DUAL MUTUALISTIC ASSOCIATIONS IN SAINFOIN

(*Onobrychis viciifolia* Scop.)

A thesis presented in partial fulfilment of
the requirements for the degree of
Master of Agricultural Science
in Agronomy at
Massey University

Kee Fui, Kon
1982

ABSTRACT

Recent studies established that many legumes, when infected with the appropriate *Rhizobium* spp. and arbuscular fungi, nodulated better and exhibited greater dinitrogen fixation than plants infected with only the rhizobia.

A similar study, therefore, was carried out in a glasshouse using sainfoin *(Onobrychis viciifolia* Scop.*)*, a legume that is rapidly gaining recognition as a potential forage crop in New Zealand and other parts of the world. Pre-germinated seeds (cv. Fakir) were planted in sterilized soils and incubated with an effective *Rhizobium* spp. (strain NZP 5301), a mixture of endophytes *(Gigaspora magarita* Becker & Hall, *Glomus fasciculata* (Thax. sensu Gerd.) Gerdemann & Trappe and *Glomus tenuis* (Greenall) Hall), or both eht rhizobia and endophytes. The experiment also included a control, without any inoculation. Endophyte infection, nodulation and dinitrogen fixation, total nitrogen and phosphorus concentrations, and plant growth and development were determined on eleven sequential samplings over about twenty weeks, up to the stage of green inflorescence.

Arbuscular mycorrhiza formation did not occur with the first endophyte inoculation, containing *Gigaspora magarita* Becker & Hall, even after 93 days of growth. This is probably because the inoculum used consisted of a low quantity of viable spores and mycelia. The second inoculation, containing the three endophyte species, produced only a low degree of infection between day 115 and 137, possibly because the extensive root lignification and relatively higher root phosphorus concentration (0.50%) restricted fungal invasion and establishment within the root cortex. Mycorrhiza formation did not increase phosphate uptake, improve nodulation and dinitrogen fixation, or increase plant growth. This is due probably to the already well-developed root systems that were efficiently exploiting the small soil volume within the bags.

Rhizobia-inoculated plants produced more nodules, larger nodules and consequently, a greater nodule dry weight than the uninoculated plants. The nodules produced in the inoculated plants were red

instead of green as in the uninoculated plants, and exhibited a greater dinitrogen fixation. As a result, these inoculated plants contained a higher concentration of shoot, root and nodule nitrogen, and a greater dry weight accumulation in the shoots and nodules. The shoot and nodule phosphorus concentrations, however, were lower in the rhizobia-inoculated than in the uninoculated plants due to the greater amount of shoot and nodule tissues which caused a dilution effect. These rhizobia effects on nodulation and dinitrogen fixation, nitrogen and phosphorus concentrations, and plant growth and development became more prominent with time.

The relatively higher nodule phosphorus concentration when compared with the shoot and root phosphorus concentrations suggests that phosphorus was presumably required in large quantities by the dinitrogen-fixing system.

PREFACE

Coexistence of organisms has long been recognised as an axism of life. In 1952, Paul R. Bulkholder formally and objectively interpreted coexistence as different biological interactions. Based on his coaction theory, these interactions were classified into and named as nine separate categories of which the most studied in agricultural ecology are competition and mutualism.

In this thesis, two examples, of mutualism, involving a forage legume *(Onobrychis viciifolia* Scop.*)*, a nitrogen-fixing bacterium *(Rhizobium* spp.*)* and three species of arbuscular fungi *(Gigaspora magarita* Becker & Hall, *Glomus fasciculatus* (Thax. sensu Gerd.) Gerdemann & Trappe and *Glomus tenuis* (Greenall) Hall), are examined. The intention of this study was to investigate the real value of coexistence of these organisms from an agricultural standpoint and, therefore, emphasis is placed on the effects of the bacterium and fungi on the nutrition, and growth and development of sainfoin. While the bulk of chapters 4, 5, 6 and 7 is devoted to these topics, the relevant background information of the research is also included in the first three chapters.

Various persons were directly and indirectly involved in the completion of this work. I am deeply indebted to Mr Angus G. Robertson for his close supervision and unceasing availability in offering advice, suggestions and practical assistance during this entire masterate programme, and his many criticisms and recommendations during editing of the manuscript. I must also acknowledge his foremost contribution to me as a research student in helping me to develop the skill of more effective thinking in scientific research.

