

Plant-herbivore interactions in natural *Brassica oleracea*
communities

Submitted by:

Erika Lucie Newton

To the University of Exeter as a thesis for the degree of
Doctor of Philosophy

April 2009

This thesis is available for library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

.....

Erika L. Newton

Abstract

Co-evolutionary interactions between plants and herbivores are suggested to be the driving force behind the high diversity observed in plant secondary metabolites. These compounds play an important role in herbivore resistance mechanisms in many plant species. An individual plant can produce and store a number of structurally different secondary compounds. Variation in plant chemical profiles is commonly observed within and between natural populations across a wide range of taxa, yet the ecological importance of this variation is still a major question in the area of plant-herbivore interactions.

In this thesis I use wild cabbage (*Brassica oleracea* var. *oleracea*) plants in twelve naturally established populations to investigate plant-herbivore interactions mediated by structural variation in aliphatic glucosinolates, a class of secondary metabolites produced by the *Brassicaceae*.

Overall, the results showed that several herbivore species respond to the genetically determined variation in glucosinolate profile, indicating that the structure of the local herbivore community can be influenced by variation in plant defence chemistry. In addition, the direction of herbivore responses to different plant chemical phenotypes differed between species. A finer scale study which focused on the interactions between an herbivore and aliphatic glucosinolate variation supported the general trend observed in the large scale study. Glucosinolate profile was also found to have an impact on plant seed set.

The findings show that glucosinolate profiles may be under selection in these natural plant populations and provide some support for the role of herbivores in the maintenance of secondary metabolite diversity.

Acknowledgements

Firstly, I'd like to thank my supervisors Dave Hodgson and James Bullock for giving me the opportunity to work on a PhD project which I have thoroughly enjoyed. Dave's incredibly supportive, approachable attitude and unfailing enthusiasm for the project have made the past few years a steep but very rewarding learning curve for me. James I thank for always making the time to respond quickly with helpful comments whenever I sent him thesis chapters or paper manuscripts, and for introducing me to Matthias Wichmann, who was a great help in selecting field sites in Dorset, and Nicole van Dam, who provided email support when I started carrying out the HPLC analysis. Jon Blount was also very helpful whenever technical problems with the HPLC equipment arose. Thanks also to two undergraduate project students, Martin Durman and Rosie Daines for fieldwork assistance.

Many thanks to the Hodgson research group for all their feedback and support, in particular ex-EXPECT team members Mairead Maclean and David Carslake. Many other friends at Tremough have been instrumental in making the last three years a really enjoyable experience and I would particularly like to thank Michelle Taylor, Sahran Higgins, Wiebke Schuett, Ruth Leeney, Lorna Shaw, Mel Smee, Lucy Wright, Stine Turner and James Grecian.

Isobel and the rest of the Giblin family I cannot thank enough for not only keeping an eye out for cabbages on the Devon coast around their house and reconnoitring a field site for me, but also for their amazing hospitality while I carried out fieldwork in Devon. I'm also very thankful for Isobel's company and help (and of course Poppy the dog's!) on some of my first field visits.

Finally, I owe so many thanks to my mum, Michéle, for all her encouragement and support.

Contents

Abstract	2
Acknowledgements	3
List of figures	9
List of tables	10
Author declaration	11
Chapter 1: Ecological mechanisms maintaining natural variation in secondary metabolites	12
Mechanisms for the maintenance of secondary metabolite diversity	13
Evidence for the role of herbivores	14
Study species	16
Glucosinolate structure and the genetic control of biosynthesis	22
Importance of field studies	24
Thesis aims	26
References	27
Chapter 2: Glucosinolate polymorphism in wild cabbage (<i>Brassica oleracea</i>) influences the structure of herbivore communities	35
Erika Newton, James Bullock, Dave Hodgson.	
Published in <i>Oecologia</i>	
Abstract	36
Introduction	37
Materials and methods	41
Field sites	41
Surveying plants and herbivores	41
Genetics of glucosinolates in <i>Brassicaceae</i>	42
Glucosinolate extraction and preparation for HPLC	45

Statistical analysis	46
Spatial structuring of glucosinolate profiles and herbivory	46
Intra-population responses of herbivores to glucosinolates	47
Inter-populations responses of herbivores to glucosinolate frequencies	47
Modelling procedure and multiple testing	48
Results	48
Geographic variation in glucosinolates	48
Geographic variation in herbivore distribution	53
Glucosinolates and herbivory: within populations	55
Glucosinolates and herbivory: between populations	57
Discussion	60
Seasonal variation in herbivore responses	62
Importance of ecological scale	62
Implications for the maintenance of secondary metabolite diversity	64
Generalist vs. specialist herbivore species	65
Acknowledgements	67
References	67
Chapter 3: Inter-annual responses of herbivores to glucosinolate polymorphisms in wild cabbage (<i>Brassica oleracea</i>).....	75
Erika Newton, James Bullock, Dave Hodgson	
Abstract	75
Introduction	76
Materials and methods	78
Statistical analysis	78
Intra-population responses of herbivores to glucosinolates	78
Inter-populations responses of herbivores to glucosinolate frequencies	79

