LETTUCE DISEASES CAUSED BY *SCLEROTINIA SCLEROTIORUM* AND *PHYTOPHTHORA PORRI* AND THEIR CONTROL

by

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A thesis submitted to the University of Adelaide
in fulfilment of the requirements for the degree
of Doctor of Philosophy

March 1984

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DECLARATION

This thesis has not previously been submitted for the award of a degree at this or any other University and is the original work of the author, except where due reference is made in the text.

Djiman Sitepu

SUMMARY

The study of vegetable diseases in smallholdings in the Northern Adelaide Plains, South Australia, revealed that lettuce has many diseases which cause substantial yield losses. The most important disease is Sclerotinia rot (*Sclerotinia sclerotiorum*) which is also very destructive to other major vegetable crops in the area. Other important diseases are: Phytophthora stem rot (*Phytophthora porri*), grey mould (*Botrytis cinerea*), downy mildew (*Bremia lactucae*), anthracnose (*Marssonina panattoniana*), and lettuce necrotic yellows (LNYV).

Phytophthora stem rot of lettuce, a disease newly discovered in this project, and causing significant yield losses, presents a serious hazard to growers in the Northern Adelaide Plains. Lettuce crops were the only vegetable plants affected by the disease, and pathogenicity tests in the laboratory and glasshouse confirmed that lettuce was the only host plant for the pathogen. *Phytophthora porri* from lettuce shows some differences from *P. porri* Foister including host plant, optimum temperature for growth and pathogenicity.

A saprophytic fungus *Fusarium lateritium* inhibited the germination of ascospores and the growth of mycelia of *Sclerotinia sclerotiorum*, and protected young plants in pot experiments in the glasshouse. Experimental evidence indicated that *S. sclerotiorum*, its ascospores in particular, requires organic matter as a prerequisite for initial infection of healthy lettuce plants. Results suggested that *F. lateritium* conidia or mycelia inhibited *S. sclerotiorum* on such media.

Several sclerotial parasites and antagonists were found in the soils of the vegetable growing areas. *Coniothyrium minitans*, was isolated from

all sampled fields, *Trichoderma* spp. including *T. harzianum*, *Gliocladium* sp. and *Fusarium* spp. were also abundant. *Trichothecium* sp. was found in only one field. *C. minitans* and *T. harzianum* possibly decompose the sclerotia of *S. sclerotiorum* in the field. One isolate of *Streptomyces* sp. inhibited the growth of *S. sclerotiorum in vitro*.

Environmental conditions such as excessive rainfall, high soil water content, cold and moist air, dense weeds together with plant condition (dense and fully grown), favoured the development of *S. sclerotiorum* which then caused severe damage. In some lettuce fields yields were reduced by more than 85%.

There were indications from a survey of vegetable crops that the incidence of disease might be reduced by: (1) intensive mechanical action by removing infected plants; (2) thorough and clean cultivation; (3) regular preventive spraying with effective fungicides; (4) crop rotation and (5) good soil drainage.

It was concluded that the two important pathogens of lettuce cause considerable yield losses in the Northern Adelaide Plains, and both pathogens were prevalent during winter crops. Improved control can probably be achieved immediately by using fungicides, sanitation and cultural practices. This project has indicated that further control can be achieved by the using of biological control agents such as *F. lateritium* against Sclerotinia rot of lettuce and the use of tolerant cultivars.

ACKNOWLEDGEMENTS

I express my sincere gratitude to Professor H.R. Wallace and Mr. T.J. Wicks, my supervisors, for their encouragement, guidance and criticism during the studies and preparation of this thesis. Also, I acknowledge assistance from Mr. M. Bumbieris and other staff of this Department for their help in this project.

The contribution from the Biometry and Photography sections in the Waite Agricultural Research Institute is also gratefully acknowledged.

Appreciation is also due to vegetable growers who kindly allowed the field surveys and experiments on their properties in the Northern Adelaide Plains and to the staff of the Department of Agriculture, also to the Fruit Growers and Market Gardeners Association of South Australia for their help.

Finally, these studies were made possible by the support of the International Agricultural Development Service (IADS) in co-operation with the Agency for Agricultural Research and Development (AARD) of Indonesia and the Department of Plant Pathology at the Waite Institute. I gratefully acknowledge their support in carrying out this project.

Chapter 1

GENERAL INTRODUCTION

The importance of disease in the vegetable industry has been appreciated for many years (Chupp and Sherf, 1960; Conners, 1967; Sherf, 1968; Channon and Maude, 1971; Morschel, 1975; O'Brien and Rich, 1976; Vock, 1978). Reports by Burdon and Magarey (1976) and Warcup and Talbot (1981) suggest that the vegetable industry in South Australia also faces serious disease problems. However, the extent of the problem has not been defined.

There are two main regions where vegetables are grown in South Australia, the Northern Adelaide Plains and the Adelaide Hills, which supply most of the vegetables for the Adelaide market. The Northern Adelaide Plains in particular is considered ideal for vegetable growing because of its strategic location; it is now the most important area for the production of cabbage, lettuce and onions (Harvey and Tugwell, 1978) and is expected to remain the major supplier for a long time. Other important crops are potato, celery and cauliflower.

The importance of the vegetable industry in South Australia is shown by the fact that it accounted for about 38 percent of the total value of horticultural products from 1971 to 1975 (Burdon and Magarey, 1976). Besides, the area and production of vegetables, lettuce in particular, have been increasing in the last few years (Fig.1).

The first aims of this project were to determine what diseases affected vegetables in the Northern Adelaide Plains, to identify the pathogens causing such diseases and to ascertain whether environmental factors such as soil water, texture, pH and salinity had any influence on the distribution and severity of the diseases.

The next aims were to find out which crop suffered most from disease, to identify the diseases of major economic importance in that crop so that they could be studied in depth and a contribution made to their subsequent control.

The approach adopted in this project was initially to survey vegetable crops in the Northern Adelaide Plains. Such a survey was considered to be an important first step because by studying the relationships between environmental factors and crop health, the major determinants of disease can be identified (Wallace, 1973; 1978; Sitepu and Wallace, 1974). Such an approach requires techniques to be devised for measuring severity of disease. Although such techniques have been used in disease assessment in many crop plants, little work has been done on vegetables (Preece, 1971). Furthermore, because vegetable crops have a high commercial value, it was realized that the economic tolerance level that a grower would accept was likely to be low.

The initial survey in the field was expected to produce useful hypothesis concerning the biology and possible control of major pathogens. To test such hypothesis it was decided to do experiments in the laboratory and glasshouse.

The results of the surveys, field work and experimental work would then be used to suggest possible control measures that could be tested in the field under farming conditions.

The use of integrated control measures, including biological control in particular, was considered to be a desirable goal (Baker and Cook, 1974).

Finally, underlying the general approach of survey work in the field to identify the pathogens causing major diseases, to elucidate the biology of such pathogens and to suggest measures for control, it was hoped that such procedures could be adopted for other crops in other places such as Indonesia, the author's home country.

Chapter 2

FIELD SURVEY

1. Introduction

1.1 *Objective of the field survey*

A field survey was made in the Northern Adelaide Plains (NAP) to understand the significance of diseases and such factors as soil, cultural practices, cropping system and climate on vegetable crops. Growers were asked a series of questions as well as to survey their crops for disease. The survey aimed to define the major problems in the vegetable industry and to provide the basis of my research programme.

1.2 *General descriptions of the areas*

The NAP area, selected for the field study, is largely planted with vegetables including: potato, onion, cabbage, cauliflower, lettuce, celery, carrot, tomato (glasshouses). Plants are irrigated by over-head sprinklers as required. Mean annual rainfall ranges from (410)-435-(550)mm. The land is flat and lies almost at the same height as sea level. Soils are mainly sandy and clay loam (Matheson and Lobbal, 1973-1975).

1.3 *Disease problems and their controls*

Many kinds of diseases caused by fungal, bacterial and virus pathogens have been reported from vegetable crops in South Australia (Talbot, 1964; Burdon and Magarey, 1976; Philp *et al.*, 1976, 1976a,b,c; Hodge, 1976; Fedderson and Philp, 1976; Feddersen, 1978; Warcup and Talbot, 1981) and under certain conditions become serious problems. Current recommendations for control of diseases in the NAP are based on general principles including:

regular sprays with fungicides, crop rotation, good cultivation, removal of infected plants, use of certified seeds and resistant cultivars.

2. The vegetable industry in the NAP

2.1 *History*

The main crops in the NAP are potato (*Solanum tuberosum* L.) lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea* var. *capitata* L. Alef.) onion (*Allium cepa* L. var. *cepa*), cauliflower (*B. oleracea* var. *botrytis* L. Alef.), celery (*Apium graveolens* L.) and carrot (*Daucus carota* L.). Lettuce and cabbage are grown mainly in autumn, winter and spring, while potato is largely produced in winter and to a lesser extent in the summer season. Growers tend to grow more than one crop each season.

The vegetable industry was started by a few growers about 30 yr ago with potato, cabbage and onion. Lettuce was introduced later, about 12 yr ago.

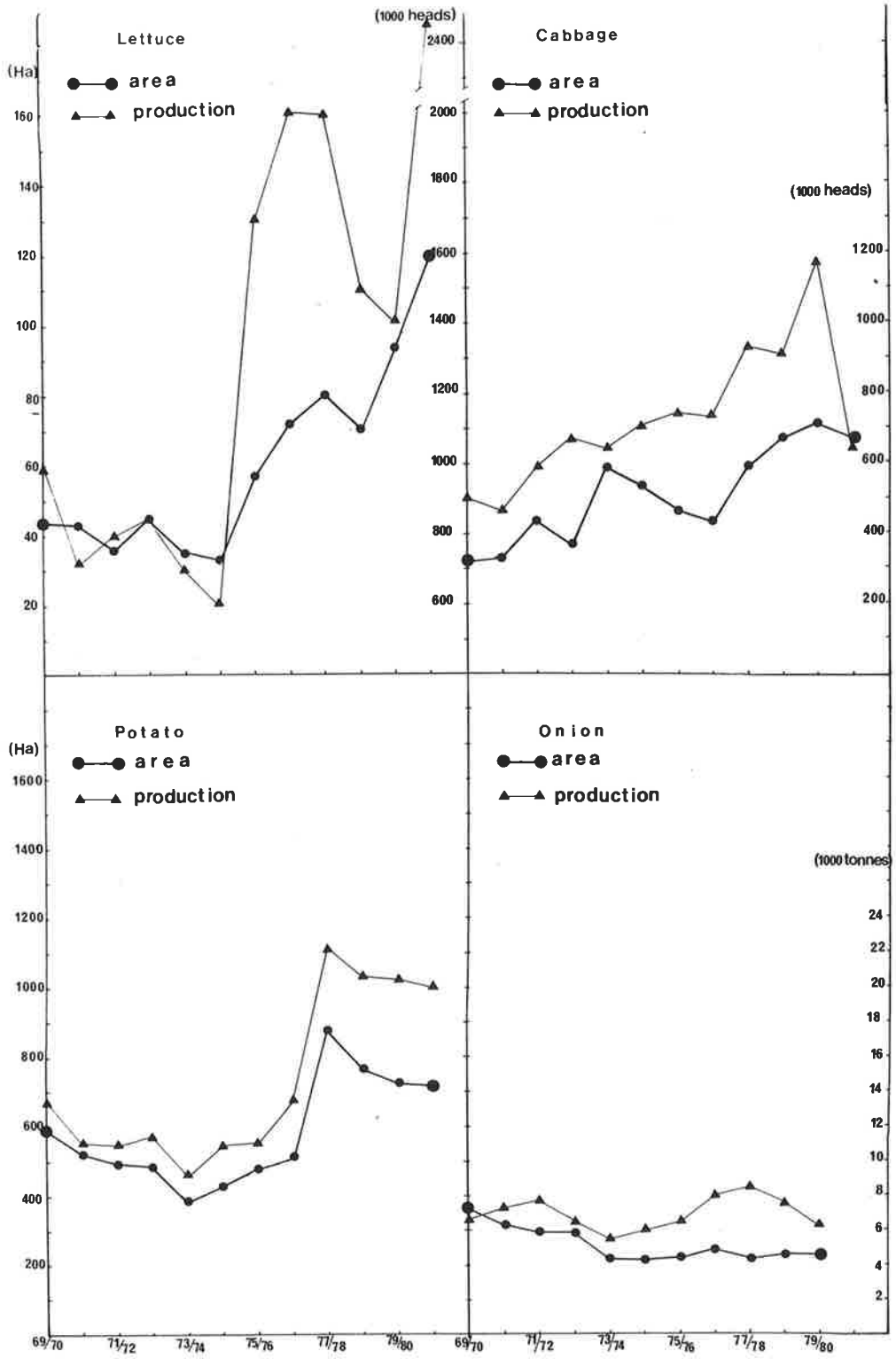
The areas and production of vegetables, lettuce in particular, have been increasing in recent years (Fig.1).

2.2 *Economic importance*

The NAP, is ideal for producing vegetables, being close to market centres. It is a very important area for cabbage, lettuce and onion and is the second most important after the Adelaide Hills Area (AHA) for potato production. The NAP supplies the local requirements for fresh vegetables in Adelaide and surrounding districts. Some of the products are exported to other States.

Fig.1: Area and production of vegetable (1969/1970 - 1979/1980) in Munno Para 1 and Munno Para 2, Northern Adelaide Plains.

(Source of data: Australian Bureau of Statistics, Adelaide).


Lettuce
(Ha)
area
production
(1000 heads)
Cabbage
area
production
(1000 heads)
Potato
(Ha)
area
production
Onion
area
production
(1000 tonnes)
69/70
71/72
73/74
75/76
77/78
79/80

2.3 *Major problems*

The main problem for the vegetable industry in the NAP is disease which causes considerable losses each year. Many important diseases occur continuously during the cool and wet weather every year. Moreover, there is no reliable way to control them.

Other problems are the fluctuation of market prices, which are beyond the control of growers. Thus, occasionally mature crops are not harvested because of very low prices. The quality of water for irrigation is good, but there is a possibility that limitation of underground water supplies may restrict the production of vegetable crops in future years.

2.4 *Agricultural practices*

The common method of cultivation is disc-harrowing. Soon after harvesting, the land is cultivated for preparation of the next crop or cover crop. All plant materials, healthy or infected, together with all kinds of weeds, are buried.

Usually, growers rotate their crops, an important procedure for the management of soil and diseases. The percentage of growers adopting the various rotation programmes are:

Fallow - crop A - crop B - fallow	(26%)
Cover crop - crop A - cover crop - crop B	(32%)
Crop A - crop B - crop C - crop A	(26%)
Fallow - crop A - fallow - crop A/B	(11%)
Without regular rotation	(5%)

Overhead sprinkling irrigation is commonly used in the NAP. Some growers use deep furrows and high ridges for better drainage of heavy soils, a procedure which seems also helps to reduce disease.

3. Materials and Methods

3.1 *Surveying crops for diseases*

Before the survey was made in the field, background information was sought from interviews with growers, a search of the literature and from visits to the vegetable growing areas.

The areas for study were chosen using the following criteria:

- Vegetable crops had been grown for at least 10 years, so that the diseases were likely to be well established.
- Vegetable crops were commercially grown in a large number of fields which made a significant contribution to the economy of local growers.
- There should be a majority of smallholdings, with variation in crops, size of fields, degree of cultivation, control of diseases and time of planting.

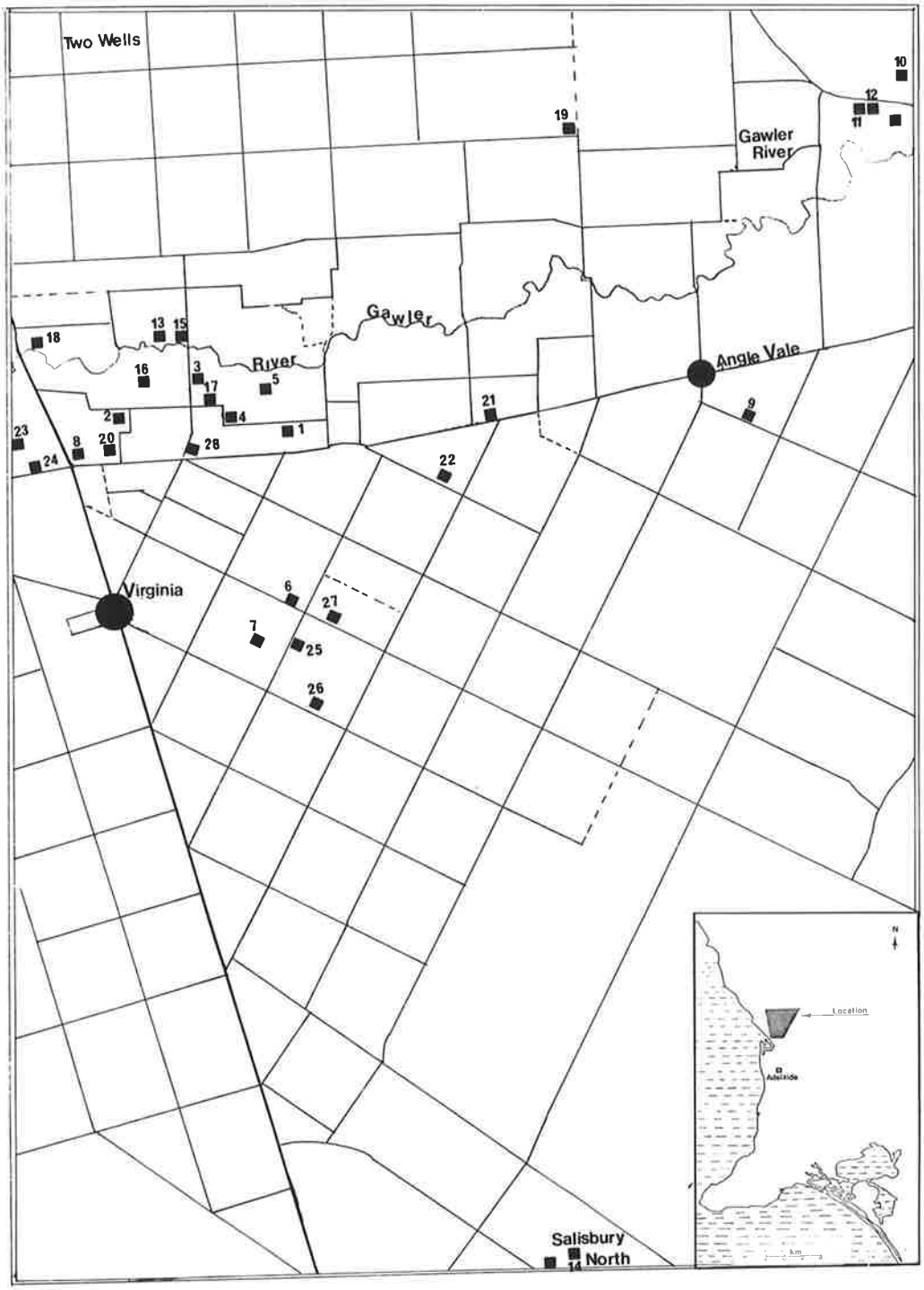
The Northern Adelaide Plains met the requirements hence sites for the study were confined to five sub-areas: Virginia, Angle Vale, Two Wells, Gawler River and Salisbury North (Fig.2).

In addition, visits were made to other areas in Athelstone and the Adelaide Hills Areas.

To collect as complete data as possible a set of questionnaires was prepared. The survey was carried out in the winter and spring of 1980. Four crops were selected for the study of their diseases: potato, cabbage, lettuce and onion. Other factors possibly associated with diseases were: the pattern of disease distribution, soil type, climatic conditions, crop rotation and fertilizer and pesticide usage. The vegetable growers were visited and interviewed.

Fig.2: Locations of study areas in the Northern Adelaide Plains, Virginia, Angle Vale, Two Wells, Gawler River and Salisbury North.

■ = location of diseases observed.


Two Wells
Gawler River
Gawler River
Angle Vale
Virginia
Salisbury North
Location
Adelaide
km
N

3.2 *Investigation, isolation and identification of pathogens*

To investigate and assess the kinds of diseases, their incidence and severity, repeated observations were conducted at several sites in the field (Fig.2). Although most of the diseases were readily identified by visual symptoms it was necessary to take samples for test in the laboratory to confirm diagnosis and toinvestigate those diseased plants where symptoms were unclear.

Isolations of pathogens were made from infected parts of the plants. All isolation routines involved surface sterilization with 1% sodium-hypochlorite for 2 min followed by thorough rinsing in distilled water and then drying. Media for the isolations were corn meal agar (CMA), potato dextrose agar (PDA), V_8 juice agar (V_8A), nutrient agar (NA), and water agar (WA), depending on the kind of pathogen. Selective media were also used for pythiaceous fungi (Tsao and Ocana, 1969). Isolates were incubated at different temperatures.

The identification of fungal isolates was based on morphological structure, mycelial characteristics, fruiting bodies and physiological aspects (Middleton, 1943; Walker, 1952; Barnett, 1955; Waterhouse, 1963; Alexopoulos, 1964; Domsch and Gams, 1972). Isolates that were thought to be pathogenic, were kept on CMA slants, for pathogenicity tests and for stock cultures. ~~Direct examination under the microscope was also made following handcut sectioning of infected plants, clearing and staining.~~ Handcut sections of infected plants were also cleared, stained and examined microscopically.

Important virus diseases of vegetables were recognized by their visual symptoms. Where diagnosis was uncertain samples of plants were brought to the laboratory for identification, by indexing on plant indicators (*Chenopodium* spp., *Nicotiana* spp.), serological tests and by using the electron microscope.

3.3 *Sampling soils from around healthy and diseased plants*

Soil ~~samples were collected~~ was sampled from around healthy and diseased ~~sites~~ plants in lettuce fields to assess various soil factors and to isolate fungi. Approximately 2 kg of soil were collected at each site and divided into sub-samples for the assessment of soil fungi, pH, percent clay, soil water and water potential.

3.3.1 *pH*

pH of soil was measured using a pH meter. Ten g of soil were placed in a 50 ml plastic container and 25 ml of 0.01 M CaCl solution was added, stirred several times for about 10 min and allowed to stand for 1 hr before it was measured by immersing the glass electrode of the pH meter. Two measurements were made per sample.

3.3.2 *Soil texture*

Soil texture was measured by using the hydrometer method to determine the proportions of clay, silt and sand. Fifty g of air-dried soil were put into 200 ml of distilled water, 20 ml of 10% Calgon solution and 3 ml of normal NaOH. The soil suspension was agitated vigorously for 20 min in an aluminium container, washed into a sedimentation cylinder and the volume made up to 1 L with distilled water. Measurements with the hydrometer were made at 5 min and 5 hr respectively after sedimentation began. The value at 5 hr gave the percentage of clay particles, then the percentage of silt and sand particles was calculated. Correction was made for temperature during the measurements.

3.3.3 *Water potential*

Water potential of soil was measured by using the filter paper method (Fawcett and Collis-George, 1967). Whatman No.42 filter paper was cut into 2 cm and 3 cm square pieces. To prevent damage by soil microorganisms, the filter papers were washed in a solution of 0.005 mercuric chloride and then dried at 90°C for 2 hr. Plastic vials 45 mm in diameter and 55 mm deep were used for containers. Half of the vial was filled with soil and then 3 pieces of the filter paper, with the smaller size in the middle, were placed on the soil and the rest of the soil placed on top, pressed firmly and then the container was closed tightly. Soil samples were kept at 25°C for 7 days, before the middle paper was recovered and its water content was assessed by weighing. By using the "Wetting characteristic of Whatman No.42 filter paper graph" (Fig.3), the corresponding water potential was calculated.

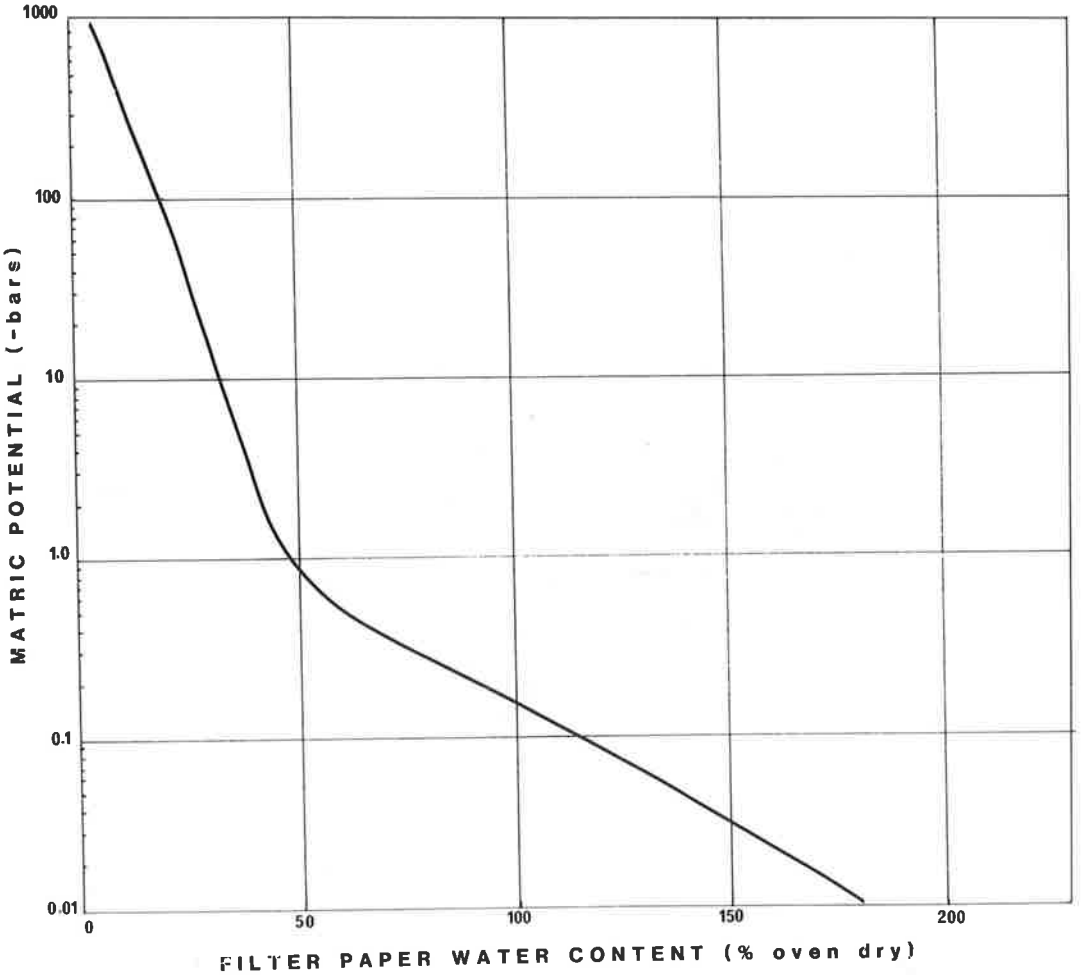
3.3.4 *Soil water*

Fifty g of soil were dried in the oven at 96°C for 24 hr, to allow the water content to evaporate. Loss of weight was calculated as a percentage of dry weight of soil.

