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A STUDY OF NITROGEN FIXATION, NITROGEN DISTRIBUTION AND SEED
YIELD OF SELECTED LEGUMES WITH TWO DIFFERENT GROWTH TYPES.

A thesis

Presented in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy

at

Massey University

Palmerston North

New Zealand.

SUWIT LAOHASIRIWONG

1986

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TABLE OF CONTENTS

	<u>Page</u>
Acknowledgement	i
List of Figures	iii
List of Tables	vii
List of Appendices	xii
Abstract	1
Chapter 1 <u>Introduction</u>	4
1.1 The importance of nitrogen in tropical agriculture	4
1.2 The importance of legumes in tropical agriculture	5
Chapter 2 <u>Review of Literature</u>	8
2.1 Nitrogen fixation	8
2.1.1 Nodulation in legumes	8
2.1.2 Nitrogen fixation in legumes	11
2.1.3 Factors influencing nodulation and nitrogen fixation	14
2.1.3.1 Environmental factors	15
2.1.3.1.1 Temperature	15
2.1.3.1.2 Light	16
2.1.3.1.3 Moisture stress	17
2.1.3.2 Nutritional factors	18
2.1.3.2.1 Combined nitrogen	19
2.1.3.2.2 Mineral nutrition of rhizobia	20
2.1.3.2.3 Effects of mineral nutrients on nodule function	21
2.1.3.3 Biological factors	22
2.1.3.3.1 Photosynthate supply	23
2.1.3.3.2 Symbiotic compatibility between rhizobium and host	26
2.1.3.3.3 Pest and disease effects	27
2.1.4 Methodology of nitrogen fixation measurements	28
2.1.4.1 Nitrogen accumulation	28
2.1.4.2 Isotopic methods	29
2.1.4.3 Acetylene reduction method	29
2.2 Nitrogen distribution in grain legumes	30
2.2.1 Nitrogen assimilation	30
2.2.2 Nitrogen distribution	32

2.2.2.1	Effects on source-sink manipulation on nitrogen distribution	33
2.2.3	Nitrogen re-distribution	33
2.2.4	Carbon: nitrogen relationships	35
2.3	Growth and development of determinate and indeterminate legumes	38
2.3.1	Growth types of grain legumes	38
2.3.2	Soybean growth and development	39
2.3.2.1	Root growth	39
2.3.2.2	Vegetative growth	40
2.3.2.3	Reproductive growth	41
2.3.2.4	The different growth types of soybeans	42
2.3.2.4.1	Vegetative growth	42
2.3.2.4.2	Reproductive growth	43
2.3.2.4.3	Nitrogen fixation	44
2.3.2.4.4	Response to less favourable environments	44
Chapter 3	<u>A study of nitrogen fixation, nitrogen distribution and yield in selected bean and soybean cultivar with either determinate or indeterminate growth</u>	46
	Abstract	46
3.1	Introduction	47
3.2	Materials and methods	48
3.3	Results	50
3.3.1	Plant growth	50
3.3.2	Nitrogen fixation and plant nitrogen	59
3.3.3	Yield and yield components	66
3.4	Discussion	75
3.4.1	Plant growth	75
3.4.2	Nitrogen fixation and nitrogen distribution	79
3.4.3	Yield and yield components	81
Chapter 4	<u>A comparative study of nitrogen fixation, nitrogen distribution and yield of two soybean cultivars with determinate and indeterminate growth types</u>	83

	Abstract	83
4.1	Introduction	84
4.2	Materials and methods	86
4.3	Results	90
	4.3.1 Plant growth	90
	4.3.2 Yield and yield components	98
	4.3.3 Nitrogen fixation	103
	4.3.3.1 The movement of ^{14}C	105
	4.3.4 Nitrogen distribution	110
	4.3.4.1 Nitrogen re-distribution	112
	4.3.5 Relative importance of plant characters in determining the difference between the two growth types	118
4.4	Discussion	119
	4.4.1 Plant growth	119
	4.4.2 Yield and yield components	123
	4.4.3 Nitrogen fixation	126
	4.4.4 Nitrogen distribution	129
	4.4.4.1 Nitrogen distribution	129
	4.4.4.2 Nitrogen re-distribution	131
	4.4.5 Relationships of nitrogen fixation and plant parameters	133
Chapter 5	<u>The effects of leaf area manipulation of an indeterminate soybean cultivar compared with the leaf area of a determinate soybean cultivar</u>	140
	Abstract	140
5.1	Introduction	141
5.2	Materials and methods	143
5.3	Results	145
	5.3.1 Plant growth and yield	145
	5.3.1.1 Plant growth	145
	5.3.1.2 Yield and yield components	162
	5.3.2 Nitrogen fixation	167
	5.3.3 Nitrogen distribution	167
	5.3.4 The relative sensitivity of plant characters to defoliation	175
5.4	Discussion	180
	5.4.1 Plant growth	180
	5.4.2 Yield and yield components	182
	5.4.3 Nitrogen fixation	184

5.4.4	Nitrogen distribution and re-distribution	186
5.4.5	Relative sensitivity of plant characters to defoliation	187
Chapter 6	<u>General discussion</u>	190
6.1	Difference between determinate and indeterminate growth types	190
6.1.1	Plant growth	190
	A] Vegetative growth	190
	B] Reproductive growth	196
	C] Seed yield	198
6.1.2	Nitrogen fixation	199
6.1.3	Nitrogen distribution	202
	6.1.3.1 Nitrogen accumulation	202
	6.1.3.2 Nitrogen re-distribution	203
6.2	The key factor determining the difference between the two growth types	205
Chapter 7	<u>Conclusions and implication for future research</u>	208
7.1	Conclusions	208
7.2	Possible implication to tropical Agriculture	211
7.2.1	Possible role of leaf area as a selection criteria for cultivars superior in nitrogen-fixing character	213
7.2.2	Future research	214
	Appendices	215
	References	246

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LIST OF FIGURES

Figure		Page
2.1	A scheme for the action of nitrogenase.	12
3.1	Time course of total plant dry weight and vegetative dry weight (leaf and stem of the determinate and indeterminate cultivars of bean and soybean.	51
3.2	Time course of relative growth rate of the determinate and indeterminate cultivars of bean and soybean.	55
3.3	Time course of leaf area per plant of the determinate and indeterminate cultivar of bean and soybean.	56
3.4	Time course of net assimilation rate of the determinate and indeterminate cultivars of bean and soybean.	58
3.5	Time course of root dry weight of the determinate and indeterminate cultivars of bean and soybean.	60
3.6	Time course of acetylene reduction activity of the determinate and indeterminate cultivar of bean and soybean.	62
3.7	Time course of nodule dry weight of the determinate and indeterminate cultivars of bean and soybean.	64
3.8	Time course of total plant nitrogen of the	

Figure		Page
	determinate and indeterminate cultivar of bean and soybean.	67
3.9	Regression lines of total plant nitrogen (log scale) and nodule dry weight of the determinate and indeterminate cultivars of bean and soybean.	69
4.1	Time course of total dry weight, pod dry weight, leaf dry weight and root dry weight of the determinate and indeterminate soybean cultivars.	91
4.2	Time course of leaf area per plant of the determinate and indeterminate soybean cultivars.	93
4.3	Time course of net assimilation rate and relative leaf expansion rate of the determinate and indeterminate soybean cultivars.	95
4.4	RLER and NAR of the determinate and indeterminate soybean cultivars compared on physiological basis.	96
4.5a	Time course of shoot:root ratio of the determinate and indeterminate soybean cultivars.	
4.5b	Shoot:root ratio on physiological age basis.	97
4.6	Ratio of dry weight of different plant components to total plant dry weight of the determinate and indeterminate soybean cultivars.	99
4.7	Time course of nodule dry weight in the determinate and indeterminate soybean cultivars.	100
4.8	Acetylene reduction activity and specific acetylene reduction activity of the determinate and	

Figure	Page
	104
4.9a	106
4.9b	108
4.10	111
4.11	113
4.12	116
4.13	117
4.14	135
4.15	
4.16	

Figure		Page
	characters.	137
5.1	Time course of total plant dry weight and leaf area per plant of different treatments of soybean.	146
5.2	Time course of stem dry weight of different treatments of soybean.	151
5.3	Root dry weight of different treatments of soybean from flowering to grain filling stage.	155
5.4	Regression lines of shoot:root ratio of different treatments of soybean.	156
5.5	Time course of nodule dry weight of different treatments of soybean.	159
5.6	Comparison of nodule:root ratio among different treatments of soybean at three growth stages.	161
5.7	Regression lines of total plant nitrogen (log scale) and nodule dry weight of different treatments of soybean.	170
5.8	Nitrogen concentration of plant components for different treatments of soybean.	171
5.9	Time course of total plant nitrogen of different treatments of soybean.	173
5.10	Time course of leaf and stem nitrogen of different treatments of soybean.	176
5.11	Time course of pod nitrogen of different treatments of soybean.	177
6.1	Relationship of total dry-matter (dependent variable) of partial defoliated indeterminate cultivar and undefoliated determinate cultivar regressed against leaf area per plant (independent variable).	192

LIST OF TABLES

Table		Page
3.1	Comparison of total plant dry weight between the determinate and indeterminate cultivars of bean and soybean, estimated from growth curves, at different growth stages.	53
3.2	Comparison of leaf & stem dry weight between the determinate and indeterminate cultivars of bean and soybean, estimated from growth curves, at different growth stages.	54
3.3	Comparison of leaf area per plant between the determinate and indeterminate cultivars of bean and soybean, estimated from growth curves, at different growth stages.	57
3.4	Comparison of root dry weight between the determinate and indeterminate cultivars of bean and soybean, estimated from growth curves, at different growth stages.	61
3.5	Comparison of acetylene reduction activity between the determinate and indeterminate cultivars of bean and soybean, estimated from growth curves, at different growth stages.	63
3.6	Comparison of nodule dry weight between the determinate and indeterminate cultivars of bean and soybean, estimated from growth curves, at different growth stages.	65

Table		Page
3.7	Comparison of total plant nitrogen between the determinate and indeterminate cultivars of bean and soybean, estimated from growth curves, at different growth stages.	68
3.8	Mean pod weight (g plant^{-1}) of bean and soybean with different growth types.	70
3.9	Mean seed weight (g plant^{-1}) of bean and soybean with different growth types.	71
3.10	Mean number of pods per plant of bean and soybean with different growth types.	72
3.11	Mean weight of 20 seeds (g) of bean and soybean with different growth types.	73
3.12	Mean number of seeds per pod of bean and soybean with different growth types.	74
3.13	Harvest indices of bean and soybean with different growth types.	76
3.14	Nitrogen harvest indices of bean and soybean with different growth types.	77
4.1	Comparison of plant dry weight components at different growth stages between the determinate and indeterminate soybean cultivars.	92
4.2	Yield, yield components, harvest index and nitrogen harvest index of the determinate and indeterminate soybean cultivars	101
4.3	Yield and yield components from main stem and branches of the determinate and indeterminate soybean cultivars.	102

Table	Page
4.4	Comparison of integrated acetylene reduction activity (m mol plant^{-1}) at two growth stages of the determinate and indeterminate soybean cultivars. 107
4.5	Recovery of ^{14}C in different plant parts of the determinate and indeterminate soybean cultivars. 109
4.6	Comparison of nitrogen from different plant parts at different growth stages between the determinate and indeterminate soybean cultivars. 114
4.7	Total plant nitrogen, total re-distributed nitrogen and proportion of re-distributed nitrogen from different plant parts in the determinate and indeterminate soybean cultivars. 115
4.8	Information from multiple discriminant analysis and structure matrix. 120
5.1	Comparison of leaf area per plant (Cm^2) among different treatments of soybean at three growth stages. 147
5.2	Comparison of total plant dry weight (g) among different treatments of soybean at three growth stages. 148
5.3	Comparison of net assimilation rate (NAR) calculated from Figure 5.1 (a,b) among different treatments of at three growth stages. 150
5.4	Comparison of total plant stem dry weight (g) among different treatments of soybean at three growth stages. 152
5.5	Comparison of branch number among different

Table	x	Page
	treatments of soybean.	154
5.6	Comparison of shoot:root ratio ($\text{g g}^{-1} \text{ plant}^{-1}$) among different treatments of soybean at three growth stages.	158
5.7	Comparison of nodule dry weight (g) among different treatments of soybean at three growth stages.	160
5.8	Comparison of total yield and yield components among different treatments of soybean at three growth stages.	163
5.9	Yield and yield components of different treatments of soybean analysed separately for branches and main stem.	164
5.10	Comparison of seed yield and number of pods per branch among different treatments of soybean.	165
5.11	Comparison of harvest index (grain yield: total above-ground biomass and nitrogen harvest index (grain nitrogen:total above-ground nitrogen) among different treatments of soybeans.	166
5.12	Comparison of acetylene reduction activity ($\mu\text{mol h}^{-1} \text{ plant}^{-1}$) among different treatments of soybean.	168
5.13	Comparison of specific acetylene reduction activity ($\mu\text{mol h}^{-1} \text{ g}^{-1} \text{ nodule dry weight}$) among different treatments of soybean.	169
5.14	Comparison of total plant nitrogen (mg) among different treatments of soybean.	174
5.15	Information from multiple discriminant analysis	

Table		Page
	and structure matrix for the four treatments of the indeterminate soybean cultivar.	179
6.1	Partition coefficient of bean and soybean from different environmental conditions at two growth stages.	197

LIST OF APPENDICES

Appendix		Page
A.	Statistical symbols.	215
B.	Acetylene reduction technique.	216
C.	Kjeldahl nitrogen content determination.	218
D.	Controlled environment room environmental conditions.	220
3.1	Information from growth curves of total plant dry weight (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $(\ln(Y) = B_0 + B_1X + B_2X^2)$	221
3.2	Information from growth curves of leaf and stem dry weight (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $(\ln(Y) = B_0 + B_1X + B_2X^2)$	222
3.3	Information from growth curves of leaf area per plant (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $(\ln(Y) = B_0 + B_1X + B_2X^2)$	223
3.4	Information from growth curves of root dry weight (dependent variable), of determinate	

- and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$ 224
- 3.5 Information from growth curves of nodule dry weight (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$ 225
- 3.6 Information from curves of acetylene reduction activity (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$ 226
- 3.7 Information from growth curves of total plant nitrogen (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$ 227
- 3.8 Information from linear regression of total plant nitrogen (dependent variable) regressed against nodule dry weight (independent variable) with the equation form $\text{Ln } (Y) = B_0 + B_1X$ 228
- 4.1 Information from growth curves of plant dry weight (dependent variable), of determinate and indeterminate soybeans, regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$ or linear equation form $(Y = B_0 + B_1X)$. 229
- 4.2 Information from growth curves of leaf area per plant (dependent variable), of determinate and indeterminate soybeans, regressed against

Appendix	Page
	time (independent variable) using quadratic equation form $(\text{Ln } (Y)= B_0 + B_1X + B_2X^2)$. 230
4.3	Information from growth curves of nodule dry weight (dependent variable), of determinate and indeterminate soybeans, regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y)= B_0 + B_1X + B_2X^2)$. 231
4.4	Information from curves of integrated acetylene reduction activity (dependent variable), of determinate and indeterminate soybeans, regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y)= B_0 + B_1X + B_2X^2)$. 232
4.5	Information from linear regression of total plant nitrogen (dependent variable) regressed against integrated acetylene reduction activity (independent variable) with equation form $Y = B_0 + B_1X$. 233
4.6	Information from linear regression of total plant nitrogen (dependent variable) regressed against nodule dry weight (independent variable) with equation form $\text{Ln } (Y) = B_0 + B_1X$. 234
4.7	Information from growth curves of plant nitrogen (dependent variable), of determinate and indeterminate soybeans, regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y)= B_0 + B_1X + B_2X^2)$ or linear equation form $(Y = B_0 + B_1X)$. 235
5.1	Information from growth curves of total plant dry

- weight (dependent variable), of determinate and four treatments of indeterminate soybeans regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$. 236
- 5.2 Information from growth curves of leaf area per plant (dependent variable), of determinate and four treatments of indeterminate soybeans regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$. 237
- 5.3 Information from growth curves of stem dry weight (dependent variable), of determinate and four treatments of indeterminate soybeans regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$. 238
- 5.4 Information from growth curves of root dry weight (dependent variable), of determinate and four treatments of indeterminate soybeans regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$. 239
- 5.5 Information from linear regression of shoot:root ratio (dependent variable) of determinate and four treatments of indeterminate soybeans regressed against time (independent variable) using linear regression form $(Y = B_0 + B_1X)$. 240
- 5.6 Information from growth curves of nodule dry weight (dependent variable), of determinate and four treatments of indeterminate soybeans regressed

- against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$. 241
- 5.7 Information from linear regression of total plant nitrogen (dependent variable) of determinate and four treatments of indeterminate soybeans regressed against nodule dry weight (independent variable) using linear regression form $(\text{Ln } (Y) = B_0 + B_1X)$. 242
- 5.8 Information from growth curves of total plant nitrogen (dependent variable), of determinate and four treatments of indeterminate soybeans regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$. 243
- 5.9 Information from growth curves of leaf + stem nitrogen (dependent variable), of determinate and four treatments of indeterminate soybeans regressed against time (independent variable) using quadratic equation form $(\text{Ln } (Y) = B_0 + B_1X + B_2X^2)$. 244
- 5.10 Information from growth curves of pod nitrogen (dependent variable), of determinate and four treatments of indeterminate soybeans regressed against time (independent variable) using linear equation form $(Y = B_0 + B_1X)$. 245

ABSTRACT

Plant growth types of the determinate and indeterminate growth forms are commonly distinguished in many legume species. However, there do not appear to be many studies where direct comparisons have been made of the two growth types in relation to nitrogen fixation and nitrogen distribution. Furthermore, there are disagreements in the literature about the yield advantage of these two growth types.

This study was initiated to identify the influence of different growth types of selected grain legumes on seed yield, nitrogen fixation, and nitrogen distribution. In addition, the emphasis was also put on finding amongst the measured parameters, one that had the greatest influence on the differences observed.

Initially determinate and indeterminate growth types of bean (Phaseolus vulgaris) and soybean (Glycine max), were studied in glasshouse conditions. The indeterminate cultivar of both species had higher leaf area and nodule dry weight, more root growth, accumulated more total dry weight and had higher yield than that of the determinate cultivar. In both species, the indeterminate cultivar accumulated more total plant nitrogen than the determinate cultivar. However, only the indeterminate soybean cultivar showed significantly more nitrogen fixation (Acetylene reduction) than that of the determinate cultivar.

Subsequently the same soybean cultivars ('Matara' =determinate and 'Amsoy' =indeterminate) were studied in controlled

environment conditions. The indeterminate cultivar produced higher vegetative dry-matter and seed yield than that of the determinate cultivar. The higher acetylene reduction activity of the indeterminate cultivar came primarily from a greater nodule mass. About 30-40% of seed nitrogen of both cultivar came from re-distribution from vegetative parts, but the stem of the indeterminate cultivar re-distributed a higher proportion of nitrogen to the seed than that of the determinate cultivar. Among several plant characters measured (viz. the dry-weights of the roots, nodules, stems, leaves, and pods, the leaf area, acetylene reduction activity and the total plant nitrogen) leaf area was identified as the key factor in determining the difference between the two growth types.

In order to determine the relative importance of leaf area as a factor influencing seed yield, nitrogen fixation and nitrogen distribution the leaf area of the indeterminate cultivar 'Amsoy' was manipulated by imposing different levels of partial leaf removal starting at the flowering stage. For one treatment, partial pod removal was also applied to induce a reduced demand of assimilate. Partial defoliation of the indeterminate cultivar reduced markedly the root growth and the number of branches, but nodule growth, acetylene reduction activity and nitrogen distribution was reduced to a lesser extent. Partial pod removal did not change the overall pattern of response. When about 60% of the leaves of the indeterminate cultivar were removed, seed yield was reduced by about 17% and it was still significantly higher than the undefoliated determinate cultivar. There was no significant difference between the rates of nitrogen accumulation in the pods under each treatment. The final seed

nitrogen concentration was not affected by defoliation treatments nor was the partitioning of nitrogen to seed.

It was concluded that there were differences between the two growth types of soybean for seed yield, nitrogen fixation, and nitrogen distribution. Leaf area was the most important parameter in determining these difference. The greater overlapping of vegetative and reproductive growth in the indeterminate cultivar seemed to be advantageous rather than disadvantageous. This longer period of vegetative growth enabled the indeterminate cultivar to produce a bigger source capacity which consequently supported more nitrogen fixation activity and produced higher seed yield.

The possible implications to tropical agriculture were discussed and some future research topics were also suggested.

CHAPTER 1

INTRODUCTION

Leguminous crops play a crucial role in agricultural production throughout history. This is attributed in part to the ability of legumes to fix atmospheric nitrogen when in symbiosis with *Rhizobium* bacteria and in part to their high protein content as food. In the coming decades increasing need for higher quantity and quality of food will place severe demand on agriculture particularly in the developing countries. The role of legumes both as a nitrogen contributor and as a source of food protein is therefore certain to increase in importance.

1.1 THE IMPORTANCE OF NITROGEN IN TROPICAL AGRICULTURE.

Nitrogen is the most important element for plants and animals and is required in large amounts for the manufacture of proteins and other nitrogenous compounds vital for growth. Despite the great abundance of dinitrogen gas in the atmosphere, this element is commonly deficient in agricultural soils in the oxidized (nitrate) and reduced (ammonium) forms which can be absorbed by plants (Novoa and Loomis, 1981). Chemical nitrogen fertilizers are costly to produce, and they are not always completely taken up by crops (Ayanaba, 1980).

Biologically fixed nitrogen has certain advantages i.e.

- i) it is a cheap source of nitrogen especially in developing countries,
- ii) nitrate pollution is minimized by the use of legumes, and

iii) gradual release of nitrogen from leguminous crop residues enables more efficient use of nitrogen by subsequent non-leguminous crops (Dobereiner and Campelo, 1977).

Nitrogen losses in tropical regions are estimated at 80-570 kg N ha⁻¹ year⁻¹ under fallow while comparable data for temperate regions are about 30 kg N ha⁻¹ year⁻¹ (Dobereiner and Campelo, 1977). The low nitrogen status is accentuated further by:

i) high precipitation with greater nitrification encouraged by alternate cycles of wetting and drying, and

ii) the low water holding and cation exchange capacity of most lateritic soils.

By and large, subsistence farmers cannot afford investment in mineral nitrogen fertilizers. Thus, in the tropics, adequate supply of nitrogen from legumes is critical for high levels of crop production.

1.2 THE IMPORTANCE OF LEGUMES IN TROPICAL AGRICULTURE.

The world production of grain legumes in 1975 was about 140 million metric tonnes slightly more than half of which came from soybean (Hardy et al., 1980). World demand for soybean alone has been projected to reach about 275 million metric tonnes by the year 2000. This is equivalent to about 27 million metric tonnes of fixed nitrogen annually, which can be translated to its current value of about US\$ 5.4 billion (1986 value US\$ 200/tonne urea) as nitrogen fertilizer.

In addition to their place as the cheapest source of food protein for human consumption, legumes have many other uses such as animal feed, soil conservation, fuel or firewood, dyes and cosmetics (Okigbo, 1978).

Although nearly half of the total area of crop legumes grown in the world is in the tropical countries, the average yield from these countries is only two-thirds of the world average. Therefore, legume production from the tropics is only about one-third of ^{the} world's production (Norman et al., 1984). Since many tropical soil are limited in their ability to produce crops due to severe deficiency in soil nitrogen (Gibson, 1982), hence, there is still need to exploit legumes for their ability to fix nitrogen which could have ^a major role in increasing seed yield or contributing as sources of nitrogen in cropping systems.

In many legume species the morphology of the two growth forms are commonly distinguished, the so-called determinate and indeterminate types. In developed countries, there is a tendency to favour the determinate plant type which is compatible with mechanical harvesting (Khan, 1977). In contrast, Elston and Bunting (1980) pointed out that an indeterminate growth type in a tropical legume should be advantageous to recovery from the water stress of either water excess or deficit which are common in most tropical developing countries. However, there do not appear to be many studies in which direct comparisons have been made on the role of these two growth types in relation to nitrogen fixation and nitrogen distribution. So, to maximize the benefit of the nitrogen contributory role of the legumes in many cropping systems we would need to understand whether the different growth types have any effects on symbiotic nitrogen fixation or the utilization and distribution of fixed nitrogen. Therefore, a series of experiments were carried out with the following objectives:

1. to identify the effects of different growth types (determinate and indeterminate) of selected grain legumes on their yield, nitrogen fixation, and nitrogen distribution.

2. to identify the plant parameter that has the greatest influence on the difference obtained.

CHAPTER 2

REVIEW OF LITERATURE

This review is divided into three main parts:

1. Nitrogen fixation: With a brief review on nodulation, nitrogen fixation in legumes and the methodology of nitrogen fixation measurements will be presented. The subject of nitrogen fixation has been extensively reviewed (*e.g.* Postgate, 1982; Broughton, 1981,1982,1983). The main emphasis of this section will therefore be focused on factors influencing nodulation and nitrogen fixation in legumes.

2. Nitrogen distribution: Apart from a brief review of nitrogen assimilation, the nitrogen distribution, nitrogen re-distribution and carbon:nitrogen relationships in grain legumes will also be reviewed.

3. Growth and development of determinate and indeterminate legumes: Apart from a brief review of determinate and indeterminate growth types of grain legumes in general, the growth and development of soybean will be discussed in more detail with emphasis on the difference between the growth types.

2.1 NITROGEN FIXATION:

2.1.1 Nodulation in legumes.

The various families, genera and species of legumes vary widely in their ability to form symbiotic relationship with the

Rhizobium bacteria. However, not all leguminous plants form nodules for example, only 28% of 258 species examined in the subfamily Caesalpinioideae were nodulated (Allen and Allen, 1961). For nodulation to occur, the host plant must be susceptible and a compatible infective strain of *Rhizobium*, capable of multiplying, must be present on the root.

The structure and functions of root nodules of legumes have been reviewed (Bergersen, 1982). In many legumes, especially temperate species, infection takes place through root hairs which are deformed in the presence of rhizobia. Successful infection occurs at defined sites in the zone of elongation near root tips and is usually associated with a particular type of deformation from which an 'infection thread' develops. This thread is in fact an invaginated tube of root hair cell-wall within which the rhizobia grow, confined in a chain along the lumen of the tube (Bergersen, 1982). Specific interactions between the host plant and the rhizobia may be expressed at the early stage, and at all subsequent stages of the nodulation process (Bergersen, 1982). In some species however, infection threads have not been observed. For example in peanut (*Arachis hypogaea*), nodules arise in the junction of lateral roots and the entry of bacteria is between the root hair and the epidermal cell (Dart, 1977)

There are two major patterns of nodule development (Chen and Thornton, 1940; Dart, 1977). One is the 'meristematic type' which is characterized by persistent apical meristems. The infected cells do not divide, but are increased in number by the ramification of infection threads into new tissue. Examples of legumes with this type of nodule development are pea (*Pisum sativum*) and clover (*Trifolium*

spp.). The second type of nodule has no persistent meristem, and is called the 'spherical type'. In spherical nodules, the central roughly-spherical masses of bacteroid-containing cells are formed by continued division of infected cells (Sutton, 1983). Examples of legumes with spherical type nodules are soybean (*Glycine max*) and peanut (*Arachis hypogaea*).

Within the developed nodules the bacteroids, containing the nitrogen-fixing enzyme system, are surrounded by a solution of the red pigment, leghaemoglobin (Bergersen and Goodchild, 1973). Nodule initiation and development are continuing processes as root development continues. Therefore, nodules of all ages may be present on a mature plant (Hicks, 1978).

It has long been recognized that the nodule shape is a characteristic of the host plant rather than the *Rhizobium* strain. Nodule shape is determined by the pattern of meristematic activity. In the round or oval nodules, such as those in soybean meristematic activity is initially spread through the nodule. After 10-20 days activity decreases and tends to be localized in pockets at the edge of the spherical bacteroid zone and much of the increase in nodule size thereafter derives from the growth of already-infected cells. Such nodules retain the capacity to increase in size even when they are about 60 days old (Dart, 1977). The mature nodule stage is characterized by high nitrogen-fixing activity and high leghaemoglobin concentrations. The expansion of plant cells and bacteroids may continue but cell division does not. The nodules continue to receive plant carbohydrate and they export combined nitrogen in return (Sutton, 1983).

2.1.2 Nitrogen fixation in legumes.

Biological fixation of nitrogen is the reduction of nitrogen gas from the atmosphere to ammonia. It takes place inside some species of bacteria and algae of which there are two broad groups. One lives freely in soil, sea and fresh water, and the other lives in a symbiotic relationship with certain plants (King, 1983). The biological nitrogen fixation had been widely reviewed (*e.g.* Bergersen, 1977; Postgate, 1978; Sprent, 1979; Havelka *et al.*, 1982).

In legumes, the symbiotic nitrogen fixation reaction is catalyzed by nitrogenase, an enzyme found in bacteroids of legume root nodules. The enzyme nitrogenase is a large molecule with several subunits. These fall into two categories: the so-called iron protein (fraction 1) consisting of two apparently identical subunits and the iron-molybdenum protein (fraction 2) of four subunits. The general structure of the nitrogenase complex is remarkably constant among the different nitrogen-fixing organisms (Roughley *et al.*, 1983). The description of how the reduction of nitrogen to ammonia ~~proceeds~~ is presented in Figure 2.1 (Postgate, 1978). The requirements for the overall reduction of nitrogen to ammonia are as follows (Roughley *et al.*, 1983):

1. A source of low redox potential reductant (approximately -430 mV). The nature of this reductant in *Rhizobium* bacteroids is probably a ferredoxin; it donates electrons singly to the nitrogenase reductase.

2. A supply of adenosine triphosphate (ATP). It is likely that 12-30 ATP molecules are necessary for the reduction of one

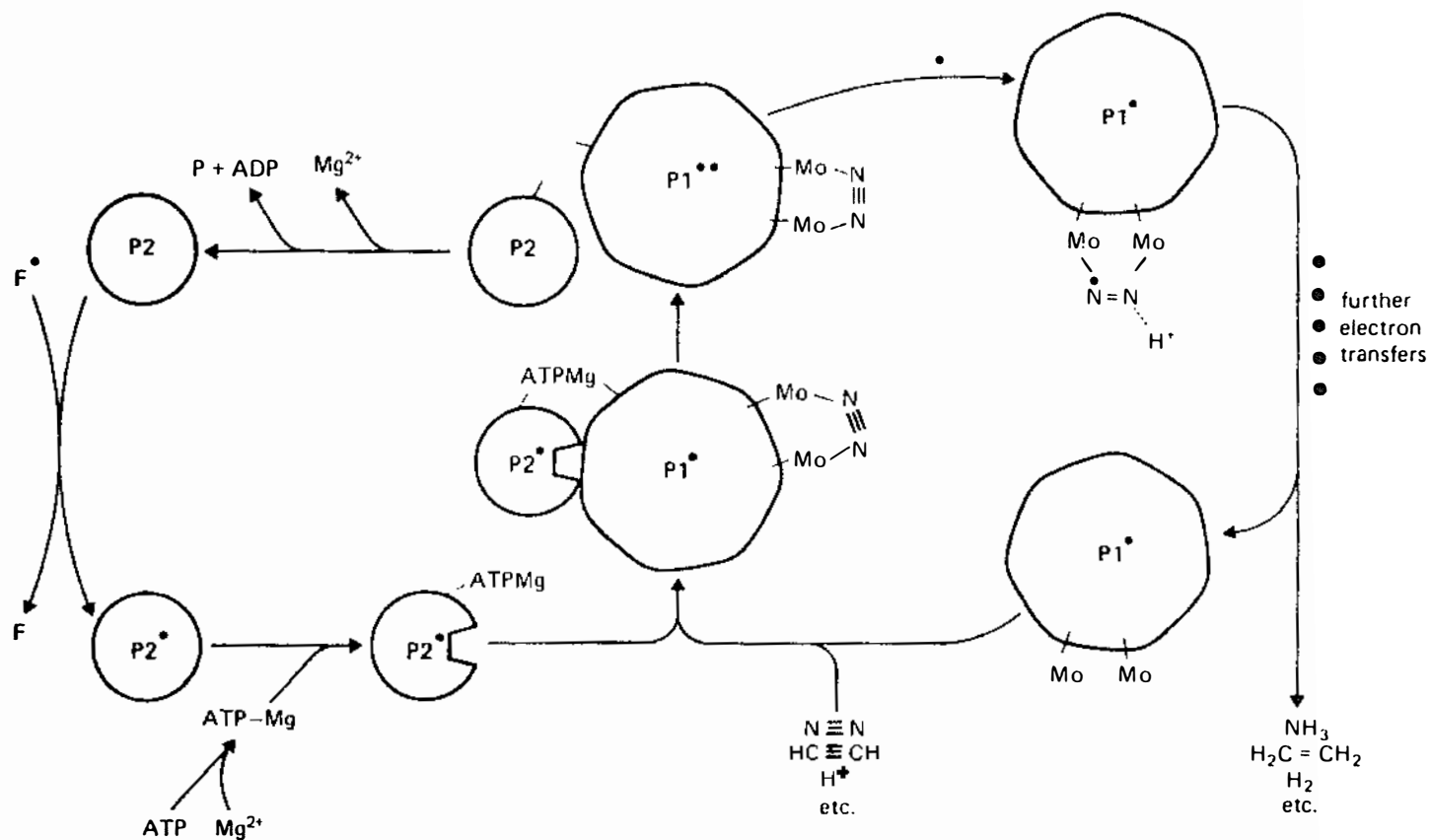
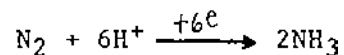


Figure 2.1 A scheme for the action of nitrogenase. P1 is the larger protein containing iron and molybdenum atoms, P2 is the smaller protein with iron atom only. F is an electron-donating substance (ferredoxin or flavodoxin) which donates an electron (a black dot) to iron atom in P2 (From Postgate, 1978).

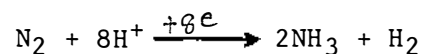
molecule of N_2 . ATP is produced by oxidative phosphorylation in bacteroids and at least some of it, in the form of its magnesium (Mg) complex, unites with the reduced nitrogenase reductase.

3. A supply of N_2 . This is not limiting under any known natural conditions. The nitrogen molecule joins with nitrogenase, almost certainly at its molybdenum (Mo)-containing centre. The N_2 -nitrogenase then unites with the reduced nitrogenase reductase-ATP-Mg to form the active nitrogenase complex. Electrons flow to the N_2 which is reduced in stages until NH_3 is formed.

Theoretically, nitrogenase consumes six electrons and six protons to reduce one molecule of dinitrogen to two molecules of ammonia.



However, Postgate (1982) points out that there is evidence that one molecule of H_2 is released during the reaction. It would therefore be more correctly represented as:



One similarity between symbiotic nitrogen fixation and the industrial production of nitrogen fertilizer is that both methods require energy before the reaction can proceed. Nitrogenase requires energy in the form of adenosine triphosphate (ATP), and it consumes about 15 moles of ATP to fix one mole of dinitrogen (Postgate, 1982). In order for the system to function properly, a supply of oxygen and carbon are essential. Oxygen is vital for the production of ATP. Carbon compounds in the nodules are used to provide energy for nitrogenase activity and that the fixed nitrogen is combined rapidly

into amino acids and amides (Bergersen, 1982). The supply of carbon originates mainly as photosynthates in the leaves, but may be supplemented by CO₂ uptake directly into nodules (Roughley *et al.*, 1983).

The symbiosis is established when the host plant provides oxidizable carbon substrates to the nodule as a source of energy for fixation. The nodules in turn export reduced nitrogen in the form of amino acids, amides, or ureides for protein synthesis in the host (Atkins, 1984). In addition, energy in the form of photosynthate is also required for nodule formation and maintenance, hydrogen loss and incorporation and transport of newly fixed nitrogen (LaRue and Patterson, 1981; Havelka *et al.*, 1982). For example, Minchin and Pate (1973) found that nodulated pea (*Pisum sativum*) used 5.9 mg of carbon for every 1 mg nitrogen fixed.

2.1.3 Factors influencing nodulation and nitrogen fixation.

The pattern of nitrogen fixation generally observed in annual grain legumes during development is of a rise in activity from the seedling stage, and during vegetative growth, to a peak somewhere during early reproductive growth, and a more or less rapid decline during pod filling (Sprent, 1979). With a growing plant, nodule initiation and development is occurring continually, but the overall pattern of nitrogen fixation by a population of nodules on a plant resembles that of individual nodules, but over a longer time span (Gibson *et al.*, 1982). The potential symbiotic nitrogen fixation of a legume can be defined as the maximum activity of that legume when it is nodulated with the most effective *Rhizobium* strain and grown under

the most favourable environmental conditions. Many biological and environmental factors can affect the nitrogen fixing potential of a legume, through effects on the nodulation pattern or on the specific nitrogenase activity of the nodules. These factors have also been widely reviewed (*e.g.* Lie, 1981; Havelka *et al.*, 1982). The objective of the following sections is to review the environmental, nutritional and biological factors limiting nitrogen fixation by legumes with emphasis especially on soybean.

2.1.3.1 Environmental factors.

2.1.3.1.1 Temperature: Soil temperature is a major factor affecting most stages of nodule formation and function. The literature on the effects of temperature on symbiotic nitrogen fixation is extensive (*e.g.* Gibson, 1977). Various combinations of host and strain of *Rhizobium* have different temperature requirements but, in general, the optimum temperature for fixation reflects the natural habitat of the host (Roughley *et al.*, 1983). Lower than optimal root temperatures are found to retard root hair infection more than nodule initiation, nodule development or nitrogen fixation (Gibson, 1971). However, relatively little is known of the effect of high soil temperatures on the survival of rhizobia under field conditions (Gibson *et al.*, 1982). In soybean, nitrogen fixation was found to increase in soil temperature up to 27° C with an optimum soil temperature of 24° C over a range of soil water potentials (Kuo and Boersma, 1971). Pankhurst and Sprent (1976) however, found a broad optimum temperature of between 15 and 30° C for nitrogen fixation by nodules of soybean (cv. Portage), and this optimum temperature range decreased with water stress.

