

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.

# **Population studies of ultraviolet-B radiation responses in white clover (*Trifolium repens* L.)**

---

A thesis presented in partial fulfilment  
of the requirements for the degree of

Doctor of Philosophy  
in  
Plant Biology

at Massey University, Palmerston North,  
New Zealand.

by  
Rainer W. Hofmann  
2000

---

To Angelika and Stephan

## Abstract

White clover growing in New Zealand is experiencing increasing levels of ultraviolet-B (UV-B) radiation as a result of ozone depletion. This thesis reports a series of investigations on morphological and physiological responses to UV-B in various white clover populations. In addition, these studies examined UV-B responsiveness in combination with drought and consequences for insect herbivores. Plants were grown in controlled environment rooms with and without supplemental UV-B radiation at a dose of  $13.3 \text{ kJ m}^{-2} \text{ d}^{-1}$ , corresponding to a 25% mid-summer ozone depletion above Palmerston North, New Zealand. Morphological measurements included numerous attributes of plant growth and morphogenesis as well as several aspects of leaf structure. Physiological studies investigated both primary and secondary metabolic functions. In general, UV-B reduced components of white clover growth. The white clover populations showed a number of constitutive and UV-B-induced differences in many morphological and physiological attributes. UV-B sensitivity was mitigated by drought and was less pronounced with increasing duration of UV-B exposure. Bioassays revealed differential dietary effects of UV-B-treated foliage on the performance of lepidopteran insects. UV-B effects were also apparent under frequent defoliation. Comparisons of morphological and physiological features showed that white clover UV-B responsiveness was mainly linked to inherent differences in morphology and growth among the populations, while on a physiological level it was more the UV-B-induced differences that conferred UV-B tolerance. In particular, UV-B tolerance of the white clover populations was related to lower constitutive productivity. This was further linked to several constitutive leaf attributes, including small leaf size, small and lens-shaped epidermal cells as well as low leaf water potential. UV-B-induced features linked to UV-B tolerance included high percentage of leaf dry mass, accumulation of UV-absorbing compounds and of total flavonols (particularly quercetin glycosides) as well as increases in leaf water potential. UV-B tolerance was greatest in white clover populations adapted to multiple and severe stresses in the habitat of origin. This series of investigations was used to propose a functional framework, linking UV-B responsiveness to underlying specialisation of the white clover populations.

This thesis has been written with a view to publish much of the presented material. Accordingly, each Chapter represents a self-contained unit, forming the basis of papers published, submitted or prepared for submission.

## Acknowledgements

Profound gratitude goes to my wife Angelika and my son Stephan. Your love and patience was vital in supporting me throughout this project. Thanks are also due to my family overseas, especially my mother who patiently accepted the fact that happiness and scientific enjoyment for one of her children happened to coincide with a study career on the other side of the world. And thanks to Keith Cederman and his family it was possible to call New Zealand our new home, resulting in the commencement of my studies and of this PhD project.

Greatest thankfulness is due to my supervisors. Associate Professor David Fountain was instrumental in the initiation of this project and a constant source of encouragement and advice throughout. From the outset of the work Dr Bruce Campbell provided invaluable intellectual, material and personal support in all aspects of the work, making the project in its entirety possible. Furthermore, thanks for helpful advice to Associate Professor Brian Jordan and to Dr Dennis Greer who accompanied the fluorescence studies.

