

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.

SOME EFFECTS OF BORON TO THE GROWTH AND CHEMICAL
COMPOSITION OF SAINFOIN (*ONOBRYCHIS*
VICIAEFOLIA SCOP.)

A thesis presented in partial fulfilment of
the requirements for the Degree of
Master of Agricultural Science
in Plant Science

at

Massey University
Palmerston North
New Zealand

Nenita Fabros Juan

1982

TABLE OF CONTENTS

	<u>Page</u>
Acknowledgements	i
List of Figures	ii
List of Tables	iii
List of Plates	vi
List of Appendices	vii
Abstract	1
<u>CHAPTER 1</u> Introduction	3
<u>CHAPTER 2</u> Review of Literature	
2.1 Introduction	5
2.1.1 The essentiality of boron	5
2.2 Sources of boron for plants	6
2.2.1 Determination of boron in plants and soil	6
2.2.2 Soil boron	8
2.2.3 Boron from commercially produced fertilizers	10
2.3 Role of boron in plants	11
2.3.1 Role of boron in the growth, development, and yield of plants	12
2.3.2 Boron in carbohydrate metabolism	18
2.3.2.1 Importance of nonstructural carbohydrates to legumes	18
2.3.2.2 Total nonstructural carbohydrate concentrations in plant parts of legumes	20
2.3.2.3 Boron in carbohydrate metabolism	21
2.3.3 Boron in nitrogen metabolism	25
2.3.3.1 Importance of nitrogen in plant growth and development	25
2.3.3.2 Sources of nitrogen for plants	26
2.3.3.3 Boron in nitrogen metabolism	28
2.3.4 Boron in nodulation and nitrogen fixation	30
2.3.4.1 Importance of inoculation to legumes	30
2.3.4.2 Boron in nodulation and in nitrogen fixation	32
2.4 Boron deficiency and toxicity symptoms in plants	33
2.4.1 Visual symptoms of boron deficiency and toxicity in plants	33
2.5 Distribution and accumulation of boron in plants	34

CHAPTER 3 Materials and Methods

3.1	Introduction	37
3.1.1	Experimental site	37
3.1.2	Experimental lay-out	37
3.2	Experimental materials, procedures and techniques	38
3.2.1	Planting materials, pregerminating the seeds and planting	38
3.2.2	Growth medium and potting procedure	38
3.2.3	Preparation of inoculant and inoculation	38
3.2.4	Nutrient solution and application	39
3.2.5	Watering and flushing	39
3.3	Analytical procedure and techniques	40
3.3.1	Harvesting	40
3.3.2	Total nonstructural carbohydrate, boron, nitrogen and phosphorus analyses	40
3.4	Statistical analysis	41
3.4.1	Relative growth rate	41
3.4.2	Analysis of variance	41
3.4.3	Curve fitting	42
3.4.4	Multiple regression	42

CHAPTER 4 Results

4.1	General description of the early establishment and growth of sainfoin plants	44
4.2	Plant growth and development	49
4.2.1	Relative growth rates	49
4.2.1.1	Plant relative growth rate	49
4.2.1.2	Shoot relative growth rate	50
4.2.1.3	Shoot relative growth rate	50
4.2.1.4	Mean relative growth rates of plant, shoot and root of eight periods of growth	51
4.2.2	Growth in total dry weight and component parts	51
4.2.2.1	Plant dry weight	66
4.2.2.2	Shoot dry weight	67
4.2.2.3	Root dry weight	68
4.2.3	Growth in number of lateral roots and leaf area	69
4.2.3.1	Number of first order lateral roots	69
4.2.3.2	Number of second order lateral roots	70
4.2.3.3	Leaf area	70