Air temperature may also have as great, if not greater, effect as soil temperature on nodule activity, perhaps because of its effect on translocation of carbohydrates from the leaves to the nodules (Brun, 1978).

2.1.3.1.2 Light: It is known that the energy required for symbiotic nitrogen fixation is derived from photosynthesis which depends on the light environment (Rabie, 1981). In white clover (*Trifolium repens*), Chu and Robertson (1974) found that shading to 50% of day light reduced the number and weight of nodules per plant but caused no detectable difference in the rate of acetylene reduction. Shading has been shown to influence growth as well as nitrogen fixation in soybean (Rabie and Kumazawa, 1979). Plants grown under full light produced more dry-matter, higher nitrogen content, greater nodule mass and higher nodule number than under shade. However, Trang and Giddens (1980) reported that in soybean total nodule activity, as measured by acetylene reduction assay, was greater under shaded plants than in unshaded plants.

Light intensity has also been reported to affect nodulation and nitrogen fixing activity. For example, Gibson (1976) found, when subterranean clover (*Trifolium subterraneum*) plants were either grown continuously under low or high light intensities for 20 days following inoculation or were transferred to alternate conditions, the nitrogenase activity of plants placed into low light decreased by 40% whereas a 50% increase in activity was recorded for plants placed into high light. Furthermore, nodule weights of plants placed into low light remained static, whereas those placed into high light increased significantly within 3 days.

2.1.3.1.3 Moisture stress: The legume nodule requires water for maintainance of turgidity of its tissues and for the export in the xylem of the fixation products. Moisture stresses due to either deficiency or excess are detrimental to ^{the} legume-*Rhizobium* symbiosis. Infection is affected in low soil water potential due to the absence of normal root hairs. Instead, short, stubby root hairs appear, which are inadequate for infection by *Rhizobium* (Worrall and Roughley, 1976). Following successful infection, reduced water supply can retard nodule development in *Vicia faba* (Gallacher and Sprent, 1978). Prolonged desiccation leads to nodule loss with a consequent reduction in the level of nitrogen fixation until new nodules form (Gibson *et al.*, 1982). Because the plant may be able to resume meristematic activity, the recovery from stress on rewatering seems to depend on the type of nodule. Plants with meristematic nodules (*i.e.* *Vicia faba*) can recover from water stress by regrowth of existing nodules, while in plants with spherical nodules (*i.e.* soybean) severe stress can cause nodule shedding and recovery is likely to be slower and to involve the formation of new nodules (Sprent, 1976).

Water-logging adversely affects plant growth, but the major factor leading to ~~depressed~~ nitrogen fixation arises from oxygen deficiency. Water-logging can also affect nodule number, size and water content (Sprent, 1976). Field observation (Lawn and Byth, 1979) and laboratory study (Stanley *et al.*, 1980) suggest that soybean, compared with other legumes is relatively tolerant to temporary water-logging. Some recent studies in Australia indicated that several legumes including soybean have substantial abilities to adjust to water-logged soils, particularly when the soil saturation occurs

during early seedling growth and the water table is maintained at the constant level *e.g.* at about 15 cm below the soil surface (Bushby, 1982). One of the ~~studies~~ showed that nodular mass was increased in high water-table culture by up to 35 times that of control plants and in some cases accounted for five percent of total plant dry-matter (Hunter *et al.*, 1980). Troedson *et al.* (1985) concluded that saturated soil culture promoted higher growth rates, more prolific nodulation, greater and more prolonged nitrogen fixation and higher seed yield than assiduous conventional irrigation.

2.1.3.2 Nutritional factors.

The mineral nutrition of legumes is somewhat more complex than that of other plant species because of the special symbiotic relationship existing between the legume host and the associated rhizobial bacteria. Particular nutritional requirements are necessary for this extra physiological process to operate efficiently. It is also important to recognise that both the types of mineral elements and the concentration required for these additional functions may not be the same as those required for the normal growth of the host plant itself (Smith, 1982).

Mineral nutrients may limit nitrogen fixation by affecting any of the following four phases, namely (i.) legume growth, (ii.) growth and survival of rhizobia, (iii.) infection and nodule development, and (iv.) nodule function (Robson, 1978).

In addition to a review of the effects of combined nitrogen, the role of mineral nutrients which are essential to each phase of symbiotic nitrogen fixation will be discussed in the following section.

2.1.3.2.1 Combined nitrogen: Combined nitrogen has been repeatedly reported to significantly inhibit and delay nodulation and nitrogen fixation in legumes (*e.g.* Ham *et al.*, 1975; Rabie *et al.*, 1979; Manhart and Wong, 1980). Three phases are affected: root hair infection, nodule growth, and nitrogenase activity. Pate (1977) reported work which suggested that root hair curling, and the subsequent formation of infection threads was more susceptible to injury than the later stages of nodulation. The effect of combined nitrogen on nitrogen fixation seems to be varied according to the nitrogen form (Rabie, 1981). For example, Mahon (1977) found that nitrate ion (NO_3^-) concentration (as 10mM potassium nitrate) decreased acetylene reduction by over 95% in pea, whereas with an equal concentration of ammonia (NH_4^+), the inhibition was estimated as only 16%. In soybean grown in nutrient solutions, Vigue *et al.* (1977) reported that nitrate inhibited nodulation more than urea and this trend was interpreted to be partially due to urea uptake by plants being slower than nitrate uptake, regardless of the urea concentration supplied.

Marked stimulation of nitrogen fixation by early supplementation with combined nitrogen has also been observed with two grain legumes. With soybean, when 7mM nitrate nitrogen was included in the nutrient solution for 14 days after sowing, a three-fold difference in total nitrogen at 36 days was attributed to the better early growth of the supplemented plants. A similar situation was found with lupins where supplementary nitrogen also considerably increased the number and length of lateral roots (Gibson, 1976).

In order to detect the significance of nitrogen fertilizer during the flowering and pod filling stages of plant growth, Streeter (1978) subjected non-nodulated soybeans to nitrogen starvation. He found that nitrogen stress during the phase of rapid seed formation was only depressive on individual seed weight and its nitrogen concentration, whereas nitrogen stress at the end of flowering or during early pod formation caused a major reduction in pod and seed numbers as well as further decreases in seed weight and in nitrogen concentration of the seed and vegetative tissues. Almost the same trend was also obtained by Egli *et al.* (1978a). It has repeatedly been reported that fixed nitrogen fails to supply all nitrogen requirements for optimum growth and maximum seed yield (*e.g.* Ham *et al.*, 1975; Rabie *et al.*, 1979; Rabie and Kumazawa, 1979; Manhart and Wong, 1980). Application of nitrogen within the range where symbiotic fixation is not inhibited may be essential, and should be based on the residual soil nitrogen (Rabie, 1981).

2.1.3.2.2 Mineral nutrition of rhizobia: The mineral nutrition of rhizobia has received little attention. Qualitative requirements for rhizobial growth have been demonstrated for phosphorus, potassium, magnesium, calcium, molybdenum, cobalt, zinc, manganese and iron (Robson, 1978). Very little has been done to relate these requirements to conditions in the rhizosphere. For most elements therefore, it is not possible to confidently assess whether the external requirement of the plant exceeds that for the growth and survival of *Rhizobium* (Smith, 1982).

2.1.3.2.3 Effects of mineral nutrients on infection and nodulation: Detailed studies on the effects of nutrients on infection

and nodulation have shown that only calcium (Ca) and boron (B) have been clearly demonstrated to be specifically involved in these processes (Robson, 1978; Smith, 1982).

In moderately acid (pH < 5.5) solutions, nodule formation appears to have a higher requirement for Ca than that of the host plant at least with some legume species *e.g.* subterranean clover (Lowther and Loneragan, 1968) and lucerne (Munns, 1970). Since, the Ca-sensitive stage occurred within 3 days after inoculation, Munns(1970) suggested that the involvement of Ca was in nodule initiation rather than in nodule development.

Studies with boron have shown a direct involvement with nodule development. Boron deficiency prevents both meristematic and vascular development (Munns, 1980). The appearance of both nitrogen and boron deficiency symptoms on boron-deficient legumes suggests that the requirement of this element for nodule development is similar to that for the growth of the host plant (Munns, 1977).

Phosphorus applications which increase plant growth commonly increase nodule number, nodule volume and nodule weight. These effects can generally be explained by indirect effects of phosphorus on nodulation associated with growth response by ^{the} legume (Robson, 1978). For example, Gates(1974) observed nodules in *Stylosanthes humilis* three days earlier in high phosphorus than in low phosphorus plants, However, differences in plant size were apparent four days earlier in high phosphorus plants. Hence nodule development may have been delayed in low phosphorus plants by limitation on host plant growth.

2.1.3.2.4 Effects of mineral nutrients on nodule function:

Several nutrients have specific roles in the nitrogen fixation process

within the nodule. However, for most nutrients this requirement is less than that for plant metabolism elsewhere in the plant (Robson, 1978).

Molybdenum and cobalt have specific roles clearly defined, respectively as a component of nitrogenase and as a nutrients for rhizobia (Munns, 1980). The requirement of molybdenum for nodule function far exceeds its requirement elsewhere in the plant (Robson,1983). Cobalt-deficient nodules of *Lupinus angustifolius* contain fewer bacteroids per unit weight of nodule and less deoxyribonucleic acid (DNA) per bacteroid (Dilworth *et al.*, 1979).

There are also indications that greater amounts of copper (Cu) and calcium (Ca) may be required for nodule function than for growth of the host plant (Robson, 1978).

Although there is no direct evidence that nodule function requires more phosphorus (P) than plant growth, increasing the amount of applied P above that required for maximum dry matter production did increase the nitrogen concentration in the shoots of several legumes species (Andrew and Robins, 1969; Andrew, 1977).

Sulphur deficiency can limit both nodulation and nitrogen fixation, reduce nodule number and reduce nodule size (Oke, 1969). Severe sulphur deficiency reduces the rate of protein synthesis more than the rate of nitrogen fixation and leads to accumulation of non-protein nitrogen (Spencer, 1959). Moderate sulphur deficiency can reduce the protein content of legumes without reducing plant growth (Jones *et al.*, 1971).

2.1.3.3 Biological factors.

2.1.3.3.1 Photosynthate supply: From the time when the free living *Rhizobium* is first attracted to and multiplies within the rhizosphere through to the stages of initiation, development, and maintenance of fully functional nodules, the host plants must supply the symbiotic organs with a wide variety of nutrient and regulatory substances in amounts and proportions matching the ever-changing requirements of ^{the} symbiosis (Pate, 1977). The importance of photosynthates for the symbiosis can be clearly shown by the results of detailed studies which have looked at the partitioning of photosynthate within the plant and how that has influenced nodule development and subsequent nitrogen fixation. For example, during the early vegetative period in pea (*Pisum sativum*), about 74% of the daily-acquired photosynthate is transported downwards and, of this, 42% is diverted to the roots and 32% to the nodules. About half of the carbon input of nodules is returned to the shoot as fixation products and the rest is mainly used for respiration (12%) and growth of the nodule (5%). In roots, approximately 35% of carbon required is used for respiration, so that nodulated roots use approximately 50% of the daily photosynthate for respiration (Minchin and Pate, 1973; Pate, 1976). The concept of photosynthate supply to the nodule from the shoot as the natural pace regulator of fixation, extends also to observations on diurnal variations in nodule activity. Since, the diurnal changes in acetylene-reducing activity usually show higher rates of fixation during daylight hours than at night (*e.g.* Bergersen, 1970; Mague and Burris, 1972), and fixation by detached nodules is consistently less than that of equivalent weights of attached nodules (Mague and Burris, 1972), nodules appear to be very dependent on

current translocate. The level of soluble carbohydrate in nodules falls and their rate of nitrogen fixation quickly declines once the photosynthesising shoot has been removed (Minchin and Pate, 1974). It had been widely recognized that the marked decline in nitrogen fixation during the early pod filling stage was coincident with the commencement of nodule senescence (*e.g.* LaRue and Kurz, 1973; Ham *et al.*, 1976). Numerous workers have attributed the decline in nitrogen fixing activity to competition from the developing pods and seeds for the limited photosynthates. In an attempt to define this more precisely Lawn and Brun (1974a) examined the seasonal pattern of nitrogen fixation relative to the physiological development of the soybean plant. They used treatments designed to alter the supply of photosynthetic assimilate to the nodules during the pod filling stage. Their main conclusions were (i) treatments to enhance the photosynthetic source-sink ratio (supplementary light and depodding) maintained nitrogenase activity well above control, (ii) those which reduced the source-sink ratio (shading and defoliation), decreased the activity below the level of the controls. So, they interpreted the results as evidence that nitrogen fixation declined during pod filling as a result of inadequate supply of assimilate to the nodules. In pea, Bethlenfalvay *et al.* (1978b) found that nitrogen fixation calculated from C_2H_2 -reduction and H_2 -evolution data, was significantly lower in the partially-defoliated plants and generally higher in the depodded plants than in the controls. However, they concluded that competition for photosynthetic products was shown to affect only the magnitude and timing, but not the apparent relative efficiency, of nitrogen fixation. Similar conclusions were reached in

the study using mung beans (*Vigna radiata*) by Chen and Sung (1982). They also indicated that partial defoliation and pod removal directly influenced the development of nodule mass. Thus these results suggest that the decline in nitrogen fixation could be due to the diversion of photosynthates into seeds resulting in a decreased supply to the nodules.

In contrast Wilson *et al.* (1978) have reported that in male-sterile mutants of soybean, nitrogen fixation declined in parallel with that in male-fertile plants. Since male-sterile plants produced 85% fewer pods than male-fertile plants, it appears that mobilization of photosynthates by developing seeds may not be the main cause for the decline in nitrogen fixation at the time of pod filling. They also observed that the leaves of male-sterile mutants remained green well after the leaves of control plants had fully senesced.

Reciprocal grafting was used to examine the relative importance of root and shoot on the symbiosis in soybeans (Lawn and Brun, 1974b; Lawn *et al.*, 1974). Differences in photosynthesis between genotypes influenced the amount of photosynthate available for export and influenced nodule activity (acetylene reduction activity). Root genotype appeared to have little effect on the amount of nodule tissue formed but did influence the ability of nodules to reduce acetylene. However, those studies were determined in the presence of only one strain of *Rhizobium*. Similar technique was used to study effect of root, shoot and *Rhizobium* strain on nitrogen fixation in four Asiatic *Vigna* species (Lawn and Bushby, 1982). They found that there were large and significant effects of root genotype, shoot genotype and *Rhizobium* strain on plant and nodule characters. Effects

of both shoot and root genotype on total nodule activity and nitrogen fixation appeared to be due to effects on nodule fresh weight, while those of *Rhizobium* strain were associated primarily with specific nodule activity. It is also suggested that shoot genotype differences in nodule fresh weight arose because of differences in leaf area.

In an attempt to see if it was possible to delay or reverse the decline of nitrogen fixation in soybean cultivars, grafting experiments were used by Malik (1983). He found that when young scions were grafted near to the base of fruiting stocks, these grafts produced a second peak of nitrogen fixation on the same root system, but the second peak was very small if the scions were grafted near to the apex of the stocks. However, these similar results could not be achieved when the scions used were the same age as the stock plant. He concluded therefore that the decline in nitrogen fixation was not simply controlled by mobilization of carbohydrates into seeds or by leaf senescence, but that a change in the physiological conditions of the different components of the shoot also regulated the rate of nitrogen fixation.

2.1.3.3.2 Symbiotic compatibility between rhizobium and host. Specificity between host legumes and rhizobia occurs at three levels, namely the ability to form nodules, the ability to fix nitrogen and the degree to which symbiotic nitrogen fixation meets the plant's nitrogen requirements (Gibson *et al.*, 1982). In general, a much wider range of rhizobia can nodulate plants than can fix nitrogen within nodules. For example, *Rhizobium leguminosarum* nodulates *Phaseolus* well *in vitro*, though it never ~~fixes~~ fixes nitrogen in the nodules (Beringer, 1984). Many tropical legumes have ability to form nodules

with a wide range of *Rhizobium* spp. strains. However, this generalization can be dangerous as strong specificity often exists with regard to the symbiotic effectiveness of the associations formed (Dart and Halliday, 1980).

2.1.3.3.3 Pest and disease effects. When nitrogen-fixing organisms bear pests or pathogens, the nitrogen fixing process is usually adversely affected. Conversely, the nitrogen-fixing symbiosis may affect the plant disease which could result from effects on plant nutrient status as well as direct effects (Sprent, 1986).

Plant viruses affect the structure, physiology and biology of both root nodules and host plant (Sprent, 1986).

Many interactions between plant pathogenic fungi and root nodules have been reported. In some cases, plant protection by rhizobia have been cited for example, Tu (1978) found that rhizobia actually colonised the tips of hyphae of *Phytophthora megasperma* which causes root rot of soybean and lucerne and in this way could affect fungal growth.

Root-knot and cyst nematodes interfere with nodulation of many legumes including soybean, peanut and cowpea (Robinson, 1961; Taha and Raski, 1969). Besides their harmful effects on nodulation and host vigour (Ko *et al.*, 1984) nematodes can also affect the specific nitrogen-fixing activity of the nodules (Gibson *et al.*, 1982).

The nodules on legumes are a favoured place for *Sitona* spp. and *Rivellia* spp. to lay their eggs, with most of the damage arising from the feeding larvae (Gibson *et al.*, 1982). *Colaspis brunnea* is reported to attack soybean nodules (Turnispeed, 1973). Legume also produce many toxins to pests, for example ureides are not utilised by

insect herbivores and thus nitrogen-fixing plants which export ureides may be at an advantage compared with plants of the same genotype utilising combined nitrogen (Wilson and Stinner, 1984).

2.1.4 Methodology of nitrogen fixation measurements. Several methods of measuring rates of nitrogen fixation have been developed. Very comprehensive comparisons of these methods have been published (Bergersen, 1980). A brief discussion of some methods is presented in the sections that follow.

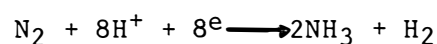
2.1.4.1 Nitrogen accumulation. The standard procedure for nitrogen analysis is the Kjeldahl determination (Bremner and Malvaney, 1982). This simplest estimate of nitrogen fixation is by total nitrogen accumulation of the crop which is based on the intuitive assumption that the crop derives all its nitrogen via symbiotic nitrogen fixation. So, many estimated values based on this methods certainly overestimate fixation (LaRue and Patterson, 1981). One promising approach using this method would be as a screening method in a breeding program. Total nitrogen production of progeny effectively nodulated by an efficient *Rhizobium* strain would be a selection criterion for high nitrogen fixation. This same approach could be used to selected for efficient rhizobia (Havelka *et al.*, 1982).

An adjusted measure of fixation by nitrogen accumulation is obtained when the contribution of soil nitrogen to the total nitrogen of legumes is estimated. The contribution of soil nitrogen is usually obtained by growing a non-fixing plant in comparison with the nitrogen fixing legume. Three versions of this method *i.e.* comparison of a legume with a non-legume, comparison of a legume with a non-nodulated legume and comparison of inoculated and uninoculated legumes were reviewed by LaRue and Patterson (1981).

2.1.4.2 Isotopic methods. The use of ^{15}N isotope as a tracer in quantifying nitrogen fixation is about the most definitive method available (Ayanaba, 1980). However, the major limitation is the high cost involved for the instruments and the isotopes and consequently the method requires skilled operators (LaRue and Patterson, 1981). There are other, less precise methods such as ^{15}N fertilizer or the 'A value' method which were also discussed by LaRue and Patterson (1981).

2.1.4.3 Acetylene reduction method. The discovery that nitrogenase has the capability of reducing acetylene to ethylene (Dilworth, 1966; Schollhorn and Burris, 1967), which, so far, is the only biological agent reported to do so (LaRue and Patterson, 1981), has provided an excellent measurement method. Innumerable variations of the method have been described (Hardy *et al.*, 1973). Typically, freshly excised roots are incubated in a chamber with 1-20% acetylene for 30-120 minutes. A sample of the gas mixture is then removed and the ethylene produced is measured by gas chromatography. This method is undoubtedly a powerful way of making comparative assessments, but it is limited as a quantitative method for the estimation of dinitrogen fixation because of the uncertainty about the dinitrogen fixing potential of nitrogenase within the nodule system (Sims *et al.*, 1983)

The principal assumption in the method involves the ratio of acetylene reduced to nitrogen fixed. The reduction of nitrogen to ammonia uses six electrons, while the production of ethylene requires two, hence the ratio 3:1 was originally assumed. However, it is now realized that the reaction of nitrogenase approximates



Furthermore, protons and acetylene compete for electrons, and with the amounts of acetylene used, only small amount of H₂ may appear. In the intact nodule the hydrogen may be metabolized by hydrogenase in appropriate strains of rhizobia and therefore not detected (LaRue and Patterson, 1981).

The acetylene reduction technique, however, has numerous advantages including its great sensitivity (10³ times more than ¹⁵N which in turn is 10³ times more sensitive than Kjeldahl or Dumas-type N determinations), portability for use in the field, and relative ease of operation (Gibson, 1974).

2.2 NITROGEN DISTRIBUTION IN GRAIN LEGUMES.

The dry matter of plants contains nitrogen in a narrow concentration range (1-7%) which can vary widely with plant species and with the type and age of plant from which it is derived. Concentrations of nitrogen in dry matter tend to be higher in shoots than in roots, and within shoots to be higher in leaves than in stems. Concentrations are usually highest in shoot apices, young leaves and fruits *i.e.* in mature seeds of soybean nitrogen may comprise up to 7% of the dry weight (Pate and Layzell, 1981).

In this section apart from a brief review of nitrogen assimilation, the nitrogen distribution, nitrogen re-distribution, and carbon:nitrogen relationships will also be reviewed.

2.2.1 Nitrogen assimilation: In general, legumes have two different systems of nitrogen assimilation (Brun, 1978). For example, firstly, legumes can absorb fixed or chemically-combined nitrogen from the soil through their roots. This nitrogen, usually in the form of

nitrate ion (NO_3^-), is then translocated through the xylem to the leaves, where energy derived from photosynthesis is utilized to reduce the NO_3^- to amino nitrogen which is then incorporated into proteins in the leaves. Alternatively, they may be translocated to other sinks and there assembled into protein. Secondly, the molecular nitrogen diffusing from the soil atmosphere into the nodule is reduced to amino nitrogen utilizing energy derived from the respiration of photosynthates translocated to the nodules from the leaves. The amino nitrogen is then transported in the xylem to the leaves where it is converted to various amino acids and then moved via the phloem to whatever nitrogen sinks there are at that stage of plant development.

Several experiments have shown that the compounds characteristic of nitrogen transport in N_2 -fixing plants are species specific, *i.e.* ureides (allantoin and allantoic acid) are the main compounds exporting fixed nitrogen in certain tropical legumes (*e.g.* soybean) (McClure and Israel, 1979; Sinclair and de Wit, 1976) while asparagine and glutamine are the translocated solutes in many temperate legumes (Pate, 1977).

Leaves play an important role in nitrogen metabolism. They are generally the most important organ in amino acid synthesis and nitrogen storage, both because large amounts of nitrogen are required in leaf growth and because nitrogen deficiencies strongly limit leaf growth and photosynthesis (Novoa and Loomis, 1981). Nitrate reductase activity of leaves shows diurnal and seasonal changes, *e.g.* the diurnal pattern in soybean leaves involved an increase in activity during the day and a decrease during the night (Nicholas *et al.*, 1976). Harper and Hageman (1972) found with soybeans that nitrate

reductase activity was maximal just after full bloom and was associated with high levels and a high nitrogen demand for grain filling. When account is taken of the distribution of nitrogen throughout the plant in a variety of annual species it is clear that leaf-bound nitrogen has considerable significance in nourishment of fruits and seeds (Pate, 1980). In the protein-rich grain legumes, the timing of nitrogen mobilization from leaves becomes especially critical in view of the effect which loss of protein from leaves may have on further photosynthetic activity of the leaves (Sinclair and de Wit, 1975). This may lead to accelerated senescence and eventually their death. This so called 'self destruction' shortens the plant's life cycle and hence limits dry matter production.

2.2.2 Nitrogen distribution: Distribution of nitrogen over the various plant parts is meaningful only at a specific time because major changes in nitrogen concentration and distribution occur during the development of the plant (deMooy *et al.*, 1973). The number of experimental reports comparing soybean cultivars for nitrogen concentrations and accumulation rates is limited. Hanway and Weber (1971 a,b,c) determined the nitrogen concentrations and accumulation rates in plant parts, positions on the plants, stage of development and the level of nitrogen applied, but the differences among cultivars were very small. They also indicated that the above-ground portion of plant accumulated nitrogen during pod filling at the rate of $18 \text{ mg d}^{-1} \text{ plant}^{-1}$, while nitrogen accumulated in the seeds at the rate of $25 \text{ mg d}^{-1} \text{ plant}^{-1}$. Spaeth and Sinclair (1983) surveyed 121 cultivars for variation in nitrogen concentrations and quantities in the vegetative parts and seeds at two stages of development (just before the

beginning of seed development and prior to physiological maturity). They found that nitrogen concentrations varied substantially among cultivars, as did total nitrogen uptake, generally in proportion to plant size. Rates of nitrogen accumulation in both the seeds and the whole plant (above ground) varied among cultivars and indicated differing rates of nitrogen translocation from the vegetative tissue to the seeds.

2.2.2.1 Effects of source-sink manipulation on nitrogen distribution: Manipulating the source and sink sizes offers another approach to assessing relationships between nitrogen requirements of vegetative and reproductive plant parts. Kollman *et al.* (1974) found that in soybean, as sink size increased from 0 to 2.7 pods per node, nitrogen concentration of leaves plus stems decreased by 72%. Alberda and Wiltage -de Boer (1983) found that in depodded soybean plants dry matter continued to accumulate in all plant parts, as did the amount of nitrogen. However, the nitrogen uptake per unit of dry matter produced was about the same for both intact and depodded plants. When the carbohydrate source was suppressed (*e.g.* shading) during the late reproductive period Neves *et al.* (1982) found in cowpea that stored foliar nitrogen was utilised to a greater degree (12%) than in unshaded plants. Kato (1980) reported that in mature leaves the rate of protein breakdown increased with increasing leaflet removal -leaflets were regarded as a source of nutrient supply to the apical sinks. He suggested that protein turnover rate, and the N-supplying capacity of leaves, could be regulated by sink size.

2.2.3 Nitrogen re-distribution: Some studies examining the uptake and distribution of nitrogen by soybean have shown that losses

of nitrogen from the vegetative tissue coincides with the accumulation of nitrogen in the seed (*e.g.* Hanway and Weber, 1971b) and with plant senescence (Sesay and Shibles, 1980). The soybean seed has a high nitrogen requirement because of its high protein content. This high nitrogen requirement, coupled with the decline in the nitrogen assimilatory processes during seed filling, suggested that the nitrogen assimilatory processes fail to meet the nitrogen requirement of the seed and, therefore, nitrogen must be re-distributed from the vegetative tissues to meet the deficit (Sinclair and de Wit, 1975). Earlier work by Hanway and Weber (1971c) established that more than 50% of the nitrogen in mature soybean seeds could be accounted for by net losses of this nutrient from other plant parts. In lupin (*Lupinus albus*) Withers and Forde (1979) estimated that leaves supplied 47% and pods wall contributed 15% of seed nitrogen requirement. Zeiher *et al.* (1982) examined the nitrogen re-distribution characteristics of soybean cultivars of varying maturities and growth types, and found that the cultivar differences in nitrogen re-distribution related to the size of the nitrogen pool which, in turn, was primarily a function of the dry weight differences associated with the differences in the times those cultivars matured. They concluded that nitrogen re-distribution did not appear to be an important factor determining the duration of seed filling or yield in soybeans. Such a re-distribution pattern seems to take place during the late pod filling stage. For example, Nelson and Weaver (1980) found that during the first 17 days of the pod filling period, nitrogen accumulation in the pods was greatest without the coincident net loss of nitrogen from the leaflets.

The role of nitrogen re-distribution after early pod filling was examined by Egli *et al.* (1978a). Using solution culture, they found that early N removal resulted in hastened leaf and petiole abscission and increased the amount of nitrogen in seeds derived from re-distribution within the plant. They concluded that re-distribution of nitrogen from vegetative to reproductive parts influenced senescence but that other factors which they did not report were also involved. A similar view was shared by Nooden (1984); in his recent review he concluded that monocarpic senescence (the degeneration leading to death of the whole organism at the end of the reproductive phase) is closely associated with pod development and both are subjected to a series of correlative controls. The nature of these controls is largely unknown but hormones as well as nutrient fluxes seem to be involved.

2.2.4 Carbon : nitrogen relationships: Although nodules have an absolute requirement for carbon (C), and photosynthetic tissues have a complementary requirement for nitrogen (N), the processes of C and N assimilation do not strictly parallel each other throughout growth (Pate and Minchin, 1980). For example, data from Herridge and Pate (1977) and Pate and Herridge (1978) showed that in cowpea and white lupin relatively more N₂ than CO₂ is assimilated during vegetative growth, but relatively reverses after flowering.

The translocation of photosynthate from leaves to pods has been found to have a specific pattern. In soybean, Stephenson and Wilson (1977a,b) found that the ¹⁴C fixed subsequent to flowering went to pods in the axil of treated leaves and to the pods at the adjacent nodes above and below fed leaves, while the more remote pods were much

less important sinks. The distribution of photosynthate, however, seems to be largely affected by the stage of plant growth; for example, Waters *et al.* (1980) found that during the pod filling stage in beans (*P. vulgaris*), over 85% of the ^{14}C activity exported from the leaf moved into pods, and less than 1% to the nodulated root system but at the flowering stage, 45% of ^{14}C was recovered from the roots and the nodules accumulated only 3.5% of the radioactivity.

Photosynthesis after anthesis is a considerably more important source of carbohydrate for seed than is mobilisation from materials assimilated during vegetative growth (Eaglesham *et al.*, 1977; Hanway and Weber, 1971a). Photosynthesis by pod walls may play an important role - to produce the same dry weight of seeds, a darkened pea fruit is estimated to require 16-20% more C from the shoot than is a fully illuminated fruit (Flinn *et al.*, 1977).

From comparisons of carbon economy of nodulated and non-nodulated root systems (Ryle *et al.*, 1979; Schubert and Ryle, 1980), the symbiotic nitrogen metabolism in nodulated roots needs higher levels ^{of} carbohydrate than that of non-nodulated systems. Ryle *et al.* (1979) found that in soybean, cowpea and white clover, plants fixing their own nitrogen respire 11-13% more of their fixed carbon each day than equivalent plants lacking nodules and utilizing nitrate nitrogen. However, this apparent advantage of non-nodulated plants is not always translated into improved seed yield. For example, although nitrate-dependent cowpea plants produce more dry matter than nodulated plants, this is used in the production of thicker stems, heavier branches, and more leaves and not for additional reproductive sites. Furthermore, the higher concentrations of N in the leaves of non-nodulated plants

remained higher than those of nodulated plants after abscission (Summerfield *et al.*, 1977).

By the end of the growth cycle, when fruits are mature, the annual grain legume is likely to have effected a net transfer of from 60-70% of its nitrogen from vegetative to reproductive parts (Pate and Atkins, 1983). According to Atkins *et al.* (1978) and Pate and Minchin (1980), grain legume species can differ widely in the pattern of nitrogen flow to their fruits. For example, in certain cultivars of mung bean (*Vigna radiata*) and white lupin (*Lupinus albus*) more than one quarter of the nitrogen fixed over the growth cycle occurs during late pod filling, suggesting that the translocation of recently-fixed nitrogen through stem and leaves to fruits is a major transport component during fruit development. On the other hand, in certain cultivars of cowpea (*Vigna unguiculata*) and pea (*Pisum sativum*), fixation declines abruptly after flowering, with the result that only 10-16% of the plant's total nitrogen is affected during pod filling. In these cases the mobilization of existing reserves of nitrogen from stem, leaf and root to fruits must obviously provide the major source for the final stages of seed ripening.

A frequently cited hypothesis (Sinclair and de Wit, 1975) is that in high protein legumes such as soybean, N required for seed must be derived from leaves, and stems, ^{and} this will result in the reduction of photosynthetic activity and lead to leaf senescence or 'self destruction'. However, the extent to which legumes show 'self destruction' can vary markedly between species and cultivars of a single species (Phillips, 1980). It is yet unclear to what extent variation in supply and demand for C and N by different organs causes

these declining leaf and nodule functions or whether the changes are mediated through specific adjustment in the gradients of particular translocated growth regulators which in turn regulate photosynthesis or the onset of senescence (Atkins, 1984).

2.3 GROWTH AND DEVELOPMENT OF DETERMINATE AND INDETERMINATE LEGUMES.

2.3.1 Growth types of grain legumes.

The basic structural components of major legume crops are similar, but the morphology of the two growth forms are commonly distinguished, the so-called indeterminate and determinate types. The theoretical distinction between the two growth types is that in the determinate type, growth in length of a main stem or branch is terminated by an inflorescence, while in the indeterminate type vegetative growth continues (Smartt, 1976).

However, many leguminous species are morphologically unstable. Some *Phaseolus* genotypes are determinate in one locality and indeterminate elsewhere. Some determinate cowpea can elongate and become indeterminate in warm (24°C) as compared with cool (19°C) night temperatures (Summerfield and Wien, 1980). It has been suggested that differences in light quality are the main cause of morphological variation (Kretchmer *et al.*, 1977).

There is a tendency amongst modern legume breeders to favour the determinate plant type, which is compatible with mechanical harvesting (Khan, 1977). However, the indeterminate plant type which has been reported to be superior in nitrogen fixation in bean (*P. vulgaris*) (Graham, 1981), or less affected by defoliation in soybean (Fehr *et al.*, 1977), should not be overlooked especially in the

tropics where the indeterminate type may provide greater yield stability in adverse growth conditions.

2.3.2 Soybean growth and development.

The soybean, *Glycine max* (L.) Merrill, is an annual plant, the developmental morphology of which has been extensively reviewed (for example Carlson, 1973; Hicks, 1978). The objective of this section is to briefly discuss its growth and development in general,

with emphasis on the differences between different growth types

2.3.2.1 Root growth: Maximum soybean seed yield depends to a large extent upon a well nodulated, extensive root system (Hicks, 1978). Mitchell and Russell (1971) reported that field grown soybeans had a major portion of the root system consisting of lateral roots arising from the upper 10-15 cm section of the primary root. These lateral roots extend outward from the plant nearly horizontally for 40-50 cm and then grow downward to depths as great as 180 cm. Root growth continues until sometime during the seed filling period when it declines and ultimately ceases prior to the seed reaching physiological maturity (Hicks, 1978).

Brouwer (1962) suggested that there was a functional equilibrium between shoots and roots. When shoot growth was rapid, there was a corresponding utilization of carbohydrates which limited root growth. When root growth was limited there was a build up of carbohydrates in the leaves owing to the lack of water and nutrients from the roots. Therefore, carbohydrates again became more available to the roots. In an attempt to differentiate between the contribution

of shoots and roots upon soybean growth, Sanders and Brown (1976) varied the shoot : root ratio by grafting to get ratios of 1:1, 1:2, 1:3, 2:1, and 3:1 respectively. Increasing the number of shoots gave increases in leaf area, number of leaves per plant, seed yield and number of seeds per plant. However, increasing the number of shoots did not increase the growth characteristics and seed yield as dramatically as increasing the number of roots. Although, the physical shoot : root ratio was varied by grafting, the shoot : root ratio based on dry weight was nearly the same (approximately 10:1) for all treatments.

2.3.2.2 Vegetative growth: In soybean, the development begins when the radicle emerges from the germinating seed and rapidly develops into a root, growing downward. Under favourable conditions for growth, the cotyledons emerge from the soil in 5-10 days after planting (Johnson and Dunphy, 1983). The lowermost node is the point of attachment for the cotyledons, the next node gives rise to the opposite unifoliolate leaves and all subsequent nodes produce single trifoliolate leaves alternately up the stem (Hicks, 1978). A new trifoliolate leaf is produced one to each node, about every 5 days during early growth and every 3 days thereafter. It takes about a week for each trifoliolate leaf to expand to its full length (Johnson and Dunphy, 1983). Most of the branch development is first order branching on the main stem. Both genetic and environmental effects such as daylength, and plant spacing will affect branching (Carson, 1973). Depending upon planting density, 0-6 branches per plant are usual. All nodes however, possess the potential for both branch and floral development (Shibles *et al.*, 1975). The number of nodes and

internodes that ultimately make up the main stem depends on the reaction of the genotype to the photoperiod in which it is grown, and the growth types (Hinson and Hartwig, 1977).

After an initial lag, leaf area production increases rapidly and nearly linearly up to the end of blooming, attaining maximal leaf area index (LAI) values of 5-8. Thereafter, LAI declines progressively, by abscission of the lower leaves (Shibles *et al.*, 1975).

2.3.2.3 Reproductive growth: Soybean cultivars differ in the critical day-length that they require for flower initiation. Temperature also influences the time required to reach floral differentiation, floral expression and subsequent reproductive stages under inductive photoperiods (Shibles *et al.*, 1975). The node producing the first flower usually is the second trifoliolate leaf node or above. The soybean is self-pollinated, pollination occurring when the flower opens or just prior to being slightly opened. Flowering occurs over a 4 to 6 week period, depending upon seasonal adaptation, but the sequence of flowering differs with growth type (Shibles *et al.*, 1975).

Pods develop slowly for the first few days following fertilization. Rapid elongation begins about the fifth day, and full length is attained by the 15th to 20th day (Shibles *et al.*, 1975). Seed development is rapid, ^{and} a mature pod may contain one to five seeds of which two to three seeds per pod are most common (Hicks, 1978). Major differences in seed yield were attributable to the length of the seed development period rather than to the rate of daily growth (Hanway and Weber, 1971d). Final seed size is influenced both by

environmental and genetic factors. Average seed weight ranges from 120-280 mg per seed (at 13% moisture). Cultivars which produce fewer seeds tend to have a large seed size (Shibles *et al.*, 1975).

