

**AN INVESTIGATION OF FACTORS INFLUENCING THE
INCIDENCE OF POTATO TUBER BLIGHT CAUSED BY
PHYTOPHTHORA INFESTANS (MONT.) DE BARY**

**A thesis submitted for the
degree of Doctor of Philosophy
in the
Faculty of Science
of the
University of Glasgow**

**by
ROBERT WILLIAM FAIRCLOUGH B. Sc.**

**Department of Plant Science
The Scottish Agricultural College
Auchincruive, Ayr**

May 1995

ProQuest Number: 11007677

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 11007677

Published by ProQuest LLC (2018). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

.....

Thesis
10202
Copy 1



This thesis is dedicated to Cairnie Le Cornu,
to my friends, and to my parents.

"1847 was the year it all began,
deadly pains of hunger drove a million from the land.
They journeyed not for pleasure,
their motive was not greed,
a journey of survival across the lonely sea."

From "The City of Chicago" by C. Moore.

ACKNOWLEDGEMENTS

Very special thanks are due to my supervisors Dr. Ruairidh A. Bain and Dr. Stephen J. Holmes for advice and encouragement during both the practical and the writing-up stages. Thanks are also due to many other SAC Auchincruive staff. In particular to Lynda Raynor, Shahida Gilani, Jackie Miller and Graeme Ligertwood, who provided help and technical assistance; to Dr. Chris Smith, Dr. Rob Finch and Dr. Andrew Walker for technical advice and to Alastair Sword (the Scottish Agricultural Statistics Service) for advice on statistics. I am also very grateful for the help of the field staff of the Plant and Environmental Science Departments, especially Donald Kiltie and Bruce Murray without whom much of this work would not have been possible. I also wish to thank Dr. Roger L. Wastie and Helen E. Stewart of SCRI for their advice and Dr. Julian Harrison (SCRI) for advice and for supplying the polyclonal anti-*P. infestans* gamma-globulin. Thanks are due to Fred Mathisen who proof-read each chapter of this thesis and corrected grammar and spelling. The help of Caledonian Produce (Geest) is also gratefully acknowledged. I wish to thank Sandoz Agro Ltd. for financial assistance.

CONTENTS	page
Abbreviations used in the text.....	1
List of Tables	2
List of Figures	4
List of Plates.....	7
Summary	8
CHAPTER 1 Literature review: the infection of potato tubers by	
<i>Phytophthora infestans</i>	
1.1 The potato crop in the U.K.	
1.1.1 The introduction of the potato into Europe and the U.K.	12
1.1.2 Potato production in the U.K.	12
1.2 The fungus <i>Phytophthora infestans</i>	
1.2.1 The first occurrence of <i>Phytophthora infestans</i> in the U.K.	13
1.2.2 Taxonomy of <i>Phytophthora infestans</i>	13
1.2.3 Life cycle	14
1.2.4 A2 mating type and the production of oospores	16
1.2.5 Tuber blight.....	17
1.2.6 Foliage and stem blight.....	19
1.3 Factors affecting the incidence of late blight	
1.3.1 Influence of the aerial environment.	19
1.3.2 Influence of cultivar....	24
1.3.3 Tuber susceptibility	25
1.3.4 Influence of edaphic factors.....	26
1.4 Control of late blight	
1.4.1 Fungicide treatment.....	28
1.4.2 Breeding for resistance	32
1.4.3 Forecasting.....	34
1.4.4 Cultural control	34

1.4.5 Harvest and post-harvest losses	35
1.5 Objectives of the study	37

CHAPTER 2 General materials and methods

2.1 Cultivars used in the experiments	39
2.2 <i>P. infestans</i> isolates and inoculation procedures	
2.2.1 Isolation and maintenance of <i>P. infestans</i>	39
2.2.2 Preparation of <i>P. infestans</i> inoculum	40
2.2.3 Isolates used in the experiments	41
2.2.4 Inoculation of plants.....	41
2.3 The soil used in the experiments.....	43
2.4 Measurement of <i>P. infestans</i> and assessment of blight symptoms	
2.4.1 Enzyme-linked immunosorbent assay (ELISA).....	43
2.4.2 Assessment of foliar and stem blight	45
2.4.3 Assessment of tuber blight	45
2.5 Field trials: planting and pesticide application	47
2.6 Statistical analyses	47

CHAPTER 3 The effect of type of fungicide on the relationship between the amount of blight on the haulm and tuber blight incidence

3.1 Introduction	50
3.2 Materials and methods.....	50
3.3 Results	
3.3.1 Effect of fungicide type on the incidence of tuber blight.....	56
3.3.2 The effect of fungicide type on the severity of foliar blight and stem blight when the incidence of tuber blight became significantly greater than zero.....	58
3.3.3 Effect of fungicide type on the incidence of foliar blight and severity of stem blight.....	61

3.3.4 Effect of fungicide type on sporangia production on leaf lesions	65
3.4 Discussion.....	68

CHAPTER 4 The role of stem blight in the infection of daughter tubers by *P. infestans*

4.1 Introduction	76
4.2 Materials and methods.....	77
4.3 Results	
4.3.1 Experiments 1 & 2 The development of stem lesions and sporangia production by <i>P. infestans</i>	87
4.3.2 Experiments 3 to 5 Stem lesions as a source of inoculum for tuber infection.....	93
4.3.3 Experiments 6 & 7 The influence of fungicide type on the relationship between leaf lesions, stem blight and tuber blight	97
4.4 Discussion.....	103

CHAPTER 5 The influence of edaphic factors on the spread of *P. infestans* inoculum in soil and on the subsequent incidence of tuber blight

5.1 Introduction	112
5.2 Materials and methods.....	113
5.3 Results	
5.3.1 Experiments 1 to 4 The effect of soil moisture content pre- and post-inoculation on the infection of daughter tubers by <i>P. infestans</i> and subsequent lesion development	124
5.3.2 Experiment 5 The effect of soil moisture content on the spread of <i>P. infestans</i> inoculum from blighted to healthy daughter tubers in the field.....	129

5.3.3 Experiment 6 The susceptibility of seed tubers to infection by <i>P. infestans</i>	132
5.3.4 Experiment 7 The contamination during planting of healthy seed tubers with <i>P. infestans</i> from infected tubers	132
5.3.5 Experiments 8 & 9 The effect of soil moisture content at planting on the sporulation on, and decay of, inoculated seed tubers	136
5.4 Discussion	
5.4.1 The spread of <i>P. infestans</i> inoculum from infected daughter tubers	141
5.4.2 The spread of <i>P. infestans</i> inoculum from infected seed tubers	147
 CHAPTER 6 The infection of ware potatoes by <i>P. infestans</i> during simulated washing and an evaluation of control measures	
6.1 Introduction	152
6.2 Materials and methods.....	153
6.3 Results	
6.3.1 Experiment 1 Estimation of the quantity of inoculum released from blighted tubers during washing	163
6.3.2 Experiment 2 The infection of immature, mature and damaged tubers by <i>P. infestans</i> during washing	165
6.3.3 Experiment 3 An investigation of the thermal death point (tdp) of isolates of <i>P. infestans</i> obtained from potato crops grown in Scotland.....	165
6.3.4 Experiment 4 The evaluation of hot water treatment as a method of minimising the infection of potato tubers by <i>P. infestans</i> during washing.....	169
6.3.5 Experiment 5 The effect of a hydrogen peroxide / peracetic acid / acetic acid mixture on the viability of a suspension of <i>P. infestans</i> containing mycelia and sporangia.....	170

6.3.6 Experiment 6 The efficacy of a hydrogen peroxide / peracetic acid / acetic acid mixture in reducing the infection of potato tubers by <i>P. infestans</i> during washing	170
6.3.7 Experiment 7 The phytotoxicity of hydrogen peroxide / peracetic acid / acetic acid mixture to potato tubers.....	172
6.4 Discussion	172
 CHAPTER 7 Concluding discussion	 185
 Literature cited	 192
 Appendices	
Appendix 2.1 Buffers used in the ELISA tests	216
Appendix 3.1 Relationship between tuber blight incidence on 18 August and haulm blight 5 to 10 days earlier	217
Appendix 3.2 Relationship between tuber blight incidence on 27 August and haulm blight 7 to 10 days earlier	218
Appendix 3.3 Relationship between tuber blight incidence on 5 September and haulm blight 5 to 16 days earlier.....	219
Appendix 3.4 Relationship between tuber blight incidence on 16 September and haulm blight 8 to 11 days earlier.....	220
Appendix 3.5 Relationship between tuber blight incidence on 2 October and haulm blight 1 to 7 days earlier	221
Appendix 4.1 Monthly rainfall at Auchincruive for 1988 and the 24 year mean	222
Appendix 5.1 Temperature at 14.00 hours GMT measured at 13 cm depth in ridges covered with polythene and in non-covered ridges	223
Appendix 6.1 Details of the processing procedure used by Caledonian Produce (Geest).....	224

ABBREVIATIONS USED IN THE TEXT

a.i.	active ingredient
ADAS	Agricultural and Development Advisory Service
ang.	angular
Am	Amplitude modules
cv.	cultivar
CMI	Commonwealth Mycological Institute
DANI	Department of Agriculture for Northern Ireland
df	degrees of freedom
ep	actual evapotranspiration rates
GMT	Greenwich Mean Time
ha	hectares
hp/paa	hydrogen peroxide / peracetic acid / acetic acid mixture
ldpe	low density polyethylene
kPa	kilopascals
mb	millibar
mr	metalaxyl resistant
ms	metalaxyl sensitive
NIAB	National Institute of Agricultural Botany
pe	predicted evapotranspiration rates
ppm	parts per million
rh	relative humidity
s.e.d.	standard error of the difference
smd	soil moisture deficit
SAC	Scottish Agricultural College
SCRI	Scottish Crop Research Institute
tdp	thermal death point

LIST OF TABLES

1.1 Classification of fungicides for the control of late blight of potatoes in the U.K.....	29
2.1 Potato cultivars used in the experiments	39
2.2 Isolate identification, source and metalaxyl sensitivity.....	42
2.3 Composition of the Bargour series soil used in field, glasshouse and laboratory experiments	44
2.4 Water content of the Bargour series soil at field capacity and at permanent wilting point.....	44
2.5 Potato foliage blight assessment key	46
3.1 Fungicide products and rates.....	52
3.2 Dates of, and intervals between fungicide applications	52
3.3 Dates of sampling plants and tubers from treatment plots	53
3.4 The severity of haulm blight when incidences of tuber blight significantly greater than zero were recorded in plots of Kingston treated with four fungicides.....	59
3.5 Area (cm ²) of foliar blight lesions on plants of cv. Kingston treated in the field with four fungicides.....	67
4.1 Target and actual soil moisture deficits for the two irrigation treatments.....	85
4.2 Stem lesion length and the incidence of tuber blight in glasshouse-grown potato plants following the inoculation of stems with <i>P. infestans</i>	94
4.3 The relationship between stem lesion length and the incidence of tuber blight in the field	94
4.4 The effect of fungicide type on blight lesion development on inoculated leaflets.....	101
4.5 The effect of leaf position on the stem on blight lesion development on inoculated leaflets.....	101

4.6 The effect of fungicide on the development of foliar, stem and tuber blight following inoculation of terminal leaflets or stems in field plots of the cv. King Edward.....	104
5.1 Water content of the sandy loam soil at field capacity and at permanent wilting point.....	114
5.2 Rainfall, long term evapotranspiration rates (pe) and target soil moisture deficits (smd) in the high, medium and low smd plots from April to October	120
5.3 Actual soil moisture deficits (smd) achieved in the high, medium and low plots from April until September.....	121
5.4 The incidence of infection for immature tubers incubated in soil at different moisture contents to which <i>P. infestans</i> sporangia had been added	126
5.5 The effect of soil moisture content on the infection of non-inoculated daughter tubers on plants that had one daughter tuber inoculated with <i>P. infestans</i>	131
5.6 The effect of soil moisture content on the spread of <i>P. infestans</i> from an inoculated daughter tuber on one plant to daughter tubers on neighbouring non-inoculated plants	131
5.7 The effect of post-planting soil moisture content on the sporulation by <i>P. infestans</i> on seed tubers inoculated at the time of harvest	138
5.8 The effect of soil moisture on the development of lesions on seed tubers inoculated with <i>P. infestans</i> on the day of planting (mean of two damaged treatments).....	140
6.1 <i>P. infestans</i> isolates used to determine the thermal death point	157
6.2 Isolates and concentrations of <i>P. infestans</i> suspensions used on three dates to evaluate hot water treatment as a method to prevent the infection of potato tubers.....	161
6.3 Lenticel damage scale	163

6.4 Correlation coefficients (r) and significance levels (p) for the regression of absorbance on concentration of <i>P. infestans</i> in the dilution series of known concentrations of <i>P. infestans</i>	165
6.5 The effect of exposure to hot water treatment for 5 minutes on the survival of a number of phenylamide-sensitive and -resistant isolates of <i>P. infestans</i> in water suspensions	168
6.6 The average number of infections per tuber for tubers dipped in suspensions of <i>P. infestans</i> at two temperatures.....	169
6.7 The survival and relative growth rates of two isolates of <i>P. infestans</i> after <i>in vitro</i> treatment with a hydrogen peroxide / peracetic acid / acetic acid mixture (hp/paa).....	171
6.8 Phytotoxic damage 5 days after tubers were dipped in a hydrogen peroxide / peracetic acid / acetic acid mixture (hp/paa).....	174

LIST OF FIGURES

3.1 The incidence of tuber blight in plots of the cv. Kingston treated with four fungicides and sampled throughout the growing season.....	57
3.2 Relationship between tuber blight incidences on 26 September and haulm blight 4 to 8 days earlier.....	60
3.3 Percentage germination of sporangia from foliage lesions sampled on 22 September from plots of the cv. Kingston treated with four fungicides	62
3.4 The percentage of foliage destroyed by <i>P. infestans</i> in plots of the cv. Kingston treated with four fungicides	63
3.5 The percentage stem area diseased in plots of the cv. Kingston treated with four fungicides.....	64
3.6 Number of sporangia released from lesions selected from field plots of the cv. Kingston treated with four fungicides.....	66
4.1 Position of plants with inoculated stems and bait plants in each plot of field experiment 4.....	81

4.2 Position of plants with inoculated stems and bait plants in each plot of field experiment 5.....	83
4.3 Position of plants with inoculated stems or leaves and bait plants in each plot of field experiment 7	88
4.4 The concentration of <i>P. infestans</i> in symptomless potato stems at different distances above the point of inoculation	89
4.5a Sporangia production in relation to stem lesion development (experiment 2a)	91
4.5b Sporangia production in relation to stem lesion development (experiment 2b)	92
4.6 The percentage gravimetric soil moisture contents of non-irrigated and irrigated plots during the experiment	95
4.7 Length of stem blight lesions in relation to soil moisture deficit (smd)	96
4.8 Incidence of tuber blight arising from stem lesions in relation to soil moisture deficit (smd).....	98
4.9 The effect of fungicide type on the incidence of infection by, and subsequent development of, <i>P. infestans</i> following leaflet inoculation.....	99
4.10 The effect of leaf position on the stem on the infection of leaflets by, and subsequent development of, <i>P. infestans</i>	102
5.1 The effect of three post-inoculation soil moisture contents on immature daughter tuber infection and lesion development 14 days after inoculation.....	127
5.2 The effect of soil moisture content post-inoculation on the infection of mature daughter tubers by <i>P. infestans</i> and subsequent lesion development (mean of three dates and three incubation temperatures).....	128
5.3 Soil moisture contents of the low, medium and high soil moisture deficit (smd) field plots over time	130

5.4 Distribution of bait plants with infected daughter tubers in the field plots at 75 % soil moisture content (combined results for both assessment dates and all replicates)	133
5.5 The relative incidences of seed tuber infection by <i>P. infestans</i> in relation to different sites of infection (mean of three inoculum concentrations and two damage treatments).....	134
5.6 The percentage of non-inoculated seed tubers infected after being mixed with a tuber which had been inoculated with <i>P. infestans</i> and incubated for 0, 2, 3, 4 or 6 days (mean value of two damage treatments)	135
5.7 The effect of soil moisture content after planting on the rotting of seed tubers that had been inoculated at harvest with <i>P. infestans</i>	137
5.8 The effect of soil moisture content on the infection of damaged and undamaged seed tubers inoculated with <i>P. infestans</i> on the day of planting and assessed 15 days later	139
5.9 The effect of soil moisture on the degree of rotting of damaged and undamaged seed tubers inoculated at planting with <i>P. infestans</i> and assessed 43 days later	142
6.1 The temperature of the bath water during the 5 minutes that <i>P. infestans</i> samples were treated.....	158
6.2 The temperature of <i>P. infestans</i> suspensions dipped in the bath water for 5 minutes	159
6.3 Calibration curves of absorbance vs. concentration of <i>P. infestans</i> in dilution series of two suspensions of known concentrations of <i>P. infestans</i>	164
6.4 Quantity of <i>P. infestans</i> released from individual blighted tubers washed on two dates after incubation for 24 hours	166
6.5 The incidence of tuber infection for immature and mature tubers, in two damage categories, when washed in a suspension of <i>P. infestans</i>	167

LIST OF PLATES

1.1 Tuber blight symptoms	18
1.2 Foliar blight symptoms	20
1.3 Stem blight symptoms.....	21
3.1 Area measurement light meter used to determine potato leaflet and foliar blight lesion areas	55
4.1 Stem lesion resulting from the growth of <i>P. infestans</i> down a leaf petiole after inoculation of the terminal leaflet.....	100
5.1 Field plots of the cv. King Edward showing separation of the plots and polyethylene covers (experiment 5).....	118
6.1 The phytotoxicity of a hydrogen peroxide / peracetic acid / acetic acid mixture to potato tubers	173

Summary

This study investigated the factors that affect the incidence of tuber blight, with particular reference to the occurrence of tuber blight in the absence of any visible foliar blight. The factors investigated were fungicide type, the severity of stem blight, edaphic factors, particularly soil moisture and the production of *P. infestans* inoculum on daughter and seed tubers. In addition, the infection of tubers during washing for the pre-pack market and its control were also studied.

The results in this thesis have demonstrated that foliar lesions are not the only source of *P. infestans* inoculum for the infection of daughter tubers. It was demonstrated that lesions on the stem base are an important source of sporangia and that in certain circumstances inoculum produced on infected seed or daughter tubers may also infect healthy daughter tubers directly. These less obvious sources of inoculum could explain the occurrence of high incidences of tuber blight in the absence of visible foliar lesions.

In a field experiment fungicide type affected the date on which incidences of tuber blight significantly greater than zero first occurred, the date on which the maximum amount of tuber blight occurred and the overall incidence of tuber blight. The effect of fungicide type on the relationships between foliar blight and the overall incidence or timing of tuber infection was modest. Fungicide type did, however, substantially affect the severity of stem blight at which incidences of tuber infection significantly greater than zero occurred. The severity of stem blight varied more with fungicide type than did the percentage foliar blight. In field and laboratory experiments the growth rate of *P. infestans* in leaflets was influenced by fungicide type and some fungicides resulted in significantly reduced incidences of subsequent petiole and stem infection. In contrast, when the stems were inoculated directly, fungicide type had little effect on stem lesion development.

The use of ELISA to monitor the growth of *P. infestans* in inoculated stems of plants in a growth cabinet showed that stem lesions continued to develop in conditions that were not suitable for the production of abundant sporangia. In this

experiment the number of sporangia produced on stem lesions, after incubation of the stems in a high humidity, did not increase substantially until the rate of lesion growth declined. Sporangia production was more closely related to the length of the lesion supporting sporulation than the lesion length *per se*. In glasshouse and field experiments incidences of tuber blight significantly greater than zero occurred following the irrigation of plants whose main stem had been inoculated with *P. infestans* but whose foliage was maintained free of blight. Another field experiment demonstrated that the incidence of tuber blight was significantly correlated with the length of stem lesions but not with the percentage of foliar blight.

Soil moisture content greatly influenced the incidence and development of blight on daughter tubers. Increasing the soil moisture content of field plots to 75 % of field capacity for approximately 12 weeks significantly increased the spread of *P. infestans* from inoculated daughter tubers to other tubers on the same plant. Healthy tubers on the same plant that were 5 cm from the source of inoculum became infected when the soil moisture content was this high. Tubers on bait plants that were up to 60 cm away from the nearest plant with an inoculated tuber were also infected. In laboratory studies increasing the soil moisture content to 79 % of field capacity pre-inoculation increased the proportion of inoculated daughter tubers which became infected whereas soil moistures above 39 % of field capacity post-inoculation reduced infection. However, post-inoculation soil moisture contents up to 79 % of field capacity considerably increased the development of lesions and sporangia production on infected daughter tubers. Soil temperature had less of an effect on infection, lesion development and inoculum production.

Increasing the soil moisture content above 79 % of field capacity immediately following inoculation decreased the infection of seed tubers inoculated 6 months after harvest. Inoculum production on the infected seed tubers increased with soil moisture content up to 79 % of field capacity although there was little effect of moisture content on lesion development. When seed tubers that had been inoculated soon after harvest were planted the following year in soil with moisture

contents below 79 % of field capacity they remained sound for a period of 58 days. A soil moisture of 79 % of field capacity increased the number of tubers supporting sporulation by *P. infestans*. The transfer of tubers, that were not supporting sporulation after 58 days, from soils at 39 % or 59 % of field capacity into soil at 79 % of field capacity resulted in the production of viable sporangia on these tubers 7 days after transfer.

When blighted seed tubers were removed from storage, incubated for 3 days or longer under conditions suitable for sporulation by *P. infestans* and then mixed with blight-free seed tubers, significant numbers of the latter became infected. Damaging the blight-free seed tubers increased the number of new infections.

Daughter tubers inoculated *in situ* on glasshouse-grown plants had *P. infestans* sporulating on their surface when harvested 9 days later. The use of ELISA to detect *P. infestans* in tuber wash samples showed that individual tubers could liberate up to 4.4 mg of *P. infestans* inoculum during washing. Significant numbers of blight-free tubers were infected following the washing of a sample of immature tubers containing 1 % of blighted tubers. The washing system was based on one used in a commercial pre-pack processing plant. Damaging the tubers increased the number infected whereas more mature tubers were less susceptible to infection.

The infection of daughter tubers dipped in a suspension of *P. infestans* for 3 minutes was completely controlled if the temperature of the suspension was 44° C. There was no indication of tuber damage at this temperature. Although concentrations of peracetic acid above 100 ppm in tuber wash water significantly reduced the survival of *P. infestans in vitro*, a concentration of 125 ppm peracetic acid did not prevent the infection of tubers during washing. In addition, experiments showed that concentrations of 250 ppm or greater resulted in severe phytotoxic damage to tubers.

CHAPTER 1

Literature review: the infection of potato tubers by *Phytophthora infestans*

1.1 The potato crop in the U.K.

1.1.1 The introduction of the potato into Europe and the U.K.

The cultivated potato was first introduced from South America into continental Europe and mainland Britain during the end of the sixteenth century (Salaman, 1949; Hawkes, 1992). Cultivation of the potato rapidly spread through much of Europe although it was a long time before the British, apart from the poorer classes, recognised its food value. In the British Isles potatoes were probably first widely grown in Scotland where, as in Ireland, they became the staple food of much of the population. For many years, however, the potato remained largely unknown in England and it was not until after the Irish potato famine of 1845-1846 that the crop was extensively grown by English farmers (Salaman, 1949).

1.1.2 Potato production in the U.K.

Although the area planted in the U.K. has been declining over the past 10 years, over 180,000 hectares of seed and ware crops (almost 3 % of the total arable land) were grown in 1992 (Anon, 1993). The percentages of the total area planted to maincrop, second early and first early cultivars in 1992 were 66.4, 24.1 and 9.5 % in England and 73.6, 19.2 and 7.2 in Scotland (Nix, 1993). One-sixth of the total mainland U.K. potato area is in Scotland. Total production of potatoes is, however, relatively stable as average yields are increasing. The average yield of maincrop potatoes for the U.K. in 1992 was 43.7 tonnes ha⁻¹ (Anon, 1993). Average yields of 36.5 and 35.4 tonnes ha⁻¹ in 1990 and 1991 respectively are, however, more typical. First and second early cultivars on average yielded 24.0 and 38.0 tonnes ha⁻¹ respectively in 1992 (Nix, 1993).

The average on-farm price for early potatoes in the U.K. during July 1993 was £80 per tonne, compared with an average price of £178 per tonne at the same time in 1994. In 1993 the average price for maincrop was £63 per tonne, compared with an average price of £122 per tonne in 1994 (Bojduniak, 1994;

1995). Exports of potatoes from the U.K. during 1992 totalled 168,432 tonnes, valued at £28.8 million, whereas imports of 354,907 tonnes were valued at £81.1 million (Anon, 1993).

1.2 The fungus *Phytophthora infestans*

1.2.1 The first occurrence of *Phytophthora infestans* in the U.K.

The potato had been cultivated in Europe for about two and a half centuries in the absence of *P. infestans*. Although *Streptomyces scabies* (common scab) and *Spongospora subterranea* (powdery scab) were known previously (Cox & Large, 1960), the year of arrival of *P. infestans* in Europe, or its source, cannot be pinpointed accurately. A review of the literature (Grainger, 1957; Lacey, 1962; Bourke, 1964; Klinkowski, 1970; Robertson, 1991) suggests that it is likely to have been between 1842 and 1845. Infections were first seen in Flanders (Belgium) in 1842 and 1843 (Klinkowski, 1970) and in Flanders, Kent, Isle of Man and possibly Ayrshire and Ireland in the 1844 season (Bourson quoted by Lacey, 1962). Bourke (1964) reported that *P. infestans* was first noted as a serious problem in 1845 in Courtrai (Belgium) towards the end of June. In 1845, unlike the previous years when it is probable that local conditions did not favour the spread of infection, the spread of the fungus was rapid. In the middle of July most of the potatoes in Flanders were affected. The fungus then spread into France and Switzerland and also southern England, southern Scotland and Ireland. The losses in 1845 were, in general, worst where the weather was wet and the attack was early.

1.2.2 Taxonomy of *Phytophthora infestans*

In 1845 the fungus on blighted leaves was described as *Botrytis infestans* although there was much controversy over whether this was the causal organism or a saprophyte (Bourke, 1964). Descriptions of the fungus published by Berkeley in 1846 and by Caspary in 1854, however, placed the fungus in the class Oomycetes and genus *Peronospora* (Lacey, 1962; Agrious, 1978). De Bary (1876) later published a comprehensive study which placed the fungus in

the new genus *Phytophthora* as a result of its special features of an indeterminate sympodial sporangiophore with ovoid, detachable papillate sporangia.

1.2.3 Life cycle

The only U.K. crop hosts of *P. infestans* are the potato (*Solanum tuberosum* L.) and the tomato (*Lycopersicon esculentum* Mill). *P. infestans* can, however, also infect and sporulate on eggplant and many other members of the Solanaceae family including tropical trees and shrubs (Cox & Large, 1960; Thurston & Schults, 1981).

In countries in which the occurrence of oospores is rare or absent potato tubers infected with *P. infestans* are essentially the only source of the fungus for infection in the following season (Boyd, 1980). Infected tubers may be found in dumps of discarded tubers, they may be planted as seed or survive over winter un-harvested (Holmes *et al.*, 1994). De Bary (1876) first demonstrated the invasion of potato sprouts from a diseased seed tuber. The occurrence of this is, however, infrequent. Murphy & McKay (1927) found that blighted seed tubers produced shoots as healthy as those from non-blighted tubers and Hirst & Stedman (1960) found that only 0.79 and 0.52 % of artificially inoculated and naturally infected tubers respectively produced infected shoots. Van der Zaag (1956) found on average one infected shoot per square km with the cultivar De Streek. Although it is widely accepted that such a low frequency of infection is sufficient to carry the disease over from year to year (Robertson, 1991), the presence of an infected shoot may not be essential for inoculum to spread from infected seed tubers. Hirst & Stedman (1960) reported outbreaks of foliar blight, in irrigated plots after heavy rain, which seemed not to originate from blighted shoots and suggested that these might have been initiated by sporangia washed up from diseased seed tubers. The evidence that this can happen is, however, meagre. When Hirst & Stedman (1960) repeated this work in only one out of three years did the race of *P.*

infestans causing the first lesions on leaves match the one used to inoculate the tubers. Lacey (1967b), in irrigation experiments similar to those of Hirst & Stedman and with known races of *P. infestans*, also failed to prove that sporangia from infected seed tubers can infect leaves which are touching the soil.

Sporangia produced on infected shoots lead to localised spread (Hirst, 1955). Long distance spread may not occur until weather conditions become favourable. Once the sporangia are deposited on the leaf or stem surface, they may germinate by means of a germ tube, or more commonly they form about eight biflagellate zoospores that swim freely in water and encyst on solid surfaces. Encysted zoospores can germinate via germ tubes that enter the host via stomata. Usually, however, an appressorium is formed and penetration hyphae enter directly through the cuticle. Once inside the plant, the nonseptate mycelium is both intercellular and intracellular by means of haustoria that extend into cells (Thurston & Schults, 1981).

In wet weather sporangia and zoospores produced on lesions are readily washed to the ground where they contaminate daughter tubers (de Bary, 1863; McIntosh, 1965; Lacey, 1962, 1965; Lapwood, 1977; Sato, 1979). These tubers may also become contaminated with sporangia or zoospores which spread underground from infected daughter tubers to healthy ones (Lacey, 1962; Lapwood, 1962) or at lifting with sporangia or zoospores produced on infected haulm or tubers (Murphy & McKay, 1925; Lapwood, 1961e).

Tuber lesions continue to develop in storage, particularly under damp conditions (Gray, 1965). Dowley & O'Sullivan (1991b) reported that viable sporangia of *P. infestans* were produced on infected potato tubers during storage and that these could infect healthy tubers during handling. Murphy (1921) and Boyd (1972), however, considered that *P. infestans* did not spread from tuber to tuber during storage.

1.2.4 A2 mating type and the production of oospores

P. infestans has two mating types, A1 and A2. Sexual reproduction can only occur between strains which are of different mating types. Until the mid-1980's the A2 mating type had not been recorded in the U.K. and was first isolated in 1984 from imported Egyptian tubers (Shaw *et al.*, 1985). Tantius *et al.* (1986) in a survey of 70 potato crops in England and Wales during 1985 isolated the A2 mating type from five crops in widely separated localities, i.e. Cumbria, Hampshire, Herefordshire, Suffolk and Gwynedd. Malcolmson (1985) found two A2 isolates among stock cultures which had been isolated from a crop of the cultivar Wilja grown commercially in the east of Scotland in 1983. One practical significance of both mating types occurring in the U.K. is that there will be increased genetic variation in the pathogen as a result of sexual reproduction. This may generate strains which are more aggressive or resistant to fungicides. Moreover as oospores have been observed in field samples in the U.K. (Shattock *et al.*, 1990; Pittis & Shattock, 1994) and as both A1 and A2 types were detected at the same source (Tantius *et al.*, 1986) abundant oospores are likely to be produced in the field if the A2 type becomes established (Tantius *et al.*, 1986). Oospores, which are thick-walled, resistant, long-lived spores will provide the fungus with a means of survival in the soil between susceptible crops. In the future this could have a considerable impact on the epidemiology of tuber blight, resulting in the direct infection of tubers, the earlier infection of crops and an increase in the spread of inoculum from tuber to tuber in the soil and from field to field with soil movement. Furthermore, effective control of oospores is likely to be very difficult and this coupled with underground spread of inoculum (Lacey, 1962; Lapwood, 1962) may lead to the frequent occurrence of high incidences of tuber blight in the absence of foliar blight.

1.2.5 Tuber blight

Tubers can become infected by *P. infestans* through eyes, lenticels, wounds (Lacey, 1967a) and directly through the skin (Walmsley-Woodward & Lewis, 1977). The first visible symptom in an infected tuber is a faint light brown line usually extending no more than 3 to 5 mm along the tuber surface. Infections sometimes fail to develop past this point and the phenomenon of lesion arrest in thread-like lesions has been reported (Lapwood 1961d, 1962, 1964; Hirst, *et al.*, 1965; Lacey, 1966). More advanced symptoms are mottled brown or purple irregular lesions on the skin which tend to become sunken during storage. Internally, infected tissue has scattered areas of dry, rusty brown, speckled patches (Plate 1.1). Infected patches generally extend in lobes towards the central pith although on some occasions they fail to penetrate the vascular ring. Blighted tubers frequently sprout in advance of other tubers, although other diseases or mechanical damage often have the same effect (Boyd, 1972).

The infection of daughter tubers by *P. infestans* is the most important phase of the disease economically (Zan, 1962). Tuber blight incidences in the U.K. are, however, generally very variable from crop to crop and from year to year (Cox & Large, 1960). Typically, in a season in which blight is common on foliage more than half the crops in England and Wales suffer little or no loss due to tuber blight whilst in others over 20 % of the tubers may become infected (Large, 1958; Cox & Large, 1960). The economic loss due to tuber blight is, however, greater than simply the percentage of tubers infected. In poor storage conditions secondary invasion by bacteria, principally *Erwinia carotovora*, may cause rapid breakdown of the tissue and further infection of previously uninfected tubers. This can lead to entire crops being lost (Cox & Large, 1960). Although grading prior to storage would remove much infection many infected tubers may be overlooked as lesions are often difficult to observe (Grainger, 1957; Gray, 1965). Furthermore, as has been stated already

infected tubers provide the inoculum that starts outbreaks the following year (Lacey, 1967b).

1.2.6 Foliage and stem blight

Leaf lesions are highly variable depending on host cultivar, relative humidity, temperature and light intensity (Thurston & Schady, 1981). Typically, however, initial symptoms on the leaves are small, pale to dark



Plate 1.1 Tuber blight symptoms

are infected leaves generally abscised prior to the infection reaching the stem. *P. infestans* rapidly colonises young stem tissue which soon turns brown and dies. Progress on mature stems is, however, much slower than on the leaves. Mature stem lesions are typically light to dark brown, long and often restricted to a narrow circumference of the stem (Plate 1.3).

1.3 Factors affecting the incidence of late blight

1.3.1 Influence of the aerial environment

The rate at which haulm blight develops is highly dependent upon the aerial environment with temperature, relative humidity and rainfall having the

infected tubers provide the inoculum that starts outbreaks the following year (Lacey, 1967b).

1.2.6 Foliage and stem blight

Leaf lesions are highly variable depending on host cultivar, relative humidity, temperature and light intensity (Thurston & Schults, 1981). Typically, however, initial symptoms on the leaves are small, pale to dark green, irregularly shaped spots. Under favourable environmental conditions, namely a high humidity with a temperature above 8° C, they grow rapidly to become large, brown to black, necrotic lesions (Plate 1.2). A chlorotic margin is often present outside the area of leaf necrosis. In humid conditions, a delicate white growth of sporangiophores and sporangia develops on the lesion generally, but not always, restricted to the chlorotic areas of recently colonised tissue, and to the underside of the leaf. However, much of the tissue which has been invaded is symptomless and sporangiophores may emerge from the underside of the leaf before chlorotic or necrotic symptoms can be detected by eye.

Stem lesions are initiated by direct infection of the stem or by expansion of a leaf lesion down the petiole (Lapwood, 1961a, b). Weihing & O'Keefe (1962) however, noted that in many cultivars infected leaves generally abscised prior to the infection reaching the stem. *P. infestans* rapidly colonises young stem tissue which soon turns brown and dies. Progress on mature stems is, however, much slower than on the leaves. Mature stem lesions are typically light to dark brown, long and often restricted to a narrow circumference of the stem (Plate 1.3).

1.3 Factors affecting the incidence of late blight

1.3.1 Influence of the aerial environment

The rate at which haulm blight develops is highly dependent upon the aerial environment with temperature, relative humidity and rainfall having the



Plate 1.2 Foliar blight symptoms



Plate 1.3 Stem blight symptoms

greatest influence. Rainfall or dew is essential for the development of an epidemic as the presence of free water on the surface of foliage is necessary for infection by *P. infestans* (Harrison, 1992). Humidity has a major effect on the development of an epidemic of foliar blight because two key stages of the life cycle of *P. infestans*, i.e. the production of sporangia and their germination, are dependent on high humidities. Prolonged survival of sporangia also requires a high humidity (Crosier, 1934; Rotem & Cohen, 1974). Rotem & Cohen (1974), however, showed that low relative humidities increased the rate of sporangia dispersal and Harrison & Lowe (1989) showed that hyphae of *P. infestans* established in leaves continued to invade tissue provided the ambient humidity was between 80 and 100 %.

Temperatures greater than 23°C (Grainger, 1979) or less than 10° C (Smith, 1956) severely restrict the development of a foliar blight epidemic. The effect of favourable temperatures on disease development can, however, be limited by dry weather whilst conversely the beneficial effect of wet weather can be countered by unfavourable temperatures (van Everdingen, reported by Harrison, 1992). Periods of weather during which the aerial environment is favourable for the development of an epidemic in the U.K. may be defined by the Beaumont period (minimum temperature of 10° C and minimum relative humidity of 75 % for 48 hours) and by the Smith period (minimum temperature of 10° C for 48 hours and minimum rh of 90 % for at least 11 hours on each of two consecutive days) (Beaumont, 1947; Smith, 1956).

Climatic conditions, particularly rainfall, also greatly affect the incidence of tuber blight. Lacey (1962, 1965) suggested that rainwater was the most probable way by which spores of *P. infestans* reach the soil to infect tubers and found that at least 6 mm of rainfall was needed to wash sporangia down to the tubers and cause infection. Hirst *et al.* (1965) found little tuber infection without rainfall and although some tubers developed blight after repeated light rain, most infections followed rain of more than 13 mm which coincided with

profuse sporulation on the foliage. Lapwood (1977) concluded that at least 5 mm of continuous rain was needed for tubers to become infected by inoculum produced on foliar blight lesions but that infection was greatest after about 10 mm of rain over a two to three-day period. Lapwood also concluded that rain was essential to keep the tuber surface wet sufficiently long for infection to occur, and that more rain was necessary for tuber infection than for leaf infection, for which a persisting dew was sufficient.

Lacey (1967b) showed that up to 30 times the amount of rain water expected could be caught in rain traps on the stems of some upright cultivars such as King Edward. Infected potato stems in growing crops and in field experiments have been observed (Lapwood, 1964; Lacey, 1967b; Steck, 1988). Both Lapwood (1964) and Lacey (1967b) demonstrated that sporangia of *P. infestans* can be washed down the stems, although never in very high numbers (Lacey, 1967b). Sporangia washed down the stem are more likely to reach the tubers than those deposited on the ridge as they are able to pass down a channel in the soil created by the stem rocking in the wind (Zan, 1962). Indeed Zan considered that, in practice, cracks in the soil surface were necessary for extensive tuber infection and Lacey (1965) showed that sporangia were moved in the soil profile by rainwater, probably through cracks, sometimes to a depth of 19.6 cm. Despite this evidence of the importance of stem blight in the epidemiology of tuber blight there has been no evaluation of the amount of inoculum produced on infected stems or the incidences of tuber blight which might arise from them.

The timing of rainfall relative to the severity of foliar blight also influences the proportion of tubers infected. Lapwood (1977), reviewing work carried out at Rothamsted Experimental Station by Hirst, Stedman, Lacey and Hide over the "blight years" of 1960 to 1967 concluded that, in general, the initial infection of tubers occurred when rain coincided with one to five percent of the foliage being destroyed by blight. Most tuber infection occurred before half of

the foliage was killed by blight and few new infections occurred after 50 to 75 % of the haulm had been destroyed. Tuber slice tests used by Lacey (1962) to determine the infectivity of surface soil showed that soil was most infective when between 25 and 75 % of the haulm had been destroyed. Furthermore, Lacey (1962) reported that two different trials in the same year (1960) gave 27 % and 48 % of infected tubers and concluded that this difference was because most of the rain fell on the former trial after 50 % of the haulm had been destroyed, when sporangia production was declining. In 1960 and 1961, the main increase in the number of tubers infected occurred at about 50 % of the haulm destroyed and in 1963 at about 30 %, probably corresponding to the period of maximum sporangia production (Lapwood, 1977). The timing of rainfall during the day is also likely to affect the incidence of tuber infection. Hirst (1953) showed that, in general, the release of sporangia as a result of a change in humidity commences at 8 am and reaches a peak at 10 am. Sporangia released in this way are, however, unlikely to give rise to tuber infection directly, unless their release coincides with rainfall, since they are more likely to be dispersed onto the haulm or outwith the crop or become desiccated.

The relationship between rainfall, foliar blight and tuber blight is, however, not always straightforward. High incidences of tuber blight have been reported when foliar blight lesions were few or absent (Murphy & McKay, 1927; Lacey, 1962; Lapwood, 1962; Boyd, 1972). Sato (1979) reported that in Japan despite extensive foliar infection and heavy rainfall incidences of tuber blight were sometimes low and concluded that tuber infection was markedly decreased in soils with temperatures above 18° C.

1.3.2 Influence of cultivar

Cultivars have race non-specific and/or race-specific resistance (see 1.4.2) to infection of the haulm or tubers by *P. infestans*. Resistance to *P. infestans* does not, however, greatly affect the commercial success of a cultivar. Only seven of the 24 cultivars fully recommended in the U.K. in 1993 by NIAB had

a rating of 6 or more for foliar blight on the NIAB 1 to 9 scale of increasing resistance. Resistance to tuber blight was poorer with only six cultivars achieving a score of 6 or greater (Anon, 1992). Van Oijen (1991) reported that yield differences between potato cultivars exposed to the same blight pressure are mainly due to the maintenance of the green leaf area in the presence of the disease. Wastie *et al.* (1987a), however, found that some cultivars appeared to be highly tolerant of the effects of loss of leaf area due to *P. infestans* whereas others were highly intolerant and had a much greater yield loss than would be anticipated from their known resistance.

1.3.3 Tuber susceptibility

Bonde *et al.* (1940), Boyd & Henderson (1953) and Boyd (1960) all found that the resistance of whole tubers to infection by *P. infestans* between tuber initiation and harvest increased with maturity. Bjor (1987), however, citing results from cultivar resistance trials, reported that there was no significant effect of the maturity of whole tubers on the susceptibility to *P. infestans*. Boyd & Henderson (1953) found that in tubers which had been scuffed an increase in resistance was associated with an increase in maturity.

The effect that maturity between tuber initiation and harvest has on the susceptibility to *P. infestans* of various infection sites on the tuber has been studied in detail. Boyd & Henderson (1953), Zan (1962), Hirst *et al.* (1965), Lacey (1967a) and Walmsley-Woodward & Lewis (1977) all found an increase in the resistance of lenticels to *P. infestans* with increasing tuber maturity. Hirst *et al.* (1965) showed the increase in resistance of lenticels to be slight, whereas Walmsley-Woodward & Lewis (1977) found that lenticels of cultivars King Edward, Record, Maris Peer and Majestic were almost completely resistant by mid-September. Lacey (1967a), however, reported that greater lenticel resistance associated with tuber maturity varied between years and associated this with the variation in suberisation which occurred in different years. Lacey (1967a) found no close association between increasing maturity and an increase

in the resistance of eyes in the cultivar King Edward, which is in contrast to Walmsley-Woodward & Lewis (1977) who found that increased resistance of eyes was associated with an increase in maturity. Walmsley-Woodward & Lewis (1977) did, however, report that eye resistance showed a temporary decrease during mid-August. This, they concluded, was related to a reduction in levels of antifungal substances within the tuber. Although Zan (1962) and Lacey (1967a) reported that infection through the periderm did not occur, Walmsley-Woodward & Lewis (1977) found that such infection occurred if tubers were immature. The association between growth cracks and tuber maturity is unclear. Growth cracks are potential sites of infection and if they increase in number and size as the tuber matures then there is an increased likelihood of infection being associated with greater maturity. No association has been made between tuber maturity and stolon infection (reported by Walmsley-Woodward & Lewis, 1977). Lohnis (reported by Lacey, 1962) related the development of a cork cambium with an increase in lesion arrest (see section 1.2.5), both of which she associated with an increase in maturity.

Lacey (1962) reported that tubers with symptoms of common scab (*Streptomyces scabies*) or black scurf (*Rhizoctonia solani*) showed no consistent or significant difference in the number of eyes or lenticels which were infected by *P. infestans* compared with healthy tubers. Bonde (reported by Boyd, 1972), however, noted that *P. infestans* sometimes infected tubers through pustules of powdery scab (*Spongospora subterranea*).

1.3.4 Influence of edaphic factors

Lapwood (1962) and Lacey (1967b) demonstrated in field trials the movement of sporangia from inoculated daughter tubers to neighbouring healthy daughter tubers. Lacey inoculated 53 tubers on separate growing plants of the cultivar Ulster Ensign on different dates. At lifting, 42 of the inoculated tubers were identified along with 11 newly infected tubers on eight plants. Similar results were found with the cultivar King Edward. All new infections

were on tubers within 1.3 cm of the one inoculated. The highest incidences of infection occurred when the soil moisture was over 20 % by weight. Lapwood (1965), however, found that 20 % soil moisture content was the lower limit required for tuber infection to occur. Soil texture may also affect the spread of *P. infestans* as both Lacey (1962) and Zan (1962) reported that spores passed more freely through vertical columns of sand than corresponding clay columns.

Lapwood (1961b) and Lacey (1962) found active sporulation by *P. infestans* on the eyes and lenticels of daughter tubers in growing crops of several cultivars, particularly Ulster Ensign. Sometimes detached sporangiophores were detected in the soil surrounding tubers, contaminating areas up to 20 mm². Murphy & McKay (1927) and Lacey (1962) have also reported sporulation on stolons in the soil and Lacey (1962) observed sporulation of *P. infestans* on the roots of potato plants grown in the laboratory. Lacey (1967b) reported that a maximum of 39,400 spores per tuber per day were produced on tubers which had been inoculated in the laboratory two weeks previously. After 2 weeks incubation sporulation rapidly declined. Tubers which were inoculated and then incubated in soil, followed by lifting and washing, produced fewer sporangia after 24 hours incubation than similar tubers incubated in the laboratory. Laboratory tests showed that sporulation was greatest on tubers of Ulster Ensign followed by King Edward, Up-to-Date and Majestic and that maximum sporulation on tubers occurred when the soil moisture was over 20 % by weight (Lacey, 1967b).

Lohnis (reported by Lacey, 1967a) found that more tubers become infected in clay soils than in sandy soils and that in clay soils an increase in resistance was associated with lenticel suberisation. Cox & Large (1960), however, reported that heavy infections occurred occasionally and negligible infection frequently in all soil types.

1.4 Control of late blight

1.4.1 Fungicide treatment

All potato blight fungicides commercially available in the U.K. are marketed as protectant fungicides. They can, however, be classified into five groups based on the mobility of their active ingredients in potato plants (Table 1.1). Differences in the control of foliar and tuber blight due to treatment with fungicides from different groups have frequently been reported (Holmes & Storey, 1962; Bock, 1981; Bain & Holmes, 1990a).

Bruck *et al.* (1980) showed that the phenylamide metalaxyl strongly inhibited sporulation of *P. infestans* at concentrations below 0.1 ppm and Coffey & Young (1984) found that the germination of zoospores was inhibited by metalaxyl. In practice blight fungicides are only applied to the foliage, however, foliar applications (Bruin *et al.*, 1982; Bhatia & Young, 1983) and soil applications (Rowe, 1982) of metalaxyl have given effective control of foliar blight. Stachewicz & Burth (1988) reported that metalaxyl treatment of seed tubers protected the germ primordia of infected tubers as well as lowering haulm susceptibility for up to 70 days after planting. Phenylamide-resistant isolates of *P. infestans* were, however, first detected on the U.K. mainland in 1981 by Holmes & Channon (1984), a year after phenylamide fungicides were made commercially available in the U.K. Subsequent surveys throughout the U.K. by SAC, DANI and ADAS have revealed that resistant isolates are now widespread (Bain & Holmes, 1990a). Furthermore, results from ADAS Trawsgoed in Wales have shown that over the years 1986 to 1989 phenylamides no longer always contributed substantially to the control of tuber or foliar blight (Slawson, unpublished).

In Israel the use of phenylamide fungicides has selected for *P. infestans* isolates which exhibit a stronger competitive ability than the sensitive population (Kadish & Cohen, 1988a; b) but this would not appear to be the case in the U.K. or Eire. Dowley (1987) found phenylamide-sensitive isolates

Table 1.1 Classification of fungicides for the control of late blight of potatoes in the U.K.

Group	mobility of active ingredients	active ingredients
1	systemic (phenylamide) + translaminar + contact	oxadixyl + cymoxanil + mancozeb
2	systemic (phenylamide) + contact	metalaxyl + mancozeb benalaxyl + mancozeb oxadixyl + mancozeb
3	systemic + contact	propamocarb + mancozeb
4	translaminar + contact	cymoxanil + mancozeb cymoxanil + chlorothalonil dimethomorph + mancozeb
5	contacts	dithiocarbamates, chlorothalonil fluazinam, fentin

in Eire to have a higher sporulation capacity and Walker & Cooke (1988) found no difference in sporulation between U.K. resistant and sensitive isolates. Although Walker & Cooke (1988) observed, in tuber inoculation and overwintering experiments, that phenylamide-resistant isolates infected more tubers of the cultivar Kerr's Pink than sensitive ones, fewer tubers infected with phenylamide-resistant isolates survived in storage due to subsequent invasion by soft rotting bacteria. Kozlovskii & Suprun (1987) found no correlation between sensitivity to metalaxyl and pathogenicity of Russian isolates.

No resistance has been reported in *P. infestans* to the translaminar fungicide cymoxanil (Schwinn & Margot, 1991). This absence of insensitivity is most likely because cymoxanil has always been marketed in a mixture, and as cymoxanil has a relatively short persistence of only a few days in plants (Douchet *et al.*, 1977) *P. infestans* has not been exposed to control by cymoxanil alone. In addition, cymoxanil is less fungitoxic than metalaxyl and therefore exerts less of a selection pressure for resistance (Schwinn & Margot, 1991).

Redbond *et al.* (1990) reported that the efficacy of the three-way mixture of mancozeb + oxadixyl + cymoxanil against foliar blight was superior to a two-way mixture of metalaxyl + mancozeb applied at the same intervals. Although the improvement in protection was small at the beginning of the season it increased as the number of applications increased. Samoucha & Gisi (1987a; b) reported that the three-way mix was more effective against both phenylamide-sensitive and resistant isolates of *P. infestans* than would have been expected from the sum of the efficacies of the individual active ingredients, representing a significant level of synergistic interaction. Compared with the two-way mix, the three-way mix has also been shown to reduce the build up of resistance to phenylamides in mixed populations of *P. infestans*. Samoucha & Gisi (1987b) reported that if the initial percentage of resistance was 10 % or higher then the two-way mixture selected a 100 % resistant population after only four to five

sporangial generations. In contrast, mixed populations containing as high as 50 % resistant isolates, remained fairly stable when treated with the three-way mixture. Indeed, Samoucha & Cohen (1988) reported that oxadixyl + mancozeb + cymoxanil mixtures were effective in controlling both phenylamide-resistant and sensitive isolates of *P. infestans* on potatoes in growth chamber studies whereas mixtures without cymoxanil were only effective against the sensitive isolates. Cooke & Little (1992), however, reported that in Northern Ireland three-way mixtures gave no better protection against foliar blight than two-way mixtures. In addition, they found that cymoxanil did not improve the protection of treated plants against metalaxyl-resistant or sensitive isolates when it was applied together with oxadixyl + mancozeb and that there was no evidence of enhanced systemicity or persistence of components of this mixture. There are no reports of the three-way mixture reducing the incidence of tuber blight compared with other fungicide treatments.

