

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.

**THE EFFECT OF WATER STRESS ON
WATER RELATIONS, CARBON ISOTOPE
DISCRIMINATION, AND SHOOT AND ROOT
GROWTH OF SAINFOIN (*Onobrychis viciifolia* Scop.)
AND LUCERNE (*Medicago sativa* L.)**

**A thesis presented
in partial fulfilment of the requirements
for the degree of Doctor of Philosophy in Department of Plant Science
at Massey University**

Seyed Reza Mir-Hosseini-Dehabadi

1994

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
لِحَمْدِ اللَّهِ رَبِّ الْعَالَمِينَ
وَالصَّلَاةِ وَالسَّلَامِ عَلَى خَاتَمِ الْأَنْبِيَاءِ وَالرَّسُلِ

**In the name of Allah,
the Compassionate, the Merciful,
Praise be to Allah, Lord of the Universe,
And Peace and Prayers be upon
His final Prophet and Messenger.**

| | |
|-------------------------------------|--------------|
| -ABSTRACT | i |
| -ACKNOWLEDGMENT | iii |
| -LIST OF ABBREVIATIONS | vi |
| -TABLE OF CONTENTS | viii |
| -LIST OF TABLES | xviii |
| -LIST OF FIGURES | xxvi |
| -LIST OF PLATES | xxxii |
| -LIST OF APPENDICES | xxxiv |

ABSTRACT

Sainfoin (*Onobrychis viciifolia* Scop.) is a useful forage legume regarded as having drought resistant attributes. Also, it does not cause bloat in ruminants and is not sensitive to alfalfa weevil (*Hypera postica* L.). Although the physiological and morphological responses to water stress of lucerne (*Medicago sativa* L.) are well known the responses of sainfoin to water stress have not been fully studied. In this study the physiological and morphological responses of sainfoin to water stress were investigated, with lucerne used as a reference plant.

The results of the indoor and outdoor studies showed sainfoin had useful characteristics for forage production in dry conditions. Relative to lucerne it had a lower yield, due to lower leaf area, lower stem number and poor regrowth. However, sainfoin responded to water stress at least as well as lucerne. Sainfoin had a higher root:shoot ratio and a lower specific leaf area ratio than lucerne, indicating a higher allocation of carbohydrate to the roots, and a lower leaf surface area for transpiration in sainfoin than for lucerne. Water stress decreased the yield of lucerne proportionally more than sainfoin mostly due to the greater reduction in the above ground dry weight of lucerne.

The indoor study of root characteristics of sainfoin and lucerne in 1m tall tubes showed that in terms of root development sainfoin responded to water stress better than lucerne. Although sainfoin had equal root mass and root length to lucerne, the root distribution of sainfoin at below 0.6 m depths was greater than for lucerne. As water stress developed sainfoin roots grew below 0.6 m earlier than lucerne roots. Sainfoin had a higher root osmotic adjustment than lucerne and also maintained higher (less negative) leaf water potential than lucerne.

The stomatal resistances (R_s) of sainfoin and lucerne were equal, but R_s was not distributed equally between adaxial and abaxial leaf surfaces. The R_s of the adaxial leaf surface of sainfoin was lower and more sensitive to water stress than the R_s of the abaxial leaf surface. The different R_s of the adaxial and abaxial leaf surfaces of sainfoin was partly due to the different stomatal frequencies of the respective surfaces.

Comparison of sainfoin cultivars in a climate room showed that the water use efficiencies (WUE) of Remont, Fakir, Cotswold-Common, and Eski, were similar. Remont was more sensitive to water stress than the other three cultivars, and Eski produced a greater root length and mass than other cultivars. The growth of Eski was initially slower than that of the Remont in both the indoor and the outdoor studies. However, lucerne grew faster than all the sainfoin cultivars. Over three harvests in the field the yields of Eski and Remont were similar but lucerne out yielded both sainfoin cultivars. Sainfoin produced a greater proportion of its yield earlier than lucerne, whereas lucerne distributed its yield throughout the whole season, indicating that sainfoin is adapted to regions with precipitation in only winter and spring.

The results of the carbon isotope discrimination (Δ) analysis for the indoor and outdoor studies showed Δ had a negative correlation with WUE, leaf water potential, osmotic potential, and stomatal resistance, but had a positive correlation with relative water content, turgor potential, transpiration rate, and photosynthetic rate. These correlations demonstrated the usefulness of this technique for evaluating the responses of plants to water stress. The stressed plants always had lower Δ than the control plants showing the higher WUE of stressed plants. The Δ of roots was higher than the Δ of the leaves suggesting that the growth of leaves occurred in conditions that were an average drier than for the growth of roots. This was supported by the lower (more negative) water potential of leaves than roots. The Δ of the roots below 0.6 m depth was higher than the Δ of roots above 0.1 m depth suggesting the roots above 0.1m grew under higher water stress than the roots below 0.6m depth. Over three harvests in the field the Δ of Eski and lucerne were similar and the Δ of Remont was higher than for Eski and lucerne.

In conclusion, sainfoin was found to have several useful attributes for growth and survival in dry regions. Of the sainfoin cultivars examined Eski was the best adapted to water stress. Relative to lucerne, sainfoin yielded less, but had a similar water use efficiency, a shorter season of growth, a greater root: shoot ratio, deeper roots and better maintenance of leaf water potential under water stress.

ACKNOWLEDGEMENT

First of all I would like to express my deepest sense of gratitude to almighty Allah, whose kindness and blessing helped me to do this study. Praise be to the final prophet Mohammad (peace upon him) and other messengers of Allah who were the righteous guidance of the human beings. I am grateful to my parents who taught me the first words and showed me the way of school on the first day of my study.

Thanks are extended to my chief supervisor Professor J. Hodgson for his excellent guidance and support during the last four years. Any time I needed his help I was provided a friendly answer. I have never felt any problems during this study due to his invaluable help and support.

I have no words to express my appreciation for the enthusiastic, helpful, friendly, and scholastic supervision of my two co-supervisors Drs. P. D. Kemp and D. J. Barker. The great patience and considerable attention of these two great men shown at regular meetings over four years is highly appreciated. Without their strong, and tireless efforts, this work could never have been finished. To me, this has been an invaluable educational experience, any errors remaining in this thesis, are entirely mine.

I acknowledge the assistance given to me by the following:

- Drs. I. L. Gordon (Department of Plant Science), and S. Ghaneshanandam (Department of Statistics) for statistical advice.
- Professor G. D. Farquhar and his group (Research School of Biological Science, Australian National University (ANU)) for their very useful discussion and comments, allowing me to work in their laboratory, analysing the samples for carbon isotope discrimination, teaching me the carbon isotope discrimination technique, and their warm hospitality during my training

course at ANU.