2.3.2.4 The different growth types of soybeans: Soybean stem growth and flowering habits are commonly divided to two types, determinate and indeterminate. There are two genetic types of determinateness, dt1, recessive and dt2, dominant to indeterminateness (Bernard, 1972).

2.3.2.4.1 Vegetative growth: The determinate type is characterized by vegetative development which ceases when the apical meristem becomes an inflorescence, while in the indeterminate type, the apical meristem continues vegetative activity during most of the growing season so that the flowering period and the time of overlap of vegetative and reproductive growth are greater for indeterminate than that of ^{the} determinate type (Hicks, 1978). Growth of the main stem and branches of the indeterminate cultivars continued after first flowering, whereas in the determinate cultivars, vegetative growth during flowering was apparently limited to the expansion of pre-existing nodes and leaves (Lawn and Byth, 1974). The vegetative canopies of indeterminate and determinate types are distinctly different. In indeterminate, the largest leaflets and the longest petioles occur in mid-plant with gradations in size toward each end of the stem. With determinates, leaflets size and petiole length are not smaller at the top of the plant, so their canopies possess poorer light distribution characteristics than do indeterminates (Shibles *et al.*, 1975). Dry matter accumulation patterns in determinate and indeterminate lines were found to differ. Of the total above ground

dry weight (not including seed), the indeterminate had produced 58% and 87% compared with 78% and 92% of the determinate at the onset of flowering and pod development respectively (Egli and Leggett, 1973).

2.3.2.4.2 Reproductive growth: Time to flowering is critical to the size of source developed in determinate cultivars,

Indeterminate cultivars can develop a large source capacity as well as long reproductive period, with an additional possible benefit that the early stage of pod-fill are accomplished when the canopy still has a large proportion of relatively young leaves, whereas with determinate type much of the yield is produced from ageing leaves (Shibles, 1980). Reproductive growth of the indeterminates begins at about the time the plants reaches one-third of its height. Flowering continues until the main stem stops elongating. Lawn and Byth (1973) reported that the indeterminate cultivars flowered for considerably longer periods than did determinate cultivars flowering at the same time. Eventhough, in the indeterminate cultivars, the pods at the bottom begin developing earlier than the pods at the top of the plants, all pod mature at about the same time due to a faster growth rate in the top pods (Johnson and Dunphy, 1983). However, it was reported that indeterminate cultivars tended to have smaller seed because the duration of the effective filling period was less for the later developed pods (Egli *et al.*, 1978b).

Schapaugh and Wilcox (1980) reported that no relationship was detected between the growth habit and the harvest index of a genotype, when they examined the relationship among harvest index and other plant characteristics of determinate, semi-determinate and

indeterminate cultivars from four maturity groups. When the harvest index of field grown soybeans of both growth types was measured in response to four types of environmental influence which resulted in considerable variability in plant size within a cultivar, Speath *et al.* (1984) found that harvest index of a cultivar was a stable characteristic irrespective of the environmental conditions.

2.3.2.4.3 Nitrogen fixation: Not much attention has been given to define the differences between growth types in their ability to fix nitrogen. Hardy *et al.* (1971) reported that determinate and indeterminate soybeans of similar maturity dates had similar fixation characteristics. Although determinate cultivars may fix up to 25% of the total nitrogen prior to flowering in contrast to 10% for indeterminate cultivars. It was also reported that several indeterminate *soybean cultivars*, which had a relatively long flowering period, had a prolonged phase of rapid fixation (Hardy *et al.*, 1971; Duke *et al.*, 1979). In bean (*Phaseolus vulgaris*), Graham (1981) concluded, from a review of field studies, that nodulation and nitrogen fixation abilities of climbing, strongly-indeterminate cultivars were consistently superior to those of most determinate bush cultivars.

2.3.2.4.4 Response to less favourable environments: Lawn *et al.* (1977) found that the indeterminate cultivars showed no yield differences for a wide range of populations under full irrigation which indicated that these cultivars can adjust to wide differences in plant density within the row under conditions favourable to growth.

The response of the two growth types of soybean to complete defoliation and half-plant cut-off at six reproductive stages was significantly different^t. Average yield reduction from defoliation for all stages was 59% for determinate cultivars, compared with 39% for the indeterminate cultivars (Fehr *et al.*, 1977).

The ability of the indeterminate growth type of soybean to continue to develop vegetative growth over several weeks has helped to reduce the yield losses due to earlier stressed conditions in the reproductive stage (Dunphy *et al.*, 1979; Laohasiriwong, 1982). This response was associated with more pods per node rather than with other yield components, or with leaf area index or leaf area duration differences (Villalobos-Rodriguez and Shibles, 1985). In cowpea, Chaturvedi *et al.* (1980) concluded that the indeterminate cultivars were better than determinate cultivar under dryland conditions.

On the basis of these studies it seems worthwhile to examine the differences between the determinate and indeterminate cultivars of selected legumes in relation to seed yield, nitrogen fixation and nitrogen distribution.

Initially, spaced plants (glasshouse and growth room) will be used to investigate the hypothesis developed in this thesis. There are a number of reasons for this:

- (a) It is much easier (from the resources and space points of view) to handle spaced plants.
- (b) Information obtained from spaced plants will still be useful because in some tropical regions farmers will plant legumes as a intercrop where the spacing is such that the plants will never form a closed canopy.

CHAPTER 3

A study of nitrogen fixation, nitrogen distribution and yield in selected bean and soybean cultivars with either determinate or indeterminate growth.

ABSTRACT.

Two commercial cultivars of bean (*Phaseolus vulgaris*) and two cultivars of soybean (*Glycine max*), one cultivar with a determinate and the other with an indeterminate growth type were studied in glasshouse conditions in relation to nitrogen fixation, nitrogen distribution and yield.

The indeterminate cultivar of both species had higher leaf area, more root growth and accumulated more total dry weight than that of the determinate cultivar. Nodule dry weight of the ~~indeterminate~~ indeterminate cultivar of both species was also higher than that of the determinate cultivar. However only the indeterminate soybean cultivar showed significantly more acetylene reduction activity than that of the determinate cultivar. In both species the indeterminate cultivar also accumulated more total plant nitrogen than the determinate cultivar. The seed yield advantage of the indeterminate cultivar over the determinate cultivar was about twice in bean and three times in soybean. However, there was no difference in the ratio of grain:above-ground biomass between growth types in term of dry weight (harvest index) or nitrogen (nitrogen harvest index) in both species.

3.1 INTRODUCTION

Although many studies have examined the nitrogen fixation of legumes, there are few which make direct comparisons of different growth types in relation to nitrogen fixation and nitrogen distribution. Also, opinions of how the different growth types could affect nitrogen fixation have varied. For example, Gibson (1980) indicated that with many species, nitrogen fixation declined soon after pod set or during early pod filling while those with a long vegetative phase (e.g. indeterminate cultivars of *Phaseolus vulgaris*) nitrogen fixation was prolonged and consequently increased seed yield. In contrast, Latimore *et al.* (1977) suggested that because there would be less vegetative competition during reproductive development in determinate soybean there would be greater quantities of available photosynthate for nodule maintenance and nitrogen fixation.

Dry-matter accumulation of soybean lines, was found to differ with either determinate or indeterminate growth. Of the total above-ground dry weight (excluding seed), the indeterminate had produced 58% compared with 78% of the determinate cultivar at initial flowering (Egli and Leggett, 1973).

It is clear therefore, that differences in dry matter accumulation and nitrogen fixation dynamics between determinate and indeterminate growth types are poorly defined. Consideration of the uses of the two types need to be based on differences at the same stage of development or similar times after planting or at physiological maturity.

This study was carried out to compare, under glasshouse conditions, the different growth types of bean and soybean at

different growth stages, in relation to nitrogen fixation, nitrogen distribution and seed yield.

3.2 MATERIALS AND METHODS

The experiment was carried out in a glasshouse at Plant Physiology Division, Department of Scientific and Industrial Research (DSIR) Palmerston North, New Zealand.

Two commercial cultivars of bean and two cultivars of soybean from Crop Research Division, DSIR, Pukekohe, New Zealand, were used:

<u>Species</u>	<u>Cultivar</u>	<u>Growth type</u>
Bean	'Tender Green'	Determinate
	'Mangere Pole'	Indeterminate
Soybean	'Matara'	Determinate
	'Amsoy'	Indeterminate (Anderson,

pers. comm.)

Plants were sown in 4.5 litre pots with a mixture of sand and pumice (75:25 v/v) under the following environmental conditions:

- Temperature; heat 18°C, vent 25°C
- Photoperiod 14 h

The experimental design was a 2x2 factorial with a randomized complete block arrangement and 3 replications.

Plants were well-watered at regular interval with nitrogen-free half strength Hoagland's A nutrient solution . The recommended *Rhizobium* cultures (NZP 5060 for bean, and NZP 5250 for soybean) from Grassland Division, DSIR, Palmerston North, New Zealand were applied several times after emergence to ensure good nodulation.

A weekly destructive harvest was carried out to determine nitrogen fixation; the dry-weights of leaves, stems, roots, nodules and pods were also determined.

The acetylene reduction technique was used to measure nitrogen fixation activity. The details of the procedure are presented in Appendix B.

Total nitrogen of different plant parts was analysed by the Kjeldahl method, with details of the procedure described in appendix C.

Statistical analysis. The growth data of each cultivar was described by a functional approach, using appropriate growth functions to fit the data against time by the least squares technique. The following growth functions were applied:

1. logistic equation $Y = Y_0 / (1 + e^{-(a+bx)})$

or $\text{Ln} (Y / (Y_0 - Y)) = B_0 + B_1 X$ in linear regression form

2. quadratic logistic equation

$$\text{Ln} (Y / (Y_0 - Y)) = B_0 + B_1 X + B_2 X^2$$

3. gompertz equation $Y = Y_0 A^{B^X}$

or $\text{Ln} (\text{Ln}(Y_0/Y)) = B_0 + B_1 X$

4. quadratic exponential equation

$$\text{Ln}(Y) = B_0 + B_1 X + B_2 X^2$$

where Y = original data

Y_0 = upper asymptote

A, B = constants

B_0, B_1, B_2 = regression estimates of the
function parameters

X = time

To make the choice of the appropriate function, several criteria of best fit were considered. The criteria included relevance of curve shape, maximization of coefficients of determination (R^2), minimization of residuals, and applicability to all set of data compared. Subprogram regression in 'SPSS' (Nie *et al.*, 1975) was used. The t-test of B_0 , B_1 and B_2 among different curves and t-test of Y estimated from curves were also done based on t-test in Draper and Smith (1966), using the Fortran program 'Regcom' (Gordon, *pers. comm.*).

Calculation of relative growth rate and net assimilation rate and their standard errors was done, using the Fortran version of the the original program by Hunt and Parsons (1974).

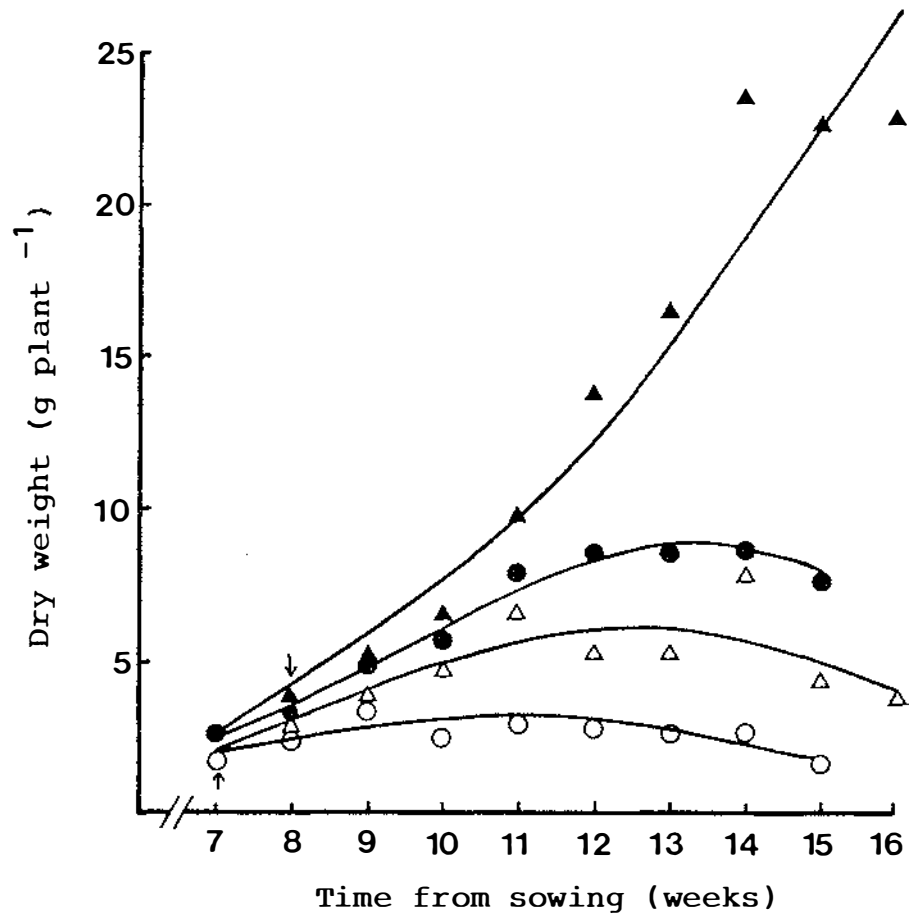
A two-factor factorial analysis of variance, incorporating the constraints of a randomized complete block design, was used. The analysis was done using the Genstat' program (Alvay *et al.*, 1977)

The statistical symbols used throughout this thesis, unless otherwise stated, are presented in Appendix A.

3.3 RESULTS

3.3.1 Plant growth: Quadratic curves were successfully used to fit the change in total dry weight and vegetative dry weight (leaf + stem) of each cultivar with time (Figures 3.1a and b). The details of these regression equations are presented in Appendices 3.1 and 3.2 . Comparisons of total and vegetative dry weight (Y) estimated from the same growth stages were made using the t-test. The results showed that in bean the indeterminate cultivar accumulated more total dry weight than that of the determinate cultivar at every growth stage after flowering, while in soybean the two growth types were different

a) Bean



b) Soybean

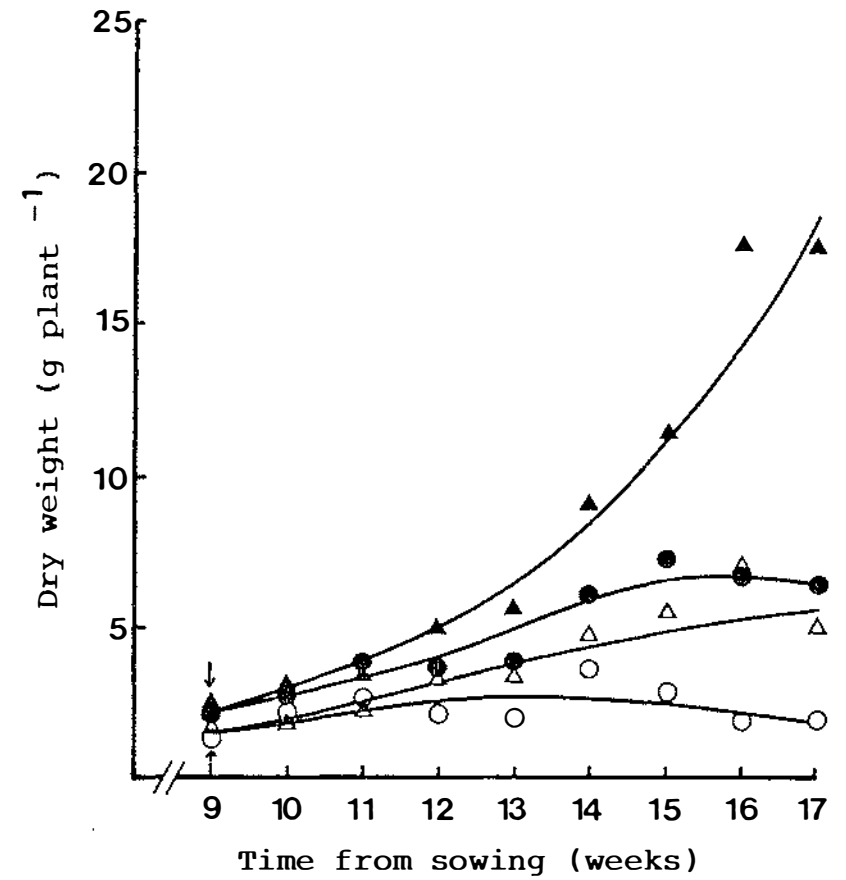


Figure 3.1 Time course of total plant dry weight (●▲) and vegetative dry weight (leaf and stem) (○Δ) of the determinate (○) and indeterminate (Δ) cultivars of bean and soybean.

(S.E. of regression coefficients are in Appendices 3.1 and 3.2)

(Arrows indicate time of first flowering)

only at the late grain filling stage (Table 3.1). The vegetative dry weight (leaf + stem) of the determinate cultivars of both species reached the maximum weight soon after flowering. In contrast, it continued to increase for a few weeks after flowering in both of the indeterminate cultivars. The indeterminate bean produced more vegetative dry weight than the determinate at every growth stage, but the superiority of the indeterminate over the determinate soybean was found only at the two late stages (Table 3.2). Relative growth rates calculated from the fitted curves, are presented in Figures 3.2a and b. Higher relative growth rates were estimated in the indeterminate growth type of both species especially at the later stages of growth.

Leaf area: Change in leaf area over time were also best described using quadratic curves (Figures 3.3a and b) although the coefficients of determination (R^2) of the determinate type of both species were quite low (Appendix 3.3); the F-tests of variance due to regression were highly significant ($P < 0.01$). Comparisons of Y-estimates indicate that the differences in leaf area at every growth stage for bean were significant between types, but in soybean significant differences were only detected at the two later stages (Table 3.3).

Net assimilation rate: The net assimilation rate was calculated from fitted curves using the formula from Buttery (1969). the results are presented in Figures 3.4a and b. The pattern of change in net assimilation rate for the same growth type in each species was similar. The main difference was at the late reproductive stages in which the determinate cultivars showed a downward trend while in the indeterminate cultivars net assimilation rate increased.

Table 3.1 Comparison of total plant dry weight between the determinate (D) and indeterminate (I) cultivars of bean and soybean, estimated from growth curves, at different growth stages.

Species	Growth type	GROWTH STAGE			
		Flowering	Pod Development	Grain Filling	Late Grain Filling
		Total plant dry weight (g plant ⁻¹)			
Bean	D	2.40	4.80	7.40	8.80
	I	4.10	7.50	12.60	19.10
	t-test	**	**	**	**
Soybean	D	2.05	3.40	4.80	6.00
	I	2.20	3.80	6.50	11.00
	t-test	ns	ns	ns	*

Table 3.2 Comparison of leaf & stem dry weight between the determinate (D) and indeterminate (I) cultivars of bean and soybean, estimated from growth curves, at different growth stages.

Species	Growth type	GROWTH STAGE			
		Flowering	Pod development	Grain filling	Late Grain filling
		Leaf & stem dry weight (g plant ⁻¹)			
Bean	D	1.80	2.70	3.10	2.70
	I	3.10	4.90	6.00	5.60
	t-test	**	**	**	**
Soybean	D	1.50	2.20	2.60	2.40
	I	1.60	2.60	3.70	4.80
	t-test	ns	ns	**	**

| S.E.

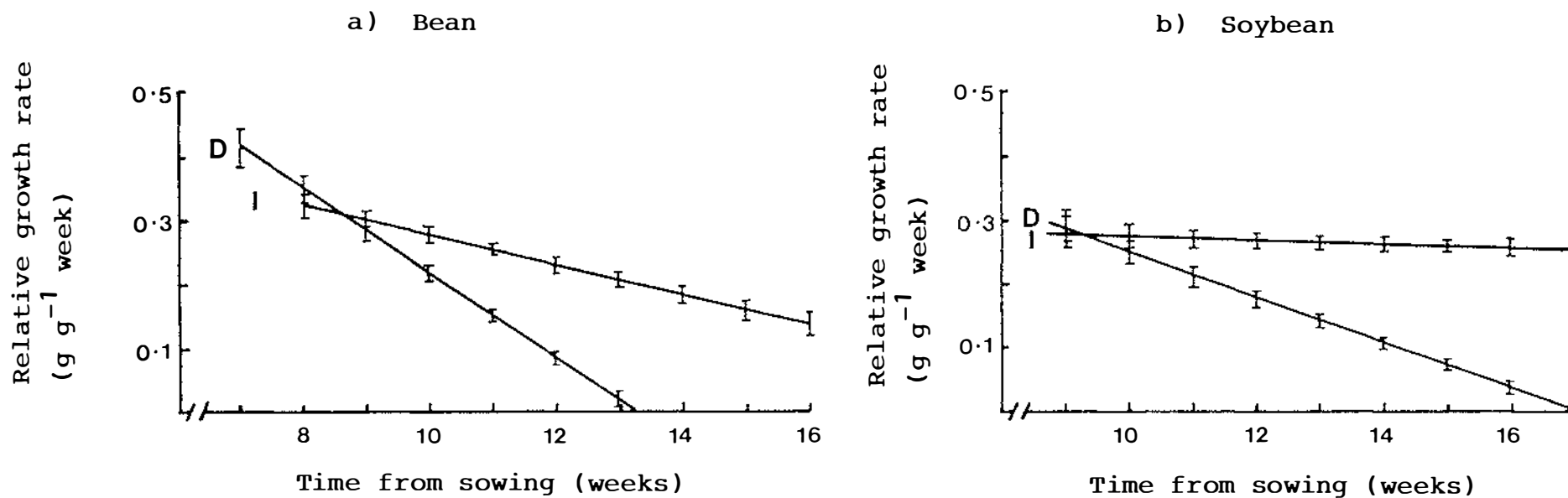


Figure 3.2 Time course of relative growth rate of the determinate (D) and indeterminate (I) cultivars of bean and soybean.

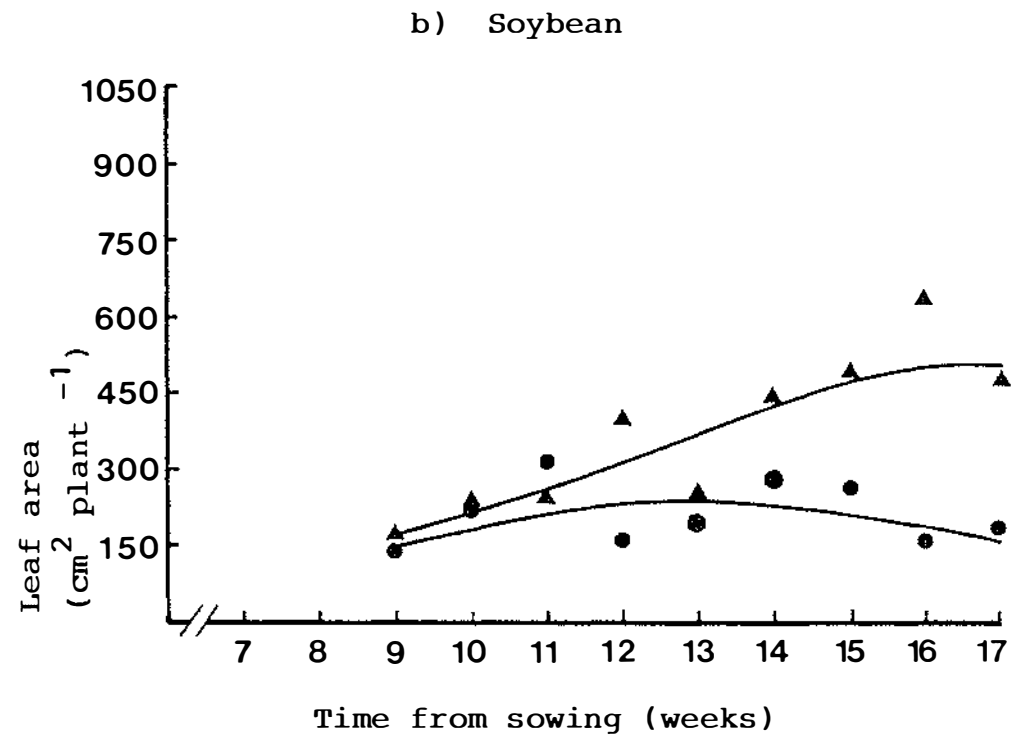
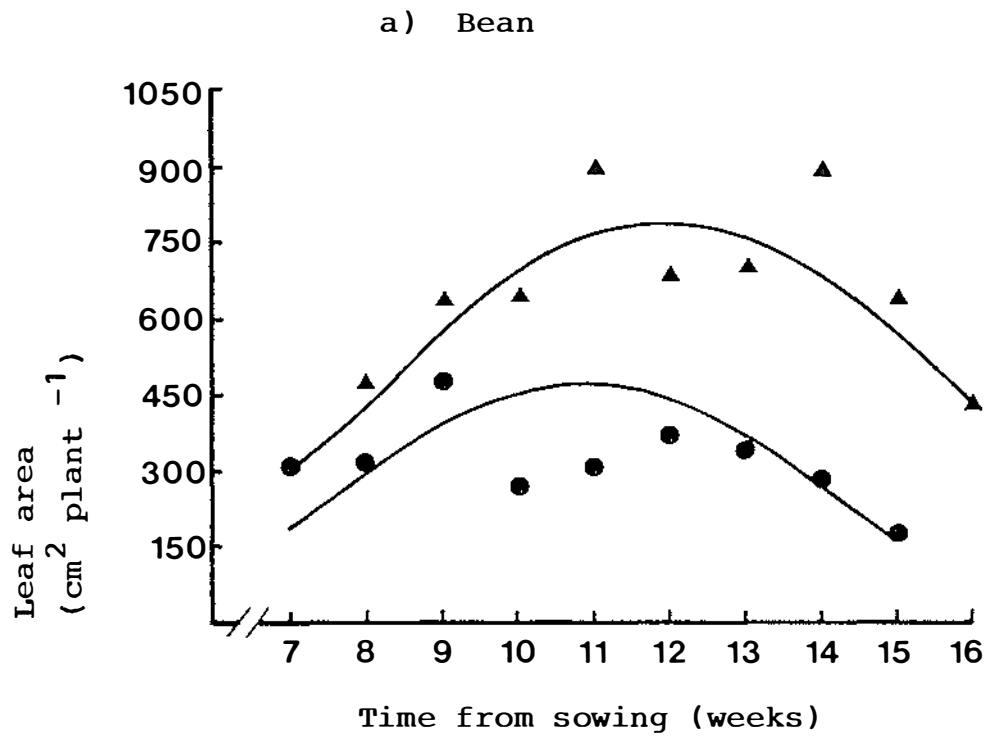


Figure 3.3 Time course of leaf area per plant of the determinate (●) and indeterminate (▲) cultivars of bean and soybean.

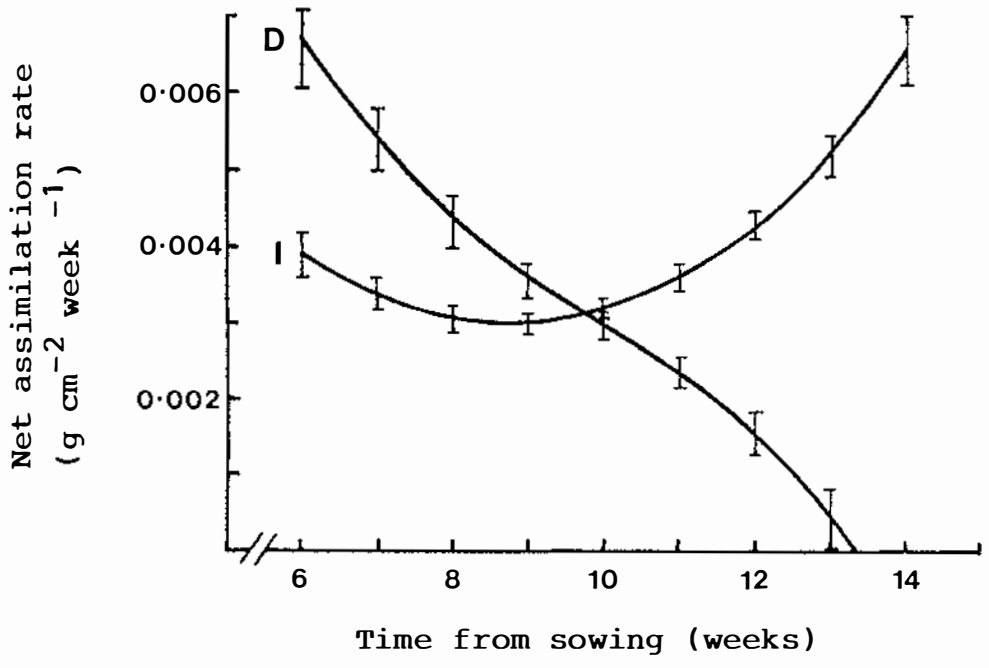
(S.E. of regression coefficients are in Appendix 3.3)

Table 3.3 Comparison of leaf area per plant between the determinate (D) and indeterminate (I) cultivars of bean and soybean, estimated from growth curves, at different growth stages.

Species	Growth type	GROWTH STAGE			
		Flowering	Pod development	Grain filling	Late grain filling
		Leaf area per plant (Cm ² plant ⁻¹)			
Bean	D	187.5	380.8	473.7	361.0
	I	437.6	691.8	805.5	686.0
	t-test	**	**	**	**
Soybean	D	150.3	204.2	228.6	210.7
	I	164.7	260.6	365.7	455.2
	t-test	ns	ns	**	**

S.E.

a) Bean



b) Soybean

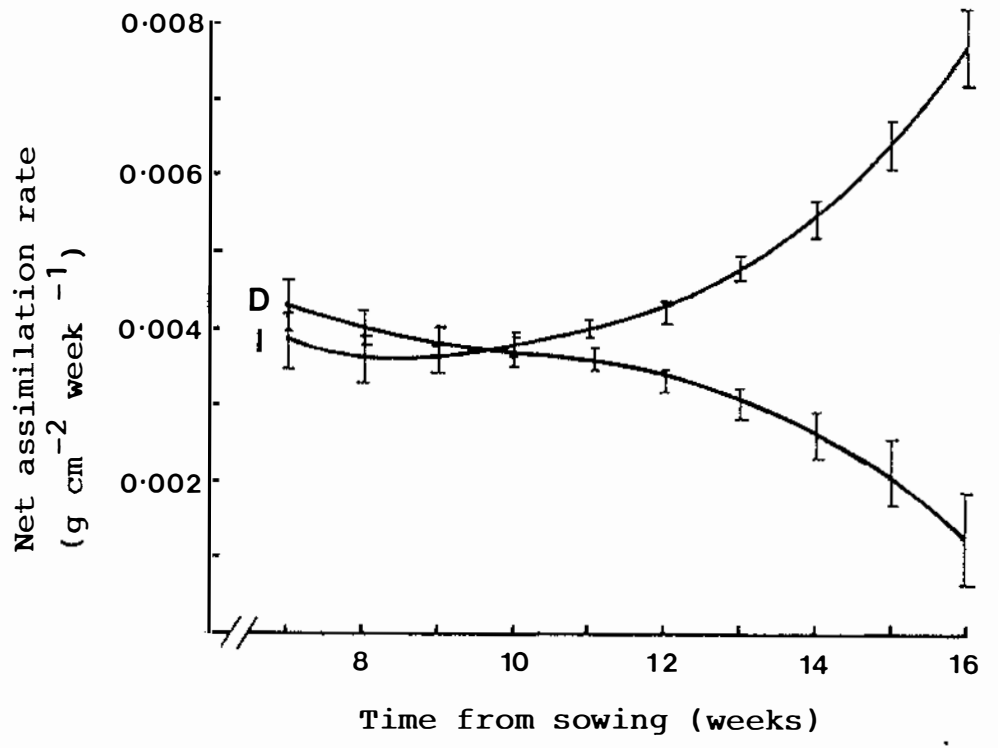


Figure 3.4 Time course of net assimilation rate of the determinate(D) and indeterminate (I) cultivars of bean and soybean.

Root growth: The root growth of the two growth types was also different in both species. Root dry weight of the indeterminate cultivars of both species reached a maximum value later than that of the determinate cultivars (Figures 3.5a and b). In other words root growth of the indeterminate cultivars continued for a longer period. The details of the root growth curves are presented in Appendix 3.4. Comparison of Y-estimates from fitted curves showed that the indeterminate cultivars had more root dry weight than those of the determinate cultivars of both species at every growth stage (Table 3.4).

3.3.2 Nitrogen fixation and plant nitrogen:

Nitrogen fixation, measured using the acetylene reduction technique, was described quite well with a quadratic equation (Figures 3.6a and b; Appendix 3.5). The comparison of values at different growth stages, estimated from fitted curves, indicated in bean that nitrogen fixation was different between the two growth types only at the flowering stage. In soybean this pattern was reversed: the difference was non-significant only during the flowering stage (Table 3.5) and at all the other growth stages the indeterminate cultivar had significantly higher nitrogen fixation activity than the determinate cultivar (Figure 3.6).

Nodule dry weight: The indeterminate cultivars of both species produced more nodule dry weight than the determinate cultivars (Figures 3.7a and b; Appendix 3.6). The difference was significant at every growth stage (Table 3.6).

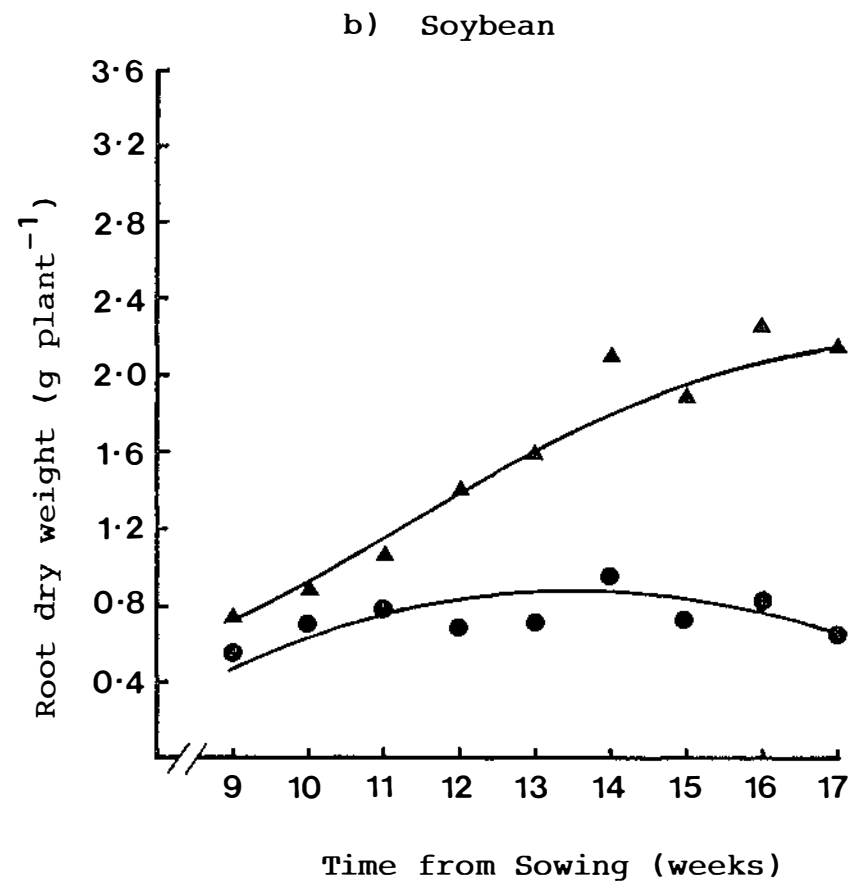
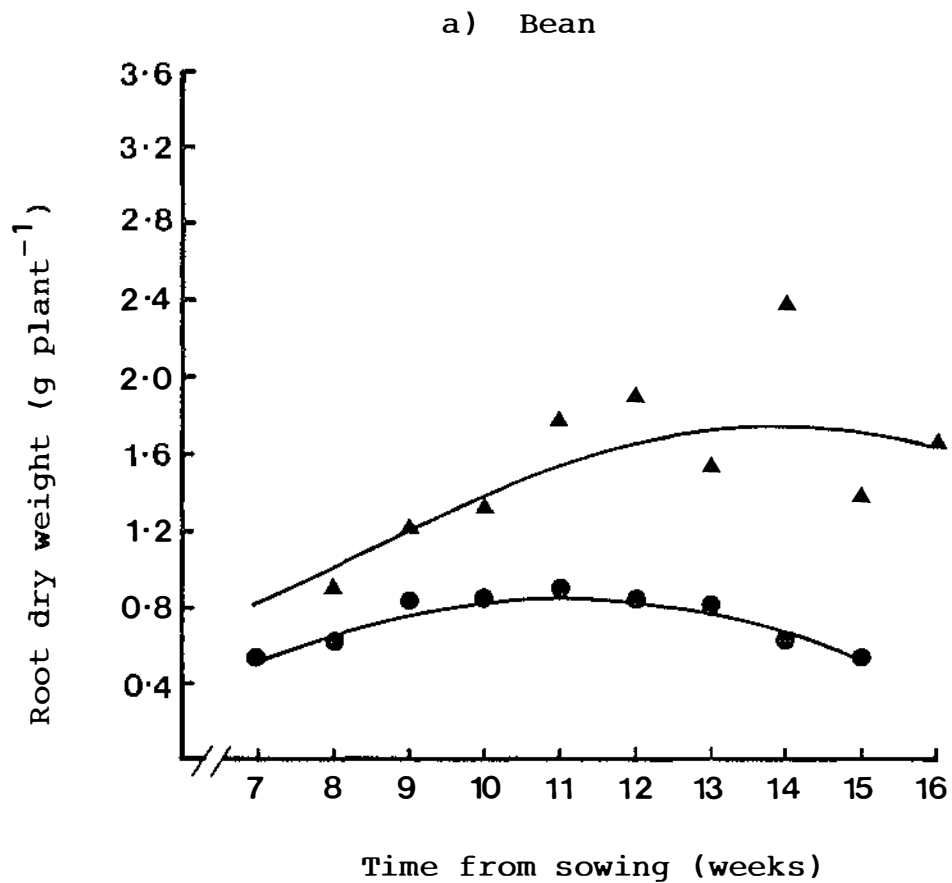


Figure 3.5 Time course of root dry weight of the determinate (●) and indeterminate (▲) cultivars of bean and soybean.