3.3.5 *Isolation of fungi*

The isolation of fungi from soil was done mainly for *Phytophthora* and *Pythium*, by using the technique of baiting (McIntosh, 1964). A subsample of about 150 g of soil was placed in a 500 ml plastic container, flooded with rain water and a pear was put into each container. The sample was incubated at room

Fig.3: Graph of wetting characteristics of Whatman No.42 filter paper.


1000
100
10
1.0
0.1
0.01
MATRIC POTENTIAL (-bars)
0
50
100
150
200
FILTER PAPER WATER CONTENT (% oven dry)

temperature (15-20°C). Infection of the pear was recognized by a brown to black colour of the skin, usually occurring at water level. All isolations from the infected pears, were done in CMA and incubated at 20°C.

3.4 *Assessing plants for diseases and yield loss in commercial lettuce fields*

The incidence of diseases and crop losses were estimated by a random sampling technique. A number of rows of plants in a unit of area were sampled, using the table of random numbers. Plants showing symptoms of disease were recorded while walking along a row to be sampled and where necessary, specimens of plant materials were taken for further investigation in the laboratory. Plants were deemed to be dead when infection was so advanced that plants would not be harvested.

4. Results and Discussion

4.1 *Diseases of vegetable crops in the NAP*

The field survey revealed that many growers in the NAP suffered crop losses due to diseases, many of which caused serious problems. Of the four crops surveyed, lettuce was the most severely affected by many kinds of diseases and seemed very susceptible to numerous pathogens. Crops were commonly infected by more than one pathogen. Table 1 shows the pathogens that were found and the diseases they cause on potato (Fig.8), lettuce (Figs.5 and 6), cabbage (Fig.7) and onion in the NAP.

There were few diseases of roots that caused significant damage. The most common root pathogens found from isolations were *Pythium* spp., *Rhizoctonia* spp., *Fusarium* spp. and *Stemphylium* sp.

4.1.1 *Fungal diseases*

Major diseases of the four crops in the NAP were caused by fungal pathogens. There were 5 pathogens that caused significant loss of yield: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Fr., *Phytophthora porri* Foister, *Bremia lactucae* Regel and *Marssonina panattoniana* (Berl.) Magn. Sclerotinia rot caused by *S. sclerotiorum*, was responsible for serious yield losses in lettuce, potato and cabbage. Lettuce was very severely affected, potato and cabbage were moderately affected. *S. sclerotiorum* was found in almost all fields of lettuce although its incidence varied from field to field (Table 2). In one field of lettuce, the incidence was so high, that the crops were not harvested.

Table 1: Results of disease investigation in commercial fields of vegetable crops in the Northern Adelaide Plains

Name of pathogens	Name of diseases in each crop			
	Potato	Lettuce	Cabbage	Onion
Fungi:				
Sclerotinia sclerotiorum	Sclerotinia rot	Sclerotinia rot	Sclerotinia rot	
Botrytis cinerea		grey mould		
Bremia lactucae		downy mildew		
Marsonnina panattoniana		anthracnose		
Phytophthora porri		stem rot		
Peronospora parasitica			downy mildew	
Peronospora destructor				downy mildew
Verticillium solani	wilt			
Alternaria solani	target spot			
Alternaria alternata				black bulb scale
Rhizoctonia solani	black dot		leaf rot	
Rhizoctonia sp.				damping off
Septoria sp.		leaf spot		
Stemphylium botryosum		leaf spot		leaf spot
Mycosphaerella brassicicola			ring spot	
Colletotrichum cocodes	black dot			
Helminthosporium solani	silver scurf			
Spongospora subterranea	powdery scab			
Aspergillus niger				black mould
Phoma lingam			black leg	
Pyrenochaeta terrestris				pink rot
Sclerotium cepivorum				white rot
Viruses:				
Lettuce necrotic yellows virus (LNYV)		Lettuce necrotic yellows		
Potato leaf roll virus (PLRV)	Potato leaf roll			
Potato virus X (PVX)	Potato virus X			
Tomato spotted wilt virus (TSWV)	Tomato spotted wilt	Tomato spotted wilt		
Alfalfa mosaic virus (AMV)	Alfalfa mosaic	Alfalfa mosaic		
Big Vein Virus (BVV)		Big vein		
Lettuce mosaic virus (LMV)		Lettuce mosaic		
Bacteria:				
Erwinia carotovora	black leg	soft rot	soft rot	

Table 2: The incidence of lettuce diseases, observed in commercial fields in NAP during the survey in 1980

Location and number of field (1)		Estimated percentage incidence						
		Phytophthora rot (2)	Sclerotinia rot	Grey mould	Anthracnose	Downy mildew	LNYV	Other diseases (3)
Virginia	1	0	4	1	7	1	17	1
	2	0	10	30	3	2	<1	1
	3	<1	2	<1	4	2	1	2
	4	0	<1	<1	30	60	3	1
	5	1	<1	<1	0	40	45	1
	6	0	4	3	5	10	2	<1
	7	0	3	2	5	11	3	<1
	8	0	4	3	0	2	10	<1
Angle Vale	9	0	2	<1	15	4	12	4
Gawler River	10	0	7	4	25	<1	<1	<1
	11	0	78	2	2	3	<1	2
	12	35	<1	<1	3	5	<1	2
Two Wells	13	0	1	1	0	5	3	1
Salisbury North	14	0	1	1	2	20	2	2

(1) - Map in Fig.2, shows the areas corresponding to each number
(2) - In winter 1981, more were found in Virginia
(3) - Minor diseases, such as: big vein virus, lettuce mosaic virus, leaf spots, bacterial wilt.

4.1.2 *Bacterial disease*

Bacterial diseases such as soft rot of lettuce (Fig.5c) and cabbage (Fig.7d,e) and black leg of potato, were rarely found during the survey. They mainly occurred in autumn, spring and summer crops. They were not considered as major problems in the NAP, as shown in Tables 2 and 3.

4.1.3 *Virus disease*

There were some viruses that caused serious diseases of lettuces (Lettuce necrotic yellows virus-LNYV, Big vein virus-BVV), and potato (Potato leaf roll virus-PLRV). Other virus diseases are shown in Table 1. LNYV was prevalent in autumn and spring seasons, while BVV was very common in winter crops. PLRV (Fig.8b) was found in summer and winter crops of potato.

4.1.4 *Other apparent abnormalities*

In addition to diseases caused by pathogens, there were abnormalities due to climatic factors. Usually, the symptoms were internal and the affected plants appeared normal from the outside, e.g. hollow-heart of potato and brown-lesion and brown-rib of lettuce (Fig.6e). These abnormalities are called physiological diseases and are not infectious. Lipton (1963) found that lettuce exposed to daytime temperatures of 30°C or more for 2 consecutive days before harvest gave brown-rib inside the lettuce head.

4.1.5 *Isolation of fungi and analysis of soil*

Isolation of *Phytophthora porri* and *Pythium*

Using green pears as bait for *Phytophthora porri* was not

Table 3: Diseases of lettuce, estimated yield loss and degree of control in commercial fields (1978, 1979, 1980)

Pathogen	Disease distribution	Estimated yield loss (%)		Degree of control achieved
		Grower's estimation (1978, 1979)	Survey (1980)	
Sclerotinia sclerotiorum	patchy, odd plant	2-85	1-80	none/poor
Phytophthora porri	patchy, odd plant	not known	1-35	not known
Botrytis cinerea	patchy, odd plant	2-40	1-25	none/poor
Bremia lactucae	patchy, large	2-20	1-20	fair
Marssonina panattoniana	patchy	1-30	1-25	poor/fair
LNYV	patchy, odd plant	1-55	1-40	none/poor
Erwinia carotovora	odd plant	1-8	1-2	none/poor
Other pathogens (minor importance)	patchy, odd plant	1-8	1-2	fair

successful, possibly because pear is an unsuitable host for the fungus, or the time of baiting was not favourable for the fungus to infect the green pear. Some *Pythium* spp. were isolated from infected pears but they were not considered to be important pathogens in any of the four crops surveyed. However, there are at least three species of *Pythium* which cause root rot and stunting of young lettuce plants, namely *P. uncinulatum*, *P. tracheiphilum* (Blok and van der Plaats-Niterink, 1978) and *P. polymastrum* (Coplin, *et al.*, 1980). Other species which have been consistently isolated from roots of lettuce are: *P. vexans*, *P. ultimum* and *P. irregulare* (Coplin *et al.*, 1980).

Soil texture

The analysis of soil texture carried out in the laboratory, indicated that the majority of soils in the NAP were sandy loams and loamy sands (Fig.4). A map of the "suitability of land for irrigation" (Matheson and Lobbal, 1973-1975) shows that most of the land used for vegetable production is sandy topsoil over permeable clay or clay loam, conditions which are eminently suitable for growth.

The association of soil factors with healthy and diseased patches

Results of soil analyses for clay content, pH, soil moisture content and water potential, suggested that patches with diseased plants were slightly but significantly wetter than patches of healthy plants (Table 4). By using the "paired-sample test" for analysis it was found that there was a significant correlation between diseased patches and low water potential ($P<0.05$) and between diseased patches and high soil moisture content ($P<0.05$). These results suggest that the wetter the soil the more likely for diseases to develop.

Table 4: Clay content, pH, water potential and moisture content of soil in healthy (H) and diseased (D) patches in some commercial lettuce fields in the Northern Adelaide Plains

Location and number of field		Clay (%)	pH		Water potential (-bar)			Moisture content (%)		
		D&H	D	H	D	H	(H-D)	D	H	(D-H)
Virginia	1	19.2	7.3	7.4	0.11	0.12	(0.01)	14.3	9.4	(4.9)
	2	20.3	7.3	6.9	0.11	0.12	(0.01)	12.3	12.2	(0.1)
	3	25.5	7.2	7.2	0.05	0.10	(0.05)	18.6	17.9	(0.7)
Angle Vale	1	13.2	6.6	5.2	0.02	0.05	(0.03)	13.0	13.2	-(0.2)
	2	13.2	5.8	5.5	0.04	0.04	(0.00)	12.0	13.3	-(1.3)
	3	NA	6.2	7.1	0.03	0.07	(0.04)	14.8	12.2	(2.6)
Gawler River	1	20.2	5.3	5.3	0.28	0.48	(0.20)	23.0	16.1	(6.9)
	2	23.4	5.1	5.0	0.05	0.24	(0.19)	16.0	16.1	-(0.1)
	3	13.6	6.1	7.5	0.05	0.04	-(0.01)	11.5	11.5	(0.0)
	4	14.9	6.6	7.3	0.09	0.09	(0.00)	12.4	9.3	(3.1)
	5	20.7	5.7	6.9	0.05	0.08	(0.03)	18.9	16.0	(2.9)
Mean							(0.05)			(1.79)
t value							2.241*			2.281*

*t values, significant at the 5% probability level, based on "paired-sample-test" analysis.

Fig.4: The composition of the textural classes of soils as used by U.S. Soil Survey (Griffin, 1972).

▲, indicates the results of soil analysis of 25 different locations at Virginia, Angle Vale and Gawler River, NAP.

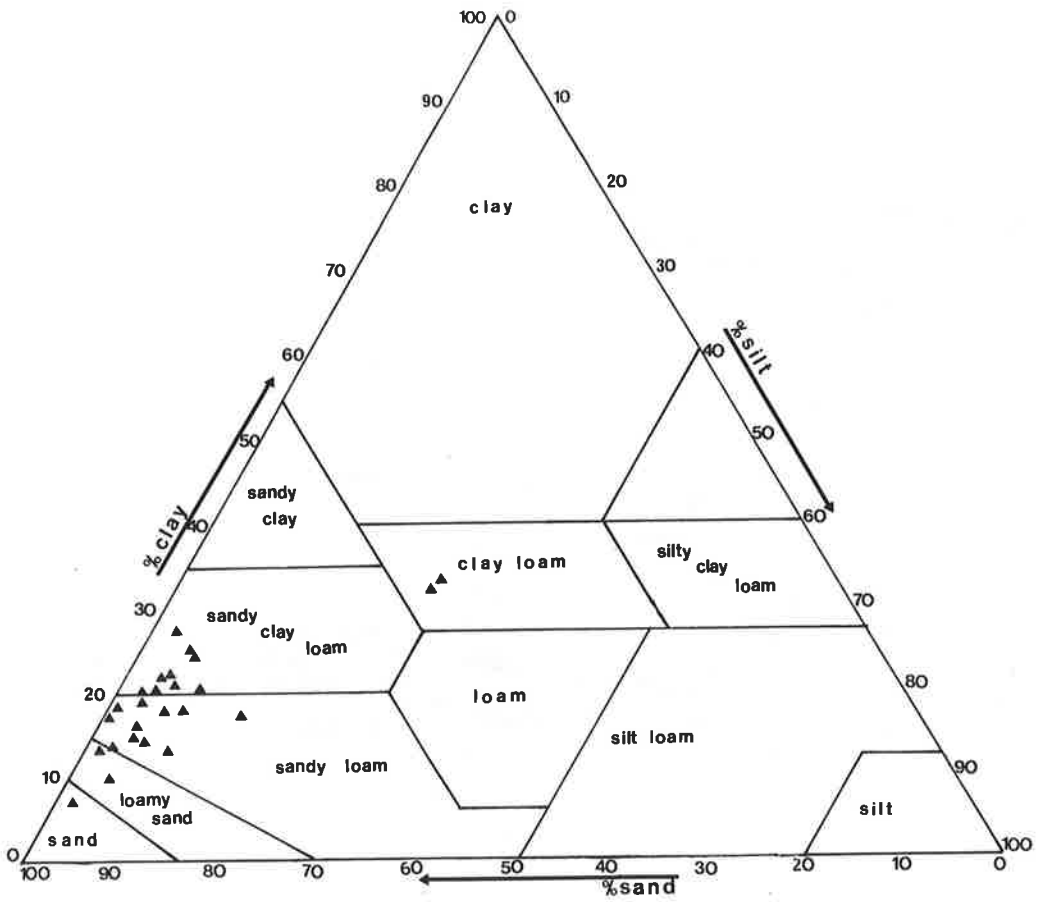
Note:

Soil fractions according to U.S. Soil Survey -

Sand ..... 2.0 - 0.05 mm

Silt ..... 0.05 - 0.002 mm

Clay ..... < 0.002 mm


clay
sandy clay
clay loam
silty clay loam
sandy clay loam
loam
silt loam
sandy loam
loamy sand
sand
silt
%clay
%silt
%sand
100
90
80
70
60
50
40
30
20
10
0

4.2 *Disease management applied by growers*

The purpose of disease management is to control or eliminate the causal factors, using resistant or tolerant cultivars, fungicides, crop rotation and appropriate cultural practices.

4.2.1 *Spraying with chemicals*

Fungicides, usually incorporated with insecticides, must be used regularly, because vegetables produce high value crops ~~with~~ have a short period of growth and ~~with~~ are affected by diseases which are often caused by several factors. Consequently vegetable crops need continuous care from the time of emerging until harvesting. Aerial spraying after the plants are large and bushy has limited effect because the chemicals do not reach the lower parts of plants which are subject to many important diseases such as: Sclerotinia rot, grey mould, stem rot, anthracnose and downy mildew. Different fungicides were used by growers (Table 5) according to manufacturers' guide lines, often in conjunction with intensive cultivation.

4.2.2 *Agricultural practices*

The source of most vegetable diseases is the soil and plant debris, so, theoretically at least, control should be achieved by using disease-free soil and good sanitation. In practice, there is very little chance to achieve pathogen-free soils and intervals between crops are too short for crop rotation. Intercropping of various vegetables can maximize land use, but may lead to the localized spread of some diseases, as well as allowing diseases to persist as most vegetable crops are infected by the same pathogen and get similar diseases, such as Sclerotinia rot and bacterial wilt.

Table 5: Fungicide usage on lettuce to control diseases in commercial fields, in NAP

Name of fungicide	Use for control(1)							Percent grower
	Php(2)	Ss	Bc	Mp	Bl	Ls	Gp	
Bromomethane (Methyl bromide)							+	5.56
Pentachloronitrobenzene (PCNB)							+	5.56
Chlorothalonil (Bravo)		+	+	+	+	+		33.33
Mancozeb (Dithane M-45)		+	+	+	+	+		55.56
Mezineb (Antracol)		+	+	+	+	+	+	11.11
Bordeaux mixture (Bordeaux)					+	+		5.56
Metalaxyl (Ridomil)		+	+		+	+	+	11.11
Benomyl (Benlate)		+	+		+	+	+	22.22
Maneb (Dithane M-22)						+		22.22
Copperoxychloride (Cuprox)					+	+		5.56

(1) Php = Phytophthora rot; Sc = Sclerotinia rot; Bc = grey mould; Mp = anthracnose; Bl = downy mildew; Ls = leaf spots; Gp = general purpose.

(2) None was known against Phytophthora rot, because the disease was found very recently.

Other cultural practices applied by growers in the NAP were to maintain fertility by applying chemical and organic fertilizers and lime, and to develop good soil drainage with deep furrows. Early and late planting of potatoes was another procedure used by growers to reduce the incidence and severity of diseases.

4.2.3 *Certified seed*

Many kinds of pathogens are seed borne and so infected seed becomes the source of many virus and bacterial diseases. Growers are advised to use certified seed from seed merchants or authorized growers.

4.3 *Yield loss due to certain diseases*

Observations indicated that various diseases differed in their effects on the plant and on yield. For example, a low incidence of Sclerotinia rot, Phytophthora stem rot, grey mould, LNYV or bacterial rot, often caused significant losses, because they destroyed the plants at any stage. In contrast, a high incidence of leaf spot, target spot, downy mildew, often caused only slight losses in yield. It was observed that *Sclerotinia sclerotiorum* on lettuce caused death, while on cabbage the same pathogen was far less damaging because, up to a certain stage of infection, damage was limited to the outer leaves of the mature cabbage. The incidence of diseases and the estimated yield losses of lettuce in the NAP, are summarized in Tables 2 and 3.

4.4 *Disease problems and growers' response*

It appears that disease is the most significant problem for the vegetable industry in the NAP. At present there is no reliable way of

eradicating or controlling important diseases such as Sclerotinia rot, grey mould, anthracnose, Phytophthora stem rot, etc, mainly because the pathogens persist in the soil along with plant debris. It was also noticed that minor diseases of major crops or major diseases of minor crops because of their low economic importance received less attention thereby causing an accumulation of inocula and so an increase in infectious potential of several diseases.

Although growers achieve some control of disease in their vegetable crops, the main effort is largely confined to crops of high value.

Generally, growers are keen to seek and to follow new methods developed for disease management. The information may come from different sources, such as: the Department of Agriculture, Growers' Associations and Pesticide Companies.

4.5 *Some important diseases in commercial lettuce fields*

The most prevalent diseases in commercial lettuce fields are Sclerotinia rot, grey mould, downy mildew, anthracnose, LNYV and Phytophthora stem rot which is described for the first time in this thesis.

4.5.1 *Sclerotinia rot or Lettuce drop*

Sclerotinia rot is the most dangerous disease in the NAP, on many kinds of vetegable crops. Purdy (1979), stated that the disease caused by *Sclerotinia* spp. is non-specific, more than 350 species of 60 families are known as hosts. The initial infection in lettuce can originate from sclerotia in the soil and from ascospores produced in apothecia of sclerotia (Adams and Tate, 1975; Adams and Ayers, 1979; Coley-Smith and Cooke, 1971). Usually infection is initiated from hyphae from sclerotia and ascospores in organic matter. The ascospores become airborne when released from

apothecia and are readily dispersed to alight on a healthy plant to cause a new infection. Infection and the spread of disease are stimulated by favourable conditions such as high soil moisture, rainfall, cool and wet weather and dense crops. The symptoms of the disease in lettuce are easily recognized. Normally, infection starts at the base of outer leaves at soil level, and then travels to the stem. Soon, the plant becomes soft and decayed and white mycelia are produced, followed by the formation of abundant black sclerotia (Figs. 5a,b). The plants can be infected at any stage, but more commonly after heads are formed. In the NAP, Sclerotinia rot was very destructive in winter.

Sclerotia of *S. sclerotiorum* in the soil and in new infected plants become sources of inocula. Approximately 90 percent of its life is in the form of sclerotia in soil (Adams and Tate, 1975). That is why, attempts to control the disease mainly depend on the success of destroying the sclerotia which may remain viable in soil for 4-10 years (McLean, 1958; Williams and Western, 1965; Adams and Ayers, 1979). The incidence and pattern of infection vary, depending on local conditions, from single plants to large patches of many plants. Yield loss can reach 85 percent.

4.5.2 *Grey mould*

Grey mould of lettuce is caused by *Botrytis cinerea*. Superficially, the symptoms of grey mould disease are similar to those caused by Sclerotinia rot, as the infected plants collapse on the soil surface. However, close examination enables the two diseases to be distinguished. Very often they are found in the same patch. An early symptom of grey mould disease is stunted

growth followed by yellowing of the outer leaves. When the infection reaches the leaf base and stem, the plant wilts and dies soon after (Smith, 1900; Campbell, 1949; Walker, 1952). The necrotic parts are a grey to greyish brown colour. *B. cinerea*, usually sporulates abundantly on the surface of infected plants and underneath the leaves (Fig.5f). The air-borne conidia are the most important agents for infection. Stall (1962) found that by applying lime to soil, grey mould of potato was reduced, indicating that soil conditions can influence the progress and severity of disease.

4.5.3 *Downy mildew*

Downy mildew in lettuce caused by *Bremia lactucae*, is widespread in commercial lettuce fields in the NAP. The pathogen can cause disease in about 30 genera with 180 species of Compositae, 24 of which are species of genus *Lactuca* (Crute and Davis, 1977). This disease was observed to be prevalent from late autumn through to late spring, during which time lettuce was grown on a large scale in the area. Infection can occur at any stage of plant growth, but is more prevalent after plants are fully grown. The under surface of infected leaves are covered with a heavy downy growth, a typical and clear symptom of the disease. Dew has been reported to play an important part in the development of downy mildew disease by aiding infection (Crute and Davis, 1977). Chlorotic or yellowish-brown spots on leaves are distinct symptoms on lettuce. It can be so abundant that leaves become dry and brittle and soon die. Usually well-exposed leaves are less severely infected.

4.5.4 *Anthracnose*

Anthracnose of lettuce caused by *Marssonina panattoniana*, is characterized by a reddish brown to purple discolouration, mainly on the lower sides of leaves. In the advanced stage, large lesions develop. Abundant accumulated lesions along the vein and stalk of the leaf cause infected leaves to turn yellow. The infection starts at the bases of outer leaves and moves ~~upward~~ inwards into younger leaves (Figs.6c,d). Anthracnose is limited to lettuce plants. It had been tested on 35 genera and over 38 species of Compositae (Couch and Grogan, 1955); only *Bellis perennis* (English daisy) and *Lactuca* spp. were susceptible. Anthracnose can spread very rapidly under favourable conditions, such as wet and sunny days. Figure 6c shows how destructive anthracnose can be; the crop shown in the Fig.6c was a write-off.

4.5.5 *Phytophthora stem rot*

The stem-rot disease caused by *Phytophthora porri* (Sitepu and Bumbieris, 1981), is characterized by wilting of the whole plant (Fig.5d). Close examination of the affected plants revealed that the stem at ground level was already infected by the pathogen which caused a brown to black discolouration (Fig.5e). The symptoms of infection were not noticed in roots or on leaves of the lettuce. This pathogen was first isolated from a commercial field in Gawler River, north of Adelaide, where I first found the disease. Later, I found it in other lettuce fields in Virginia.

4.5.6 *Lettuce necrotic yellows*

The disease was first reported in Australia in 1963 (Stubbs

and Grogan, 1963). Before it was confirmed as LNYV it had already existed in lettuce in Victoria where it caused considerable losses in autumn and winter crops.

In the NAP, the disease was very occasional in winter, but very prevalent in autumn and spring when it was very destructive in some fields. Table 3 shows that the loss in yield can be up to 40%. The virus is transmitted by the aphid *Hyperomyzus lactucae* (Stubbs and Grogan, 1963; Randles and Carver, 1971). Infections become more severe when conditions are favourable for the vector. When a plant is infected, the colour of the leaves changes rapidly from green to dull green, then yellow; in the final stages most of the leaves are necrotic (Fig.6a). High temperature appears to stimulate wilting and death. In mature plants there is an internal slimy rot.

4.5.7 *Big vein*

Scattered lettuce plants with big vein caused by BVV were found in some fields but damage was not severe. Some of the infected plants could still form heads. Big vein virus is transmitted by a soil-borne fungus *Olpidium brassicae* (Campbell *et al.*, 1980). The ensuing disease causes plants to grow upright and to have thicker leaves with clear veins (Fig.6b).

4.5.8 *Minor virus diseases*

Minor virus diseases were also noticed such as lettuce mosaic virus, tomato spotted wilt virus and alfalfa mosaic virus. The latter has not previously been reported in South Australia.

4.6 *Questions that arose during the survey*

Under natural conditions, diseases showed differences in distribution and severity. However, the nature and cause of the variations were not clear. The question to be answered is, which biotic and abiotic environmental factors have the greatest influence on the incidence and severity of disease and to what extent does inoculum potential play a part.

Outbreaks of disease in vegetable crops that frequently occur suddenly following a change in the weather can best be controlled by effective fungicides. Decisions whether to use fungicides and insecticides or not, should be supported by continuous experiments and a disease monitoring system, so that a fungicide is used at appropriate times on the right target to maximize control and to minimize side effects and costs.

Crop rotation, good cultivation and removing diseased plants probably increase control of vegetable diseases, but to what degree such agricultural practices should be employed is a difficult question as the cost of operation is high and laborious.

5. Summary and Conclusions

Observations in the Northern Adelaide Plains indicate that intensification of vegetable growing has accelerated the build up of soil-borne pathogens and created significant problems. Some of the most destructive diseases are: Sclerotinia rot, grey mould, downy mildew, anthracnose, Phytophthora stem rot and Lettuce necrotic yellows.

Lettuce plants from seedling to mature stages, seem to be very vulnerable to many kinds of diseases. Yields were reduced in many fields, losses ranging from 1 to 85 percent.

Many growers were concerned about these seasonal problems of disease and were anxious to seek better methods of disease management. There were indications that disease management by spraying, crop rotation, sanitation and deep ploughing, reduced the incidence and severity of Sclerotinia rot, grey mould and anthracnose. The use of biological agents and tolerant cultivars had not been tried.

Phytophthora stem rot of lettuce, a disease newly discovered in this project, causing significant losses, was found in commercial fields in Virginia and Gawler River. It presented a serious potential hazard to growers.

The two most important diseases: Sclerotinia rot (*Sclerotinia sclerotiorum*) and the new Phytophthora stem rot (*Phytophthora porri*) of lettuces were selected for further study as they presented some useful biological questions and they were both of economic importance.

