243-2246.

62. Huang, H.C. 1980. Control of sclerotinia wilt of sunflower by hyperparasites. Can. J. Plant Pathol. 2: 26-32.

63. Huang, H.C. 1981. Distribution of *Coniothyrium minitans* in Manitoba sunflower fields. Can. J. Plant Pathol. 3: 219-222.

64. Huang, H.C. and J.A. Hoes. 1976. Penetration and infection of *Sclerotinia sclerotiorum* by *Coniothyrium minitans*. Can. J. Bot. 54: 406-410.

65. Hubbard, J.P., G.E. Harman, and C.J. Eckenrode. 1981. Biocontrol of seed attacking fungi and insects. New York's Food and Life Sciences Quarterly. 13: 15-17.

66. Imolehin, E.D. and R.G. Grogan. 1980. Factors affecting survival of sclerotia, and effects of inoculum density, relative position and distance of sclerotia from the host on infection of lettuce by *Sclerotinia minor*. Phytopathology 70: 1162-1167.

67. Johnson, L.F. and E.A. Curl. 1972. Methods for Research on the Ecology of Soil-Borne Plant Pathogens. Burgess Publishing Co. Minneapolis. 247 pp.

68. Kerr, A. 1980. Biological control of crown gall through production of agrocin 84. Plant Disease 64: 24-30.

69. Keyworth, W.G. and L.J.R. Milne. 1969. Induced tolerance of *Sclerotium cepivorum* to antibiotics in the presence of onion exudates. Ann. Appl. Biol. 63: 415-424.

70. King, J.E. and J.R. Coley-Smith. 1969. Production of volatile alkyl sulphides by microbial degradation of synthetic allin and allin-like compounds, in relation to germination of sclerotia of *Sclerotium cepivorum* Berk. Ann. Appl. Biol. 64: 303-314.

71. King, J.E. and J.R. Coley-Smith. 1969. Suppression of sclerotium germination in *Sclerotium cepivorum* Berk. by water expressed from four soils. Soil Biol. Biochem. 1: 83-87.

72. Kommedahl, T. and I-pin Chang Mew. 1975. Biocontrol of corn root infection in the field by seed treatment with antagonists. Phytopathology 65: 296-300.

73. Kommedahl, T. and C. E. Windels. 1978. Evaluation of biological seed treatment for controlling root diseases of pea. Phytopathology 68: 1087-1095.

74. Kommedahl, T. and C. E. Windels. 1981. Introduction of microbial antagonists to specific courts of infection:

Seeds, seedlings and wounds. Pages 227-248 in G.C. Papavizas Biological Control in Crop Production. Allanheld, Osmun and Co. New Jersey. 450 pp.

75. Linderman, R.G. and R.G. Gilbert. 1973. Behavior of Sclerotium rolfsii produced in soil or in culture regarding germination stimulation by volatiles, fungistasis and sodium hypochlorite treatment. Phytopathology 63:359 362.

76. Littley, E.R. 1982. Specific tolerance of S. cepivorum to dicarboximide fungicides. Can.J.Plant Pathol. 4:307(Abstract).

77. Makkonen, R. and O. Pohjakallio. 1960. On the parasites attacking the sclerotia of some fungi pathogenic to higher plants and on the resistance of these sclerotia to their parasites. Acta. Agric. Scand. 9: 105-126.

78. Marx, D.H. 1975. The role of ectomycorrhizae in the protection of pine from root infection by Phytopthora cinnamomi. Pages 112-115 in G.W. Bruehl ed. Biological and Control of Soil-Borne Plant Pathogens. Amer. Phytopathol. Soc., St. Paul, Minnesota.

79. McCain, A.H. 1967. Quantitative recovery of sclerotia of Sclerotium cepivorum from field soil. Phytopathology 57: 1007.

80. Merriman, P.R. 1976. Survival of sclerotia of Sclerotinia sclerotiorum in soil. Soil Biol. Biochem. 8: 385-389.

81. Merriman, P.R. and S. Isaacs. 1978. Evaluation of onions as a trap crop for Sclerotium cepivorum. Soil Biol. Biochem. 10: 339-340.

