EFFECTS OF *BRASSICA JUNCEA*, GERMINATION STIMULANTS - DIALLYL DISULPHIDE AND DIPROPYL DISULPHIDE, AND WARM-SEASON FLOODING, ON SURVIVAL OF SCLEROTIA OF *SCLEROTIUM CEPIVORUM* IN B.C

by

Judith Terry
B.Sc. Biology, 1993, Simon Fraser University, B.C., Canada

THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF PEST MANAGEMENT
in the Department
of
Biological Sciences

SIMON FRASER UNIVERSITY
August 1996

APPROVAL

Name: JUDITH A. TERRY

Degree: Master of Pest Management

Title of Thesis:
EFFECTS OF *BRASSICA JUNCEA*, GERMINATION STIMULANTS, DIALLYL DISULPHIDE AND DIPROPYL DISULPHIDE AND WARM-SEASON FLOODING, ON SURVIVAL OF SCLEROTIA OF *SCLEROTIUM CEPIVORUM* IN B.C.

Examining Committee:

Chair: Dr. A. Plant, Assistant Professor

Dr. J. Rahe, Professor, Senior Supervisor
Department of Biological Sciences, SFU

Mr. D. Ormrod, Extension Plant Pathologist
British Columbia Ministry of Agriculture, Fisheries and Food

Dr. Z. Punja, Associate. Professor
Department of Biological Sciences, SFU

Dr. R. Utkhede, Adjunct Professor
Department of Biological Sciences, SFU
Public Examiner

Date Approved: August 12, 1996

Title of Thesis/Project/Extended Essay

EFFECTS OF BRASSICA JUNCEA, GERMINATION STIMULANTS - DIALLYL DISULPHIDE AND DIPROPYL DISULPHIDE, AND WARM-SEASON FLOODING, ON SURVIVAL OF SCLEROTIA OF SCLEROTIUM CEPIVORUM IN B.C.

Author:

(signature)

Judith Terry

(name)

15 August, 1996

(date)

ABSTRACT

The potential of *Brassica juncea* as an amended cover crop, two germination stimulants (diallyl disulphide, DADS and dipropyl disulphide, DPDS) and warm-season flooding, to reduce the survival of sclerotia of *Sclerotium cepivorum* in onion-growing areas of B.C. was investigated.

The effect of *B. juncea* was studied as a spring and fall amended cover crop on muck soils under field conditions in Cloverdale, B.C. in 1994. In the spring amendment, the treatments were *B. juncea* cv. Cutlass, natural weed growth, and bare soil, which were replicated thrice in a complete randomized design. Survival of introduced sclerotia was assessed 2 months after treatments were initiated. There was no significant difference (P=0.05) between treatments in the decay of sclerotia. In the fall trial, 12 L pots containing muck soil from Cloverdale, B.C. were seeded with *B. juncea* line 94-20726J2 or unamended and kept weed-free. There were four replications of each treatment. Survival of introduced sclerotia was assessed 5 weeks after amendment. There was no significant difference (P=0.05) between treatments in the decay of sclerotia.

Allylisothiocynate (AICT), a compound released during the decomposition of *B. juncea*, was also evaluated for its effect on sclerotia of *S. cepivorum*. Low concentrations (0.05 uL) stimulated germination, while concentrations of 0.4 uL were

not different from the control (water and Tween 20).

The effects of DADS and DPDS was evaluated under field conditions in 1995 in Oliver, B.C. Fall application to sandy soil resulted in a significant (P=0.0002) decline in the number of intact indigenous sclerotia in the DADS treatment compared to untreated controls. Similarly, spring application of DADS to muck soil in Cloverdale, B.C significantly reduced the number of intact, introduced sclerotia. However, DPDS did not significantly affect the population of introduced sclerotia in either the spring or fall application.

The effect of warm-season flooding on survival of introduced sclerotia in muck soils was assessed in pots. The average number of viable sclerotia was significantly (P=0.05) reduced by 52 % and 94 % compared to the control after 6 and 12 weeks, respectively.

The results from this research indicate that the use of DADS and warm-season flooding can reduce the survival of sclerotia of *Sclerotium cepivorum* in onion-growing areas of B.C.

DEDICATION

To my loving husband, Charles Terry

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1 Distribution of the Pathogen

White rot is one of the most important and destructive fungal diseases specific to *Allium* species such as onion (*A. cepa*), garlic (*A. sativum*), chives (*A. schoenoprasum*), and leeks (*A. ampeloprasum*). The disease was first discovered in England by Rev. M.J. Berkely in 1841 and has since been reported worldwide. Areas affected by this disease include the United Kingdom, Holland, Australia, United States, and Canada (Crowe, 1994b). In the United States, white rot has been reported from major and minor *Allium* production areas in the states of Washington, Idaho, Oregon, Nevada, Montana, and California (except for the hot, desert areas). The pathogen has not, however, been reported from Arizona, Colorado, or Utah (Crowe, 1994a). The pathogen affects *Allium* production wherever susceptible host plants are grown during a cool season, providing conducive temperatures for growth and reproduction of the pathogen. Under these conditions, white rot can become the major limiting factor for continued commercial production of *Allium* crops (Crowe, 1994b).

1.2 Economic losses

In 1980, the total areas of land affected by white rot in Australia, the Netherlands and the United Kingdom were estimated to be 5300, 3400, and 700 ha, respectively (Merriman et al, 1980). Currently in the United Kingdom, the number of fields infested with the white rot pathogen continues to increase, especially in established onion-growing areas.

Growers of dry bulb onions are having increasing difficulty finding land which is free of inoculum, and several farmers have stopped growing the crop due to the disease (Davies, 1994).

The pathogen was first recorded in the commercial onion-growing area of the Lower Fraser Valley of British Columbia in 1970 (Ormrod and Conroy, 1970). By 1974, the pathogen had spread throughout most of the Fraser Valley onion-growing areas (Ormrod et al, 1977). In an attempt to restrict the spread of the pathogen, regulations of the Provincial Plant Protection Act were introduced in 1972 to prohibit onion production on known infested fields, but new infestations continued to appear and the regulations were later rescinded.

1.3 Chemical Control

Two fungicides, iprodione (Rovral^{R}) and dichloran (Botran^{R}) are registered for use against white rot on onions in Canada, but neither provides satisfactory control. The dicarboximide fungicide, iprodione, has significantly reduced white rot in field trials in the Fraser Valley (Utkhede and Rahe, 1979). However, iprodione cannot be relied upon in the long term for managment of white rot because there is evidence that the pathogen can develop resistance to this group of fungicides (Littley and Rahe, 1984), and there is a loss of efficacy due to enhanced microbial degradation in the soil (Walker *et al*, 1986). The use of dichloran to control white

rot is economically and environmentally questionable (D. Ormrod, personal communication, BCMAFF). Currently the only viable management option for this disease has been to avoid growing onions in known infested areas.

1.4 The Pathogen

The causal agent of white rot is *Sclerotium cepivorum* Berk., (subdivision Deuteromycetes; class Mycelia Sterilia; order Myceliales) a fungus with ascomycetous affinities, but no described perfect stage. In nature, its pathogenic activities are restricted to *Allium* species (Coley-Smith, 1959). The fungus has no known functional spores, but does produce microconidia which have never been seen to germinate, and their function remains unknown (Coley-Smith, 1960). The only reproductive structures formed are sclerotia, which are usually uniformly round and black, with diameters between 0.35-0.50 mm. They have a characteristic rubbery texture when squeezed gently with fine pointed forceps. *Botrytis cinerea* sclerotia can be similar in size, shape, and color, but are more usually 0.25 to 1.0 cm in length and irregular in shape. In the absence of a host plant, *S. cepivorum* persists in the soil as these sclerotia. Mature sclerotia possess an outer rind of one to two layers of thickened, heavily pigmented rounded cells which enclose a large medullary region comprised of closely-packed elongated hyphae (Coley-Smith, 1960). Mature sclerotia from infected plants in the field are black

and firm, but immature and soft sclerotia with no or an imperfectly-formed rind also are found. Entwistle (1994) observed that the rind of immature sclerotia may continue to develop after their removal from the host, and hence their capacity for survival may be substantial. Softness of sclerotia can also indicate the early stages of germination or decay.

1.5 Survival in Soil

Sclerotia of *S. cepivorum* have been known to persist for 10 years with little loss of viability (Crowe *et al*, 1980). Recently, Crowe (1994a) reported large disease losses in an onion crop due to *S. cepivorum* in a field near Tulelake, California that had not been planted to onions for at least 40 years. Onions were grown in 1950 or 1951 and one of the first incidences of white rot in the Tulelake Basin occurred then.

In contrast, sclerotia in muck soils in the Fraser Valley of British Columbia decay rapidly due to a combination of environmental conditions (flooding) and increased microbial activity (Leggett and Rahe, 1985).

1.6 Sclerotial Germination

Sclerotia of *S. cepivorum* are subject to the phenomenon of soil mycostasis (Dobbs and Hinson, 1953). Sclerotia are constitutively dormant for 1-3 months after formation, and then can be stimulated to germinate in non-sterile soil by

Allium root exudates (Coley-Smith, 1960). Roots of *Allium* spp. exude small quantities of alkyl cysteine sulphoxides, which are thermostable, water soluble, and diffusible compounds. These compounds are metabolized by bacteria in the soil to produce a stimulatory mixture of volatile alkyl sulphides (King and Coley-Smith, 1969). Six common soil bacteria were shown to be capable of degrading synthetic alkyl cysteine sulphoxides, resulting in the evolution of the corresponding volatile alkyl sulphides (King and Coley-Smith, 1969). Thus, the soil microflora appear to play an important role in the induction of sclerotial germination of *S cepivorum*.

Three factors are reported to influence the germination of sclerotia of *S. cepivorum*: host plant exudates, biotic soil factors, and abiotic soil factors. The importance of the soil microflora is not limited to converting non-volatile compounds to volatile stimulants, however. In the absence of volatile stimulants, sclerotia of *S. cepivorum* do not normally germinate in non-sterile soil, while they do germinate on sterile soil, agar, and silica gels (Coley-Smith *et al*, 1967). This suggests that components of the soil microflora may also play an important role in the dormancy of sclerotia. Sclerotial germination may also be affected, directly or indirectly, by many other groups of organisms. These organisms have shown potential as biological control agents of white rot, either through antagonism to mycelial growth or via

parasitism of the sclerotia (Utkhede and Rahe, 1980; Leggett, 1983; Ayers and Adams, 1979)

Abiotic soil conditions such as temperature, moisture, pH, and soil structure also may play an important role in the germination process. Tan (1982) found that germination of sclerotia of *S. cepivorum* in mineral soil at 28% moisture content (-285 mbar) was greatest between 14 and 17 C, after 15 days of incubation with a germination stimulant. No germination was observed at 10 C. These observations are in agreement with the temperature response reported by Crowe and Hall (1980b).