(S.E. of regression coefficients are in Appendix 3.4).

Table 3.4 Comparison of root dry weight between the determinate (D) and indeterminate (I) cultivars of bean and soybean, estimated from growth curves, at different growth stages.

Species	Growth type	GROWTH STAGE			
		Flowering	Pod development	Grain filling	Late grain filling
		Root dry weight (g plant ⁻¹)			
Bean	D	0.5	0.8	0.9	0.8
	I	1.0	1.4	1.6	1.7
	t-test	**	**	**	**
Soybean	D	0.5	0.7	0.9	0.8
	I	0.7	1.1	1.6	2.0
	t-test	**	**	**	**

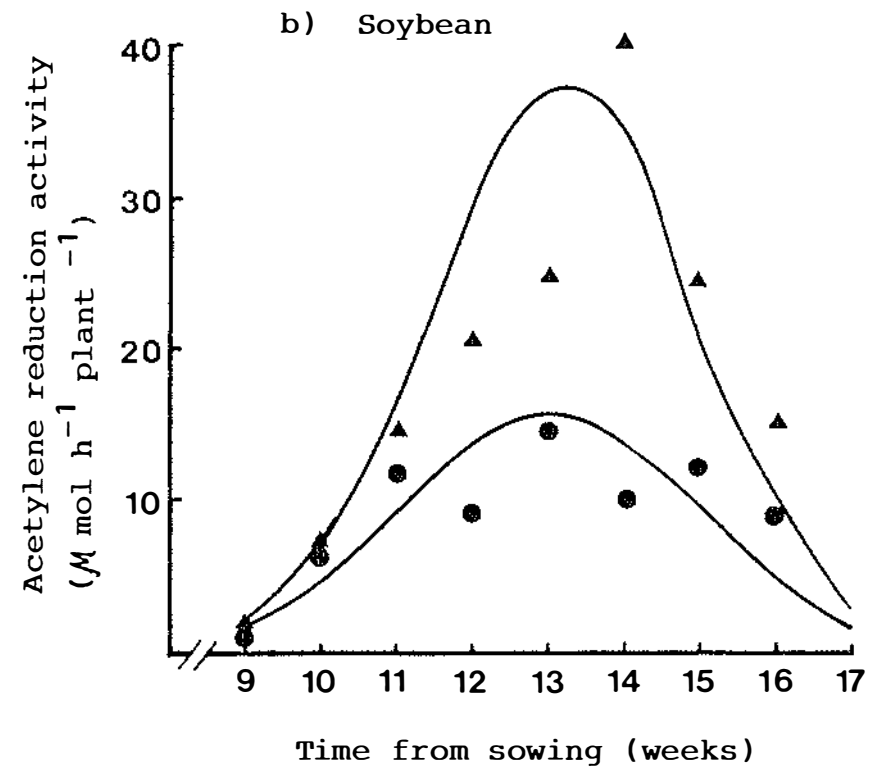
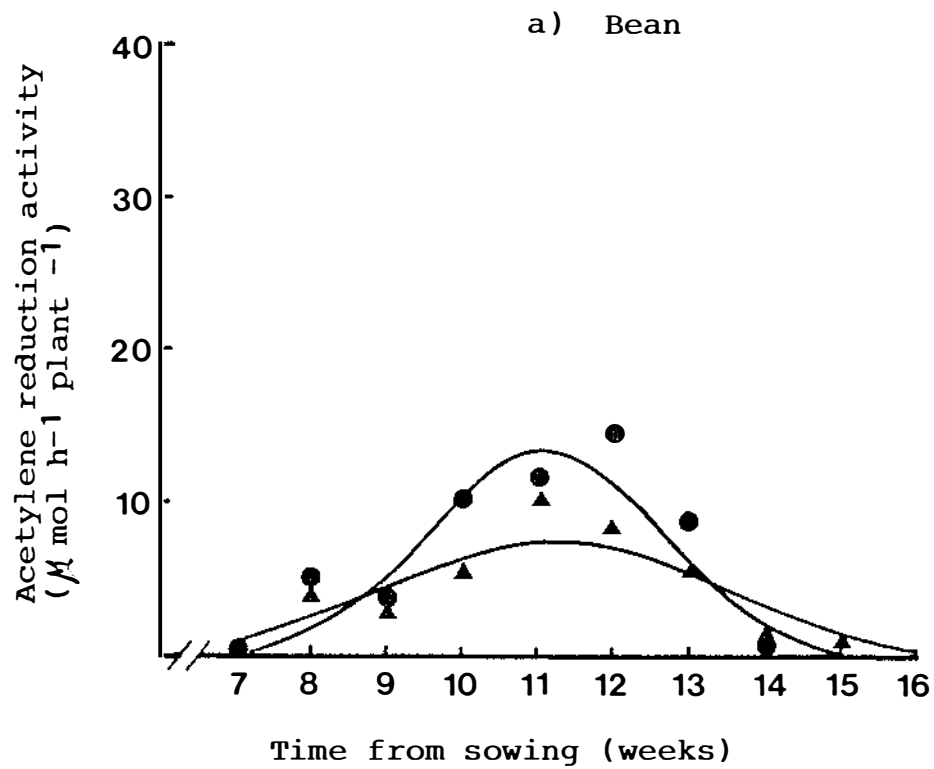
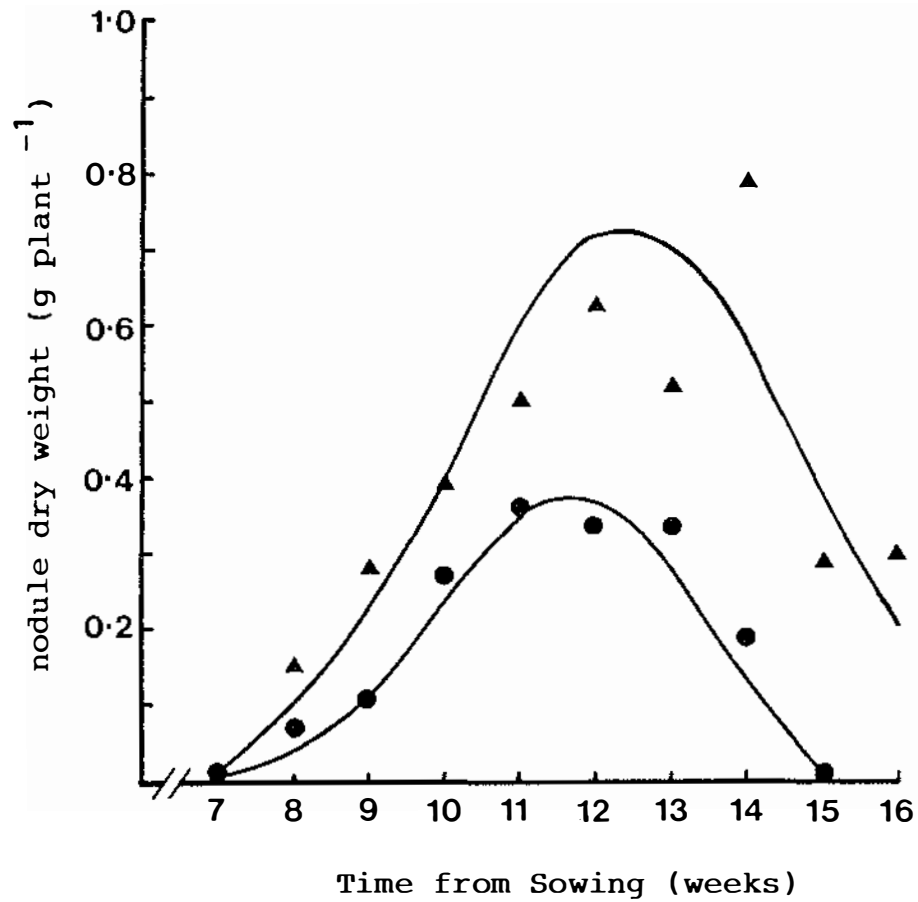


Figure 3.6 Time course of acetylene reduction activity of the determinate (●) and indeterminate (▲) cultivars of bean and soybean. (S.E. of regression coefficients are in Appendix 3.5).

Table 3.5 Comparison of acetylene reduction activity between the determinate (D) and indeterminate (I) cultivars of bean and soybean, estimated from growth curves, at different growth Stages.

Species	Growth Types	GROWTH STAGES			
		Flowering	Pod development	Grain filling	Late grain filling
		Acetylene reduction activity ($\mu\text{mol C}_2\text{H}_4\text{h}^{-1}$)			
Bean	D	0.5	4.4	13.5	5.1
	I	2.5	6.3	6.8	3.0
	t-test	**	ns	ns	ns
Soybean	D	1.5	8.5	15.6	8.9
	I	1.5	15.4	37.6	21.5
	t-test	ns	*	**	**

a) Bean



b) Soybean

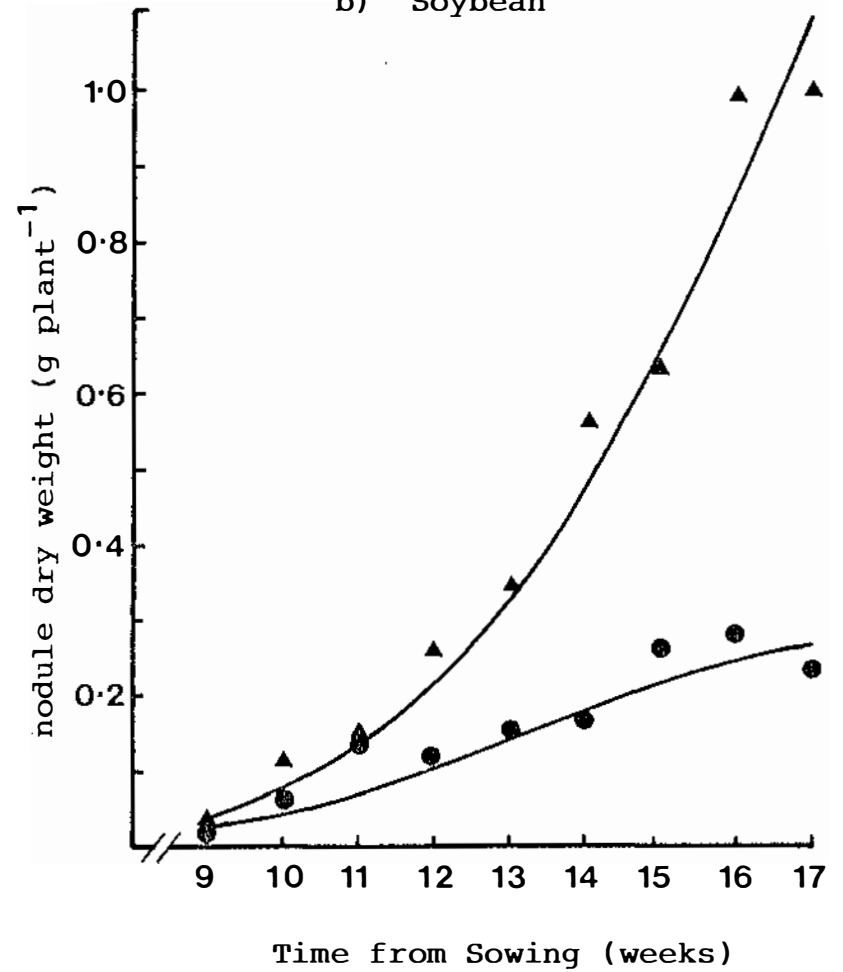


Figure 3.7 Time course of nodule dry weight of the determinate (●) and indeterminate (▲) cultivars of bean and soybean. (S.E. of regression coefficients are in Appendix 3.6).

Table 3.6 Comparison of nodule dry weight between the determinate (D) and indeterminate (I) cultivars of bean and soybean, estimated from growth curves, at different growth stages.

Species	Growth types	GROWTH STAGES			
		Flowering	Pod development	Grain filling	Late grain filling
		nodule dry weight (g plant ⁻¹)			
Bean	D	0.01	0.10	0.43	0.33
	I	0.10	0.40	0.73	0.58
	t-test	**	**	*	*
Soybean	D	0.03	0.08	0.14	0.22
	I	0.05	0.14	0.33	0.66
	t-test	*	**	**	**

Total plant nitrogen: The indeterminate cultivars of both species accumulated about three times more total plant nitrogen than those of the determinate cultivars by the final harvest (Figures 3.8a and b; Appendix 3.7). The comparison of values estimated from curves showed that the difference was significant at every growth stage compared in bean, while it was so only at the two later stages in soybean (Table 3.7).

When total plant nitrogen (log scale) was fitted against nodule dry weight, before nodule dry weight started to decline, the intercept represented the amount of nitrogen in the plant before nitrogen fixation commenced and the slope indicated the nitrogen fixing activity in the nodule (Dobereiner 1966). It was found in both of the determinate cultivars of bean and soybean that there was a significantly steeper slope (*i.e.* higher activity) than for the indeterminate cultivars (Figure 3.9; Appendix 3.8). In other words, nodules of the determinate cultivars performed better in terms of the ability to fix nitrogen per unit weight of nodule than indeterminate cultivars.

3.3.3 Yield and yield components:

The indeterminate cultivar of bean produced about twice the seed yield of the determinate cultivar, while the yield advantage of the indeterminate soybean cultivar was about three times more than that of the determinate cultivar (Tables 3.8 and 3.9). The major contribution to the difference came from the number of pods per plant in soybean and number of seeds per pod in bean (Tables 3.10, 3.11 and 3.12). However, the ability of the two growth types to convert

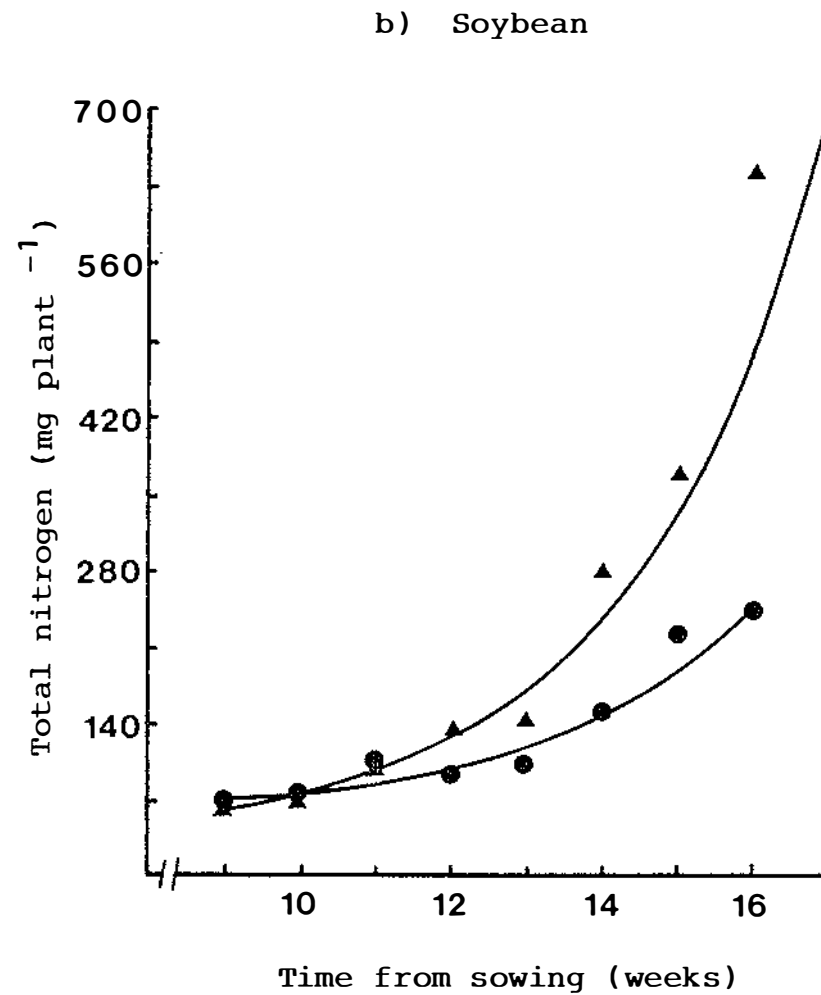
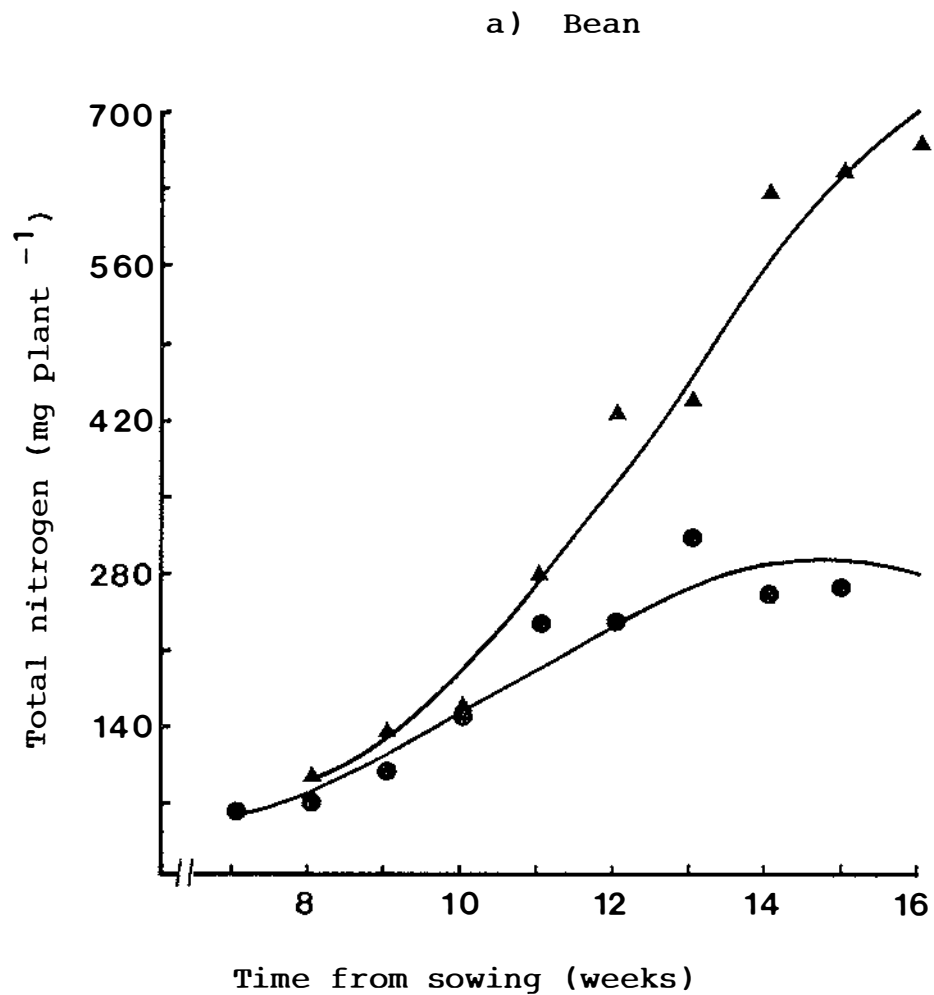
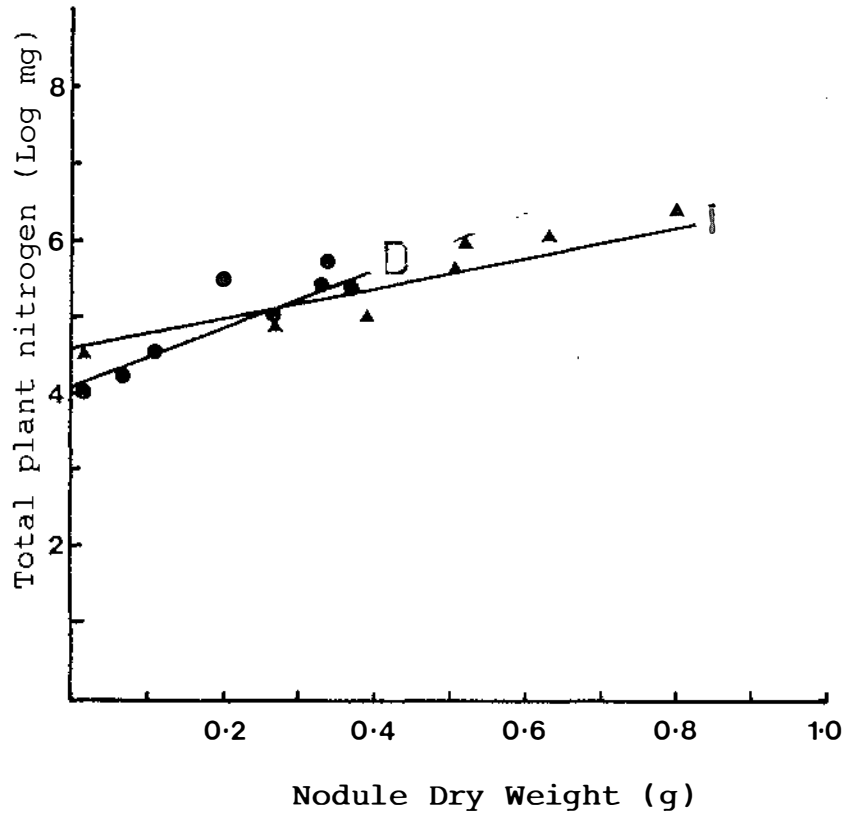


Figure 3.8 Time course of total plant nitrogen of the determinate (●) and indeterminate (▲) cultivars of bean and soybean. (S.E. of regression coefficients are in Appendix 3.7).

Table 3.7 Comparison of total plant nitrogen between the determinate (D) and indeterminate (I) cultivars of bean and soybean, estimated from growth curves, at different growth stages.

Species	Growth type	GROWTH STAGE			
		Flowering	Pod development	Grain filling	Late grain filling
		Total Plant Nitrogen (mg plant ⁻¹)			
Bean	D	48.0	113.3	195.9	248.0
	I	83.6	192.0	361.2	556.2
	t-test	**	**	**	**
Soybean	D	60.5	87.9	124.0	170.0
	I	58.5	96.6	173.2	337.3
	t-test	ns	ns	**	**

a) Bean



b) Soybean

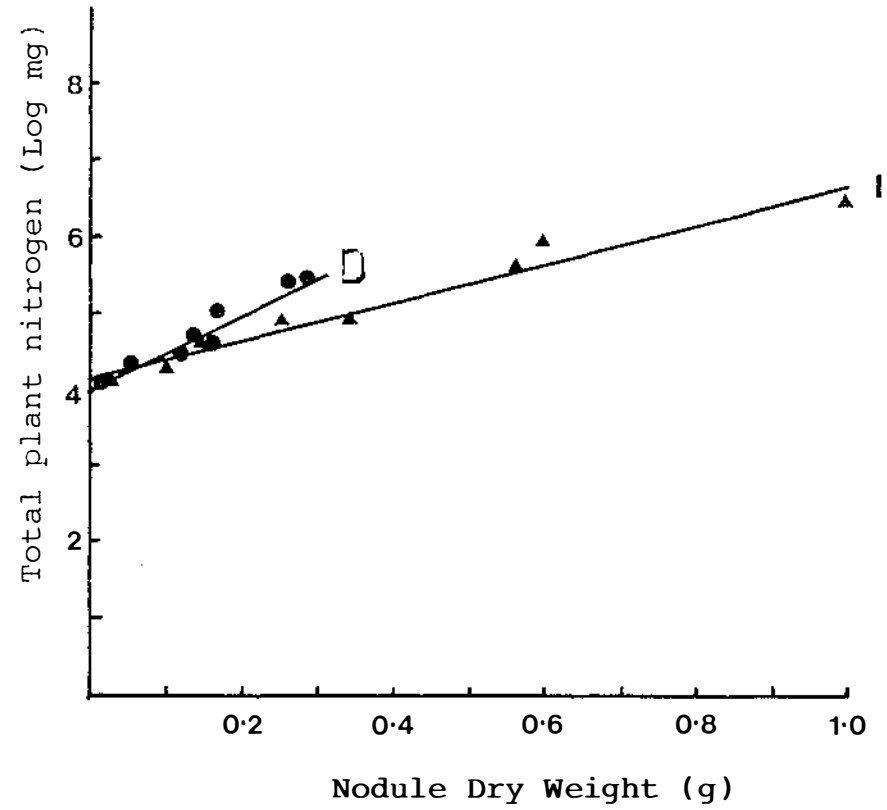


Figure 3.9 Regression lines of total plant nitrogen (log scale) and nodule dry weight of the determinate(D) and indeterminate (I) cultivars of bean and soybean. (S.E. of regression coefficients are in Appendix 3.8)

Table 3.8 Mean pod weight (g plant ⁻¹) of bean and soybean with different growth types.

Species	Growth type		Mean
	Determinate	Indeterminate	
Bean	7.43	15.51	11.47
Soybean	4.99	15.05	10.02
Mean	6.21	15.28	
F-test	type **	Species ns	Type * Species ns
cv (%) = 18.2			

Table 3.9 Mean seed weight (g plant⁻¹) of bean and soybean with different growth types.

Species	Growth type		Mean
	Determinate	Indeterminate	
Bean	5.54	10.60	8.07
Soybean	3.19	9.75	6.47
Mean	4.37	10.17	
F-test	Type	Species	Type * Species
cv (%) = 17.7	**	ns	ns

Table 3.10 Mean number of pods per plant of bean and soybean with different growth types.

Species	Growth Type		Mean
	Determinate	Indeterminate	
Bean	3.25	3.78	3.51
Soybean	11.67	24.83	18.25
Mean	7.46	14.31	

	Type	Species	Type * Species
F-test	**	*	*
cv (%) = 29.7			

Table 3.11 Mean weight of 20 seeds (g) of bean and soybean with different growth types.

Species	Growth Type		Mean
	Determinate	Indeterminate	
Bean	9.73	10.62	10.17
Soybean	3.97	3.96	3.97
Mean	6.85	7.29	
	Type	Species	Type * Species
F - test	ns	**	ns
cv (%) = 6.8			

Table 3.12 Mean number of seeds per pod of bean and soybean with different growth types.

Species	Growth Type		Mean
	Determinate	Indeterminate	
Bean	3.51	5.36	4.43
Soybean	1.34	2.00	1.67
Mean	2.43	3.68	

	Type	Species	Type * Species
F-test	**	**	ns

cv (%) = 18.8

biological yield to seed yield was very similar as indicated by the similar harvest indices (Table 3.13). Nitrogen utilization expressed as nitrogen harvest indices was also similar between the two types (Table 3.14).

3.4 DISCUSSION

3.4.1 Plant growth: Growth curves have been used as an approach to plant growth analysis in this experiment because it offered a number of advantages (Hunt, 1982). Some of the advantages which are directly relevant to this experiment are i) the difficulties involved in the pairing of plants when applying the classical formulae can be avoided, ii) information from all sampling occasions have been used in determining the value of the derived quantities, whereas in the classical method only data from two consecutive harvests can be used.

The difference between the two growth types of both species in their dry matter accumulation was quite similar (Figure 3.1a and b; Table 3.1) *i.e.* the indeterminate cultivars accumulated significantly more dry-matter than the determinate cultivars at the late grain filling stage. However, there was not a complete cessation of accumulation of dry-matter in the vegetative portion of the plant at the beginning of flowering for the determinate cultivars. The result is similar to that reported by Egli and Leggett (1973) in their experiment using field-grown soybeans over two seasons.

Since total dry-matter yield depends on the development of leaf area and the rate of net photosynthesis (Turner and Begg, 1981), the higher leaf area of the indeterminate types (Figure 3.3a and b) reflects directly the advantage of the indeterminate types in

Table 3.13 Harvest indices of bean and soybean with different growth types.

Species	Growth type		Mean
	Determinate	Indeterminate	
Bean	0.66	0.52	0.59
Soybean	0.61	0.55	0.58
Mean	0.64	0.54	
	Type	Species	Type * Species
F-test	ns	**	ns
cv (%) = 5.2			

Table 3.14 Nitrogen harvest indices of bean and soybean with different growth types.

Species	Growth type		Mean
	Determinate	Indeterminate	
Bean	0.88	0.77	0.82
Soybean	0.74	0.75	0.74
Mean	0.81	0.76	
	Type	Species	Type * Species
F-test	ns	ns	ns
cv (%) = 9.9			

producing dry-matter. Consequently, the vegetative dry weight (leaf + stem) of the indeterminate cultivars of both species was about twice that of the determinate cultivars at physiological maturity (Figure 3.1a and b). A comparison of total dry weight between the two growth types at each growth stage (Table 3.1) showed significant differences only in the later growth stages of soybean. Similarly there was no difference in leaf area between the two types during the early growth stages (Table 3.3). These differences between the two growth types were similar to those reported by Chaturvedi *et al.* (1980); in cowpea the indeterminate cultivars produced more dry-matter in leaves, stem and total plant than those of determinate cultivars.

The net assimilation rate (NAR) calculated from fitted curves showed an interesting pattern (Figure 3.4a and b). The NAR values of indeterminate cultivars of both bean and soybean increased at the later stage of reproductive growth. Koller *et al.* (1970) interpreted the increase in NAR during the latter part of the growing season as a response of the photosynthetic apparatus to an increased demand for assimilates, which was due to the rapid growth of the seed. Sharma *et al.* (1982) reported that NAR's from pod development to maturity of sixteen soybean cultivars were strongly correlated with yield.

One of the major difference between the two growth types was root growth. The indeterminate cultivars produced more root dry weight than those of the determinate cultivars at every growth stage compared (Figure 3.5; Table 3.4). Cultivars of soybeans were found to differ considerably in root growth, especially in proliferation during late development (Raper and Barber, 1970; Mitchell and Russell, 1971). However these workers did not find any relationship between yielding

ability and nutrient assimilation due to differences in rooting behavior. More recently Silberbush and Barber (1984) reported that cultivars with more extensive root systems absorbed more P and K and this was found to be associated with higher grain yield in soybean.

3.4.2 Nitrogen fixation and nitrogen distribution:

Variation in the ability of grain legumes to fix nitrogen has been reported both between and within species (Graham, 1982). In this experiment, beans had a lower activity than soybeans (Figure 3.5a and b). ^{However, at the end total nitrogen of both types was similar (Figure 3.8).} Similar observations were quoted by Graham and Halliday (1977) both in terms of total activity and specific activity. A difference in total nitrogen fixation activity after flowering between the two growth types was found only in soybean (Table 3.5). There have been only a few studies which compared nitrogen fixation directly between the two growth types; Hardy *et al.* (1971) reported that determinate and indeterminate soybeans of similar maturity dates had similar nitrogen fixation activity. In contrast Graham (1981) quoted field studies which indicated that the indeterminate cultivars of bean (*Phaseolus vulgaris*) were consistently superior to that of most determinate bush types in total nitrogen fixation.

When total plant nitrogen (dependent variable) is regressed with nodule dry weight (independent variable), the intercept represents the amount of nitrogen actually absorbed from the original seed and the soil and the regression coefficient (B_1) is an 'index of nitrogen fixing efficiency'. Pioneering this method, Dobereiner (1966) found that this 'index of nitrogen fixing efficiency' seemed to be constant for each plant species and appeared to be independent of most environmental effects, *Rhizobium* strains and plant varieties. In this

experiment the determinate cultivars of both species had significantly higher regression coefficients than those of the indeterminate cultivars (Figure 3.9). This would suggest that the nodules of the determinate cultivar of both bean and soybean in this experiment are more efficient than those of the indeterminate cultivar in fixing nitrogen.

Since in this experiment no mineral nitrogen was added after the first week from seedling emergence, and the pot mixture had very low organic nitrogen, the change in total plant nitrogen with time should be a relatively accurate means of determining the total amount of nitrogen fixed as well. In this case the indeterminate cultivars of both species had significantly higher total plant nitrogen than the determinate cultivars (Figure 3.10).

The similarity between acetylene reduction rate, but difference in total plant nitrogen, between the two bean cultivars could partly be due to the actual acetylene reduction data which showed high plant to plant variability. Nodule weight (fresh or dry) has traditionally been used to indicate dinitrogen fixation in legumes (Bell and Nutman, 1971; Brockwell, 1971). In soybean the higher nitrogen fixation of the indeterminate cultivar was due to a higher nodule dry weight (Figure 3.7), since the 'nitrogen fixing efficiency', as indicated by the regression technique, was lower in the indeterminate cultivar (Figure 3.9). On the other hand, the superior nodule growth in the indeterminate bean cultivar did not result in higher nitrogen fixation rate. This could, in part, be due to the nodule activity in the bean being more sensitive to environmental variability e.g. temperature than nodule dry weight (Rennie and Kemp, 1981).

The total plant nitrogen in each cultivar was highly dependent on the total plant biomass (correlation coefficients > 0.94). A similar relationship in soybean was previously reported by Hanway and Weber (1971a); Spaeth and Sinclair (1983); and Anderson and Vasilas (1985). Many studies examining the distribution in nitrogen by soybean have shown that the loss of nitrogen from vegetative tissue coincides with the accumulation of nitrogen in seed (i.e. Hanway and Weber 1971c; Derman *et al.*, 1978). The ratio of nitrogen in the grain to total above ground plant nitrogen or 'nitrogen harvest index' was not significantly different between the two growth types (Table 3.14). This indicates that the distribution of nitrogen to grain was related to the size of the nitrogen pool, which was, in turn, closely related to the dry weight rather than to the difference in nitrogen translocation.

3.4.3 Yield and yield components: Comparisons of yield differences between determinate and indeterminate legume types have recorded similar values in soybean (Egli and Leggett, 1973), higher values in indeterminate type of cowpea (Chaturvedi *et al.*, 1980) or higher values in the determinate type of soybean (Hicks *et al.*, 1969). The results from this present experiment showed that the indeterminate type of both species out-yielded the determinate counterpart (Tables 3.8 and 3.9). This advantage mainly came from a higher number of pods per plant (Table 3.10). The production of yield in a grain crop depends upon the accumulation of dry-matter in seeds. The amount of seed yield produced is the product of the number of seeds and their size, determined by the rate and duration of dry-matter accumulation (Wein and Ackah, 1978). In this experiment the reproductive period of

the two growth types of each species was the same, so this indicates that the rate of dry-matter accumulation into the seed was likely to be the key factor in causing the yield difference. The indeterminate types could achieve higher yield because they produced a larger source capacity, as well as benefiting during the early stages of pod filling from the canopy which still had a large proportion of relatively young leaves. In contrast, determinate types have to derive much of their photosynthate from ageing leaves (Shibles, 1980). Assessment of the 'harvest index' could identify a pattern in the relationships between the total biomass of the mature plant and the fractions contributed from the seeds. There was no difference in the harvest index between the two growth types of both species in the present experiment (Table 3.13). Previous work had indicated that the harvest index of a cultivar was a stable characteristic irrespective of substantial differences in environmental conditions during plant growth (Spaeth *et al.*, 1984).

Since plants in this experiment were largely dependent on symbiosis to satisfy their nitrogen demand, then the difference in total nitrogen between two growth types (Figure 3.8) could only be due to the difference in nitrogen fixation. It had been reported that where cowpea is dependent on symbiotically-fixed nitrogen, superior nitrogen fixing genotypes were also higher yielding (Graham and Scott, 1983). The results in this experiment could also draw similar conclusion.

CHAPTER 4

A comparative study of nitrogen fixation, nitrogen distribution and yield of two soybean cultivars with determinate and indeterminate growth types.

ABSTRACT

Two soybean cultivars Matara' (determinate) and Amsoy' (indeterminate) were studied in controlled environment conditions to identify the differences in nitrogen fixation, nitrogen distribution, nitrogen re-distribution and yield. The emphasis in this experiment was to determine the key factors that may contribute towards the difference between the two growth types.

The indeterminate cultivar produced twice as much seed yield as the determinate cultivar. Vegetative dry-matter components of the indeterminate cultivar were significantly higher than those of the determinate cultivar. This was largely because the indeterminate cultivar had more leaf area per plant, a longer period of root growth and more nodules than that of the determinate cultivar.

The superiority of the indeterminate cultivar in acetylene reduction activity came primarily from a rapid increase in the nodule mass, since the specific acetylene reduction activity of the determinate cultivar was generally higher than that of the indeterminate cultivar. There was a linear relationship between 'integrated nitrogen fixation activity' and the nitrogen accumulation of both cultivars.

About 30-40% of seed nitrogen of both cultivars came from re-distribution. The stem of the indeterminate cultivar re-distributed a higher proportion of nitrogen to the seed than that of the determinate cultivar.

Among several plant characters tested (viz. dry-weight of roots, nodules, stems leaves and pods, leaf area per plant, acetylene reduction activity and total plant nitrogen) leaf area was identified as one of the key factors in determining the difference between the two growth types.

4.1 INTRODUCTION

From the previous experiment (reported in Chapter 3), it was evident that the indeterminate cultivars of both bean and soybean produced approximately three times more total plant nitrogen than the determinate cultivars. However, only the indeterminate soybean showed significantly more acetylene reduction activity than that of the determinate cultivar. The indeterminate cultivars of both bean and soybean also had higher nodule dry weights than those of the determinate cultivars. With these advantages, the indeterminate bean and soybean cultivars produced two and three times more seed yield respectively than in the determinate cultivars.

However, there are disagreements in the literature about which growth types had an advantage in yield. For example, Egli and Leggett (1973) and Beaver *et al.* (1985) recorded similar values of grain yield between the two growth types in soybean, whereas Hicks *et al.* (1969) reported higher values in the determinate type. Chaturvedi *et al.* (1980) found higher a value for the indeterminate type of cowpea.

Furthermore, Beaver and Johnson (1981) found that the determinate cultivars performed less predictably than the indeterminate cultivars when they compared the yield stability of the two growth types adapted to the northern United States. So the yield difference between the two growth types may not only be due to genetic differences but also to other factors such as environmental conditions.