Good control of foliar blight with fungicides does not necessary result in good tuber blight control and, as demonstrated by Hirst *et al.* (1965), good control of foliar blight can often lead to high numbers of sporangia being deposited on the soil. Also, there is little information on the effect of different fungicides on the relationship between foliar blight and tuber blight. Bain & Holmes (1990a) reported that although fentin hydroxide gave about equal control of foliar blight as some other fungicides only fentin hydroxide significantly reduced the incidence of tuber blight. The widely accepted mechanism of this improved tuber blight control is that fentin fungicides kill (by inhibiting respiration) spores and mycelium of *P. infestans* in the soil before tuber infection can occur. The scientific evidence for this is, however, meagre and is based largely on the evidence obtained by Aldridge (reported by Corbett, 1974) that fentin compounds are active inhibitors of oxidative phosphorylation in isolated mammalian mitochondria. Fentin fungicides are also reported to inhibit sporulation by *P. infestans* (Schwinn & Margot, 1991).

1.4.2 Breeding for resistance

To improve the resistance of *S. tuberosum* cultivars after the *P. infestans* epidemics during the 19th century, crosses with *S. demissum* from Mexico were made. In addition to race non-specific resistance the progeny of these crosses exhibited race-specific resistance, the genetic basis for which is the gene-for-gene relationship first proposed by Flor (Wastie, 1991). Depending upon which resistance and virulence genes are present in the host and pathogen respectively race-specific resistance will be effective against one or more races of *P. infestans*. It is not effective against races with virulence genes which correspond to the host's resistance genes (R genes) (Wastie, 1991). There are currently eleven known ex-demissum genes. These genes prevent *P. infestans* development following infection by conditioning a hypersensitive necrotic response in the plant cells. However, once cultivars carrying R gene resistance are widely grown and are exposed to a high disease pressure they can become susceptible to the new races of *P. infestans* with novel combinations of virulence genes which they select for (Boyd, 1972). Complex races of *P. infestans* were detected soon after the introduction, in the early part of the 20th century, of cultivars which carried R genes (Roer & Toxopeus, 1961). One example of this was in 1968 when tubers of the cultivar Pentland Dell which were previously resistant due to the cultivar having the resistance genes R₁, R₂ and R₃ became badly blighted over a wide area of the U.K. (Malcolmson, 1969). Within any particular cultivar R gene resistance does not always operate in both the tubers and haulm and in general, the resistance of whole tubers to infection by *P. infestans* is not necessarily related to that of the haulm (Roer & Toxopeus, 1961). There are many complex races of *P. infestans* currently in the U.K., however, as virulence analysis is time consuming and sometimes inaccurate, very little work has been performed to determine their frequency and type (Wastie, 1991).

The limited success of race specific resistance in preventing blight epidemics has led breeders to concentrate on race non-specific resistance and breeding material is generally selected without considering R genes (Wastie, 1991). Race non-specific resistance is a complex of many characteristics that combine to limit the development of all isolates of a given pathogen (Colon & Budding, 1990). Umaerus (reported by Wastie, 1991) found that this resistance inhibited penetration into, and spread of the pathogen through, the plant tissue. In addition, Umaerus found that race non-specific resistance increased the intensity of sporulation and the length of time required to initiate new infections. Many host and environmental factors affect race non-specific resistance. Race non-specific resistance to foliar blight is expressed as factors which affect the ability of *P. infestans* to infect and develop, such as the nature of the leaf surface, the maturity and growth habit of the plant, and its canopy density and structure. Race non-specific resistance to tuber blight includes the depth in the soil at which tubers are produced; for example tubers of the cultivar King Edward are often formed on short stolons near the stem base or near the soil surface (Boyd, 1972) and so are more prone to infection. Cultivars whose tubers are formed early before the risk of blight becomes too great may escape much infection whereas those for which sporangia and zoospores are readily washed into the soil may become heavily infected.

Breeding for resistance in tubers has in general received less attention than breeding for resistance to foliar blight (Wastie, 1991). Langton (reported by Wastie, 1991) suggested that this was due to the belief that a high degree of resistance in the foliage would deny inoculum to the tubers beneath. Toxopeus (1958), however, showed that in practice a slowly developing leaf infection on a resistant cultivar produced inoculum over a much longer period of time than a more rapidly spreading and heavily sporulating infection on a susceptible genotype. Furthermore, as van der Zaag (1959) found that infected tubers of cultivars with good tuber resistance were less likely to produce infected stems,

breeding for tuber resistance may effectively reduce the overall source of the inoculum for the following year.

1.4.3 Forecasting

Older forecasting systems, produced empirically using meteorological records by Beaumont (1947) and Smith (1956), predict the initial occurrence of foliar blight in crops and identify when the first treatment should be applied. These systems are still used in the U.K., e.g. a spray warning is issued by SAC following the occurrence of the first high risk period of the season (Smith or Beaumont periods; see section 1.3.1). If further high risk periods are recorded additional spray warnings are issued with the recommendation that fungicide spray intervals are reduced.

Recently more sophisticated forecasting systems have been produced and the features of these have been reviewed by Harrison (1992). In general, however, it is considered that sophisticated forecasting systems offer little benefit over the simpler systems in predicting the occurrence or development of blight primarily due to the large variation in the micro-climate within the crop canopy (Harrison, 1992). Furthermore, with the exception of one forecasting system developed in Germany by Sparr & Ebert (quoted in Harrison, 1992), they do not forecast the first occurrence or overall incidence of tuber blight.

1.4.4 Cultural control

Careful attention to good agronomic practice may also reduce the overall incidence of blight. Although tubers as deep as 15.2 cm below the crest of the ridge may be infected (Grainger, 1957), Boyd (1972) reported that King Edward tubers in the top 5.1 cm are more frequently infected than those which are deeper. Well-formed ridges may therefore protect many of the developing tubers.

High concentrations of soil nitrogen have been associated with increased severity of foliar blight (Carnegie & Colhoun, 1983) and Herlihy (reported by Boyd, 1972) found that the incidence of infected tubers increased with

increased nitrogen but was reduced with extra phosphate. Nitrogen applications may encourage lush haulm growth and as a result greater sporulation on the haulm, promoting a higher incidence of foliar and tuber blight. In addition, as high soil nitrogen tends to retard tuber initiation and maturity it may indirectly increase the susceptibility of tubers to infection (Boyd, 1972).

Irrigation generally increases the overall incidence of foliar blight (Rotem *et al.*, 1970) and tuber blight (Rotem & Palti, 1969) and should not be applied in excess of that required to maintain adequate soil moisture for crop growth and to prevent common scab.

When the percentage foliar blight reaches a certain level, typically 5 % of the leaf area, dessication of the crop will minimise the risk of tuber infection.

1.4.5 Harvest and post-harvest losses

Murphy (1921) showed that if lifting occurred while the infected haulm was still green, infection of tubers by sporangia from the haulm could be greater than that which occurred before lifting. An interval of 10 to 14 days between death of the haulm and lifting is generally recommended. Murphy & McKay (1925), however, found that tubers became infected even after periods of longer than 14 days and Lacey (1962) reported that although the infectivity of sporangia in soil declined rapidly after 7 days, the sporangia remained infective for up to 32 days after burning off the haulm with sulphuric acid. Cox & Large (1960) concluded that further work was required to determine whether a 10 to 14 day period between death of the haulm and lifting was sufficient. In the U.K. crops are frequently not harvested for 3 weeks after desiccation to allow skin set so that tuber damage inflicted during harvest and moisture loss during storage are reduced.

Blighted tubers may also be a source of sporangia for the infection of healthy tubers during handling and processing. The possibility of eliminating this source, and of reducing infection by other fungal pathogens, by chemical application has been looked at recently by various researchers. Stachewicz &

Burth (1988) reported that, in field and laboratory experiments, applications of metalaxyl within 24 hours of infection resulted in greatly reduced tuber blight and consequently storage losses were considerably less. Turkensteen *et al.* (1990), using a technique of green crop harvesting, found that the application of chlorothalonil to the tubers at lifting reduced the incidence of tuber blight from 6.7 % or 3.7 %, depending on green crop harvesting method used, to 0.7 %. This compared with 0.5 % tuber blight when a conventional herbicide was used to desiccate the haulm. Similar results were found when mancozeb and zineb were applied to the tubers. However, Turkensteen gave no indication of the disease pressure in the trial or of the fungicide programme applied to the haulm. Both factors may affect the success of such an approach. Kassim (1986), Hide & Cayley (1987) and Harrison & Franc (1988) all found that the application of chemicals to tubers post-harvest reduced the incidence of other tuber diseases.

For the control of tuber infection that occurs during processing non-chemical treatments such as the temperature or pH of the wash water used during washing may also be a possibility. Martin *et al.* (1987) reported that water with a pH of 2.4 almost completely inhibited direct and indirect germination of *P. infestans* sporangia. Scholey *et al.* (1968) found that the pre-packing of immature tubers in polyethylene bags led to an increased incidence of tuber rotting by *Erwinia carotovora*. Limited trials by Zan (1962) suggest that reduced oxygen concentration, i.e. 10.5 %, had no effect on the germination of *P. infestans* sporangia, whereas carbon dioxide at twice the normal concentration found in air decreased tuber infection.

1.5 Objectives of the study

The overall objective of this study was to investigate the circumstances under which a high incidence of tuber blight could occur in the apparent absence of foliar blight. The subsidiary objectives were to examine:

- a. the effect of fungicide type on the overall incidence of tuber blight,
- b. whether fungicide type affects the relationship between the amount of blight on the haulm and the number of blighted tubers,
- c. the influence of the infection of potato stems by *P. infestans* on the overall incidence of tuber blight,
- d. the influence of fungicide type on the initiation and development of stem blight and on the subsequent incidence of tuber blight,
- e. the influence of edaphic factors, especially soil moisture content, on the infection of potato tubers by *P. infestans* and on the spread of inoculum underground from infected tubers,
- f. the potential for spread of *P. infestans* during washing of potatoes for the pre-pack market and to evaluate potential control measures.

CHAPTER 2

General materials and methods

2.1 Cultivars used in the experiments

Three potato cultivars were used in these experiments. They were selected on the basis of maturity type and blight resistance rating (Table 2.1). All the cultivars used were on the U.K. Recommended List either for general or special use (Anon, 1992). Seed tubers of the Super Elite grade in the Scottish Seed Potato Classification Scheme were used in most experiments. Progeny tubers produced at Auchincruive from Super Elite seed were used in experiments in which potato tubers were not planted to produce plants.

Table 2.1 Potato cultivars used in the experiments

cultivar	maturity type	blight resistance rating ¹	
		foliage	tuber
Home Guard	early	3	3
King Edward	maincrop	3	3
Kingston	maincrop	4	3

¹1 to 9 scale on which 9 indicates a very high level of resistance

2.2 *P. infestans* isolates and inoculation procedures

2.2.1 Isolation and maintenance of *P. infestans*

Following the surface sterilisation of washed blighted tubers for 30 seconds using cotton wool soaked in industrial methylated spirits (Holmes & Channon, 1984), conical segments were cut from the cortex with a sterilised scalpel. These segments, or in some cases infected leaf material, were placed onto the surface of rye B agar which had been prepared using a technique adapted from that of Hodgson & Grainger (1964). Sixty grams of organic rye grains were partially ground before soaking in 1 l of distilled water at 50° C for 3 hours. The grains were then removed by filtration and 20 g of Oxoid No. 3 agar and

20 g of sucrose added to the filtrate. The pH of the mixture was adjusted to between 6.5 and 7 prior to autoclaving at 120° C and 103 kPa for 15 minutes. The agar was amended with rifamycin (0.03 g), pimarinic acid (0.05 g) and benomyl (Benlate) (0.02 g) before being poured into 5 cm diameter Petri dishes. After inoculation, the agar plates were sealed with Parafilm (American National Can) and kept at 15° C in the dark for approximately 5 days. A sterilised 4 mm diameter cork borer was used to transfer agar plugs with hyphal tips of *P. infestans* from inoculated plates to new agar plates in order to obtain pure cultures. Isolates were sub-cultured onto fresh rye B agar plates at intervals of 3 to 5 weeks throughout the course of these experiments. In order to avoid any loss of pathogenicity, reported to occur as a result of continued culturing on agar media (Hohl, 1991), isolates were grown at intervals on potato leaves.

2.2.2 Preparation of *P. infestans* inoculum

Leaves from plants of the cv. King Edward growing in the glasshouse were placed on corrugated damp tissue in plastic trays with the abaxial (lower) surface uppermost and inoculated with several 30 µl droplets of a dilute suspension of *P. infestans* (mycelia and sporangia) harvested from rye B agar. On occasions, a hand-held, gas-propelled sprayer (Humbrol) was used to apply finer droplets of the *P. infestans* suspensions to the leaves. The trays of leaves were then sealed in polyethylene bags (Samson, Idpe) and incubated at 15° C with a 16-hour daylength (Stewart, 1990) of an approximate light intensity of c. 1000 µmol. m⁻² s⁻¹ for 12 to 17 days. The incubation period was reduced to 9 to 12 days for leaves that had been inoculated using the Humbrol sprayer. Following incubation sporangia were washed from the leaves with distilled water, the concentration checked using a haemocytometer and adjusted to that required. Earlier experiments (results not presented), in accord with Lacey (1962), had shown that tap water was toxic to *P. infestans* sporangia. To ensure a continuity of supply of leaf material seed tubers of the cv. King Edward were

periodically planted in 8 cm square pots containing compost in a blight-free glasshouse. During the winter, plants were exposed to a 16-hour daylength under fluorescent lights with an approximate intensity of $2000 \mu\text{mol. m}^{-2} \text{ s}^{-1}$. On occasions sporangia were produced on rye B agar by incubating inoculated plates at 15°C in the dark for 7 to 19 days.

2.2.3 Isolates used in the experiments

Thirteen isolates of *P. infestans*, all from the U.K., were used in these experiments (Table 2.2). The sensitivity to metalaxyl of the isolates most frequently used was determined using the floating leaf disc technique (Carter *et al.*, 1982).

2.2.4 Inoculation of plants

Tubers were wounded by removing a small piece of tissue from the tuber surface with a flamed scalpel so that cortical tissue was exposed (Wigginton, 1974). The technique used to inoculate the tubers was based on those used by Lacey (1966), Malcolmson (1969) and Bhatia & Young (1985). A single 5 mm square of Whatman No. 1 filter paper, onto which a 20 μl droplet containing between 500 and 1000 *P. infestans* sporangia had been placed, was inserted into the wound. On occasions, a droplet of *P. infestans* sporangia was inserted directly into the wound (Lacey, 1967b). For stem or leaf inoculation the filter paper was sealed onto the stem or leaf surface with Parafilm to prevent drying out. Paper clips were used on the leaves to ensure that the impregnated filter paper and Parafilm remained in place. On occasions, the centres of leaflets were inoculated with a 150 μl droplet of sporangial suspension using a micropipette (Stewart, 1990). As the viability of sporangia decreases with age (unpublished results), inoculations were always performed with inoculum which was less than 20 days old. Harrison (1992) reported that there was a marked decline in the ability of sporangia to release zoospores as cultures of *P. infestans* aged. When zoospores were used for plant inoculation the sporangia

Table 2.2 Isolate identification, source and metalaxyl sensitivity

isolate identification	source		metalaxyl sensitivity (100 ppm)
	potato tissue	location	year
SA91/ SA92	foliage	Strathclyde	1990
TSE1/ TSE2	tuber	Strathclyde	1990
RF1	tuber	Strathclyde	1991
2.1	tuber	U.K.	1992
2.7	foliage	Dumfries	1992
3.8	foliage	U.K.	1992
4.1	tuber	Borders	1992
FI	foliage	Strathclyde	1992
T1A1	tuber	Northern Ireland	1992
T69	tuber	U.K.	1992
TSI	tuber	Strathclyde	1992

suspensions were chilled at 10° C for 2 hours prior to inoculation (Stewart, 1990).

2.3 The soil used in the experiments

A sandy loam of the Bargour series (Table 2.3) was used for all field experiments and all of the glasshouse and growth cabinet experiments in which soil moisture was a factor. Although unusual for Ayrshire, sandy loam soils are typical of much of the lowland potato production area of eastern Scotland (C. Smith, personal communication). To ensure that soil moisture contents in these experiments corresponded to those occurring in a field situation a pressure membrane technique, similar to that described by Heining (1963), was used to determine the moisture contents (% gravimetric water content) of the soil at field capacity and at permanent wilting point (Table 2.4).

Plants used in glasshouse experiments, other than when soil moisture was a factor, were grown in either a 1:1 v/v mixture of sandy loam soil and sphagnum peat (Finnfibre Horticulture) or a 7:3:2 v/v mixture of sandy loam soil, sphagnum peat and Perlite.

2.4 Measurement of *P. infestans* and assessment of blight symptoms

2.4.1 Enzyme-linked immunosorbent assay (ELISA)

P. infestans in plant material (experiment 3 in Chapter 4) and in tuber wash water (experiment 1 in Chapter 6) was assayed using PTA-ELISA as described by Clark & Adams (1977), Moham (1981) and by Harrison *et al.* (1990). Polystyrene immunological microtitre plates (Nunc Immunological II; Gibco Ltd) were used. For each sample, six 200 µl aliquots were placed in predetermined duplicate plate wells leaving the outer plate wells blank. The plates were then incubated at 4° C for 16 hours before washing using an automatic plate washer (Dynatech) which soaked the plates for three consecutive cycles of 60 seconds with PBS-Tween (Appendix 2.1) and with sterile distilled water. The washing process was repeated three times. Once the

Table 2.3 Composition of the Bargour series soil used in field, glasshouse and laboratory experiments

<u>fraction</u>	<u>%</u>
sand (total)	54.3
silt	20.6
clay	25.1
organic matter ¹	(7.7)
organic carbon ¹	(2.3)

¹ percentage of total sand, silt and clay fraction

Table 2.4 Water content of the Bargour series soil at field capacity and at permanent wilting point

<u>soil stratum</u>	<u>gravimetric water content (%)</u>	
	<u>field capacity</u>	<u>permanent wilting point</u>
	<u>(0.05 bar)</u>	<u>(15 bar)</u>
topsoil	26.72	8.21
subsoil	23.87	10.06
mean	25.30	9.14

plates had dried, 200 µl of a 1 in 1000 dilution of polyclonal anti-*P. infestans* gamma globulin in PBS-Tween (supplied by J. Harrison, SCRI) was added to each plate well. The plates were then incubated for 3 hours at 37° C following which they were washed as previously described and then allowed to dry. Two hundred microlitres of goat anti-rabbit gamma globulin-alkaline phosphatase conjugate (Sigma Chemical Company, A-8025) diluted in PBS-Tween at 1 in 8000 was then added to each plate well and the plates incubated for a further 3 hours at 37° C. The plates were then washed and allowed to dry as previously described. Four phosphatase substrate tablets (Sigma Chemical Company, 104 - 105) were then dissolved in 30 ml of substrate buffer (Appendix 2.1) and 200 µl of this suspension were added to each plate well. After 2 hours incubation at 20° C the absorbance value of each well at 405 nm was determined using a Titertek Multiskan Photometer (Flow Laboratories).

2.4.2 Assessment of foliar and stem blight

Foliage disease in field plots was assessed by the same person approximately twice weekly using a modified version of a widely used key (Anon, 1947) (Table 2.5). Stem blight was assessed as the percentage of the surface area of individual stems with symptoms. To confirm that stem and foliage lesions were due to *P. infestans* approximately 5 % of lesions were incubated at 15° C in the dark for c. 48 hours, and then examined for sporangia of *P. infestans*.

2.4.3 Assessment of tuber blight

Tubers were assessed for the blight symptoms described in section 1.2.5. The percentage of blighted tubers at lifting was assessed in terms of both tuber number and weight, but to avoid repetition only the percentages based on tuber number are presented in this thesis. Confirmation that lesions were due to *P. infestans* was obtained by the incubation and examination of tubers as described in section 2.4.2.

Table 2.5 Potato foliage blight assessment key

percentage	description ¹
0	blight not seen in plot
0.025	1 or 2
0.05	3 or 4
0.1	5 to 8
0.2	9 to 16
0.3	17 to 31
0.4	32 to 63
0.5	64 to 127
0.6	128 to 255
0.7	1 or 2
0.8	3 or 4
0.9	5 to 9
1.0	10
5.0	50
25	nearly every leaflet with lesions
50	every plant affected and about one-half of leaf area destroyed by blight
75	approximately three-quarters of the leaf area destroyed by blight
95	only a few leaves left green
100	all leaves dead from blight infections

¹0 - 0.6 % foliar blight based on lesions per plot. 0.7 - 100 % based on number of lesions per plant.

2.5 Field trials: planting and pesticide application

All field trials (experiments) were carried out in a field in which potatoes had not been grown since 1986. Seed tubers were planted using a tractor-mounted Reekie four-row planter. The drills were 70 cm wide with 30 cm between the tubers. Drills were ridged up and the herbicide Opogard (Ciba Agriculture) sprayed before emergence. All fungicides were applied in 300 l of water ha⁻¹ using a Horstine Farmery Powerdrive tractor-mounted sprayer in 1991 and 1992 and a tractor-mounted AZO compressed-air sprayer in 1993. The operating pressure of both sprayers was between 2 and 3 bar which gave a medium spray quality. A 3.5 m spray boom with flat fan nozzles set at 50 cm centres was used in all years. To prevent damage to the trial plots by tractor wheels (reported by Large *et al.*, 1954) fungicides and herbicides were applied from tramlines which bypassed the plots.

2.6 Statistical analyses

The data obtained from all but one of the experiments were continuous. All proportion or percentage data were transformed by means of the angular transformation (i.e. $\arcsin \sqrt{p}$) as used by Roberts (1992). Counts were transformed using the square root transformation (Mead & Curnow, 1983). To test that all transformed data satisfied the necessary assumption of normality (Mead & Curnow, 1983) two diagnostic tests were carried out. Plots of the residuals vs. fitted values were made to demonstrate that the assumption of homogeneity of variance was satisfied. Plots in which the values of the residuals remained relatively constant as the fitted values increased indicated that the transformation was appropriate. Plots of quantile vs. quantile were performed to indicate the normality of the distribution of the residuals. A linear relationship indicated that the residuals were distributed normally. Where the data were not continuous, i.e. experiment 7 in Chapter 6, plotted residuals approximated a normal distribution and further transformations of the data did not improve this distribution. Linear and multiple linear regression were

performed to determine the correlations between different variates. The analyses were performed using Genstat5 (Payne *et al.*, 1987) or Minitab, Version 6.2 (Anon, 1989).

CHAPTER 3

The effect of type of fungicide on the relationship between the amount of blight on the haulm and tuber blight incidence

3.1 Introduction

The relationship between the amount of foliar blight in a crop and the subsequent incidence of tuber blight is complex. Hirst *et al.* (1965) established that when the fungicides copper oxychloride, zineb, maneb and mancozeb were applied to trial plots of the cultivar King Edward the first infected tubers generally occurred when between 1 and 5 % of the foliage was destroyed by *P. infestans* and that most infections arose before 50 % of the haulm had been destroyed. No significant differences in the control of tuber blight by different fungicide types and no effect of fungicide on the relationship between foliar blight and tuber blight were noted. On the other hand high incidences of tuber blight in the absence of foliar blight were observed in some potato crops in the U.K. in 1988 (Bain & Holmes, 1990b). The field experiment in this chapter aimed to investigate to what extent fungicide type affected the relationship between the amount of blight on the haulm and the timing and overall incidence of tuber infection. Fentin hydroxide and metalaxyl + mancozeb were selected because they were reported to give very good control of tuber blight whereas mancozeb was reported to give poor control (Schwinn & Margot, 1991). The mancozeb + oxadixyl + cymoxanil mix was also included, although it was not marketed in the U.K. until 1990.

3.2 Materials and methods

The trial was situated in Diamond Field at Auchincruive and was planted with the cv. Kingston (see section 2.1) on 26 April 1991. Details of the field soil are given in section 2.3. The design of the trial, taken from Fisher & Yates (1963), was intended to ensure that disease pressure from the control plots across the fungicide treated plots was as uniform as possible. The trial consisted of five treatments with six replicate plots, each plot comprising approximately 160 plants in four drills of 40 plants.

Inoculation of the trial with *P. infestans* was carried out on 11 July before any natural infection was observed in the locality. Small blocks of 12 plants at each end of the treatment plots served as infector plots and two plants in every infector plot were inoculated. The first plant was inoculated with nine 150 μ l droplets of an equal mixture of two different metalaxyl-sensitive (ms) isolates (TSE1 & TSE2) (see section 2.2.3) at a concentration of 1.5×10^4 sporangia ml^{-1} and the second plant with one droplet, of the same concentration and volume, of an equal mixture of two different metalaxyl-resistant (mr) isolates (SA91 & SA92). This was intended to give an initial inoculum ratio of 10:90 mr:ms isolates. Immediately following inoculation, the plants were covered with clear polyethylene bags for 24 hours to maintain a high relative humidity and to prevent the inoculum from being washed off. Eight days later the inoculated plants were assessed for infection and 88 % of the plants inoculated with ms isolates and 74 % of those inoculated with the mr isolates were found to be infected. The true ratio of mr:ms isolates at the start of the season was therefore 10:107.

In order to maintain disease pressure during dry periods, overhead irrigation was applied. The system was calibrated to apply 1.3 mm of water per minute. Measurements were made to ensure that the distribution of irrigation water over the trial was approximately even.

The fungicide products used and their rates of application are listed in Table 3.1. There was also an unsprayed (control) treatment. The first fungicide sprays were applied on 3 July, which was just before the plants met across the drills. A modified, tractor-mounted, Horstine Farmery Powerdrive sprayer (see section 2.5) was used to apply products in 300 l of water ha^{-1} . Additional dates of spraying are given in Table 3.2. Towards the end of the season the recommended spray intervals were extended to try to increase the overall amount of blight in the trial. The nearest synoptic weather station, which was

Table 3.1 Fungicide products and rates

product	active ingredient (% a.i.)	product application rate (kg ha ⁻¹)
Dithane 945	mancozeb (80 % w/w)	1.7
Fubol 75	mancozeb + metalaxyl (67.5: 7.5 % w/w)	2.0
Du-ter 50	fentin hydroxide (47.5 % w/w)	0.56
Trustan	cymoxanil + mancozeb + oxadixyl (3.2:56:8 % w/w)	2.5

Table 3.2 Dates of, and intervals between, fungicide applications

spray number	date	interval (days)
1	3 July	
2	16 July	13
3	30 July	14
4	14 August	15
5	30 August	16
6	19 September	20
7	7 October	18

located at Prestwick Airport (approximately 2 miles from Auchincruive), recorded only one high risk period (Smith) during the season, on 28 and 29 August. As the spray interval at the start of this high risk period was 16 days,

the plots were poorly protected at this stage which probably further increased disease pressure within the trial.

The percentage foliar blight per plot was measured approximately twice weekly from the introduction of *P. infestans* onwards, using a modified version of a widely used key (Anon, 1947) (see section 2.4.2). Plants and tubers from each of the treatment plots were destructively sampled on nine occasions throughout the season (Table 3.3). On each date nine plants (three randomly

Table 3.3 Dates of sampling plants and tubers from treatment plots

sample date	weather conditions during lifting
25 July	dry
31 July	dry
8 August	dry
18 August	dry
27 August	rain
5 September	rain
16 September	rain
26 September	dry
2 October	dry

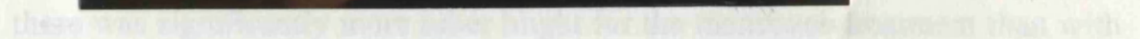
selected blocks of three adjacent plants in the same drill) were dug from each plot. There were guard plants on either side of each block of three plants. Only one stem from each harvested plant was retained for assessment. The percentage surface area of the stem with blight symptoms was either assessed on the day of sampling or within two days. The tubers harvested from any one plot were bulked prior to assessment. On each sampling date tuber blight incidence per plot was assessed by randomly selecting 54 tubers per plot,

washing them, examining them visually and recording the sites of infection and their areas.

In addition, some more detailed assessments were made. To determine if the type of fungicide influenced the number of sporangia produced on lesions, 10 infected leaflets per plot were randomly selected on 19 and 31 August, 7 and 22 September and 1 October. Leaflets were washed with sterile distilled water from a 500 ml capacity polyethylene squeeze bottle to remove as many sporangia as possible and then incubated at 16° C for 48 hours to allow further sporulation. The 10 leaflets per plot were then bulked and washed for 30 seconds in 7 ml of sterile distilled water in order to wash off any newly formed sporangia. Estimates of the concentrations of these sporangia were made from 10 samples of each washing using a haemocytometer slide. The influence of fungicide type on the size of foliar lesions (and on the size of the leaflets) was examined on 31 August and 7 September by photocopying the sampled leaflets prior to the incubation period described above. The leaflet and lesion areas on the photocopies, which could generally be distinguished clearly, were measured at a later date using an area measurement light meter (Plate 3.1). To determine if fungicide type had any effect on sporangial germination, a 2 ml sample from each of the sporangial suspensions produced from blighted leaflets selected on 22 September was diluted to approximately 5×10^3 sporangia ml⁻¹. These samples were chilled at 10° C for 2 hours (Stewart, 1990) and left at 16° C for a further 24 hours to encourage germination. Counts of the numbers of sporangia that had germinated, either directly or by the release of zoospores, and the number that had failed to germinate were made by counting all the sporangia visible under the field of view of the microscope. Twenty different fields of view were examined for each suspension.

The trial was desiccated on 30 September by applying diquat (Reglone) at a rate of 800 g a.i. in 300 l water ha⁻¹.

For all treatments other than those indicated by the asterisk, the growing



and foliar blight lesion areas. Higher tuber blight incidence on the plots was also greater than in the control plots, but not significantly so. Significantly less tuber blight occurred with the mancozeb + metalaxyl, or mancozeb + amoxyl + cymoxanil treatments than in the control plots, but not significantly so, than in the mancozeb + metalaxyl treatment plots. By 2 October, although tuber blight incidences for all treatments were still significantly greater than zero, the significant differences between the treatments had disappeared.

3.3 Results

3.3.1 Effect of fungicide type on the incidence of tuber blight

For all treatments tuber blight appeared relatively early in the growing season, peaked and then, with the exception of the mancozeb + oxadixyl + cymoxanil and the fentin hydroxide treatments declined. Fungicide type, however, affected the date on which incidences of tuber blight significantly greater than zero first occurred. A significant incidence of tuber blight occurred in the control plots on 18 August, in the mancozeb- and mancozeb + metalaxyl-treated plots on 16 September and in the fentin hydroxide- and mancozeb + oxadixyl + cymoxanil-treated plots on 26 September (Fig. 3.1).

Fungicide type affected the date on which the maximum amount of tuber blight occurred and the overall incidence of tuber blight (Fig. 3.1). For the control, mancozeb and mancozeb + metalaxyl treatments incidences of tuber blight peaked on 26 September. Incidences of tuber blight in plots treated with fentin hydroxide or mancozeb + oxadixyl + cymoxanil were, however, highest at the final assessment on 2 October. Overall, for the four fungicides used, fentin hydroxide generally gave the lowest incidence of tuber blight followed by mancozeb + oxadixyl + cymoxanil, mancozeb + metalaxyl and mancozeb. On 16 September significantly more tuber blight was recorded in plots treated with mancozeb than in those treated with fentin hydroxide. On 26 September there was significantly more tuber blight for the mancozeb treatment than with the other fungicides, between which there were no significant differences. On this date the incidence of tuber blight in the mancozeb-treated plots was also greater than in the control plots, but not significantly so. Significantly less tuber blight occurred with the fentin hydroxide and mancozeb + oxadixyl + cymoxanil treatments than in the control and less, but not significantly so, than in the mancozeb + metalaxyl treatment plots. By 2 October, although tuber blight incidences for all treatments were still significantly greater than zero, the significant differences between the treatments had disappeared.

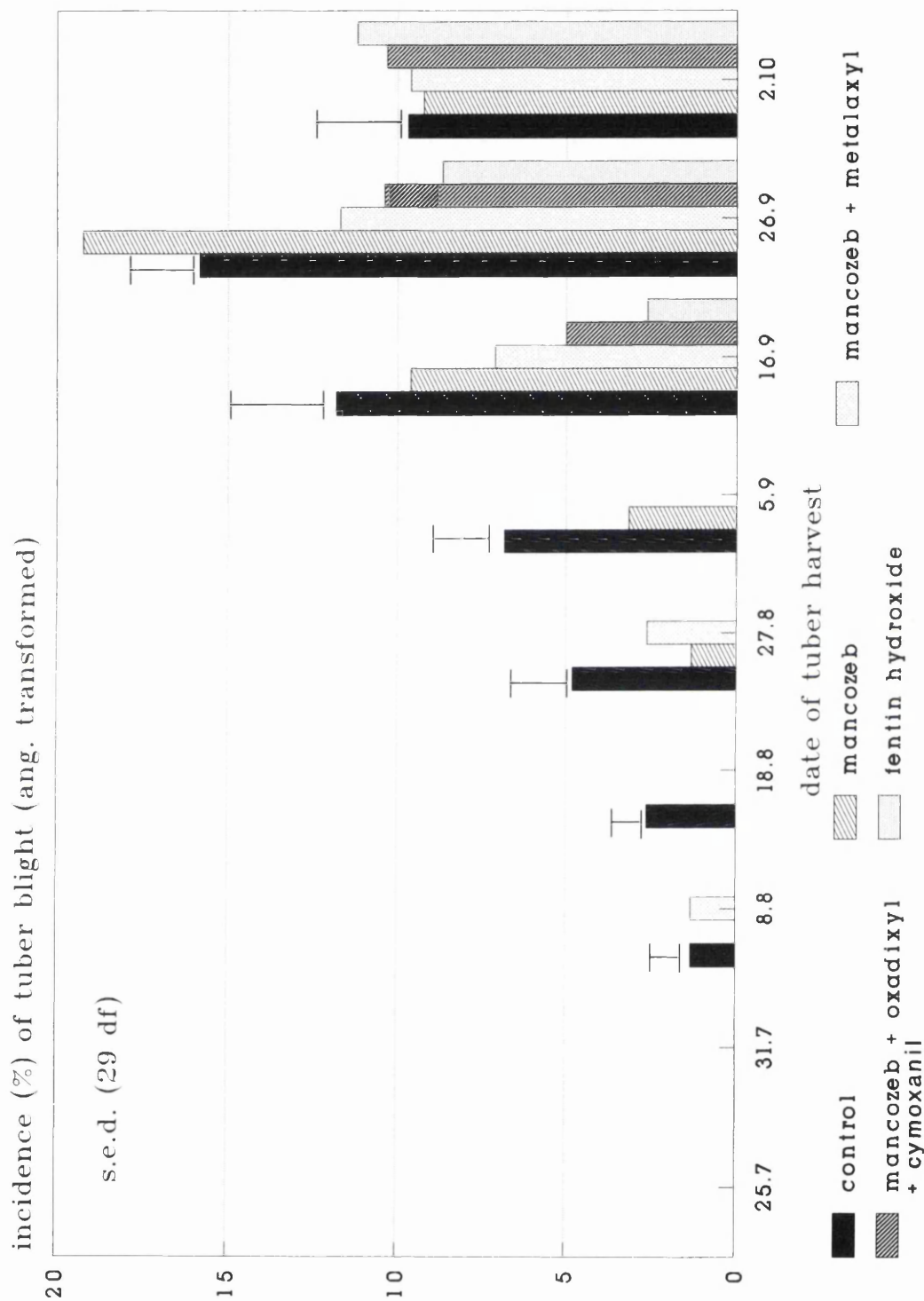


Fig.3.1 The incidence of tuber blight in plots of the cv. Kingston treated with four fungicides and sampled throughout the growing season

3.3.2 The effect of fungicide type on the severity of foliar blight and stem blight when the incidence of tuber blight became significantly greater than zero

All fungicides, compared with the control, increased the percentage foliar and stem blight at which incidences of tuber blight significantly greater than zero occurred. For the four fungicides tested fungicide type had some effect on the amount of stem blight at which significant incidences of tuber infection occurred but relatively little effect on the corresponding percentage foliar blight (Table 3.4). Plots treated with fentin hydroxide, compared with the other fungicide treatments, had a relatively small amount of stem blight when the incidence of tuber blight became significant. However, the corresponding incidence of foliar blight was highest for fentin hydroxide-treated plots.

Fungicide type had only a minor effect on the relationships between the amount of haulm blight or lesion sporulation and tuber blight. On 26 September (when the greatest differences in tuber blight between fungicide treatments were detected) the ranking orders for foliar and stem blight and the cumulative sporangia number for the mancozeb, mancozeb + metalaxyl and mancozeb + oxadixyl + cymoxanil treatments corresponded to that for tuber blight (Fig. 3.2). Fentin hydroxide, however, despite having the lowest incidence of tuber blight did not have the lowest incidence of foliar blight or cumulative sporangia number. Stem area infected was, however, smallest in the plots treated with fentin hydroxide. The incidence of tuber blight in the control plots was intermediate, in spite of the control having the highest values for cumulative sporangia number and foliar and stem blight.

From the data which are available (Appendices 3.1 to 3.5) it is not possible to determine which of the three variates, i.e. foliar or stem blight or cumulative sporangia number, has the greatest influence on the incidence of tuber blight. Although there was some indication that treatment with fentin hydroxide

Table 3.4 The severity of haulm blight when incidences of tuber blight significantly greater than zero were recorded in plots of Kingston treated with four fungicides

treatment	date of first significant incidence of tuber blight	corresponding haulm blight on that date ¹	
		foliar blight	stem blight
control	18 August	3.1	0.0
mancozeb	16 September	9.8	19.0
mancozeb + metalaxyl	16 September	4.3	9.1
mancozeb + oxadixyl + cymoxanil	16 September	8.9	10.0
fentin hydroxide	26 September	11.5	2.9

¹ values calculated from regressions of haulm blight over time

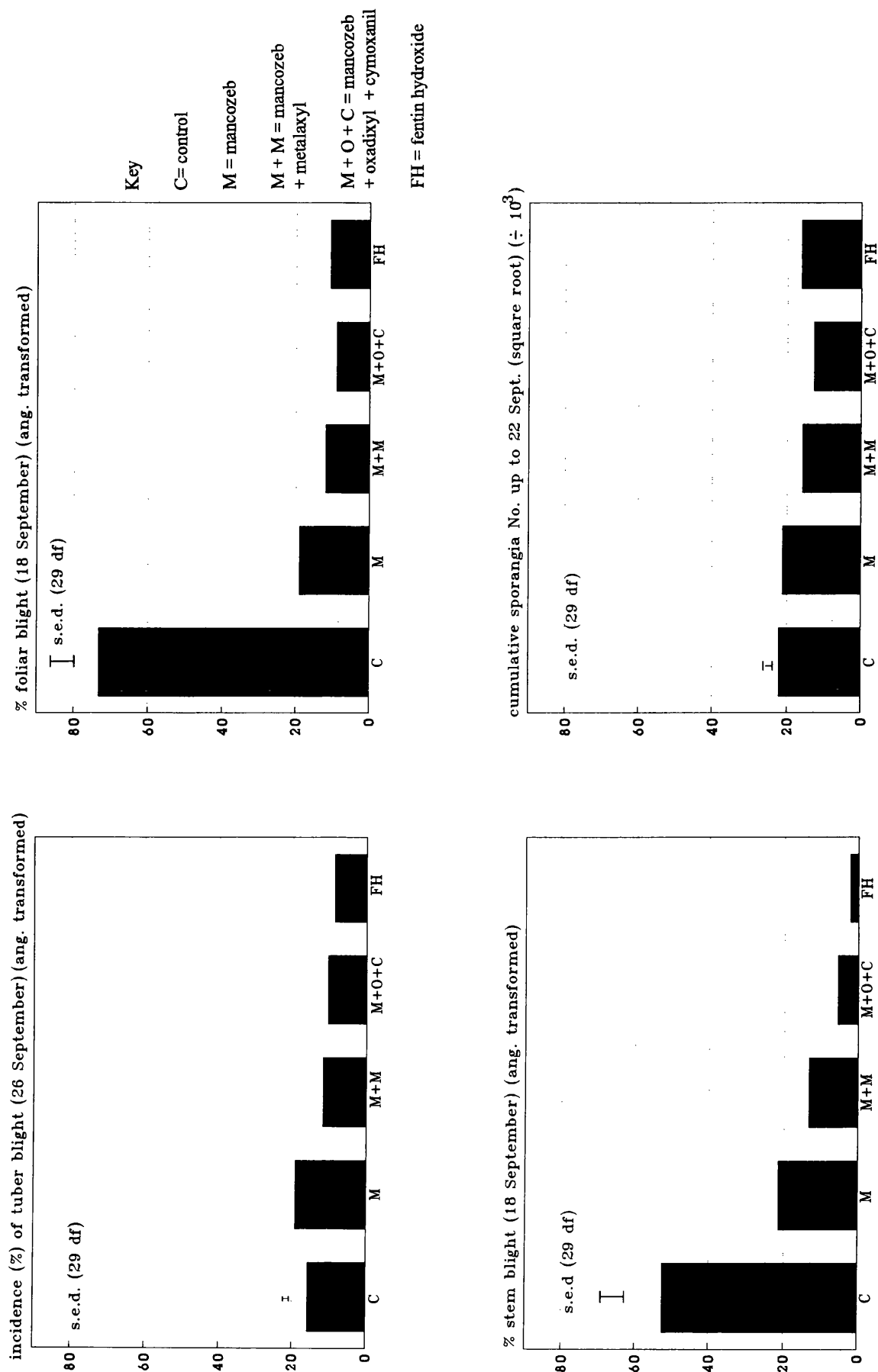


Fig. 3.2 Relationship between tuber blight incidences on 26 September and haulm blight 4 to 8 days earlier

reduced the ability of sporangia to germinate indirectly, incidences of tuber blight for the different fungicides were not, in general, closely related to the germination of sporangia from leaf lesions (Fig. 3.3).

3.3.3 Effect of fungicide type on the incidence of foliar blight and severity of stem blight

Percentages of foliar blight significantly greater than zero first occurred in the control and mancozeb treatment plots on 26 July and in the plots treated with other fungicides on 5 August (Fig. 3.4). All fungicide treatments, between which there were no significant differences, gave significantly less foliar blight than the control from 26 July onwards. Fungicide type affected the severity of stem blight (Fig. 3.5) to a greater extent than it affected the incidence of foliar blight. By the final assessment, on 28 September, the severity of stem blight ranged between 10.1 and 43.3 % for the fentin hydroxide and mancozeb treatments respectively whereas for foliar blight (on 25 September) the corresponding range was 19.6 to 22 % (Figs. 3.4 & 3.5). Overall for the four fungicides used fentin hydroxide generally gave the least severe stem blight followed by mancozeb + oxadixyl + cymoxanil, mancozeb + metalaxyl and mancozeb. Significant stem blight first occurred in the mancozeb-treated plots on 28 August but the value for this treatment was not significantly greater than for other fungicides. Similarly, on 18 September, stem blight significantly greater than zero only occurred in the mancozeb-treated plots. Control of stem blight on 18 September was markedly superior, but not significantly so, in the plots treated with fentin hydroxide or mancozeb + oxadixyl + cymoxanil compared with those treated with mancozeb + metalaxyl (Fig. 3.5). On 28 September significantly more stem blight occurred with the mancozeb and mancozeb + metalaxyl treatments, between which there was no significant difference, than with the fentin hydroxide treatment. Markedly more stem blight was also recorded in the mancozeb- and the mancozeb + metalaxyl-

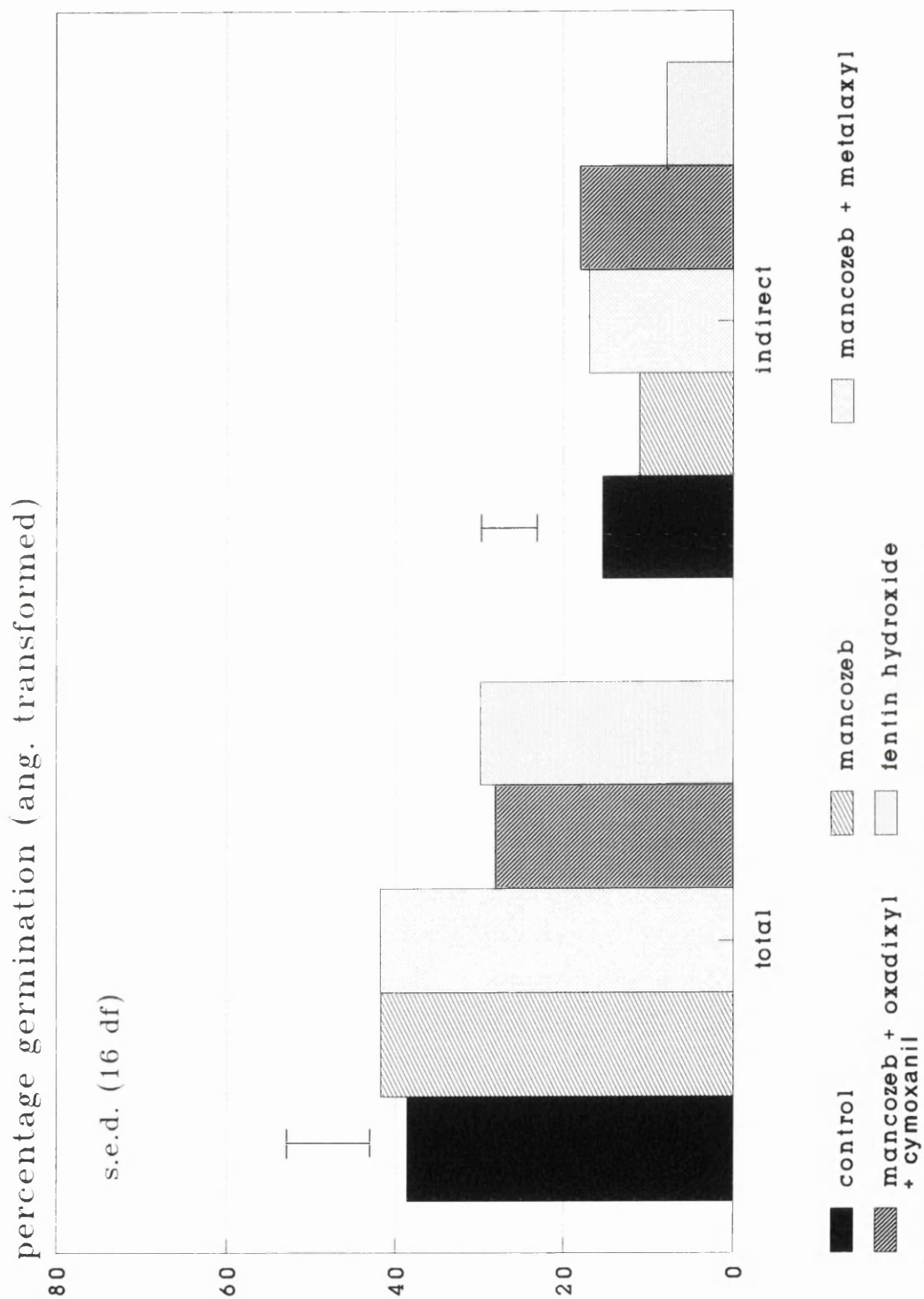


Fig. 3.3 Percentage germination of sporangia from foliage lesions sampled on 22 September from plots of the cv. Kingston treated with four fungicides

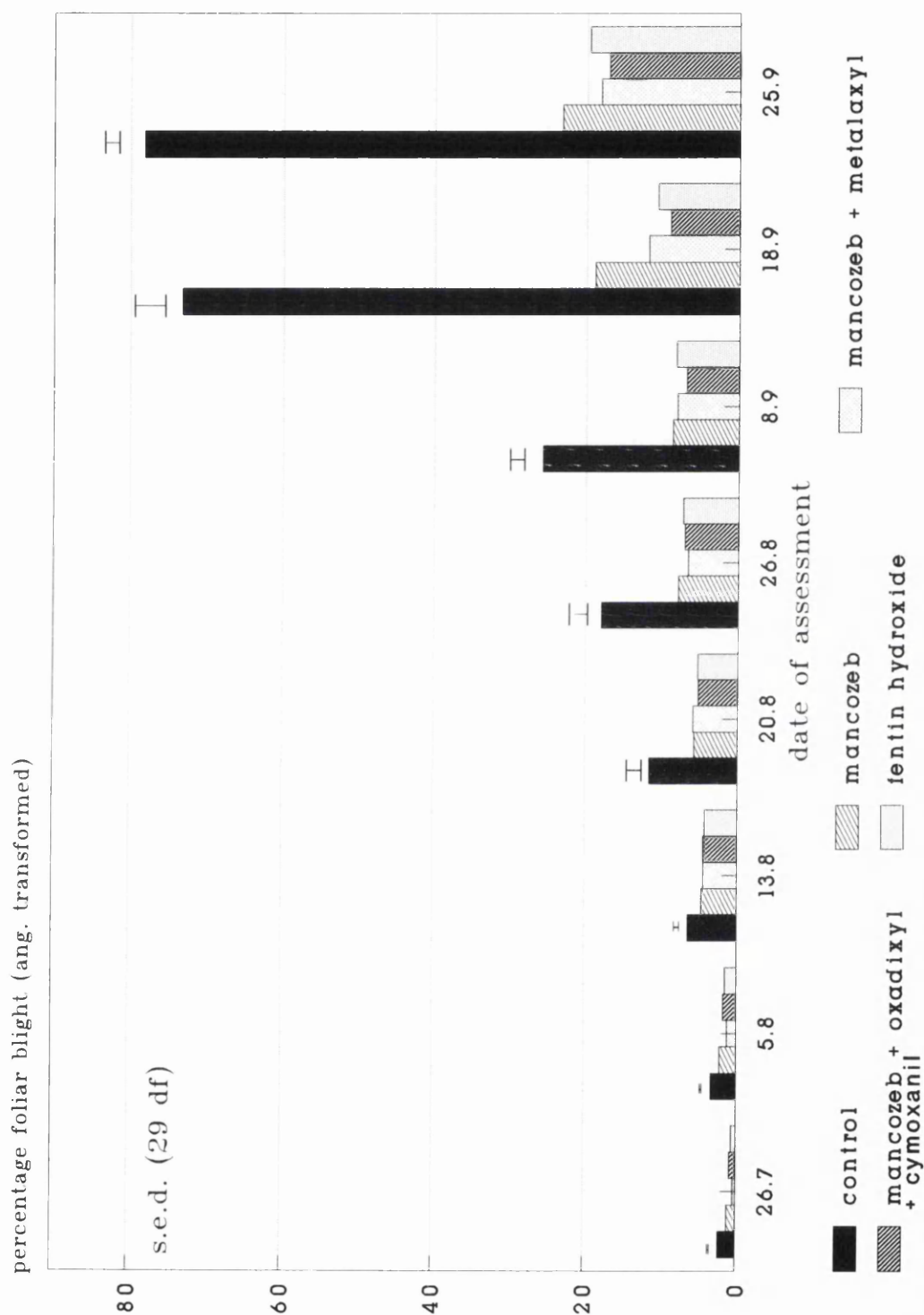


Fig 3.4 The percentage of foliage destroyed by *P. infestans* in plots of the cv. Kingston treated with four fungicides

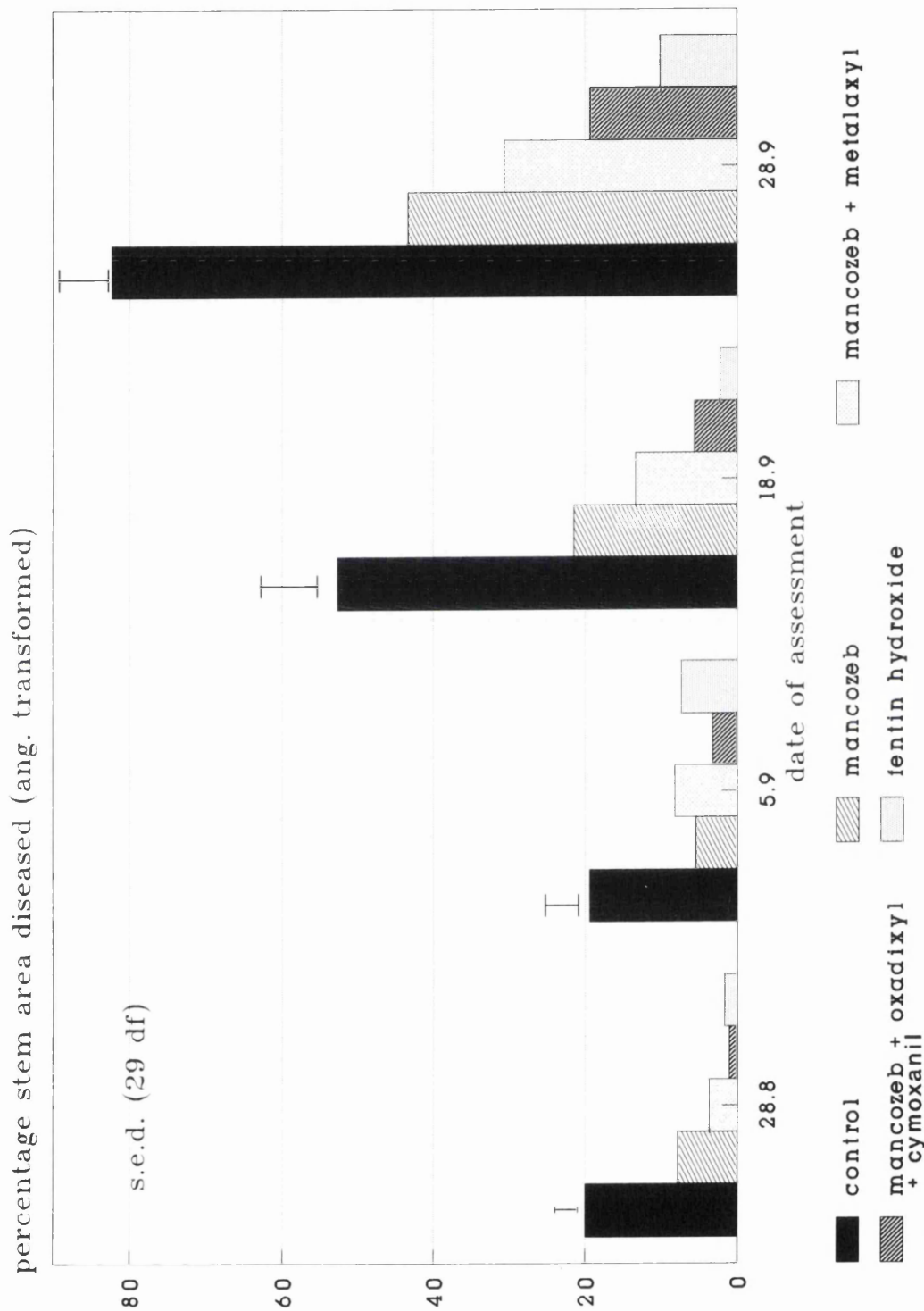


Fig 3.5 The percentage stem area diseased in plots of the cv. Kingston treated with four fungicides

treated plots than in those treated with the mancozeb + oxadixyl + cymoxanil mixture.

