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.

.

# A Study of Two Seed-Borne Alternaria Diseases

.

on Choumoellier

A Thesis presented in partial fulfilment of the requirements for the Degree of Master of Agricultural Science at Massey University

bу

Franklin Henry Wood November 1966.

## TABLE OF CONTENTS

CHAPTER

	INTE	ODUCTION
ī		TIFICATION OF THE <u>ALTERNARIA</u> PATHOGENS HOUMOELLIER
	A.	INTRODUCTION
	в.	MATERIALS AND WETHODS
	C.	RESULTS
		(1) <u>A.brassicae</u> 8 (2) <u>A.brassicicola</u> 10
	D.	DISCUSSION 12
$\mathbb{T}\mathbb{I}$	A.BR	ASSICAE AND A.BRASSICICOLA AS SEED-BORNE
	PATH	<u>OGENS</u>
	Α.	INTRODUCTION
	в.	MATERIALS AND METHODS 24
	С.	INOCULUM ASSOCIATED WITH SEED LINES 26
		(1) Seed infection
	D.	VIABILITY OF SEED-BORNE INOCULUM 32
		<ul> <li>(1) Seed infection</li></ul>
	E.	THE EFFECTS ON GERMINATION OF THE ELIMINATION OF <u>A.BRASSICICOLA</u> FROM SEED 39
		(1) Identification of an effective hot
		water treatment 41 (2) Effect of hot water treatment on
		germination 45
	F.	DISCUSSION 49

CHAPTER

III	EFF	ECT OF THE PATHOGENS ON SEEDLING DEVELOPMENT
	А.	INTRODUCTION
	В.	MATERIALS AND METHODS
	С.	EFFECTS ON EVERGENCE
		(1) Potential effect of contamination in emergence
		(2) The potential effect of seed infection by <u>A.brassicicola</u> on emergence
		(3) Effect of seed-borne inoculum on
		emergence in commercial seed lines 64 (4) Effect of seedling infection by A.brassicicola on seedling develop-
		ment 65
	D.	MECHANISM INVOLVED IN SEEDLING ATTACK . 69
	11 <b>.</b>	DISEASED SEEDLINGS AS A SOURCE OF INOCULUM IN THE SEED BED
		(1) Glasshouse trials
	⊮ •	DISCUSSION
IV	THE	EFFECT OF THE PATHOGENS IN SEED CROPS
	A.	INTRODUCTION 80
	В.	MATERIALS AND METHODS
	С.	SYMPTOMOLOGY 84
		(1) Foliage symptoms 84 (2) Seed head symptoms
	D.	THE YIELD AND QUALITY OF SEED HARVESTED FROM ARTIFICIALLY INOCULATED SILIQUAS . 88
		(1) Effect of siliqua infection on seed yield
		(2) Effect of siliqua infection on the
		size of seed harvested 93 (3) Discussion 102
	Ë.	EXAMINATION OF NATURALLY INFECTED FIELD CROP SAMPLES

CHAPTER

V

F.	MECH. LOSS	ANISM •	OF S	EED • •	IN:	FE( •	стэ •	101 •	∛", ∎	1N] •	) (	SEI •	ED.	۰		10
	(1) (2)	<u>A.bra</u> A.bra			. <u>a</u> •	0 6	•	•	د •	•	4 9	•	•	* *	•	1( 1·
G.	DISC	USSION	••	• •	٠	•	٠	•	۰	•	•		•	•	•	1
A CO	DNSIDE	RATION	OF	HEAT	<u>TH</u>	S	CRI	:EF	<u>II</u>	\G	M	<u>c'r</u> i	101	DS		
A.,	INTR	ODUCTI	ON .	• •	•	•	٠	•	٠	٠	ه	٠	٠	٠	•	1
з.	THE I TEST	BLOTTEN ING .	R ME	THOD	• F( •	DR •	S I	•	) <u>}</u>	fe <i>i</i>	• Ll	РН •	o	•		1.
	(1) (2) (3)	Specie Contae Germin	ct i	nfec	tic	on	ir	ł	lo						•	1 1
		tests	• •	<b>9</b> 0	۰	٠	٥	a	٩	۵	•	•	٠	•	0	1
С.		PLATI	VG M.	ETHO	DS	۰	٠	٠	٠	٠	4	a	Þ	۰	٠	1
	(1)	Compar			ຣບ	ırf	ac	0	st	ser	ri]	19	sat	tic	on	4
	(2)	treatr Modif: screen	icat	ions			:he			sic •		<u> </u>		•	•	1
D.	COMB	INATION	TE(	CHNI	QUE	5		•		•			•		•	1
	(1)	Antibi	loti	cs a	st	oac	ete	ri	.a]	_						
	(	suppre			-	٥		٠		•	•	•	•	٠	٠	1
	(2)	Use in	1 Se	ed s	cre	er	in	£	٠	•	,	۰	¢	٠	•	1
E.	DISCU	JSSION	• •	1 O	۰	•	٠	•	•	•	٠	a	•	۵	•	16
SÜMM	ARY .	~ <b>~</b> ~		5 8	•	۰	•	-	•	ŗ	٩	•	•	•	ø	16
BIBI	JOGRAI	ЭНY														