Several workers have estimated that between 50 and 60% of the nitrogen in seed comes from the re-distribution of nitrogen from vegetative tissue (Hanway and Weber, 1971b; Egli *et al.*, 1978a). Increasing the quantity of nitrogen available for grain development in soybean could be accomplished, therefore, by increasing whole plant nitrogen accumulation and/or increasing the proportion of vegetative nitrogen translocated to the developing grain (Jeppson *et al.*, 1978). In a previous experiment, the indeterminate cultivars had a greater mass of vegetative plant parts (*i.e.* leaf and stem) and hence they also had bigger nitrogen pools. It would also be of interest to know if the re-distribution of nitrogen in the two growth types was different: variability for nitrogen translocation from vegetative tissues to the developing grain may exist between the two cultivars or conversely, differences in seed nitrogen content may be mainly due to differences in sizes of the nitrogen pools.

The objectives of this experiment were

- 1) To identify the differences in nitrogen fixation, nitrogen distribution and yield between the two growth types (with the emphasis on finding the factor that has the greatest influence on the difference obtained).

2) To compare nitrogen re-distribution rates to the developing seed from the vegetative plant parts between the two growth types.

In order to obtain optimum growth conditions, in which the experiment could be repeated with minimal variation, controlled environmental techniques were used.

4.2 MATERIALS AND METHODS

The experiment was carried out in controlled environment rooms at the Climate Laboratory, Plant Physiology Division, Department of Scientific and Industrial Research (DSIR), Palmerston North, New Zealand.

The two soybean cultivars from the previous experiment were used; i.e.

'Matara'	Determinate
'Amsoy'	Indeterminate

Plants were sown in 4.5 litre pots with a mixture of sand and pumice (75:25 v/v) under the environmental conditions described in Appendix D.

The experimental design was a randomized complete block design with 5 replicates.

Plants were given complete Hoagland's A nutrient solution until emergence, then the same *Rhizobium* culture used in the previous experiment was applied several times to ensure good nodulation. After inoculation, plants were well watered at regular interval with nitrogen-free, half-strength Hoagland's A nutrient solution.

Five weekly destructive harvests were carried out from the flowering stage, to determine nitrogen fixation and the dry-weights of different plant components (*viz* leaf, stem, root, nodule and pod).

The acetylene reduction assay (Hardy *et al.*, 1971) was used to measure nitrogen fixation activity. The details of the procedure are presented in Appendix B.

Total plant nitrogen of different plant parts was analysed by the Kjeldahl method (Bremner and Mulvaney, 1982) with details of the procedure described in Appendix C.

The amount of nitrogen re-distributed was estimated from changes in total nitrogen (mg plant^{-1}) for each plant part between early grain filling stage and the final harvest at maturity. The estimation of nitrogen-redistribution was similar to that described by Egli *et al.* (1978a)

Radioactive ^{14}C was used to identify whether there were any differences in C movement at the time when nitrogen fixation of the two cultivars was markedly different. At three weeks after flowering one plant per replication was exposed to ^{14}C . Labelling took place at the same time of the day for both cultivars which had one week difference in reaching flowering stage. The ^{14}C , with an activity of 20 micro Ci, was applied to a single leaf at the same position, *i.e.* third node from the unifoliolate leaf, at the same growth stage for both cultivars. The leaf was labelled by enclosing it in a small perspex leaf chamber (approximate volume 500 ml) which had a hinged lid with foam rubber around its edge to ensure that the connecting petiole was undamaged. It also had a small internal fan to ensure rapid circulation of the labelled air inside the chamber.

After 24 h the plants were harvested and dissected into leaf, stem and pod and also categorized according to their position relative to (below or above) the labelled leaf. Plant parts were then dried at 80°C for 24 h weighed and analysed for ^{14}C activity. The sample preparation for the assay was modified Shimshi's method (Shimshi, 1969) the same as that described by Withers (1979). The activity of the sample was determined using a Beckman L1700 liquid scintillation counter.

Statistical analysis

The growth data of each cultivar was described by a functional approach, using appropriate growth functions to fit the data against time by the least squares technique (Hunt, 1982). The details of the growth functions and the analysis procedures are the same as those described in ~~Chapter~~ 3.

A randomized complete block design was used for the analysis of variance. The analysis was done using the 'Genstat' program (Alvay *et al.*, 1977).

A path analysis was used to find a 'plausible interpretation' of the relationship between variables. A path diagram derived from the analysis is a network with the directions specified. It therefore enables an evaluation of the direct effect of one cause on a result and the indirect effects due to other causes. In a path diagram, arrows indicate the assumed direction of influence of one variable on another. The numbers, or path coefficients associated with the arrows, are path coefficients (p) which measure the importance of a given path of influence from cause to effect. Arrows

coming from outside the diagram, and their associated coefficients, represent external sources of variation. The theory and detailed analysis are fully described by Li (1975). In this study, the analysis was done using the Fortran program 'Correl' (Gordon *pers. comm*).

A multiple discriminant analysis technique was used to combine several plant characters into an index of cultivar or treatment performance. This analysis aims at distinguishing between two or more groups on the joint basis of several attributes. It partitions the dispersion (i.e. all variance and covariance) into treatment and error components and discriminates on the basis of maximizing the ratio of treatment to error (as in an F-test). The model of this analysis is the same as described by Cooley and Lohnes (1971) and Laohasiriwong (1982).

Once the discriminant functions have been derived, it may be possible to obtain satisfactory discrimination with only one or two of the functions, by using only those which takes into account a large proportion of total dispersion of the original data. The main objective for this analysis in the present study was to identify the original attributes which contributed most to distinguish between cultivars or treatments using those functions which had the most discriminating power. This could be done by interpreting the 'structure matrix', which is the matrix of pooled within-group correlations between the discriminant functions, and the discriminating variables to find the variable which contributes most to differentiation along the particular function (Klecka, 1975). The analysis was done using the 'SPSS' program (Nie *et al.*, 1975).

4.3 RESULTS

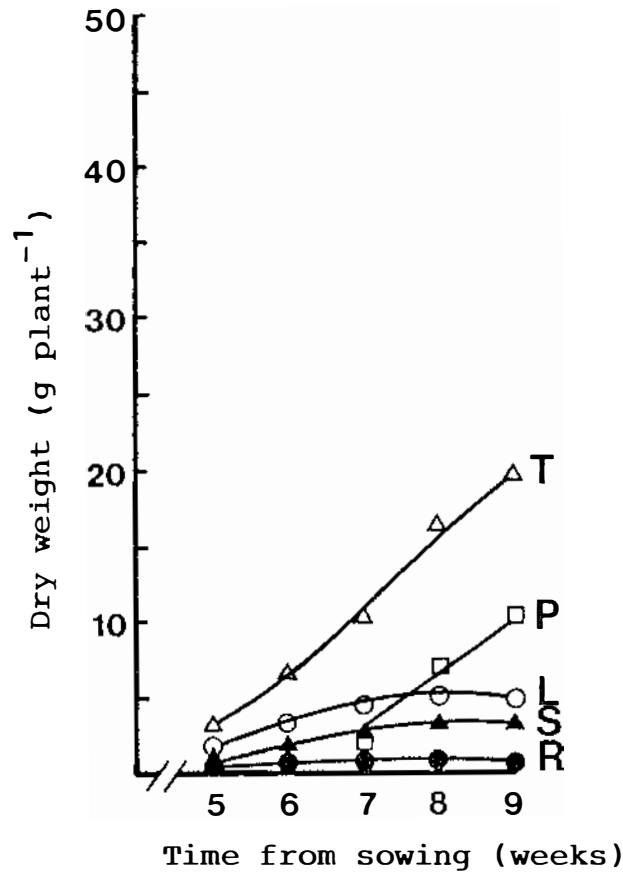
4.3.1 PLANT GROWTH: The determinate cultivar 'Matara' reached the flowering stage in 5 weeks, a week earlier than the indeterminate cultivar 'Amsoy'. The data sets for total, leaf, stem and root dry weights were fitted with quadratic growth curves (Figure 4.1, Appendix 4.1). Except for the root dry weight of the determinate cultivar, which had a rather low R^2 value (0.47), all the other curves had R^2 values > 0.75 . However, the analysis of variance on the regression for root dry weight was still highly significant ($P < 0.01$).

The data set for the pod dry weight during the sampling period was fitted with a linear equation (Figure 4.1, Appendix 4.1)

From fitted curves it can be seen that by the grain filling stage the total plant dry weight of the indeterminate cultivar was three times heavier than that of the determinate cultivar. The growth curves for the leaf, stem and root dry weights indicate that the growth of these components in the determinate cultivar reached their maximum values about 1 week before the end of the sampling period, while the values for the indeterminate cultivar showed an increasing trend throughout. The indeterminate cultivar was 2-3 times heavier than the determinate cultivar in all the dry weight components both on a time scale (Figure 4.1) and on a growth stage scale (Table 4.1).

The maximum leaf area per plant of the determinate cultivar was only about one-third that of the indeterminate cultivar (Figure 4.2, Appendix 4.2). This was reached in week 8 for the determinate cultivar and in week 10 for the indeterminate cultivar. The relative leaf area expansion rate (RLER) and net assimilation rate (NAR) were

Determinate cultivar



Indeterminate cultivar

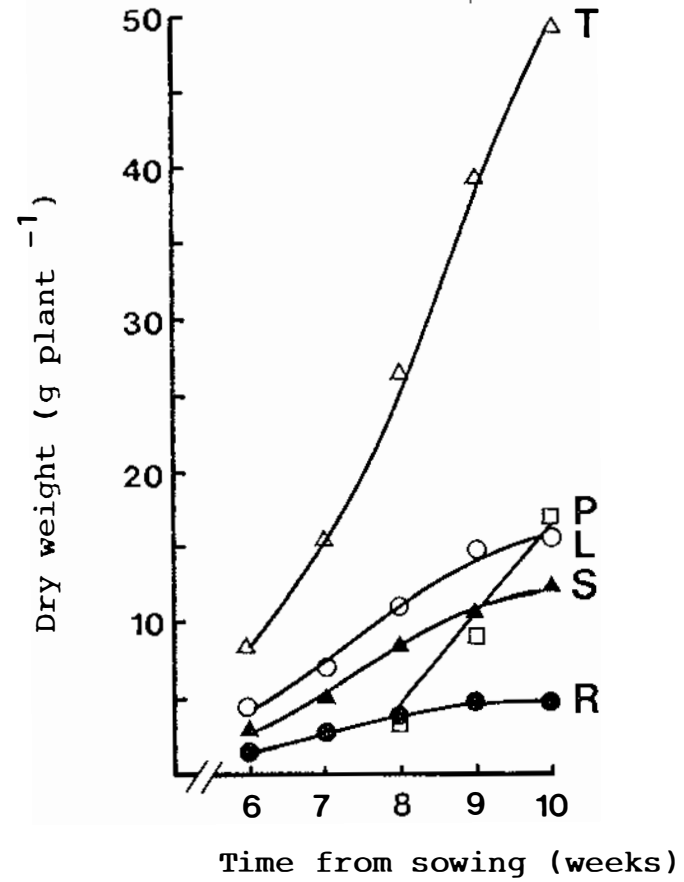


Figure 4.1 Time course of total dry weight (T), Pod Dry weight (P), Leaf Dry weight (L), Stem Dry weight (S) and Root Dry weight (R) of the determinate and indeterminate soybean cultivars (plotted values are means of 5 replicates). (S.E. of regression coefficients are in Appendix 4.1).

Table 4.1 Comparison of plant dry weight components at different growth stages between the determinate (D) and indeterminate (I) soybean cultivars.

Plant Character	Growth Type	Growth Stage		
		Flowering	Pod Development	Grain Filling
		(g plant ⁻¹)		
Root	D	0.56	0.89	0.94
	I	1.49	3.69	4.54
	t-test	**	**	**
Stem	D	0.93	2.83	3.20
	I	2.52	8.44	11.47
	t-test	**	**	**
Leaf	D	1.70	4.63	4.88
	I	4.22	10.93	15.47
	t-test	**	**	**
Total wt.	D	3.19	10.86	19.57
	I	8.23	26.02	49.55
	t-test	**	**	**

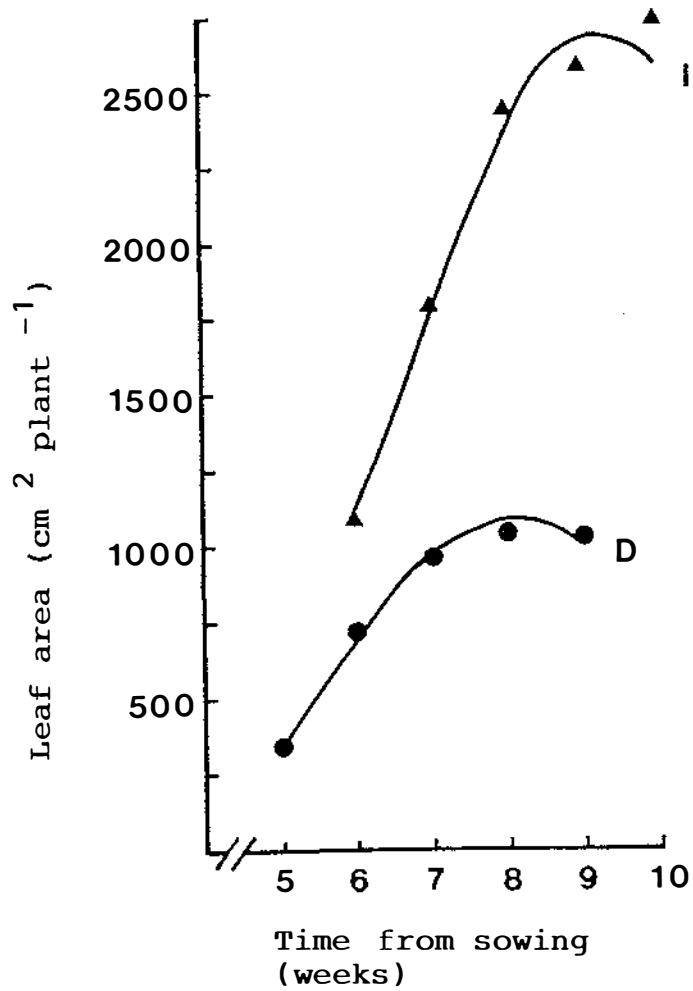


Figure 4.2 Time course of leaf area per plant of the determinate (D) and indeterminate (I) soybean cultivars (Plotted values are mean of 5 replicates). (S.E. of regression coefficients are in Appendix 4.2)

estimated from the respective growth curves. The indeterminate cultivar had a higher relative leaf area expansion rate from week 6 than the determinate cultivar and leaf area expansion persisted for about 2 weeks longer (Figure 4.3). The pattern of NAR response also differed between the two growth types; in the determinate cultivar NAR decreased rapidly from $0.007 \text{ g cm}^{-2} \text{ week}^{-1}$ at week 5 to $0.0027 \text{ g cm}^{-2} \text{ week}^{-1}$ by week 9, while in the indeterminate cultivar NAR was maintained at between $0.005\text{-}0.004 \text{ g cm}^{-2} \text{ week}^{-1}$ between week 6 to week 8, before decreasing gradually to $0.003 \text{ g cm}^{-2} \text{ week}^{-1}$ by week 10 (Figure 4.3).

However, if the results were evaluated on the basis of physiological age (*i.e.* according to different growth stages), it was clear that the determinate cultivar had a higher RLER than the indeterminate cultivar during flowering and that there was no difference by the pod development stage (Figure 4.4a). Similarly the determinate cultivar had a higher NAR value than the indeterminate cultivar during flowering but the values became similar during pod development and NAR was slightly higher in the indeterminate cultivar during grain filling (Figure 4.4b).

In spite of higher total plant and root dry weight in the indeterminate cultivar (Figure 4.1), the shoot:root ratio of the indeterminate cultivar was significantly lower than that of the determinate cultivar (Figure 4.5a). This difference remained even after adjusting for physiological age (Figure 4.5b).

When the dry-weight of each component was expressed as a percentage of total plant dry weight it was clear that the indeterminate cultivar had a higher ratio of dry-matter accumulation

| S.E.

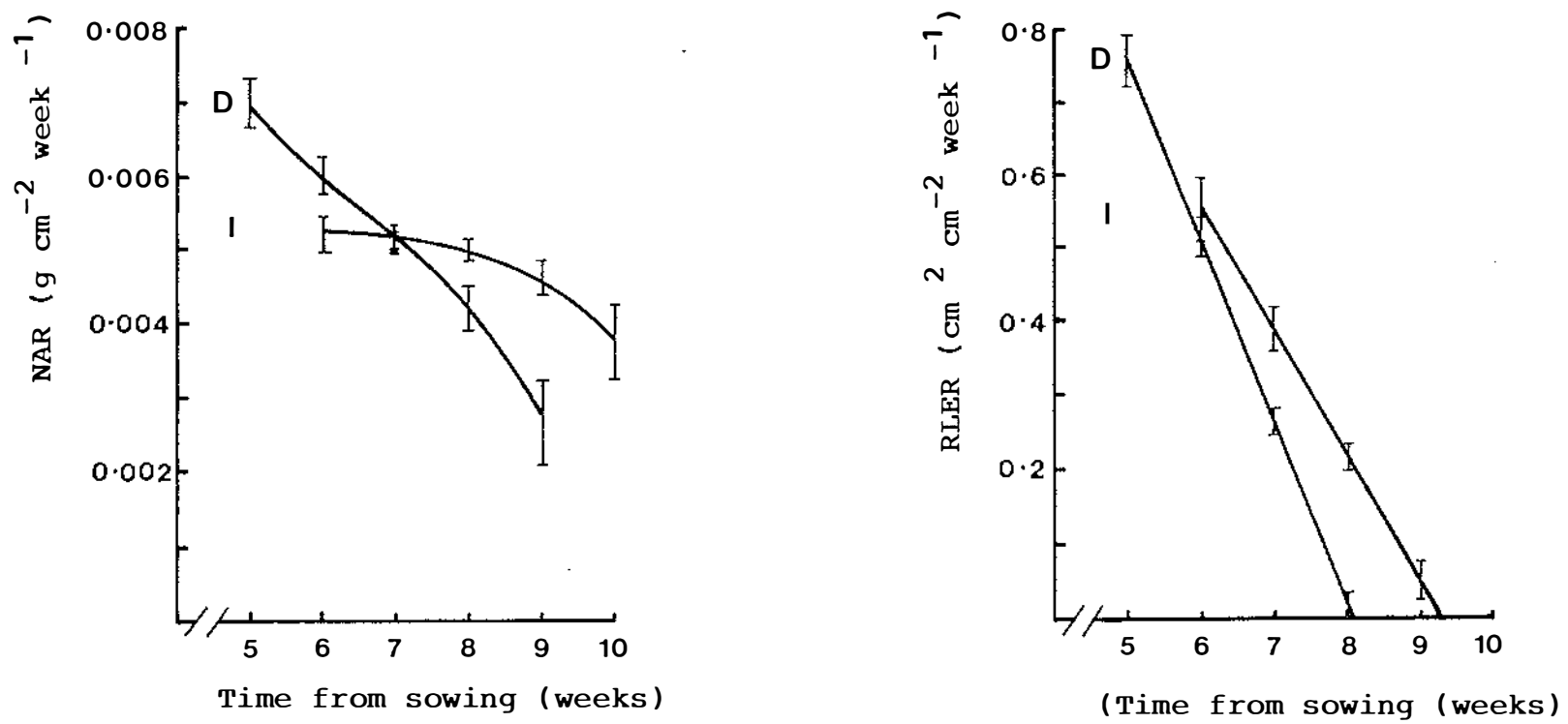


Figure 4.3 Time course of net assimilation rate (NAR) and relative leaf expansion rate (RLER) of the determinate (D) and indeterminate (I) soybean cultivars.

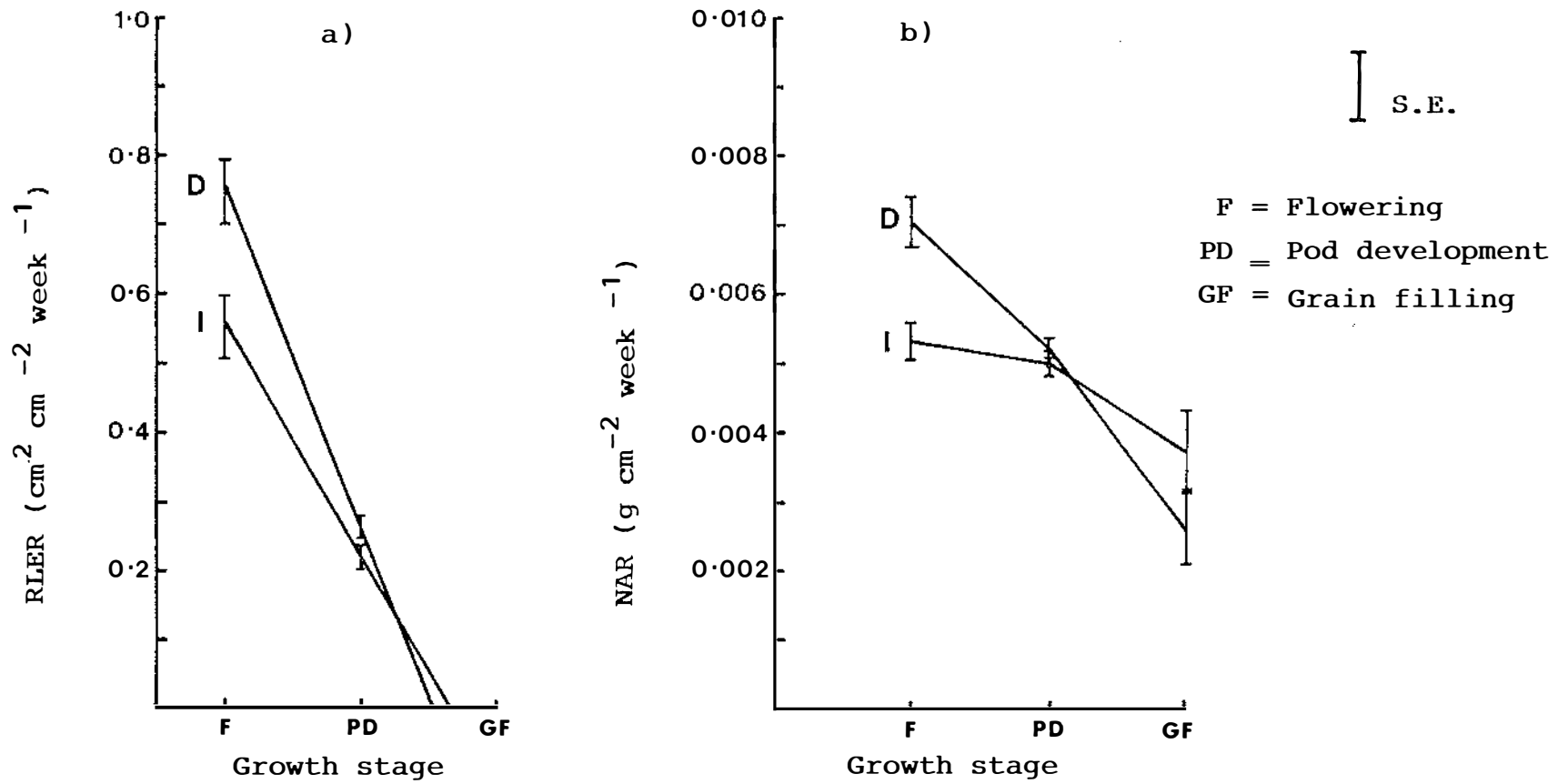


Figure 4.4 RLER and NAR of determinate cultivar (D) and indeterminate (I) soybean cultivars compared on physiological age basis.

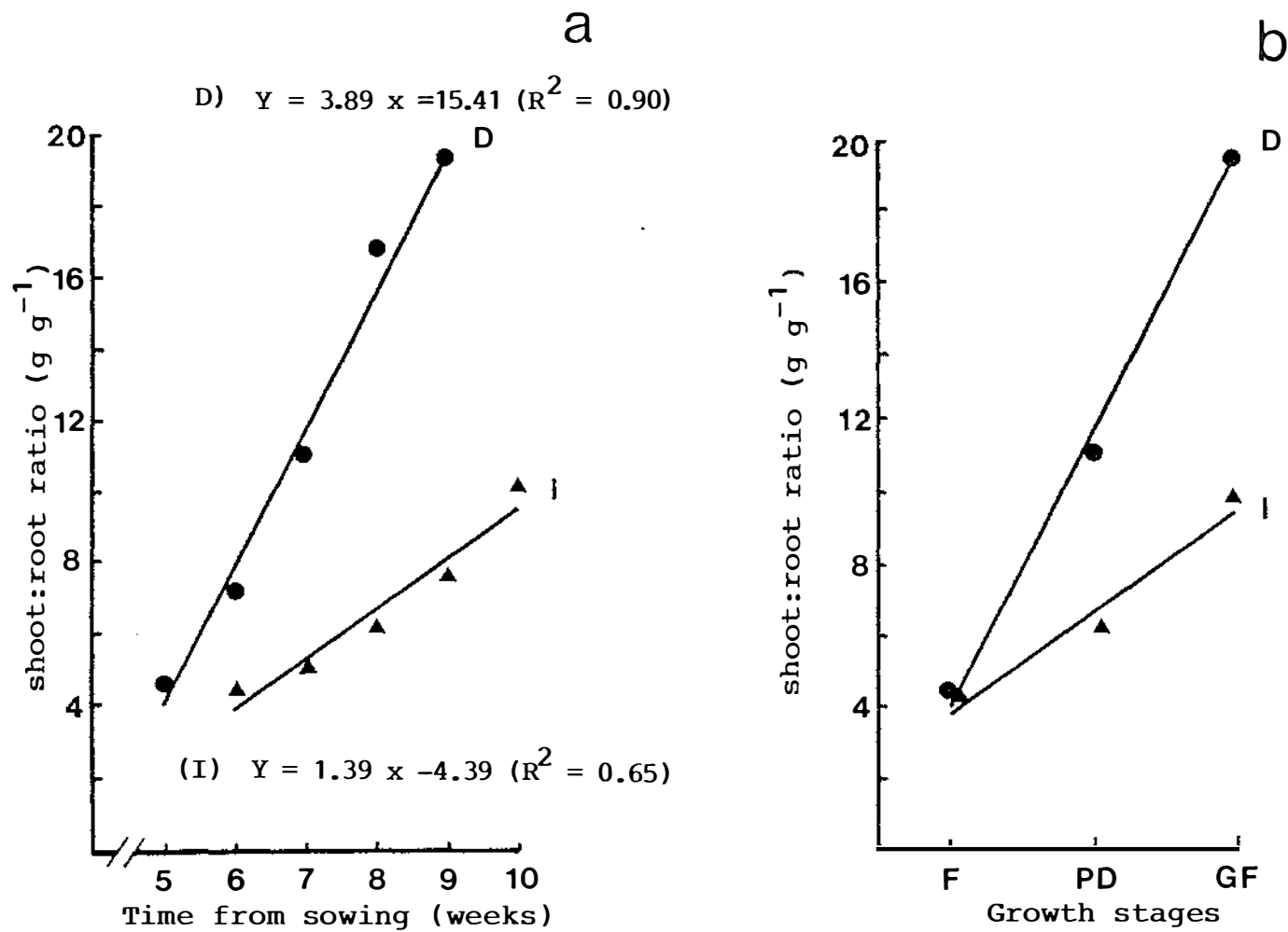


Figure 4.5 a Time course of shoot:root ratio of the determinate (D) and indeterminate (I) soybean cultivars (plotted values are mean of 5 replicates).
b shoot:root ratio on physiological age basis (growth stages as for Figure 4.4)

in the root and stem components from pod development stage (3 weeks from first flowering) to the end of the sampling period than the determinate cultivar (Figure 4.6). The leaf dry-weight ratio however, was quite similar between the two growth types. In contrast the pod dry-weight ratio of the determinate cultivar was significantly higher than that of the indeterminate cultivar for all three of the sampling periods.

The pattern of nodule growth of the two cultivars contrasted significantly (Figure 4.7 , Appendix 4.3). In the determinate cultivar nodule dry weight reached a peak by the pod development stage (at week 7; 0.6 g plant^{-1}) and started to decrease gradually as the plant developed towards the grain filling stage. The indeterminate cultivar had an average of 0.1 g plant^{-1} higher nodule dry weight than the determinate cultivar during the first 7 weeks of growth and thereafter this difference increased rapidly to 1.2 g plant^{-1} by the grain filling stage at week 10.

4.3.2 YIELD AND YIELD COMPONENTS: The indeterminate cultivar produced $34.31 \text{ g plant}^{-1}$ of seed which was approximately twice that of the determinate cultivar ($14.69 \text{ g plant}^{-1}$) (Table 4.2). This was due mainly to the number of pods per plant ($60 \text{ pods plant}^{-1}$ in the indeterminate cultivar compared to $29 \text{ pods plant}^{-1}$ in the determinate cultivar), and was primarily due to higher number of pods from the branches in the indeterminate cultivar (Table 4.3). On average the determinate cultivar had three and the indeterminate cultivar had five branches plant^{-1} . The mean seed weight (20 seeds weight) and number of seeds per pod were similar between the two cultivars (Table 4.3).

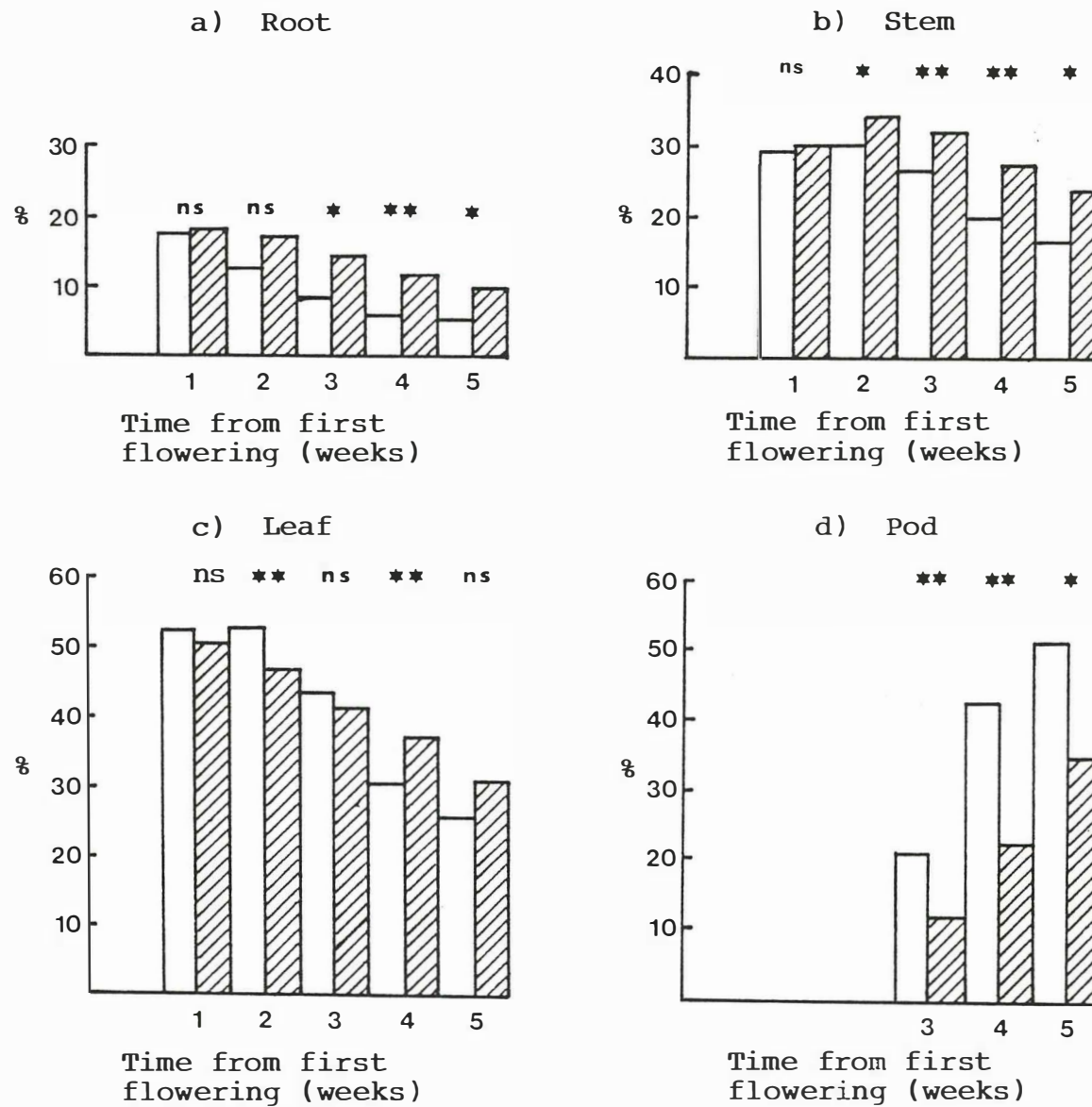


Figure 4.6 Ratio of dry weight of different plant components to total plant dry weight of determinate (□) and indeterminate (▨) soybean cultivars a) root b) stem c) leaf d) pod.
(Sowing was five weeks before flowering in the determinate and 6 weeks in the indeterminate cultivars).

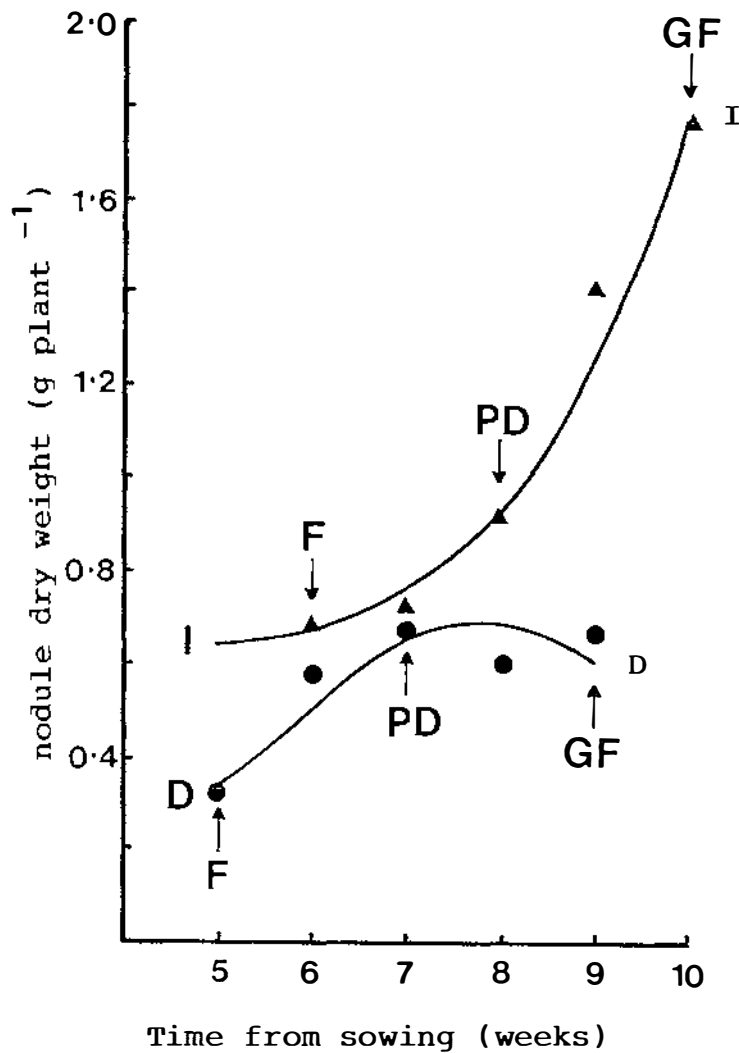


Figure 4.7 Time course of nodule dry weight in determinate (D) and indeterminate (I) soybean cultivars (Arrows indicated growth stages - description of growth stages as for Figure 4.4) (S.E. of regression coefficients are in Appendix 4.3).

Table 4.2 Yield, yield components, harvest index and nitrogen harvest index of the determinate and indeterminate soybean cultivars.

Growth type	Plant characters						
	Pod & seed wt. g plant ⁻¹	Total seed wt.	No. of pods	No. of seed pod ⁻¹	Av. seed wt. (g 20 seeds ⁻¹)	Harvest index	Nitrogen harvest index
Determinate	19.89	14.69	29.20	2.21	4.56	65.0	91.0
Indeterminate	47.81	34.31	59.80	2.39	4.85	61.1	90.0
F-test	**	**	**	ns	ns	*	ns
cv (%)	10.87	9.15	10.57	13.77	11.03	3.11	5.30

Table 4.3 Yield and yield components from main stem and branches of the determinate (D) and indeterminate (I) soybean cultivars.

Growth type		Seed wt.	No. of pods	Seeds pod ⁻¹	Av. seed wt.
		(g plant ⁻¹)			(g 20 seeds ⁻¹)
Main stem	D	9.47	16.4	2.3	5.11
	I	10.40	18.2	2.3	4.99
	F-test	ns	ns	ns	ns
	cv (%)	17.05	9.32	14.29	21.12
Branches	D	5.22	12.8	2.2	3.09
	I	23.91	41.6	2.4	4.79
	F-test	**	**	ns	**
	cv (%)	11.33	11.54	16.65	4.73

However, when the mean seed weights from the branches were compared, the results indicated that the indeterminate cultivar had significantly bigger seed than the determinate cultivar.

Final harvest indices from the two cultivars were significantly different. The harvest index was 61.1% for the indeterminate cultivar and 65.0% for the determinate cultivar (Table 4.2).

