POTENTIAL FOR REDUCING SURVIVAL OF SCLEROTIA OF SCLEROTIUM CEPIVORUM IN THE FRASER VALLEY OF BRITISH COLUMBIA

by

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Potential for reducing survival of sclerotia of Sclerotium-cepivorum in the Fraser Valley of B.C.

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ABSTRACT

The potential of using agronomic practices for reducing the survival of sclerotia of *Sclerotium cepivorum* in the field and hence protecting onions from white rot, a disease caused by this pathogen was investigated.

Indigenous sclerotial populations of *S. cepivorum* were estimated in a commercial onion field near Cloverdale, B.C. after harvest of a crop which had suffered 25-30% losses to white rot in 1986. The effect of the use of *Brassica juncea* as a winter cover crop and its residue incorporation on the survival of sclerotia of *S. cepivorum* in this field was studied. Survival of indigenous and introduced sclerotial populations was assessed at different times of the year. There was a significant decline in number of sclerotia with time in all plots. A significantly greater decline in the numbers of intact firm sclerotia occurred in *B. juncea* treatment plots than in control plots.

The level of disease in a commercial onion crop grown on the study site the following season was assessed. The results obtained showed that despite the substantial decrease in inoculum levels of sclerotia in treated plots, the disease incidence was not significantly different in treated and control plots.

In another experiment the effect of flooding on survival of sclerotia of *S. cepivorum* in non-amended soil and soil amended with *B. juncea* or natural vegetation was assessed. Flooding reduced sclerotial survival but the magnitude of this effect was related to the presence and nature of the vegetative amendments. The results obtained lead to a conclusion that a combination of flooding and specific crop residue incorporation can reduce survival of sclerotia in the soil under the type of conditions (mild winter with high precipitation) experienced in the Fraser Valley of B.C. The study did not detect a reduction in white rot of onions associated with reduced survival of sclerotia. The

implications of the utilization of flooding and crop residue incorporation in reducing sclerotial survival are discussed in relation to other disease management methods.

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DEDICATION

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DISEASE HISTORY AND INTRODUCTION TO THE THESIS

1.1 WHITE ROT

White rot is a serious disease of onion and other *Allium* spp. caused by the soil borne fungus, *Sclerotium cepivorum* Berk. (Coley-Smith 1959; Walker 1969; Jones and Mann 1963). It was first discovered in England by M.J. Berkeley in 1841 and has since been reported in most commercial onion production areas in many countries (Walker 1969; Scott 1956; Adams & Papavizas 1971). It can cause severe yield losses (Adams 1981; Crowe *et al.* 1980; Croxall *et al.* 1953).

Dry bulb and green bunching onions are grown in the Fraser Valley of British Columbia. Spring seeded dry bulb onions are one of the leading vegetable crops grown on muck soils near Cloverdale, British Columbia (Rutherford & Ormrod 1985). White rot was first recorded in the commercial onion growing areas of the Fraser Valley in 1970 (Ormrod & Conroy 1970). By 1974 the disease had spread to every onion field on the farm where it was first recorded resulting in a loss of 50% of the crop (Ormrod *et al.* 1977). In the spring of 1975, regulations were passed under the British Columbia Plant Protection Act forbidding the growing of onions on land known to contain *S. cepivorum* and restricting the movement of soil and machinery from such lands (Ormrod *et al.* 1977). However the disease has continued to spread and is a major problem to the local commercial onion industry (Utkhede *et al.* 1978).

White rot of onions is a major problem for the growers because it continues to spread and increase in severity where onions are grown in infested soil. It causes substantial yield losses and no completely effective chemical or biological control is yet available (Utkhede 1982).

1.2 PATHOGEN

S. cepivorum is a facultative saprophytic fungus (class Deuteromycetes; order Mycelia Sterilia) and grows in soil from an infected food base (Scott 1956). Its pathogenic activities under natural conditions are restricted to *Allium* spp. (Coley-Smith 1959, 1979). The fungus produces microconidia in nature (Coley-Smith 1960) and in culture (Asthana 1947) but these spores have not been observed to germinate and their function is unknown. In the absence of the host, the pathogen usually persists in soil as a sclerotium for long periods without any known vegetative growth or functional sporulating stage (Coley-Smith 1959; Walker 1969; Crowe *et al.* 1980).

1.3 SURVIVAL OF THE PATHOGEN

The only means by which the pathogen is known to survive in the absence of a host is as small black (0.2 to 0.6 mm diam.) sclerotia (Coley-Smith 1959; Crowe *et al.* 1980; Walker 1969). These propagules are extremely persistent and remain viable in soil for many years even in the absence of host *Allium* spp., either on or beneath the soil surface (Coley-Smith 1959; Walker 1969; Crowe *et al.* 1980). Coley-Smith (1979) recovered sclerotia from soil even after 10 years burial, and Crowe *et al.* (1980) recovered viable sclerotia from fields in California where no onions had been grown for 15 years. In contrast Leggett *et al.* (1983) reported a substantial decay of sclerotia within one year of burial in muck soil in the Fraser Valley of B.C. The rapid decay of sclerotia was presumably due to the local environmental conditions.

The sclerotium has an outer rind of one or two layers of rounded, thick cells enclosing a large tissue of hyphae. They are formed in diseased tissue on the onion stem base and are released into the soil either when the infected plant decays or at harvest

time (Coley-Smith 1959; Entwistle & Munasinghe 1978). Sclerotia can withstand the extremes of wetness and dryness in the soil (Coley-Smith *et al.* 1974; Papavizas 1977). The sclerotia remain dormant in nonsterile soil until stimulated to germinate by root exudates from *Allium* spp. (Coley-Smith *et al.* 1968; Coley-Smith & Hickman 1957; Coley-Smith & King 1969; Elnaghy *et al.* 1971; King & Coley-Smith 1969a).

1.4 INFECTION AND DISEASE DEVELOPMENT

Primary infection of the host plant is by hyphae originating from germinating sclerotia of *S. cepivorum* (Coley-Smith 1959; Walker 1969; Crowe *et al.* 1980). Sclerotia germinate within a temperature range of 14°C-20°C (Adams & Papavizas 1971; Entwistle & Munasinghe 1978). Two types of germination have been reported: hyphal germination (Adams & Papavizas 1971; Coley-Smith *et al.* 1967), and eruptive or plug germination (Coley-Smith 1960). The eruptive germination is generally more infective than hyphal germination (Scott 1956; Coley-Smith 1960).

Roots of onion bulbs are attacked by the fungus, which subsequently invades and forms a superficial white, fluffy mycelium on the lower portion of the bulb. After the primary infection, the mycelial growth can spread from one plant to another, thus causing secondary infections (Crowe & Hall 1980a; Scott 1956). Hyphae of *S. cepivorum* spread intercellularly and intracellularly through roots and cortical tissue of stems, eventually destroying the parenchymatous tissue (Abd-El-Razik *et al.* 1973). New sclerotia are formed within and on the host tissue and become distributed across the fields and through the soil profile in subsequent years (Crowe *et al.* 1980).

1.5 SYMPTOMS OF THE DISEASE

The above ground symptoms of white rot are yellowing and dying of the outer leaves of the onion plant beginning at the tips and progressing downwards (Walker 1969). The disease may occur early in the season and cause death of seedlings, but significant losses are more commonly observed in late summer as a bulb rot. The root system is rapidly destroyed by the fungus and infected bulbs become soft and watersoaked in the area colonized by the pathogen. Example of an infected bulb is shown in Fig. 1.

1.6 CHEMICAL CONTROL

Many control measures for white rot have been tried in different parts of the world, with limited success. In some cases where infestation of the soil is severe, onion production has had to be abandoned (Adams and Papavizas 1971; Merriman *et al.* 1980). Neither chemical or biological treatment has reduced the disease to economically acceptable levels in the Fraser Valley of B.C.

Control of white rot by chemicals, including calomel (Booer, 1945; Croxall *et al.* 1953), dichloran (Fletcher *et al.* 1971), pentachloronitrobenzene (PCNB) (Rushdi *et al.* 1974), iprodione (Entwistle & Munasinghe 1980; Utkhede & Rahe 1979) and vinclozolin (Utkhede & Rahe 1979) has been reported but none of these is considered to provide reliable economic control. Among these chemicals, only dichloran is registered for use on onions in Canada. The dicarboximide fungicides iprodione and vinclozolin have significantly reduced white rot in field trials in the Fraser Valley (Utkhede & Rahe 1979) and might provide a short term solution to the white rot problem. Dicarboximides likely could not be relied upon in the long term because there is already evidence that the pathogen can develop resistance to them (Littley & Rahe 1984), and loss of efficacy of iprodione due

Figure 1: An onion bulb showing symptoms of white rot

to enhanced degradation in soil was reported by Walker *et al.* (1986).

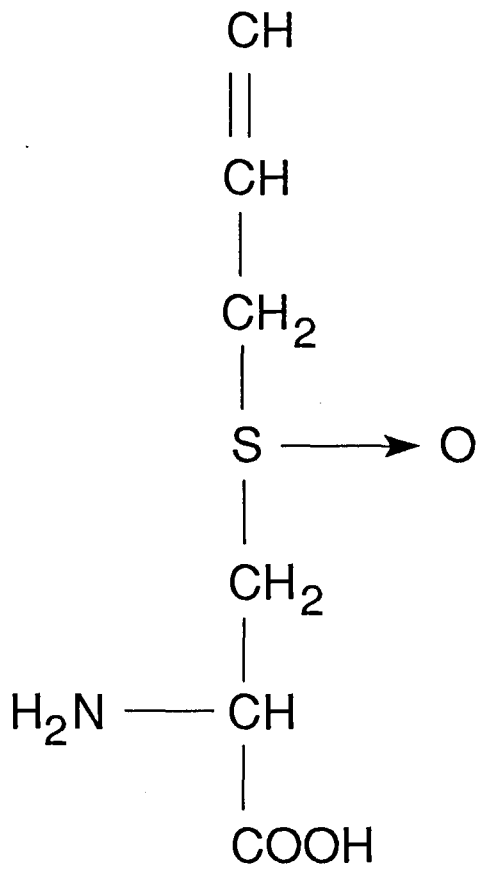
In view of these problems, other control methods such as breeding for resistance and biological control are being sought. Work done at Simon Fraser University has identified sources of resistance to white rot (Utkhede & Rahe 1978). It would take several years before a commercially acceptable cultivar could be developed, however, and it is unlikely that the level of resistance that can be achieved will provide complete control. There is thus need for increased effort to achieve the control of white rot of onions by biological means.

