

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.

*Graphania mutans* (Walker)  
and  
*Acremonium lolii* (Latch)

The Relationship Between an Insect  
Herbivore and a Fungal Endophyte  
of Perennial Ryegrass.

A thesis presented in partial fulfilment  
of the requirements for the degree of  
Master of Science in Zoology  
at Massey University

IAN ROBERT MCGEE

1987

## ABSTRACT

In examining the relationship between *Graphania mutans* (Walker) (Lepidoptera: Noctuidae) and perennial ryegrass infected with the fungal endophyte *Acremonium lolii* (Latch in press), the biology of *G. mutans* was investigated. Two types of larval development are identified: 'fast-track' larvae develop more rapidly through fewer instars and grow much larger than 'slow-track' larvae when reared on both artificial diet and perennial ryegrass. The complexity of *Graphania* speciation is discussed.

The presence of endophyte is shown to confer on perennial ryegrass resistance to *G. mutans* larvae in the laboratory, with strong antixenosis and possible antibiosis effects exhibited. Feeding preference tests show that neonate and sixth instar fast-track larvae significantly prefer excised endophyte-free ryegrass to endophyte-infected leaves. The effects of endophyte on the development of fast-track and slow-track larvae are to decrease larval weight, head capsule width, and the number of successful pupations.

The same methods were used to determine the effects of peramine (an antifeedant compound for Argentine stem weevil extracted from endophyte-infected perennial ryegrass) on fast-track *G. mutans* larvae. Incorporated into artificial diet at 10ppm, peramine has no effect on neonate and sixth instar larval feeding preference. Peramine does affect larval development, causing reduced larval weight, delayed pupation, and increased mortality.

The role of peramine in endophyte-induced resistance, and the possible adaptive significance for perennial ryegrass of endophyte infection is considered. The interactions between *G. mutans*, endophyte and perennial ryegrass within the pasture ecosystem are discussed, and suggestions and hypotheses presented for future investigation.

## ACKNOWLEDGEMENTS

I am indebted to many people for the assistance and advice which helped make this thesis possible. Many thanks to my supervisor Professor Brian Springett, who provided constructive criticism and advice throughout the study, and to Dr. Peter Fenemore who critically appraised the draft of the thesis.

I am particularly grateful for the help, encouragement and facilities provided by the staff of the Entomology Division substation of the DSIR at Palmerston North. Peter McGregor was instrumental in guiding me into research on *Graphania mutans*, and his assistance in matters scientific, statistical, and photographic is greatly appreciated. I am also obliged to Dr. Jenny Dymock, Dr. Darryl Rowan and Steve Pilkington for much useful discussion.

John Dugdale confirmed the identity of my moths, and supplied helpful information about *Graphania* taxonomy. Advice on statistical analyses was provided by Dr. Ian Henderson, and Mike Moffat helped in the production of the figures. I am also most grateful for the interest shown by the staff and post-graduate students of the Botany and Zoology Department, Massey University.

Finally, I thank my family for their invaluable support and encouragement, and in particular I thank Keitha Eichstaedt for her assistance, understanding, and above all, tolerance.

TABLE OF CONTENTS

		PAGE
Abstract.....		ii
Acknowledgements .....		iii
Table of Contents .....		iv
List of Tables .....		vii
List of Figures .....		viii
List of Plates .....		ix
Chapter I	Introduction.....	1
Chapter II	Biology of <i>Graphania mutans</i> .....	5
	<i>Introduction</i> .....	5
	<i>Section 1</i> <i>G. mutans</i> Life history .....	7
	Introduction.....	7
	(1) Eggs.....	8
	(2) Larvae.....	9
	(3) Pupae.....	11
	(4) Adults.....	13
	<i>Section 2</i> Fast-track and slow-track <i>G. mutans</i> .....	16
	Introduction.....	16
	Methods .....	16
	Results.....	17
	Discussion.....	22

	PAGE
<b>Chapter III</b> <b>Endophyte and <i>Graphania mutans</i></b> .....	25
<i>Introduction</i> .....	25
<i>Section 1</i> Endophyte choice tests .....	26
Introduction.....	26
Methods .....	27
Results.....	29
Discussion.....	32
<i>Section 2</i> Endophyte and fast-track larvae .....	33
Introduction.....	33
Methods .....	34
Results.....	35
Discussion.....	42
<i>Section 3</i> Endophyte and slow-track larvae .....	43
Introduction.....	43
Methods .....	43
Results.....	44
Discussion.....	50
<i>Section 4</i> Conclusion .....	53
 <b>Chapter IV</b> <b>Peramine and <i>Graphania mutans</i></b> .....	 55
<i>Introduction</i> .....	55
<i>Section 1</i> Peramine choice tests.....	56
Introduction.....	56
Methods .....	56
Results.....	57
Discussion.....	57

	PAGE
<i>Section 2</i> Peramine and fast-track larvae.....	59
Introduction.....	59
Methods .....	59
Results.....	59
Discussion.....	64
 <i>Section 3</i> Conclusion .....	 66
 Chapter V   General discussion and conclusions .....	 68
 Appendix I.....	 73
Appendix II.....	74
Appendix III.....	75
Appendix IV .....	76
Appendix V.....	77
Appendix VI.....	78
 References.....	 79