3.3.4 Effect of fungicide type on sporangia production on leaf lesions

Fungicide treatment delayed the time of maximum sporulation. Sporangia production on lesions from the control and mancozeb-treated plots, in general, decreased over time, whilst sporulation on lesions from the mancozeb + metalaxyl-treated plots increased over time (Fig. 3.6). Sporangia production on lesions from the mancozeb + oxadixyl + cymoxanil and fentin hydroxide treatments continued to increase until 22 September, after which a slight decline was observed. Significant differences in sporulation, as a result of fungicide treatment, were recorded on the different assessment dates. On 19 August, sporangia production on lesions treated with mancozeb was significantly greater than on those treated with mancozeb + oxadixyl + cymoxanil. Sporangia production for all fungicide-treated lesions was significantly reduced compared with the control. On 31 August sporulation on the lesions from the control and the mancozeb-treated plots was significantly greater than on those from plots treated with fentin hydroxide or mancozeb + oxadixyl + cymoxanil. By 7 September, although significant differences between the four fungicide treatments had disappeared, all fungicides significantly reduced sporangia production compared with the control. On 22 September, sporulation on the control lesions was, however, significantly reduced compared with the fungicide-treated lesions, between which there were no significant differences. On 1 October sporangia production from the mancozeb-treated lesions was significantly reduced compared with those lesions treated with the other fungicides.

From the data that are available, sporangia production within sampling dates was generally related to the area of the lesion (Table 3.5; Fig. 3.6). On 31 August the largest lesions were on plants treated with mancozeb

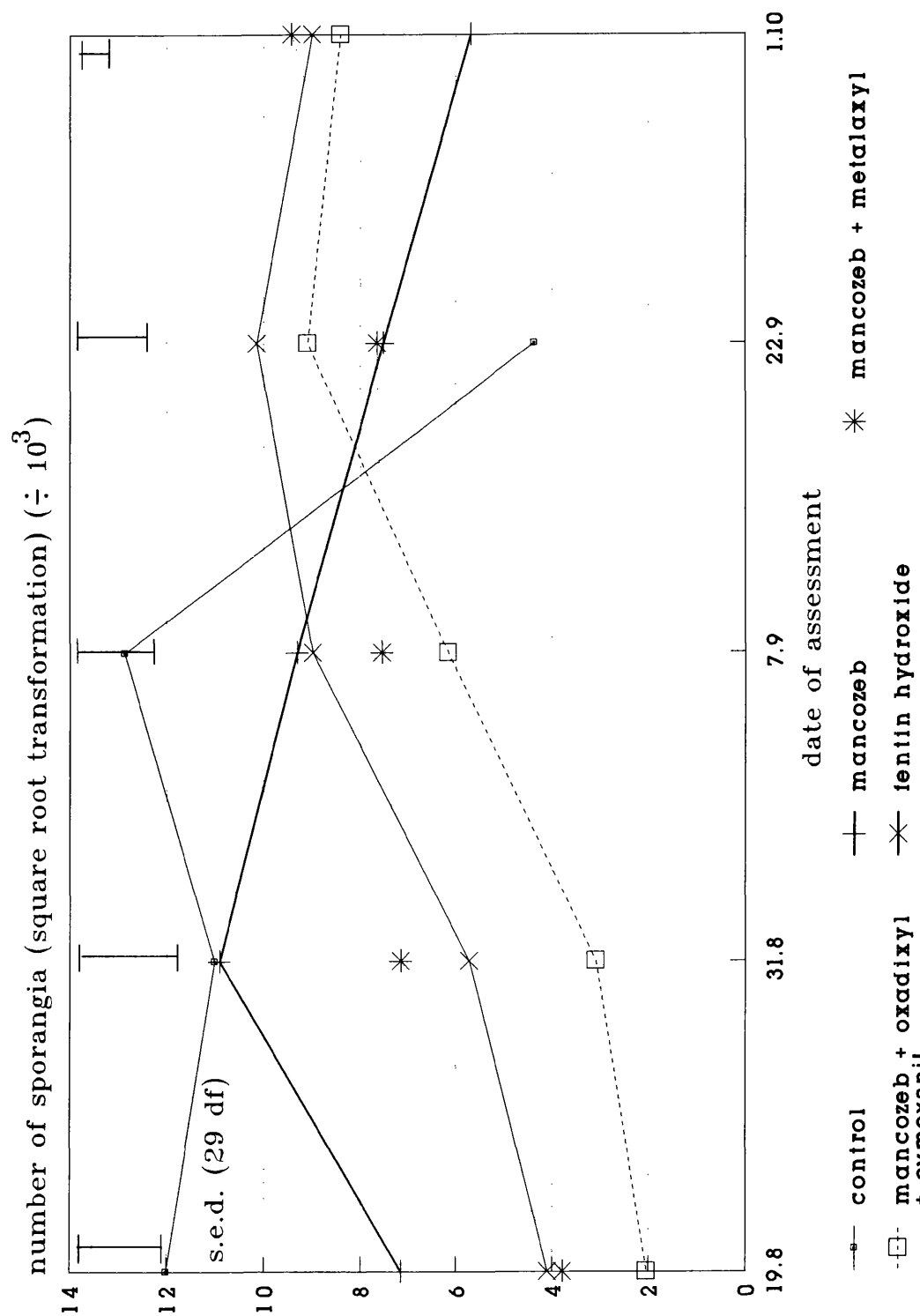


Fig.3.6 Number of sporangia released from lesions selected from field plots of the cv. Kingston treated with four fungicides

+ metalaxyl or mancozeb, or on untreated plants. Sporangia production on 31 August was greater for these three treatments than for fentin hydroxide or mancozeb + oxadixyl + cymoxanil. A significant reduction in sporulation on lesions from plants treated with mancozeb + oxadixyl + cymoxanil compared with those treated with mancozeb was associated with significantly smaller lesions. Significantly reduced sporangia production from lesions treated with

Table 3.5 Area (cm²) of foliar blight lesions on plants of cv. Kingston treated in the field with four fungicides

treatment	sampling date	
	31 August	7 September
control	7.44 (16.57) ¹	3.75 (12.85)
mancozeb	8.65 (22.33)	3.17 (16.00)
fentin hydroxide	5.81 (18.85)	2.59 (16.43)
mancozeb + metalaxyl	8.67 (22.74)	2.68 (17.53)
mancozeb + oxadixyl + cymoxanil	4.13 (16.87)	2.42 (18.10)
s.e.d. (29 df)	1.97 (2.52)	0.40 (1.98)

¹ values in parentheses are corresponding leaflet areas (cm²)

mancozeb + oxadixyl + cymoxanil compared with control lesions, and from fentin hydroxide treated-lesions compared with control or mancozeb-treated lesions, was related to smaller, but not significantly smaller, lesions. Although the lesions from plants treated with mancozeb + metalaxyl were significantly larger than those treated with mancozeb + oxadixyl + cymoxanil they did not produce a significantly greater number of sporangia. The ranking orders of lesion area and sporangia production from lesions on 7 September were generally similar. A significant reduction in sporulation on lesions from the

fungicide-treated plots, compared with the control, was associated with a reduced lesion size. Only the lesions from plots treated with mancozeb + oxadixyl + cymoxanil, fentin hydroxide and mancozeb + metalaxyl were, however, significantly smaller than control lesions.

Checks were made to determine if differences in foliar lesion area between treatments were genuine or an artefact of fungicide influencing leaflet size. On 31 August the ranking orders of the fungicide treatments for lesion and leaflet area were generally similar. For example, significantly smaller lesions on plants treated with mancozeb + oxadixyl + cymoxanil compared with those treated with mancozeb or mancozeb + metalaxyl were associated with significantly smaller leaflets (Table 3.5). On 7 September, however, the ranking orders for lesion and leaflet area were not related. There were no significant differences in leaflet area between fungicide treatments on 7 September.

3.4 Discussion

Fungicide type affected the incidence of tuber blight and fentin hydroxide generally gave the lowest incidence, with the exception of the final assessment. This result is in general agreement with the findings of Last (1961), Holmes & Storely (1962), Pieters (1962), Jarvis *et al.* (1967), Bock (1981) and Bain & Holmes (1990a) that fentin fungicides can be very effective at controlling tuber blight. Holmes & Storely (1962) proposed that significant reductions in the incidence of tuber blight for fentin acetate compared with copper oxychloride, copper hydroxide, zineb and maneb were attributable to a suppressive effect on foliar sporulation or to protection of the tubers by the fungicide reaching the soil. Treatment of soil with fentin fungicides has, however, been only partly successful in reducing the incidence of tuber blight. Applications of fentin acetate to the soil significantly reduced tuber blight in only one out of two years (McIntosh, 1965, 1966) and only in one of two experiments conducted

during 1962 by Bruin (reported by Bock, 1981). Schwinn & Margot (1991) reported that fentin hydroxide inhibits the release and germination of zoospores which might also explain the lower incidences of tuber blight. The results reported here also show that sporangia harvested from lesions treated with fentin hydroxide, compared with lesions treated with other fungicides, have a reduced ability to germinate indirectly.

Foliar applications of the phenylamide metalaxyl have been reported to protect tubers from infection by *P. infestans* (Bhatia *et al.*, 1979; Smith, 1979; Cooke *et al.*, 1981; Bruin *et al.*, 1982; Bhatia & Young, 1983; Cooke & Little, 1986, unpublished). Similarly, Easton & Nagle (1985) found that metalaxyl, when applied either to the foliage or to the soil, significantly reduced the incidence of tuber blight. Bruck *et al.* (1980) reported that metalaxyl significantly reduced the sporulation capacity of leaf lesions and Bock (1981) proposed that the protection of tubers was due to metalaxyl dramatically reducing the number of zoospores being produced on foliar lesions. In addition Bruin *et al.* (1982) and Barak *et al.* (1984) detected biologically active residues of metalaxyl, of 0.02 to 0.04 and 0.002 to 0.01 ppm respectively, in tubers following foliar applications. Barak *et al.* (1984) suggested that this conferred on the tubers general resistance to fungal pathogens through the stimulation of the host's resistance. In the results reported here, however, reductions in the incidence of tuber blight in the plots treated with mancozeb + metalaxyl compared with those treated with mancozeb alone were generally modest. These results indicate that the contribution of metalaxyl to the control of tuber blight in situations where a significant proportion of phenylamide-resistant *P. infestans* is present is limited. Slawson (unpublished) showed that since 1984 the control of tuber blight by metalaxyl at the Trawsgoed Experimental Station in Wales has been minimal and recent surveys by SAC and the Department of Agriculture, Ireland have shown that the occurrence of

phenylamide resistant isolates in the U.K. and Ireland is widespread (Bain & Holmes, 1990b; Dowley & O'Sullivan, 1991a).

In the trial reported here there was little difference in the incidence of tuber blight for mancozeb + oxadixyl + cymoxanil compared with the mancozeb + metalaxyl treatment. These results are, however, only for one year and, as there is no other published data on the efficacy of the three-way mix in tuber blight control, further work is needed to confirm these results.

Holmes & Storey (1962) found in trials over 4 years that tuber blight incidences in plots treated with the dithiocarbamate fungicides zineb and maneb were essentially the same as in the control plots. In the trials reported here mancozeb gave very poor control of tuber blight and at one sampling time the incidence was greater than in the control plots. This is in agreement with results from Bain & Holmes (1990a) which showed that in trials in two years incidences of tuber blight in mancozeb-treated plots were higher than in the unsprayed plots. Likewise, Lloyd *et al.* (1962) found that treatment with zineb increased the incidence of tuber blight compared with the untreated control and Brase & Baumann (reported by Bock, 1981) found in three years that the incidence of tuber blight in plots treated with dithiocarbamate fungicides was greater than in control plots. The results reported here suggest that higher incidences of tuber blight in plots treated with mancozeb compared with the untreated control may be due to the limited effect of mancozeb, compared with other fungicides, on sporulation by *P. infestans*. With the exception of mancozeb, all fungicide treatments delayed the peak sporulation on leaf lesions. This may have led to a reduced incidence of tuber infection since tuber resistance to infection increases with tuber maturity. Fungicides maintain green leaf area, and therefore sporangia production, for longer therefore the risk of tuber infection in plots treated with fungicides compared with un-treated plots is increased.

At the end of the season incidences of tuber blight declined in all of the treatment plots with the exception of those treated with mancozeb + oxadixyl + cymoxanil or fentin hydroxide. This decline, which was most pronounced in the control plots and in those treated with mancozeb, was probably the result of blighted tubers breaking down. Indeed lesions on the tubers from the control and mancozeb-treated plots were observed to be far larger (unpublished results), possibly because they had been infected earlier or because they had been exposed to a higher concentration of inoculum. In addition, by 2 October fewer new infections of healthy tubers probably occurred in these plots as the remaining healthy tubers were less accessible to the inoculum released from the foliage or stems and probably less susceptible to infection (see section 1.3.3). Furthermore, results from this experiment have shown that less inoculum was produced at the end of the season on haulm lesions from the control plots and from those treated with mancozeb. Results from field trials carried out over 4 years by Hirst *et al.* (1965) also showed that in some years the incidence of tuber blight in both fungicide-treated and untreated plots decreased at later sampling dates.

The results of this field experiment have shown that even when very different types of blight fungicide were used they did not greatly influence the relationship between the incidence of foliar blight and the initial occurrence of tuber blight. This agrees with the conclusion reached by Hirst *et al.* (1965), using contact fungicides only, that fungicide type had little effect on the incidence of foliar blight at which tuber infection first occurred. It therefore seems unlikely that the occurrence of tuber blight in the absence of foliar blight (Murphy & McKay, 1927; Grainger, 1957; Hirst & Stedman, 1962; Lacey, 1962; Boyd, 1972) is due to fungicide type altering the epidemiology of the disease. The results reported here have, however, shown that fentin hydroxide, compared with the other fungicide treatments, dramatically reduced the severity of stem blight at which tuber infection occurred. These results are,

however, only from a single year, in which conditions were generally not favourable for the spread of *P. infestans*, and as a result incidences of haulm blight were relatively low for all fungicide treatments. A season more conducive to haulm blight may have resulted in a greater influence of fungicide on the relationship between the incidence of haulm blight and the timing of the initial occurrence of tuber blight. In addition, comparison of the results from this experiment is complicated as fentin hydroxide and mancozeb + oxadixyl + cymoxanil compared with mancozeb and mancozeb + metalaxyl delayed the occurrence of significant incidences of tuber blight by 10 days. All fungicide treatments increased the percentage of foliar blight and severity of stem blight at which tuber infection arose compared with the control. This is in agreement with the results of Hirst *et al.* (1965) which showed that in untreated plots the first tubers became infected when only 1-2 % of the unprotected foliage had symptoms. Tuber infections in plots treated with copper oxychloride, zineb, maneb or mancozeb were, however, seldom abundant until at least 5 % of the foliage was blighted. Hirst *et al.* (1965) concluded that this was presumably because the fungicides killed many sporangia before they reached the tubers.

When the percentage foliar blight was greatest, fungicide type had only a minor influence on the relationship between foliar blight and tuber blight. On 26 September significantly lower incidences of tuber blight in the plots treated with fentin hydroxide, mancozeb + oxadixyl + cymoxanil or mancozeb + metalaxyl compared with those treated with mancozeb were not associated with significantly less foliar blight. This is in agreement with the results of Holmes & Storey (1962) which showed that a significant reduction in the incidence of tuber blight in plots treated with fentin acetate compared with those treated with zineb and maneb was not associated with a reduction in the incidence of foliar blight. Bain & Holmes (1990b) also found that although fentin hydroxide and mancozeb gave about equal control of foliar blight, the incidence of tuber blight was only significantly reduced in the plots treated

with fentin hydroxide. Bock (1981), however, reported that fentin hydroxide and fentin acetate showed superior control of both foliar and tuber blight compared with dithiocarbamate fungicides. The results reported here suggest that the significantly better control of tuber blight by fentin hydroxide compared with mancozeb was, at least in part, a result of the superior control of stem blight. The potential for high incidences of tuber blight to arise from sporangia produced on stem lesions has been noted previously (Lapwood, 1964, 1965, 1977). The influence of fungicide treatment on the incidence of stem blight is covered in more detail in Chapter 4.

The results reported here have shown for the first time that fungicide treatment affected the severity of stem blight to a greater extent than it affected the percentage of foliar blight. Although no significant differences in foliar blight were recorded, the severity of stem blight was on three occasions significantly less for fentin hydroxide than for mancozeb. The control of stem blight was also markedly superior in plots treated with mancozeb + oxadixyl + cymoxanil compared with mancozeb + metalaxyl. These results suggest that separate blight assessments for the foliage and stems may be necessary in fungicide evaluation trials and perhaps in assessments of the resistance of breeding material.

Treatment with mancozeb + oxadixyl + cymoxanil generally gave little improvement in the control of foliar blight compared with mancozeb + metalaxyl. Although Samoucha & Gisi (1987a), Samoucha & Cohen (1988), Samoucha *et al.* (1988) and Redbond *et al.* (1990) reported that the efficacy of the three-way phenylamide mixture against foliar blight was superior to that of the two-way mix, the results obtained in this experiment are in accord with those obtained in Northern Ireland by Cooke & Little (1992) that the addition of cymoxanil to oxadixyl + mancozeb gave no better control of foliar blight than the two-way mixture.

Fungicide type affected sporangia production on blight lesions. Fentin hydroxide, mancozeb + metalaxyl and mancozeb + oxadixyl + cymoxanil, compared with mancozeb, delayed the maximum sporulation on leaf lesions by 3 to 4 weeks. Cumulative sporangia numbers were, however, generally higher in plots treated with fentin hydroxide than in those treated with mancozeb + oxadixyl + cymoxanil or mancozeb + metalaxyl, although incidences of tuber blight were generally lower with fentin hydroxide.

Phytotoxic damage to potato foliage by fentin fungicides has been reported. Bock (1981) reported that foliar applications of fentin hydroxide were phytotoxic and Pieters (1962) noticed severe leaf burns after seven applications of fentin acetate, although applications of fentin hydroxide showed very little phytotoxicity. There was, however, no indication of any phytotoxicity of the fungicides used in the field experiment reported here.

CHAPTER 4

The role of stem blight in the infection of daughter tubers by *P. infestans*

4.1 Introduction

As stated in the previous chapter, Hirst *et al.* (1965) found that in fungicide treated field trials the first blighted tubers were found when the percentage of leaf area infected reached about 1 to 5 % and that most tuber infections had occurred by the time 50 % foliar blight was reached. The relationship between foliar blight and tuber blight is not, however, always straightforward and incidences of up to 30 % tuber blight have been reported when foliar blight lesions were few (Cotton, 1922; Lapwood, 1962, 1964) or absent (Murphy & McKay, 1927; Grainger, 1957; Hirst & Stedman, 1962; Lacey, 1962; Boyd, 1972). One possible explanation for this phenomenon is that under certain circumstances stem lesions are able to initiate high incidences of tuber blight. Indeed the potential of the inoculum produced on infected stems to cause tuber blight has been noted (Lapwood, 1964, 1965, 1977; Lacey, 1966). Although the number of blighted stems which develop from blighted seed tubers is very low (Boyd, 1980), stem lesions which originate from a different inoculum source have frequently been reported in the growing crop (Crosier, 1934; van der Zaag, 1956; Lapwood, 1961a, b, c, 1964; Zan, 1962; Lacey, 1966; Rotem & Cohen, 1974; Kable & MacKenzie, 1980; Steck, 1988). High incidences of sporulating lesions on stems have also been observed in field trials on occasions (Bain, personal communication) and Robertson (1991) reported that sporangia were produced at stomata on stem lesions. There has, however, been little research to determine which factors influence the infection of stems or to examine the development of stem lesions under a closed foliage canopy where penetration by fungicides is limited. Similarly the incidence of tuber blight which might result from inoculum produced on blighted stems has not been evaluated.

One objective of Chapter 4 was to investigate the rate of mycelial growth in the stems of potato plants inoculated with *P. infestans* (experiment 1) and the associated production of sporangia (experiment 2). Further objectives were to

determine, in glasshouse and field experiments, the incidence of tuber blight resulting from inoculum produced on infected stems and to determine the influence of rainfall and soil moisture on this incidence (experiments 3 to 5). Further glasshouse and field experiments (6 & 7) investigated the effect of fungicide type on the initiation and development of stem blight and on the subsequent incidence of tuber blight.

4.2 Materials and methods

4.2.1 Experiments 1 & 2 The development of stem lesions and sporangia production by *P. infestans*

Experiment 1 The growth of *P. infestans* within stems of potato plants

The stems of five week-old plants of the cv. Home Guard, grown in the glasshouse in small pots containing a sandy loam and sphagnum peat mixture (see section 2.3) were inoculated with sporangia of *P. infestans*, isolate SA91 (see section 2.2.3), which had been washed, with sterile distilled water, from potato leaves inoculated 9 days earlier. Non-inoculated plants served as controls. In total, six plants were inoculated approximately 5 cm above the soil surface using the technique described in section 2.2.4. Following inoculation the plants were placed in a growth cabinet at 10° C with a 16-hour daylength of approximate light intensity c. 1000 $\mu\text{mol. m}^{-2} \text{s}^{-1}$ and a low relative humidity of c. 70 %. The progression of *P. infestans* in the inoculated stem tissue was monitored using ELISA. Samples were prepared as detailed by Moham (1981), Harrison *et al.* (1990) and Harrison & Perry (1990). Seven days after inoculation and before any lesions were visible the inoculated stems were sectioned transversely with a sterilised scalpel into six separate 10 mm lengths from the point of inoculation upwards. Sections taken from the same distance above the inoculation point were bulked and then ground for 5 minutes using a pestle and mortar. Following grinding 0.5 g of tissue from each sample were extracted and suspended in 5 ml of carbonate buffer (see appendix 2.1).

Samples of non-inoculated potato stem were similarly prepared. All of the suspensions were then sonicated (M.S.E. Soniprep 150) for 10 minutes (55 seconds on & 5 seconds off in each minute) at 18 amplitude modules (Am). Following sonication 0.05 g of polyclar (Sigma Chemical Company) and 0.95g of sodium metabisulphate (Sigma Chemical Company) were added to each sample before leaving the samples to incubate for 2 hours at room temperature. After incubation the samples were centrifuged for 10 minutes at 18000 rpm. Replicates of extracted supernatant were then diluted by 1 in 500 in carbonate buffer before assaying by ELISA as described in section 2.4.1. The increase in absorbance readings due to *P. infestans* in the test samples was calculated by subtracting the mean absorbance reading of the control samples from the mean absorbance readings of the test samples. The concentration of *P. infestans* in the test samples was then calculated by comparing the mean sample absorbance value (A_{405}) (see section 2.4.1) due to *P. infestans* with those given by a series of five-fold dilutions of a 0.75 mg ml^{-1} extract of *P. infestans* mycelium, isolate SA91, peeled from 10 day-old cultures on rye B agar ground and sonicated as described above.

Histological studies were carried out in order to determine in which stem tissue *P. infestans* was present. Inoculated stems were cross sectioned either freehand or with a microtome after fixing in formalin-acetic acid-alcohol as described by O'Brian & McCully (1981). Sectioned samples were then stained in either glycerine-toluidine blue O (Hächler & Hohl, 1984) or acid fuchsin (Crucefix *et al.*, 1984) prior to microscopic examination.

Experiment 2 Sporangia production in relation to stem lesion development

The main stem on nine lots of six plants of the cv. Home Guard grown in a sandy loam and sphagnum peat mixture (see section 2.3) was inoculated and the plants incubated as in experiment 1. A similar number of control plants were inoculated with washings from non-inoculated leaves which had also been incubated for 9 days. At 7, 14, 22, 28, 34 and 40 days post-inoculation one

plant per lot was selected at random. Stem lesion length, visible from the second assessment date onwards, was measured from the point of infection to its highest point. Each plant was then sealed in a polyethylene bag and incubated at 10° C at a relative humidity close to 100 % for 48 hours. Following incubation the length of stem on which sporulation was visible was measured and any leaves which were attached to this area were removed. As earlier attempts to use a stem trap (Corke, 1958) to collect water coming down the stem were unsuccessful, inoculated stems were cut off at the base and suspended with fine pins above test-tubes. The equivalent of 4 mm of rainfall over two 5 minute periods, separated by an interval of 5 minutes, was then applied to the cut stems using a misting bench. The volume of water caught below each stem was measured and the concentration of sporangia assessed using a haemocytometer slide. This experiment was repeated once using similar techniques.

4.2.2 Experiments 3 to 5 Stem lesions as a source of inoculum for tuber infection

Experiment 3 Stem lesion development and sporulation on glass-house grown plants

The main stem of 24 week-old plants of the cv. Home Guard grown in the sandy loam and sphagnum peat mixture (see section 2.3) was inoculated as in experiment 1. A similar number of control plants were inoculated with washings from non-inoculated leaves which had been incubated for 9 days. Following inoculation the plants were kept in a glasshouse with an ambient temperature of between 16 and 26° C. The foliage of the plants was kept dry by watering the pots from below and the soil in the pots was kept close to field capacity. Ten, 22 and 30 days after inoculation four lots of two plants were selected at random and the stem lesion length measured. The plants were then sealed in polyethylene bags and incubated at a humidity close to 100 % for 48 hours. After incubation the simulated rainfall treatment was carried out as in

experiment 2 but a total of 6 mm of rainfall was applied to the plants which were still in their pots. To check for leaflet infection the haulm of the plants was removed following misting and the lower leaves still attached to the inoculated stem were incubated at 16° C for c. 24 hours. The soil containing the tubers was maintained at approximately field capacity for a further 10 days after which the tubers were harvested and assessed for infection by *P. infestans*. This experiment was repeated once using similar timings and techniques.

Experiment 4 The relationship between stem blight and tuber blight in field plots

Field plots of the cv. King Edward were planted on 15 May 1992. Plots were separated from each other by a minimum distance of 6 m of cultivated and rolled soil. There were three replicate plots of two treatments, i.e. plots with inoculated stems and control plots in a randomised block design. The freedom from blight of the seed was ensured by the careful visual inspection of samples which were incubated for 7 days at 10° C and at a relative humidity close to 100 % to reveal any infection. The trial was inoculated on 27 July, as in experiment 1, with c. 200 *P. infestans* sporangia of an equal mixture of isolates SA91 (phenylamide-resistant) and TSE1 (sensitive) (see section 2.2.3) per stem. Two isolates were used in this and in some of the following experiments in case one isolate was not sufficiently pathogenic. The trial was inoculated before there was any natural infection in the locality and when the crop canopy was meeting along the rows. Each plot had two inoculated (or control) plants per drill, each with five bait plants on either side (Fig 4.1). Rainfall at Auchincruive was measured from the time of inoculation onwards. Additional overhead irrigation, to approximate the amount of rainfall in 1988 at Auchincruive, was applied twice weekly to all the plots. Rainfall in 1988, measured at Auchincruive, during July, August and September had been higher

Drill

1 2 3 4

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

* * * *

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

* * * *

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

● ● ● ●

Key

* = inoculated or control plant

● = bait plant

Fig. 4.1 Position of plants with inoculated stems and bait plants in each plot of field experiment 4

than normal (Appendix 4.1) and a survey of commercial crops in Scotland established that although incidences of foliar blight were low (as reported by growers), high incidences of tuber blight were found.

Mancozeb (1360 g a.i. ha⁻¹ in Dithane 945) was sprayed, using a tractor-mounted sprayer (see section 2.5), at 10-day intervals from soon after emergence and the foliage regularly checked for blight. Suspect lesions were incubated at 16° C and a high relative humidity for 24 hours. Thirty-nine and 59 days after inoculation the two inoculated or control plants from each of the two centre rows of each plot were harvested. For each plant the stem lesion length was measured and the incidence of tubers with blight assessed on the day of harvest. Three bait plants on either side of each of the inoculated and control plants were separately harvested and assessed for tuber blight.

Experiment 5 The effect of soil moisture on the development of stem lesions and the incidence of tuber blight on field-grown plants

Plots of the cv. King Edward consisting of four drills each were planted with blight-free seed on 15 May 1992. There were three replicate plots of two soil moisture treatments and an equal number of control plots. Plots were separated from each other by 12 m of cultivated and rolled soil in the direction of the drills and 5.1 m in the direction perpendicular to the drills. For selected plants one stem was inoculated, using the method described in experiment 1, on 10 July with c. 160 *P. infestans* sporangia of a mixture of isolates SA91 and TSE1 in the ratio 9:11 respectively. Each plot had six inoculated (or control) plants per drill with two non-inoculated bait plants on either side (Fig 4.2). The bait plants on the edge of the subplots were separated from each other by a 90 cm gap, in the centre of which an additional bait plant served as an indicator of underground movement of sporangia. The two target soil moisture treatments were no irrigation and irrigation equivalent to a season of high rainfall. Rainfall

data for Auchincruive from 1971 to 1991 were ranked by month from the lowest to the highest values and the third Quartile (Q3) (Anon, 1989) calculated for each month. These data, in conjunction with long term evapotranspiration rates (pe) were used to establish target soil moisture deficit (smd) for the trial plots (Table 4.1). From April 1 rainfall at the trial site was measured daily and the actual evapotranspiration rates (ep) were calculated weekly. From these data the smds of all the trial plots were calculated and water was applied to the irrigation treatment plots at three to five-day intervals through seep hose (Access Irrigation). The smd for the non-irrigated plots was lower than that targeted due to greater than expected rainfall (Table 4.1). To check that irrigation gave lower soil moisture deficits, 10 soil cores were taken from each plot at regular intervals and oven-dried at 110° C for 36 hours (Johnson & Curl, 1972) to determine the percentage gravimetric soil moisture content.

All plots were treated with mancozeb (1360 g a.i. ha⁻¹ in Dithane 945) at c. 10-day intervals to protect the foliage from infection by *P. infestans*. Suspect lesions were incubated at 16° C in a high relative humidity for 24 hours as a check for any foliar blight. At 17, 33, 49 and 64 days after inoculation one randomly selected block of four inoculated, or four control, plants was harvested from each plot. At each sampling the lesion length on each inoculated stem was measured and the incidence of blighted tubers was determined on the day of harvest. In addition, for the second sampling only, the sporulation capacities of the stem lesions were determined by incubating each stem at 10° C and c. 100 % rh for 48 hours. After incubation all the visible *P. infestans* mycelium was carefully removed using a clean, mounted needle and suspended in 0.5 ml of water. The concentration of sporangia in each of the suspensions was assessed using a haemocytometer slide.

Table 4.1 Target and actual soil moisture deficits for the two irrigation treatments

month	soil moisture deficit ¹ (mm of water m ⁻¹ depth of soil)			
	target		actual	
	no irrigation	irrigation	no irrigation	irrigation
April	-12.2	0.0	0.0	0.0
May	-21.6	0.0	0.0	0.0
June	-54.3	-12.8	-47.8	0.0
July	-65.1	-5.8	-56.3	0.0
August	-42.7	0.0	-0.6	0.0
September	0.0	0.0	0.0	0.0

¹calculated at the end of the month

4.2.3 Experiments 6 & 7 The influence of fungicide type on the relationship between leaf lesions, stem blight and tuber blight

Experiment 6 Stem blight development on glasshouse-grown plants in relation to fungicide type

Single seed tubers of the cv. King Edward were planted in pots in the glasshouse in June 1993 in the sandy loam soil and sphagnum peat mixture (see section 2.3). Soon after emergence the plants were allocated to 15 lots of eight plants which were arranged in a randomised block design. The foliage of the plants was kept dry by watering the pots from below. Six weeks after planting, three replicate lots were treated with one of five fungicide treatments, i.e. cymoxanil + mancozeb + oxadixyl (80 + 1400 + 200 g a.i ha⁻¹ in Trustan), cymoxanil + mancozeb (90 + 1360 g a.i ha⁻¹ in Curzate M), mancozeb (1360 g a.i ha⁻¹ in Dithane 945), fentin hydroxide (266 g a.i ha⁻¹ in Du-ter 50) or no

fungicide (control) using a Mardrive precision bench sprayer calibrated to apply the equivalent of 253 l of water ha⁻¹. The plants were then returned to the glasshouse for 10 days following which they were re-sprayed so that the quantities of active ingredient on the plants would be more typical of a commercially grown crop. After a further two days in the glasshouse single leaves were cut from the upper, middle and lower sections of the main stem of each plant. Each leaf was cut so that approximately 10 cm of stem remained attached to the leaf. Single leaves were then placed in containers, with the lower end of the stem in a small vial of water. The containers were randomly arranged between 18 trays. A 20 µl droplet, containing approximately 100 sporangia of *P. infestans*, isolate T1A1 (see section 2.2.3), was then placed on the centre of the adaxial (upper) surface of each terminal leaflet. Immediately following inoculation the trays were sealed in polyethylene bags and incubated for a further 3 days at 16° C with a 24-hour daylength to help delay leaf senescence. Following incubation, the number of infected leaflets and petioles were assessed. The leaves were then incubated for a further 4 days under the same conditions, after which symptoms of blight in the stem were assessed.

Experiment 7 The growth of stem blight lesions and incidence of tuber blight following stem and leaf inoculations of plants in field plots treated with mancozeb or fentin hydroxide

Plots of the cv. King Edward, consisting of four drills each, were planted with blight-free seed on 8 May 1993. Plots were separated from each other by 10 m of cultivated and rolled soil in the direction of the drills and 5 m in the direction perpendicular to the drills. There were three replicate plots of six treatments, i.e. plots with inoculated stems or inoculated leaves or non-inoculated control plants, treated with either mancozeb (1360 g a.i. ha⁻¹ in Dithane 945) or fentin hydroxide (266 g a.i. ha⁻¹ in Du-ter 50). The trial was inoculated, using the method described in section 2.2.4, on 28 July as the foliage was meeting along the drills and before there were reports of natural

infection in the locality. On the main stem either the stem base or the terminal leaflet of a leaf mid-way up the stem was challenged with c. 5000 *P. infestans* sporangia, isolate T1A1. Paper clips were used to ensure that the impregnated filter paper and Parafilm stayed in place. Each drill had six plants with inoculated stems or leaves or which were non-inoculated controls and had two guard plants on either side (Fig. 4.3). Plots were sprayed with the fungicides from soon after plant emergence at intervals of 10 to 14 day depending on blight risk. Rainfall at the site was recorded from soon after inoculation and additional overhead irrigation was applied to the plots twice weekly to correspond to the amount of rainfall at Auchincruive in 1988. Plots were assessed at regular intervals for the percentage of foliar blight using a modified version of a widely used key (see section 2.4.2). Fifty-nine days after inoculation three randomly selected rows of four inoculated or control plants were harvested from each plot. The stem lesion length, or the percentage of the inoculated leaf area which had symptoms, and the number of blighted tubers at harvest were assessed. Assessments were also made of the number of blight lesions developing at sites which were not inoculated.

4.3 Results

4.3.1 Experiments 1 & 2 The development of stem lesions and sporangia production by *P. infestans*

Experiment 1 The growth of *P. infestans* within stems of potato plants

ELISA tests on symptomless stems, carried out 7 days after inoculation showed that the concentration of *P. infestans* mycelium was greatest 30-40 mm above the point of inoculation (Fig. 4.4). At this height the concentration of *P. infestans* was approximately 10 times greater than that of the control. Although the concentration of *P. infestans* in the symptomless stems was lower above 30-40 mm, concentrations of almost three times greater than that of the

Drill

1 2 3 4

● ● ● ●

● ● ● ●

* * * *

* * * *

* * * *

* * * *

* * * *

* * * *

● ● ● ●

● ● ● ●

Key

* = inoculated or control plant

● = bait plant

Fig. 4.3 Position of plants with inoculated stems or leaves and bait plants in each plot of field experiment 7

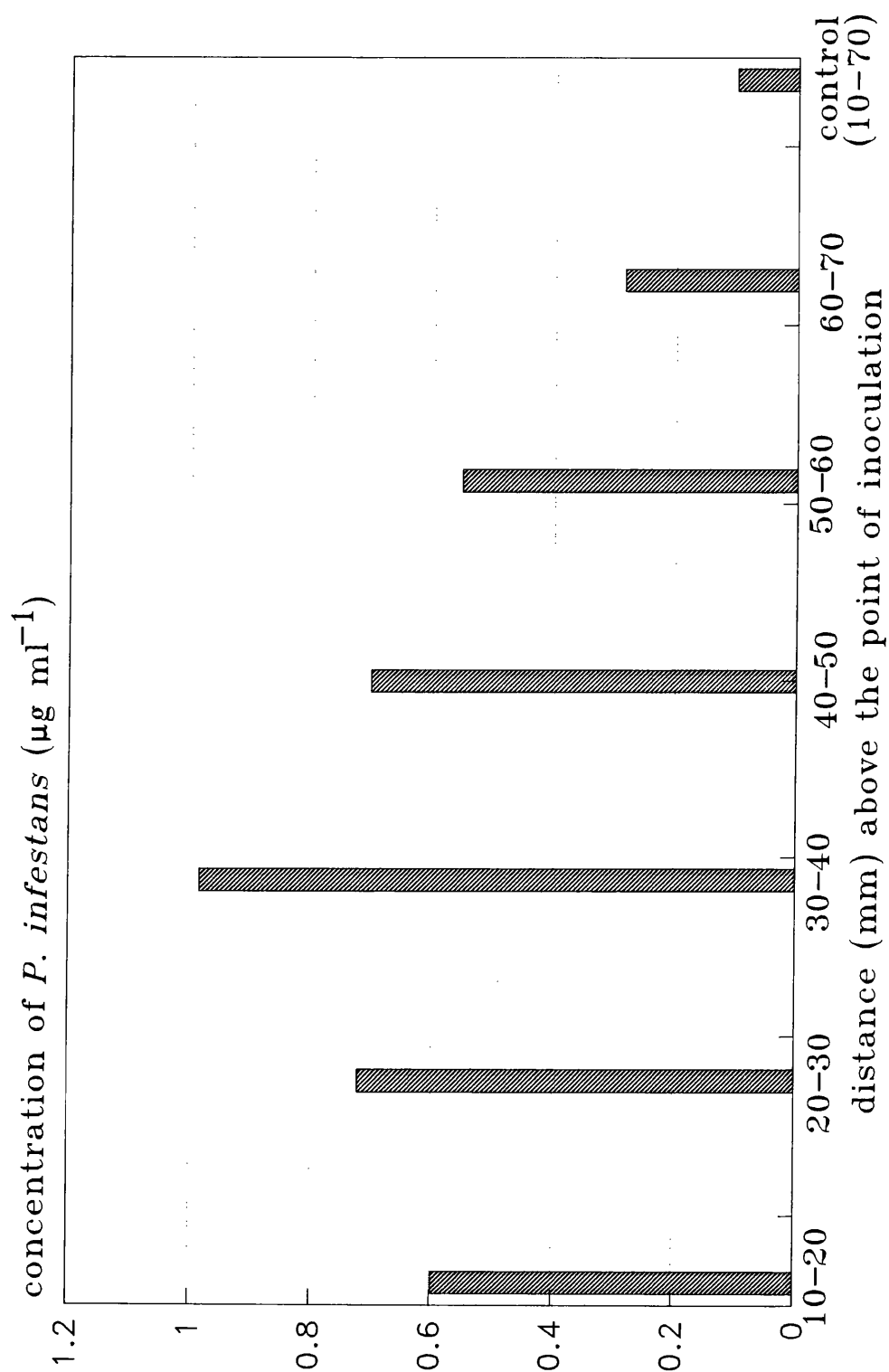


Fig.4.4 The concentration of *P. infestans* in symptomless potato stems at different distances above the point of inoculation

control were still detected at distances of 60-70 mm above the point of inoculation.

The results of the histological studies were inconclusive due to unsatisfactory differences in staining of stem tissue and *P. infestans* mycelium. However, cross sections of stem lesions indicated that the mycelium extended only into the epidermal layer and outermost cortex and to a depth of less than 2 mm.

Experiment 2 Sporangia production in relation to stem lesion development

Lesion length, i.e. length of necrotic tissue, on stems inoculated with *P. infestans* increased significantly between each of the assessment dates in both experiments (Figs. 4.5a and 4.5b). However, the number of sporangia produced after the incubation of stems at c. 100 % rh did not increase significantly until 30 and 28 days after inoculation in experiment 2a and 2b respectively. In experiment 2a the increase in sporangia number coincided with a reduction in the rate of lesion growth and an increase in the length of the lesion which supported sporulation, i.e. from the advancing margins of the lesion only to most of the lesion length. In experiment 2a sporangia numbers and lesion length were not significantly correlated but sporangia production and the length supporting sporulation after incubation were ($r = 0.998$, $p = 0.002$). In both experiments the increase in sporangia number coincided with the start of senescence of the plants. Nine days after inoculation abundant sporangia were produced on stems which before incubation at c. 100 % rh for 48 hours had been symptomless (Fig. 4.5a and 4.5b). In both experiments inoculated stems became completely girdled by *P. infestans* after 40 days and lesion development ceased. No lesions were observed on control stems inoculated with washings from non-inoculated leaves.

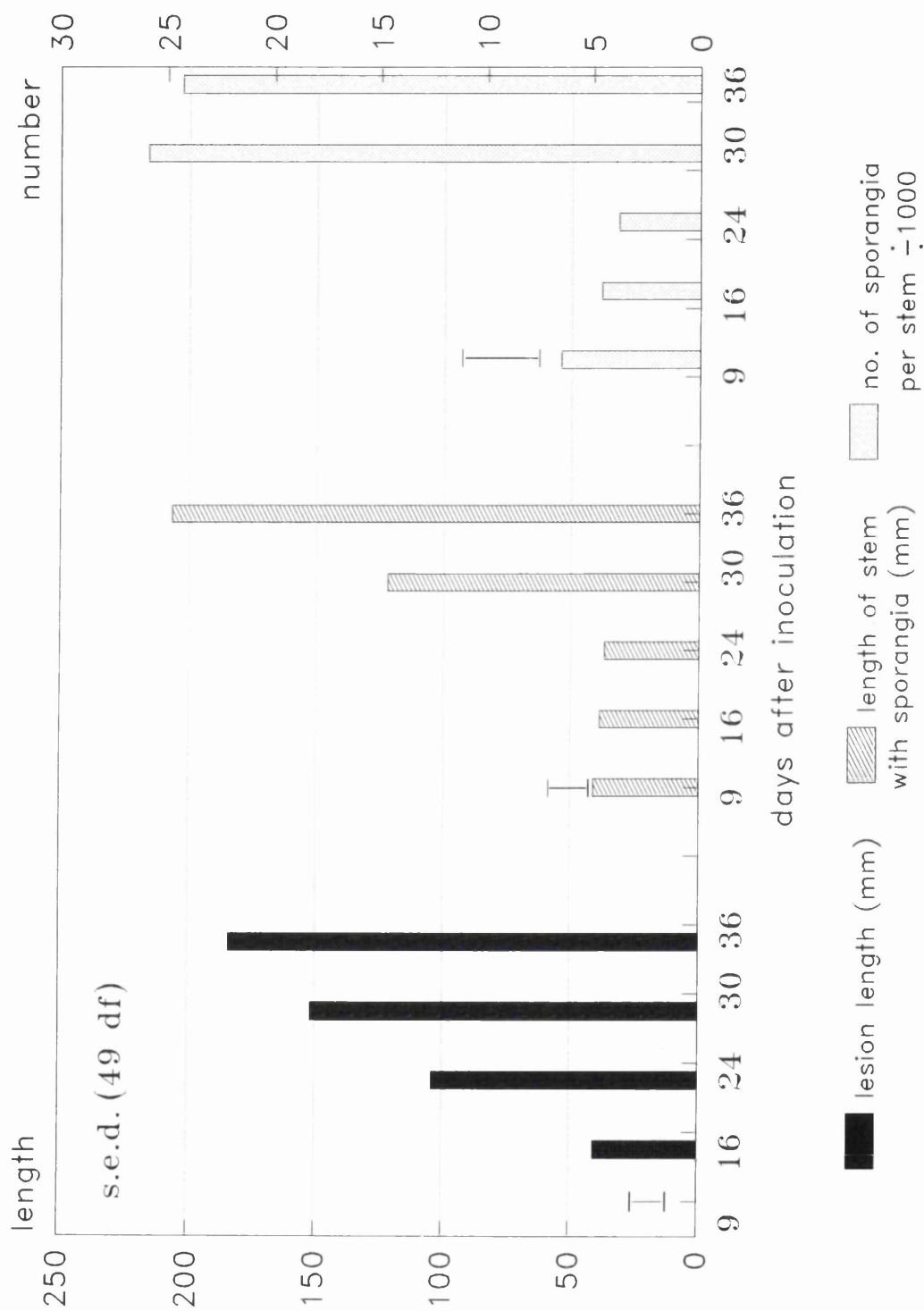


Fig. 4.5a Sporangia production in relation to stem lesion development (experiment 2a)

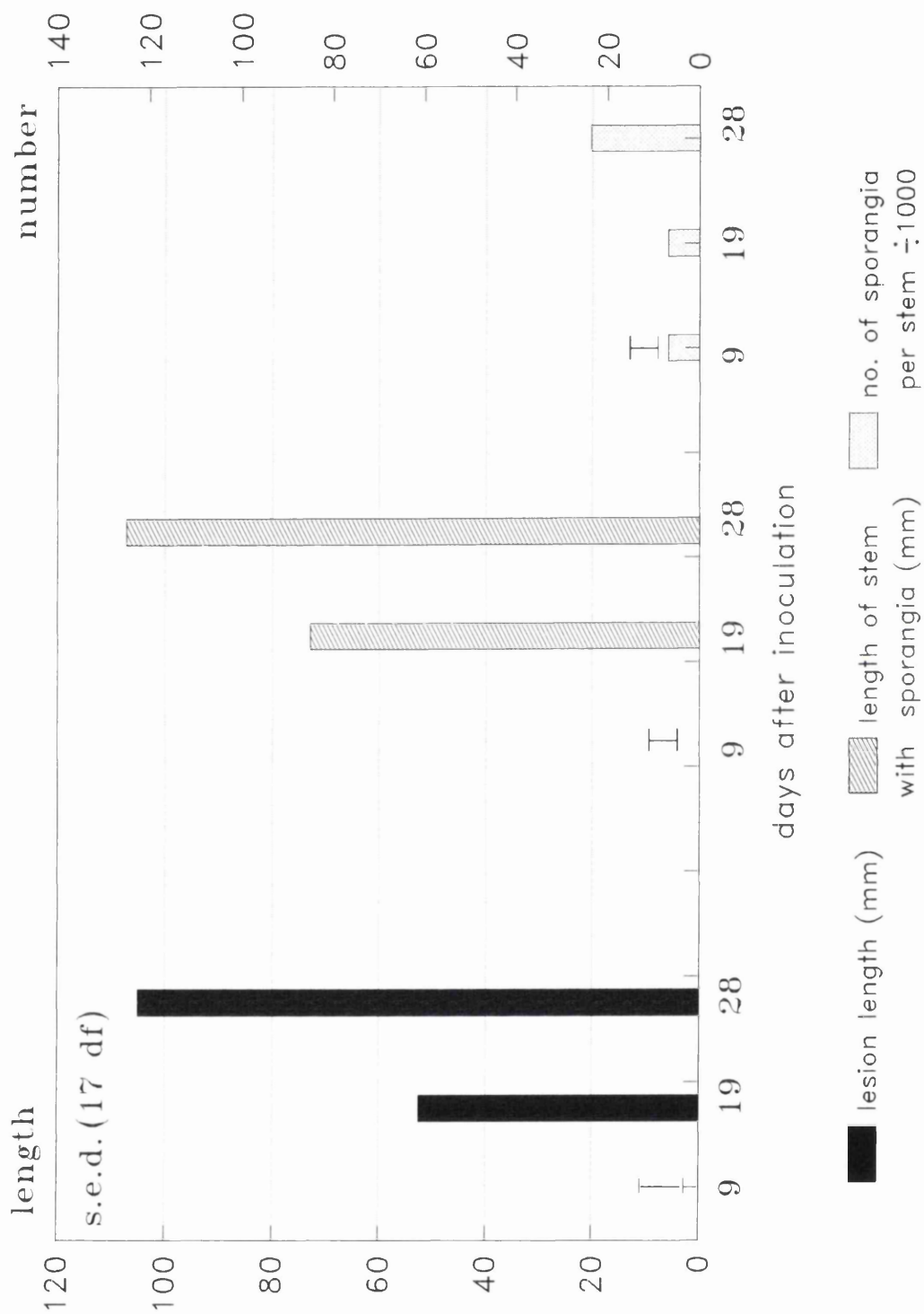


Fig. 4.5b Sporangia production in relation to stem lesion development (experiment 2b)

4.3.2 Experiments 3 to 5 Stem lesions as a source of inoculum for tuber infection

Experiment 3 Stem lesion development and sporulation on glass-house grown plants

A significant increase in the length of stem lesions over time was recorded in both experiments (Table 4.2). The incidence of tuber blight was, however, only once significantly greater than zero; in experiment 3a 10 days after inoculation. No *P. infestans* infection was observed on the control plants or on the incubated lower leaves of inoculated stems.