- Professors W. Silvester, and A. Rajendram Carbon Isotope Unite (University of Waikato,) for analysing samples for carbon isotope discrimination.
- Professor R. G. Thomas (Department of Plant Biology) for his advice and comments on stomatal frequency reported in this thesis.
- Drs. C. A. Cornford and D. W. Fountain (Department of Plant Biology) for teaching me plant water relations.
- Associated Professor A.C.P. Chu and Dr. C. Matthew (Department of Plant Science) for their comments, discussions, and sympathetic support.
- Drs. D. R. Scotter, and D. J. Horne (Department of Soil Science) for teaching me soil water relationships and allowing me to work in their laboratory.
- Mr L. D. Currie (Department of Soil Science) for measurements of soil fertility used in this study.
- Technicians of the Plant Science Departments: Ms. F. Brown for her excellent assistance with laboratory work on growing rhizobium and rhizobium inoculation, and Mrs. C. Mckenzie for drawing the templates and providing facilities.
- Mrs. S. Cleland for patiently and carefully estimating leaf area, and for measurements of leaf water potential.
- The Manager of Plant Growth Unit (P.G.U.) Mr. R. Johnston and staffs of P.G.U. in particular technical assistance of C. Forbes, and G. Russell during the experiment. I will never forget the immediate and positive response of Mr. Johnston to all of my requests and his famous sentence: Nothing is impossible.
- Mr. B. Mckay for writing the computer programs for the manipulation of the data from the porometer, and Li-Cor 6200.
- Ag-Research for permission to use the facilities which made this study possible.
- The staff of Hort-Research in particular those in the Climate Room Services, and the Technical Service team for prompt and efficient service.
- Mr. T. Lynch and M. Osborne (Plant Science Department) for Technical

-
- assistance with the field experiment and refurbishment of the Rain-out shelter.
 - Dr. D.J. Barker for lending the Decagon, and TDR, and his assistance with the for preparation of the figures in this thesis.
 - Dr. H. Behboudian for lending the Wescor, and his excellent advice and comments.
 - The late Dr. M. Forde and Montana State University for providing sainfoin seed.
 - All postgraduates and members of Plant science Department who provided me an excellent and friendly environment and removed all difficulties.
 - The staff of the Massey University Computer Center for their cooperation.
 - All members of the International Muslim Association at Massey, in particular Iranian, Pakistanian, and Indonesian brothers and their families who created a pleasant and religious work environment for me and my family.

Special thanks are extended to my wife Nosrat for her patience, loyal support, sacrifices and understanding in allowing me to pursue my study during last decade in tranquillity. I am also grateful to my beloved daughter Zeinab and my son Ali for their patience, forbearance and submissiveness during this study.

Finally the financial support (full scholarship) of the Ministry of Jihad Sazandegi (Iran) for undertaking this study is highly appreciated.

This dissertation is dedicated to the martyrs of the Islamic Republic of Iran who presented their life to Allah and irrigated the tree of Islam by their blood.

LIST OF ABBREVIATIONS:

- A= Assimilation rate ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)
ABA= Abscisic Acid
ANOVA= Analysis of variance
 C_a = CO_2 concentration of the air (ppm)
 C_i = CO_2 concentration inside of the leaf (ppm)
 C_s = Stomatal conductance (cm/s)
D= drainage
DAP= Days after planting
DS= Days of stress
DW= Dry weight
E= Transpiration rate ($\text{mol H}_2\text{O}/\text{m}^2/\text{s}$)
 e_a = Vapour pressure of the air
 e_i = Vapour pressure of the leaves
FR= Fine roots (<0.3 mm diam.)
GSWC= Gravimetric soil water content (%)
I= Interception of rainfall by crop canopies
LA= Leaf area (cm^2)
LAI= Leaf area index
LDW= Leaf dry weight (g)
P= Turgor potential (MPa)
 P_a = Partial pressure of CO_2 concentration of the air (MPa)
 P_i = Partial pressure of CO_2 concentration of the leaf (MPa)
 P_n = Net photosynthetic rate ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)
R= run-off
 R_s = Stomatal resistance (s/cm)
RH= Relative humidity (%)
RSE= Relative stem elongation (mm/mm/day)
RWC= Relative water content of the leaf (%)
RWD= Root weight density (g/m^3)

SDW= Stem dry weight (g)

SLA= Specific leaf area (cm^2/g)

SEM= Standard error of the mean

S/R= shoot:root ratio

TAC= Total available carbohydrate

TDR= Time domain reflectometer

TR= Thick roots (>0.3 mm diam.)

Tr= Transpiration rate ($\text{ml H}_2\text{O}$)

VPD= Vapour pressure deficit

VSWC= Volumetric soil water content (%)

W= Transpiration efficiency $\{(\mu\text{mol CO}_2/\text{m}^2/\text{s})/(\text{mol H}_2\text{O}/\text{m}^2/\text{s})\}$

WUE= Water use efficiency

Y_{ec} = Economic yield

Δ = Carbon isotope discrimination

π = Osmotic potential (MPa)

π_{100} = Osmotic potential at full turgor (MPa)

v = Water vapour pressure difference between the intercellular spaces and the atmosphere

ϕ = Loss of carbon or water not through stomata

Ψ = Leaf water potential (MPa)

Table of contents

| | |
|--|----------|
| Chapter 1 | 1 |
| 1. Introduction and objectives | 2 |
| | |
| Chapter 2 | 4 |
| 2 Review of literature | 5 |
| 2.1 Plant adaptation to water stress | 5 |
| 2.1.1 Definition and concepts | 5 |
| 2.1.2 Categories of drought resistance | 5 |
| 2.1.2.1 Drought escape | 6 |
| 2.1.2.2 Avoiding stress | 7 |
| 2.1.2.3 Dehydration tolerance (low lethal water status) | 10 |
| 2.2 Water use efficiency | 11 |
| 2.2.1 Introduction | 11 |
| 2.2.2 Definitions | 11 |
| 2.2.3 Factors effecting water use efficiency | 13 |
| 2.2.3.1 Plant factors | 13 |
| 2.2.3.2 Environmental factors | 14 |
| 2.2.4 Improving water use efficiency | 15 |
| 2.3 Water status of the plant | 16 |
| 2.3.1 Water potential | 16 |
| 2.3.2 Components of water potential | 16 |
| 2.4. Osmotic adjustment | 16 |
| 2.4.1 Definition | 16 |
| 2.4.2 Osmotic potential (π) | 17 |
| 2.4.3 Components of osmotic adjustment | 17 |
| 2.4.4. Importance of osmotic adjustment in dry conditions | 18 |
| 2.5 Stomatal resistance | 20 |
| 2.5.1 Stomatal resistance and water stress | 20 |