Fig.5:

a. Lettuce crops infected by Sclerotinia rot, were left unharvested

b. Typical symptoms of Sclerotinia rot in lettuce, with white mycelia and black sclerotia of *S. sclerotiorum*

c. Bacterial rot of lettuce

d. Field view of wilting lettuce plants caused by Phytophthora stem rot

e. Cross-section of infected stem, with brown to black discolouration, caused by Phytophthora stem rot

f. Grey mould on lettuce. Notice the sporulation of the pathogen *B. cinerea*.


a
b
c
d
e
f

Fig.6:

a. Lettuce necrotic yellows caused by LNYV in a commercial lettuce field in Virginia

b. Big vein of lettuce caused by BVV. Typical symptoms: thicker and smaller leaves and vein clearing

c. Young lettuce plants must be removed because of severe infection by anthracnose

d. A leaf showing typical lesions of anthracnose

e. Brown lesions and brown rib of mature lettuce, caused by hot summer weather. Usually the symptoms are noticed at harvesting time.


a
b
c
d
e

Fig.7:

a. Cabbage, fully covered by infected leaves with black sclerotia of *S. sclerotiorum*

b. Cross-section of infected cabbage shown in (a) the inner part is healthy and edible

c. Black leg of cabbage, caused by *Phoma lingam*

d. Young cabbage infected by bacterial soft rot *(Erwinia carotovora)*

e. Cross-section of infected cabbage (d), showing soft rot of stem and head

f. Rhizoctonia leaf rot on cabbage

g. A simple pathogenicity test with *Rhizoctonia* isolate.

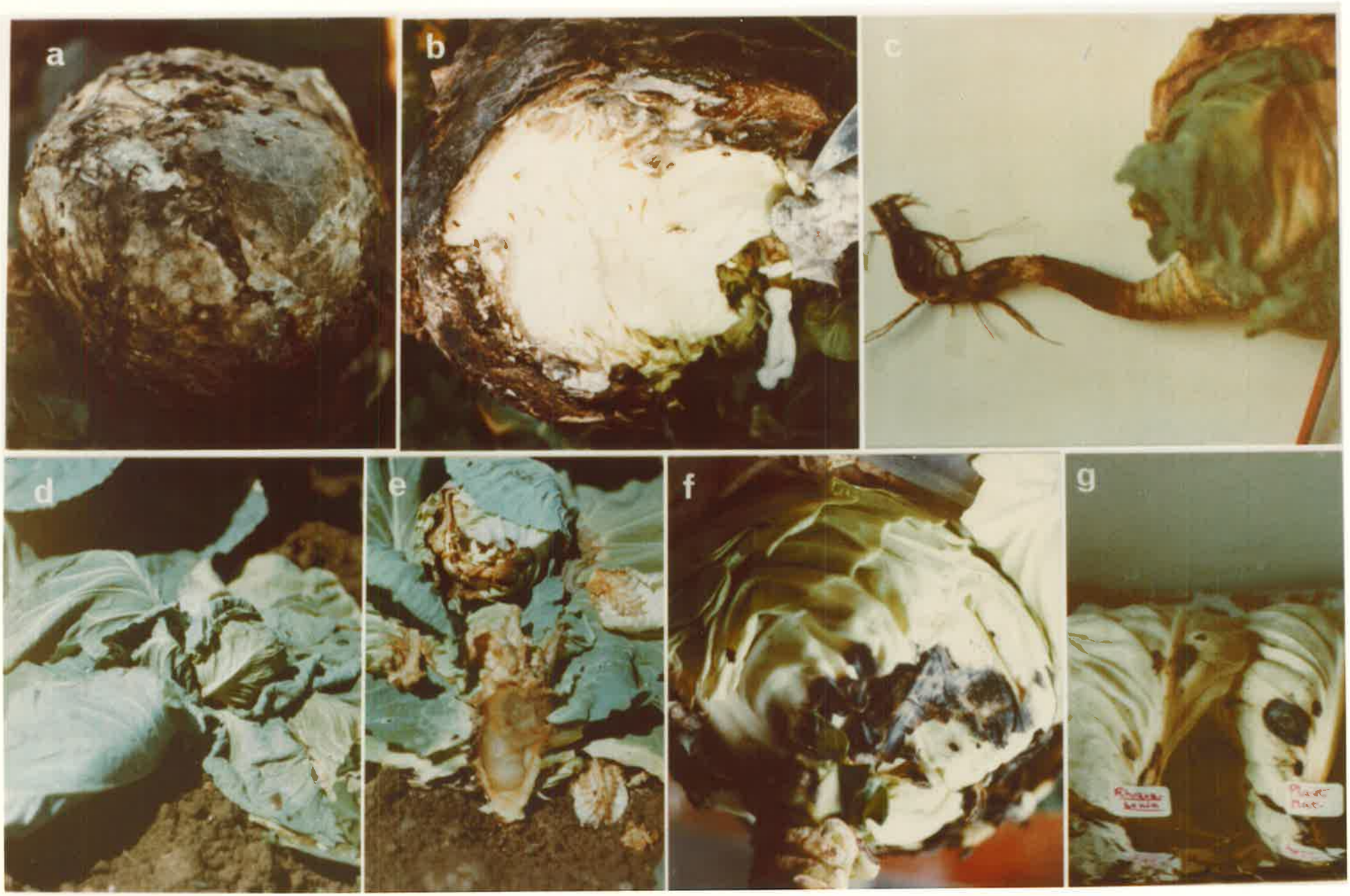

a
b
c
d
e
f
g

Fig.8:

a. Potato stems, infected by Sclerotinia rot. Sclerotia were formed inside dead stems

b. Potato infected by potato leaf role (PLRV). Younger leaves become smaller, erect and pale in colour

c. Potato mosaic on potato leaves, caused by PMV

d. Target spot, a general view of leaf spots caused by *Alternaria solani*

e. Silver scurf (*Helminthosporium solani*) on potato tubers

f. Common scab (*Streptomyces scabies*) on potato tuber

g. Phytophthora rot (*Phytophthora* sp.) on potato tuber.


a
b
c
d
e
f
g

Chapter 3

STEM ROT OF LETTUCE CAUSED BY *PHYTOPHTHORA PORRI*

1. Introduction

1.1 *Objectives*

A stem rot disease of lettuce observed in a commercial field during the winter of 1980 at Gawler River and Virginia, Northern Adelaide Plains, caused significant losses to some lettuce growers.

It appeared to be a new pathogen of which nothing was known of the factors involved in its development, pathogenicity, survival and reproduction, all important aspects for the better understanding of its pathogenesis and possible control.

The study investigated the etiology of the disease, identified the causal organism and examined the influence of some physiological and cultural factors.

1.2 *A previously undescribed pathogen in commercial lettuce fields*

The new stem rot disease is caused by *Phytophthora porri*, a fungus that has never been recorded before in lettuce plants.

From studies on other species of *Phytophthora* the following factors were considered likely to influence *P. porri* of lettuce: temperature (Waterhouse, 1931; Hyre and Cox, 1953; Brasier, 1969; Bumbieris, 1974, 1981), light (Fawcett and Klotz, 1934; Aragaki and Hine, 1963; Harnish, 1965; Berg and Gallegly, 1966; Brasier, 1969; Plourde and Green, 1982), medium (Waterhouse, 1931; Rao *et al.*, 1966), nitrogen and carbon

(Mehrotra, 1952; Wills, 1954; Cameron and Milbrath, 1964), soil moisture (Duniway, 1975, 1976; Sterne *et al.*, 1976; Bumbieris, 1981), chemical treatment (Staub and Young, 1980).

1.3 *Yield loss in commercial field*

Estimated yield losses due to stem rot disease in the winters of 1980 and 1981 in some fields were as high as 35% and 42% respectively. The highest loss was found in a field where the lettuces were 3-4 weeks old, suggesting that under favourable conditions the stem rot disease could cause considerable losses to growers.

1.4 *Incidence and distribution of the disease*

The disease was mainly confined to winter crops, when temperatures were low and when rainfall was high. The level of incidence varied depending on plant age and environmental conditions such as waterlogging.

The distribution of stem rot disease was patchy, infection being most severe where plant growth was inhibited due to excess soil water.

The fact that the stem rot disease was observed in several separate fields at Virginia and Gawler River, suggested that it was widespread in the area.

2. Materials and Methods

2.1 *Recovery, isolation and identification of* P. porri

During the field survey of vegetable diseases in the North Adelaide Plains (NAP), a disease of lettuce was observed in commercial lettuce fields at Virginia and Gawler River. The diseased plants wilted and collapsed completely within 2-4 weeks. Examination of infected plants showed a central, dark, firm rot of the stem extending from soil level upwards while the roots appeared healthy.

Because examination of diseased tissue had revealed the presence of oogonia and sporangia, characteristic of *Phytophthora*, both cornmeal (CMA) and selective $P_{10}VP$ medium (Tsao and Ocana, 1969) were used to isolate the organism from diseased lettuce stems. Small pieces were surface sterilized in 1% sodium-hypochlorite for 30 sec, washed in distilled water and placed on the media. They were incubated at 15, 20 and 25°C.

For production of sporangia, the fungus was grown on V-8J agar containing 100 ml Campbell's V_8-juice, 900 ml distilled water, 0.02 $CaCO_3$, 2 ml β-sitosterol. The technique of Mehrlich (1935) was used to stimulate sporangial production in non-sterile soil extract. Squares, approximately 2 x 2 cm were cut from a 7 day old culture, submerged in 1% soil extract and incubated at room temperature (14-17°C) for 48 h.

To induce release of zoospores, the cultures of *P. porri* were chilled in the refrigerator (ca. 4°C) for 2 h and then observed under the microscope. The key of Waterhouse (1963) was used to identify *P. porri*. This identification was substantiated by the C.M.I. A culture has been deposited at the C.M.I. with the number I.M.I. 251374.

2.2 *Experimental*

2.2.1 *Pathogenicity test*

To examine the pathogenicity of *P. porri* to lettuce cultivars and other vegetable crops, a series of experiments were done in the laboratory, growth chamber and glasshouse. Seeds were sown in sterile vermiculite, incubated at 25°C for 1 week. They were transplanted into U.C. mix potting soil ($\frac{1}{2}$ cubic metre of coarse sand and $\frac{1}{2}$ cubic metre of "Dedrof" peatmoss are mixed with nutrients: Potassium nitrate, 0.12 kg; magnesite, 0.24 kg; reverted super, 0.14 kg; plaster of Paris, 0.92 kg; potassium sulphate, 0.12 kg; blood meal, 0.14 kg; hydrated lime, 0.18 kg), in 15 x 15 cm pots, unless otherwise indicated.

Plants were inoculated with *P. porri*, by placing the inoculum around the stem of each plant. Plants were watered once a day as required.

2.2.2 *Physiological and cultural factors*

Liquid and solid media used to study the influence of some physiological and cultural factors, were prepared as follows: 125 ml Erlenmeyer flasks containing 75 ml media were used for liquid cultures and 9 cm sterile Petri dishes containing 20 ml media for agar (1.5%) cultures. All media were autoclaved for 15 min at 120°C.

One plug, 5 mm in diameter, of actively growing mycelium of *P. porri* was placed into each flask and at the centre of each agar plate. The cultures were incubated at 15°C in darkness, unless otherwise indicated.

Mycelial growth in liquid cultures was assessed by filtering mycelium through Whatman No.2 filter paper, rinsing with distilled water, drying to constant weight in an oven at 96°C for 2 h and weighing. The growth and characteristics of mycelial colonies and the production of sporangia and oogonia were studied in solid cultures. In each plate, four radii of the colony were measured and the mean of all plates in each treatment gave a measure of colony growth. Numbers of sporangia and oogonia within the microscopic field were counted at random in each plate.

2.2.3 *Most susceptible part of the lettuce to* P. porri

To determine the most susceptible part of the lettuce plant to *P. porri*, the inoculum of the fungus was placed on cotyledons, stems and roots of lettuce seedlings, incubated at 15°C for 1 week and then observed under the microscope.

2.2.4 *Effect of soil water and temperature on infection*

The lettuce cultivar Green Dale was sown in sterilized field soil and UC (1:1, V/V), in 11 x 7 cm containers. Three levels of temperature and two levels of soil water were studied. Inoculum of *P. porri* was prepared in sterilized field soil enriched with cornmeal extract and incubated at 15°C for 40 days. They were incubated in dew chambers. Soil water was maintained at the required level by weighing the pots at each watering. Each treatment was replicated five times.

2.2.5 *Efficacy of some fungicides to control* P. porri

To determine the efficacy of some fungicides against *P. porri*, tests were done on CMA media and in soil, according to the method of Zentmyer (1955). For the bio-assay in CMA each fungicide (Table 17) was mixed with CMA prior to pouring into 9 cm Petri dishes, each containing 20 ml medium. A plug, 5 mm in diameter, of actively growing mycelium was placed at the centre of each Petri dish, then incubated at 15°C for 10 days. Growth of mycelium was observed and measured. Treatments were replicated three times.

For the soil drench, field sandy loam soil was used, air dried, put through a sieve with an apperture of 1 mm and sterilized for 1 h at 120°C. Plastic vials, 25 mm in diameter and 80 mm deep, were used as containers. Ten ml of soil were placed in the vial and a plug of *P. porri*, 7.5 mm in diameter was placed on the soil and 10 ml of the soil placed on the top of it, then another plug of mycelium was put on the soil. A 5 ml solution of each fungicide (Table 17), enough to wet the soil, was poured into the vial. They were incubated at 20°C for 24 h.

All plugs from the soil were taken and washed with distilled water, dried on Kleenex tissues then placed on CMA and incubated at 15°C, to determine the growth of *P. porri*.

Results were assessed from the CMA as presence or absence of mycelial growth. It was replicated three times.

3. Results and Discussion

3.1 *Description of P. porri*

Growth of non-septate *Phytophthora* like hyphae from diseased tissue was observed after 2 days on both CMA and selective $P_{10}VP$ media, incubated at 15 and 20°C but not at 25°C. No other fungus was seen in any of the Petri dishes.

On CMA, the hyphae were 3-7 µm wide and non-septate. After about 1 week they became septate and their contents began to disappear. Abundant terminal and intercalary hyphal swellings up to 35 µm in diameter developed on the CMA or when the plugs of the fungus from CMA cultures were submerged in distilled water or soil extract. Terminal and intercalary structures were considered to be chlamydospores (Figs.9/4,5), because they were delimited from the supporting hyphae by septa (Blackwell, 1949) which measured 20-40 µm in diameter.

Most sporangia were semi-papillate or papillate (Figs.9/6,7) but sporangia with a shallow apical thickening were also present. Shape of the sporangia varied from ellipsoid or broadly ovoid (Figs. 9/6.7.8). Occasionally their shape was somewhat distorted and they had two papillae (Fig.9/9). Sometimes sporangia were laterally attached or intercalary. The size of sporangia was (32)-64.7-(87) x (22-45.4-(57) µm with a mean L/B ratio of 1.42. Sporangia sometimes germinated directly and new sporangia were formed at the tips of germ tubes. Longer chains of sporangia were never observed under experimental conditions. Sporangia were non-caducuous although septa were sometimes observed 5-6 µm below the sporangia. False pedicels as described by Blackwell (1949) were also seen.

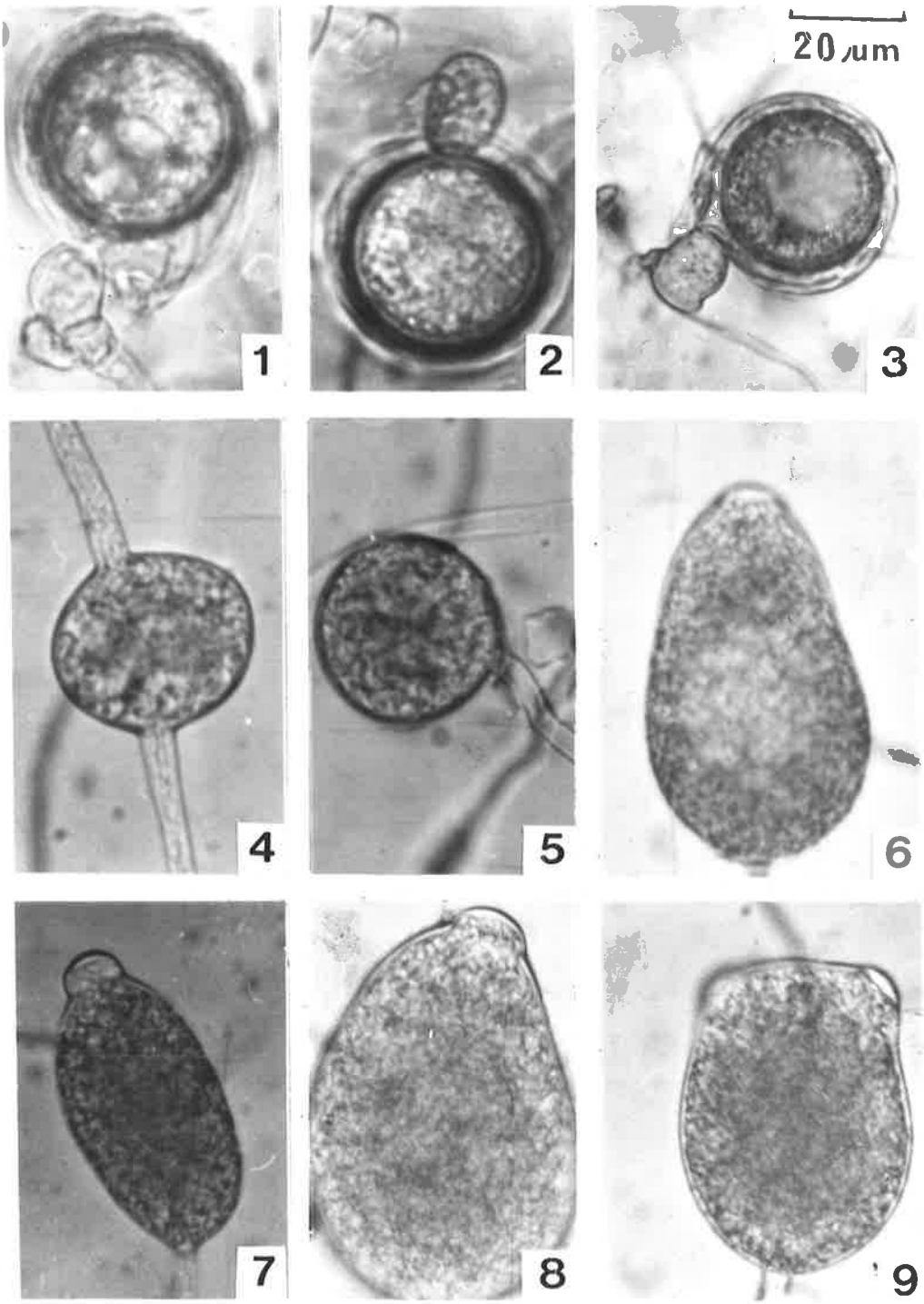
Sporangiophores were undifferentiated and variable in length. Besides very long ones (>300 µm), very short ones (3-4 µm) were also

Fig.9: Characteristics of fruiting bodies of *P. porri*

1,2,3: cogonia

4,5: chlamydospores

6,7,8,9: sporangia


20 um
1
2
3
4
5
6
7
8
9

observed. In some cases the sporangiophores broadened below the sporangia. Sympodial branching of sporangiophores was seen only occasionally.

For production of zoospores, sporangia of *Phytophthora* are often chilled for 20-30 min and then returned to room temperature. In *P. porri*, release of zoospores occurred only when sporangia were left at 4°C for 2-3 h. Production of zoospores continued for a while at room temperature but soon ceased. Zoospores were released from sporangia without formation of vesicles; exit pores were approximately 8-10 µm wide. Production of new sporangia by internal proliferation was not observed.

P. porri of lettuce produced oogonia in host tissue and in culture. Production of oogonia occurred in V-8J agar. Oogonia on the media measured (35)-41.1-(46) µm in diameter. They were spherical or somewhat distorted with smooth or undulated surfaces (Figs.9/1,2,3). Oospores were distinctly aplerotic, smooth and (26)-31.7-(35) µm in diameter. Antheridia were mostly paragynous but sometimes amphigynous, mostly diclinous and occasionally monoclinous (Fig.9/3). Their shape varied from broadly ellipsoid to nearly spherical and sometimes one or two appendices were seen at the base of the antheridial cell (Fig.9/1).

3.2 *Pathogenicity of P. porri*

Lettuce cultivars Ace Field and Green Dale, were planted in potting soil and grown for 7 weeks. Inoculation was made by placing five plugs, 5 mm in diameter, taken from a 7 day old CMA culture, at the base of each stem. Pure CMA plugs were added to uninoculated plants. All pots were kept in a growth chamber where the temperature fluctuated between 16 and 19°C. Soil in the pots was kept wet by placing the pots in plastic containers 6 cm deep filled with water.

Table 6 shows that two of the inoculated plants wilted within 7 days after inoculation while seven plants of the cultivar Ace Field and nine plants of the cultivars Green Dale wilted within 28 days. All uninoculated plants remained healthy. Examination of the wilted plants showed dark rotting of the stems, while the roots appeared healthy. Longitudinal sections showed that rotting extended to the centre of the stem, but not into the roots or leaves. Thus, the disease symptoms in the wilted plants were similar to those observed in the field. However, the disease appeared less severe in the pathogenicity test than in the field as only isolated brown lesions which in some cases extended 2-3 mm into the tissue, were observed on the stems of the remaining inoculated plants. This may be due to different environmental conditions, especially temperature which was considerably lower in the field during the winter months. The records of the Bureau of Meteorology show that the mean minimum temperature for June, July and August in the area were 7.4, 6.7 and 6.4°C respectively, while the mean maximum temperatures for the same months were 16.6, 15.6 and 17.7°C. The fungus was isolated from all wilted plants and its identity confirmed.

Young seedlings of lettuce, cabbage, leek, carrot, tomato, cauliflower, broccoli and Brussels sprout, were used to examine the pathogenicity of *P. porri* to various vegetable crops. Seeds of the plants were surface sterilized with 0.5% $HgCl_2$ for 1 min and washed in distilled water, then placed in sterile Petri dishes on moist filter paper and incubated at 25°C for germination. After 5 days the seedlings were arranged in rows on moist filter paper in sterile Petri dishes and 20 seedlings of each plant were inoculated by placing 5 mm diameter plugs of a 10 day old culture of the fungus on the stem and the root tip of the seedlings. Plugs of CMA were added to uninoculated seedlings of each plant. They were incubated at 15°C.

Table 6: Pathogenicity of *P. porri* on two cultivars of lettuce

Period after Inoculation	Number of plants wilted(1)			
	Ace Field		Green Dale	
	Uninoculated	Inoculated	Uninoculated	Inoculated
7 days	0	1	0	1
9 days	0	2	0	5
21 days	0	4	0	7
28 days	0	7	0	9

(1) Each cultivar was sown in ten pots and each pot contained 5 plants. Inoculation was made when plants were 7 weeks old.

All lettuce cultivars showed disease symptoms 8 days after inoculation, while of the other plants only cauliflower seedlings showed a slight browning of the stem. All uninoculated seedlings remained healthy. Thus the *P. porri* of lettuce was not pathogenic to any of the tested plant species other than lettuce under the experimental conditions (Table 7).

Similar results were obtained in a later pot test with 5 week old lettuce, leek and cabbage (Fig.10a).

Furthermore in Table 8, the result of the pathogenicity of *P. porri* on 17 lettuce cultivars in potting soil in the glasshouse, shows identical results with the test on young seedlings in the laboratory.

There was an indication that some cultivars were resistant to *P. porri*, i.e.: Green Mignonette, Great Lakes, Salinas and Yara Lake.

It is important to note that there are some differences between *P. porri* of lettuce and *P. porri*.

(1) The description of *P. porri* (Waterhouse, 1963), does not mention the presence of chlamydospores, while structures considered to be chlamydospores were present in the lettuce *P. porri*.

(2) The optimum and maximum growth temperatures of *P. porri* are 8-10°C higher than those of the lettuce isolate. Although the temperature requirements for growth of a fungus may be influenced to a certain degree by the medium, the differences in this case are too large to be attributed to this factor.

(3) The lettuce *P. porri* failed to infect leek and cabbage which are listed as host plants of *P. porri* (Stamps, 1978).

(4) The lettuce *P. porri* was able to infect potato tuber (Fig.10b), while *P. porri* originated from carrot failed to infect potato tuber (Ho, 1983).

Table 7: Pathogenicity of *P. porri* on young seedling of lettuce, leek, tomato, carrot, cabbage, broccoli, cauliflower and Brussels sprout

Cultivar	Percentage seedlings infected (1)
Lettuce:	
Green Dale	100
Original Great Lakes	70
Salinas	40
Imperial Triumph	40
Winter Lake	20
Butter Crunch	15
Ace Field	5
Leek:	
Musselburg	0
Tomato:	
K.Y. 1	0
Grosse lisse	0
Carrot:	
Early horn	0
Cabbage:	
Early Ball	0
Ball Head	0
Savoy King	0
Cauliflower:	
Deep Heart	(5)?
Phenomenal Early	0
Broccoli:	
Green Duke	0
Brussels sprout:	
Top Score	0

(1) Each cultivar consisted of 20 seedlings.