Experiment 4 The relationship between stem blight and tuber blight in field plots

Despite treatment of the plots with mancozeb at regular intervals, stem lesion length had increased significantly 39 days after inoculation and a high incidence of tuber blight occurred in plots in which stems had been inoculated (Table 4.3). Incidences of tuber blight were significantly greater than zero for inoculated plants assessed 39 days after inoculation but not at the second assessment. Incidences of tuber blight were not significantly greater than zero for the bait plants at either assessment. No *P. infestans* sporangia were observed on incubated, suspect leaf lesions. None of the daughter tubers sampled from the plots in which stems had not been inoculated were blighted.

Experiment 5 The effect of soil moisture on the development of stem lesions and the incidence of tuber blight on field-grown plants

The irrigated and non-irrigated plots were at different soil moisture contents from soon after stem inoculation although the difference decreased with time (Fig. 4.6). There was no significant difference in the incidence of stem infection in the non-irrigated (74.7 %) and irrigated (70.1 %) plots. In general, soil moisture deficit had little effect on stem lesion length, however, 33 days after inoculation the stem lesions in the non-irrigated plots were significantly longer than those in the irrigated plots (Fig. 4.7). In addition, at 33 days

Table 4.2 Stem lesion length and the incidence of tuber blight in glasshouse-grown potato plants following the inoculation of stems with *P. infestans*

		days after inoculation			s.e.d. (11 df)
		10	22	30	
experiment					
3a	lesion length (mm)	64.2	140.5	159.1	30.80
	incidence (%) of tuber infection ¹	7.6	0.0	0.0	3.44
3b	lesion length (mm)	83.7	136.3	135.1	15.57
	incidence (%) of tuber infection ¹	0.0	6.6	0.0	5.42

¹ang. transformed data

Table 4.3 The relationship between stem lesion length and the incidence of tuber blight in the field

	inoculated plots		control plots		
	days after inoculation				s.e.d.
	39	59	39	59	(23 df)
stem lesion length (mm)	180.0	208.0	0.0	0.0	26.90
incidence (%) of tuber infection ¹	20.0	9.0	0.0	0.0	8.10
incidence (%) of tuber infection ²	4.9	4.2	0.0	0.0	2.75

¹for inoculated or control plants (ang. transformed data)

²for neighbouring bait plants (ang. transformed data)

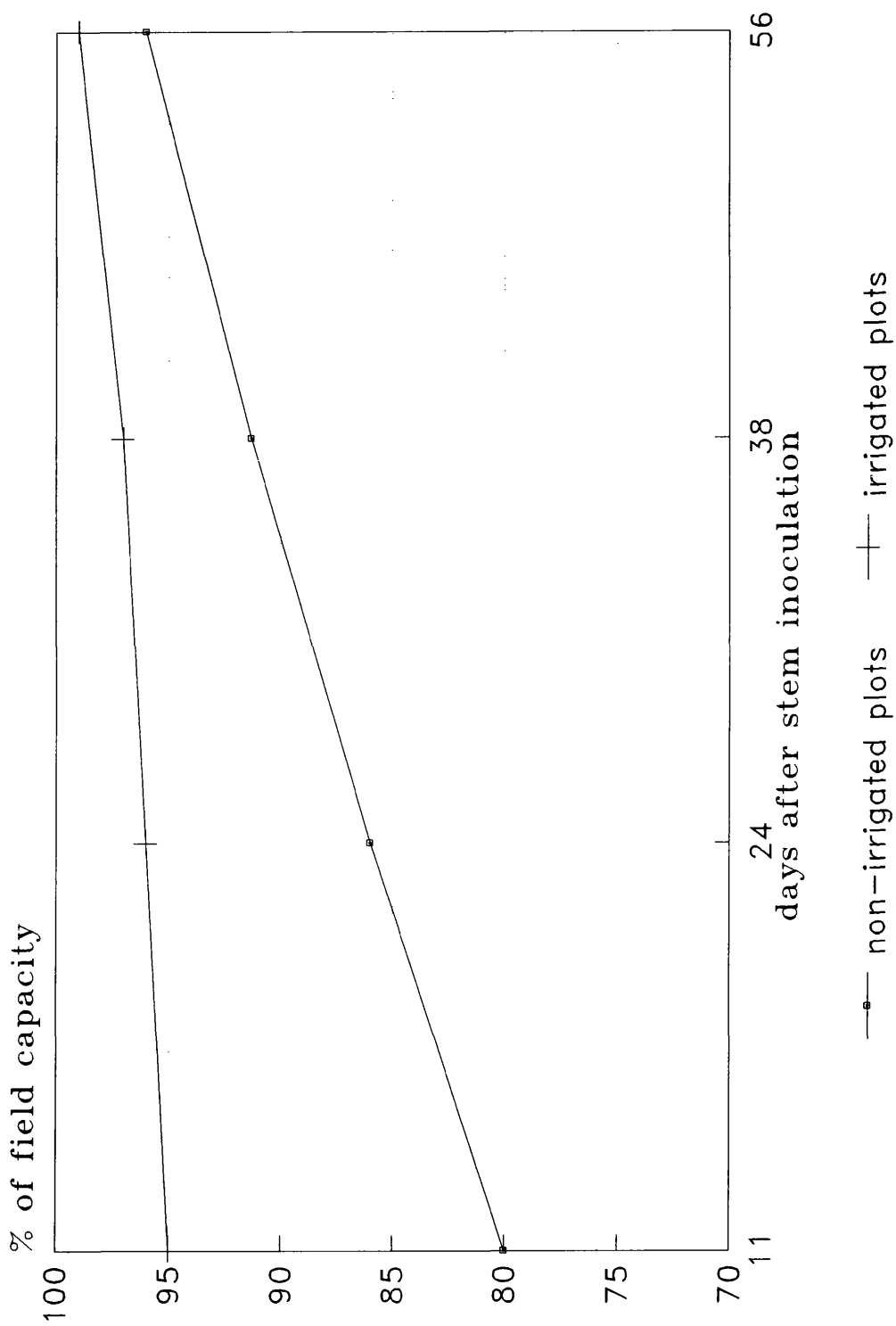


Fig.4.6 The percentage gravimetric soil moisture contents of non-irrigated and irrigated plots during the experiment

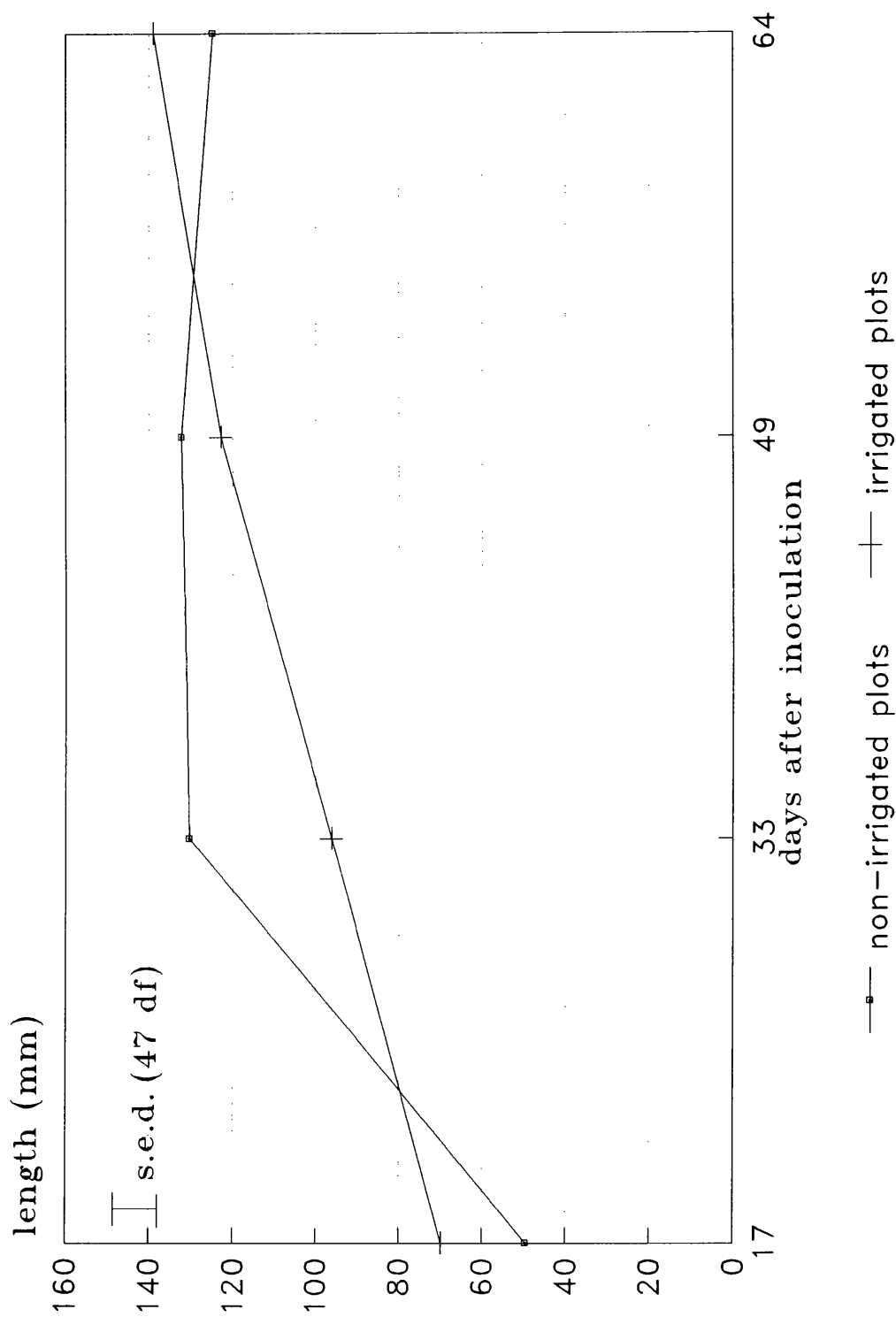


Fig.4.7 Length of stem blight lesions in relation to soil moisture deficit (smd)

significantly more sporangia per stem were harvested after incubation of lesions from the non-irrigated plots (14.4×10^4) than lesions from the irrigated plots (1.2×10^4) (s.e.d. 5.90×10^4 , 47 df). The increase in sporangia production was far greater than would have been expected from the increase in lesion length alone and represented a nine-fold increase in the sporangia production per unit length of lesion. A consistently higher incidence of tuber blight developed in the non-irrigated plots, although the incidence was only significantly greater than zero in tubers harvested 64 days after inoculation (Fig. 4.8). The bait plants were not harvested at any of the assessment dates since only low incidences of tuber blight occurred on the inoculated plants. No *P. infestans* sporangia were observed on suspect foliar lesions, even after incubation.

4.3.3 Experiments 6 & 7 The influence of fungicide type on the relationship between leaf lesions, stem blight and tuber blight

Experiment 6 Stem blight development on glasshouse-grown plants in relation to fungicide type

All four fungicide treatments gave significantly fewer infected leaflets than the control (Fig. 4.9). There were, however, no significant differences between the fungicides. The four fungicide treatments significantly reduced the growth rates of lesions in leaflets (Table 4.4) but again there were no significant differences. Preliminary experiments (data not presented) demonstrated that when glasshouse-grown plants were sprayed with a suspension containing 500 *P. infestans* sporangia ml⁻¹ of isolate TSE1, over 90 % of the stem lesions which developed were the result of mycelial growth down a leaf petiole from an infected leaf lamina (Plate 4.1). In experiment 6 significantly fewer leaflet lesions progressed into the petiole on plants treated with fenitrothion compared with the other fungicides, between which there were no significant differences (Fig. 4.9). However, compared with the control all the fungicide treatments significantly reduced the number of leaflet lesions which spread into

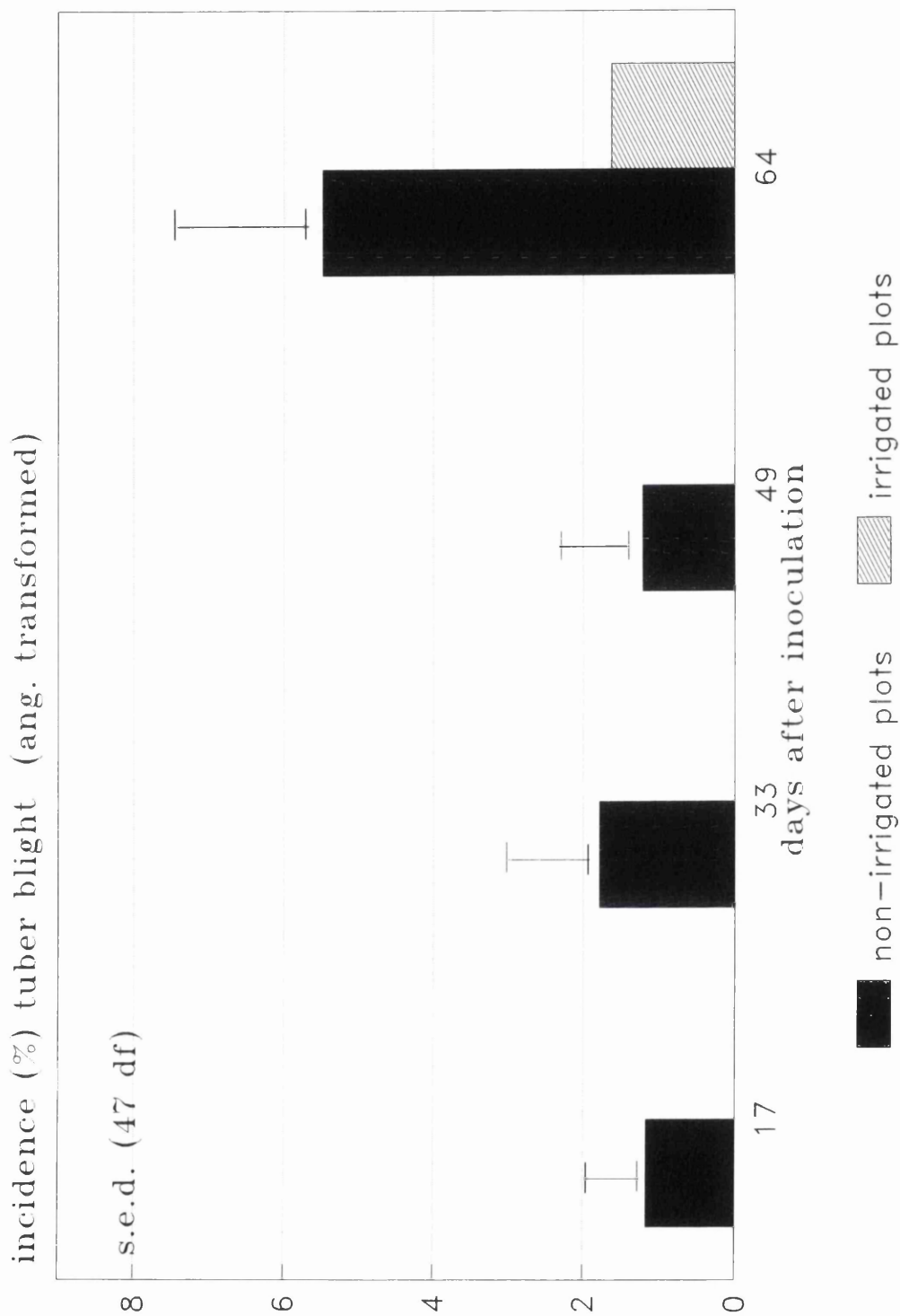


Fig. 4.8 Incidence of tuber blight arising from stem lesions in relation to soil moisture deficit (smd)

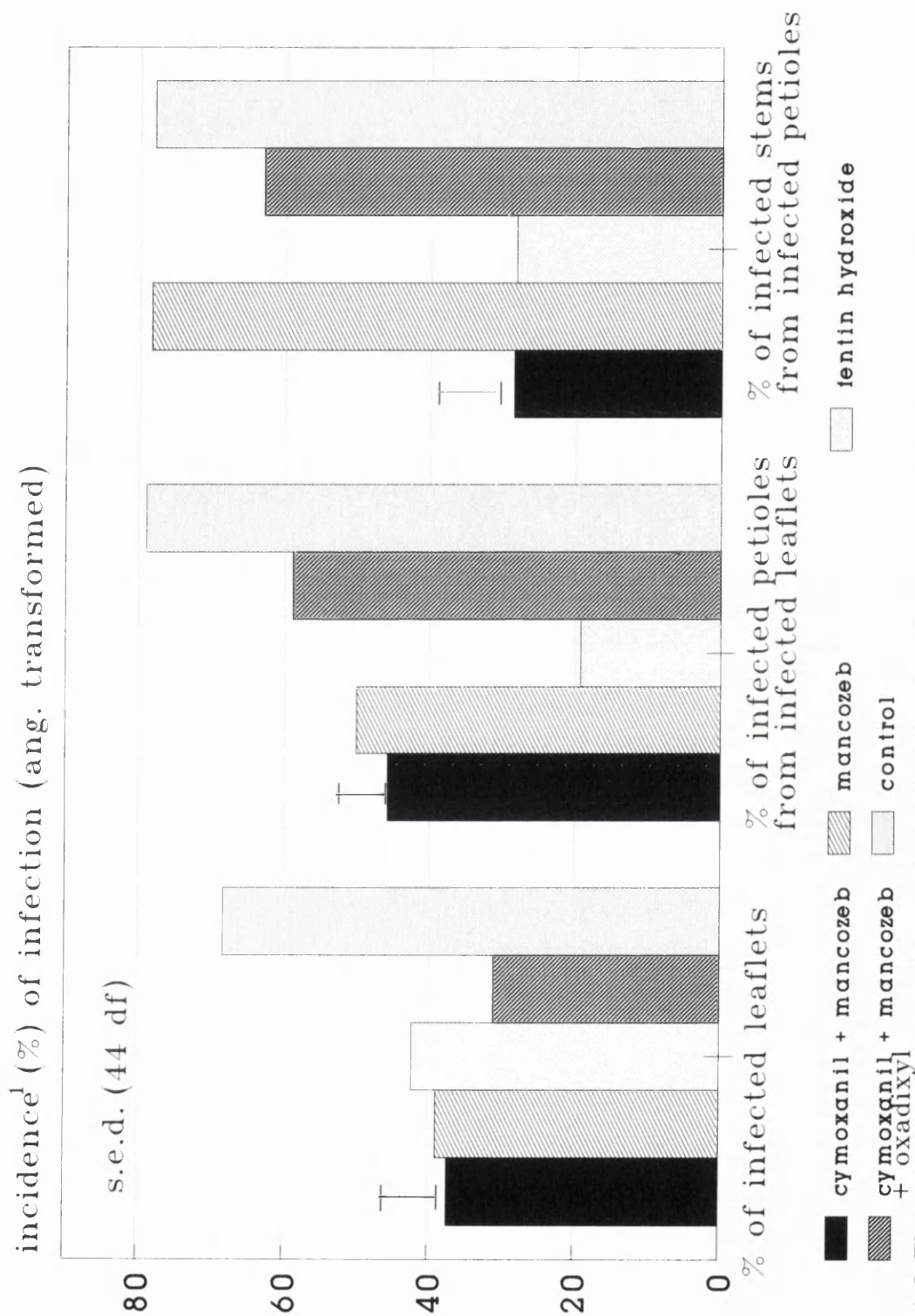


Fig.4.9 The effect of fungicide type on the incidence of infection by, and subsequent development of, *P. infestans* following leaflet inoculation ¹(means for the three leaflet positions)

the petiole. A significantly smaller number of petiole lesions expanded sufficiently to infect the stems of plants treated with either ferric hydroxide or the cymoxanil + mancozeb mixture compared with the other three treatments which were not significantly different from each other.

Table 4.4 The effect of fungicide type on night lesion development on inoculated leaflets



Plate 4.1 Stem lesion resulting from the growth of *P. infestans* down a leaf petiole after inoculation of the terminal leaflet

% of leaflet area diseased (area transformed)			
upper	middle	lower	s.e.d. (48 d.f.)
46.9	53.5	57.9	5.55

the petiole. A significantly smaller number of petiole lesions expanded sufficiently to infect the stems of plants treated with either fentin hydroxide or the cymoxanil + mancozeb mixture compared with the other three treatments which were not significantly different from each other.

Table 4.4 The effect of fungicide type on blight lesion development on inoculated leaflets

% of leaflet area diseased (ang. transformed)					
cymoxanil + mancozeb	mancozeb	fentin hydroxide	cymoxanil + mancozeb + oxadixyl	control	s.e.d. (44 df)
37.9	47.1	35.2	48.8	64.7	7.16

Significantly more leaflets from the upper than the middle or lower sections of the stem became infected (Fig. 4.10), but there was no significant interaction between fungicide treatment and leaflet position. Lesion development was greater on upper and middle leaflets, significantly for the middle compared with the lower leaflets (Table 4.5). For the upper stem sections significantly more petiole lesions developed into stem lesions compared with the lower sections (Fig. 4.10).

Table 4.5 The effect of leaf position on the stem on blight lesion development on inoculated leaflets

% of leaflet area diseased (ang. transformed)			
upper	middle	lower	s.e.d. (44 df)
46.9	53.5	39.9	5.55

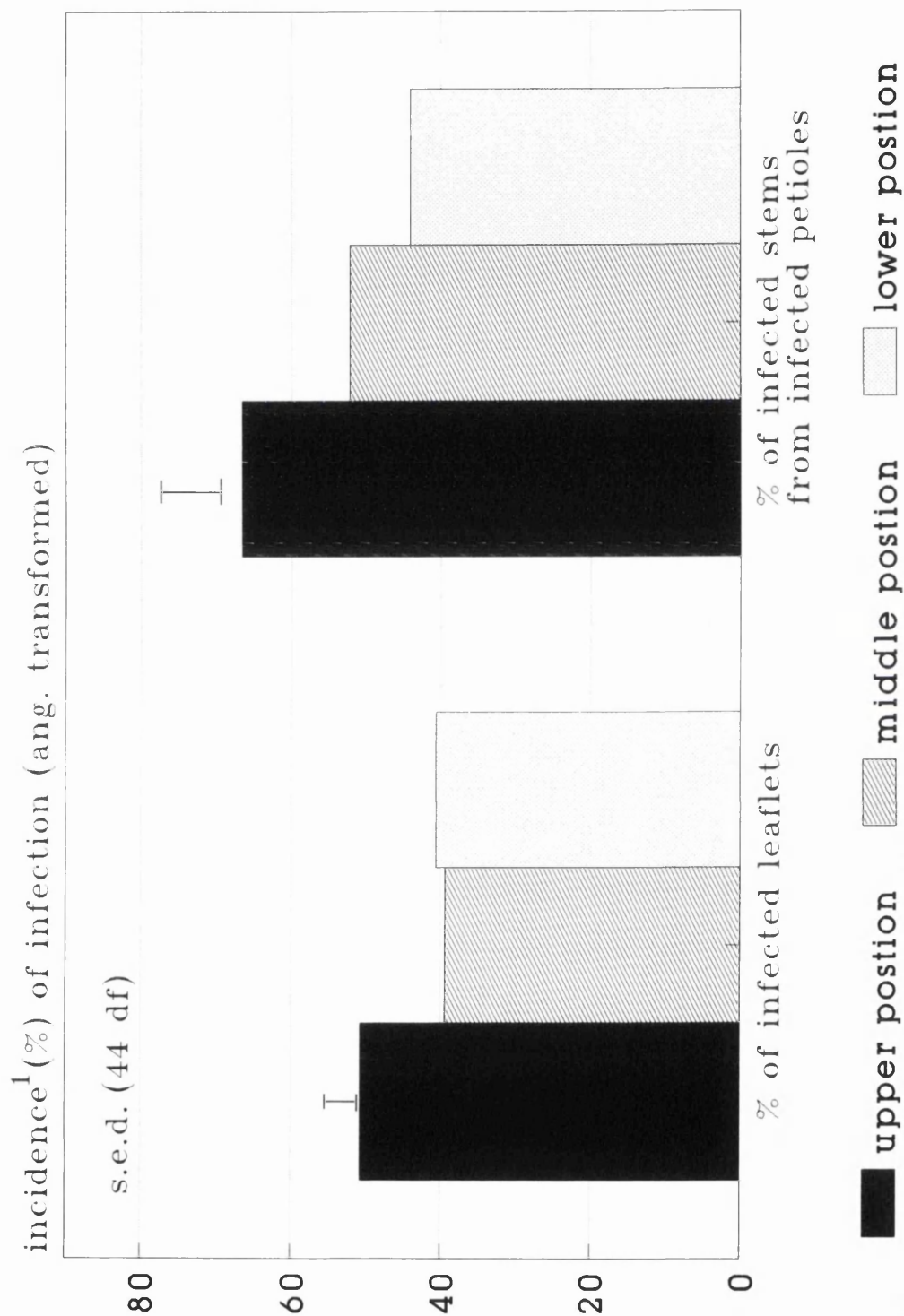


Fig 4.10 The effect of leaf position on the stem on the infection of leaflets by, and subsequent development of, *P. infestans* (mean of the five fungicide treatments)

Experiment 7 The growth of stem blight lesions and incidence of tuber blight following stem and leaf inoculations of plants in field plots treated with mancozeb or fentin hydroxide

Neither fungicide treatment nor inoculation site significantly affected the incidence of haulm infection. Treatment with fentin hydroxide compared with mancozeb significantly reduced the growth rate of lesions in inoculated leaflets (Table 4.6). This resulted in a significantly reduced stem lesion length at the junction of the petiole and stem for the fentin hydroxide-treated plants. In contrast, where the stems had been inoculated directly, fungicide had little effect on stem lesion length. For both leaflet and stem inoculations treatment with fentin hydroxide resulted in significantly reduced incidences of tuber blight compared with mancozeb. For the control the incidence of tuber blight was higher, but not significantly so, in the mancozeb-treated plots. Multiple linear regression revealed that the percentage tuber blight was significantly correlated with the length of stem lesions ($r=0.488$; $p<0.001$) but that it was not significantly correlated with foliar blight ($r=0.488$; $p=0.211$). Foliar infection sites that were not inoculated were higher, but not significantly so, in the plots treated with mancozeb compared with fentin-hydroxide.

4.4 Discussion

Sporangia produced on the leaves are generally regarded to be the main source of inoculum for the infection of tubers by *P. infestans*. The experiments reported here, however, have demonstrated for the first time that *P. infestans* lesions on the stem base may result in significant incidences of tuber blight when foliar lesions are absent. In addition, as stem lesions were concealed by the crop canopy, these results suggest that infected stems might have been the cause of the high incidences of tuber blight which have been reported to occur in the absence of any visible foliar blight (Murphy & McKay, 1927; Grainger,

Table 4.6 The effect of fungicide on the development of foliar, stem and tuber blight following inoculation of terminal leaflets or stems in field plots of the cv. King Edward

	leaflet inoculation		stem inoculation		control		s.e.d. (71 df)
	fentin hydroxide	mancozeb	fentin hydroxide	mancozeb	fentin hydroxide	mancozeb	
% of leaf area with symptoms ¹	27.6 ²	47.1 ²	-	-	-	-	4.72
length of stem lesion (mm)	0.6	43.3	125.0 ²	132.0 ²	-	-	17.43
incidence (%) of tuber blight ¹	6.4	14.2	14.0	24.2	0.0	2.2	3.31
number of other foliar infection sites	0.5	1.1	0.6	1.3	0.8	0.7	0.47

¹ang. transformed data

²for inoculated sites only

1957; Hirst & Stedman, 1962; Lacey, 1962; Boyd, 1972). Although inoculum from seed tubers or from hidden infected leaf material may also explain this phenomenon, checks confirmed that these inoculum sources were absent from the experiments reported here. These results provide evidence to support the suggestion by Lapwood (1964, 1965) and Lacey (1966, 1967a, b) that stem lesions are an important source of inoculum for tuber infection. These results also suggest that a better estimate of the risk of tuber blight incidence may be obtained from assessments of both foliar and stem blight.

Experiments in this chapter and one experiment in Chapter 3 have shown that fungicide type can significantly influence the development of stem blight and subsequent incidence of tuber blight. Treatment with fentin hydroxide, compared with mancozeb, significantly reduced the development of stem lesions and the incidence of tuber blight in field experiments in which leaves were inoculated. However, when stems were inoculated directly, treatment with fentin hydroxide, compared with mancozeb, did not significantly reduce the development of stem lesions, although tuber blight incidences were significantly lower. This suggests that the control of tuber blight obtained with fentin hydroxide in these and other trials (Bock, 1981) may be due to a reduction in stem blight as well as its antispore effect on all lesions and the formation of a fungicidal layer on the soil surface (Hartel, 1962; Schwinn & Margot, 1991). In addition, fentin hydroxide is reported to inhibit the release and germination of zoospores (Schwinn & Margot, 1991) and although Schöber & Ullrich (reported by Harrison, 1992) found that zoospores were not essential for the infection of tubers, Stewart (personal communication) was of the opinion that zoospores were necessary for the infection of tubers *in situ*.

Preliminary glasshouse experiments demonstrated that if inoculum was sprayed over the whole plant, infected stems generally arose from *P. infestans* growing down the leaf petiole from initial infections of the leaf lamina. Lapwood (1961a, b) also found that stem blight in field trials was often the

result of *P. infestans* advancing from infected leaflets into the leaf petiole and then into the stem. Weihing & O'Keefe (1962), however, noted that with some cultivars infected leaves abscised prior to the lesion reaching the stem. In view of this further experiments are required to establish the effect of cultivar on the development of stem blight.

Fentin hydroxide, unlike mancozeb, significantly reduced the progression of *P. infestans* in petioles and also the proportion of stem lesions which developed from lesions on the petioles compared with the control. Although this result was obtained with detached stems it is in agreement with the results of the field experiment which showed that fentin hydroxide, compared with mancozeb, reduced the development of stem lesions when leaf laminae were inoculated. Furthermore, Malcolmson (1969) concluded that, in general, lesion development in detached leaves was similar to that in leaves remaining attached to the plant. This effect of fentin hydroxide is surprising as, like mancozeb, it is classified as a contact fungicide, the action of which is evaded by the pathogen once infection has taken place (Schwinn & Margot, 1991). Some curative effect (Schwinn & Staub, 1987) and translaminar movement (Schwinn & Margot, 1991) has, however, been reported for the fentin fungicides and this might account for the reduction in mycelial growth of *P. infestans* in leaves following treatment with fentin hydroxide.

Wash off by rain or irrigation water may reduce the effectiveness of contact fungicides in the field. However, van Bruggen *et al.* (1986) found that the tenacity of fentin hydroxide was greater than that of mancozeb when upper leaves were exposed to simulated rain of different acidities. In addition, Bruhn & Fry (1982) and Spadafora *et al.* (1984) showed that the wash off of fungicides depended greatly on the cultivar. Further work is therefore required to determine the significance of precipitation or irrigation on the efficacy of fentin hydroxide in preventing the establishment of stem blight in different cultivars. Fentin hydroxide can, however, cause phytotoxicity in young potato

leaves (Bock, 1981) and as a result it is usually only applied to commercial crops as the final two sprays. Results from the experiments reported here, however, suggest that application of fentin hydroxide from the first observations of blight in the crop would limit the occurrence of stem blight and subsequent tuber blight.

In glasshouse experiment 6 although the cymoxanil + mancozeb mixture did not significantly limit the growth rate of *P. infestans* in leaflets compared with the control, the proportion of stem lesions which developed from petiole lesions was significantly reduced. This suggests that cymoxanil may be concentrated within the leaf petiole, possibly as a result of its translaminar properties (Douchet *et al.*, 1977). Whereas Samoucha & Gisi (1987a) reported that cymoxanil moved systemically within tomato and grapevine plants into unsprayed leaves, Cohen & Grinberger (1987) found that cymoxanil showed only translaminar movement in potato. As cymoxanil is rapidly broken down in potato plant and persists for only 6 days under field conditions (Douchet *et al.*, 1977), or 7 days under glasshouse conditions (Samoucha & Gisi, 1987a), it is likely to protect the stems of field-grown plants for only the first few days after spraying. Although this protection period could be maintained for longer by reducing the spray interval current U.K. statutory recommendations limit applications of products containing cymoxanil to a minimum interval of 10 days. For reasons which are not clear, cymoxanil + mancozeb + oxadixyl failed to protect the stem from infection to the same degree as cymoxanil + mancozeb. Although an improved control of foliar blight as a result of the synergy between cymoxanil + mancozeb + oxadixyl has been reported (Samoucha & Gisi, 1987a, b; Redbond, 1990) ^{*et al*} there have been no investigations into the effect of this synergy on the control of stem blight. In view of this, and of the results reported here which have highlighted the importance of stem blight in causing tuber blight, the benefits of using cymoxanil in a mixture with a fully systemic phenylamide fungicide need further evaluation.

Lacey (1967b) showed that when the canopy was complete much rain water was deflected outwards into the furrows but with the onset of senescence an increased amount of water was channelled down the stem. The coincidence of sporulation on necrotic tissue and channelling of rain or irrigation water down the stem would account for the high incidences of tuber blight recorded in the experiments reported here. In addition Lacey (1967b) and Lapwood (1977) noted that cultivar also affected the distribution of rainwater which had fallen on potato plants and that more water was directed down the stems of the cultivar Up to Date than King Edward. High incidences of tuber blight may therefore be avoided in crops in which stem blight is observed through judicious use of irrigation water, particularly towards the end of the season, or by early dessication of the crop. Additional field experiments are required to determine the influence of senescence on the sporulation of *P. infestans* on stem lesions and on the channelling of irrigation and rain water.

In the field experiments reported here overhead irrigation clearly increased the incidence of tuber blight that arose from inoculum produced on infected stems. Lapwood (1965) also observed that sporangia produced on stem lesions could be washed down to the tubers when it rained or when irrigation was applied. In two consecutive years the incidences of over 20 % tuber blight recorded in some plots were associated with a single application of over 12 mm of irrigation water. In 1988, when there were reports of over 30 % tuber blight in some commercial crops in Scotland, daily rainfall of over 12 mm occurred on occasions during July and August. Lapwood (1977) found that at least 5 mm of continuous rain was needed for tubers to become infected from foliar blight lesions, but that infection was greatest after about 10 mm of rain over a two to three-day period. Tuber blight incidences for glasshouse-grown plants (experiment 3), which received the equivalent of 6 mm of rainfall only, were significantly different from zero on only one occasion despite the development of large stem lesions. However, the absence of wind to rock the stem and create

gaps (Lacey, 1967b) between the stem and compost and of cracks in the compost to allow spores easy access to the tubers may also have reduced the number of tubers infected. In addition, the results from experiment 2 indicate that lesion length may not be a good indicator of sporangia production.

Crosier (1934) reported that the optimum temperature for the formation of *P. infestans* sporangia *in vivo* was 18 to 22° C. Harrison & Lowe (1989) found that 90 to 100 % rh was needed for many sporangia to be formed. Results from growth chamber studies (experiment 2) showed that stem lesions continued to expand in conditions which were not suitable for sporulation by the fungus. Keay (1953) showed that *P. infestans* could grow for months within stem tissue of the host without sporulating and that the pathogen did not produce sporangia until conditions became suitable. Similarly Clayson & Robertson (1956) reported that during hot, dry conditions over a period of 31 days post-inoculation, stem lesions were observed to slowly grow vertically in both directions. Harrison & Lowe (1989) also reported that established infections of *P. infestans* in leaves continued to invade tissue regardless of ambient humidities between 80 and 100 % and were possibly unaffected by even lower humidities. Clearly the implication for the development of *P. infestans* on potato stems in the field is that greater numbers of sporangia will eventually be formed if sporangia are produced along the whole length of the lesion when conditions become favourable. *P. infestans* is, however, generally considered to be a biotrophic fungus and would therefore be expected to sporulate on healthy green tissue only. In addition, on infected leaves in the field most sporulation is seen as an annulus surrounding the dying necrotic centre of the lesion (Lapwood, 1961a, b, c). Hammond & Lewis (1987), however, suggested that the nutritional mode of a pathogen in plant tissue cannot always be clearly categorised as biotrophic or necrotrophic and that it may change from one mode to the other as the infection develops. Furthermore, Rotem *et al.* (1978) reported that sporangia of *P. infestans* were produced on necrotic tissue and

suggested that *P. infestans* was an intermediate between biotrophic and necrotrophic fungi. Observations of inoculated stems in growth chamber studies (experiment 2) have shown that sporulation by *P. infestans* does occur on necrotic tissue as the plant senesces. The ability of the stem lesion to support sporulation on necrotic tissue may be due to haustoria which penetrate non-necrotic tissue beneath the lesion. This would not occur for leaflet lesions since *P. infestans* generally destroys the full depth of leaflet tissue. Lowings & Acha (1959), however, found that under glasshouse conditions growth of hyphae of *P. infestans* was stimulated in leaves after the onset of host senescence. The mechanisms which initiate sporulation on necrotic tissue need further elucidation, but may be associated with a reduced supply of nutrients as a result of the onset of senescence. Although in the growth cabinet experiments the stems were completely girdled c. 40 days after inoculation they were abnormally thin and, as was shown in the field experiments, plants with thicker stems were not girdled by *P. infestans*. Lapwood (1961b), however, found that by September natural infections in the field had girdled many of the stems in some cultivars. This suggests that thin, very young, or in some cases, mature stems which become infected may not survive to produce inoculum for the infection of daughter tubers.

The results from field experiment 5 show that tuber blight incidences in the field were significantly reduced in plots in which the soil moisture was kept close to field capacity using seep hose. This was probably due to the reduced length of, and sporulation by, the stem lesions in the high soil moisture plots, as a result of water from the seep hose landing directly on the diseased stem as well as on the soil. Abundant water on the foliage was considered by Rotem *et al.* (1978) to suppress sporulation by *P. infestans*, possibly due to impeded gaseous exchange (Duniway, 1979). The effects of soil moisture on tuber blight is examined in Chapter 5.

CHAPTER 5

The influence of edaphic factors on the spread of *P. infestans* inoculum in soil and on the subsequent incidence of tuber blight

5.1 Introduction

The possibility that sources of *P. infestans* inoculum other than foliar blight lesions may sometimes be responsible for the infection of daughter tubers has been raised in previous chapters. Another alternative source of *P. infestans* inoculum is sporangia produced on blighted daughter tubers. Sporulation by *P. infestans* on blighted daughter tubers underground has been demonstrated in the past (Lapwood, 1961d, e; Lacey, 1962) and the underground spread of *P. infestans* between daughter tubers over a distance of 1.3 cm has been reported by both Lapwood (1962) and Lacey (1962, 1967b). Soil temperature and soil moisture have been reported to affect the severity of disease caused by several species of *Phytophthora* (Duniway, 1975; Pratt & Mitchell, 1976; Kuan & Erwin, 1980). The objectives of this chapter were to determine the influence of soil moisture on the infection of healthy daughter tubers by *P. infestans* (experiments 1 & 2) and to investigate the influence of soil moisture and temperature, pre- and post-inoculation, on tuber infection and subsequent lesion development (experiments 3 & 4). The effect of soil moisture pre- and post-inoculation on the degree of spread of *P. infestans* from daughter tubers was examined in a field experiment (experiment 5).

Although oospores of *P. infestans* have been observed in samples of potato plants from fields in the U.K. (Shattock *et al.*, 1990; Pittis & Shattock, 1994) and produced *in vitro* by mating U.K. A1 and A2 field isolates (Malcolmson, 1985; Tantius *et al.*, 1986) the blighted, overwintering tuber has long been identified as the main source of inoculum by which the disease carries over between years (van der Zaag, 1956; Hirst & Stedman, 1960; Boyd, 1980). Seed tubers which are evidently blighted may be removed prior to planting, however, the contamination of healthy tubers by inoculum produced on the surface of blighted seed remains a possibility. Dowley & O'Sullivan (1991b) found viable sporangia on the surface of diseased tubers after short term storage. Experiments 6 and 7 investigated the conditions that may lead to the

contamination and infection of healthy seed tubers by inoculum produced on infected seed. Once the diseased tuber is planted the exact pathway by which the fungus progresses to the new foliage and daughter tubers is not always clear. The growth of *P. infestans* mycelium from the infected tuber into the sprout and then to the stem was first suggested by Berkeley in 1846 (Wallin & Polhemus, 1956) and de Bary (1876) later established that this pathway did exist. However, although stem infection from infected seed tubers was accomplished relatively easily by Murphy & McKay (1927) at temperatures of around 25° C in the glasshouse, stem infections which originate from blighted seed tubers are rarely seen in the field (Hirst, 1955; van der Zaag, 1956; Grainger, 1957). Boyd (1972) suggested that the occurrence of tuber blight in the absence of foliar blight may be due to transmission from diseased seed tubers on which sporulation occurred in the soil. To date, however, there have been no reports of sporulation on naturally infected mother tubers after planting. Glasshouse studies (experiments 8 & 9) were performed to determine the effect of soil moisture on inoculum production and, more importantly, the length of time after planting that mother tubers produced viable inoculum.

5.2 Materials and methods

5.2.1 Experiments 1 to 4 The effect of soil moisture content pre- and post-inoculation on the infection of daughter tubers by *P. infestans* and subsequent lesion development

Experiment 1 Pre-inoculation soil moisture treatment / immature tubers

A pressure membrane technique similar to that described by Heining (1963) was used to determine the moisture content (% gravimetric water content) of a sandy loam soil (see section 2.3) at field capacity and at permanent wilting point (Table 5.1). Blight-free, immature tubers of the cv. King Edward, harvested from mancozeb-treated field plots in September 1992, were carefully washed to limit any damage and allocated at random to nine lots of 20. After

washing, three lots of tubers were buried in replicate trays containing oven-dried sandy loam soil adjusted to either 39, 59 or 79 % of field capacity. The basis for the selection of soil moisture percentages was to encompass a range of soil moistures between permanent wilting point and field capacity which were likely to occur in the field. Soil moistures of 39, 59 and 79 % of field capacity broadly represent soil moistures in low, medium and high rainfall years respectively (see experiment 5). The trays were then covered with polyethylene bags to limit water loss and incubated in the glasshouse for 8 days. Kaufman & Williams (1962) showed that polyethylene was suitable to maintain a relatively constant soil water content in microbial studies and that it was readily permeable to carbon dioxide and to other gases.

Table 5.1 Water content of the sandy loam soil at field capacity and at permanent wilting point

	gravimetric water content (%)	
	field capacity	permanent wilting point
	(0.05 bar)	(15 bar)
topsoil	26.72	8.21
subsoil	23.87	10.06
mean	25.30	9.14

Ambient temperature in the glasshouse, monitored using a thermograph, ranged between 12 and 29° C but was on average 20° C. Although evaporation through the polyethylene was low the soil moisture contents of the trays were reassessed, by weight, at regular intervals and any moisture loss was rectified. A stock suspension of *P. infestans* sporangia, isolate FI (see section 2.2.3), was prepared from slices of King Edward tubers which had been inoculated 10 days

previously. Tubers from the different soil moisture treatments were dipped for 2 minutes in 3 l of a *P. infestans* suspension containing 500 sporangia ml⁻¹ and single tubers then placed in individual containers randomly arranged between nine trays. These trays were then covered with polyethylene, to maintain a high relative humidity, and kept at 16° C in the dark. Nine days later the incidence of tuber infection and the site and number of infections were assessed.

Experiment 2 Pre- and post-inoculation soil moisture treatments / immature tubers

Blight-free, immature tubers of the cv. King Edward were harvested from mancozeb-treated field plots on 3 and 28 August and 13 September 1992 and washed as described previously. Ten tubers were then buried in three replicate trays containing the sandy loam soil adjusted to the same three moisture contents used in experiment 1. The trays were then covered with polyethylene bags to limit water loss and arranged in a randomised block design within a glasshouse in which the temperature was on average 20° C and ranged between 10° C and 30° C. Evaporation loss was assessed and moisture replaced as necessary as in experiment 1. Eight days later the tubers were carefully removed and the soil from each tray spread over a 1 m² surface. A stock suspension of *P. infestans* sporangia, isolate TSE1, was prepared from previously inoculated King Edward tuber slices. This suspension was adjusted to a concentration of c. 2×10^4 sporangia ml⁻¹ and 15 ml sprayed onto the surface of the soil from each tray using a Humbrol hand-held, precision aerosol sprayer. After spraying, the soil was thoroughly mixed prior to the tubers being reburied. The trays were returned to the glasshouse and after 10 to 12 days the tubers were assessed as in experiment 1.

Experiment 3 Post-inoculation soil moisture treatment / immature tubers

Blight-free, immature tubers of the cv. King Edward were harvested from mancozeb-treated field plots during August 1992 and were inoculated at a single damage site with a 30 µl droplet containing c. 900 sporangia of isolate

TSE1. Ten tubers were placed in each of three replicate trays containing soil at 39, 59 and 79 % of field capacity. The trays were then covered with polyethylene bags and arranged in a randomised block design within a glasshouse in which the temperature was on average 20° C and ranged between 10° C and 32° C. Evaporation loss was assessed and moisture replaced as necessary as in experiment 1. All of the tubers were sampled after incubation for 14 and, following re-burial, at 22 days. At both assessments, and after carefully brushing away any soil, tubers were assessed for the incidence of infection, percentage tuber surface area diseased and the number of sporulating sites per tuber. An assessment of the number of sporangia per sporulating site was made 22 days after inoculation. For that assessment the mycelial mats from five randomly selected sporulating sites per tuber were carefully removed using a mounted needle, bulked and suspended in 0.25 ml of sterile water in plastic vials. These suspensions were then thoroughly shaken on a Jencons Mixmatic mixer for 30 seconds prior to extracting 10 aliquots of 5 µl from each, placing them on microscope slides and counting the number of sporangia and zoospores in the field of view.

Experiment 4 Post-inoculation soil moisture and temperature treatments / mature tubers

Blight-free, mature daughter tubers of the cv. King Edward were inoculated with isolate TSE1 during December 1991 using the technique given in experiment 3. Following inoculation, lots of six randomly selected tubers were buried in trays containing sandy loam soil at three moisture contents, i.e. 40, 95 and 140 % of field capacity. The basis for the selection of soil moistures in this experiment was to encompass a range of soil moistures between permanent wilting point and soil saturation. Three replicate trays of each soil moisture were incubated in a randomised block design at 8, 12 or 16° C in three growth cabinets. The temperatures were selected using records of soil temperature at 10 cm depth at Auchincruive from July to October for the years 1981 to 1991.

The mean monthly temperatures at 09.00 GMT were 15.6, 14.5, 11.6 and 8.2° C respectively. In each of the growth cabinets the temperature was monitored using a thermograph and rarely varied by more than one degree either side of the target temperature. The soil moisture contents were maintained by weighing the trays daily and replacing water lost through evaporation as necessary as in experiment 1. Two tubers per tray were sampled after 17, 24 and 28 days of incubation and assessed, as in experiment 3, except that number of sporangia per sporulating site was not assessed

5.2.2 Experiment 5 The effect of soil moisture content on the spread of *P. infestans* inoculum from blighted to healthy daughter tubers in the field

Plots of the cv. King Edward consisting of four drills, each 0.7 m wide and containing 24 plants at 0.3 m spacing, were planted on 15 May 1992 so as to have a minimum distance of 6 m of bare ground between any two plots. The freedom from blight of the seed tubers was ensured by the careful inspection, before planting, of samples incubated to reveal any infection. Soon after emergence all the plots were covered with polyethylene (600 gauge, Clydeside Trading Society) at soil-level and the plants trained through small incisions made in it. The purpose of the polyethylene, which was left on the plots for the duration of the experiment (Plate 5.1), was to prevent rain landing on the soil surface in the plots. This, in conjunction with the application of known volumes of water through seep hose (Access Irrigation), situated on the outside of the two centre drills, from 2 June allowed plots of three soil moisture deficits (smds) to be created.

To measure any increase in soil temperature resulting from the plots being covered with polyethylene a Grant multichannel instrument was used from June to August to record temperature hourly at c. 13 cm depth in nine polyethylene-covered ridges and nine ridges not covered with polyethylene. Lapwood (1977) reported that the average depth of daughter tubers was 13 cm from the crest of the ridge. Rainfall data for April to September at Auchincruive for the years



Plate 5.1 Field plots of the cv. King Edward showing separation of the plots and polyethylene covers (experiment 5)

1971 to 1991 were ranked by month from the smallest to the largest values and the overall mean, the mean of the first quartile (Q1) and the mean of the third quartile (Q3) (Anon, 1989) calculated and selected to represent respectively a medium, low and high rainfall for each month (Francis, 1981) (Table 5.2). These data, in conjunction with long term (1971-1991) predicted evapotranspiration rates (pe) (Francis, 1981) were used to calculate the target smd values of the high, medium and low plots at the beginning of each month. Weekly actual evapotranspiration rates (ep) from the different smd plots were calculated from Morecs real time data, ep and smd for maincrop potatoes in Ayrshire (square 62). These data in conjunction with the data on the quantities of water applied to each plot through seep hose were used to calculate the smd actually achieved in the high, medium and low smd plots (Table 5.3). Comparison of Tables 5.2 and 5.3 shows that the target smds were largely achieved from early July onwards. To check that the different irrigation schedules were resulting in plots with different soil moisture deficits, 10 soil cores were taken from each plot at regular intervals and oven-dried at 110° C for 36 hours to determine the % gravimetric soil moisture content (Johnson & Curl, 1972).

There were six plots of each smd treatment, i.e. three with inoculated daughter tubers and three non-inoculated controls. In order to keep the haulm free from blight, mancozeb (1360 g a.i. ha⁻¹ in Dithane 945) was sprayed at 10-day intervals from soon after emergence throughout the growing season. On 17 July in each "inoculated" plot, one daughter tuber on each of two plants per drill was inoculated with an equal mixture of isolates TSE1 and SA91. These daughter tubers were uncovered to allow inoculation *in situ* using the technique described in section 2.2.4. After inoculation the tubers were immediately re-covered with soil and the polyethylene repaired. Each inoculated plant had five

Table 5.2 Rainfall, long term predicted evapotranspiration rates (pe) and target soil moisture deficits (smd) in the high, medium and low smd plots from April to October

month	rainfall ¹ (mm)			pe (mm)	smd on the 1 st of the month (mm of water m ⁻¹ of soil)		
	Q1	mean	Q3		high	medium	low
April	21.2	49.7	58.2	-37.5	0.0	0.0	0.0
May	24.3	50.6	69.2	-60.0	-16.3	0.0	0.0
June	39.5	57.3	77.6	-90.0	-52.0	-9.4	0.0
July	46.5	69.2	86.6	-80.0	-102.5	-42.1	-12.4
August	41.3	87.4	135.8	-65.0	-136.0	-52.9	-5.8
September	70.7	112.7	140.7	-40.0	-159.0	-30.5	0.0
October	nc	nc	nc	nc	-128.3	0.0	0.0

¹first quartile mean (Q1), mean and third quartile mean (Q3) of monthly rainfall

nc not calculated

non-inoculated bait plants on either side in the drill. Incidences of tuber blight for the non-inoculated tubers from eight inoculated plants were assessed on 7 and 21 August and on 14 September. This involved carefully removing the soil and mapping the position of all the tubers of the plant using a grid and map system similar to that used by Lacey (1966). Three bait plants, from either side of each harvested inoculated plant, were harvested on 7 August and on 15 September and the incidence of tuber blight assessed on individual plants but the positions of the tubers were not mapped.

