

WAITE INSTITUTE
4. 11. 85
LIBRARY

POTATO DISEASES IN SOUTH AUSTRALIA;
STUDIES ON LEAFROLL, EARLY BLIGHT AND
BACTERIAL WILT

by

E.B. AKIEW

Department of Plant Pathology
Waite Agricultural Research Institute
The University of Adelaide
South Australia

A thesis submitted to the University of Adelaide
in partial fulfillment of the requirement for
the degree of Doctor of Philosophy

March 1985

Awarded 15.8.85

	Page
F. Effect of granular systemic-insecticides on the spread of PLRV	44
G. Assesment of yield losses due to PLRV	48
III. Discussion and Conclusions	60
Chapter 4 Studies on <i>Alternaria solani</i>	
I. Introduction	70
II. Experiments and Results	
A. Mycelial growth of <i>A. solani</i> on various synthetic media	72
B. Light and temperature interaction on the sporulation of <i>A. solani</i>	73
C. Sporulation of <i>A. solani</i> on different synthetic media	75
D. Effect of temperature on sporulation of <i>A. solani</i>	77
E. Relationship between length of growth period and sporulation	80
F. Sporulation of <i>A. solani</i> on PDA plates arranged in layers	82
G. Tolerance of <i>A. solani</i> to fungicides	82
III. Discussion and Conclusion	86
Chapter 5 Studies on <i>Pseudomonas solanacearum</i>	
I. Introduction	88
II. Experiments and Results	
A. Selection of bacteriocin-producing, and bacteriocin-sensitive strains of <i>P. solanacearum</i>	91
B. Evaluation of selective media for isolation and quantification of <i>P. solanacearum</i> in soil	92
C. The effect of adding organic amendments to soil on persistence of <i>P. solanacearum</i>	98
D. The influence of soil moisture and temperature on the persistence of <i>P. solanacearum</i>	102
E. The role of presumed non-host crops on the survival of <i>P. solanacearum</i> in soil	104

	Page
F. Influence of <i>Meloidogyne javanica</i> on bacterial wilt of Exton potatoes	105
G. Protection of potato plants from bacterial wilt with avirulent bacteriocin-producing strains of <i>P. solanacearum</i>	107
III. Discussion and Conclusion	109
Chapter 6 General Discussion	115
Bibliography	119
Appendices	128

SUMMARY

Surveys of potato crops showed that virus diseases are widely distributed in the three principal potato-producing areas of South Australia. Of the thirty eight kinds of potato diseases recorded, potato leaf-roll virus (PLRV) and early blight caused by *Alternaria solani* are the most prevalent. South Australia's hot dry summers appear to inhibit the development of some fungal and bacterial diseases and prevent the establishment of diseases caused by *Phytophthora infestans*, and *Pseudomonas solanacearum*.

The use of mineral oils and insecticides to control the spread of PLRV in seed-potato crops was studied. The best results with spray compounds and granular systemic-insecticides were obtained with Dimethoate and Aldicarb, respectively. In a field trial with the potato cultivar Exton, secondary leaf-roll infection caused significant yield reduction. Yield loss was correlated with the percentage of infected plants and distance of planting. The data also confirm the opinion that healthy plants adjoined by diseased ones compensate, in part, for the yield loss of the infected plants.

Experimental evidence indicated that *Alternaria solani* requires light for conidiophore production and a dark period at low temperature for subsequent formation of conidia. The fungus formed more conidia on potato-dextrose-agar than on any of the other agar media used. Of the seventeen fungicides assayed in the laboratory, Captafol and Mancozeb effectively inhibited conidial germination and mycelial growth.

The influence of temperature, moisture, organic matter, and non-host crops on the persistence of *Pseudomonas solanacearum* in artificially infested soil was investigated. High soil moisture, lower temperature, and presence of presumed non-host crops were related with long term survival of the bacterium in soil. The high temperature strain of the bacterium, biotype-III (race-1), survived longer than the potato strain, biotype-II (race-3). Experimental results suggest that bacterial wilt in potato plants is not controlled by avirulent bacteriocin-producing strains of *Pseudomonas solanacearum*, and that initial wilting is not accelerated by *Meloidogyne javanica*.

DECLARATION

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, no material described herein has been previously published or written by another person except when due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

(Candidate's signature)

.....21/3/85.....

Date

ACKNOWLEDGEMENTS

To Professor H. R. Wallace, my academic supervisor, I express my profound gratitude for his encouragement, guidance and criticism during the studies and preparation of this thesis. I also gratefully acknowledge the assistance of Drs. R. I. B. Francki and J. W. Randles (Virologists), Dr. J. W. Warcup (Mycologist), and of Professor A. Kerr (Bacteriologist).

I thank the South Australian Potato Industry Committee, the staff and fellow students at the Department of Plant Pathology, Waite Agricultural Research Institute for their assistance.

Last but not least, I gratefully acknowledge the Mountain State Agricultural College (Philippines), Australian Development Assistance Bureau, and the Australian Centre for International Agricultural Research for the scholarships.

CHAPTER 1

GENERAL INTRODUCTION

The potato, *Solanum tuberosum* L., is characteristically a crop of the cool, temperate regions, or of elevations of about 5,000 ft. or more in the tropics. It is one of the best sources of nourishment, and an important food crop besides wheat, rice, and corn. Potato is one of the major food crops in Australia, and the leading vegetable crop, in terms of area and production, in South Australia (Table 1). Between 1980-1984, the average potato production in South Australia was about 97,000 tons valued at over 21 million dollars (Crop and Fruit Statistics, Australia).

Unlike most cultivated plants, potatoes are prone to numerous kinds of plant pathogens, and are intolerant to adverse environment, nutrient imbalance, and other disease-causing entities. About 137 kinds of potato diseases can be found in the literature so far (Horvath, 1967 ; Hodgson et al., 1976 ; Miska and Nelson, 1975 ; Marschel, 1975 ; Obrien, 1976 ; Hooker, 1981).

Some potato diseases are considered economically important in South Australia. For instance, Williams (1984) indicated that the prevalence of potato leafroll virus (PLRV) has been the major problem right from the beginning of the first seed-production scheme (1950) to the present. Despite its significance in potato production, potato diseases are little studied in South Australia. A literature review reveals that the study of Francki and McLean (1966) and the recent work of Francki (1979)

Table 1 : Leading vegetables grown for human consumption, South Australia.

Vegetable	Area (Hectares)	Production (Tonnes)	Value (\$ Million)
Potatoes	3,748.6	97,479.3	21.68
Onions	3,456.0	33,623.0	8.73
Carrots	388.6	10,624.0	4.51
Tomatoes	381.0	12,197.3	8.9

Notes: Area, production and value are Average for 1980 - 1983

- Data obtained from the Australian Bureau of Statistics, Canberra.

were the only published research on potato diseases in South Australia, apart from the identification work of Warcup and Talbot (1980).

This project was, therefore, conducted to determine the occurrence of potato diseases in South Australia, to identify the causes of such diseases, and to find out those that are of major economic importance so that they could be studied in depth and contributions made to the present knowledge about potato diseases. The approach adopted was initially to survey potato crops because surveys provide important data on incidence and prevalence of diseases necessary for direction and planning of a research programme.

Diseases such as PLRV and early blight were studied further as they are considered economically important in South Australia. Moreover, the survival of *Pseudomonas solanacearum* in soil was investigated to substantiate the concept that the bacterium cannot survive for long periods in South Australian soil, and to contribute to knowledge on the ecology of the bacterium.

The diseases mentioned so far, including rootknot nematodes (*Meloidogyne spp.*), are also widespread and destructive in many developing countries in the tropics (Thurston, 1980). Hence, this project was also designed so that the procedures could be used in other places such as the Philippines, the authors home country, where potato production and many diseases associated with the crop are becoming increasingly important.

CHAPTER 2

POTATO PATHOGENS AND DISEASES DETECTED IN SOUTH AUSTRALIA

I. Introduction

The incidence, distribution, and economic importance of potato diseases in South Australia are little understood except for powdery scab *Spongospora subterranea* as described by Wicks (1980). The reports of Philp et al. (1976) are limited to short descriptions of symptoms and treatments of some potato diseases and pests and the records of Warcup and Talbot (1980) are confined to a list of plant diseases, including those of potatoes, reported from South Australia.

South Australia has a dry mediterranean type of climate with mild winters and warm summers, and the prevailing weather conditions during the main cropping season (November–March) are generally warm and dry (Figure 1). It is only during the spring, autumn, and early part of the winter season that potato crops are exposed to short periods of cool wet weather. Since weather has such a profound influence on disease development, it was considered likely that most of the known potato diseases, particularly those caused by fungi and bacteria, would not be found in the commercial potato-producing areas of the state, and that aphid-borne virus diseases, such as potato leafroll virus and potato virus Y, would be widespread in the area because of the mild winters which favour the prevalence of aphids early in the cropping season.

Since no information was available to support such assumptions, the principal potato-producing areas of South Australia were surveyed for

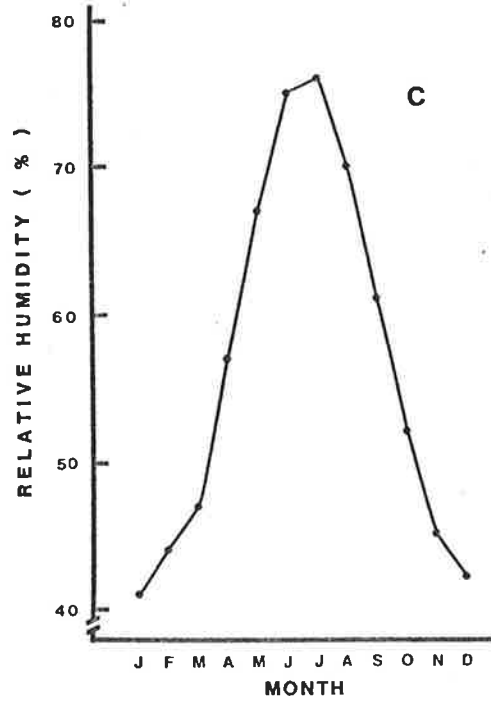
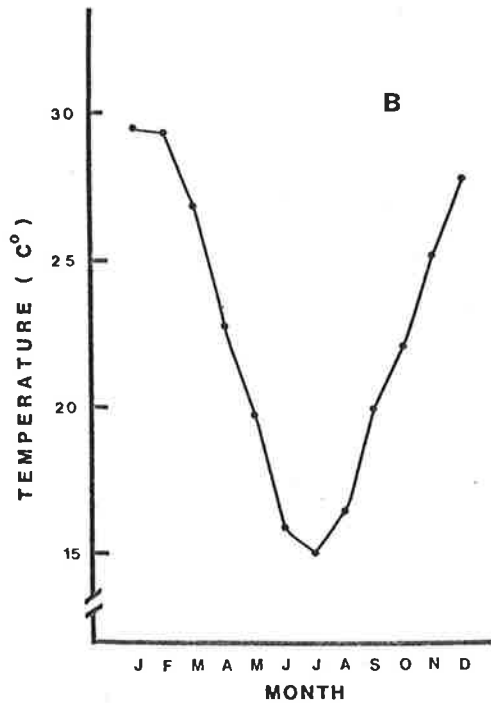
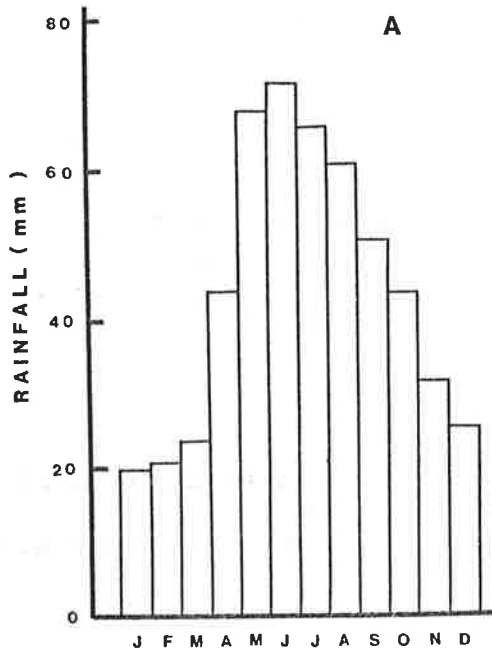
Figure 1: Climatological data, South Australia:

A = mean monthly rainfall based on all years of records

B = mean monthly temperature

C = mean relative humidity based on composite records of Grennwich and Stevenson screen observation

C = mean days of rainfall



diseases for a period of three years. The study was also conducted to confirm and supplement the reports previously mentioned, and indicate where further investigation might be useful.

II. Materials and Methods

The selective and general purpose media used for isolating and growing pathogenic fungi and bacteria are listed in Appendix 1. The plant species used for pathogenicity tests, and for virus indexing, including antisera are listed in Appendix 2-3. For disease identification, the following publications were used as references; CMI, AAB Descriptions of Plant Viruses, CMI Descriptions of Plant Pathogenic Fungi and Bacteria, CIH Descriptions of Plant Parasitic Nematodes, and a Compendium of Potato Diseases (Hooker, 1981).

A. Disease survey

The incidence, severity, distribution, and economic importance of diseases affecting seed and commercial ware crops in South Australia were determined during the growing seasons of 1981-1984. The study was conducted with the cooperation of the South Australian Potato Industry Committee, and the South Australian Department of Agriculture.

A stratified sampling method (Church, 1972) was followed in planning the survey. The sampling area was established by dividing the potato growing area into three districts as shown in Figure 2. Based on the total number of farmers involved in potato growing, the required number of sampling units (farms) per district was calculated. There were

Figure 2: Principal potato producing districts of South Australia; Adelaide Plains (I), Adelaide Hills (II), and Southeast of South Australia (III).

fifteen, nine, and eight sampling units in districts I, II and III, respectively.

During each cropping season, potato crops were inspected, aphid populations monitored, and diseased specimens collected. The seed and ware crops in district-III were inspected at least twice per year and samples of leaves or tubers were collected from seed crops for virus indexing. Washing and packing centres in each area were also inspected for the occurrence of post-harvest diseases. The cold store in Balhanna (district-II) was visited to inspect seed-potatoes before they were taken out for planting.

Disease incidence was estimated by determining the proportion of infected plants or tubers. The following data were also gathered: kind and variety of potatoes planted, kind of pesticides and frequency of spraying, method and frequency of irrigation and the purpose of the crop whether it was for seed, processing, or for the the fresh market.

B. Diagnosis

Preliminary identification of potato diseases was done during the survey and it was based mainly on symptoms and signs of infection. Samples of infected plants, leaves, or tubers, and soil samples were collected for laboratory study.

Virus diseases

Suspected virus diseases were transmitted to a range of indicator plants and grown in the glasshouse at 15-21°C until symptoms appeared. Inoculated plants that remained symptomless after 4 weeks were tested

for possible latent infection by inoculating other host plants or potatoes. The methods used for virus transmission were: mechanical and graft transmissions, insect transmission with aphids, leafhoppers, and mirids. The duration of virus acquisition and inoculation feeding by aphids were 3 minutes to two days, and one to three days, respectively. For the other two kinds of insects, acquisition and inoculation feeding times were five days.

Leaf-dip preparations of crude sap, extracted from infected leaves, were used for observing virus particles. The sap was prepared by macerating a 2mm² piece of infected leaf tissue in two drops of 0.5% glutaraldehyde. Copper grids (400 mesh), previously coated with formvar, were ionized and dipped in the sap just enough to smear the surface. After blotting the excess sap on the grid, the preparation was negatively stained with tungstophosphoric acid for about 10 seconds and then observed for the presence of virus particles in a JEM 100 x Electron Microscope.

Serology by means of either the Gel-diffusion or Enzyme-Linked-Immunosorbent-Assay (ELISA) was also used to identify viruses. The procedure of Clark and Adams (1971), and the methods indicated in the Potascreen-TM assay kits were followed in doing the ELISA test. To detect potato spindle-tuber viroid by means of electrophoresis, the procedure of Schumann et al. (1978) was adopted.

Fungal Diseases

The pathogens causing potato diseases were established using Koch's postulates. Infected plant material was examined for the presence of

fungus fructifications and the primary causal agent isolated and tested for pathogenicity.

Infected parts of the plant were washed free of adhering dirt and then 3 mm² pieces of tissue were cut along the advancing margin of the lesion. The pieces of tissue were immersed in a solution of sodium-hypochlorite for 2-3 minutes to sterilize the surface, rinsed three times in sterile water, blotted dry, and plated on agar medium. Infected stems or tubers in which the pathogen had penetrated deep into the vascular tissues, were surface sterilized with absolute ethanol. The surface of the plant material was flamed before it was aseptically cut into two parts. From the inner lesion, bits of tissue were cut out and plated on agar medium, or aseptically sliced potato tuber disk. Fungal fructifications such as *sclerotia* were sometimes used to obtain pure cultures.

Spore suspensions of fungal isolates from leaves or stems were used to inoculate potato plants. The plants were kept under moist conditions for 2-3 days before they were transferred to the glasshouse at a temperature of 25°C. For isolates obtained from plant parts below or just above the soil surface, sprouted potato tubers or the soil were inoculated with suspensions of fungal fructifications just before planting. In some cases, detached potato leaves or whole tubers were inoculated instead of potato plants.

Bacterial Diseases

Specimens on which no fungal fruit bodies could be observed were examined for gross signs of bacterial infection such as bacterial ooze. After the presence of bacterial ooze had been confirmed, the specimens

were washed free of adhering soil, blotted dry, and bits of tissue were cut out and suspended in 10 ml of sterile distilled water. After 30-60 minutes, loopfuls of the resulting suspension were streaked on a suitable agar medium. The inoculated agar plates were incubated at temperatures of 25-30°C and observed daily for the appearance of bacterial colonies. Pure cultures were obtained from single colonies of bacteria and tested for pathogenicity to complete the requirements of Koch's postulates.

Bacterial inoculum was prepared by suspending fresh growth from 48 hour agar cultures in distilled water at concentrations between 10^7 - 10^8 cells per ml. Aseptically sliced potato-tuber disks were inoculated by placing a drop of inoculum on the cut surface which was pricked with a flamed needle and placed in a sterile Petri dish. Inoculated materials were normally incubated at temperatures of 27-30°C. For isolates obtained from potato leaves, either tobacco leaves were infiltrated with inoculum using a 25-gauge syringe, or potato plants were sprayed with inoculum, and kept in a moist chamber at a temperature of about 30°C for three days. For isolates obtained from potato stems, a drop of inoculum was placed in the leaf axil and pricked into the stem with a flamed needle. Inoculated plants were maintained in a glasshouse at 25-30°C.

C. Extraction and identification of Nematodes

Nematodes such as the root knot nematode *Meloidogyne sp.*, found in tubers or roots, were extracted directly from the infected tissue. A modified Baermann funnel method was used to extract nematodes found in soils. Soil samples were collected in the field and after mixing, about 50 g were sprinkled over a layer of 'Kleenex tissue' paper supported by

wire mesh. The wire mesh was placed in a Petri dish and then filled with water until the soil was wet. The following day, the nematodes were collected using a sieve. The morphological characteristics of the juvenile stage were used as criteria in identifying the nematodes.

III. Results and Discussion

Thirty eight kinds of potato disease were found during the survey, and among those that were identified, the following diseases have not previously been reported in South Australia: potato virus S, tomato big bud (aster yellows), pink rot *Phytophthora cryptogea*, leaf spot caused by *Phoma* sp., *Alternaria alternata*, or *Stemphylium botriosum*, stem rot *Sclerotium rolfsii*, tuber leak caused by either *Pythium ultimum* or *P. irregulare*, leaf gray mould *Botrytis cinerea*, and bacterial wilt *Pseudomonas solanacearum*.

The diseases caused by *Phoma*, *A. alternata*, *B. cinerea*, and *S. botriosum* are not included in the list of potato diseases recorded in Australia by Morschel (1975). Potato leak caused by *Pythium irregulare* is considered as a new disease of potatoes since it has not been reported in the literature.

The incidence of potato diseases in the three principal potato-producing areas of South Australia is presented in Table 2. Some diseases found during the survey are illustrated in Appendix 9 (Plates 1 to 8).

Table 2 : Potato diseases recorded in South Australia in 1981-84

Disease	% of plants with symptoms	
	Range	Mean
Alfalfa mosaic virus	0-1	nil
Potato leafroll virus	1-45	19
Potato virus S	0-7	2
Potato virus X	0-10	4.5
Potato virus Y	0-1	nil
Tomato big bud (Aster yellows)	0-5	2.5
Tomato spotted wilt virus	0-27	3.0
<i>Alternaria solani</i>	10-80	31.0
<i>Alternaria alternata</i>	0-11	2.5
<i>Botrytis cinerea</i>	0-3	nil
<i>Colletotrichum coccodes</i>	not determined	
Fusarium dry rot	not determined	
<i>Helminthosporium solani</i>	not determined	
<i>Phoma foveata</i>	not determined	
<i>Phoma sp.</i> (leaf spot)	0-65	10
<i>Phytophthora cryptogea</i>	0-2	nil
<i>Pythium ultimum</i>	0-1	nil
<i>Pythium irregulare</i>	0-1	nil
<i>Rhizoctonia solani</i>		
- black scurf	not determined	
- stem rot	2-12	7
<i>Sclerotium rolfsii</i>	0-8	nil
<i>Sclerotinia sclerotiorum</i>	0-11	2
<i>Spongospora subterranea</i>	0-19	3
<i>Stemphylium botriosum</i>	0.1-17	3.5
<i>Streptomyces scabies</i>	0-13	5
<i>Verticillium dahliae</i>	0-80	2
<i>Erwinia atroceptica</i>	0-0.1	nil
<i>Erwinia carotovora</i>	not determined	
<i>Pseudomonas solanacearum</i>	nil	
<i>Meloidogyne hapla</i>	0-16	7.8 (South east district)

A. Diseases caused by Viruses, Mycoplasma and Viroids

Among the virus diseases, potato leaf-roll virus (PLRV) was the most prevalent and widespread in the potato growing districts. Symptoms of either primary or secondary infections were frequently observed from seed or ware crops and incidence of over 10% was common. The incidence of PLRV was more variable within a district than between two districts perhaps due to the different classes of seeds planted. Although an incidence of more than 1% was seldom recorded from crops grown from certified seeds (Certified in Victoria, Australia), leafroll incidence as high as 45% was found, particularly from crops planted with other classes of seeds derived either from a previous crop or produced in the Southeast of South Australia.

Foundation seeds produced in Victoria, Australia, are grown and multiplied in the Southeast of South Australia for the production of 'mother' seed which are used in growing crops to be certified as seed. Although this scheme was still in operation during the survey, most farmers preferred to plant certified seed from Victoria (CSV) because of its higher quality, e.g. lower disease incidence. A common practice among farmers was to grow a first crop of CSV and then to use a certain portion of the harvested tubers to grow a second or subsequent crop. In the Adelaide plains (district-I), the spring, autumn, and winter crops are planted in September, March, and April, respectively. The main and seed crops, which are chiefly grown in the Southeast of the State and in the Adelaide Hills, are planted in November.

Aphids were found to be more abundant in the beginning and towards the end of the cropping seasons particularly from October to December

(Figure 3). Several aphid species were also identified, the most abundant being *Aulacorthum solani*, *Rhopalosiphum padi*, *Myzus persicae* and *Macrosiphum euphorbiae* (Table 3). Thus, it seems likely that the presence of such aphids and their abundance in potato crops, particularly during the early part of the cropping season, accounted for the high incidence of PLRV in both ware and seed crops. It is not known whether *R. padi* and some of the other aphids identified can transmit PLRV.

R. padi, a well known vector of barley yellow dwarf virus, can transmit potato virus Y (PVY) but not as efficiently as *M. persicae* (Kennedy et al., 1962). Despite the abundance of these two aphid species, the incidence of PVY was very low. In most ware crops, where the number of PLRV-infected plants was more than a quarter of the healthy ones, no PVY or fewer than 1.0% was recorded. It appears that PVY spreads less extensively than PLRV in South Australia. Moreover, the low incidence of PVY could have been due to a limited source of inoculum or because the virus was not efficiently transmitted by the aphids. Many strains of PVY are known and aphids, especially *M. persicae*, can spread PVYⁿ in the field more rapidly than PVY^o (Beemster and Rozendaal, 1972). Potato virus Y strains belonging to the type virus C (PVY^c) differs from the other two strains in not being transmitted by *M. persicae* (Delgado-Sanchez and Grogan, 1966). Thus, the PVY found in this survey probably belongs to the PVY^c group and could be the main reason why it is not prevalent.

The effect of PLRV on PVY transmission by aphids is little understood whereas the inhibiting effects of potato virus M (PVM), and potato virus S (PVS) on the efficiency of infection by potato viruses X,

Figure 3 Mean weekly counts of aphids; *Myzus persicae*, *Macrosiphum euphorbiae* and *Aulacorthum solani*, in districts I (---●---) and II (---▲---).

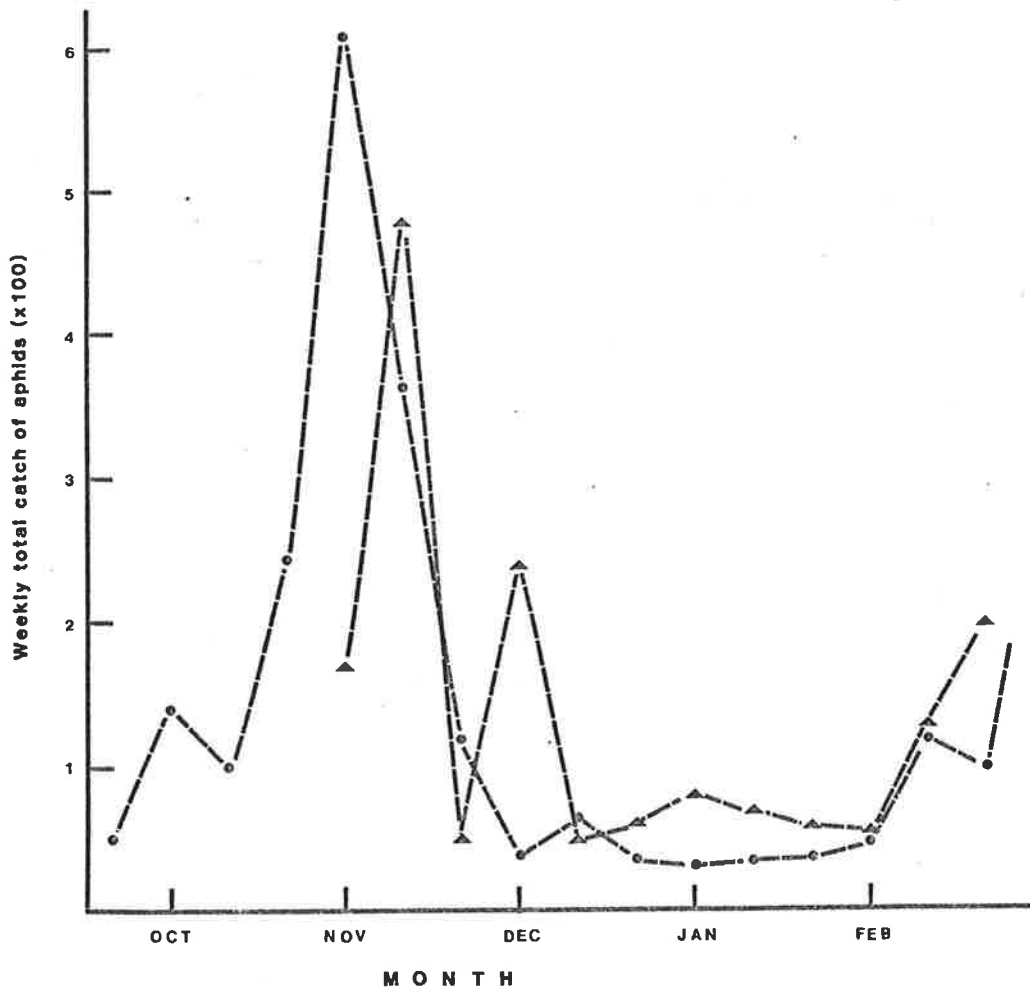


Table 3 : Species of aphids caught in a Moericke water trap.

Aphid species	Relative abundance (%)
<i>Acyrtosiphon pelargonii</i>	4.0
<i>Aulacorthum solani</i>	32.5
<i>Aphis criticola</i>	nil
<i>Aphis craccivora</i>	2.3
<i>Aphis gossypii</i>	nil
<i>Brachycaudus helichrysi</i>	nil
<i>Brevicoryne brassicae</i>	7.5
<i>Capitophorus elaeagni</i>	2.3
<i>Cavariella algopodii</i>	2.4
<i>Hyperomyzus lactucae</i>	nil
<i>Macrosiphum euphorbiae</i>	2.2
<i>M. miscanthi</i>	nil
<i>M. rosae</i>	nil
<i>Myzus persicae</i>	20.9
<i>Rhopalosiphum padi</i>	23.5
<i>Taxoptera miscanthi</i>	nil
<i>Lipaphis erysimi</i>	nil

and Y have been established by Pietrak (1980). He found that PVS decreased infection of PVX and PVY by 14% and 20%, respectively, whereas PVM decreased infection of both PVX, and PVY by 25%. As PVS was detected in ware and seed crops, although not extensively, its presence may have also contributed to the low incidence of PVY.

The potato seeds from the Southeast of South Australia were not extensively indexed for PVS during the study and its incidence might have been higher than that reported here. In a survey of potato viruses X, S, and Y in Queensland, PVS was considered the most prevalent and infections of 0-100% have been detected from crops and seeds produced in New South Wales, Tasmania, and Victoria (Holmes and Teakle, 1980).