The most active volatile compounds in bulb tissue extracts appear to be n-propyl and allyl sulphides (Coley-Smith and King, 1969). It is interesting that sclerotia show a low response to methyl compounds but a high response to n-propyl and allyl sulphides. Methyl sulphides are produced by a wide range of plants, whereas n-propyl and allyl sulphides are characteristic compounds of *Allium* species, but are also found in small quantities in members of the Cruciferae (Coley-Smith and Cooke, 1971).

The mechanism by which mycostasis is broken in not known, although the specific response of the sclerotia suggests that volatile compounds in some way remove the restraint on the metabolic pathway responsible for the initiation of germination (Coley-Smith and Cooke, 1971).

1.7 Infection of the host

The sclerotia germinate in soil by producing a plug of mycelium which erupts through the sclerotial rind. Hyphae can grow out from the plug for distances of several millimetres and then anastomose (Coley-Smith, 1960). Stimulated germination is extremely slow below 9 C, is most rapid between 14-18 C, and terminates abruptly near 24 C. Sclerotial germination also proceeds most rapidly at soil moisture levels of -300 mbar (Crowe and Hall, 1980b).

The roots and leaf sheaths of the host are penetrated directly and the invading hyphae advance inter- and intracellularly through underground host tissue. The first symptoms of infection are yellowing and dying of the outer leaves of the plant, beginning at the tips and progressing downwards. Roots and bases of scales are attacked and the fungus becomes evident on the infected bulbs as superficial, white fluffy mycelium (Figure 1). The mycelium later produces sclerotia at the base of the plant. Crowe and Hall (1980a) found that symptoms were not evident until the pathogen had reached and partially rotted the stem plate or leaf sheaths. At infections 2-4 cm or more below the stem plate, extensive plant-to-plant spread of mycelium occurred. The pathogen spread among roots of the same and neighbouring plants as the mycelium grew along roots, radiated from active sites of colonization and infected other roots up to 1-2 cm away.

FIGURE 1: Onion bulbs showing symptoms of white rot.

1.8 Antagonists of *Sclerotium cepivorum*

Utkhede and Rahe (1980) isolated six bacteria (all *Bacillus subtilis*) and a fungus *(Penicillium nigricans)* from sclerotia recovered from naturally-infested field soils that were antagonistic to mycelial growth of *S. cepivorum*. The *B. subtilis* isolates were evaluated in replicated pot and field trials, and some showed significant suppression of white rot development when applied as seed treatments. The *P. nigricans* isolate was strongly inhibitory to growth of *S. cepivorum* in dual culture tests, but it did not significantly reduce white rot in a field test when applied as a massive spore dose to seeds.

In an evaluation of fungi from bulk and rhizosphere soils collected from the Fraser Valley of British Columbia, Leggett (1983) found that 184 of 310 fungal isolates in dual culture tests exhibited some type of antagonism against mycelial growth of *S. cepivorum*. These isolates included *P. nigricans*, five other *Penicillium* species, *Trichoderma harzianum, T. hamatum,* and two additional *Trichoderma* species, *Gliocladium* species, *Fusarium* species, *Cladosporium* species, and two *Ulocladium* species. However, none of these isolates significantly reduced disease when evaluated in field trials.

1.9 Biological Control

Two sclerotial parasites, *Coniothyrium minitans* and *Sporidesmium sclerotivorum* (Ayers and Adams, 1979) have potential for biological control of white rot. Huang (1980) and Trutman *et al* (1980) found that *C. minitans* reduced numbers of sclerotia of *Sclerotinia sclerotiorum* in the field, and in greenhouse trials it controlled *S. cepivorum* (Ahmed and Tribe, 1977). Leggett (1983) found that *C. minitans* was able to parasitize sclerotia of *S. cepivorum* in the laboratory but was not able to significantly reduce the population of sclerotia in the field. Leggett suggested that *C. minitans* may have been unable to compete with other micro-organisms present in the soil or that the temperature and moisture conditions may have been unfavourable.

1.10 Germination Stimulants for Disease Control

The specific interaction between sclerotia of *S. cepivorum* and alkyl-cysteine sulphoxides or their breakdown products suggests a possible use of germination stimulants for control of white rot. If germination of sclerotia was stimulated in the absence of host tissue, the resultant hyphal growth could be lysed and become vulnerable to various forms of microbial antagonism that would reduce the inoculum potential of the pathogen (Gilbert and Griebel, 1969). To date, three compounds have been evaluated as germination stimulants of *S. cepivorum*.

The use of artificial onion oil was evaluated by Merriman *et al* (1980) and Utkhede and Rahe (1982) and partial disease control was obtained. Merriman *et al* reported that at one site in Australia, the injection of onion oil reduced the number of sclerotia in the 0-20 cm soil profile, which resulted in a reduced incidence of white rot. At another test site, onion oil reduced sclerotial numbers by 50% but there was no reduction in disease incidence. The differences between sites may have been due to differences in soil structure and absorption capacity, and to a higher population of sclerotia at the second site. Alternatively, differences in the composition of the soil microflora at the two sites may have been involved. The level of white rot is not necessarily proportional to the population of sclerotia of *S. cepivorum* in the soil (Utkhede *et al,* 1978)

Utkhede and Rahe (1982) reported that the percent infection of onions in a muck soil near Cloverdale, B.C. was significantly reduced in plots treated with onion oil. The numbers of sclerotia in samples from treated and untreated plots were not significantly different prior to application of onion oil, but were significantly lower in samples one month after treatment compared to untreated plots.

An allyl compound, diallyl disulphide (DADS), which occurs naturally in garlic but not in onion, was significantly more effective than onion oil in stimulating sclerotial germination (Coley-Smith and King, 1969). Germination of

sclerotia of *S. cepivorum* in bags injected with DADS was 91-96%. Coley-Smith and Parfitt (1986) also reported a marked effect of the concentration of DADS used, and in field tests done in the United Kingdom, there were seasonal differences in the effect of DADS. Summer treatments (June, July and August) resulted in poor germination compared to applications in the spring and fall. This seasonal difference in germination is believed to be related to the rate of breakdown of DADS from the soil, which in turn is related to the soil temperature (Coley-Smith and Parfitt, 1986).

The third compound reported to stimulate germination of sclerotia is allylisothiocyanate, AITC (Coley-Smith and Parfitt, 1983). When applied to soil, AITC stimulated up to 66% germination compared to 3% in the control. Interestingly, AITC is not a constituent of *Allium* species but is found in certain crucifers. Studies have shown that during the decomposition of crucifers in soil, volatile compounds with phytotoxic as well as mycotoxic properties are released (Lewis and Papavizas, 1970a). Cruciferous amendments, such as leaves and stems of cabbage, kale, and mustard have been shown to control root rot of peas caused by the soil-borne phycomycete *Aphanomyces euteiches* (Lewis and Papavizas, 1970b). The same types of amendments have also been shown to suppress root rot of bean caused by *Thielaviopsis basicola* (Papavizas, 1968). In solarized soil amended with dried, ground cabbage residue, the viability of *Pythium ultium* and *Sclerotium rolfsii* was

reduced when the soil was heated in a controlled environment system siulating a diurnal solarization temperature curve at a sublethal maximum of 38 C (Gamliel and Stapleton, 1993).

In a preliminary study, Joshi (1988) found that sclerotia of *S. cepivorum* decayed more rapidly in plots seeded with *Brassica juncea*, a mustard that has a high AITC content (Wallbank and Wheatly, 1976). This effect was observed for the indigenous soil population and for two introduced populations of sclerotia. It was not clear, however, whether the observed decay of sclerotia was also due to natural flooding that occurred in the winter months in the study plots, or due to the presence of organic matter which may have increased microbial activity or accelerated oxygen consumption in the soil, or to the specific stimulatory action of volatiles from *B. juncea*, or some combination of these factors.

In coastal B.C, onion white rot is the most serious disease of onions and threatens to make onion production uneconomical. White rot has already been a major contributor to the decline of onion production in the B.C Interior. The onion crop is worth more than $2 million annually to the B.C. growers and is also a valuable disease-reducing rotation crop for other vegetables such as carrots and celery.

The objectives of this research were to examine the effects of (i) a cruciferous cover crop (*B. juncea*) in non-flooded soil, (ii) two germination stimulants (diallyl disulphide, and dipropyl disulphide), and (iii) warm season flooding, on the survival of sclerotia of *S. cepivorum*.

CHAPTER II: EVALUATION OF *BRASSICA JUNCEA* AS AN AMENDED COVER CROP TO STIMULATE SCLEROTIAL GERMINATION.

2.1 Introduction

The agronomic practice of cover cropping is used primarily to prevent erosion and to incorporate organic matter into the soil. Cover crops can also improve soil structure, enhance soil fertility and suppress pests such as weeds, insects and pathogens (Lal *et al*, 1991). Most cover crops are grown during the cold season in northern latitudes and during the dry season in tropical climates. They can be managed as living mulches, which remain wholly or partly alive during the row crop's growing season, or as dead mulches that are chemically or mechanically killed and usually amended into the soil before planting the 'cash' crop (Lal *et al*, 1991).

Experimental work devoted to crop residues and soil amendments in relation to soil-borne plant pathogens has shown that the direct or indirect use of amendments may give rise to some biological or cultural control of root-infecting fungi (Papavizas, 1973). With the increasing public concern over pesticides, the use of cover crops may become more widespread, thus increasing the importance of studying plant tissue amendments in relation to disease reduction. One promising strategy for soil-borne plant pathogens is to trigger germination of dormant propagules in the absence of a host crop. The resultant vegetative growth will then be followed by lysis of hyphae, microbial antagonism or other processes

that reduce the 'inoculum potential' of the pathogen (Gilbert and Griebel, 1969). Inoculum potential as described by Garret (1970) is defined as "the energy of growth of a parasite per unit area of host surface available for infection of a host," at the surface of the host to be infected.

Many factors may be involved in the suppression of plant pathogens by cover crops and their residues. These include increased soil fungistasis (Adams and Papavizas, 1969) and microbial activity (Papavizas and Adams, 1969), production of mycotoxic volatiles from decomposing amendments (Lewis and Papavizas, 1970a), stimulation of fungal propagules in the absence of the host followed by subsequent lysis before formation of secondary propagules occurs (Adams and Papavizas, 1969), and the strategy of 'trap' cropping (Harling and Kennedy, 1991). Trap crops are used to induce resting spore germination in advance of a 'cash' crop. Data from field and glasshouse experiments have shown that 'trap' cropping with oilseed rape seedlings may reduce the incidence of club root (*Plasmodiophora brassicea)* and increase yield in calabrese, a susceptible *Brassica* species (Harling and Kennedy, 1991).