Table 5.3 Actual soil moisture deficits (smd) achieved in the high, medium and low plots from April until September

month	smd on the 1 st of the month (mm of water m ⁻¹ of soil)		
	high	medium	low
April	0.0	0.0	0.0
May	0.0	0.0	0.0
June	0.0	0.0	0.0
July	-97.0	-51.0	-23.0
August	-145.0	-69.0	0.0
September	-242.0	-100.0	-7.1

5.2.3 Experiment 6 The susceptibility of seed tubers to infection by *P. infestans*

Super Elite seed tubers of the cv. King Edward were incubated during February 1993 at 12° C and c. 80 % relative humidity to help break dormancy. After 10 days incubation the tubers were washed and assessed to ensure freedom from blight and allowed to dry for c. 5 minutes. Tubers were then allocated at random to 12 lots of 20 tubers and 10 tubers per lot were

artificially damaged by manually shaking them for 30 seconds in a plastic box containing c. 70 g of washed and dried stone chippings. A sporangial stock suspension of *P. infestans*, isolate SA91, was prepared from 12 day-old rye B agar cultures by dispersing the sporangia in sterile distilled water and shaking on a Griffen flask shaker for 1 hour. Three lots of damaged and three lots of undamaged tubers were then separately dipped for 60 seconds in 2 l of one of four treatment suspensions, i.e. 0 (control), 500, 1000 or 2000 sporangia ml⁻¹. Single tubers were then placed in containers which were randomly arranged between eight trays. These trays were covered with polyethylene, to maintain a high relative humidity, and kept at 16° C in the dark. Seven days later the number and sites of any infections were recorded for individual tubers.

5.2.4 Experiment 7 The contamination during planting of healthy seed tubers with *P. infestans* from infected tubers

Blight-free daughter tubers of the cv. King Edward were harvested from mancozeb-treated field plots and during December 1992 inoculated at a single damage site with a 20 µl droplet containing c. 100 sporangia of isolate TSE1. Single tubers were then placed in containers and stored at 10° C in the dark; conditions which are optimal for the survival of blighted tubers (van der Zaag, 1959).

During March 1993 non-inoculated seed tubers of the cv. King Edward were incubated for 10 days and checked for freedom from blight as described in experiment 6. These tubers were then allocated at random to 15 lots of 50 tubers, after which 25 tubers per lot were artificially damaged as described in experiment 6. Damaged tubers were marked with indelible red ink. In turn each of three lots of tubers was placed in a container with a single tuber which had been inoculated the previous December and incubated in a high relative humidity at 16° C and in the dark for one of five time periods, i.e. 0, 2, 3, 4 or 6 days. The containers were inverted 20 times to simulate the tuber mixing which may occur pre-planting (Dowley & O'Sullivan, 1991b). Following

mixing the inoculated tubers were removed and single tubers from the remainder placed in containers which were randomly arranged between 30 trays. These trays were then covered and incubated as described in experiment 6. Seven days later the trays were removed from the incubator and the individual tubers assessed for infection by *P. infestans*.

5.2.5 Experiments 8 & 9 The effect of soil moisture content at planting on the sporulation on, and decay of, inoculated seed tubers

Experiment 8 Inoculation at the time of harvest of the seed tubers

Blight-free daughter tubers of the cv. King Edward, harvested from mancozeb-treated field plots in October 1992, were inoculated and stored as described in experiment 7. During March 1993, after dormancy break, sound tubers were selected and allocated at random to 20 lots of six. Three tubers per lot were damaged as in experiment 6 after which all of the tubers were planted in four replicate trays of the sandy loam soil adjusted to 39, 59, 79, 99 and 119 % of field capacity. These trays were then covered with polyethylene and arranged in a randomised block design within a glasshouse in which the temperature was on average 16° C and ranged between 12 and 21° C. Evaporation loss was assessed and moisture replaced as necessary as in experiment 1. All of the tubers were harvested 14, 35 and 58 days after burial and, after carefully brushing away any soil, assessed for the presence of sporulating sites of *P. infestans*. An assessment of the percentage of the tuber surface area rotted, principally as a result of *P. infestans*, was also made. Following the assessments after 14 and 35 days sound tubers were re-buried in their original trays and incubated as before. After 58 days sound tubers from the 39 and 59 % soil moisture treatments were re-buried in soil at a moisture of 79 %. These tubers were then assessed for the presence of sporulating sites 7 days later. The effect of soil moisture content on the percentage of the tuber surface area blighted was not assessed since it was generally c. 100 % by the time the tubers were first buried.

Experiment 9 Inoculation shortly before planting the seed tubers

Super Elite seed tubers of the cv. King Edward, previously stored at 4° C, were incubated for 15 days at 12° C during January 1993 in order to help break dormancy. Following incubation the tubers were assessed to ensure freedom from blight. Tubers were then allocated at random to 20 lots of six following which three tubers per lot were artificially damaged as described in experiment 6. All of the tubers were then inoculated using the technique described in experiment 7. Inoculated tubers were then buried on the same day and incubated in soils at the same moisture contents as in experiment 8. These trays were then covered with polyethylene and arranged in a randomised block design within a glasshouse in which the air temperature was on average 12° C and ranged between 8 and 18° C. Evaporation loss was assessed and moisture replaced as necessary as in experiment 1. All tubers were harvested at 15, 26 and 43 days after inoculation. After carefully brushing away any soil, tubers harvested at 15 days were assessed for infection by *P. infestans*, and at all assessments, for the percentage of the tuber surface covered by the lesion and the number of sporulating sites. Sound tubers were re-buried after the assessments at 15 and 26 days, and incubated as before. An additional assessment of the percentage of tuber surface area rotted, principally as a result of *P. infestans*, was made on tubers harvested 43 days after inoculation.

5.3 Results

5.3.1 Experiments 1 to 4 The effect of soil moisture content pre- and post-inoculation on the infection of daughter tubers by *P. infestans* and subsequent lesion development

Experiment 1 Pre-inoculation soil moisture treatment / immature tubers

Although more tubers became infected if they had been incubated at the highest compared with the lowest soil moisture content, the differences were not significant. However, the number of tubers which became infected via the

lenticels was significantly affected by pre-inoculation soil moisture content. Twenty, 7.2 and 5.4 % (ang. transformed) of tubers which had been in the soils at 79, 59 and 39 % of field capacity respectively (s.e.d. 5.56, 8 df) became infected via the lenticels. The percentages of infection for 59 and 39 % of field capacity were not significantly greater than zero. Although the number of eyes which became infected was also higher at 79 compared with 39 % of field capacity the differences were not significant.

Experiment 2 Pre- and post-inoculation soil moisture treatments / immature tubers

The incidence of tuber infection was only significantly greater than zero for the soil at 79 % of field capacity and if the tubers were harvested in August (Table 5.4). With the exception of the assessment on 28 August, no tubers became infected at soil moistures of either 39 or 59 % of field capacity. There was no evidence of a decline in tuber susceptibility as tubers matured. For all three soil moisture contents the incidence of infection was greater for tubers harvested on 28 August compared with 3 August and 13 September. In general, the majority of infections took place at lenticels.

Experiment 3 Post-inoculation soil moisture treatment / immature tubers

The incidence of tuber infection decreased with increasing soil moisture content and was significantly lower at 79 compared with 39 % of field capacity when assessed 14 days after inoculation (Fig. 5.1). The incidence of infection remained unchanged 22 days after inoculation (results not presented). When assessed 14 days after inoculation the percentage of the tuber surface area diseased was significantly greater for the highest compared with the lowest soil moisture content (Fig. 5.1). By 22 days after inoculation, however, these differences had disappeared (results not presented). At the earlier assessment the number of sporulating sites per tuber was significantly greater for the highest compared with the two lower soil moistures, between which there was no significant difference. Although significant differences in the number of

sporulating sites per tuber still occurred after 22 days, differences were reduced (data not presented). When assessed 22 days after inoculation the relative number of sporangia and zoospores were significantly greater for the highest compared with the two lower soil moistures, between which there was no significant difference.

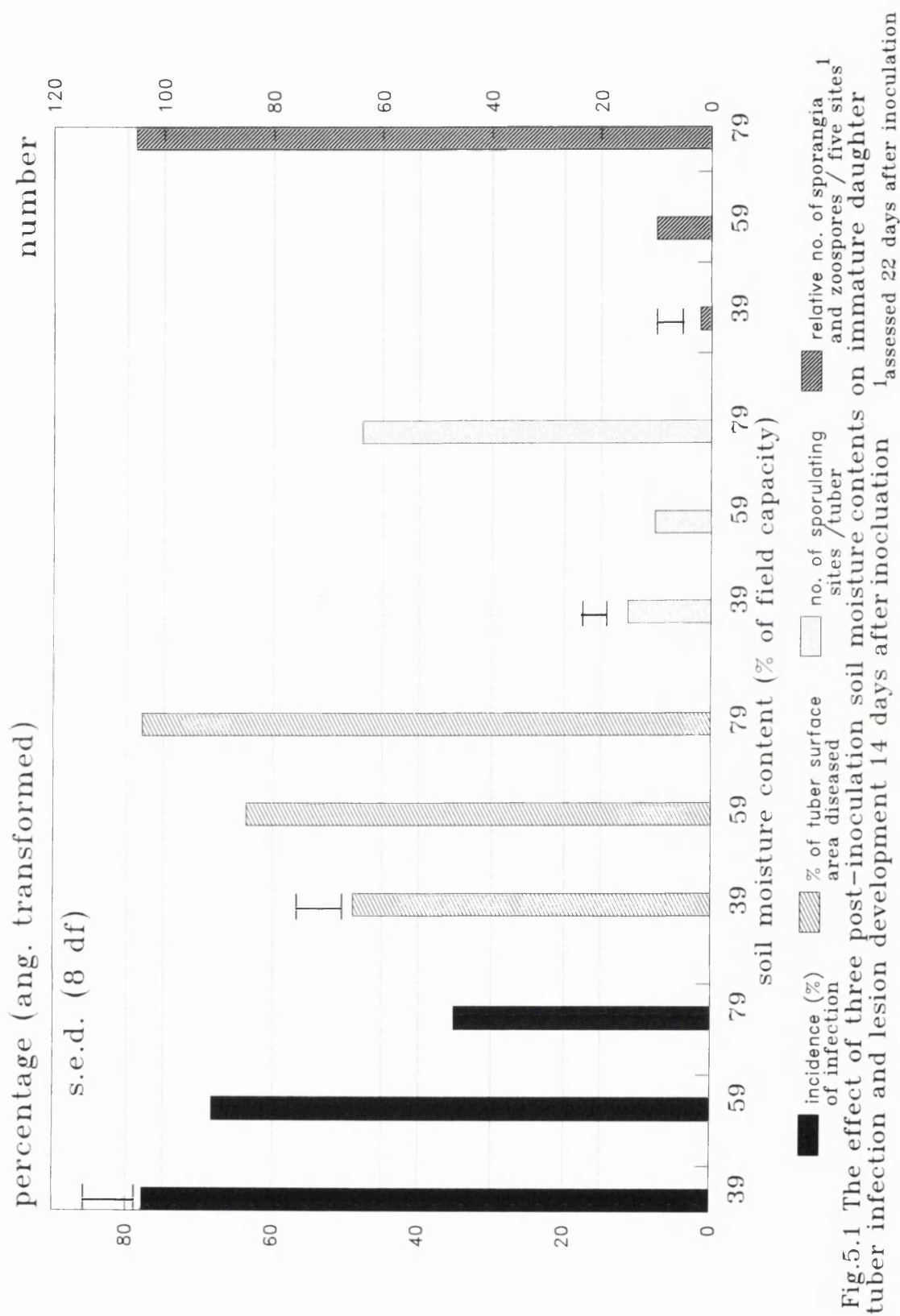
Table 5.4 The incidence of infection for immature tubers incubated in soil at different moisture contents to which *P. infestans* sporangia had been added

date of tuber harvest	incidence (%) of tuber infection ¹			
	soil moisture content (% of field capacity)			s.e.d. (8 df)
	39	59	79	
3 August	0.0	0.0	8.6	3.52
28 August	6.1	10.5	12.9	5.33
13 September	0.0	0.0	0.0	-

¹ ang. transformed data

Experiment 4 Post-inoculation soil moisture and temperature treatments / mature tubers

The effect of temperature and the interaction between temperature and soil moisture content on lesion development were not significant. The incidence of infection of daughter tubers decreased significantly with increasing soil moisture content (Fig. 5.2). The percentage of the tuber surface which was diseased and the number of sporulating sites per tuber were also significantly lower at the higher moisture contents. Soil temperature had less of an effect than soil moisture and the relationship was less straightforward. The percentages (ang. transformed) of tuber infection at 8, 12 and 16° C were 76.2,



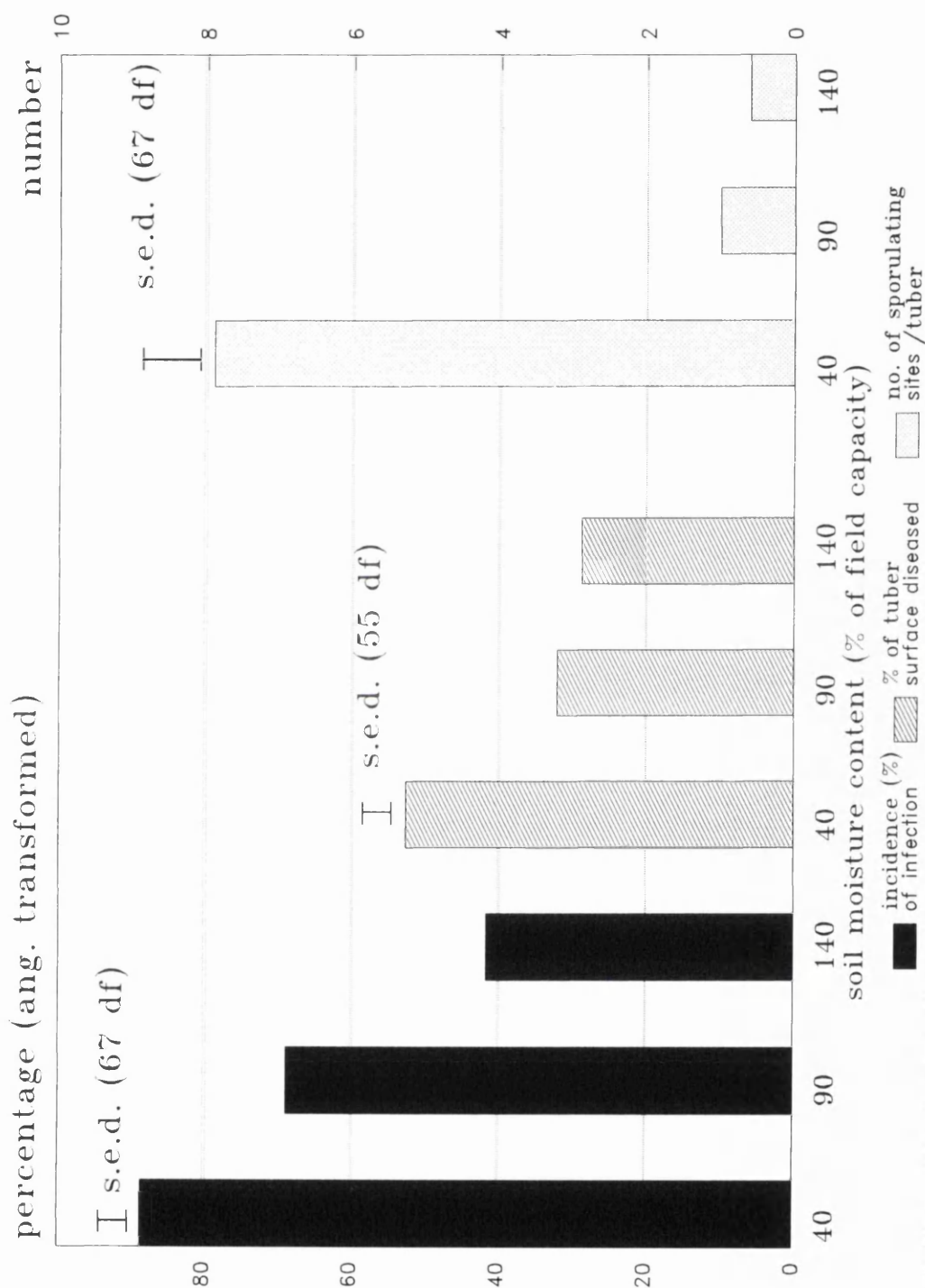


Fig. 5.2 The effect of soil moisture content post-inoculation on the infection of mature daughter tubers by *P. infestans* and subsequent lesion development (mean of three dates and three incubation temperatures)

56.2 and 65.8 respectively with a s.e.d. of 6.74 (67 df).

5.3.2 Experiment 5 The effect of soil moisture content on the spread of *P. infestans* inoculum from blighted to healthy daughter tubers in the field

Figure 5.3 shows that the ranking order of the three soil moisture content treatments in the field was consistent from tuber inoculation until the end of the experiment. On average the high smd plots were at 52 %, the medium smd plots at 62 % and the low smd plots at 75 % of field capacity.

From the time of daughter tuber inoculation onwards the temperature increase as a result of the polyethylene sheeting was generally only 1° C (Appendix 5.1). Prior to the inoculation of the tubers, however, soil temperature at 13 cm depth was often 3° C higher under the polyethylene compared with drills not under polyethylene. The reduced difference in temperature seen later coincided with the crop canopy approaching complete ground cover towards the end of July.

Fewer of the inoculated daughter tubers became infected in the high moisture content plots than in the plots of the other treatments but the differences were not significant (data not presented). Increasing the soil moisture content of field experiment plots to an average of 75 % of field capacity significantly increased the spread of blight from inoculated daughter tubers to other tubers on the same plant (Table 5.5) whereas in plots with average moisture contents of 62 % or 52 % of field capacity there was some spread of *P. infestans* from inoculated daughter tubers but it was not significantly greater than zero. Infection of healthy tubers was recorded up to 5 cm away from the daughter tuber which had been inoculated, although most of the additional infections were found within 2.5 cm of the initial inoculum source. Regular checks of the leaves and stems and incubation of suspect lesions during the growing season failed to detect blight on the haulm. At all assessments no blighted tubers were found in any of the plots in which tubers had not been inoculated.

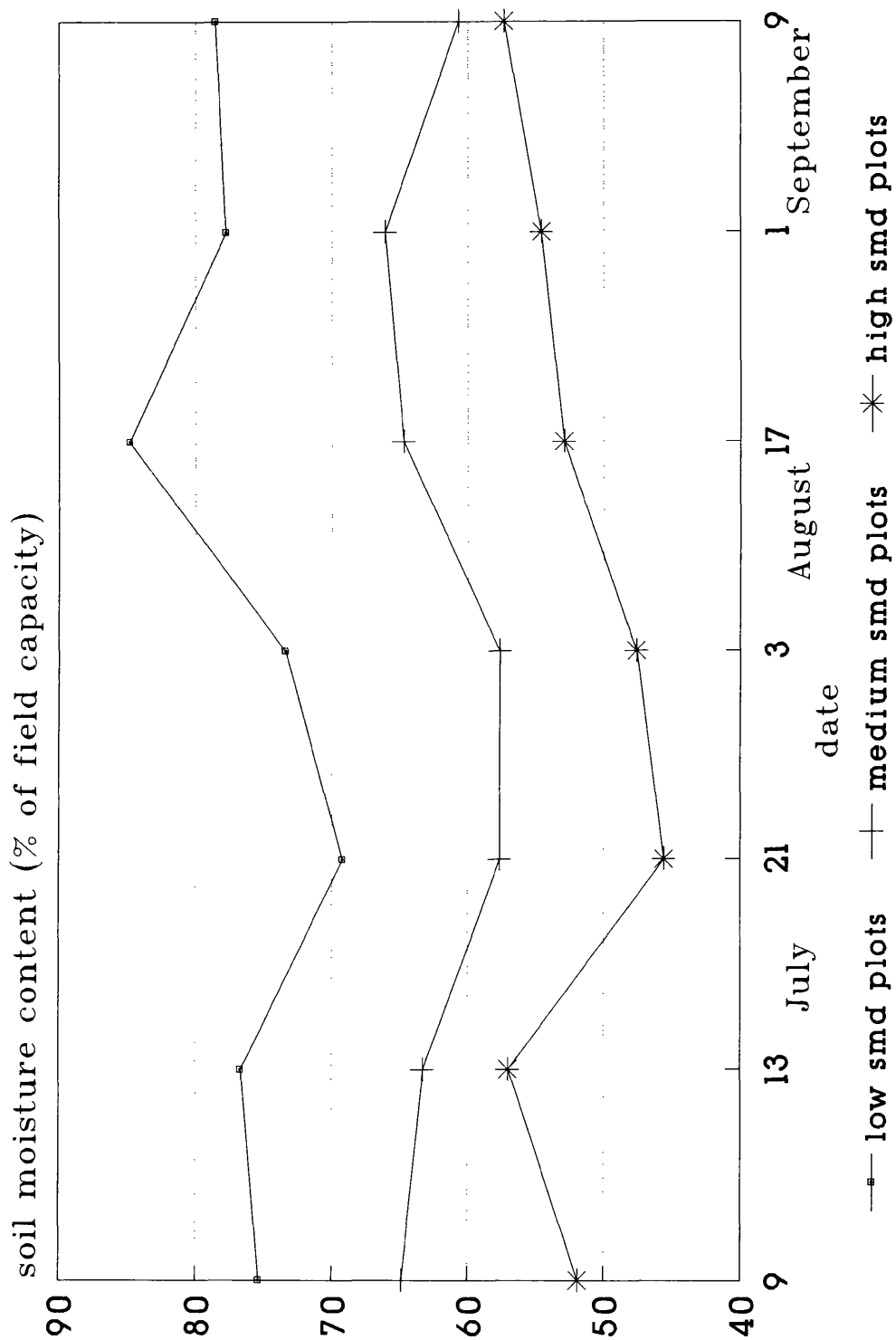


Fig.5.3 Soil moisture contents of the low, medium and high soil moisture deficit (smd) field plots over time

Table 5.5 The effect of soil moisture content on the infection of non-inoculated daughter tubers on plants that had one daughter tuber inoculated with *P. infestans*

date of harvest	incidence (%) of tuber infection ¹			
	soil moisture content (% of field capacity)			s.e.d. (17 df)
	52	62	75	
7 August	0.0	0.0	18.0	0.98
21 August	0.0	0.0	4.1	2.36
14 September	6.1	3.9	11.2	5.57

¹ ang. transformed data

The spread of *P. infestans* from inoculated daughter tubers to tubers on surrounding plants occurred most frequently in the treatment with an average soil moisture of 75 % (Table 5.6). Only for this treatment did significant numbers of daughter tubers become infected. In two plots at 75 % moisture

Table 5.6 The effect of soil moisture content on the spread of *P. infestans* from an inoculated daughter tuber on one plant to daughter tubers on neighbouring non-inoculated plants

date of harvest	incidence (%) of tuber infection ¹			
	soil moisture content (% of field capacity)			s.e.d. (17 df)
	52	62	75	
7 August	0.0	0.0	6.6	1.44
15 September	1.7	0.0	7.0	1.00

¹ data are ang. transformed

content tuber infection occurred two plants away from the nearest infected tubers, equivalent to a distance of 0.6 m (Fig. 5.4). No tubers on any of the bait plants in plots at 62 % moisture content became infected but one infected tuber on a bait plant was detected in a plot at 52 % moisture content next to, and up the slope from, an inoculated plant. The slight slope within the plots does not appear to have greatly influenced the distribution of new infections although slightly more tubers and plants were infected over a greater distance up the slope than down the slope (Fig. 5.4).

5.3.3 Experiment 6 The susceptibility of seed tubers to infection by *P. infestans*

The effects of the damage treatment and the interaction between damage and sporangia concentration were not significant. Suspensions containing 500 or more sporangia ml^{-1} resulted in a significant number of tubers becoming infected. A suspension of 1000 sporangia ml^{-1} gave a significantly greater number of infections per tuber than 500 sporangia ml^{-1} (1.7 and 0.6 respectively, s.e.d. 0.22, 23 df). The highest concentration of sporangia, i.e. 2000 ml^{-1} , gave an intermediate number of infections, i.e. 1.3, but this was not significantly fewer than for 1000. No infections were recorded in the control treatment. Infection occurred most frequently through sprouted eyes compared with wound sites and lenticels (Fig. 5.5).

5.3.4 Experiment 7 The contamination during planting of healthy seed tubers with *P. infestans* from infected tubers

No infections occurred in healthy seed tubers mixed with inoculated tubers which had either not been incubated or had been incubated for only 2 days (Fig 5.6). However, a number significantly greater than zero became infected following mixing with an inoculated tuber incubated for 3 days. Incubation of inoculated tubers for 4 or 6 days resulted in significantly more non-inoculated tubers becoming infected. However, the increase in the incidence of seed tuber

		drill				
		1	2	3	4	
↑		1	0	0	0	key
		0	0	1	0	*= plant with inoculated daughter tuber
		2	0	1	1	0-2= number of infected tubers per bait plant
up		*	*	*	*	
slope		0	1	0	0	
		0	1	0	2	
		0	0	0	0	

Fig. 5.4 Distribution of bait plants with infected daughter tubers in the field plots at 75 % soil moisture content (combined results for both assessment dates and all replicates)

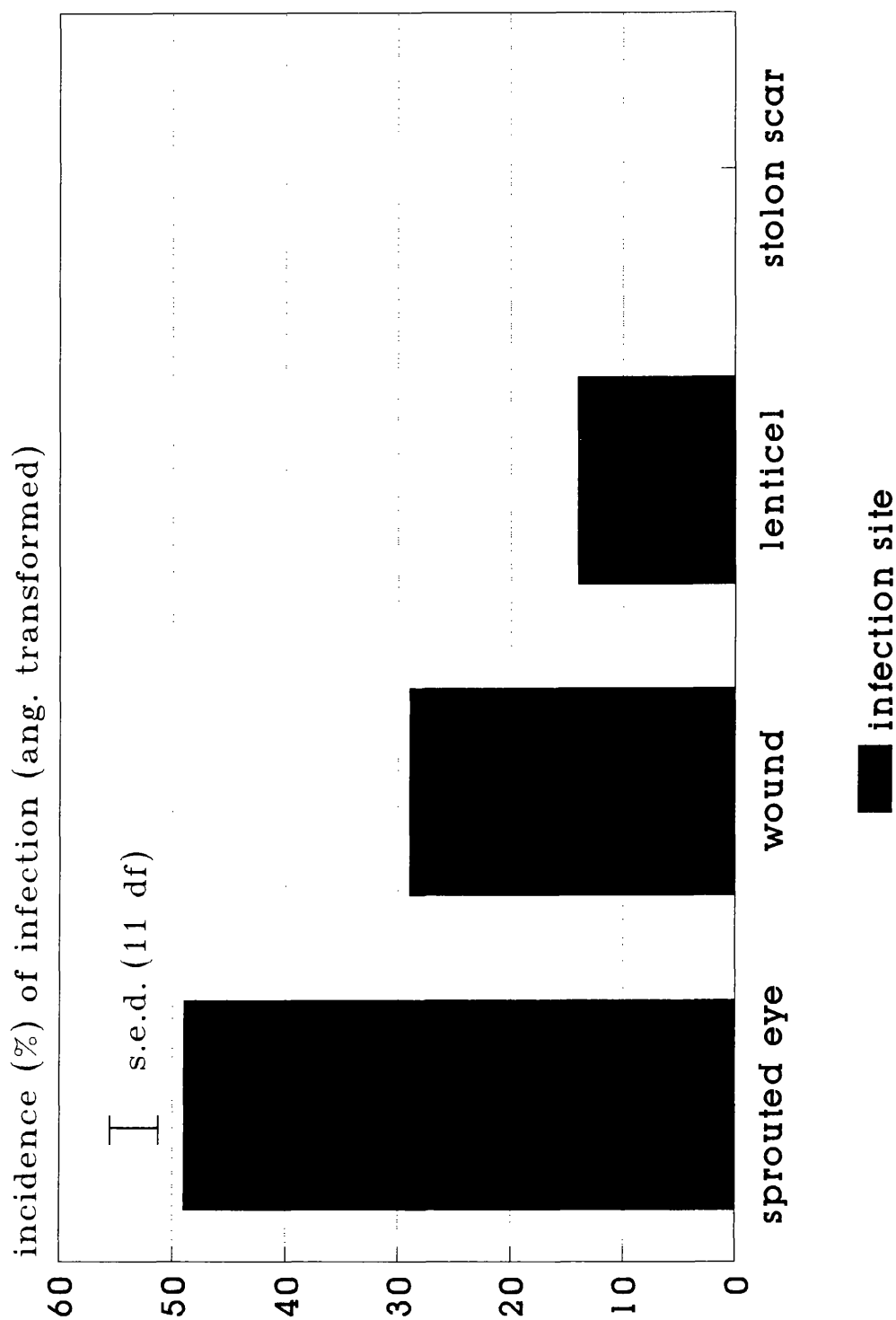


Fig 5.5 The relative incidences of seed tuber infection by *P. infestans* in relation to different sites of infection (mean of three inoculum concentrations and two damage treatments)

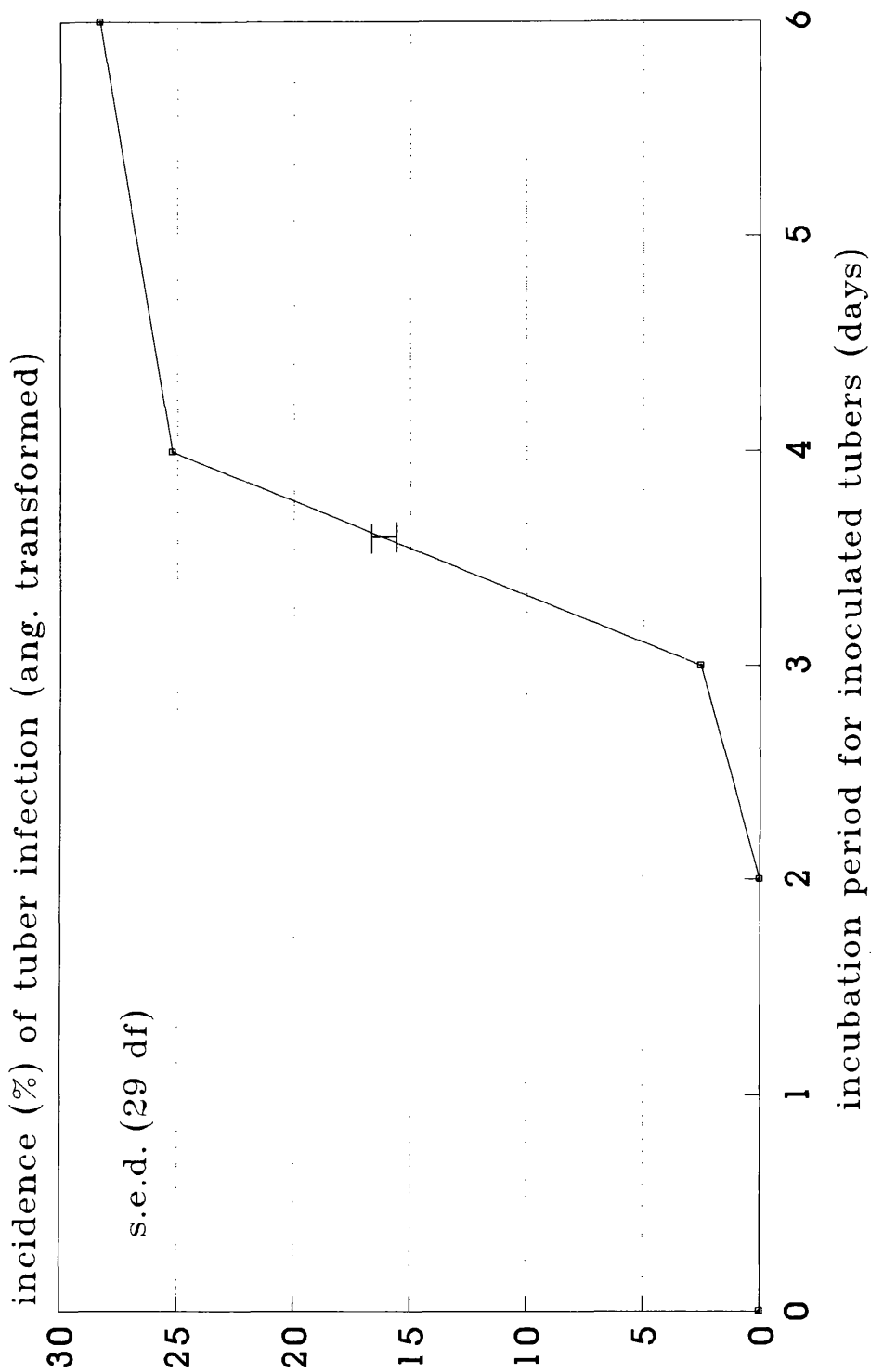


Fig.5.6 The percentage of non-inoculated seed tubers infected after being mixed with a tuber which had been inoculated with *P. infestans* and incubated for 0, 2, 3, 4 or 6 days (mean value of two damage treatments)

infection tailed off after 4 days of incubation. More damaged than undamaged tubers became infected but the effects of damage treatment and the interaction between damage and length of incubation period were not significant. Infection normally occurred via the sprout which was generally killed as a result.

5.3.5 Experiments 8 & 9 The effect of soil moisture content at planting on the sporulation on, and decay of, inoculated seed tubers

Experiment 8 Inoculation at the time of harvest of the seed tubers

Increasing soil moisture and damaging the tubers generally resulted in a significant increase in seed tuber rotting due to *P. infestans* (Fig. 5.7). In general, decay increased progressively with soil moisture for both damaged and undamaged treatments. All seed tubers were completely rotted in soils at 119 and 99 % field capacity by 14 and 35 days respectively. When assessed after 35 days, undamaged tubers were significantly less decayed than damaged ones for each soil moisture below 99 % of field capacity. However, by 58 days differences were only significant for tubers in soil at 39 and 59 % of field capacity. Bacterial soft rot, principally due to *Erwinia carotovora*, also increased noticeably with increasing soil moisture content (results not presented).

The number of tubers supporting *P. infestans* sporulation was very low on each assessment date and for all of the soil moisture treatments. As a result of this, and because different numbers of tubers were assessed for each treatment at the last two assessment dates, these results were not statistically analysed. When assessed 14 and 35 days after burial the number of tubers supporting sporulation by *P. infestans* was greatest at 79 and 59 % of field capacity respectively (Table 5.7). No sporulation by *P. infestans* was observed on any of the tubers which were assessed 58 days after burial. The transfer, after 58 days, of sound tubers (all of which had not been previously damaged) from the soils at 39 or 59 % to soil at 79 % of field capacity, however, resulted in sporulation on one and four tubers respectively 7 days after the transfer. The viability of

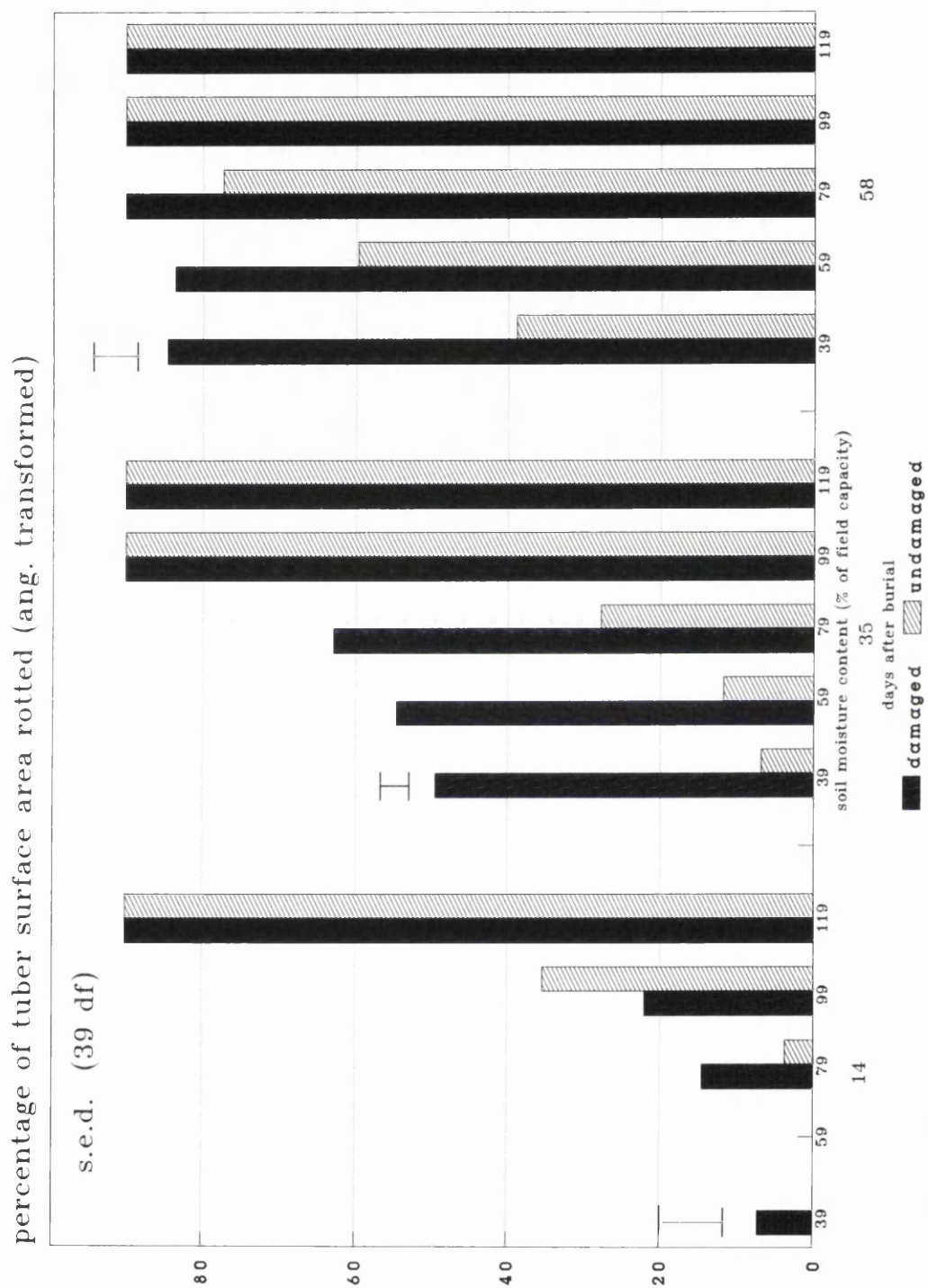


Fig. 5.7 The effect of soil moisture content after planting on the rotting of seed tubers that had been inoculated at harvest with *P. infestans*

sporangia harvested from these tubers was confirmed by the successful infection of potato leaflets.

Table 5.7 The effect of post-planting soil moisture content on sporulation by *P. infestans* on seed tubers inoculated at the time of harvest

days after planting	number of tubers supporting sporulation soil moisture content (% of field capacity)				
	39	59	79	99	119
14	0 ¹	0	2	1	0
35	1	2	1	0	0

¹number of tubers out of a total of 24

Experiment 9 Inoculation shortly before planting the seed tubers

The soil moisture content of 119 % of field capacity completely prevented tuber infection. The incidence of infection for undamaged seed tubers was generally lower at the higher soil moisture contents; significantly for the highest compared with the lowest (Fig. 5.8). The incidence of infection for damaged tubers peaked at 59 and 79 % of field capacity. Damaging the tubers increased the number which became infected, except for the soil at 39 % of field capacity. The impact of damage was particularly pronounced and significant for the 99 and 79 % of field capacity treatments.

The effect of damage and the interaction between damage and moisture content on lesion development were, with the exception of percentage of tuber surface area rotted, not significant at any of the assessment dates. The number of tubers supporting sporulation and the number of sporulating sites per tuber were generally greatest at 79 % of field capacity 15 and 26 days after inoculation and significantly greater for 79 compared with 39 % of field capacity 15 days after inoculation (Table 5.8). Significant differences between

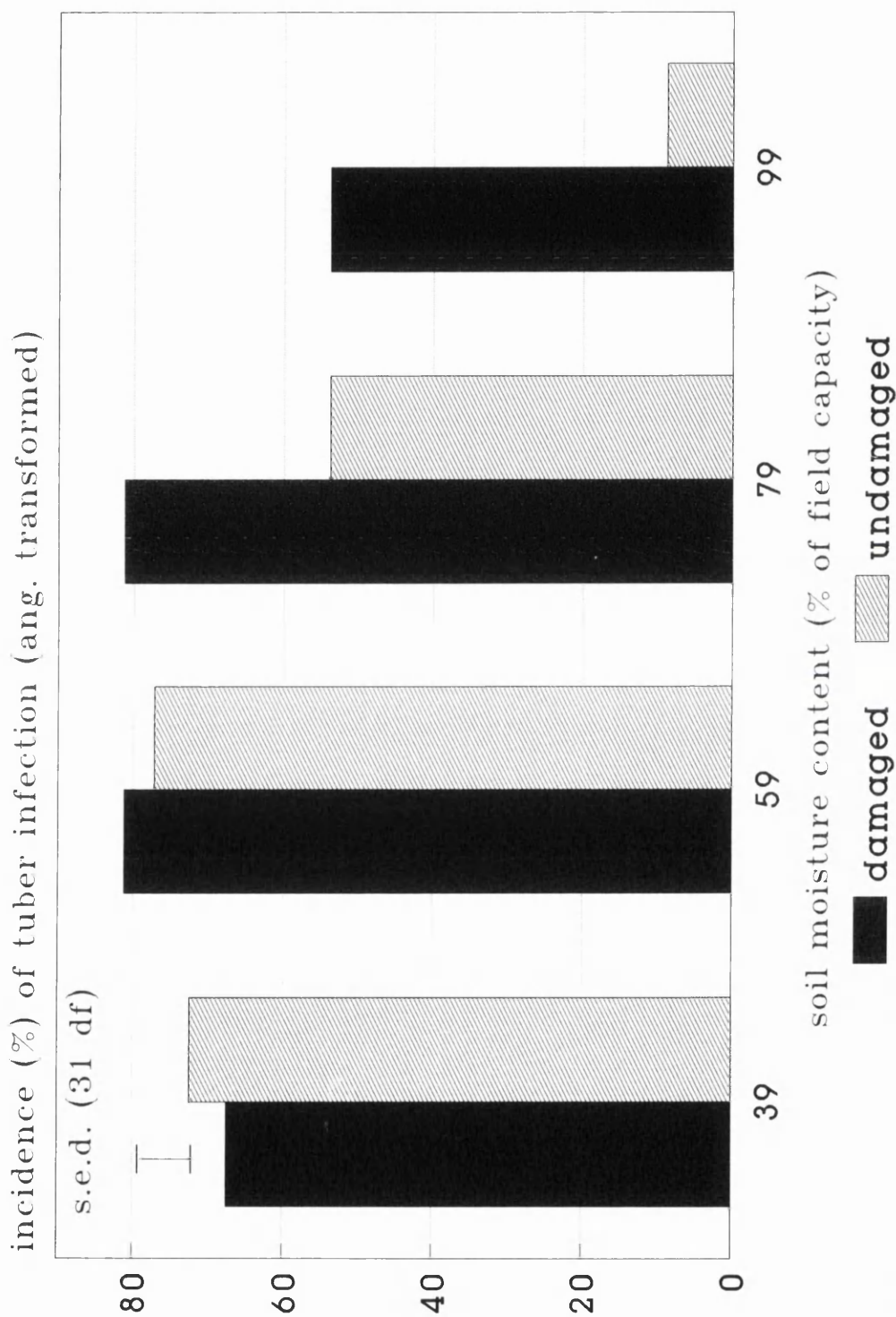


Fig. 5.8 The effect of soil moisture content on the infection of damaged and undamaged seed tubers inoculated with *P. infestans* on the day of planting and assessed 15 days later

Table 5.8 The effect of soil moisture on the development of lesions on seed tubers inoculated with *P. infestans* on the day of planting (mean value of two damage treatments)

	days after inoculation	soil moisture (% of field capacity)				s.e.d. (31 df)
		39	59	79	99	
number (%) of tubers supporting sporulation ¹	15	4.1	45.1	47.7	38.0	15.81
	26	0.0	4.1	20.6	0.0	12.32
	43	0.0	0.0	0.0	0.0	-
number of sporulating sites per tuber	15	0.1	0.7	1.4	1.1	0.52
	26	0.0	0.4	0.4	0.0	0.20
	43	0.0	0.0	0.0	0.0	-
percentage of tuber surface covered by the lesion ¹	15	17.5	23.8	22.9	17.7	3.69
	26	22.1	29.7	38.3	44.7	4.19
	43	73.6	73.9	68.3	64.6	8.63

¹ang. transformed data

treatments were not, however, detected 26 days after inoculation. Forty-three days after inoculation no tubers supported sporulation. Soil moisture had no consistent or significant effect on the percentage of the tuber surface diseased, except for the assessment 26 days after inoculation at which it increased with soil moisture content up to 99 %. For each soil moisture, the area of the tuber surface with symptoms increased progressively over the 43 days of the experiment.

When assessed 43 days after inoculation the degree of rotting of infected tubers, principally by *P. infestans*, was greater at higher soil moistures (Fig. 5.9). The increase in rotting with increased soil moisture content was much greater for damaged tubers. The impact of damage was significant for the soil at 99 % of field capacity.

5.4 Discussion

5.4.1 The spread of *P. infestans* inoculum from infected daughter tubers

Experiments reported here have demonstrated for the first time that the spread of *P. infestans* from diseased to healthy daughter tubers occurs between plants. In addition the distance of spread is much greater than previously thought. Healthy tubers on bait plants up to 60 cm away from the nearest infected plant were infected if soil moistures were high. These experiments have also demonstrated the spread of *P. infestans* from diseased to healthy daughter tubers on the same plant over a distance of 5 cm when soil moisture was high. Lapwood (1962) and Lacey (1962, 1967b) reported the spread of *P. infestans* over a distance of 1.3 cm from tuber to tuber on the same plant. Lacey (1965) concluded that although the underground movement of *P. infestans* and infection of tubers was possible it was unlikely to be significant. These experiments have, however, demonstrated for the first time that when soil moistures are high significant incidences of tuber blight can arise as a result of underground spread of *P. infestans* between daughter tubers. This phenomenon

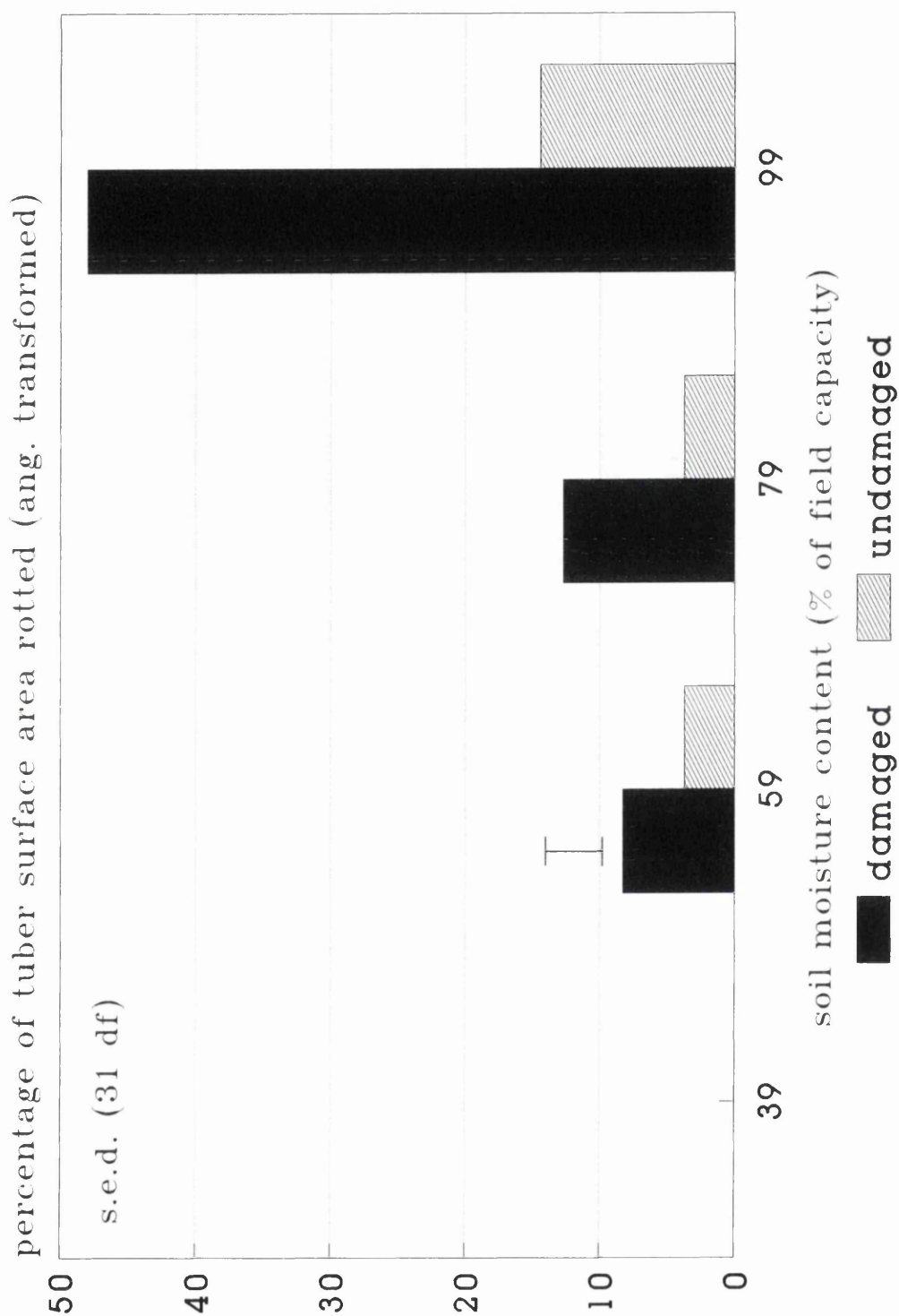


Fig. 5.9 The effect of soil moisture on the degree of rotting of damaged and undamaged seed tubers inoculated at planting with *P. infestans* and assessed 43 days later

may partly explain the reported high incidences of tuber blight at the harvest of crops which had no foliar blight (Murphy & McKay, 1927; Grainger, 1957; Hirst & Stedman, 1962; Lacey, 1962; Boyd, 1972). There is evidence that infected seed tubers could be the source of *P. infestans* inoculum for the initial infection of daughter tubers. This route of disease transmission is discussed in more detail in the second section of this discussion.

Sporangia production on inoculated daughter tubers generally increased with soil moisture up to 79 % of field capacity. This is likely to be a contributing factor to the significantly greater tuber to tuber spread which occurred at higher soil moisture contents in the field. Lacey (1967b) reported that harvested tubers, which were then incubated for 24 hours, sporulated most when the soil moisture content in the field had exceeded 20 % by weight. Gisi *et al.* (1980) showed that the maximum number of *Phytophthora cinnamomi* sporangia was produced in a sandy loam soil at a matrix potential of 80 to 160 mb, i.e. just below field capacity, and that the number decreased to zero as the soil became drier. Similar results were obtained for *Phytophthora palmivora*. Pfender *et al.* (1977) reported that *Phytophthora megasperma* sporulated in a sandy loam soil at 50 mb, i.e. field capacity, but ceased to sporulate as the soil became drier.