Special acknowledgements go to Chris Hunt for design and operation of the UV-B enrichment facilities and to Derryn Hunt and *The 3 Herbageladies* Yvonne Gray, Christine van Meer and Margaret Greig for technical support. Thanks also to Greig Cousins, Sheryl and Paul Doyle, Laurie Kennedy, Joce Tilbrook, Dave Scammel, Helen Little, Joanne Morris, Bruce Jackson, Linda Robinson, Charlotte Madie, Lyn Watson and Mush Williamson for technical advice and help. Greatly appreciated is helpful advice and discussion provided by Drs Grant Abernethy, Dave Barker, Richard Biggs, Janet Bornman, John Brock, John Caradus, Harry Clark, Sandra Diaz, John Hunt, Geoff Lane, William Laing, Soheila Mackerness, Cory Matthew, Chris Mercer, Tessa Mills, Paul Newton, Tony Parsons, Jelte Rozema, Derek Woodfield, Warren Williams and Sang Dong Yoo. Inspiring debate was provided by Professor Rod Thomas who taught me and participated in the dissection of white clover stolon tips. Thanks also to all staff at AgResearch Grasslands, who were extremely friendly and accommodating to their students, treating Todd White and me completely as their own. And Todd, it was a pleasure to share this intense time of learning with you.

The PhD work also allowed me to experience a number of fruitful, productive collaborations. This included extensive work together with Professor Richard Lindroth (University of Wisconsin) during the insect bioassay studies, resulting in a published paper written jointly with Rick (Chapter 7). Great appreciation also goes towards the plant chemistry group at IRL in Lower Hutt, including Drs Ken Markham, Stephen Bloor, Ewald Swinny and Ken Ryan with whom it was not only a pleasure and privilege to extract flavonoids and conduct HPLC analyses but who also provided many inspirational discussions. Further acknowledgement is due to Ewald for NMR studies identifying the structure of the flavonoid compounds detected in HPLC. I am also particularly grateful to Wilhelmina Martin of AgResearch and Doug Hopcroft and 'Crunch' of HortResearch for showing me the techniques involved in the preparation of samples and the conducting of analyses for plant nutrient levels and electron microscopy. Valuable statistical advice is acknowledged from Dr Sam Beckett and Duncan Hedderley at Massey University and from the site statisticians at AgResearch Grasslands, Dr David Baird, Robert Fletcher and Fred Potter.

The following institutions are gratefully acknowledged for financial support:

PhD Fellowship:

- The Agricultural and Marketing Research and Development Trust (AGMARDT).

Operational funding:

- AgResearch Grasslands.
- Massey University.
- New Zealand Foundation for Research, Science and Technology (contract number C10632).

# Table of Contents

<b>Chapter 1. Introduction .....</b>	<b>1</b>
The solar UV environment of New Zealand .....	1
<i>Solar radiation and ozone</i> .....	1
<i>Hemisphere comparisons and New Zealand-related factors</i> .....	2
<i>Past and future trends</i> .....	4
The New Zealand pasture ecosystem .....	5
UV-B radiation and plants.....	6
<i>History</i> .....	6
<i>Legumes and white clover</i> .....	7
<i>Population studies</i> .....	9
<i>Underlying mechanisms</i> .....	10
<i>Interaction with other factors</i> .....	12
General description of the experimental approaches.....	17
Aims .....	18
<b>Chapter 2. <i>Trifolium repens</i> responses to ultraviolet-B radiation: integrated analysis of 17 traits and 26 populations.....</b>	<b>20</b>
Abstract.....	21
Introduction .....	22
Materials and Methods .....	24
<i>Experimental design</i> .....	24
<i>Plant cultivation and UV-B irradiation</i> .....	24
<i>Plant material</i> .....	25
<i>Whole plant growth measurements</i> .....	28
<i>Leaf measurements</i> .....	28
<i>Epidermal studies</i> .....	29
<i>Statistical analysis</i> .....	29
Results .....	30
<i>Univariate UV-B responses</i> .....	30
<i>Plant trait responses in the first principal component</i> .....	31
<i>Population responses in the first principal component</i> .....	33
<i>Plant trait responses in the second principal component</i> .....	34
<i>Population responses in the second principal component</i> .....	34
<i>Correlation analyses</i> .....	35