	<u>Page</u>
4.3 Chemical composition of sainfoin cv Fakir	93
4.3.1 Total nonstructural carbohydrates	93
4.3.1.1 Plant total nonstructural carbohydrates	93
4.3.1.2 Shoot total nonstructural carbohydrates	94
4.3.1.3 Root total nonstructural carbohydrates	94
4.3.2 Total boron concentration, and boron uptake	95
4.3.2.1 Plant total boron concentration and boron uptake	95
4.3.2.2 Shoot total boron concentration and boron uptake	98
4.3.2.3 Root total boron concentration and boron uptake	97
4.3.3 Total nitrogen concentration and nitrogen uptake	98
4.3.3.1 Shoot total nitrogen concentration and shoot nitrogen uptake	98
4.3.3.2 Root total nitrogen concentration and root nitrogen uptake	98
4.3.4 Total phosphorus concentration and phosphorus uptake	99
4.3.4.1 Shoot total phosphorus concentration and shoot phosphorus uptake	99
4.3.4.2 Root total phosphorus concentration and root phosphorus uptake	100
4.4 Relationship of the plant dry weight, plant total nonstructural carbohydrates and plant boron content to the chemical composition of sainfoin	100
4.4.1 Relative importance of some sainfoin chemical composition in determining plant dry weight, plant total nonstructural carbohydrates and plant boron content	100
 <u>CHAPTER 5</u> Discussion	
5.1 General description of the early establishment and growth of sainfoin	114
5.2 Plant growth and development	115
5.3 Effects of boron on the chemical composition of sainfoin cv Fakir	117
5.3.1 Total nonstructural carbohydrates	117
5.3.2 Boron concentrations and boron uptake in sainfoin	119

	<u>Page</u>
5.3.3 Nitrogen and phosphorus concentrations and uptake	120
5.4 Total plant dry weight, plant total nonstructural carbohydrates, and boron concentration and chemical components of sainfoin	122
<u>CHAPTER 6</u> Conclusion	124
Bibliography	125
Appendices	140

ACKNOWLEDGEMENTS

I wish to extend my sincere gratitude and appreciation to my supervisor, Mr Angus G. Robertson for his constant and unrestricted help and guidance throughout the study.

The joint support of the External Aid Division of the Ministry of Foreign Affairs, New Zealand, Tarlac College of Agriculture, Philippines, and Agronomy Department at Massey University is gratefully acknowledged.

Special thanks are due to the following:

Mr Jim M. Fortune, Massey University for supplying the sainfoin (cv Fakir) seeds used in the research.

The Plant Growth Unit for the use of their laboratory facilities. I would like to thank Mr Gary Cranfield, in particular.

Mr Mike Speirs, Department of Agriculture and Fisheries, Levin, and Dr R.M. Haslemore, DSIR, Plant Physiology Division for their technical assistance in the boron and sugar analyses, respectively.

Dr Ian L. Gordon, Massey University for his help in the statistical analysis of the experiment.

Mr Steve Black, Computer Center, Massey University for his time and help liberally given in the computer and programming works involved in the study.

The staffs and postgraduate students of the Agronomy Department for their friendship and help offered.

Mrs Patricia Fleet for her fine and competent typing, and Mr Peter Ellingham for his skilful illustrations.

My grateful acknowledgements are also sincerely extended to my parents, Mr & Mrs Marcelino J. Juan, and my brothers and sisters, and my friends, Mr C.P. Ramos and his family, in particular, without whose love, encouragements and prayers, this work would not have been completed.

Above all, to Him who made this work possible, in sincerity, I gratefully acknowledge the faithfulness and sustenance that I have received from my Heavenly Father throughout my study.

LIST OF FIGURES

		<u>Page</u>
Figure 1	Relative growth rate of whole plant (mg/mg/day) for eight periods of growth	53
Figure 2	Relative growth rate of shoot (mg/mg/day) for eight periods of growth	55
Figure 3	Relative growth rate of root (mg/mg/day) for eight periods of growth	57
Figure 4	Total plant dry weight (mg/plant) for nine harvest times	73
Figure 5	Growth curves of the whole plant from the six treatments	74
Figure 6	Total shoot dry weight (mg/shoot) for nine harvest times	79
Figure 7	Growth curves of shoot from the six treatments	80
Figure 8	Total root dry weight (mg/root) for nine harvest times	85
Figure 9	Growth curves of root from the six treatments	86
Figure 10	Plant nonstructural carbohydrates (TNC) (%) for eight harvest treatments	103