Tubers can become infected by *P. infestans* through eyes, lenticels, wounds (Lacey, 1967a) and directly through the skin (Walmsley-Woodward & Lewis, 1977). Lapwood (1977), however, reported that lenticels and eyes were the most frequent sites for tuber infection by *P. infestans* in the field. Meinl (reported by Cutter, 1978) showed that higher soil moistures increased tuber lenticel initiation and development and therefore the number of potential sites for infection. In the experiments reported here significantly more lenticels became infected on tubers which had been buried in soils with a high moisture content compared with low moisture content prior to inoculation. Meinl (reported by Cutter, 1978) and Lohnis (reported by Zan, 1962) reported that

under dry conditions a suberised layer forms below the complementary cells of the lenticel and suberisation has been associated with the greater resistance of lenticels to *P. infestans* in clay soils by Lohnis (Lacey, 1967) and to *Erwinia carotovora* var. *atroseptica* (Fox *et al.*, 1971). In moisture-saturated soils water is absorbed by the tuber (Perombelon & Lowe, 1975), the suberised layer of cells below the lenticels swells and ruptures and the lenticels open and proliferate (Cutter, 1978). Lacey (1962) and Adams (1975) suggested that proliferation might increase the susceptibility of lenticels to infection by *P. infestans*. When tubers were buried in soil with a high moisture content compared with a low moisture content significantly greater incidence of infection only occurred when tubers were harvested in early August and not when they were harvested later (experiment 2). Adams & Lapwood (1978) found that lenticels proliferated less readily as the tubers aged and that they did not proliferate in the field after mid-August. Although results obtained by Stewart *et al.* (1993) showed that tubers from drier compost were significantly more susceptible to infection by *P. infestans* than those from wet or moist compost, they suggested that this might be a result of the adverse effect of the dry compost on potential antagonists of *P. infestans*.

In contrast to their effect on the susceptibility of lenticels to infection by *P. infestans* high soil moisture contents prior to inoculation reduced the infection of damaged daughter tubers. This may have been the result of the beneficial effect of soil moisture and therefore humidity on the formation of wound periderm. The speed of wound periderm development, which confers an important degree of resistance to some fungal diseases (Cutter, 1978), has been shown to increase with relative humidity (Artschwager, 1927). Alternatively, the reduced tuber infection may reflect increased soil fungistasis or bacterial competition at damage sites. Compared with the number of proliferating lenticels on a tuber the number of tuber wounds occurring *in situ* is likely to be small, therefore the impact of soil moisture content on the susceptibility of

wounds is likely to be small compared with its overall effect on tuber susceptibility. The most likely cause of damage to tubers in the field is pest attack but, although in some cultivars this can be high (Winfield *et al.*, 1967), insecticides, resistant cultivars and cultural control methods (Gibson, 1978) generally keep the incidence of pest damage low. The wounding of tubers *in situ* by quartz sand particles as a result of growth (Hooker & Page, 1960) and by secondary growth (Whitehead *et al.*, 1953) has also been reported but this is also unlikely to be at significant levels in practice.

In experiment 5 high soil moistures increased the distance moved by *P. infestans* from inoculated daughter to healthy daughter tubers. Stolzy *et al.* (1965) reported that only at a high moisture content were the pores in a sandy loam soil of sufficient size and continuity to allow zoospores, but not sporangia, of *Phytophthora* spp. to move with the infiltrating waters to the vicinity of the root surfaces of *Citrus sinensis*. Boyd (1980) found in field experiments that the first haulm infections by *P. infestans* occurred on potato stems at soil level when rainfall, and presumably soil moistures, were high. He suggested that these infections were initiated by motile zoospores of *P. infestans*, from infected seed tubers, which were carried in soil water a distance of, perhaps, 10 to 20 cm to the soil surface. Furthermore, Pfender *et al.* (1977) reported that the indirect germination of sporangia of *P. megasperma* in a sandy loam soil was only observed when the soil was at field capacity. Stolzy *et al.* (1965) found higher incidences of root decay by *Phytophthora* spp. in *Citrus sinensis* at higher soil moisture contents and they suggested that this was because conditions were more favourable for the production of zoospores. Zan (1962) noted that zoospores of *P. infestans* penetrated further into the soil profile than did sporangia when washed down by rain. It is likely that the blighted tubers which occurred on bait plants in the experiments reported here were infected by zoospores, which had moved in free water in the soil profile or at the soil surface.

Soil type also influences the movement of fungal propagules through soil. Pfender *et al.* (1977) found that although zoospores of *P. megasperma* were able to migrate upwards through 65 mm of a sandy loam soil they rarely moved more than 24 mm through a silt loam soil. Allen & Newhook (1973) noted that the collision of *P. cinnamomi* zoospores with solid surfaces produced a disorienting effect which made zoospore movement through pores less than 190 μ m in diameter unlikely. Pore necks bigger than this were calculated to be 44.9 % more frequent in a sandy loam than in a silt loam soil (Pfender *et al.*, 1977). Soil structure also affects pore size and water movement. Soils with good structures and which are well drained may prevent much of the lateral movement of water contaminated with *P. infestans*. In contrast, however, gleying between the topsoil and subsoil layers probably impeded the downward movement of water in the soil used in these experiments.

Crosier (1934) reported that the optimum temperature for the production of *P. infestans* sporangia on infected leaves and tuber slices was between 18 and 22° C. In the experiments reported here, however, soil temperatures of 8 compared with 16° C did not reduce the sporulation on infected tubers and although tubers were less susceptible at 12 than at 8 or 16° C it is unlikely that the incidence of tuber blight would be greatly affected by the range of soil temperatures which occur during the growing season in northern Britain. Sato (1979), however, reported that in Japan despite extensive foliar infection and heavy rainfall the incidence of tuber blight was low and attributed this to unfavourable temperatures for zoospore germination. Soil temperatures during the growing season remained above 18° C, even during and immediately after rainfall.

The results of this thesis indicate that tuber blight could be minimised by the development of methods to reduce the tuber to tuber spread of *P. infestans*. Breeding cultivars with greater tuber resistance to blight would prevent much of the spread of infection. More resistant tubers should reduce the number of

spores produced on the surface of diseased tubers as Lapwood found that tubers which are resistant to infection are also generally resistant to the development of *P. infestans* within the tuber (Wastie, 1991). Wastie (1991), however, noted that, in general, breeding for tuber resistance to *P. infestans* has been somewhat neglected compared with breeding for foliage resistance. Furthermore, few genotypes are actually tested as blight resistance assessments often do not begin until between the 5th and 7th year of selection in a typical breeding programme (Wastie, 1991). More rapid progress in selecting for better tuber resistance would, however, be possible if glasshouse progeny tests (Wastie *et al.*, 1987b) were used in breeding programmes. Alternatively the spread of *P. infestans* from tuber to tuber in the field might be reduced by careful irrigation management aimed at preventing the channelling of water, and movement of spores, along the soil surface. Many growers irrigate on the basis that potatoes are shallow rooting and highly susceptible to water stress and that maximum yields are only obtainable if soil moisture deficits are kept low for much of the season. Allen & Scott (1992), however, reported that rooting depth is in general much greater than previously thought and concluded that many potato crops receive too much water, particularly if unexpected rainfall occurs. Improvement of drainage in fields with impeded water movement, i.e. pans or gleys, may also prevent much of the channelling of water along the soil surface. Applications of fungicides as soil drenches to control tuber to tuber spread of *P. infestans* would be too expensive to be of practical use and in addition would contravene the Fungicide Resistance Action Committee's anti-resistance strategy of not using a soil-applied fungicide for the control of late blight.

5.4.2 The spread of *P. infestans* inoculum from infected seed tubers

In countries in which the occurrence of oospores is rare or absent *P. infestans* survives over the winter as mycelium in infected tubers and it is widely accepted that the initial sources of inoculum for new infections are the infected stems produced by these tubers (Salmon & Ware, 1926; van der Zaag,

1956; Hirst & Stedman, 1960). Van der Zaag (1956) found that the incidence of infected stems was much lower in cultivars with blight-resistant foliage than in cultivars with susceptible foliage. However, Murphy & McKay (1927) reported that 99 % of tubers infected with *P. infestans* failed to produce infected stems and that those infected sprouts which did reach the surface died soon after emergence. Similarly, Hirst (1955) noted that fewer than 1 % of artificially inoculated tubers produced infected stems and Hirst & Stedman (1960) found only 21 infected stems after planting 3260 infected tubers. Boyd (1980), in experiments over six years in which up to 1000 blighted seed tubers were planted, observed no infected stems arising from the direct growth of *P. infestans* from the seed tuber into the base of the sprout either when the seed tubers were naturally or artificially infected. Wallin & Polhemus (1956) observed no blighted stems from inoculated seed tubers of the cultivar Irish Cobbler. The observation by Roer & Toxopeus (1961) that the shoot and stem may be completely resistant to the *P. infestans* originating from the seed tuber because of different R genes in the haulm and tuber might, for some cultivars, explain the very low incidences of infected stems observed in practice. As has been stated earlier in this discussion, Boyd (1980) suggested that zoospores released from the blighted seed tuber could infect stems indirectly after they had moved in soil water away from the seed tuber upwards through the soil and onto the surface of the stem. Hirst & Stedman (1960) also suggested that spores or mycelium from diseased seed tubers may cause epidemics in the absence of an invaded stem and Lacey (1967b) noted that there is much evidence compatible with the idea that leaves touching the soil in furrows might be infected by zoospores produced on blighted seed tubers. For these proposals to be acceptable it is necessary to demonstrate that zoospores are produced on the surface of blighted seed tubers. The results of this thesis have demonstrated for the first time that sporangia of *P. infestans* can be formed at the time of

planting on the surface of seed tubers which had been infected the previous season.

Another consequence of the formation of sporangia on seed tubers around the time of planting is that tuber to tuber transmission of *P. infestans* could occur from diseased to healthy seed tubers when the tubers are handled at planting. Other results in this thesis have demonstrated this for the first time. Seed infected in this way would be impossible to identify and remove at planting. However, such transmission is only likely to occur infrequently because specific environmental conditions are required. In the experiments reported here infected seed tubers had to be incubated in a high relative humidity for a period of 48 hours for tuber to tuber transmission of *P. infestans* to occur. High relative humidities might, however, occur in practice. Sprouting increases the respiration rate of tubers (Burton *et al.*, 1992) and this could lead to increased relative humidities in store. An increased relative humidity around tubers may also develop if the seed gets wet just before planting and planting is then delayed. A reduction in the production of sporangia on the surface of diseased tubers might be achieved by keeping seed stocks well ventilated prior to planting and by limiting the handling of seed.

Other results reported here have demonstrated that in dry soils blighted seed tubers can remain sound for a period of at least 58 days after planting and that sporulation can occur when soil moisture content is subsequently increased. This raises the possibility of transmission of *P. infestans* directly from the blighted seed tuber to the daughter tubers. Indeed, immature tubers are particularly susceptible to infection by *P. infestans* (Walmsley-Woodward & Lewis, 1977). This is another alternative explanation of the phenomenon of high incidences of tuber blight which sometimes occur in the absence of any visible foliar blight. However, further work is required to determine the extent of, and factors which might influence, such spread in practice. The direct spread of *P. infestans* from mother to daughter tuber would only occur in

cultivars which formed daughter tubers while inoculum was being produced on the infected mother tuber. Allen & Scott (1992) reported that for the cultivar Estima planted on 1 April, tuber initiation occurred approximately 8 weeks later whereas for the cultivar Cara it was not until 11 weeks after planting. For most cultivars the timing of tuber initiation has not been studied in detail. Low temperature is reported to induce tuber initiation (Ivins & Bremner, 1964) whereas high temperatures (Menzel, reported by Cutter, 1992) or high rates of nitrogen (Ivins & Bremner, 1964) delay it. Potato cultivar influences sporulation by *P. infestans* on daughter tubers in the glasshouse (Lacey, 1962) and may therefore also influence the length of time that infected seed tubers support sporulation. Isolate may also affect the period of sporulation and the rate of decay of infected tubers. Harrison (1992) noted that isolate affected the proportion of sporangia that released zoospores after chilling. Walker & Cooke (1988) found in overwintering experiments that fewer tubers of the cultivar Kerr's Pink survived in storage if infected with phenylamide-resistant compared with sensitive isolates due to subsequent invasion by soft rotting bacteria. In this thesis the production of sporangia on seed tubers was examined in one soil type only and different soil types are likely to influence the rate of rotting of infected tubers and the timing of sporangia production on them.

CHAPTER 6

The infection of ware potatoes by *P. infestans* during simulated washing and an evaluation of control measures

6.1 Introduction

Despite rigorous quality assessments of potato crops in the field and of the tubers harvested from them to try to ensure a virtually blight-free sample for processing, there have been reports of high incidences of tuber blight in samples of pre-packed potatoes (Caledonian Produce, personal communication). These may be explained by the presence of a few blighted tubers among the harvested crop which during washing supply inoculum for the infection of healthy tubers. Water in wash and dump tanks was identified as one of the main sources of plant pathogens of washed produce as early as 1932 when Barker & Heald (1932) reported the infection of apples by *Penicillium expansum* (blue mould) during cleaning and packing. Likewise the transmission of *Botrytis cinerea* and *Mucor piriformis* during the processing of pears has been demonstrated (Spotts & Cervantes, 1986, 1992). Agrios (1978) reported that post-harvest losses were predominantly caused by the Ascomycetes and Fungi Imperfecti but that a few Oomycetes, e.g. *Pythium* and *Phytophthora* spp., can also cause post-harvest soft rots of fleshy fruits and vegetables. The tuber to tuber spread of *P. infestans* during processing of the ware crop has, however, received little attention. Indeed it was not until 1991 that the production of viable sporangia on tubers that had been stored for a short period and the spread of infection from diseased to healthy tubers during handling was reported (Dowley & O'Sullivan, 1991b). As it is virtually impossible to detect every infected tuber in a sample for processing, tubers with lesions may be washed along with healthy tubers. Indeed final quality assessments are often performed after washing the sample (Caledonian Produce, personal communication). Washed tubers, which are often still wet, are placed in plastic bags; conditions which are ideal for infection to occur.

The aims of the experiments in Chapter 6 were first to quantify the release of *P. infestans* inoculum from blighted tubers during washing (experiment 1) and to assess the potential of this inoculum to cause new infections during the

simulated bulk washing of tubers (experiment 2). Further studies were performed to determine if either heat treatment (experiments 3 & 4) or the application of a disinfectant to the wash water (experiments 5, 6 & 7) might prove an effective means of preventing the spread of *P. infestans* during tuber washing. Heat treatment is a well-established technique for controlling plant pathogens (Phillips & Austin, 1982) and has been used to destroy *P. infestans* in seed tubers (van der Zaag, 1956). Peracetic acid is amongst the most powerful microbiocides known and is reported to be effective against a wide spectrum of micro-organisms including viruses, bacteria and fungal spores (Anon, 1988). Furthermore, the development of resistance to peracetic acid by any micro-organism has not been recorded.

6.2 Materials and methods

6.2.1 Experiment 1 Estimation of the quantity of inoculum released from blighted tubers during washing

Seed tubers of the cv. Home Guard were planted in a sandy loam soil (see section 2.3) in pots in the glasshouse in December 1990. The temperature in the glasshouse, which was recorded daily, was maintained within the range 9 to 22° C from the time of planting onwards. Higher temperatures have been shown to inhibit tuber initiation (Slater, 1968). From emergence, the plants were exposed to a 16-hour day under fluorescent SON-T lamps with an approximate intensity of 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The inoculation of immature daughter tubers *in situ* was performed on two occasions using essentially the same method as Lacey (1967b). The soil covering the daughter tubers was removed to expose the tuber surface and a 20 μl droplet containing c. 500 sporangia of *P. infestans*, isolate TSE1 (see section 2.2.3), was placed on a small wound made in the tuber as described in section 2.2.4. The tops of the pots were then covered with polyethylene film for 24 hours prior to replacing the soil. Following tuber inoculation the soil was kept moist by watering daily.

Tubers on other plants were not inoculated to serve as controls. Soil temperature at 10 cm depth, measured daily from the time of tuber inoculation, varied between 12 and 22° C and was on average 17.5 and 17.9° C for the first and second tuber inoculations respectively. The average depth of daughter tubers in the field was reported to be 13 cm from the crest of the ridge (Lapwood, 1977).

Control and inoculated tubers were harvested 9 days after inoculation. Excess soil was removed and the tubers incubated at 15° C in conditions close to 100 % relative humidity for 24 hours before weighing. These conditions are not untypical of those which might occur within stacks of tubers in commercial packing stations prior to tuber washing. To ensure that only the inoculum which would normally be washed off in a commercial washing plant was removed, the procedure for simulated washing was as close to that used by Caledonian Produce as possible (Appendix 6.1). Individual tubers were shaken in 400 ml of carbonate buffer (Appendix 2.1) at approximately 10° C for 3 minutes. The resulting sporangia suspensions were thoroughly shaken on a Griffin flask shaker for 15 minutes, after which a 10 ml sample was extracted from each suspension and retained. The remainder of each sporangia suspension was discarded. Earlier attempts to ascertain the concentration of *P. infestans* in tuber wash samples using the tuber slice method (Lapwood, 1961e; Lacey, 1962, 1967b) failed to give consistent results as slices often became infected by soft rot bacteria. Each sample was sonicated (M.S.E. Soniprep 150) for 35 minutes (55 seconds on, 5 seconds off in each minute) at 14 amplitude modules (Am). Immediately following sonication each of the samples was assayed for *P. infestans* using the enzyme-linked immunosorbent assay (ELISA) technique described in section 2.4.1. The absorbance due to *P. infestans* in the wash water samples was calculated by subtracting the mean absorbance reading of the control samples from the absorbance readings of the wash water samples. Absorbance values (A_{405}) for a series of five-fold dilutions

of a 3.3 mg ml⁻¹ extract of *P. infestans* mycelia peeled from 10 day-old cultures on rye B agar (see section 2.2.1) and prepared as above were determined. These absorbance values (after transformation to log₁₀ values) were regressed (linearly and quadratically) on the concentration of *P. infestans* to provide a calibration curve of absorbance against known concentration of *P. infestans*. The concentration of *P. infestans* in each of the wash samples was then calculated from its absorbance value (after transformation to log₁₀ values) using the quadratic calibration curve which gave a better fit than the linear curve.

6.2.2 Experiment 2 The infection of immature, mature and damaged tubers by *P. infestans* during washing

On 17 December 1990 single seed tubers of the cv. Home Guard were planted in pots containing a mixture (7:3:2 by volume) of sandy loam soil, sphagnum peat and Perlite (see section 2.3) in the glasshouse. Single superphosphate fertiliser had been added to the mixture at the rate of 3g l⁻¹. Temperature and lighting regimes were maintained as in experiment 1. A liquid foliar feed of N, P & K in the ratio 2:1:4 was applied to run off daily from emergence onwards. Regular checks were made to ensure that the haulm was free from blight. Tuber skin set was induced on 20 April by cutting and removing the haulm of the plants.

Daughter tubers were harvested from randomly selected plants on April 12, 16, 19 and 30 and May 8 and 15. Tubers harvested after the haulm had been cut had noticeably thicker skins than those harvested prior to the removal of the haulm. At each harvest 75 tubers, between c. 35 and 85 mm in diameter, were harvested and carefully examined to ensure freedom from any blight infections. Tubers were then allocated at random to five lots of 15 tubers. Two of these lots, one of which was the control, were then artificially damaged by manually shaking them for 60 seconds in a plastic box containing c. 70 g of surface-sterilised stone chippings. The remaining three lots were undamaged replicates. Each of the five lots, with the exception of the control, was then washed for 3

minutes in a bin containing a mycelial and sporangial suspension of *P. infestans*, isolate TSE1, in tap water at a concentration of $1.648 \mu\text{g ml}^{-1}$. The concentration of *P. infestans* in the water was equivalent to the concentration of sporangia and mycelia calculated to be in commercial washing apparatus after 4 hours of washing a potato stock with approximately 1 % of tubers infected with blight. This and the ratio of water to tuber weight, which was 12:1, were based on the results of experiment 1 and on information obtained during the visit to Caledonian Produce (Appendix 6.1). The 15 control tubers were washed for 3 minutes in the same volume of water without any *P. infestans* added. The temperature and pH of the water used for each wash were measured. Following washing, the tubers were left to drain for approximately 3 minutes prior to individual tubers being placed in containers. These containers were then randomly arranged between four trays, covered with polyethylene and kept at 16°C in the dark. Ten to 14 days later the trays were removed and the tubers assessed for lesions and sites of sporulation. Any sites of sporulation were confirmed as *P. infestans* by microscopic examination. Tubers that were considered to be blighted but on which no sporulation occurred were kept for a further 10 to 14 days after which a confirmatory second assessment was made.

The design of the experiment was unbalanced therefore no s.e.d. could be calculated for the comparison of damaged and undamaged tubers.

6.2.3 Experiment 3 An investigation of the thermal death point (tdp) of isolates of *P. infestans* obtained from potato crops grown in Scotland

For six *P. infestans* isolates fungal growth (mycelia and sporangia) was harvested from 18 plates of rye B agar which had been inoculated 10 days previously. The isolates used were obtained from various locations in the west of Scotland and included metalaxyl-sensitive and metalaxyl-resistant ones (Table 6.1). Three replicate suspensions of each isolate containing 0.17 mg ml^{-1} were prepared and then shaken on a Griffen flask shaker for 1 hour. Aliquots of 3 ml of each suspension were then dispensed under aseptic conditions into

seven sterilised test tubes. Three tubes for each isolate, one from each replicate suspension, were then dipped for 5 minutes in a gyratory bath containing water at 25 (control), 36, 40, 44, 48, 52 or 56° C to an accuracy of $\pm 0.5^\circ$ C. The temperature of the bath water and the temperature of sample suspensions (which reached the appropriate treatment temperature within 1 minute) were recorded over the 5 minutes (Figs. 6.1 & 6.2 respectively). Following heating, the suspensions were allowed to cool to room temperature. Eight 10 μ l aliquots, each containing approximately 60 sporangia as well as mycelia of *P. infestans*, were then spotted onto rye B agar. These plates were sealed then incubated at 16° C in the dark for 9 days. After incubation the number of spots where *P. infestans* was growing was recorded.

Table 6.1 *P. infestans* isolates used to determine the thermal death point

code	origin	sensitivity to phenylamide
SA91	Strathclyde	resistant
RF1	Strathclyde	not determined
2.7	Dumfries	sensitive
4.1	Borders	resistant
TSE1	Strathclyde	sensitive
FI	Strathclyde	not determined

6.2.4 Experiment 4 The evaluation of hot water treatment as a method of minimising the infection of potato tubers by *P. infestans* during washing

Single seed tubers of the cv. Home Guard were planted in pots containing a 50:50 mixture of sandy loam soil and sphagnum peat in the glasshouse on 23 December 1992. The temperature and light regimes were as described in experiment 1. Immature daughter tubers were harvested before skin set from

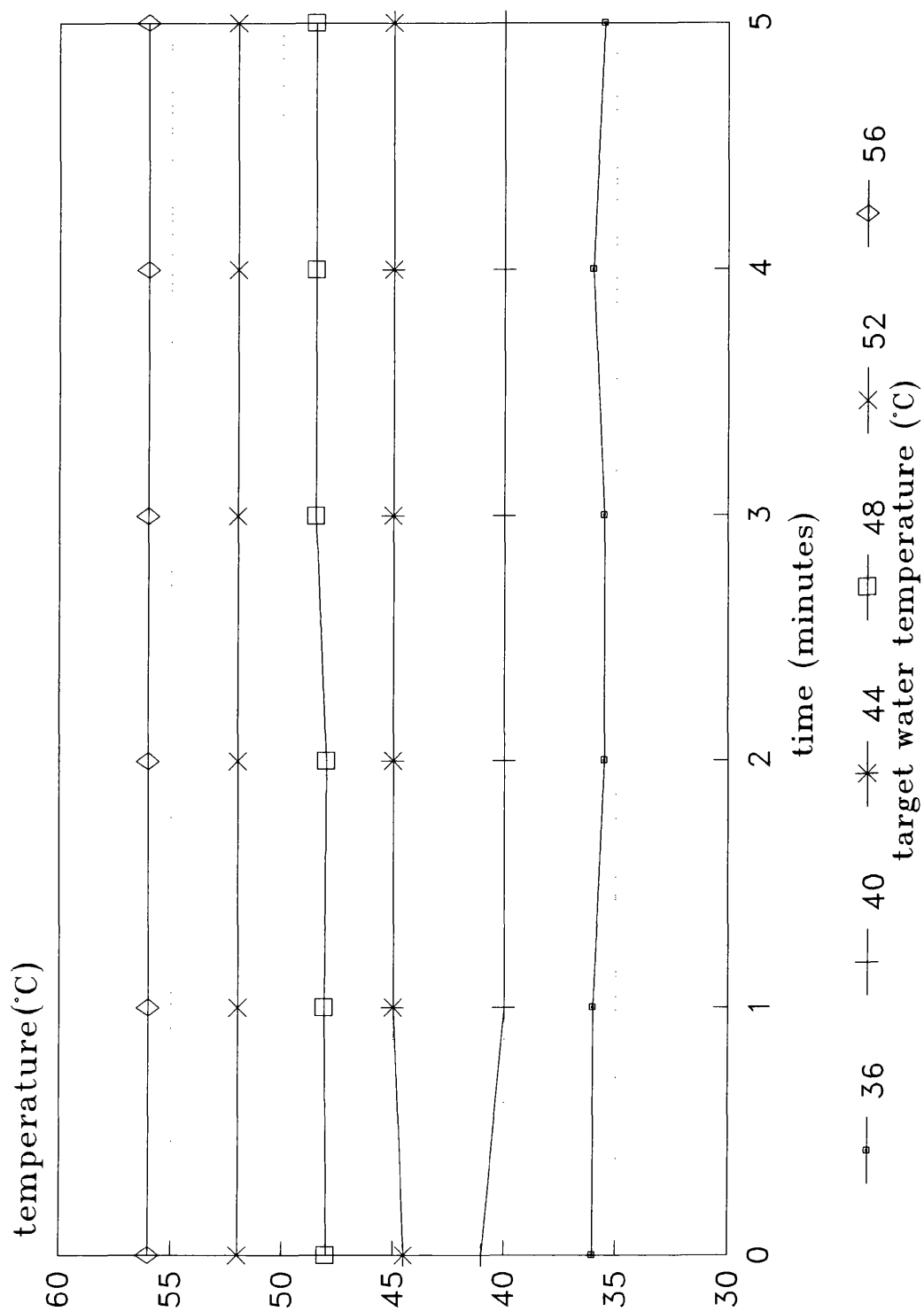


Fig. 6.1 The temperature of the bath water during the 5 minutes that *P. infestans* samples were treated

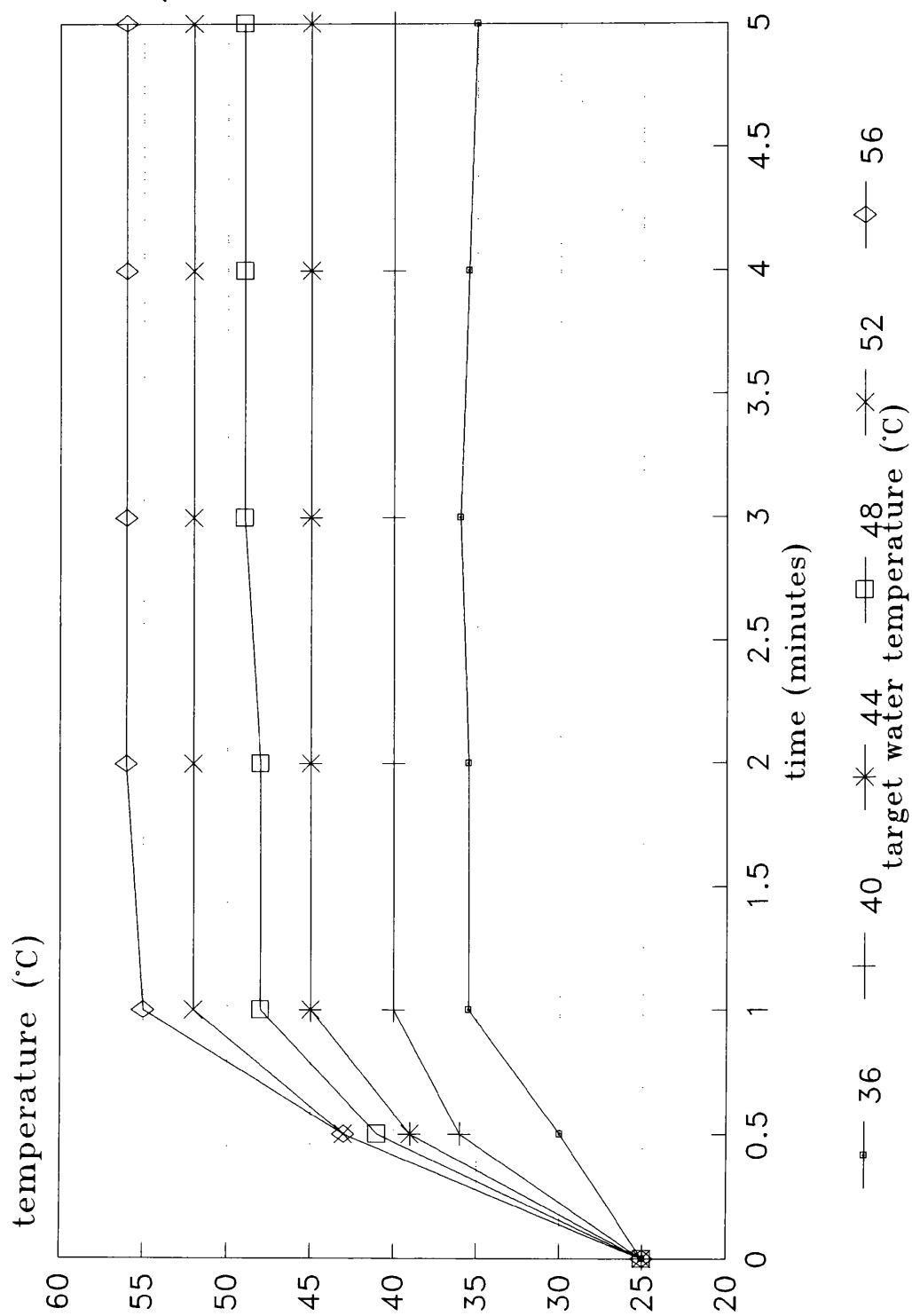


Fig. 6.2 The temperature of *P. infestans* suspensions dipped in the bath water for 5 minutes

approximately 45 randomly selected plants on April 27, May 17 and June 4. On each date 240 tubers of between c. 35 and 85 mm diameter were harvested and carefully examined to ensure that none had symptoms of blight. Tubers were then allocated at random to 12 lots of 20. A mycelial and sporangial suspension of a single isolate of *P. infestans* (Table 6.2) was prepared at each date from c. 10 day-old rye B agar cultures by dispersing the mycelia and sporangia in 5 ml of sterile water and shaking on a Griffen flask shaker for one hour. On each date three replicate lots of tubers were then dipped for 3 minutes in one of four treatment suspensions, i.e. water at 44° C without and with *P. infestans* and water at room temperature (19-22° C) without and with *P. infestans*. The water was heated in a Gallenkamp bath to an accuracy of $\pm 1^\circ \text{C}$ and its temperature was monitored throughout the experiment. No temperature drop was recorded when the tubers were first immersed in the water. For the first evaluation the ratio of water to tuber weight and the concentration of *P. infestans* were as in experiment 2 but for the other two evaluations the concentration of *P. infestans* in the suspension was increased to $8.0 \mu\text{g ml}^{-1}$ (Table 6.2). Following treatment tubers were incubated at c. 15° C in conditions close to 100 % relative humidity and 8 to 10 days later assessed for the number of infections per tuber.

6.2.5 Experiment 5 The effect of a hydrogen peroxide / peracetic acid / acetic acid mixture on the viability of a suspension of *P. infestans* containing mycelia and sporangia

For the two isolates TSE1 and 4.1 (see section 2.2.3) three lots of 48 plugs were removed, using a 5 mm diameter corkborer, from the growing edge of 15 day-old *P. infestans* cultures on rye B agar. The mycelial mat was allowed to remain attached to the agar. Microscopic examination of sample pieces established that the mycelial mat had not penetrated into the agar to any great extent. For each of the two isolates eight plugs were selected at random and submerged for 5 minutes in separate Petri dishes containing the different concentrations of the hydrogen peroxide / peracetic acid / acetic acid (hp/paa)

product. The product used was Proxitane 0510 containing 20.5 % hydrogen peroxide, 5 % peracetic acid, 10 % acetic acid and 64.5 % water (Solvay Interlox Ltd). Treatments were 0 (control), 25, 50, 100, 200 and 400 ppm of peracetic acid. Following treatment the plugs were rinsed twice in sterile distilled water, on each occasion for 5 minutes, and after drying single plugs were placed on rye B agar in a Petri dish. These dishes were sealed and incubated as in experiment 3 and the number showing any *P. infestans* growth after 12 days recorded. Additional assessments were made of the growth rate of cultures from each of the treatments and from the control.

Table 6.2. Isolates and concentrations of *P. infestans* suspensions used on three dates to evaluate hot water treatment as a method to prevent the infection of potato tubers

date	isolate	<i>P. infestans</i> concentration
		($\mu\text{g ml}^{-1}$)
April 27	T1A1	1.65
May 17	TSI	8.0
June 4	T1A1	8.0

6.2.6 Experiment 6 The efficacy of a hydrogen peroxide / peracetic acid / acetic acid mixture in reducing the infection of potato tubers by *P. infestans* during washing

Seed tubers of the cv. Home Guard were planted on 23 December 1992 and the resulting plants maintained as described in experiment 4. Immature daughter tubers were harvested before skin set on 6 April from 25 randomly selected plants and carefully examined to ensure that none had symptoms of blight. Tubers were then allocated at random to 12 lots of 15. A mycelial and

sporangial suspension of a single isolate of *P. infestans*, SA91, was prepared from 19 day-old rye B agar cultures by dispersing the fungal growth in sterile water and shaking on a Griffen flask shaker for one hour. Three replicate lots of tubers were then dipped for 3 minutes in 5 l of one of four treatments, i.e. 125 ppm peracetic acid without and with *P. infestans* at a concentration of $8.0 \mu\text{g ml}^{-1}$ and water without and with *P. infestans* at the same concentration. Following treatment individual tubers were placed in containers which were then randomly arranged between six trays, covered with polyethylene and incubated as in experiment 2. Four days later the trays were removed and the tubers assessed for blight lesions and for sites of *P. infestans* sporulation.

6.2.7 Experiment 7 The phytotoxicity of a hydrogen peroxide / peracetic acid / acetic acid mixture to potato tubers

Seed tubers of the cv. Home Guard were planted on 23 December 1992 and the resulting plants maintained as described in experiment 4. Immature daughter tubers were harvested on 31 March from randomly selected plants and allocated at random to 21 lots of 7 tubers. Three lots were then dipped for 3 minutes in 1 l of one of seven treatment suspensions, i.e. 0 (control), 125, 250, 500, 1000, 2000 and 4000 ppm peracetic acid. Following treatment individual tubers were placed in containers which were then randomly arranged between five trays, covered with polyethylene and incubated as in experiment 2. Five days later the amount of lenticel browning or pitting was assessed using a scale of 0 to 5 (Table 6.3).

Additional assessments were made of the percentage of the tuber surface area which was discoloured. Although the lenticel browning and pitting data are categorical by nature, diagnostic tests (see section 2.6) performed on the data showed that the residuals were approximating normal distribution and that the assumptions necessary for an analysis of variance (Mead & Curnow, 1983) were satisfied.

Table 6.3 Lenticel damage scale

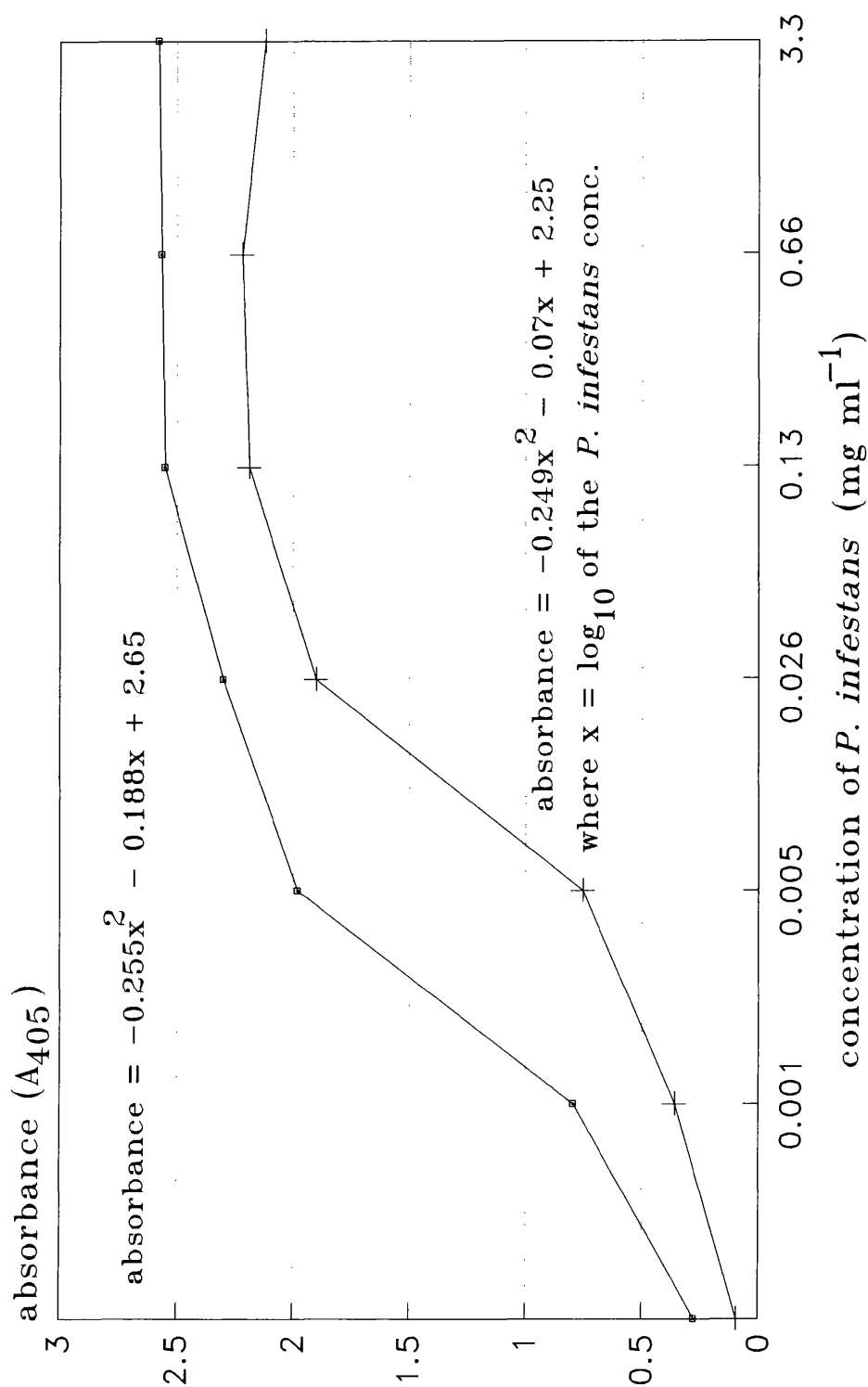
	lenticel symptoms
0	no browning
1	slight browning
2	moderate browning
3	slight pitting
4	moderate pitting
5	severe pitting

6.3 Results

6.3.1 Experiment 1 Estimation of the quantity of inoculum released from blighted tubers during washing

The calibration curves produced from the results of the assays of the concentration of *P. infestans* in the five-fold dilution series are presented in Fig. 6.3. Although the curves were similar, the same concentration of *P. infestans* gave different absorbance values in each assay. The correlation between absorbance and concentration of *P. infestans* for the mean of the two calibration curves was closer for the quadratic compared with the linear equation (Table 6.4).

When inoculated daughter tubers were harvested they were supporting sporulation on wounds, eyes and lenticels. Assays on 27 March showed that six tubers inoculated with *P. infestans* and maintained in conditions similar to those found in a packhouse could liberate on average 1.80 mg of *P. infestans* inoculum per tuber during washing (Fig. 6.4). However, on average only 1.12 mg of *P. infestans* per tuber were released from nine tubers assayed on 13 May.



—■— Assay on 27 March —+— Assay on 13 May
Fig. 6.3 Calibration curves of absorbance vs. concentration of *P. infestans* in dilution series of two suspensions of known concentrations of *P. infestans*

Table 6.4 Correlation coefficients (r) and significance levels (p) for the regression of absorbance on concentration of *P. infestans* in the dilution series of known concentrations of *P. infestans*

equation		
linear	r	0.88
	p	0.05
quadratic	r	0.96
	p	0.01

6.3.2 Experiment 2 The infection of immature, mature and damaged tubers by *P. infestans* during washing

Significantly fewer mature, compared with immature, tubers were infected for both the undamaged and damaged treatments (Fig. 6.5). For each assessment of immature tubers the number of undamaged tubers infected was significantly greater than zero but there were no significant differences between the three individual assessment times. Damaging immature tubers gave complete infection. For mature, undamaged tubers the percentage infection was not significantly greater than zero. Damaging the tubers substantially increased infection at each assessment. As with immature tubers, no significant differences were found between the three assessments. No infections were recorded in the control tubers at any of the assessment dates.

6.3.3 Experiment 3 An investigation of the thermal death point (tdp) of isolates of *P. infestans* obtained from potato crops grown in Scotland

All of the isolates responded similarly to heat treatment (Table 6.5). At temperatures up to 40° C there was, with the exception of isolate 4.1, no significant reduction in viability. However, at 44° C significantly reduced viability of all isolates occurred. Death of all *P. infestans* isolates did

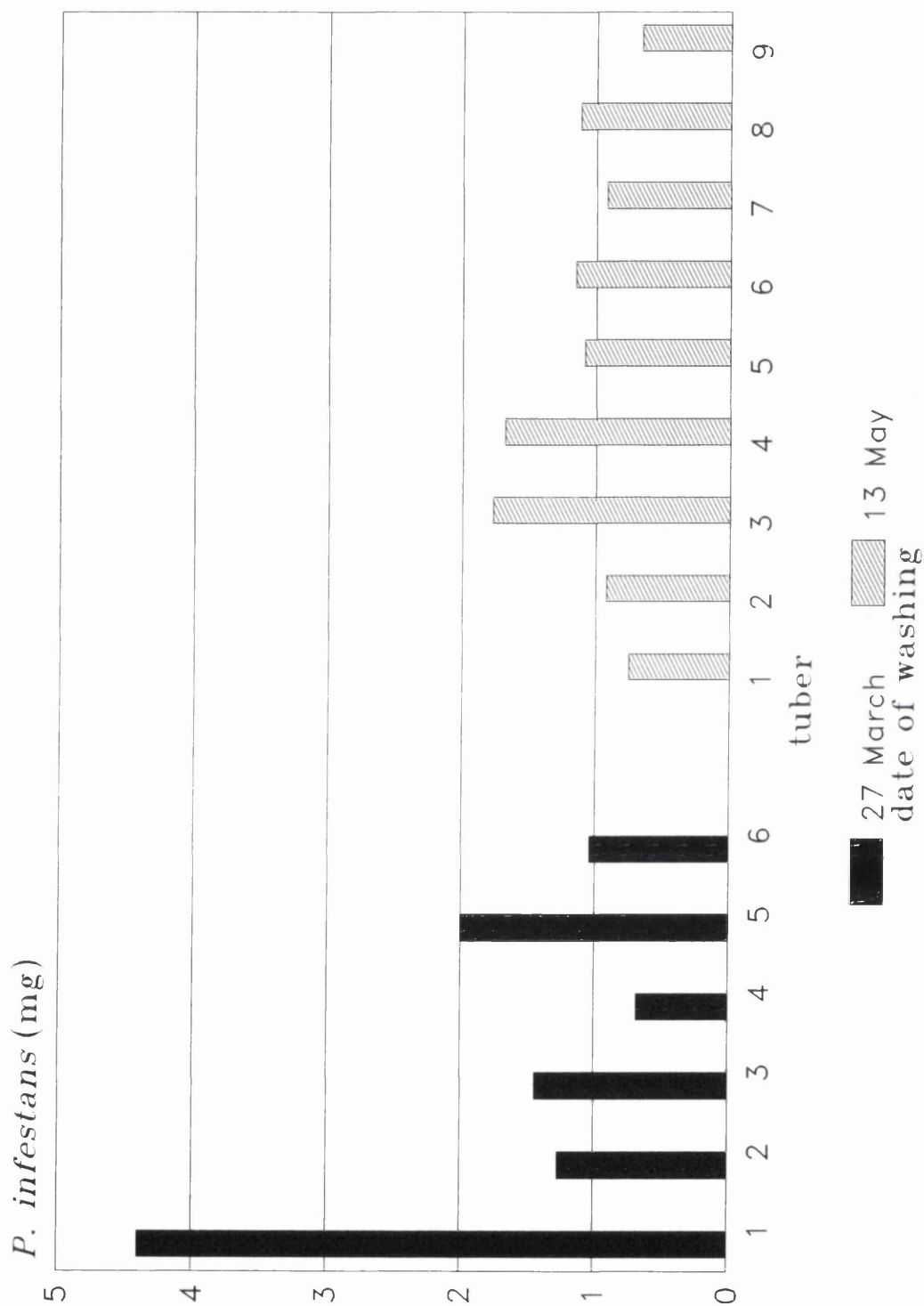


Fig. 6.4 Quantity of *P. infestans* released from individual blighted tubers washed on two dates after incubation for 24 hours

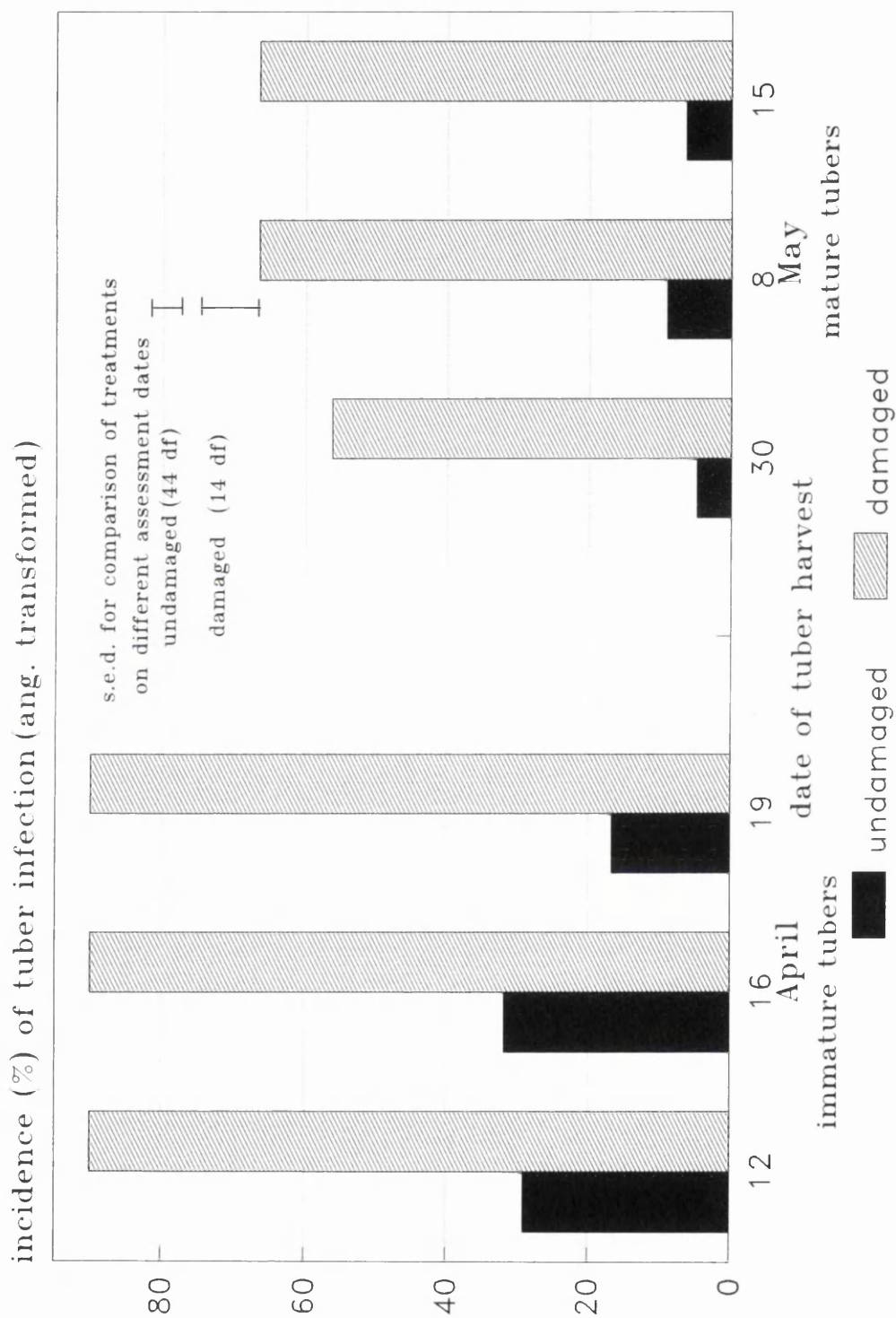


Fig. 6.5 The incidence of tuber infection for immature and mature tubers, in two damage categories, when washed in a suspension of *P. infestans*

Table 6.5 The effect of exposure to hot water for 5 minutes on the survival of a number of phenylamide-sensitive and -resistant isolates of *P. infestans* in water suspensions

isolate	temperature of water (°C)								s.e.d. (167 df)
	25	36	40	44	48	52	56		
SA91	82.5 ¹	86.2	90.0	0.0	0.0	0.0	7.5	5.43	
RF1	86.2	90.0	89.3	0.0	3.7	3.7	7.5	5.60	
2.7	86.2	90.0	90.0	7.5	3.7	0.0	0.0	3.66	
4.1	90.0	90.0	82.5	0.0	0.0	3.7	0.0	2.95	
TSE1	90.0	90.0	90.0	18.7	7.5	0.0	0.0	2.95	
FI	56.3	75.0	88.4	0.0	0.0	0.0	0.0	13.70	

¹percentage of samples surviving (ang. transformed data)

not occur even at the highest temperature of 56° C but the percentage of samples surviving the four highest temperatures was generally not significantly greater than zero.

6.3.4 Experiment 4 The evaluation of hot water treatment as a method of minimising the infection of potato tubers by *P. infestans* during washing

The infection of potato tubers dipped for 3 minutes in a mycelial and sporangial suspension of *P. infestans* at a concentration of 8.0 µg ml⁻¹ was completely controlled by a water temperature of 44° C (Table 6.6). In an earlier evaluation no infection occurred for tubers dipped in a *P. infestans* suspension containing 1.65 µg ml⁻¹. Although surprising, the need to increase the concentration of *P. infestans* from 1.65 to 8.0 µg ml⁻¹ before infection of tubers occurred was probably due to the low pathogenicity of the isolates at that time. There was no indication in any of the three evaluations of tuber damage as a result of hot water treatment.

Table 6.6 The average number of infections per tuber for tubers dipped in suspensions of *P. infestans* at two temperatures

		<u>temperature of water (°C)</u>				
		<u>19- 22</u>		<u>44</u>		
date	isolate	<i>P. infestans</i> suspension	water	<i>P. infestans</i> suspension	water	s.e.d. (11 df)
May 17	TSI	0.6	0.0	0.0	0.0	0.11
June 4	T1A1	0.8	0.0	0.0	0.0	0.19

6.3.5 Experiment 5 The effect of a hydrogen peroxide / peracetic acid / acetic acid mixture on the viability of a suspension of *P. infestans* containing mycelia and sporangia

The highest concentration of the mixture killed both isolates of *P. infestans* (Table 6.7). However, the maximum concentration at which isolates TSE1 and 4.1 survived treatment differed. Although the viability of isolate TSE1 was significantly reduced at 100 ppm peracetic acid, its viability was significantly greater than zero at this concentration. Survival of isolate 4.1 was significantly reduced at 50 ppm peracetic acid and although samples survived concentrations up to 200 ppm the percentage of survival at concentrations above 50 ppm was not significantly greater than zero. The relative growth rates of isolates TSE1 and 4.1 after treatment with sub-lethal concentrations of peracetic acid were reduced (Table 6.7). The reduction was greater for the higher doses of the mixture. In another experiment (data not presented) using three different isolates, i.e. 2.1, RF1, and T69 (see section 2.2.3), treated with concentrations up to 4000 ppm peracetic acid, survival was rare at 250 and nil at 500 ppm peracetic acid.