1.7 FACTORS AFFECTING GERMINATION OF SCLEROTIA

The primary functions of the sclerotium are to perrenate the fungus, and to bring about successful contact between the pathogen and host tissue. Therefore, sclerotial germination is an important phase at which the control of *S. cepivorum* might be implemented. There are three groups of factors which are known to influence the germination of sclerotia of *S. cepivorum.*

1. Host plant exudates
2. Soil microflora as biotic soil factors
3. Abiotic soil factors.

Coley-Smith (1979) demonstrated that the dormancy of sclerotia of *S. cepivorum* in the field is a condition imposed by the soil microflora, since they germinate readily in sterile soil. They are held in a dormant condition by soil fungistasis (Allen & Young 1968; Coley-Smith *et al.* 1967; King & Coley-Smith 1969b) and are stimulated to germinate by compounds associated with aqueous extracts and exudates of *Allium* spp. (Coley-Smith 1960; Coley-Smith & Hickman 1957; Coley-Smith & Holt 1966). The active volatile compounds in extracts of *Allium* spp. appear to be alkylsulphides and in particular



ALLYLCYSTEINE SULPHOXIDE
(ALLIIN)

FIGURE 2: Chemical structure of *alliin*, a component of root exudates of *Allium* spp.

(King and Coley-Smith, 1969a)

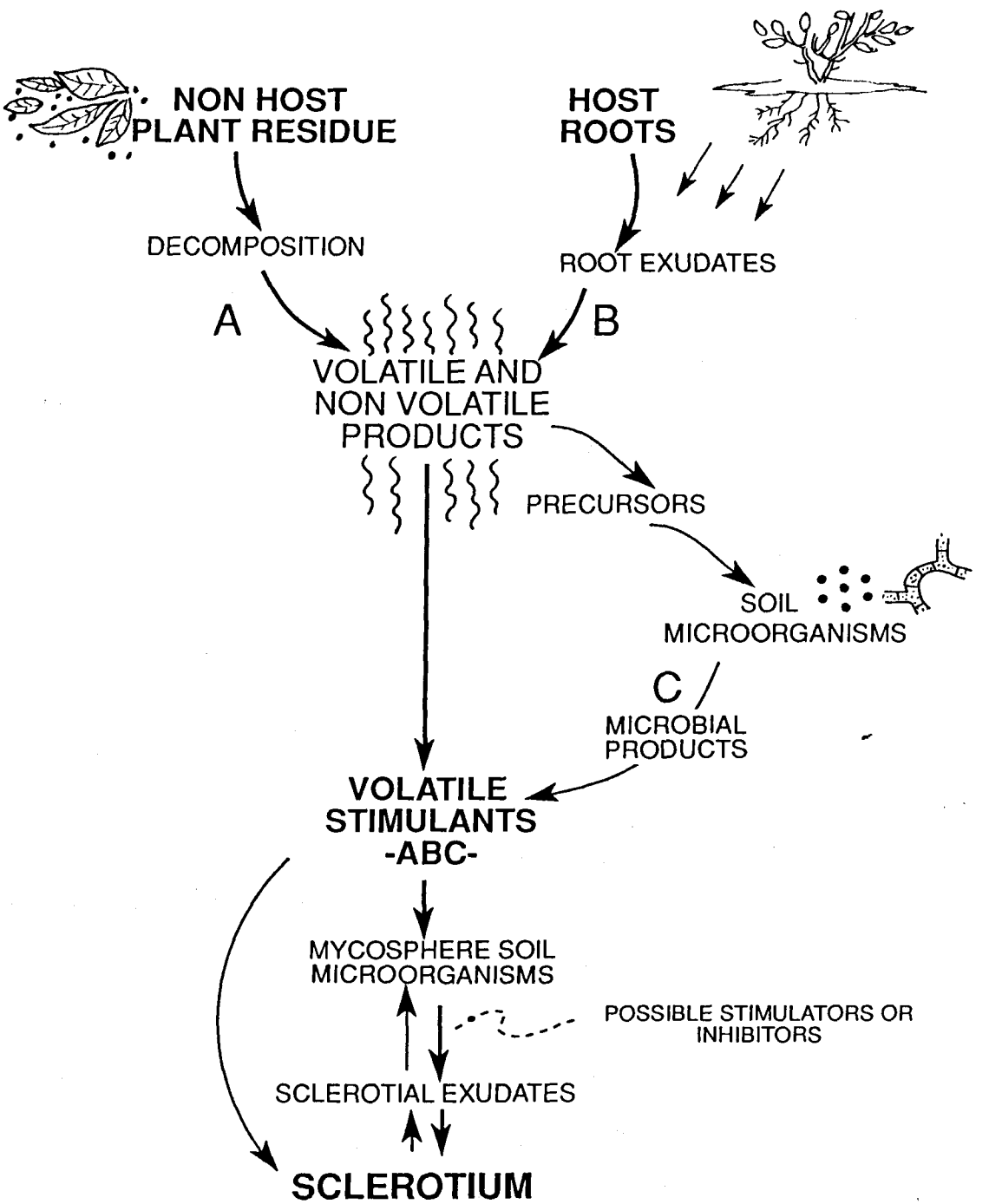
n-propyl and allylsulphides (Coley-Smith & Cooke 1971). Sclerotia show a low response to methyl sulphides which are produced by a wide variety of plants but a high response to n-propyl and allylsulphides produced only by *Allium* spp. These volatile compounds are not produced by intact onion plants. Intact onion plants exude small quantities of allyl cysteine sulphoxides. These latter (Fig. 2) compounds are thermostable, water soluble and diffusible, and are metabolized by bacteria to generate stimulatory volatile alkylsulphides (King & Coley-Smith 1969a).

The use of onion oil to induce germination of sclerotia in the absence of host plants and thereby reduce the number of sclerotia has been evaluated (Merriman *et al.* 1980; Utkhede & Rahe 1982). Partial control of the disease was achieved presumably because a substantial proportion of sclerotia germinated in the absence of the host, decayed quickly and did not form secondary sclerotia. However control was incomplete and implementation of this method would be prohibitively expensive unless a germination stimulant much cheaper than onion oil could be produced.

An onion flavour component, namely diallyl-disulphide (DADS) has also been evaluated for control of white rot under laboratory and field conditions (Entwistle *et al.* 1982; Coley-Smith & Parfitt 1986). Variable efficacy was obtained and it was found that there was a marked seasonal response of *S. cepivorum* to treatment with DADS (Coley-Smith & Parfitt 1986). There are a number of features of DADS treatment which have to be resolved before it could be recommended to farmers for control of white rot.

Volatile stimulants for sclerotial germination are produced from their precursors in onion root exudates by bacterial degradation, thus soil microflora may play an important role in the induction of sclerotial germination (Coley-Smith and Dickinson 1971; Dickinson and Coley-Smith 1970; Reddy 1986) It has also been reported that sclerotia of *S. cepivorum* enhance microbial activity (Coley-Smith & Dickinson 1971; Dickinson & Coley-Smith 1970). It seems that soil microflora and/or microflora associated with sclerotia

Figure 3: Hypothetical scheme showing three sources of volatile organic compounds produced in soil (A,B,C) and their possible effect and modes of action on propagules of soil-borne fungal pathogens in soil.


NON HOST
PLANT RESIDUE
HOST
ROOTS
DECOMPOSITION
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A
B
VOLATILE AND
NON VOLATILE
PRODUCTS
PRECURSORS
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MICROORGANISMS
C
MICROBIAL
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VOLATILE
STIMULANTS
-ABC-
MYCOSPHERE SOIL
MICROORGANISMS
POSSIBLE STIMULATORS OR
INHIBITORS
SCLEROTIAL EXUDATES
SCLEROTIUM

may mediate the stimulatory effect of *Allium* root exudates.

The possible sources and interactions of volatile stimulants, sclerotia and the soil microflora are summarized in Fig. 3.

1.8 BIOLOGICAL CONTROL

Biological control by protection of host plant surfaces involves the establishment of antagonists in or near the infection court of the host, thereby providing a biological barrier against the target pathogen (Cook 1981c). Biological control of this type may involve the use of antagonists as biological pesticides, some of which are commercially available (De Trogolf & Ricard 1976; Greigg 1975; Kerr 1980; Rishbeth 1963). Several other diseases have been controlled biologically in field trials but these biological agents have not yet been developed for commercial use (Backman & Rodrigues-Kabana 1975; Harman *et al.* 1980; Kommendahl & Chang 1975).

Several soil microorganisms have been found to be antagonistic to mycelial growth of *S. cepivorum.* Some *Fusarium* species, *Penicillium nigricans* (Moubasher *et al.* 1970) and *Streptomyces griseus* (Merriman & Borkenhead 1977) have been used to control *S. cepivorum* with varying success. Other soil fungi such as *Trichoderma viridae* and *Coniothyrium minitans* (Coley-Smith & Cooke 1971) are parasitic to sclerotia of *S. cepivorum* and have been effective in control of *S. cepivorum* (Ahmed & Tribe 1977; Abd-El-Moity *et al.* 1982).

The microorganisms that show substantial antagonistic activity against *S. cepivorum* in laboratory or greenhouse experiments, few that have been tested in the field have given disappointing results. Field trials in the Fraser Valley showed that *Bacillus subtilis* could reduce the levels of white rot significantly in some cases (Utkhede & Rahe 1980) but the results obtained from 4 years experimentation were not consistent. A preliminary study

of the effect of vesicular arbuscular mycorrhizal (VAM) fungi on white rot did not reveal potential for disease control (Tardif 1987), but much remains to be studied about the possible interaction between VAM and onion white rot.

Abiotic soil factors such as temperature, moisture, pH and soil structure are other factors which may affect either directly or indirectly the germination process. Changes in these abiotic variables may affect both the activity of soil microflora and the host plant exudation, thus influencing indirectly the germination of sclerotia.

The microbial activity of soil bacteria has been shown to increase when dry sclerotia sclerotia of *S. cepivorum* are placed in unsterile soil (Coley-Smith & Dickinson 1971; Dickinson & Coley-Smith 1970). Smith (1972a, 1972b, 1972c) claimed that air-dried sclerotia of a number of fungi including *S. cepivorum* and *S. rolfsii* rot more quickly than do non air dried sclerotia when placed in moist soil. He attributed the enhanced decay to the intensified microbial activity caused by nutrient leakage from the dried sclerotia. In contrast to Smith's results, Coley-Smith (1959) found that when dried sclerotia of *S. cepivorum* were buried in field soil, a large proportion survived for 4 years.

Crowe & Hall (1980b) reported that wet and warm soil conditions promoted decay of sclerotia. Their results supported those of Scott (1956) who indicated that flooding might eradicate sclerotia from infested soil. Leggett (1983) suggested that the unusually high rate of decay of sclerotia within one year may be due to winter flooding usually encountered in Fraser Valley muck soils, althogh no direct evidence was presented. Adams (1987) found that high soil temperatures (40–50°C) and low soil moisture reduced survival and activity of sclerotia of *S. cepivorum* and *S. minor*.