LIST OF TABLES

	<u>Page</u>	
Table 1	Mean relative growth rate (mg/mg/day) of the whole plant as affected by the different combinations of treatments for 8 periods of growth	52
Table 2	Mean relative growth rate of shoot (mg/mg/day) as affected by the different combinations of treatments for 8 periods of growth	54
Table 3	Mean relative growth rate of root (mg/mg/day) as affected by the different combinations of treatments for 8 periods of growth	56
Table 4	Summary of relative growth rate of the whole plant (mg/mg/day) for 8 periods of growth	58
Table 5	Mean relative growth rate of sainfoin plant at different periods of growth: (a) Harvest 1 (b) Harvest 3 (c) Harvest 5	59
Table 6	Summary of relative growth rate of shoot (mg/mg/day) for 8 periods of growth	60
Table 7	Mean relative growth rate of shoot at different periods of growth: (a) Harvest 1 (b) Harvest 3 (c) Harvest 5 (d) Harvest 8	61
Table 8	Summary of relative growth rate of root (mg/mg/day) for 8 periods of growth	62
Table 9	Mean relative growth rate of the root at different periods of growth: (a) Harvest 1 (b) Harvest 3 (c) Harvest 4	63
Table 10	Summary of the mean relative growth rate of the whole plant, shoot and root of eight periods of growth	64
Table 10a	Mean relative growth rate of the root (mg/mg/day) of eight periods of growth	65
Table 11	Summary of total dry matter yield (mg/plant) for the whole plant for 9 harvest times	71
Table 12	Mean dry weight of sainfoin (mg/plant) at different harvest times: (a) Harvest 1 (b) Harvest 4 (c) Harvest 8	72
Table 13	Information from linear regression	75
	$\ln \frac{\hat{Y}}{Y_0 - Y} = \beta_0 + \beta_1 x$ for the plant dry weight of the six treatments	

Table 14	Summary of total dry matter yield of shoot (mg/mg/shoot) for 9 harvest times.	76
Table 15	Mean dry weight of sainfoin shoot (mg/plant-shoot) at different harvest times: (a) Harvest 1 (b) Harvest 4 (c) Harvest 6 (d) Harvest 8 (e) Harvest 9	77
Table 16	Information from linear regression $\ln \frac{Y}{\hat{Y}_0 - Y} = \beta_0 + \beta_1 x$ for the shoot dry weight of the six treatments.	81
Table 17	Summary of total dry matter yield of root (mg/root) for 9 harvest times	82
Table 18	Mean dry weight of root (mg/plant-root) at different harvest times: (a) Harvest 1 (b) Harvest 3 (c) Harvest 4 (d) Harvest 5 (e) Harvest 8 (f) Harvest 9	83
Table 19	Information from linear regression dry $\ln \frac{Y}{\hat{Y}_0 - Y} = \beta_0 + \beta_1 x$ for the root dry weight of the six treatments	87
Table 20	Summary of number of first order lateral roots for the first 6 harvest times	88
Table 20a	Mean number of first order lateral roots at harvest 4	89
Table 21	Summary of number of second order lateral roots for four harvest times	90
Table 21a	Mean number of the second order lateral roots at harvest 4	91
Table 22	Summary of the total leaf area (cm ²) for the last 3 harvest times	92
Table 23	Summary of total nonstructural carbohydrates (%) of the whole plant for 8 harvest times	101
Table 24	Mean total nonstructural carbohydrates of the plant at harvest (a) 3 and (b) 6	102
Table 25	Summary of the total nonstructural carbohydrates of the shoot at three harvest times	104
Table 25a	Mean total nonstructural carbohydrates of the shoot at harvest 7	105
Table 26	Summary of the total nonstructural carbohydrates (%) of the root at three harvest times	106

Table 27	Summary of the boron concentration ($\mu\text{g/g}$) and boron uptake ($\mu\text{g/plant}$) of the whole plant, shoot, and root respectively	107
Table 27a	Mean boron concentration ($\mu\text{g/g}$) of the whole plant at harvest 9	108
Table 27b	Mean boron uptake ($\mu\text{g/shoot}$) of the shoot at harvest 9	109
Table 27c	Mean boron concentration ($\mu\text{g/g}$) of the root at harvest 9	110
Table 28	Summary of the nitrogen concentration (mg/g) and nitrogen uptake (mg/plant) of the shoot, and the root, respectively	111
Table 29	Summary of the phosphorus concentration (mg/g) and phosphorus uptake (mg/plant) of the shoot, and the root, respectively	112