6.3.6 Experiment 6 The efficacy of a hydrogen peroxide / peracetic acid / acetic acid mixture in reducing the infection of potato tubers by *P. infestans* during washing

A concentration of 125 ppm peracetic acid did not prevent the infection of potato tubers during simulated washing in a suspension of *P. infestans*. Thirteen percent of tubers became infected after being dipped in the *P. infestans* suspension with or without the hp/paa mixture. Two percent of the tubers dipped in the solution containing peracetic acid only were also infected, most likely due to contamination of these tubers with *P. infestans* from the suspensions. The assessment of tubers for blight caused by *P. infestans* was made difficult by symptoms of other pathogens, notably *Alternaria solani*, *Rhizoctonia solani* and soft rot bacteria. Consequently

Table 6.7 The survival and relative growth rates of two isolates of *P. infestans* after *in vitro* treatment with a hydrogen peroxide / peracetic acid / acetic acid mixture (hp/paa)

		concentration of peracetic acid (ppm)						s.e.d. (133 df)
	isolate	0	25	50	100	200	400	
percentage survival ¹	TSE1	90.0	90.0	86.2	56.2	0.0	0.0	7.81
	4.1	90.0	67.5	48.2	15.0	15.0	0.0	11.43
relative growth rate ^{1,2}	TSE1	90.0	90.0	75.0	48.7	mv	mv	10.90
	4.1	90.0	71.6	78.2	60.2	47.6	mv	12.83

¹ data are ang. transformed

² for the cultures which survived treatment with lower concentrations of the hp/paa mixture, expressed as a percentage of the control

mv = missing value

tubers needed to be assessed for infection by *P. infestans* sooner after treatment than was ideal and as a result the percentage of lesions on which sporangia of *P. infestans* were detected was less than 10 %.

6.3.7 Experiment 7 The phytotoxicity of a hydrogen peroxide / peracetic acid / acetic acid mixture to potato tubers

High concentrations of peracetic acid resulted in severe tuber phytotoxicity (Plate 6.1). Concentrations of 250 ppm peracetic acid and above resulted in a significantly greater area of the tuber surface being discoloured compared with the control (Table 6.8). Concentrations of 500 ppm peracetic acid and above gave significantly greater lenticel pitting than in the control. The severity of both surface area browning and lenticel pitting increased with dose of peracetic acid.

6.4 Discussion

The experiments in this chapter have demonstrated for the first time that *P. infestans* can spread from diseased to healthy tubers and cause infection during tuber washing in a system based on those used in commercial processing plants. This may explain the high incidences of tuber blight that occasionally occur following the processing of ware tubers when pre-wash inspections revealed no symptoms in the sample. The occurrence of such spread in practice, however, is dependent on the presence of viable sporangia on the surface of diseased tubers at the time of washing. Viable sporangia have been detected on the surface of recently-harvested tubers (Murphy & McKay, 1927; Lapwood, 1961d, e, 1962, 1965; Hirst & Stedman, 1962; Lacey, 1962 and Boyd, 1972) and also on blighted tubers stored for a limited period (Dowley & O'Sullivan, 1991b). It is also the case that recently harvested tubers for processing are often stored in large stacks within the packing house for up to 48 hours prior to washing (Caledonian Produce, personal communication) and as a result of tuber

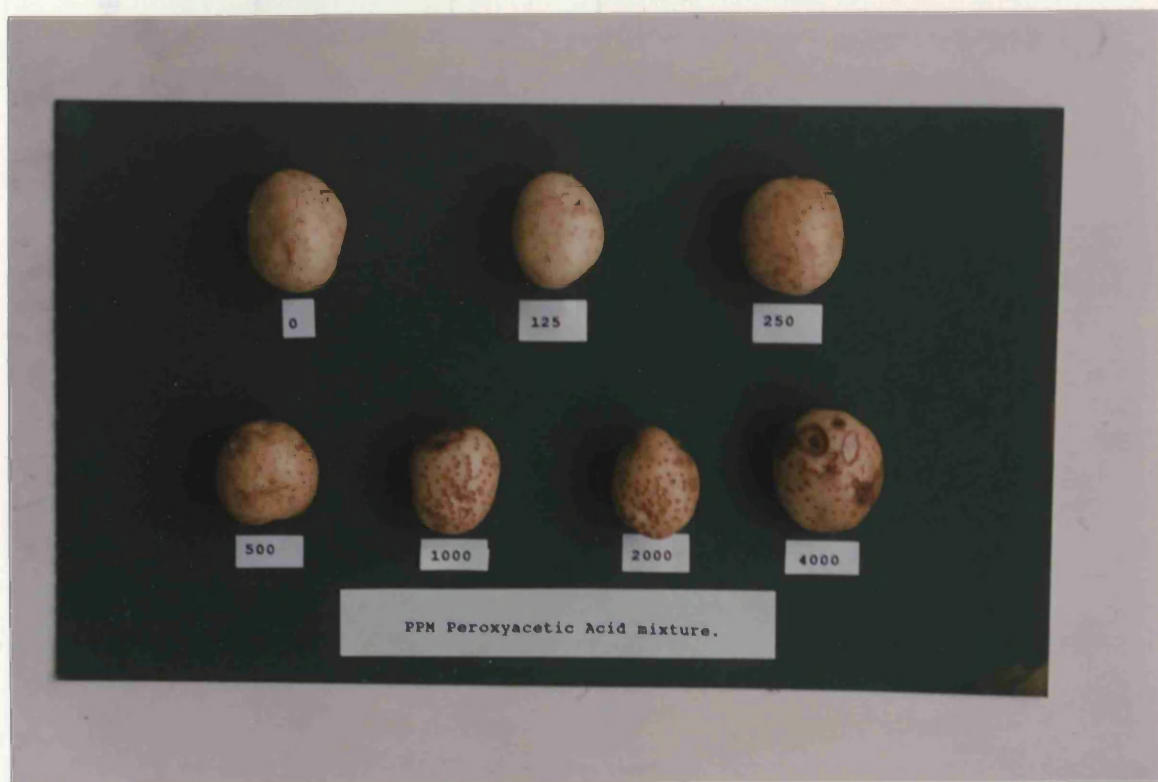


Plate 6.1 The phytotoxicity of a hydrogen peroxide / peracetic acid / acetic acid mixture to potato tubers

Table 6.8 Phytotoxic damage 5 days after tubers were dipped in a hydrogen peroxide/ peracetic acid / acetic acid mixture (hp/paa)

	concentration of peracetic acid (ppm)					
	0	125	250	500	1000	2000 4000 s.e.d. (20 df)
% of surface area browned ¹	13.34	14.29	29.66	40.66	38.33	45.13 46.12 3.72
lenticel pitting ²	0.48	0.48	1.57	2.48	3.29	4.29 4.24 0.619

¹ang. transformed data

²on a scale of 0-5

respiration the relative humidity and temperature within these stacks normally increase (Hide & Boorer, 1991). This is likely to increase sporulation by *P. infestans* on the surface of diseased tubers. Furthermore, the respiration rates of tubers will increase with the handling of the tubers (Isherwood & Burton, 1975) and any increase in ambient temperature (Burton, 1974, 1978). Burton (1974) measured increases in respiration rate of 30 to 100 % in freshly harvested tubers brought into a warm packing house.

Schippers (1977) reported that the respiration rate of tubers harvested soon after tuber initiation was high and decreased throughout the remainder of the growing season. Potatoes harvested when they are immature respire more rapidly than mature tubers, to an extent associated with the degree of immaturity. As a result the relative humidity within stacks of immature tubers is likely to be particularly high and this will promote sporulation by *P. infestans* on the surface of diseased tubers. Burton (1978) reported that cultivar may also affect the respiration rate of immature tubers. Crosier (1934) demonstrated that a relative humidity of greater than 91 % was required before sporulation by *P. infestans* occurred on infected leaflets but results from Harrison & Lowe (1989) suggest that this would be influenced by air speed. Although numerous sporangia were formed on infected leaflets in a relative humidity of 80 % at air speeds of 0.3 m s^{-1} , sporangia production was dramatically reduced as the air speed increased. Air speeds within stacks of tubers stored indoors can be assumed to be relatively low and relative humidities above the minimum needed for sporulation by *P. infestans* on infected tubers are likely to occur. Further experiments are needed to measure the changes in relative humidity within a potato stack over time and to determine the conditions under which sporulation on infected tubers commences. Results from such experiments could be used to develop measures, for example improved ventilation and reduced holding time prior to

washing, to minimise the production of sporangia on the surface of diseased tubers.

Even although the extent of sporulation by *P. infestans* on infected tubers will decline if tubers are cooled, the temperature within a potato stack is unlikely to be sufficiently low to prevent sporulation. Rotem *et al.* (1978) reported that sporulation occurred on infected leaf tissue even when temperatures were too low for the growth of lesions and Crosier (1934) reported the formation of sporangia on infected tubers at temperatures between 3 and 26° C.

The number of tubers which become infected during washing will largely depend on the number of diseased tubers and the amount of inoculum produced per infected tuber. Although counts of the number of sporangia released from infected daughter tubers after a short period of incubation have been used as estimates of inoculum production from tubers (Lacey, 1962, 1965; Lapwood, 1962), Agrios (1978) stated that "inoculum is any part of the fungal pathogen that can initiate infection; thus for fungi inoculum may be spores, sclerotia or fragments of mycelia". The possibility that fragments of *P. infestans* mycelium released from blighted tubers during washing could infect healthy tubers should also be considered. Lacey (1962) reported that sterilised soil on which mycelia but not sporangia of *P. infestans* were observed could infect tuber tissue. Hirst & Stedman (1962) suggested that spores or mycelia of *P. infestans* might cause infection of potato foliage. Similarly, mycelia of *Phytophthora cinnamomi* were found to infect the roots of forest pine in the absence of any fungal spores (Marx & Bryan, 1969). Experiments carried out for this thesis to resolve whether mycelia of *P. infestans* can cause tuber infection in the absence of sporangia were inconclusive, due to unsuccessful separation techniques. Filtering out sporangia through mesh of 45 µm diameter (Henry Simon Group Ltd.) was not effective and although the propagation of mycelia without sporangia on V8 agar (Galindo & Gallegly, 1960) was successful, the

pathogenicity of mycelia produced in this way was considered to be atypical and so further experiments with it were discontinued.

Harrison *et al.* (1990) used an ELISA technique to ascertain the total concentration of *P. infestans* in leaf tissues. This technique was considered suitable for use in the experiments in this thesis which assayed the release of *P. infestans* from infected tubers during washing since Harrison *et al.* (1990) showed that the anti-*P. infestans* gamma-globulin did not react with extracts of nine unrelated fungi found on potatoes or the bacterium *Erwinia carotovora*. Although Harrison *et al.* (1990) found a strong cross reaction with an extract of *Phytophthora erythroseptica* no symptoms of this disease were seen on inoculated tubers in the experiments reported here. This ELISA technique, however, did not distinguish between viable and non-viable *P. infestans* and although a technique was developed which enabled such a distinction to be made (results not presented) the poor correlation between *P. infestans* concentration and absorbance obtained did not justify its use. Moreover, since the inoculum in the relevant experiments reported in this thesis was a maximum of 9 days old the inability of Harrison *et al.*'s ELISA technique to detect viable *P. infestans* only was not considered too important a problem. The viability of cultures growing on rye B agar was found to be reduced only after 10 days at 17° C (Harrison, 1992). On average more inoculum was released from the tubers assayed on 27 March than those assayed on 13 May. This result may be due to the former tubers being kept in a soil with a slightly higher moisture content. Greater sporulation by *P. infestans* on tubers from soil with high moisture contents and greater sporangia production by other *Phytophthora* spp. in soils with high moisture contents have been found by other researchers (see Chapter 5). Results from experiment 3 in this chapter show that a relatively small increase in soil moisture has a large influence on the number of sporangia produced on infected daughter tubers. High soil moistures just prior to harvesting may therefore result in a large amount of

inoculum being produced on infected tubers and greater incidences of infection by *P. infestans* during processing. The tubers assayed on 27 March and 13 May were assayed 9 days after inoculation therefore the amount of inoculum released was likely to have been close to the maximum possible for the respective conditions. Lapwood (1962) showed that sporulation on tubers artificially inoculated in the field then incubated at 15° C in a humid chamber for 24 hours was greatest between 8 and 13 days when up to 40,000 sporangia per tuber per day were produced. Lacey (1962) showed that inoculum production on tubers inoculated in the laboratory and incubated at room temperature began approximately 4 days after inoculation and peaked at around 10 days when c. 15,600 sporangia per tuber per day were produced. Although sporangia production declined after 10 days, up to c. 4600 sporangia per day were still produced 21 days after inoculation. These results suggest that tuber infections which occur within 7 to 13 days of harvest have the potential to result in a higher incidence of infection by *P. infestans* during processing than those infected earlier in the season. This highlights the importance of good control of tuber blight at the end of the growing season.

At 1100 GMT the average soil temperature at a depth of 15 cm in the pots containing the inoculated tubers during the 9 days prior to the assessments of inoculum production were 17.5 and 17.9° C for 27 March and 13 May respectively. However, no comparison of temperatures in the glasshouse with those in the field can be made because comparable values at 15 cm depth in field soil around the time of harvest were not available. Harrison *et al.* (1990) found that temperature had a marked effect on the growth rate of *P. infestans* hyphae in leaves of potato plants and that leaflets incubated at 12 or 17° C for 13 days contained 5.3 and 18.9 mg fresh weight of mycelia per gram of leaf respectively. Crosier (1934) reported that the production of *P. infestans* mycelia in culture increased with temperature up to 21° C. Results from experiment 4 in Chapter 5, however, showed that variation in soil temperature

between 8 and 16° C had little effect on the development of tuber lesions or the number of sporangia produced on them.

Further work is required to establish the influence of some other factors on the relationship between the incidence of tuber blight in the potato stock before and after washing. The experiments in this chapter only examined the spread of *P. infestans* during bulk washing of tubers of one cultivar, i.e. Home Guard. Home Guard tubers have a blight resistance rating of 3 on a 1 to 9 scale, on which 9 indicates the highest resistance (Anon, 1992) and spread of tuber blight might be expected to be lower during the washing of tubers with higher resistance ratings. Having said this, published tuber resistance ratings may not, however, accurately reflect the extent of infection which would occur during bulk washing particularly if immature tubers are washed. Bhatia & Young (1985) found that tubers of some cultivars became temporarily highly resistant when freshly harvested whereas others remained susceptible. Furthermore, some cultivars are far more susceptible to tuber damage (Anon, 1992) and this can greatly influence differences in disease incidence between cultivars. Further experiments need to be carried out using cultivars with a range of tuber blight resistances to establish the relative importance of the initial incidence of tuber blight in the stock and of cultivar resistance to both damage and infection on the extent of tuber to tuber spread during bulk washing. In addition the fungistatic effects of the soil and debris that tend to accumulate in wash water should be considered. Lingappa & Lockwood (1961) attributed general soil fungistasis to the production of antibiotics by a mixture of soil micro-organisms including *Streptomyces* spp. and *Pseudomonas* spp. on or near the surface of individual spores. Lacey (1965) further identified that soil-borne *Rhizoctonia solani*, *Trichoderma viride* and *Mucor spinosus* caused the disintegration of the cytoplasm of *P. infestans*.

The skins of immature tubers are thin and incompletely set (Scott & Wilcockson, 1978) and are penetrated more easily by *P. infestans* than those of

mature tubers. The results reported here show that immature tubers are more prone than mature tubers to infection by *P. infestans* during bulk washing. Bonde *et al.* (1940) and Boyd & Henderson (1953) found that the resistance of whole tubers to *P. infestans* increased as they matured from early August to October. More specifically, Walmsley-Woodward & Lewis (1977) found that the resistance of eyes and lenticels of several cultivars of potato tubers to infection by *P. infestans*, lifted at intervals of two weeks from mid-July onwards, gradually increased as the tubers matured. It follows therefore that washing stocks of immature tubers before mature tubers may reduce the spread of tuber blight. Hide & Lapwood (1978) reported that the susceptibility of immature tubers to bacterial rotting subsequent to washing was reduced by allowing the skins to set prior to washing. However, this is not a practical measure to control the spread of blight as skin set, as determined by a significant reduction in the rate of water loss from the tuber surface, takes approximately 2 weeks (Burton, 1978) and the delay in marketing the crop would be unacceptable. Control of the spread of *P. infestans* during washing would be more easily achieved by additional inspections of the foliage and tubers of crops which are intended for processing while immature and changing the wash water between stocks.

Various types of tuber damage can occur at harvest including bruises, scuffs, deep or shallow cuts and tissue crushing (Gray & Hughes, 1978; Adams, 1979). Adams (1979) found that wounds which incorporated crush damage were most susceptible to infection by *Phoma exigua* var. *foveata*. This, he suggested, was because exudates from the crushed cells assisted in the initial infection process and because such wounds sometimes had fissures running deeper into the tuber where the tissue was more susceptible. There is, however, no evidence to suggest that wounds with crushed tissue would be more susceptible to *P. infestans* than other types of damage. Indeed, compared with scuff wounds, crush wounds would probably damage less of the tuber

periderm, which has been shown to protect the tuber from infection by *P. infestans* (Zan, 1962; Lacey, 1967a; Walmsley-Woodward & Lewis, 1977). In this chapter the difference in the incidence of tuber infection for damaged and undamaged tubers was proportionately greater with mature tubers, which is in agreement with the observations of Lacey (1967a) that direct penetration of the periderm by *P. infestans* is more difficult as the tuber matures. Walmsley-Woodward & Lewis (1977) observe that the penetration of *P. infestans* through undamaged periderm only occurred with immature tubers. Zan (1962), however, reported that the direct penetration of the undamaged periderm by *P. infestans* did not occur. Measures to reduce damage may reduce the incidence of tuber blight in the processed crop after washing. Good seedbed preparation and destoning is estimated to reduce severe tuber damage in some instances by between 2 to 5 % (McRae & Fleming, 1989) and Jarvis (1978) reported that wounds were most severe in tubers from dry, stony soils. Tuber damage during harvest has been shown to be reduced by the correct adjustment of the harvester to give the best lifting conditions and the padding of machinery to reduce the impact of drops (Jarvis, 1978; McRae & Fleming, 1989). The adoption of wider rows or the use of row-crop wheels will also reduce much of the damage caused by the crushing of tubers on the sides of ridges (Jarvis, 1978; McRae & Fleming, 1989). Post-harvest damage during grading, estimated to be up to 4 % of the total yield, is reported to be substantially reduced where machinery is padded and plastic grading screens rather than wire screens are used (McRae & Fleming, 1989). In addition, handling tubers only when they are at temperatures of above 8° C will reduce damage during grading (McRae & Fleming, 1989).

Results in this chapter have shown that hot water treatment (44° C for 5 minutes) can minimise the infection of healthy tubers by *P. infestans* during washing. Hide (1975) reported that the immersion of tubers in water at 45° C for 10 minutes arrested infections by *Polyscytalum pustulans*. Dashwood *et al.*

(1991), however, showed that temperatures of 45 and 50° C for 5 minutes had little effect on the viability of one week-old cultures of *Helminthosporium solani*, *Rhizoctonia solani*, *Colletotrichum coccodes* and *Phoma exigua* var. *foveata* but temperatures between 53 and 57° C reduced their viability, although not always by 100 %. MacKay & Shipton (1983) demonstrated that dipping tubers in water at 52° C for 10 minutes or 55° C for 5 minutes was required to reduce the numbers of *Erwinia carotovora* to below the limit of detection on naturally contaminated potato tubers. The only post-harvest pathogens which require to be controlled on pre-packed potatoes are *P. infestans* and *Erwinia* spp. since pre-packed potatoes should all be consumed within 7 to 10 days of processing (Caledonian Produce, personal communication). These results suggest that although a hot water treatment of 44° C for 3 minutes would not control *Erwinia* spp. it would considerably reduce the spread of tuber blight in samples of pre-packed tubers. Rotting of tubers by *Erwinia* spp. could be minimised by adequately drying the tubers after washing and by improving ventilation following packing to prevent anaerobiosis which is a major factor in soft rot development (de Boer & Kelman, 1978).

Although Dashwood *et al.* (1991) reported that *Penicillium* spp. rapidly recolonised tuber eyes after water treatment at 56° C, in the experiments described here contamination by *Penicillium* spp. was not visible following treatment at 44° C. It is likely that 44° C was not high enough to increase the saprophytic food base from heat-damaged eyes or kill competitive saprophytes which Dashwood *et al.* (1991) suggested were the causes of the rapid re-colonisation by *Penicillium* spp. following treatment at 56° C. Water temperatures cooler than 44° C might have been expected to control the infection of tubers by *P. infestans* as sporangia and zoospores of *P. infestans* have been shown to be unable to survive dry air temperatures as low as 20° C (Wallin, 1953). However, in air in which the humidity is below saturation,

desiccation of the sporangia would appear to be an important factor influencing effect of temperature on the viability of sporangia (Harrison, 1992). Indeed, sporangia in contact with a leaf surface have been shown to survive temperatures up to 30° C for 24 hours in relative humidities of 80 % but for only 8 hours when the relative humidity was only 50 % (Rotem & Cohen, 1974).

In vitro tests, performed as part of this thesis, demonstrated that the thermal death point (in water) of the majority of *P. infestans* isolates tested was 44° C but that, with the exception of one isolate, some samples of each isolate survived temperatures greater than 44° C. This suggests that rather than isolates differing in their heat tolerance, heat-tolerant mycelia or sporangia are present in the population as a whole. Some variation in heat tolerance between different isolates might have been expected if the isolates had been selected from a larger geographical area. Martin (1949) reported that the length of time that eight isolates of *P. infestans* survived 30° C *in vivo* varied from 6 hours to 6 days depending on whether isolates were from northern or southern states of the USA. Larance & Martin (1954) found that although all of the 13 isolates of *P. infestans* tested *in vivo* survived 48 hours exposure to a temperature of 32.5° C, only five survived 35° C for the same period.

Dashwood *et al.* (1991) reported that the temperature increase in tuber tissue during treatment with water at 56° C extended to 2 to 3 mm below the tuber surface. In circumstances in which high incidences of infection by *P. infestans* at lifting might be expected hot water treatment could be used to arrest the development of *P. infestans* infections in tubers if the treatment was performed soon enough after lifting.

Results have shown that peracetic acid can kill *P. infestans* in wash water but that the concentrations required to be effective also result in tuber damage. Shirsat *et al.* (1991) found that treatment with salicylic acid (1000 and 2000 ppm) and sodium hypochlorite (0.1 % and 0.2 %), when used as a post-harvest

wash in an attempt to control the number of *Erwinia* spp., increased the amount of microbial spoilage in irradiated ware tubers. Although Shirsat *et al.*(1991) suggested that this was the result of superficial abrasions caused by the extra handling of the tubers, there may also have been some phytotoxic effect of the chemicals. For disinfectants to be successfully used to control the spread of *P. infestans* during bulk washing the problems of tuber phytotoxicity will have to be overcome. The experiments reported in this thesis need to be repeated to determine the efficacy of other disinfectants in preventing the spread of *P. infestans* during washing. Any effective product which is shown not to cause phytotoxic damage will also have to be effective at concentrations which do not result in unacceptable residues on the treated tubers.

CHAPTER 7

Concluding discussion

A recurring theme in this thesis has been the investigation of factors that could explain the high incidences of tuber blight that can occur at lifting in the absence of any visible foliar blight. Results from this work indicate that the spread of inoculum from late blight lesions on stem bases may, at least in part, account for this phenomenon. Closure of the crop canopy conceals from view stem lesions that develop early in the season, prevents most fungicides from reaching these lesions and can create a more humid microclimate under the closed crop canopy which is likely to increase sporulation on stem lesions. Although closure of the crop canopy will also conceal from view and promote sporulation on lower leaf lesions sporangia produced on these are unlikely to be as important a source of inoculum for the infection of daughter tubers. Sporangia produced on basal stem lesions are more likely to be moved to the daughter tubers by irrigation or rainwater. Stem lesions are also more likely to continue developing during dry periods than leaf lesions and the survival of *P. infestans* during periods of prolonged dry weather will also be greater in stem lesions since blighted leaves can abscise under such conditions. In addition, results reported in this thesis show that sporulation can occur over the whole length of stem lesions therefore the number of sporangia produced in relation to lesion area will be greater for stem compared with leaf lesions.

The importance of stem lesions as a source of inoculum for the infection of daughter tubers was demonstrated by another field experiment in which the main stem was inoculated and the foliage maintained blight-free through the use of mancozeb. Significant incidences of tuber blight occurred after overhead irrigation was applied. An increased awareness by growers of the relationship between stem and tuber blight together with appropriate action such as the judicious use of irrigation water and the use of fungicides that are particularly effective against stem blight, such as fentin hydroxide, would reduce the incidence of tuber blight. In order to minimise the risk of tuber blight in commercial crops desiccation is recommended when the percentage foliar

blight reaches approximately 5 %. However, since this thesis indicates that inoculum produced on stems rather than leaves is responsible for many tuber infections further work is required to establish the threshold for stem blight at which desiccation should be carried out.

Fungicide type influenced the progression of *P. infestans* from leaf lesions into the stem. An experiment using stem sections with one attached leaf showed that fentin hydroxide and cymoxanil + mancozeb, unlike mancozeb or cymoxanil + mancozeb + oxadixyl, significantly reduced the number of leaflet lesions growing into the stem compared with the untreated control. Further work is needed to confirm the discrepancy between cymoxanil + mancozeb and cymoxanil + mancozeb + oxadixyl since it is assumed that the protection of stems is largely effected by cymoxanil. Fentin hydroxide protected stems from infection only if leaflets were inoculated and not if the stems were inoculated directly. This suggests that in order to minimise the risk of tuber blight, fungicides that protect the stem should be used before stem infection occurs and not restricted to the last two sprays by which time stems will probably be infected already. Currently, growers use fentin hydroxide for the final two sprays only, largely because of concerns over phytotoxicity. Ideally a fungicide that protects the stem from infection without causing phytotoxic damage needs to be developed.

The treatment of field plots with fungicides delayed the onset of tuber blight and restricted its development. Treatment of plots with fentin hydroxide or mancozeb + oxadixyl + cymoxanil compared with mancozeb + metalaxyl or mancozeb delayed by 10 days the occurrence of incidences of tuber blight which were significantly greater than zero. At later assessments, however, fungicide type had little effect on the incidence of tuber blight, although fentin hydroxide delayed the peak incidence of tuber blight by 6 days compared with the other fungicide treatments. These results indicate that incidences of tuber blight in commercial crops may be lower where fentin hydroxide was the main

fungicide used. In general, however, better control might be achieved by improving cultivar resistance to tuber blight. In agreement with previous work the results reported here show that where mancozeb alone was used the incidence of tuber blight was higher than in the untreated control plots. A possible explanation is that since mancozeb can maintain green leaf area, and therefore sporangia production, for longer tuber infection is increased.

Although it is widely accepted that tubers become infected by *P. infestans* inoculum washed down from the haulm, this may not be the only source of inoculum for the infection of daughter tubers. In experiments in this thesis viable sporangia were produced on the surface of blighted seed tubers 65 days after planting. This indicates that seed tubers may on occasions be a direct source of inoculum for the infection of daughter tubers. However, as the spread of *P. infestans* from seed tuber to daughter tuber is dependent upon several specific conditions occurring successively, seed tubers are unlikely to be a consistent direct source of inoculum for the infection of daughter tubers. The conditions required are a storage temperature suitable to maintain the viability of both *P. infestans* and the blighted tuber, a relatively low soil moisture content after planting to minimise seed tuber decay and once daughter tubers are formed a higher soil moisture content to encourage sporulation.

The role of infected daughter tubers as an underground source of inoculum for the infection of healthy daughter tubers was examined in relation to soil moisture content. The effect of soil moisture content on the infection of daughter tubers was complex. Incubating tubers in high soil moistures before inoculation tended to increase infection whereas the opposite was true for such treatments applied after inoculation. Soil moisture contents up to 79 % of field capacity after inoculation considerably increased lesion development and inoculum production on infected tubers. Increasing the soil moisture content of field plots to 75 % of field capacity significantly increased the spread of inoculum from infected daughter tubers to other tubers on the same plant.

Healthy tubers up to 5 cm away on the same plant became infected. Healthy tubers on neighbouring plants up to 60 cm away from the nearest plant with an inoculated tuber were also infected. These results suggest that where there is an initial underground source of inoculum, i.e. blighted daughter tubers, a substantial incidence of tuber blight could occur if a prolonged high soil moisture content prevailed. Although such conditions are infrequent they occur in seasons of high prolonged rainfall, such as the summer of 1988 in Scotland. However, further work is needed to confirm the results reported here, especially as the soil moisture treatments were artificial in that they were approximately constant for a prolonged period whereas in most years soil moistures fluctuate more widely. Such fluctuations may actually increase the incidence of tuber blight since, although high soil moistures are necessary to move *P. infestans* to the tubers, lower soil moisture post-inoculation favours infection. In addition, periods of dry weather would also create cracks in the soil providing easier access to the tubers for sporangia and zoospores of *P. infestans* in rain or irrigation water. Further work is also required to determine to what extent different soil textures or structures affect the incidence of tuber infection.

Experiments in this thesis have also demonstrated that seed tubers can become contaminated with *P. infestans* inoculum from infected seed tubers during the handling which occurs prior to planting. For such spread to occur sporulation by *P. infestans* on the seed tubers prior to handling is essential. Results suggest that this requires a high relative humidity for a minimum of 3 days therefore except under exceptional circumstances seed tubers are unlikely to be a consistent source of inoculum for the spread of *P. infestans* by this route. Further work in this area is, however, required and if spread of *P. infestans* inoculum between seed tubers is found to occur during handling it will be necessary to develop techniques that minimise or eliminate it.

Results in this thesis have shown for the first time that *P. infestans* can spread from diseased to healthy tubers during washing. Infection was demonstrated for tubers washed in water with a concentration of *P. infestans* equivalent to that estimated to be in a commercial washing system after 4 hours of washing a potato stock with approximately 1 % of tubers infected with blight. In practice the number of tubers that become infected during washing will depend on the number of blighted tubers in the stock, the amount of *P. infestans* inoculum produced per infected tuber and the susceptibility to infection of the tubers. The amount of inoculum produced per infected tuber will be influenced by the pathogenicity of the *P. infestans* present, tuber resistance to blight, the soil moisture content prior to harvest and by the relative humidity and temperature of the potato stock between harvest and washing. Tuber susceptibility to infection is particularly influenced by tuber maturity and by cultivar susceptibility to tuber damage as well as the susceptibility of the cultivar's tuber tissue to infection.

Other results in this thesis indicate that hot water treatment may be of practical value in reducing the spread of *P. infestans* inoculum from infected daughter tubers to healthy tubers during washing for the pre-pack market. Hot water treatment could prevent the high incidences of tuber blight that occasionally occur when pre-wash inspections revealed no symptoms in the stock. Previous work on the use of hot water treatment has concentrated on the eradication of tuber-borne pathogens on seed stocks and consequently has required relatively high temperatures. This has sometimes resulted in impaired sprout growth. In the experiment performed here, however, it was only necessary to control *P. infestans* whilst it was in water and as a result a relatively low water temperature of 44° C was effective. There was no indication of tuber damage as a result of hot water treatment at 44° C. These results also show that where hot water treatment is used to control tuber-borne diseases such as *Erwinia carotovora* subsp. *atroseptica* and *Phoma exigua* var.

foveata, inoculum of *P. infestans* released as a result of washing will also be controlled. The success of hot water treatment in controlling *P. infestans* in wash water justifies further work to establish its potential to reduce the incidence of tuber blight where contamination of tubers with *P. infestans* has occurred at lifting.

Literature cited

- Adams, M.J. (1975) Potato tuber lenticels: development and structure. *Annals of Applied Biology* **79**, 265-273.
- Adams, M.J. (1979) The significance of tuber damage and inoculum concentration of *Phoma exigua* var. *foveata* in the development of gangrene in stored potato tubers. *Annals of Applied Biology* **95**, 31-40.
- Adams, M.J. & Lapwood, D.H. (1978) Studies on the lenticel development, surface micro-flora and infection by common scab of potato tubers growing in wet and dry soil. *Annals of Applied Biology* **90**, 335-343.
- Agrios, G.N. (1978) *Plant Pathology*. New York: Academic Press, 172-434.
- Allen, R.N. & Newhook, F.J. (1973) Chemotaxis of zoospores of *Phytophthora cinnamomi* to ethanol in capillaries of soil pore dimensions. *Transactions of the British Mycological Society* **61** (2), 287-382.
- Allen, E.J. & Scott, R.K. (1992) Principles of agronomy and their application in the potato industry. In: Harris, P.M., ed. *The Potato Crop*. New York: Chapman & Hall, 816-881.
- Anon. (1947) The measurement of potato blight. *Transactions of the British Mycological Society* **31**, 140-141.
- Anon. (1988) Proxitane 0510: product data booklet. Warrington, Interlox Chemicals Limited, 1-11.
- Anon. (1989) *Minitab Statistical Handbook*. Pennsylvania: Minitab Inc.
- Anon. (1992) *Classified list of potato varieties*. Cambridge: National Institute of Agricultural Botany.

Anon. (1993) *Annual yearbook of statistics- Production and Trade*. Rome: Food and Agricultural Organisation of the United Nations.

Artschwager, E. (1927) Wound periderm formation in the potato as affected by temperature and humidity. *Journal of Agricultural Research* **35**, 995- 1000.

Bain, R.A. & Holmes, S.J. (1990a) Phenylamide insensitivity in *P. infestans* and control of potato late blight with fungicides. *Proceedings of the British Crop Protection Conference - Pests and Diseases, 1990*. Brighton: BCPC, 1121-1126.

Bain, R.A. & Holmes, S.J. (1990b) Phenylamide use on Scottish seed potato crops and resistance in *Phytophthora infestans* isolated from progeny tubers. *Proceedings of the Crop Protection in Northern Britain Conference, 1990*, Dundee: ACPNB, 373-378.

Barak, E., Edgington, L.V. & Ripley, B.D. (1984) Bioactivity of the fungicide metalaxyl in potato tubers against some species of *Phytophthora*, *Fusarium* and *Alternaria* related to polyphenoloxidase activity. *Canadian Journal of Plant Pathology* **6**, 304-308.

Barker, K.F. & Heald, F.D. (1932) Some problems concerning blue mould in relation to cleaning and packing of apples. *Phytopathology* **22**, 879-898.

Beaumont, A. (1947) The dependence on the weather of the dates of outbreak of potato blight epidemics. *Transactions of the British Mycological Society* **31**, 45-53.

Bhatia, S.K., Young, R.J. & Pencis, S.I. (1979) Detection of fungitoxic activity in potato tubers field-treated with CGA 48988 - a systemic fungicide. *Phytopathology* **69** (5), 534 (abstract).

Bhatia, S.K. & Young, R.J. (1983) Detection of antifungal activity in potato tubers field-treated with metalaxyl, a systemic fungicide. *Plant Disease* **67**, 1075-1079.

Bhatia, S.K. & Young, R.J. (1985) Reaction of potato tuber slices to *Phytophthora infestans* in relation to physiological age. *American Potato Journal* **62**, 471-478.

Bjor, T. (1987) Testing the resistance of potato genotypes to tuber late blight. *Potato Research* **30**, 525-532.

Bock, R. (1981) Triphenyltin compounds and their degradation products. *Residue Review* **79**, 1-230.

Bojduniak, R. (1994) Average potato prices 1989-1994. *Farm Brief* **9(27)**, 2.

Bojduniak, R. (1995) Average potato prices 1989-1994. *Farm Brief* **10(3)**, 2.

Bonde, R., Stevenson, F.J. & Clark, C.F. (1940) Resistance of certain potato varieties and seedling progenies to late blight in the tubers. *Phytopathology* **30**, 733-748.

Bourke, P.M.A. (1964) Emergence of potato blight, 1843-46. *Nature* **203**, 805-808.

Boyd, A.E.W. (1960) Size of potato tubers and natural infection with blight and pink rot. *Plant Pathology* **9**, 99-101.

Boyd, A.E.W. (1972) Potato storage diseases. *Plant Pathology* **51(5)**, 297-321.

- Boyd, A.E.W. (1980) Development of potato blight (*Phytophthora infestans*) after planting infected seed tubers. *Annals of Applied Biology* **95**, 301-309.

- Boyd, A.E.W. & Henderson, M.J. (1953) Susceptibility of immature potato tubers to blight. *Plant Pathology* **2**, 113-117.
- Bruck, R.I., Fry, W.E. & Apple, A.E. (1980) Effect of metalaxyl, an acylalanine fungicide, on developmental stages of *Phytophthora infestans*. *Phytopathology* **70**, 597-60.
- Bruhn, J.A. & Fry, W.E. (1982) A mathematical model of the spatial and temporal dynamics of chlorothalonil residues on potato foliage. *Phytopathology* **72**, 1306-1312.
- Bruin, G.C.A., Edgington, L.V. & Ripley, B.D. (1982) Bioactivity of the fungicide metalaxyl in potato tubers after foliar sprays. *Canadian Journal of Plant Pathology* **4**, 353-356.
- Burton, W.G. (1974) The oxygen uptake, in air and in 5 % oxygen, and the carbon dioxide output, of stored potato tubers. *Potato Research* **7**, 113-137.
- Burton, W.G. (1978) The physics and physiology of storage. In: Harris, P.M., ed. *The Potato Crop*. New York: Chapman & Hall, 545-606.
- Burton, W.G., van Es, A. & Hartmans, K.J. (1992) The physics and physiology of storage. In: Harris, P.M., ed. *The Potato Crop*. New York: Chapman & Hall, 608-727.
- Carnegie, S.F. & Colhoun, J. (1983) Effects of plant nutrition on susceptibility of potato leaves to *Phytophthora infestans*. *Phytopathologische Zeitschrift* **108**, 242-250.
- Carter, G.A., Smith, R.M. & Brent, K.J. (1982) Sensitivity to metalaxyl of *Phytophthora infestans* populations in potato crops in south-west England in 1980 and 1981. *Annals of Applied Biology* **100**, 433-441.

- Clark, M.F. & Adams, A.N. (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34**, 475-483.
- Clayson, A.M. & Robertson, N.F. (1956) Survival of *Phytophthora infestans* in potato stem lesions. *Plant Pathology* **5**, 30.
- Coffey, M.D. & Young, L.H. (1984) Responses to metalaxyl of sensitive and resistant isolates of *Phytophthora infestans*. *Phytopathology* **74**, 615-620.
- Cohen, Y. & Grinberger, M. (1987) Control of metalaxyl-resistant causal agents of late blight in potato and tomato and downy mildew in cucumber by cymoxanil. *Phytopathology* **77**, 1283-1288.
- Colon, L.T. & Budding, D.J. (1990) Components of field resistance to *Phytophthora infestans* in *Solanum* spp. *Proceedings of the EAPR Conference, 1990*. Edinburgh: EAPR, 84-85.
- Cooke, L.R., Clifford, D.R. & Holgate, M.E. (1981) Control of potato late blight (*Phytophthora infestans*) with systemic fungicides. *Pesticide Science* **12**, 678-684.
- Cooke, L.R. & Little, G. (1992) Persistence of fungicidal protection of potato leaves against blight after treatment with two- and three-way fungicide mixtures. *Annals of Applied Biology* **120**, pp 24-25 (supplement).
- Corbett, J.R. (1974) *The biochemical mode of action of pesticides*. London: Academic Press.
- Corke, A.T.K. (1958) A trap for water-borne spores. *Plant Pathology* **7**, 56-57.
- Cotton, A.D. (1922) Fungus disease of crops- Potatoes 1920-1921. *Miscellaneous Publication of the Agricultural Fisheries London*, **38**, 21-24.

Cox, A.E. & Large, E.C. (1960) *Potato blight epidemics throughout the world*. United States Department of Agriculture; USDA publication no. 174 (Agriculture Handbook Series).

Crosier, W. (1934) *Studies in the biology of Phytophthora infestans (Mont.) de Bary*. Cornell University Agricultural Experiment Station Memoir No. 155.

Crucefix, D.N., Mansfield, J.W. & Wade, M (1984) Evidence that determinants of race specificity in lettuce downy mildew disease are highly localised. *Physiological Plant Pathology* **24**, 93-106.

Cutter, E. (1978) Structure and development of the potato plant. In: Harris, P.M., ed. *The Potato Crop*. New York: Chapman & Hall, 70-147.

Cutter, E. (1992) Structure and development of the potato plant. In: Harris, P.M., ed. *The Potato Crop*. New York: Chapman & Hall, 65-161.

Dashwood, E.P., Burnett, E.M. & Perombelon, M.C.M. (1991) Effect of a continuous hot water treatment of potato tubers on seed-borne fungal pathogens. *Potato Research* **34**, 71-78.

de Bary, A. (1876) Researches into the nature of the potato-fungus *Phytophthora infestans*. *Journal of the Royal Agricultural Society, England, Series 2* (12), 239-269.

de Boer, S.H. & Kelman, A. (1978) Influence of oxygen concentration and storage factors on susceptibility of potato tubers to bacterial soft rot (*Erwinia carotovora*). *Potato Research* **21**, 65-79.

Douchet, J.P., Absi, M., Hay, S.J.B., Muntan, L. & Villani, A. (1977) European results with DPX 3217, a new fungicide for the control of grape downy mildew and potato late blight. *Proceedings of the British Crop Protection Conference - Pests and Diseases, 1977*. Brighton: BCPC, 535-540.

- Dowley, L.J. (1987) Factors affecting the survival of metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. *Potato Research* **30**, 473-45.
- Dowley, L.J. & O'Sullivan, E. (1991a) Changes in the distribution of phenylamide-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland 1985-89. *Potato Research* **34**, 67-69.
- Dowley, L.J. & O'Sullivan, E. (1991b) Sporulation of *Phytophthora infestans* on the surface of diseased potatoes and tuber to tuber spread of infection during handling. *Potato Research* **34**, 295-296.
- Duniway J.M. (1975) Limiting influence of low water potential on the formation of sporangia by *Phytophthora drechsleri* in soil. *Phytopathology* **65**, 1089-1093.
- Duniway J.M. (1979) Water relations of water molds. *Annual Review of Phytopathology* **17**, 431-460.
- Easton, G.D. & Nagle, M.E. (1985) Timing and root absorption affecting efficiency of metalaxyl in controlling *Phytophthora infestans* on potato in north-western Washington State. *Plant Disease* **69**, 499-500.
- Fisher, A. & Yates, A. (1963) *Statistical Tables for Biological, Agricultural and Medical Research*. Edinburgh: Oliver and Boyd.
- Fox, R.T.V., Manners, J.G. & Myers, A. (1971) Ultrastructure of entry and spread of *Erwinia carotovora* var. *atroseptica* into potato tubers. *Potato Research* **14**, 61-73.
- Francis, P.E. (1981) *The climate of the agricultural areas of Scotland*. Climatological Memorandum No. 108. Edinburgh: Meteorological Office.

- Galindo, J. & Gallegly, M.E. (1960) The nature of sexuality in *Phytophthora infestans*. *Phytopathology* **50**, 123-128.
- Gibson, R.W. (1978) Pest aspects of potato production, Part 2. Pests other than nematodes. In: Harris, P.M., ed. *The Potato Crop*. New York: Chapman & Hall, 470-503.
- Gisi, U., Zentmyer, G.A. & Klure, L.J. (1980) Production of sporangia by *Phytophthora palmivora* in soils at different matrix potentials. *Phytopathology* **70**, 301-308.
- Grainger, J. (1957) Blight - The potato versus *Phytophthora infestans*. *Agricultural Review (London)* **3(5)**, 10-26.
- Grainger, J. (1979) Scientific proportion and economic decisions for farmers. *Annual Review of Phytopathology* **17**, 223-252.
- Gray, D. & Hughes, J.C. (1978) Tuber quality. In: Harris, P.M., ed. *The Potato Crop*. New York: Chapman & Hall, 504-544.
- Gray, E.G. (1965) The problem of tuber blight in Northern Scotland. *European Potato Journal* **8 (3)**, 178.
- Hächler, H. & Hohl, H. R. (1984) Temporal and spatial distribution patterns of collar and papillae wall appositions in resistant and susceptible tuber tissue of *Solanum tuberosum* infected by *Phytophthora infestans*. *Physiological Plant Pathology* **24**, 119-128.
- Hammond, K.M. & Lewis, B.G. (1987) The establishment of systemic infection in leaves of oilseed rape by *Leptosphaeria maculans*. *Plant Pathology* **36**, 135-147.

- Harrison, J.G. (1992) Effects of the aerial environment on late blight of potato foliage - a review. *Plant Pathology* **41**, 384-416.
- Harrison, J.G., Barker, H., Lowe, R. & Rees, P.A. (1990) Estimation of amounts of *Phytophthora infestans* mycelium in leaf tissue by enzyme linked immunosorbent assay. *Plant Pathology* **39**, 274-277.
- Harrison, J.G. & Lowe, R. (1989) Effects of humidity and wind-speed on sporulation of *Phytophthora infestans* on potato leaves. *Plant Pathology* **38**, 585-591.
- Harrison, J.G. & Perry, D.A. (1990) Detection of *Phytophthora infestans* and *Spongospora subterranea* in potato tissue by ELISA. *Proceedings of the EAPR Conference, 1990*. Edinburgh: EAPR, 82-83.
- Harrison, M.D. & Franc, G.D. (1988) Post-harvest chemical treatment for control of tuber infection by *Alternaria solani*. *American Potato Journal* **65**, 247-253.
- Hartel, K. (1962) Triphenyl-tin compounds. A new fungicidal group of active substances- their biological properties and their application to agriculture. *Journal of Agricultural & Veterinary Chemicals* **3**, 19-24.
- Hawkes, J.G. (1992) History of the potato. In: Harris, P.M., ed. *The Potato Crop*. NewYork: Chapman & Hall, 1-12.
- Heining, B. (1963) A pressure membrane apparatus. *Journal of Agricultural Engineering Research* **8**, 48-49.
- Hide, G.A. (1975) Effect of heat treatment of potato tubers on *Oospora pustulans*. *Plant Pathology* **24**, 233-236.

- Hide, G.A. & Boorer, K.J. (1991) Effects of drying potatoes after harvest on the incidence of disease after storage. *Potato Research* **34**, 133-137.
- Hide, G.A. & Cayley G.R. (1987) Effects of delayed fungicide treatment and of curing and chlorpropham on the incidence of skin spot on stored potato tubers. *Annals of Applied Biology* **110** (3), 617-627.
- Hide, G.A. & Lapwood, D.H. (1978) Disease aspects of potato production. In: Harris, P.M., ed. *The Potato Crop*. New York: Chapman & Hall, 407- 439.
- Hirst, J.M. (1953) Changes in atmospheric spore content: diurnal periodicity and the effects of weather. *Transactions of the British Mycological Society* **36**, 375-393.
- Hirst, J.M. (1955) The early history of a potato blight epidemic. *Plant Pathology* **4**, 44-50.
- Hirst, J.M. & Stedman, O.J. (1960) The epidemiology of *Phytophthora infestans*. II. The source of inoculum. *Annals of Applied Biology* **48**, 489-517.
- Hirst, J.M. & Stedman, O.J. (1962) The infection of potato foliage and tubers by *Phytophthora infestans*. *Rothamsted Experimental Station Report for 1961*. Harpenden: Rothamsted Experimental Station, 113-114.
- Hirst, J.M., Stedman, O.J., Lacey, J. & Hide, G.A. (1965) The epidemiology of *Phytophthora infestans*. IV. Spraying trials, 1959 to 1963, and the infection of tubers. *Annals of Applied Biology* **55**, 372-395.
- Hodgson, W.A. & Grainger, P.N. (1964) Culture of *Phytophthora infestans* on artificial media prepared from rye seeds. *Canadian Journal of Plant Science* **44**, 583-584.

Hohl, H.R. (1991) Nutrition. In: Ingram, D.S. & Williams, P.H., eds. *Advances in Plant Pathology* vol. 7. London: Academic Press 53-83.

Holmes, S.J., Fairclough, R., Bain, R.A. (1994) Managing late blight of potatoes in the 1990s. *Proceedings of the British Crop Protection Conference - Pests and Diseases, 1994*. Brighton: BCPC, 367-374.

Holmes, S.J.I. & Channon, A.G. (1984) Studies on metalaxyl-resistant *Phytophthora infestans* in potato crops in south-west Scotland. *Plant Pathology* **33**, 347-354.

Holmes, T.D. & Storey, I.F. (1962) Comparative trials of copper, dithiocarbamate and organo-tin fungicides for potato blight control in Lincolnshire, 1958-1961. *Plant Pathology* **11**, 139-146.

Hooker, W.J. & Page, O.T. (1960) Relation of potato tuber growth and skin maturity to infection by common scab, *Streptomyces scabies*. *American Potato Journal* **37**, 414-423.

Isherwood, F.A. & Burton, W.G. (1975) The effect of senescence, handling, sprouting and chemical sprout suppression on the respiratory quotient of stored potato tubers. *Potato Research* **18**, 98-104.

Ivins, J.D. & Bremner, P.M. (1964) Growth, development and yield in the potato. *Outlook on Agriculture* **4**, 211-217.

Jarvis, R.H., Short, J.L. & Shotton F.E. (1967) Copper, dithiocarbamates and organotin compounds for the control of potato blight, 1962-65. *Plant Pathology* **16**, 49-53.

Jarvis, R.H. (1978) Mechanisation and crop performance. In: Harris, P.M., ed. *The Potato Crop*. New York: Chapman & Hall, 355-375.

Johnson, L.F. & Curl, E.A. (1972) *Methods for the research of soil-borne plant pathogens*. Minneapolis: Burgess Publishing Company.

Kable, P.F. & MacKenzie, D.R. (1980) Survival of *Phytophthora infestans* in potato stem lesions at high temperatures and implications for disease forecasting. *Plant Disease* **64**, 165-167.

Kadish, D. & Cohen, Y. (1988a) Fitness of *Phytophthora infestans* isolates from metalaxyl-sensitive and resistant populations. *Phytopathology* **78**, 912-915.

Kadish, D. & Cohen, Y. (1988b) Competition between metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* in the absence of metalaxyl. *Plant Pathology* **37**, 558-564.

Kassim, M.J. (1986) Chemical control of post-harvest disease of potato and tomato. *Indian Phytopathology* **39** (2), 243-244 (abstract).

Kaufman, D.D. & Williams, L.E. (1962) Polyethylene bags for the study of soil micro-organisms. *Phytopathology* **52**, 16 (abstract).

Keay, M.A. (1953) Delayed sporulation of *Phytophthora infestans* on infected potato shoots. *Plant Pathology* **2**, 68-71.

Klinkowski, M. (1970) Catastrophic plant diseases. *Annual Review of Phytopathology* **8**, 37-60.

Kozlovskii, B.E. & Suprun, V.I. (1987) Comparative estimation of biological properties of field isolates of *Phytophthora infestans* (Mont.) de Bary differing in their sensitivity to Ridomil. *Mikologiya i Fitopatologiya* **21** (6), 559-562 (English abstract).