Cruciferous amendments, such as cabbage, kale, collards, Brussel sprouts, kohlrabi, mustards, turnips and cress, appear to have an adverse effect on *Aphanomyces euteiches* (Lewis and Papavizas, 1970b) and *Thielaviopsis basicola* (Papavizas, 1967). Crucifers contain an abundance of sulphur-containing substances. The sulphur-containing volatiles released from

cabbage during decomposition include mercaptans such as methylmercaptan (CH_3SH), dimethyl sulfide ($[CH_3]_2S$), dimethyl disulfide ($[CH_3]_2S_2$), and isothiocyanates (Lewis and Papavizas, 1970; Tollsten and Bergstrom, 1988). Another characteristic of the Cruciferae family are glucosinolates, which are sulphur-containing glucosides found in all parts of the plant. Glucosinolates, when hydrolysed by an enzyme present in *Brassica* seeds and vegetative tissues, yield oxazolidinethiones, nitriles, thiocyanates, and fungicidal and bactericidal isothiocyanates (Tollsten and Bergstrom, 1988).

Allylisothiocyanate has been reported to stimulate germination of sclerotia of *S. cepivorum* in non-sterile soil (Coley-Smith and Parfitt, 1983). This compound is released by three species of mustards, *Brassica nigra, B. carinata, and B. juncea*, during tissue decomposition (Tollsten and Berstrom, 1988)

The purpose of this experiment was to evaluate the effect of *B. juncea* as a spring- and fall-amended covercrop on the survival of sclerotia of *S. cepivorum* in field soil in the Fraser Valley, B.C.

2.2 Materials and Methods:

2.2.1 Spring amendment field trial

The study site was situated in a mucksoil vegetable growing area near Cloverdale, B.C. Soils in this area are characterized by organic matter from 50 to 80% and soil pH vales of 4.8-6.5. The site was 6m X 30m in size and was divided into nine plots, each measuring 1.3m X 5m. Three treatments were used: *B. juncea*, natural weed growth and bare soil. Each treatment was replicated three times in a complete randomized design. The soil was rotovated and shaped into beds. Seeds of *B. juncea* cv. Cutlass (provided by Paul Garvin, Garvin Farms, Cloverdale), were handseeded on April 22, 1994 at 17 g/plot and the seed was raked in. On control plots, weeds were either allowed to grow or controlled by hand-weeding and use of glyphosate (Round-up). Glyphosate was applied on 11 May and 17 July, 1994 at a rate of 50 ml to 2.5 l of water.

After 2 months (20 June, 1994), plant height was measured, and three random quadrats (.3 m X .3 m) were sampled in each plot to estimate plant biomass. In each quadrat, whole plants (root and shoots) were removed, washed, and weighed. After biomass measurements were taken, all *B. juncea* plants and weeds were mowed to a height of 15 cm with a weed-eater and rototilled into the soil to a depth of 20 cm.

The sclerotia used in this study were produced axenically on potato dextrose agar (PDA). The sclerotia were buried in

clay pots containing a sandy loam type soil from Aldergrove and kept outside, undercover with infrequent watering for 4 months before use. Sclerotia (in groups of 20) were placed within nylon mesh bags with 25 g of uninfested moist muck soil. The muck soil was sampled for sclerotia before use. The bags were attached to wooden stakes (three per stake) with nylon string and buried at a 6-8 cm depth in the field plots (three stakes per plot) prior to seeding of *B. juncea*. Three bags from each plot were recovered at 10 days (zero-time) after burial, and 2 months after amending with *B. juncea*. Three extra bags per plot were buried in case any were lost or missing at the end of 2 months. Sclerotia were recovered using the soil sieving-sucrose centrifugation technique described by Vimard *et al* (1986). Data (average number recovered out of twenty per plot) were analyzed using analysis of variance (P=0.05). The sclerotia were surfaced sterilized for 3 min in 1% sodium hypochlorite solution, and plated on dilute PDA (PDA:water agar 1:4, v/v) to test for viability. Viability was defined as the ability of a plated sclerotium to produce mycelium and new sclerotia on the agar medium after 14 days in a dark incubator at 16 C .

Weekly soil temperatures were measured with a soil temperature probe throughout the trial at depths of 5, 10 and 20 cm.

2.2.2 Fall amendment pot trial

The study site was situated in an outdoor compound at Simon Fraser University, Burnaby, B.C. In this trial, four pots (12 L each) were filled with muck soil (characterized by organic matter from 50 to 80% and soil pH vales of 4.8-6.5) from Cloverdale, B.C and seeded (5.5 g/pot) on 13 August, 1995 with *B. juncea*, line 94-20726JS seed supplied by Vipan Bansal, University of Alberta. Four control pots (12 L each) contained muck soil only and were kept weed-free by hand weeding.

The same batch of axenic sclerotia produced for the spring trial and stored in claypots outside with infrequent watering was used . The sclerotia (20 sclerotia/25g moist soil) were placed in nylon bags with muck soil obtained from a white rot-free area. These bags (four bags per pot) were buried to a depth of 6-8 cm in all the pots on 02 October, 1995. Ten days later, *Brassica* plant height was measured, and the plants were uprooted, washed in water, and weighed to determine total biomass. The bags of sclerotia were also removed from all pots. The *Brassica* plants, roots and shoots, were cut into 8-10 cm long pieces and 400 g fresh weight was amended to each pot comprising the treatment, along with the bags of sclerotia (Figure 2). In control pots, the bags of sclerotia were also removed, the soil was mixed and the bags were reburied.

FIGURE 2: Fall amendment of *Brassica juncea* with bags of sclerotia in muck soil.

One bag was recovered from each of the replicate pots at the time of amendment of the *Brassica* plants (zero-time) and 5 weeks after amendment. Weekly soil temperatures in the pots were taken throughout the trial at depths of 5 cm and 10 cm. Daily precipitation was measured by a weather station in the outdoor compound.

2.3 RESULTS

2.3.1 Spring amendment field trial

Percent seedling emergence and growth of *B. juncea* plants was good and average plant height at 2 months after seeding ranged from 50-85 cm. Biomass measurements showed an average of 10.3 $\pm$ 4.4 kg of fresh plant material per m2 in the *Brassica* plots. In the weedy plots, biomass was 9.7 $\pm$ 3.9 kg per m2, and the predominant weed species were *Polygonum pensylvanicum* (smartweed), *Amaranthus retroflexus* (redroot pigweed), and annual grasses. In the weeded control plots, bare soil was maintained throughout the trial. Weekly soil temperatures taken at 5, 10 and 20 cm are shown in Table 1.

Very little precipitation occurred after incorporation of the plant material. In July only 2 days had rain with a total precipitation of 16.8 mm. August was even drier with a total of 9.4 mm of precipitation that fell over 6 days. Weekly cumulative precipitation for the duration of the trial is shown in Table 1.

TABLE 1: Weekly soil temperatures at Cloverdale, British Columbia taken at 5, 10, 20 cm depth and weekly cummulative rainfall for 01 May, 1994 to 31 August, 1994.

Dates	Weekly Temp. (^{0}c)			Weekly Cummulative ppt (mm)[1]
	5 cm	10 cm	20 cm	
03/05	20.0	16.7	12.0	3.4
09/05	26.7	18.3	15.0	0.7
16/05	22.8	18.9	15.0	5.8
24/05	26.7	24.4	19.4	trace
31/05	18.3	17.2	15.0	22.4
08/06	23.3	15.0	15.0	5.6
14/06	21.1	17.8	17.2	19.5
21/06[2]	**26.7**	**18.9**	**18.3**	**18.1**
28/06	28.3	18.9	18.9	2.0
06/07	32.2	22.8	21.1	12.6
15/07	28.9	26.7	21.1	0.0
21/07	32.2	28.3	25.0	4.2
26/07	28.3	26.1	21.1	0.0
06/08	21.1	20.6	20.0	0.4
17/08	25.6	22.2	21.7	4.3
24/08	26.7	22.3	21.5	0.7
31/08	18.9	16.7	15.0	4.0

1 - Data supplied by Environment Canada, Station Cloverdale East.
2 - Mustard plants were incorporated on 21 June, 1994.

At the zero-time sampling of buried sclerotia (09 May, 1994), the average number recovered out of 20 was 16 $\pm$ 2.0 from the *B. juncea* seeded plots, 15.5 $\pm$ 1.8 from the weedy control plots, and 14.8 $\pm$ 2.8 from the weeded control plot (Table 2). These values were not significantly different from one another ($P \leq 0.05$). Viability of recovered sclerotia was 98%.

At 2 months after incorporation, the average number of viable sclerotia recovered out of 20 was 14.6 $\pm$ 2.2 from *B. juncea* seeded plot, 15.4 $\pm$ 1.5 from the weedy control plots, and 14.8 $\pm$ 1.9 from the weeded control plot. These values were not significantly different from the zero-time sampling (Table 2).

2.3.2 Fall amendment pot trial

The mean fresh weight of *Brassica* plants grown in the pots after two months was 437.5 $\pm$ 75 g (8.6 kg/m2), and the average plant height was 40 cm. Weekly soil temperatures from October to December ranged from frozen ground to 12.1 C (Table 3). Precipitation during the trial was quite high, with 448 mm in Oct, 564 mm in November and 384 mm in December (Table 3). The soil was well drained and there was no standing water in the pots.

At 'zero-time' (12 October 1995) the average number of sclerotia recovered out of 20 was 9.5 $\pm$ 2.4 for *Brassica* pots and 11.8 $\pm$ 2.5 for control pots, and at 5 weeks (15 November,

TABLE 2: Number of sclerotia of *Sclerotium cepivorum* recovered out of 20 at zero-time (10 days after burial) and 2 months after incorporation (20 August 1994) of *Brassica juncea*.

	Zero-time (9 May 1994)	2 months after incorporation (20 August 1994)
B. juncea	16.0 ± 2.0 a[1]	14.6 ± 2.2 a
Weedy Control	15.5 ± 1.8 a	15.4 ± 1.5 a
Control	14.8 ± 2.8 a	14.8 ± 1.9 a

1 - numbers followed by the same letter do not differ ($P \leq 0.05$) according to SNK Multiple Range Test.

TABLE 3: Weekly soil temperatures in the outdoor compound at Simon Fraser University, taken at 5, and 10 cm and weekly cummulative rainfall for 02 October, 1995 to 18 December, 1995.

Dates	Soil Temperature (^{0}C)		Weekly Cummulative ppt (mm)[1]
	5 cm	10 cm	
09/10	12.1	11.4	37
16/10	11.2	11.0	172
23/10	5.8	6.4	170
30/10	4.6	3.8	60
06/11	11.4	10.8	13
13/11	9.8	8.6	205
20/11	6.8	5.4	95
27/11	6.4	5.3	183
04/12	6.2	5.0	123
11/12	ground frozen		70
18/12	7.0	5.8	169

1 - Recorded at SFU.