82. Merriman, P.R., S. Isaacs, R.R. MacGregor and G.B. Towers. 1980. Control of white rot in dry bulb onions with artificial onion oil. Ann. Appl. Biol. 96: 163-168.

83. Merriman, P.R., R.D. Price and K.F. Baker. 1974. The effect of inoculation of seed with antagonists of Rhizoctonia solani on the growth of wheat. Aust. J. Agric. Res. 25: 213-218.

84. Merriman, P.R., I.M. Samson and B. Schippers. 1981. Stimulation of germination of sclerotia of Sclerotium cepivorum at different depths in soil by artificial onion oil. Neth. J. Plant Pathol. 87: 49-57.

85. Moody, A.R. and D. Gindrat. 1977. Biological control of cucumber black root rot by Gliocladium roseum. Phytopathology 67: 1159-1162.

86. Moubasher, A.H., M.A. Elnaghy and S.E. Megala. 1970. Fungi isolated from sclerotia of _Sclerotium cepivorum_ and from soil and their effects upon the pathogen. _Plant and Soil_ 33: 305-312.

87. Nair, N.G. and P.C. Fahy. 1976. Commercial applications of biological control of mushroom bacterial blotch. _Aust. J. Agric. Res._ 27: 417-422.

88. Ormrod, D.J. and J.R. Conroy. 1970. First record of white rot of onion in coastal British Columbia. _Can. Plant Dis. Surv._ 50: 110-111.

89. Papavizas, G.C. 1972. Isolation and enumeration of propagules of _Sclerotium cepivorum_ from soil. _Phytopathology_ 62: 545-549.

90. Papavizas, G.C. 1977. Some factors affecting survival of sclerotia of _Macrophomina phaseolina_ in soil. _Soil Biol. Biochem._ 9: 337-341.

91. Papavizas, G.C. 1977. Survival of sclerotia of _Macrophomina phaseolina_ and _Sclerotium cepivorum_ after drying and wetting treatments. _Soil Biol. Biochem._ 9: 343-348.

92. Papavizas, G.C. and R.D. Lumsden. 1980. Biological control of soil borne fungal propagules. _Ann. Rev. Phytopathol._ 18: 389-413.

93. Porter, C. L.. 1924. Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. _Amer. J. Bot._ 11: 168-187.

94. Pullman, G.S. and J.E. DeVay. 1981. Effect of soil flooding and paddy rice culture on the survival of _Verticillium dahliae_ and incidence of Verticillium wilt in cotton. _Phytopathology_ 71: 1285-1289.

95. Rai, J.N. and V.C. Saxena. 1975. Sclerotial mycoflora and its role in the natural biclogical control of white rot disease. _Plant and Soil_ 43: 509-513.

96. Rishbeth, J. 1963. Stump protection against _Fomes annosus_ III inoculation with _Peniophora gigantea_. _Ann. Appl. Biol._ 52: 63-77.

97. Rushdi, M., M.N. Shatla, A. Abd-El-Razik, F.A. Darwish, A. Ali and E. El-Yamani. 1974. Effect of cultural practices and fungicides on control of white rot disease of onion. _Zeitschrift fur Pflanzenkrankheinten und Pflanzenschutz_ 81: 337-340.

98. Scott, M.R. 1956. Studies on the biology of _Sclerotium_

cepivorum Berk. I. Growth of the mycelium in soil. Ann. Appl. Biol. 44: 576-583.

99. Scott, M.R. 1956. Studies on the biology of Sclerotium cepivorum Berk. II. The spread of white rot from plant to plant. Ann. Appl. Biol. 44: 584-589.

100. Sheridan, J.J. 1973. The survival of Mycospharella pinoides on pea haulm buried in soil. Ann. Appl. Biol. 75: 195-203.

101. Short, G.E., T.D. Wyllie and P.R. Bristow. 1980. Survival of Macrophomina phaseolina in soil and in residue of soybean. Phytopathology 70: 13-17.

102. Sivasithamparam, K. and C.A. Parker. 1978. Effects of certain isolates of bacteria and actinomycetes on Gaeumannomyces graminis var tritici and Take all of wheat. Aust. J. Bot. 26: 773-782.