APPENDICES

## FIGURES

	After page
1.	A.brassicae - distribution of spore length 19
2.	A.brassicae - distribution of spore width 19
3.	A.brassicae - distribution of beak length 19
ų,	Spore germination of <u>A.brassicae</u>
5.	A.brassicicola - distribution of spore length 19
6.	A.brassicicola - distribution of spore width 19
7.	Temperature effects on growth
8.	Temperature effects on growth (Neergaard, 1945) . 19
9.	Definition of terminology used in describing symptoms expressed during germination by abnormal seedlings and infected seedlings parasitised by <u>A.brassicicola</u>
10.	Design of glass-faced seed boxes used to enable close observation of symptoms on diseased seedlings
11.	Method of artificial inoculation of choumoellier seed heads with atomised spore suspensions $83$
12.	A choumoellier siliqua illustrating the structures referred to in the text
13.	Apparatus used in seed washing trials 137
14.	The effect of various concentrations of sodium 2,4-D in MA and PDA on colony growth of <u>A.brassicicola</u>
15.	The effect of various concentrations of sodium 2,4-D in MA and PDA on colony growth of <u>A.brassicae</u> 146
16.	The effect of various concentrations of streptomycin sulphate in PDA on colony growth of <u>A.brassicae</u> and <u>A.brassicicola</u>

## PLATES

1.	Colony characteristics of <u>A.brassicae</u> and <u>A.brassicicola</u>	19
2.	Conidial production by <u>A.brassicae</u>	19
3.	Leaf penetration by A.brassicae	19
4.	Conidiophore characteristics of A.brassicicola .	19
5.	Conidial characteristics of A.brassicicola	19
6.	Temperature effects on colony growth	19
7.	Spore germination and penetration by A.brassicicola	19
8.	Apparatus used in hot water treatment of seed	26
9.	Effect on emergence of seed artificially contaminated with <u>A.brassicicola</u>	52
10.	Hypocotyl lesions caused by A.brassicicola	58
11.	Infection cabinets used in artificial infection of seed	62
12.	Effect of infection on seedling development	68
13.	Early stage symptoms of damping-off caused by <u>A.brassicicola</u>	71
14.	Cotyledon infection of <u>A.brassicae</u> originating from the attached testa	71
15.	Wire-stem symptoms and cortical splitting of the hypocotyl caused by <u>A.brassicicola</u>	72
16.	Microtome sections of hypocotyl tissue infected with <u>A.brassicicola</u>	72
17.	Apparatus for measuring the effective dispersal of spores	76
18.	Symptoms expressed by choumoellier leaves artificially inoculated with <u>A.brassicae</u> and <u>A.brassicicola</u>	85
19.	Field symptoms on choumoellier leaves caused by <u>A.brassicae, A.brassicicola</u> and <u>M.brassicicola</u> .	85
20,	Plant artificially inoculated with <u>A.brassicae</u> showing advanced stages of infection	85
21.	Plant artificially inoculated with <u>A.brassicicola</u> showing advanced stages of infection	85

After page

22.	Artificially inoculated racemes of choumoellier .	87
23 <b>.</b>	Symptoms on siliquas artificially inoculated with <u>A.brassicae</u>	87
24.	Symptoms on siliquas artificially inoculated with <u>A.brassicicola</u>	87
25.	The extent of lesion development on siliquas artificially inoculated with <u>A.brassicae</u> and <u>A.brassicicola</u>	87
26.	Effect of siliqua infection on the quantity of seed harvested from 100 pods $\ldots$	93
27.	Mechanism of seed loss from siliquas due to <u>A.brassicicola</u> infection	111
28.	Spread of A.brassicicola within infected siliquas	111
29.	Seedling symptoms caused by <u>A.brassicicola</u> during a blotter test	122
30.	"Contact" infection between seedlings during a blotter test	124
31.	Effect of varying concentrations of sodium 2,4-D on germination in a blotter test	128
32.	Seed germinator used in seed health trials	128
33.	Development of <u>A.brassicicola</u> colonies from infected seeds following incubation for six days at 22°C on PDA	130
34.	Effect on varying the concentration of sodium 2,4-D in MA on colony growth of <u>A.brassicae</u> and A.brassicicola	149
35.	Effect of varying the concentration of sodium 2,4-D in FDA on colony growth of <u>A.brassicae</u> and A.brassicicola	149
36.	Effect of varying the concentration of sodium 2,4-D in FDA on germination of seed	149
37.	Comparison of agar methods used in screening choumoellier seed for <u>A.brassicae</u> and	140
38.	<u>A.brassicicola</u>	1 <u>49</u> 157