1995) after amendment of *Brassica*, 10.5 ± 3.5 for *Brassica* pots and 10.5 ± 1.9 for control pots respectively (Table 4). *Brassica* pots and control pots were not significantly different from each other at 5 weeks. Viability of recovered sclerotia was 98%.

2.4 DISCUSSION

Under the conditions of this experiment, amendment of muck soil with *B. juncea* had no affect on survival of sclerotia of *S. cepivorum* during the period of observation in both the spring and fall trials. This finding contrasts with the report of Joshi (1988) who concluded that *B. juncea* reduced the survival of sclerotia when incorporated in the fall. That study was conducted in a muck soil which was water-saturated for much of the fall and winter months. The differences between the results of the two studies could be due to differences in moisture levels, soil temperatures, or in the amount of allylisothiocynate released from the decomposing plant matter in the soil (not measured in this study). Joshi (1988) showed that volatiles released from decomposing *B. juncea* contained a number of constituents, including a distinct peak of AITC. In this study, soil temperatures were generally above 16 C, which is optimal for germination of sclerotia of *S. cepivorum*. In the previous study by Joshi (1988), flooded pots showed a clear differentiation for sclerotial recovery between *B. juncea*

TABLE 4: Number of sclerotia of *Sclerotium cepivorum* recovered out of 20 at zero-time and 5 weeks after incorporation of *Brassica juncea*.

	Zero-time (12 October 1995)	5 weeks (16 November 1995)
B. juncea pots	9.5 ± 2.4	10.5 ± 3.5
Control pots	11.8 ± 2.5	10.5 ± 1.9

amended pots and pots amended with general vegetation (grass and chickweed). Since the soil in the present study was not flooded, it is difficult to conclude whether the differences in results are due to flooding or variable soil temperatures.

The use of *Brassica* as a soil amendment could have some drawbacks. *B. juncea* might attract certain pests, and could serve as an overwintering haven for other broad host range pests. *Brassica* spp. can be infested with turnip aphid (*Hyadaphis erysimi*), whereas cabbage aphid (*Brevicoryne brassicae*) and green peach aphid (*Myzus persicea*) attack various Brassicaceae, including mustard (*Brassica hirta*) and canola (*Brassica napus)* (Bugg, 1991). Also, growing *Brassica* could increase the population of *Plasmodiophora brassicae*, the club root pathogen, in the soil, and overwintering crucifers could increase overwintering of turnip mosaic virus, which has a broad host range and could pose a threat to other vegetables.

Another similar study by Villar *et al* (1990) reported that incorporation of cruciferous residues (cabbage, *B. oleracea* L. Capitata group, and broccoli, *B oleracea* L Italica group) at 5% w/v, to soil infested with *S. cepivorum* (four sclerotia/g of soil) resulted in a significant reduction in the number of dead plants and in the disease index (40% and 25 - 65%, respectively) as compared to the control under greenhouse conditions. The best results were obtained with the incorporation of cruciferous plants plus soil

solarization, and solarization alone which reduced the number of dead plants (93-100% and 78-83%, respectively) and the disease index (78-83% and 67-79%, respectively).

This is interesting, in that the cruciferous plant residues used release insignificant amounts of allylisothiocyanate (Wallbank and Wheatly, 1976). The major component released by *B. oleracea* was identified by gas liquid chromatography and mass spectrometer as *cis*-hex-3-enyl acetate.

Although incorporation of *B. juncea* alone into muck soil did not reduce sclerotial survival in this study, using *Brassica* residue in conjunction with flooding may have potential for white rot management and further research is needed.

CHAPTER III: EVALUATION OF ALLYLISOTHIOCYANATE AS A GERMINATION STIMULANT FOR SCLEROTIA OF *SCLEROTIUM CEPIVORUM*

3.1 INTRODUCTION

Approximately 100 different glucosinolates have been identified in plant tissue (Duncan, 1991). Enzymatic hydrolysis of glucosinolates by membrane-bound thioglucosidase produces numerous compounds, including isothiocyanates, nitriles, thiocyanates, epinitriles, and glucose. Some of the hydrolytic breakdown products have antimicrobial, fungicidal, and insecticidal properties (Chew, 1988). Allyl glucosinolate is one of the predominant glucosinolates in *B. nigra* (L) W. Koch, *B. carinata* A. Braun, and *B. juncea* (L) Czernj and Coss., and is generally converted to allyl isothiocyanate (AITC) at a pH of 4.0 or greater (Borek *et al*, 1994). Borek *et al* (1994) reported that, in soil, AITC was the predominant hydrolytic byproduct formed from allyl glucosinolate. AITC, a volatile compound, is as toxic to fungi as methyl isothiocyanate, an active ingredient in commercial soil fumigants (Vaughn *et al*, 1993)

Interestingly, allylisothiocyanate (AITC) was reported by Coley-Smith and Parfitt (1983) to stimulate germination of sclerotia of *S. cepivorum*. In laboratory soil-tube experiments 66% germination of sclerotia was obtained at a concentration of 10 mg/L of AITC .

The objective of this study was to determine if AITC applied directly to muck soils would stimulate germination of sclerotia of *S. cepivorum*.

3.2 MATERIALS AND METHODS

The sclerotia used in this study were axenically produced and stored for 1 year outdoors undercover with infrequent watering in clay pots with sandy loam type soil from Aldergrove. AITC was purchased from Aldrich Chemicals. Germination tests were conducted in 9 cm diameter petri plates. Each plate contained 60 cc (40 g) of muck soil collected from Cloverdale at 104% moisture content. Twenty sclerotia were placed on a fine nylon mesh on the soil surface. Four concentrations of AITC were used (0.4 ul, 0.2 ul, 0.1 ul and 0.05 ul) by mixing in 1.0 ml of distilled water containing 0.08 % Tween 20 (v/v). The control consisted of 1 ml of the water-Tween mix. Each treatment was replicated five times. Treatments were applied by micropipette to the soil surrounding the mesh that supported the sclerotia. After application of the treatments to the soil with a micro pipette, the plates were individually sealed in polyurethane bags. The replicate plates of each treatment were placed in individual cardboard boxes located approximately 2.5 m equidistant from each other in a well-ventilated greenhouse. The temperature in the greenhouse was recorded continuously during the experiment by a weather recorder. The number of

sclerotia that had germinated was rated under a dissecting microscope at 5 X magnification. The data collected from the plates were analyzed using analysis of variance and Student-Newman-Keuls multiple comparison tests. Any sclerotial contaminants were also noted and an attempt to isolate them was made by plating spores suspension on PDA.

3.3 RESULTS

The number of sclerotia germinating in each treatment is shown in Figure 3. Treatment effects were highly significant (P=0.0001). The multiple comparison test separated the treatments into three groups. The proportion of sclerotia germinating was highest in the 0.05 ul treatments, and least in the control and 0.4 ul treatment (Figure 3). The average daily temperature during the trial (23 January, 1995 to 06 February, 1995) was 8 C with maximum of 25 C and minimum of 0 C.

3.4 DISCUSSION

Maximal response of sclerotia was seen with the lowest concentration (0.05 ul), whereas the highest concentration (0.4 ul) did not differ from control, with 4% germination. In the field, the volume of soil (1 ha, 25 cm depth) that this compound would be applied to is 2.5×10^9 cm^3. Assuming the bulk density of muck soil is 1g/cc, then the dry weight of soil in 1 ha to a 25 cm depth is 2.5×10^9 g. If 0.05 mg is

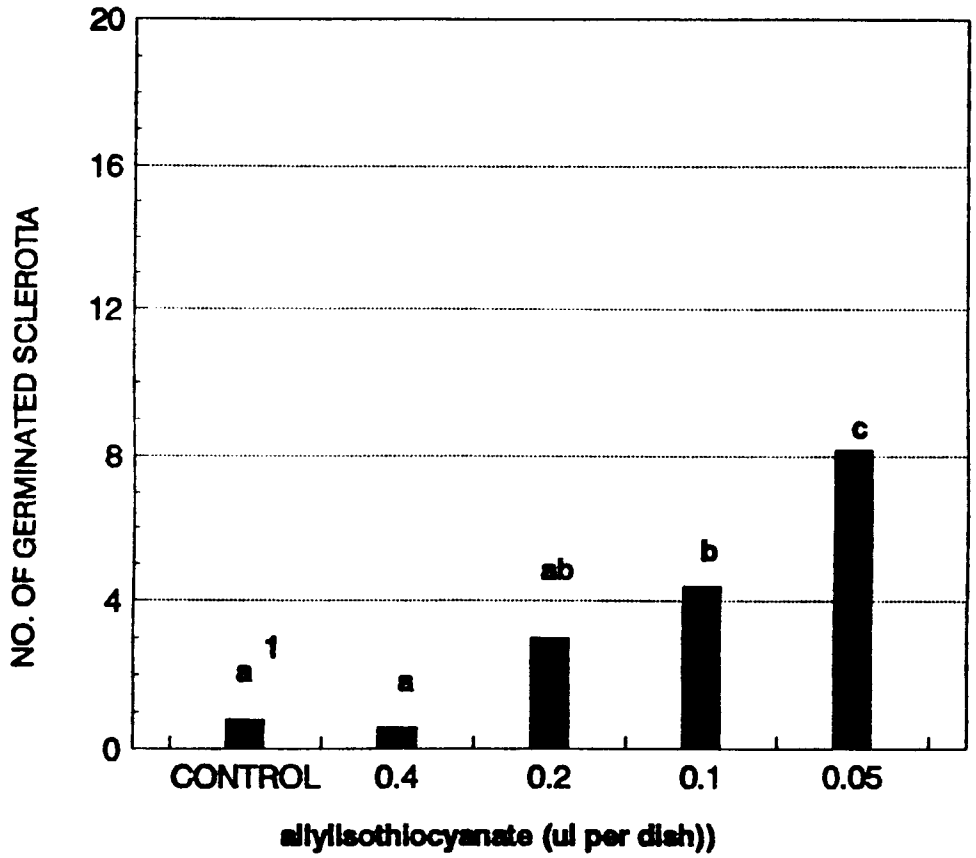

FIGURE 3: Number of sclerotia germinated out of 20 after 14 days of exposure to four concentrations of allyl-isothicyanate in petri dishes. Bars with the same letter do not differ (p=0.0001) according to SNK Multiple Range Test. Data are the average of five replicates.

applied to 20 g, this would make the lowest rate (0.05 ul) equivalent to an application rate of 6.25 L/ha.