Discussion .....	37
<i>Plant trait responses in the first principal component</i> .....	37
<i>Population responses in the first principal component</i> .....	38
<i>Plant trait responses in the second principal component</i> .....	40
<i>Population responses in the second principal component</i> .....	41
<i>Relationship between PC1 and plant features</i> .....	42
Conclusions .....	44
<b>Chapter 3. Growth responses to ultraviolet-B radiation in <i>Trifolium repens</i> populations depend on water availability, productivity, and duration of stress .....</b>	<b>45</b>
Abstract.....	46
Introduction .....	46
Materials and Methods .....	49
<i>Experimental design</i> .....	49
<i>Plant cultivation and UV-B irradiation</i> .....	49
<i>Harvesting of plant material and drought application</i> .....	52
<i>Statistical analysis</i> .....	53
Results .....	53
<i>Influence of drought on UV-B sensitivity</i> .....	53
<i>Influence of population differences and time on UV-B sensitivity</i> .....	57
Discussion .....	60
<i>Influence of drought on UV-B sensitivity</i> .....	60
<i>Influence of population differences on UV-B sensitivity</i> .....	61
<i>Influence of time on UV-B sensitivity</i> .....	63
<b>Chapter 4. Effects of ultraviolet-B radiation on <i>Trifolium repens</i> morphology: mediation by drought and relationships to UV-B sensitivity .....</b>	<b>65</b>
Abstract.....	66
Introduction .....	66
Materials and Methods .....	69
<i>Biomass production and leaf attributes</i> .....	69
<i>Stolon growth and apical bud dissection</i> .....	70
<i>Statistical analysis</i> .....	70
Results .....	71
<i>Between-population comparisons</i> .....	73

Discussion .....	77
<i>Leaf and stolon morphology</i> .....	77
<i>Relative leaf dry mass allocation</i> .....	79
<i>Between-population comparisons</i> .....	79
Conclusions .....	81
<b>Chapter 5. Effects of ultraviolet-B radiation on <i>Trifolium repens</i> physiology: mediation by drought and relationships to UV-B sensitivity .....</b>	<b>82</b>
Abstract.....	83
Introduction .....	83
Materials and Methods .....	86
<i>UV-absorbing compounds</i> .....	87
<i>Flavonoid analysis</i> .....	87
<i>Chlorophyll concentration</i> .....	88
<i>Chlorophyll fluorescence</i> .....	88
<i>Leaf water potential and proline</i> .....	89
<i>Statistical analysis</i> .....	90
Results .....	90
<i>UV-absorbing compounds and flavonoids</i> .....	90
<i>Photosynthetic pigmentation and photochemistry</i> .....	92
<i>Leaf water potential and proline</i> .....	93
<i>Intraspecific relationships to biomass production and UV-B sensitivity</i> .....	96
Discussion .....	99
<i>UV-absorbing compounds and flavonoids</i> .....	99
<i>Photosynthetic pigmentation and photochemistry</i> .....	101
<i>Leaf water potential and proline</i> .....	102
<i>Intraspecific relationships to biomass production and UV-B sensitivity</i> .....	104
Conclusions .....	106
<b>Chapter 6. Responses of nine <i>Trifolium repens</i> L. populations to ultraviolet-B radiation: differential flavonol glycoside accumulation and biomass production .....</b>	<b>108</b>
Abstract.....	109
Introduction .....	109
Materials and methods.....	112
<i>Flavonoid isolation and identification</i> .....	112