Potato diseases caused by tomato spotted wilt virus (TSWV), and mycoplasma (purple top wilt) were found but not as extensively as PLRV. Plants infected by TSWV were often seen during the main cropping seasons (November-March) particularly in the Adelaide Hills where an incidence of 27% was recorded in 1982. The disease was rarely seen in the Adelaide plains probably because its vector is not abundant in the area during the cropping seasons (October-December, March-June). The known insect vector of the virus is a species of thrips (Thysanoptera) whose abundance is favoured by warm, dry weather (Norris, 1951) rather than wet conditions. For purple top wilt, an incidence of 19% was recorded only once from a crop in the Adelaide plains. The seeds which were used to grow the crop, were obtained in the Adelaide Hills where leafhoppers, the vector of the virus, were found to be abundant.

Potato plants infected by alfalfa mosaic virus (AMV) were frequently seen especially from crops near lucerne pastures, but only

few plants were affected. Although the virus is transmitted by *M. persicae*, *M. euphorbiae* and eleven other species of aphids (Kennedy et al., 1962), it does not spread as extensively as other aphid-borne and stylet-borne viruses. Thus, the disease is not considered to be economically important due to its limited occurrence.

Figures 4a and 4b show a disease that was tentatively identified as tomato big bud (aster yellows). Although the disease was present in most areas, there was only one crop on the Adelaide plains that was almost completely affected by the disease. Infected plants were stunted and aerial tubers formed at the leaf axil. The disease was not associated with infection by *Rhizoctonia*, as the underground stem appeared healthy. Tubers and stem cuttings taken from infected and healthy looking plants in the same area, when grown in the glasshouse, produced similar symptoms. Furthermore, healthy potato plants were stunted after *Greontiades sp.*, a sucking insect abundant in the crop, was allowed to feed on it. The insect, known locally as Rutherglen bug, is a common pest of potatoes in South Australia, and abundant during hot summer days. Although no reports are available to show that such an insect is a vector of potato viruses, and no virus particles were detected from leaf dip preparations of infected leaves, there is a possibility that an infectious pathogen and the insect are associated with the disease.

In the Adelaide Hills, potato tubers showing symptoms of infection by potato spindle-tuber viroid (PSTV) were encountered. Infected tubers have pointed ends, are elongated and some have growth cracks (Figure 5). Although this disease appears to have been introduced into South Australia (Cartwright, 1984), no viroid was detected in any of the tuber samples, using tomato bio-assay and electrophoresis.

Figure 4a A potato crop in the Adelaide Plains (District I) showing severely stunted plants suspected to be caused by a Mycoplasma

Figure 4b A plant from the same field showing aerial tubers, enlarged stem bud, and stunted growth.



of being
Figure 5 Potato tuber (right) suspected to be infected by potato spindle tuber viroid (PSTV).

In the case of the infected tuber



Virus diseases caused by cucumber mosaic virus (CMV), potato aucuba mosaic virus (PAMV), potato vein bonding virus, potato viruses M, and A were not detected during the survey although they have been recorded in Australia by Morschel (1975).

B. Diseases caused by Fungi

Several diseases affecting the aboveground parts of the potato plant were found but only early blight caused by *Alternaria solani* was prevalent. In the Adelaide plains, severe outbreaks of early blight frequently occurred during the autumn and the early part of the winter seasons. On the other hand, the occurrence of the disease in the main potato growing districts was dependent upon the prevailing weather conditions. Thus, potato crops which were not harvested before the onset of rainy days were often severely affected. The occurrence and severity of the disease from one field to another was variable and is attributed to the different potato varieties planted and the kinds of pesticides applied. For instance, the cultivar 'Red Pontiac' appeared to be more susceptible than Kennebek, or Exton, and the fungicide Chlorothalonil was considered very effective against the fungus.

Leaf blight caused by *Phoma* sp. was seen only in the Southeast of South Australia. The symptoms of the disease are similar to those of early blight except for the presence of pycnidia on the lesion. Severe infection which caused defoliation particularly of the cultivar 'Red Pontiac' was observed towards the end of the cropping season when heavy rainfall occurred. A similar disease was reported by Turkensteen (1981) and he identified the causal fungus as *Phoma andina*.

and only

Potato diseases caused by *Sclerotinia sclerotium* (white mould), *Sclerotium rolfsii*, *Botrytis cinerea*, and *Stemphylium botriosum* were found in most of the potato crops but generally these diseases were not prevalent. However, high incidence of white mould was sometimes recorded in the Adelaide Plains particularly in fields where crops like lettuce and cabbage were planted in rotation with potatoes. Usually, plants near irrigation sprinklers were killed before digging, and tubers were often rotten.

The following pathogens were found affecting mainly the underground parts of potato plants: *Rhizoctonia solani* (black scurf and stem rot), *Helminthosporium solani* (silver scurf), *Spongospora subterranea* (powdery scab), *Phytophthora cryptogea* (pink rot), *Pythium ultimum* and *P. irregulare* (tuber leak), and *Verticillium dahliae* (wilt). Blemished tubers due to the combined infection of *R. solani* and *H. solani* were very common and winter crops that were not harvested but kept in the ground until the following spring were often severely affected. The pathogen also caused stem rot and tuber deformation especially of the cultivar 'Red Pontiac' but both of these diseases were not as prevalent as black scurf. Although black and silver scurf do not directly affect the yield of potatoes, the high demand for washed and premium grade potatoes in the market has elevated the significance of these diseases.

The occurrence of powdery scab was sporadic and only two cases with an incidence of 2% and 19% were recorded during the survey. In both infestations, the seeds used came from Victoria (Australia) and the fields that were planted were previously used for pasture. Powdery scab is becoming increasingly important in South Australia due to its adverse effect on tuber quality and value. It seems that the pathogen can

survive for a long time in some parts of the State. For instance, the disease recurred in a field which had been fallowed and used for pasture over four years.

Pink rot and tuber-leak were occasionally found in processing centres and water-logged areas of the field. Tuber rot caused by *Pythium irregulare* (Plate 2e, Appendix 10) was detected from rotting tubers in the Adelaide Hills, and was found in a field which was frequently irrigated. The field was previously used for pasture. This pathogen has not been reported in the literature as a pathogen of potatoes although it is highly pathogenic to cereals. The fungus was identified by the Commonwealth Mycological Institute and its reference number is IMI 285723. Although the fungus is known to be widespread in South Australian soils, it is not certain whether the disease is more prevalent than *P. ultimum*.

Potato wilt due to *Verticillium dahliae* was often seen and incidences of 14, 18, and 80% have been recorded on three separate occasions from ware-crops in the Adelaide Hills (district-II). The fields which had 14, and 18% wilt were planted with local seed, while the field with 80% wilt was planted with seed which was not certified in Victoria. Soil samples were taken from the three sites to determine if the pathogen was endemic in the area. In addition to the fungus, very large numbers of parasitic nematodes, mostly *Heterodera sp.* and *Pratylenchus sp.*, were found in the soil samples which suggest that the severity of the wilt was influenced by the nematodes.

The significance of nematodes in increasing the severity of wilt due to *Verticillium dahliae* in numerous crops has been recognized. For

example, Krikun and Orion (1979) found a positive correlation between *Pratylenchus thornei* and severity of *Verticillium* wilt in potatoes, and even relatively resistant cultivars sustained losses of about 30-40% in the presence of nematodes.

Botrytis cinerea, *Fusarium sulphureum* and *Phoma foveata* (gangrene) were the most common pathogens isolated from diseased tubers in cold stores. Tubers that sustained mechanical injury during harvesting and grading were often affected by such pathogens. Several of the cold-stored potato seeds were also affected by gangrene which suggest that the occurrence of the disease in storage is a consequence of planting diseased tubers. Gangrene was first reported by Feddersen (1978) in a number of certified seeds originating from Victoria and also from potatoes grown using such seeds. It is becoming increasingly important in most commercial potato-growing areas of South Australia.

C. Diseases caused by Bacteria

The four most common bacterial disease of potatoes ; black leg, common scab, soft rot, and brown rot or bacterial wilt, were found during the survey. However, their incidence, except common scab, was generally low.

Black leg caused by *Erwinia atroseptica* was the most widespread among the bacterial disease, but its incidence never exceeded 0.1%. The disease was found to occur at any stage of plant development, causing severe decay of stems. The occurrence of black leg in South Australia was attributed to the use of infected tubers for planting, and frequent irrigation during hot days. Thus, infected plants were often seen during

the summer months (main cropping season) whereas they were practically absent during the autumn and winter seasons.

The occurrence of common scab (*Streptomyces scabies*) was sporadic. The pitted type of scab was often found in the Adelaide Hills whereas the russet or raised scab was more or less common throughout the districts. The occurrence of scab was found to be related to soil type, e.g. sandy soils, and irregular irrigation. Common scab is considered an important disease of potatoes in the Adelaide Plains district where market gardens, which supply washed potatoes, are primarily located.

Soft rot due to *Erwinia carotovora* was often found only in waterlogged areas of the field, in stored potatoes, and in packing centres. It is not a serious disease of potatoes in South Australia perhaps due to the dry climate.

Brown rot or bacterial wilt caused by *Pseudomonas solanacearum*, not previously been recorded in South Australia, was first detected in 1982 from tubers intended for virus indexing. The bacterium was isolated and identified as, *P. solanacearum*, biotype-II, based on the classification of Hayward (1964) or Race 3, the potato strain (Budenhagen et al., 1962). It is pathogenic only to potatoes and tomatoes. The bacterium was not detected in the infected area or in other commercial fields in South Australia in the year following its first detection which strongly suggests that the bacterium cannot survive for long periods in South Australian soil even if introduced. On separate occasions (1984), the disease was detected for the second time from a crop grown from two kinds of seeds obtained from two different sources. The absence of bacterial wilt in one of the crops in the same area suggests that the

occurrence of the disease in South Australia is associated with the use of infected ^{seeds} seeds. Thus, it is believed that bacterial wilt (*P. Solanacearum*) is not endemic in South Australia unlike Queensland, New South Wales and Victoria.

D. Disease caused by nematodes

Root knot nematode (*Meloidogyne sp.*) was found in two locations ; Penong (district-II), and the South-east of South Australia. *Meloidogyne hapla* is prevalent in the South-east where an incidence of about 16% was recorded. Plate 7f (Appendix 10) shows an infected tuber that was also collected from the South-east. It was first suspected as potato rot caused by *Ditylenchus destructor* but the nematode found in the lesion did not conform to the descriptions of *D. destructor* (Thorne, 1945). The disease was quite prevalent and it had been observed in the area even before this survey.

E. Miscellaneous Diseases

Physiological diseases, little examined in South Australia, were not included in the survey. However, some of those that were considered important, but not identified, are illustrated in Plate 8.

IV. Conclusions

The presence of most diseases previously recorded in South Australia by Warcup and Talbot (1980) were confirmed in this survey and some new diseases identified. Potato leafroll and early blight (*A. solani*) were omnipresent and believed to be the two most destructive

diseases occurring in commercial ware and seed crops in the state. Foundation and mother seed-crops are frequently reinfected with potato leafroll virus and that is why potato growers prefer to plant Victorian seeds rather than those produced locally. The abundance of a number of Aphid species and other insects in potato crops probably accounted for the prevalence of potato leafroll virus. There were indications that virus diseases other than those identified during the survey, occur in South Australia, thus, it would be worthwhile to investigate further the etiology of virus diseases of potatoes in South Australia. Moreover, studies on resistance to potato viruses should be emphasized, although introduction of potatoes into Australia is strictly controlled.

The relatively low incidence of fungal and bacterial diseases, except early blight and black scurf, indicates the profound influence of weather on the epidemiology of these diseases. The fact that late blight (*Phytophthora infestans*), the most destructive disease of potatoes in other parts of the world, has not been found, and the absence of *Pseudomonas solanacearum* from the field, where it was first detected, during subsequent years indicate strongly the inability of most fungal and some bacterial diseases to survive under South Australia's hot dry summers. Moreover, about 50% of the fungal and bacterial diseases of potatoes recorded from the other states were present in South Australia but only 10% of these were prevalent. Disease-forecasting systems, like those for late blight (*P. infestans*), are based on a detailed knowledge of a critical period during which the weather is conducive to sporulation, dispersal, and subsequent infection. Epidemiological research in South Australia should prove useful in formulating forecasting systems for those diseases of economic importance.

Species of nematodes other than rootknot have been detected in potato fields. As nematodes have been shown to influence the severity of diseases caused by soil-borne pathogens, e.g. *Verticillium* and *Pseudomonas solanacearum*, and transmit some virus diseases, their role in decreasing yield of vegetables is worth studying further.

The economic importance of potato diseases in South Australia is greatly influenced by market demands. For example, diseases that directly affect yield and storage quality of potatoes are considered major problems in areas that produce processing potatoes. On the other hand, diseases producing blemishes reduce the market value of potatoes for the market gardener.

Having surveyed the potato diseases of South Australia, it was decided to study in greater detail potato leaf roll virus and early blight because they are widespread, not only in South Australia but also in the tropics (CMI Map 89), and destructive wherever potatoes are grown (CMI AAB No. 36). Furthermore, studies on the ecology of *P. Solanacearum*, with emphasis on the effects of soil factors, have been conducted to provide some basis for developing an effective control procedure.

CHAPTER 3

STUDIES ON POTATO LEAFROLL VIRUS

I. Introduction

Quanjer and his group (1916) were the first to demonstrate the infectivity of potato leafroll, and proposed that the disease is due to a virus (Murphy, 1922 ; Whitehead, 1945). The properties of the virus remained long obscure until Peters succeeded in revealing its shape and dimension by electron-microscopy some years ago (Van der Want, 1972). As described by Peters (1970), potato leafroll virus (PLRV) has isometric particles of about 24 nm in diameter, affects a narrow range of hosts, is not sap transmissible but is transmitted by either grafting or aphids. Of the ten aphid species that can transmit the virus in a persistent (circulative) manner, *Myzus persicae* (Sulz.) appears to be the most efficient (Kennedy, Day and Eastop, 1962). Although several strains of PLRV have been detected, differing in symptom severity on *Solanum tuberosum* and *Physalis floridana*, all such strains induce a similar type of symptom (Beemster and Rozendaal, 1972).

In potatoes, primary symptoms develop and tubers are infected following aphid-transmission of the virus. The symptoms appear mainly on the plant's shoots which stand upright, roll, and turn slightly pale-green. In some cultivars, the young leaves turn pink or purple starting at the margins. Primary symptoms may be lacking when plants are infected late in the growing period. Secondary symptoms become evident when an infected tuber produces a plant. The plant as a whole appears stunted and rigid with pale-green, stiff, leathery, and rolled leaves. In

certain cultivars, the disease causes internal necrosis of the phloem known as "net necrosis".

Chemical Control of PLRV

Soon after it was demonstrated that aphids are the principal, if not the only agent by which the virus spreads in potato crops, different kinds of chemicals have been tested in an attempt to control the vector and prevent the spread of the disease.

Field trials with nicotine, DDT, and Demeton sprays have been conducted in the Netherlands since 1938 (Schepers, 1972). With the advent of granular formulations of systemic-insecticides, Burt et al. (1960) found that the application of such formulations promises to be a useful way of controlling the disease. Nevertheless, the use of granulated systemic-insecticides in the epidemiology of PLRV has, in some cases, produced conflicting results. For instance, in Austria (Wenzle, 1964), France (Robert, 1976), and Japan (Tamada et al., 1971) the spread of PLRV from infected plants in the same crop was minimized by application of granular systemic-insecticides, whereas in New Zealand (Till, 1971), and the U.S.A. (Powell and Mondor, 1973 ; Bacon et al., 1976), similar treatment did not always prevent the spread of PLRV although the aphid population was controlled.

With the use of oils to control transmission and spread of persistent viruses, conflicting results were also reported. Hein (1970) demonstrated that oils do not inhibit transmission of PLRV by *Myzus persicae*, whereas Simons and Zitter (1980) obtained evidence that oil

can interfere with transmission of tomato yellows virus (TYV), a virus similar to PLRV.

Yield losses due to PLRV

Among the virus diseases of potatoes, leafroll has always been considered the most important because it causes high yield-loss. The effect of leafroll on yield of potatoes has been well documented by Murphy (1922), Loughnane (1941), Kirkpatrick and Blodgett (1943), Bald et al. (1946), Watson and Wilson (1956), Broadbent et al. (1962), Murphy et al. (1966), Reestman (1970), and Marco, 1984). Losses from secondary infection were shown to vary from 10% to over 90% depending on the potato variety, virus strain, severity of symptoms, cultural, and environmental conditions. Yield loss per unit area was also shown to be indirectly proportional to the percentage of infected plants due to compensation. Furthermore, the amount of compensation was dependent upon plant density, soil fertility, and water supply. Based on partial compensation by healthy plants, Kirkpatrick and Blodgett (1943), Reestman (1970), and Harper et al. (1975) formulated equations to estimate yield loss from crops with a certain percentage of infected plants. Such equations have not been tested, so far, on experiments involving potato crops with different percentages of infected plants at different distances of planting.

As potato leafroll has received little attention in Australia where it appears to be a serious disease, a number of experiments were conducted. The first part of this chapter describes experiments aimed at controlling the spread of PLRV in seed-crops by application of mineral oils and insecticides, and the last part deals with yield loss

assessments due to leafroll. The effect of a severe strain of PLRV, isolated in South Australia, on the yield of a susceptible potato cultivar, interactions between percentage infection and distance of planting are emphasized. Moreover, the accuracy of mathematical equations to estimate yield loss was tested using the yield data obtained in this study.

II. Experiments and Results

A. Detection and selection of a PLRV strain

Fifty eight potato leaf samples were collected from potato crops in the Northern Adelaide plains. Each leaf was washed, blotted dry, placed in Petri dishes with moist filter paper, and infested with five apterous aphids (*Myzus persicae*). After a virus acquisition-feeding period of two days, the aphids were transferred to individually caged *Datura stramonium* plants. The aphids were then killed by spraying the plants with Pyrethrum insecticide after four days of inoculation-feeding. From each of the infected plants, the virus was re-transmitted, first to *D. stramonium* and then, after 39 days, to *Physalis floridana* seedlings. The seedlings were maintained in a glasshouse at 20-24°C and 21 days later, five plants with severe symptoms of PLRV were selected and serologically tested for PLRV and potato virus Y (PVY) by means of ^{the} ELISA ^{test}. Virus transmission was also performed on *Capsella bursa-pastores* (Shepherd's purse) plants to detect the presence of Beet Western Yellow Virus (BWYV).

The result of the transmission experiments indicated that the leaf samples from the field were infected with either mild or severe strains

of PLRV (Figure 6). Based on serological tests, the selected plants were infected by PLRV only, although PVY was detected in some of the leaf samples. Furthermore, the inoculated sheperds purse plants did not show symptoms of BWYV as described by Duffus (1970), thus, further tests was not carried out.

From one of the *Physalis* plants, with severe symptoms of PLRV-infection, the virus was transmitted to and maintained on *Datura stramonium*, and served as inoculum in subsequent experiments.

B. Response of potato cultivars to PLRV infection

Tolerance to PLRV infection was evaluated on the following potato cultivars ; Coliban, Exton, Kennebek, Sebago, Sequoia, and Tasman. Seed-tubers were propagated from pathogen-free tubers obtained from the tissue-culture laboratory in Victoria, Australia. Previously cold-stored seeds were sprouted in a glasshouse and then cut into sections such that each cut-piece had one sprout.

Adult aphids (*M. persicae*), previously reared on chinese cabbage plants (*Brassica pekinensis*), were allowed to larviposit on healthy and leafroll-infected *D. stramonium* plants and then removed the following day so that the nymphs did not vary in age by more than 24 hours. Two weeks later, groups of eight aphids were transferred from the colony to each potato sprout and after five days of inoculation-feeding, the sprouts were sprayed with metasystox + malathion. Each tuber was planted in pots (10 inch diameter) containing 7 kg of Recycled Soil (R.C. soil, Appendix 4), and grown in a glasshouse at 20-24°C. As severity of symptom and yield are related (Harper et al., 1975), only the weight of

Figure 6 *Physalis floridana* plants showing mild (Top) and severe (Bottom) symptoms of PLRV-infection. Seedlings which had developed cotyledons were infested with three viruliferous *M. persicae*.



V5
4/10/00

PERU
V5A
4/10/00

tubers, harvested from each plant after 90 days from planting, was recorded. Tolerance of the potato cultivars to leafroll infection was based on the percentage reduction of yield.

All the inoculated plants showed rolling of the lower leaves, a typical symptom of secondary PLRV infection. The severity of stunting and leaf-rolling differed considerably among cultivars, being most pronounced on Exton (Figure 7). Purple discoloration of rolled leaves was very distinct on Exton and Tasman, and slight or absent on the other cultivars. Decreased productivity of infected plants was more evident in Exton, Coliban, Tasman, and Sebago, than on Sequoia, Kennebek, and Katahdan (Table 4).

Exton was used in subsequent experiments because it produces a very distinct symptom of PLRV infection and is widely grown in South Australia.

C. Biological tests of Mineral oils

1. Phytotoxic effects of oil-sprays

Oils have been used to control virus diseases in potatoes (Peters, 1977) and burning of leaves after oil spraying has been observed (Bradly et al., 1966 ; Quemener, 1976 ; Schepers et al., 1977). To avoid such undesirable side effects of oil application, five samples of mineral oils were tested on *Physalis floridana* and potato plants.

The oils were supplied by Golden Fleece Petroleum Ltd., and Mobil Oil Australia Ltd. The characteristics of these oils are presented in Appendix 5.

Figure 7 Reaction to a severe strain of potato leafroll virus (PLRV) in Sequoia, Katahdin, Kennebec, Sebago, Coliban, Tasman, and Exton. Plants are 60-days old, and were grown in a glasshouse at 20-24°C. Plants on the left, and on the right hand side are infected, and healthy, respectively.





EXTON

Table 4 : Yield of potato cultivars infected with a severe strain of PLRV.

Cultivar	Yield per plant (g) ¹		Reduction in yield (%)
	Healthy	infected	
Katahdin	374.8	262.5	29.9
Kennebec	389.8	265.1	31.7
Sequoia	354.1	195.4	44.8
Sebago	310.0	144.4	50.8
Tasman	297.8	134.4	53.2
Coliban	381.3	163.0	56.5
Exton	325.6	121.3	62.9
	H.S.D. (P = 0.05)		16.58

¹ Mean yield of twelve plants

H.S.D. - Tukey's w-procedure (honestly significant difference).
coefficient of variation (C.V.) = 12.3%

Spray emulsions of summer spray oil (SSO) and Lovis spray oil (LSO) were prepared by suspending them in water. As all the Mobil Oil samples were without emulsifiers, Agral-60 (ICI Australia Ltd.) was added to the water at 0.1% concentration before spray emulsions were prepared. Test plants were thoroughly sprayed with 0.75% emulsions using a jet-pack power unit (National Paint Product Ltd.). Test plants sprayed with water, and 0.1% water suspension of Agral-60 served as control. The treated plants were kept in a glasshouse at 20-25°C.

The result of the tests showed that the Mobil Oil sprays are phytotoxic on potatoes and *P. floridana* plants. The leaves of the oil treated plants were scorched after 2-3 days from spraying. On the other hand, all plants sprayed with either SSO or LSO were not burned, thus, both oils were evaluated further.

2. Effect of oil spray on potato yield

To determine the range of oil-concentration for field tests, a trial was conducted in a glasshouse at temperatures of 25-27°C during the day, and 17-20°C at night. Potato (Exton) stem-cuttings were treated with 'ceradex' (ICI) and rooted in vermiculite. Each plant was then transplanted in 10 inch pots containing 10 kg of R.C. soil. A week after planting, and every week thereafter, the plants were sprayed with SSO and LSO at 1.0, 1.5, and 2.0% concentration. The experimental design was a randomized complete block with 4 treatments and five replications. The yield per plant was recorded at ninety days after planting.

Based on the average yield from each treatment, both oils caused a reduction of tuber yield despite the absence of phytotoxic symptoms in

the form of leaf burning (Table 5). Plants sprayed with 2.0% concentration of either SSO or LSO yielded significantly less than the control. Insignificant reduction of yield was recorded from plants treated with oil up to 1.5% concentration.

D. Effect of oil spray on aphid-transmission of PLRV

Colonies of viruliferous aphids (*Myzus persicae*) were reared in the same manner as indicated in Experiment-B. Seeds of *Physalis floridana* were germinated on moist filter paper in petri dishes, and germinated seeds were transplanted in pots containing R.C. soil. The resulting seedlings were selected for uniformity of fully expanded cotyledons. Plants sprayed with 1.0% oil emulsion, and those with water were infested with three viruliferous aphids one day after spraying, and then caged separately. The aphids were removed from the plants after three days of inoculation-feeding, discarding all plants on which the aphids died. The number of plants that became infected after 21 days was recorded.

Based on three trials, the oil sprays slightly inhibited transmission of PLRV by aphids (Table 6), LSO being less effective than SSO. Treatments were statistically insignificant ($P > 0.05$).

E. Comparative effects of oil and insecticide sprays on the spread of PLRV

The experiment was conducted during November-February to ensure an abundance of aphids while the plants were growing. The field was rotavated twice and then furrows made at a depth and distance of 24 and 75 cm, respectively. Just before planting, ammonium sulfate (21%N),

Table 5 : Yield of Exton potatoes sprayed with mineral oil in glasshouse condition.

Oil Concentration (%)	Yield per plant (g) ¹	
	S.S.O.	L.S.O.
0	190.4	200.4
1.0	185.6	196.2
1.5	185.0	192.2
2.0	179.1	188.9
	H.S.D. (P = 0.05)	9.1
	C.V. (%)	1.7
		2.5

¹ Mean yield of fifty plants

S.S.O. - Summer spray oil

L.S.O. - Lovis spray oil

Table 6 : Transmission of PLRV by *Myzus persicae* to *Physalis floridana* seedlings with or without oil-spray.

Trial	Number of Plants/Treatment	Number of Infected Plants (%)		
		Control	S.S.O.	L.S.O.
I	80	81.6	76.6	80.0
II	80	90.0	82.7	88.2
III	100	95.0	85.0	87.0

Superphosphate (8.6%P), and potassium sulfate (41%K) were applied in the furrows at the rates of 170, 40, 400 kg per hectare, respectively. The area was partitioned into plots, each plot consisting of four 3.5-metre long furrows. Foundation seeds, supplied by the Potato Research Station (Victoria, Australia), were prepared in the same manner as indicated in Experiment-B and then planted at 30 cm distance along the furrows. As sources of inoculum, infected tubers were planted in the middle of each row, and infected plants, colonized by aphids (*Myzus persicae*), were placed along the boarder of the experimental field. Sprinklers were used to irrigate the field, and a moericke water-trap, placed at the centre of the field, was used to monitor aphid flights. The equipment used for spraying was fitted with four nozzles, adjustable to a height of 30-40 cm from the plant canopy, and with a cylinder of compressed CO₂ as source of spray pressure (7 psi).

Summer spray oil and lovis spray oil were applied at 1.0% concentration, while Maldeson (V-10 Malathion; Ag. Chem. Pty. Ltd.), Dimethoate (Lane Rogor, Rocke-Moog Ltd.), and Demeton-S-Methyl (Metasystox, Bayer Australia Ltd.), were applied at 625, 300, and 250 mg a. i. per litre of water, respectively.

Before each weekly spraying, plants from the control plots were sprayed with water to determine the quantity of spray-solution required. Spraying began when the sprouts started to emerge and ended about 2 weeks before harvest. At 120 days from planting, the tubers of each plant were harvested separately, and one tuber per plant was indexed for PLRV. Indexing was done by following the method described by Hiddema (1970). The 'eyes' from the bud end of the tuber were excised with a melon scoop, placed in a coarsely woven bag, and immersed in aqueous

gibberellic acid (1 mg/litre) for 10 minutes. The treated tuber-pieces were then planted, at uniform depth of 2.5 cm, in seed boxes containing R.C. soil, and were grown in a glasshouse at 20-24°C. Five weeks later, the resulting plants were examined for symptoms of PLRV-infection, and those with vague symptoms were checked for PLRV by means of ELISA.

The experimental design was a randomized complete block with five treatments and four replications.

The symptoms of PLRV as observed on plants grown in the glasshouse for virus indexing are illustrated in Figure 8. Based on the percentage of infected plants, the insecticides, except Maldison, were significantly more effective than the oil sprays in controlling the disease, the most effective being Dimethoate (Table 1). The aphid population, particularly during the early growth of the plants, was generally high (Figure 9).

F. Effect of granular systemic-insecticides on the spread of PLRV

The experiment was conducted during September-December when the aphid population was high. The materials and methods used in the experiment were similar to those in Experiment-E, except for the insecticides. The following granular systemic-insecticides were evaluated ; Carbofuran (Furadan 10G, Union Carbide), Disulfoton (Disyston 5-G, Bayer Company), Aldicarb (Temik 10-G, Union Carbide), and Terbufos (Counter 15-G, Cyanamid Company).

Figure 8 Symptoms of PLRV-infected Exton potatoes grown in the glasshouse for virus indexing. Sprouts were treated with gibberellic acid (1mg/litre), and were grown for 4-weeks at 20-24°C.