Merriman *et al* (1981) reported an inhibition of germination with increasing doses of artificial onion oil (5% in water). They postulated that changes in the soil microflora and inhibition of germination by toxic compounds occurred at high concentration of onion oil. In contrast, Coley-Smith and Parfitt (1986) found that high concentrations of dially disulphide (DADS) were more effective stimulants of sclerotial germination than lower doses. They used 10 different concentration of DADS (0.05, 0.10, 0.25, 0.5, 1.0, 2.5, 5.0 10.0 25.0 and 50.0 g/l of water) applied as aqueous suspensions by injection into polyester bags containing 25 sclerotia in a small quantity of sand. The enhancement of germination was similar for all concentrations except the two lowest.

Alternatively, *Brassica* species, applied as a green manure to soil, can suppress other soilborne plant pathogens and nematodes. Mayton *et al* (1995) found that two *Brassica* species (*B. nigra*, and *B. juncea*) are able to suppress the growth of *Fusarium sambucinum*, potato dry rot pathogen. A modification of an assay designed for volatile fungicides, developed by Richardson and Munnecke, was used. Treatments were considered 'suppressive' if the radial growth at 7 days was <50% of the unamended control.

In experiment 1, five *Brassica* species were compared for suppressive activity using 10,20, and 40 g of leaf tissue. The *Brassica* species differed greatly in their ability to suppress the growth of *F. sambucinum*. Both *B. nigra*, cultivar Type 1 and *B. juncea*, cultivar Cutlass, were suppressive with >50% inhibition of radial growth compared to an unamended control. In contrast, the cultivars of *B. napus*(cv. Midas), *B. carinata* (cv. Dodolla), and *B. campestris* (c.v Torch), were not suppressive at any dose. Inhibition of radial growth of the fungal pathogen was greatest at the highest dose, 40g of plant tissue, for both the *B. nigra* and *B. junceas* cultivars. A small, and likely insignificant, level of growth inhibition was observed for *B. carinata,* and *B. nigra* treatments at the highest doses.

However, reduction of population densities of *S. cepivorum* in soil due to the incorporation of *Brassica* tissue with high concentrations of allyl glucosinolate remains to be demonstrated.

CHAPTER IV: EVALUATION OF THE EFFECTS OF TWO GERMINATION STIMULANTS ON THE SURVIVAL OF SCLEROTIA OF *SCLEROTIUM CEPIVORUM* IN THE FIELD

4.1 INTRODUCTION

During growth of *Allium* species, sulphur-containing amino acid conjugates specific to this plant genus are secreted from the roots (King and Coley-Smith, 1968). These conjugates are broken down into a number of volatile sulphur compounds, which include many of the flavour and odour compounds characteristic of *Allium* species (King and Coley-Smith, 1969). These same volatile sulphur compounds specifically induce the dormant sclerotia of *S. cepivorum* to germinate (Coley-Smith, 1960).

Two host volatiles, diallyl disulphide (DADS) and dipropyl disulphide (DPDS), have been synthetically produced to mimic host volatiles. These compounds induce sclerotia of the pathogen to germinate even though there is no host tissue present. If a large proportion of sclerotia could be stimulated to germinate in infested field soil, the use of synthetic sulphide germination stimulants could be an effective method to reduce inoculum levels.

Product efficacy depends upon a number of factors which relate to both the physical behaviour of the germination stimulants and to the biological responses of the pathogen (Crowe, 1993). In trials, volunteer *Allium* plants and related weed species should be eliminated as the pathogen may continue to reproduce on these hosts. Further, sclerotia of the

pathogen may not be responsive to germination stimulants for several months after formation on diseased plants. For this reason, an adequate germination response may not occur if germination stimulants are used too soon after a white rot disease episode. Applications should not be made until at least 6 - 12 months after the previous *Allium* crop is harvested, and after all crop volunteer plants are eliminated (Crowe, 1993).

Germination stimulants must reach the surface of the sclerotia to be effective (Crowe, 1993). The stimulants are volatile and tend to redistribute after application, which enhances product effectiveness. Product efficacy depends upon (1) how well distributed the initial application is; (2) whether the soil structure and moisture allow for good volatilization and redistribution; and (3) how long the volatile material can be contained in the soil.

Soil moisture is important for sclerotial germination. Soil which is too dry may be well permeated by the stimulants but lack sufficient moisture to elicit germination. The white rot pathogen is maximally responsive to stimulants when soil moisture is at -300 mbar, or field capacity (Crowe and Hall, 1980b). Soils which are too wet may inhibit volatilization and permeation of the stimulants.

Soil temperature affects both the biological response of the pathogen and the behaviour of the product (Crowe, 1993). The sclerotia of *S. cepivorum* germinate between 9 - 24 C,

with the optimal response around 14 - 17 C (Crowe and Hall, 1980b). The germination response seems to be inhibited when soil temperatures at 10 cm depth fluctuate into the mid-20's C or higher at any time. Also, at higher soil temperatures, the germination stimulants are more volatile. Although high soil temperatures enhance volatilization and increase the stimulants' ability to permeate the soil, they also accelerate their loss from the soil surface at a time when the fungus is becoming less likely to respond. The volatility of germination stimulants declines to negligible levels at about 7-10 C, thus the product is not lost from soil by volatilization during the winter (Crowe, 1993). When temperature and moisture conditions are met, the effective duration of response to germination stimulants is about 2.5 months (Crowe, 1993).

Although onion oil has been tested as a germination stimulant in B.C. (Utkhede and Rahe, 1982), no work has been done with the two host volatiles, DADS and DPDS. The objective of this study was to determine the degree to which DADS and DPDS applied to muck (high organic) soil and to sandy (low organic) soil would stimulate sclerotial germination and subsequently reduce sclerotial numbers in these soils.

4.2 MATERIALS AND METHODS

4.2.1 Fall Application; Sandy Interior Soil

The study site was situated in a field that was heavily infested with *S. cepivorum* near Oliver, B.C. The site consisted of two areas both of which had been were planted to onions in 1993 and fallow in the 1994 season. Each area (9m X 156m) was divided into twelve 9m X 13m plots. Before treatment (07 September, 1994), each plot was sampled to estimate the initial population of sclerotia. The plots were sampled again on 24 May, 1995. Two composite samples per plot were taken at each sampling time. Each composite sample consisted of 20 randomly chosen subsamples of approximated 25 cc taken from the top 10 cm of the soil profile with a trowel. Each composite sample was dry sieved through a No. 12 (1.70 mm) sieve and thoroughly mixed twice in a large plastic bag. Triplicate 50 cc quantities of soil from the mixed samples were individually washed through stacked 0.600 mm and 0.210 mm sieves. The residue on the 0.210 mm sieve was added to 2.5 M sucrose solution in a 50 ml centrifuge vial and centrifuged for 5 min at high speed (3,200 G). The supernatant from the vial was transferred to a 0.210 mm sieve, washed with water and examined under the dissecting microscope at 5X. Twenty randomly selected sclerotia from one of the triplicate samples were plated on 20% PDA to test viability as described previously. The data collected was analyzed using the t-test procedure.

In the first area, plots were either treated with DADS or not treated (control), and in the second area, plots were treated with DPDS or not treated (control). Treatment and control plots were replicated six times and randomly assigned within each of the two areas. A buffer zone of 2.5 m was established between application areas, and a buffer zone of 1 m was established between plots for sampling. Both germination stimulants were applied at the recommended rate of 10 L per hectare in a minimum of 500 L of water (0.11 L of stimulant/plot) and were injected at 25 cm and 10 cm from shanks 5 cm apart with commercial equipment by MGM Fumigating, 33865 Farmer Road, Abbotsford, B.C., on 08 September, 1994. Soil temperatures at the time of application were 18.9 C and 21.1 C at 20 cm and 10 cm respectively. The soil was irrigated before and after application as required to maintain soil moisture at approximately 50-70 % of field capacity. Weekly soil temperatures were taken throughout the experiment at 10 cm and 20 cm depth using a soil temperature probe.

4.2.2 Spring Application; Coastal Muck Soil

A trial in Cloverdale, B.C. was conducted on the Garvin farm which is located in the muck soil flood plain area of Cloverdale, B.C. The field used in this trial was planted to onions in 1994 and had a very high incidence of white rot. On April 22, 1995, a total area of 0.44 ha (1.10 acre) was treated with the germination stimulants, DADS and DPDS. The

products were applied with the same type of equipment as was used in the sandy soil trial (Figure 4), at the recommended rate of 10 L of product in a minimum of 500 L of water per ha at a depth of 25 cm and 10 cm. Treated plots (11 m X 120 m) and control plots (9 m X 120 m) were replicated twice within the field and randomly assigned. Weekly soil temperatures were taken at 5 cm and 10 cm.

Sclerotia from the same population described in Chapter 2 were buried in nylon bags one day before application of the stimulants. There were 10 bags, each with 20 sclerotia in 20 g of moist muck soil. The bags were removed on July 07, 1995 and the number of sclerotia in each bag was estimated using the method described previously. The data were arcsine transformed and analyzed using analysis of variance and Student-Newman-Keuls multiple comparison tests.

4.3 RESULTS

4.3.1 Fall Application

The average number of sclerotia recovered from 50 g of soil from control and treatment plots was 17.4 and 15.6 for the DADS trial and 44.6 and 41.6 for the DPDS trial at the beginning of the trial, respectively (Figure 5). After the application, soil temperatures ranged from 9 C to 24 C for approximately one month before dropping below the threshold for germination. Weekly temperatures are shown in Table 5. When sampled on 24 May, 1995, two more composite samples were

FIGURE 4: Application of diallyl disulphide (DADS) and dipropyl disuphide (DPDS) in Cloverdale, B.C. by MGM Fumigating.