Table 8: Results of pathogenicity of *P. porri* on 17 cultivars of lettuce

Cultivar	Percentage plants infected (1)
Original Great Lakes	60
Green Dale	50
Penlake (medium all seasons type)	40
Imperial 847	40
Imperial 847 (medium type)	40
Ace Field	30
Early Mature Lake	30
Imperial Triumph	20
Sunny Lake	20
Winter Lake	10
Buttercrunch	10
Brown Mignonette	10
Cos Lettuce	10
Green Mignonette	0
Great Lakes	0
Salinas	0
Yara Lake	0

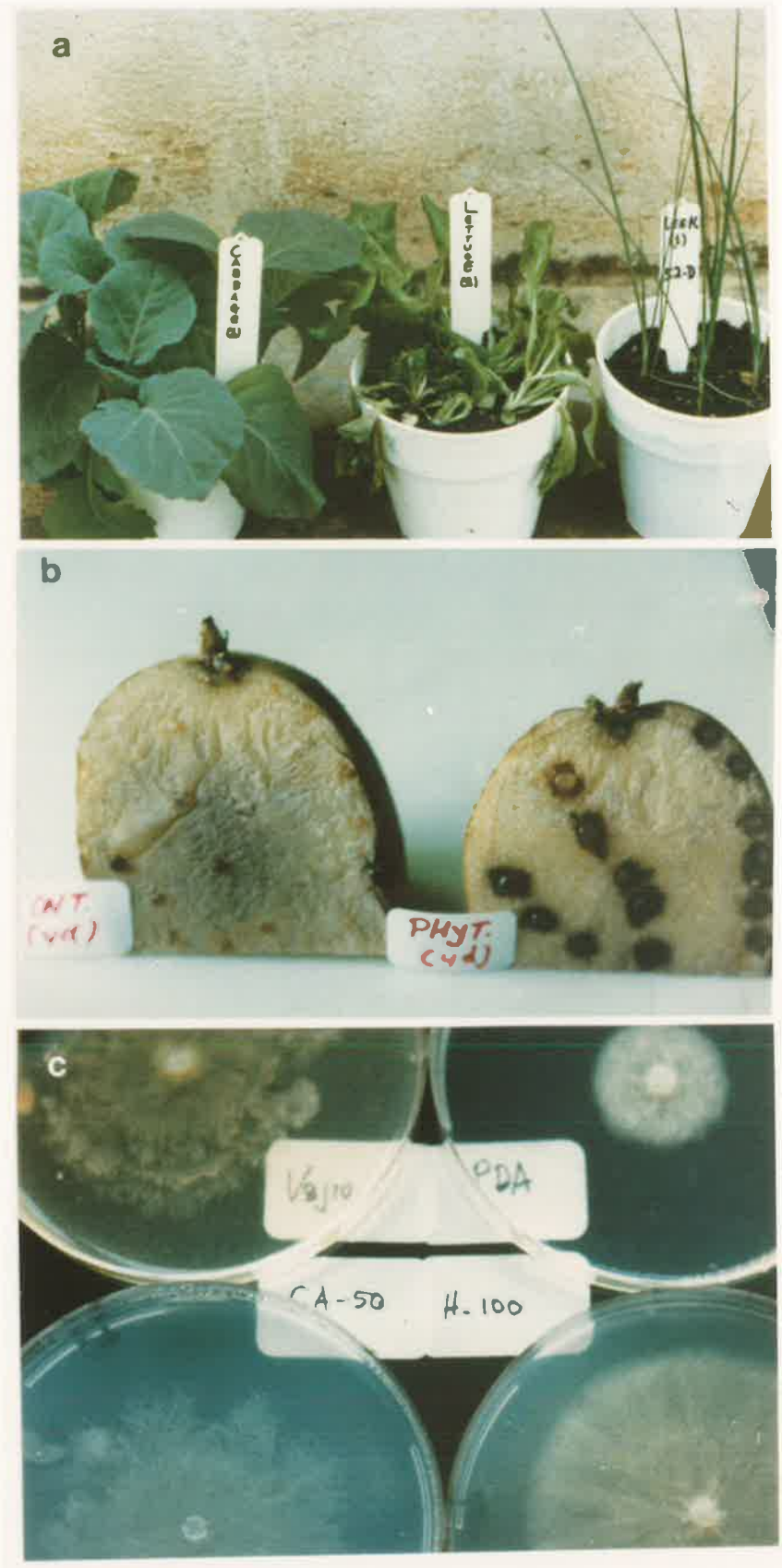
(1) Each value is the mean of 10 plants (two pots each containing five plants)

Fig.10:

a. Pathogenicity of *P. porri* in cabbage, lettuce and leek. Lettuce plants wilt as the result of infection by *P. porri*, while cabbage and leek remain healthy. Other than wilting there were no symptoms of infection

b. *P. porri* infected potato tubers when plugs 7 days old from CMA cultures were placed on the surface of the cutting tuber and incubated at 15°C for 4 days

c. Different characteristics of mycelial growth in colonies of *P. porri* on different media: Vgjuice agar, Difco dehydrated PDA, corn meal agar and lettuce stem extract agar. Media were incubated at 15°C for 15 days.


a
b
PHYT.
c
V8J10
PDA
CA-50
H-100

Although Stamps (1978) mentioned some physiological specialization in *P. porri*, the above described differences suggest that the lettuce *P. porri* may be a specific lettuce pathogen.

3.3 *The occurrence of P. porri in commercial lettuce fields and yield loss*

In the winter of 1980, the disease caused by *P. porri* was first observed in 3 separate commercial lettuce fields at Viriginia and Gawler River, causing yield losses ranging from 1 to 35% (Table 2). Furthermore in the winter of 1981 more severe infection by *P. porri*, in addition to the previous year, was observed in three further fields. Estimated yield loss in each field ranged from 6 to 42%. The occurrence of the disease in several fields indicated that the fungus was widespread in the area.

3.4 *Factors that favour the infection in the field*

The pathogen occurred in different types of soil, such as sandy loam and loamy sand. The pattern of infection was patchy and appeared to be associated with soil water content and plant age. Most severe infection occurred in young plants in places where water accumulated after rain or irrigation.

The fact that the disease was observed only during the cold winter months suggested that it is favoured by low temperature.

3.5 *Effect of physiological and cultural factors*

3.5.1 *Effects of kinds of media*

Both synthetic and non-synthetic types of media (Table 9) were tested. The media were prepared in liquid and solid forms using methods commonly used for *Phytophthora* and other soil-borne fungi (Commonwealth

Table 9: Dry weight of mycelia and production of sporangia and oogonia of *P. porri* in different media

Media	Dry weight (mg) of mycelia (1)	Production of sporangia (2)	Production of oogonia (2)
LSE (lettuce stem extract)	39.92[a]	+	+++
CME (corn meal extract)	38.83[ab]	0	+++
V-8J (V-8 juice)	34.67[bc]	+	+++
Pt.E (potato extract)	33.53[bc]	+	+++
CSE (cabbage stem extract)	31.60[c]	+	++
GPS (glucose peptone solution)	30.50[cd]	0	0
LBE (lima bean extract)	29.16[cde]	0	++
NS (nutrient solution)	24.16[de]	0	0
Ct.E (carrot extract)	23.53[de]	0	+
NDY (Czapek-Dox + yeast)	23.50[de]	0	0
FV-8J (filtered V-8 juice)	23.17[e]	-	-
SE (clarified soil extract)	6.00[f]	+	0
DW (distilled water)	3.13[f]	+	0
R'S (Richards' solution)	2.13[f]	0	0
CMA (Difco corn meal agar)	-	+++	0
PDA (Difco potato dextrose agar)	-	0	0

(1) Mean value of three replicates, incubated at 15°C for 40 days. Values followed by similar letter are not significantly different ($P<0.05$)

(2) Grown in solid media (1.5% agar); 0 = no sporangia or oogonia; + = few; ++ = moderate; +++ = abundant sporangia or oogonia; - = not tested.

Mycological Institute, 1968; Johnson and Curl, 1972; Ribeiro, 1978). Lettuce stem extract (LSE), was obtained from 100 g of chopped lettuce stems, autoclaved for 20 min in 1 L of distilled water, filtered through Whatman No.2 filter paper. For solid cultures 15 g "Difco" agar powder were added to 1 L extract solution. Other media were prepared by similar procedures: Potato extract (Pt.E), 200 g/l; cabbage stem extract (CSE), 100 g/l; lima bean extract (LBE), 200 g/l; cornmeal extract (CME), 75 g/l; carrot extract (Ct.E), 200 g/l; V_8juice (V-8J), 200 ml/l of V_8 juice (Campbell Soup Co.); clarified soil extract (SE), 1000 g/l (autoclaved for 30 min left overnight, filtered with cheese cloth, then centrifuged for 20 min at 4000 rpm).

P. porri grew in both synthetic and non-synthetic media. Mycelial growth in solid cultures was closely similar to that in liquid cultures. In general, faster growth was obtained from plant extracts such as LSE, CME and V-8J, while growth was slower in SE, DW and Richards' solution (Table 9). The production of sporangia and oogonia varied with the medium. Abundant oogonia were found in plant extract cultures whereas none occurred in synthetic and dehydrated "Difco" media. Production of sporangia was greatest in CMA.

These characteristics are similar to other species of *Phytophthora*. For example, *P. palmivora* Butler grows better on non-synthetic media (Rao *et al.*, 1966), *P. parasitica* sporulates abundantly in green papaya fruit (Aragaki and Hines, 1963), while in less precise terms Hyre and Cox (1953) stated that size and number of sporangia of *P. phaseoli* varied, depending on the medium.

3.5.2 *Effect of concentration of plant extract medium*

The growth of mycelium and production of oogonia in different concentrations of lettuce stem extract were determined (Fig.11). Mycelium was grown in liquid culture, incubated at 15°C for 40 days and replicated four times; oogonia were produced in solid culture for 10 days and it was replicated twice.

Figure 11 shows that the mycelial growth (a) and the oorgonial production (b) increased linearly with concentration of LSE in cultures.

3.5.3 *Effects of pH*

The effect of pH of the medium was studied in two different liquid cultures, LSE and non-sterilized 5% soil extract. The pH was adjusted by adding citric acid and/or potassium hydroxide solution to the extracts. The values of pH used in the experiment are shown in Fig.12 for the LSE-culture and in Table 10 for the soil extract culture. Dry weight of mycelia in the LSE-culture was measured after 40 days incubation. There were five replicates for each pH treatment.

Three plugs of growing mycelium of *P. porri* from a 10 days LSE-agar culture were submerged in a 6.5 cm Petri dish containing 20 ml soil extract and incubated at room temperature (20-23°C) for 48 h. The number of sporangia around the end of each plug was counted.

Phytophthora porri grew over a wide range of pH in medium cultures. Figure 12 shows the relationship between pH and the mycelial growth. The fastest growth occurred in media with pH 5 to 7. At pH 3.5 and 12 the fungus did not grow. Production of sporangia was greatest at pH 9 and 9.5 (Table 10).

Fig.11: The effect of concentration of lettuce stem extract (LSE)-medium on mycelial growth and oogonial production of *P. porri*

a. Regression of dry weight of mycelium on concentration of LSE-medium, grown in liquid medium ($r = 0.99$; $p<0.001$)

b. Regression of number of oogonia on concentration of LSE-medium, grown in solid medium ($r = 0.95$; $p<0.005$).

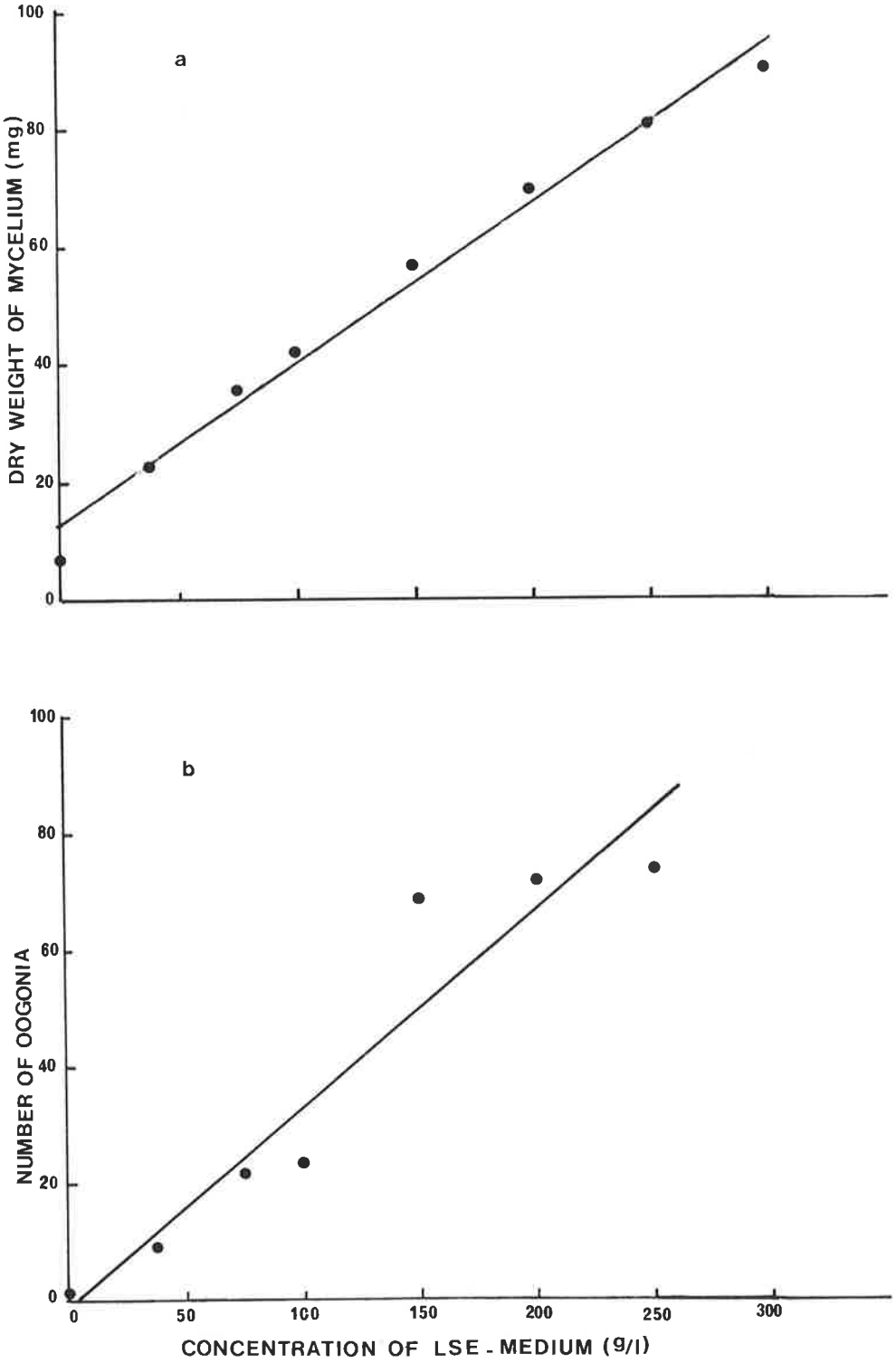

a
DRY WEIGHT OF MYCELIUM (mg)
100
80
60
40
20
0
b
NUMBER OF OOGONIA
100
80
60
40
20
0
0
50
100
150
200
250
300
CONCENTRATION OF LSE - MEDIUM (g/l)

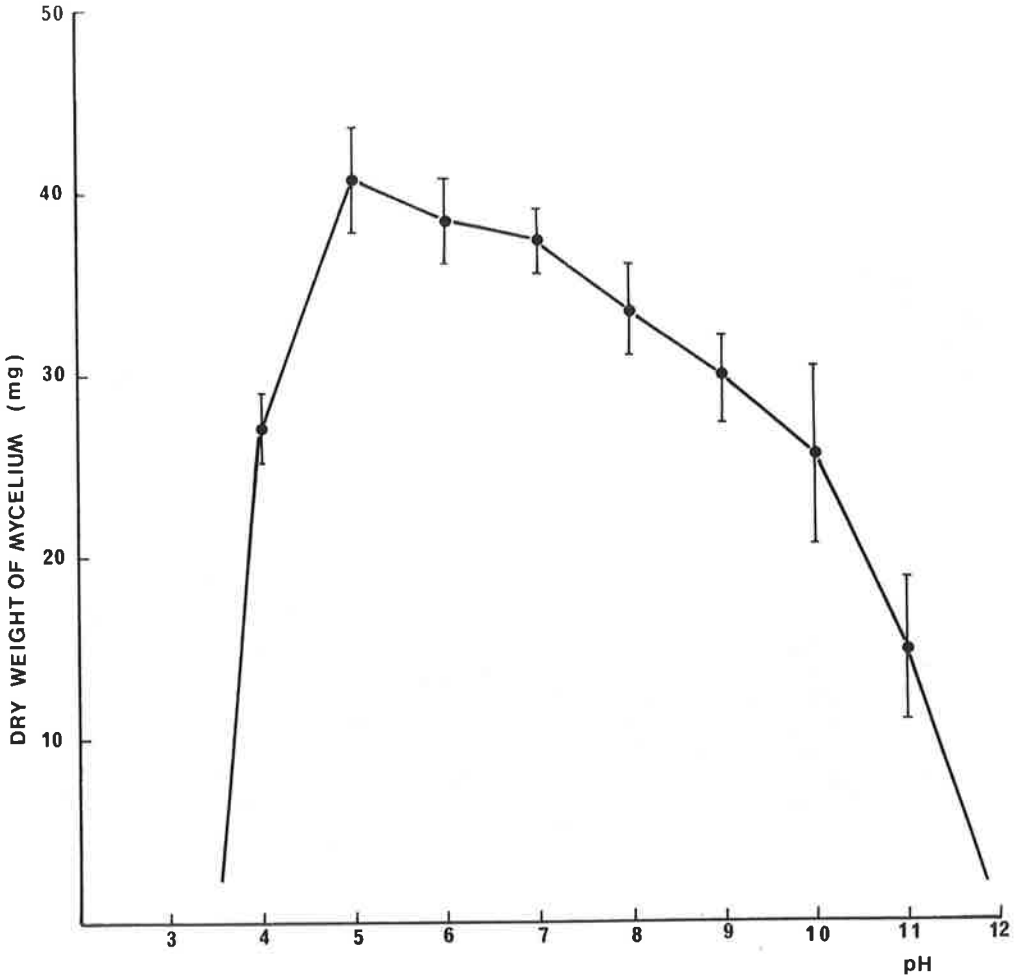
Table 10: Effect of pH in non-sterile 5% soil extract on production of sporangia of *P. porri*

pH	Number of sporangia (transf. $\sqrt{X + \frac{1}{2}}$)
3.0	0.71[g]
4.0	0.71[g]
4.5	3.64[e]
5.0	5.59[ed]
6.0	5.68[c]
7.0	6.17[bc]
8.0	5.76[c]
9.0	7.31[a]
9.5	7.68[a]
10.0	4.91[d]
10.5	3.92[a]
11.0	1.47[f]

Values followed by similar letters are not significantly different ($P<0.05$). Mean value of ~~six~~ five replicates.

Fig.12: Dry weight of mycellium of *P. porri* after 40 days at different pH (mean value of five replicates)

Vertical lines show 95% confidence limits.


DRY WEIGHT OF MYCELIUM (mg)
50
40
30
20
10
3
4
5
6
7
8
9
10
11
12
pH

3.5.4 *Effects of temperature and light*

To determine the influence of temperature and light, two kinds of media were used: LSE-agar (LSA) and cornmeal agar (CMA) as *P. porri* only produces oogonia in LSA and sporangia in CMA. They were incubated for 10 days at 5, 10, 15, 20, 25, 30°C and room temperature (20-23°C) in the dark; at 15°C under alternating 12 h light and 12 h darkness; and at room temperature and 25°C in continuous light. There were two replicates per treatment.

Temperature had a marked effect on both mycelial growth and production of sporangia and oogonia (Table 11). Sporangia and oogonia were more abundant in conditions that favoured mycelial growth, i.e. at 15°C. Light had no marked effect.

3.5.5 *Effects of nitrogen and carbon sources*

The aim of this experiment was to study the ability of *P. porri* to use nitrogen and carbon from different sources. The following basal medium (Abeygunawardena and Wood, 1957) was used: NH_4Cl - 1.16 g; KH_2PO_4 - 1 g; $MgSO_4.7H_2O$ - 1 g; yeast extract - 2 mg; micro element mixture (MEM) - 1 ml in 1 L distilled water. For N source treatments, NH_4Cl was replaced by sucrose - 25 g. (MEM = $FeSO_4.7H_2O$ - 0.1 g; $CuSO_4.5H_2O$ - 0.01g; $ZnSO_4.7H_2O$ - 0.1 g; $MnSO_4.4H_2O$ - 0.1 g in 100 ml distilled water).

Nitrogen sources tested were: L-asparagine, ammonium sulphate, ammonium nitrate, ammonium chloride, ammonium tartrate, sodium nitrate, potassium nitrate, calcium nitrate and peptone at a concentration of 0.09% nitrogen in basal medium. The sources of carbon: glucose, sucrose, D(-) fructose (laevulose), lactose, maltose, mannitol,

Table 11: Mycelial growth and production of sporangia and oogonia of *P. porri* at different temperature and light

Temperature (°C) L = light D = darkness	Mean radius (mm) of colony in CMA (1)	Production of sporangia in CMA (2)	Production of oogonia in LSE (2)
5 (D)	10.00^{e}	0	0
10 (D)	19.25^{b}	+	++
15 (D)	24.62^{a}	+++	+++
22 (D)	17.50^{c}	+	+
25 (D)	1.50^{f}	0	0
30 (D)	0.00^{g}	0	0
20-23 (D)	19.00^{b}	++	+
20-23 (L)	14.87^{d}	+	+
15 (D/L)	20.00^{b}	++	+++
25 (L)	1.25^{f}	0	0

(1) Mean value of four radii at right angles to each other with two replicates. Values followed by similar letter are not significantly different ($P<0.05$).

(2) 0 = no sporangia or oogonia produced; + = few, ++ = moderate; +++ = abundant sporangia or oogonia.

starch, citric acid, glutamic acid and glycerol, had amounts of carbon equivalent to 2.5% glucose. Each treatment was replicated twice.

Of the compounds supplying nitrogen, fastest growth occurred in peptone, followed by L-asparagine, calcium nitrate and sodium nitrate (Table 12).

Of compounds supplying carbon, mycelial growth was fastest in maltose (Table 13). The fungus did not grow in media containing D(-)fructose, citric acid and glutamic acid. Tables 12 and 13 also show that few sporangia and oogonia were produced in any of the media.

The ability of *P. porri* to utilize nitrogen and carbon from different sources, can be judged from a comparison with the basal medium. Of the nitrogen sources, three compounds stimulated growth whereas in the carbon sources, seven compounds were stimulatory. Few sporangia or oogonia were produced. An aspect worth studying further is the suggested association between nitrogen and oogonial production and carbon and sporangial production.

Peptone as the best source of nitrogen for *P. porri* in this experiment, was also reported by Saksena and Bhargave (1963, *cit.* Rao *et al.*, 1966) as a good source for *P. phaseoli* Thaxter. According to Cameron and Milbrath (1964), most species of *Phytophthora* grow more rapidly in media containing organic nitrogen than inorganic nitrogen. However, Wills (1954) found that *P. parasitica* var. *nicotianae* (Breda de Haan) Tucker, grew quicker in media containing sodium nitrate but slowly in asparagine; the opposite result was found in this experiment. The results of the growth studies using a carbon source supported the general statement of

Table 12: Dry weight of mycelium and production of sporangia and oogonia of *P.porri* on different sources of nitrogen

Source of nitrogen	Dry weight (mg) of mycelium (1)	Production of (2)	
		sporangia	oogonia
Peptone	44.00[a]	0	+
L-Asparagine	36.70[b]	0	0
Calcium nitrate	34.00[b]	0	0
Sodium nitrate	24.45[c]	0	0
Ammonium nitrate	21.45[c]	0	0
Ammonium chloride	19.05[c]	0	0
Potassium nitrate	18.50[c]	0	0
Ammonium tartrate	18.45[c]	0	0
Ammonium sulphate	18.10[c]	0	0
Basal medium	14.80[c]	0	0

(1) Mean value of two replicates, assessed at 40 days. Values followed by similar letter are not significantly different ($P<0.05$)

(2) Counted under microscopic fields (10 x ocular by 10 x objective) at random; mean value of two replicates each with 4 fields;
0 = no sporangia or oogonia; + = very few oogonia.

Table 13: Dry weight of mycelium and production of sporangia and oogonia of *P. porri* on different sources of carbon

Source of carbon	Dry weight (mg) of mycelium (1)	Production of (2)	
		sporangia	oogonia
Maltose	21.50[a]	0	0
Glycerol	16.60[b]	0	0
D-Glucose	15.70[b]	0	0
Starch	15.70[b]	0	0
Sucrose	14.15[b]	0	0
Lactose	11.95[b]	+	0
Mannitol	11.50[b]	+	0
Basal medium	5.15[c]	+	0
D(-)Fructose	0.00[c]	0	0
Citric acid	0.00[c]	0	0
Glutamic acid	0.00[c]	0	0

(1) Mean value of two replicates, assessed at 40 days. Values followed by similar letter are not significantly different ($P<0.05$)

(2) Counted under microscopic fields (10 x ocular by 10 x objective) at random; mean value of two replicates each with 4 fields;
0 = no sporangia or oogonia; + = very few sporangia.

Mehrotra (1952), that maltose, sucrose, dextrose, soluble starch and glycerol were most rapidly utilized by several species of *Phytophthora*.

3.5.6 *Effects of soil extract concentration*

The aim of this study was to examine the effect of soil extract on production of sporangia of *P. porri*, as this method is commonly used to stimulate sporangia production of the species of *Phytophthora* (Mehrlich, 1935; Bumbieris, 1978). Sterilized and non-sterilized soil extracts with different concentrations (Table 14) were studied. Plugs of *P. porri*, 7.5 mm in diameter from a 10 day old culture were submerged in 20 ml soil extract in 6.5 cm Petri dishes. They were incubated at room temperature (20-23°C) for 48 h, then the number of sporangia was counted around the edge of each plug. There were four replicates.

Table 14 shows that sporangial production was greatest in cultures containing sterilized 10 g/100 ml water, followed by non-sterilized 2 g soil, sterilized 2 g soil, non-sterilized 10 g soil and 5 g soil/100 ml water.

The results suggest that soil extract induced sporangial production of *P. porri* at concentrations of 2 to 10 g soil in 100 ml distilled water.

3.5.7 *Survival of* P. porri *in culture*

To study the survival of *P. porri* in agar culture at different temperatures, a 7 day old culture on CMA was kept at -4, 25 and 32°C for 3, 6, 10, 15, 25 and 35 days. Plugs, 5 mm in diameter, were taken

Table 14: Effect of soil extract concentration on sporangial production of *P. porri*

Soil (g/100 ml water)	Number of sporangia (trans. $\sqrt{X + \frac{1}{2}}$)
0 (non-sterile extract)	3.97^{c}
1 (non-sterile extract)	5.33^{bc}
2 (non-sterile extract)	7.21^{ab}
5 (non-sterile extract)	6.33^{ab}
10 (non-sterile extract)	6.75^{ab}
15 (non-sterile extract)	5.98^{bc}
0 (sterile extract)	5.13^{bc}
2 (sterile extract)	6.99^{ab}
10 (sterile extract)	8.03^{a}

Values followed by similar letter are not significantly different ($P<0.05$). Mean value of four replicates.

from the margin of the colony after storage for the required time and placed singly at the centre of CMA in Petri dishes and incubated at 15°C for 10 days. There were four replicates.

Table 15 shows that *P. porri* kept at -4°C for 35 days still grew, but it did not grow after 15 days at 25°C. The fungus did not survive at 32°C.

3.6 *The most susceptible tissues of lettuce plants*

Examination of the infected seedlings showed that in most cases infection occurred at the stem and extended upwards. Very rarely were the roots infected. This suggested that *P. porri* preferred certain infection sites on the lettuce plant.

In a second experiment the fungus was grown in liquid medium containing extracts of different parts of the lettuce plant (Table 16). One hundred g of each part were chopped, extracted in 1 L distilled water and autoclaved. A plug, 5 mm in diameter of mycelium was put into 75 ml of medium and incubated at 15°C for 40 days. The treatments were replicated three times.

Table 16 shows that best growth was found in cultures containing stem extract, followed by main root, shoot and leaf stalk extracts. However the differences were not statistically significent. Poorest growth of mycelium was found in cultures containing pith, leaf blade and root extracts.

3.7 *Efficacy of some fungicides*

Of the 18 fungicides tested in agar, 14 were effective in preventing growth of *P. porri* at the lowest concentration recommended for each

Table 15: Effect of temperature of storage on the survival of *P. porri* in culture

Length of storage (day)	Mean growth of mycelium (mm/24 h)		
	-4°C	25°C	32°C
3	1.59	1.33	0
6	1.61	1.42	0
10	1.69	1.43	0
15	1.70	0	0
25	1.67	0	0
35	1.55	0	0

Mean value of four replicates.

Table 16: Dry weight of mycelium of *P. porri* grown in media containing extracts from different parts of the lettuce plant

Kind of medium	Mean dry weight of mycelium (mg)
LSE (lettuce stem extract)	38.61[a]
MRE (lettuce main root extract)	31.34[ab]
ShE (lettuce shoot extract)	30.76[ab]
LLE (lettuce leaf stalk extract)	29.68[ab]
LPE (lettuce pith extract)	27.94[b]
LBE (lettuce leaf blade extract)	27.86[b]
LRE (lettuce root extract)	22.90[b]

Values followed by similar letter are not significantly different ($P<0.05$). Mean value of three replicates.

fungicide (Table 17, column A). However the results in agar plates did not correspond to those found when they were applied as drenches in soil. There were six fungicides (fosetyl + mancozeb, captan, mancozeb, fenaminosulf, metiram and metalaxyl) which killed the fungus on the soil surface, while only metalaxyl gave absolute prevention at a depth of 20 mm in the soil (Table 17, column B).