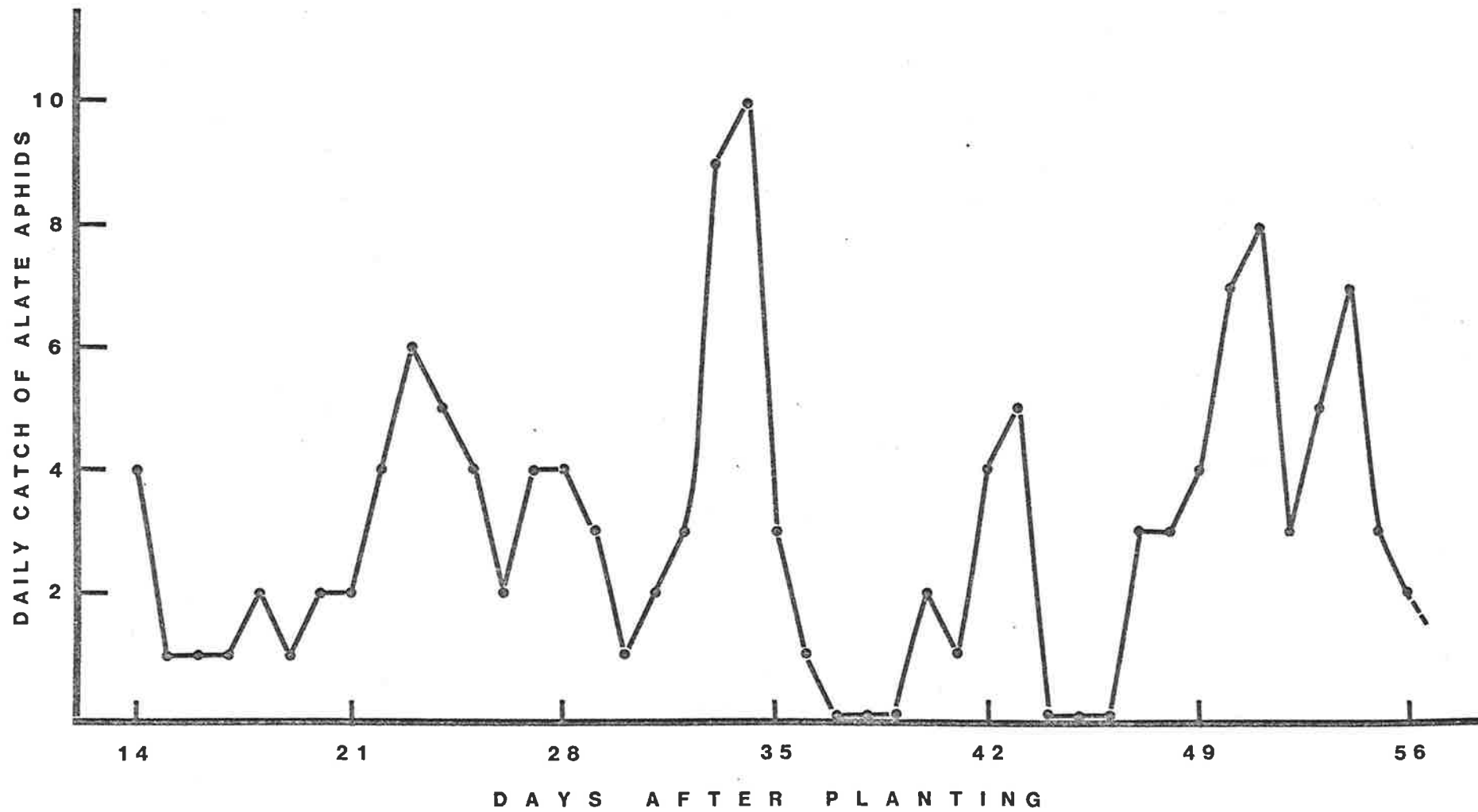


Table 7 : Percentages of leafroll-infected tubers from untreated and treated potato plants.

Treatment	Per cent Infection	
	Range	Mean
Untreated	88.8 - 100	90.6
S.S.O. (1%)	76.6 - 87.5	80.3
L.S.O. (1%)	78.6 - 87.2	82.6
Maldeson	72.2 - 94.4	88.8
Demeton-S-Methyl	70.6 - 72.2	71.3
Dimethoate	56.6 - 68.9	65.0
	H.S.D. P = 0.05	15.3
	P = 0.01	19.3

Coefficient of Variation = 8.5%

Figure 9 Daily catches of alate *Myzus persicae* (Sulz.) in one Moericke water-trap. (Note days indicated, 14 to 56 coincided with the first to the 7th spraying). Aphid catches after day 56, not included in the graph, followed a similar trend but with very low numbers.



At planting, the granules were applied in the furrows at 2, 3 and 4 kg active ingredient (a.i.) per hectare. The seeds were then planted in the furrows such that the sprouts were not in contact with the insecticides. Cultural management, similar to that in previous experiments, was followed. At 120 days from planting, each plant was numbered so that subsequent classification of healthy and infected plants or tubers was possible. From each plant, the tubers were harvested and one tuber was checked for PLRV adopting the indexing method described in experiment E.

The experimental design was a split-plot with three replications ; the insecticides, and insecticide-levels were assigned in the main plots, and sub-plots, respectively.

As a pre-emergence treatment to control the spread of PLRV, Aldicarb was significantly more effective than any of the other insecticides tested (Table 8). In untreated plots, most of the tubers harvested and indexed for PLRV were infected, whereas in plots treated with insecticides, incidence of PLRV was much reduced. The incidence of PLRV decreased as the level of insecticide application was increased, except for Terbufos (Table 9). Aphids were abundant throughout the duration of the experiment (Figure 11).

G. Assessment of yield losses due to PLRV

1. Comparative yield between healthy and leafroll plants

Random field sampling was used to estimate potential yield of healthy and PLRV-infected potato plants grown on a commercial farm. From

Table 8 : Effect of Granular insecticide treatment on the spread of PLRV.

Insecticide	Range of Infection (%)	
	Untreated	Treated
Aldicarb	90 - 100	13 - 20
Disulfoton	92 - 100	25 - 47
Carbofuran	96 - 100	35 - 60
Terbufos	90 - 94	42 - 68

Table 9 : The influence of different levels of Granular systemic - insecticide on the spread of PLRV.

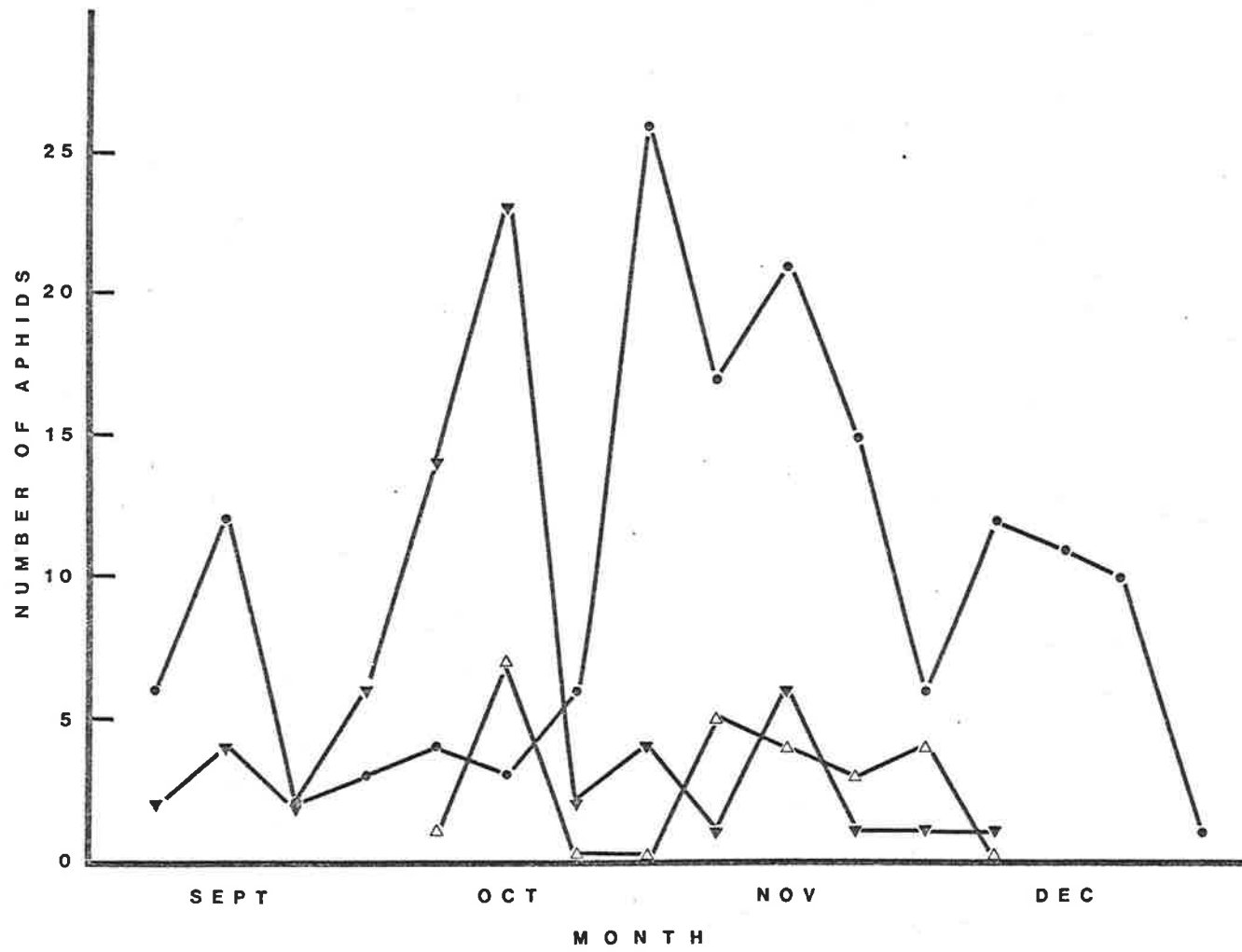
Insecticide	Plants infected (%) ¹			
	Kg a.i. per hectare			
	0	2.0	3.0	4.0
Aldicarb	94.0	18.9	16.2	15.7
Disulfoton	94.8	37.9	33.9	29.4
Carbofuran	98.4	55.7	44.5	43.5
Terbufos	91.6	58.7	63.3	45.4
Mean	94.8	42.8	39.5	33.5

¹ Mean value of 48 plants

	Standard Error (%)	L.S.D. (P = 0.05)
Two rate means	2.05	5.7
Two rate means of the same insecticide	4.11	11.5
Two insecticide means at the same rate	3.79	7.9

C.V. (Rates) = 9.5%

Figure 11 Weekly catches of alate *Myzus persicae* (- solid dot -), *Macrosiphum euphorbiae* (- ▼ -), and *Aulacorthum solani* (- Δ -), in one Moericke water-trap. The curve for *M. euphorbiae*, and *A. solani* represent catches from the first week of October to the first week of December.



A crop of potatoes (Var. Exton.) which had 20% leafroll incidence, plants with, and without symptoms of leafroll, and having two or three main stems, were collected at random. The total tuber weight and haulm weight of each plant were recorded.

Based on the frequency distribution of tuber yield per plant, leafrolled plants yielded 66.3% less than non-leafrolled plants (Figure 12). There was a significant regression between yield and weight of haulm (Figure 13), a higher degree of linear relationships was measured in leafrolled plants than in plants without leafroll.

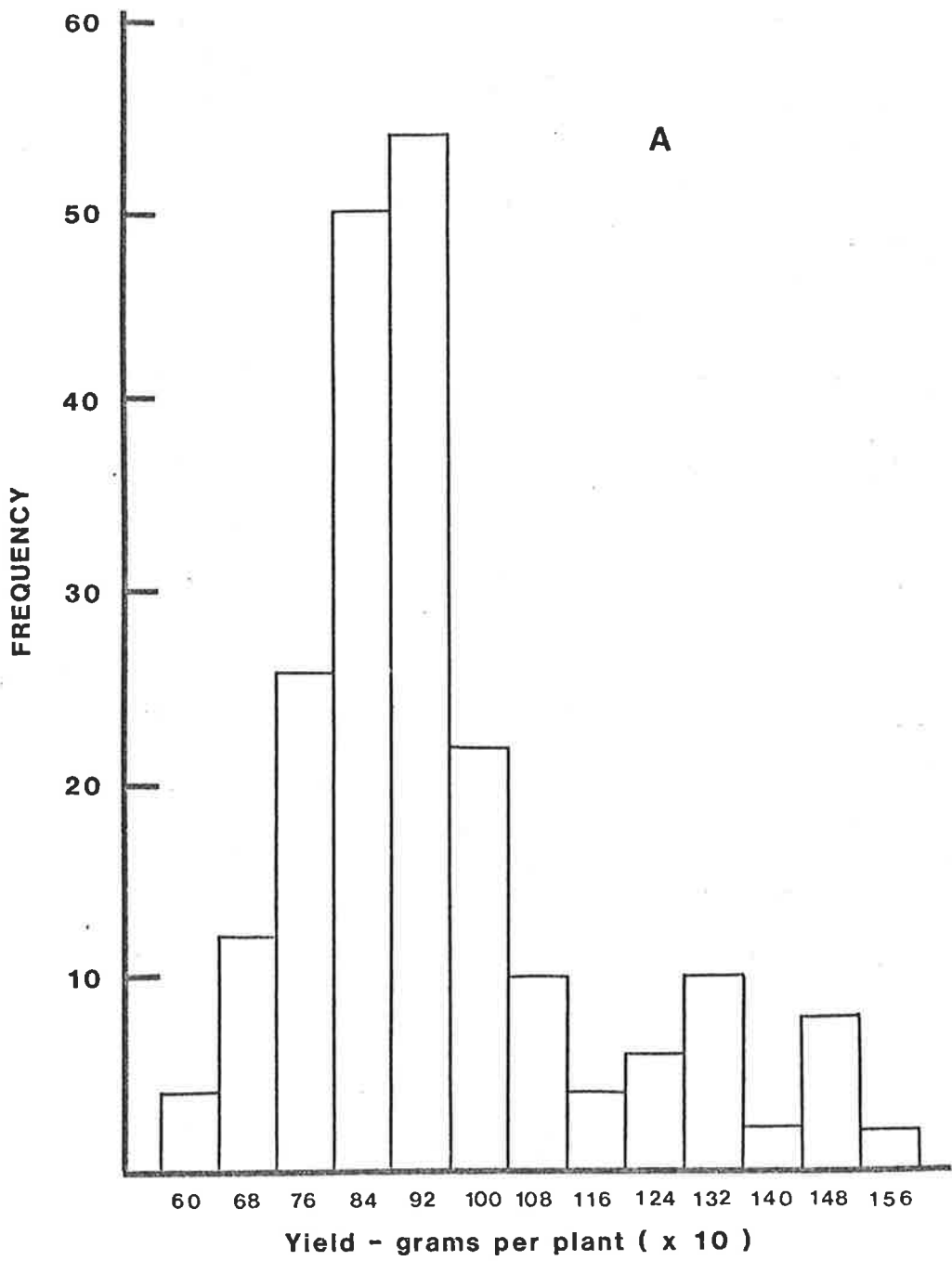
2. Effect of secondary leafroll and planting distance on potato yield

The response of Exton potatoes to different levels of leafroll (% of plants infected) and two distances of planting was investigated to complement the field sampling data previously recorded. The experiment, consisting of ten treatment combinations (5 x 2 factorial) arranged in a complete block design with three replications, was set in mid-December as the population of alate-aphids started to decline.

Potato tubers, previously harvested from the crop in Experiment-F and indexed for PLRV, were treated with 2-chloroethanol (Quik Sprout, AG CHEM PTY. LTD.) to break dormancy, and kept in diffuse light at about 20°C after the treatment. Seeds with uniform sprouts were selected, cut into sections so that each seed-piece had two sprouts, and marked as H (healthy) or D (infected).

Seedlots of 0, 25, 50, 75 and 100% infected or healthy tubers were set out in field plots (80 cm apart, 20 metres long) at planting

Figure 12 Distribution of tuber yield per plant of healthy (A), and infected (B) Exton potatoes. Plants were gathered from a commercial crop.



A

Handwritten note: Distribution of yield

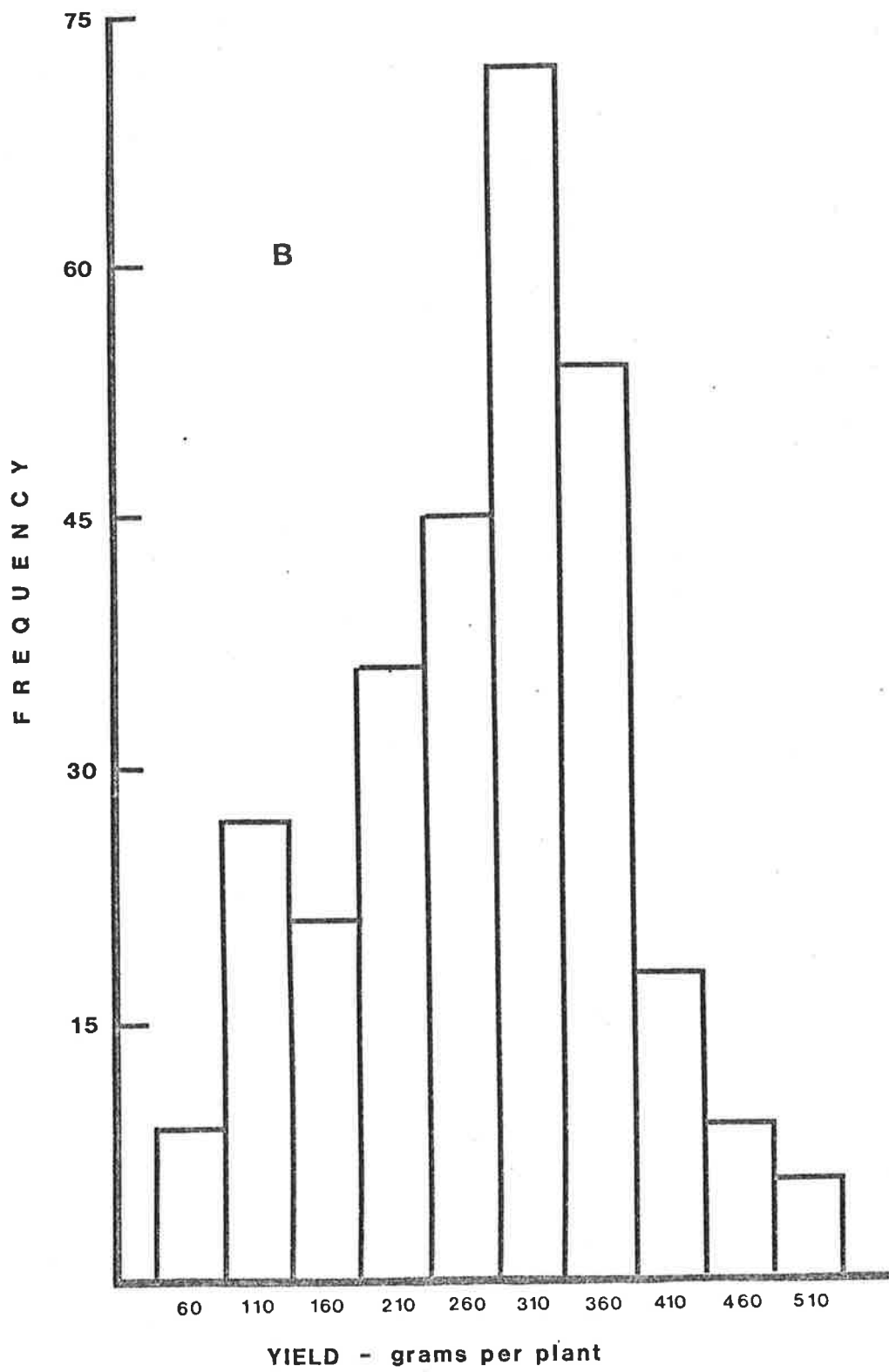
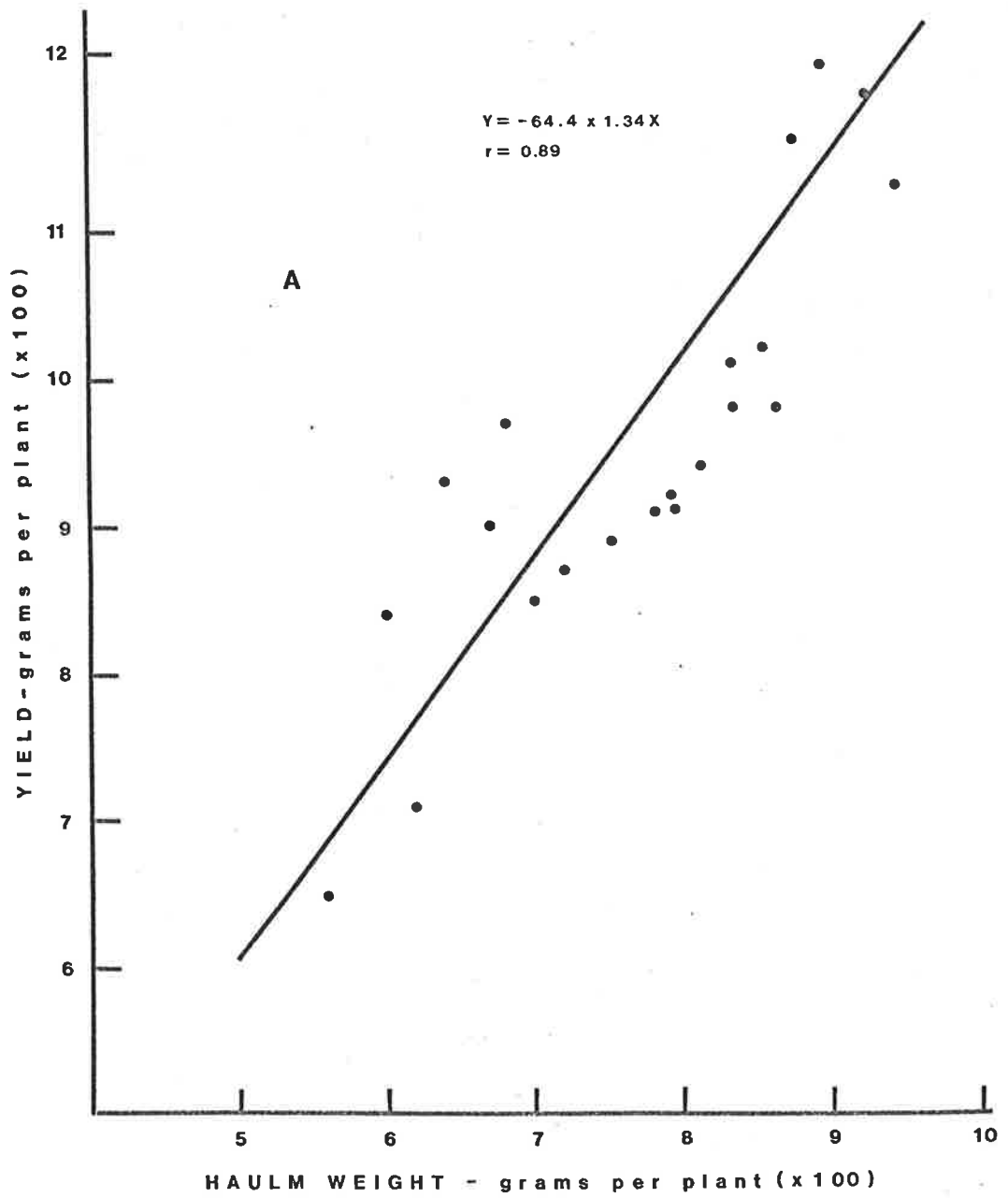
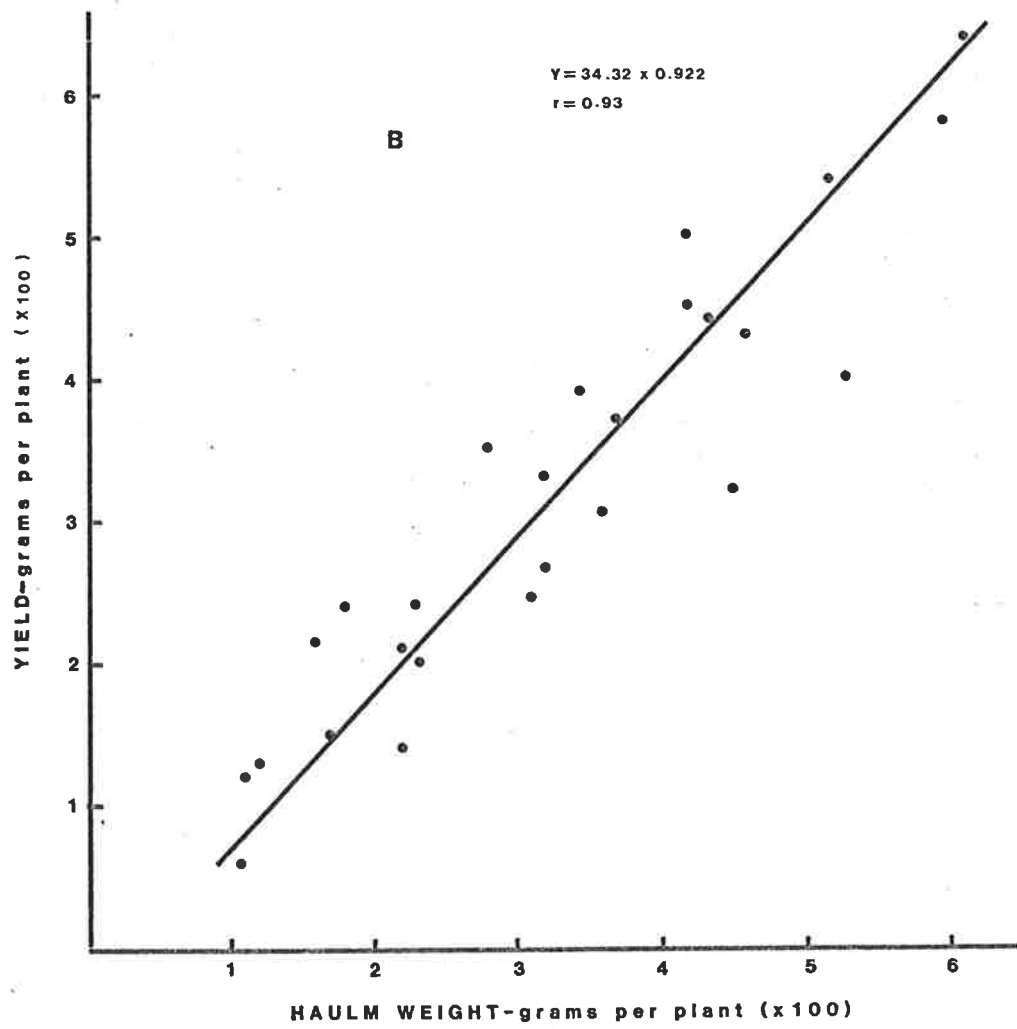


Figure 13 Regression of tuber yield of healthy (A), and PLRV-infected (B) plants on weight of Haulm.





distances of 20 and 30 cm in the row. The location of H and D tubers in the plots, except those at 100% disease or healthy, was recorded so that subsequent comparison of position and yield for each plant was possible.

To prevent the spread of leafroll virus from infected to healthy plants, Aldicarb (2 kg a.i. per ha.) was applied at planting, and plants sprayed with Dimethoate every week starting from 30 days after planting. Chlorothalonil (Bravo, AG CHEM PTY. LTD.), applied at 72 g a.i. per hectare in combination with the insecticide sprays, was used for the control of early blight (*A. solani*). Other materials and methods were similar to those in Experiment-E.

The potatoes were harvested by hand after 120 days from planting and the tuber weight of the plants in the centre of each class was recorded. The different classes used were:

- (1) healthy with a healthy plant on each side (H-H-H)
- (2) healthy with a leafroll plant on one side and healthy on the other (H-H-D, D-H-H)
- (3) healthy with leafroll plants on both sides (D-H-D)
- (4) leafroll with healthy plants on both sides (H-D-H)
- (5) leafroll with a healthy plant on one side and a leafroll on the other (H-D-D, D-D-H), and
- (6) leafroll with leafroll plants on both sides (D-D-D).

One tuber from each of the healthy plants was indexed for PLRV to determine the extent of virus spread among healthy plants, and to evaluate the effectiveness of the insecticides applied. Total yield per treatment (20-linear metre plot) was recorded and yield loss measured.

Yield loss was also estimated by means of the following mathematical models :

(1) Equation developed by Reestman (1970) ;

$$\text{yield loss} = M_H - (Y_H + Y_L) \times 100$$

where:

M_H = yield for a 100% healthy crop

$$Y_H = K_{hi} \times Z_h (K_{hi} \times Z_h + Z_i)^{-1} \times M_H$$

$$Y_L = Z_i (K_{hi} \times Z_h + Z_i)^{-1} \times M_L$$

M_L = yield of a 100% diseased crop

Z_h and Z_i = the proportion of healthy and infected plants, respectively.

K_{hi} = relative crowding coefficient (Appendix 6)

(2) Equation developed by Blodgett (1941) and modified in this study ;

$$\text{yield loss (\%)} = (M_H - Y) \times 100$$

where:

M_H = yield of a 100% healthy crop

Y = yield at certain percentage of disease, estimated by means of a frequency table (Appendix 7)

A comparative yield between healthy and infected potato plants is illustrated in Figure 14, and the ranges of tuber-sizes produced by such plants are shown in Figure 15. Among the healthy plants, those planted at 30 cm intervals in the row yielded 3.5% more of large-tubers (over 86 g) than those planted at 20 cm, whereas diseased plants yielded almost the same sizes of tubers regardless of planting distance (Figure 16).

Figure 14: Tubers of healthy (left), and PLRV-infected (right)
Exton potatoes. Most plants developed two main stems.



Figure 15 Sizes of tubers produced by healthy, and diseased plants.

A = over 230 g
B = 86-230 g
C = 35-85 g
D = less than 35 g

Bar = 10 cm.