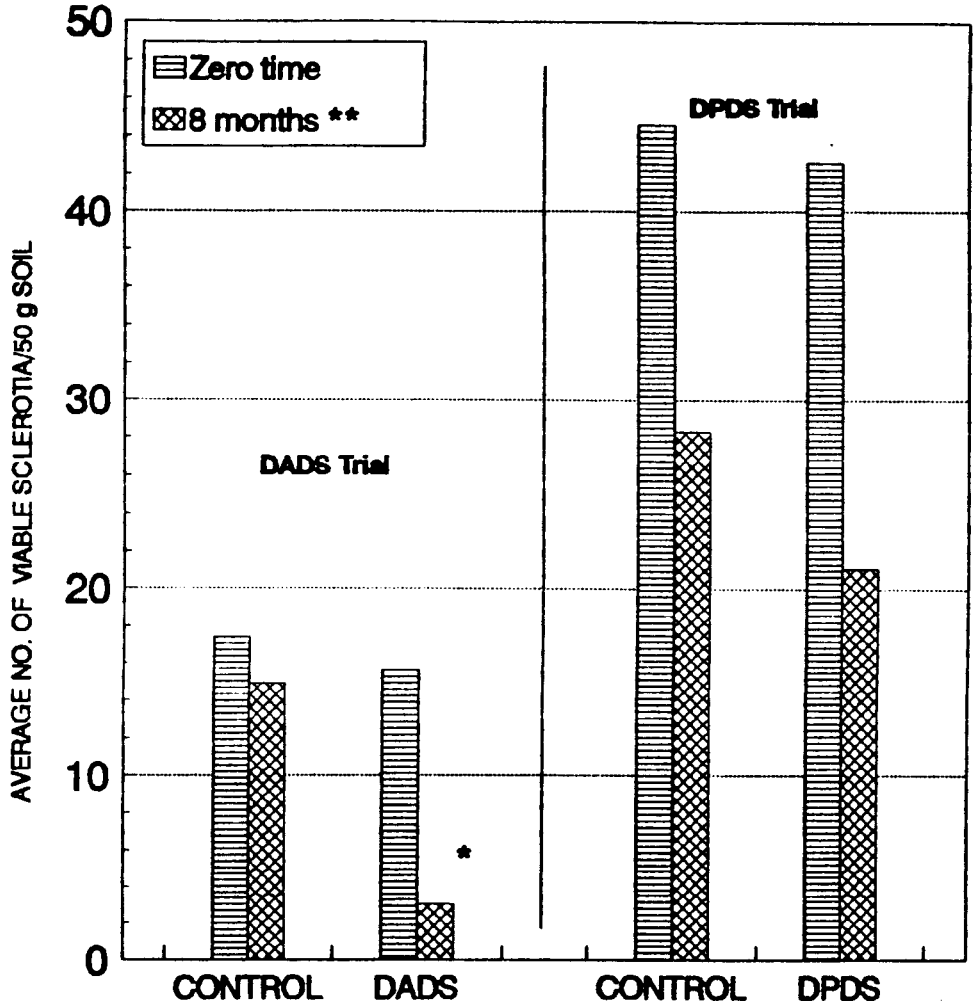

FIGURE 5: Average number of viable sclerotia recovered from control and treatment plots in diallyl disulphide (DADS) and ddipropyl disulphide (DPDS) trials at Oliver, B.C. just prior to (zero time) and 8 months after treatment on 08 September 1994. * Significantly different from control at three months (p=0.0002). ** Includes 3 months with mean temperatures above 9 C.

TABLE 5: Soil temperatures at Oliver, B.C taken at weekly intervals at 10 and 20 cm depths from 08 September, 1994 to 15 October 1994 and from 27 March 1995 to 15 May 1995.

Dates	Soil Temperature ^{0}C	
	10 cm	20 cm
08/09	21.1	18.9
16/09	18.9	16.7
24/09	20.0	17.8
01/10	16.1	13.9
08/10	12.2	10.0
15/10	10.0	9.4
	no data	
27/03	13.3	8.3
03/04	14.4	11.1
10/04	13.3	10.0
17/04	12.8	9.4
24/04	13.3	10.0
01/05	20.0	15.0
08/05	18.9	15.6
15/05	20.6	16.1

taken from each plot, and the group means of control and treatment plots were 14.9 and 3.0 sclerotia /50 g soil, respectively in the DADS trail and 28.3 and 21.1 sclerotia/50 g soil, respectively in the DPDS trail (Figure 5). For DADS, treatment effects were highly significant with P= 0.0002 (Figure 5). For DPDS, treatment effects were not significant. Viability of recovered sclerotia was 98% and did not differ significantly among sclerotia recovered from the various treatments.

4.3.2 Spring Application

Bags were recovered from plots 11 weeks after application on 07 July, 1995. Recovery of bags varied between plots. It is possible that the injection shanks may have destroyed or moved some of the bags. A total of 13 bags were recovered from control plots, 4 bags were recovered from DADS plots, and 14 bags were recovered from DPDS plots. The mean number of sclerotia recovered out of 20 was 10.0 $\pm$ 6.0 for control, 5.1 $\pm$ 3.8 for DPDS and 1.8 $\pm$ 2.9 for DADS (Table 6). The overall model of treatment effects was significant with P=0.0056. Significantly fewer sclerotia were recovered from the DADS plots than from the control plots, while recoveries of sclerotia in DPDS and control plots were not significantly different.

Weekly soil temperatures ranged from 15.0 to 25.3 C at 5 cm depth and from 13.7 to 22.0 C at 10 cm (Table 7).

TABLE 6: Mean numbers of introduced sclerotia recovered out of 20, 3 months after application of two germination stimulants, DADS and DPDS.

Treatment	Mean	S.D.	n	Range
Control	10.0 a[1]	6.0	13	2-17
DPDS	5.1 ab	3.8	14	0-12
DADS	1.8 b	2.9	4	0-6

1 - means followed by same letter do not differ (P = 0.0056) according to SNK Multiple Range Test.

TABLE 7: Weekly soil temperatures at Cloverdale, B.C taken at 10 and 20 cm depth and accumulated rainfall from 22 April 1995 to 04 July 1995.

Date	Soil Temperature ^{0}C	
	10 cm	5 cm
04/05	13.7	17.1
10/05	14.8	15.0
17/05	15.8	18.1
26/05	18.9	23.0
31/05	19.0	24.2
07/06	17.6	20.8
14/06	18.0	21.0
21/06	18.4	21.5
28/06	22.0	25.3
04/07	21.7	25.2

4.4 DISCUSSION

The results obtained for DADS in both the spring and fall applications confirm previous work conducted by Coley-Smith and Parfitt (1986) and Crowe *et al* (1994). DADS applied to the soil significantly reduced sclerotial numbers in both coastal muck and sandy interior soil. In both the fall and spring applications, weekly soil temperatures at 10 cm did not exceed 24 C. I am unable to explain the poor results obtained with the DPDS application.

Coley-Smith and Parfitt (1986) found little difference in the behaviour of five isolates of *S. cepivorum,* with >90% germination for each in the presence of DADS. In their study, DADS was injected (50g/L) directly into polyester bags containing sclerotia.

Crowe *et al* (1994) reported that sclerotial populations in an Oliphant silt loam and on Panoch sandy loam declined by 97.5 -100% from pretreatment level following application of DADS 75+, DADS 90+, and POLY (a mixture of 20-40% DADS, 30-69% diallyl trisulfide, 10-30% diallyl tetrasufide and traces of diallyl sulfide, diallyl penta sulfide, pentane and other sulphur materials). Differences among products and rates of application(50 L and 500 L/ha) were not statistically different. For DNPDS (98% di-N-propyl disulphide and a trace of related compounds), sclerotial recovery declined by 93.5-95.7% in the Oliphant silt loam (Walla Walla, Washington) and declined by 70% and 19.9 % for rates of 500 L/ha and 50 L/ha

on the Panoch sandy loam (Five Points, California).

Other field trials at Walla Walla, first treated in fall, 1989 and at Nampa, Idaho (sandy loam), first treated in the spring of 1990 were maintained through the summer of 1992 (Crowe *et al* ,1994). Treatments included DADS 75+ at three rates of application and an untreated control. At Walla Walla, mean sclerotial recoveries in May 1990 were 81.0, 3.1, 1.2, and 1.2 % of pre-treatment levels for rates of 0, 5, 50, and 500 L /ha, respectively. At Nampa, mean sclerotial recoveries in June 1990 were 91.8, 12.3, 0, and 1.2 % of pre-treatment levels for DADS 75+ rates of 0, 5, 50, and 500 L/ha, respectively.

These plots were retreated in the fall of 1990 (Walla Walla) or the spring of 1991 (Nampa) just as in the previous year. At Walla Walla, mean sclerotial recoveries in May 1991 were 59.6, 0, 0, and 0 % of original (1989) pretreatment levels for DADS 75+ rates of 0, 5, 50, and 500 L/ha, respectively. At Nampa, mean sclerotial recoveries in June 1991, were 80.2, 0, 0, and 0 % of original (1990) pretreatment levels for DADS 75+ rates of 0, 5, 50, and 500 L/ha, respectively.

In a subsequent planting of virus-free onions (cv 'California Early') the incidence of white rot was reduced to 9-16% at Walla Walla and 0-2% at Nampa compared to 100% and 65%, respectively in the untreated plots at these sites. The disease in the treated plots in which no sclerotia recovered

occurred very late, and was claimed to result from deeply-located inoculum that was not controlled by the treatments (Crowe *et al*, 1994).

Although DADS and related products are effective in reducing soil populations of sclerotia, this does not necessarily suggest that the disease will be controlled in the field. If soil populations are extremely high (10,000 sclerotia/kg of soil), a reduction of 90% would still leave an inoculum base of 1,000 sclerotia/kg soil. In California, a mean population of 1.5 sclerotia/kg soil resulted in 40% white rot in garlic (Crowe *et al*, 1980). In contrast, a population of 6,000 sclerotia/kg soil resulted in 40 % white rot in garlic in Egypt (Amein *et al*, 1980). In organic muck soils of the Fraser Valley, there is no clear correlation between the occurrence of viable, potentially virulent sclerotia of *S. cepivorum* and the occurrence of white rot on onions planted in the same soil (Utkhede, Rahe and Ormrod, 1978). In 1976, they found that white rot occurred on two of the 10 farms represented by 13 fields from which *S. cepivorum* was isolated in 1977. Also, white rot infected onions were found in 1977 in five fields where *S. cepivorum* sclerotia were not found in the corresponding soil samples. In all cases, the populations of sclerotia were much lower (20-250 sclerotia/kg soil) than those reported by Crowe *et al* (1980) and Amein *et al* (1982).

In all of the above studies, the raw products have been applied to the soil. Undoubtedly, dosage varied greatly over

time as the products dissipated from the soil, were chemically changed, or was bound to soil components (Coley-Smith and Parfitt, 1986). As suggested by others (Crowe *et al*, 1994), the formulation of germination stimulants into temperature-controlled and/or moisture-controlled slow release products in which dosage may be more consistent and extend over time might greatly alter the fungal response and enhance efficacy of the products.

If germination stimulants prove to be highly effective for control of white rot, it may be possible to eventually bring highly infested fields back in to production of *Allium*, and use the stimulants as periodic 'preventive' treatment on fields which are at risk of white rot infestation.

