

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.

**CONTRACTILE GROWTH OF  
THE HYPOCOTYL IN WHITE  
CLOVER SEEDLINGS**  
*(Trifolium repens L.)*

**Carla Anne Maine**

**1998**

**A thesis presented in partial fulfilment of the  
requirements for the degree of  
Master of Science  
at  
Massey University,  
Palmerston North, New Zealand**

## ABSTRACT

White clover (*Trifolium repens* L.), like many epigeal emerging legumes, displays a phenomenon known as contractile growth. This process occurs in the weeks following seedling emergence and involves the longitudinal shortening and radial expansion of tissues in the hypocotyl and upper primary root. During routine trials at AgResearch Grasslands, Palmerston North, it was noted that some seedlings of cv. Grasslands Huia displayed an aberrant growth form characterized by a failure to display normal contractile growth. These seedlings were intermated for one to two generations to form the 'non-contractile' populations used in this thesis. The 'contractile' populations used were formed through the random selection and intermating of plants which showed normal contraction. The non-contractile phenotype was found to occur naturally in populations of white clover at a frequency of 2-22%. When compared to tissue from normal contractile seedlings, hypocotyl tissue from non-contractile seedlings showed reduced radial expansion which in turn led to decreased contraction.

Since the plant hormone ethylene has been implicated in the promotion of radial expansion in many plant tissues and systems (Abeles 1973 b), the effects of applying silver thiosulphate and the ethylene-releasing compound Ethrel, were examined in contractile and non-contractile seedlings. Endogenous ethylene production by contractile and non-contractile seedlings was also examined. Seedlings from the contractile line showed an overall higher production of ethylene during early seedling growth than did seedlings from the non-contractile line. When treated with silver thiosulphate (which blocks ethylene perception), hypocotyls from seedlings of the contractile line failed to display contractile growth and hypocotyls from seedlings of the non-contractile line displayed non-contractile growth to a greater degree. When treated with Ethrel hypocotyls from seedlings of the non-contractile line displayed contractile growth but hypocotyls from seedlings of the contractile line failed to display contractile growth. These results indicate that ethylene is required for normal contractile growth of the hypocotyl in white clover seedlings, and that non-contractile growth of the hypocotyl is caused by a decreased production of, rather than a decreased sensitivity to, the plant hormone ethylene.

## ACKNOWLEDGEMENTS

I would like to thank:

- my supervisors Paula Jameson (Massey University) and Derek Woodfield (AgResearch Grasslands) – for their help, advice, and never-ending patience.
- AgResearch Grasslands – as this research was partially supported by AgResearch through Foundation for Research Science and Technology Grant C 10 640
- Derek Woodfield - for kindly providing the plant material for this project
- John Caradus – for his advice and encouragement during the initial stages of this work
- every single person working at AgResearch Grasslands – as much for their overall friendliness as for the practical help, advice and resources I received, and
- my family, friends and flatmates – for their love and support over the past four years.

# CONTENTS

	<b>PAGE</b>
Abstract	ii
Acknowledgements	iii
List of Figures	vi
List of Tables	vii

## **CHAPTER 1 : INTRODUCTION**

1.1 White Clover	1
1.2 Contractile growth	2
1.3 Why implicate ethylene?	5
1.4 Ethylene/auxin interactions and cellular growth	8
1.5 Biosynthesis, regulation and perception of ethylene	10
1.6 The ethylene-mediated 'triple response' and ethylene mutants	15
1.7 Light and the triple response	21
1.8 Aims	23

## **CHAPTER 2 : MATERIALS AND METHODS**

2.1 Contractile growth in a range of cultivars	24
2.2 Comparison of contractile vs non-contractile growth	26
2.3 Application of Ethrel and silver thiosulphate to contractile and non-contractile seedlings :	
2.3.1 Experiment one	27
2.3.2 Experiment two	28
2.4 Ethylene production by contractile and non-contractile seedlings grown in culture	28
2.5 Measurement of cortical and epidermal cells from contractile and non-contractile seedlings grown in culture	30