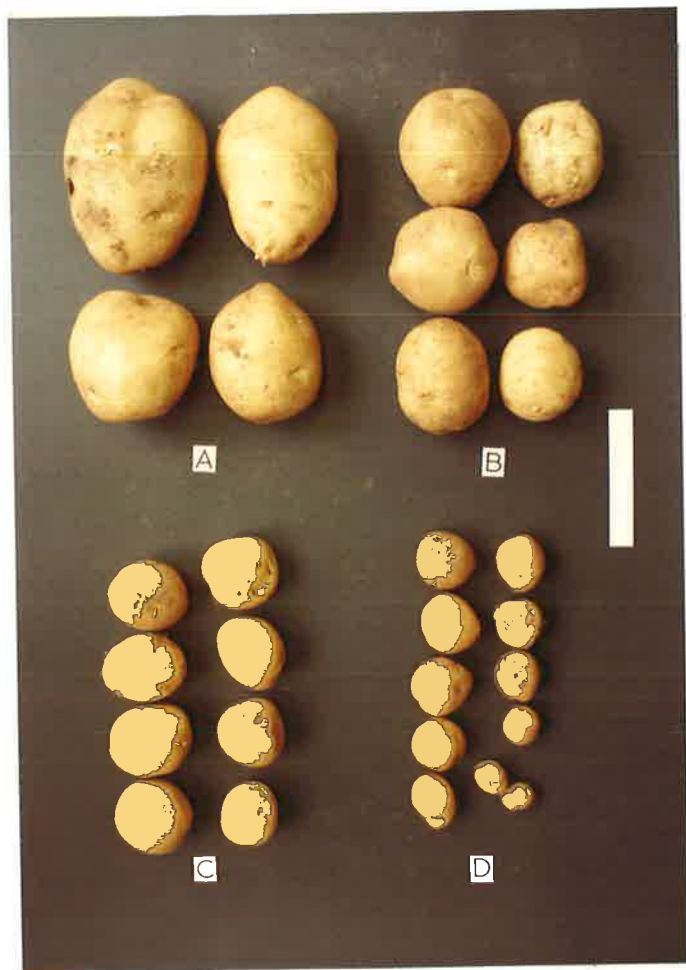
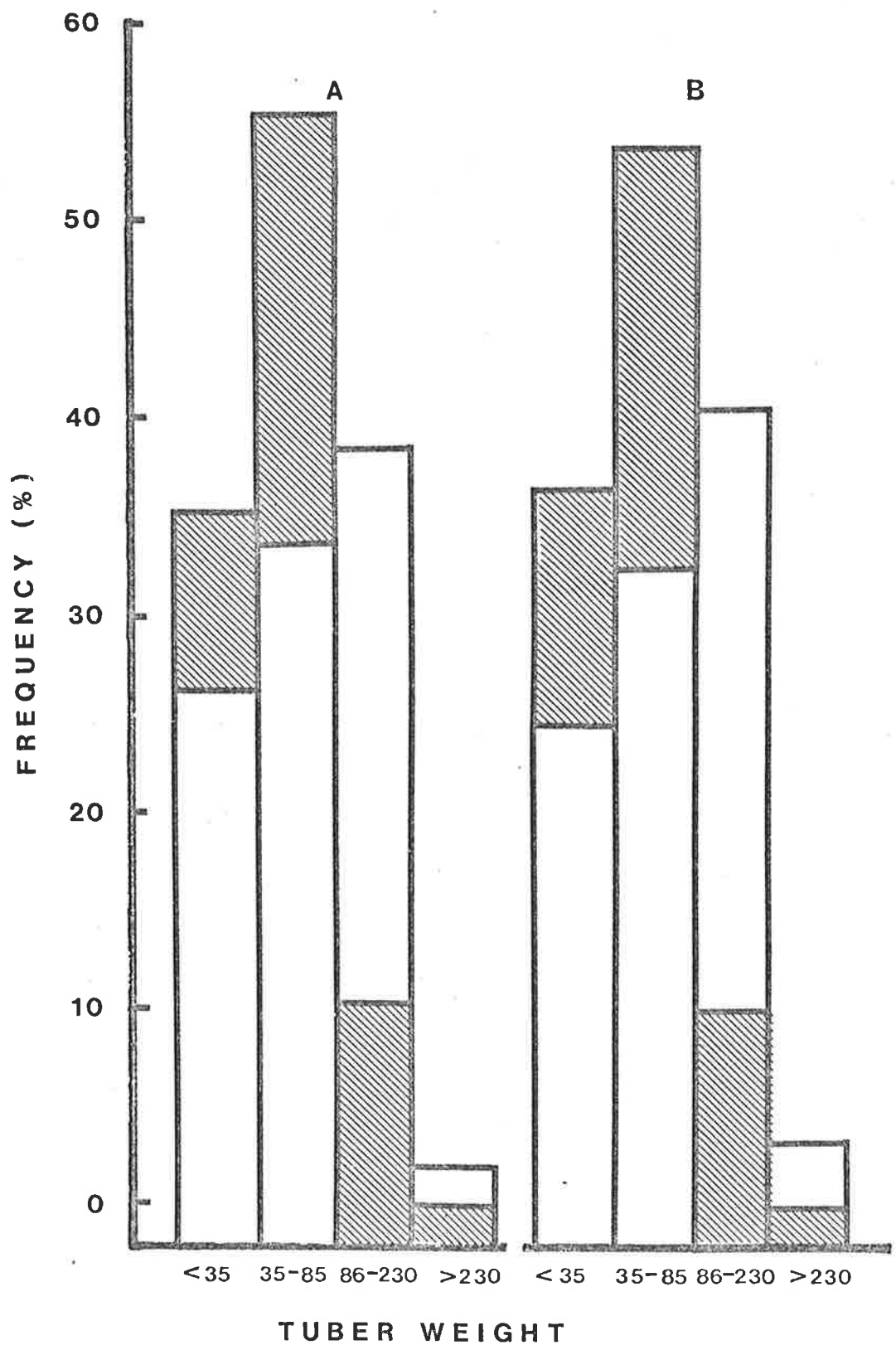


Figure 16 Percentage of tuber-sizes from healthy (white bars) and infected (shaded bars) Exton potatoes planted at 20 cm (A) and 30 cm (B) in the row. Tubers came from plots with 100% healthy, and 100% infected plants.



Yield compensation, as measured from the different classes of healthy (H) and infected (D) plants, was higher at closer than at wider distance of planting, healthy plants situated between two diseased ones (D-H-D) being the most productive (Table 10). The total yield per 20 m plot diminished as the percentage of plants infected by leafroll increased (Figure 17). Between 20 and 30 cm spacings at the same level of leafroll incidence, the yield of plants with 0, 25 and 50% leafroll differed significantly ($P < 0.05$), whereas those with 75 and 100% leafroll were the same ($P > 0.05$). The same yields ($P > 0.05$) were also recorded between treatment-combinations 0% at 30 cm and 25% at 20 cm, and between 25% at 30 cm and 50% at 20 cm.

The yield losses actually measured, and those estimated by using equations are presented in Table 11. From 25 to 75% leafroll, the equation-I developed by Reestman (1970) estimated the actual loss to within 0.7 - 1.7%, and 1.5 - 1.8% for the 20 and 30 cm planting distance, respectively, whereas that of Blodgett (1941) estimated loss to within 2.9 - 3.3, and 0.6 - 2.6% for 20 and 30 cm spacings, respectively.

III. Discussion and Conclusion

Field grown potatoes are often affected by more than one virus and to separate such viruses, a number of plant species can be used. Horvath (1967) listed several test plants which he used to separate potato viruses. For instance, he indicated that PVY can be eliminated and PLRV separated from PVY by ; aphid (persistent), and *Cuscuta* transmission using *Datura stramonium*, or root transmission using *Solanum tuberosum* L. as host. In this study, *Datura stramonium* appears to be a suitable host

Table 10 : Yield of Exton potatoes from the center of three, in various sequences of healthy (H) and leafroll-infected (D) plants

Sequence	Mean yield (Grams)	
	Distance between plants	
	20 cm	30 cm
H-H-H	550	640
H-H-D	670	772
D-H-D	751	785
H-D-H	34.6	37.8
H-D-D	45.1	49.0
D-D-D	71.5	70.2
	L. S. D.	
	P=0.05	58.2
	P=0.01	97.1

Figure 17 Relation between yield of Exton potatoes and percentages of leafroll-infected plants at planting distance of 20 cm. (-●-) and 30 cm (-●-) in the row.

A = L.S.D. at 1%
B = L.S.D. at 5%

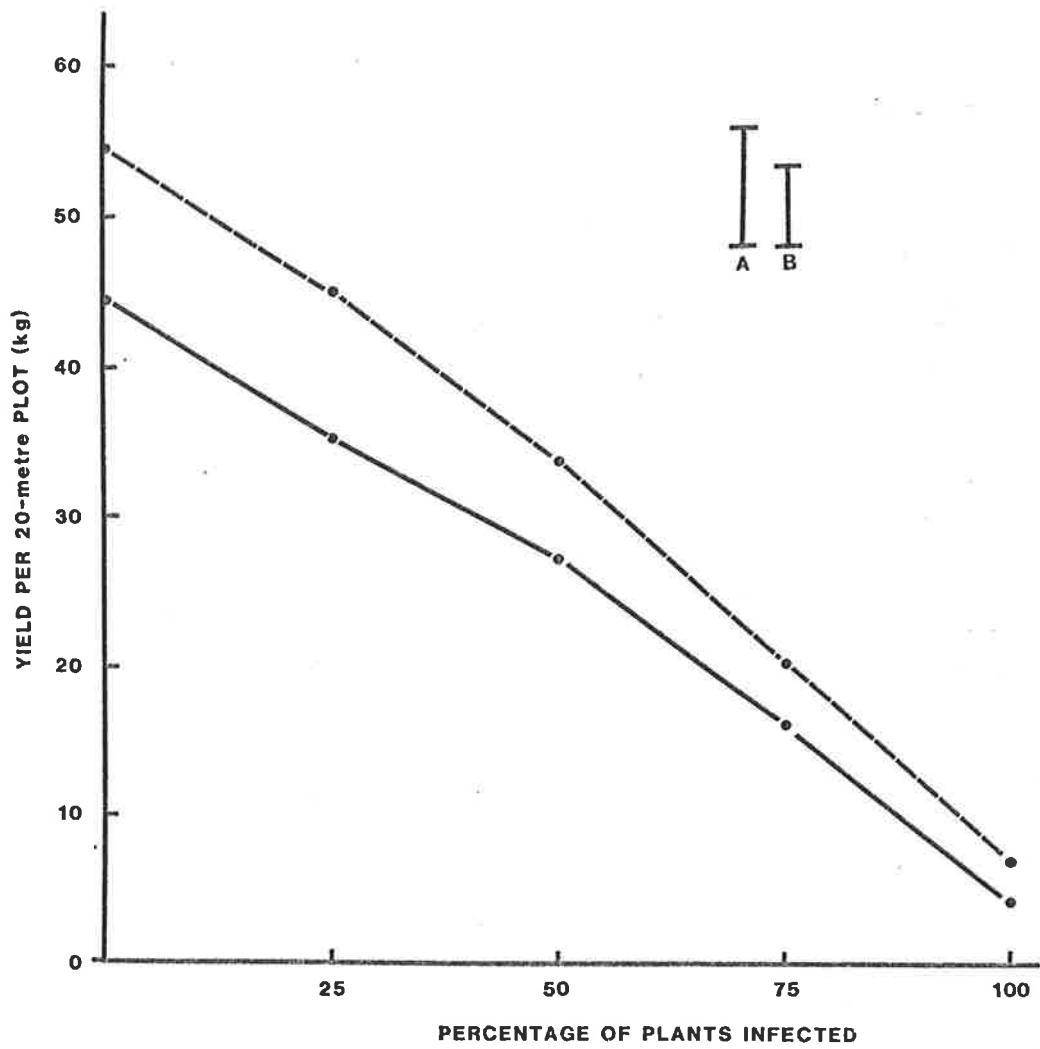


Table 11 : Actual, and estimated yield losses of Exton potatoes due to leafroll at 20 and 30 cm planting distance.

Percentage of plants infected	Yield Losses					
	20 cm			30 cm		
	A	B	C ¹	A	B	C ¹
25	17.5	16.8	14.6	20.8	19.3	18.2
50	37.8	39.5	34.8	38.7	40.5	39.3
75	62.8	63.5	66.1	62.2	63.7	63.6
100	86.9	86.9	86.	89.1	89.1	88.9

- ¹ A - yield loss actually measured
 B - yield loss estimated by equation-1
 C - yield loss estimated by equation-2

for obtaining or for propagating PLRV isolate, and being immune to PVY infection, the plant can be used to maintain pure isolates of PLRV. In glasshouse tests, leafroll-infected *D. stramonium* plants were found to be good sources of the virus. Moreover, it was observed that aphids (*M. persicae*) multiplied more rapidly on such plants than on potatoes, or on *Physalis floridana*, hence, *D. stramonium* was used routinely in this project as a source of inoculum. However, strains of PLRV are best distinguished on *P. floridana* plants. That PLRV could be transmitted from potatoes to *Capsella-bursa pastores* (Shepherd's purse), an indicator host of Beet Western Yellow Virus, has not been confirmed in this study, suggesting that not all PLRV - isolates can infect this test plant.

The method adopted in this project to infect potato sprouts with leafroll virus seems to be efficient, though a number of viruliferous aphids (8 per sprout) and a long acquisition feeding period (14 days) are required. Nevertheless, inoculated potato seeds produced plants that showed typical symptoms of secondary leafroll-infection such as stunting, discoloration and rolling of lower-mature leaves. Such symptoms are important criteria for evaluating tolerance or resistance of potato varieties or clones to PLRV infection.

That mineral oil sprays have a phytotoxic effect in the form of leaf burning and reduce the yield of potatoes has been confirmed. De Wijs (1980) found that mineral oils with unsulfonated residue (USR) under 90 are, in general, phytotoxic due to the presence of unsaturated olefines and aromatics. As the mobile oil samples were phytotoxic to potatoes, they probably contain lower USR. On the other hand, the reduction in yield due to summer-spray-oil and lovis-spray-oil, suggested that oils can interfere with the physiological process of

plants though leaf burning may not be evident. The action of mineral oils in the physiology of potato plants is little understood, hence studies on this subject should prove useful.

That oil does not reduce aphid transmission of leafroll in glasshouse tests has been confirmed. The possibility that both SSO and LSO are not effective against stylet-borne viruses can be excluded because such oils were tested, and found effective, against PVY (Appendix 8). Hein (1970) examined a number of virus-vector combinations for the prevention of virus transmission by spraying plants with mineral oils and he found that the transmission of all tested viruses was strongly reduced except PLRV.

The relation between the physico-chemical properties of mineral oils and their inhibiting properties, especially on transmission of non-persistent viruses are known. Wyman (1971) found that a low viscosity oil (6 cSt at 37°C) was more effective than oil with higher viscosity (16, 30, 67 cSt at 37°C) against beet mosaic virus, whereas De Wijs (1980) showed that the activity of mineral oil against PVY decreases with decreasing oil viscosity. The result presented in Appendix 8 supports that of De Wijs.

The mechanism or mode of action by which oil interferes with aphid transmission of viruses is not known so far. However, several suggestions have been made (Vanderveken, 1977). For instance, Bradley (1963) suggested that oil may either remove virus particles from the stylet or cause virus particles to adhere more firmly on the stylet. Simons et al. (1977) treated the labium of aphids with Huber's clay, which absorbs oil, and found that the removal of oil restored

transmission to the level found with similar untreated control aphids. Inhibition of aphid transmission of plant viruses by oil was observed with viruses of varying morphology such as flexuous rods, icosahedral, and pleomorphic bacilliform particles, which suggest that viral structure does not play an obvious role in the inhibition action of oil (Peters, 1977). Oil does not inactivate viruses as Loebenstein et al. (1964) showed that the original infectivity of TMV, mixed with oil, was restored by breaking the emulsion. Moreover, Zschiegner et al. (1974) concluded that the inhibition of virus transmission could not be attributed to the inactivation or denaturing of the virus. However, oil has been shown and confirmed to interfere in the infection process. For instance, the number of local lesions on *Nicotiana glutinosa*, after inoculation with intact particles of TMV, was reduced by a mineral oil treatment of the leaves (Peters and Lebbink, 1975). Similar results were observed with PVY on *Chenopodium quinoa* using SS0 and LS0.

Simons and Zitter (1980) observed good control of tomato yellows virus (TYV) in field grown tomatoes by weekly application of oil, and they stated that both TYV and PLRV are similar in host range, symptomatology, and virus-vector relationships. In view of such a report, weekly application of oil-emulsions was compared with that of insecticides to control leafroll in field grown potatoes. As indicated previously, oil sprays seems to be ineffective against the virus, and causes yield reduction, in this case up to 35%. High temperature (35-43°C) recorded during the experiment probably accounted for such a high reduction of yield.

Although the insecticides, Dimethoate and Demeton-S-Methyl, reduced the spread of leafroll, the highest level of control (65%) achieved with

such insecticides was considered unsatisfactory. The high aphid population before the first spraying, and throughout the duration of the experiment probably accounted for the high incidence of leafroll. As the potato sprouts did not emerge all at the same time, those that emerged earlier, before the first spraying, could have been colonized by viruliferous aphids. Furthermore, there was sufficient time for aphids, coming from the infection-source, to feed on the sprouts which emerged one day before the first, second, or third spraying. It is also possible that the insecticides did not kill viruliferous aphids quickly enough to prevent transmission of leafroll, as shown by Till (1970). Although Broadbent et al. (1956) showed that Systox (Demeton-O + Demeton-S) prevented the spread of leafroll virus, Marco (1980) found that the commonly used aphicide, Metasystox (Oxydemeton-methyl) was less effective, and that Dimethoate (Rogor) gave good results in the laboratory test, but was less efficient in the field trials.

The granular form of systemic-insecticide appears to be effective against leafroll probably because such formulations, being absorbed and translocated to the different parts of the potato sprouts prior to emergence, rendered the plants lethal to aphids. Of the four insecticides used Aldicarb reduced virus spread to as much as 87%, whereas in the control plots, 94-100% of the plants were infected with the virus. Marco (1980) showed that when leafroll infection in the control plot was about 12.6%, single treatments of Aldicarb and Ethiofencarb reduced infectivity to 4.1 and 3.2%, respectively, whereas two treatments with Aldicarb reduced infectivity to 1.7%. Thus, it is likely that the insecticides ; Carbofuran, Disulfoton and Terbufos, (registered in South Australia for horticultural use), can give better control of leafroll if disease incidence is low. Populations of

viruliferous aphids, particularly *M. persicae*, weather conditions in relation to the population dynamics of aphids, and susceptibility of potato cultivars seem to be of great importance in adapting control methods.

Yield losses in potatoes due to secondary leafroll-infection seems to vary depending on the virus strain, susceptibility of the cultivar, and environmental conditions. Most investigators from other countries have found that the mean tuber-yield of secondary leafroll-infected plants is reduced by ; 27% (Murphy et al., 1966), 57% (McMillan, 1968), 59, 69, and 79% (Robertson, 1976). Furthermore, Harper et al. (1975) showed that netted gem potatoes with mild, moderate, and severe symptoms yielded 65, 80 and 92% less than those with no symptoms. The yield loss measured on Exton potatoes by field sampling (66%) and that recorded from the experimental plots (87-89%) are comparable to the data of Harper et al. Most of the diseased plants gathered from the field, as indicated in Figure 12, showed mild symptoms whereas those from the experimental plots showed severe symptoms, because the original seeds were infected with severe leafroll.

The higher degree of linear relationship measured in leafroll infected plants indicates that symptom severity is related to yield. Yield loss is not directly proportional to the percentage of diseased plants because of mutual competition between plants. As indicated in the yield of the different classes of healthy and infected plants, vigorously growing healthy plants compensate for adjacent poorly growing plants infected by leafroll. Thus, yield loss determined by comparing fields containing all diseased plants with fields in which all plants

are healthy gives an inaccurate measure of the yield loss at a certain percentage of diseased plants in a field.

The mathematical equation of Reestman (1970), seems to be a reliable tool for estimating yield loss at certain percentages of disease. Thus, the equation estimated the actual loss at 25% leafroll incidence to within 0.7%. The other equation (Blodgett, 1941 ; Kirkpatrick and Blodgett, 1943) estimated the actual loss less accurately and Harper et al. (1975) found this equation to be inadequate. It is likely that such equations can be used to estimate yield loss in the field.

CHAPTER 4

STUDIES ON *ALTERNARIA SOLANI*

I. Introduction

Early blight (target spot) caused by *Alternaria solani* Sorauer (Syn. *Macrosporium solani* Ellis and Martin) is a serious disease of potatoes, and is found worldwide wherever the crop is grown (CMI Map 87, ed. 4, 1972). The disease affects leaves, stems, and tubers, being often more severe when plants have been predisposed by injury, poor nutrition, or other types of stress. Field resistance to foliage infection is associated with plant maturity, and late maturing varieties of potatoes are more resistant (Waingartner, 1980).

Ellis and Gibson (1975) described *Alternaria solani* as follows:

- colony - effuse, grayish brown or black and hairy.
- conidiophore - arising singly or in small groups, straight or flexuous, septate, rather pale brown or olivaceous brown, up to 110 μ long, 6-10 μ thick.
- conidia - usually solitary, rarely in short chains, straight or slightly flexuous, obclavate or with the body of the conidium oblong or ellipsoidal, tapering to a beak which is commonly the same length as or rather longer than the body, pale or mid pale golden or olivaceous brown, smooth, overall length usually 150-300 μ , 15-19 μ thick in the broadest part with 9-11 transverse and 0 or a few

longitudinal or oblique septa ; beak flexuous, pale, sometimes branched, 2.5-5 μ thick, tapering gradually.

The fungus has been a subject of a number of studies because it does not sporulate readily on culture media without special treatment. As abundant spores, produced aseptically, are often required when screening potato clones for resistance to the disease, or when evaluating fungicides in the laboratory or glasshouse, considerable attention has been directed towards improving in vitro conidial production. Rands (1917) found that stimulation of sporulation occurred when a 10-12 day-old potato agar culture of *A. solani* was cut up and exposed to sunshine. Cultures of the fungus was also induced to sporulate by exposure to ultra-violet light (McCallan and Chan, 1944 ; Charlton, 1953), sunlight (Padhi and Rath, 1974), or to flourescent light (Luken, 1960 ; Barksdale, 1968). Recently, Shahin and Shepard (1978) induced profuse sporulation of *A. solani*, and two other species of *Alternaria*, by placing blocks of agar-culture on the surface of a sporulation medium. To some workers, such methods consistently produced high numbers of spores while to others, none of these methods was useful.

The relationships among radiation, media, and temperature on sporulation of *Alternaria solani* in pure culture was investigated, and such factors were considered in developing a simple method of inducing sporulation of the fungus. Furthermore, fungicides were tested against the fungus, in vitro using spores obtained with such methods.

II. Experiments and Results

A. Mycelial growth of *A. solani* on various synthetic media

Fungal isolate - isolates of *A. solani* were obtained from typical leaf lesions on potatoes gathered from the Adelaide plains, South Australia, and were grown on potato dextrose agar (PDA) - plates. Pure cultures of the fungus were grown on PDA - plates for seven days at 20°C. Then, Petri dishes containing 1.5% water agar were each covered with a culture plate, and left overnight in the laminar flow cabinet with the lights off. Dislodged conidia on the surface of the water-agar were allowed to germinate for 24 hours before they were isolated as single spores.

Medium - Several media were used : Corn meal agar (CMA), Lima bean agar (LBA), Malt extract agar (MEA), potato dextrose agar (PDA), yeast extract agar (YEA), all Difco products ; V-8 juice agar (V8A), and prune agar.

Plates (9 mm diameter) containing 20 ml medium were seeded with a centrally positioned 5 mm diameter plug taken from the edge of an actively growing 3-day old single-spore culture. The inoculated plates were incubated at 25°C, and the radial growth of colonies was assessed at 84 hours after inoculation by measuring the radial length of colonies at four different positions.

The result presented in Table 12 shows that *A. solani* produced significantly more growth ($P < 0.01$) on PDA than on any of the other media tested. The fungus grew at the same rate ($P > 0.05$) on Prune agar and V8 - juice agar. On all the media, conidiophores and aerial hypha developed but no conidia were formed.

B. Light and temperature interaction on the sporulation of *A. solani*

The isolate used in Experiment - A was grown on PDA and kept at 25°C for 3-4 days after which it was used as inoculum. Petri dishes containing 20 ml of PDA were inoculated in the same manner as described previously, and incubated under constant light at temperatures of 18, and 25°C. The light source was 40-W white fluorescent tubes that were located 15 inches above the cultures, and the light intensity at plate level was 4000 Lux. Comparable plates were covered with two layers of black polyethylene plastic sheets to exclude light. Seventy eight hours after seeding, half the number of plates which were grown at 25°C were transferred and placed overnight (12-14 hours) in an incubator at 18°C without supplementary lighting. After the spore induction period, the conidia produced on each plate were washed off the mycelia with water, spore suspensions centrifuged at 400 g for 5 min, and the resultant pellets diluted to 10 ml of distilled water. The concentration of conidia per plate was determined with a haemocytometer.

The conidia were also tested for pathogenicity by inoculating potato plants (Var. Red Pontiac) regenerated from stem cuttings. The plants were sprayed with conidial suspension (10-11,000 conidia per ml), and covered with a transparent plastic bag for 3 days to maintain high humidity. Infection was recorded from 5-10 days after inoculation.

Table 12 : Effect of media on mycelial growth of *A. solani*

Media	Mean colony diameter (mm)
Potato Dextrose Agar	58.0
Prune Agar	50.16
V-8 juice Agar	48.66
Lima Bean Agar	46.83
Yeast Extract Agar	44.66
Corn Meal Agar	42.83
Malt Extract Agar	39.33
L.S.D.	
	P = 0.05 2.24
	P = 0.01 3.01

C.V. = 4.0%

The result presented in Figure 18 shows that the cultures of *Alternaria solani* sporulated at 18°C in either light or dark conditions, and did not sporulate at 25°C in the same condition. Sporulation was highest on cultures grown and illuminated for 72 hours before being incubated at 18°C in the dark (Figure 18A), and was lowest on those grown in the dark (Figure 18B). At 18°C, continuous illumination of cultures during growth and incubation reduced sporulation, whereas similar treatment but without light drastically reduced sporulation (Figure 18C).

Harvested spores were highly pathogenic when inoculated on potato plants. Symptoms of infection were observed 4 days after inoculation, and were typical of early blight under field conditions 10 days later.

C. Sporulation by *A. solani* on different synthetic media

The fungal isolate, media, and seeding method were similar to those described in Experiment A. Petri dishes containing 20 ml of medium were seeded with the fungus and grown continuously for 72 hours at 25°C under fluorescent lamps with a light intensity of 4000 Lux at the plate level. During this period, the plates were not inverted, and no spores were formed.

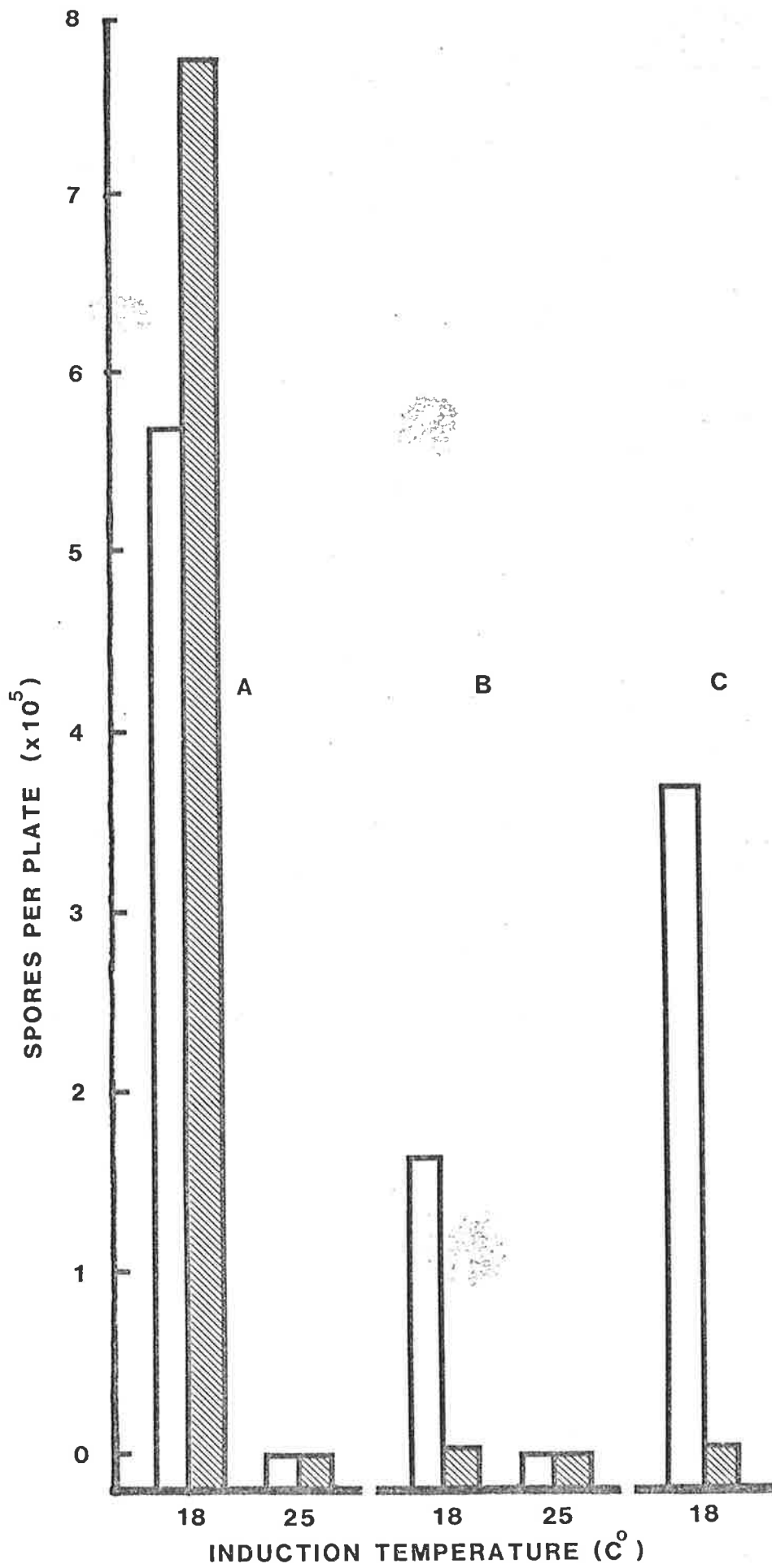
After the initial growth period, the cultures were transferred and placed overnight (12-14 hours) in a growth cabinet. To induce sporulation, the temperature inside the incubator was set at 18°C and the lights were switched off. Spores were collected and concentrations determined by adapting the method described previously.