- Kuan, T.L. & Erwin, D.C. (1980) Predisposition effect of water saturation of soil of *Phytophthora* root rot on alfalfa. *Phytopathology* **70**, 981-986.
- Lacey, J. (1962) *The activity of Phytophthora infestans (Mont.) de Bary in soils*. Ph.D. Thesis. University of Reading.
- Lacey, J. (1965) The infectivity of soils containing *Phytophthora infestans*. *Annals of Applied Biology* **56**, 363-380.
- Lacey, J. (1966) The distribution of healthy and blighted tubers in potato ridges. *European Potato Journal* **9(2)**, 86-90.
- Lacey, J. (1967a) Susceptibility of potato tubers to infection by *Phytophthora infestans*. *Annals of Applied Biology* **59**, 257-264.
- Lacey, J. (1967b) The role of water in the spread of *Phytophthora infestans* in the potato crop. *Annals of Applied Biology* **59**, 245-257.
- Lapwood, D. H. (1961a) Potato haulm resistance to *Phytophthora infestans*. I. Field assessment of resistance. *Annals of Applied Biology* **49**, 140-151.
- Lapwood, D. H. (1961b) Potato haulm resistance to *Phytophthora infestans*. II. Lesion production and sporulation. *Annals of Applied Biology* **49**, 316-330.
- Lapwood, D. H. (1961c) Potato haulm resistance to *Phytophthora infestans*. III. Lesion distribution and leaf destruction. *Annals of Applied Biology* **49**, 704-716.
- Lapwood, D. H. (1961d) Haulm and tuber resistance to *Phytophthora infestans*. In: *Rothamsted Experimental Station Report for 1960*. Harpenden: Rothamsted Experimental Station, 123.
- Lapwood, D. H. (1961e) Sporulation of blight on potato tubers within ridges. *Plant Pathology* **10**, 40.

- Lapwood, D. H. (1962) Soil activity of *Phytophthora infestans* and haulm and tuber resistance to blight. *In: Rothamsted Experimental Station Report for 1961*. Harpenden: Rothamsted Experimental Station, 115-117.
- Lapwood, D. H. (1964) Haulm and tuber resistance to *Phytophthora infestans*. *In: Rothamsted Experimental Station Report for 1963*. Harpenden: Rothamsted Experimental Station, 111-112.
- Lapwood, D. H. (1965) Tuber susceptibility to potato blight *In: Rothamsted Experimental Station Report for 1964*. Harpenden: Rothamsted Experimental Station, 129-131.
- Lapwood, D. H. (1977) Factors affecting the field infection of potato tubers of different cultivars by blight (*Phytophthora infestans*). *Annals of Applied Biology* **85**, 23-42.
- Larance, R.S. & Martin, W.J. (1954) Comparison of isolates of *Phytophthora infestans* at different temperatures. *Phytopathology* **44**, 495.
- Large, E.C. (1958) Losses caused by potato blight in England and Wales. *Plant Pathology* **7**, 39-48.
- Large, E.C., Taylor, E.R., Storey, I.F. & Yule, A.H. (1954) Spraying trials in the potato-growing area around the Wash, 1948-53. *Plant Pathology* **3**, 40-48.
- Last, F.T. (1961) Results of spraying trials for *Phytophthora infestans*. *In: Rothamsted Experimental Station Report for 1960*. Harpenden: Rothamsted Experimental Station, 152-155.
- Lingappa, B.T. & Lockwood, A.S. (1961) The nature of the widespread soil fungistasis. *Journal of General Microbiology* **26**, 473-485.

- Lloyd, G.A., Otaci, C. & Last, F.T. (1962) Triphenyltin acetate residues on potato leaves in blight spraying trials. *Journal of Science, Food and Agriculture* **13**, 353.
- Lowings, P.H. & Acha, I.G. (1959) Some factors affecting growth of *Phytophthora infestans* (Mont.) de Bary. *Transactions of the British Mycological Society* **42**(4), 491-501.
- MacKay, J.M. & Shipton, P. (1983) Heat treatment of seed tubers for control of potato blackleg (*Erwinia carotovora* subsp. *atroseptica*) and other diseases. *Plant Pathology* **32**, 385- 393.
- Malcolmson, J.F. (1969) Factors involved in resistance to blight (*Phytophthora infestans* (Mont.) de Bary) in potatoes and assessment of resistance using detached leaves. *Annals of Applied Biology* **64**, 461-468.
- Malcolmson, J.F. (1985) *Phytophthora infestans* A2 compatible types recorded in Great Britain. *Transactions of the British Mycological Society* **85**, 531.
- Martin, W.J. (1949) Strains of *Phytophthora infestans* capable of surviving high temperatures. *Phytopathology* **39**, 14 (abstract).
- Martin, S.B., Campbell, C.L. & Bruck, R.I. (1987) Influence of acidity levels in simulated rain on disease progress and sporangial germination, infection efficiency, lesion expansion, and sporulation in the potato late blight system. *Phytopathology* **77** (6), 969-974.
- Marx, D.H. & Bryan, W.C. (1969) Effect of soil bacteria on the mode of infection of pine roots by *Phytophthora cinnamomi*. *Phytopathology* **59**, 614-619.

McIntosh, A.H. (1965) An experiment on control of potato tuber blight. *Proceedings of the British Crop Protection Conference -Pests and Diseases, 1965*. Brighton: BCPC, 199-207.

McIntosh, A.H. (1966) Fungicides. *Rothamsted Experimental Station Report for 1966*. Harpenden: Rothamsted Experimental Station, 184.

McRae, D.C. & Fleming, J. (1989) *Potato damage-where it occurs-how to avoid it*. London: PMB.

Mead, R. & Curnow, R.N. (1983) *Statistical methods in agriculture and experimental biology*. London: Chapman & Hall.

Moham, S.B. (1981) Evaluation of antisera raised against *Phytophthora fragariae* for detecting the red core disease of strawberries by enzyme-linked immunosorbent assay (ELISA) *Plant Pathology* **37**, 206-216.

Murphy, D.A. (1921) The sources of infection of the potato tuber with the blight fungus, *Phytophthora infestans*. *Scientific Proceedings, Royal Dublin Society* **16**, 353-368.

Murphy, D.A. & McKay, R. (1925) Further experiments on the sources and development of blight infection in potato tubers. *Journal Dept. Lands and Agriculture* **25**, 10-24.

Murphy, D.A. & McKay, R. (1927) Some further cases of the production of diseased shoots by potato tubers attacked by *Phytophthora infestans*, and a demonstration of alternative sources of foliage and tuber infection. *Scientific Proceedings, Royal Dublin Society* **18**, 413-422.

Nix, J. (1993) *Farm Management Pocketbook*. London: Wye College.

O'Brian, T.P. & McCully, M.E. (1981) *The study of plant structure: principles and selected methods*. Melbourne: Termacarphi Ltd.

Payne, R.W., Lane, P.W., Ainsley, A.E., Bicknell, K.E., Digby, P.G.N., Harding, S.A., Leech, P.K., Simpson, H.R., Todd, A.D. Verrier, P.J., Grower, J.C., Tunnicliffe Wilson, G. & Paterson, L.J. (1987) *Genstat5 Reference Manual*. Oxford: Clarendon Press.

Perombelon, M.C.M. & Lowe, R. (1975) Studies on the initiation of bacterial soft rot in potato tubers. *Potato Research* **18**, 64-82.

Pfender, W.F., Hine, R.B. & Stanghellini, M.E. (1977) Production of sporangia and release of zoospores by *Phytophthora megasperma* in soil. *Phytopathology* **67**, 657-663.

Phillips, D.J. & Austin, R.K. (1982) Changes in peaches after hot water treatment. *Plant Disease* **66**, 487-488.

Pieters, A.J. (1962) Triphenyltin hydroxide- a fungicide for the control of *Phytophthora infestans* on potatoes, and some other fungal diseases. *Proceedings of the British Crop Protection Conference-Pests and Diseases, 1962*. Brighton: BCPC, 461-470.

Pittis, J.E. & Shattock, R.C. (1994) Viability, Germination and infection potential of oospores of *Phytophthora infestans*. *Plant Pathology* **43**, 387-396.

Pratt, R.G. & Mitchell, J.E. (1976) Interrelationships of seedling age, inoculum, soil moisture level, temperature and host and pathogen genotype in *Phytophthora* root rot of alfalfa. *Phytopathology* **66**, 81-85.

- Redbond, M. R., Devey, J-P. & Barlow, J. N. (1990) The development of a three-way fungicide mixture based on synergistic interaction, for effective control of *Phytophthora infestans*. *Proceedings of the British Crop Protection Conference - Pests and Diseases 1990*. Brighton: BCPC, 1115-1120.
- Roberts, S.J. (1992) Effects of soil moisture on the transmission of pea bacterial blight from seed to seedling. *Plant Pathology* **41**, 136-140.
- Robertson, N.F. (1991) The challenge of *Phytophthora infestans*. In: Ingram, D.S. & Williams, P.H. eds. *Advances in Plant Pathology* vol. 7. London: Academic Press, 1-31.
- Roer, L. & Toxopeus, H. J. (1961) The effect of R-genes for hypersensitivity in potato-leaves on tuber resistance to *Phytophthora infestans*. *Euphytica* **10**, 35-42.
- Rotem, J., Palti, J. (1969) Irrigation and plant disease. *Annual Review of Phytopathology* **7**, 267-288.
- Rotem, J. & Cohen, Y. (1974) Epidemiological patterns of *Phytophthora infestans* under semi-arid conditions. *Phytopathology* **64**, 711-714.
- Rotem, J., Cohen, Y. & Bashi, E. (1978) Host and environmental influences on sporulation *in vivo*. *Annual Review of Phytopathology* **16**, 83-101.
- Rotem, J., Palti, J. & Lomas, J. (1970) Effects of sprinkler irrigation at various times of the day on development of potato late blight. *Phytopathology* **60**, 839-843.
- Rowe, R.C. (1982) Translocation of metalaxyl and RE26745 in potato and comparison of foliar and soil application for the control of *Phytophthora infestans*. *Plant Disease* **66**, 989-993.

Salaman, R.N. (1949) The history and social influence of the potato. Cambridge University Press, Cambridge.

Salmon, E.S. & Ware, W.M. (1926) Note on the occurrence of diseased shoots arising from potato tubers infected by *Phytophthora infestans*. *Annals of Applied Biology* **13**, 289-300.

Samoucha, Y. & Cohen, Y. (1988) Synergistic interactions of cymoxanil mixtures in the control of metalaxyl-resistant *Phytophthora infestans* of potato. *Phytopathology* **78**, 636-640.

Samoucha, Y. & Gisi, U. (1987a) Systemicity and persistence of cymoxanil in mixture with oxadixyl and mancozeb against *Phytophthora infestans* and *Plasmopara viticola*. *Crop Protection* **6**, 393-398.

Samoucha, Y. & Gisi, U. (1987b) Use of two- and three-way mixtures to prevent build-up of resistance to phenylamide fungicides in *Phytophthora* and *Plasmopara*. *Phytopathology* **77**, 1405-1409.

Samoucha, Y., Levy, R.S. & Cohen, Y. (1988) Efficacy over time of cymoxanil mixtures in controlling late blight in potatoes incited by a phenylamide-resistant isolate of *Phytophthora infestans*. *Crop Protection* **7**, 210-215.

Sato, N. (1979) Effect of soil temperatures on the field infection of potato tubers by *Phytophthora infestans*. *Phytopathology* **69**, 989-993.

Schippers, P.A. (1977) The rate of respiration of potato tubers during storage. 1. Review of the literature. *Potato Research* **20**, 173-188.

Scholey, J., Marshal, C. & Whitbread, R. (1968) A pathological problem associated with pre-packing of potato tubers. *Plant Pathology* **17**, 135-139.

Schwinn, F.J. & Margot, P. (1991) Control with chemicals. In: Ingram, D.S. & Williams, P.H. eds. *Advances in Plant Pathology* vol. 7. London: Academic Press, 225-265.

Schwinn F.J. & Staub, T (1987) Phenylamides and other fungicides against oomycetes. In: Lyr, H. eds. *Modern Selective Fungicides*. Jena: Gustav Fischer Verlag, 259-273.

Scott, R.K. & Wilcockson, S.J. (1978) Application of physiological and agronomic principles to the development of the potato industry. In: Harris, P.M., ed. *The Potato Crop*. New York: Chapman & Hall, 678-704.

Shattock, R.C., Shaw, D.S., Fyfe, A.M., Dunn, J.R., Loney, K.H. & Shattock, J.A. (1990) Phenotypes of *Phytophthora infestans* collected in England and Wales from 1985 to 1988: mating type, response to metalaxyl and isoenzyme analysis. *Plant Pathology* **39**, 242-248.

Shaw, D.S., Fyfe, A.M., Hibberd, P.G. & Abdel-Sattar, M.A. (1985) Occurrence of the rare A2 mating type of *Phytophthora infestans* on imported Egyptian potatoes and the production of sexual progeny with A1 mating types from the U.K. *Plant Pathology* **34**, 552-556.

Shirsat, S.G., Thomas, P. & Nair, P.M. (1991) Evaluation of treatments with hot water, chemicals and ventilated containers to reduce microbial spoilage in irradiated potatoes. *Potato Research* **34**, 227-231.

Slater, J.W. (1968) The effect of night temperature on tuber initiation of the potato. *European Potato Journal* **11**, 14-22.

Smith, J.M. (1979) The use of metalaxyl for the control of downy mildew diseases. *Proceedings of the British Crop Protection Conference- Pests and Diseases, 1979*. Brighton: BCPC, 331-340.

Smith, L.P. (1956) Potato blight forecasting by 90 percent humidity criteria. *Plant Pathology* **5**, 83-87.

Spadafora, V.J., Bruhn, J.A. & Fry, W.E. (1984) Influence of selected protectant fungicides and host resistance on simple and complex potato late blight forecasts. *Phytopathology* **74**, 519-523.

Spotts R.A. & Cervantes L.A. (1986) Populations, pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* spp. and *Mucor piriformis* in packinghouses. *Plant Disease* **70**, 106-108.

Spotts R.A. & Cervantes L.A. (1992) Effects of ozonated water on postharvest pathogens of pear in laboratory and packing house tests. *Plant Disease* **76** (3), 256-259.

Stachewicz, H. & Burth, U. (1988) Dressing of seed potatoes with metalaxyl against *Phytophthora infestans* (Mont.) de Bary. *Archiv für Phytopathologie und Pflanzenschutz* **24**, 35-43 (abstract in English).

Steck, U. (1988) Potato late blight- all problems solved. *Gesunde Pflanzen* **49** (10), 421-424 (abstract in English).

Stewart, H.E. (1990) Effect of plant age and inoculum concentration on expression of major gene resistance to *Phytophthora infestans* in detached potato leaves. *Mycological Research* **94**, 823-826.

Stewart, H.E., Wastie, R.L. & Bradshaw, J.E. (1993) The effect of the moisture level of potting compost on the susceptibility of glasshouse-grown tubers to late blight. *Potato Research* **36**, 29-33.

Stolzy, L., Letey, J., Klotz, L., Labanauskas, K. (1965) Water and aeration as factors in root decay of *Citrus sinensis*. *Phytopathology* **55**, 270-275.

- Tantius, P.H., Fyfe, A.M., Shaw, D.S. & Shattock, R.C. (1986) Occurrence of the A2 mating type and self-fertile isolates of *Phytophthora infestans* in England and Wales. *Plant Pathology* **35**, 578-581.
- Thurston, H.D. & Schults, O. (1981) Late blight. In: Hooker, W.J., ed. *Compendium of Potato Diseases*. Minnesota: American Phytopathological Society, 40-42.
- Toxopeus, H. J. (1958) Some notes on the relations between field resistance to *Phytophthora infestans* in leaves and tubers and ripening time in *Solanum tuberosum* subsp. *tuberosum*. *Euphytica* **7**, 123-130.
- Turkensteen, L.J., Mulder, M. & Bouman, A. (1990) Control of gangrene and late blight on seed potatoes by a green crop harvesting method and application of fungicides and antagonists. *Proceedings of the EAPR Conference, 1990*. Edinburgh: EAPR, 86-87.
- van Bruggen, A.H.C., Osmeloski, J.F. & Jacobson, J.S. (1986) Effects of simulated acidic rain on wash-off of fungicides and control of late blight on potato leaves. *Phytopathology* **76**, 800-804.
- van der Zaag, D.E. (1956) Overwintering epidemiologie van *Phytophthora infestans*, tevensenige nieuwe bestrijdingsmogelijkheden. *Tijdschrift over Plantenziekten* **62**, 89-156 (abstract in English).
- van der Zaag, D.E. (1959) Some observations on breeding for resistance to *Phytophthora infestans*. *European Potato Journal* **2**, 278-286.
- van Oijen, M. (1991) Light use efficiencies of potato cultivars with late blight (*Phytophthora infestans*). *Potato Research* **34**, 123-132.

Walker, A.S.L. & Cooke, L.R. (1988) The survival of phenylamide-resistant strains of *Phytophthora infestans* in potato tubers. *Proceedings of the British Crop Protection Conference - Pests and Diseases, 1988*. Brighton: BCPC, 353-358.

Wallin, J.R. (1953) The production and survival of sporangia of *Phytophthora infestans* on tomato and potato plants in the field. *Phytopathology* **43**, 505-508.

Wallin, J.R. & Polhemus, D.N. (1956) The growth and development of *Phytophthora infestans* from potato tubers in steamed soil. *Plant Disease Reporter* **40**(6), 534-537.

Walmsley-Woodward, D.J. & Lewis, B.G. (1977) Laboratory studies of potato tuber resistance to infection by *Phytophthora infestans*. *Annals of Applied Biology* **85**, 43-49.

Wastie, R.L. (1991) Breeding for resistance. In: Ingram, D.S. & Williams, P.H. eds. *Advances in Plant Pathology* vol. 7. London: Academic Press, 193-224.

Wastie, R.L., Caligari, P.D.S. & Stewart, H.E. (1987a) Effects of late blight on yield of potatoes in the presence and absence of metalaxyl + mancozeb. *Proceedings of the Crop Protection in Northern Britain Conference, 1987*, Dundee: ACPNB, 186-191.

Wastie, R.L., Caligari, P.D.S., Stewart, H.E. & MacKay, G.R. (1987b) A glasshouse progeny test for resistance to tuber blight (*Phytophthora infestans*). *Potato Research* **30**, 533-538.

Weihing, J.L. & O'Keefe, B. (1962) Epidemiological patterns of potato varieties in relation to late blight. *Phytopathology* **52**, 1268-1273.

Whitehead, T., McIntosh, T.P. & Findlay, W.M. (1953) *The potato in health and disease*. Edinburgh: Oliver & Boyd.

Wigginton, M.J. (1974) Effects of temperature, oxygen tension and relative humidity on the wound-healing process in the potato tuber. *Potato Research* **17**, 200-214.

Winfield, A.L., Wardlow, L.R. & Smith, B.F. (1967) Further observations on the susceptibility of main crop cultivars to slug damage. *Plant Pathology* **16**, 136-138.

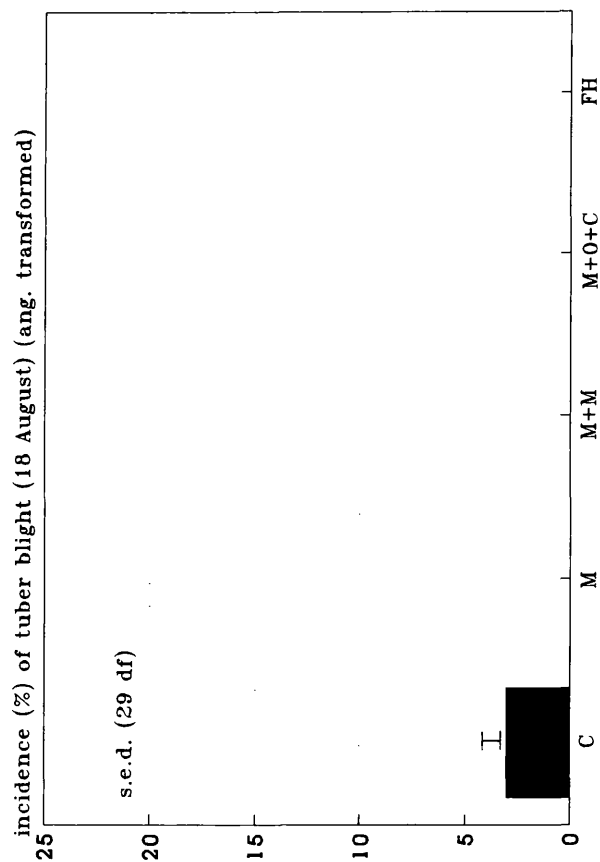
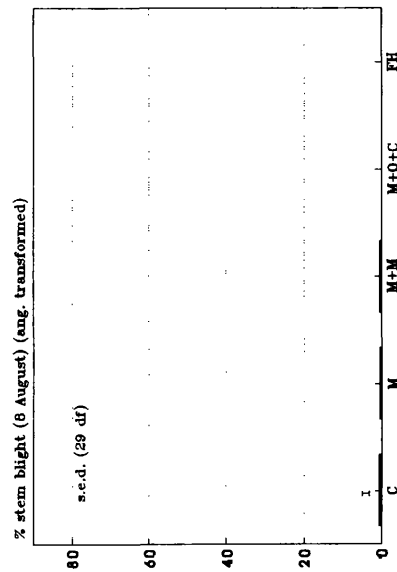
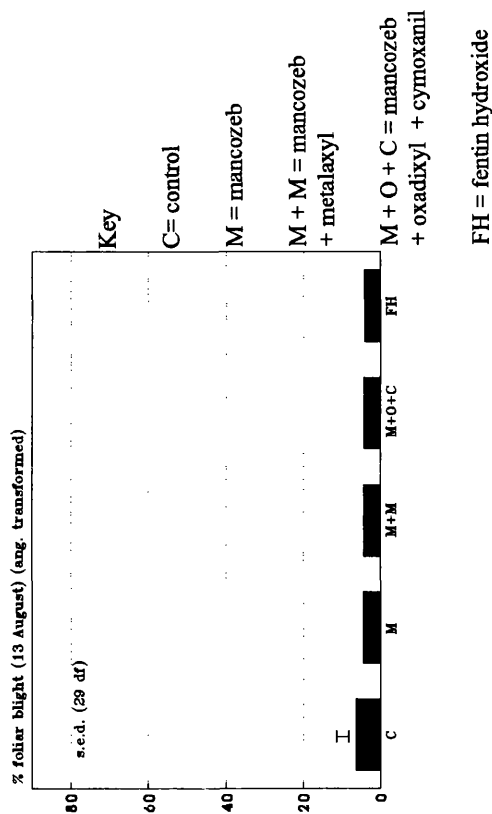
Zan, K. (1962) Activity of *Phytophthora infestans* in soil in relation to tuber infection. *Transactions of the British Mycological Society*. **45(2)**, 205-221.

Appendix 2.1 Buffers used in the ELISA tests

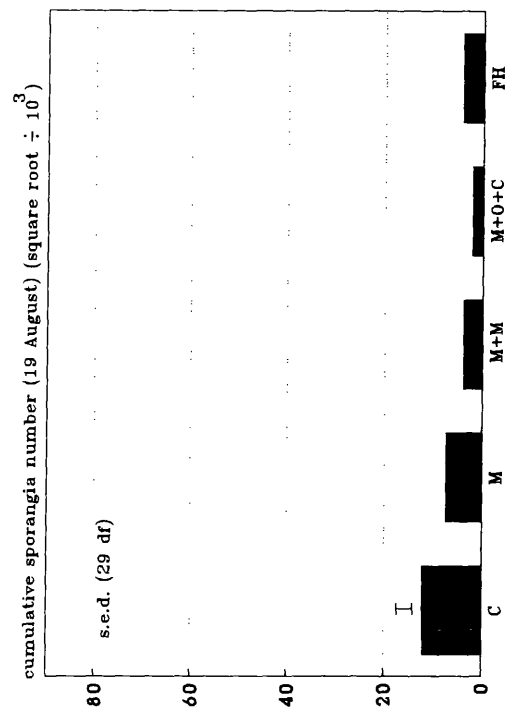
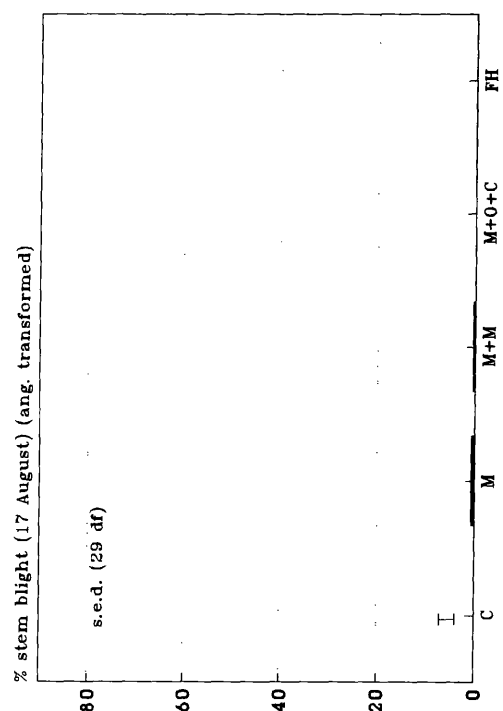
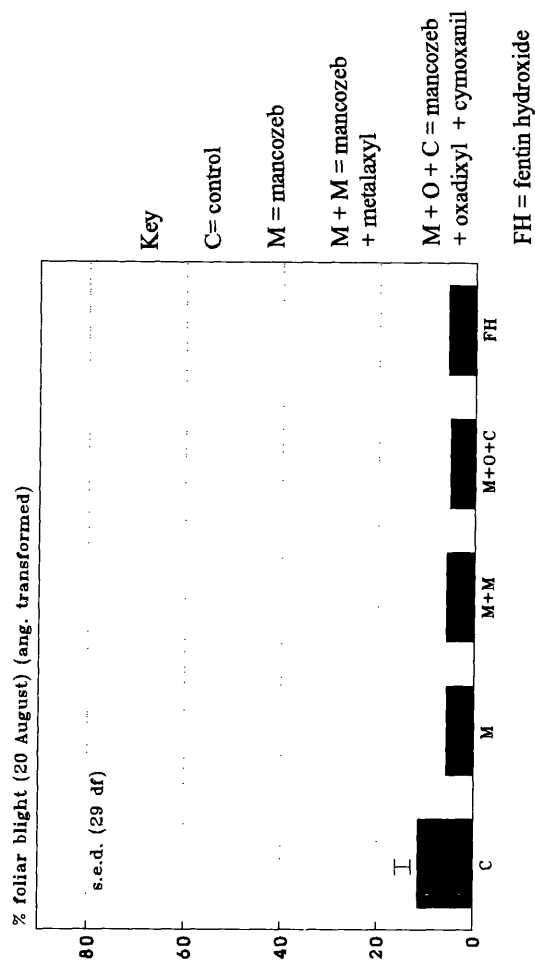
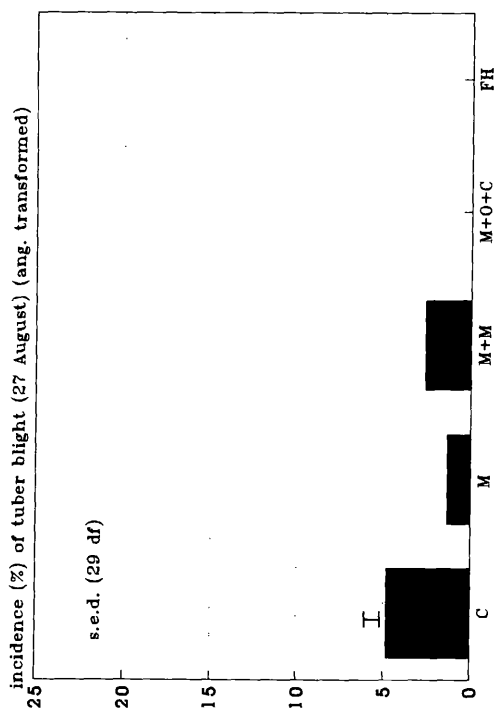
	carbonate buffer	phosphate buffer saline with Tween (PBS-Tween)	substrate buffer
chemicals	1.59 g Na ₂ CO ₃ 2.93 g NaHCO ₃ 0.2 g NaN ₃	80 g NaCl ¹ 2 g KH ₂ PO ₄ ¹ 29 g Na ₂ HPO ₄ ¹ 2 g KCl ¹ 5 ml Tween 20 2 g NaN ₃	2 g NaN ₃ 97 ml diethanolamine 4-8 ml 0.3 M HCl ²
distilled water	1 l	10 l	800 ml

¹these chemicals were initially dissolved in 1 l of distilled water

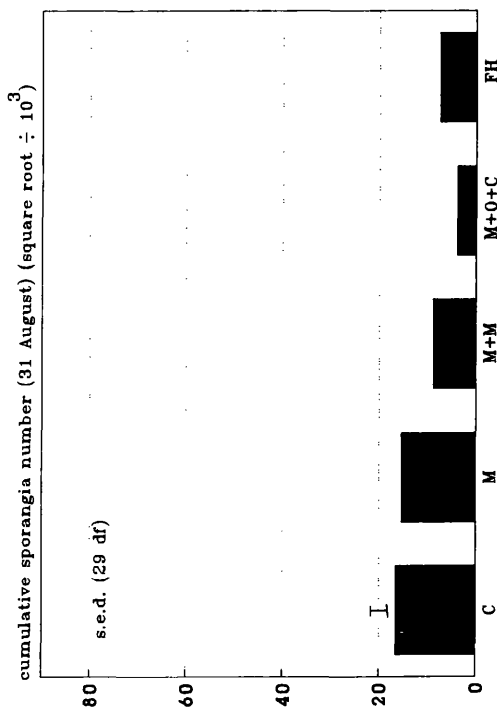
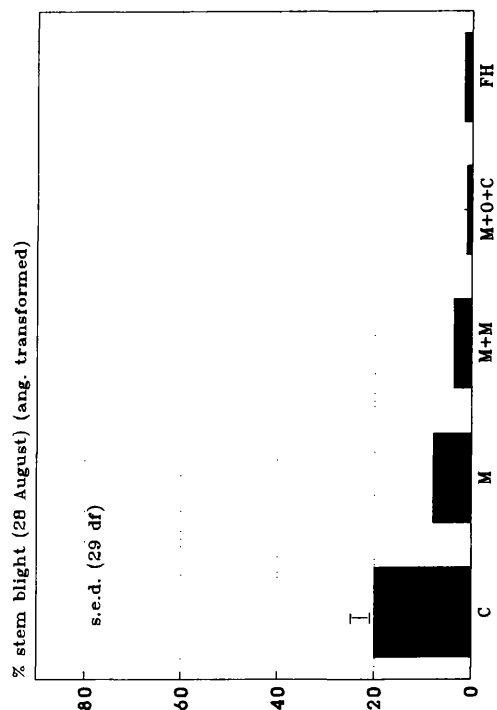
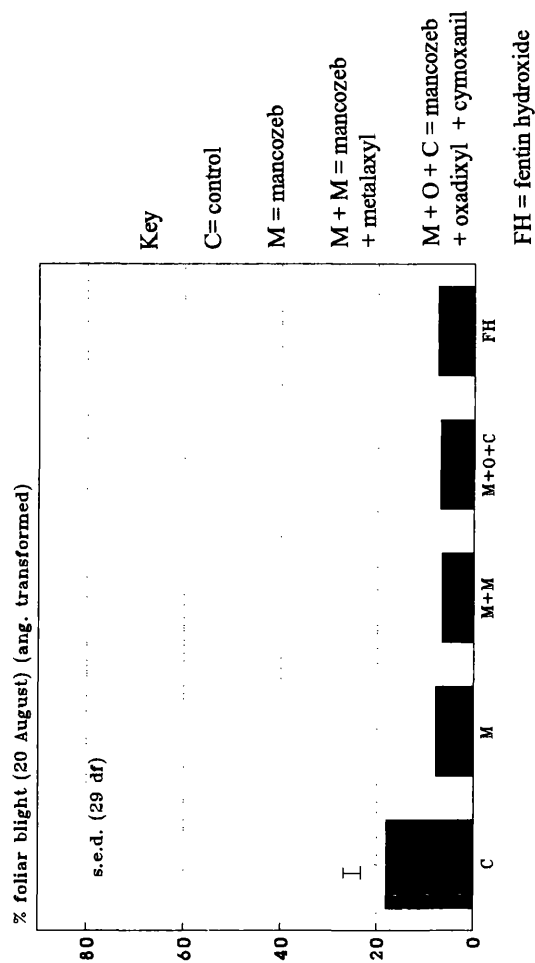
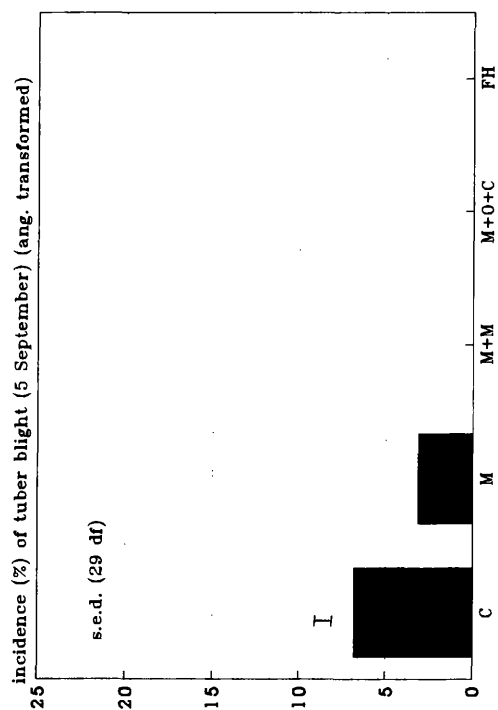
²added as necessary to obtain a pH of 9.8



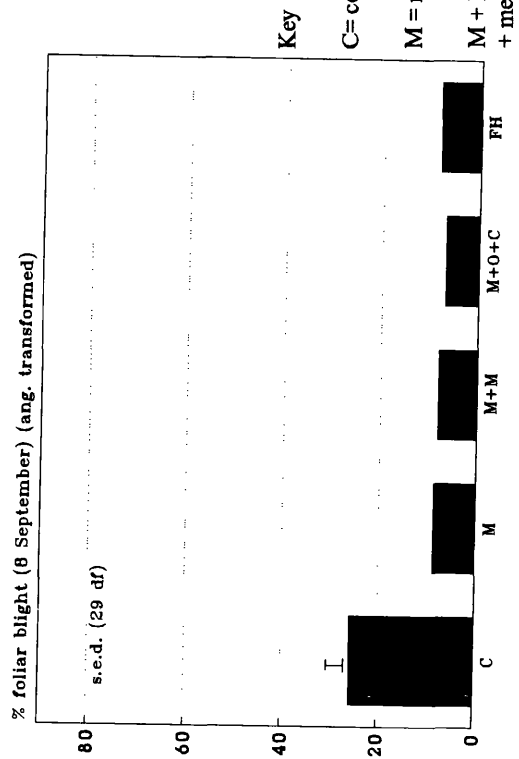
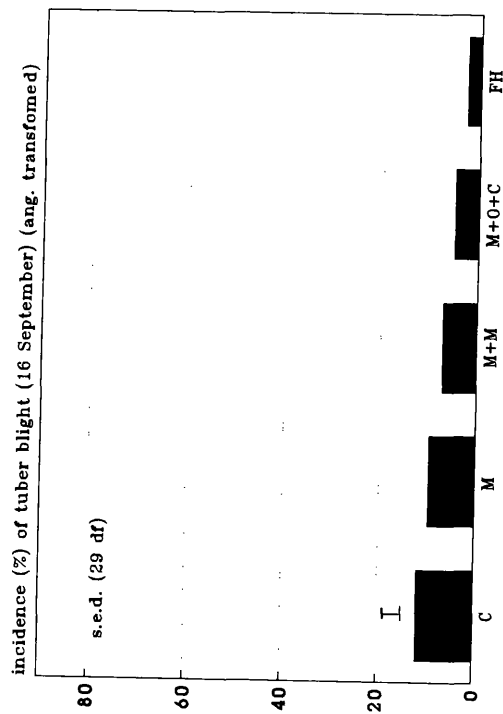
Appendix 3.1 Relationship between tuber blight incidence on 18 August and haulm blight 5 to 10 days earlier



Appendix 3.2 Relationship between tuber blight incidence on 27 August and haulm blight 7 to 10 days earlier



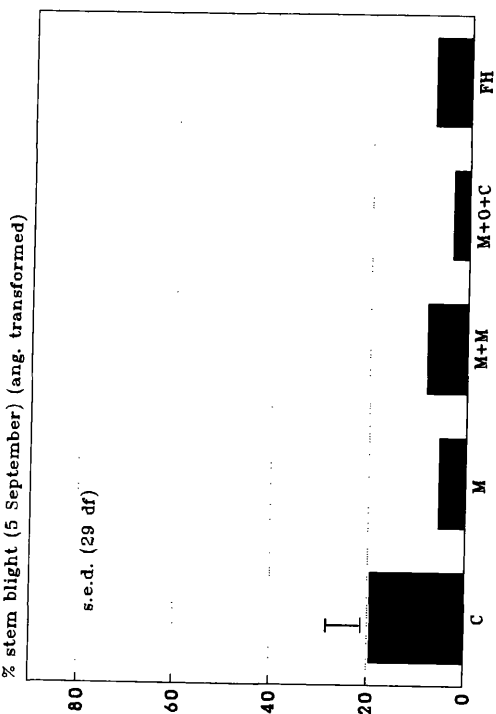
Appendix 3.3 Relationship between tuber blight incidence on 5 September and haulm blight 5 to 16 days earlier



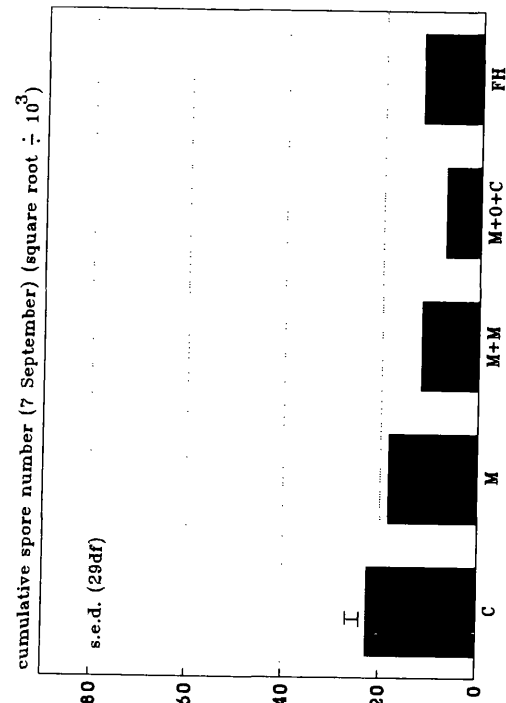
Key
C= control
M = mancozeb
M + M = mancozeb
+ metalaxyl

M + O + C = mancozeb
+ oxadixyl + cymoxanil

% stem blight (5 September) (ang. transformed)

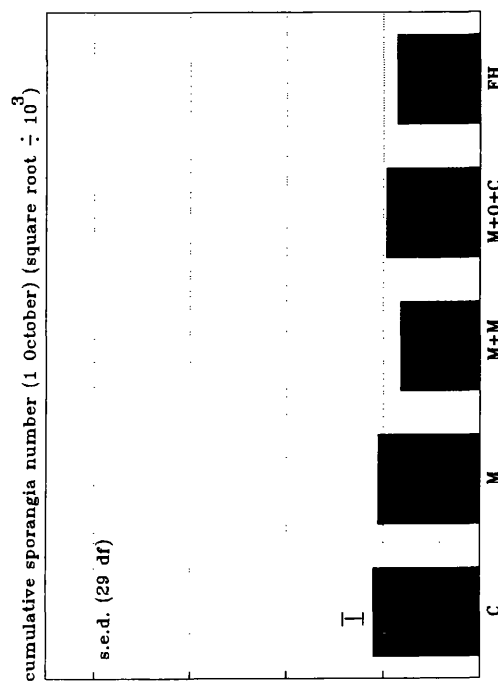
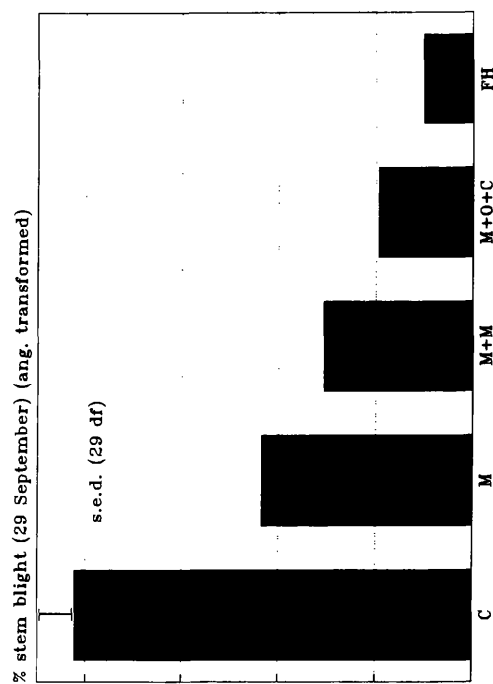
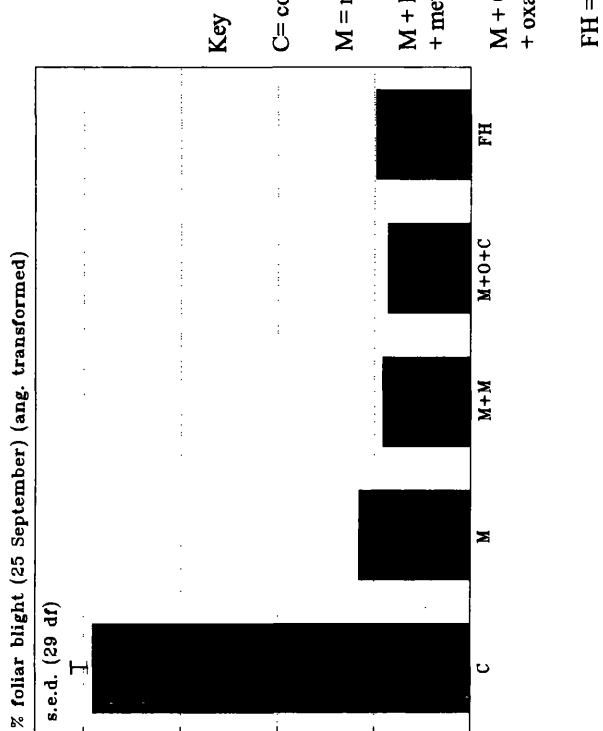
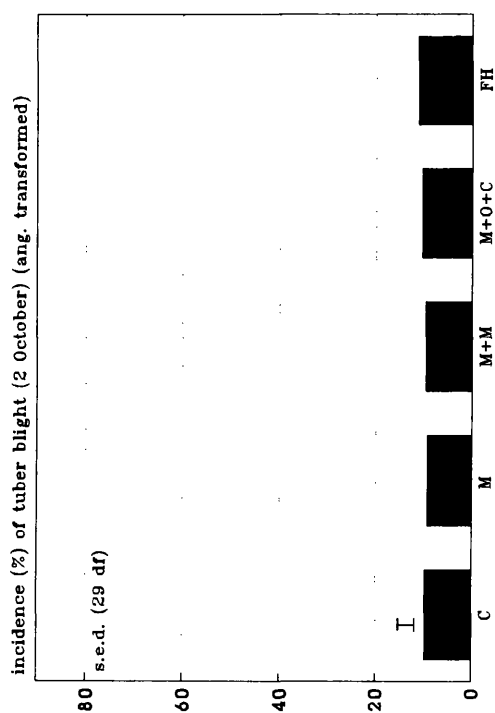


cumulative spore number (7 September) (square root $\div 10^3$)

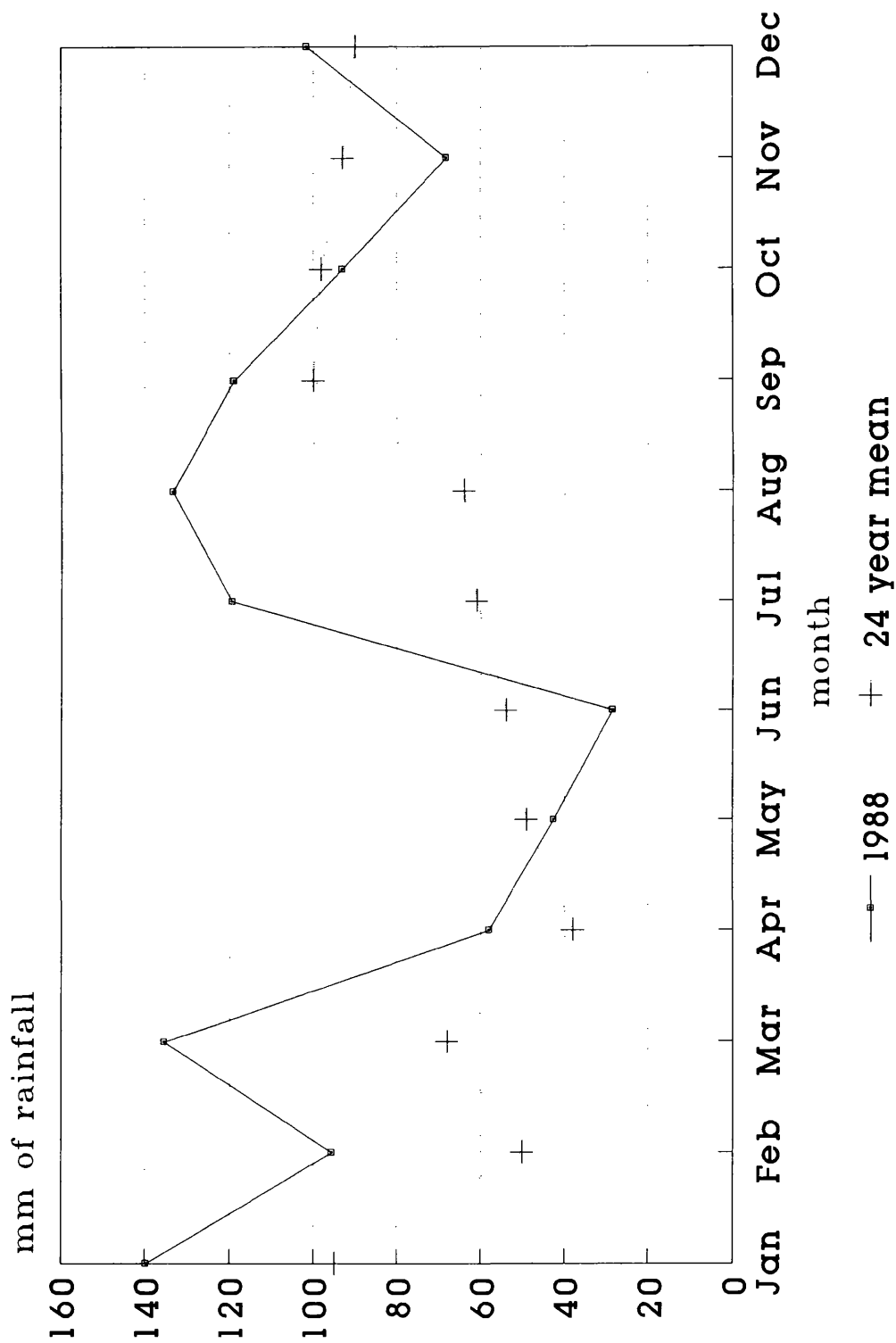


FH = fenitrothion hydroxide

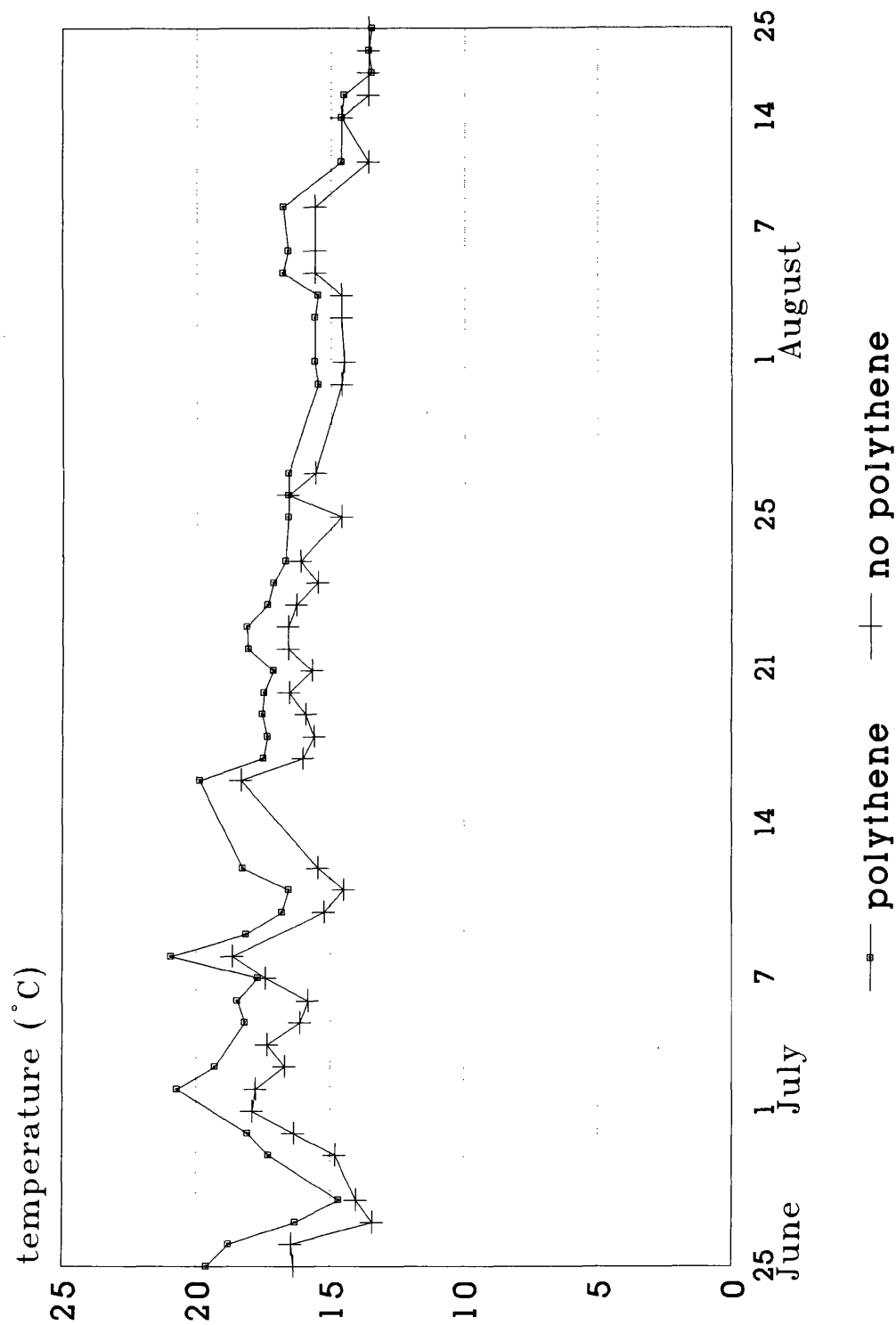
Appendix 3.4 Relationship between tuber blight incidence on 16 September and haulm blight 8 to 11 days earlier



Appendix 3.5 Relationship between tuber blight incidence on 2 October and haulm blight 1 to 7 days earlier



Appendix 4.1 Monthly rainfall at Auchincruive for 1988 and the 24-year mean



Appendix 5.1 Temperature at 14.00 hours GMT measured at 13 cm depth in ridges covered with polythene and in non-covered ridges

Appendix 6.1

The table below gives the details of the commercial bulk potato washing plant used by Caledonian Produce (Geest) at Lanark. These details were used to develop the tuber washing methods used in Chapter 6.

type of washer	Pylbro barrel washer
water volume in the tank (l)	1755
temperature of wash water (°C)	9-10
estimated volume of water change per day (l)	1755
length of wash cycle (minutes)	3
throughput of tubers (kg hour ⁻¹)	3000
average weight of tubers per cycle (kg)	150
tuber size (mm)	45-65
length of time between harvest and washing (hours)	> 24