| | |
|--|-----------|
| 2.5.1.1 Stomatal resistance and transpiration. | 20 |
| 2.5.1.2 Stomatal response to drought and plant water status | 21 |
| 2.5.2 Stomatal response to humidity | 21 |
| 2.5.3 Stomatal response to CO ₂ concentration | 22 |
| 2.5.4 Stomatal response to phytohormones | 23 |
| 2.6 Root and water stress | 23 |
| 2.6.1 Rooting depth | 24 |
| 2.6.2 Hydraulic conductance of the root | 25 |
| 2.7 Carbon isotope discrimination | 26 |
| 2.7.1 Definition | 26 |
| 2.7.2 Drought, soil strength and discrimination (Δ) | 26 |
| 2.7.3 Water use efficiency and discrimination (Δ) | 27 |
| 2.8 Sainfoin (<i>Ononbrychis viciifolia</i> Scop.) | 28 |
| 2.8.1 Sainfoin: potential as a forage legume | 28 |
| 2.8.2 Agronomy of sainfoin | 29 |
| 2.8.3 Sainfoin in dry conditions | 30 |
| Chapter 3 | 32 |
| 3. Adaptation of sainfoin cultivars and lucerne to water stress | 33 |
| 3.1 Abstract | 33 |
| 3.2 Introduction | 34 |
| 3.3 Materials and Methods | 34 |
| 3.3.1 Experimental | 34 |
| 3.3.2 Measurements | 35 |
| 3.4 Statistical analysis | 36 |
| 3.5 Results and Discussion | 36 |

| | |
|---|-----------|
| Chapter 4 | 39 |
| 4. Physiological and morphological responses of lucerne to soil moisture stress | 40 |
| 4.1 Abstract | 40 |
| 4.2 Introduction | 41 |
| 4.3 Materials and Methods | 42 |
| 4.3.1 Experimental | 42 |
| 4.3.2 Measurements: | 42 |
| 4.3.2.1 Transpiration (Tr) | 42 |
| 4.3.2.2 Relative water content (RWC) | 43 |
| 4.3.2.3 Stomatal resistance (Rs) | 43 |
| 4.3.2.3 Leaf water potential (Ψ), Leaf osmotic potential (π) | 43 |
| 4.3.2.4 Leaf area development, and leaflet number .. | 43 |
| 4.3.2.5 Plant height and relative stem elongation | 44 |
| 4.3.3 Plant harvest | 44 |
| 4.4 Statistical analysis | 44 |
| 4.5 Results | 45 |
| 4.6 Discussion: | 53 |
| 4.6.1 Physiological responses | 53 |
| 4.6.2 Morphological responses | 53 |
| 4.7 Conclusion | 55 |
| Chapter 5 | 56 |
| 5. Comparison of sainfoin cultivars and lucerne, with an emphasis on sainfoin responses to water stress. | 57 |
| 5.1 Abstract | 57 |
| 5.2 Introduction | 58 |
| 5.3 Materials and Methods | 59 |
| 5.3.1 Glasshouse experiment | 59 |

| | |
|--|-----------|
| 5.3.2 Field experiment | 59 |
| 5.4 Results | 62 |
| 5.4.1 Glasshouse experiment | 62 |
| 5.4.2 Field experiment | 63 |
| 5.5 Discussion | 67 |
| 5.5.1 Glasshouse experiment | 67 |
| 5.5.2 Field Experiment | 68 |
| 5.6 Conclusion | 69 |
| | |
| Chapter 6 | 70 |
| 6. Plant water status, and shoot and root growth of sainfoin cultivars at constant water stress levels. | 71 |
| 6.1 Abstract | 71 |
| 6.2 Introduction | 72 |
| 6.3 Materials and Methods | 74 |
| 6.3.1 Experimental | 74 |
| 6.3.2 Measurement | 76 |
| 6.3.2.1 Transpiration rate (Tr) | 76 |
| 6.3.2.2 Relative water content (RWC) | 76 |
| 6.3.2.3 Stomatal resistance (Rs) | 76 |
| 6.3.2.4 Leaf water potential (Ψ), Leaf osmotic potential (π) | 77 |
| 6.3.2.5 Photosynthesis | 77 |
| 6.3.2.6 Leaf area development, and leaflet number | 79 |
| 6.3.2.7 Plant harvest | 79 |
| 6.3.2.8 Water use efficiency (WUE) | 79 |
| 6.3.2.9 Specific leaf area (SLA) | 80 |
| 6.3.3 Statistical analysis | 80 |
| 6.4 Results | 82 |
| 6.4.1 Roots | 82 |
| 6.4.2 Yield | 82 |

| | |
|---|-----|
| 6.4.3 Leaf area | 86 |
| 6.4.4 Specific leaf area (SLA) | 86 |
| 6.4.5 Relative water content (RWC) | 88 |
| 6.4.6 Stomatal resistance (Rs) | 90 |
| 6.4.7 WUE | 90 |
| 6.4.8 Leaf water potential at dawn | 93 |
| 6.4.8.1 Glasshouse | 93 |
| 6.4.8.2 Climate room | 93 |
| 6.4.9 Osmotic potential (π) at dawn | 94 |
| 6.4.9.1 Glasshouse | 94 |
| 6.4.9.2 Climate room | 94 |
| 6.4.10 Turgor potential (P) | 95 |
| 6.4.10.1 Glasshouse | 95 |
| 6.4.10.2 Climate room | 95 |
| 6.4.11 Total, osmotic, and turgor potential of the leaf at midday | 96 |
| 6.4.11.1 Leaf water potential | 96 |
| 6.4.11.2 Osmotic potential | 97 |
| 6.4.11.3 Turgor potential | 97 |
| 6.4.11.4 Midday leaf water potential by pressure bomb | 98 |
| 6.4.11.5 Leaf water potential: Wescor vs Pressure Chamber | 98 |
| 6.4.12 Photosynthesis (P_n) | 101 |
| 6.4.13 Leaflet number | 103 |
| 6.4.14 Estimated leaf area | 103 |
| 6.4.15 Transpiration rate | 105 |
| 6.5 Discussion | 108 |
| 6.5.1 Yield and its components | 108 |
| 6.5.2 Relative water content | 109 |
| 6.5.3 Water status of sainfoin | 110 |

| | |
|--|------------|
| 6.5.4 Stomatal resistance | 112 |
| 6.5.5 Transpiration and stomatal resistance | 113 |
| 6.5.6 Photosynthesis and WUE response to water stress | 114 |
| 6.5.7 Cultivar response to water stress | 114 |
| 6.6 Conclusion | 116 |
| Chapter 7 | 117 |
| 7. Root and shoot responses of sainfoin and lucerne to water stress | 118 |
| 7.1 Abstract | 118 |
| 7.2. Introduction | 119 |
| 7.2. Materials and Methods | 123 |
| 7.2.1 Plant materials and culture | 123 |
| 7.2.2 Plant growth container | 123 |
| 7.2.3 Design and treatments | 124 |
| 7.2.4 Soil moisture. | 126 |
| 7.2.5 Morphological measurement | 129 |
| 7.2.6 Physiological measurement | 129 |
| 7.2.7 Statistical analysis | 131 |
| 7.2.7.1 Analysis of morphological measurements | 131 |
| 7.2.7.2 Analysis of physiological measurements: | 131 |
| 7.2.7.3 Analysis of soil moisture | 132 |
| 7.3. Results | 134 |
| 7.3.1 Soil moisture | 134 |
| 7.3.1.1 Average soil moisture (0-70 cm depth) | 136 |
| 7.3.1.2 Water content of the pots measured by weighing | 136 |
| 7.3.2 Morphological measurements | 138 |
| 7.3.2.1 Leaf area | 138 |
| 7.3.2.2 Leaf dry weight (LDW) | 138 |
| 7.3.2.3 Stem dry weight (SDW) | 142 |
| 7.3.2.4 Specific leaf area (cm ² /g) | 142 |