There is less known about the optimum soil conditions for germination of sclerotia of *S. cepivorum* than might seem to be the case because most of the optimum conditions have been determined for disease development, rather than for sclerotial

germination (Entwistle & Munasinghe 1976; Walker 1926). Walker (1926) studied the relation of soil temperature and soil moisture to onion white rot disease development. The optimum temperature was found to be 20–24°C. Adams & Papavizas (1971) found that the optimum temperature for onion white rot development was 15°C, whereas for germination of sclerotia of *S. cepivorum* in autoclaved soil it was 20°C. Optimum pH for growth of mycelium on agar has been found to be 4.8 to 5.3 but in the soil more than 50% of the sclerotia germinated at pH between 4.5 and 7.8. Entwistle & Munasinghe (1976) demonstrated that sclerotial germination, mycelial growth and seedling infection were all markedly influenced by temperature. Crowe & Hall (1980b) reported that germination varied with matric potential rather than with moisture content of two different soil types. They found that decay of sclerotia of *S. cepivorum* was greatest at soil saturation but this occurred only at high temperature. Sclerotial survival would appear to be affected by the interaction of temperature and moisture levels.

As sclerotia are able to survive for many years in the absence of a host (Coley-Smith 1959; Walker 1969; Crowe *et al.* 1980), crop rotation does not seem to have much potential for reducing populations of sclerotia. Some cultural practices such as the use of organic amendments, altering sowing times (Rushdi *et al.* 1974) and the use of onions as a trap crop for reducing the numbers of sclerotia of *S. cepivorum* in soil have been tried but have been of very little success in controlling white rot (Merriman & Issacs 1978).

Coley-Smith and Parfitt (1983) reported that allylisothiocyanate (ACN) was quite effective in stimulating sclerotial germination. Surprisingly this chemical is not found in *Allium* spp., but does occur in certain species of the family Cruciferae (Itoh *et al.* 1984, 1985; Kozima *et al.* 1987; Lewis & Papavizas 1971; Lin & Hua 1986) and specifically in *Brassica juncea* (mustard, Wallbank and Wheatly 1976). Studies have shown that during the decomposition of crucifers in soil, some volatile compounds with phytotoxic as well as

mycotoxic properties are produced (Lewis & Papavizas 1971; Patrick *et al.* 1964). It is possible that certain crucifers may be able to influence the behaviour or survival of *S. cepivorum* under field conditions.

The purpose of this thesis was to examine the effects of *Brassica juncea* and flooding on the survival of indigenous and artificially introduced populations of sclerotia of *Sclerotium cepivorum.* Subsequent effects of the cover crop residue incorporation on the incidence of white rot and some other commonly occurring diseases in a succeeding crop of commercial onions were also examined.

CHAPTER 2

EFFECT OF BRASSICA JUNCEA AS A WINTER COVER CROP ON THE SURVIVAL OF SCLEROTIUM CEPIVORUM

2.1 INTRODUCTION

Crop rotation can affect the incidence of plant diseases (Curl, 1963; Griffin *et al.* 1981). Diseases caused by pathogens that decline rapidly in the absense of a host may be controlled by enforcing an appropriate interval between successive occurrences of the host crop. The same strategy can be used to slow the rate of population increase of an introduced pathogen in the soil.

Some plant species can directly or indirectly affect the survival of pathogens of other plants. Various researchers have found that the immediately preceeding crop in a rotation, or certain organic amendments derived from crops, can have a substantial effect on the incidence of diseases caused by different organisms (Butterfield *et al.* 1978; Kollmorgen *et al.* 1983; Cook 1981c; Ramirez-Villapudua and Munnecke 1988). Other studies have shown that during decomposition of crop residues in soil, volatile compounds with phytotoxic as well as mycotoxic properties are produced (Lewis and Papavizas 1971; Papavizas and Lumsden 1980; Patrick *et al.* 1964). Jarvis and Thorpe (1981) showed that the use of lettuce as a cover crop between successive spring-sown greenhouse tomato crops, and incorporation of the remaining lettuce residues into the soil controlled the foot and root rot (crown rot) disease of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici.*

A modification of crop rotation for disease control is the use of a host species as a 'trap crop' for a pathogen. The objective is to exploit the potential of the trap crop to deplete the reservoir of resting propagules of the pathogen by stimulating their

germination, and then destroy the 'trap' or otherwise prevent reproduction of the pathogen.

Sclerotium cepivorum Berk., the fungus causing white rot of *Allium* spp., is potentially an ideal candidate for the trap crop approach. Sclerotia of the pathogen are able to survive in the field for many years in the absence of host *Allium* spp. (Coley-Smith 1979; Crowe *et al.* 1980), so that conventional crop rotation would seem to be of little value for reduction of inoculum. Sclerotia of *S. cepivorum* germinate in response to volatile sulfide compounds derived from enzymatic or microbial action on non-volatile precursors found uniquely in root exudates of *Allium* spp. (Coley-Smith & King 1969; Coley-Smith & Cooke 1971). Merriman and Issaacs (1978) tested onions as a trap crop for *S. cepivorum*. Despite the apparent potential of the approach, they found that onions did not significantly reduce populations of sclerotia in soil, even though disease occurred in 19% of the trap crop plants and these were destroyed with a herbicide prior to the appearance of new sclerotia.

Coley-Smith and Parfitt in 1983 reported that germination of sclerotia of *S. cepivorum* in non-sterile soil can be stimulated by allylisothiocyanate (ACN). This compound does not occur in *Allium* spp., but is found in certain crucifers. Decomposing crucifers are known to produce substances with mycotoxic properties (Patrick 1986), and such substances might also contribute to the death of germinated sclerotia. The purpose of this experiment was to evaluate whether *Brassica juncea* (mustard), a crucifer, grown as a winter cover crop might enhance the decay of sclerotia of *S. cepivorum* under field conditions. *Brassica juncea* was chosen for study because it is known to produce ACN (Itoh *et al.* 1984, 1985; Kozima *et al.* 1987), and also for its ability to produce large amounts of leafy biomass during the relatively mild fall and winter conditions that typically occur in the Fraser Valley of British Columbia.

2.2 MATERIALS AND METHODS

The study site was in a field that is part of a commercial muckland vegetable farm near Cloverdale, B.C. The field was cropped to onions and suffered losses of 25–30% due to white rot in 1986 (J.E. Rahe, personal communication). The field was rototilled and rolled after harvest of the previous year crop. The study site was 270x36 m overall, and was divided into six plots of 45x36 m Seed of *B. juncea, cv.* Cutlass, was kindly provided by Dr. G.F.W. Rokow, Agriculture Canada Research Station, Saskatchewan. Alternate plots in the study site were seeded to *B. juncea* on 4 September, 1986 at 2.3 kg/plot using a Cyclone Little Giant hand seeder, and seeding in both directions. Plants were disced down around 15 May, 1987.

Three random sites were selected in each of the *B. juncea* seeded plots on 22 February, 1987 for the purpose of estimating the amount of plant biomass. At each site plants were pulled from a 929 cm^2 area and carried to the laboratory in plastic bags. All the soil was shaken off the plants, and the contents of each bag were weighed separately.

After about two months storage of these mature plants in the refrigerator, volatiles collected from rotting mature plant leaves were trapped on Porapak Q trap over a three day period and analysed by gas liquid chromatography by H.D. Pierce, Jr, (Department of Chemistry, Simon Fraser University).

Each plot was subdivided into 10 subplots for the purpose of estimating populations of indigenous sclerotia. Composite soil samples representing each of the 60 subplots, each consisting of a minimum of 35–40 g of soil collected from the surface and at approximately 25–30 equidistant locations in a subplot, were taken on 4 September, 1986. The study site was sampled in a similar fashion on 22 February, and 3 June, 1987 after the *Brassica* plants had been disced down. Only 24 samples (Four subplots from each

treatment plot) were taken on 22 February, 1987. Each composite sample was mixed in a rotary tumbler for 5 min at 48 revolutions/min. After mixing, a single 20 g (wet wt.) subsample was taken from each sample and the number of sclerotia contained was determined by wet seiving and centrifugation in 70% sucrose (Vimard *et al.* 1986). Moisture content of the soil was determined by drying 25 g of soil from each sample (second and third sampling times) at 110°C in an oven for 24 hours and reweighing. Percent moisture was calculated as follows.

$$\% \text{ moisture} = \frac{\text{weight of wet soil} - \text{weight of dry soil}}{\text{weight of dry soil}} \times 100$$

Moisture contents of the soil samples at the first sampling time (September, 1986) were not determined. The soil samples taken in September, 1986 were considered to have the same moisture content as those obtained in June, 1987. Consequently the mean moisture content of June samples was used for estimation of propagules on dry weight of soil basis for the September, 86 samples.

Introduced sclerotia used in the study were obtained from naturally infected onions from this farm. Sclerotia were harvested from bulbs and kept in soil in clay pots in the laboratory at room temperature, and watered infrequently. Sclerotia were recovered from the soil in pots by wet sieving when needed. Two populations of sclerotia were used, one from onions produced in 1985 and aged in the laboratory for 1 year, and the other from onions of the 1986 crop. Two hundred sixteen nylon mesh bags, each containing 20 intact firm sclerotia in 25 g of soil, were prepared. The soil was taken from a headland of the field in which the study site was situated and was found by analysis to be free of indigenous sclerotia. On the following day, one month after seeding, three bags from each population were placed at each of six replicate sites in each plot. The bags were buried 5-6 cm deep in the soil and attached to a central wooden stake at each site by

color coded nylon ribbons (Fig. 4).

Six replicate bags of each population (one from each stake) were recovered after 8, 19 and 25 weeks of burial. Sclerotia in each bag were recovered by wet sieving and centrifugation, and their numbers and condition assessed. Intact, firm sclerotia were surface sterilized in 1% NaOCl for 4 min, rinsed six times with sterile deionized water, plated onto potato dextrose agar (PDA) and kept at 17°C. The plates were observed for 15 days, and those sclerotia producing colonies typical of *S. cepivorum* were recorded as viable.

Tests of viability for broken, germinating, soft or hollow sclerotia were not reliable therefore for analysis of data only intact firm sclerotia were considered. The sclerotia recovered from all samples taken for indigenous population estimation were recorded as numbers/kg of dry soil. Data were analysed by analysis of variance and covariance (using time as a covariate). Selected means were compared by using Student's T-Test (P=0.05).

FIGURE 4: Field experiment showing arrangement of nylon mesh bags containing sclerotia of *Sclerotium cepivorum* in a study plot.

2.3 RESULTS

Excellent emergence of *B. juncea* occurred, and dense stands were present in the seeded plots by October. These plants continued to grow during milder portions of the fall and winter. By late February, above ground stems ranged from 39–65 cm in length, and overall plant height from 56–84 cm. Typical stems were 2–3 cm in diameter at the crown. Fresh weight biomass (roots and shoots) averaged 438±60g/929 cm^2 (47 tonnes/ha) on February 22, 1987. Sparse growth of winter annuals, primarily chickweed (*Stellaria media*) and some unidentified grasses occurred on the unseeded plots.