The fungicidal effects decreased in the soil application, suggesting that the active ingredient (a.i.) of some fungicides was inactivated or adsorbed by soil particles or chemicals. However, Klomparens and Vaughan (1952) reported good correlation between laboratory results of fungicides in malt agar and field control of several turf pathogens. Zentmyer (1955) on the other hand, found that the fungitoxicity of many fungicides that are highly effective in other types of tests was reduced when they were tested as a soil drench.

Metalaxyl was also reported to be effective against *P. infestans* of tomato either as a soil drench or foliar spray (Cohen *et al.*, 1979), *P. infestans* in potato leaves (Bruck *et al.*, 1980) and *P. parasitica* var. *nicotianae* of tobacco (Staub and Young, 1980).

In the case of the lettuce *P. porri*, because its activity in nature appears to be on or near the soil surface, the penetration of fungicides into the soil is not essential, therefore several fungicides which were effective in soil surface application, may possibly control *P. porri* i.e.: metalaxyl, metiram, captan, mancozeb, fenaminosulf and mixture of fosetyl and mancozeb.

4. Summary and Conclusions

4.1 *Phytophthora porri*

A stem rot disease of lettuce of various ages, which had not previously

Table 17: Efficacy of some fungicides against *P.porri* in agar culture and in soil

Fungicide	A. On CMA		B. In soil		
	a.i. (ppm) (1)	Mean growth of mycelium (mm/24 h) (2)	a.i. (ppm) (3)	Mean growth of mycelium (mm/24h) 0 mm	20mm deep (4)
Fosetyl (Aliette)	800	0	2400	1.24	1.24
Fosetyl + mancozeb (Mikal)	980	0	2100	0	1.25
Benomyl (Benlate)	500	0.1	1000	1.42	1.42
Chlorothalonil (Bravo)	1225	0	1630	1.25	1.53
Captan (Orthocide)	830	0	1245	0	1.24
Captafol (Difolatan)	500	0	1250	1.38	1.53
Mancozeb (Dithane M 45)	960	0	1600	0	0.99
Fenaminosulf (Dexon)	595	0	1190	0	0.25
Metiram (Polyram 2000)	1200	0	1600	0	1.18
Prochloraz (Sportack)	320	0	640	0.28	1.35
Metalaxyl (Ridomil)	400	0	750	0	0
Vinclozolin (Ronilan)	750	0.18	750	1.28	1.49
Iprodione (Rovral)	500	0.63	750	1.51	1.79
Carbozoline (Serinal)	750	0.15	1000	1.79	1.47
SN.78.314	200	0	300	0.17	0.22
Procymidone (Sumisclex)	500	0	1000	1.04	1.29
Quintozene (Terraclor)	2250	0	3750	1.68	1.52
Etridiazole (Terrazole)	84	0	140	0.44	1.28
Control (Distilled water)	0	2.03	0	1.98	2.00

(1) Concentration recommended at lowest rate for each fungicide

(2) Mean value of three replicates after 10 days

(3) Concentration at higher rate of each fungicide

(4) Mean value of three replicates after 6 days.

been recorded, was discovered at Virginia and Gawler River, Northern Adelaide Plains. A *Phytophthora*, morphologically similar to *P. porri* was isolated and identified from infected plant tissues.

There are differences between *P. porri* of lettuce and *P. porri*. The *P. porri* of lettuce did not infect leek and cabbage under experimental conditions, two of the host plants of *P. porri*, its optimum and maximum growth temperature is 8-10°C lower than *P. porri* and it produces sporangia, oogonia and chlamydospores in both articifical media and plant tissues.

However, the differences between *P. porri* of lettuce and *P. porri* are of some significance, suggesting that *P. porri* of lettuce is likely to be a specific pathogen to lettuce.

4.2 *Conditions for better growing of P. porri*

Waterlogging, wet and cold conditions appeared to favour the incidence and severity of stem rot disease in the field. Such conditions may favour the pathogen's ability to grow and to infect healthy plants and at the same time may inhibit the host plant.

In the laboratory, *P. porri* grew in synthetic and non-synthetic media. Media containing plant extracts were best for mycelial growth and oogonial production, while only CMA gave abundant sporangia. The optimum temperature for growth and sporulation was 15°C. Mycelial growth was most rapid at pH 5 to 6 and sporangial production was highest at pH 9 and 9.5. Three compounds of nitrogen stimulated growth, which the fastest was peptone followed by L-asparagine and calcium nitrate. Of the carbon sources tested, growth was stimulated most by maltose followed by glycerol, D-glucose, starch, sucrose, lactose and mannitol.

It is concluded that peptone at pH 5 to 7 and 15°C provide the most favourable conditions for mycelial growth.

4.3 *Possible control measure*

The nature of the infection on the stem and the pattern of distribution of infected plants in the field suggested that the inoculum of stem rot disease was soil-borne.

The only plants known so far to be hosts of *P. porri* are lettuce cultivars. Thus cultural practices to eliminate inoculum and stimulate plant growth, such as good drainage, avoidance of waterlogging and crop rotation, together with regular sprays with effective fungicides, should provide good control of *P. porri*. The search for resistant cultivars is likely to be profitable as some of the lettuce cultivars tested were not infected in the pathogenicity tests in the glasshouse.

Chapter 4

SCLEROTINIA ROT IN LETTUCE PLANTS

1. Introduction

1.1 *Objectives*

Sclerotinia rot of lettuce caused by *S. sclerotiorum* is a most destructive disease in commercial lettuce fields in the Northern Adelaide Plains. It causes high losses in yield not only for lettuce but also in other crops such as potato and cabbage.

So far there is no reliable way to control the disease in the field. However, differences that were found in occurrence and distribution in several commercial fields, indicated that there were some factors which favoured and some which restricted its potential for infection under natrual conditions.

The aim of the study was to determine those factors that had the greatest influence on the distribution and severity of Sclerotinia rot in commercial lettuce fields and to study the roles of some natural enemies (parasites and antagonists) in controlling the pathogen.

1.2 *Its occurrence and importance*

Under natural conditions the interaction between the pathogen and its host plants appeared to be influenced by a combination of several factors. Based on the conditions and the cropping background of the vegetable areas, the following assumptions were made in developing a hypothesis to explain the variation in distribution of the fungal pathogen in lettuce fields.

(1) Inoculum: The main source of inoculum of *S. sclerotiorum* is sclerotia, which can survive for many years in the soil.

(2) The major environmental factors influencing the fungus are: pH, salinity, texture, water content, matric potential, field capacity of soil and temperature.

(3) Mycoparasites and antagonists; influence the survival of sclerotia and other stages of the fungus.

(4) Cultural practices: such as cultivation, crop rotation, control measures, fertilizer usage are also important.

The approach consisted of sampling of soil, collecting data of diseased plants and analysing the data to ascertain whether there were any correlations between the incidence of disease and the factors mentioned above.

1.3 *Host plants and its distribution*

All main vegetable crops grown in the Northern Adelaide Plains including: cabbage, potato, cauliflower and celery, are susceptible and are good hosts for *S. sclerotiorum*. Thistle, a common weed in the area is also a good host of the fungus. *S. sclerotiorum* has a wide range of hosts, more than 350 species of 60 families of plants (Purdy, 1979). The most important host plants are: French beans, lettuce, potato, crucifers, celery, sunflower and rapeseed.

Its distribution is world-wide. In the Northern Adelaide Plains, it was found in all kinds of soil in commercial fields. It was most prevalent during winter.

1.4 *Biological control*

Routine sprays with fungicides has failed to control the disease in the fields, hence the search for a procedure of biological control seemed worthwhile. Several hyperparasites have been found on sclerotia of *Sclerotinia* species. *Coniothyrium minitans* is an effective parasite (Campbell, 1947; Merriman, 1976; Huang, 1977; Trutmann *et al.*, 1980). *Sporidesmium sclerotivorum* has been discovered in the soils of the United States of America, causing natural destruction of sclerotia of *Sclerotinia* spp. (Ayers and Adams, 1979; Adams and Ayers, 1981). *Trichoderma* spp. are also parasitic and antagonistic to many sclerotial fungal pathogens (Wells *et al.*, 1972; Merriman, 1976; Dos Santos and Dhingra, 1982). Other saprophytic fungi reported as antagonists or parasites of sclerotia are species of *Fusarium*, *Mucor*, *Alternaria*, *Epicoccum* (Merriman, 1976), *Penicillium*, *Aspergillus*, *Stachybotrys atra* (Rai and Saxena, 1975), *Gliocladium roseum* (Tribe, 1957), *Trichothecium roseum* (Boning, 1933, *cit.* Willetts and Wong, 1981).

2. Materials and Methods

2.1 *Assessment of disease in commercial lettuce fields*

2.1.1 Sampling procedure

To investigate the relationships between the incidence of Sclerotinia rot of lettuce, soil factor and the pathogen (sclerotia), soil samples and records of diseased plants were obtained from Virginia, Angle Vale and Gawler River, three localities in the Northern Adelaide Plains.

Five commercial lettuce fields, previously surveyed for vegetable disease, were chosen for the study. Two of the fields

were continuously cultivated with lettuce, while the other three alternated between lettuce and other vegetable crops or cover crops, at two to three year intervals.

Soil samples were collected at random from five sites in each field. Each site, approximately 10 x 10 m, was capable of containing 800 to 900 lettuce plants. About 2.5 kg of soil from the top 20 cm were collected from each site and divided into sub-samples for the assessment of numbers of sclerotia of *S. sclerotiorum*, pH, soil texture, soil water, water potential and salinity.

2.1.2 *Assessment of diseased plants*

The numbers of infected and uninfected plants were counted at each site. Diseases other than Sclerotinia rot were excluded from the count.

2.1.3 *Factors measured*

Under natural conditions the distribution, density and severity of the disease varied in commercial fields, suggesting that several factors such as number of sclerotia, soil factors (pH, soil texture, soil water, soil water potential, salinity), cultural practices and mycoparasites or antagonists in the soil were involved.

2.1.3.1 *Number of sclerotia of S. sclerotiorum*

Sclerotia were obtained from soil using the modified "Wet sieving technique" (Hoes and Huang, 1975). Four replicates of 200 g soil were washed with running tap water on a sieve with an aperture of 2 mm. The washed residue was transferred into a glass Petri dish. Sclerotia were counted, using a low power microscope

when necessary, and picked up with forceps and surface sterilized with a mixture of 1:1 (v/v) 1% sodium hypochlorite-absolute alcohol. All sclerotia, except those which were apparently rotten, were placed on PDA and incubated at 25°C for the examination of healthy and dead sclerotia.

2.1.3.2 *Soil factors*

Soil texture, water potential, pH and soil water content were measured using the methods described in Chapter 2.

Salinity was measured using a Conductivity Meter. Air dried soil was made into a solution in double glass distilled water, in the ratio of 5:1 (v/v) water-soil. The solution was thoroughly mixed by stirring it in a plastic container, 10 cm deep and 4 cm in diameter, then allowed to stand for 30 min. Then it was stirred again and the electrode with all three platinum sheets was immersed into the solution. Readings were made and the values expressed in micromho.

Field capacity. Approximately 50 ml of soil were placed in a funnel with very fine pores. Water was poured onto the soil until saturated. The water from the soil was allowed to drip; when it stopped the soil was considered to be at field capacity. The soil was placed in a Petri dish and oven dried at 96°C for 24 h. The loss of water content was calculated as a percent of dry weight of soil.

2.1.3.3 *Cultural practices*

Cultural practices were recorded according to the cropping system and cultivation of a field, including degree of cultivation,

soil fertility, soil drainage, type of furrows, fungicide and fertilizer usage. They were categorized as good, fair or bad practices.

2.2. *Recovery, isolation and identification of sclerotial parasites or antagonists*

Mycoparasites and antagonists of sclerotia were isolated from decomposing sclerotia and from soil. Baits of laboratory-produced sclerotia and selective media were used for the isolation from soil. Twenty five sclerotia were mixed with field soil in a container, each containing 100 g soil and incubated at 20°C for 8 weeks. Sclerotia were taken and surface-sterilized with a mixture of 1:1 (v/v) 1% sodium hypochlorite-absolute alcohol and washed several times with distilled water. Then they were placed on sterile moist filter paper in a Petri dish and incubated at 20°C. Each treatment was replicated five times. The growth of mycoparasites on sclerotia was examined periodically for four weeks. Infected sclerotia were recorded and pure cultures of fungal isolates were made.

Actinomycete antagonists were isolated from five commercial lettuce fields, using the dilution plate method with a specific medium for actinomycetes (agar - 40g; K_2HPO_4 - 2 g; $MgSO_4.7H_2O$ - 2 g; NaCl - 2 g; $(NH_4)_2SO_4$ - 4 g; $CaCO_3$ - 4 g; soluble starch - 20 g). One ml of a $1:10^5$ soil dilution was applied to each Petri dish containing 20 ml of the medium and incubated at 25°C for six days; the colonies were then examined under the microscope. Each treatment was replicated four times.

Identifications were based on morphological structure, mycelial growth, fruiting bodies and physiological aspects (Waksman, 1952; Gilman, 1957; Burges, 1958; Rifai, 1969; Domsch *et al.*, 1980). Tests on

parasitic and antagonistic potential were done against sclerotia of *S. sclerotiorum in vitro*.

2.3 *Laboratory and Glasshouse experiments*

2.3.1 *Production of sclerotia of* S. sclerotiorum

Sclerotia were produced on autoclaved carrot disc cultures in Petri dishes or Erlenmeyer flasks, inoculated with plugs of mycelium from a PDA culture and incubated at 25°C for two weeks. Sclerotia were extracted from the media by washing in a 2 mm sieve, surface sterilized with a mixture of 1:1 (v/v) 1% sodium hypochlorite-absolute alcohol, rinsed several times with distilled water and air dried on a bench in a laminar flow cabinet.

2.3.2 *Pathogenicity tests*

Experiments were done in the glasshouse. Seeds were sown in sterile vermiculite and incubated at 25°C for one week. They were transplanted into U.C. mix potting-soil in 15 x 15 cm pots. Water and fertilizer were added when required.

2.3.3 *Efficacy of some fungicides against* S. sclerotiorum

To determine the efficacy of some fungicides to control *S. sclerotiorum*, tests were done on CMA media and in soil, according to the method described in Chapter 3. The treatments were incubated at 25°C. Assessments with the CMA medium were recorded as presence or absence of mycelial growth. Each treatment was replicated three times.

2.3.4 *Physical and cultural factors*

Liquid and solid media used to study theinfluence of sources of nitrogen and carbon were prepared as follows: 125 ml Erlenmeyer flasks containing 75 ml media were used for liquid cultures and 9 cm sterile Petri dishes containing 20 ml media for agar (1.5%) cultures. One plug, 5 mm in diameter of actively growing mycelium of *S. sclerotiorum* was placed into each flask and at the centre of each agar plate. The cultures were incubated at 25°C for four days for liquid cultures and two weeks for agar plates.

Mycelial growth in liquid culture was assessed by filtering the mycelium, rinsing with distilled water, drying to constant weight in an oven at 96°C for 6 h and weighing.

2.3.5 *A prerequisite for infection by ascospores*

To study the factors initiating the initial infection by ascospores on healthy plants, seeds of the lettuce cultivar Imperial Triumph were sown in sterile vermiculite for one week. They were transplanted into U.C. mix potting soil in 15 x 15 cm pots and grown in the glasshouse for six weeks.

2.3.6 *Factors effecting sclerotial viability and germination*

To determine the effect of some factors on sclerotial viability and germination (carpogenic germination), laboratory-grown sclerotia were used throughout. To test for the effect of duration of storage, sclerotia were placed in a cool room (4°C) in sterile Petri dishes. To test for the effect of burial and

duration of storage, sclerotia were kept at different depths in soil in glass containers, at 15°C under alternating 12 h light and 12 h darkness in a growth chamber.

2.3.7 *Biological control of* S. sclerotiorum *by* F. lateritium

2.3.7.1 *In the laboratory and glasshouse*

Sclerotia were obtained from a two week carrot disc culture. Apothecia were produced by placing sclerotia on sterile coarse sand in closed plastic containers, 7.5 cm high and 7.5 cm in diameter. The sand was periodically moistened with distilled water during incubation at 15°C under alternating light and dark or at room temperature during winter. Six to seven weeks later many apothecia with mature ascospores were obtained from the cultures.

Fusarium lateritium produced macroconidia abundantly after a short period of incubation on PDA, CMA or autoclaved brown rice grains (Carter, 1983). After 7 days at 25°C macroconidia were harvested and stored. In addition to the media already mentioned, dilute Czapek-Dox plus yeast extract (NDY/20) was made up according to the formula of the Commonwealth Mycological Institute (1968) and glucose peptone agar (GPA/20) as formulated by Cook *et al.* (1949). Lettuce stem extract (LSA) was obtained from 100 g of lettuce stems which were chopped, boiled in 1 L distilled water, filtered through Whatman filter paper No.2 and 15 g agar powder added, then autoclaved for 15 min. Lettuce leaf extract (LLA) and cabbage stem extract (CSA) were obtained by a similar procedure.

2.3.7.2 *In the field*

The experiments were carried out in two commercial lettuce fields in Virginia, during the winter of 1983. It was a randomized block design, each block containing four treatments:

(a) *Fusarium lateritium* macroconidia
(b) Fungicide: benomyl (Benlate, 50% W.P.)
(c) Alternating between *F. lateritium* and benomyl
(d) Untreated-control

The plot size per treatment was equivalent to 45-50 plants. A suspension of *F. lateritium* macroconidia was sprayed thoroughly on the plants and soil surfaces. Benomyl solution equivalent to 500 g/1000l per hectare was used. An automatic "Silo knapsack sprayer" was used throughout. Sprays were done four times at intervals of ten days. In the first experiment the lettuce cultivar was Salinas and in the second one it was Imperial Triumph. Insect damage was prevented by spraying the blocks with insecticide (Malathion). During the experiments there were no routine sprays by growers.

Assessments on the incidence of Sclerotinia-rot and other diseases of lettuce were made every ten days prior to spraying. At the end of the experiments, numbers of plants harvested were recorded and the effects of each treatment were expressed as percentage diseased plants caused by *S. sclerotiorum*. Because the blocks were in commercial fields, cultivation, irrigation and fertilization were done by the growers according to their usual practice.

3. Results and Discussion

3.1 *Correlation between disease and environmental factors*

Percent diseased plants and mean values of numbers of sclerotia of *S. sclerotiorum*, percent clay, salinity, pH, water potential, water field capacity and soil water content are shown in Table 18. The correlation coefficients between the factors are shown in Table 19.

3.1.1 *Correlation between diseased plants and sclerotia*

The results of two successive samplings suggested that the numbers of sclerotia of *S. sclerotiorum* in field soil were positively correlated with percent diseased plants in lettuce crops (Figs.13a,b). Because the sclerotium is the primary survival structure in the life cycle of *S. sclerotiorum*, their presence in field soil has always been a major concern. Little is known about the relationship between inoculum (sclerotia) level and the level of disease and severity with Sclerotinia rot of lettuce. However, the data obtained from 25 sampling sites of five commercial lettuce fields in the Northern Adelaide Plains, showed that the highest number of sclerotia per Kg of soil was about twelve. This number is considered high for *S. sclerotiorum*, although an even higher number was expected in a field that was heavily infested in the previous season (number of sclerotia per plant in the lettuce field ranged between 166-(430)-618).

Adams and Tate (1975) found the inoculum level of *S. sclerotiorum* (small sclerotia, about 1 mm, recognized as *S. minor* Jagger), ranged from 0-(3.9)-20/100 g soil, in New Jersey lettuce

Table 18: Mean values of percentage diseased plants, number of sclerotia and soil factors at 25 sampling sites in commercial lettuce fields

Sampling site	Percentage diseased plants (trans. $\sin.^{-1}\sqrt{y}$) (1)	Number of sclerotia in 1000 g of soil (transf. $\sqrt{X+\frac{1}{2}}$) A (2)	Number of sclerotia in 1000 g of soil (transf. $\sqrt{X+\frac{1}{2}}$) B (2)	Salinity (micro-mho/cm)	pH	Water field capacity %	Soil water content (%) A (3)	Soil water content (%) B (3)	Soil water potential (-bars) A (4)	Soil water potential (-bars) B (4)	Clay (%) (5)
A_1	20.62	1.53	1.48	374.75	7.6	23.35	15.74	14.36	0.07	0.06	17.38
A_2	25.77	1.53	1.77	321.75	7.0	24.51	15.18	14.10	0.07	0.06	
A_3	58.44	1.98	1.22	256.50	7.3	28.15	13.74	14.36	0.06	0.13	
A_4	44.48	2.35	1.73	288.75	6.7	25.94	14.78	12.01	0.04	0.07	
A_5	64.60	3.94	3.59	178.25	7.7	21.58	12.41	16.09	0.04	0.05	
B_1	0.57	1.53	0.71	385.25	7.0	23.22	9.43	12.46	0.08	0.05	7.83
B_2	0 57	0.71	0.71	400.00	6.9	23.11	9.79	10.99	0.06	0.06	
B_3	9.81	0.71	1.22	461.00	7.1	23.78	10.38	12.01	0.07	0.04	
B_4	12.25	2.35	1.53	476.25	7.0	21.14	11.83	14.60	0.05	0.03	
B_5	14.30	0.71	0.96	363.25	6.9	22.83	9.29	16.33	0.08	0.02	
C_1	14.65	1.53	1.22	551.25	7.4	25.39	16.58	16.77	0.04	0.06	18.94
C_2	22.71	1.53	1.53	212.50	7.4	24.51	13.97	14.68	0.06	0.05	
C_3	37.94	2.79	2.04	361.25	6.9	28.56	14.65	13.51	0.06	0.11	
C_4	38.53	2.35	0.71	346.15	7.1	18.27	14.26	16.28	0.04	0.06	
C_5	53.73	3.24	2.80	264.75	7.3	23.24	16.88	13.22	0.11	0.06	
D_1	0.57	0.71	0.71	1077.50	7.1	20.69	9.39	12.21	0.12	0.06	9.88
D_2	0.57	0.71	0.71	967.50	6.9	23.76	9.31	12.21	0.12	0.07	
D_3	5.44	0.71	0.96	975.00	7.2	22.77	8.62	11.28	0.10	0.06	
D_4	9.98	0.71	0.96	995.00	7.3	23.07	9.96	14.34	0.11	0.04	
D_5	14.18	0.71	0.71	942.5	7.3	27.45	9.27	12.26	0.11	0.13	
E_1	3.63	0.71	0.96	644.75	7.2	24.43	15.77	18.65	0.10	0.05	26.46
E_2	0.57	0.71	0.71	597.5	7.2	20.20	16.39	17.90	0.04	0.10	
E_3	0.57	0.71	0.71	630.00	7.4	27.53	20.86	17.59	0.08	0.08	
E_4	3.63	0.71	0.71	827.5	7.4	35.66	19.56	20.63	0.11	0.12	
E_5	0.57	0.71	0.71	650.00	7.2	33.05	19.59	19.47	0.10	0.17	

(1) Percentage diseased plant was estimated approximately two weeks before harvest.

(2), (3), (4) - A = first sampling; B = second sampling

(5) Percent clay was measured as a combined sample, one for each field.

Table 19: Correlation coefficients of factors measured in 25 sampling sites of commercial lettuce fields in the Northern Adelaide Plains

Factors	Correlation coefficients (r) for associated factors							
	No. of sclerotia							
	1st sampling	2nd sampling	Salinity (u mho/cm)	Water potential (-bar)	Water field capacity (%)	Soil water content (%)	pH	Clay (%)
Percentage diseased plant	***	***	***	*	NS	NS	NS	NS
Number of sclerotia			***	*	NS	NS	NS	NS
Salinity				***	NS	NS	NS	NS
Water potential					NS	NS	NS	NS
Water field capacity						**	NS	NS
Soil water content							NS	NS
pH								NS

*** P<0.001; ** P<0.01; * P<0.05; NS = not significant.

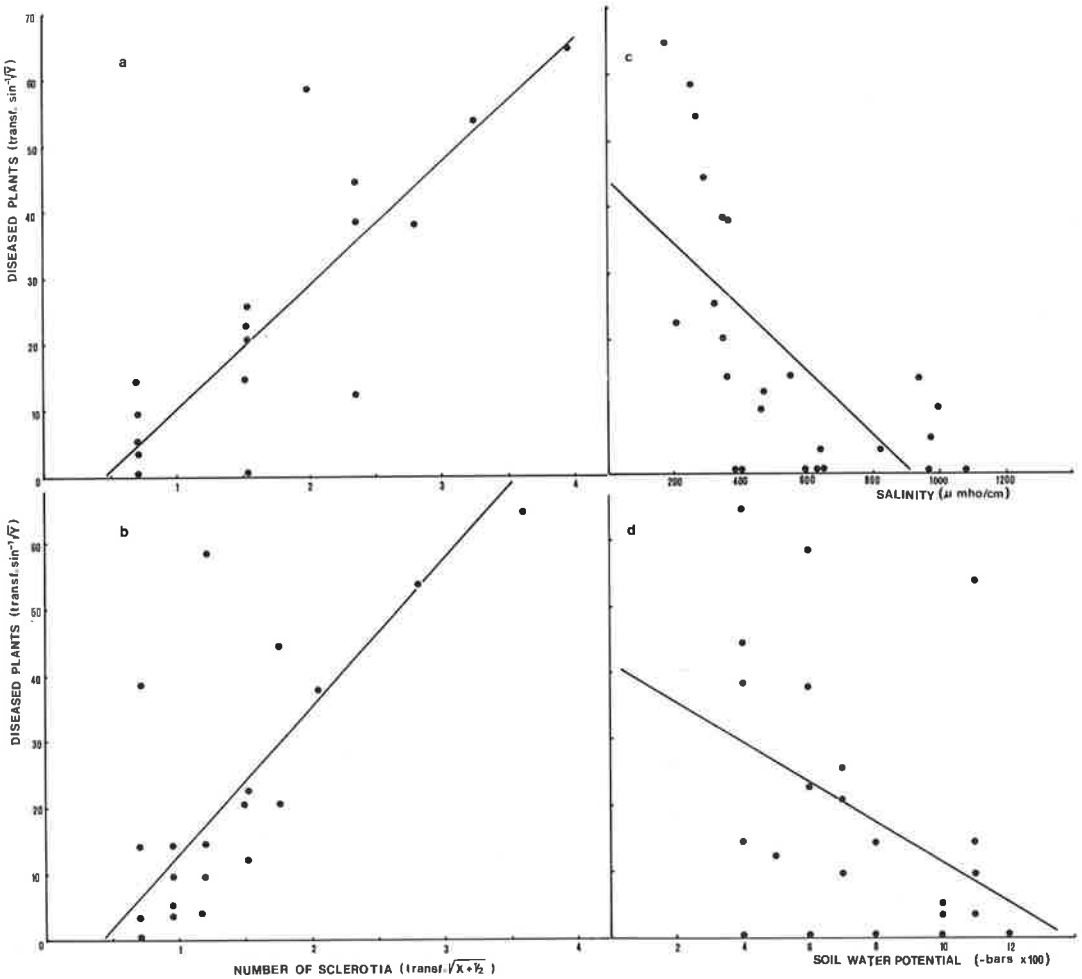
Fig.13: The relationship between percent diseased plants and

a. the number of sclerotia of *S. sclerotiorum* (first sampling; $r = 0.868$; $P<0.001$)

b. the number of sclerotia (second sampling; $r = 0.798$; $P<0.001$)

c. soil salinity ($r = -0.655$; $P<0.001$)

d. soil water potential ($r = -0.426$; $P<0.05$).


a
b
c
d
DISEASED PLANTS (transf. sin⁻¹√Y)
DISEASED PLANTS (transf. sin⁻¹√Y)
NUMBER OF SCLEROTIA (transf. √X+½)
SALINITY (μ mho/cm)
SOIL WATER POTENTIAL (-bars x100)

fields. They also suggested that the losses from disease in the field increased when the inoculum level increased. Hoes and Huang (1975) recovered 24 sclerotia/kg soil from the rhizosphere of sunflower plants with root rot. These observations suggest that the incidence and severity of Sclerotinia rot in lettuce fields can be predicted from the number of sclerotia in the field soil.