Figure 18 Sporulation response of 72 hour cultures of *A. solani* to light and temperature.

A = Cultures were illuminated for 72 hours at 25°C and then subjected to a 12 hour sporulation period in light (□), and in dark (▨), at 18°C and 25°C.

B = Cultures grown in complete darkness for 72 hours at 25°C and then incubated in similar conditions as in A.

C = Cultures continuously grown and incubated in light (□) and in dark (▨).



The experimental design was a randomized complete block with 7 treatments replicated six times.

The result presented in Table 13 shows that *A. solani* grown on PDA produced significantly more spores ($P < 0.01$) than on any of the other media used. A similar result was achieved with cultures on Lima Bean Agar (LBA) compared with those on other media except PDA. On all the media, aerial hyphae were sparse, abundant conidiophores developed, and no conidia were formed during the 72-hours initial growth period.

D. Effect of temperature on sporulation of *A. solani*

That the fungus sporulates more readily at low than at high temperatures was investigated to determine the optimum temperature at which maximum sporulation can be achieved. The materials and methods used were similar to those described in Experiment - C, except that cultures were grown on PDA, and were induced to sporulate at temperatures ranging from 5 to 30°C. Spores were collected and concentrations determined by adapting the previously described method.

The experiment was repeated twice and both experiments were conducted following a randomized complete block design with 6-treatments replicated six times.



The result presented in Figure 19 shows that the cultures sporulated at temperatures between 5-20°C, sporulating at its maximum potential at 15°C. A significantly higher number of spores was produced ($P < 0.01$) at 15°C than at other temperature. Despite the presence of conidiophores during the 72 hour initial growth period, cultures

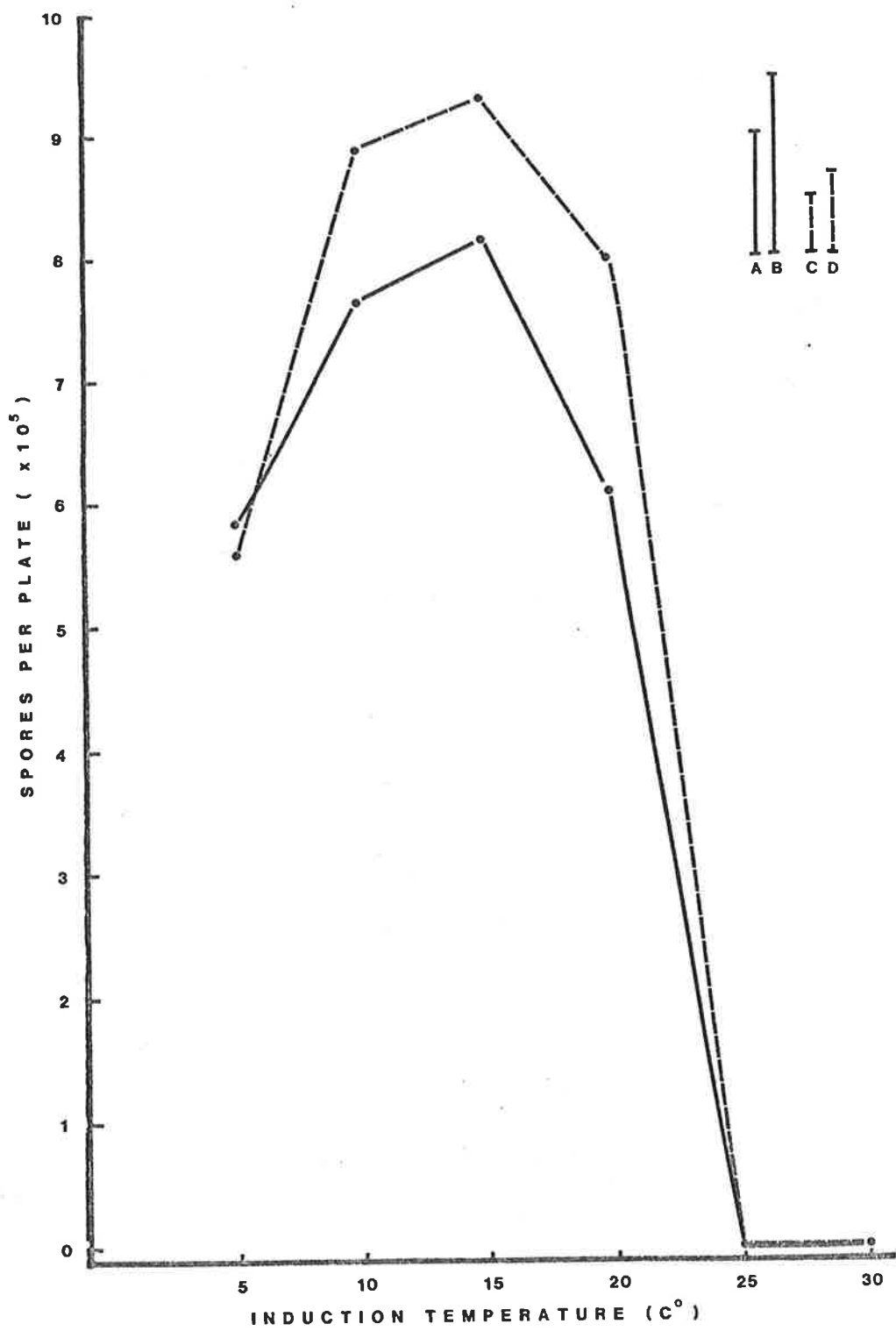
Table 13 : Influence of medium on sporulation of *A. solani*.

Media	Spores per plate ($\times 10^5$)
Potato Dextrose Agar	4.88
Lima Bean Agar	3.77
V8-juice Agar	1.97
Prune Agar	1.46
Malt Extract Agar	0.964
Corn Meal Agar	0.126
Yeast Extract Agar	0.125
L.S.D	
	P = 0.05
	P = 0.01

Coefficient of Variation = 10%

Figure 19 Sporulation response of 72 hour cultures of *A. solani* to temperature.

	=	First trial
	=	Second trial
A	=	L.S.D. (P = 0.05)
B	=	L.S.D. (P = 0.01)
C	=	L.S.D. (P = 0.05)
D	=	L.S.D. (P = 0.01)



cultures that were induced to sporulate at 25°C and 30°C did not sporulate which indicated that at temperatures higher than 20°C, sporulation by *A. solani* is inhibited.

E. Relationship between length of growth period and sporulation

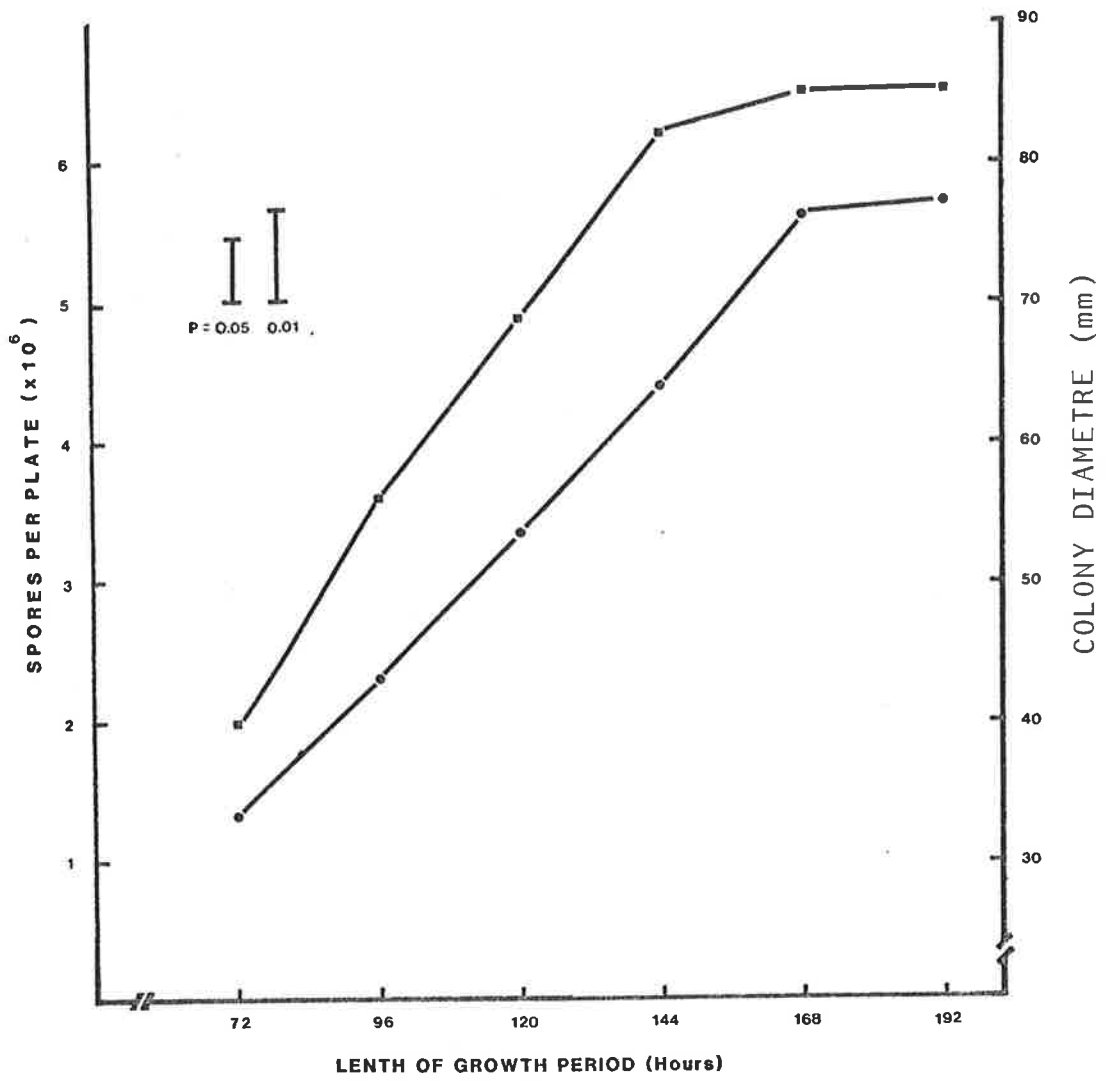
Petri dishes containing 20 ml of PDA were seeded with a 5 mm diameter agar plug taken from the edge of an actively growing 4 day old single-spore culture. The inoculated plates were placed under fluorescent lamps in a constant 25°C temperature room. Seventy two hours after inoculation, and every 24 hours thereafter, six culture-plates were transferred and left overnight (12-14 hours) in a growth cabinet, (without light) which was set at 15°C, to induce sporulation.

Spores were counted by adapting the previously described method except that plates were flooded with 1% triton X-100 (Sigma chemical Co.) to prevent the conidia from sticking to either the agar surface or the petri dish walls during collection. The experimental design was a randomized complete block with six treatments and five replications.

Figure 20 shows that the cultures progressively produced more spores as the length of growth period was increased from 72 to 192 hours. Optimum sporulation was achieved with cultures grown for 168 hours (7 days), and a growth period longer than these did not significantly ($P > 0.05$) improve sporulation. Growth periods longer than 9 days reduced sporulation levels. The differences between numbers of spores produced between periods from 72 to 168 hours were highly significant ($P < 0.01$).

Figure 20 Relationship between length of growth period and sporulation of *A. solani*.

●————● = Number of spores
■————■ = Mycelial growth



F. Sporulation of *A. solani* on PDA-plates arranged in layers

As culture plates in an inverted position during the initial growth period were observed to contain fewer conidia than those which were not inverted, sporulation in culture plates stacked to a height of four plates was investigated. Seeded PDA-plates were arranged in four layers and kept in a growth room with constant light and temperature (25°C) for four days. After the growth period, the culture plates were transferred and left overnight in a sporulating cabinet described previously. Spore counts were recorded 14 hours after incubation.

Table 14 shows that sporulation in the middle and bottom plates were 95 to 99% less than that in the top plate. Differences in the number of conidia produced in each layer of culture plate were statistically significant ($P < 0.01$) except between the two bottom plates. Aerial hyphae were densely produced in the middle-lower, and bottom plates, whereas these were sparse and practically absent in the middle-upper and top plates, respectively.

G. Tolerance of *A. solani* to fungicides

A cellophane-transfer bioassay described by Neely (1978) was adapted to test the effect of 17 kinds of fungicides on spore germination and growth of the fungus in vitro.

Fungicide solutions were prepared with de-ionized water and concentrations of 500 ppm, and 20 ppm were used to test fungicidal, and fungistatic properties of the chemicals, respectively. About 20-24 hours after the cellophane disks were seeded, the fungistatic properties of

Table 14 : Sporulation of *A. solani* in PDA plates arranged in four layers during the growth period.

Position of Plate	Conidia per plate ($\times 10^5$)
Top	44.01
Middle-upper	2.13
Middle-lower	0.58
Bottom	0.27
L.S.D.	
	P = 0.05
	P = 0.01

C.V. = 3.2%

Arathi

the chemicals, respectively. About 20-24 hours after the cellophane disks were seeded, the fungistatic property of each chemical was determined by recording the percentage of spores that germinated. When 99% or more of the spores had not germinated, or had germ tubes that were less than half the spore length, the chemical was considered fungistatic. The fungicidal property of each chemical was determined four days after seeding. If fungal growth was observed on the disk which were placed on PDA-plate, the chemical was considered non-fungicidal.

Among the fungicides tested, captafol, chlorothalonil, sprodione, and orthocide were fungistatic, whereas Mancozeb, Benomyl, Procymidone, Ferraminosulf, and Vinclozolin were fungicidal (Table 15). The rest of the fungicides did not affect spore germination or mycelial growth of *A. solani*.

Table 15 : Effect of some fungicides on *A. solani* in vitro.

Fungicide	Inhibition of Spore Germination (%)	Mycelial growth ¹
Captafol (Difolatan)	99.0	-
Chlorothalonil (Bravo)	99.1	-
Iprodione (Rovral)	99.0	-
Orthocide (Captan)	99.0	-
Mancozeb (Dithane M-45)	97.8	-
Benomyl (Benlate)	97.2	-
Procymidone (Sumisclex)	96.0	-
Ferraminosulf (Dexon)	94.1	-
Cabozoline (Serinal)	88.1	-
Fosetyl + Mancozeb (Mikal)	66.33	+
Fosetyl (Alliette)	12.5	+
Vinclozolin (Ronilan)	9.0	+
Metalaxyl (Ridomil)	7.2	+
Etridiazole (Terrazole)	7.1	+
TCMTB	5.45	+
Quintozene (Terroclor)	3.05	+
S.N. 78.314	2.2	+
Control	0	+

¹ - = No growth on PDA after 4 days.

+ = The fungus grew on PDA after 4 days.

III. Discussion and conclusion

The strain of *Alternaria solani* used in this study appears to grow most rapidly on Potato-dextrose-agar but sporulates sparingly unless given special treatment. An initial growth period at 25°C with supplementary light, followed by a spore-induction period of darkness at low temperature (15-18°C) appear to be sufficient treatments to induce PDA-cultures of the fungus to sporulate profusely. However, culture plates must neither be in an inverted position nor arranged in layers during the growth period as sporulation will be drastically reduced. Nevertheless, conidia which do not vary in age by more than the length of spore-induction period can be obtained with such a method. The age of conidia (inoculum) may be an essential factor to be considered when screening fungicides, or evaluating potato varieties under controlled conditions.

As with other fungi, sporulation in *A. solani* depends on light and temperature which can be separated into inhibitory and stimulatory processes by manipulating such factors. Sporulation of some dematiaceous fungi, particularly the large-spored forms, is inhibited by light at high temperatures (Barnett and Lilly, 1950 ; Houston and Oswald, 1946 ; Leach, 1961 ; Reid, 1958 ; Witsch and Wagner, 1955). Among the genera with species exhibiting this phenomenon are *Alternaria*, *Stemphyllium*, *Helminthosporium* and *Heterosporium*. The action of light on inhibiting conidial^{iv m} formation in *A. solani* is similar to that in *A. tomato* (Agaraki, 1964).

The photo-inhibition of sporulation in *A. solani* was investigated by Lukens (1963). He has shown the positive effect of riboflavin-5-

phosphate mononucleotide (FMN) in overcoming the inhibitory effect of light, and suggested that a flavin acts in 2-ways : it is a receptor through which light inhibits sporulation, and it is required also for conidial^{um} formation which is rendered ineffective by light. However, he later (1966) reported that conidiophores can bear spores in light when the temperature is below 23°C, and suggested that only the biochemical pathway of sporulation which predominates above 23°C is sensitive to light. An action spectrum for photo-induced conidium formation in *A. solani* was investigated by Honda and Yuroki (1981). They found that the most effective wavelength in inducing conidium formation is 230 nm, wavelength greater than 356 nm being ineffective.

Some of the fungicides tested, particularly captafol and chlorothalonil, appear to be effective against *A. solani*. Although the chemicals have not been tested in the field, observations made on commercial potato farms reveal that chlorothalonil is effective in controlling early blight caused by *A. solani*. In the literature, Lahman et al. (1982) showed that Difolatan 4F (captafol), Difolatan 4F + DMSO (dimethyl sulfoxide) reduced populations of viable *A. solani* spores on the soil surface when applied as a pre-harvest chemical to control tuber infection. Moreover, Platt (1983) studied the effects of metalaxyl, mancozeb and chlorothalonil on early, and late blight (*Phytophthora infestans*) and he found that the incidence of early blight was reduced on plants treated with chlorothalonil or mancozeb but not with metalaxyl. Although chemicals have been shown to be effective against the fungus (Goss, 1923 ; Potter and Hooker, 1960 ; Ohms and Fenwick, 1961 ; Harrison et al., 1965 Douglas and Groskopp, 1974), the use of resistant or tolerant potato varieties is likely to be more economical especially in places where pesticides are costly.

CHAPTER 5

STUDIES ON *PSEUDOMONAS SOLANACEARUM*

I. Introduction

Bacterial wilt caused by *Pseudomonas solanacearum* (Smith, 1896) Smith 1914 is one of the most important and lethal diseases affecting a number of economic crops such as potato, tobacco, tomato, and bananas. The host range of the bacterium is exceptionally wide including more than 30 families ^{including} such as Solanaceae, Musaceae, Asteraceae and Fabaceae (Kelman, 1953). The bacterium is widespread in tropical, subtropical, and warm-temperate parts of Asia, Africa, Australasia, Europe, North America, Central America, and the West Indies (CMI Map 138 Ed. 2), and the disease limits growing of potatoes in Southeast Asia particularly at lower, warmer elevations.

In potatoes, symptoms of bacterial wilt such as wilting, stunting, and yellowing of the foliage may appear at any stage in the growth of the plant. All leaves of infected plants may wilt quickly without much change in colour when disease development is rapid. On cut tubers, white droplets of bacterial slime ooze out of the infected vascular ring when pressure is applied. The eyes, often at the bud or apical end, become grayish brown, and a sticky exudate may form on them or at the stolon connection.

Three races of *P. solanacearum* have been proposed on the basis of pathogenic specialization and certain cultural properties : Race 1 affects tobacco, tomato, many solanaceous and other weeds and certain

diploid banana ; Race 2 causes bacterial wilt of triploid bananas (Moko disease) and *Heliconia* ; Race 3 affects potato and tomato, but is not highly virulent on other solanaceous crops (Buddenhagen and Kelman, 1964). On the otherhand, Hayward (1964) classified the bacterium into 4 biovars according to its capacity to oxidize 3 disaccharides (lactose, maltose and cellobiose), and 3 hexose alcohols (mannitol, sorbitol and dulcitol) : Biovar 1 strains oxidize neither group of carbohydrates ; Biovar 2 strains oxidize the disaccharides but not the hexose-alcohols ; Biovar 3 strains oxidize both groups of carbohydrates, Biovar 4 strains oxidize the hexose alcohols but not the disaccharides. Such systems of classification are not easily correlated except for the fact that Race 3, the potato strain, is equivalent to Biovar 2.

In naturally infested soils, *P. solanacearum* can survive on : infected host-plant debris (Lloyd, 1978) alternative wild hosts (French, 1983), in the rhizosphere of some weeds and economic crops (Quimio and Chan, 1978), and in the roots of presumed non-host crops (Granada and Sequeira, 1981). Moreover, the bacterium can survive for a long time in deep soil layers (McCarter et al., 1969). The effect of periodic dry soil on bacterial viability appears to be a major factor in the absence of wilt or its failure to increase in hot, dry areas even after they are introduced. In general, the effects of differing soil factors on the survival of *P. solanacearum* in soil have not been studied by direct techniques probably due to lack of a suitable selective media. For instance, Nesmith and Jenkins (1979) found that most of the media developed to detect *P. solanacearum* in soil were unsatisfactory because they allow growth of too many background bacteria, or they are appropriate only for certain strains of the bacterium.

Recently, some selective media have been developed to detect *P. solanacearum* in soil. Nesmith and Jenkins (1979) developed a selective medium for monitoring the population of the bacterium from naturally and artificially infested soil. They used a basal medium derived from a modification of the standard triphenyl tetrazolium chloride medium (TZC) and a final medium with anti-microbial compounds. A bacteriocin technique was also developed by Chen and Echandi (1982) to detect, isolate, identify and quantify *P. solanacearum* in soils. They also used TZC with chloramphenicol and pentachloronitrobenzene as basal medium, and used a wide spectrum bacteriocin-sensitive strain of the bacterium as an indicator to distinguish *P. solanacearum* colonies from other soil bacteria on the basal medium. More recently, Granada and Sequeira (1984) developed a modified TZC medium, with good plating efficiency and high selectivity.

This chapter deals with an ecological study of *P. solanacearum* with emphasis on the survival of biotypes 2 and 3 in artificially inoculated soil. The interaction between soil moisture and temperature, and the effect of organic matter amendment on the persistence of the bacterium in soil were investigated to provide some basis for an understanding of factors accelerating the destruction of soil borne inoculum and their application to control. Moreover, the survival of *P. solanacearum* in soil planted with non-host crops was investigated to provide the basis for controlling the disease by crop rotation. The influence of *Meloidogyne javanica*, and avirulent bacteriocin-producing strains of *P. solanacearum* on bacterial wilt of potatoes were also investigated.

II. Experiments and Results

A. Selection of bacteriocin-producing, and bacteriocin-sensitive strains of *P. solanacearum*.

As the selective medium developed by Chen and Echandi (1982) requires a bacteriocin-sensitive strain of the bacterium, the following Australian isolates of *P. solanacearum* were tested :

Strain	Biotype	Host	Origin	Source of Culture
C605	2	Potato	S. Australia	
015B	2	Potato	Queensland	Hayward, A.C.
025	2	Potato	Queensland	Hayward, A.C.
0127	2	Potato	Queensland	Hayward, A.C.
0223	2	Potato	W. Australia	Hayward, A.C.
0966	2	Potato	New South Wales	Lloyd, A.B.
023A	2	Potato	Queensland	Lloyd, A.B.
006	3	<i>Xanthium pungens</i>	Queensland	Hayward, A.C.
010	3	Tomato	Queensland	Hayward, A.C.
012B	3	<i>Rapistrum rugosom</i>	Queensland	Hayward, A.C.
0131	3	Potato	Queensland	Hayward, A.C.
0170	3	Tobacco	Queensland	Hayward, A.C.
0171	3	<i>Solanum melongena</i>	Queensland	Hayward, A.C.
0647B	3	Tomato	Queensland	Lloyd, A.B.
QT1	3	Tomato	Queensland	Lloyd, A.B.
QT2	3	Tomato	Queensland	Lloyd, A.B.
QT3	3	Tomato	Queensland	Lloyd, A.B.
003	4	<i>Zingiber officinale</i>	Queensland	Hayward, A.C.
009	4	<i>Zingiber officinale</i>	Queensland	Hayward, A.C.
0280B	4	Tomato	Darwin, N.T.	Hayward, A.C.

Each strain was tested as a bacteriocin indicator (sensitive) against each strain as a bacteriocin producer. Petri plates containing Kelman's tetrazolium chloride agar medium (TZC) were spot-seeded with different test strains and incubated for 40-hours at 30°C. After incubation, the plates were inverted, exposed to the vapor of 3-4 ml. of chloroform for 1-hour, and allowed to stand with the lids off for another hour. A 0.2

ml sample of the indicator strain (10^7 cells/ml) was added to 4 ml of 0.7% melted water agar (45°C) and poured over the seeded agar-plate. Plates were incubated for an additional 24-hours at 30°C. A strain sensitive to bacteriocin from the greatest number of strains was chosen and used in subsequent experiments.

Of the 20 isolates of *P. solanacearum* tested, 12 and 9 were bacteriocin-producer and bacteriocin-sensitive, respectively (Table 16). Bacteriocinogenicity in this test was indicated by the formation of inhibition zones around bacterial colonies (Figure 21). The biovar 2 strains, except 023A, and biovar 4 strains were all bacteriocin-sensitive, whereas all the biovar 3 strains were bacteriocin-producers. Strain 023A (biovar 2) produced a bacteriocin against other strains belonging to the same biovar, whereas most of the isolates were sensitive to bacteriocin produced by strain 0280B, a biovar 4 isolate from the Northern Territory of Australia.

B. Evaluation of selective media for isolation and quantification of *P. solanacearum* in soil.

The selective (semi-selective) media developed by Nesmith and Jenkins (1979), Chen and Echandi (1982), and by Granada and Sequeira (1983) were evaluated. The composition of each medium is indicated in Appendix 1. The South Australian isolate (C605) was used as the bacteriocin indicator complete Chen and Echandi's medium, and strain-170 was used to inoculate the soil.

Table 16 : Sensitivity of *P. solanacearum* strains to strains of the same bacterium.

Biovar	Bacteriocin Producer Strain	No. of Bacteriocin sensitive strains		
		Biovar 2 (7) ²	Biovar 3 (10)	Biovar 4 (3)
2	023A	6	0	3
3	006	6	0	3
	010	6	0	3
	012B	6	0	3
	0131	5	0	3
	0170	6	0	2
	0171	5	0	3
	0647B	5	0	3
	QT1	5	0	3
	QT2	5	0	3
	QT3	6	0	3
4	C280B	1	10	2

² Numbers in parentheses indicate the number of strains in each biotype tested for bacteriocin sensitivity.

Figure 21 Bacteriocin reaction of *P. solanacearum* isolates to an indicator strain (of *P. solanacearum*) layered over the colonies in water agar. Colonies were initially grown 40-hours on TZC-medium and killed with chloroform before the indicator strain was introduced. The inhibition zones indicate the presence of bacteriocin to which the indicator strain is sensitive.



B.1 Per cent reduction of soil bacteria

A red-brown Urrbrae loam soil (Appendix 9, Figure 22) from the Waite Institute experiment field was used to evaluate the effectiveness of the media in reducing the numbers of background bacteria. A 10 g sample of soil was placed in a flask, and distilled water was added to the 100 ml mark and shaken for 30 min, then aliquotes of 0.1 ml from 10-fold dilutions were spread on plates containing the medium being tested. Dilutions were also spread on Casamino-acids-peptone-glucose agar (CPG) medium to determine the total number of bacteria in the soil. To calculate the per cent reduction of soil bacteria, the equation formulated by Cuppels and Kelman (1974) was adopted ;

$$\text{Per cent Reduction} = \frac{\text{No. of bacteria recovered with test medium}}{\text{Total no. of bacteria on CPG-medium}}$$

As indicated in Table 17 the total soil bacteria was significantly reduced ($P < 0.01$) to about 50% on TTC medium, and to over 96% on the selective media. Bacterial and fungal growth were completely inhibited on the medium developed by Granada and Sequeira (GS), whereas a few fungal and bacterial colonies grew on either the Nesmith and Jenkins (NJ) or the Chen and Echandi's (CE) medium. Since both the GS and CE-media were simpler to prepare than NJ, they were evaluated further.

B.2 Recovery of *P. solanacearum* from an artificially infested soil.

An Urrbrae soil described previously was used in the experiment. To compare the efficiency of the GS with the CE-medium in recovering *P. solanacearum* from soil, 1 ml of a bacterial suspension ($10^4 - 10^5$ cfu/ml) was added to a gram of unsterilized soil (oven dry weight) and

Figure 22 Moisture characteristics curve of experimental (Urrbrae) soil.

Samples of soil passed through a 2 mm sieve were moistened to -0.98 KPa, placed on moist filter paper inside sintered glass funnels and suctions of -0.98 to -19.6 KPa (10 to 200 cm of water) were applied for two weeks before estimation of soil moisture contents. Suctions of -100 , -500 , and -1500 KPa were obtained using pressure chambers. Plastic conduits with the moist soils were placed on top of a high pressure ceramic plate in the chamber and pressure applied from a compressed air cylinder for two weeks.