Distribution of sclerotia was patchy in each plot and variation among samples was high in control and seeded subplots (Table 1). None of the sclerotia recovered were found to be in a germinating stage. Temperature and rainfall data for the period September, 86 to June, 87 are shown in Table 2.

Mean populations of indigenous sclerotia at the time of seeding were 535 and 445 per kg dry soil in seeded and control plots, respectively, but the difference was not statistically significant. The population of sclerotia decreased significantly between the first (4 September) and second (22 February) samplings in seeded but not in control plots (Fig. 5, Table 1). Populations of sclerotia in both seeded and control plots appeared to decline markedly between the second (22 February) and third (3 June) samplings. Much of the apparent decline may have been due to dilution of the surface population of sclerotia with subsurface soil caused by discing and rototilling that occurred about 15 May. Estimated populations of indigenous sclerotia on 3 June, 1987 were 24.5 and 49.5 per kg dry soil in seeded and control plots, respectively, and the difference between these was not significant. Less than one percent of recovered sclerotia were found to be soft and/or germinating when sampled on 3 June.

The numbers of intact firm sclerotia recovered from bags containing known numbers of sclerotia obtained from infected onions declined significantly with time in both seeded and control plots (Fig. 6). Decline was slight until December, and accelerated during the winter months. Most of the decline was due to missing or decomposed sclerotia. The combined total of soft, broken and germinating sclerotia recovered at each sampling time ranged from 0.8 to 2.9 percent of total numbers of intact sclerotia introduced. Survivorship of sclerotia of the 1985 and 1986 populations buried in the test plots did not differ (P=.4841). Numbers of introduced sclerotia in buried nylon mesh bags declined more rapidly in the seeded plots than in the control plots. After 25 weeks, populations of sclerotia buried in plots without *B. juncea* had declined by 38%, while those buried in plots with *B. juncea* had declined by 56% (Fig. 6) and were significantly different ($P<0.05$). Viability of recovered sclerotia ranged from 91–100%. Most of the recovered sclerotia that failed to produce colonies of *S. cepivorum* on PDA following immersion for 4 min in 1% NaOCl were contaminated with a bacterium that produced inhibition zones against *S. cepivorum* when subsequently tested in dual cultures (Fig. 7).

Analysis of the volatiles released by decomposing plants of *B. juncea* harvested in February showed a number of constituents including a distinct peak of allylisothiocyanate (H.D. Pierce, Jr, personal communication).

Table 1: **Indigenous populations of sclerotia of *Sclerotium cepivorum* estimated at 0, 171 and 272 days after seeding with *Brassica juncea*.**

TREATMENT	Sept. 4, 1986 0 days	Feb. 22, 1987 171 days	June 3, 1987 272 days
	Sclerotia recovered per kg dry weight of soil**		
BRASSICA	535±76.49 a (30, 0–1489.0)	201±55.36 b (12, 0–517.5)	24.8±9.78 c (30, 0–106.5)
CONTROL	445±113.48 a (30, 0–2872.5)	431±95.95 a (12, 0–1043.0)	49.5±11.10 c (30, 0–212.5)

• Data are MEAN ± SE, with n and range in parentheses.

*Means followed by same letter do not differ significantly as tested by student's T–Test (P=0.05).

Table 2: **Temperature and rainfall data* for the period September, 86 to June, 87.**

MONTH	TEMPERATURE (°C)		RAINFALL
	mean	range	mm/month
Sep, 86	13.7	3.0–28.5	79.2
Oct	11.2	2.0–22.0	59.8
Nov	6.1	−3.0–16.0	259.8
Dec	4.6	−4.0–14.5	114.4
Jan, 87	3.8	−5.0–14.0	94.1
Feb	10.4	−2.0–15.5	31.4
Mar	8.1	−2.0–20.5	131.9
Apr	10.9	2.0–28.5	130.0
May	13.0	4.0–27.0	139.1
Jun	16.6	6.5–30.5	20.3

* Data obtained from Environment Canada, Surrey Municipal Hall, B.C.

Figure 5: Effects of *Brassica juncea*, natural conditions and rototilling on persistence of indigenous populations of sclerotia of *Sclerotium cepivorum* in Fraser Valley muck soil.

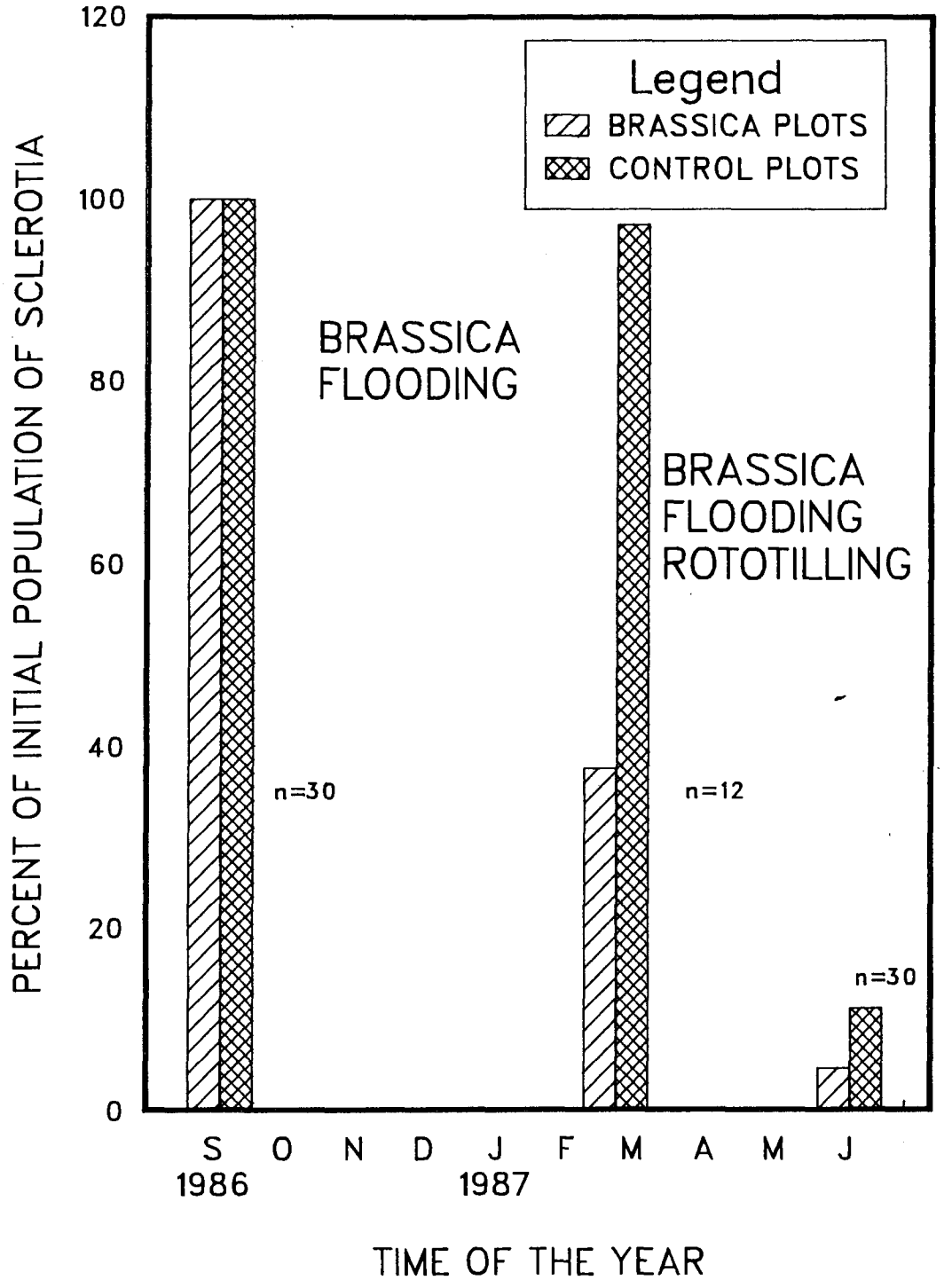

120
100
80
60
40
20
0
PERCENT OF INITIAL POPULATION OF SCLEROTIA
Legend
BRASSICA PLOTS
CONTROL PLOTS
BRASSICA
FLOODING
BRASSICA
FLOODING
ROTOTILLING
n=30
n=12
n=30
S
O
N
D
J
F
M
A
M
J
1986
1987
TIME OF THE YEAR

Figure 6: Effects of *Brassica juncea* and natural conditions on the survival of introduced sclerotia of *Sclerotium cepivorum* in Fraser Valley muck soil.