Even though sclerotia of *S. sclerotiorum* seem unlikely to cause direct infection through soil, they probably produce ascospores which are the primary source of inoculum (Abawi and Grogan, 1979; Cook *et al.*, 1975).

3.1.2 *Correlation between disease and factors other than sclerotia*

Significant negative correlations were found between percent diseased plants and soil salinity and soil water potential (Figs.13c,d). No significant correlations were found between diseased plants and pH, water field capacity, soil water content and percent clay (Table 19). The results show that percent diseased plants decreased as salinity increased. In terms of plant health, salinity may reduce plant vigour and yield without visible symptoms (U.S. Salinity Laboratory Staff,1954), which is contrary to the results found in my experiment. The more likely explanation for the negative correlation is probably the effect of salinity on the number of sclerotia (Table 19) rather than an effect on the plant.

Soil water potential appeared to influence disease incidence, as percent diseased plants decreased linearly with soil water potential (-bars) as shown in Fig.13d. Once again it is possible that lack of water inhibits sclerotia and, therefore, the incidence of diseased plants.

3.1.3 *Correlation between factors other than percent diseased plants*

Correlation coefficients of factors in Table 19 show significant correlations (negative) between number of sclerotia and salinity and soil water potential, between salinity and soil water potential (positive) and between water field capacity and soil water content (positive).

Increasing salinity and soil water potential probably influenced the viability and degradation of sclerotia and so reduced their numbers in the soil. The report by Williams and Western (1975) that soil moisture above 30% of the soil water holding capacity favoured survival of sclerotia supports my results.

3.1.4 *Cultural practices*

From the survey (Chapter 2) there was some indication that the degree of cultural practice such as cultivation, crop rotation, removal of infected plant, fungicide and fertilizer usage, influenced the occurrence and severity of Sclerotinia rot in commercial lettuce fields. Thus, clean and thorough cultivation followed by proper drainage of soil decreased the incidence and severity of disease. The most likely explanation is that under good cultivation plants not only grow better, but there is less chance for the pathogen to germinate and infect healthy plants because there is less organic matter and soils are drier.

Crop rotation gave positive results as long as the interval between crops was sufficient to reduce the inoculum in the soil to a level below economic importance. In the Northern Adelaide Plains

crop rotation was not sufficient to control Sclerotinia rot, mainly because the length of the rotation, 2 to 3 years, was not long enough and because the crops that were used are also alternative hosts of the pathogen. Rotation appears to be aimed more at the fertility of soil than to permit esscape from disease. Furthermore, the same agricultural tools are used in different fields thus encouraging spread of the pathogen.

The number of sclerotia of *S. sclerotiorum* surviving in the soil can be reduced by deep ploughing (Chambers and Hardie, 1964; Merriman, 1976).

3.2 *Sclerotial parasite and antagonist*

Several fungal parasites and antagonists were isolated from soil and parasitized sclerotia, such as *Coniothyrium minitans*, *Trichoderma harzianum*, *Trichoderma* sp., *Trichothecium* sp., *Gliocladium* sp., *Fusarium* spp., *Penicillium* sp., *Mucor* sp. and some unidentified saprophytic fungi (Table 20). *C. minitans* was isolated from all sampled fields, suggesting that the parasite contributes to the degradation of sclerotia of *S. sclerotiorum* in the fields. *Gliocladium* sp. was also abundant. *Trichothecium* sp. was found in only one field.

Sporidesmium sclerotivorum, which is found over a wide area of the United States as an effective parasite against sclerotia (Ayers and Adams, 1979; Adams and Ayers, 1981), was not found in the Northern Adelaide Plains.

There were several isolates of Actinomycetes isolated from soil of commercial fields in the NAP, but only an isolate of *Streptomyces* sp. appeared to show an antagonistic reaction to *S. sclerotiorum* (Fig. 14).

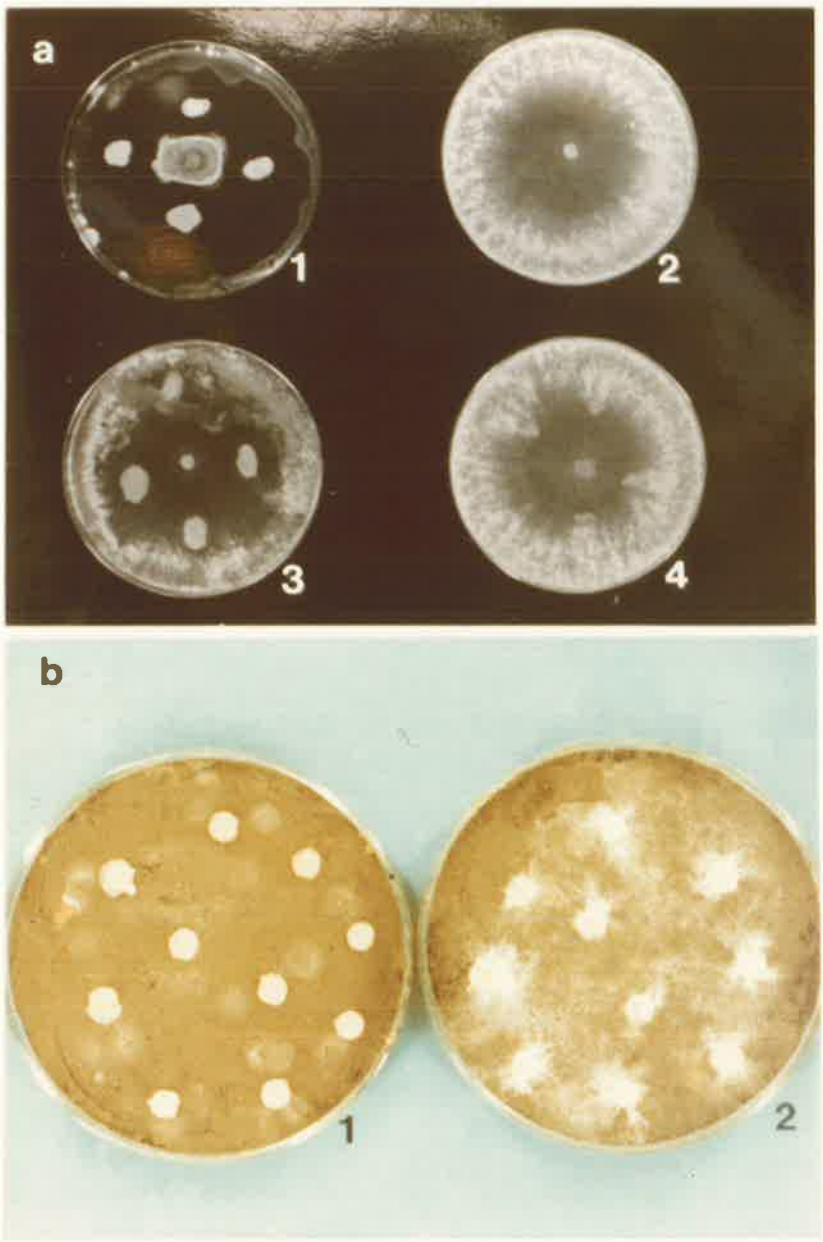
Table 20: Mycoparasites on sclerotia of *S. sclerotiorum* isolated from 5 commercial fields in the NAP

Field with Sclerotinia rot	Number of sclerotia	Number of parasitized sclerotia (%)(1)							
		None	*C.minitans*	*Trichoderma* spp.	*Trichothecium* sp.	*Gliocladium* sp.	*Fusarium* spp.	*Penicillium* sp.	Other fungi
Lettuce (Gawler River)	65	24.6	27.7	4.6	0	35.4	0	0	7.7
Lettuce (Virginia)	60	26.7	51.67	5.0	0	0	0	5	11.6
Lettuce (Virginia)	78	10.3	30.8	0	0	35.9	11.5	3.8	7.7
Potato (Virginia)	72	11.1	38.9	0	9.7	27.9	2.8	6.9	2.7
Cabbage (Salisbury North)	60	11.7	18.3	8.3	0	55.0	10.0	0	6.7

(1)Usually more than one fungus colonizing the sclerotia.

Fig.14: One isolate (a_1) of Ascomycetes inhibited the mycelial growth of *S. sclerotiorum* (at the centre) while the other three did not.

Similar inhibition occurred in clay culture (b_1), compared to control plate (b_2).


a
1
2
3
4
b
1
2

3.3 *Pathogenicity of S. sclerotiorum*

Cultivars of lettuce, prickle lettuce, marigold, barley, wheat, rye-grass, rye-corn and lucerne, were grown for eight weeks in potting soil in pots, each containing two plants. Inoculum of *S. sclerotiorum* was grown on sterilized carrot discs for five days and then blended to homogenize. Each pot received 10 ml of inoculum which touched the plants. All pots were put in metal trays (75 x 50 x 5 cm) containing water, to keep the soil wet. They were kept in the glasshouse where the temperature ranged between 19 and 27°C. The treatments were replicated three times.

Infection in lettuce cultivars was observed three days and in marigold cultivars seven days after inoculation. Thus, under the experimental conditions, although some lettuce cultivars escaped such as Early Mature Lake, Original Great Lakes, Great Lakes (50%), Green Dale (67%) and KOK (83%), all cultivars were infected (Table 21). There is no report in the literature of resistance in lettuce to *S. sclerotiorum*. Steadman (1979) stated that *S. sclerotiorum* has an extremely wide host range. Newton and Sequeira (1972) reported that the escape from infection by *S. sclerotiorum* may be associated with the type of growth of the lettuce cv. However, there is evidence of field resistance in lucerne (Elgin and Beyer, 1968).

The results suggest that two cultivars of marigold, barley, wheat, rye corn, rye grass and lucerne are resistant to *S. sclerotiorum*. They could possibly be used as alternative crops in a rotation programme.

3.4 *Effect of inoculum density on disease incidence in lettuce*

Autoclaved brown rice was inoculated with *S. sclerotiorum* and incubated at 25°C for 5 days. During the incubation the culture was agitated

Table 21: Results of pathogenicity of *S. sclerotiorum* on young lettuce, prickle lettuce, marigold, barley, rye grass, rye corn and lucerne

	Cultivar	Percentage plant infected(1)
Lettuce:		
1	Imperial Triumph	100.0
2	Imperial 847	100.0
3	Imperial 847 (medium type)	100.0
4	Butter Crunch	100.0
5	Cos Lettuce	100.0
6	Brown Mignonette	83.3
7	Green Mignonette	83.3
8	Sunny Lake	83.3
9	Winter Lake	83.3
10	Ace Field	83.0
11	Oak Leaf Lettuce	83.0
12	Salinas	83.0
13	Yates Dale	66.7
14	Early Mature Lake	50.0
15	Original Great Lakes	50.0
16	Great Lakes	50.0
17	Yara Lake	50.0
18	Green Dale	33.3
19	KOK-60	16.7
Prickle Lettuce:		
20	Prickle Lettuce	33.3
Marigold:		
21	Jubilee	16.7
22	Flamenco	16.7
23	Petite Yellow	0.0
24	Honey Comb	0.0
Barley:		
25	Clipper	0.0
Wheat:		
26	Halberd	0.0
Rye-Grass:		
27	Rye-grass	0.0
Rye-Corn:		
28	Rye-corn	0.0
Lucerne:		
29	Lucerne	0.0

(1) Each value is the mean of six plants (three pots each containing two plants).

once a day. Rice grains were mixed with sterile U.C. soil mixture to obtain 0, 0.15, 0.3, 0.6, 1.2, 2.5, 5, 10 and 20% of inoculum by weight (Table 22). One hundred g of the mixture were spread evenly on the soil surface in pots (15 x 15 cm) containing young lettuce plants (3 weeks old). All pots were put in metal trays (75 x 50 x 5 cm) containing water. The experiment was designed in blocks, each containing nine treatments with five replicates. The pots in each block were randomized on the bench in the glasshouse. Temperature ranged between 18 and 26°C. Assessments were made each day, two days after inoculation. Infected plants were removed from pots, in order to maintain the initial inoculum density.

There was a high correlation between time after inoculation and inoculum density and the incidence of infection (Table 22). All plants were killed at 2, 4 and 10 days by 20, 10 and 5% inoculum respectively. The % of infected plants was high (44%) at ten days after inoculation with an inoculum density as low as 0.6%.

Thus the results confirm observations in the field that the higher the inoculum the more plants are infected (Figs.13a,b).

3.5 *Efficacy of some fungicides*

Of the 18 fungicides tested on agar plates, seven prevented growth of *S. sclerotiorum* at the lowest concentration recommended for each fungicide (Table 23, column A). The results in agar cultures did not always correspond with the results when the fungicides were tested as a soil drench. One fungicide (vinclozolin) gave absolute control both on agar plates and on the soil surface or at a depth of 20 mm in the soil. For control on the soil surface there were eight effective fungicides namely: benomyl, captan, fenaminosulf, prochloraz, vinclozolin, iprodione, carbozoline and procymidone (Table 23, column B).

Table 22: Effect of inoculum density in disease incidence on lettuce plants

Inoculum density (%)	Percentage plant infected (1)						
	2D	3D	4D	5D	6D	7D	10D
20	*100*	100	100	100	100	100	100
10	72	92	*100*	100	100	100	100
5	36	56	60	80	96	96	*100*
2.5	28	44	60	72	76	76	84
1.2	0	12	16	24	24	28	40
0.6	0	12	12	12	12	12	44
0.3	0	0	4	4	16	16	24
0.15	0	4	4	4	4	4	4
0.0	0	0	0	0	0	0	0

(1) Each value is the mean of 25 plants (five pots each containing five plants); 2D, 3D, ... = 2 days, 3 days, ... after inoculation.

Table 23: Efficacy of some fungicides against *S. sclerotiorum* in agar culture and in soil

Fungicide	A. On CMA		B. In soil		
	a.i. (ppm) (1)	Mean growth of mycelium (mm/24 h) (2)	a.i. (ppm) (3)	Mean growth of mycelium (mm/24 h) 0 mm (4)	Mean growth of mycelium (mm/24 h) 20 mm deep (4)
Fosetyl	800	6.51	2400	6.28	6.70
Fosetyl + mancozeb	980	5.31	2100	7.17	9.88
Benomyl	500	0	1000	0	1.37
Chlorothalonil	1225	2.66	1630	8.06	7.83
Captan	830	0	1245	0	7.06
Captafol	500	1.29	1250	8.11	7.72
Mancozeb	960	2.08	1600	3.74	5.85
Fenaminosulf	595	1.31	1190	0	4.31
Metiram	1200	1.31	1600	6.74	8.00
Prochloraz	320	0	640	0	4.04
Metalaxyl	400	3.27	750	6.93	7.48
Vinclozolin	750	0	750	0	0
Iprodione	500	0	750	0	5.19
Carbozoline	750	0	1000	0	3.49
SN.78.314	200	6.77	300	6.44	7.11
Procymidone	500	0	1000	0	7.50
Quintozene	2250	1.58	3750	4.67	7.48
Etridiazole	84	1.66	140	5.06	6.70
Control	0	9.16	0	10.1	9.98

(1) Concentration recommended at lowest rate for each fungicide

(2) Mean value of three replicates after 3 days

(3) Concentration at higher rate of each fungicide

(4) Mean value of three replicates after 3 days.

Utkhede and Rahe (1979) reported that vinclozolin and iprodione gave promising results in controlling the sclerotial fungus *Sclerotium cepivorum*. They also suggested that the major aspect of the control was against mycelial growth in the soil. Vinclozolin was also reported effective against Sclerotinia rot and grey mould in lettuce (Heimes and Locher, 1979).

Spraying the soil surface and the timing of applications gives better protection to lettuce (Marcum *et al.*, 1977; Merriman *et al.*, 1978; Steadman, 1979), as Sclerotinia rot originates from organic matter or senescent plant material on the soil surface. However alternating sprays with recommended fungicides would probably increase the efficacy of the fungicides. The results reported here suggest that vinclozolin, benomyl, carbozoline and iprodione are likely to control *S. sclerotiorum* in soil possibly under field conditions.

3.6 *Physiological and cultural factors*

3.6.1 *Effects of pH*

The effects of pH were studied on PDA in 9 cm Petri dishes. The pH value was adjusted by adding citric acid and/or potassium hydroxide solution to the medium prior to sterilization. The values used in this experiment are shown in Fig.15. A plug of actively growing mycelium of *S. sclerotiorum* from a 2 day culture was placed at the centre of each agar plate and incubated at 25°C for seven days. Mycelial growth was measured every two days and counting of sclerotia every seven days after inoculation. There were five replicates for each treatment.

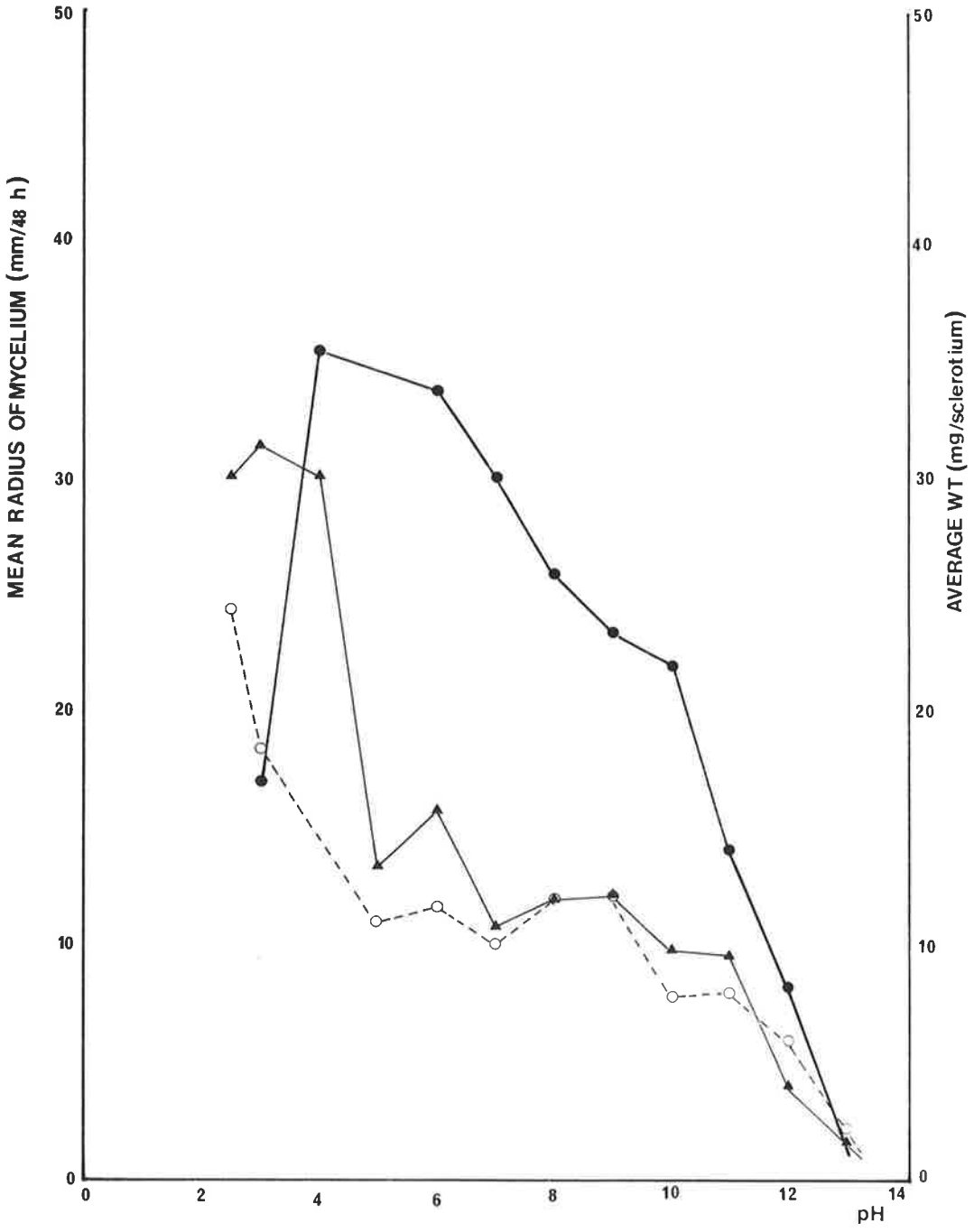
Although *S. sclerotiorum* grew over a wide range of pH it's mycelium grew quickest in cultures at pH 4 to 6 (Fig.15). Pro-

Fig.15: Effect of pH of media on mycelial growth and production of sclerotia of *S. sclerotiorum*

●——● mycelial growth (mm) after 48 h

○——○ number of sclerotia per Petri dish

▲——▲ average weight (mg) of 1 sclerotium.


MEAN RADIUS OF MYCELIUM (mm/48 h)
AVERAGE WT (mg/sclerotium)
pH
0
10
20
30
40
50
0
2
4
6
8
10
12
14

duction of sclerotia was greatest at pH 2 to 4. Fig.15 also shows that the average weight of sclerotia varied depending on the pH of the medium; heaviest sclerotia occurred at pH 2. The optimum pH for mycelial growth was very low, agreeing with other reports (Tanrikut and Vaughan, 1951; Rai and Agnihotri, 1971).

3.6.2 *Effects of nitrogen and carbon sources*

The aim of this experiment was to study the ability of *S. sclerotiorum* to utilize nitrogen and carbon from different sources. Basal medium of Abeygunawardena and Wood (1957) was used throughout. Nitrogen sources tested were: L-asparagine, peptone, ammonium sulphate, ammonium nitrate, ammonium chloride, ammonium tartrate, sodium nitrate, potassium nitrate and calcium nitrate at a concentration of 0.09% nitrogen in basal medium; the source of carbon: glucose, sucrose, D(-)fructose (laevulose), lactose, maltose, mannitol, starch, citric acid, glutamic acid and glycerol at amounts of carbon equivalent to 2.5% glucose. They were incubated at 25°C for eight days. Each treatment was replicated twice.

Of the compounds supplying nitrogen, fastest growth occurred in media containing potassium nitrate, followed by calcium nitrate and sodium nitrate. Of compounds supplying carbon, mycelial growth was fastest in glucose, lactose, sucrose and starch. In general, the production of sclerotia was stimulated most by compounds of nitrogen as shown in Table 24 which also shows that media promoting mycelial growth did not necessarily promote the production of sclerotia. High production was found in L-asparagine, sodium nitrate and potassium nitrate for nitrogen and maltose, D(-)fructose, lactose and glucose for carbon sources.

Table 24: Dry weight of mycelium and sclerotial production of *S. sclerotiorum* on different sources of nitrogen

Source of nitrogen	Dry weight (mg) of mycelium (1)	Production of sclerotia in 9 cm Petri dish (2)
Potassium nitrate	579.80	44.5
Calcium nitrate	576.93	33.5
Sodium nitrate	529.17	46.5
Ammonium nitrate	431.60	19.0
Ammonium sulphate	400.50	24.5
Ammonium chloride	395.63	25.0
L-asparagine	365.83	46.5
Peptone	329.93	32.0
Ammonium tartrate	272.73	18.0
Basal medium	189.20	0

(1) Mean value of three replicates

(2) Mean value of two replicates.

The data found in the experiment suggested that compounds supplying nitrate were better than ammonium, which agreed with the findings of Newton (1946) and Demetrides (1953, *cit.* Willetts and Wong, 1980). Willis (1968) found that *S. sclerotiorum* grew equally well on media containing nitrate and ammonium compounds. According to Rai and Agnihotri (1971), potassium nitrate produced maximum growth but poor sclerotia, while sodium nitrate and calcium nitrate gave good growth and sclerotial formation. Hacskaylo *et al.* (1954) reported that glucose, maltose and sucrose were good sources for carbon for mycelial growth, while glycerol and mannitol were poor sources. Such findings are in agreement with the results of this experiment, except for maltose which was inadequate for mycelial growth but gave the highest number of sclerotia (Table 25).

3.7 *A prerequisite for infection by ascospores*

The purpose of the experiment was to study organic matter or enriched solution as prerequisites for initial infection by ascospores of *S. sclerotiorum* on healthy lettuce plants. Green leaves of lettuce, potato and soursob (*Oxalis pes-caprae* L.) were oven dried for 24 h and each was crushed in a Petri dish. Plant extracts and other solutions used in the experiment were similar to those used in cultural tests *in vitro* (Table 26). Six weeks-old lettuce plants in pots each containing 4 plants were used.

Each treatment was applied evenly to the upper surface of lettuce leaves by using a power "Jet Pak" sprayer for liquid and by hand for plant materials. Then a mass of ascospore suspension was applied using a similar sprayer. All treatments were incubated at 20°C for four days in a dew chamber. It was replicated four times.

Table 25: Dry weight of mycelium and sclerotial production of *S. sclerotiorum* on different sources of carbon

Source of carbon	Dry weight (mg) of mycelium (1)	Production of sclerotia in 9 cm Petri dish (2)
Glucose	435.93	22.0
Lactose	422.73	23.5
Sucrose	388.37	18.0
Starch	380.27	17.0
D(-)fructose	304.73	27.0
Glycerol	166.93	2.5
Maltose	158.73	29.0
Mannitol	148.87	10.0
Basal medium	83.03	0
Glutamic acid	18.53	0
Citric acid	16.17	0

(1) Mean value of three replicates

(2) Mean value of two replicates.

Table 26: Effect of substances as a prerequisite for initial infections by ascospores of *S. sclerotiorum* in healthy young lettuce

Substance (1)	Number of lesions (2)	Scoring appearance (3)
Lettuce leaf	20 - L	+++
Potato leaf	15 - L	+++
Peptone solution	11 - L	+++
Soursobs leaf	12 - L/M	+++
Starch solution	12 - M	+++
NDY	11 - L/S	+++
CSE (Cabbage stem extract)	9 - L	+++
LSE (Lettuce stem extract)	8 - L	+++
Sucrose solution	8 - L/S	++
Sodium nitrate solution	8 - L/S	+
Potato extract	4 - S	+
Glycerol solution	0	0
Soil extract	0	0
SDW (sterile distilled water)	0	0

(1) Leaves of lettuce, potato and soursobs were oven dried and then crushed and spread over wetted leaves of healthy lettuce. Others were sprayed over lettuce plants.