## TABLES

1.	Pathogenic fungi recorded on choumoellier in	
	New Zealand	2
2.	Spore dimensions of <u>A.brassicae</u>	14
3.	Spore dimensions of <u>A.brassicicola</u>	15
1 <u>4</u> o	Previously recorded spore dimensions of <u>A.brassicae</u> • • • • • • • • • • • • • • • • • • •	16
5.	Previously recorded spore dimensions of <u>A.brassicicola</u>	17
6.	Spore dimensions of <u>A.brassicicola</u> in microns (after Neergaard, 1945)	18
7.	Spore dimensions of <u>A.brassicae</u> in microns (after Neergaard, 1945)	19
8.	I.S.T.A. health tests on distributed samples of brassica seed	21
9.	Prevalence of <u>A.brassicicola</u> in choumoellier seed lines screened at G.S.T.S	22
10.	Prevalence of <u>A.brassicae</u> in choumoellier seed lines screened at G.S.T.S.	28
11.	The prevalence of <u>A.brassicicola</u> in choumoellier seed lines screened in 1965 and 9166	28
12.	The prevalence of <u>A.brassicae</u> in choumoellier seed lines screened in 1965 and 1966	28
13.	Percentage of infecting seedlings resulting from contaminating inoculum	31
14.	Viability of seed infections after three years . four months storage	34
15.	Viability of seed infections after two years five months storage	35
16.	Viability of seed infections after one year six months storage	36
18.	Examination of hot water treated seed by an agar plate method	44
19.	Examination of the germination of hot water treated seed	44
20.	Examination of the emergence of hot water treated seed	4 <b>4</b>

21.	Effect of hot water treatment on the germinability of six months old seed	47
22.	Effect of hot water treatment on the germinability of 18 months old seed	48
23.	The potential effect of seed contamination with <u>A.brassicae</u> and <u>A.brassicical</u> on emergence $\ldots$	57
24.	Pre- and post-emergence seedling death in emergence trials	61
25.	The effect of artificial infection of seed with <u>A.brassicicola</u> on emergence	64
26.	Effect of seed infection on the emergence of commercial seed lines	66
27.	The effect of seed contamination on seedling development	68
28.	Effects of watering on scedling development	75
29.	Comparison of the mean weight of 100 siliquas harvested from inoculated seed heads	89
30.	Comparison of the mean weight of seed harvested from 100 siliquas	90
31.	Comparison of the mean germination percentage of seed harvested from infected siliquas	91
32.	Comparison of the mean germination percentage of seed harvested from infected siliquas	91
32.	Percentage infection of seed harvested from inoculated siliquas	92
33.	Comparison of the mean percentage weight of seed from inoculated siliquas retained by the 2.007 mm sieve	94
34.	Comparison of the mean percentage weight of seed from inoculated siliquas retained at the sieve size groupings >1.651 mm <2.007 mm	95
35.	Comparison of the mean percentage weight of seed retained at the sieve size groupings >1.270 mm <1.651 mm	96
36.	Comparison of the mean percentage weight of seed retained at the sieve size groupings >0.838 mm <1.270 mm	97
37•	Comparison of the mean germination percentage of seed from inoculated siliquas retained by the 2.007 mm sieve	99

38.	Comparison of the mean germination percentage of seed from inoculated siliquas retained by the >1.651 mm <2.007 mm sieve size groupings	100
39•5	Comparison of the mean germination percentage of seed from inoculated siliquas retained by the >1.270 mm <1.651 mm sieve size groupings	101
40.	Effect of siliqua infection on seed infection for various seed size groupings	103
41.	Isolation from field samples of choumoellier seed	107
42.	Isolation from siliquas infected with <u>A.brassicicola</u>	113
43.	Isolation from siliquas infected with A.brassicae	116
46.	Identification of <u>Alternaria</u> infections of seedlings to species level	124
47.	Contact infection in a blotter health test $\ldots$	126
48.	Percentage infection from plating seed after surface sterilisation by three different methods	132
49.	Comparative surface sterilisation of artificially infected seed	139
50.	Effect of chemical and non-chemical surface sterilisation on the infection percentage recorded for naturally infected seed	141
51.	Effect of varying 2,4-D/MA concentrations on seed germination	146
52.	Effect of autoclaving 2,4-D/MA on colony growth .	148
53.	Effect of autoclaving 2,4-D/PDA on colony growth	149
54.	Health screening using four agar plate methods .	152
55.	Health screening lines by the "combination method"	160

### ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Mr H.T. Wenham for his guidance, encouragement and constructive criticism throughout the course of the study and in preparation of this thesis.