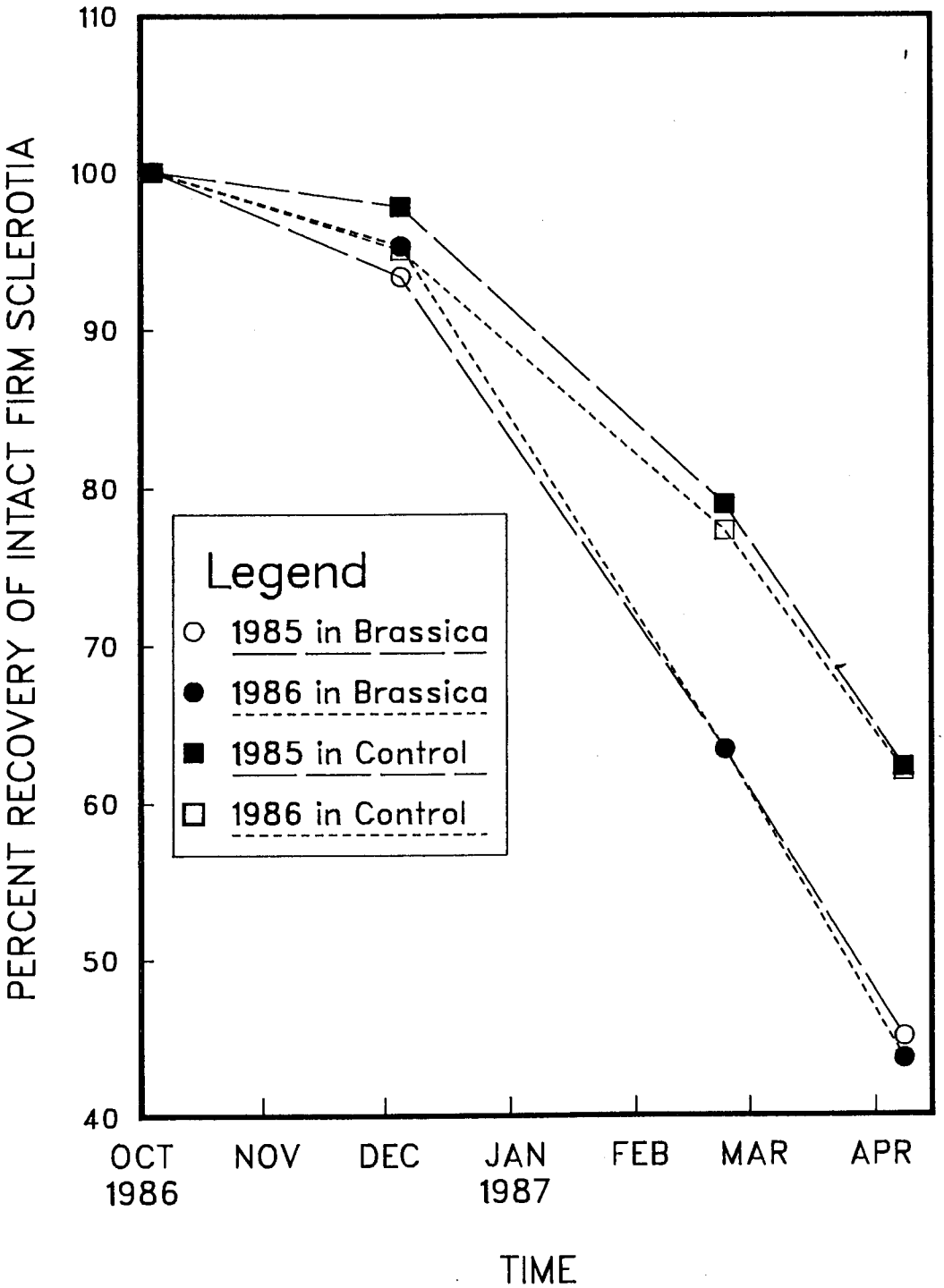

PERCENT RECOVERY OF INTACT FIRM SCLEROTIA
110
100
90
80
70
60
50
40
Legend
1985 in Brassica
1986 in Brassica
1985 in Control
1986 in Control
OCT
1986
NOV
DEC
JAN
1987
FEB
MAR
APR
TIME

Figure 7: Dual culture test on potato dextrose agar showing antagonism between *Sclerotium cepivorum* and an unidentified bacterium isolated from the surface of sclerotia of *S. cepivorum.*

2.4 DISCUSSION

The results reported here show that sclerotia of *S. cepivorum* decayed substantially during a single winter season in the Fraser Valley mucklands. This supports the findings of Leggett *et al.* (1983) and Leggett and Rahe (1985) but are in contrast to the observations of Coley-Smith (1979) and Crowe *et al.* (1980). Leggett (1983) suggested that the high rate of decay of sclerotia in the Fraser Valley might be associated with winter flooding typically encountered in Fraser Valley muck soils. However, she presented only indirect evidence for this contention.

The results of the present study showed that sclerotia decayed significantly with time in all plots and more rapidly in plots seeded with *B. juncea* (Fig. 6). This effect was observed for the indigenous sclerotial population and for two populations of introduced sclerotia. This suggests that the presence of the *B. juncea* crop influenced survival of sclerotia of *S. cepivorum.*

The addition of organic matter or crop residues has been used to control many diseases (Huber *et al.* 1970). Lewis and Papavizas (1977) studied the effect of decomposing immature and mature residues of rye, oat, soybean, sorghum, barley, buckwheat, timothy and corn on *Fusarium* root rot of bean and found that most of these residues significantly reduced the disease. Toussan, Patrick and Snyder (1963) studied the effect of crop residue decompostion products on the germination of *Fusarium solani* f. sp. *phaseoli* chlamydospores in soil and found that these reduced the inoculum levels by inhibiting chlamydospore formation. Many studies have shown that during decomposition of crucifers in the soil, compounds with phytotoxic and/or mycotoxic properties are produced (Patrick, 1986). Some of these substances had strong inhibiting effects on mycelial growth, zoospore motility and germination of infective propagules of *Aphanomyces euteiches* and reduced pea root rot (Lewis and Papavizas 1971).

The results of this study would suggest that *B. juncea* released certain compounds which stimulated sclerotial germination of *S. cepivorum* in the absence of host *Allium* spp. Coley-Smith and Parfitt (1983) studied the response of sclerotia of *S. cepivorum* to artificial stimulants and found that allylisothiocyanate applied to soil stimulated up to 66% germination of sclerotia compared to 3% germination in the control. Allylisothicyanate and diallyldisulphide were comparable in their ability to promote germination of sclerotia of *S. cepivorum.* Surprisingly, allylisothiocyanate is not a component of *Allium* spp. *B. juncea* has been found to contain the highest levels of allylisothiocyanate among all the crucifers studied so far, and its presence was confirmed in the vapors emanating from rotting plants produced in this study (H.D. Pierce, Jr, Personal communication). Accordingly it seems possible that the presence of the *B. juncea* crop in the field might have induced or stimulated germination of sclerotia, perhaps because of the presence or release of allylisothiocyanate and the germinated sclerotia decayed rapidly in the absence of a suitable host.

The recovery of intact firm sclerotia decreased significantly with time (Fig. 5 and Fig. 6) in all cases. It is not known yet if this decay or disappearance of sclerotia was due to natural flooding encountered in the field (Leggett & Rahe 1985), the presence of organic matter, the effect of which could be general because of increased microbial activity resulting in increased antagonism, or specific due to certain volatiles from *B. juncea* that stimulated the germination of sclerotia as well there is the possibility that decomposition of organic matter or crop residue from *B. juncea* might have accelerated oxygen consumption by soil microflora in the saturated muck soil. In such soil sclerotia might have died as a consequence of oxygen depletion. A detailed study on the effect of flooding and oxygen requirements for survival of sclerotia under natural environmental conditions is needed to evaluate the above hypothesis.

CHAPTER 3

SURVEY OF DISEASE LEVEL IN COMMERCIAL ONION FIELD

3.1 INTRODUCTION

The ultimate aim of efforts made to reduce inoculum levels of soil-borne pathogens is to reduce the diseases caused by those pathogens to economically acceptable levels. It is difficult to eradicate the inoculum completely, particularly if it is soil associated. Papavizas & Lumsden (1980) noted out that if inoculum levels are reduced, the efficacy of other control methods (cultural practices, resistant or tolerant cultivars, fungicides) may be enhanced. However, it is, difficult to state what is an acceptable level of inoculum because it differs among different host species, pathogens, soil and environmental conditions.

The study described in the previous chapter demonstrated that *B. juncea* grown as a winter cover crop in 1986-87 reduced the survival of sclerotia of *Sclerotium cepivorum* significantly compared to controls. So next step was to see if this reduction in population of sclerotia in treated plots, reduce the level of disease in these plots in the succeeding onion crop. This chapter describes an analysis of the survey done on the estimation of the diseases like white rot and smut and the yield of healthy marketable onions in a crop of commercial onions grown on the previous study site in 1987.

3.2 METHODS FOR ESTIMATING DISEASE LEVEL

The experimental site described in the previous chapter (Brassica trial) was seeded with the dry bulb onion cultivar "Aries" on 28 May, 1987. The onions were planted in five rows on raised beds with a Stanhay precision seeder. There were nine beds running the length of the study site and crossing each of the six subplots. Indigenous sclerotial population in the study site was assessed 19 days after incorporating the cover crop

residue as described in chapter 2.

Six of the nine beds of the onion crop were assessed for white rot, smut and other diseases at various intervals beginning 5 August, 1987. A systematic pattern of sampling was adopted for this survey by taking four, approximately equidistant samples from each bed in each subplot. All sampling sites were located at least 3 m from the boundries separating the subplots. At each sampling site a plant in a row was chosen at random, and 10 contiguous plants were taken and rated for smut, white rot and other diseases such as maggot and other unidentified diseases of onions. Samples were taken on 5, 12, 20, and 26 August, and 4 September, 1987. For analysis of white rot data, sample sites were scored as uninfected, or infected, if any plants with white rot were found among the 10 contiguous plants. In the case of smut, the number of plants with smut was recorded. Data were subjected to analysis of variance with nested design using time as a covariate. Selected means were compared by using a student's T-test (P=0.05).

On 21 and 22 September, 1987, a more extensive sampling was done. At each of five sampling sites in each of the six beds in each subplot, 0.25 m^2 quadrats were assessed for total number of plants present, the number of plants with white rot, smut and other unidentified diseases including maggot, and the yield of healthy marketable bulbs. Data were analysed by analysis of variance, and means were separated by using DMRT.

Immediately after the onions were harvested, on 17 October, 1987, composite soil samples were taken for estimating populations of indigenous sclerotia from the experimental site and from an adjacent area that had been cropped to potatoes in 1987. Twenty composite soil samples were taken from each field (40 total). Each sample consisted of 20–25g of soil taken 0–6 cm deep from 25–30 approximately equidistant locations in each plot. Each composite sample was mixed in a rotary tumbler for 5 min at 48 revolutions/min. After mixing, 25g and 20g (wet weight) subsamples were taken from each

sample, the former for estimating moisture content and the latter for estimating sclerotial populations. The numbers of sclerotia contained in the soil samples were determined by wet sieving and centrifugation in 70% sucrose (Vimard *et al.* 1986). Moisture content was estimated as described in chapter 2. Recovered sclerotia were assessed for condition, and intact firm sclerotia were surface sterilised with 1% NaOCl for 4 min, and plated onto potato dextrose agar. Their viability and identity was assessed after incubation at 17-18°C for 2 weeks. Numbers of sclerotia were calculated on dry wt basis and converted to numbers/kg dry soil, and tested for difference in populations of sclerotia between the onion and potato fields by using a student's T test (P=0.05)..

3.3 RESULTS

Preliminary sampling by Dr. J.E. Rahe on 20 July 87 (personal communication), and the samples taken on August 5, 1987 detected very few plants with white rot (maximum four plants in 1440 plants sampled), but smut was widespread. The level of white rot increased dramatically in samples taken after 5 August. Analysis of data from samples taken August 12 to September 4, 1987 (4 sampling times) showed that the level of white rot did not differ significantly with time between August 12 and September 4 (Table 3, T-test). Contrary to expectations based on the significantly enhanced decline of introduced sclerotia observed in *Brassica* subplots compared to control subplots (Fig. 6, Chapter 2), analysis of the pooled data for the period August 12 to September 4 showed that significantly higher levels of white rot occurred in treatment subplots than in control subplots (Table 4, T-test).

Smut levels appeared to decline with time after the first sampling (Table 5), presumably due to drying and loss of the small plants affected by smut. Percent smut was numerically less in treatment than in control plots on four of the five sampling dates, but the difference was significant (T-test) only for the 12 August sampling. There was a significant block effect for both white rot and smut. The gradient of smut and white rot in blocks was found opposite to each other.