(2) L = large lesion; M = medium; S = small.

(3) +++ = infection occurred rapidly and large; ++ = less rapid; + = in some cases it did not spread; 0 = no infection.

The results in Table 25 show that ascospores required organic matter or other substances for initial infection on lettuce plants. Oven dried leaf materials (lettuce, potato, soursob), peptone, starch and NDY supported the initial infection, while glycerol, soil extract and distilled water did not.

3.8 *Factors affecting sclerotial viability*

The aim of the experiments was to study the effect of some factors influencing the survival and germination of sclerotia under laboratory conditions.

3.8.1 *Effect of storage duration in cool room*

Air dried sclerotia were placed in a sterile Petri dish and stored in a cool room (ca. 4°C) until they were used. To induce sclerotial germination (carpogenic germination), the sclerotia were placed on moist sterile field soil in a container 7 cm deep and 7.5 cm in diameter and kept in an outside shade house during winter. The number of stipes was recorded periodically and ended after two months.

Table 27 shows that, after 16 months in storage, a high percentage of sclerotia still germinated (53.3%), compared with the highest one (80%) when they were stored for 5 months. The number of stipes per sclerotium ranged from 0 to 8. Tests of mycelial growth on CMA showed that 95-100% sclerotia were viable.

3.8.2 *Effect of burial in storage*

In a separate test, sclerotia were kept in sterile field soil

Table 27: Effect of duration in storage on the survival of sclerotia *S. sclerotiorum*

Length in storage (month)	Percent germinated sclerotia (1)	Number of stipes per sclerotium
16	53,3	0 - 5
14	53.3	0 - 5
9	76.7	0 - 6
8	40.0	0 - 5
7	53.3	0 - 5
5	80.0	0 - 8
4	70.0	0 - 7
2	63.3	0 - 6
1	76.7	0 - 8

(1) Mean values of 30 sclerotia.

Test of mycelial growth on CMA, showed that 95 - 100% sclerotia were viable.

at different depths (Table 27). The water content of the soil was 12.5%. They were stored at 15°C under alternating 12 h light and 12 h darkness for 16 months in a growth chamber. Then the sclerotia were germinated as described in 3.8.1.

There was no sclerotial germination during storage. The highest percentage stipes occurred when sclerotia were buried 5 cm deep (Table 28). The number of stipes per sclerotium ranged from 0 to 12.

3.8.3 *Effect of depth on sclerotial germination*

Newly harvested sclerotia were placed at the following depths in sterile field soil: 0, 2.5, 5, 7.5, 10, 12.5 cm. They were germinated after 16 months (see 3.8.1).

No sclerotia germinated when they were buried. However, Cooke (1970) and Smith (1972) reported that apothecia are formed from sclerotia buried not deeper than 5 cm in soil. In the present experiment there was no indication of stipes even after three months.

The loss of viability of sclerotia is influenced by several factors. High temperature and high soil water content (Williams and Western, 1965; Smith, 1972; Cook *et al.*, 1975), depth of burial (Chambers and Hardie, 1964; Cook *et al.*, 1975; Merriman, 1976), structure and pH of soil (Merriman, 1976) all reduce viability.

Table 28: Effect of depth in soil during storage on the sclerotial germination of *S. sclerotiorum*

Depth in soil in storage (cm)	Percent germinated sclerotia (Mean of 20 sclerotia)	Number of stipe per sclerotium
0 (soil surface	75	0 - 5
2.5	95	0 - 12
5.0	100	0 - 10
10.0	90	0 - 7
12.5	80	0 - 6

Fig.16: Sclerotia of *S. sclerotiorum*

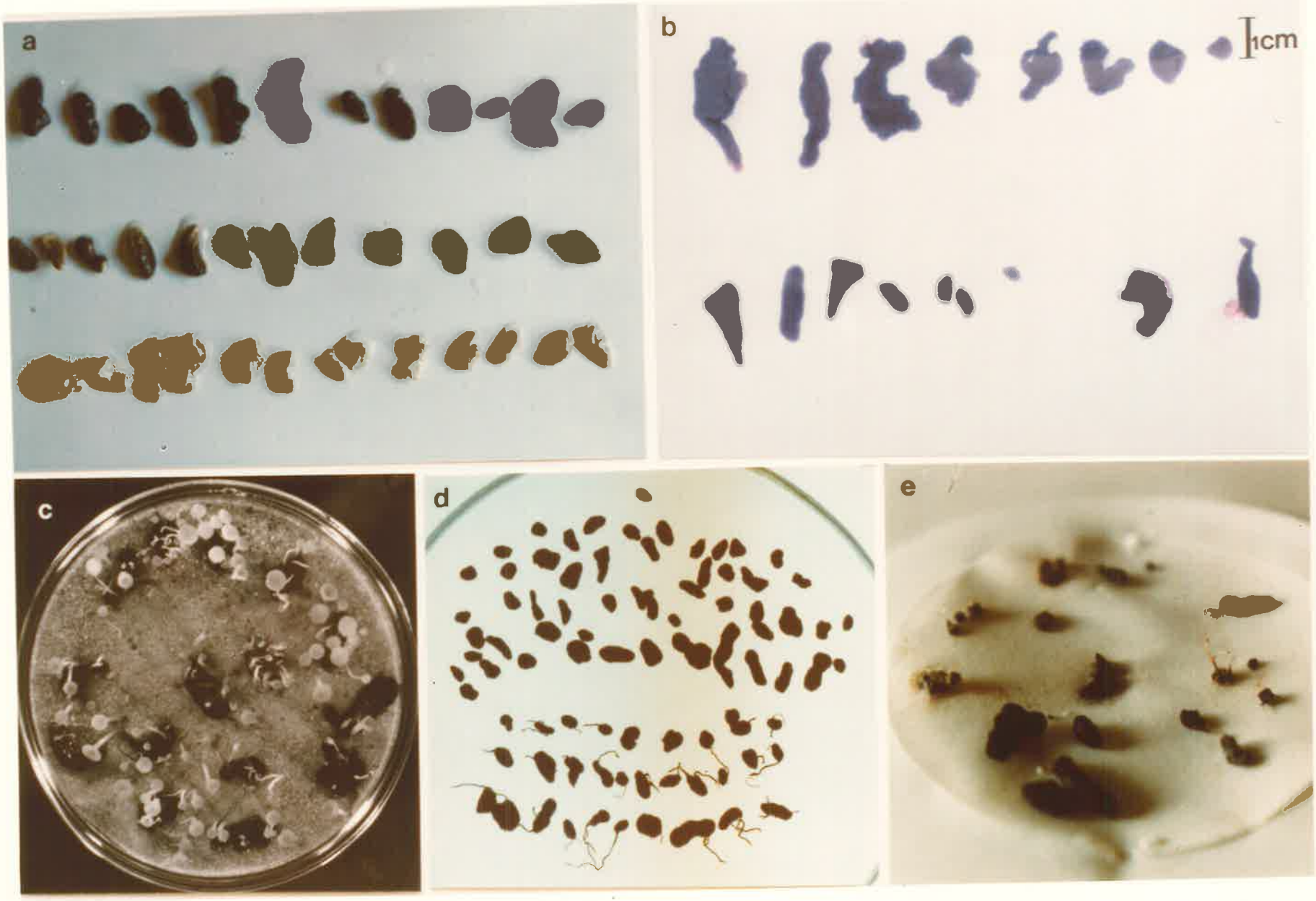
a. The visual stages of sclerotial formation, collected from natural infected lettuce:
Back row: mature, black, compact, hard
Middle row: half-mature, gelatinous, soft
Front row: newly formed, white, gelatinous soft.

b. The size and form of sclerotia from natural infected lettuce; from largest 3.3 x 2.4 to the smallest 0.6 x 0.4 cm.

c. Production of apothecia on moist sterile coarse sand under laboratory condition at 15°C. Sclerotia were produced in autoclaved carrot discs at 25°C for 2 weeks.

d. Sclerotia from lettuce field soil, some germinated (with stipes), some did not.

e. Examples of sclerotia colonized by mycoparasites or antagonists. They were kept at 25°C for 2 weeks on wet sterile filter paper.


a
b
1cm
c
d
e

3.9 Biological control of *S. sclerotiorum*

3.9.1 *Biological control by* Fusarium lateritium

3.9.1.1 *Inhibition of mycelial growth*

The effect of *F. lateritium* on mycelial growth of *S. sclerotiorum* was studied on six media: NDY/20, PDA, CMA, LSA, LLA and CSA (see Section 2.3.7.1). An agar plug of *F. lateritium* was placed near the edge of each plate and incubated at 25°C. Two days later, an agar plug of *S. sclerotiorum* was placed at the opposite side of the plate and incubated for eight days before measurements were made of any inhibition zones. There were two replicates per treatment.

F. lateritium inhibited mycelial growth on all six media being greatest on NDY/20 as shown by the mean radius of the inhibition zone in Table 29.

3.9.1.2 *Inhibition of ascospore germination*

The fungitoxicity of *F. lateritium* against *S. sclerotiorum* was studied using the method of Carter and Price (1974). Two media (NDY/20 and GPA/20) were used. Fifteen ml of each medium were poured into sterile 90 mm glass Petri dishes. Plugs, 5 mm in diameter, taken from the margin of an actively growing *F. lateritium* colony on consecutive days were placed singly at the centre of a Petri dish and incubated at 25°C. After ten days, all the *F. lateritium* colonies were killed by exposure to chloroform vapour for 30 min and the chloroform allowed to evaporate. Each plate was then sprayed with a heavy suspension of *S. sclerotiorum* ascospores and incubated at 25°C for three days before measurements were made. The extent of the inhibition zones

Table 29: Inhibition of mycelial growth of *S. sclerotiorum* in different media previously inoculated with *E. lateritium*

Medium	Radius (mm) of inhibition zone (1)	Radius (mm) of *F. lateritium*
NDY/20	45.0[a]	8,5
CMA	40.0[b]	11.5
CSA	37.7[bc]	10.9
LLA	36.8[c]	11.0
LSA	36.7[c]	11.5
PDA	34.0[c]	9.3

(1) Values followed by the same letter are not significantly different ($P<0.05$). Each value is the mean of two replicates.

was expressed as the mean of four radial measurements. There were two replicates per treatment.

As shown in Fig.17a the radius of the inhibition zone after one day on NDY/20 was 15 mm. Thereafter, the radius of inhibition increased linearly with time as shown by the statistically significant regression line ($P<0.001$). A similar result was obtained on GPA/20 (Fig.17b).

3.9.1.3 *The influence of medium on the inhibition of ascospore germination*

To ascertain whether inhibition of ascospore germination was effective on other media, six kinds in addition to NDY/20 and GPA/20 were used: PDA, CMA, LLA, LSA, CSA (see Section 2.3.7.1) and ALL (autoclaved lettuce leaf). *F. lateritium* colonies were killed after 4 days incubation, then sprayed with a suspension of *S. sclerotiorum* ascospores. There were six replicates per treatment and measurements of inhibition zones were made after three days.

Inhibition of ascospore germination was effective on six other media. Greatest inhibition occurred in NDY/20 and least in ALL as shown by the mean values of the inhibition zone (Table 30).

3.9.1.4 *The influence of temperature on inhibition of ascospore germination*

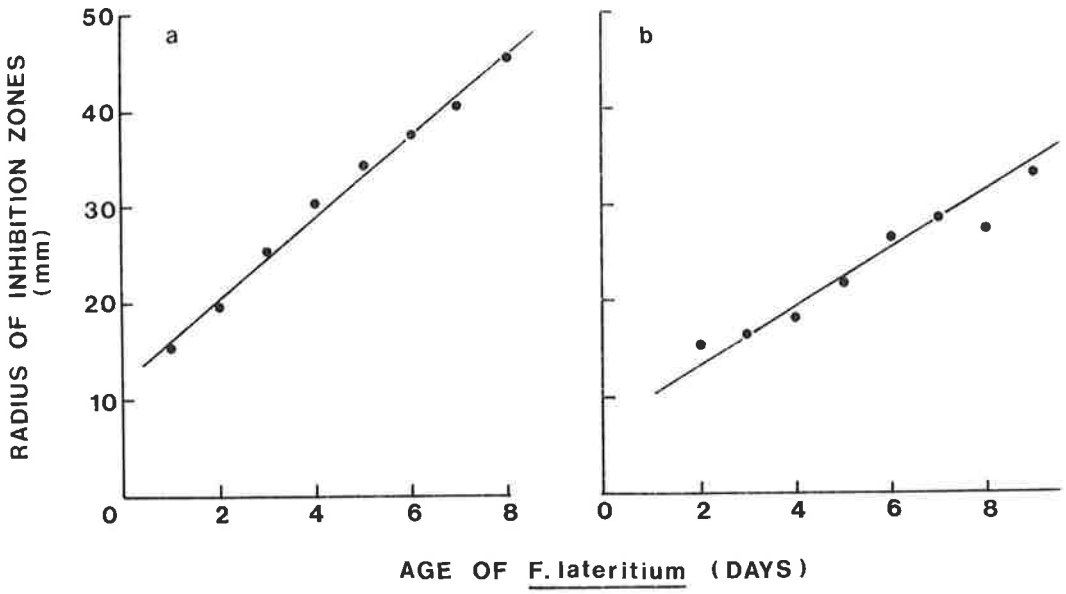
A 5 mm disc of *F. lateritium* from a seven day culture on CMA was placed at the centre of an NDY/20 plate and incubated for three days at each of the following: 5, 10, 15, 20, 25 and 30°C in darkness; 7.5 and 15°C with alternating 12 h light and 12 h darkness; 25°C in continuous light. Colonies of *F. lateritium* were killed by

Fig.17: The inhibitory influence of *F. lateritium* against *S. sclerotiorum in vitro*

a. Regression of radius of inhibition zone on age of *F. lateritium* colonies on NDY/20 ($r = 0.994$); $P<0.001$)

b. Regression of radius of inhibition zone on age of *F. lateritium* colonies on GPA/20 ($r = 0.969$; $P<0.001$)

c. Effect of temperature of incubation on radius of zones of inhibition of ascospore germination by 3 day culture of *F. lateritium*. Open circles: continuously dark. Closed circles: 7.5 and 15°C 12 h light - 12 h dark, and 25°C continuous light. Vertical lines show 95% confidence limits.


a
b
RADIUS OF INHIBITION ZONES (mm)
50
40
30
20
10
0
2
4
6
8
AGE OF F. lateritium (DAYS)

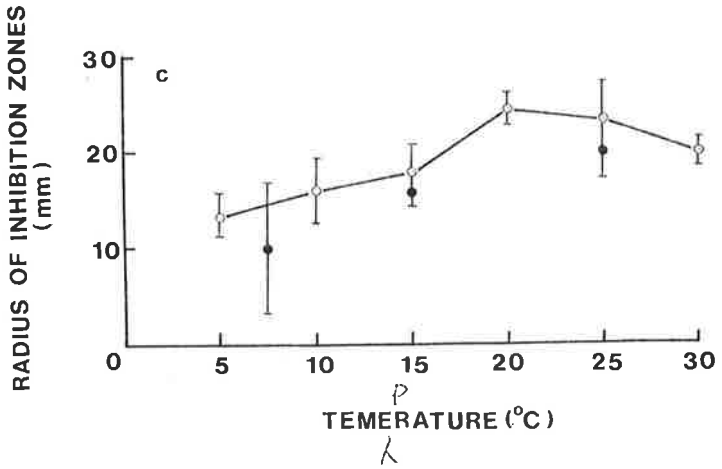

c
RADIUS OF INHIBITION ZONES (mm)
30
20
10
0
5
10
15
20
25
30
TEMERATURE (°C)

Fig.18: Control of *S. sclerotiorum* by *F. lateritium in vitro*.

a. Inhibition zone increased as the age of *F. lateritium* (4,5,6,7,8 and 9 days) increased on NDY/20

b. Autoclaved lettuce leaves inoculated with a plug of mycelium of *F. lateritium*, were incubated at 25°C for four days then killed with chloroform vapour. All leaves were then sprayed with a suspension of ascospores of *S. sclerotiorum*. The inhibition zones show the protection against *S. sclerotiorum* by *F. lateritium*, after four days incubation at 25°C.

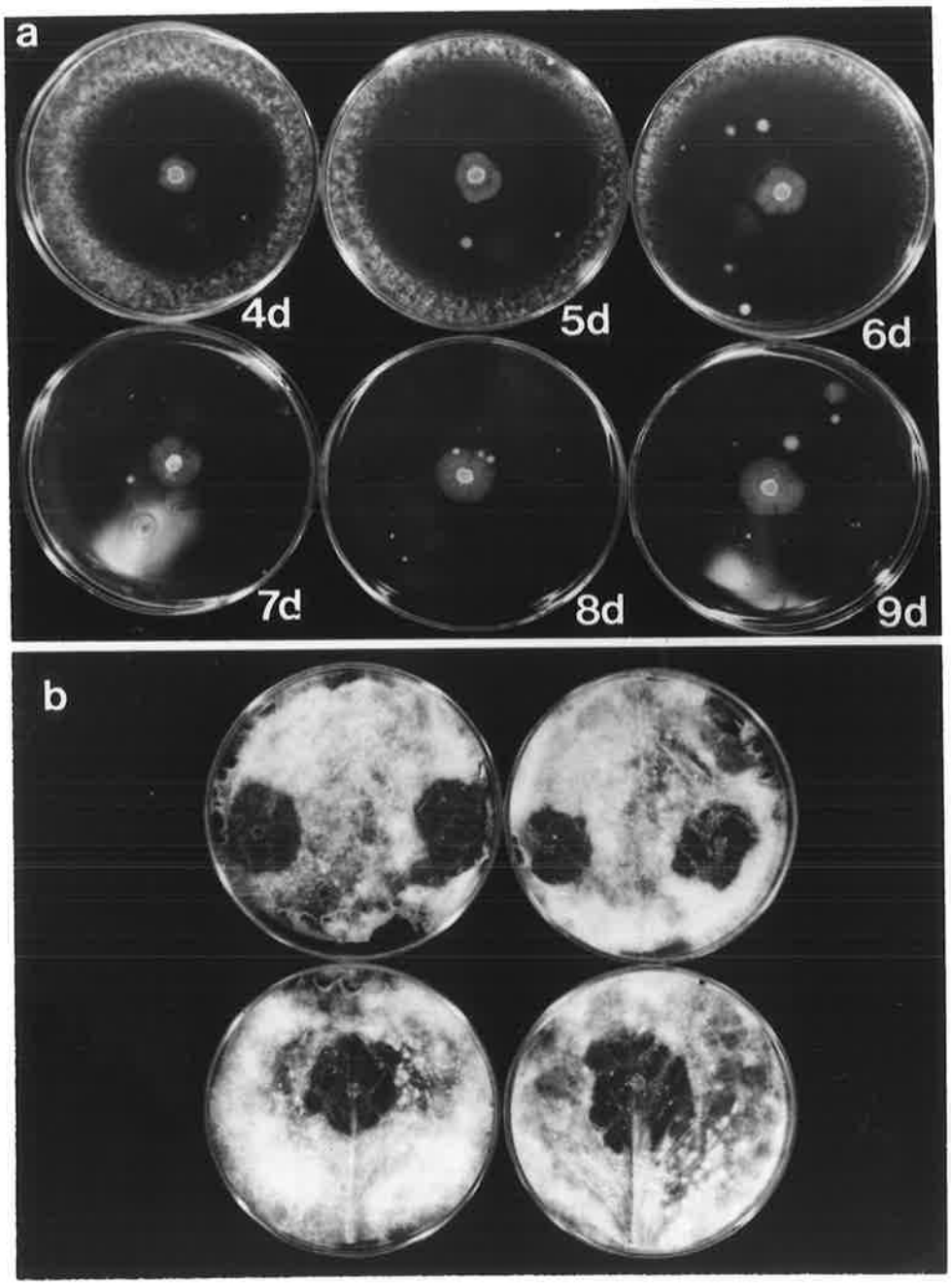

a
4d
5d
6d
7d
8d
9d
b

Table 30: Radius of zone of inhibition of ascospore germination in different media. Measurements were made after three days incubation at 25°C

Medium	Radius (mm) of inhibition zone (1)
NDY/20	30.21[a]
LSA	27.54[b]
LLA	25.96[bc]
CMA	23.83[cd]
CSA	22.83[d]
PDA	19.13[e]
FPA/20	18.13[e]
ALL	14.92[f]

(1) Values followed by the same letter are not significantly different ($P<0.05$). Each value is the mean of six replicates.

chloroform vapour and the plate sprayed with a suspension of *S. sclerotiorum* ascospores. Plates were incubated at 25°C for four days before measurements of inhibition zones were made. There were three replicates per treatment.

As indicated in Fig.17c, the extent of inhibition was greatest at about 20°C under continuous dark incubation. Although very little mycelial growth of *F. lateritium* occurred at 5, 7.5 and 30°C, nevertheless the inhibition zones were substantial.

3.9.1.5 *Glasshouse experiments on the protection of young lettuce plants*

The lettuce cultivar Imperial Triumph was used throughout. Seeds, surface sterilized in 1% sodium-hypochlorite-absolute alcohol 2:1 (v/v) were sown in sterile vermiculite. After six days at 25°C, five seedlings were transplanted into each of a series of pots (15 x 15 cm) containing steam sterilized UC soil mixture and allowed to grow for four weeks in a glasshouse (20-30°C). The experiment was designed in three blocks, each containing three treatments with five replicates. The pots in each block were randomized on the bench. Mature apothecia from sand cultures were placed in open containers and positioned centrally in each of the three blocks so that released ascospores could reach any plant in the experiment. The "puff" of ascospores occurred naturally and air currents dispersed and deposited them on plant leaves and soil surfaces. The release of ascospores continued until all apothecia dried and wilted. The three treatments were arranged as follows: The first treatment consisted of autoclaved lettuce leaves, as organic matter, placed on the surface of the soil in contact with each plant. The second treatment was the same as the first but

sprayed with a suspension of *F. lateritium* macroconidia 7 h before inoculation with *Sclerotinia* ascospores. In the third treatment neither organic matter nor *Fusarium* macroconidia were applied.

Each of the three blocks of 15 pots was arranged in a metal tray (75 x 50 x 5 cm) and covered with a plastic cage 50 cm high to maintain humidity and to prevent dispersal of ascospores beyond the pots in each block. Plants were watered and fertilized at intervals. Disease was assessed by observing symptoms of Sclerotinia rot and the experiment ended when all plants in the first treatment had been killed by *S. sclerotiorum*.

First symptoms of Sclerotinia rot were observed in pots with organic matter on the soil surface but no *Fusarium*; after seven days all plants were dead. When organic matter as well as *Fusarium* was absent none of the plants showed symptoms.

The effect of adding *F. lateritium* to pots containing *S. sclerotiorum* and organic matter was most marked: few plants were infected even after 14 days (Table 31 and Fig.19).

The results of these studies show that *F. lateritium* inhibits the germination of ascospores and the growth of mycelia of *S. sclerotiorum in vitro*. The antagonistic property of *F. lateritium* was first reported by Carter (1971) and further studies under field and laboratory conditions revealed its efficacy in preventing the invasion of apricot trees by *Eutypa armeniacae* (Carter, 1971; Carter and Price, 1974). These experiments indicate the inhibitory properties of *F. lateritium* and confirm the observations of Carter and Price (1974) that the nature of the medium influences the extent of the inhibition zone.

Table 31: The influence of organic matter and *F. lateritium* on the infection of lettuce plants by ascospores of *S. sclerotiorum* after 6 and 14 days after inoculation

Treatment	Plants infected by *S. sclerotiorum*[1] %	
	6 days	14 days
Organic matter only	98.7	100.0
Organic matter plus *F. lateritium*	5.3	33.3
No organic matter, no *F. lateritium*	0.0	5.3

[1] Each value is the mean of 75 plants (15 pots each containing five plants).

Fig.19: Control of *S. sclerotiorum* by *F. lateritium*.

Front row: organic matter only; all plants were killed by *S. sclerotiorum*

Middle row: organic matter plus *F. lateritium* which controlled the *S. sclerotiorum*

Back row: neither organic matter nor *F. lateritium* applied

Pots were originally arranged at random on the bench. They have been rearranged here to show the effects of the three treatments (See also Table 31).

The results indicate that organic matter on the surface of soil and in contact with lettuce plants is a prerequisite for infection by *S. sclerotiorum*. It is possible that *F. lateritium* prevents the establishment of the pathogen in the organic matter.

3.9.1.6 *Field experiment*

The results of the first experiment showed no infection of Sclerotinia rot and the incidence of other lettuce diseases was also very low.

In the second experiment, the incidence of Sclerotinia rot was low, not only in the experiment sites but also in the entire field and in the adjacent fields. Table 32 shows that only the plots with a combination of *F. lateritium* and benomyl had plants free from infection with Sclerotinia rot compared with 3.66% in the control plots.

Only few diseases such as LNYV and downy mildew caused problems during the experiment. Downy mildew was found mainly on mature leaves but it did not cause significant loss.

F. lateritium did not infect lettuce plants, thereby confirming the results in the laboratory and glasshouse.

3.9.2 *Biological control by sclerotial parasites*

Laboratory-grown sclerotia were used in tests for the efficacy of some parasites and antagonists on sclerotia of *S. sclerotiorum*. Twenty five sclerotia were mixed with 200 g of sterile, moist field soil in closed plastic containers, 7 cm deep and 7.5 cm in diameter, each previously inoculated with a suspension of

Table 32: Percentage plants harvested and diseased plants in commercial lettuce field treated with *F. lateritium* and benomyl (second experiment)

	Percentage plant harvested (1)	Percentage diseased plants by(2)	
		a. all kinds (3)	b. Sclerotinia rot
Fusarium lateritium	64.45	5.77^{ab}	1.12^{a}
Benomyl	64.39	7.10^{ab}	0.30^{a}
F. lateritium and benomyl	71.19	2.03^{b}	0.00^{a}
Control (non treated)	61.83	9.86^{a}	3.66^{b}

(1) Mean value of eight replicates. Unharvested plants were due to disease and bad quality

(2) Mean value of eight replicates. Values followed by similar letter are not significantly different ($P<0.05$)

(3) LNYV (majority), grey mould (*B. cinerea*), Sclerotinia rot (*S.sclerotiorum*), bacterial soft rot (*Erwinia* sp.). Downy mildew (*B. lactucae*) was found throughout, infecting mature leaves, but it did not kill the plants.

C. minitans, *T. harzianum*, *Fusarium* sp. and *Streptomyces* sp. They were incubated at 25°C for 10 weeks in darkness. Each treatment was replicated twice.

All sclerotia inoculated with *C. minitans* were infected, covered with numerous black pycnidia of *C. minitans*. The parasitized sclerotia lost their viability and became soft and degraded. The sclerotia inoculated with *T. harzianum*, *Fusarium* sp. and *Streptomyces* sp. kept a firm and normal form and appeared to be healthy. There was no indication of infection of the sclerotia. However, attempts to grow the treated sclerotia on CMA, after they were surface sterilized with 1:1 (v/v) sodium hypochlorite-absolute alcohol, showed that 28% of sclerotia were overgrown by *T. harzianum* which probably infected them. All sclerotia inoculated with *Fusarium* sp. and *Streptomyces* sp. produced mycelial growth.