Dr Conway Ll. Powell, of the MAF Ruakura Soil and Plant Research Station in Hamilton, was most generous in supplying a substantial quantity of fungal inocula as my initial cultures. Throughout the study, he, being an outstanding world authority on mycorrhiza research, showed a deep interest in the work and provided many prompt suggestions which were invaluable.

Sainfoin seeds (cv. Fakir) were kindly supplied by Mr Jim A. Fortune, of the Agronomy Department. I am also grateful for the

permission to sample some sainfoin plants from his experimental plots, and his many suggestions.

The methodology of acetylene reduction assay was kindly introduced and demonstrated by Dr Jim A. Crush and Mr Paul Yarrell, of the DSIR Grasslands Division, Palmerston North. Owing to certain unavailable glassware, the assaying procedure was slightly modified, but the value of their contributions remains. I am thankful for the privilege to use the Pye gas chromatograph and other facilities in the Botany Department as well as the technical assistance given by Dr David W. Fountain and his technician, Mr Chong Loong Kan.

The colorimetric autoanalysis of both total plant nitrogen and phosphorus was kindly conducted by Mr Russell W. Tillman, of the Soil Science Department and, therefore, a considerable amount of routine work was reduced, enabling me to concentrate on other aspects of the study. His instructions on the preparation of the Kjeldahl digest reagent and Kjeldahl digestion are also fully appreciated.

I am exceedingly grateful to Mr Hugh Nielson, of the Horticulture and Plant Health Department, for the supply of some chemical reagents and classware, and his assistance in compound-microscope photography. All the micrographs in this volume are his fine work.

Appreciation is expressed to Dr Murray J. Hill for the permission to use the weighing facilities in the Seed Technology Centre and the assistance received from his technician, Mrs Karen Johnstone.

I wish to thank Dr Ian L. Gordon, of the Agronomy Department and Mr Greg C. Arnold, of the Mathematics and Statistics Department, for advice in statistical methods. I am also exceedingly grateful to Dr Neil A. Macgregor for his general recommendations and the great interest he took in this research. To my typist, Mrs Cecily Willbond, I wish to extend my sincere appreciation for her efficient and excellent work.

Very special appreciation must be made to my wife, Lih Ju, for her long-suffering, financial assistance and unsacrificial contribution of her time and energy in helping me in the experimental work, while also fulfilling the role of a homemaker and breadwinner. Financial awards from Helen E. Akers (two years), John Alexander Hurley, William Hudson and the Christian Centre Palmerston North are also gratefully acknowledged.

Finally, I wish to acknowledge the inspiration from the Holy

Spirit and God's gift of the ineffable awesome creation which I intimately worked with for over five months. The opportunity is here for me to return the magnificent glory of His ingenious design which aptly speaks of His omniscience.

TABLE OF CONTENTS

Page

ABSTRACT ii.

PREFACE iv.

TABLE OF CONTENTS vii.

LIST OF TABLES xi.

LIST OF FIGURES xv.

LIST OF PLATES xvii.

LIST OF APPENDICES xviii.

CHAPTER

1. INTRODUCTION 1.

2. TERMINOLOGY 5.
  - 2.1 Introduction 5.
  - 2.2 Terms Used in Dinitrogen Fixation 5.
    - 2.21 Infection, invasion and infectivity 5.
    - 2.22 Mutualism and symbiosis 5.
    - 2.23 Rhizobia, *Rhizobium* and *Rhizobium* spp. 6.
  - 2.3 Terms Used in Mycorrizae 6.
    - 2.31 Endophyte 6.
    - 2.32 Mycorrhiza types 6.

3. REVIEW OF LITERATURE

  - 3.1 Introduction 7.
  - 3.2 Sainfoin as a Crop 8.
    - 3.21 General distribution and history 8.
    - 3.22 Botanical description 10.
    - 3.23 Some agricultural characteristics 12.
    - 3.24 Suitability in New Zealand conditions 15.
  - 3.3 Dinitrogen-Fixing System of Legumes 17.
    - 3.31 Development of the legume-*Rhizobium* system 17.
    - 3.32 Dinitrogen fixation 20.
    - 3.33 Methods of improving dinitrogen fixation 25.