Harvest data taken on 21 and 22 September, 1987 did not show a significant treatment or block effect for white rot, nor a significant treatment effect for smut (Table 6, T-test). Other pests including maggot and some unidentified onion diseases were detected on 4.55 percent of the total plants present and were not significantly affected by treatments but did show significant differences among blocks. Yield was significantly different in the three blocks, but the treatment and control plots did not show significant differences (P=0.05). On average the yield of healthy marketable onions from treated and

control plots was 31.58 and 28.85 tonnes per hectare, respectively (Table 6).

Mean populations of indigenous sclerotia when sampled on 17 October were 37.3 and 39.8 per kg dry weight of soil in the potato and onion (treated and control) fields, respectively, and the difference between these was not significant. Numbers of sclerotia recovered in individual samples ranged 0–3/20 g wet weight of soil for both fields.

Table 3: **Comparison of proportions of sites* with white rot at different sampling times in Brassica treated and control subplots.**

TREATMENT	Aug.5	Aug.12	Aug.20	Aug.26	Sept.4	Sept.22
BRASSICA	.00	.28	.55	.50	.25	.86
CONTROL	.04	.19	.43	.33	.22	.85

*The values are means of three blocks and six beds.

Table 4: **Comparison of percentage* of plants with white rot at different sampling times in Brassica treated and control plots.**

TREATMENT	Aug.,5	Aug.,12	Aug.,20	Aug.,26	Sept.,4
BRASSICA	0.00	5.00	10.69	9.44	3.33
CONTROL	0.42	3.33	6.38	6.25	2.63

* Values are means of three blocks and six beds.

Table 5: **Comparison of percent smut* at different times in Brassica treated and control plots.**

TREATMENT	Aug.,5	Aug.,12	Aug.,20	Aug.,26	Sept.,4
BRASSICA	20.41*	12.91	8.61	9.44	8.61
CONTROL	27.36	21.94	8.61	12.08	8.75

• Values are means of three blocks and six beds.

* Means were significantly different (T-test, P=0.05) only for 12 August sampling.

Table 6: **Comparison of disease levels and yield of onions at harvest* in Brassica treated and control plots.**

TREATMENTS	Percent White rot	Percent Smut	Percent Other Diseases	Yield in Tonnes Per Hectare
BRASSICA	11.64	7.43	4.5	31.58
CONTROL	12.95	9.38	4.61	28.85

* Values are means of three blocks and six beds.

3.4 DISCUSSION

The results obtained during this survey show that the level of white rot was higher in the treated *(Brassica)* plots than in control plots from 12 August to 4 September. This difference apparently disappeared late in the season, as the harvest data obtained on 21 and 22 September, 1987 showed no significant differences among treated and control plots on white rot incidence (Table 6).

The data show that visible signs of white rot on onion bulbs developed within a two weeks period (Aug., 5 to Aug., 20). The sudden, late season appearance of white rot has generally been observed in the Fraser Valley, as has also the general observation that if the crop is sown early, white rot shows up early and if it is sown late, white rot shows up late (Dr. J.E. Rahe, personal communication). Moreover, on this particular farm, a field adjacent to the study site that was seeded in late March of 1987 developed white rot in early June (M. Tardif & J.E. Rahe, unpublished observation). These observations suggest that infections resulting in visible signs of disease may occur at a particular stage of host development or that latent infections may be present in an apparently healthy crop, and that development of visible mycelium on the bulb may await certain physiological changes of the host.

A possible explanation for the increased incidence of white rot in the treated plots during the early stages of disease development is that the *Brassica* residue may have served to activate propagules of the pathogen. The 1987 onion crop was seeded within two weeks of incorporating the *B. juncea* crop residue into the soil. Its decomposition could have stimulated germination of sclerotia via release of allylisothiocyanate (ACN, Coley-Smith and Parfitt 1983). Without a source of ACN in the control plots, sclerotial germination there would depend on production of volatile stimulants by the host, and hence occur later and possibly at lower levels than in the treated plots.

The effect of treatment (*B. juncea* cover crop and its subsequent incorporation into the soil) in reducing the incidence of smut uncovers some other unknown, possible influences of *B. juncea* on other diseases of onions. It is hard to say at this stage if there is any negative correlation between smut and white rot incidence. As blocks showed significant differences, it shows that the experimental field was not homogenous, which could be due to many environmental factors.

The means of populations of sclerotia did not show significant differences between field cropped to onion and potato, which shows that the subsequent host crop (onion) did not influence the inoculum levels of *S. cepivorum* in 1987. This unexpected result is likely related to the restricted development of disease in 1987. Overall, white rot infections in 1987 were found to be on 12.3% of the plants sampled whereas it was 25–30% in 1986 (J.E. Rahe, personal communication). The major difference between disease development in 1986 and 1987, however, was that in 1986 most of the bulbs that were infected with *S. cepivorum* had black sclerotia formed already by early August, whereas in 1987 even at the time of harvest (Sept., 21, 22) there were very few bulbs (approximately 1%–2% of infected bulbs) that had black sclerotia formed on them. It appears that very few sclerotia were formed as a result of the white rot that developed in 1987. This could be due to some environmental conditions (high temperature and hence low moisture) that occurred in 1987.

B. juncea as a winter cover crop, did not reduce white rot or improve the subsequent onion crop despite its effect on the rate of decline of sclerotia of *S. cepivorum*, neither did it have an adverse effect, as the yields obtained from treated and control plots did not differ significantly. If the hypothesis that the *B. juncea* residue served to enhance germination of sclerotia and hence infection of onion seedlings is correct, it is possible that reduced disease might result if the *Brassica* were incorporated in early winter of the preceding year, or if seeding of onions were delayed for one

year.

CHAPTER 4

EFFECT OF FLOODING AND ORGANIC AMENDMENTS ON SURVIVAL OF SCLEROTIA OF SCLEROTIUM CEPIVORUM

4.1 INTRODUCTION

Changes in the physical and chemical environment of the soil can directly or indirectly affect the infection and development of disease in plants. They may directly influence plant health, or affect the survival and/or infection abilities of pathogens, hence influencing disease incidence. Properties of the soil environment include soil texture, organic and biological composition, pH, temperature, aeration and gas exchange, water and nutrient availability, and other chemical factors; these can be effected by crop rotation, tillage practices, and crop residue management.

Soil water can influence the incidence of disease both directly and indirectly. It is one of the most poorly understood aspects of the soil environment as regards its influence on microbial interactions in soil. Infection and development of disease in plants can be altered by changes in soil resulting from waterlogging. Cook and Papendick (1972) stated that fungi are favoured by a relatively dry environment and bacteria by wet conditions. Excessive soil water is often harmful to higher fungi and waterlogging could eliminate some sensitive species (Mengenot & Dien 1979). The lower fungi are an exception and require free water for zoospore movement. Stover (1979) reported that only one species of bacteria, *Pseudomonas solanacearum*, on tobacco *(Nicotiana)* and bananas *(Musa)* was controlled by flooding. Several diseases caused by fungi have been controlled by maintaining a flooded soil for varying periods of time. The Chinese have long recognized the benefits of rotations of paddy rice with cotton for control of *Fusarium oxysporum* f. sp. *vasinfectum* (Cook, 1981b). Moore (1949) found that sclerotia of *Sclerotinia sclerotiorum* decayed completely within 23–45 days when marl, muck or sandy

soils were flooded. Another classic example of control of disease by flooding is that of banana wilt, caused by *Fusarium oxysporum* f. sp. *cubense* in Central America (Stover 1953).

The rate at which a soil becomes anaerobic when saturated with water (flooding) is largely related to the rate of oxygen utilization which in turn is related to the level of aerobic microbial activity. Microbial activity is generally enhanced by addition of organic amendments. The availability of nutrients or organic matter in a soil is often related to pathogen suppression (Menzies 1962 & 1970; Moore 1949; Smith & Restall 1971 and Watson 1964). For example, 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 (Menzies 1962). These results suggest that addition of organic matter or a food supplement might enhance decay of a pathogen, caused by flooding.

Fraser Valley muck soils are often flooded during the winter season (Leggett & Rahe, 1985), and the unusually high rate of decay of sclerotia in these soils was attributed to winter flooding (Leggett *et al.* 1983). Valdes and Edgington (1987) also observed reduced incidence of white rot in flooded soil. The purpose of this experiment was to study the effect of flooding and organic matter incorporation on the survival of sclerotia under conditions typical of the winter months in Fraser Valley. Earlier studies showed that recovery of sclerotia from plots seeded with *Brassica juncea* was less than from unseeded plots (Fig. 6), *B. juncea* and indigenous winter annuals were compared as organic amendments.

4.2 MATERIALS AND METHODS

This study was conducted outdoors at Simon Fraser University, Burnaby, B.C. Styrofoam cups of 350 ml capacity were used as experimental pots. The bottoms were cut open to allow drainage in half of the cups and half of the cups were kept intact for the flooding treatment (Fig. 8).

Nylon mesh bags were prepared, each containing 20 intact firm sclerotia with 20 g of unsterilised, sclerotia free muck soil. The sclerotia were of the 1986 population described in chapter 2.

Brassica plants brought from the experimental field site described in chapter two were chopped into about 5 cm pieces and added to each cup at a rate equivalent to the amount of biomass present in the field, which turned out to be 20 g fresh wt per cup. Indigenous winter vegetation from the control plots (grass and chickweed) was prepared in a similar manner and added to other pots, also at 20 g/cup. Into each cup some unsterilised muck soil was placed, followed by half of the plant material (where added), the nylon bag containing sclerotia, the remaining plant material (where added), and finally the cup was filled with soil to approximately 3/4 of its volume (Fig. 9). Pots for nonflooded treatment were prepared on site.

There were total of 6 treatments (3 flooded and 3 nonflooded) and 8 replications of each treatment and the pots were arranged in a Randomized Complete Block design (Fig. 10) outdoors under natural environmental conditions. The experiment was set up on February 23, 1987. At the beginning of the experiment, the viability of sclerotia from the population used for the experiment was estimated on potato dextrose agar by the method described in chapter 2.

Figure 8: Styrofoam cups to show the cut and intact bottom used for drained and flooded condition, respectively.

