

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

SELECTED STUDIES ON STRAINS OF
BOTRYTIS CINEREA

A dissertation presented in fulfilment
of the requirements for the
Master of Science
at
Massey University

TERRENCE MARK STEWART

MARCH 1986

ABSTRACT

Some characteristics of benzimidazole and dicarboximide resistant and susceptible strains of Botrytis cinerea and the chemicals that control them were studied.

Difference in sporulation or sclerotial production could be not be used to differentiate between fungicide resistant or susceptible strains. Generally, mycelial growth of dicarboximide low-level resistant strains was slower than that of susceptible strains on unamended malt extract agar and was considerably so on media amended with 0.68 M NaCl. No benzimidazole low-level resistance was detected in the benzimidazole susceptible strains tested.

Fourteen strains of B. cinerea were screened for the ability to sporulate in the dark to assess the feasibility of using material which filtered Ultra-violet light as a glasshouse covering. Eleven of these strains sporulated in complete darkness.

Chlozolate showed a high degree of protectant and systemic activity against dicarboximide susceptible strains but was poor on low-level resistant strains. PP192 showed high protectant but no systemic activity on both susceptible and low-level resistant strains.

Sub-lethal doses of vinclozolin and iprodione on plant surfaces were shown to stimulate the sporulation of B. cinerea from an inoculum source such as an agar plug.

ACKNOWLEDGEMENTS

My sincere thanks to all who have contributed and assisted me in the course of this research. In particular, I wish to thank Dr Peter Long for his supervision and assistance in the preparation of this manuscript. Thanks also to Mrs Lorraine Davis for her technical assistance, Dr Ross Beever for supplying the cultures used in this study, and Mrs Lois Mather for growing the experimental plants.

Finally, thanks to my wife Alison, whose encouragement and support throughout this study was as welcoming as it was necessary.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
CONTENTS	iv
LIST OF TABLES	x
LIST OF FIGURES	xiv
LIST OF PLATES	xvi
CHAPTER 1. INTRODUCTION	1
1.1 An overview of <u>Botrytis cinerea</u>	1
1.2 Light requirements for the sporulation of <u>B. cinerea</u>	6
1.3 Variation in cultural characteristics of <u>B. cinerea</u>	8
1.4 Fungicide resistance	12

CHAPTER 2. GENERAL MATERIALS AND METHODS	23
2.1 Isolates	23
2.2 Growth media	24
2.3 Incubation conditions	24
2.4 Plant material	25
2.5 Fungicides	25
2.6 Assessment methods	28
2.7 Statistical analysis	28
CHAPTER 3. MORPHOLOGICAL CHARACTERISTICS OF ISOLATES	31
3.1 Introduction	31
3.2 Materials and methods	34
3.3 Results	35
(a) Colony morphology	35
(b) Morphology and resistance	36
(c) Effect of peptone	37
3.4 Discussion	50

CHAPTER 4. LIGHT REQUIREMENTS FOR THE SPORULATION
OF B. CINEREA

4.1	Introduction	54
4.2	Materials and methods	56
	(a) Ability of <u>B. cinerea</u> isolates to sporulate in the dark	56
	(b) Effect of type of inoculum on sporulation in the dark	57
4.3	Results	59
	(a) Ability of <u>B. cinerea</u> isolates to sporulate in the dark	59
	(b) Effect of type of inoculum on sporulation in the dark	60
4.4	Discussion	65

CHAPTER 5. FUNGICIDE RESISTANCE	68
5.1 Introduction	68
5.2 Materials and methods	71
(a) Resistance to carbendazim	71
(b) Growth on high osmotic media supplemented with iprodione	71
5.3 Results	73
(a) Resistance to carbendazim	73
(b) Growth on high osmotic media supplemented with iprodione	74
5.4 Discussion	81
(a) Resistance to carbendazim	81
(b) Growth on high osmotic media supplemented with iprodione	82

CHAPTER 6. FUNGICIDES FOR THE CONTROL OF	84
<u>B. CINEREA</u>	
6.1 Introduction	84
6.2 Materials and methods	86
(a) EC ₅₀ determinations	86
(b) Protectant activity	86
(c) Systemic activity	88
(d) Translaminar activity	88
(e) Eradicant activity	89
6.3 Results	91
(a) EC ₅₀ determinations	91
(b) Protectant activity	92
(c) Systemic activity	93
(d) Translaminar activity	93
(e) Eradicant activity	93
6.4 Discussion	106

CHAPTER 7. EFFECT OF SUB-LETHAL CONCENTRATIONS OF DICARBOXIMIDE FUNGICIDES ON THE SPORULATION OF <u>B. CINEREA</u>	108
7.1 Introduction	108
7.2 Materials and methods	109
7.3 Results	110
7.4 Discussion	112
CHAPTER 8. GENERAL DISCUSSION	114
CHAPTER 9. REFERENCES	118