2.6	Examination of hypocotyls from contractile and non-contractile plants grown in a glasshouse – light microscopy part I	31
2.7	Examination of hypocotyls from contractile and non-contractile plants grown in a glasshouse – light microscopy part II	32
2.8	Statistical analysis	33

### **CHAPTER 3 : RESULTS**

3.1	Contractile growth in a range of cultivars	34
3.2	Comparison of contractile vs non-contractile growth	40
3.3	Application of Ethrel and silver thiosulphate to contractile and non-contractile seedlings :	
3.3.1	Experiment one	42
3.3.2	Experiment two	47
3.3.3	Experiment two (petiole comparisons)	52
3.4	Ethylene production by contractile and non-contractile seedlings grown in culture	54
3.5	Measurement of cortical and epidermal cells from contractile and non-contractile seedlings grown in culture	58
3.6	Examination of hypocotyls from contractile and non-contractile plants grown in a glasshouse – light microscopy part I	59
3.7	Examination of hypocotyls from contractile and non-contractile plants grown in a glasshouse – light microscopy part II	65

<b>CHAPTER 4 : DISCUSSION</b>	<b>68</b>
-------------------------------	-----------

<b>REFERENCES</b>	<b>78</b>
-------------------	-----------

# LIST OF FIGURES

<b>CHAPTER 1 :</b>		<b>PAGE</b>
1.1	Final steps in the biosynthetic pathway of ethylene	10
1.2	Proposed model for ethylene signal transduction in <i>Arabidopsis</i>	13
1.3	Position of loci in ethylene response pathway	18
<b>CHAPTER 3 :</b>		
3.1	Hypocotyl growth trends of a range of cultivars ( <i>contractile phenotype</i> )	35
3.2	Mean length and width of leaves in a range of cultivars	37
3.3	Maximum mean hypocotyl height versus mean leaf area	39
3.4	Hypocotyl growth of contractile and non-contractile seedlings	41
3.5	Hypocotyl growth of seedlings from the contractile and non-contractile lines treated with Ethrel and silver thiosulphate	43
3.6 A-D	Hypocotyl growth of contractile and non-contractile seedlings treated with Ethrel and silver thiosulphate	45
3.7	Hypocotyl growth of seedlings from the contractile and non-contractile lines treated with Ethrel and silver thiosulphate	48
3.8 A-D	Hypocotyl growth of contractile and non-contractile seedlings treated with Ethrel and silver thiosulphate	50
3.9	Mean petiole lengths of plants from the contractile and non-contractile lines	53
3.10 A-D	Petiole lengths of contractile and non-contractile plants treated with Ethrel and silver thiosulphate	55
3.11	A-E Ethylene produced by contractile and non-contractile seedlings	57
3.12	Transverse sections of hypocotyls from contractile and non-contractile seedlings	67

## LIST OF TABLES

<b>CHAPTER 2 :</b>	<b>PAGE</b>
2.1      Number of vials sampled and total number of samples taken	30
<b>CHAPTER 3 :</b>	
3.1      Percentage of plants that survived and percentage of plants that displayed non-contractile growth	36
3.2      Maximum hypocotyl height attained by different clover cultivars	38
3.3      Percentage of plants that survived and percentage of plants that displayed non-contractile growth	42
3.4      Percentage of plants that survived and percentage of plants that displayed non-contractile growth	47
3.5      Percentage of plants that survived and percentage of plants that displayed non-contractile growth	52
3.6      Height, width and area of cortical and epidermal cells from hypocotyls of contractile and non-contractile seedlings	59
3.7      Type and number of cells present in each layer of hypocotyl tissue	61
3.8      Height and width of hypocotyls from contractile and non- contractile seedlings	62
3.9      Number of cortical and epidermal cells along the length of contractile and non-contractile seedling hypocotyls	62
3.10     Number of parenchyma cells along the length of contractile and non-contractile seedling hypocotyls	63
3.11     Height, width and area of cortical and epidermal cells from the hypocotyls of contractile and non-contractile seedlings	64
3.12     Height, width and area of parenchyma cells from the hypocotyls of contractile and non-contractile seedlings	65
3.13     Width of tissue layers in the hypocotyls of contractile and non-contractile seedlings	66