Results .....	112
<i>Flavonoid identification</i> .....	112
<i>Total flavonols</i> .....	115
<i>Specific flavonols</i> .....	115
<i>Q:K ratio</i> .....	117
<i>Flavonol relationships to biomass production</i> .....	118
Discussion .....	121
<i>Total flavonols</i> .....	121
<i>Differential UV-B effects on quercetin and kaempferol glycosides</i> .....	122
<i>Population differences in the accumulation of total flavonols</i> .....	123
<i>Population differences in the accumulation of quercetin and kaempferol</i> .....	125
<i>Relationship of flavonol levels to UV-B tolerance</i> .....	126
Conclusions .....	127
<b>Chapter 7. Differences in <i>Trifolium repens</i> L. responses to ultraviolet-B radiation: foliar chemistry and consequences for two lepidopteran herbivores .....</b>	<b>128</b>
Abstract.....	129
Introduction .....	129
Materials and methods.....	131
<i>Experimental design</i> .....	131
<i>Plant cultivation and UV irradiation</i> .....	131
<i>Sampling procedure for plant nutrient analyses</i> .....	132
<i>Nitrogen</i> .....	132
<i>Available carbohydrates</i> .....	133
<i>Neutral detergent fibre</i> .....	133
<i>Cyanogenesis</i> .....	134
<i>UV-absorbing compounds</i> .....	134
<i>Spodoptera litura bioassays</i> .....	135
<i>Graphania mutans bioassays</i> .....	136
<i>Statistical analysis</i> .....	136
Results .....	137
<i>Foliar chemistry</i> .....	137
<i>Spodoptera litura performance</i> .....	139
<i>Graphania mutans performance</i> .....	145
Discussion .....	145
<i>Foliar chemistry related to primary metabolism</i> .....	145

<i>Foliar chemistry related to secondary metabolism</i> .....	146
<i>Insect Responses</i> .....	147
<b>Chapter 8. Responses to ultraviolet-B radiation in frequently defoliated <i>Trifolium repens</i> populations: differential growth, photochemistry and anatomy</b> .....	<b>150</b>
Abstract.....	151
Introduction .....	151
Materials and Methods .....	153
<i>Leaf material</i> .....	153
<i>Chlorophyll fluorescence</i> .....	153
<i>Structural studies</i> .....	154
Results .....	155
Discussion .....	160
<i>Leaf growth and photochemistry</i> .....	160
<i>Structural studies</i> .....	161
<b>Chapter 9. Discussion</b> .....	<b>164</b>
A functional framework to synthesise results .....	164
Comparisons between experiments .....	174
Context and limitations of the findings .....	178
Perspectives and future studies.....	181
<i>Organ-specific studies</i> .....	184
<i>Population-specific studies</i> .....	186
<i>Interaction of UV-B with additional stress, disturbance and competition</i> .....	187
<b>Chapter 10. References</b> .....	<b>189</b>

## List of Figures

Fig. 1-1. Cyanide production in the white clover cultivar 'Huia' .....	17
Fig. 1-2. Scheme of the research project based on plant attributes .....	19
Fig. 2-1. Principal components analysis.....	32
Fig. 2-2. Relationships between UV-B sensitivity and plant attributes .....	36
Fig. 3-1. Biomass production under UV-B and drought .....	55
Fig. 3-2. Relative changes in the rate of biomass production in response to UV-B. ....	56
Fig. 3-3. Relationship between constitutive productivity and relative growth.....	58
Fig. 3-4. Time-course of the UV-B response .....	59
Fig. 4-1. Leaf initiation in apical buds under UV-B and drought .....	72
Fig. 4-2. Leaf size under UV-B and drought.....	74
Fig. 4-3. Percentage leaf dry mass under UV-B and drought. ....	74
Fig. 4-4. Stolon elongation rate under UV-B and drought.....	75
Fig. 4-5. Relationships of constitutive productivity to UV-B sensitivity and leaf attributes .....	76
Fig. 5-1. UV-absorbing compound levels under UV-B and drought .....	91
Fig. 5-2. Flavonol glycoside accumulation under UV-B and drought .....	91
Fig. 5-3. Leaf water potential under UV-B and drought .....	94
Fig. 5-4. Proline concentration under UV-B and drought.....	95
Fig. 5-5. Relationships of constitutive productivity to quercetin accumulation and leaf water potential .....	97
Fig. 5-6. Relationship between UV-B-induced relative changes in growth and in UV- absorbing compounds.....	98