LIST OF TABLES

Table		Page
2.1	Isolates of <u>B. cinerea</u> obtained from D.S.I.R. Plant Protection division.	30
3.1	Sporulation and sclerotial production of strains of <u>B. cinerea</u> isolated from grapes, with 4 levels of peptone added to the media.	43
3.2	Sporulation and sclerotial production of strains of <u>B. cinerea</u> isolated from cucumber, with 4 levels of peptone added to the media.	44
3.3	Sporulation and sclerotial production of strains of <u>B. cinerea</u> isolated from tomato, with 4 levels of peptone added to the media.	45
3.4	Sporulation and sclerotial production of strains of <u>B. cinerea</u> isolated from phaseolus with 4 levels of peptone added to the media.	46

- 3.5 Sporulation and sclerotial production of 47
a $D^S B^S$ strain of B. cinerea isolated
from strawberry, with 4 levels of peptone
added to the media.
- 3.6 Sporulation and sclerotial production of 47
a $D^S B^S$ strain of B. cinerea isolated
from kiwifruit, with 4 levels of peptone
added to the media.
- 3.7 Growth over three days of subcultures of 48
7569 ($D^1 B^r$) taken from Sc or Sp sectors of
two colonies growing on MA with 5 g/l
peptone. Growth measured in mm.
- 3.8 Means and standard errors of spore and 49
sclerotial production over different levels
of peptone.
- 4.1 Spore counts from representative cultures 62
to ascertain the reliability of visual
assessment procedure.
- 4.2 Visual assessment of sporulation of 14 63
strains of B. cinerea on a 0 - 3 scale.
Means of three replicates.

4.3	Visual assessments of sporulation on a 0 - 3 scale of 4 strains of <u>B. cinerea</u> taken from 2 different sources of inoculum. Means of three replicates.	64
5.1	Mycelial growth in mm/hr of benzimidazole susceptible isolates of <u>B. cinerea</u> on unamended MEA.	76
5.2	Mycelial growth in mm/hr of B ^r D ¹ isolates of <u>B. cinerea</u> on fungicide amended MEA	77
5.3	Mycelial growth in mm/hr of B ^r D ^S isolates of <u>B. cinerea</u> on fungicide amended MEA	78
5.4	Mycelial growth in mm/hr with 4 isolates on OMEA and MEA	79
5.5	Mycelial growth in mm/hr of isolates on fungicide and NaCl amended MEA. - Comparison of treatments.	80
6.1a	Protectant activity of chlozolate against dicarboximide resistant isolates.	101

6.1b	Protectant activity of PP192 on dwarf bean leaves.	102
6.2	Systemic activity of fungicides applied as a soil drench to pots of dwarf beans 3 days before inoculation of leaves with agar plugs.	103
6.3	Translaminar activity of fungicides applied to undersides of dwarf bean leaves 2 days before inoculation on the top surface with agar plugs.	104
6.4	Eradicant activity of fungicides on established infections of isolate 7663 (D ^S B ^S) on dwarf bean leaves. All fungicides were used at a rate of 0.5 mg/ml.	105
7.1	Average number of spores/plug x 10 ⁶ from MEA plugs which showed sporulation on dicarboximide treated bean leaves.	111

LIST OF FIGURES

Figure		Page
2.1	Diagram of petri plate to show the measurement of mycelial growth rate of <u>B. cinerea</u> .	29
3.1	Sporulation of D ^S and D ^L strains of <u>B. cinerea</u> on peptone amended MA.	40
3.2	Sporulation of B ^S and B ^L strains of <u>B. cinerea</u> on peptone amended MA.	41
3.3	Average sclerotial production of <u>B. cinerea</u> on peptone amended MA.	42
5.1	Average mycelial growth of B ^r isolates at different carbendazim concentrations.	75
6.1a	Dose response curve for mycelial growth in chlozolate amended OMEA.	96
6.1b	Dose response curve for mycelial growth in PP192 amended OMEA.	97
6.2	EC ₅₀ values in ug/ml for chlozolate and PP192 over 18 isolates of <u>B. cinerea</u> .	99

- 6.3 Graph showing % infection of dwarf bean 100
leaves 3 days after inoculation with agar
plugs of B. cinerea on to fungicide
treated surface.

LIST OF PLATES

	Page
PLATE 1.1: Grape bunches infected by <u>B. cinerea</u> .	22
PLATE 1.2: Chrysanthemum flowerheads infected by <u>B. cinerea</u> .	22
PLATE 3.1: Isolate 7551 showing an example of a typical sclerotial (Sc) strain of <u>B. cinerea</u> over 4 peptone levels in MA.	38
PLATE 3.2: Isolate 7516 showing an example of a typical sporulating (Sp) strain of <u>B. cinerea</u> over 4 peptone levels in MA.	38
PLATE 3.3: Isolate 7569 on MA showing saltation.	39
PLATE 4.1: A series of isolates showing scale of sporulation.	61
PLATE 4.2: Comparison of two isolates grown under light and dark conditions.	61

- PLATE 6.1: Detached inoculated bean leaves in humid chamber before incubation. 90
- PLATE 6.2: Colony margin of B. cinerea on OMEA plate supplemented with 25 ug/ml PP192 after 7 days growth. 94
- PLATE 6.3: Colony margin of B. cinerea on unamended OMEA plates after 7 days growth. 94
- PLATE 6.4: Germ tubes from spores after 7 days on OMEA supplemented with 25 ug/ml of PP192. 95