4.3.3 NITROGEN FIXATION: The maximum acetylene reduction activity of the determinate cultivar was approximately half that of the indeterminate cultivar (Figure 4.8a). Furthermore, the pattern of acetylene reduction activity for the two growth types of soybean was also quite different and fluctuated over a wide range. After flowering, the indeterminate cultivar showed marked decrease in acetylene reduction activity from 50 micro mol C_2H_4 h^{-1} to 20 micro mol C_2H_4 h^{-1} within a week, then increased almost linearly with time to 124 micro mol C_2H_4 h^{-1} over the following three weeks. In contrast, acetylene reduction activity in the determinate cultivar increased from 18 micro mol C_2H_4 h^{-1} at flowering stage to 60 micro mol C_2H_4 h^{-1} over the following two weeks but dropped sharply back to 18 micro mol C_2H_4 h^{-1} within a week before returning to about 60 micro mol C_2H_4 h^{-1} a week later. However, when the data were expressed as micro mol C_2H_4 h^{-1} g^{-1} nodule dry weight, i.e. as specific nitrogen fixation activity, the fluctuations were reduced and the determinate cultivar had generally higher activity than the indeterminate cultivar (Figure 4.8b).

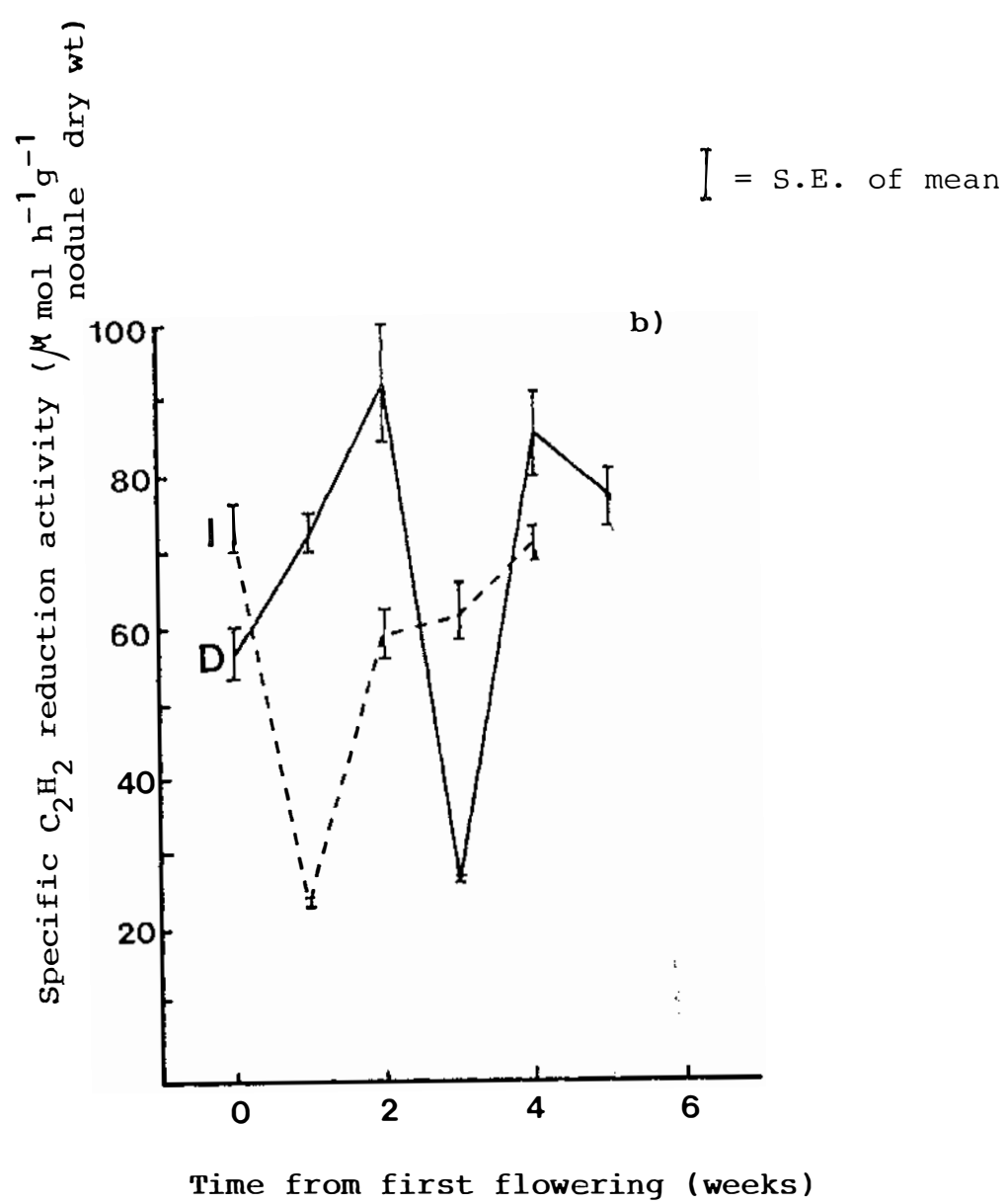
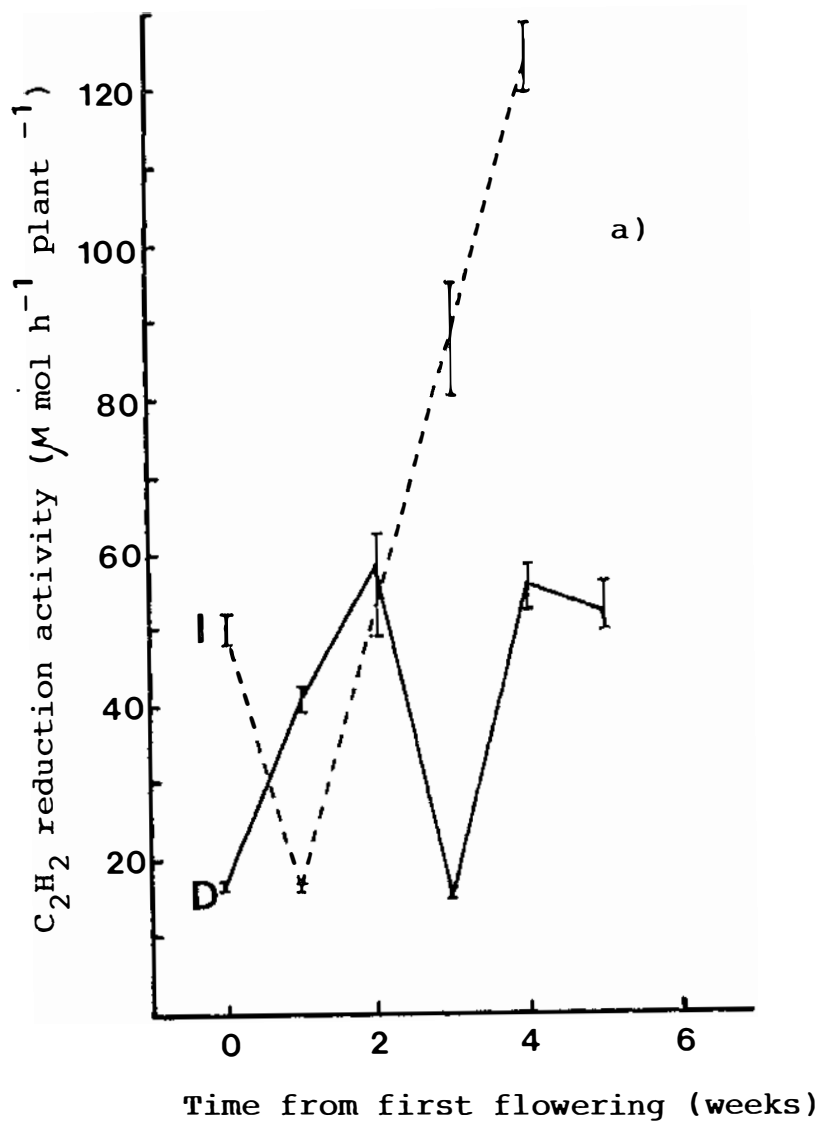


Figure 4.8 Acetylene reduction activity (a) and specific acetylene reduction activity (b) of the determinate (D) and indeterminate (I) soybean cultivars.

The area under the curve of the acetylene reduction activity data in Figure 4.8 was integrated to represent, as a first approximation, the amount of nitrogen fixed between flowering and grain filling stages. When values at the same growth stage were compared, only the difference at the grain filling stage was significantly different; with the indeterminate cultivar fixing about one-and-a-half times more nitrogen than the determinate cultivar (Figure 4.9a; Appendix 4.4). In other words, the difference in nitrogen fixation between the two cultivars came mainly after the pod development stage (Table 4.4).

The nitrogen fixing efficiency of nodules could also be compared by regressing total plant nitrogen (log scale) with nodule dry weight, with the regression coefficient representing the rate of nitrogen fixing efficiency (Dobereiner, 1966). When the slopes of the two regression lines ($b_1 = 3.5$ and $b_2 = 0.9$ for the determinate and the indeterminate cultivar respectively) were compared, they were significantly different at the 5% level of probability (Figure 4.10, Appendix 4.5)

4.3.3.1 The movement of ^{14}C : The carbon movement results showed that for both cultivars very little ^{14}C moved away from the node (leaf + pod) of the labelled leaf (Table 4.5); approximately two-thirds of the ^{14}C was retained within the labelled leaf and most of the remaining one-third was found in the pods at the same node as the labelled leaf. More ^{14}C was found immediately above and below the labelled leaf in the indeterminate cultivar (1.2%) than in the determinate cultivar (0.33%), and the difference was significant for the internode immediately below the labelled leaf (0.94% for the

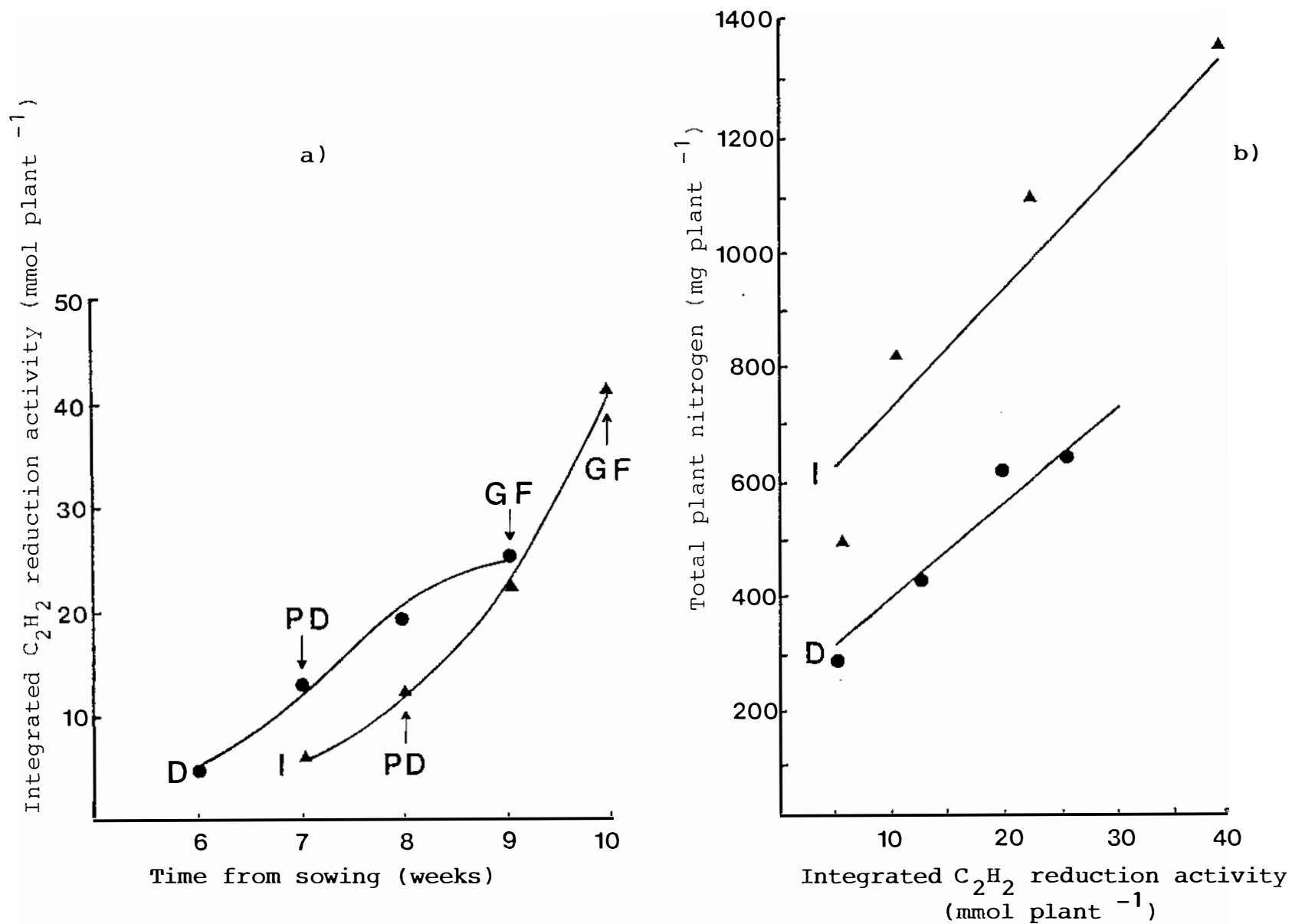


Figure 4.9a) Integrated acetylene reduction curves of the determinate (D) and indeterminate (I) soybean cultivars. (S.E. of regression coefficients are in Appendix 4.4).
b) Linear regression lines of total plant nitrogen (dependent variable) regressed against integrated acetylene reduction activity (independent variable). (S.E. of regression coefficients are in Appendix 4.5)

Table 4.4 Comparison of integrated acetylene reduction activity (m mol plant⁻¹) at two growth stages of the determinate (D) and indeterminate (I) soybean cultivars.

Growth type	Growth stages	
	Pod Development	Grain Filling
	m mol plant ⁻¹	
D	12.3	25.0
I	11.8	41.0
t-test	ns	**

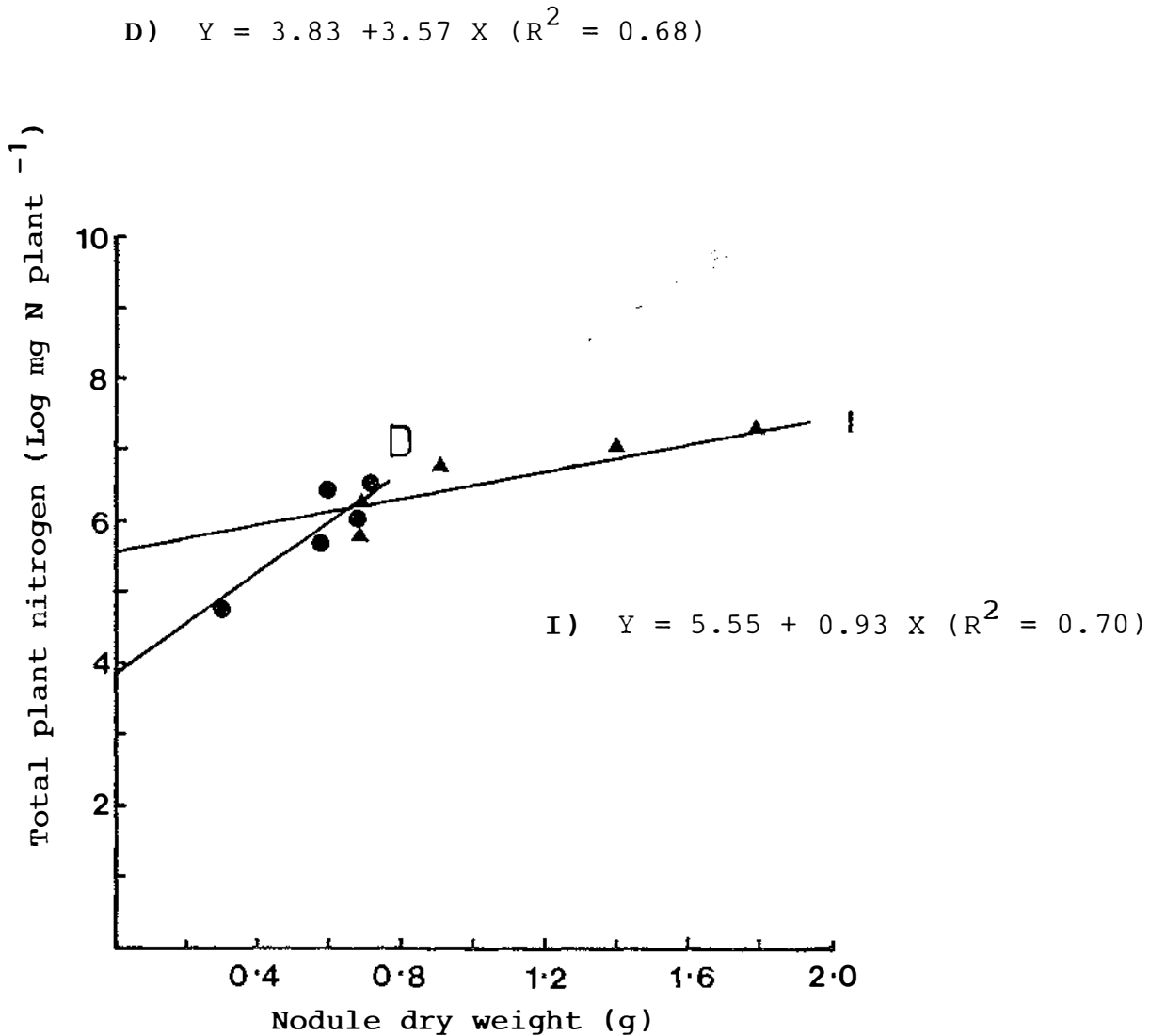


Figure 4.10 Regression lines of total plant nitrogen (log scale) as the dependent variable regressed against nodule dryweight (independent variable) of the determinate (D) and indeterminate (I) soybean cultivars. (Plotted values are mean of 5 replicates) (S.E. of regression coefficients are in Appendix 4.6).

Table 4.5 Recovery of ^{14}C in different plant parts of the determinate and indeterminate soybean cultivar (%).

Plant Parts	Determinate	Indeterminate	F-test	cv (%)
1 st Internode above labelled leaf	0.01	0.04	ns	23.25
The labelled leaf	67.00	62.40	ns	25.68
Pods at the same node of labelled leaf	31.60	33.00	ns	37.68
Pods at 1 st node below labelled leaf	0.16	0.22	ns	19.08
1 st Internode below labelled leaf	0.16	0.94	**	19.83

indeterminate cultivar and 0.16% for the determinate cultivar, respectively).

4.3.4 NITROGEN DISTRIBUTION: Nitrogen concentration, expressed as percentage nitrogen for total plant and plant components (*viz.* root, stem, leaf and pod), for the different growth stages including final harvest are presented in Figure 4.11.

By and large, at the flowering stage, the indeterminate cultivar had a higher nitrogen concentration in all components other than the stem component than the determinate cultivar (Figure 4.11 b, c & d). This resulted in a slightly higher total plant nitrogen concentration in the indeterminate cultivar than the determinate cultivar for the flowering stage, although the difference was not significant (Figure 4.11 a). Thereafter total plant nitrogen concentration was significantly higher in the determinate cultivar than the indeterminate cultivar during both the pod development and grain filling stages. Nitrogen concentration of the individual components was less consistent; concentration values for the root and leaf were similar between the two cultivars whereas those for the stem were different and the relationship between types changed with time. The leaf nitrogen concentration had the greatest change with time (development stage) from about 5-6% at flowering to 3% at the grain filling stage (Figure 4.11 d). The pattern of change in leaf nitrogen concentration was also different between the two cultivars; in the indeterminate cultivar leaf nitrogen concentration steadily declined with time from the flowering stage, whereas in the determinate cultivar it was maintained at about the same level from

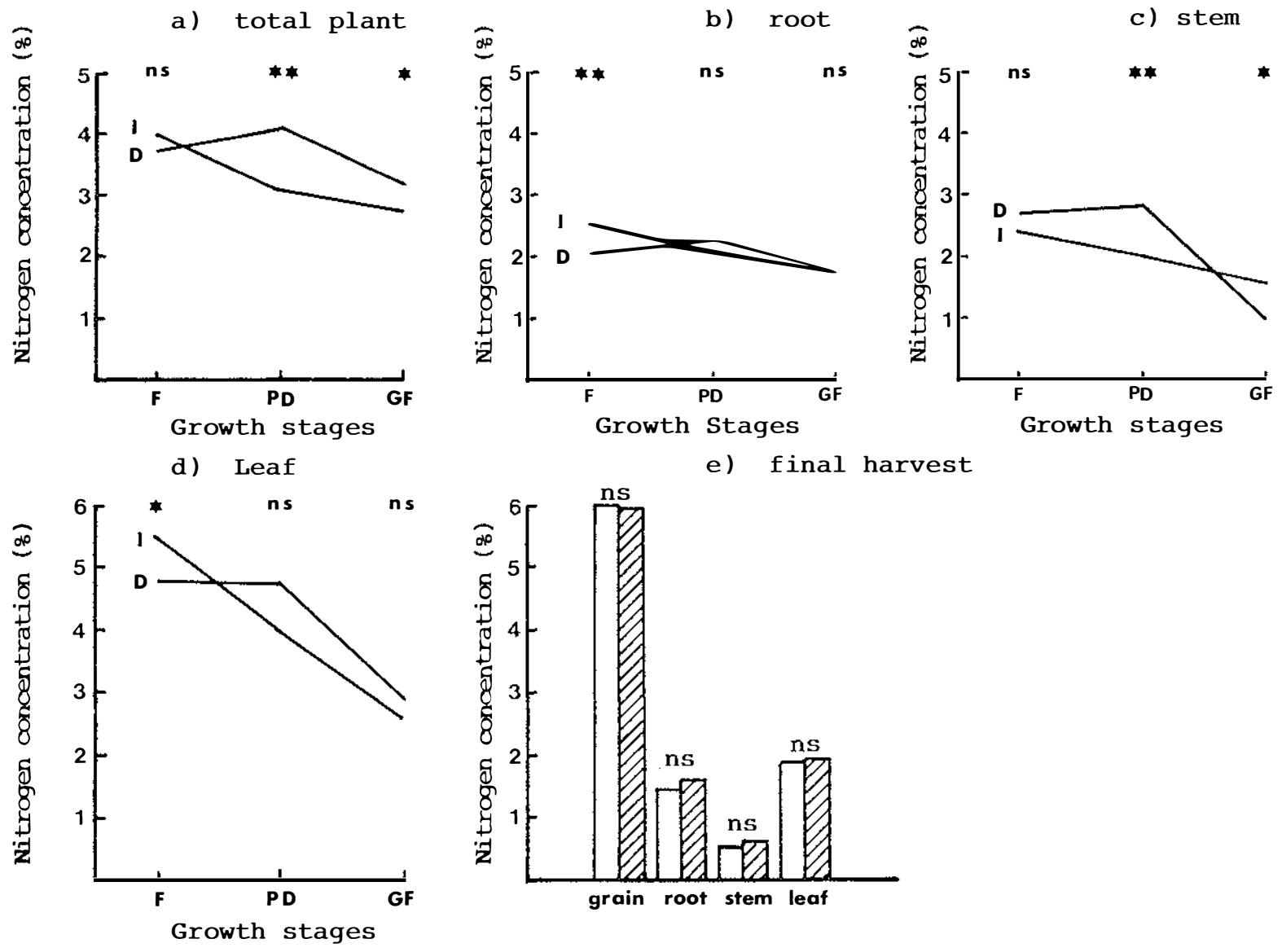


Figure 4.11 Nitrogen concentration of different plant parts of the determinate (D, □) and indeterminate (I, ▨) soybeans. Growth stages as for Figure 4.4

the flowering stage until pod development stage before decreasing quite sharply as it approaches the grain filling stage.

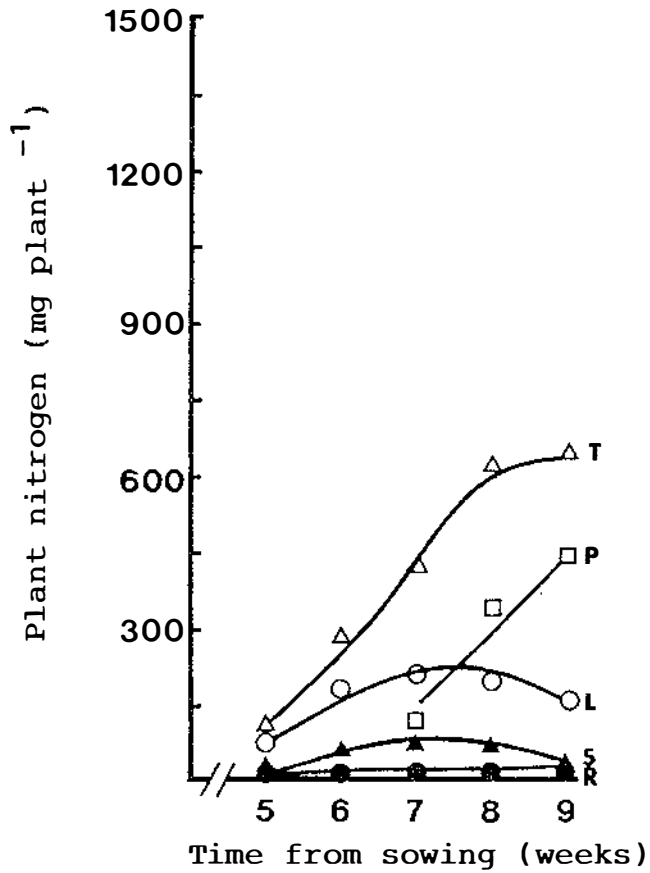
The pattern of nitrogen accumulation with time was similar to that for dry-matter accumulation (Figure 4.12 and Appendix 4.7). Except for total root nitrogen in the determinate cultivar, all the other nitrogen accumulation patterns were fitted well with a quadratic equation. Nitrogen accumulation in the leaf, stem and total plant of the indeterminate cultivar was higher than that of the determinate cultivar and this difference was maintained at all growth stages (Table 4.6). At final harvest the indeterminate cultivar had accumulated about three times more nitrogen than the determinate cultivar (Table 4.7).

The relative accumulation rate of nitrogen (RRNA) was first used by Gibson (1965) to compare the relative efficiency of nitrogen accumulation. In this experiment RRNA was calculated from the nitrogen accumulation curves in Figure 4.12 using the same formula as that for calculating RGR (reported in Materials and Methods section). Comparison of RRNA values between the two cultivars, showed that the determinate cultivar had a higher RRNA than the indeterminate cultivar at the flowering stage but this was reversed by the grain filling stage (Figure 4.13).

The indeterminate cultivar had higher ratio of nitrogen accumulated in the root, stem and leaf from the pod development stage (3 weeks from first flowering) until the end of the sampling period than the determinate cultivar (Figure 4.14).

4.3.4.1 Nitrogen re-distribution: Nitrogen re-distributed from different plant parts was calculated using the same method

a) Determinate cultivar



b) Indeterminate cultivar

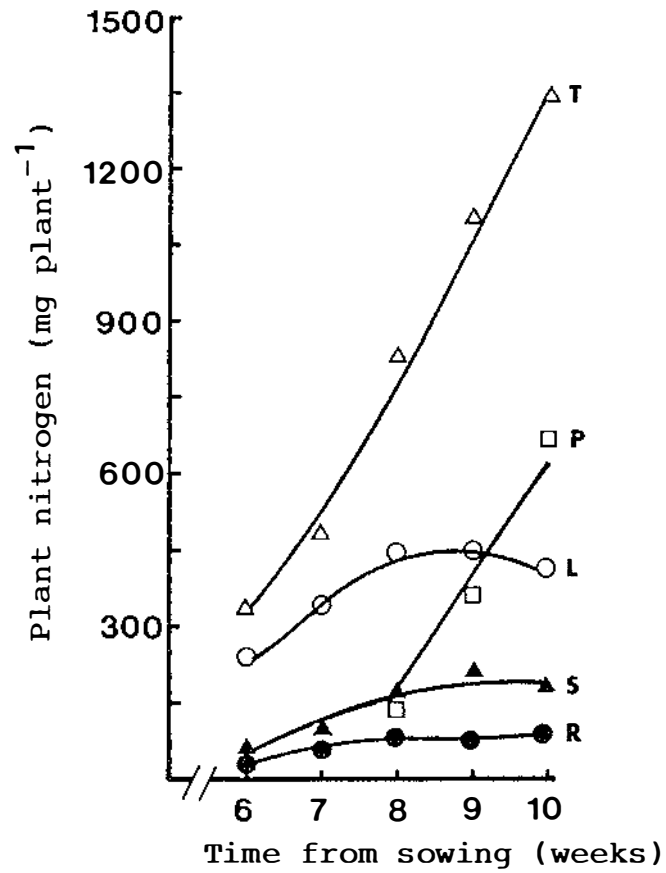


Figure 4.12 Time course of nitrogen accumulation of stem (S) leaf (L) pod (P) and total plant nitrogen (T) of the determinate and indeterminate soybean cultivars. (Plotted values are mean of 5 replicates). (S.E. of regression coefficients are in Appendix 4.7)

Table 4.6 Comparison of nitrogen of different plant parts at different growth stages between the determinate (D) and indeterminate (I) soybean cultivars.

Plant Characters	Growth type	Growth stages		
		Flowering	Pod Development	Grain Filling
		mg plant ⁻¹		
Stem	D	24.46	83.04	31.79
	I	53.83	160.87	182.47
	t-test	**	**	**
Leaf	D	82.10	220.43	139.85
	I	231.83	428.38	396.39
	t-test	**	**	**
Total	D	115.90	451.65	621.60
	I	321.31	785.80	1361.71
	t-test	**	**	**

Table 4.7 Total plant nitrogen, total re-distributed nitrogen and proportion of re-distributed nitrogen from different plant parts in determinate and indeterminate soybean plants grown under controlled environment conditions.

	Determinate	Indeterminate	F-test cv (%)	
Total plant nitrogen (mg plant ⁻¹)	918.09	2661.09	**	10.73
Total re-distributed N. (mg plant ⁻¹)	235.45	552.55	**	19.86
Proportion of re-distributed N. from leaves (%)	60.76	54.54	ns	20.02
Proportion of re-distributed N. from stem (%)	23.04	30.94	*	15.30
Proportion of re-distributed N. from pod wall (%)	16.20	14.52	ns	30.25
Ratio of re-distributed N./seed N.	40.51	31.89	ns	18.24

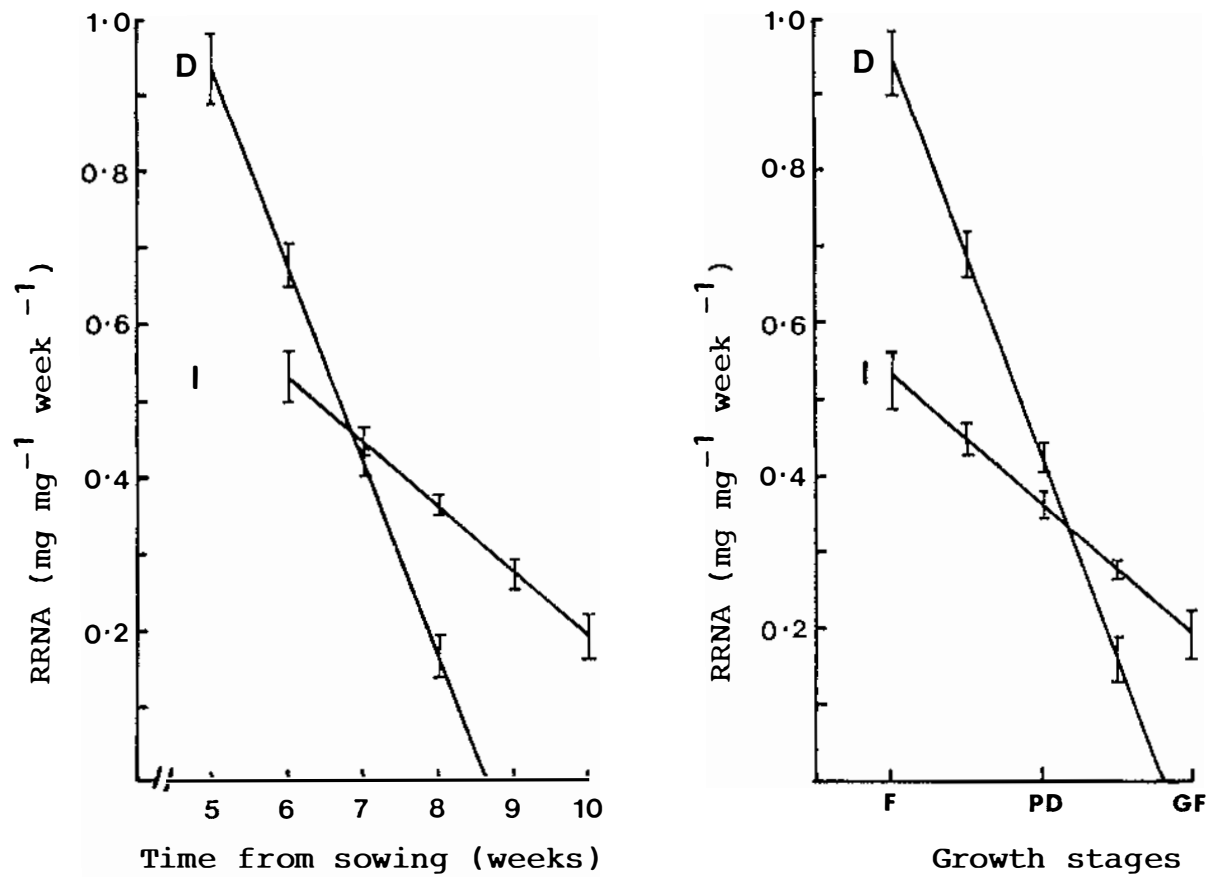


Figure 4.13 Relative accumulation rate of nitrogen (RRNA) calculated from total plant nitrogen curves of the determinate (D) and indeterminate (I) soybean cultivars. (Growth stages as for Figure 4.4)

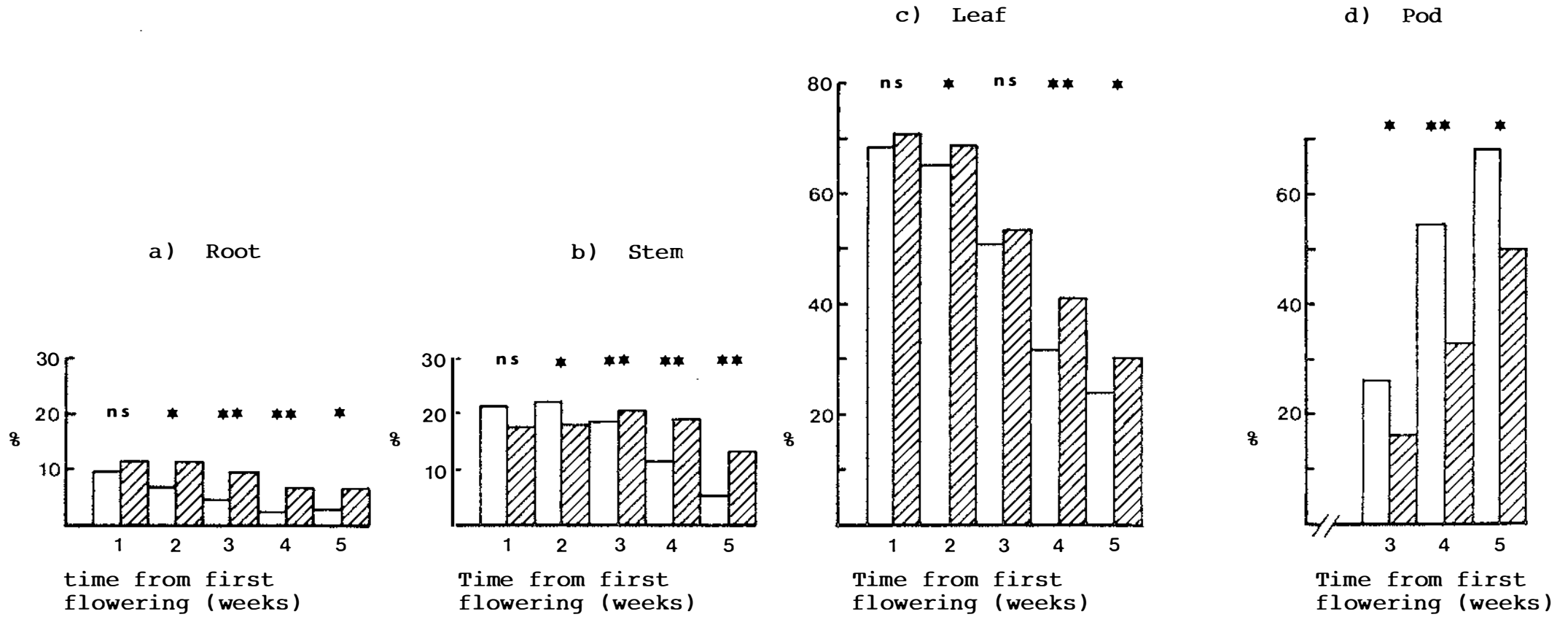


Figure 4.14 Ratio of nitrogen content of different plant parts to total plant nitrogen (%) of the determinate (□) and indeterminate (▨) soybean cultivars.

described by Egli *et al.* (1978). The indeterminate cultivar re-distributed more than twice as much nitrogen as that of the determinate cultivar. However, this was only 32% of the total seed nitrogen compared with 41% in the determinate cultivar (Table 4.7). A similar trend was also found in the proportion of nitrogen re-distributed from leaves and pod wall, although the differences were not statistically significant. In contrast, in the indeterminate cultivar, the stem contributed a significantly higher amount of the re-distributed nitrogen component than occurred in the determinate cultivar. Leaves were major source of nitrogen re-distribution in both cultivars, with about 55 and 61% of the re-distributed nitrogen from leaves of the indeterminate and determinate cultivars respectively.

4.3.5 RELATIVE IMPORTANCE OF PLANT CHARACTERS IN DETERMINING THE DIFFERENCE BETWEEN THE TWO GROWTH TYPES: One of the objectives of this experiment is to determine the key plant parameters that may influence the differences between the two soybean growth types. It is possible to use a multiple discriminant analysis to statistically distinguish between two and more groups on the joint basis of several attributes, and identify the attribute which contributes most to distinguish between the groups as explained in the Materials and Methods section.