CHAPTER Page

3.4 Mycorrhiza System of Legumes 28.

3.41 Occurrence 28.

3.42 Development of arbuscular mycorrhizae 29.

3.43 Arbuscular mycorrhizae and phosphorus nutrition 33.

3.44 Arbuscular mycorrhizae and nitrogen nutrition 40.

3.5 Agricultural Significance of Dual Mutualistic Associations 42.

4. EXPERIMENTAL MATERIALS AND METHODS 44.

4.1 Introduction 44.

4.2 Preliminary Experiments 44.

4.21 Glasshouse test 44.

4.22 Field test 45.

4.3 The Principal Experiment 45.

4.31 Experimental design and layout 45.

4.32 Schedule of destructive sampling 47.

4.33 Experimental measurements 48.

4.4 Preparation of Materials 48.

4.41 Growth containers 48.

4.42 Growth medium 48.

4.43 Sainfoin seedlings 48.

4.44 Rhizobia inoculum 50.

4.45 Endophyte inoculum 50.

4.46 Nutrient solutions 50.

4.5 Experimental Procedures 51.

4.51 Control of glasshouse conditions 51.

4.52 Potting 51.

4.53 Inoculation of endophyte 51.

4.54 Planting 52.

4.55 Inoculation of rhizobia 52.

4.56 Thinning and replacement of seedlings 52.

4.57 Watering 53.

4.58 Nutrient application 53.

4.6 Analytical Techniques 53.

4.61 Sampling method 53.

4.62 Removal and washing of plants 54.

CHAPTER Page

- 4.63 Determination of plant dry weight 54.
- 4.64 Estimation of endophyte infection 54.
- 4.65 Determination of dinitrogen fixation 55.
- 4.66 Determination of total nitrogen and phosphorus 56.

4.7 Problem with Endophyte Infection 57.
- 4.71 Reisolation of Fm 57.
- 4.72 Reinoculation with MX 57.
- 4.73 Detection of condensed tannins 57.
- 4.74 Determination of root morphology 58.

4.8 Statistical Methods 58.
- 4.81 Data transformation 58.
- 4.82 Analysis of variance 59.
- 4.83 Coefficient of variation and standard error 61.
- 4.84 Duncan's new multiple range test 61.
- 4.85 Regression analysis 61.

5. EXPERIMENTAL RESULTS 64.

5.1 Introduction 64.

5.2 Preliminary Results 64.
- 5.21 Glasshouse test 64.
- 5.22 Field test 64.

5.3 Development of Arbuscular Mycorrhizae 66.
- 5.31 First inoculation with Fm 66.
- 5.32 Condensed tannins and root morphology of sainfoin 66.
- 5.33 Second inoculation with MX 66.

5.4 Development of Dinitrogen-Fixing System 69.
- 5.41 Infection 69.
- 5.42 Nodulation 69.
- 5.43 Dinitrogen fixation 75.
- 5.44 Regression analysis 80.

5.5 Total Nitrogen and Phosphorus in Plant Tissue 80.
- 5.51 Total nitrogen 80.
- 5.52 Total phosphorus 90.
- 5.53 Correlation studies 98.

5.6 Growth and Development of Sainfoin 101.

CHAPTER Page

5.61 Plant dry weight 101.
5.62 Root-shoot ratio 107.
5.63 Secondary stem production 107.
5.64 Regression analysis 114.

6. DISCUSSION 115.
6.1 Introduction 115.
6.2 Development of Arbuscular Mycorrhizae 115.
6.21 First inoculation with Gm 115.
6.22 Second inoculation with MX 117.
6.3 Development of Dinitrogen-Fixing System 118.
6.31 Infection 118.
6.32 Nodulation 118.
6.33 Dinitrogen fixation 120.
6.4 Total Nitrogen and Phosphorus in Plant Tissue 122.
6.41 Total nitrogen 122.
6.42 Total phosphorus 124.
6.5 Growth and Development of Sainfoin 126.
6.51 Plant dry weight 126.
6.52 Root-shoot ratio 127.
6.53 Secondary stem production 128.

7. CONCLUSIONS 129.

APPENDICES 131.
REFERENCES CITED 175.