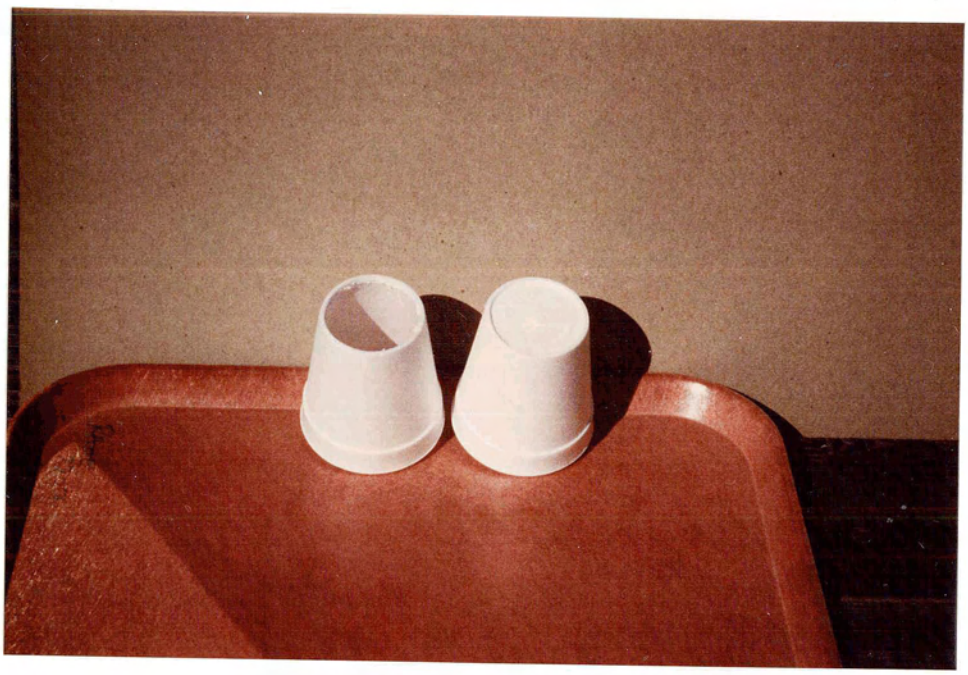
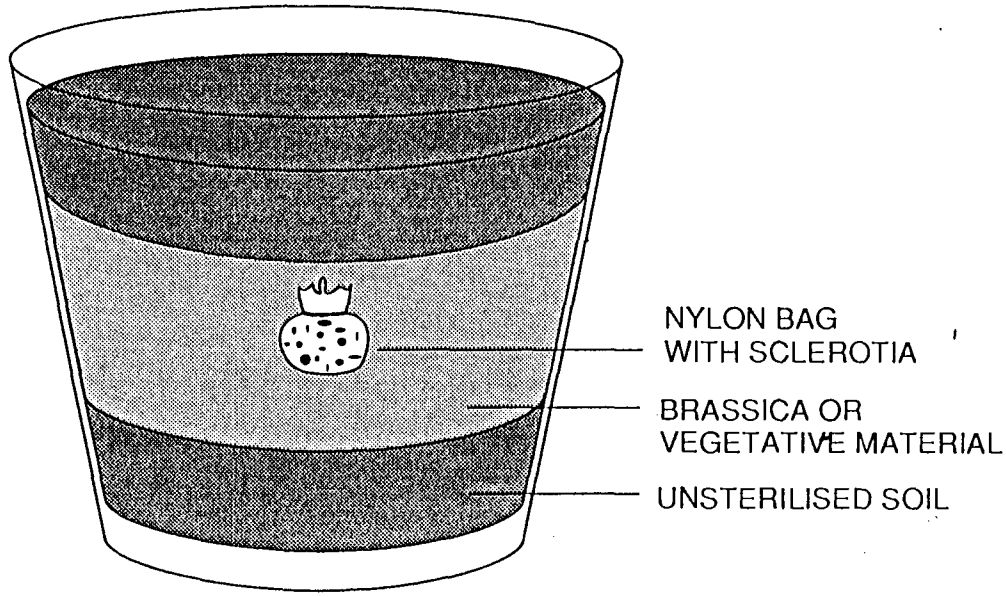


Figure 9: Diagramatic representation of arrangement of nylon mesh bags containing sclerotia of *Sclerotium cepivorum* and plant material in the soil in cups.


NYLON BAG
WITH SCLEROTIA
BRASSICA OR
VEGETATIVE MATERIAL
UNSTERILISED SOIL

After 90 days, the bags were removed from the cups and the contents were analysed for percent recovery and viability of sclerotia. All bags were analysed within a 4 day period. Only intact firm sclerotia were considered for data analysis. Data were subjected to analysis of variance and means were separated using Duncan's Multiple Range test.

Figure 10: Experimental setup showing flooded & nonflooded treatments in a randomized complete block design.

4.3 RESULTS

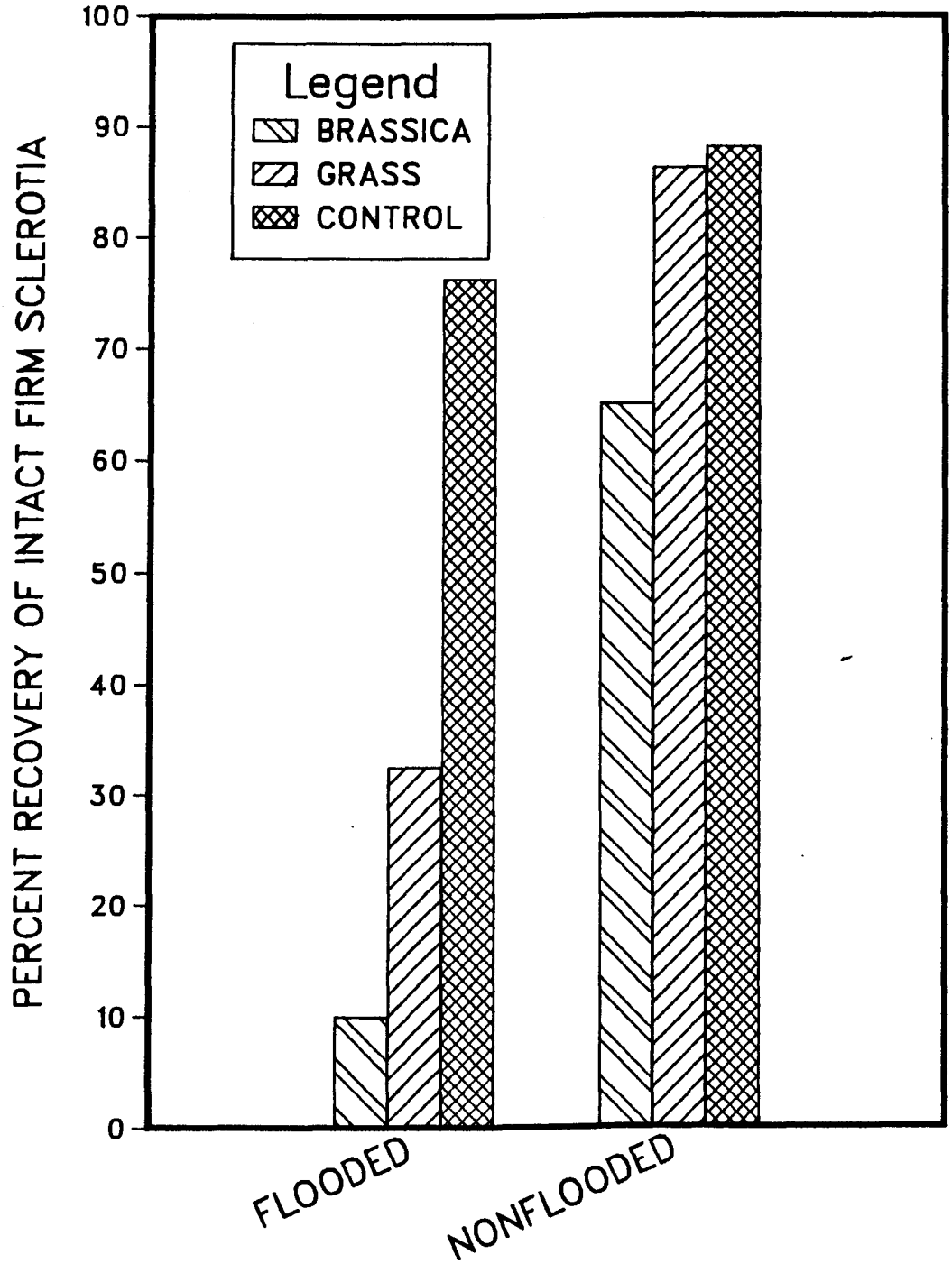
The results of this study are presented in Fig. 11. The percent recovery of intact firm sclerotia decreased significantly ($P<0.05$) within 90 days in all treatments except unflooded unamended and unflooded but amended with indigenous vegetation. The results showed significant effect of flooding and *Brassica* or vegetation incorporation in reducing survival of sclerotia over controls.

This study showed that flooding favours decay or disappearance of sclerotia (Fig. 11). It also suggested that decay of sclerotia was enhanced when some organic matter was added to the flooded pots, in comparison to the nonamended treatment. The results indicate that organic matter from *B. juncea* added in flooded soil significantly decreased the percent recovery of sclerotia. In this treatment the population of sclerotia was reduced to 10% of its original population, where as in nonflooded replication of this treatment, it declined to 64.8%.

There was a clear difference in sclerotial recovery between *Brassica* treated and general vegetation treated pots. Many sclerotia recovered from flooded treatments lost their integrity and were very soft. Some plug germinating sclerotia were also recovered. Viability of sclerotia recovered from all six treatments ranged from 87.5% to 97.8%. Viability of sclerotia at zero time was 100%.

As was found in the field study, most of the recovered sclerotia that did not germinate on potato dextrose agar were contaminated by a bacterium that appeared to be one of the type which survived surface sterlization with NaOCl solution and produced inhibition zones against sclerotia when tested in dual cultures PDA.

Figure 11: Effects of organic amendments on survival of introduced populations of sclerotia of *S. cepivorum* under the influence of flooded or nonflooded soil.


PERCENT RECOVERY OF INTACT FIRM SCLEROTIA
100
90
80
70
60
50
40
30
20
10
0
Legend
BRASSICA
GRASS
CONTROL
FLOODED
NONFLOODED

4.4 DISCUSSION

The use of flooding to control plant diseases was attempted in the muck soils of Southern Florida where the root knot nematode is a problem in vegetable production (Kincaid 1946). Although flooding has been known to cause a rapid decline in the fungal population of the soil (Stover *et al.* 1953), there are only three documented cases of it being used for fungus disease control. The diseases are: tobacco black shank in Sumatra, *Sclerotinia* on celery in Florida and Fusarium wilt of bananas in Central America (Stover 1979; Stover *et al.* 1953).

Sewell (1965) pointed out that the responses of soil fungi to changes in soil moisture are seldom attributable solely to the direct effect of water on the fungus of concern. He stated that inadequate gas exchange, resulting in depletion of O_2 and increase in CO_2 concentration, probably was the primary cause of the inhibition observed in saturated and very wet soils, both low O_2 and high CO_2 concentrations have been shown to inhibit the production of microsclerotia by *Verticillium dahliae* (Ioannou *et al.* 1977). The Chinese have practiced rotation of paddy rice with cotton for control of *Fusarium oxysporum* f. sp. *vasiinfectum* (Cook 1981b). European grape growers have also used flooding to control the *Phylloxera* root aphid (Newhall 1955).