Dos Santos and Dhingra (1982) reported that *T. harzianum*, *T. koningii* and *T. pseudokoningii* parasitized the sclerotia of *S. sclerotiorum in vitro* and killed 62-100% of sclerotia. While Steadman (1982) suggested that *Trichoderma* sp. and *C. minitans* has been less effective on *S. sclerotiorum* in the United States. Tests of antagonistic microorganisms in soil by Broadbent *et al.* (1971) suggested that some Actinomycetes inhibited fungal root pathogens. They found that three isolates of *Bacillus* and one *Streptomyces* isolate effectively controlled damping-off by *R. solani* in pot trials.

4. Summary and Conclusions

4.1 *Sclerotinia rot a major disease of lettuce*

Sclerotinia sclerotiorum is the most destructive pathogen of lettuce in the Northern Adelaide Plains. Yield loss due to this particular pathogen can be as high as 85 percent. The progressive pathogenicity of the fungus under favourable conditions and the ability of sclerotia to survive in soil, enable the pathogen to cause infection and loss in a large area. Plants can be infected at any stage of growth and always results in death.

4.2 *Factors affecting the incidence and severity of disease in lettuce fields*

There is a significant positive correlation between inoculum level of sclerotia and the incidence of Sclerotinia rot. Widely scattered inoculum can promote fast and wide infection in commercial lettuce fields. Waterlogging, wet and cold weather and dense weeds and plants favour the incidence and severity of the disease. Thus, when conditions are not favourable for Sclerotinia rot, the infections are often scattered, but when the conditions are favourable the disease seems to break out explosively almost simultaneously in several fields. Cultural practices such as good cultivation, sanitation, soil drainage, crop rotation and fungicide usage appear to restrict the incidence and severity of Sclerotinia rot.

4.3 *Possible control measures*

At present there is no effective commercial control of Sclerotinia rot in the Northern Adelaide Plains. There was an indication that the disease might be reduced, when measures were taken to prevent the build up of inocula (sclerotia) by intensive mechanical action that removed

infected plants by thorough and clean cultivation, regular preventive spraying with effective fungicides, crop rotation and good soil drainage.

The frequent outbreaks of Sclerotinia rot in lettuce fields which occur suddenly after a change of the weather, can only be controlled by effective fungicides.

In the laboratory and glasshouse, some parasites and antagonists such as *C. minitans*, *T. harzianum* and *Streptomyces* sp. showed effective control of sclerotia of *S. sclerotiorum* and inhibited mycelial growth.

F. lateritium inhibited ascospore germination and mycelial growth of *S. sclerotiorum* in various agar media and organic matter. The introduction of *F. lateritium* macroconidia before introducing *S. sclerotiorum* gave marked protection to young lettuce plants in the glasshouse. Thus, *F. lateritium* appeared to be effective in controlling Sclerotinia rot. Field experiments, although hampered by lack of natural infection, nevertheless supported the glasshouse results.

In the long term, tolerant and resistant cultivars of lettuce to Sclerotinia rot should be sought by selection and breeding.

Chapter 5

GENERAL DISCUSSION

The aims of this project: to identify and describe the diseases affecting vegetables in the Northern Adelaide Plains and their causal pathogens, to ascertain whether environmental factors influence disease, to determine which crops are most affected by disease and to identify the major diseases in them, have been largely achieved.

Studies revealed that the vegetable industry in smallholdings in the Northern Adelaide Plains has been suffering from destructive diseases for many years. The most important one is Sclerotinia rot which, a survey indicated, could cause losses in yield of lettuce of up to 85%. Sclerotinia rot also caused significant losses to other major crops including potato, cabbage, celery and cauliflower.

Lettuce, from the seedling to mature stage, is the most susceptible crop to many kinds of disease, including, in addition to Sclerotinia rot, Phytophthora stem rot, grey mould, anthracnose, downy mildew and lettuce necrotic yellows.

According to Channon and Maude (1971) extensive monoculture and adoption of new cultural techniques increase the incidence and severity of diseases. Furthermore, while old diseases persist, new ones have appeared in vegetable crops. My studies confirm these views. Thus, I discovered two diseases in lettuce, Phytophthora stem rot previously unrecorded anywhere, and alfalfa mosaic previously unrecorded in the Northern Adelaide Plains. Unlike Phytophthora stem rot, alfalfa mosaic has been found only in a few plants and was considered to be unimportant.

Because lettuce was such an economically important crop and

Phytophthora was such a destructive pathogen in that crop, the disease was studied in depth.

Natural infection of lettuce by *Phytophthora* species has not been recorded before. The species I found caused considerable yield losses in lettuce as high as 35 and 45% in the winters of 1980 and 1981 respectively in some fields. Disease was most pronounced in young plants especially under wet and cold conditions.

Newhook *et al.* (1978) listed four low temperature *Phytophthora* species: *P. syringae* (Kleb.) Kleb., *P. primulae* Toml., *P. hibernalis* Carne and *P. lateralis* Tucker and Milbrath. Clancy and Kavanagh (1978) also reported a low temperature *Phytophthora* which they intend to name *P. eriugena*. Examination of the morphological characteristics of the above species (Waterhouse, 1963, 1970; Clancy and Kavanagh, 1978) clearly shows that my lettuce isolate is a different species. When the characteristics of the lettuce *Phytophthora* were compared to the high temperature species, they corresponded most closely to those of *P. porri* Foister (Waterhouse, 1963). However, there are distinct differences between the *P.porri* from lettuce and the high temperature *P. porri*, including optimal temperature for growth, host plant and pathogenicity. A culture of the *P.porri* from lettuce has been deposited at the Commonwealth Mycological Institute with the number IMI.251374.

Laboratory tests showed that *P. porri* from lettuce survived at -4°C but not at 25°C or more. However, the survival of the fungus in the soil needs to be further investigated as many *Phytophthora* species survive under adverse conditions either as mycelia or particularly as chlamydospores (Mircetich and Zentmyer, 1966; Zentmyer and Erwin, 1970).

The highly important pathogen *Sclerotinia sclerotiorum* was also chosen for detailed study. As expected, the number of sclerotia of *S. sclerotiorum* in the soil had the greatest effects on the incidence and severity of Sclerotinia rot disease.

My studies suggested that soil salinity and water content influenced the percentage of infected plants and the number of sclerotia in the field. The influence of such key environmental factors further suggests that such cultural practices as good drainage and appropriate crop rotation play an important role in the control of vegetable diseases.

Further indications of how control of Phytophthora stem rot and Sclerotinia rot could be achieved was gained from experiments in the laboratory and glasshouse. Thus, some cultivars of lettuce were tolerant to both pathogens and there were also clear indications that fungicides were effective. The possibility that biological control might be achieved was enhanced by experiments that showed that the saprophytic fungus, *Fusarium lateritium* clearly controlled *Sclerotinia sclerotiorum* on young lettuce plants under laboratory and glasshouse conditions. My results indicated that such control was achieved through the inhibition of ascospore germination and mycelial growth of *S. sclerotiorum* by *F. lateriium*. Maximum control was achieved when *F. lateritium* was applied in the presence of organic matter which is also a prerequisite for the initial infection by ascospores of *S. sclerotiorum* in healthy lettuce crops.

Unfortunately, levels of infection by *S. sclerotiorum* in field lettuce crops was unexpectedly low in 1983, although the winter was wet and cold. Nevertheless results from field experiments done at that time did indicate that *F. lateritium* was not pathogenic to lettuce plants and that disease was less in the plots with *F. lateritium* alone or in combination with

the fungicide, benomyl. Clearly, further experiments are needed to test what appears to be a promising method of biological control.

The existence of several important sclerotial parasites and antagonists in the soil of the Northern Adelaide Plains, in particular, *Coniothyrium minitans* and *Trichoderma harzianum*, suggested that these organisms together with others (*Trichoderma* spp., *Fusarium* spp., *Mucor* sp., *Trichothecium* sp., *Gliocladium* sp., *Penicillium* sp. and *Streptomyces* sp.) might have controlled the disease. Further studies in this area would be worthwhile.

From the initial field survey, there was some indication that the degree of cultural practice such as cultivation, crop rotation, removal of infected plants, fungicide and fertilizer usage, influenced the occurrence and severity of Sclerotinia rot in commercial lettuce fields. Thus, clean and thorough cultivation followed by proper drainage of soil decreased the incidence and severity of disease. The most likely explanation is that, under good cultivation, plants not only grow better and are therefore more tolerant to disease, but also that there is less chance for the pathogen to germinate and infect plants because there is less organic matter and soils are drier.

The survey also indicated that crop rotation is useful as long as the interval between crops is sufficient to reduce the inoculum in the soil to a level below economic importance. In the Northern Adelaide Plains crop rotation on most farms is not sufficient to control Sclerotinia rot, mainly because the length of the rotation, 2 to 3 years, is too short and because the crops that are used are also alternative hosts of the pathogen. Rotation appears to be aimed more at improving the fertility of soil than permitting escape from disease. Furthermore, the same agricultural tools are used in different fields thus encouraging spread

of the pathogen. It is possible that the number of sclerotia of *S. sclerotiorum* surviving in the soil could be reduced by deep ploughing (Chambers and Hardie, 1964; Merriman, 1976).

The results achieved in this project indicate that increased control of disease in lettuce might be achieved immediately by a more rational approach to cultivation practices such as crop rotation, drainage and fungicide application.

In the short term increased control of disease is likely to be achieved by the use of tolerant cultivars and the application of *F. lateritium* as a biological control agent. In the long term, the possibility of using sclerotial parasites and inhibitors needs to be explored ~~explained.~~

Finally, with such a range of available control measures it should be possible to achieve integrated control whereby the level of control for each method used is adjusted to optimize crop yields.

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APPENDIX 1: PUBLICATION

Stem rot of lettuce caused by a low temperature *Phytophthora porri* in South Australia.

Australasian Plant Pathology 10: 59-60.

Sitepu, D., & Bumbieris, M. (1981). Stem Rot of lettuce caused by a low temperature Phytophthora porri in South Australia. *Australasian Plant Pathology, 10*(3), 59-60.

APPENDIX 2: PUBLICATION

Biological control of *Sclerotinia sclerotiorum* in lettuce by *Fusarium lateritium*. *Aust. J. Expt. Agric. Anim. Husb.* 24: (In press).

02 Biological control of Sclerotinia sclerotiorum in 03 lettuce by Fusarium lateritium

004 D. Sitepu and H. R. Wallace

005 Summary—*Fusarium lateritium* inhibited ascospore germination and mycelial growth of *Sclerotinia* 006 *sclerotiorum* in various agar media. Although the inhibition zones produced by *F. lateritium* were most 007 extensive at 20°C, they were still substantial at 5 and 30°C. *S. sclerotiorum* requires organic matter on 008 the soil surface for subsequent infection of lettuce stems in pots. The introduction of *F. lateritium* into 009 such pots, 7 h before introducing *S. sclerotiorum*, gave protection. In infected pots with organic matter, 010 99% of plants were infected after 6 d, whereas only 5% were infected when *F. lateritium* was 011 introduced. After 14 d these values increased to 100 and 33%, respectively.

12 *Sclerotinia sclerotiorum* (Lib.) de Bary, a fungal 013 pathogen of vegetable crops, causes severe losses in 014 many commercial lettuce fields of the Northern 015 Adelaide Plains in South Australia where vegetable 016 crops have been grown continuously for the past 20 017 years. Fungicides have not controlled the pathogen, 018 hence the search for and improvement of biological 019 control seem worthwhile.

020 Several hyperparasites have been found on sclerotia 021 of *Sclerotinia* spp. *Coniothyrium minitans* is an effective 022 parasite on sclerotia (Campbell 1947; Merriman 1976; 023 Huang 1977; Trutmann *et al.* 1980). *Sporidesmium* 024 *sclerotivorum* has been discovered in soils of the USA, 025 causing natural destruction of sclerotia of *Sclerotinia* spp. 026 (Ayers and Adams 1979; Adams and Ayers 1981). Other 027 saprophytic fungi reported as antagonists or parasites of 028 sclerotia are species of *Trichoderma* (Dos Santos and 029 Dhingra 1982), *Fusarium*, *Mucor*, *Alternaria*, *Epicoccum* 030 (Merriman 1976), *Penicillium*, *Aspergillus* and *Stachybotrys* 031 *atra* (Rai and Saxena 1975). The antagonistic property of 032 *F. lateritium* to *Eutypa armeniacae* was first reported by 033 Carter (1971) and further studies under field and 034 laboratory conditions revealed its efficacy in preventing 035 the invasion of apricot trees by this fungus (Carter 1971; 036 Carter and Price 1974).

037 To explore the possibility that *F. lateritium* might 038 control *S. sclerotiorum*, a series of *in vitro* experiments was 039 done to test the hypothesis that mycelial growth and 040 ascospore germination of *S. sclerotiorum* would be 041 inhibited by *F. lateritium*. As *in vitro* experiments do not 042 necessarily reflect events in a soil-plant environment, 043 the efficacy of *F. lateritium* as a biological control agent 044 was also tested in pots containing soil and lettuce 045 plants.

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The culture of *F. lateritium* used was provided by Dr M. V. Carter who originally isolated the fungus from wood tissues of apricot branches and referred it to the Commonwealth Mycological Institute for identification. Its reference number at the CMI, which keeps a culture of this fungus, is IMI 148025.

Materials and methods

Sclerotinia sclerotiorum was isolated from an infected lettuce taken from a commercial field at Virginia, South Australia. Media for isolations and stock cultures were potato dextrose agar (PDA), commeal agar (CMA) (Commonwealth Mycological Institute 1968) and autoclaved carrot discs. All cultures were incubated at 25°C in darkness. Sclerotia were obtained from autoclaved carrot discs about 5 mm thick inoculated with plugs of mycelium from a PDA culture and incubated for two weeks. Sclerotia were extracted from the media by washing on a 2 mm sieve, surface sterilized with a mixture of 2:1 (v/v) 1% sodium hypochlorite–absolute alcohol, rinsed several times with distilled water and air dried.

Apothecia were produced by placing sclerotia on sterile coarse sand in closed plastic containers 7·5 cm high and 7·5 cm in diameter. The sand was periodically moistened with distilled water during incubation at 15°C under alternating light and dark or at room temperature during winter. Six to seven weeks later, many apothecia with mature ascospores were obtained from the cultures.

Fusarium lateritium produced macroconidia abundantly after a short incubation on PDA, CMA or autoclaved brown rice grains (Carter 1983). After 7 d at 25°C, macroconodia were harvested and stored.

In addition to the media already mentioned, dilute Czapek-Dox plus yeast extract (NDY/20) was made up according to the formula of the CMI (1968) and glucose peptone agar (GPA) as formulated by Cook *et al.* (1949). Lettuce stem extract (LSA) was obtained from 100 g of chopped lettuce stems boiled in 1 litre of distilled water for 30 min. After filtering through Whatman filter paper No. 2 and adding 15 g agar powder, the extract was autoclaved for 15 min. Lettuce leaf extract (LLA) and cabbage stem extract (CSA) were obtained by a similar procedure.

Experiment 1: Inhibition of mycelial growth

The effect of *F. lateritium* on mycelial growth of *S. sclerotiorum* was studied on six media: NDY/20, PDA, CMA, LLA, LSA and CSA. An agar plug of *F. lateritium* was placed near the edge of each plate and incubated at 25°C. Two days later, an agar plug of *S. sclerotiorum* was placed at the opposite side of the plate and incubated for 8 d before measurements were made of any inhibition zones. There were two replicates per treatment.

Experiment 2: Inhibition of ascospore germination

The action of *F. lateritium* against *S. sclerotiorum* was studied using the method of Carter and Price (1974). Two media (NDY/20 and GPA/20) were used. Fifteen ml of each medium were poured into sterile 90 mm glass Petri dishes. Plugs 5 mm in diameter, taken from the margin of an actively growing *F. lateritium* colony, were placed singly on consecutive days at the centre of Petri dishes and incubated at 25°C. After 10 d, all *F. lateritium* colonies were killed by exposure to chloroform vapour for 30 min. and the chloroform allowed to evaporate. Each plate was then sprayed with a heavy suspension of *S. sclerotiorum* ascospores and incubated at 25°C for 3 d before measurements were made. The extent of the inhibition zones was expressed as the mean of four radial measurements. There were two replicates per treatment.

Experiment 3: The influence of medium on inhibition of ascospore germination

To ascertain whether inhibition of ascospore germination was effective on other media, six other media were used in addition to NDY/20 and GPA/20: PDA, CMA, LLA, LSA, CSA and ALL (autoclaved lettuce leaf). *Fusarium lateritium* colonies were killed after 4 d incubation, then sprayed with a suspension of *S. sclerotiorum* ascospores. There were six replicates per treatment and measurements of inhibition zones were made after 3 d.

Experiment 4: The influence of temperature on inhibition of ascospore germination

To determine the influence of temperature on inhibition of ascospore germination, a 5 mm disc of *F. lateritium* from a 7-d culture on CMA was placed at the centre of an NDY/20 plate and incubated for 3 d at each of the following: 5, 10, 15, 20, 25 and 30°C in darkness; 7·5 and 15°C with alternating 12 h light and 12 h darkness; 25°C in continuous light. Colonies of *F. lateritium* were killed by chloroform vapour and the plate sprayed with a suspension of *S. sclerotiorum* ascospores. Plates were incubated at 25°C for 4 d before measurements of inhibition zones were made. There were three replicates per treatment.

Experiment 5: Protection of young lettuce plants

The lettuce cultivar Imperial Triumph was used throughout. Seeds, surface sterilized in 1% sodium hypochlorite–absolute alcohol 2:1 (v/v), were sown in sterile vermiculite. After 6 d at 25°C, five seedlings were transplanted into each of a series of pots (15 cm × 15 cm) containing steam-sterilized soil and allowed to grow for four weeks in a glasshouse (20–30°C). The experiment was designed in three blocks, each containing three treatments with five replicates. The pots in each block were randomized on the bench. Mature apothecia from sand cultures were placed in open containers and positioned centrally in each of the three blocks so that released ascospores could reach any plant in the experiment. The 'puff' of ascospores occurred naturally and air currents dispersed and deposited them on plant leaves and soil surfaces. The release of ascospores continued until all apothecia dried and wilted. The three treatments were arranged as follows. The first treatment comprised autoclaved lettuce leaves, as organic matter, placed on the surface of the soil in contact with each plant. The second treatment was the same as the first but sprayed with a suspension of *F. lateritium* macroconidia 7 h before inoculation with *S. sclerotiorum* ascospores. In the third treatment, neither organic matter nor *F. lateritium* macroconidia were applied.

Each of the three blocks of 15 pots was arranged in a metal tray (75 cm × 50 cm × 5 cm) and covered with a plastic cage 50 cm high to maintain humidity and to prevent dispersal of ascospores beyond the pots in each block. Plants were watered and fertilized at intervals. Disease was assessed by observing symptoms of Sclerotinia rot and the experiment ended when all plants in the first treatment had been killed by *S. sclerotiorum*.

Results

Experiment 1: Inhibition of mycelial growth

Fusarium lateritium inhibited mycelial growth on all six media (figure 1), inhibition being greatest on NDY/20, as shown by the mean radius of the inhibition zone (table 1).

TABLE 1

Inhibition of mycelial growth of S. sclerotiorum *in media previously inoculated with* F. lateritium *(experiment 1). Measurements were made after 8 d incubation at 25°C.*

Medium	Radius of inhibition zone	Radius of *F. lateritium*
	mm	mm
NDY/20	45·0a	8·5
CMA	40·0b	11·5
CSA	37·7bc	10·9
LLA	36·8c	11·0
LSA	36·7c	11·5
PDA	35·0c	9·8

Values not followed by a common letter differ significantly ($P < 0·05$). Each value is the mean of two replicates.

Experiment 2: Inhibition of ascospore germination

As shown in figure 2*a*, the radius of the inhibition zone after one day on NDY/20 was 15 mm. Thereafter, the radius of inhibition increased linearly with time ($P < 0·001$). A similar result was obtained on GPA/20 (figure 2*b*).

TABLE 2

Radius of inhibition zone of ascospore germination in media (experiment 3). Measurements were made after 3 d incubation at 25°C.

Medium	Radius of inhibition zone
	mm
NDY/20	30·21a
LSA	27·54b
LLA	25·96bc
CMA	23·83cd
CSA	22·83d
PDA	19·13e
GPA/20	18·13e
ALL	14·92f

Values not followed by a common letter differ significantly ($P < 0·05$). Each value is the mean of six replicates.

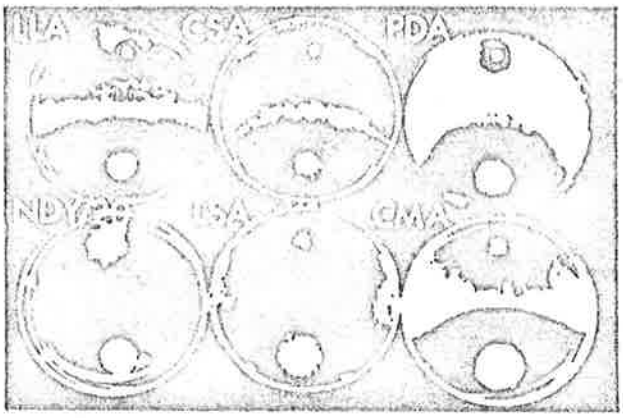

Figure 1—(experiment 1) – Interaction between F. lateritium *and* S. sclerotiorum, *8 d after inoculation on six kinds of media (see text).*

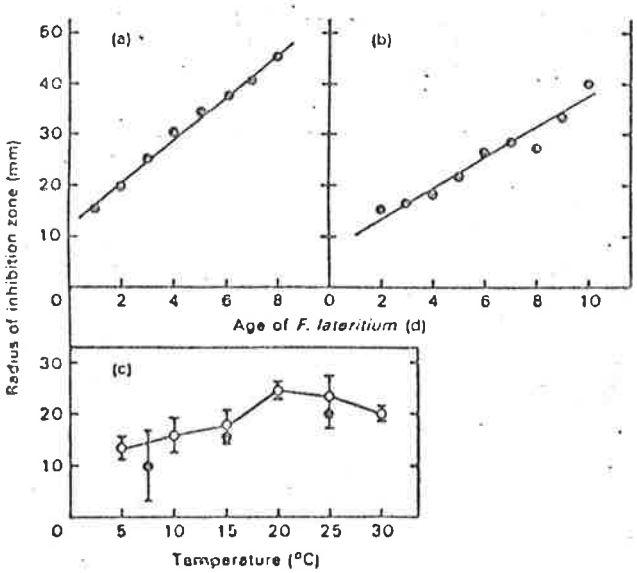

Figure 2—The inhibitory influence of F. lateritium *on* S. sclerotiorum *in vitro.*

(a) Regression of radius of inhibition zone on age of F. lateritium *colonies on NDY/20 in experiment 2. ($r = 0·994$; $P < 0·001$).*

(b) Regression of radius of inhibition zone on age of F. lateritium *colonies on GPA/20 in experiment 2. ($r = 0·969$; $P < 0·001$).*

(c) Effect of incubation temperature on radius of inhibition zones of ascospore germination by 3-d cultures of F. lateritium *(experiment 4). Open circles: continuously dark. Closed circles: 7·5 and 15°C 12 h light–12 h dark, and 25°C continuous light. Vertical bars show 95% confidence limits.*

Experiment 3: Influence of medium on inhibition of ascospore germination

Inhibition of ascospore germination was effective on six other media in addition to NDY/20 and GPA/20. Greatest inhibition occurred in NDY/20 and least in ALL, as shown by the mean values of the inhibition zones (table 2).

Experiment 4: Influence of temperature on inhibition of ascospore germination

The extent of inhibition was greatest at about 20°C under continuous dark incubation (figure 2*c*). Although very little mycelial growth of *F. lateritium* occurred at 5, 7·5 and 30°C, nevertheless the inhibition zones were substantial. There is an indication that inhibition may be lower with alternating light and dark than under continuous darkness.

Experiment 5: Protection of young lettuce plants

First symptoms of Sclerotinia rot were observed in pots with organic matter on the soil surface but no *F. lateritium*; after 7 d, all plants in this treatment were dead. When organic matter was also absent, none of the plants showed symptoms at this time.

TABLE 3

The influence of organic matter and F. lateritium *on the infection of lettuce plants by ascospores of* S. sclerotiorum *after 6 and 14 d (experiment 5). Each value is the mean of 75 plants (15 pots each containing five plants). Temperature in glasshouse ranged from 20 to 30°C.*

Treatment	Plants infected by *S. sclerotiorum*	
	6 d	14 d
	%	
Organic matter only	98·7	100·0
Organic matter + *F. lateritium*	5·3	33·3
No organic matter, no *F. lateritium*	0·0	5·3

Discussion

Our results show that *F. lateritium* inhibits the germination of ascospores and the growth of mycelia of *S. sclerotiorum in vitro*. As well as indicating the inhibitory properties of *F. lateritium*, our experiments confirm the observations of Carter and Price (1974) that the nature of the medium influences the extent of the inhibition zone.

The effect of adding *F. lateritium* to pots containing *S. sclerotiorum* and organic matter was most marked: very few plants were infected even after 7 d, although one-third were infected after 14 d (table 3).

Our results also indicate that *F. lateritium* inhibits *S. sclerotiorum* in a soil-plant system as well as *in vitro*. Organic matter on the surface of soil and in contact with lettuce plants strongly favours infection by *S. sclerotiorum*. It is possible that *F. lateritium* prevents the establishment of the pathogen in the organic matter.

Experiments using *F. lateritium* to control *S. sclerotiorum* under field conditions are in progress.

Acknowledgments

We thank Dr M. V. Carter for the isolate of *F. lateritium* and for useful advice.

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Received December 31, 1982; accepted August 19, 1983

APPENDIX 3: Number and characteristics of sclerotia of *S. sclerotiorum* on PDA

Number of Petri dish	Position of sclerotia in P. dish			Total number of sclerotia	Average weight (mg) of 1 sclerotium	Percent water content	Size (mm) of sclerotia	
	centre	in a circle (r=appr. 1 cm)	periphery				largest	smallest
(1)						(2)	(3)	
1	1	2	20	23	18.8	58.1	7 x 3	2 x 1.5
2	2	1	26	29	12.4	59.7	4 x 3	1 x 1
3	1	0	19	20	15.1	57.6	5 x 4	1.5 x 1.5
4	2	0	20	22	15,8	57.4	6 x 3	1.5 x 1
5	2	6	25	33	12.1	58.9	7 x 3.5	1 x 1
6	1	0	31	32	11.4	56.7	9 x 2	2 x 2
7	1	2	25	28	14.4	58.7	6 x 4	1.5 x 1.5
8	1	0	30	31	15.8	57.2	9 x 4	2 x 2
9	1	0	28	29	14.6	56.3	7 x 3	2.5 x 2
10	1	0	24	25	15.0	57.5	5 x 4.5	1.5 x 1

(1) 90 mm in diameter Petri dishes, each containing 20 ml PDA. Incubated at 25°C for two weeks

(2) Oven dried at 96°C for 24 hours (constant weight)

(3) l = length; w = width.