CHAPTER V: THE EFFECT OF WARM SEASON FLOODING ON SURVIVAL OF SCLEROTIA OF SCLEROTIUM CEPIVORUM

5.0 INTRODUCTION

Factors of the physical and chemical environment in the soil can affect infection levels, development of disease, and survival of fungal pathogens. Soil flooding is used to control diseases caused by *Sclerotinia sclerotiorum* (Coley-Smith and Cooke, 1971) and *Verticillium dahliae* (Pullman and DeVay, 1981) by enhancing the decay of their survival structures. Sewell (1965) postulated that the responses of soil fungi to changes in soil moisture are seldom attributed solely to the direct effect of water on the fungi and that inadequate gas exchange resulting in depletion of oxygen and increase in the CO_2 concentration was probably the primary cause of disease inhibition. In contrast, Littley (1992) found that neither excess water nor anaerobiosis alone mimicked flooding. He concluded that the lethal effect of flooding probably involved an anaerobic microflora adapted to survive in flooded, anaerobic soils.

While sclerotia of *S. cepivorum* are quite persistent under normal field conditions, they may die more quickly under flooded soil conditions. Crowe and Hall (1980b) found that decay of sclerotia of *S. cepivorum* in saturated soil increased with increasing temperature, at temperatures of 6 C and higher. No decay was seen at 0 C and 4 C. For all temperatures above 6 C, decay was greatest at soil saturation

and decreased with decreasing soil moisture. However, Leggett and Rahe (1985) observed decay in the field at temperatures that ranged predominantly from 0 to 10 C. Joshi (1988) found that flooding enhanced decay of sclerotia of *S. cepivorum* which supports the hypothesis of Leggett and Rahe that flooding is the factor responsible for the atypically high rate of natural sclerotial decay in the muck soils of the Lower Fraser Valley.

The objective of this study was to determine the effect of warm season flooding on the survival of sclerotia of *Sclerotium cepivorum* in muck soil.

5.1 MATERIALS AND METHODS

The trial commenced on May 02, 1994. Plots were situated in the same field as the *B. juncea* trial described in Chapter I. Three plots (1.3m X 5m) with three subplots were used. Subplots consisted of 12 L plastic pails that were buried so that their tops were level with the field soil surface. Each subplot contained three bags of sclerotia (20 sclerotia/25 g soil). These subplots were flooded on May 02, 1994 until the water level was 2 cm above the soil level in the pail. The water level was maintained at 2 cm above the soil surface for the entire trial, with water being added as needed. Weekly soil temperatures in the flooded pails were taken at three depths, 5,10,and 20 cm. Bags of sclerotia were recovered at zero-time, and after 6 weeks and 12 weeks of flooding.

Sclerotia were recovered from the soil in the bags by wet sieving and centrifugation with 2.5M sucrose (Vimard *et al*, 1986). The sclerotia were surface-sterilized with 1% sodium hypochlorite and plated on 20% PDA (PDA:water agar 1:4, v/v) to determine viability.

5.2 RESULTS

'Zero'-time samples were recovered on May 09, 10 days after burial and 7 days after flooding. The average numbers of sclerotia recovered from flooded and non-flooded plots at zero, 6 weeks and 12 weeks are shown in Table 8.

At both 6 and 12 weeks, the average number of viable sclerotia recovered from the flooded plots was significantly different from the controls ($P< 0.05$), and the average number of viable sclerotia recovered from the flooded plots at 12 weeks was significantly less than the number recorded at 6 weeks. The average temperatures (and ranges) recorded from 03 May to 26 July, 1994 at 20 cm, 10 cm, and 5 cm were 17.5 C (11.7-22.2 C), 19.7 C (13.3-26.7 C) and 23.0 C (15.6-28.3 C).

5.3 DISCUSSION

In this trial, warm season flooding was very effective at decreasing the number of viable sclerotia of *S. cepivorum* under field conditions. After 12 weeks of flooding at soil temperatures above 15 C at 20 cm, the number of sclerotia recovered was significantly reduced. Based on their own

TABLE 8: Mean numbers of sclerotia of *Sclerotium cepivorum* recovered out of 20 from muck soil in nylon mesh bags buried for various lengths of time in flooded and non-flooded muck soil.

Sampling Time	Average no. of viable sclerotia	
	Control	Flooded
Zero time	14.7 ± 2.8	16.8 ± 1.8
6 weeks	15.6 ± 2.5	8.2 ± 4.0 *
12 weeks	13.6 ± 1.6	0.9 ± 1.8 *

* - Significantly different from control (P=0.05)

finding, Crowe and Carlson (1994) concluded that seasonal flooding has potential for control of *Allium* white rot. During a single season of flooding in 1992, with a relatively average summer, sclerotial populations were decreased by 98%. In 1993, with a near record cool season for the area, a single season flooding reduced sclerotial populations by 96%.

Banks and Edginton (1989) combined spring flooding with crop rotation. The field used was naturally infested with sclerotia of *S. cepivorum*, was easy to flood and sloped so that only one half of the field was flooded. Flooding occurred in the spring of 1986 from 15 March to 07 April. During flooding, the water was kept at approximately 5 cm above ground level. After a 3-week flooding period, the water was pumped from the field, and in mid-April carrots were planted in both the flooded and nonflooded areas. There was a decrease in survival of sclerotia in both the control and the flooded plots over time. For the period December 1985 until April 1986, 29% of the sclerotia in the nonflooded plot were lost to natural winter decay. In the flooded plot, during the same period of time, there was an 84% decrease in the sclerotial population. Therefore, approximately 55% of sclerotial decay could be attributed to flooding. The July soil sampling (with a crop of carrots) showed no further reduction in the population of sclerotia in flooded and nonflooded plots. At the November sampling, no sclerotia were found in the flooded plots and a significant reduction was

detected in the non-flooded plot. This reduction was attributed to the carrot rotation.

The addition of organic matter to flooded soil has also been reported to enhance sclerotial decay (Joshi, 1988), which may act through providing an energy base to increase the anaerobic microbial population, as well as by accelerating the development of anaerobic conditions. Menzies (1962) found that when microsclerotia of *Verticillium dahliae* were incubated in saturated soil under nitrogen, the microsclerotia were eliminated in 3 weeks. When 1% alfalfa meal or 0.1% glucose was added, the microsclerotia were eliminated in 5 days. These results suggest that addition of organic matter or a food supplement might enhance decay of a pathogen in a flooded environment.

Flooding, although successful, would be restricted to areas where flooding can be performed, and for best results should be done during the warmer summer months. The cost of a single full season flooding in central Oregon, including lost cropping opportunity costs, was estimated to be similar or somewhat less than the cost of tarped methyl bromide fumigation (Crowe and Carlson, 1994).

Flooding also has some positive and negative attributes (Dreihuyzen, 1987). The advantages of flooding include control of some disease organisms, control of some weeds, reduction of wind erosion, halting of subsidence, and reduced oxidation of organic matter. Negative attributes include reduced access to

land, danger of puddling at the surface and danger of water erosion and loss of a crop producing season while flooded. The relative importance of both positive and negative attributes are dependent on soil type and environmental conditions when the soil is flooded; for example, oxidation is limited at lower temperatures. Therefore flooding during the winter has no effect on reducing oxidation of organic matter because little oxidation occurs at low temperatures (Ms. Mary Margaret Gaye, B.C. Min. of Agriculture, Fisheries and Food, Cloverdale, B.C).

CHAPTER IV: OVERALL DISCUSSION

This study evaluated three potential methods, the use of *Brassica juncea* as an amended cover crop, two germination stimulants (diallyl disulphide and dipropyl disulphide) and in-season flooding to decrease the population of sclerotia of *Sclerotium cepivorum* in onion growing areas of B.C.

Overall, the most effective treatment for reduction of sclerotia was warm season flooding in muck soils. Populations of sclerotia in flooded pots were reduced by 52% at 6 weeks and 94 % at 12 weeks. This may be a feasible management tool for Cloverdale growers. Flooding of the field following an early crop such as carrots which are harvested in July, would allow 2 to 2.5 months of warm temperatures. The addition of organic matter to the flooded field could possibly enhance the decay of sclerotia as was seen by Joshi (1988). This strategy may also reduce levels of phialospores of *Chalara elegans*, the causal agent of black root rot of carrot, in muck soil. Chittaranjan and Punja (1994) reported that with increasing temperatures (15, 20, or 25 C), recovery of phialopores from flooded soil was significantly reduced ($P \leq 0.01$) and no propagules were detected after 12 weeks.

Application of DADS to the soil, injected at 25 cm and 10 cm from shanks 5 cm apart, also was effective in reducing sclerotial numbers in both muck and sandy soil by 80%. Total

eradication with germination stimulants is unlikely with one treatment. Sclerotia from deep in the soil, that escape treatment, and sclerotia from the untreated edges that find their way back into treated soils may cause disease and result in the formation of 'new' sclerotia (Crowe, 1993). But with repeated treatment, if economical, it may be possible to bring white rot infested fields back into production. Formulation of germination stimulants into temperature-controlled and/or moisture-controlled slow release granulated products in which dosage may be more consistent and extended over time might greatly alter the fungal responses (Crowe *et al*, 1994)

Both field trials with *B. juncea* as an amended cover crop were unsuccessful in reducing sclerotial numbers of *S. cepivorum*. This result is in contrast to the result obtained by Joshi (1988) who found that *B. juncea* did influence the survival of sclerotia when incorporated in the fall. The field used for her study was a muck soil that was naturally water saturated for much of the fall and winter months. There are many possible explanations for the differences in our results, such as differences in moisture levels, soil temperatures, or in the presumed release of allylisothiocynate from the decomposing plant matter in the soil (not measured in either study). It seems likely, however, that much of the decline in sclerotial population attributed by Joshi to the *B. juncea* amendment may have been caused by natural winter flooding.

With this pathogen, control practices based on inoculum reduction are limited because inoculum density may return to high levels from very low levels within a single season due to prolific reproduction of sclerotia on the few plants decayed during recropping of *Allium* species (Crowe *et al*, 1980).

REFERENCES:

Adams, P.B. and Papavizas, G.C. 1969. Survival of root-infecting fungi in soil. X. Sensitivity of propagules of *Thielaviopsis basicola* to soil fungistasis in natural and alfalfa amended soil. Phytopathology 59:135-138

Amein, A.M., El-Shabrawy, A.M., and Abd-Elrazik, A.A. 1982. Density of *Sclerotium cepivorum* sclerotia in soil in relation to severity of white rot of Garlic. Assiut Journal of Agricultural Sciences 13:29-38.

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

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

Banks, E., and Edgington, L.V. 1989. Effects of integrated control on the onion white rot pathogen in organic soils. Can. J. Plant. Pathol. 11:268-272.

Borek, A., Morra, M.J., Brown, P.D., and McCaffrey, J.P. 1994. Allelochemicals produced during sinigrin decomposition in soil. Journal of Agriculture Food Chemistry 42:1030-1034.

Bugg, R.L. 1991. Cover crops and control of arthropod pest of agriculture. Pp. 157-163. *In*: Hargrove, W.L. (ed), Covercrops for Clean Water. Proceedings of International Conference at Tennessee Experimental Station, April 9-11, 1991. Soil and Water Conservation Society, 7515 Northeast Ankeny Rd., Ankeny, Iowa. 50021-9764.