The results of this study provide direct evidence that flooding enhances decay of sclerotia of *S. cepivorum*, and supports the hypothesis of Leggett and Rahe (1985) that flooding is the factor responsible for high rate of decay of sclerotia in local muck soils. Watson (1964) found that addition of several crop residues to *Pyrenochaeta terrestris* infested soils resulted in reduction of the incidence of onion pink root. He pointed out that a combination of a proper residue and anaerobic fermentation was required for eradication. He reported that sphagnum moss and dried soybean were not as effective as green soybean, sweet clover, corn, timothy, alfalfa, oat, straw and sugar beet residues in

eradicating *P. terrestris.* Results from my study also indicate that the presence and nature of organic residue interacts with flooding. Decay of sclerotia of *S. cepivorum* was higher in soil amended with *Brassica juncea* residue compared with general vegetation (Fig. 11). Flooding alone did not reduce the survival of sclerotia to the extent that it did when the soil was amended with organic residue. Valdes and Edgington (1987) studied the effect of crop rotation and flooding on the incidence of white rot and found a significant decrease in flooded plots. They reported that when flooding was followed by rotation with carrots, comparatively better control of white rot was obtained in organic soil than by flooding alone.

The results of the studies conducted here demonstrated that the population of sclerotia was reduced to almost 10% of the original population in pots that were flooded with *Brassica* amendments as compared with flooded pots with grass amendment where the population was reduced to about 32%. It wouls seem that *B. juncea* might have some stimulatory effect on germination of sclerotia, and that its effect is more pronounced when the soil is flooded. It is possible that a combination of these two treatments could provide a good control of white rot in the Fraser Valley muck soil. However, before this technique can be applied in disease management in the field, a detailed study needs to be done in the field by flooding the field followed by crop rotation with *B. juncea.* There is possibility that flooding might have enhanced anaerobic decomposition of *B. juncea* which released allylisothiocyanate and stimulated sclerotial germination in the soil in the absence of the host plant. Another aspect to be considered in this soil experiment is the possible effect of antagonistic or competitive microflora. As has been found in this study a bacterial isolate dominated as sclerotial surface microflora and was antagonistic to *S. cepivorum* in dual culture test; it might be due to increase in this particular bacterium that resulted in enhanced decay of sclerotia.

The decline in recovery of sclerotia over 90 days of intermittent flooding observed in this study could be due to anaerobic conditions due to a depletion of oxygen and/or increase in carbon dioxide, increased antagonism, competition, release of a specific germination stimulant or a combination of all these factors. There seems to be a potential for using *B. juncea* as a rotation crop combined with flooding the field for the control of *S. cepivorum* on onion. As temperature is another important factor influencing the effect of flooding in reducing plant pathogens, a study dealing this relationship needs to be done.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Biological and cultural control techniques have been used effectively to control certain plant diseases (Huang 1980; Papavizas and Lumsden 1980; Curl 1963; Katan 1981; Sewell 1965; and Stover 1979). This area of plant disease control is receiving increasing attention relative to chemical control. Chemical controls are generally expensive, can pose health hazards in handling and product consumption, and may become ineffective after repetitive use if the pathogens develop resistance to them. Reliable alternative methods of disease control are being sought.

Biological and cultural methods have provided effective control of white rot of onions in some cases (Merriman and Borkenhead 1977; Smith 1972c; Utkhede & Rahe 1980) but results have been disappointing in other cases (Merriman *et al.* 1980; Utkhede & Rahe 1982) Germination of sclerotia of *Sclerotium cepivorum*, has been found to be stimulated in field soil by host root exudates (Coley-Smith & King, 1969). Crop rotation with non-host crops does not seem to have much potential for reducing the disease severity because sclerotia of *S. cepivorum* can survive for many years in the absence of the host (Coley-Smith 1979; Crowe *et al.* 1980) Attempts have been made to use onions as a trap crop for control of white rot (Merriman & Issacs 1978) but the results were not very encouraging. However, the persistent population of sclerotia of *S. cepivorum* remains an obvious target for biological/cultural control.

This study evaluated ways to reduce the populations of sclerotia of *S. cepivorum* in naturally infested soil representative of commercial onion producing areas of the Fraser Valley of B.C. using different agronomic practices. An analysis of the effects of *Brassica juncea* grown as a winter cover crop on survival of sclerotia of *S. cepivorum* showed that it reduced the survival of sclerotia significantly. The presence of *B. juncea* might

have stimulated germination of sclerotia which in the absence of a host decayed within a short period of time. Obviously parasitized sclerotia were not recovered and viability of the recovered sclerotia throughout the experiments was greater than 90%. Thus the treatments did not reduce the viability of the remaining sclerotia. They presumably induced germination of sclerotia which, in the absence of a host, decayed rapidly due to lysis or increased activity of antagonists or by some other mechanism. Direct evidence concerning the mechanism of population reduction is lacking, however, and further studies should be conducted to get a better understanding of the specific action of *B. juncea* on *S. cepivorum.*

The results from the survey of the indigenous sclerotial population raise certain questions. They showed that there was a marked reduction of sclerotia of *S. cepivorum* in treated plots between the first and second samplings (Table 1). There was even greater reduction in both treated and control plots between the second and third sampling, such that populations in the treated and control plots did not differ significantly when last sampled in June. The study site was rototilled just before the last soil samples were taken. It is possible that the unexpected precipitous decline in numbers of sclerotia in both treated and control plots between the February and June samplings was due to dilution of surface sclerotia with sub-surface soil as a result of rototilling. If this is true, then it suggests that most of the sclerotia of *S. cepivorum* situated ≥10 cm below the soil surface decay during the winter in our local muck soils. Furthur studies should be directed to this hypothesis, and the effect of rototilling the land to different depths on survival of sclerotia could also be evaluated.

Leggett and Rahe (1985) suggested that the high rate of decay of sclerotia of *S. cepivorum* in Fraser Valley muck soils was presumably due to winter flooding. My study suggests that the sclerotia decayed under the influence of flooding as well as crop residue incoporation from the cover crop. Watson (1964) reported that the addition of several crop

residues to soil infested with *Pyrenochaeta terrestris* resulted in reduction of the incidence of onion pink root. Valdes and Edgington (1987) also reported that crop rotation of onions with carrots reduced incidence of white rot in their muck soils.

The results of a survey done on the level of white rot and other diseases occurring in the onion crop seeded immediately after incorporation of the *B. juncea* cover crop suggested that despite the significant reductions of introduced populations of sclerotia of *S. cepivorum*, white rot incidence was not significantly different in treatment and control plots. The results also showed an unexpected effect of *B. juncea* in reducing smut on onions. Yields of healthy marketable onions did not differ significantly in treated and control plots.

Populations of sclerotia in the onion field and an immediately adjacent potato field did not differ significantly after harvest of these crops, suggesting that the onion crop did not increase the inoculum level in 1987. An unusual observation in the 1987 onion crop was that the bulbs affected with white rot did not have black sclerotia formed on them. Late planting and/or comparatively high summer temperatures in 1987 might have been responsible for this. It is possible that as the fungal mycelium on bulbs developed late in the season, the mycelium might not have had time or experienced the conditions necessary to form sclerotia. It has been generally observed that if the onions are seeded early, white rot shows up early, and if they are seeded late, white rot shows up late in the Fraser Valley (Dr. J.E. Rahe, personal communication). A detailed study dealing with the optimum temperature conditions for white rot development in Fraser Valley muck soils needs to be done.

The effect of intermittent flooding on survival of sclerotia in non-amended and soil amended with *B. juncea* or natural vegetation was assessed. Flooding reduced the survival of sclerotia but the magnitude of this effect was related to the presence and nature of the vegetative amendments. This study did not provide direct evidence regarding the effect

of flooding and *B. juncea* residue incorporation on white rot incidence. Furthur studies on this direction might help to improve our understanding of the interaction of undecomposed organic matter and flooding on white rot in local muck soils.

My results agree with the findings of Valdes and Edgington (1987) who reported that a combination of flooding with crop rotation provided better control of white rot compared to flooding alone. Cook and Papendick (1972) stated that wet soil favours the development of many diseases but completely saturated soil can have a detrimental effect on the survival of many pathogens. Sclerotia of *Sclerotinia sclerotiorum* has been reported to lose their viability within 60 days in saturated muck soil (Brooks 1939). Flooding of banana land to eradicate *Fusarium oxysporum* f. *cubense* in Central America is another example of good disease control (Stover 1979).

My studies show that flooding and incorporation of a specific residue can be used to control the population of sclerotia of *S. cepivorum* in the soil. Garrett (1944) pointed out that adequate control of some pathogens could be accomplished by reduction of the numbers of the pathogen to a low level rather than its complete elimination. The results from this study have clearly shown that flooding enhances sclerotial decay and the effect is significantly greater when the soil is amended with specific crop residues. If the hypothesis that *B. juncea* residue served to enhance germination of sclerotia and hence infection of onion seedling is correct, it is possible that reduced disease might result if the *Brassica* were incorporated in early winter of the preceding year, or if seeding of onions were delayed for one year. Unfortunately I could not follow up with this evaluation as the farmer who provided the experimental plot decided not to grow onions on the study site in 1988.

A considerable reduction of population of sclerotia of *S. cepivorum* could possibly be achieved in Fraser Valley muck soils by growing *B. juncea* as a winter cover crop, incorporating the crop residue in the soil and then flooding the field. Temperature is also

an important factor influencing the interaction of residue amendments and flooding and a detailed study on the effect of flooding and *Brassica* residue incorporation at different temperatures is needed.

Flooding, particularly during the winter months could easily be implemented in the Fraser Valley. *B. juncea* grows well under the local conditions, produces heavy biomass and is an economically feasible crop to be used as a winter cover crop. For practical application of this approach, consideration must be given to the facts that *B. juncea* is highly susceptible to club root (*Plasmodiophora brassicae*), and is a very good host for turnip mosaic virus.

In summary, I have shown that flooding enhances decay of sclerotia of *S. cepivorum*. When specific organic materials are incorporated into the soil which is flooded, a significant reduction in sclerotial populations can be achieved. This effect might be used to reduce yield losses to white rot of onions under our local environmental conditions, although I did not demonstrate this potential in my study. Results from my study are largely preliminary and represent the first initiative taken towards the possible effect of flooding and *B. juncea* on survival of sclerotia of *S. cepivorm* in the Fraser Valley muck soils.

These findings open some new areas for furthur studies. More work is needed to fully understand the mechanism of action of flooding and *B. juncea* on survival of sclerotia of *S. cepivorum*, and the interaction of these factors with temperature. Subsequent research in these areas may lead to a better and more reliable approach to white rot control than presently available.

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