| | |
|---|-----|
| 7.3.2.5 Shoot:Root ratio | 142 |
| 7.3.2.6 Root Length Density | 146 |
| 7.3.2.7 Root weight density (RDW) | 150 |
| 7.3.3 Physiological measurements | 153 |
| 7.3.3.1 Relative water content (RWC) | 153 |
| 7.3.3.2 Transpiration rate | 153 |
| 7.3.3.3 Stomatal resistance (Rs) | 156 |
| 7.3.3.4 Leaf water potential (Ψ) by Pressure Bomb . . . | 160 |
| 7.3.3.5 Leaf water potential by Wescor (Ψ) | 162 |
| 7.3.3.6 Osmotic potential (π) | 164 |
| 7.3.3.7 Turgor potential of the leaf | 166 |
| 7.3.3.8 Osmotic potential at full turgor | 169 |
| 7.3.3.9 Total, osmotic, and turgor potential of roots . . | 171 |
| 7.3.3.10 Root osmotic potential at full turgor | 175 |
| 7.3.3.11 Comparison of total, osmotic and turgor potential of root and leaf | 177 |
| 7.3.3.11.1 Total potential of leaf-root | 177 |
| 7.3.3.11.2 Osmotic potential of leaf-root | 180 |
| 7.3.3.11.3 Osmotic potential of leaf and root at full turgor (π_{100}) | 182 |
| 7.5. Discussion | 184 |
| 7.5.1 Soil moisture | 184 |
| 7.5.2 Morphology | 184 |
| 7.5.2.1. Root growth | 186 |
| 7.5.3 Physiology | 187 |
| 7.5.3.1 Relative water content | 187 |
| 7.5.3.2 Stomatal resistance. | 188 |
| 7.5.3.3 Leaf water potential | 188 |
| 7.5.3.4 Osmotic potential (π) | 189 |
| 7.5.3.5 Root water status | 191 |
| 7.5. Conclusion | 193 |

| | |
|--|------------|
| 8.4.3.9 Stomatal frequency | 241 |
| 8.5 Discussion | 243 |
| 8.5.1 Soil moisture | 243 |
| 8.5.2 Morphology | 244 |
| 8.5.3 Physiological factors: | 246 |
| 8.3 Conclusions: | 249 |
| Chapter 9 | 250 |
| 9. Carbon Isotope Discrimination of leaves and roots of water stressed sainfoin | 251 |
| 9.1 Abstract | 251 |
| 9.2 Introduction | 252 |
| 9.3 Materials and Methods | 253 |
| 9.3.1 Technique | 253 |
| 9.3.2 Measurements | 254 |
| 9.3.3: Statistical analysis | 254 |
| 9.4 Results | 255 |
| 9.4.1 Climate Room experiment | 255 |
| 9.4.2 Glasshouse results | 256 |
| 9.4.2.1 Carbon isotope discrimination of leaves in the glasshouse experiment (Chapter 7). | 256 |
| 9.4.2.2 Carbon isotope discrimination of the roots | 256 |
| 9.4.3 Carbon isotope discrimination in the field. | 257 |
| 9.4.3.1 Carbon isotope discrimination of Remont | 257 |
| 9.4.3.2 Carbon isotope discrimination of Eski, Remont, and lucerne in the field. | 258 |
| 9.4.4 Relationships between Δ and Ψ , π , P, RWC, Rs, Tr, C _i /C _a and P _n | 260 |
| 9.4.5 Relationships of Δ with yield and SLA | 260 |
| 9.5 Discussion | 267 |
| 9.5.1 Discrimination and WUE | 267 |
| 9.5.2: Discrimination and Roots | 269 |

| | |
|---|------------|
| 9.5.3: Yield, water status of the leaf and Δ | 270 |
| 9.6 Conclusion: | 272 |
| Chapter 10 | 273 |
| 10. General Discussion | 274 |
| 10.1 Responses to water stress of sainfoin | 274 |
| 10.3 Comparison of the methods used in this study | 278 |
| 10.4 Conclusion | 280 |
| | |
| Literature cited | 282 |
| | |
| Appendixes | 323 |

List of tables:

| | |
|---|----|
| Table 3.1 : Root, leaf and above ground dry matter (g/pot) for three sainfoin cultivars and lucerne after 100 days, with no watering over the last 30 days. | 36 |
| Table 4.1: Dry weight (g DW) of total shoot, stem, leaf, and root, root length (m), leaf area (cm ²), and specific leaf area (cm ² /g) per pot of lucerne at different levels of soil water available (133 DAS) | 46 |
| Table 4.2: Stomatal resistance (Rs), relative water content (RWC), transpiration rate (TR), leaf water potential (Ψ), and osmotic potential (π) of lucerne at three soil moisture levels. | 52 |
| Table 5.1: Leaf area (LA), leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), and specific leaf area (SLA) of eight glasshouse-grown sainfoin cultivars and species, and lucerne, at 65 days after planting. | 62 |
| Table 5.2: Leaf area (LA), leaf dry weight (LDW), stem dry weight (SDW), and specific leaf area (SLA) of field grown Remont, for stressed (rain-out shelter) and non-stressed (rain-fed control) treatments. | 65 |
| Table 6.1 : The length and dry weight of fine and thick roots of four sainfoin cultivars at three soil moisture.(180 day after planting) | 83 |
| Table 6.2 : The interaction between cultivars and soil moisture for total root length (m). (SEM = 288.7) | 84 |
| Table 6.3: Yield, cumulative yield (CU yield), and leaf area (LA), of sainfoin | |

| | |
|--|-----|
| cultivars per pot at different soil moisture levels 200 day after planting (DAP) | 85 |
| Table 6.4 (a) Leaf dry weight (DW), leaf area (LA), and specific leaf area (SLA) of two sampled plants per pot. | 87 |
| Table 6.4 (b): Interaction of cultivar by soil moisture treatment for SLA (SEM= 12.85) (P < 0.05). | 87 |
| Table 6.5: Water use efficiency (g/kg) (by 5 methods, see text) of four sainfoin cultivars at three soil moisture levels. | 92 |
| Table 7.1: Leaf area (cm ² /pot) of Eski and Grasslands G35 sainfoin, and Grasslands Oranga lucerne, at the early harvest (45 days after planting (DAP)) late harvest (75 DAP), and regrowth harvest (30 days regrowth, 105 DAP) at two soil moisture levels. | 140 |
| Table 7.2: Leaf dry matter (g/pot) of Eski and Grasslands G35 sainfoin and Grasslands Oranga lucerne at early harvest (45 days after planting, DAP) late harvest (75 DAP) and regrowth harvest (30 days regrowth and 105 DAP) at two soil moisture levels. | 141 |
| Table 7.3: Stem and petiole dry matter (g/pot) of Eski, and Grasslands G35 sainfoin and Grasslands Oranga lucerne at the early harvest (45 days after planting), late harvest (75 DAP) and the regrowth harvest (30 days regrowth, 105 DAP) at two soil moisture levels. | 144 |
| Table 7.4: The specific leaf area (cm ² /g) and shoot:root ratio of two sainfoin cultivars and lucerne at the early harvest (45 days after planting, DAP), late harvest (75 DAP), and the regrowth harvest (30 days, 105 DAP) at two soil moisture levels. | 145 |