Modelling procedure and multiple testing	79
Results	80
Within populations	80
Between populations	84
Discussion	89
Inter-annual variation in the responses of herbivores	89
Implications for the maintenance of secondary metabolite diversity	94
Acknowledgements	95
References	95
Chapter 4: Bottom-up vs. top-down effects of glucosinolate variation on aphid colony dynamics in wild cabbage populations.....	99
Erika Newton, James Bullock, Dave Hodgson	
Submitted to Ecological Entomology	
Abstract	99
Introduction	99
Study system	102
Materials and methods	104
Field sites	104
Glucosinolate extraction and analysis	104
Experimental design	105
Statistical analysis	107
Results	109
Effect of plant phenotype on aphids	109
Responses of natural enemies	113
Discussion	116
Differential effects of glucosinolates on aphid colony dynamics	117

No evidence for differential top-down regulation	119
Why no evidence for top-down regulation?	121
The importance of winged aphids	122
Conclusions	123
Acknowledgements	124
References	124
Chapter 5: The impact of glucosinolate profile and aphid attack on wild cabbage seed set.....	134
Erika Newton, James Bullock, Dave Hodgson	
Abstract	134
Introduction	135
Materials and methods	139
Field sites	139
Glucosinolate extraction and analysis	140
Experimental design	141
Statistical analysis	142
Results	143
Effect of aphid and natural enemy presence on seed set	143
Effect of aphid colony size on seed set	146
Discussion	149
Effect of environment and phenotype but no evidence for G-by-E	149
Effect of aphid attack on seed set	149
Effect of natural enemies on seed set	151
Conclusions	152
Acknowledgements	153
References	153

Chapter 6: Does herbivore attack have a differential effect on the fitness of glucosinolate phenotypes?	161
Erika Newton, James Bullock, Dave Hodgson	
Abstract	161
Introduction	162
Materials and methods	163
Effects of herbivores and glucosinolates on seed set	164
Statistical analysis	164
Results	165
Effects of herbivores and glucosinolates on seed set	165
Discussion	173
Glucosinolates and plant fitness	173
Effects of herbivores on the fitness of different phenotypes	173
Effect of environment and phenotype but no evidence for G-by-E	174
Detecting costs in natural populations	175
Conclusions	176
Acknowledgements	177
References	177
Chapter 7: Discussion.....	180
Wider implications of results and future work	185
References	187

List of Figures

Chapter 1:

Figure 1.1. General locations of field sites in south west England	18
Figure 1.2. Locations of Dorset field sites	19
Figure 1.3. Locations of Devon field sites	20
Figure 1.4. Locations of Cornwall field sites	21
Figure 1.5. Diagram of aliphatic glucosinolate loci	23

Chapter 2:

Figure 2.1. Diagram of aliphatic genetics	44
Figure 2.2. Frequencies of aliphatic glucosinolate phenotypes	50
Figure 2.3. Intra-population responses of herbivore species to sinigrin	56
Figure 2.4. Herbivore responses to glucosinolates at the inter-population scale.	59

Chapter 3:

Figure 3.1 Responses of herbivore species to glucosinolates	82
Figure 3.2. Mean probability of snail infestation	83
Figure 3.3. Herbivore responses to sinigrin at the inter-population scale	86

Chapter 4:

Figure 4.1. Mean counts of unwinged aphids	111
Figure 4.2 .Mean proportions of winged aphid	112
Figure 4.3. Mean proportions of <i>Diaeretiella rapae</i> -parasitised aphids	114
Figure 4.4. Mean counts of unwinged aphids on plants in each treatment	115

Chapter 5:

Figure 5.1. The effect of genotype and environment on plant fitness	137
Figure 5.2. Mean seed set produced by plants in each treatment	145
Figure 5.3. The effect of the cumulative number of aphids on plant seed set	147
Figure 5.4. The relationship between basal stem diameter and seed set	148

Chapter 6:

Figure 6.1. Mean seed set of plants differing in aliphatic glucosinolate profile	166
Figure 6.2. The correlation between plant basal stem diameter and seed set	167
Figure 6.3. Effect of <i>B. brassicae</i> , phenotype and population on seed set	169
Figure 6.4. Effect of progoitrin on seed production, results from <i>B. brassicae</i>	170
Figure 6.5. Effect of snails, phenotype and population on seed set in 2008	171
Figure 6.6. Effect of progoitrin on seed production, results from snail analysis	172

List of Tables

Chapter 2:

Table 2.1. Significance of all fixed factors	51
Table 2.2. Variance components for each ecological scale	54

Chapter 3:

Table 3.1. Significance of all fixed factors	87
--	----

Author's declaration

Statement of contribution to co-authored papers:

I carried out all data collection for each of the chapters, including herbivore surveys, seed collection and HPLC analysis of glucosinolates. I carried out all statistical analysis under the guidance of Dave Hodgson and James Bullock and I followed a methodology developed by Dave Hodgson for the spatial inter-population statistical analysis (Chapters 2 and 3). I initially drafted all sections of the chapters and amended later drafts following the advice of D. J. Hodgson, J. M. Bullock and, for Chapters 2 and 4, several anonymous journal reviewers.