LIST OF TABLES

Table		Page
1.1	Concentration of sixteen elements in complex plants	2.
1.2	Efficiency and contribution from various dinitrogen-fixing systems	2.
3.1	Carbon and nitrogen in the nodules of three legumes	24.
4.1	Schedule of destructive sampling	47.
4.2	Computed variables used in statistical analyses	49.
4.3	Equations of transformation of various variables	60.
5.1	Means of endophyte-infected root cortex of sainfoin (var. *bifera* Hort.) in the glasshouse	65.
5.2	Means of endophyte-infected root cortex of sainfoin (cvs. Fakir and Remont) in the field	65.
5.3	Means of infected root cortex of corn with *Gigaspora magarita* Becker & Hall	67.
5.4	Means of condensed tannin score of different sainfoin plant components	67.
5.5	Mean root diameter, mean extension of the root hair cylinder from the epidermis and root hair abundance for tertiary rootlets of four legumes	68.
5.6	Means of infected root cortex of corn and sainfoin with a mixture of endophytes (MX)	70.
5.7	Transformed treatment means of endophyte infection per field of view (x 10^{-2})	70.
5.8	Transformed treatment means of nodule number per plant	73.
5.9	Transformed treatment means of nodule dry weight per plant	74.
5.10	Transformed treatment means of nodule dry weight per nodule (X10^{-2})	76.
5.11	Transformed treatment means of acetylene reduced per hour per plant	77.

Table		Page
5.12	Original treatment means of acetylene reduced per hour per gram nodule dry weight	78.
5.13	T-test values for homogeneity of regression slopes between nodule dry weight and nodule number, and nodule dry weight and nodule size	81.
5.14	Summary of stepwise multiple regression analysis for different treatments	82.
5.15	Transformed treatment means of total cotyledon nitrogen	84.
5.16	Transformed treatment means of total shoot nitrogen	85.
5.17	Transformed treatment means of total root nitrogen	86.
5.18	Transformed treatment means of total nodule nitrogen	87.
5.19	Original treatment means of total plant nitrogen	88.
5.20	Transformed treatment means of total cotyledon phosphorus	93.
5.21	Original treatment means of total shoot phosphorus	94.
5.22	Original treatment means of total root phosphorus	95.
5.23	Transformed treatment means of total nodule phosphorus	96.
5.24	Original treatment means of total plant phosphorus	97.
5.25	Correlations of tissue nitrogen and acetylene reduction	99.
5.26	Correlations of tissue phosphorus and acetylene reduction	100.
5.27	Original treatment means of cotyledon dry weight per plant ($x10^{-3}$)	103.
5.28	Transformed treatment means of shoot dry weight per plant	104.
5.29	Transformed treatment means of root dry weight per plant	105.

Table Page

5.30 Transformed treatment means of total plant dry weight per plant 106.

5.31 Transformed treatment means of root-shoot ratio 109.

5.32 Transformed treatment means of secondary stem number per plant 110.

5.33 T-test values for homogeneity of regression slopes between shoot dry weight and root dry weight and between shoot dry weight and nodule dry weight 111.

5.34 Summary of stepwise multiple regression analysis for different treatments 113.

LIST OF FIGURES

Figure Page

3.1 Factors affecting the energy supply for dinitrogen fixation in a legume nodule 26.

3.2 Model of soil phosphates and inorganic phosphorus in different pools 34.

3.3 Root hairs and extramatrical mycelium in the soil 34.

4.1 Experimental layout in the glasshouse 46.

5.1 Mycorrhiza infection of sainfoin measured as score of infected cortex per microscope field of view over time (zero infection obtained for all treatments before day 115) 72.

5.2 Nodule number per sainfoin plant over time (zero data set for all treatments before day 36) 72.

5.3 Nodule dry weight per sainfoin plant over time (zero data set for all treatments before day 36) 72.

5.4 Nodule dry weight per sainfoin nodule over time (zero data set for all treatments before day 36) 72.

5.5 Acetylene reduced per hour per sainfoin plant over time (no assay made before day 77) 79.

5.6 Acetylene reduction per hour per gram nodule dry weight of sainfoin over time (no assay made before day 77) 79.

5.7 Simple regression plots of nodule dry weight against nodule number per sainfoin plant for treatments C, E, R and B 79.