Thanks are also due to :

the Staff of the Palmerston North Seed Testing Station, and especially Miss D. Matthews and Mr M. Hill for their co-operation in providing seed samples and records;

Miss D. Scott and Miss C. Mitchell of the Photographic Unit for their technical advice and the care taken in the preparation of illustrations;

the Staff of the University Library for obtaining publications;

Mr A. Bryant for advice on statistical analysis; Miss S. Cooper for her patience and diligence in typing this thesis;

Mrs. M. Hudson and Mrs. E. Robertson for laboratory assistance.

Finally, I wish to record the debt of gratitude I owe my wife for her unfailing support and willing help throughout the study.

### INTRODUCTION

The livestock industry in New Zealand is based on a pastoral farming economy, an important aspect of which is the utilisation of fodder crops as supplementary feed during seasons of poor pasture growth. Playing an important role in this regard are the two selections of choumoellier (Brassica oleracea var. acephala D.C.) usually referred to as giant choumoellier and medium stemmed choumoellier. The growing importance of choumoellier in feed crop rotations is indicated by the increased area sown, from 8,000 acres in 1933 to 130,000 acres in 1963 (New Zealand Farm Production Statistics, 1962-63). In the past, choumoellier seed has been imported from the United Kingdom, but more recently the trend has been to promote locally grown seed. At the present time New Zealand's requirements are met in most years by South Island growers producing seed under the authority of a Government seed certification scheme.

In discussing brassica crops in New Zealand, Palmer (1966) stated that - "apart from weather variations, the main uncontrollable causes of yield variations are fungus diseases, insect pests and associated virus diseases". Seven fungus diseases are recorded in New Zealand on choumcellier (Table 1), five of which are evidenced by foliage lesioning in field stands. TABLE 1

Pathogenic fungi recorded on choumoellier in New Zealand

	Pathogen	Authority	
*	Alternaria brassicicola (Schw.)Wilt.	Brien and Dingley,	1957
*	Alternaria brassicae (Berk.)Sacc.	Morton,	1964
*	<u>Peronospora parasitica</u> (Pers.)Tul.	Brien and Dingley,	1959
*	Phoma lingum (Tode ex Fr.)Desm.	Neill and Brien,	1933
\$	Mycosphaerella brassicicola (Fr.)Lind.	Brien,	1939
	Sclerotinia sclerotiorum (Lib.)Mass.	Cunningham,	1927
	<u>Plasmodiophora</u> brassicae Woron.	Cunningham,	1922

Preliminary surveys in the Manawatu established that leaf infections caused by <u>A.brassicae</u> and <u>A.brassicicola</u> are prevalent and on the basis of the extent of the disease development these two fungi are considered to be of some significance in affecting yields of local choumoellier crops. Both pathogens are world-wide in distribution and have been studied on a number of brassica crops (Weimer, 1924, 1926; Neergaard, 1945; Rangel, 1945; Domsch, 1957; McDonald, 1959; Changsri and Weber, 1963). In general, published work reveals both species to be seed-borne and frequently of economic significance in :

\* Species which cause foliage symptoms

- (a) causing seed and seedling death and thereby considerably affecting stand establishment;
- (b) directly reducing yields in seed crops by infecting siliquas and seed.

To the author's knowledge there has been no formal study conducted on the seed-borne nature of the pathogens in choumcellier, but seed health surveys reported in the 1963 and 1964 annual reports of the Government Seed Testing Station (Palmerston North), show that the <u>Alternaria</u> species were present in a high percentage of the seed lines screened, thus indicating the prevalence of the diseases in choumcellier crops in this country.

In view of the lack of detailed research relating to the seed-borne nature of both pathogens in choumoellier seed, and the fact of both fungi being recorded in a high percentage of New Zealand seed lines, studies were undertaken, the main objectives of which were :

- (i) to determine the extent to which both pathogens are associated with New Zealand certified seed lines;
- (ii) to investigate the significance of <u>A.brassicae</u> and <u>A.brassicicola</u> in causing seed bed losses;
- (iii) to investigate the role of both pathogens in causing reductions in yields of choumcellier seed crops;

3.

 (iv) to critically examine methods of health screening for <u>A.brassicae</u> and <u>A.brassicicola</u> in brassica seed.