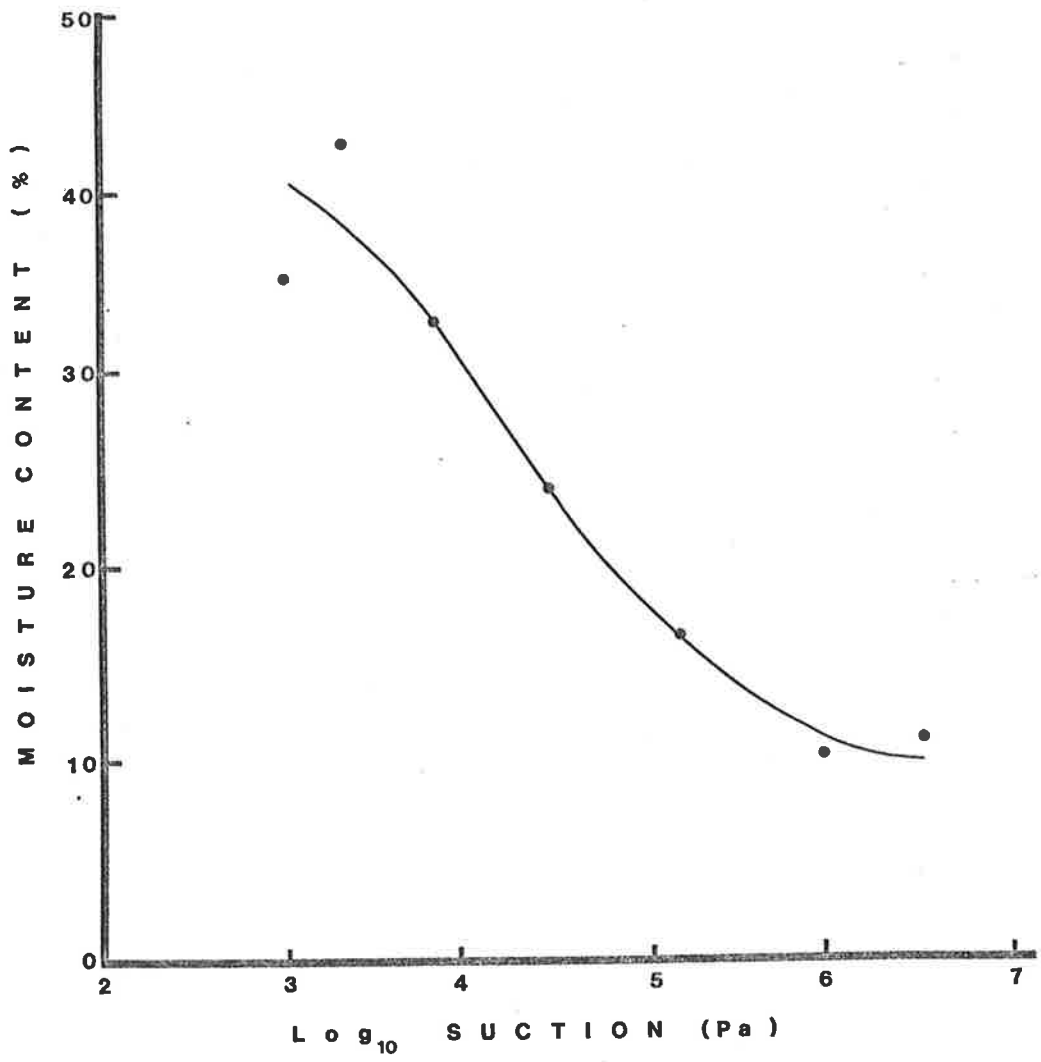


Table 17 : Populations of soil bacteria on non-selective and selective media.

Media ¹	Total Bacteria Per Gram Dry Soil	Reduction of soil bacteria (%)
CPG	2.75×10^6	-
TZC	1.3×10^6	52.72 a ¹
NJ	2.7×10^4	99.0 b
CE	8.9×10^4	96.8 c
GS	0	100.0 d

Means followed by different letters are significantly different ($P = 0.01$) after being transformed ($X + 1/2$).

¹ Casamino-acids-peptone-glucose agar (CPG), Triphenyl tetrazolium chloride agar (TZC), Nesmith and Jenkins (NJ), Chen and Echandi (CE), Granada and Sequeira (GS).

later, 0.1 ml samples of the 10^{-2} and 10^{-3} dilutions were spread, with a bent pasteur pipette, on each plate containing the medium being evaluated. Aliquots (0.1 ml) from the inoculum suspension were spread on TZC-medium so that subsequent comparison of plating efficiency was possible. Plates were incubated at 30°C , and were examined after the required incubation period for each selective medium was completed.

As illustrated in Figure 23-A, the medium developed by Chen and Echandi recovered 100% *P. solanacearum* from the artificially infested soil, and only 1.5% of other soil bacteria. Neither bacteria nor fungi grew on the GS-medium even after plates were re-inoculated with pure cultures of *P. solanacearum* (Figure 23B). However, when the medium was modified by not including the antibiotic, thiomersal, *P. solanacearum* was recovered from the soil diluted 10^{-2} (Figure 23-C). At a soil dilution of 10^{-2} however, 1.8% of other soil bacteria grew on the modified GS-medium recovered. Colonies of the pathogen on the modified medium were milky white 2-3 days after plating and colony type was similar to that observed on TZC-medium 4-5 days later (Figure 23-D).

C. The effect of adding organic amendments to soil on persistence of *P. solanacearum*.

As Tanaka (1978) found that addition of manure to infested subsoil reduced considerably the population of *P. solanacearum* and wilt severity, the effect of adding fowl manure to soil artificially infested with *P. solanacearum* on the survival of the bacterium was investigated.

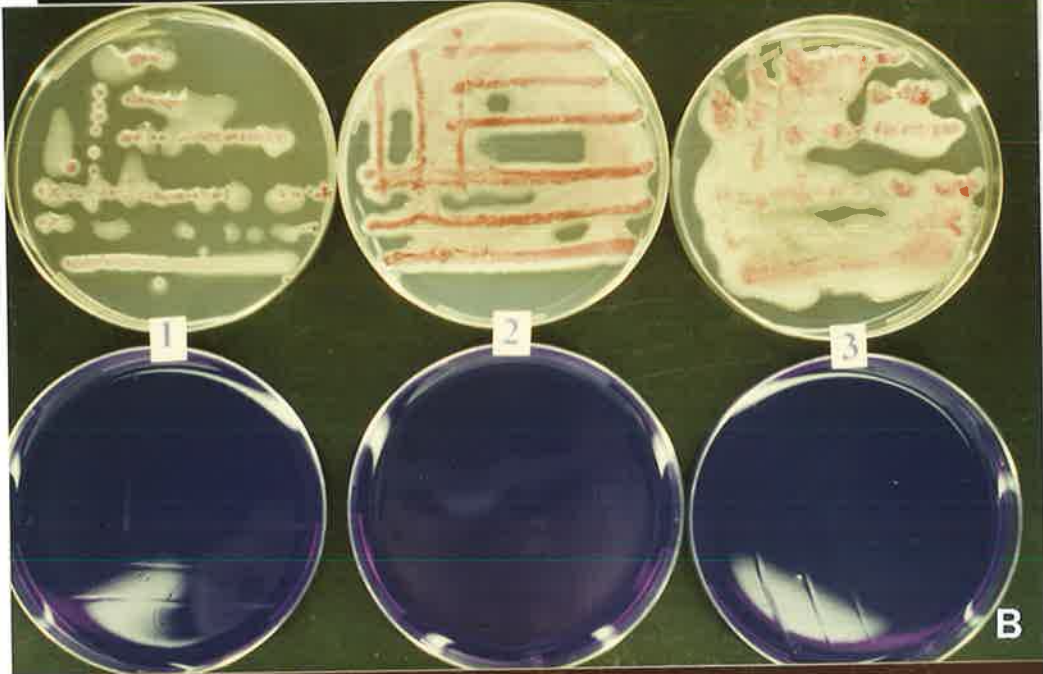
The soil type described in the previous experiments was used. Soil samples were divided into two lots, labelled as soil-A and soil-B. A

Figure 23 Comparative efficiency of selective media for recovery of *P. solanacearum* from artificially infested soil.

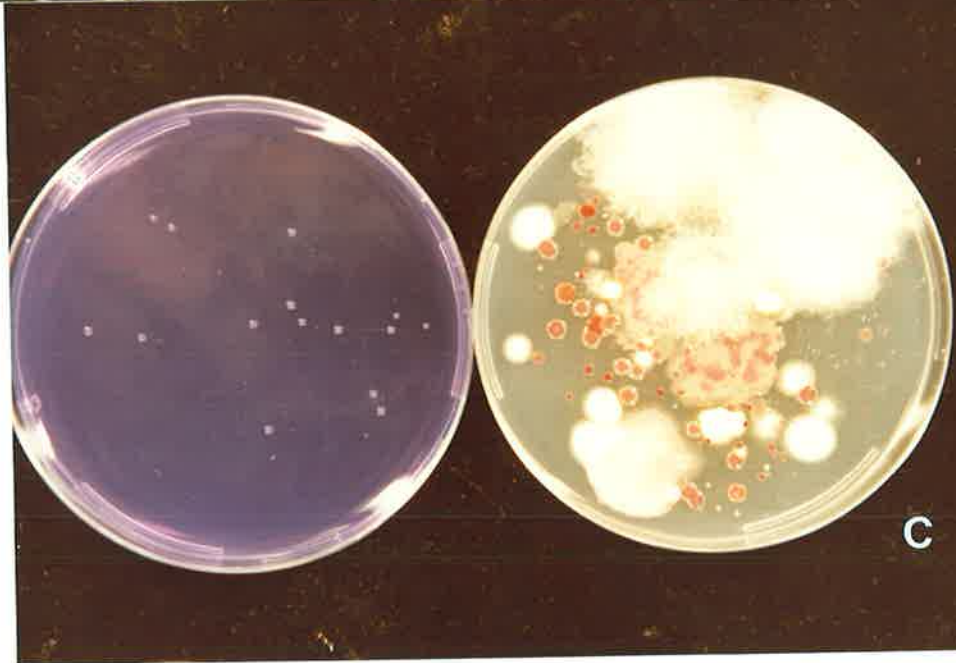
- (A) Plate (right) shows clear inhibition zones around *P. solanacearum* colonies, and left plate shows a colony of other soil bacteria with no inhibition zone around it (arrow).
- (B) Top plates show growth of *P. solanacearum* on TZC-medium, bottom plates with no bacterial growth contains Granada and Sequeira's (GS) selective medium.
- (C) 3-day old colonies of *P. solanacearum* on modified GS-medium (left plate) and on TZC-medium (right plate).
- (D) 2-3 days old (top), and 4-5 days old (bottom) colonies of *P. solanacearum* on GS-medium (modified).



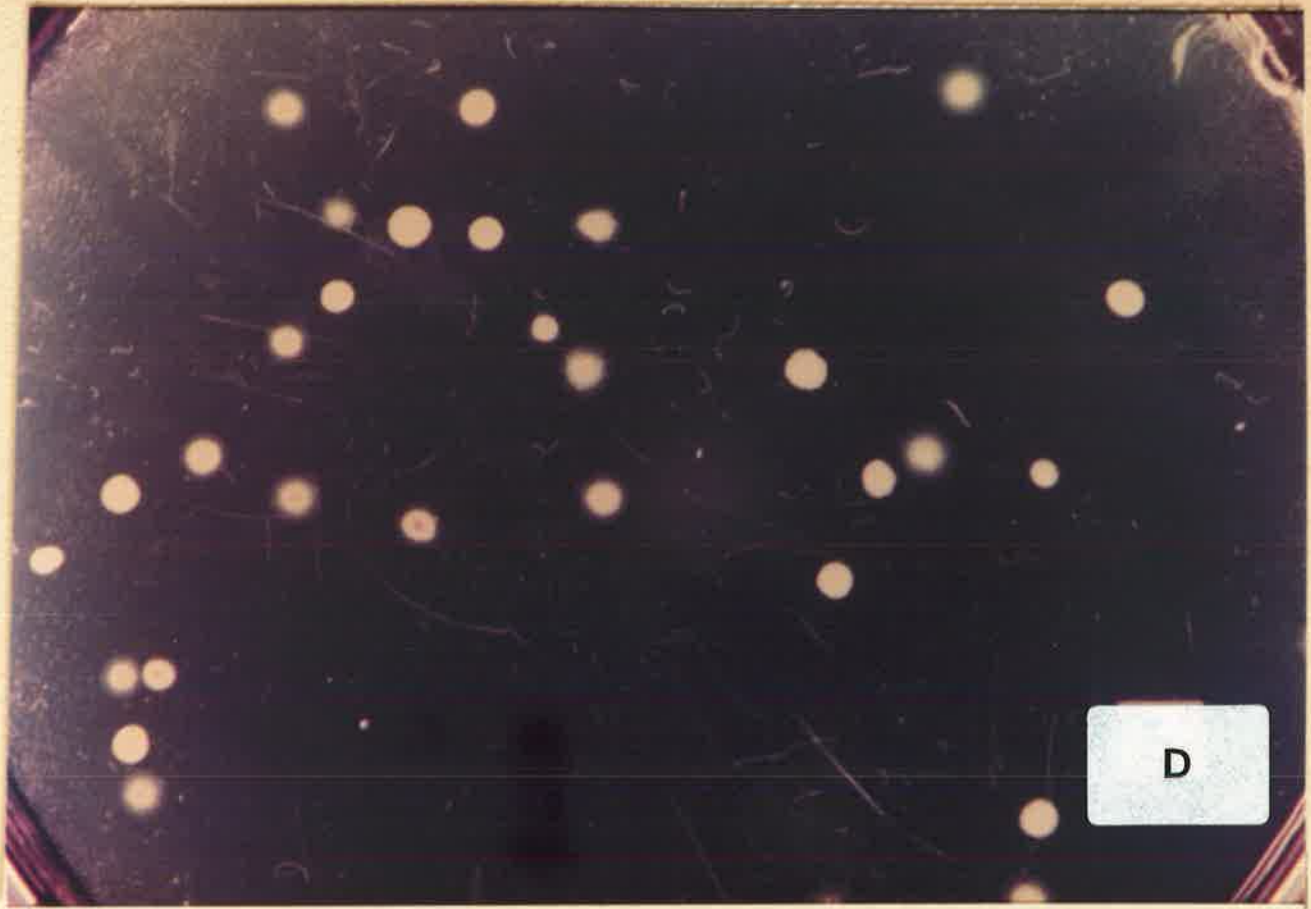
A



B



C



D



D

fully decomposed fowl manure was added to soil-A at a ratio of one-part manure to four-parts soils. Plastic containers (12 cm diameter x 6 cm deep) were filled with equal quantities of the soil preparations and infested with strain-170 at an inoculum density of 5×10^7 cells per g oven dry soil. After adjusting the moisture content of the soil to about 15%, they were kept in a growth cabinet at 30°C. The following day and every 14-days thereafter, the number of *P. solanacearum* and other soil bacteria per gram of soil (oven dry weight) were determined. Procedures for isolating the bacterium were similar to those described in Experiment-B2. The medium used was a modified GS-medium.

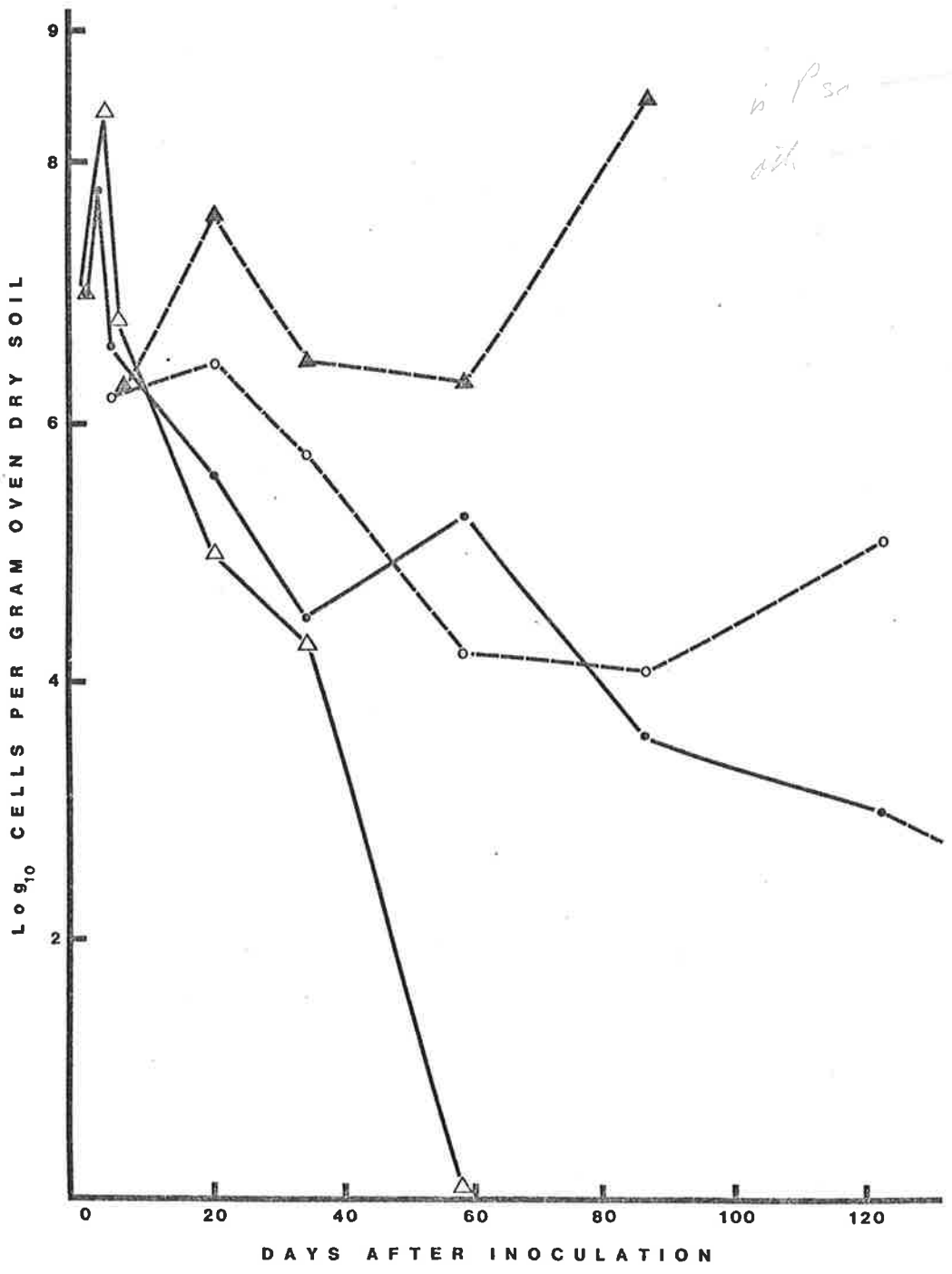
The result presented in Figure 24 shows that the initial population (P_0) of *P. solanacearum* in both soil-types increased a few days after inoculation and then progressively declined thereafter. At soil dilutions of 10^{-1} and 10^{-2} , *P. solanacearum* in the soil without manure was still detectable at 126 days from inoculation, and was undetectable beyond that period. Populations of the bacterium in soil amended with manure declined at a faster rate and the bacterium was not detectable 34-days after inoculation. The population of other soil bacteria was comparatively high particularly in the soil with manure. A bacterium and an actinomycete, which were dominant in and isolated from the manured soil, inhibited the growth of *P. solanacearum* when tested on CPG-medium by means of a modified bacteriocin technique; the organisms were spot seeded on CPG-plates and strain-0170 was used as an indicator. The antagonistic organisms were also present in the fowl manure but not in the soil without manure.

Figure 24 Population density trends of *P. solanacearum*, and of other soil bacteria (-----), in soil + manure (Δ) and in soil - manure (\bullet). Soil moisture content was about 15%. (Field capacity = 24%). The initial inoculum level (P_0) was Log 7.

cannot follow

what is 0 = Δ

is P₃₀
at



D. The influence of soil moisture and temperature on the persistence of *P. solanacearum*

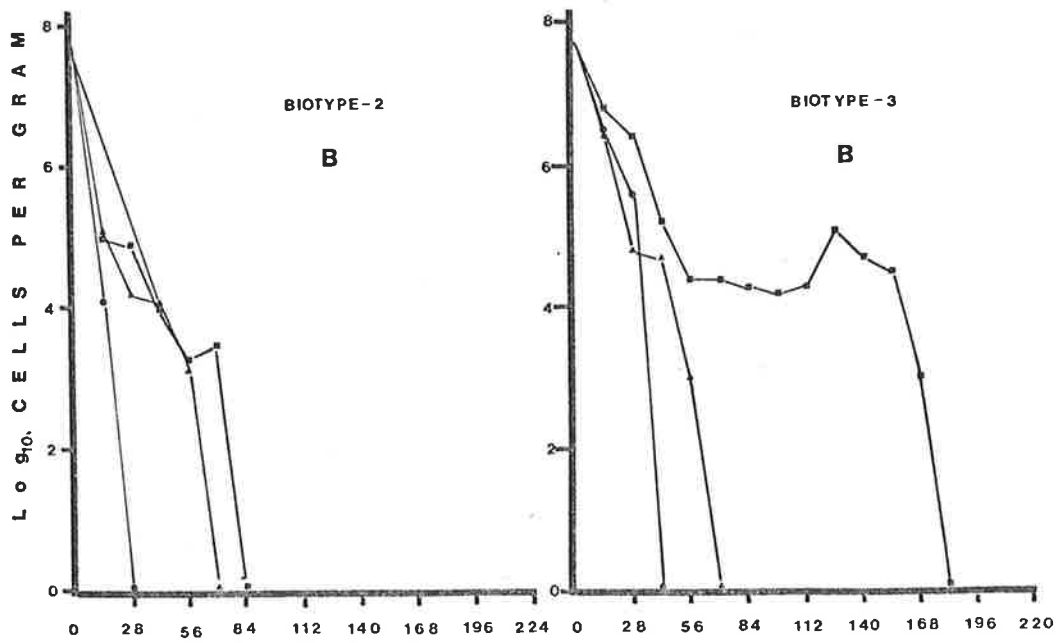
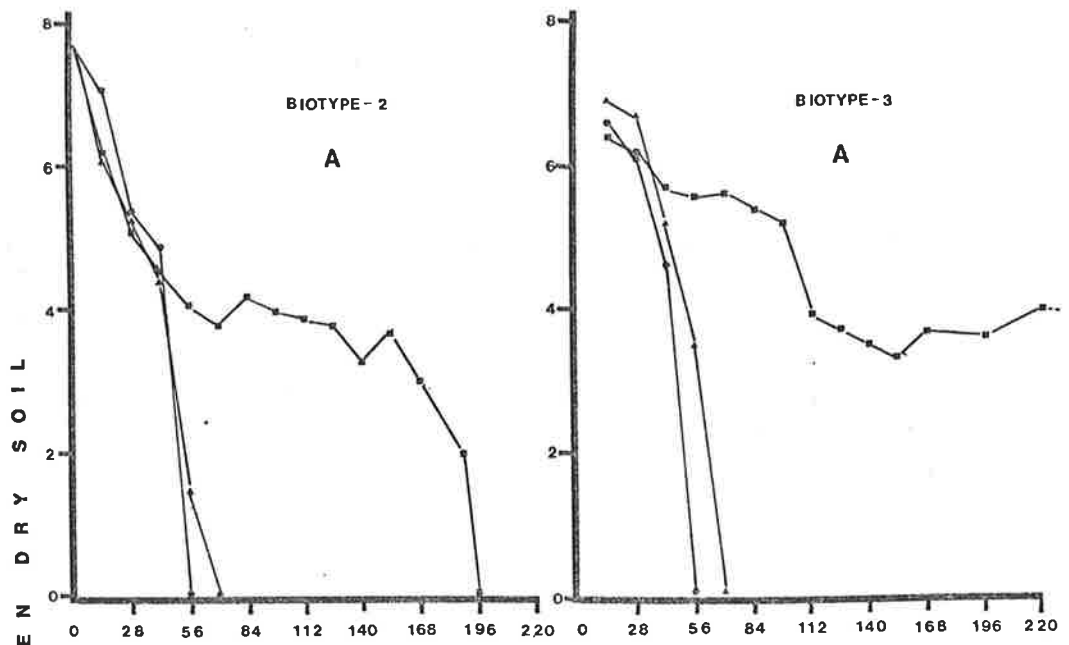
Changes in populations of *P. solanacearum*, biovar 2 and 3, with time were monitored in artificially infested Urrbrae soil. Strains 025 (biovar 2) and 170 (biovar 3), potato and tomato isolates respectively, were used to inoculate soils in plastic containers (12 cm diameter x 12 cm deep) at an inoculum density of 10^7 cells per g oven-dry soil. Soil moisture (100% field capacity = 24% moisture content) was adjusted to 15-25, 40-50, and 90-100% field capacity and was maintained at approximately the same level by constantly weighing the soil and adding sterile distilled water when necessary. The inoculated soil samples were kept in growth cabinets set at $20^\circ\text{C} \pm 3$, and at $30^\circ\text{C} \pm 2$.

Bacterial populations in the soil were determined during the first, second, third and fourth day after inoculation, and every 14 days thereafter. The procedure of measuring bacterial numbers in soil and the selective media used were similar to those described in a previous experiment.

The experimental design was a randomized complete block with 12-treatment combinations (2 strains x 2 temperature x 3 moisture levels) and three replications.

Figure 25 shows that populations of *P. solanacearum* decreased sharply with increase in temperature, and with decrease in soil moisture. In general, the biovar 3 strain survived longer and its density declined at a lower rate than the biovar 2 strain. At 20°C and at field capacity, the biovar 2 strain survived for a period of about

Figure 25 Population density trends of *P. solanacearum* in soils at 20°C (A), 30°C (B), and at soil moisture of 25% (●), 50% (▲), and 100% (■) of field capacity. Soil moisture content at field capacity was 24%.



DAYS AFTER INOCULATION

24-weeks whereas the biovar 3 strain was still detectable in soil dilutions of 10^{-1} - 10^{-2} 28-weeks after inoculation (populations of the pathogen were not monitored after that date). With the same moisture level but at 30°C, the biovar 3 strain persisted in the soil 12-weeks longer than the other strain, which indicates that biovar 3 is adapted to a wider range of environmental conditions. The persistence of both pathogens in similar conditions was investigated further.

E. The role of presumed non-host crops on the survival of *P. solanacearum* in soil

The experiment was conducted to investigate the role of crops, generally used in crop rotation, in maintaining high populations of the pathogen; the implication of presumed non-host crops on survival of the pathogen was reported (Granada and Sequeira, 1981).

Seeds of corn (*Zea mays* L.), soybeans (*Vigna sinensis* L.); and stem cuttings of potato (*Solanum tuberosum*) and sweet potato (*Ipomoea batatas* (L.) Lam.) were planted in vermiculite and when the seedlings/cuttings were almost ready for transplanting, a previously air dried Urrbrae loam soil was prepared. Plastic bags were each filled with four kilograms of soil and suspensions of a 48-hour culture of *P. solanacearum* were mixed with the soil. Strains 170, and 0235 were used at an inoculum density of 2×10^5 cells per g oven dry soil. With a gypsum block soil moisture meter (ENVIRONDATA, Queensland, Australia), the moisture tension of the soil was adjusted to 5-5.5 bars (moist soil condition) and was maintained during the experiment by regulating the amount of water added. Three seedlings/cuttings were transplanted into each pot with inoculated soil and grown in the glasshouse at 32°C. The

1
14/10/81

population of the pathogen was monitored every 28-days using a modified GS-medium described in previous experiments.

The population of *P. solanacearum* remained constantly high in soils planted with potatoes, whereas the bacterium was not detected in soil without plants at 56 days after inoculation (Table 18). Eighty four days after soil inoculation/planting, the pathogen was detected in soil with sweet potato, potato, or soybean, and was not detected in the other treatments by the modified selective-medium. The biovar 3 strain persisted longer than the biovar 2 strain.

F. Influence of *Meloidogyne javanica* on bacterial wilt of Exton potatoes

As nematodes, particularly *Meloidogyne* spp., have been shown to enhance bacterial wilt of potatoes (Jatala et al., 1976 ; Jatala and Martin, 1977 ; Tschanz, 1978 ; Mai et al., 1981), the influence of *Meloidogyne javanica* on bacterial wilt of potatoes was investigated. Strain C605 of *P. solanacearum* was used at an inoculum density of 10^2 , 10^4 , and 10^7 cfu per g oven dry soil. Each 3 kg soil was inoculated, mixed in a plastic bag, and placed in 15 cm diameter plastic pots. *Meloidogyne javanica* was extracted from the roots of tomato plants previously inoculated and grown in the glasshouse. Roots were cut into 1-2 cm pieces and placed in a funnel with stainless steel mesh. The funnels were then placed under the spray in a mist chamber, each draining into a test tube. Nematodes collected the following day were discarded, and those collected during subsequent days were used to infest the soil. Soil per pot was inoculated by pouring 20 ml suspension containing 650, 600, and 1,300 nematode larvae at planting, 3 days and 6

Table 18 : Population changes of *P. solanacearum* in soils with different plants.

Plant	Biovar ¹	Log ₁₀ cfu/g oven dry soil (days after planting)		
		28	56	84
None	2	2.954	-	-
	3	3.300	-	-
Potato	2	4.477	5.176	5.505
	3	5.176	5.204	5.431
Corn	2	3.000	4.300	-
	3	3.397	4.439	-
Sweet potato	2	3.778	-	3.00
	3	4.151	3.954	3.653
Soybean	2	3.00	-	-
	3	4.698	2.477	5.579

Biovar 2 = strain 025

Biovar 3 = strain 0170

- = *P. solanacearum* was not recovered using the modified GS-medium at 10⁻¹ dilution.

days after planting, respectively. The potato plants (12 replicates) were maintained in a glasshouse at 30°C, observed daily for the occurrence of wilt, and the percentage of leaves that wilted were recorded.

The result, presented in Table 19, shows that the nematode did not accelerate initial wilting at bacterial inoculum densities of 10^2 and 10^7 cfu/g oven dry soil. At 10^4 , the first plant to show wilting was observed in treatments with nematodes. The nematode apparently accelerated severity of wilting at this particular bacterial concentration. The severity of wilting was enhanced by the nematode.

G. Protection of potato plants from bacterial wilt with avirulent bacteriocin-producing strains of *P. solanacearum*

Bacteriocin-producing strains of *P. solanacearum* were selected from those in Experiment-A based on the diameter of inhibition zones on strain C605. Strains 674B, 131, QT, and 170 were cultured in glucose-proteose peptone-yeast extract broth at 25°C for 5 to 10 days, streaked on TZC plates and uncubated at 30°C for 48-hours (Kelman and Hruschka, 1973). The procedure was repeated until single colonies of avirulent bacteriocin-producing strains (ABPS) were isolated. The isolates were tested for pathogenicity by inoculating tomato plants (Var. Rutgers). If avirulent, the strains were retested in vitro against strain C605 to confirm bacteriocinogenic activity.