| | |
|--|-----|
| Table 7.5: Total root length density (m/m^3)* 10^3 of Eski, Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels and three harvests. | 149 |
| Table 7.6: Total root dry weight density (g/m^3) of Eski, Grasslands G35, and lucerne (Grasslands Oranga) at two different soil moisture levels for three harvests. | 152 |
| Table 7.7: Relative water content (%) of two sainfoin cultivars and lucerne at the early (45 days after planting, DAP) late, (75 DAP) and regrowth (30 days after cutting, 105 DAP) harvests at two soil moisture levels. | 155 |
| Table 7.8 (a): Total stomatal resistance (s/cm) of sainfoin cultivars and lucerne at two soil moisture levels for early (45 days after planting), late (75 days after planting), and regrowth (30 days after cutting) harvests. | 157 |
| Table 7.8 (b): Stomatal resistance (s/cm) of abaxial and adaxial surfaces of leaves of sainfoin cultivars and lucerne in two soil moisture levels at early harvest (45 days after planting), late harvest (75 days after planting), and regrowth. | 158 |
| Table 7.9: Total leaf water potential (MPa) by pressure bomb of early (45 days after planting, DAP) late (75 DAP) and regrowth (30 days after cutting, 105 DAP) harvests of two sainfoin cultivars and lucerne at two levels of soil moisture, at dawn and midday. | 161 |
| Table 7.10 : Total leaf water potential by Wescor (MPa) of early (45 days after planting, DAP)and late harvest (75 DAP) and regrowth (30 days after cutting, 105 DAP) harvest of two sainfoin cultivars and lucerne, | |

| | |
|--|-----|
| at two levels of soil moisture, at dawn and midday | 163 |
| Table 7.11: Osmotic potential by Wescor (MPa) of early (45 days after planting, DAP) late (75 DAP) and regrowth (30 days after cutting, 105 DAP) harvests of two sainfoin cultivars and lucerne, at two levels of soil moisture, at dawn and midday. | 165 |
| Table 7.12: Turgor potential (MPa) of early (45 days after planting, DAP) late (75 DAP) and regrowth (30 days after cutting, 105 DAP) harvests of two sainfoin cultivars and lucerne, at two levels of soil moisture, at dawn and midday. | 168 |
| Table 7.13: The full turgor osmotic potential (MPa) using the Decagon of Eski, Grasslands G35 and lucerne (Grasslands Oranga) at dawn and midday at early harvest (45 days after planting, DAP), late (75 DAP), and regrowth (30 days after cutting, 104 DAP) harvests at two levels of soil moisture. | 170 |
| Table 7.14: The total potential (MPa) of roots of sainfoin cultivars and lucerne at two depths, and two soil moisture levels at early (45 days after planting, DAP), late (75 DAP), and regrowth (30 days after cutting, 105 DAP) harvests | 172 |
| Table 7.15: The osmotic potential (MPa) of roots of sainfoin cultivars and lucerne at two soil moisture levels at early (45 days after planting, DAP), late (75 DAP), and regrowth (30 days after cutting, 105 DAP) harvests | 173 |
| Table 7.16: The turgor potential (MPa) of roots of sainfoin cultivars and lucerne at different depths at two soil moisture levels at early (45 days after planting, DAP), late (75 DAP), and regrowth (30 days after | |

| | |
|---|-----|
| cutting, 105 DAP) harvests. | 174 |
| Table 7.17: The root osmotic potential at full turgor (MPa) of two sainfoin cultivars and lucerne at two depths and two soil moisture levels at early (45 DAP), late (75 DAP), and regrowth (30 days after cutting, 105 DAP) harvests. | 176 |
| Table 7.18 : The total water potential (MPa) of roots and leaves of sainfoin cultivars and lucerne at different depths for two soil moisture levels at early (45 days after planting), late (75 days after planting), and regrowth harvests. | 179 |
| Table 7.19: The osmotic potential (MPa) of the roots and leaves of sainfoin cultivars and lucerne at two soil moisture levels at the early (45 days after planting), late (75 days after planting), and regrowth harvests. | 181 |
| Table 7.20: The osmotic potential (MPa) of roots and leaves of sainfoin cultivars and lucerne at full turgor at different depths at two soil moisture levels at the early (45 days after planting), late (75 days after planting), and regrowth harvests. | 183 |
| Table 8.1: The average volumetric soil moisture (cm^3/cm^3 %) to 0-70 cm depth for the stressed and non-stressed experiments for three harvests, and regrowth following the second harvest. | 204 |
| Table 8.2: Leaf area (cm^2/m^2) of Eski, Remont, and lucerne at five harvests under non-stressed and stressed experiments. | 207 |
| Table 8.3: Leaf dry weight (g/m^2) of Eski, Remont, and lucerne at five harvests under non-stressed and stressed experiments. | 208 |

| | |
|---|-----|
| Table 8.4: Stem dry weight (g/m^2) of Eski, Remont, and lucerne at five harvests under stressed and non-stressed experiments. | 210 |
| Table 8.5: The stem density (stems/m^2) of Eski, Remont, and lucerne at five harvests under non-stressed and stressed experiments. | 211 |
| Table 8.6: Yield (g/m^2) of Eski, Remont, and lucerne at five harvests under non-stressed and stressed experiments. | 215 |
| Table 8.7: The specific leaf area (cm^2/g) of Eski, Remont, and lucerne at five harvests under non-stressed and stressed experiments. | 216 |
| Table 8.8 : Relative water content (%) of Eski, Remont, and lucerne at dawn for five harvests under stressed and non-stressed experiments. | 218 |
| Table 8.9: Relative water content (%) of Eski, Remont, and lucerne at midday for five harvests under stressed and non-stressed experiments. | 219 |
| Table 8.10: Leaf water potential (MPa) measured by Pressure Bomb for Eski, Remont, and lucerne at five harvests at dawn under non-stressed and stressed experiments | 222 |
| Table 8.11: Leaf water potential (MPa) measured by Pressure Bomb for Eski, Remont, and lucerne at five harvests at midday under non-stressed and stressed experiments | 223 |
| Table 8.12: Leaf water potential (MPa) measured by Wescor for Eski, Remont, and lucerne at dawn from five harvests under non-stressed and stressed experiments | 224 |
| Table 8.13: Leaf water potential (MPa) measured by Wescor for Eski, | |