5.8 Simple regression plots of nodule dry weight per sainfoin plant against nodule dry weight per nodule for treatments C, E, R and B 79.

5.9 Total cotyledon nitrogen concentration of sainfoin over time (inadequate tissue for analysis at day 93, while zero data sets after day 104) 83.

5.10 Total shoot nitrogen concentration of sainfoin over time 83.

Figure Page

5.11 Total root nitrogen concentration of sainfoin over time 83.

5.12 Total nodule nitrogen concentration of sainfoin over time (inadequate tissue for analysis at day 36, 45 and 61, while zero data set before day 27) 83.

5.13 Total cotyledon phosphorus concentration of sainfoin over time (inadequate tissue for analysis at day 93, while xero data sets after day 104) 91.

5.14 Total shoot phosphorus concentration of sainfoin over time 91.

5.15 Total root phosphorus concentration of sainfoin over time 91.

5.16 Total nodule phosphorus concentration of sainfoin over time (inadequate tissue for analysis at day 36, 45 and 61, while zero data set before day 27) 91.

5.17 Total plant nitrogen concentration of sainfoin over time 92.

5.18 Total plant phosphorus concentration of sainfoin over time 92.

5.19 Cotyledon dry weight per sainfoin plant over time (zero data sets after day 104) 102.

5.20 Shoot dry weight per sainfoin plant over time 102.

5.21 Root dry weight per sainfoin plant over time 102.

5.22 Total plant dry weight per sainfoin plant over time 102.

5.23 Root-shoot ratio of sainfoin over time 108.

5.24 Secondary stem number per sainfoin plant over time (zero data set before day 77) 108.

5.25 Regression plots between shoot dry weight and root dry weight of sainfoin (R_1 and B_1 are plotted from day 18 to 77, while R_2 and B_2 are plotted from day 93 to 137) 108.

5.26 Regression plots between shoot dry weight and nodule dry weight of sainfoin (C_1 and E_1 are plotted from day 36 to 93; C_2 and E_2 are plotted from day 93 to 137; R and B are plotted from day 77 to 137) 108.

Figure Page

5.27 Scatterplots of acetylene reduction against nodule dry weight from day 77 to 137 112.

5.28 Scatterplots of acetylene reduction against shoot dry weight from day 77 to 137. 112.

LIST OF PLATES

Plate Page

4.1 Incubation and gas sampling in the first step of acetylene reduction assay 56a.

4.2 Gas chromatography in the second step of acetylene reduction assay 56a.

4.3 Auto-analyser used in the analysis of total nitrogen and phosphorus 56b.

4.4 Auto-analyser recorders 56n.

5.1 General view of infection with *Gigaspora magarita* Becker & Hall in corn (x 1 220) (HY, hypha; AR, arbuscle; VE, vesicle). 68a.

5.2 General view of infection with MX in sainfoin at day 137 (X 380) (AP, appressorium; MY, mycelium; AR, arbuscle; RH, root hair) 68a.

5.3 Endophyte entry points in sainfoin roots at day 137 (X 1 220) (AP, appressorium; HY, hypha) 68b

5.4 Arbuscles at differing stages of development in sainfoin at day 137 (X 1 220) (E, early; I, intermediate; L, late) 68b.

5.5 A sainfoin root hair infected with *Rhizobium* spp. (X 1 910) (RH, root hair; IT, infection thread) 71a.

5.6 General view of a region with many infected root hairs (X 1 910) (RH, root hair; IT, infection thread) 71a.

5.7 Sainfoin nodules of various sizes, shapes and colour 71b.

5.8 Green (left) and red (right) nodules from the uninoculated (C and E) and rhizobia-inoculated (R and B) treatments 71b.