Chew, F.S. 1988. Biological effects of glucosinolates. Pp. 155-181. *In*: Biological Active Natural Products. Potential use in Agriculture. H.G. Culter, ed. American Chemical Society, Washinton, D.C.

Chittaranjan, S. and Punja, Z.K. 1994. Factors influencing survival of phialospores of *Chalara elegans* in organic soil. Plant Disease 78:411-415

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.

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

Coley-Smith, J.R. and Cooke, R.C. 1971. Survival and germination of fungal sclerotia. Annual Review of Phytopathology 9: 65-92.

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

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

Coley-Smith J.R. and Parfitt, D. 1983. Response of sclerotia to artificial stimulants at different times of the year. Proceedings of the 2nd International Workshop on *Allium* White Rot, June 22-24, 1983. Horticultural Research International, Wellesbourne, Warwick CV35 9EF, UK.

Coley-Smith, J.R. and Parfitt, D. 1986. Some effects of diallyl disulphide on sclerotia of *Slerotium cepivorum*: Possible novel control method for white rot disease of onions. Pesticide Science 37:587-594.

Crowe, F. 1993. Management of *Allium* white rot (*Sclerotium cepivorum*). A work plan for treatment of infested fields using sclerotial germination stimulants and how it works.
Contact: Dr. F. J Crowe, Oregon State University.

Crowe, F. 1994a. Current known distibution of white rot in the western U.S. In: Proceeding of Fifth International Workshop on *Allium* White Rot. 11-13 May 1994, Cordoba, Spain. Horticultural Research International, Wellesbourne, Warwick CV35 9EF, UK.

Crowe, F. 1994b. White Rot of *Allium* species. Garlic and Onion Disease Symposium, Reno, Nevada, March 31, 1994.
Contact: Dr. F. J Crowe, Oregon State University.

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

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

Crowe F.J. and Carlson, H. 1994. Continued investigation of flooding as a means of Allium white rot control. In: Proceeding of Fifth International Workshop on *Allium* White Rot. 11-13 May 1994, Cordoba, Spain. Horticultural Research International, Wellesbourne, Warwick CV35 9EF, UK.

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

Crowe, F.J., Debons, J. Darnell, T., Thornton, M., McGrath,D., Koepsell, P. Ladorde, J, and Redondo Jaures, E. 1994. Control of *Allium* white rot disease with DADS and related products. In: Proceedings of the Fifth International Workshop on *Allium* White Rot, 11-13 May 1994, Cordoba, Spain. Horticultural Research International, Wellesbourne, Warwick CV35 9EF, UK.

Davies, J.M.l. 1994. *Allium* white rot (*Sclerotium cepivorum*) of bulb onions in the UK 1991-1993. In: Proceedings of the Fifth International Workshop on *Allium* White Rot, 11-13 May 1994, Cordoba, Spain. Horticultural Research International, Wellesbourne, Warwick CV35 9EF, UK.

Driehuyzen, M.G. 1987. Water Management of Organic Soils. Proceedings of the 11th B.C. Soil Science Workshop 1987.

Dobbs, C. G. and Hinson, W. H. 1953. A widespread fungistasis in soils. Nature, Lond. 172: 197.

Duncan, A., 1991. Glucosinolates. Pages 127-147 in: Toxic Substances in Crop Plants. J.P. D'Mello, C.M. Duffus, and J.H Duffus, eds. Royal Society of Chemistry, Cambridge, UK

Entwistle, A.R. 1994. The dynamics of *Sclerotium cepivorum* inocula: a theoretical framework. In: Proceedings of the Fifth International Workshop on *Allium* White Rot, 11-13 May 1994, Cordoba, Spain. Horticultural Research International, Wellesbourne, Warwick CV35 9EF, UK.

Gamliel, A. and Stapleton, J.J. 1993. Characterization of antifungal volatile compounds evolve from solarized soil amended with cabbage residues. Phytopathology 83:899-905.

Garrett, S.D. 1970. Inoculum Potential. Pp. 9-10. *In*: Pathogenic Root-Infecting Fungi. Cambridge at the University Press.

Gilbert, R.G. and Griebel, G.E. 1969. The influence of volatile substances from alfalfa on *Verticillium dahliae* in soil. Phytopathology 59:1400-1403.

Hall, D.H. and Somerville, P. A. 1983. Effect of inoculum potential on control of white rot by fungicides. In: Proceeding of Second International Workshop on Allium White Rot, 22-24 June 1983, Beltsville USA, 113-114. Horticultural Research International, Wellesbourne, Warwick CV35 9EF, UK.

Harling, R. and Kennedy, S.H. 1991. Biological control of *Plasmodiophora brassicae* using a bait crop. Med. Fac. Landbouww. Rijksuniv. Gent 56:159-170.

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

Joshi, V.K. 1988. Potential for reducing survival of sclerotia of *Sclerotium cepivorum* in the Fraser Valley of British Columbia. MSc Thesis, Biol. Sci., Simon Fraser University, Burnaby, BC, Canada.

King, J.E and Coley-Smith, J.R. 1968. Effects of volatile products of *Allium* species and their extracts on germination of sclerotia of *Sclerotium cepivorum* Berk. Ann. Appl. Biol. 61:407-414.

King, J.E. and Coley-Smith, J.R. 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.

Lal, R., Regnier, E., Eckert, D.J., Edwards, W.M., and Hammond. R. 1991. Expectation of cover crops for sustainable agriculture. Pp. 1-11. *In* Hargrove, W.L. (ed), Covercrops for Clean Water. Proceeding of International Conference at Tennessee Experimental Station, April 9-11, 1991. Soil and Water Conservation Society, 7515 Northeast Ankeny Rd., Ankeny, Iowa. 50021-9764.

Leggett, M. 1983. Potential for the biological control of onion white rot in the Fraser Valley of British Columbia. PhD Thesis, Biol. Science, Simon Fraser University, Burnaby, B.C. Canada.

Leggett, M.E. and Rahe, J.E. 1985. Factors affecting the survival of sclerotia of *Sclerotium cepivorum* in the Fraser Valley of British Columbia. Ann. Appl. Biol 106:255-263.

Lewis, J. A. and Papavizas, G.C. 1970a. Evolution of volatile sulfur-containing compounds from decomposition of crucifers in soil. Soil Biol Biochen 2: 239-246

Lewis, J.A. and Papavizas, G.C. 1970b. Effect of sulfur-containing volatile compounds from cabbage decomposition on *Aphanomyces euteiches*. Phytopathology 61:208-213.

Littley, E.R. 1992. Sclerotia of *Sclerotium cepivorum*: Factors affecting their formation, survival and infection of onions. PhD thesis, Biol. Science, Simon Fraser University, Burnaby, B.C.

Littley, E.R. and Rahe, J.E. 1984. Speciic tolerance of *Sclerotium cepivorum* to dicarboximide fungicides. Plant Disease 68:371-374

Mayton, H.S., Oliver, C., Vaughn, S.F. and Loria, R. 1996. Correlation of fungicidal activity of *Brassica* species with allyl isothiocynate production in macerated leaf tissue. Phytopathology 86:267-271.

Menzies, J.D. 1962. Effects of anaerobic fermentation in soil on survival of sclerotia of *Verticillium dahliae*. Phytopathology 52:743

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

Merriman, P.R., Samson I.M., and Schippers, B. 1981 Stimulation of germination of sclerotia of *Sclerotium cepivorum* at different depths in soil by artifical onion oil. Netherlands Journal of Plant Pathology 87:45-53.

Ormrod, D. J. 1977. Report of the 1977 Fraser Valley Onion Disease Survey. B.C. Ministry of Agric., Cloverdale, BC.

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

Ormrod, D.J., Swanson, T.A., and Smith, G.N. 1977. Field diseases of onions in costal British Columbia. Can Plant Dis. Surv. 57:49-51.

Papavizas, G.C. 1968. Survival of root-infecting fungi in soil VI. Effect of amendment on bean root rot caused by *Thielaviopsis basicola* and on inoculum density of the causal organism. Phytopathology 58:421-428.

Papavizas, G.C. 1973. Crop residues and amendments in relation to survival and control of root-infecting fungi: an introduction. Page 76. *In* G.W. Bruehl (ed.). Biology and Control of Soil-Borne Plant Pathogens. The American Phytopathological Society, St. Paul, Minnesota.

Papavizas, G.C. and Adams, P. B. 1969. Survival of root-infecting fungi in soil. X11. Germination and survival of endoconidia and chlamydospores of *Thielaviopsis basicola* in fallow soil and in soil adjacent to germinating bean seed. Phytopathology 58:371-378.

Pullman, G.S. and Devay, J.E. 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.

Richardson, L.T. and Munnecke, D.E. 1964. A bioassay for volatile toxicants from fungicides in soil. Phytopathology 54:836-839.

Sewell, G.W. F. 1965. The effect of altered physical conditions of soil on biological control pp. 479-494. In: Ecology of Soil-Borne Plant Pathogens. K.F. Baker and W.C. Snyder eds. Univ. Calif. Press. Berkley. CA.

Tan, M. 1982. A bioassay for germination of sclerotia of *Sclerotium cepivorum*. MSc thesis, Biology, Free University, Amsterdam, The Netherlands.

Tollsten, L., and Bergstrom, G. 1988. Headspace volatiles of whole and macerated plant parts of *Brassica* and *Sinapis*. Phytochemistry, 27:2073-2077.

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

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

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

Utkhede, R.S. and Rahe, J.E. 1982. Treatment of muck soil with onion oil to control onion white rot. Can. J. Plant Path. 4: 78-80.

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

Vaughn, S.F. Spenser, G.F., and Loria, R. 1993. Inhibition of *Helminthosporium solani* strains by natural isothiocynates. Amer. Potato Journal 70:852-853.

Villard, L.A.C. Zavaleta-Mejia, E. and Garcia, E.R. 1990. Effect of the incorporation of cruciferous (*Brassicae*) residues on soil-borne pathogens. II. Effects of the incorporation of cabbage and broccoli on white rot (*Sclerotium cepivorum* Berk.) of onion, under greenhouse conditions. Revista Mexicana de Fitopatologia 8:160-165.

Vimard, B., Leggett, M.E. and Rahe, J.E. 1986. Rapid isolation of sclerotia of *Sclerotium cepivorum* from muck soil by sucrose centrifugation. Phytopathology 76:465-467.

Walker, A., Brown, P.A. and Entwistle, A.R. 1986. Enhance degradation of Iprodione and Vinclozolin in soil. Pesticide Science 17:183-193

Wallbank, B.E, and Wheatley, G.A. 1976. Volatile constituents from cauliflower and other crucifers. Phytochemistry 54:1437.