LIST OF PLATES

		<u>Page</u>
Plate 1	Representative sainfoin plants from the six treatments	46
Plate 2	Boron deficiency symptoms in sainfoin. Margins of leaves turn yellow then take on reddish tinge	47
Plate 3	Reddening petioles of a boron-deficient sainfoin	48

LIST OF APPENDICES

	<u>Page</u>	
Appendix 1	Experimental lay-out	140
Appendix 2	Preparation and handling of media used in Rhizobial culture	141
Appendix 3	Determination of soluble sugars (TNC) in plant material	143
Appendix 4	Determination of boron	145
Appendix 5	Nitrogen determination by Kjeldahl technique	147
Appendix 6.1	Analysis of variance for relative growth rate of whole plant (mg/mg/day) for eight periods of growth	149
Appendix 6.2	Analysis of variance for relative growth rate of shoot (mg/mg/day) for eight periods of growth	152
Appendix 6.3	Analysis of variance for relative growth rate of root (mg/mg/day) for eight periods of growth	155
Appendix 6.4	Analysis of variance for mean relative growth rate of (1) the whole plant (2) the shoot (3) the root of eight periods of growth	158
Appendix 6.5	Analysis of variance for the total dry weight of the whole plant (mg/plant) for nine harvest times	159
Appendix 6.6	Analysis of variance for the total dry weight of shoot (mg/shoot) for nine harvest times	162
Appendix 6.7	Analysis of variance for the total dry weight of root (mg/root) for nine harvest times	165
Appendix 6.8	Analysis of variance for the number of first order lateral roots for six harvest times	168
Appendix 6.9	Analysis of variance for the number of second order lateral roots for four harvest times	170
Appendix 6.10	Analysis of variance for total leaf area (cm ²) for the last three harvest times	172

Appendix 6.11	Analysis of variance for total nonstructural carbohydrates of the whole plant (%) for eight harvest times	173
Appendix 6.12	Analysis of variance for total non-structural carbohydrates of shoot (%) for the last three harvest times	176
Appendix 6.13	Analysis of variance for total non-structural carbohydrates of root (%) for the last three harvest times	177
Appendix 6.14	Analysis of variance for (a) boron concentration ($\mu\text{g/g}$), and (b) boron uptake ($\mu\text{g/plant}$) of the whole plant at final harvest	178
Appendix 6.15	Analysis of variance for (a) shoot boron concentration ($\mu\text{g/g}$), and (b) shoot boron uptake ($\mu\text{g/shoot}$) at final harvest	179
Appendix 6.16	Analysis of variance for (a) root boron concentration ($\mu\text{g/g}$), and (b) root boron uptake ($\mu\text{g/root}$) at final harvest	180
Appendix 6.17	Analysis of variance for (a) shoot nitrogen concentration (mg/g), and (b) shoot nitrogen uptake (mg/shoot) at final harvest	181
Appendix 6.18	Analysis of variance for (a) root nitrogen concentration (mg/g), and (b) root nitrogen uptake (mg/root) at final harvest	182
Appendix 6.19	Analysis of variance for (a) shoot phosphorus concentration (mg/g), and (b) shoot phosphorus uptake (mg/shoot) at final harvest	183
Appendix 6.20	Analysis of variance for (a) root phosphorus concentration (mg/g), and (b) root phosphorus uptake (mg/root) at final harvest	184
Appendix 7	Summary of multiple regression of plant dry weight, plant total nonstructural carbohydrates, and plant boron content with some chemical components of sainfoin	185

ABSTRACT

Some effects of boron on the growth and chemical composition of sainfoin (*Onobrychis viciaefolia* Scop.) plants cv Fakir were evaluated in a glasshouse.

The growth and development of sainfoin plants was not affected by the different levels of boron applied but was affected by nitrogen application and inoculation due to the nodulation failure of the latter. Generally, the root showed the highest dry matter yield and the fastest relative growth rate.

Similarly, the total nonstructural carbohydrates of the sainfoin plants were not affected by the different levels of boron. Nitrogen application reduced the total nonstructural carbohydrates of the whole plant. Moreover, when 1 ppm boron was applied, both the shoot and the root yielded the highest total nonstructural carbohydrates. Likewise, root and shoot total nonstructural carbohydrates were reduced by the application of nitrogen. Roots gave a higher total nonstructural carbohydrate yield than the shoot.