5.9 Sainfoin plants at day 93 (C, control; E, endophyte; R, rhizobia; B, both inoculations) 101a.

5.10 Sainfoin plants at day 137 (C, control; E, endophyte; R, rhizobia; B, both inoculations) 101a.

5.11 Nitrogen deficiency symptoms of sainfoin plants in treatment C and E at day 115 101b.

5.12 Secondary stem initiation at the end of rosette leaf stage at day 77 101b.

LIST OF APPENDICES

Appendix Page

Appendix		Page
A	SI and SI-derived units and other common abbreviations	131.
B	Effects of endophytes on nodulation and dinitrogen fixation	133.
C	Programme summary	135.
D	Endophyte culture	137.
E	Soil-test results	139.
F	Yeast-mannitol medium	141.
G	Nutrient solutions	142.
H	Glasshouse temperatures	145.
I	Development of endophyte infection assessment key	146.
J	Gas chromatography	148.
K	Colorimetric automated analysis	151.
L	Pooled ANOVA for endophyte infection	153.
M	Pooled ANOVA for nodulation and dinitrogen fixation variables	154.
N	Pooled ANOVA for total nitrogen and phosphorus concentrations	159.
O	Pooled ANOVA for plant growth variables	169.

CHAPTER 1

INTRODUCTION

Nitrogen is an essential element for plant growth and reproduction as it is required in the synthesis of proteins, enzymes, deoxyribonucleic acids and many intermediate metabolic compounds. It is, therefore, a key constituent of all plant cells. Dry matter of vascular plants contains about 15 000 $\mu g\ N\ g^{-1}DM$ (1.5% nitrogen), making it the most abundant soil element in plant tissue (Table 1.1).

The supply of nitrogen in current and future agricultural systems is a major determinent of adequate food production to meet the ever-expanding human population. During the green revolution, many agricultural plants were selected and bred for responsiveness to fertilizer supply, resulting in the necessity for intensive application of fertilizers particularly nitrogen (Cummings and Gleason, 1971; Engibous, 1975; Jackson *et al,* 1975; Cox and Atkins, 1979). Scrimshaw and Taylor (1980) have worked out that the primary factor responsible for increases in crop production between 1961 and 1976 was the increasing use of fertilizers. Between the same period, the world's annual consumption of nitrogen fertilizers rose sharply from 11 to 45 Gkg, a dramatic 309% increase (*Fertilizers - Annual Review,* 1968; *FAO Fertilizer Yearbook,* 1980). In 1980, it reached a record of 57 Gkg year^{-1} (*FAO Fertilizer Yearbook,* 1981). With this increasing trend in nitrogen fertilizer usage, it is perhaps not an exaggeration to reaffirm the statement of Viets (1965) that more crops are deficient of nitrogen than of any other element.

The primary supplement of nitrogen to crop plants is from industrial nitrogen fertilizers. However, the existing method of industrial synthesis of nitrogen fertilizers requires a high input of expensive fossil energy. For instance, in the manufacture of ammonia, the precursor for various types of nitrogen fertilizers, a temperature of 400 to 500°C and a pressure of 15 to 35 MPa must be created to drive the Haber-Bosch process in a modern plant, with a production capacity of about 900 Mkg day^{-1} (Bridger *et al,* 1979). In this process alone, the natural gas feed and fuel cost contributes 25% of the total manufacturing cost (Finneran and Czuppon, 1979). With the addition of other fuel expenditure as in the conversion of ammonia into nitrogen fertilizers, in transport and in application, the final nitrogen fertilizer applied to

TABLE 1.1

CONCENTRATION OF SIXTEEN ELEMENTS IN COMPLEX PLANTS (AFTER STOUT, 1961)

Element	Concentration ($\mu g\ g^{-1}$ DM)
From the atmosphere and water,	
carbon	450 000
oxygen	450 000
hydrogen	60 000
From the soil,	
nitrogen	15 000
potassium	10 000
calcium	5 000
magnesium	2 000
phosphorus	2 000
sulphur	1 000
chlorine	100
iron	100
manganese	50
boron	20
zinc	20
copper	6
molybdenum	0.1

TABLE 1.2

EFFICIENCY AND CONTRIBUTION FROM VARIOUS DINITROGEN-FIXING SYSTEMS (AFTER BURNS AND HARDY, 1975, WITH ALTERATIONS IN PARENTHESIS FROM PAUL, 1978)

Dinitrogen-fixing system	Land use (Mha)		Rate of fixation (kg N_2ha^{-1} year^{-1})	Total contribution (Gkg N_2year^{-1})
Legume-*Rhizobium*	legume	250	140 (80)	35 (20)
Legume-*Rhizobium*	permanent grassland	3 000	15 (8)	45 (24)
Blue-green algae	rice	135	30	4
Free-living and "loose" associations	other crops	5	5	5

the field is an expensive item for many farmers.