| | |
|--|-----|
| Remont, and lucerne at midday from five harvests under non-stressed and stressed experiments | 225 |
| Table 8.14: Osmotic potential (MPa) of Eski, Remont, and lucerne at dawn from five harvests for non-stressed and stressed experiments. | 227 |
| Table 8.15: Osmotic potential (MPa) of Eski, Remont, and lucerne at midday from five harvests for non-stressed and stressed experiments. | 228 |
| Table 8.16: The osmotic potential at full turgor π_{100} (MPa) of Eski, Remont, and lucerne at dawn from five harvests for non-stressed and stressed experiments. | 230 |
| Table 8.17: The leaf osmotic potential at full turgor π_{100} (MPa) of Eski, Remont, and lucerne at midday from five harvests for non-stressed and stressed experiments. | 231 |
| Table 8.18: Turgor potential (MPa) of Eski, Remont, and lucerne at dawn from five harvests for non-stressed and stressed experiments. | 233 |
| Table 8.19: Turgor potential (MPa) of Eski, Remont, and lucerne at midday from five harvests for non-stressed and stressed experiments. | 234 |
| Table 8.20: Photosynthesis ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$) of Eski, Remont, and lucerne at midday from five harvests for non-stressed and stressed experiments. | 236 |
| Table 8.21: Stomatal resistance (s/cm) of Eski, Remont, and lucerne at midday from five harvests for non-stressed and stressed experiments, measured by a Li-Cor 6200. | 237 |

| | |
|---|-----|
| Table 8.22 (a): Stomatal resistance (s/cm) of adaxial and abaxial leaf surfaces measured by Delta porometer for Eski, Remont, and lucerne for stressed and non-stressed experiments. | 239 |
| Table 8.22 (b): Pooled analysis of variance results ($P > F$) for stomatal resistance measured by Delta Porometer over the second and third harvests for adaxial, abaxial and total leaf surfaces. | 240 |
| Table 9.1: Carbon isotope discrimination with Cotswold-Common, Eski, Fakir, and Remont, at three levels of soil moisture in a climate room. | 255 |
| Table 9.2: Carbon isotope discrimination with leaves of Eski and Grasslands G35 at two soil moisture levels in the glasshouse. | 256 |
| Table 9.3: Carbon isotope discrimination of roots of Eski, at two depths and two soil moisture levels in the Glasshouse. | 257 |
| Table 9.4: Carbon isotope discrimination of Eski, Remont, and lucerne for non-stressed and stressed experiments at the second (60 days after imposing water stress, DS), third (90 DS), and regrowth (65 DS) harvests in the field. | 259 |

List of figures

- Fig. 3.1: Stomatal resistance, transpiration and relative water content during 31 days water stress. Symbols are the average of four replicates for three sainfoin cultivars and lucerne. Bars show \pm SEM 38
- Fig 4.1: Lucerne height at different levels of soil water available. Vertical bars show \pm SEM. 47
- Fig. 4.2: Lucerne relative stem elongation per pot at different levels of soil water available. 47
- Fig 4.3: The estimated leaf area per plant for lucerne at different levels of soil water available. Vertical bars show \pm SEM. 48
- Fig 4.4: The leaflet numbers per pot for lucerne at different levels of soil moisture available. Vertical bars are \pm SEM. 48
- Fig 4.5: The total dry weight components per pot for lucerne at different levels of soil moisture available. Vertical bars show \pm SEM. 49
- Fig. 4.6: The root dry weight and root length per pot for lucerne at different levels of soil moisture available. Vertical bars show \pm SEM. 49
- Fig 4.7: The relationship between transpiration rate and leaf area at different soil moisture levels. 52
- Fig. 5.1: Volumetric soil water content (VSWC, cm^3/cm^3 %) for 0-15 (O), and 50-70 cm (■) depth under a rain-out shelter. Symbols are means of three replicates. 64

- Fig. 5.2 Relationships between Remont petiole water potential (Ψ , -MPa), relative water content (RWC, %), or stomatal resistance (adaxial surface, closed symbols; abaxial surface, open symbols) and volumetric soil water content (0-15 cm, VSWC, cm^3/cm^3 %), for rain-fed (non-stressed) plots (\square, \blacksquare) and stressed plots (\circ, \bullet). Symbols are means of three replicates. Vertical bars show \pm SEM. 66
- Fig. 6.1: soil water retentivity curve for the growth medium 75
- Fig. 6.2 (a) : Relative water content of sainfoin at three soil moisture levels. The Vertical bars show \pm SEM. 89
- Fig. 6.2 (b): Relative water content of four sainfoin cultivars. Vertical bars show \pm SEM. 89
- Fig. 6.3: Stomatal resistance of abaxial and adaxial leaf surfaces. Vertical bars are \pm SEM. 91
- Fig 6.4: Osmotic potential (π), Leaf water potential (Ψ), and turgor potential (P), of sainfoin at three soil moisture levels at dawn. Vertical bars show mean s.e.m. 99
- Fig 6.5: Relationship between leaf water potential measured by Pressure Chamber and Wescor. 100
- Fig 6.6: Photosynthesis rate $\mu\text{mol Co}_2/\text{m}^2/\text{s}$ of sainfoin at three soil moisture levels. Vertical bars show \pm SEM. 102
- Fig 6.7 (a): Leaflet number of sainfoin at three soil moisture levels. Vertical bars show \pm SEM. Markers are mean of four replicates. 104

| | |
|--|-----|
| Fig 6.7(b): Estimated leaf area of sainfoin at three soil moisture levels. Vertical bars show \pm SEM. Markers are mean of four replicates. . . . | 104 |
| Fig 6.8 (a): Transpiration rate ml/pot/day of sainfoin at three soil moisture levels. Vertical bars show \pm SEM. Markers are mean of four replicates. | 106 |
| Fig 6.8 (b): Relationship between leaf area and transpiration rate. Vertical bars show \pm SEM. Markers are mean of four replicates. | 106 |
| Fig. 6.9 (a): Relationship between stomatal resistance of sainfoin and transpiration rate. | 107 |
| Fig. 6.9 (b): Relationship between stomatal conductance of sainfoin and transpiration rate. | 107 |
| Fig 7.1: Plant growth container (Drawn by Cally McKenzie) | 125 |
| Fig. 7.2: Relationship between gravimetric soil water content determined from sampling and volumetric soil water content determined by TDR . . . | 133 |
| Fig. 7.3: Volumetric soil water content (cm^3/cm^3) of two moisture treatments (M) (control and stressed) at 0.2, 0.5, and 0.85 m depth for three harvests (M) (early, late, and regrowth harvests). *,**, and **** show significance at the 0.05, 0.01, 0.0001 levels, respectively | 135 |
| Fig 7.4: Root length density (m/m^3)* 10^3 of the Eski, Remont, and lucerne at different depths for two soil moisture levels. Bars show \pm SEM. | 148 |
| Fig 7.5: Root mass density (g/m^3) of Eski, Remont, and lucerne at different depths for two soil moisture levels. Bars show \pm SEM. | 151 |