Seed-tubers were dipped for 1-hr in suspensions of ABPS at 10^9 cfu/ml, and planted in pots containing R.C. soil previously inoculated with Strain C605 at 5×10^7 cfu/g oven dry soil; ABPS-treated seeds

Table 19 : Average days when initial wilting was observed on plants grown in soil with and without *Meloidogyne javanica*.

INOCULUM DENSITY cfu/g soil of <i>P. solanacearum</i>	DAYS AFTER PLANTING	
	+ NEMATODE	- NEMATODE
10 ²	51.3 (36.3)	28.0 (11.2)
10 ⁴	29.6 (88.8)	37.4 (78.0)
10 ⁷	18.0 (88.9)	18.0 (55.6)

Values in parenthesis are the mean percentage of leaves that wilted. Data was not statistically analyzed because a number of plants at inoculum density of 10² did not wilt.

Not good enough
should not be done
in this

planted in R.C. soil without the pathogen served as controls. The treatments were replicated six times, each replicate consisting of 4 plants (4 plants per pot). The plants were maintained in a growth chamber at 25-27°C, and were observed daily for the appearance of wilt symptoms.

The result of the experiment shows that the ABPS did not protect the plants from bacterial wilt (Table 20). All the ABPS-treated seeds, planted in infested soil, produced plants that wilted 26 days after planting, whereas those in uninfested soil (-C605) remained healthy.

III. Discussion and conclusion

Twenty isolates of *Pseudomonas solanacearum*, representing a range of origin within Australia, and belonging to biovars 2, 3 and 4, were tested for bacteriocin production. The biovar 3 isolates seem to produce bacteriocins that selectively inhibit biovars 2 and 4 isolates. However, strain 023A, originally isolated and classified by Lloyd (University of New England, Australia) as biovar 2 was not affected; instead it showed inhibiting properties similar to those of a biovar 3 strain. Biochemical tests (Hayward, 1964) were carried out and results suggest that the strain should have been classified as type-3, because both dissacharides and hexose alcohols were oxidized. Thus, all the type-3 isolates are considered bacteriocin producers and inhibit either a biovar 2 or a biovar 4 strain. The result of the experiment seems to support the suggestion of Cuppels et al. (1978) that bacteriocins produced by *P. solanacearum* could be useful in the development of a bacteriocin-typing scheme for the same bacterium.

A selective medium appears to be indispensable when dealing with the ecology of *P. solanacearum* particularly the soil borne phase of the pathogen's life cycle. The three media evaluated are very effective for reducing contaminating soil bacteria and fungi but such media must be tested using tropical soils wherein organisms are usually abundant and diverse. Furthermore, prior testing of strains for susceptibility to the antibiotic components of such media is required as some strains may be susceptible. For instance, the selective medium developed by Granada and Sequeira (1983) had to be modified by eliminating thiomersal, an antibiotic component of the medium, because it inhibited the growth of all the strains used in this project. The modified medium can ^{be used to} recover the pathogen from the soil but ^{does not} totally eliminate other soil bacteria particularly in soil dilutions of 10^{-1} . Nevertheless, by incorporating a bacteriocin-sensitive strain of *P. solanacearum*, colonies of the pathogen, which form inhibition zones around them, can be distinguished from those of other soil bacteria. Though zones of inhibition also occur as a result of the action of soil antagonistic bacteria, such as those from the soil amended with manure, they can easily be distinguished. These techniques could probably be used to find out whether the non-virulent butyrous mutants, which usually develop in culture media, can persist in the soil and have the potential to revert to the virulent fluidal form especially when a host plant is present.

Knowledge of the ecology of *P. solanacearum* particularly the soil-borne phase of the pathogen's life history is lacking probably because adequate techniques have not been available to monitor the bacterium in the soil. However, experimental results indicate that techniques (selective media) recently developed can be used with good or excellent results. Two such media have been satisfactorily used to investigate the

influence of soil moisture and temperature, non-host plants, and organic matter amendment on the persistence of *P. solanacearum* in artificially infested soil. High soil moisture and low temperature, and presence of presumed non-host plants appear to favour long term survival of the bacterium in the soil, whereas organic matter (fowl manure) added to infested soil seems to have an eradicating effect on the pathogen.

Although the biovar 3 (Race 1) isolate survived longer and was more tolerant of desiccation than the biovar 2 (Race 3) isolate, the ability of both types to survive for 190 days, or longer in soil devoid of host-plant debris but with high moisture content and at low temperature suggests that *P. solanacearum* can persist in deep soil layers where those conditions are likely to occur. McCarter et al. (1969) investigated the vertical distribution of *P. solanacearum* in several soil layers, and found that a high infestation of the pathogen occurred in the top 30 cm, and a low infestation at deeper levels (60-75 cm). Moreover, he suggested that the absence of the bacterium in the top 15 cm of one soil was due to the dryness of the soil. The results obtained in this project seem to support his findings.

Granada and Sequeira (1981) found that corn, sorghum, bean, peas and soybean planted in soil infested with *P. solanacearum*, became infected but remained symptomless. Moreover, they have recovered the pathogen (Race-1) from the roots of such plants, whereas I unsuccessfully attempted to do the same from roots of corn, soybean, and sweet potato which were planted in pots containing soil with a Race-1. Nevertheless, my experimental results indicated that the pathogen can persist longer in the soil when those plants are present, and seems to support the findings of Jackson and Gonzales (1981) that such crops do

not reduce incidence of bacterial wilt when planted in rotation with potatoes.

The high microbial activity and presence of antagonistic organisms in the soil amended with fowl manure probably caused the fast decline of the pathogen's population. Suppression of plant pathogens with organic amendments have been attempted by a number of workers as indicated in sections of books and other publications such as those of Baker and Cook (1974), Papavizas (1974) and of Cook and Baker (1983). The effect of adding fowl manure to infested soil on the incidence of bacterial wilt is worth investigating. Furthermore, the addition of copious quantities of organic matter has been shown to increase the population of *Dactylla oviparasitica*, a nematode trapping fungus, and of *Bacillus penetrans*, a hyperparasite of nematodes (Corke and Rishbeth, 1981). Since the development of bacterial wilt on potatoes is enhanced by *Meloidogyne* spp. (Mai et al., 1981), the effect of organic matter amendments on incidence of bacterial wilt in fields where both pathogens are present is also worth investigating.

Interactions of *Meloidogyne incognita*, *M. incognita acrita* and *P. solanacearum* on potatoes were investigated by Jatala and Martin (1977). They found that these pathogens markedly interact, and that wilt-resistant cultivars can become susceptible when attacked by these nematodes. The result obtained in this project suggests that in soils infested with *P. solanacearum* and *Meloidogyne javanica*, wilt symptoms on potatoes are enhanced though initial wilting is not accelerated. However, further studies need to be done particularly on inoculum levels of both pathogen, potato cultivar, and temperature interactions. Moreover, the interactions among strain, potato cultivar (clone) and

Amount of
wilt.

environment are little studied, hence require further investigation. Such studies should prove useful especially in breeding for resistance to both pathogens.

From the results obtained in this project, it seems unlikely that avirulent bacteriocin-producing strains (ABPS) of *P. solanacearum* can be used to control bacterial wilt of potatoes. Because not one of the four ABPS tested delayed wilting, it is possible that the ABPS did not colonize, hence did not protect the growing roots of the potato plants. Thus, to be effective, ABPS must multiply profusely, and should be able to colonize the root system of potatoes. Moreover, the ABPS must attach to root infection sites and produce bacteriocins that will kill the pathogen. In the interim progress report of the International Potato Centre (1983), it was stated that under growth-room conditions, treatment with ABPS caused a significant reduction in disease severity, and the same treatment under glasshouse conditions delayed symptom expression. In this experiment, seed pieces were dipped in bacterial suspension, planted in pots, and one month later, the plants were challenged by pouring a bacterial suspension on the soil, thereby probably accounting for the delay of symptom expression. As indicated previously, our method involved dipping seed tubers in a suspension of ABPS and planting them in soils previously inoculated with the pathogen to simulate field condition. It is believed that many studies must be conducted before ABPS can be considered as biological control agents for bacterial wilt of potatoes.

CHAPTER 6

GENERAL DISCUSSION

Several aspects of potato disease research ^{has} been dealt with in this project. The survey and diagnostic work were considered an important first step because by studying the relationships between environmental factors and crop health, the major determinants of disease can be identified (Wallace, 1978). A number of experiments were also conducted principally to confirm reports from other countries, where the climate differ from that of South Australia, to point out which problems need to be investigated further, and to contribute to knowledge on potato diseases and their control.

The survey conducted in 1981-84 revealed that climatic conditions have a profound influence on the occurrence and severity of diseases on potatoes such as leafroll (PLRV), early blight (*A. solani*), and bacterial wilt (*P. solanacearum*). For instance, owing to the mild winters, aphids are abundant early in the growing season hence PLRV is widespread in South Australia. On the other hand, as most rapid progress of early blight occurs during periods of alternating wet and dry weather (Weingartner, 1981), the disease seems to be prevalent during the early and towards the end of the cropping season when rainfall generally occur. The dry weather and high, desiccating temperatures probably account for the absence of late blight (*P. infestans*), and the limited occurrence of *P. solanacearum* in South Australia. As a result of the survey, the importance of potato disease in potato production in South Australia has become known particularly to the growers, and a potato disease booklet has been published.

The results of experiments on potato leafroll virus indicate that the disease can spread quickly, is satisfactorily controlled with a combination of granular insecticides and insecticide sprays and can substantially reduce yield. As some granular insecticides are highly toxic, however, they could probably be used to control leafroll only in seed-potato crops. The discrepancies between the results obtained and those published elsewhere indicate the need to adapt measures applicable to certain conditions or areas. Moreover, since insecticide application would certainly affect the population of aphid-predators, other means of control, such as resistant cultivars or clones, must be considered. The assessments made on yield loss due to leafroll, and yield loss data given by other workers suggest that the amount of loss due to the disease can vary depending on cultivars, climatic condition, and strain of the virus. Thus, to develop economical control measures, whether by breeding resistant cultivars or using insecticides, disease-loss appraisal is believed to be an essential step.

In South Australia, losses due to potato leafroll virus could be avoided if growers used certified seed from Victoria each year and avoided the practice of saving seed from a previous year for planting in a current year.

The method used to induce sporulation of *A. solani* may be useful because it is comparatively simpler than most other techniques used so far. However, the method is probably suitable only for *A. solani* since it was not tested on other species of *Alternaria*. Nevertheless, the technique should prove useful for obtaining abundant spores with similar age which can be used to inoculate potato plants to be evaluated for early blight resistance. Fungicides, though effective, are sometimes

costly and uneconomical to use for the control of the disease. Hence, the use of tolerant cultivars should be considered as an alternative. Little is known about resistance to *A. solani* but CIP scientists believe that efforts to identify and utilize resistance will be necessary to control the disease in many countries where it is a problem (Thurston, 1980). It has been observed that the cultivar Tasman, bred in 1962 by the Tasmanian Department of Agriculture from a cross between (Duke of York x B Co/4) and (Crana x 11-79), selected by the Department of Agriculture, Victoria, is more tolerant to early blight than any of the other cultivars presently grown in Australia.

Bacterial wilt caused by *P. solanacearum* is probably the most destructive bacterial disease of potatoes. For instance, the most recent outbreak of the disease in South Australia, which was attributed to the use of contaminated seeds imported from other States, caused about 50% incidence in one form. The experimental results seem to support the belief that the bacterium cannot persist for so long in South Australian soil because of the dry weather and high desiccating temperatures that often prevail during the summers months. Because the pathogen can apparently persist in moist, cool soil devoid of host plant debris, the use of resistant potatoes seems to be the most practical method of controlling the disease. About 17 potato clones have been tested in a number of countries and found to be resistant (International Potato Center, 1983). It appears that more investigations on the ecology of the pathogen are required to provide information^s that are relevant to the breeding programme of developed countries, such as Australia, and to the developing countries in the tropics where the disease is a major problem.

Finally, it is hoped that the suggestions and questions raised during the conduct of this project will be considered for further study.

BIBLIOGRAPHY

- AGARAKI, M. (1964). Relation of radiation and temperature to the sporulation of Alternaria tomato and other fungi. *Phytopathology* 54 (5) : 565-569.
- BACON, O.G., BURTON, V.E., McLEAN, D.L., JAMES, R.H., RILEY, W.D., BAGHOTT, K.G. and KINSEY, M.G. (1976). Control of the green peach aphid and its effect on the incidence of potato leafroll virus. *Journal of Economic Entomology* 69 : 410-414.
- BAKER, K.F. and COOK, R.J. (1974). Biological control of Plant Pathogens. W.H. Freeman, San Francisco. Reprinted ed., 1982. Am. Phytopathology Soc., St. Paul, MN. 433 pp.
- BALD, J.G., NORRIS, D.D. and HELSON, G.A.H. (1946). Transmission of potato virus diseases. 5. Aphid populations, resistance, and tolerance of potato varieties to leafroll. *Bulletin of the Council of Scientific and Industrial Research, Melbourne*, No.196.
- BARKSDALE, T.H. (1968). A method of screening for resistance to early blight on tomato seedlings. *Phytopathology* 58 : 883 (Abstr.)
- BARNETT, H.L. and LILLY, V.G. (1950). Influence of nutritional and environmental factors upon asexual reproduction of Choanephora cucurbitarum in culture. *Phytopathology* 40 : 80-89.
- BEEMSTER, A.B.R. and ROZENDAAL, A. (1972). Potato viruses : properties and symptoms. In "Viruses of potatoes and seed-potato production", pp. 115-142. (Ed.) J.A. de Bokx). Wageningen : Center for Agricultural Publishing and Documentation.
- BLODGETT, F.M. (1941). A method for the determination of losses due to diseased or missing hills. *American Potato Journal* 18 (5) : 132-135.
- BRADLEY, R.H.E. (1963). Some ways in which a paraffin oil impedes aphid transmission of potato virus Y. *Canadian Journal of Microbiology* 9 : 369-380.
- BRADLEY, R.H.E., MOORE, C.E. and POND, D.D. (1966). Spread of potato virus Y curtailed by oil. *Nature*, 207 : 1370-1371.
- BROADBENT, L., BURT, P.E. and HEATHCOTE, G.D. (1956). The control of potato virus diseases by insecticides. *Ann. Appl. Biol.* 44 (2) : 256-273.
- BROADBENT, L., HEATHCOTE, G.D. and WRIGHT, R.C.M. (1962). Home-production of seed for early potatoes. 4. Loss of yield in virus-infected plants. *Experimental Horticulture*, 1 : 4-6.
- BUDDENHAGEN, J.W. and KELMAN, A. (1964). Biological and physiological aspects of bacterial wilt caused by Pseudomonas solanacearum. *Ann. Rev. Phytopathology* 2 : 204-230.

- BUDDENHAGEN, J.W., SEQUEIRA, L. and KELMAN, A. (1962). Diagnosis of races of Pseudomonas solanacearum. *Phytopathology* 52 : 726.
- BURT, P.E. Broadbent, L. and Heathcote, G.D. (1960). The use of soil insecticides to control potato aphids and virus diseases. *Ann. Appl. Biol.* 48 (3) : 580-590.
- CARTWRIGHT, D.N. (1984). Potato spindle tuber viroid survey - South Australia. *Australasian Plant Pathology* 13 (1) : 4-5.
- CHARLTON, K.M. (1953). The sporulation of Alternaria solani in culture. *Brit. Mycol. Soc. Trans.* 36 : 349-355.
- CHEN, W. and ECHANDI, E. (1982). Bacteriocin production and semi-selective medium for detection, isolation and quantification of Pseudomonas solanacearum in soil. *Phytopathology* 72 : 310-313.
- CHURCH, B.M. (1971). The place of sample survey in crop loss estimation. *Crop loss assessment methods : FAO manual on the evaluation and prevention of losses by pests, diseases, and weeds.* Rome : FAO. 2.2/1-12 (looseleaf).
- CLARK, M.F. and ADAMS, A.M. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34 : 475-483.
- COOK, R.J. and BAKER, K.F. (1983). The nature and practice of biological control of plant pathogens. The American Phytopathological Society. St. Paul, Minnesota. 539 pp.
- CORKE, A.T.K. and RISHBETH, J. (1981). Introduction of antagonists for biological control. In "The Nature and Practice of Biological Control of Plant Pathogens". pp. 283-311. (Eds. R.J. Cook, and K.F. Baker).
- CUPPELS, D.A., HANSON, R.S. and KELMAN, A. (1978). Isolation and characterization of a bacteriocin produced by Pseudomonas solanacearum. *J. Gen. Microbiology.* 109 : 295-303.
- DE WIJS, J.J. (1980). The characteristics of mineral oils in relation to their inhibitory activity on the aphid transmission of potato virus Y. *Netherland Journal of Plant Pathology* 86 : 291-300.
- DELGADO-SANCHEZ, S. and GROGAN, R.G. (1966). Purification and properties of potato virus Y. *Phytopathology* 56 (12) : 1397-1404.
- DOUGLAS, D.R. and GROSKOPP, M.D. (1974). Control of early blight in eastern and south central Idaho. *Am. Potato J.* 51 : 361-368.
- DUFFUS, J.E. (1972). Beet Western yellows virus. C.M.I. A.A.B. Descriptions of Plant Viruses No. 89. Kew, England : Commonwealth Mycological Institute, Association of Applied Biology.
- ELLIS, M.B. and GIBSON, J.A.S. (1975). Alternaria solani Sorauer. CMI Descriptions of Pathogenic fungi and Bacteria No. 475. KEW, Commonwealth Mycological Institute.

- FEDDERSEN, H. (1978). Phoma of potatoes. Fact Sheet 262/633. Department of Agric. and Fisheries, South Australia, 1 p.
- FRANCKI, R.J.B. (1980). Limited value of the thermal inactivation point, longevity in vitro and dilution endpoint as criteria for the characterization, identification and classification of plant viruses. *Intervirology* 13 : 91-98.
- FRANCKI, R.I.B. and McLEAN, G.D. (1968). Purification of potato virus X and preparation of infectious Ribonucleic acid by degradation with Lithium chloride. *Australian Journal of Biol. Sci.* 21 : 1311-1318.
- FRENCH, E.R. (1983). Ecological behaviour of Pseudomonas solanacearum. In "Abstracts of Papers, Fourth International Congress of Plant Pathology", p. 35. Melbourne, Australia : University of Melbourne.
- GOSS, R.W. (1923). Potato diseases in Nebraska. *Nebr. Agr. Exp. Sta. Bull.* 186.
- GRANADA, G.A. and SEQUEIRA, L. (1983). A new selective medium for Pseudomonas solanacearum. *Plant Disease* 67 : 1084-1088.
- HAYWARD, A.C. (1964). Characteristics of Pseudomonas solanacearum. *Journal of App. Bact.* 27 (2) : 265-277.
- HARPER, F.R., NELSON, G.A. and PITTMAN, U.J. (1975). Relationship between leafroll symptoms and yield in Netted Gem Potato. *Phytopathology* 65 : 1242-1244.
- HARRISON, M.D. and LIVINGSTON, C.H. (1965). Control of potato early blight in Colorado. I. Fungicidal spray schedules in relation to the epidemiology of the disease. *Am. Potato J.* 42 : 319-327.
- HEIN VON, A. (1971). A contribution to the influence of oil on the virus transmission by aphids. *Phytopath Z.*, 71 : 42-48 (in German with English Summary).
- HIDDEMA, J. (1972). Inspection and quality grading of seed potatoes. In "Viruses of potatoes and seed-potato production", pp. 206-214. (Ed. J.A. de Bokx). Wageningen, Centre for Agricultural Publishing and Documentation.
- HODGSON, W.A., POND, D.D. and MUNRO, J. (1974). Diseases and pests of potatoes. Canada Dept. of Agric. Publ. 1492 : 69 pp.
- HOLMES, I.R. and TEAKLE, D.S. (1980). Incidence of potato viruses S, X and Y in potatoes in Queensland. *Australasian Plant Pathology* 9 (3) : 3-4.
- HONDA, Y. and YUNOKI, T. (1981). An action spectrum for photoinduced conidium formation in Alternaria solani (Ellis and Martin) Sorauer. *Annals of the Phytopathological Soc. of Japan* 47 (3) : 327-334.
- HOOKE, W.J. (1980). Compendium of potato diseases. The American Phytopathological Society. 125 pp.

- HORVATH, J. (1967). Separation and determination of viruses pathogenic to potatoes with special regard to potato virus Y. *Acta Phytopathologica Academiae Scientiarum Hungaricae* 2 : 319-360.
- HOUSTON, B.R. and OSWALD, J.W. (1946). The effect of light and temperature on conidium production by Helminthosporium gramineum in culture. *Phytopathology* 36 : 1049-1055.
- INTERNATIONAL POTATO CENTER. (1983). Annual Report CIP. 1982. Lima, Peru. pp. 41-47.
- INTERNATIONAL POTATO CENTRE. (1983). Interim progress report -1983, Grant-in-Aid, University of Wisconsin, Madeson.
- JACKSON, M.T. and GONZALEZ, L.C. (1981). Persistence of Pseudomonas solanacearum (Race 1) in a naturally infested soil in Costa Rica. *Phytopathology* 71 : 690-693.
- JATALA, P., GUTARRA, L., FRENCH, E.R. and ARANGO, J. (1976). Interaction of Heterodera pallida and Pseudomonas solanacearum on potatoes. *Nematology* 8 : 289-290. (Abstr.)
- JATALA, P. and MARTIN, C. (1977). Interaction of Meloidogyne incognita acrita and Pseudomonas solanacearum on field grown potatoes. *Proc. APS* 4 : 177-178.
- KELMAN, A. (1953). The bacterial wilt caused by Pseudomonas solanacearum. *N.C. Agric. Exp. Stn. Tech. Bull.* 99 : 194 pp.
- KENNEDY, J.S. DAY, M.F. and EASTOP, V.F. (1962). A conspectus of aphids as vectors of plant viruses. London, Commonwealth Institute of Entomology.
- KIRKPATRICK, H.C. and BLODGETT, F.M. (1943). Yield losses caused by leafroll of potatoes. *American Potato Journal*, 20 : 53-56.
- KRIKUN, J. and ORION, D. (1979). Studies of the interaction of Verticillium dahliae and Pratylenchus thornei on potato. *Phytoparasitica* 7 (1) : 42.
- LAHMAN, L.K., (1982). Pre-harvest chemical application for control of tuber infection by Alternaria solani. *Am. Potato Journal* 59 (11) : 523-532.
- LEACH, C.M. (1961). The sporulation of Helminthosporium oryzae as affected by exposure to near ultraviolet radiation and dark periods. *Can. J. Botany* 39 : 705-715.
- LLOYD, A.B. (1978). Survival of the Potato strain of Pseudomonas solanacearum in soil. In "Proceedings of the 4th International Conference on Plant Pathogenic Bacteria", pp. 875-878. Angers: Institut National de la Recherche Agronomique.
- LOEBENSTEIN, G., ALPER, M. and DEUTSCH, M. (1964). Preventing aphid-spread cucumber mosaic virus with oil. *Phytopathology* 54 : 960-962.

- LOUGHNANE, J.B. (1941). The susceptibility to leafroll of certain potato varieties and its effect on this yield. *Journal of the Department of Agriculture, Republic of Ireland* 38 : 48-67.
- LUKENS, R.J. (1960). Conidial production from filter paper cultures of Helminthosporium vagans and Alternaria solani. *Phytopathology* 50 : 867-868.
- LUKENS, R.J. (1963). Photo-inhibition of sporulation in Alternaria solani. *American J. of Botany* 50 : 720-724.
- LUKENS, R.J. (1966). Interference of low temperature with the control of tomato early blight through use of nocturnal illumination. *Phytopathology* 56 : 1432-1433.
- MAI, W.F., BRODIE, B.B. and HARISSON, M.B. (1981). Nematodes. In "Compendium of potato diseases". (Ed. W.J. Hooker). pp. 93-101. The American Phytopathological Society.
- MARCO, S. (1980). The use of insecticides to control potato leafroll virus in seed-potato crops on the Golan Heights. *Phytoparasitica* 8 (1) : 61-71.
- MARCO, S. (1984). Effect of potato leafroll virus on autumn potatoes in Israel. *Potato Research* 27 : 15-162.
- McCALLAN, S.E.A. and CHAN, S.Y. (1944). Inducing sporulation of Alternaria solani in culture. *Contrib. Boyce Thompson ent.* 13 : 323-335.
- McCARTER, S.M., DUKES, P.D., JAWORSKI, C.A. (1969). Vertical distribution of Pseudomonas solanacearum in several soils. *Phytopathology* 59 : 1675-1677.
- McMILLAN, Jr. R.T. (1968). Yield from current season infected potato leafroll virus resistant and susceptible potato varieties. In "Proceedings of the Florida State Horticultural Society 81 : 192-194.
- MISKA, J.P. and NELSON, G.A. (1975). Potato seed-piece decay : a bibliography, 1930-1975. *Canadian Plant Disease Survey* 55 : 126-139.
- MORSCHER, J.R. (1975). Plant disease recorded in Australia and Overseas (Part 1), Canberra, Australia. Dept. of Health : pp. 57-60.
- MURPHY, P.A. (1922). Investigations on the leaf-roll and mosaic disease of the potato. *J. Dept. Agric. and Tech. Instr., Ireland*, xiii
- MURPHY, H.J., GOVEN, M.J. and MERIAM, D.C. (1966). Effect of three viruses on yield, specific gravity, and chip color of potatoes in Maine. *American Potato Journal* 43 : 393-396.

- NEELY, D. (1978). A cellophane-transfer bioassay to detect fungicides. In "Methods for Evaluating plant fungicides, nematocides, and bactericides, pp. 15-18 (Ed. E.I. Zehr et al). The American Phytopathological Soc.
- NESMITH, W.C. and JENKINS, E.F. (1979). A selective medium for the isolation and quantification of Pseudomonas solanacearum from soil. *Phytopathology* 69 : 182-185.
- NORRIS, D.O. (1951). Spotted wilt of potato. *Australian Journal of Agricultural Research* 2 : 221-260.
- OBRIEN, M.J. and RICH, A.E. (1976). Potato diseases. U.S. Dept. Agric., Agri. Res. Serv. Agriculture Handbook No. 474 : 79 pp.
- OHMS, R.E. and FENWICK, H.S. (1961). Potato early blight, symptoms cause, and control. *Idaho Agr. Ext. Service Bull.* 346.
- OKABE, N. (1954). Studies on Pseudomonas solanacearum V. Antagonism among strains of P. solanacearum. *Rept. Fac. Agric. Shizuoko University* 4 : 37-40.
- PADHI, N.N. and RATH, G.R. (1974). Sporulation of Alternaria solani in pure culture. *Indian Phytopathology* 7 : 316-317.
- PAPAVIZAS, G.C. (1974). Effect of organic amendments on root disease. In "The relation of soil microorganisms to plant disease. South Coop. Ser. Bull. 183 : 25.
- PIETRAK, J. (1982). The effect of potato viruses S and M on infection by potato viruses X and Y in simultaneous inoculation of potato plants (Abstract). *Review of Plant Pathology* 61(6) : 252.
- PETERS, D. (1970). Potato leafroll virus. C.M.I. Description of plant viruses No. 36. Kew : Commonwealth Mycological Institute, Association of Applied Biology.
- PETERS, D. (1977). The use of oils to prevent the spread of virus diseases and its mode of action. *Proc. Br. Crop. Prot. Conf.* : 823-830.
- PETERS, D. and LEBBINK, G. (1973). The effect of oil on the transmission of pea enation mosaic virus during short inoculation probes. *Entomologia Experimentalis et applicata* 16 : 185-190.
- PHILIP, W.P., FEDDERSEN, H.D., WICKS, T.J. and MADGE, P.E. (1976). Pests and diseases of potatoes. Fact sheet 262/600. Dept. Agric. and Fisheries, South Australia : 3 pp.
- PLATT, H.W. (1983). Effects of metalaxyl, mancozeb and chlorothalonil on blight, yield and tuber rot of potato. *Can. J. Plant Pathology* 5 (1) : 38-42.
- POTTER, H.S. and HOOKER, W.J. (1960). Early blight of potato. *Michigan Agr. Expt. Fact Sheet* No. 573.

- POWELL, D.H. and MONDOR, T.W. (1973). Control of the green peach aphid and suppression of leafroll on potatoes by systemic soil insecticides and multiple foliar sprays. *Journal of Economic Entomology* 66 : 170-177.
- QUEMENER, J. (1976). Essais de protection contre le virus Y par des traitements a base d'huile Minerale. *Le pomme de Terre Francaise*, 371 : 5-16.
- QUIMIO, A.J. and CHAN, H.H. (1978). Survival of Pseudomonas solanacearum E.F. Smith in the rhizosphere of some weeds associated and economic plants rotated with tomato. In "Proceedings of the 4th International Conference on Plant Pathogenic Bacteria", p. 883. Angers : Institut National de la Recherche Agronomique.
- RANDS, R.D. (1917). The production of spores of Alternaria solani in pure culture. *Phytopathology* 7 : 316-317.
- REESTMAN, A.J. (1970). Importance of the degree of virus infection for the production of ware potatoes. *European Potato Journal* 5 : 166-172.
- REID, J. (1958). Studies on the fusaria which cause wilt in melons. II. The effect of light, nutrition and various chemicals on the sporulation of certain fusarial isolates, and preliminary investigations on the etiology of wilting of the muskmelon fusarium. *Can. J. Botany* 36 : 507-537.
- ROBERT, Y. (1976). Action de traitements insecticides endotherapiques sur la dissemination du virus de l'enroulement de la pomme de terre en Bretagne. *Phytiatrie-Phytopharmacie* 25 : 187-200.
- ROBERTSON, D.G. (1976). Virus diseases. *Potato Research in Kenya 1970-1976*, pp. 16-27. Technical Report Ministry of Overseas Development, London.
- SCHEPERS, A. (1972). Control of aphid vectors in the Netherlands. In "Viruses of potatoes and seed-potato production." Wageningen : Center for Publishing and Documentation.
- SCHEPERS, A., BUS, C.B., DE BOXX, J.A., and CUPERUS, C. (1977). De verspreiding Van Y^N virus in aardappelen. *Landbouwkundig Tijdschrift* : 89-5 (in Dutch).
- SCHUMANN, G.L., THURSTON, H.D., HURST, R.K., KAWAMOTO, S.O. and NEMOTO, G.I. (1978). Comparison of tomato bioassay and slab gel electrophoresis for detection of potato spindle tuber viroid in potato. *Phytopathology* 68 : 1256-1259.
- SHANIN, E.A. and SHEPARD, J.F. (1979). An efficient method for inducing profuse sporulation of Alternaria species. *Phytopathology* 69 : 618-620.
- SIMONS, J. and ZITTER, T.A. (1980). Use of oils to control aphid-borne viruses. *Plant Disease* 64 (6) : 542-546.