In conjunction with this high cost, tracer studies reveal that the use of applied nitrogen fertilizers in the field by crop plants is inefficient. Depending on the type of crop, agricultural practices, fertilizer, climate and soils (Winteringham, 1980), between 20 to 60% of the total applied nitrogen is absorbed by crop plants (Allison, 1965, 1966; Bartholomew, 1971; Gutschick, 1980; Hauck, 1981). The work of Myers and Paul (1971) showed that wheat *(Triticum aestivum* L.*)* grown in a sandy loam and a clay soil, recovered about 25% and 50% of the applied ammonium nitrate respectively in the shoots. In two other studies, a first year maize (*Zea mays* L.) crop utilized only about 22% of the labelled urea, this being from grain and straw (Arora *et al,* 1980), while a first year dwarf bean (*Phaseolus vulgaris* L.) crop removed about 30% of the labelled ammonium sulphate (Cervellini *et al,* 1980). The unrecovered nitrogen is "lost" through immobilization, leaching, erosion, denitrification and volatilization of which leaching and erosion, if excessive, pose a serious threat to environmental pollution and public health (Mulder *et al,* 1977; Wild and Cameron, 1980).

From the foregoing discussion, it is apparent that the continual heavy reliance on nitrogen fertilizers in the future is becoming a questionable proposition. The emphasis of current nitrogen and crop research is, therefore, strongly orientated towards improving biological dinitrogen fixation (Evans, 1975; Hardy *et al,* 1975; Brill, 1980; Hardy, 1980a, b; Lambourg, 1980; Subba Roa, 1980). Several biological dinitrogen fixing systems are available for incorporation into agricultural production as shown in Table 1.2. The most important and efficient of which, in relative terms, is the legume-*Rhizobium* mutualistic association. The data in Table 1.2 indicate that this type of association fixes an average of between 80 to 88 kg N_2 $ha^{-1}year^{-1}$ and, thus, contributes between 20 to 44 Gkg N_2 $year^{-1}$ to cultivated land under legumes and permanent grassland. However, a fixation as high as 171 kg N_2 $ha^{-1}year^{-1}$ has been obtained in the developed New Zealand pastures in which the principal legume component is white clover (*Trifolium repens* L.) (Hoglund *et al*, 1979).

Although the legume-*Rhizobium* association is the most efficient by comparison, it is widely recognised that its dinitrogen-fixing activity seldom attains the optimal rate. For example, the clovers in the New Zealand pastures are capable of fixing a potential of 215 to 336 kg

N_2 ha^{-1}year^{-1} (Sears *et al*, 1965; Levy; 1970). Improvement on the rate of dinitrogen fixation is, therefore, an imperative research endeavour in order to sustain the necessary agricultural production levels.

The physical and biological factors that directly and indirectly influence the legume-*Rhizobium* relationship have been identified and comprehensively reviewed by various authors (Lie, 1974; Gibson, 1977; Munns, 1977; Pate, 1977; Parker *et al*, 1977; Dommergues, 1978; Vincent, 1980; Grandhall, 1981). One of these factors is soil phosphorus, an essential element for the growth and nodulation of legumes (van Schreven, 1958; Andrew, 1977; Andrew and Jones, 1978). Many legumes, when infected with arbuscular fungi, show an enhanced phosphate absorption and, subsequently, an associated increase in growth, nodulation and dinitrogen fixation (Crush, 1974; Daft and El-Giahmi, 1974, 1975,1976; Powell, 1976; Mosse *et al*, 1976; Mosse, 1977; Abbott and Robson, 1977; Smith and Daft, 1977; Carling *et al*, 1978; Azcon-G. de Aguilar *et al*, 1979; Smith *et al*, 1979). Similar studies on sainfoin (*Onobrychis viciifolia* Scop.) have yet to be carried out and since it is a legume which is gradually gaining world-wide recognition as a potential forage crop, the purpose of this study is to examine the endophyte-phosphate interaction, and its effects on the nodulation and dinitrogen fixation in sainfoin.