- Fig. 7.6: Relationships between volumetric soil water content, and (A) relative water content% , and (B) turgor potential for Eski, Grasslands G35, and lucerne. Symbols are mean of four replicates, vertical bars represent \pm SEM 154
- Fig 7.7: Stomatal resistance of (A) adaxial, and (B) abaxial leaf surface of Eski, Grasslands G35, and lucerne at different soil moisture. Symbols are mean of four replicates. Vertical bars are \pm SEM. 159
- Fig 7.8: Relationship between turgor potential and relative water content of Eski, Grasslands G35, and lucerne. Points are mean of four replicates. Respective regression equations and standard error of slope (SE) are $Y = -0.68 + 0.01X$, $R^2 = 95\%$, $SE = 0.0016$, $Y = -0.9 + 0.01X$ $R^2 = 92\%$, $SE = 0.0029$, $Y = -0.79 + 0.012X$ $r^2 = 89\%$, $SE = 0.1148$ 167
- Fig. 7.9(a): Relationship between leaf and root water potential of lucerne, Eski, and Grasslands G35. Respective regression equations are $Y = 0.28 + 1.1X$, $R^2 = 92.3\%$, $Y = 0.47 + 1.5X$ $R^2 = 80\%$, $Y = -0.031 + 0.99X$ $R^2 = 99\%$ 178
- Fig. 7.9(b): Relationship between leaf and root osmotic potential at full turgor for lucerne, Eski, and Grasslands G35. Respective regression equations are $Y = -0.56 + 0.23X$ $R^2 = 42\%$, $Y = 2.4 + 3.3X$ $R^2 = 87\%$, $Y = 0.11 + 0.99X$ $R^2 = 33\%$ 178
- Fig 8.1a: The volumetric soil water content of stressed and non-stressed experiments at 0-15, and 15-30 cm depths during 140 days water stress. The regression equations for 0-15 cm depth of non-stressed and stressed are $Y = 47 - 0.1 * X$ $R^2 = 30\%$, $Y = 39 - 0.25 * X$ $R^2 = 40\%$, $Y = 29.4 - 0.1 * X$ $R^2 = 67\%$ respectively. Markers are means of eight replicates. 203

Fig 8.1b: VSWC of the stressed and non-stressed experiment at 30-50 cm and 50-70 cm depth during 140 days water stress. The regression equations for 30-50 and 50-70 cm depths for non-stressed and stressed experiment are $Y=57-0.2*X$, $R^2=63\%$, $Y=52-0.24*X$ $R^2=855$, $y=34+0.03*X$, $R^2=11.3\%$, $Y=39-0.06*X$ $R^2=60\%$. Markers are means of eight replicates. 203

Fig 8.2: Leaf and stem dry weight of Eski, Grasslands G35, and lucerne in the stressed and non-stressed (control) experiments for (a) first harvest (30 days after imposing water stress, Ds), (b) second harvest (60 DS), and (c), third harvest (90 DS). Vertical bars present the \pm SEM. 214

Fig 8.3: Relationships between relative water content, and (a) leaf water potential at midday, (b) osmotic potential at midday, and (c) leaf water potential at dawn, and (d) osmotic potential at dawn. Points are means of four replicates. Bars represent \pm SEM. 220

Fig. 9.1 : Correlation of Δ and a) turgor potential b) leaf water potential c) osmotic potential d) relative water content of four sainfoin cultivars (Cotswold-Common, Eski, Fakir, and Remont) under three constant soil moisture levels (C is control and M and S are moderately and severely stressed). Vertical and horizontal bars represent \pm SEM. Markers are means of four replicates. 261

Fig 9.2: Correlation of Δ and stomatal resistance of a) abaxial leaf surface b) adaxial leaf surface c) whole leaf of the four sainfoin cultivars (Cotswold-Common, Eski, Fakir, and Remont) under three constant soil moisture levels (C is control, and M and S are moderately and severely stressed respectively, vertical and horizontal bars represent \pm SEM. Markers are means of four replicates. 262

- Fig. 9.3 : Correlation of Δ and a) transpiration b) net photosynthesis, and c) instantaneous transpiration of Cotswold-common, Eski, Fakir, and Remont under three constant soil moisture levels (C is control, and M and S are moderately and severely stressed, respectively). Vertical and horizontal bars represent \pm SEM. Markers are mean of four replicates. 263
- Fig. 9.4 Correlation of Δ and water use efficiency (g dry weight/kg water) of the four sainfoin cultivars (Cotswold-Common, Eski, Fakir, and Remont) under three constant soil moisture levels (C is control and M and S are moderately and severely stressed). Vertical and horizontal bars represent \pm SEM. Markers are mean of four replicates. 264
- Fig. 9.5: Correlation of Δ and ratio of internal (C_i), and external (C_a) partial pressure of CO_2 concentration of leaves of Eski, Remont, and lucerne under stressed (S) and non-stressed (NS) conditions over the third and regrowth harvests in the field. 265
- Fig. 9.6: Correlation of Δ and yield of four sainfoin cultivars (Cotswold-Common, Eski, Fakir, and Remont) under three constant soil moisture levels. Vertical and horizontal bars represent \pm SEM. Markers are the mean of four replicates. 266
- Fig. 9.7: Correlation of yield and transpiration rate of four sainfoin cultivars (Cotswold-Common, Eski, Fakir, and Remont) under three constant soil moisture levels. Vertical and horizontal bars represent \pm SEM. Markers are the mean of four replicates. 266

List of plates

| | |
|---|-----|
| Plate 5.1: A sainfoin (left), and lucerne plant (right) 60 days after planting. | 61 |
| Plate 6.1: A view of the plants in the climate room. | 78 |
| Plate 6.2: Measurement of total and osmotic potential by Wescor. | 78 |
| Plate 6.3: Control (left), moderately (middle), and severely (right), stressed sainfoin plants. | 81 |
| Plate 6.4: The roots mass of the Eski for control (left), moderately (middle), and severely (right) stressed treatments. | 81 |
| Plate 7.1: A view of a single sainfoin plant grown in the pot (1.5m tall) . . . | 120 |
| Plate 7.2: (a) Plastic chips used for minimising evaporation, (b) Internal view of plant growth container. | 127 |
| Plate 7.3 Watering by hoses at seven depths (left), and location of hoses (right). | 128 |
| Plate 7.4 (a): A view of experiment in the glasshouse and measurements of soil moisture using TDR, (b) seven segments of roots for measurements of root length and root mass at seven depths. | 130 |
| Plate 8.1: a) Rain out shelter for imposing water stress in the field b) A view of field experiment. | 198 |
| Plat 8.2: A view of a) non-stressed plants and b) stressed plants in the field. | 199 |

Plate 8.3: Stressed plants under rain-out shelter 140 days after imposing water stress (top) and, soil cracking and impaired sainfoin growth (bottom). 205

Plate 8.4: The stomatal frequencies of a) adaxial and b) abaxial surfaces of leaf of sainfoin (Eski). 242