Eight plant characters *viz.* the dry-weight of the roots, nodules, stems, leaves and pods, the leaf area, acetylene reduction activity and the total plant nitrogen from all five samplings were used as attributes in this analysis. Information from the analysis is

presented in Table 4.8 . From the test for statistical significance of discriminating information it showed that the first discriminant function was significant and could explain up to 100% of the variance between the two cultivars. When the structure matrix was examined (Table 4.8), it indicated that leaf area was the parameter contributing the most to differentiation between the two cultivars along the first discriminant function. This was followed, in order of importance, by root dry weight, leaf dry weight, stem dry weight, and nodule dry weight.

4.4 DISCUSSION

For clarity in discussion this section will be divided into 5 sub-sections according to the objectives of this experiment *viz.* to identify the differences between the two soybean cultivars in

- i. plant growth
- ii. yield and yield components
- iii. nitrogen fixation
- iv. nitrogen distribution and nitrogen re-distribution
- v. relationships between plant characters and nitrogen

fixation.

4.4.1 PLANT GROWTH: The theoretical distinction between these two growth types of legumes is that in the determinate type, growth in length of a main stem or branch is terminated by an inflorescence, while in the indeterminate type vegetative growth continues independently of flowering which occurs at axillary sites (Smartt,

Table 4.8 Information from multiple discriminant analysis (a) and structure matrix (b).

a)

Function	Eigenvalue	Percent of variance	Canonical correlation	After function	Chi-square	df	Significance
1	2.35788	100	0.8379	0	53.298	8	****

b) structure matrix

plant character	function 1
Leaf area	0.744
Root dry weight	0.729
Leaf dry weight	0.620
Stem dry weight	0.617
Nodule dry weight	0.497
Total plant. Nitrogen	0.427
c ₂ H ₂ reduction activity	0.290
P _{od} ² dry weight	0.113

1976). Although, there was greater competition between vegetative and reproductive growth in the indeterminate cultivar than in the determinate cultivar, Egli and Leggett (1973) found no difference between soybean cultivars in the total amount of dry-matter produced.

In the present experiment however, vegetative dry-matter components of the indeterminate cultivar viz. leaf, stem and root were significantly higher than those of the determinate cultivar (Table 4.1). This could have partly been due to the indeterminate cultivar reaching the flowering stage one week later than the determinate cultivar. However, calculations to assess the impact of this extra week showed that the indeterminate cultivar had produced only about 4.4 g dry-matter in that time whereas, by the end of the sampling period the indeterminate cultivar accumulated about 20 g dry-matter more than that of the determinate cultivar. Hence, the later flowering can not fully account for the difference. In a growing plant, where photosynthetic tissues make the major contribution to dry-matter production, the absolute growth rate at any time is the product of the rate of increase in weight per unit of leaf (net assimilation rate, NAR) and the amount of leaf present (Jackson, 1963). The difference in dry-matter yield in this present experiment therefore, was largely the result of the indeterminate cultivar having more leaf area per plant than the determinate cultivar at every growth stage from first flowering onwards (Table 4.1). Furthermore, the indeterminate cultivar also had a higher NAR at the grain filling stage (Figure 4.4b). The NAR values in this experiment were also very similar to the values reported by Sivakumar and Shaw (1978) using the same calculation technique.

Root growth was also different between the two cultivars. Root dry weight of the determinate cultivar began to plateau soon after flowering, whereas for the indeterminate cultivar it continued to increase for a further two weeks after initial flowering before levelling off by the final sampling date (Figure 4.1). This higher root growth in the indeterminate cultivar was also reflected in the lower shoot:root ratio at the two growth stages compared after flowering (Figure 4.5). The shoot:root ratio values from the indeterminate cultivar were quite similar to the values from field grown soybean cultivar 'Wayne' (Sivakumar *et al.*, 1977) which had values ranging from 6.8 to 9.5. When the ratio of root dry-matter to total dry-matter was compared, it indicated that more dry-matter was partitioned to the root component of the indeterminate cultivar than that of the determinate cultivar (Figure 4.6). This would suggest that the indeterminate cultivar had more photosynthate available to promote more root growth over a longer period of time than the determinate cultivar. This could have implications for nitrogen fixation, as will be discussed below (4.4.3). There is evidence that the root system may be a yield-limiting factor in soybean. For example, Sanders and Brown (1976) found in their grafting experiment that increasing the root:shoot ratio resulted in increased growth, nutrient uptake and yield of Lee 68' soybeans; increasing the number of shoots per root (increasing the shoot:root ratio) has much smaller effects. Silberbush and Barber (1984) also reported that soybean cultivars with more extensive root systems were found to absorb more P and K and these cultivars were those with highest yields.

Nodule dry weight changes in this experiment (Figure 4.7) indicated that the indeterminate cultivar still had high nodule growth after the pod development stage, while it began to decline at this time in the determinate cultivar. In legumes, either a reduced photosynthate supply or an increased level of combined nitrogen can cause the senescence of previously active nodules (Sutton, 1983). In the present experiment, the early nodule senescence of the determinate cultivar was probably due to the reduction of photosynthate supply. This could in part be supported by the fact that there was significantly less dry-matter partitioned to the roots of the determinate cultivar as compared with that of the indeterminate cultivar (Figure 4.6).

4.4.2 YIELD AND YIELD COMPONENTS: It has been suggested that competition between vegetative and reproductive growth during flowering and pod development may reduce the number of pods set (Greer and Anderson, 1965). This led to the suggestion that cultivars with a determinate growth type may exhibit less competition during this period and thus produce higher yields (Shibles *et al.*, 1975). However, there are disagreements over these points in the literature. For example, Egli and Leggett (1973) and Beaver *et al.* (1985) recorded similar values of grain yield between the two growth types in soybean, whereas Hicks *et al.* (1969) reported higher values in the determinate type of soybean and Chaturvedi *et al.* (1980) found a higher value in an indeterminate type of cowpea. Furthermore, Beaver and Johnson (1981) found that the determinate cultivars possessed less predictable performances than the indeterminate cultivars when they compared yield

stability of two growth types adapted to the northern United States. Thus the yield difference between the two growth types may not only be affected by genetic factors but also by other factors such as environmental conditions, cultural practices and their interactions as well. In the present experiment, the indeterminate cultivar produced twice as much seed yield as the determinate cultivar (Table 4.2).

A comparison of the yield components indicated that the main yielding advantage of the indeterminate cultivar over the determinate cultivar came from the number of pods set. Pod number has previously been reported as the most important component in determining grain yield in soybean (e.g. Greer and Anderson, 1965; Shibles *et al.*, 1975; Laohasiriwong, 1982). When the yield and yield components from the main stem and the branches were compared separately between the growth types, it was clear that the difference was mainly due to the difference in branching (Table 4.3). Beaver *et al.* (1985) also found that the determinate cultivars set as many pods on the main stem as the indeterminate cultivar. In contrast to the result of this present experiment, they found that the determinate cultivars ('Elf' and 'Clark') produced a greater number of pods and more seed weight from branches than the indeterminate cultivar 'Williams'. However, their experiment was carried out in the field where they noted that the conditions were 'less than productive'. The morphological behavior of the two growth types in their experiment may not be directly comparable to those of the present experiment which was conducted under controlled environment conditions.

Several authors have concluded that the difference in the duration ^{over} which seed accumulates dry matter [^] accounts for more yield

variation among cultivars than do differences in the rate of seed dry matter accumulation (e.g. Hanway and Weber, 1971a; Egli and Leggett, 1973). In the present experiment both cultivars had a similar duration of seed dry-matter accumulation (4 weeks from grain filling stage). Hence the yield advantage of the indeterminate cultivar was mainly due to more pods developing from a larger source capacity (leaves). As Tanaka (1980) explained, in grain legumes during the period from flowering to the early stage of pod development, the source capacity is the key factor of pod setting. This is because the vegetative, reproductive and ripening phases overlap each other after the commencement of flowering and pods start to grow while both new leaves and flower primordia continue to differentiate on the growing stems for some time. The sink (young pods) of this growth stage, and the potential sink (flowers) and source (leaves) of the next growth (ripening) phase grow simultaneously, and these organs may compete with each other when the source capacity is insufficient to meet all the demands of these sinks. The sink capacity during the ripening phase (pod number) is decided by the condition of this competition during a short period at or after flowering.

The proportion of the dry-matter of a grain crop that is harvested (seed) relative to the total above ground biomass, *i.e.*, harvest index, is an important parameter describing the efficiency of grain yield (Gifford and Evans, 1981). It has been suggested that future improvements in yield may come primarily from improvements in the partitioning of assimilate into the harvested fraction (Loomis *et al.*, 1979). The results in this experiment indicated that the proportion of dry-matter which was partitioned to the pods was higher

in the determinate cultivar than in the indeterminate cultivar (Figure 4.6). The final harvest indices of the two cultivars were also significantly different (Table 4.2). However, the difference in the final harvest indices was relatively smaller than the proportion of dry-matter which was partitioned to the pods at the grain filling stage. This could be due to different strategies of reproductive growth between the two growth types, *i.e.*, the determinate soybeans produce most of their vegetative growth first, and then their reproductive growth while the indeterminate soybeans overlap their vegetative and reproductive periods (flowering continues until the plant stops producing new nodes and by then seed development has already begun on the lower nodes). Even though pods at the basal nodes begin developing earlier than pods at the distal nodes all the pods mature at about the same time due to a faster growth rate in the upper pods (Johnson and Dunphy, 1983).

4.4.3 NITROGEN FIXATION: There are relatively few published studies on the effect of growth types on nitrogen fixation. Bello *et al.* (1980) found that indeterminate cultivar 'Williams' fixed more nitrogen after pod filling than other cultivars with semi-determinate or determinate growth types. However, they did not discuss the change of acetylene activity in relation to the other growth stages of the different growth types. In general, the values of acetylene reduction activity in the present experiment were higher than those reported by Skrdleta *et al.* (1978) when compared at similar growth stages with their glasshouse-grown soybean.

The patterns of acetylene reduction activity of the two growth types in this experiment were markedly different. The sharp decrease in activity of the indeterminate cultivar after flowering probably came from the strong competition for photosynthate between vegetative and reproductive organs at this time (Figure 4.8). In contrast the acetylene reduction activity of the determinate cultivar increased markedly until the plant reached the pod development stage after which the rate declined, subsequently recovered and then declined again at ^{the} grain filling stage. However, it is not clear why these changes occurred as will be shown in the next Chapter that in the determinate cultivar when the activity declined, it did not go up again.

There are numerous studies indicating that the nitrogen fixation potential of the plant is limited by its ability to provide photosynthate to the nodules (see Bergersen, 1975; Edwards, 1975). The results from the present experiment indicated that the total nitrogen fixed by the indeterminate cultivar was greater than that of the determinate cultivar (e.g. higher 'integrated acetylene reduction activity' Figure 4.9). Since the acetylene reduction activity per unit nodule dry weight of the determinate cultivar was generally higher than those of the indeterminate cultivar (Figure 4.8), it could be concluded that the superiority of the acetylene reduction activity in the indeterminate cultivar came largely from a rapid increase in the nodule mass, which certainly came from the indeterminate cultivar's ability to supply more photosynthate to the nodules. The results of ^{14}C movement, which showed that a higher percentage of ^{14}C moved downward from the source leaf of the indeterminate cultivar than

that of the determinate cultivar supports that conclusion indirectly (Table 4.5).

The distribution of assimilates in soybean is predominantly localized about the source leaf (Stephenson and Wilson, 1977a). The major portion of the assimilate accumulates in the axillary pods of the source leaf, with leaves lower in the canopy contributing very little elsewhere (Stephenson and Wilson, 1977a, 1977b). The results from this experiment supported their finding, with about 30% of ^{14}C found in the axillary pods *and* less than 1% elsewhere (Table 4.5).

The significantly higher amount of ^{14}C found in the internode below the labelled leaf in the indeterminate cultivar (Table 4.5) may be an indication that there is a greater downward movement of photosynthate in this than in the determinate cultivar. Similar trends had been reported in other species. For example, Baker *et al.* (1983) found a 4.6% distribution of ^{14}C in the indeterminate field bean (*Vicia faba*) compared with only 2.5% in the determinate cultivar when the leaf at ^{the} first flowering node was labelled.

Acetylene reduction activity is an instantaneous estimate of nitrogen fixation . One way of estimating the total activity is by summing the area under the values for instantaneous acetylene reduction activity determined at each successive harvest (Patterson and LaRue, 1983); this is the 'integrated acetylene reduction activity'. For plants that depended solely on symbiotic nitrogen, the relationship of this 'integrated acetylene reduction activity ' and total plant nitrogen should be linear. A linear relationship was found between the two parameters for each cultivar in this experiment (Figure 4.9b). Patterson and LaRue (1983) also reported significant

correlations between total plant nitrogen, 'integrated acetylene reduction activity', isotope dilution and N-difference when they studied the seasonal nitrogen fixation of 21 soybean cultivars. Their 'integrated acetylene reduction activity' values, however, were less than the values reported in this experiment (5-20 mmol plant⁻¹ compared with 20-50 mmol plant⁻¹). This may be due to the difference in environmental conditions (e.g. temperature), since their experiment was conducted in the field whereas this experiment was conducted in controlled environmental conditions.

4.4.4 NITROGEN DISTRIBUTION: The soybean seed has a high nitrogen requirement of which a substantial proportion comes from symbiotic nitrogen (Warembourg and Fernandez, 1985). However, nitrogen fixation declines early in the pod-filling phase (e.g. Lawn and Brun, 1974a). This high nitrogen requirement, coupled with the decline in the nitrogen assimilatory processes during seed filling, suggests that the nitrogen assimilatory processes may fail to meet the nitrogen requirement of the seed. Nitrogen must therefore be re-distributed from vegetative tissues to the pods to meet this deficit (Sinclair and de Wit, 1975; 1976). Sinclair and de Wit called this a 'self destruction' hypothesis.

4.4.4.1 Nitrogen distribution: There are some suggestions that differences in nitrogen concentration between cultivars is environmentally induced rather than due to genetic differences (Zeiger *et al.*, 1982). Those authors found that the nitrogen concentration differences between cultivars were not consistent across years or between plant parts when they examined eight soybean

cultivars for two seasons. However, Speath and Sinclair (1983) found in their survey of 121 soybean cultivars that nitrogen concentrations varied substantially among cultivars as did total nitrogen uptake, and was generally in proportion to plant size.

In the present experiment, nitrogen concentration of the two cultivars was significantly different for the total plant and the stem component at the pod development and grain filling stages (Figure 4.11). The pattern of change in nitrogen concentration of the stem component of both cultivars was also interesting. At the pod development stage the nitrogen concentration of the determinate cultivar was significantly higher than that of the indeterminate cultivar. This was mainly due to the fact that the stem component of the determinate cultivar had stopped growing by this stage (Figure 4.1). In contrast, at the grain filling stage, the stem component of the indeterminate cultivar had a higher nitrogen concentration than that of the determinate cultivar (1.5% and 1.0%, respectively). However, at plant maturity no difference was found in nitrogen concentration for any plant component (Figure 4.11). This could be a consequence of a difference in nitrogen re-distribution (see next subsection).

Nitrogen accumulation in the leaf, stem and total plant of the indeterminate cultivar were higher than those of the determinate cultivar and this applied at all of the growth stages (Table 4.6). From a comparison of the regression coefficients of the nitrogen accumulation curves (Figure 4.12), it was evident that significant differences existed between the two cultivars in all three curves (viz. total, leaf and stem nitrogen). This might be regarded as an

indication that the indeterminate cultivar could accumulate nitrogen faster than the determinate cultivar. The higher acetylene reduction activity of the indeterminate cultivar, as compared with the determinate cultivar, discussed in the previous section tends to support this suggestion.

The partitioning pattern of nitrogen to different plant parts for each of the two cultivars followed a very similar pattern to that of dry-matter partitioning (Figure 4.6 vs Figure 4.14). The proportion of nitrogen in the vegetative parts of the determinate cultivar was significantly lower than that in the indeterminate cultivar after the pod development stage. This could also be interpreted as evidence to support the suggestion that existing reserves of nitrogen from vegetative parts of the determinate cultivar were needed to provide nitrogen to the seed much earlier than was the case for the indeterminate cultivar.

Comparisons of the final nitrogen distribution to the grain as represented by the 'nitrogen harvest index' (ratio of seed nitrogen and total above ground nitrogen) between the two growth types was not significantly different (Table 4.2). A similar conclusion was also found in the previous experiment reported in Chapter 3. This is in agreement with Jeppson *et al.* (1978), who concluded that nitrogen harvest index was a very stable characteristic among cultivars.

4.4.4.2 Nitrogen re-distribution: Zeiher *et al.* (1982) examined the nitrogen re-distribution characteristic of soybean cultivars of varying maturities and growth types, and found that cultivar differences in nitrogen re-distribution were related to the size of the nitrogen pool, which was primarily a function of the dry

weight and was closely associated with cultivar maturity. The results from the present experiment supported this hypothesis, *i.e.* with bigger size of nitrogen pool, the indeterminate cultivar re-distributed more than twice as much nitrogen as that of the determinate cultivar (Table 4.7). The proportion of nitrogen re-distributed was similar for both the leaf and pod wall components of both cultivars. In contrast, the nitrogen re-distributed from the stem component was significantly higher in the indeterminate cultivar than in the determinate cultivar (Table 4.7). However, leaves were still the most important source of nitrogen re-distribution. Similar conclusion were also reported in lupin (Withers and Forde, 1979).

Speath and Sinclair (1983) reported that the indeterminate cultivar 'Amsoy' continued to accumulate nitrogen at a high, constant rate throughout the pod-fill stage, but retained much of this nitrogen in the stem so that it was not available for seed development. Evidence from the present experiment, which also used 'Amsoy' as the indeterminate type, indicated that 'Amsoy' continued to accumulate nitrogen at a high rate during the pod filling stage (Figure 4.13). However, in contrast to the result obtained by Speath and Sinclair (1983), more nitrogen was re-distributed for seed development (Table 4.6). This was probably because Speath and Sinclair carried out their experiment in the field, where the plants may not have reached the full yield producing potential. The seed nitrogen of 'Amsoy' from their experiment was only about half that of the seed nitrogen from the 'Amsoy' used in the present experiment. This could also suggest that 'Amsoy' may have the ability to store nitrogen in the vegetative parts if it has already met the nitrogen requirement of the seed.

4.4.5 RELATIONSHIPS OF NITROGEN FIXATION AND PLANT PARAMETERS: The evaluation of the effects of various plant morphological variables on acetylene reduction rates could help to determine their relative significance in influencing the nitrogen fixation ability of the plant, and hence might provide an indirect means of selecting for superior nitrogen-fixing genotypes.

When studying linearly related variables, a correlation coefficient only describes and measures the degree of association between two variables. The multiple regression equation is useful when the best or closest prediction of Y (dependent variable) based on the information in $X_1 \dots X_k$ (independent variables) is needed. However, when the objective is not so much concerned with prediction as to the proposal of a 'plausible interpretation' of the relationship between the variables, the path analysis which is concerned with erecting a causal structure compatible with the observed data (Li, 1975) may be more useful. Accordingly, a path analysis has been chosen for this experiment to determine the contribution of various plant parameters to nitrogen fixation.

The plant parameters used in constructing the path analysis diagram were those frequently indicated in the literature as being related to or associated with nitrogen fixation directly (*e.g.* Rennie and Kemp, 1981; Graham and Setter, 1983; Denison *et al.*, 1985). These parameters are root dry weight, nodule dry weight, leaf area per plant, acetylene reduction activity, and total plant nitrogen. The path diagram showed that in both growth types leaf area was more important as a link to nodule dry weight than root dry weight (Figure

4.15). For *acetylene reduction*, the path coefficient of leaf area was very small in both growth types thus did not indicate any strong direct relationship between leaf area and acetylene reduction activity (Figure 4.15). Hume (1981) reported good relationship between acetylene reduction activity and leaf area, but he only examined acetylene reduction activity during the vegetative stage.

Of the plant morphological parameters considered, nodule weight per plant had been reported to be most closely correlated with acetylene reduction activity (Johnson and Hume, 1973; Lawn *et al.*, 1974; Denison *et al.*, 1985). There was a marked difference in the relationship between nodule dry weight and acetylene reduction activity between the two cultivars (Figure 4.15). In the indeterminate cultivar, the result indicated that variation of acetylene reduction activity came mainly from the change of nodule dry weight ($p = 0.98$), whereas in the determinate cultivar this relationship was lower than that of the unexplained indicated by ^{the} _^ arrow from ^{the} _^ outside. This would support the conclusion from ^{the} _^ previous section that ^{the} _^ superiority of the acetylene reduction activity in the indeterminate cultivar came largely from a rapid increase in the nodule mass.

Leaf area of both growth types was also important to total plant nitrogen. The close relationship between leaf area and nodule dry weight found in this experiment was also reported in the other species e.g. in sainfoin (*Onobrychis viciifolia*) (Hume, 1981) and cowpea (Graham and Setter, 1983). ~~The~~ relationship between acetylene reduction activity and total plant nitrogen in both cultivars was poor ($p=0.15$). Since acetylene reduction is an instantaneous estimate of

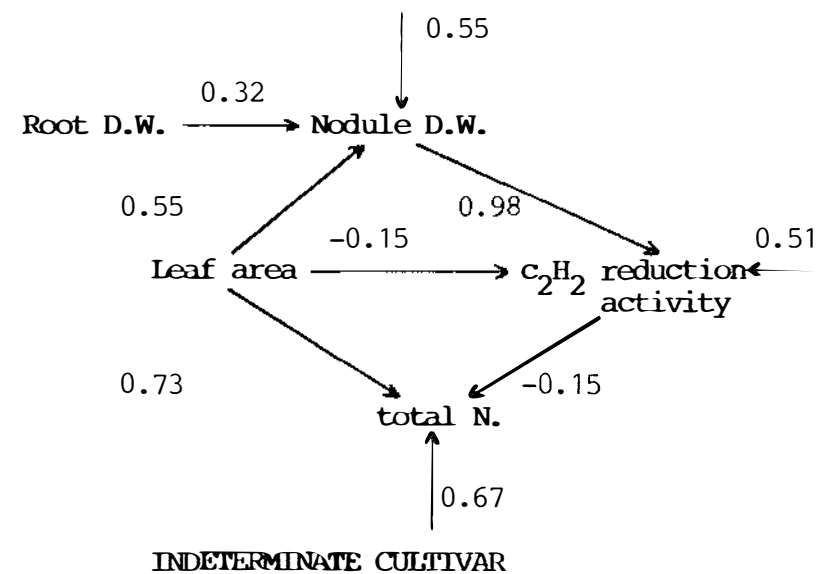
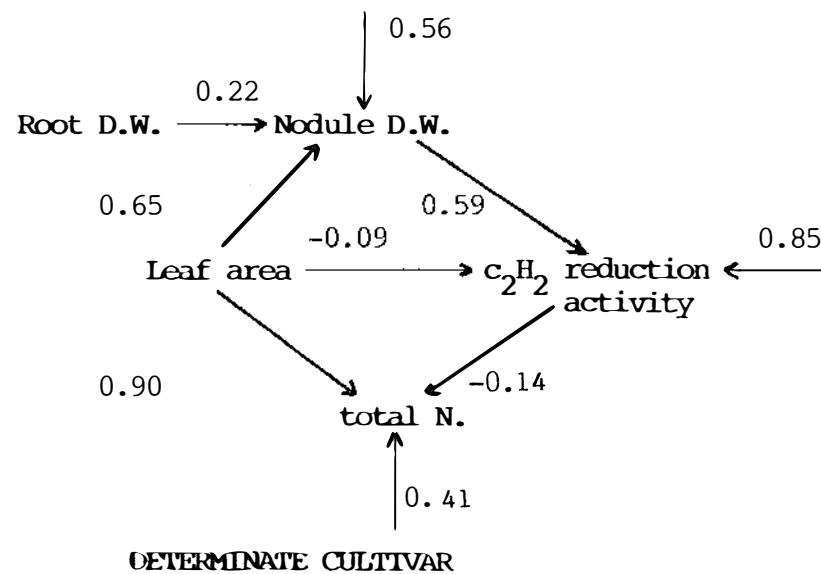


Figure 4.15 Path diagrams of the relationships of acetylene reduction activity and total plant nitrogen with some plant characters.

nitrogen fixation which could vary according to growth stage of the plant and the environmental conditions at the time of sampling, this may explain the low relationship link between the acetylene reduction activity and total plant nitrogen. Although the external source of variation in some path was relatively large (values associated with arrows coming from outside), the inclusion of the other plant parameters did not help to reduce these values very much. This was the indication that there were some factors ^{other} than these plant parameters alone that could ^{have} influenced their relationship with nitrogen fixation.

As discussed earlier in section 4.4.3 the 'integrated acetylene reduction activity' (summation of area under the curves of acetylene reduction activity) was closely related to total plant nitrogen (Figure 4.9b). Hence it may represent the overall nitrogen fixation better than the acetylene reduction activity which is an instantaneous measurement. The good linear relationship with total nitrogen as reported in the previous section supported this argument. The path diagram linking the 'integrated acetylene reduction activity' with the same plant characters as used in Figure 4.15 was then constructed to identify the relationship between leaf area, nodule dry weight, and the 'integrated acetylene reduction activity' (Figure 4.16). The results indicated that contrasting relationships existed among these parameters between the two growth types. In the determinate cultivar 'integrated acetylene reduction activity' was closely linked to leaf area ($p=0.71$) while in the case of the indeterminate cultivar it was mainly linked to nodule dry weight ($p=0.92$). The strong link between leaf area and the

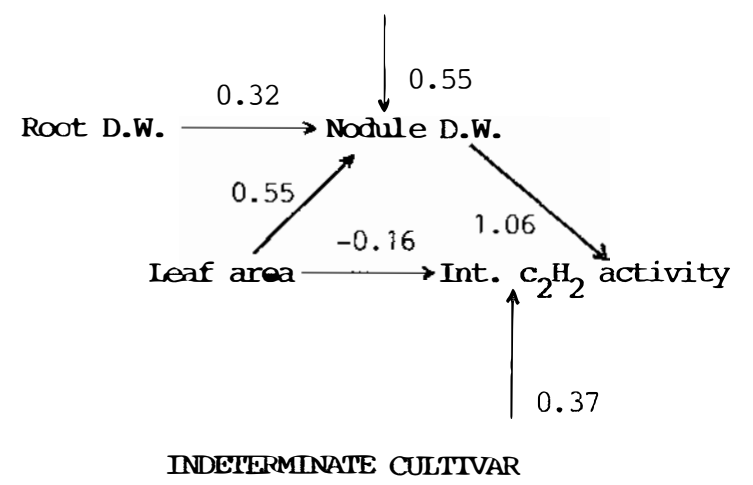
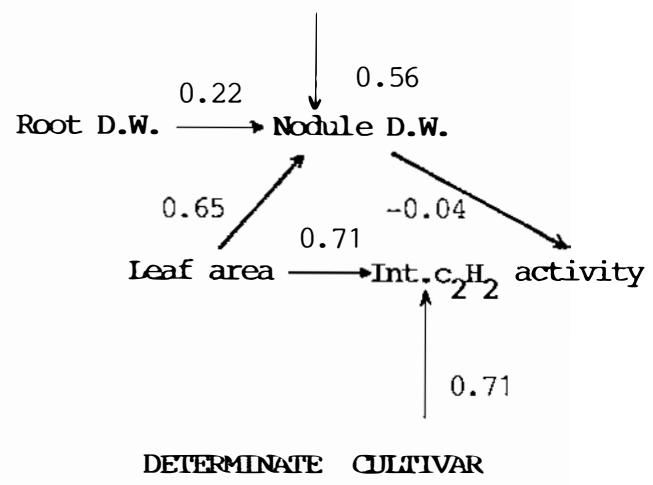


Figure 4.16 Path diagrams of the relationships of integrated acetylene reduction activity with some plant characters.

'integrated acetylene reduction activity' in the determinate cultivar is interesting, because it means that a plant with a larger leaf area will have greater photosynthetic capacity (Ham *et al.*, 1976; Hardy *et al.*, 1977) and should be capable of supporting greater nitrogen fixation activity. The evidence from the present experiment implies that leaf area may be the major limiting factor for the absolute amount of nitrogen fixation in the determinate cultivar. In contrast, the result indicated that nodule dry weight may be the major limiting factor in the indeterminate cultivar. In other words, photosynthetic capacity to support nitrogen fixation activity in the indeterminate cultivar probably was not as limiting as in the case for the determinate cultivar.

The path analysis results (Figures 4.15, 4.16) together with the results from the discriminant analysis in Table 4.8 indicate that leaf area was the most important plant character discriminating the differences between the two growth types. It was also likely that leaf area was the major limiting factor for nitrogen fixation activity in the determinate cultivar. Bethlenfalvay *et al.* (1978b) found that the absolute rates of acetylene reduction were significantly reduced in pea when leaf area was altered by partial defoliation.

The leaf area of the indeterminate cultivar in this experiment was about twice that of the determinate cultivar. Hence the interesting question would be 'what would happen to the nitrogen fixation of this indeterminate cultivar if its leaf area was reduced to approach that of the determinate cultivar ?'

To answer this question, the next experiment was carried out in which leaf area of the indeterminate cultivar was manipulated to see the extent to which defoliation could have an influence on nitrogen fixation, nitrogen distribution and seed yield relative to that of the determinate cultivar.

CHAPTER 5

The effects of leaf area manipulation of an indeterminate soybean cultivar compared with the leaf area of a determinate soybean cultivar.

ABSTRACT.

In previous experiments, the indeterminate soybean cultivar 'Amsoy' produced twice as much seed yield as the determinate cultivar 'Matara'. One particular difference between the plant characteristics of the two cultivars was leaf area, where the indeterminate cultivar had about twice the leaf area per plant as the determinate cultivar.

In this experiment, the leaf area of the indeterminate cultivar was manipulated by imposing different levels of partial leaf removal from the flowering stage onwards. The objective was to find out the extent to which leaf area is important in contributing towards the advantage of the indeterminate cultivar with regards to nitrogen fixation, nitrogen distribution and grain yield. In one treatment, partial pod removal was also applied to induce a reduced demand for assimilate.

Partial defoliation of the indeterminate cultivar had a marked effect on root growth and branch number and a lesser effect on nodule growth, acetylene reduction activity and nitrogen distribution. Even where leaf area was similar to that of the determinate cultivar from pod development stage, the indeterminate cultivar still had significantly higher seed yield and total plant nitrogen. The results

suggested that the advantage of the indeterminate cultivar 'Amsoy' over the determinate cultivar 'Matara' in yield was primarily from higher leaf area per plant which enhanced more number of branches, hence made possible a large number of nodes and subsequently number of pods. It also suggested that root and stem growth were more sensitive to defoliation than acetylene reduction activity and nitrogen distribution. Partial pod removal did not change the overall pattern of the responses.

5.1 INTRODUCTION.

Seed production in most grain legumes appears to depend largely on photosynthate produced after the onset of flowering (Pate and Minchin, 1980). The source-sink strategy differs substantially between the determinate and the indeterminate types of soybean. In the determinate type, vegetative growth ceases at about the time flowers first appear, hence time to flowering is critical to the size of source developed. In contrast, in the indeterminate type considerable overlap occurs in the flowering, pod fill and vegetative growth periods allowing for the development of large source and sink capacities (Shibles, 1980). From the previous two experiments, both nitrogen fixation (as expressed by acetylene reduction activity) and grain yield of the indeterminate cultivar were consistently higher than for the determinate cultivar. Furthermore, leaf area was identified as the plant character that had the greatest influence on these two parameters (Chapters 3 and 4); the larger leaf area of the indeterminate cultivar had undoubtedly contributed by being greater source.

However, not all increases in seed yield have been accredited with a larger leaf area. For example, Tanner and Ahmed (1974) found that when soybean cultivars were sprayed at flowering with TIBA (2,3,5 - tri-iodobenzoic acid), seed yield was increased even though leaf area indices were decreased. They concluded that the anti-auxin effect of TIBA apparently suppressed vegetative growth and brought about a more favourable balance between vegetative and reproductive growth without adversely affecting seed yield. It is now widely recognized that the role of vegetative parts (such as leaves and stem) as nitrogen reservoirs for developing seeds, at the time when symbiotic fixation activity is falling rapidly, is of great importance (Sinclair and de Wit, 1975; 1976; Eglesham *et al.*, 1977). Furthermore, reduction in nitrogen fixation activity has been attributed to the stronger partitioning of available photosynthate in favour of the host plant rather than to nodule tissues of the symbiotic system (Hardy and Hevelka, 1975; Bethlenfalvay and Phillips, 1977a,b; Bethlenfalvay *et al.*, 1978a, b).

In the present experiment the objective was to determine the extent to which leaf area is important in contributing towards the yield and nitrogen fixation advantages of the indeterminate cultivar. The manipulation of leaf area was achieved by imposing different levels of leaf defoliation on the indeterminate cultivar and partial pod removal was also imposed in one treatment.

5.2 MATERIALS AND METHODS.

The experiment was carried out in the controlled environment rooms at Plant Physiology Division, Department of Scientific and Industrial Research (DSIR), Palmerston North, New Zealand.

The same two soybean cultivars from the previous experiments were used, i.e.:

'Matara'	Determinate
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'Amsoy'	Indeterminate
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Plants were grown in 4.5 litre pots in a medium of sand and pumice (75:25 v/v) under the same environmental conditions as used in the previous experiment (Appendix D).

The experimental design was a randomized complete block design with 5 replications.

Plants were given complete Hoagland's A nutrient solution until ~~EMERGENCE~~. The same *Rhizobium* strain which was used in previous experiments was applied several times after germination to ensure good nodulation. After inoculation the plants were well watered at regular intervals with half strength nitrogen-free Hoagland's A nutrient solution.

The defoliation treatments were imposed on the indeterminate cultivar from the flowering stage. The five treatments were:

- UD - The undefoliated determinate cultivar.
- UI - The undefoliated indeterminate cultivar.
- I2 - Two leaflets of each trifoliolate leaf of the indeterminate cultivar were removed from the third node upwards.
- I1 - One leaflet of each trifoliolate leaf of the

indeterminate cultivar was removed from the third node upwards.

I2P - The same level of defoliation as in I2 plus 20% pod removal. Pods were removed at each node to maintain a similar pod number per node as that of the determinate cultivar at the same growth stage.

Five weekly destructive harvests were carried out from the flowering stage and the dry weights of leaves, stems, roots, nodules and pods were determined at each. Nitrogen fixation was determined weekly using the acetylene reduction technique (Appendix B) and total nitrogen of the different plant parts was determined by the Kjeldahl method (Appendix C).

Statistical analysis.

The growth data of each treatment was described by the functional approach, *i.e.* using the appropriate growth function to fit the data against time by the least squares technique (see Chapter 3).

Analysis of variance: A randomized complete block design was used and the analysis was done using the Genstat program (Alvey *et al.*, 1977).

Discriminant analysis: This multivariate analysis was used to analyse the relative effects of defoliation on each of the different plant characteristics. The purpose of this analysis was to distinguish between the four defoliation treatments of the indeterminate cultivar on the joint basis of several attributes which could ^{be} affected by defoliation. The main objective was to identify the attribute which contributes most to distinguish treatments, which in this case would be the attribute affected most by

the defoliation. The methods of this analysis were described in more details in Chapter 4.

5.3 RESULTS.

5.3.1 Plant growth and yield.

5.3.1.1 Plant growth: Growth curves of total plant dry weight and leaf area per plant for each treatment were fitted by quadratic equation (Figures 5.1a,b). The information on the regression equations is shown in Appendices 5.1 and 5.2.

The defoliation treatments successfully created three levels of leaf area for the indeterminate cultivar. The three levels were (i) UI - undefoliated indeterminate cultivar, (ii) I2 and I2P - with leaf area similar to that of the undefoliated determinate cultivar (UD) from pod development stage, in I2P some pods were also removed, and (iii) I1 - with leaf areas intermediate between UD and UI (Figure 5.1b and Table 5.1).

Relative to UI, defoliation significantly reduced total plant dry weight by 30% in I1 and 40% in I2 and I2P at both the pod development and grain filling stages (Table 5.2). However, even with a similar leaf area to that of UD from pod development stage, plants in I2 and I2P were still able to accumulate approximately 24% and 27% more total plant dry weight than did UD at the grain filling stages respectively.