Boron content of the whole sainfoin plant, the shoot and the root ranging from 0 - 55 $\mu\text{g/g}$ increased in proportion with the increment of boron applied. Similar results were obtained from boron uptake of the whole plant, the shoot and the root. There was a depression of boron concentrations and boron uptake of the whole plant, the shoot and the root, when nitrogen was applied, implying a deficiency situation.

Although nonsignificant effects of boron levels were obtained from nitrogen and phosphorus concentration and uptake, respectively, of both shoot and root, application of 2 ppm boron reduced the concentration of nitrogen but not nitrogen uptake, and reduced phosphorus concentration and phosphorus uptake. Application of nitrogen increased shoot and root nitrogen contents and nitrogen uptake but decreased root and shoot phosphorus concentrations and phosphorus uptake.

It was concluded that levels of 2 ppm boron concentration were not adequate to support satisfactory growth when plants were supplied with sufficient levels of other nutrients.

Keywords: Boron, nitrogen, *Rhizobium*, total nonstructural carbohydrates (TNC)

INTRODUCTION

Sainfoin (*Onobrychis viciaefolia* Scop.), a newly reintroduced forage crop in New Zealand, has drawn considerable interest in the research field. It is thought to have a nutritional value, no bloating tendencies as a livestock feed and a potential substitute for alfalfa (*Medicago sativa* L.) (Clarke & Reid, 1974; Jones & Lyttleton, 1971; Usman *et al.*, 1968) and known to be a drought and winter hardy crop (Koch *et al.*, 1972; Spedding & Diekmahns, 1972). However, it has not been accepted agriculturally because of its excessive coarseness, poor leafiness and probable low palatability (Eslick, 1968), visual characters which can be misleading. In addition, it has been found to have poor establishment with *Rhizobium* infection, leading to poor nodulation, and thus to inefficient nitrogen fixation. In some cases, plants are nodulated abundantly but nodules are ineffective and are inefficient in fixing dinitrogen (Ross & Delaney, 1971; Burton & Curley, 1968; Sims *et al.*, 1968). Sainfoin, however, was reported to outyield alfalfa consistently in areas where production is limited to one cutting (Hanna & Smoliak, 1968). Likewise, it also grows better on drylands where precipitation is sufficient to grow one hay crop (Cooper, 1965). In addition, sainfoin cotyledons were found to contribute as much as 100, 54, and 18% of total seedling photosynthesis at 7, 11, and 19 days, respectively (Fransen & Cooper, 1974). Cotyledons of sainfoin, therefore, provide a substantial contribution to the nourishment of newly emerged seedlings, giving them a better establishment while the seedlings have no nodules yet to supply their nitrogen requirement.

Several legumes, especially forage species that are potentially capable of fixing dinitrogen efficiently have not been well studied. Due to the limited research that had been conducted on these forage legumes, i.e., cicer milkvetch (*Astralagus cicer* L.) and sainfoin, there is also limited knowledge on the proper management of these forage crops

(Major *et al.*, 1979). This includes water relations, temperature, light, soil and nutrient requirements and also proper cultural management practices, conditions that must be favourable for a successful plant growth and development. Likewise, the efficiency of the symbiotic relationship depends also on the host's capacity to provide its partner, i.e., *Rhizobia* the necessary energy requirement for dinitrogen fixation. Hence, a healthy growing legume plant has a greater capacity to provide the *Rhizobia* their energy requirement.

Despite the numerous disadvantages of growing sainfoin as mentioned previously, it has a greater potential as a livestock feed in the future. Research is needed to meet the need for more managerial and agronomic information on yield responses in terms of hay and total dry matter production as well as responses to symbiotic relationship.

One aspect of study which this project was designed to investigate is the role of micronutrient boron which is essential in plant growth and development, reproduction, carbohydrate metabolism, nitrogen metabolism, nodulation and nitrogen fixation.

The purpose of this study was to examine some of the effects of boron in plant growth and development. It included two components namely:

1. The effects of boron on the early growth and development of sainfoin in terms of dry matter production of both root and shoot, relative growth rate, root number, and leaf area, and
2. the effects of boron on the chemical composition of the sainfoin plant on the whole, specifically on boron concentration and uptake and total non-structural carbohydrate (TNC) content.

Analyses of both root and shoot tissues were completed to determine nitrogen and phosphorus contents.