LIST OF TABLES

	PAGE
<i>Table 1:</i> Comparison of fast-track and slow-track <i>G. mutans</i> reared on artificial diet.....	23
<i>Table 2:</i> Results of fast-track larval feeding preference tests with endophyte-free and endophyte-infected perennial ryegrass leaves (a) Neonate larvae (b) Sixth instar larvae.....	31
<i>Table 3:</i> Comparison of the development of fast-track larvae reared on endophyte-free and endophyte-infected perennial ryegrass leaves .....	39
<i>Table 4:</i> Comparison of the mortality of fast-track <i>G. mutans</i> reared on endophyte-free and endophyte-infected perennial ryegrass leaves .....	40
<i>Table 5:</i> Comparison of the development of slow-track larvae reared on endophyte-free and endophyte-infected perennial ryegrass leaves .....	48
<i>Table 6:</i> Comparison of the pupae of slow-track <i>G. mutans</i> reared on endophyte-free and endophyte-infected perennial ryegrass leaves .....	49
<i>Table 7:</i> Comparison of the mortality of slow-track <i>G. mutans</i> reared on endophyte-free and endophyte-infected perennial ryegrass leaves .....	49

	PAGE
<i>Table 8:</i>	Results of fast-track larval feeding preference tests with peramine-free and peramine-treated artificial diet
	(a) Neonate larvae
	(b) Sixth instar larvae..... 58
<i>Table 9:</i>	Comparison of the development of fast-track larvae reared on peramine-free and peramine-treated artificial diet ..... 61
<i>Table 10:</i>	Comparison of the pupae of fast-track <i>G. mutans</i> reared on peramine-free and peramine-treated artificial diet ..... 65
<i>Table 11:</i>	Comparison of the mortality of fast-track <i>G. mutans</i> reared on peramine-free and peramine-treated artificial diet ..... 65

LIST OF FIGURES

		PAGE
<i>Figure 1:</i>	Age at pupation of fast-track and slow-track <i>G. mutans</i> reared on artificial diet or endophyte-free perennial ryegrass leaves (a) Artificial diet (b) Endophyte-free ryegrass .....	6
<i>Figure 2:</i>	Mean larval weight $\pm$ SEM of fast-track and slow-track <i>G. mutans</i> reared on artificial diet.....	19
<i>Figure 3:</i>	HCW regression of fast-track and slow-track <i>G. mutans</i> reared on artificial diet.....	20
<i>Figure 4:</i>	Mean HCW for each instar of fast-track and slow-track <i>G. mutans</i> reared on artificial diet.....	21
<i>Figure 5:</i>	Mean larval weight $\pm$ SEM of fast-track larvae reared on endophyte-free and endophyte-infected perennial ryegrass leaves .....	37
<i>Figure 6:</i>	HCW regressions of fast-track larvae reared on endophyte-free and endophyte-infected perennial ryegrass leaves .....	41
<i>Figure 7:</i>	Mean larval weights $\pm$ SEM of slow-track larvae reared on endophyte-free and endophyte-infected perennial ryegrass leaves (a) Eight instar larvae (b) Nine instar larvae (c) Ten and eleven instar larvae.....	46

## PAGE

<i>Figure 8:</i>	Total amount of leaf material consumed daily by slow-track larvae reared on endophyte-free and endophyte-infected perennial ryegrass leaves .....	51
<i>Figure 9:</i>	Mean larval weight $\pm$ SEM of fast-track and slow-track <i>G. mutans</i> larvae reared on endophyte-free and endophyte-infected perennial ryegrass leaves .....	54
<i>Figure 10:</i>	Mean larval weights $\pm$ SEM of fast-track larvae reared on peramine-free and peramine-treated artificial diet (a) Six instar larvae (b) P+6NOP and seven instar larvae.....	62
<i>Figure 11:</i>	HCW regressions of fast-track larvae reared on peramine-free and peramine-treated artificial diet.....	63

List of Plates

		PAGE
<i>Plate 1:</i>	<i>G. mutans</i> egg batch laid on filter paper. The pale green eggs are infertile .....	10
<i>Plate 2:</i>	First instar <i>G. mutans</i> larvae surrounding the remains of their eggshells .....	10
<i>Plate 3:</i>	Fourth instar fast-track <i>G. mutans</i> larva reared on perennial ryegrass leaves. Feeding damage is also shown .....	12
<i>Plate 4:</i>	Seventh instar fast-track <i>G. mutans</i> larva reared on perennial ryegrass leaves .....	12
<i>Plate 5:</i>	Recently moulted sixth instar fast-track <i>G. mutans</i> larva reared on artificial diet.....	14
<i>Plate 6:</i>	<i>G. mutans</i> pupa. The gap between abdomen and thorax on the ventral surface eventually closed over.....	14
<i>Plate 7:</i>	Male <i>G. mutans</i> adult.....	15
<i>Plate 8:</i>	Female <i>G. mutans</i> adult .....	15
<i>Plate 9:</i>	Endophyte feeding preference test with a sixth instar fast-track <i>G. mutans</i> larva. Feeding damage is also shown .....	30