- SIMONS, J.N., McLEAN, D.L. and KINSEY, M.G. (1977). Effects of mineral oil on probing behaviour and transmission of stylet-borne viruses by Myzus persicae. *Journal of Economic Entomology* 70 : 309-315.
- TAMADA, T., TAKAKUWA, M. and NARITA, T. (1971). Effects of granulated systemic insecticides on the time of spread of potato leaf roll virus and on the transmission behaviour of aphid Myzus persicae sulz. *Bulletin of Hokkaido Prefectural Agricultural Experiment Station*, No 23, pp. 80-89 (in Japanese with English summary).
- THORNE, G. (1945). Ditylenchus destructor, N. sp., the potato rot nematode, and Ditylenchus dipsaci (Kuhn, 1857) Filipjev, 1936, the teasel nematode (nematoda : Tylenchidae). *Proceedings of the Helminthological Soc. of Washington* 12 (12) : 27-33.
- THURSTON, H.D. (1980). International potato disease research for developing countries. *Plant Disease* 64 (3) : 252-258.
- TILL, B.B. (1971). The effect of insecticides on the spread of potato leafroll virus in seed potato fields at Pukekohe. *New Zealand Journal of Agricultural Research* 14 : 458-468.
- TURKENSTEEN, L.J. (1981). Phoma leaf spot. In "Compendium of potato diseases", pp. 47-48. (Ed. W.J. Hooker). The American Phytopathological Society.
- VANDERVEKEN, J.J. (1977). Oils and other virus transmission. In "Aphids as virus vectors", pp. 435-454. (Ed. K.F. Harris and K. Maramorosch). New York, Academic Press.
- VAN der WANT, J.P.H. (1972). Introduction to plant virology. In "Viruses of potatoes and seed-potato production", pp.19-25. (Ed. J.A. de Bokx). Wageningen : Centre for Agricultural Publishing and Documentation.
- WALLACE, H.R. (1978). The diagnosis of plant disease of complex etiology. *Ann. Rev. Phytopathol.* 16 : 379-402.
- WARCUP, J.H. and TALBOT, P.H.B. (1981). "Host-pathogen index of plant diseases in South Australia". Dept. of Plant Pathology, Waite Agric. Res. Inst. Univ. of Adelaide, 114 pp.
- WATSON, D.J. and WILSON, J.H. (1956). An analysis of the effects of infection with leafroll virus on the growth and yield of potato plants and of its interactions with nutrient supply and shading. *Ann. Appl. Biol.* 44 : 390-409.
- WEINGARTNER, D.P. (1981). Early blight. In "Compendium of potato diseases", pp. 43-44. (Ed. W.J. Hooker). The American Phytopathological Society.
- WENZL, H. (1964). An advance in seed potato growing - granular insecticides against virus transmitting aphids. *Pflanzenarzt* 17 (2): 20-21.
- WHITEHEAD, T. et al. (1945). "The potato in health and disease". 2nd. ed. Oliver and Boyd, London : 388 pp.

- WICKS, T.J. (1980). Powdery scab now widespread. The grower (official journal of the S.A. Fruit Growers and Market Gardeners Ass.) 31 : 1 p.
- WILLIAMS, C. and MOSS, D. (1984). Potato seed certification in South Australia. In "National Cultivar Workshop", pp. 73-78. Terrigal, New South Wales, Australia.
- WITSCH, J.V. and WAGNER, F. (1955). Beobachtungen iiber deri Einfluss des lichtetes auf Mycelund conidien-bildung bei Alternaria Crassicae var. dauci. Archiv f. Mikrobiologie 22 : 307-312.
- WYMAN, J.A. (1971). The use of oils and other materials in the reduction of aphid transmitted plant viruses. Thesis, Univ. Wisconsin, U.S.A., No. 109 : 71-16.
- ZSCHIEGNER, H.,J., EISENBRANCT, K.G. and FRITZSCHE, R. (1974). Beitrog zum Wirkungsmechanismus von Mineralolen bei der Verhinderung der Virusubertragung durch Vektoren. Acchir fur Phytopathlogie und Pflanzenschutz 10 : 371-345.

APPENDICES

Appendix 1

Culture media

V-8 JUICE AGAR (Miller, 1955)

V-8 juice	200 ml
CaCO ₃	3.0 g
Agar	20.0 g
Distilled water	800 ml

Prune Agar (Talboys, 1960) : Selective medium for *Verticillium* spp.

Prune extract	100 ml
Lactose	5 g
Yeast extract (Difco)	1 g
Agar	30 g
Distilled water	900 ml

pH = 5.8 - 6.0

Crystal Violet Pectate Medium (Cuppels and Kelman, 1974) : Selective medium for pectolytic *Erminia*.

Crystal Violet (0.075%)	1 ml
NaOH (IM)	4.5 ml
CaCl ₂ .2H ₂ O (10%)	3 ml
NaNO ₃	1 g
Agar	2 g
Sodium polypectate	9 g
Sodium lauryl sulphate (10%)	0.5 ml

TZC Agar (Kelman, 1954) : General growth and isolation medium for *P. solanacearum*

	g/lit
Peptone	10
Casein hydrolysate (Difco)	1.0
Glucose	5.0
Agar	15.0

Tetrazolium chloride is added as 1 ml of a filter - sterilized 0.5% solution per 100 ml of molten (60°C) medium.

Selective medium for *P. solanacearum* (Nesmith and Jenkins, 1979).

	Final concentration per litre of medium
K ₂ HPO ₄	1.18 g
KH ₂ PO ₄	0.44 g
(NH ₄) ₂ SO ₄	1.32 g
MgSO ₄ ·7H ₂ O	0.2 g
MnSO ₄ ·H ₂ O	1.5 mg
ZnSO ₄ ·7H ₂ O	1.6 mg
Ferric citrate (FeC ₆ H ₅ O ₇ ·5H ₂ O)	3.0 mg
plus Citric Acid	1.9 mg

	Final concentration (PPM)
Penicillin G	1.0
Tyrothricin	20
Chloromycetin	5.0
2,3,5 Triphenyl Tetrazolium Chloride	500
Polymyxin B	100
Vancomycin	10
Bacitracin	50
Benomyl (50W)	500
Chloroneb (Tersan SP 65W)	100
Cycloheximide (Actidione)	50
Pentachloronitrobenzene (Terraclor 75W)	30
Pimaricin	20
Dichloran (Botran 75W)	100

	Final Concentration (G) per litre of medium
Dextrose	4.0
Peptone	10.0
Yeast Extract	1.0
Casamino Acids	1.0
Agar	18.0

Stocks of chemicals and antimicrobial compounds are not used after 90 days. The basal medium is made by adding appropriate amounts of each stock salt solution of boiling deionized water. The other ingredients are added to the basal medium.

Semi-selective medium for *P. solana* ceorum (Chen and Echandi, 1982).

	G/lit
Chloramphenicol	10.0
PCNB	37.5

The antimicrobial compounds are added to TZC agar. A 1.5% water agar (45°C) is poured on seeded plates and after 24-28 hr incubation. A 4 ml of melted 0.7% water agar containing 10^7 cfu/ml of bacteriocin-sensitive strain is poured over the agar surface of plates. Plates are incubated on additional 24 hr at 30°C.

Selective medium for *P. solanacearum* (Granada and Sequeira, 1983).

	mg. per litre
Crystal Violet	50
Thiomersal	5.0
Polymyxin B	100.0
Tyrothricin	20.0
Chloromycetin	5.0

The antimicrobial compounds are sterilized in 70% ethanol (1 ml), diluted to desired concentration, and stored at 4°C. Crystal violet is sterilized for 7 min at 121°C. Thiomersal is added without treatment. TZC agar is used as basal medium and each antimicrobial compound is added just before dispensing the medium.

Appendix 2

Test Plants

Nicotiana tobaccum (white burley)
Nicotiana glutinosa L.
Nicotiana rustica L.
Gomphrena globosa L.
Capsicum annum L.
Chenopodium amaranticolor Coste et Reyn
Chenopodium quinoa L.
Physalis floridana Rydb.
Phaseolus vulgaris L.
Datura stramonium L.
Lycopersicon esculentum Mill var. Rutgers
Capsella bursa-pastoris
Solanum tuberosum

Appendix 3

Antisera

ELISA TESTS – KITS (Ogdia Inc. Ind. U.S.A.) for PVY and PLRV

Antisera for PLRV and BWYV were supplied by Agriculture Canada. Research Station, Vancouver, B.C.

PVX antisera were supplied by the Department of Plant Pathology, Waite Agricultural Research Institute, South Australia.

PVS antisera were supplied by the plant Research Institute, Burnley, Victoria, Australia.

Appendix 4

R.C. Soil

Per 0.5 M³ of soil

Peatmoss	0.1 M ³
Blood meal	500 gm
Potassium Sulfate	200 gm
Superphosphate	100 gm
Ground Limestone	200 gm

The soil consists of decomposed plant materials and soil from previous experiments, sterilized at 100°C for 45 minutes and then quickly cooled before adding the other ingredients.

Appendix 5

Mineral oils and their characteristics

Mobil oil stock No. 146 (Sample 1)

- Highest grade of finished mineral oil
- Viscosity in Soybolt Universal Seconds (SUS) at 100°F = 100
- Viscosity in centistokes (cSt) at 40° = 20
- Turbine grade, hydrogen treated to saturate olefines and improve oxidation stability

Mobil oil stock No. 236 (Sample 2)

- Viscosity in SUS = 300
- Viscosity in cSt = 55
- Automotive grade of mineral oil, vacuum distilled from crude oil, aromatics removed by extraction with furfural and then wax removed by selective solvent dewaxing using 50/50 Methyl Ethyl Ketone/Toluene.

Mobil oil stock No. 302.5 (Sample 3)

- Heavier grade for automotive use.
- Viscosity in cSt = 130

Golden Fleece Oil Samples Summer-spray-oil (SSO)

- Viscosity in SUS = 70
- Unsulfunated Residue = 90

Lovis-spray-oil (LSO)

- Viscosity = 60
- Unsulfunated Residue = 90

Both oils are commercially used in citrus orchards for the control of spider mites.

Appendix 6

Relative Crowding Coefficient
(De Wit, 1960)

$$\begin{array}{l} \text{Relative Crowding} \\ \text{Coefficient of I} \\ \text{with J} \end{array} = \frac{\begin{array}{l} \text{Mean yield per plant of} \\ \text{I in mixture} \end{array}}{\begin{array}{l} \text{Mean yield per plant of} \\ \text{J in mixture} \end{array}} \frac{\begin{array}{l} \text{Mean yield of I in pure} \\ \text{stand} \end{array}}{\begin{array}{l} \text{Mean yield of J in pure} \\ \text{stand} \end{array}}$$

Appendix 7

Frequency Table
(Blodgett, 1941)

Estimated percentage of plants in a field situated like the central plant in the various classes for fields with different percentage of disease.

Disease in the Field (%)	HHH	DHD	HHD DHH	DDD	HDH	HDD DDH
0	100	-				
20	51.2	3.2	25.6	0.8	12.8	6.4
40	21.6	9.6	28.8	6.4	14.4	19.2
60	6.4	14.4	19.2	21.6	9.6	28.8
80	0.8	12.8	6.4	51.2	3.2	25.6
100	-	-	-	100	-	-

Appendix 8

A comparison of the virus transmission inhibiting properties of SSO and LSO.

Treatment	Number of Plants Infected ¹ (%)		Number of Local Lesion ²	
	Trial I	Trial II	Trial I	Trial II
Control	66.6	79.2	180	78
SSO	20.4	21.9	20	7
LSO	47.3	36.6	29	15

1 *Physalis floridana* plants with well developed cotyledons were inoculated with PVY with the aid of 4 aphids (*Myzus persicae*) which had previously fasted for two hours. The plants were previously sprayed with oil, or water (control), at a concentration of 0.2%.

2 *Chenopodium guinoa* plants were sprayed with 0.2% oil, dusted with carborondum powder, and then mechanically inoculated with PVY. Mean value of ten leaves per treatment.

Appendix 9

Characteristics of a red brown Urrbrae loam soil

pH	5.4
Total C	1.48%
Total N	0.15%
Total P	316 ppm
Clay	19.4%
Silt	31.3%
Fine sand	43.8%
Coarse sand	2.0%

Appendix 10

Plate 1 :

- a. Potato virus S
- b. Gel-diffusion in agar, PVS antiserum in the centre well and infected sap in the top wells.
- c. Purple top wilt
- d. Phoma leaf spot showing symptoms similar to early blight
- e. Pink rot caused by *Phytophthora cryptogea* (f).
- g. Stem rot showing mycelia and sclerotia of *Sclerotium rolfsii*

PLATE 1



Plate 2 :

- a. Red Pontiac potatoes severely infected by *Phoma* sp., and pycnidia releasing spores (b).
- c. *Alternaria alternata*, and conidia (d).
- e. Leak caused by Pythium irregulare, and fruiting bodies of the fungus (f).
- g. Stemphyllium leaf spot.

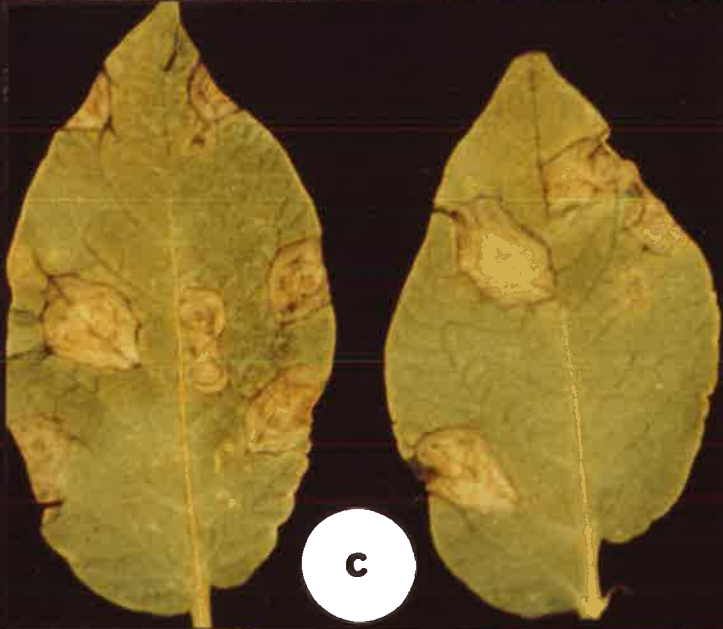


Plate 3 :

- a. Potato crop severely infected by a disease tentatively identified as tomato big bud (aster yellows).
- b. A closeup of infected plant showing enlarged leaf nodes and aerial tubers.
- c. Severe mosaic caused by potato virus X.
- d. PVX particles from a leaf dip preparation.
- e. Alfalfa mosaic virus.
- f. Tubers showing symptoms of potato spindle tuber viroid infection but was not proven.

PLATE 3



Plate 4 :

- a. Brown rot or bacterial wilt caused by *Pseudomonas solanacearum*, showing bacterial ooze in the vascular tissue.
- b. Potato leafroll virus.
- c. *Physalis floridana* plants, inoculated with PLRV (left) and healthy (right).
- d. Potato virus Y.
- e. Underside of PVY infected leaf showing veinal necrosis.
- f. Tomato spotted wilt virus showing characteristic symptom.

PLATE 4



Plate 5 :

- a. Early blight caused by *Alternaria solani* showing the charactersitic ring or target appearance of the lesion.
- b. Stem rot caused by *Sclerotinia sclerotiorum*, with sclerotia.
- c. Blight caused by *Botrytis cinerea*.
- d. Tubers showing the black sclerotia of *Rhizoctonia solani* on the surface (black scurf).
- e. Silver scurf of potatoes caused by *Helminthosporium solani*.

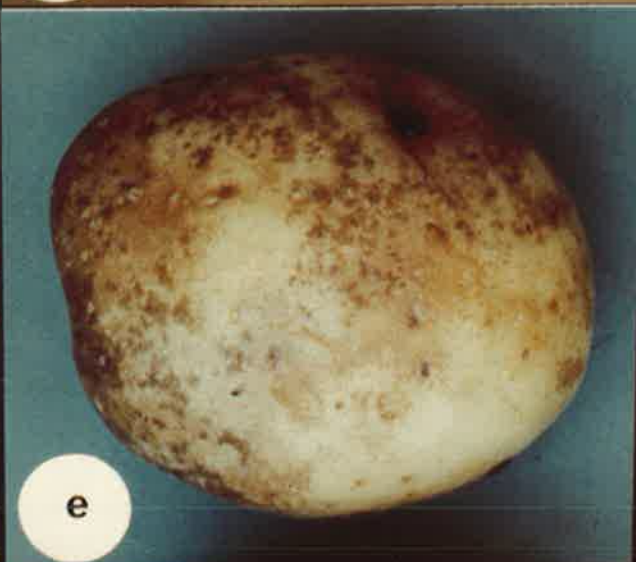
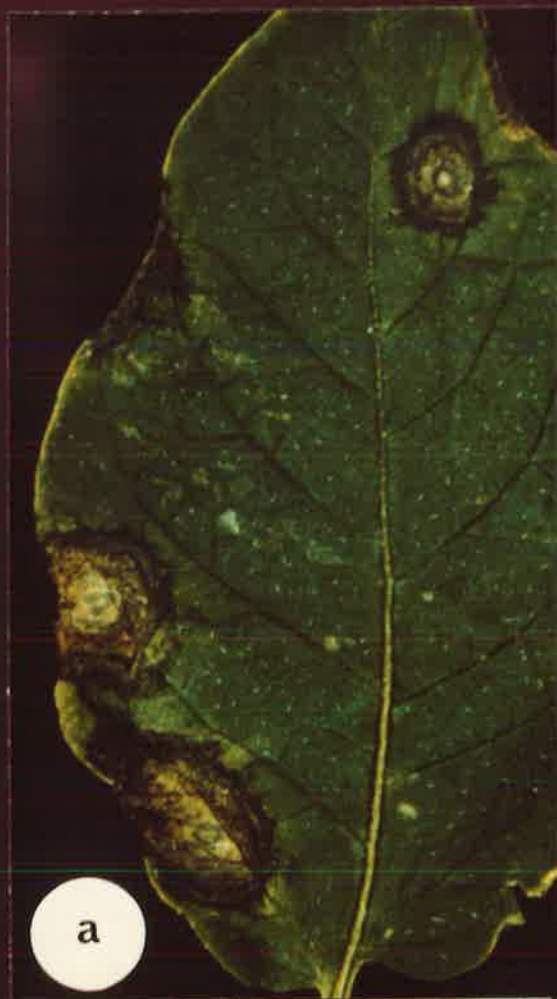


Plate 6 :

- a. Powdery scab caused by *Spongospora subterranea*.
- b. Stem rot due to infection of *Rhizoctonia solani*.
- c. Verticillium wilt (*Verticillium dahliae*).
- d. Gangrene on stored potatoes caused by *Phoma foveata*.
- e. Tuber rot on stored potatoes caused by *Botrytis cinerea*.

PLATE 6



Plate 7 :

- a. Dry rot on stored potatoes due to *Fusarium solphureum*.
- b. Pitted type of common scab (*Streptomyces scabies*).
- c. Raised pustules on tubers caused by root knot nematode (*Meloidogyne sp.*).
- d. Deformed tubers of the cultivar 'red pontiac' due to infection by *Rhizoctonia solani*.
- e. Black leg of potatoes caused by *Erwinia atroceptica*.
- f. Tubers showing nematode injury which leads to tuber rot.

PLATE 7



a



d



b



e



c



f

Plate 8 :

- a. Leaf showing mosaic and distortion, virus?
- b. Leaf showing veinal chlorosis.
- c. Leaf blight suspected to be a bacterial disease.
- d. Veinal necrosis on the underside of the leaf which was suspected to be PVY.
- e. 'Dimple ends', perhaps caused by a virus, or a physiological disorder.



The Objectives of the Research Programme

The project was conducted initially to determine what diseases occurred in the potato crops of South Australia, to identify the pathogens associated with those diseases and to define two or three aspects which could be studied in depth.

South Australia has a dry Mediterranean type of climate with mild winters and warm to hot summers with high light intensity. The prevailing weather conditions during the main cropping season (November to March) are generally warm and dry (Fig. 1). It is only during the spring, autumn and early part of winter that potato crops are exposed to short periods of cool wet weather. As weather is known to have a profound influence on disease development, it was considered likely that most potato diseases, particularly those caused by fungi and bacteria, would not be found in the commercial potato-producing areas of South Australia. On the other hand, it seemed likely that diseases caused by aphid-borne viruses were more widespread in S.A. because mild winters favoured the prevalence of aphids early in the cropping season.

To test these hypotheses it was decided to conduct surveys and experiments on three diseases and their associated pathogens: potato leaf roll and potato leaf roll virus, early blight and Alternaria solani, bacterial wilt and Pseudomonas solanacearum.

A further objective involving the three diseases and their associated pathogens was to explore the possibility of control. Thus, for PLRV, the possibility of inhibiting transmission and spread of the virus by aphids through the application of mineral oils to potato plants was thought to be a useful approach. For early blight, it was decided that a study of the

influence of fungicides would be worthwhile and for bacterial wilt, recent successes with avirulent bacteriocin-producing strains of bacterial pathogens, prompted a study of such strains of P. solanacearum and their protective qualities.

In summary then, the objectives of this research project were to survey and identify the major diseases and their causal pathogens in potato crops in South Australia, to test the hypothesis that weather is a major determinant of the development of disease in potatoes in S.A. and to contribute to knowledge of how the three pathogens selected for study might be controlled.

The Influence of Weather

The high yield loss of 66% measured in the field supports the hypothesis that potato leaf roll is probably the most serious disease of potatoes in South Australia. Experiments in the field from November to February confirmed that populations of the aphid, Myzus persicae, were high at that time and were associated with a high incidence of the disease, potato leaf roll in the potato crop.

The hypothesis that the disease 'early blight' caused by Alternaria solani does not increase in South Australia because of the inhibitory effect of climate during the main potato growing season, was tested by experiments on the influence of environmental factors on the sporulation of A. solani. It was found that the optimum temperature for sporulation was 15°C and was markedly inhibited above 20°C. Furthermore the requirement for darkness or low light intensity to induce sporulation is additional evidence that the high temperatures and light intensities in South Australia from December to February probably inhibit the development of A. solani and hence reduce the severity of 'early blight'.

The sporadic and rare occurrence of bacterial wilt caused by Pseudomonas solanacearum is attributed, once again, to the hot dry conditions in S.A. during the potato growing season. To test this hypothesis it was decided to study the interaction between soil moisture and temperature and the effect of organic matter on the survival of the bacterium in soil. The results showed unequivocally that populations decreased as temperature increased and soil moisture decreased. It was concluded that high soil moisture and low temperatures favoured survival; such environmental characteristics do not occur in S.A. soils.

In summary, it is likely that in South Australia, the prevalence and economic importance of diseases and their associated pathogens may be closely related to climate. Potato leaf roll, its causative virus and the aphid vector represent the group that can tolerate the harsh conditions in South Australia whereas the less tolerant pathogens such as A. solani and P. solanacearum represent the other group.

Control

An important objective in the research project was to explore the possibility of controlling the three pathogens. This was considered to be a worthwhile objective as each pathogen required a different approach. With potato leaf roll, the virus causing the disease could be controlled through control of its aphid vector; with early blight, chemical control with fungicides seemed appropriate; with bacterial wilt, biological control using avirulent strains seemed an interesting possibility.

Potato leaf roll has received little attention in Australia although it is considered to be a serious disease. Its seriousness was confirmed by the yield loss assessments in this study. The use of oil sprays to control the disease required studies on the most suitable potato cv for experimentation, the phytotoxic effect of oil sprays, the choice of oils and the management of aphids under experimental conditions. Results of experiments failed to indicate that oil sprays were effective against PLRV and furthermore they could cause yield reductions of the order of 35%. The granular form of some systemic insecticides gave better control where aphid populations were low but the influence of weather is again important as aphid populations and the susceptibility of potato cultivars are probably influenced by this environmental factor.

Studies on the influence of a range of fungicides on spore germination of A. solani indicated that captafol and chlorothalonil were likely candidates for field experimentation in the control of early blight. However such fungicides are costly, hence it may be more economical to search for resistant or tolerant cvs of potato especially in developing countries.

Bacterial wilt disease caused by Pseudomonas solanacearum is one of the most important diseases of potato and other crops. This disease limits the growing of potatoes in S.E. Asia particularly at lower, warmer

elevations. It is an extremely important disease in the Philippines and as my future work will involve a search for ways of controlling that disease, it was decided to combine an ecological study with the use of bacteriocin-producing strains of P. solanacearum. Unfortunately, control by this means was not achieved and further studies are required. The ecological studies emphasised the importance of soil moisture, temperature and non-host plants on the survival of the bacterium, and possibly account for the distribution of the disease in the Philippines where potatoes in the cool wet highlands are less affected than those on the plains. The significance of races of P. solanacearum with different tolerances to desiccation deserved further study.

General Discussion

Any attempt to increase yields of a crop in a particular area requires adequate survey and diagnostic work to identify the main constraints on the crop. Thus, although potato leaf roll appears to be the most serious disease in potatoes in South Australia, other virus diseases were found but not identified, so the etiological story is incomplete. Factors, other than pathogens may influence yield and the results presented in this thesis point to the profound importance of climate in determining how damaging a pathogen will be. The data support the hypothesis that many diseases in potatoes caused by fungi and bacteria are either not found or are of little consequence in South Australia because of the hot dry conditions during the main potato growing season from December to February. The absence of late blight caused by Phytophthora infestans and bacterial wilt caused by Pseudomonas solanacearum are probably due to such conditions. Thus, 50% of fungal and bacterial diseases of potatoes recorded in other states occur in South Australia and of these only 10% are prevalent.

The serious nature of potato leaf roll is probably related to South Australia's mild winters which favour the prevalence of the virus' aphid vectors in the cropping season. Epidemiological research on diseases of economic importance in potato crops would be worthwhile as it would enable critical periods for aphid flights and for fungal sporulation, dispersal and infection to be determined in relation to weather. Simulation models for forecasting disease outbreaks could then be devised to assist in the more economic application of insecticides and fungicides.

One of the original objectives was to explore control of the three diseases described in this thesis. All three diseases are prevalent in the tropics and potato wilt is particularly serious in the Philippines. The data presented here confirm the view that a knowledge of the ecology of a disease and its causal pathogen is necessary. For example, the influence

of weather on the aphid vectors of PLRV is important as well as the need to determine which strains of the virus are most prevalent and if the potato cvs. that are grown in an area are tolerant and susceptible to the virus. Similarly, the effect of weather on the tolerance of potato plants to fungicides and the importance of non hosts and soil conditions as influenced by weather, on the survival of P. solanacearum also suggest that of all the environmental components, weather is probably the most important.

Although control of P. solanacearum by crop rotation and organic amendments may seem to be the most likely method at present, the lack of success with bacteriocin-producing strains of P. solanacearum should not deter further studies. A more intensive search for more effective strains seems justified.

Potato crops are complex ecosystems and the possibility that decreased yields may be the result of interactions between pathogens, climatic factors and potato cvs. needs investigation. Sociological factors may even play a part. In South Australia, losses due to potato leaf roll could be avoided if growers used certified seed from Victoria each year and avoided the practice of sowing seed from a previous year for planting in a current year.

Corrections and Amendments

Page 1 The statement "Unlike most cultivated plants, potatoes are prone to numerous kinds of plant pathogens....." should be replaced by "Most staple crop plants are beset by many pathogens and are sensitive to nutrient imbalance and adverse environment".

Page 12, Para 2. Reference to "disease" and "pathogen" is confusing. Pathogen is defined as the causal agent of disease. Disease is the physiological malfunction of the plant caused by the pathogen. Unfortunately in the literature diseases are sometimes referred to in terms of the causal pathogen, a symptom or a combination of the two e.g. Fusarium dry rot.

Page 19, Lines 3-4 "...as other aphid-borne and stylet-borne viruses".
Should read "as other viruses borne on the stylets of aphids".

Page 19 Lines 14-16 "...healthy potato plants were stunted..." should read "healthy potato plants were subsequently stunted.....".

Page 19, Line 21. Add "For example, the Rutherglen bug may itself produce a toxin".

Page 25-26 Reference to Streptomyces scabies under the heading "Bacteria" is incorrect. S. scabies is an actinomycete.

Page 36, Line 5. The word "secondary" refers to infection that occurs after infection in a previous season.

Page 41, Table 5; Page 46; Table 7. The term H.S.D. is unfamiliar in plant pathology literature. As explained in Table 4 (Page 38) it is an abbreviation for "Honestly Significant Differences" in Tukey's w-procedure. This procedure is used in one-way analyses of variance where multiple comparisons need to be tested.

Page 108, Table 19 As the data were not statistically analysed, conclusions from this experiment can only be tentative. Thus, comments on the effects of nematodes on wilting (Pages 107, 113) should be treated with caution.