Fig. 6-1. HPLC profiles of 'Tienshan' grown with and without UV-B .....	114
Fig. 6-2. Total flavonol concentrations .....	116
Fig. 6-3. Quercetin and kaempferol concentrations .....	116
Fig. 6-4. Ratio of quercetin to kaempferol (Q:K).....	118
Fig. 6-5. Relationship between quercetin accumulation and productivity .....	119
Fig. 6-6. Relationship between quercetin accumulation and relative growth .....	120
Fig. 7-1. Foliar chemical composition.....	138
Fig. 7-2. Growth of <i>Spodoptera litura</i> and <i>Graphania mutans</i> larvae.....	140
Fig. 7-3. Development times and pupal weights of <i>S. litura</i> .....	141
Fig. 7-4. Stadium duration and growth rates of <i>S. litura</i> and <i>G. mutans</i> .....	142
Fig. 7-5. Consumption rates and food utilisation of <i>S. litura</i> and <i>G. mutans</i> .....	143
Fig. 7-6. Development times and pupal weights of <i>G. mutans</i> .....	144
Fig. 8-1. Light micrographs of transverse white clover leaflet sections .....	158
Fig. 8-2. TEM micrographs of transverse white clover leaflet sections.....	159
Fig. 9-1. Model.....	165
Fig. 9-2. Scheme of the research project based on attributes .....	166
Fig. 9-3. Relationship of growth responses .....	176
Fig. 9-4. Relationship of constitutive growth.....	177
Fig. 9-5. Relationship of UV-absorbing compound responses.....	177
Fig. 9-6. Morpho-physiological attributes in 'Huia' and 'Red' .....	180

## List of Tables

Table 1-1. Comparison of UV-B irradiances in New Zealand and Germany .....	3
Table 2-1. Characteristics of 26 white clover populations .....	26
Table 2-2. UV-B-induced univariate percent changes .....	31
Table 2-3. Correlations of plant attributes to the principal components .....	33
Table 3-1. Description of nine white clover populations .....	50
Table 3-2. REML analysis of aerial biomass production .....	54
Table 4-1. Attributes of growth and morphology under UV-B and drought.....	72
Table 5-1. Biochemical and photochemical attributes under UV-B and drought .....	93
Table 7-1. Effects on cyanogenesis and UV-absorbing compounds.....	139
Table 8-1. Morphological attributes in frequently defoliated plants.....	156
Table 8-2. Photochemical parameters in frequently defoliated plants .....	157
Table 9-1. Overall features of the series of investigations .....	167
Table 9-2. Key aspects from large-scale screening .....	168
Table 9-3. Key aspects from examinations of UV-B susceptibility in interaction with drought and flavonoid responses.....	169
Table 9-4. Key aspects from bioassays and studies of frequently defoliated plants .....	171
Table 9-5. General comparisons between experiments.....	175

## Abbreviations

AC	Available carbohydrates
ACR	Average consumption rate
AD	Approximate digestibility
AGR	Average growth rate
$\Delta F/F_m$	Photochemical yield
DM	Dry mass, dry matter
ECI	Efficiency of conversion of ingested food
$F_v/F_m$	Intrinsic efficiency of PSII
HCN	Hydrogen cyanide
LAR	Leaf appearance rate
LM	Light microscopy
LWR	Leaf weight ratio
NDF	Neutral detergent fibre
NGP	Number of growing points
NPQ	Non-photochemical quenching
PC	Principal component
PCA	Principal components analysis
PDM	Percent leaf dry mass
PPF	Photosynthetic photon flux
PSII	Photosystem II
$q_P$	Photochemical quenching
RGR	Relative growth rate
RSR	Root:shoot ratio
SER	Stolon elongation rate
SLM	Specific leaf mass
TEM	Transmission electron microscopy
UV-A	Ultraviolet-A radiation
UV-B	Ultraviolet-B radiation
UV-C	Ultraviolet-C radiation
$\psi_L$	Leaf water potential