List of appendices

| | |
|--|-----|
| Appendix 4.1: Template used to estimate leaf area of lucerne during growth. | 324 |
| Appendix 6.1: Relative water content (RWC) of four sainfoin cultivars at three soil moisture levels. | 325 |
| Appendix 6.2: Stomatal resistance (s/cm) of the abaxial leaf surface of four sainfoin cultivars and three soil moisture levels. | 326 |
| Appendix 6.3: Stomatal resistance (s/cm) of the adaxial leaf surface of four sainfoin cultivars and three soil moisture levels. | 327 |
| Appendix 6.4: Leaf water potential (-MPa) of four sainfoin cultivars at three soil moisture levels at dawn in the glasshouse. | 328 |
| Appendix 6.5: Osmotic potential (-MPa) of four sainfoin cultivars at dawn under three soil moisture levels in the glasshouse | 329 |
| Appendix 6.6: Turgor potential (-MPa) of four sainfoin cultivars at dawn under three soil moisture levels in the glasshouse | 330 |
| Appendix 6.7: Leaf water potential (-MPa) of four sainfoin cultivars at dawn in the climate room under three soil moisture levels. | 331 |
| Appendix 6.8: Osmotic potential (-MPa) of four sainfoin cultivars at dawn under three soil moisture levels in the climate room | 332 |
| Appendix 6.9: Turgor potential (-MPa) of four sainfoin cultivars at dawn under three soil moisture levels in the climate room | 333 |

Appendix 6.10: Leaf water potential (-MPa) of four sainfoin cultivars at three soil moisture levels at midday in the climate room. 334

Appendix 6.11: Osmotic potential (-MPa) of four sainfoin cultivars at three soil moisture levels at midday in the climate room. 335

Appendix 6.12: Turgor potential (-MPa) of four sainfoin cultivars at three soil moisture levels at midday in the climate room. 336

Appendix 6.13: Leaf water potential (-MPa) (measured by pressure bomb) of four sainfoin cultivars at three soil moisture levels at midday in the climate room 337

Appendix 6.14: Photosynthesis ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$) of four sainfoin cultivar at three soil moisture levels during the last month in the climate room. . 338

Appendix 6.15: Leaflet numbers/pot of four sainfoin cultivars at three soil moisture levels. 339

Appendix 6.16: Estimated leaf area of four sainfoin cultivars at different soil moisture levels. 340

Appendix 6.17: The monthly transpiration rate (ml/pot/day) of four sainfoin cultivars at three soil moisture levels. 341

Appendix 6.18: Repeated measures analysis of morphological and physiological characters of sainfoin cultivar under three soil moisture levels 342

Appendix 6.19: The interaction between time * soil moisture * cultivar' Ψ at dawn in the climate room. 343

| | |
|---|-----|
| Appendix 6.20: The interaction between Time * Soil moisture * cultivar'π at dawn in the climate room. | 344 |
| Appendix 6.21: Template used to estimate leaf area of sainfoin during growth. | 345 |
| Appendix 7.1: Formulas used to calculate the moisture demand (I1...I7) for 125, 275, 425, 587.5, 762.5, 925, mm depths respectively, at regrowth harvests according to VSWC of 20 cm (A), 50 cm (B), 85 cm (C) depth. | 346 |
| Appendix 7.2: The average volumetric soil water content (cm^3/cm^3 , %) of pots to 70 cm depth measured by TDR at three harvests three plant types and two soil moisture treatments. | 347 |
| Appendix 7.3: Volumetric soil water content (cm^3/cm^3 %) of pots at early, late, and regrowth harvests under two soil moisture treatments at 0.2, 0.5, and 0.85 m depth at harvesting times. | 348 |
| Appendix 7.4: Root length density (m/m^3)* 10^3 at seven depths at the early harvest (45 DAP) of Eski, Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels. | 349 |
| Appendix 7.5: Root length density (m/m^3)* 10^3 at seven depths at the late harvest (75 DAP) of Eski, and Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels. | 350 |
| Appendix 7.6: Root length density (m/m^3)* 10^3 at seven depths at regrowth harvest (30 days cutting, 105 DAP) of Eski, and Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels. | 351 |
| Appendix 7.7: Root weight density (g/m^3) at seven depths at the early | |

| | |
|--|-----|
| harvest (45 DAP) of Eski, Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels. | 352 |
| Appendix 7.8: Root weight density (g/m^3) at seven depths at the late harvest (75 DAP) of Eski, and Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels. | 353 |
| Appendix 7.9: Root weight density (g/m^3) at seven depths, at regrowth harvest (105 DAP) of Eski, Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels. | 354 |
| Appendix 7.10: $P > F$ from pooled ANOVA over harvests for leaf water potential measured by pressure bomb (Ψ_p), leaf water potential measured by Wescor (Ψ), osmotic potential of the leaf measured by Wescor (π), turgor potential of the leaf (P), and osmotic potential of the leaf at full turgor measured by Decagon (π_{100}), over all three harvests. | 355 |
| Appendix 7.11: $Pr > F$ for the effect of time (dawn and midday) and time interactions for leaf water potential measured by Pressure bomb (Ψ_p), leaf water potential measured by Wescor (Ψ), osmotic potential of the leaf measured by Wescor (π), turgor potential of the leaf (P), and osmotic potential of the leaf at full turgor measured by Decagon ($\pi_{(100)}$), over three harvests. | 356 |
| Appendix 7.12: $Pr > F$ for pooled ANOVA over three harvests for root water potential (Ψ), osmotic potential (π), turgor potential (P), and root osmotic potential at full turgor (π_{100}), at two depths. | 357 |
| Appendix 7.13: $Pr > F$ for pooled ANOVA over three harvests for roots and leaves for water potential (Ψ), osmotic potential (π), turgor potential | |

(P), and root osmotic potential at full turgor (π_{100}). 358

Appendix 8.1: Soil water content (cm^3/cm^3) to 1.7m depth at soil water potentials of -1.5, -0.1, and -0.0005 MPa. (Adapted from Scotter et al. 1979a). 359

Appendix 8.2: Mean rainfall, temperature of the air and earth, and relative humidity (RH) (measured at Ag-Research, Grasslands 500 m from the Experimental area) during experiment in the field. 360

Appendix 8.3: The volumetric soil moisture at depth 0-0.15, 0.15-0.30, 0.30-0.50, 0.50-0.70 m for the stressed and non-stressed experiments for all three harvests, and regrowth of second harvest. 361

Appendix 8.4: Repeated measures analysis for comparison of the first (30 days after stress) and second (60 days after stress) harvests and their subsequent harvests for morphological characteristics. 362

Appendix 8.5: The results of repeated measures analysis for comparison of the first (30 days after stress) and second (60 days after stress) harvests and their subsequent harvests for physiological characteristics at dawn. 363

Appendix 8.6: The results of repeated measures analysis for comparison of the first (30 days after stress) and second (60 days after stress) harvests and their subsequent regrowth harvest for physiological characteristics at midday. 364

Appendix 8.7: Probability of significance for comparison of stomatal resistance (R_s)(s/cm) of adaxial, abaxial surfaces and total leaf R_s from the second harvest and related regrowth harvest by repeated measures analysis. 365