103. Smith, A.M. 1972. Drying and wetting sclerotia promotes biological control of Sclerotium rolfsii Sacc. Soil Biol. Biochem. 4: 119-123.

104. Smith, A.M. 1972. Nutrient leakage promotes biological control of dried sclerotia of Sclerotium rolfsii Sacc. Soil Biol. Biochem. 4: 125-129.

105. Smith, A.M. 1972. Biological control of fungal sclerotia in soil. Soil Biol. Biochem. 4: 131-134.

106. Suslow, T.V., M.N. Schroth, and M. Isaka. 1982. Application of a rapid method for gram differentiation of plant pathogenic and saphrophytic bacteria without staining. Phytopathology 72: 917-918.

107. Swinburne, T.R. 1978. The potential valve of bacterial antagonists for the control of apple canker. Ann. Appl. Biol. 89: 94-95.

108. Takashi, N. and U. Tadao. 1978. Biological and morphological characteristics of the sclerotia of Rhizoctonia solani Kuhn. produced in soil. Soil Biol. Biochem. 10: 471-478.

109. Tronsmo, A. and J. Ystaas. 1980. Biological control of Botrytis cinerea on apple. Plant Disease 64: 1009

110. Trutman, P., P. J. Keane, and P. R. Merriman. 1980. Reduction of sclerotial inoculum of Sclerotinia sclerotiorum with Coniothyrium minitans. Soil Biol. Biochem. 12: 461-465.

111. Trutmann, P., P. J. Keane and P. R. Merriman. 1982.

Biological control of *Sclerotinia sclerotiorum* on aerial parts of plants by the hyperparasite *Coniothyrium minitans*. *Trans. Brit. Mycol. Soc.* 78: 521-529.

112. Tu, J.C. 1980. *Gliocladium virens*, a destructive mycoparasite of *Sclerotinia sclerotiorum*. *Phytopathology* 70: 670-674.

113. Turner, G.J. and H.T. Tribe. 1975. Preliminary field plot trials on biological control of *Sclerotinia trifoliorum* by *Coniothyrium minitans*. *Plant Pathol.* 24: 109-113.

114. Utkhede, R.S. and J.E. Rahe. 1978. Screening commercial onion cultivars for resistance to white rot. *Phytopathology* 68: 1080-1083.

115. Utkhede, R.S., J.E. Rahe and D.J. Ormrod. 1978. Occurrence of *Sclerotium cepivorum* sclerotia in commercial onion farm soils in relation to disease development. *Plant Dis. Reptr.* 62: 1030-1034.

116. Utkhede, R.S. and J.E. Rahe. 1979. Evaluation of chemical fungicides for control of onion white rot. *Pestic. Sci.* 10: 414-418.

117. Utkhede, R.S. and J.E. Rahe. 1979. Wet-sieving floatation technique for isolation of sclerotia of *Sclerotium cepivorum* from muck soil. *Phytopathology* 69: 295-297.

118. Utkhede, R.S. and J.E. Rahe. 1980. Biological control of onion white rot. *Soil Biol. Biochem.* 12: 101-104.

119. Utkhede, R.S. and J.E. Rahe. 1982. Treatment of muck soil with onion oil to control onion white rot. *Can. J. Plant Pathol.* 4: 79-80.

120. Walker, J.C. 1969. *Plant Pathology* McGraw-Hill Book Co., New York. 819 pp.

121. Watson, R.D. 1960. Soil washing improves the value of soil dilution and the plate count method of estimating populations of soil fungi. *Phytopathology* 50: 792-794.

122. Wells, M.D., D.K. Bell and C.A. Jaworski. 1972. Efficacy of *Trichoderma harzianum* as a biocontrol for *Sclerotium rolfsii*. *Phytopathology* 62: 442-447.

123. Willetts, H.J. 1971. The survival of fungal sclerotia under adverse environmental conditions. *Biol. Rev.* 46: 387-407.

124. Wood, R.K.S. and M. Tveit. 1955. Control of plant diseases by use of antagonistic organisms. *Bot. Rev.* 21: 441-537.

125. Wright, J.M. 1955. The production of antibiotics in soil. II Production of *Griseofulvin* by *Penicillium nigricans*. *Ann. Appl. Biol.* 43: 288-296.