STUDIES ON THE ROLE OF THE GLUCOSINOLATE-MYROSINASE SYSTEM IN RESISTANCE OF OILSEED RAPE TO 
Sclerotinia sclerotiorum

by

Siamak Rahmanpour Ozan

Bachelor of Science in Plant Protection- Tehran University, IRAN
M.Sc. in Plant Pathology- Tehran University, IRAN

A thesis submitted for the degree of 
Doctor of Philosophy
of the
University of New England
Armidale, NSW
Australia

March 2008
Acknowledgements

I am indebted to my principal supervisor, Dr. David Backhouse, and co-supervisor, Dr. Heather Nonhebel for their great encouragement, guidance and wonderful insights on the topic of my research. I am grateful to David and Heather for their patience and help with editing the product.

Thank you to all Botany academic and technical staff for supporting me, especially Dr. Mohammad Fatemi, Richard Willis, and Ian Simpson. I will never forget the help from postgraduate students particularly Amar Pandey and Dalvinder P. Singh Lakhesar.

Appreciation is extended to the BBMS group where I conducted the enzyme assays and gel photography. The assistance of Kim Quinn on working with the NanoDrop spectrophotometer in the Beef Industry Centre is appreciated.

Thank you to Iranian and Australian friends and families who supported my family and me physically and emotionally.

I am grateful especially to my great and lovely family, my wife, Sarira Fasihian and my son, darling Radin. Sarira has supported me anytime emotionally and physically while she was tolerating loneliness in family duties. I was seeing that Radin in the last months of my thesis project needed my presence more at home. I really appreciate both your patience. During Radin’s birth and his first months growing period, my mother in law, Mrs. Mahbobeh Ranji kindly supported my family and gave me the opportunity to continue my project with confidence. Her presence was appreciable and I am grateful to her.
Summary

The glucosinolate-myrosinase (GSL-M) system in oilseed rape and other members of the family Brassicaceae produces toxic products which can limit fungal pathogen attacks on the host. The role of this system in resistance of oilseed rape to *Sclerotinia sclerotiorum*, causal agent of stem rot, was investigated.

Mustard powder was used as a GSL and myrosinase source in bioassays. The effect of toxic volatiles derived from hydrolysis of glucosinolates was observed as inhibition of fungal growth. Oxalic acid, a pathogenicity factor of the pathogen, did not affect production of toxic volatiles and inhibition occurred only at very acidic pH levels, regardless of the presence of oxalic acid. This indicated that oxalic acid at physiological concentrations and pH did not affect the GSL-M defense system.

Exposure of *S. sclerotiorum* colonies to inoculated leaves or leaf discs of host species or cultivars revealed that volatiles derived from infected leaf tissues have a toxic effect. This suggested that the GSL-M system is activated during infection of leaves and disease development. Freeze-dried powders of shoot parts of brassica species and cultivars including leaf, petiole, and stem demonstrated significant differences in producing toxic volatiles through their inhibitory effects on *S. sclerotiorum* mycelial growth in vitro, indicating that GSL contents in brassica species and even cultivars have different potentials for toxic products.

Tolerance of *S. sclerotiorum* to toxic volatiles derived from mustard powder and also synthetic isothiocyanates developed during repeated exposure of mycelium to these biocidal chemicals. Applying sublethal concentrations of mustard powder toxic volatiles and allyl isothiocyanate upregulated three putative glutathione-S-transferase enzyme encoding genes after 1h exposure. This is the first report of *S. sclerotiorum* response to isothiocyanates. There was significantly higher GST enzyme activity in treated mycelium compared with the control. GSTs may be involved in detoxification of isothiocyanates.

The resistance of oilseed rape varieties and brassica species was evaluated with leaf disc and intact plant inoculations under controlled conditions. No significant differences between varieties were observed in the leaf disc evaluations. In contrast, intact plant inoculations resulted in significant differences between genotypes and an oxalic acid assay followed the same pattern as for fungal inoculations. AV-Sapphire and AG-Castle among the oilseed rape cultivars were the most resistant and susceptible genotypes, respectively. The evaluated brassicas responded to the disease independently of their potential to produce toxic
volatile derived from GSL hydrolysis. Moreover, there was no correlation between quantity of epicuticular wax and resistance.

Histopathological studies also revealed that the main sites of the GSL-M system in leaves of brassica plants were invaded without any appreciable signs of toxicity to the fungus of ITCs, which are produced upon cellular damage.

The study established that toxic volatiles, particularly isothiocyanates, released during hydrolysis of glucosinolates in brassica plants and oilseed rape, despite their toxic effect on *Sclerotinia sclerotiorum* in vitro, are detoxified by the pathogen. Therefore, these preformed antimicrobial products do not prevent the disease during host-pathogen interactions.
Declaration

This thesis contains the results of studies conducted at the Plant Pathology Research Laboratories in the discipline of Botany, School of Environmental and Rural Science at the University of New England.

I certify that all the work reported in this thesis is original and my own research work. The text of this thesis does not contain material which has been accepted as part of the requirements for any other degree in any university or any material previously published unless due reference is made.

Signed

Siamak Rahmanpour Ozan
Dedication

Dedicated to

my wife, Sarira, spring of kindness

and

my son, Radin, my shining little star
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Acknowledgements</th>
<th>ii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summery</td>
<td>iii</td>
</tr>
<tr>
<td>Declaration</td>
<td>v</td>
</tr>
<tr>
<td>Table of contents</td>
<td>vii</td>
</tr>
</tbody>
</table>

### Chapter 1 General introduction

1

### Chapter 2 Literature review

5

### Chapter 3 Bioassay system, and effect of oxalic acid on the glucosinolate-myrosinase system

| Introduction | 21 |
| Materials and Methods | 22 |
| Results | 24 |
| Discussion | 30 |

### Chapter 4 Effect of volatiles derived from brassica shoot tissues on *Sclerotinia sclerotiorum*

| Introduction | 33 |
| Materials and Methods | 34 |
| Results | 35 |
| Discussion | 39 |

### Chapter 5 Adaptation of *S. sclerotiorum* to volatiles from mustard powder and synthetic isothiocyanates

| Introduction | 42 |
| Materials and Methods | 42 |
| Results | 43 |
| Discussion | 50 |
Chapter 6 Induction of GSTs in *Sclerotinia sclerotiorum* in response to exposure to mustard powder volatiles and allyl-isothiocyanate

Introduction 53
Materials and Methods 54
Results 58
Discussion 62

Chapter 7 Responses of brassica species and cultivars to *Sclerotinia sclerotiorum* in controlled environment studies

Introduction 66
Materials and Methods 67
Results 70
Discussion 84

Chapter 8 Histopathological studies on infection of brassica species and varieties by *Sclerotinia sclerotiorum*

Introduction 87
Materials and Methods 88
Results 89
Discussion 93

Chapter 9 General Discussion 96

References 100