When the net assimilation rates (NAR) of the different treatments were calculated from the fitted curves, the results showed that NAR of the defoliated plants in I2 and I2P (about 60% defoliation) increased drastically from $0.005 \text{ g cm}^{-2} \text{ week}^{-1}$ at flowering (week 7) to 0.009

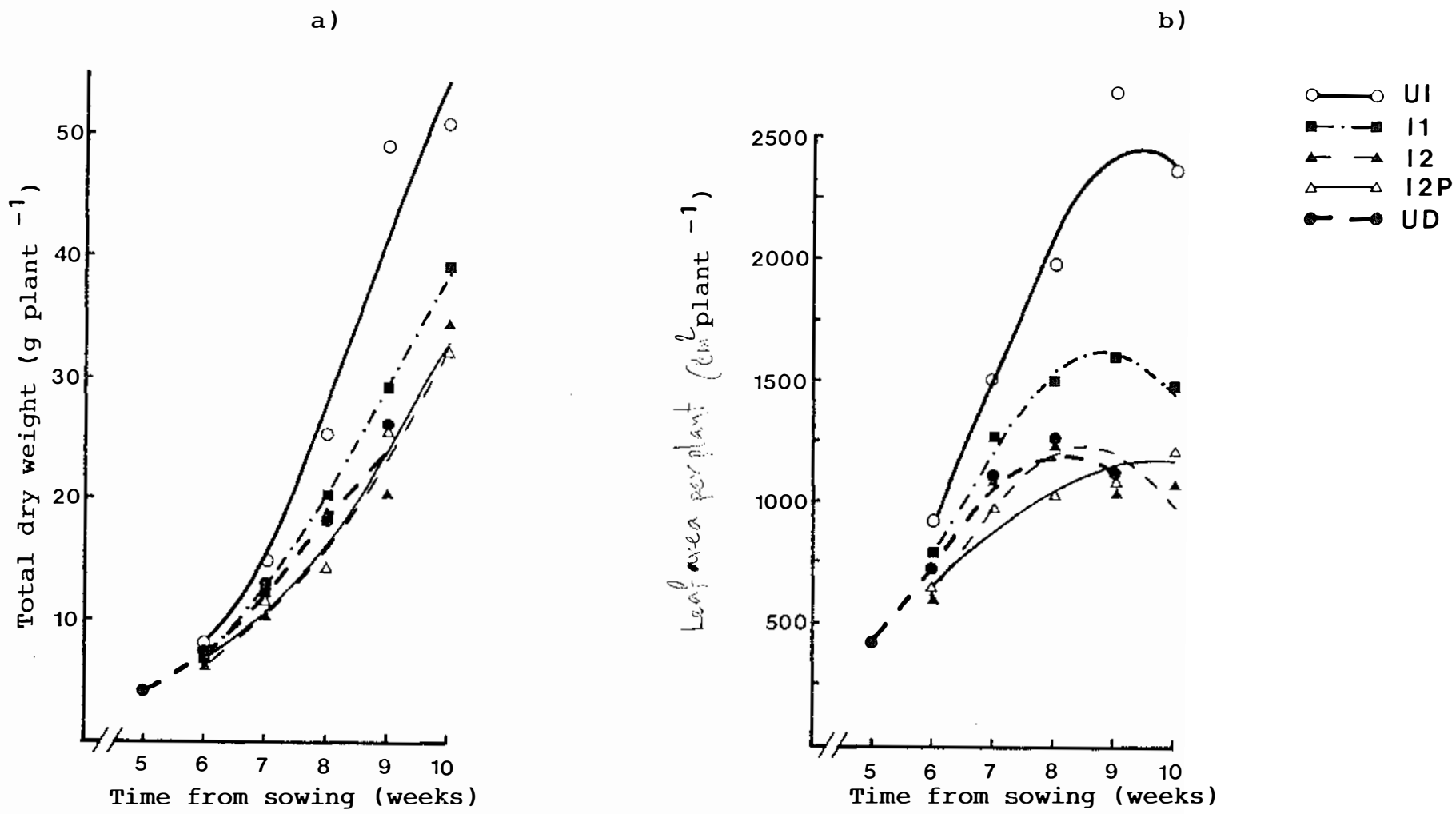


Figure 5.1 Time course of (a) total plant dry weight and (b) leaf area per plant of different treatments of soybean. (Plotted values are means of 5 replicates; S.E. of regression coefficients are in Appendix 5.1 and 5.2).

Table 5.1 Comparison of leaf area per plant (cm²) among different treatments of soybean at three growth stages.

Treatment	Growth Stage					
	Flowering		Pod Development		Grain Filling	
	(cm ²)	(%)	(cm ²)	(%)	(cm ²)	(%)
UD	418.3 ^d	(47)	1050.6 ^c	(50)	1119.9 ^c	(47)
UI	885.9 ^a	(100)	2084.1 ^a	(100)	2380.8 ^a	(100)
I2	609.4 ^c	(69)	1192.4 ^c	(57)	964.3 ^c	(41)
I1	781.4 ^{ab}	(88)	1532.3 ^b	(74)	1437.7 ^b	(60)
I2P	663.7 ^{bc}	(75)	1037.0 ^c	(50)	1152.6 ^c	(48)

Means in the same column sharing the same letter are not significantly different compared by t-test (P≤0.05)

Numbers in brackets are % of UI values.

Table 5.2 Comparison of total plant dry weight (g) among different treatments of soybean at three growth stages.

Treatment	Growth Stage					
	Flowering		Pod Development		Grain Filling	
	(g)	(%)	(g)	(%)	(g)	(%)
UD	4.1 ^b	(54)	12.1 ^d	(44)	25.8 ^c	(48)
UI	7.6 ^a	(100)	27.2 ^a	(100)	54.1 ^a	(100)
I2	6.2 ^a	(82)	16.2 ^c	(60)	32.1 ^b	(59)
I1	6.9 ^a	(91)	19.7 ^b	(72)	38.3 ^b	(71)
I2P	6.9 ^a	(91)	16.0 ^c	(59)	32.7 ^b	(60)

Means in the same column sharing the same letter are not significantly different compared by t-test ($P \leq 0.05$).

Numbers in brackets are % of UI values.

g cm⁻² week⁻¹ by ^{the} grain filling stage (week 10) (Table 5.3). In contrast the undefoliated UD and UI plants showed a decreasing trend from approximately 0.0065 g cm⁻² week⁻¹ to 0.0045 g cm⁻² week⁻¹ at the same period. In I1 (about 30% defoliation), NAR values remained within the range of 0.0055 g cm⁻² week⁻¹ to 0.0065 g cm⁻² week⁻¹ during the entire period. In general, all the indeterminate cultivar treatments including the undefoliated (UI) plants showed higher NAR than that of the undefoliated determinate cultivar (UD) at pod development stage. However, due to large standard error of estimate, the results were not significantly different at 5% level of probability.

Stem growth was also affected by defoliation. Quadratic growth curves of stem dry weight are presented in Figure 5.2 and Appendix 5.2. A comparison of the stem dry weight between different treatments showed that defoliation had significantly reduced stem dry weight in I2, I1 and I2P treatments by about 30-40% relative to that of the undefoliated (UI) treatment (Table 5.4). There was no difference amongst I2, I1 and I2P even though I1 had about 15-20% more leaf area than that of I2 or I2P from the pod development stage onwards. However, I2 and I2P still produced significantly higher stem dry weight than that of the undefoliated determinate cultivar (UD) at grain filling stage even though their leaf areas were similar from pod development stage onwards. Stem dry weights in the determinate cultivar were only 52%, 39% and 36% of UI in the flowering, pod development and grain filling stages respectively.

One significant effect of defoliation on stem growth was the affect on branch number. At plant maturity, the number of branches in

Table 5.3 Comparison of net assimilation rate (NAR) calculated from Figure 5.1 (a,b) of different treatments of soybean at three growth stages.

Treatment	Growth stage		
	Flowering	Pod Development	Grain Filling
	NAR ($\text{g cm}^{-2} \text{ week}^{-1}$)		
UD	0.0061 ^{ab}	0.0053 ^a	0.0047 ^a
UI	0.0067 ^a	0.0064 ^a	0.0045 ^a
I2	0.0056 ^{ab}	0.0056 ^a	0.0092 ^a
I1	0.0055 ^{ab}	0.0055 ^a	0.0063 ^a
I2P	0.0047 ^b	0.0060 ^a	0.0092 ^a

Means in the same column sharing the same letter are not significantly different compared by t-test (P = 0.05).

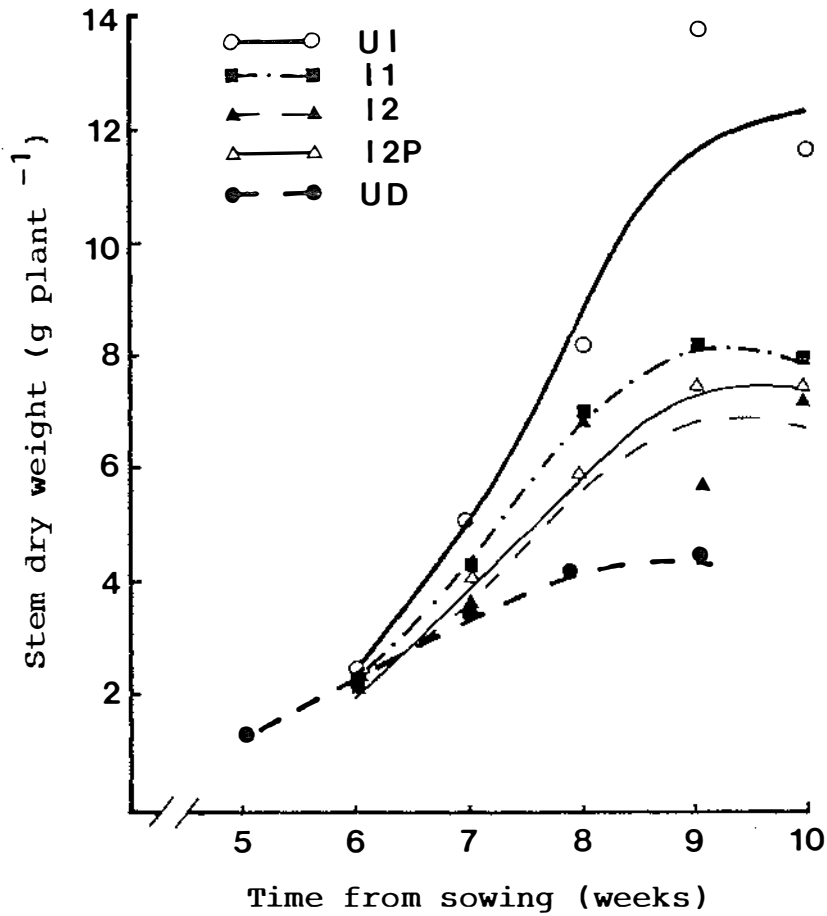


Figure 5.2 Time course of stem dry weight of different treatments of soybean. (Plotted values are mean of 5 replicates; S.E. of regression coefficients are in Appendix 5.3).

Table 5.4 Comparison of total plant stem dry weight (g) among different treatments of soybean at three growth stages.

Treatment	Growth Stage					
	Flowering		Pod Development		Grain Filling	
	(g)	(%)	(g)	(%)	(g)	(%)
UD	1.2 ^b	(52)	3.4 ^c	(39)	4.4 ^c	(36)
UI	2.3 ^a	(100)	8.8 ^a	(100)	12.3 ^a	(100)
I2	1.9 ^a	(83)	5.7 ^b	(65)	6.8 ^b	(55)
I1	2.1 ^a	(91)	6.8 ^b	(77)	7.8 ^b	(63)
I2P	1.9 ^a	(83)	5.9 ^b	(67)	7.5 ^b	(61)

Means in the same column sharing the same letter are not significantly different compared by t-test ($P \leq 0.05$).

Numbers in brackets are % of UI values.

I2 had been significantly reduced to the same level as in UD (Table 5.5). However, there was no effect on the number of branches in I2P which had similar level of defoliation but in addition had 20% of the pods removed. Although the number of branches in I1 had been reduced by about 23%, the difference (relative to UI) was not statistically significant.

Root dry weights of UD and I2 could not be satisfactorily fitted with any one of the functions used (the best R^2 are presented in Appendix 5.4). Accordingly, the mean values of root dry weight at each harvest were plotted and a comparison of the data at each sampling was done using analysis of variance (Figure 5.3). Severe defoliation (I2) reduced root dry weight within a week after it was imposed. At the fourth harvest (about 1 week after the pod development stage) the root dry weight of plants in treatment I2P was significantly higher than that of plant in I2 indicating significant competition over this period between pod and root growth. At the final harvest all of the plant in the defoliated treatments had similar root dry weights, but these were reduced to about two-thirds of the values for the undefoliated indeterminate cultivar (UI). However, the values in the defoliated treatments were still significantly higher than that of the undefoliated determinate cultivar (UD).

The shoot:root ratio of all the treatments increased linearly with time (Figure 5.4, Appendix 5.5). A test of the differences among the regression coefficients showed that there were no differences in the rates of change (slopes) of shoot:root ratios amongst all the treatments. In general, the undefoliated determinate cultivar had a

Table 5.5 Comparison of branch number among different treatments of soybean.

	Treatment				
	UD	UI	I2	I1	I2P
Branch Number	3.0 ^b	5.2 ^a	3.6 ^b	4.0 ^{ab}	5.0 ^a
% of UI	58	100	69	77	96

Means sharing the same letter are not significantly different compared by LSD (P = 0.05).

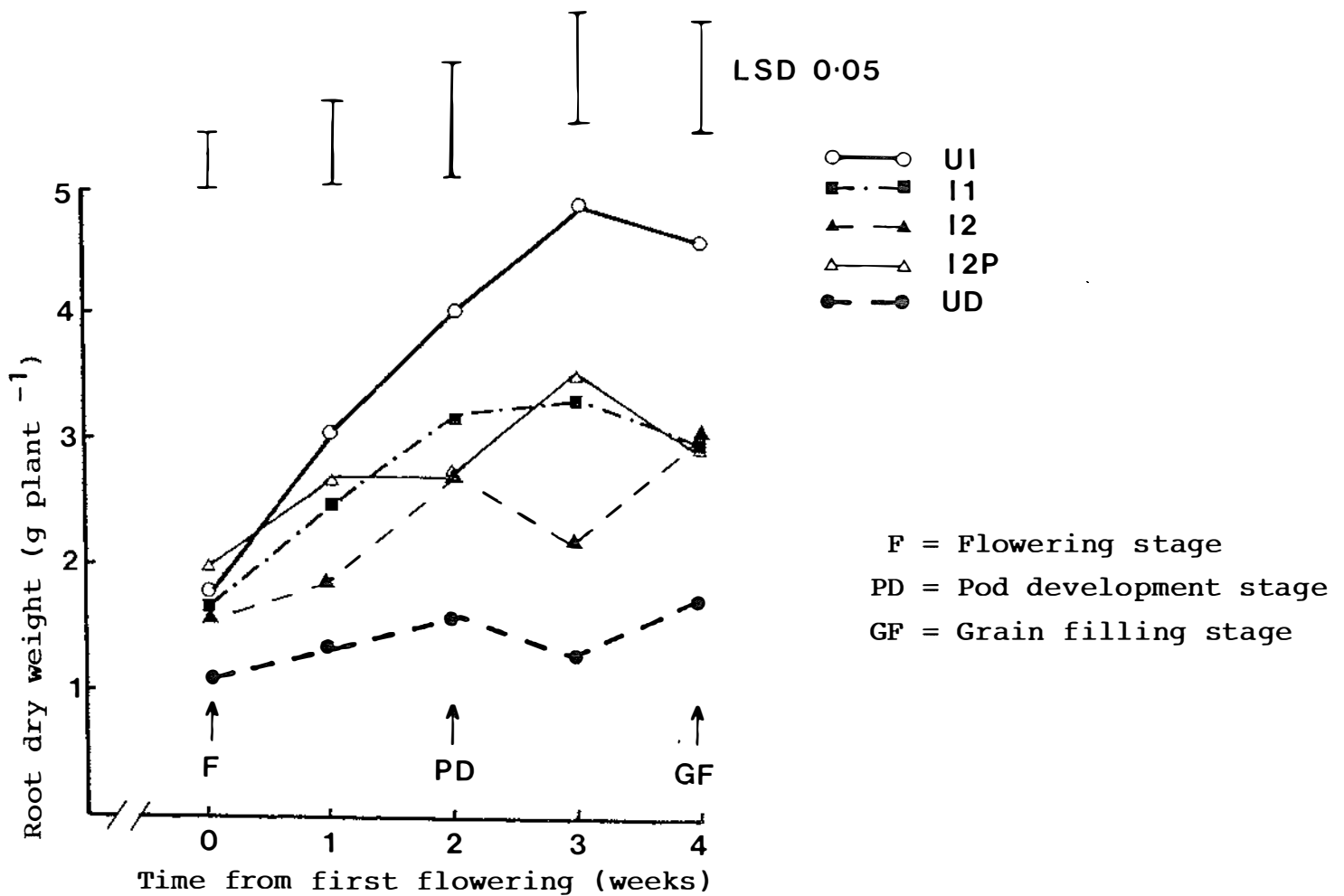


Figure 5.3 Root dry weight of different treatments of soybean from flowering stage to grain filling stage. (Sowing was 5 weeks before flowering in UD and 6 weeks in the other treatments).

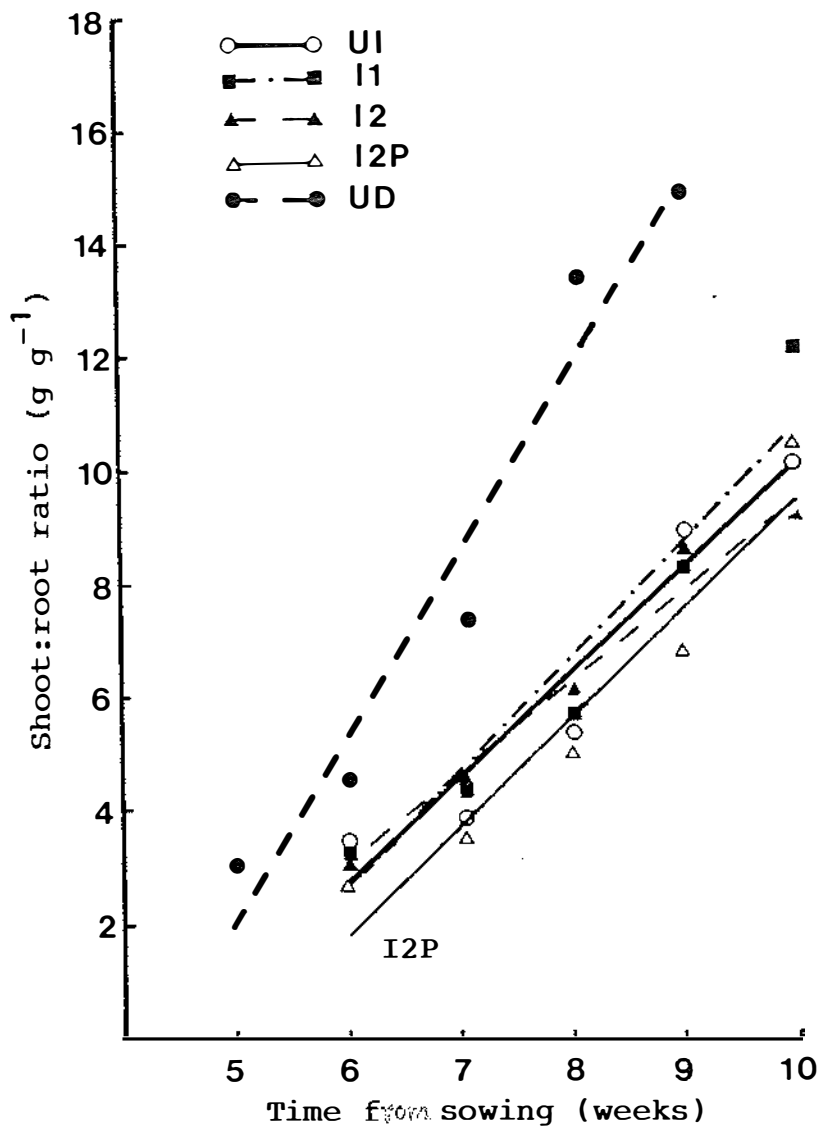


Figure 5.4 Regression lines of shoot:root ratio of different treatments of soybeans. (Plotted values are means of 5 replicates; S.E. of regression coefficients are in Appendix 5.5)

higher shoot:root ratio than that of the four treatments of the indeterminate cultivar after flowering (Table 5.6). There was no difference amongst the four indeterminate cultivar treatments at the grain filling stage.

The effect of defoliation on nodule growth was considerably less than that on root growth. At the beginning of the defoliation treatment (flowering stage), The determinate cultivar had produced significantly less nodule weight than that of the indeterminate cultivar (Figure 5.5, Appendix 5.6, Table 5.7). Although plants from I2 and I2P had lower nodule dry weights than UI and I1 at the pod development stage, the difference was not statistically significant except for that between I1 and I2P. However, by the grain filling stage, all of the defoliation treatments had lower nodule dry weights than the undefoliated UI plants although statistically only I2 was significantly lower than UI. In all growth stages the indeterminate cultivar irrespective of treatment, had a significantly higher nodule dry weight than the determinate cultivar.

When nodule dry weight was expressed on a per unit root dry weight basis, there were no significant difference amongst the treatments at the flowering and grain filling stages (Figure 5.6). However, at the pod development stage, the undefoliated determinate cultivar (UD) had significantly higher, and the undefoliated indeterminate cultivar (UI) had significantly lower, nodule:root ratios than the other treatments. Among the three defoliated treatments, the differences were not significant.

Table 5.6 Comparison of shoot:root ratio ($\text{g g}^{-1} \text{ plant}^{-1}$) among different treatments of soybean at three different growth stages.

Treatment	Growth Stage		
	Flowering	Pod Development	Grain Filling
UD	3.02 ^{ab}	7.17 ^a	15.03 ^a
UI	3.63 ^a	5.37 ^{bc}	10.24 ^b
I2	2.99 ^b	6.02 ^{ab}	9.17 ^b
I1	3.51 ^a	5.54 ^{bc}	12.15 ^{ab}
I2P	2.61 ^b	4.75 ^c	10.67 ^b
F-test	*	*	*
cv (%)	16.9	15.2	22.5

Means in the same column sharing the same letter are not significantly different compared by LSD ($P \leq 0.05$).

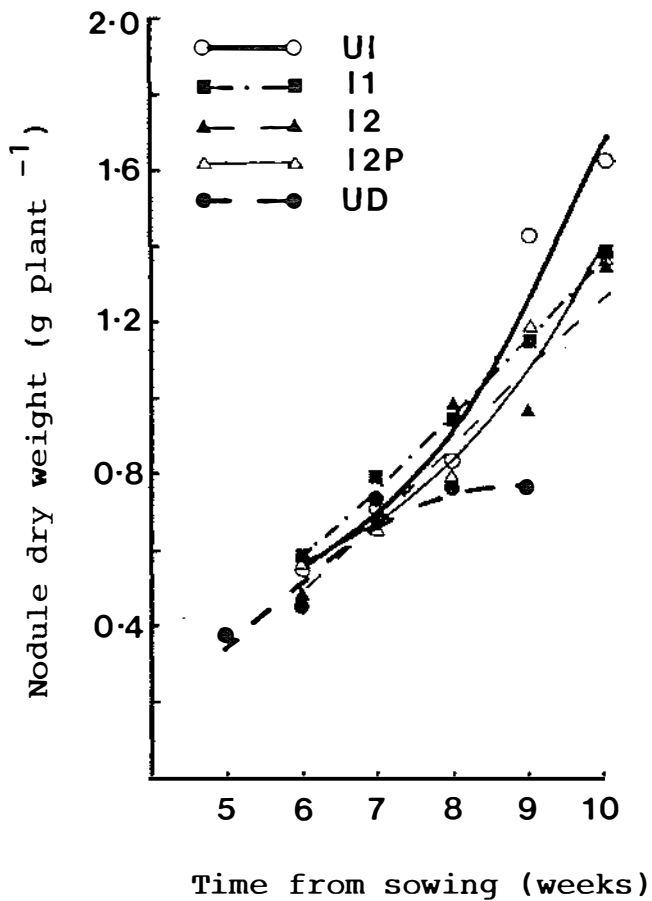


Figure 5.5 Time course of nodule dry weight of different treatments of soybean. (Plotted values are means of 5 replicates; S.E. of regression coefficients are in Appendix 5.6)

Table 5.7 Comparison of nodule dry weight (g) among different treatments of soybean at three different stages.

Treatment	Growth Stage					
	Flowering		Pod Development		Grain Filling	
	(g)	(%)	(g)	(%)	(g)	(%)
UD	0.34 ^b	(64)	0.66 ^c	(73)	0.76 ^c	(45)
DI	0.53 ^a	(100)	0.91 ^{ab}	(100)	1.69 ^a	(100)
I2	0.49 ^a	(92)	0.88 ^{ab}	(97)	1.28 ^b	(76)
I1	0.59 ^a	(111)	0.95 ^a	(104)	1.36 ^{ab}	(80)
I2P	0.56 ^a	(106)	0.83 ^b	(91)	1.46 ^{ab}	(84)

Means in the same column sharing the same letter are not significantly different compared by t-test ($P \leq 0.05$).

Numbers in brackets are % of UI values.

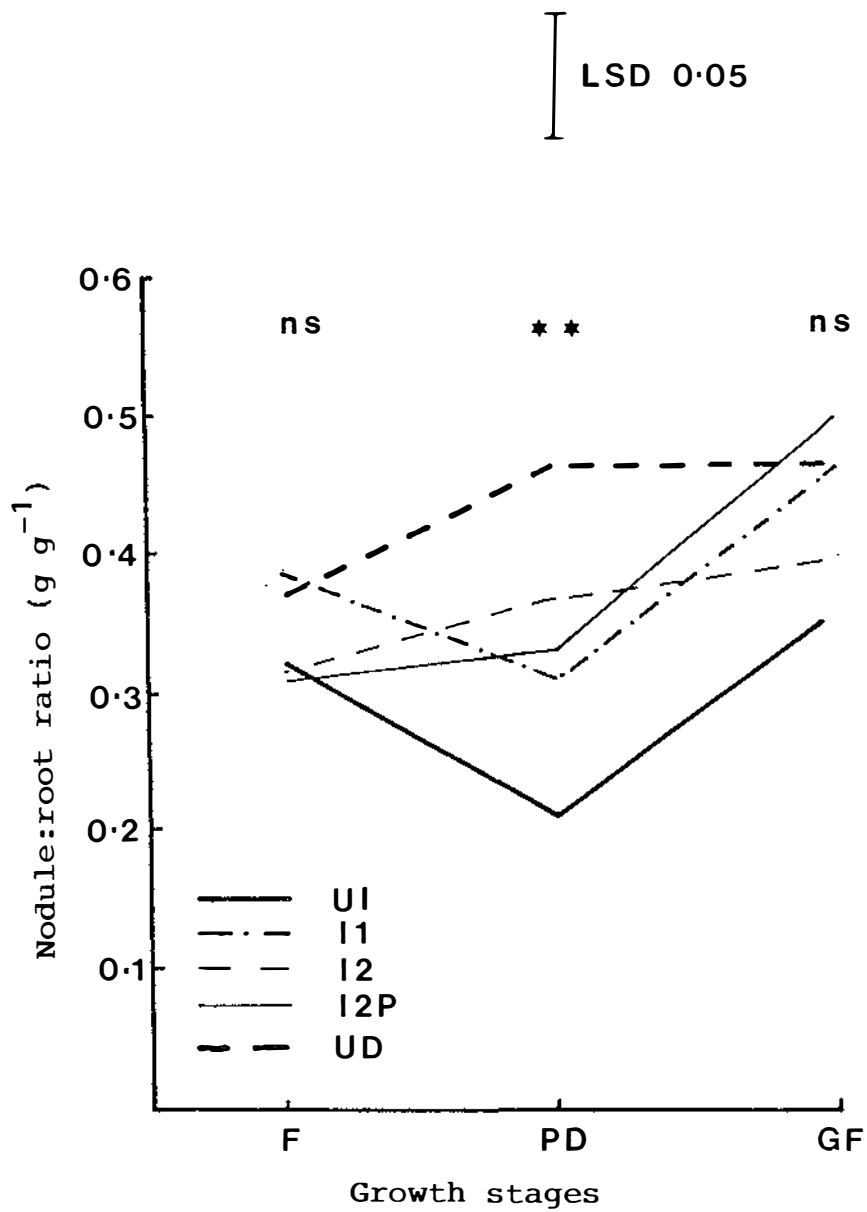


Figure 5.6 Comparison of nodule:root ratio among different treatments of soybean at three growth stages. (Growth stages as for Figure 5.3).

5.3.1.2 Yield and yield components: When about 60% of the leaves of the indeterminate cultivar were removed (i.e. I2 and I2P), seed yield was reduced by about 17% (from 27 g to 23 g) (Table 5.8). In contrast, there was no significant reduction in final seed yield in the 30% defoliation treatment (I1).

When seed yield, yield components (number of pods, number of seeds per pod and average seed weight) and number of nodes with pods from either the branches or the main stem were analysed the results indicated that the seed yield differences came largely from differences in branch node number (Table 5.9). This difference was mainly due to the difference in pod number and in the number of nodes with pods. In contrast, amongst the yield components from the main stem, only seed size was significantly affected by defoliation.

When seed yield and pod number were analysed on a per branch basis, there was no difference amongst treatments within the indeterminate cultivar. Although the determinate cultivar (UD) had lower values of both seed yield and number of pods, these values were not statistically different from the others (Table 5.10). Hence, the yield difference in this experiment was primarily the result of a difference in the number of branches per plant.

A comparison of harvest indices (ratio of seed yield to total above-ground biomass) indicated that I2 plants had significantly higher harvest index than UI (Table 5.11). The undefoliated determinate cultivar (UD) had the highest harvest index (0.65) and it was also significantly higher than those of the four treatments of the indeterminate cultivar.

Table 5.8 Comparison of total yield and yield components among different treatments of soybean.

Treatment	Seed Yield	No. of Pods	Seeds per Pod	Seed Weight
	g plant ⁻¹			g(20 seeds)
UI	15.62 ^c	30.8 ^b	2.41	4.00
UI	27.67 ^a	53.6 ^a	2.64	4.28
I2	23.07 ^b	49.4 ^a	2.43	3.84
I1	25.46 ^{ab}	55.2 ^a	2.40	3.86
I2P	22.94 ^b	47.4 ^a	2.46	3.98
f- test	**	**	ns	ns
cv (%)	14.4	17.2	10.2	6.1

Means in the same column sharing the same letter are not significantly different compared by LSD ($P \leq 0.05$).

Table 5.9 Yield and yield components of different treatments of soybean analysed separately for branches and main stem.

Treatment	Seed Yield		No. of Pods		No of Nodes with Pods		Seed No per Pod		Seed Weight (20 seeds)	
	Branches	Main Stem	Branches	Main Stem	Branches	Main Stem	Branches	Main Stem	Branches	Main Stem
UD	6.31 ^c	9.31	11.2 ^c	19.6	7.8 ^d	9.2	2.4	2.4	4.14	3.92 ^b
UI	17.92 ^a	9.75	34.8 ^a	18.8	20.8 ^b	9.6	2.4	2.4	4.27	4.23 ^a
I2	13.76 ^b	9.31	28.4 ^b	21.0	16.0 ^c	10.8	2.9	2.5	3.88	3.78 ^b
I1	17.89 ^a	7.56	39.0 ^a	16.2	25.4 ^a	8.2	2.4	2.5	3.89	3.86 ^b
I2P	12.58 ^b	10.36	26.4 ^b	21.0	14.6 ^c	9.6	2.5	2.6	3.96	4.05 ^b
F-test	**	ns	**	ns	**	ns	ns	ns	ns	*
cv (%)	28.9	21.6	31.8	20.7	31.9	15.5	10.3	10.6	9.1	5.5

Means in the same column sharing the same letter are not significantly different compared by LSD ($P \leq 0.05$).

Table 5.10 Comparison of seed yield and number of pods per branch for different treatments of soybean.

Treatment	Seed Yield (g branch ⁻¹)	Number of Pods per Branch
UD	2.59	5.36
UI	3.41	6.64
I2	3.89	7.80
I1	3.68	8.02
I2P	3.10	6.52
F-test	ns	ns
cv (%)	32.26	36.18

Table 5.11

Comparison of Harvest index (grain yield:total above-ground biomass) and nitrogen harvest index (grain nitrogen:total above-ground nitrogen) among different treatments of soybean.

Treatment	Harvest Index	Nitrogen harvest index
UD	0.65 ^a	0.94
UI	0.56 ^c	0.93
I2	0.61 ^b	0.94
I1	0.59 ^c	0.93
I2P	0.59 ^c	0.94
F-test	**	ns
cv (%)	4.11	3.08

Means in the same column sharing the same letter are not significantly different compared by LSD ($P \leq 0.05$).

5.3.2 Nitrogen fixation.

Acetylene reduction activities were significantly different between the different treatments in the four weeks following the defoliation treatments (Table 5.12). During the first week after flowering the highest activity ^{was} detected in I1 plants (i.e. where there was only minor defoliation). However, three weeks beyond flowering the activities in UI and I1 were higher than those in I2 and I2P (Table 5.12). The acetylene reduction activity per unit nodule dry weight (specific acetylene reduction activity) showed no difference among the four indeterminate cultivar treatments (Table 5.13) indicating that the difference in total acetylene reduction activity among the indeterminate cultivar treatments (UI to I2P) came mainly from different amounts of nodule tissue in each treatment. The undefoliated determinate cultivar (UD) showed higher specific acetylene reduction activity than those of the indeterminate cultivar in UI to I2P at first flowering and two weeks from first flowering.

The 'nitrogen fixing efficiency', as used in the previous experiments (regression of total plant nitrogen with nodule dry weight) showed that the slope (B_1) of UD was significantly higher than those of the other four treatments (Figure 5.7, Appendix 5.7). However, there was no difference amongst the four treatments of the indeterminate cultivar.

5.3.3 Nitrogen distribution.

Nitrogen concentration in the plant components was quite different among treatments (Figure 5.8a-f). At the flowering stage

Table 5.12

Comparison of Acetylene reduction activity ($\mu\text{mol h}^{-1} \text{ plant}^{-1}$) among different treatments of soybean.

Treatment	Time from first flowering (weeks)				
	0	1	2	3	4
UD	31.35	26.24 ^b	48.60 ^a	29.93 ^d	29.89
UI	24.75	36.76 ^{ab}	33.23 ^c	73.14 ^a	58.37
I2	16.92	35.67 ^{ab}	43.07 ^{ab}	46.71 ^{cd}	59.88
I1	21.74	51.78 ^a	42.11 ^{abc}	67.21 ^{ab}	61.37
I2P	23.47	27.86 ^b	38.37 ^{ab}	50.38 ^{bc}	54.85
F-test	ns	*	*	**	ns
cv (%)	31.1	35	17.5	25.22	33.2

Means in the same column sharing the same letter are not significantly different compared by LSD ($P \leq 0.05$).

Table 5.13

Comparison of Specific acetylene reduction activity ($\mu\text{mol h}^{-1} \text{g}^{-1}$ nodule dry weight) among different treatments of soybean.

Treatment	Time from first flowering (weeks)				
	0	1	2	3	4
UD	84.38 ^a	59.82	69.34 ^a	39.18	42.22
UI	46.47 ^b	52.05	40.42 ^b	51.69	35.21
I2	35.37 ^b	49.65	44.63 ^b	48.43	43.75
I1	36.48 ^b	65.43	46.00 ^b	58.01	45.32
I2P	40.76 ^b	41.08	48.65 ^b	42.85	39.91
F-test	**	ns	**	ns	ns
cv (%)	27.9	28.7	25.2	20.8	33.2

Means in the same columns sharing same letter are not significantly different compared by LSD ($P \leq 0.05$).

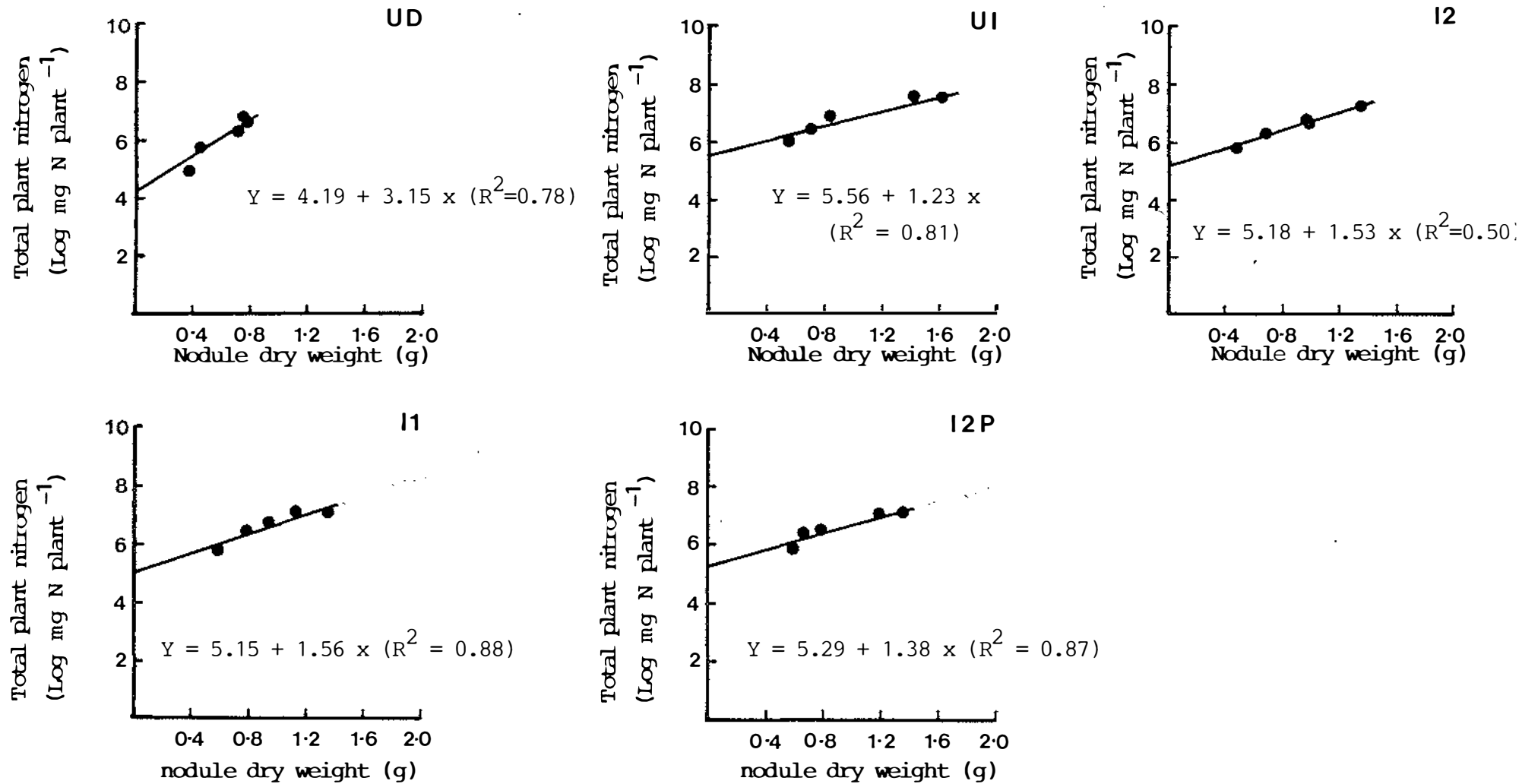


Figure 5.7 Regression lines of total plant nitrogen (log scale) and nodule dry weight of different treatments of soybean (plotted values are mean of 5 replicates). (S.E. of regression coefficients are in Appendix 5.7).

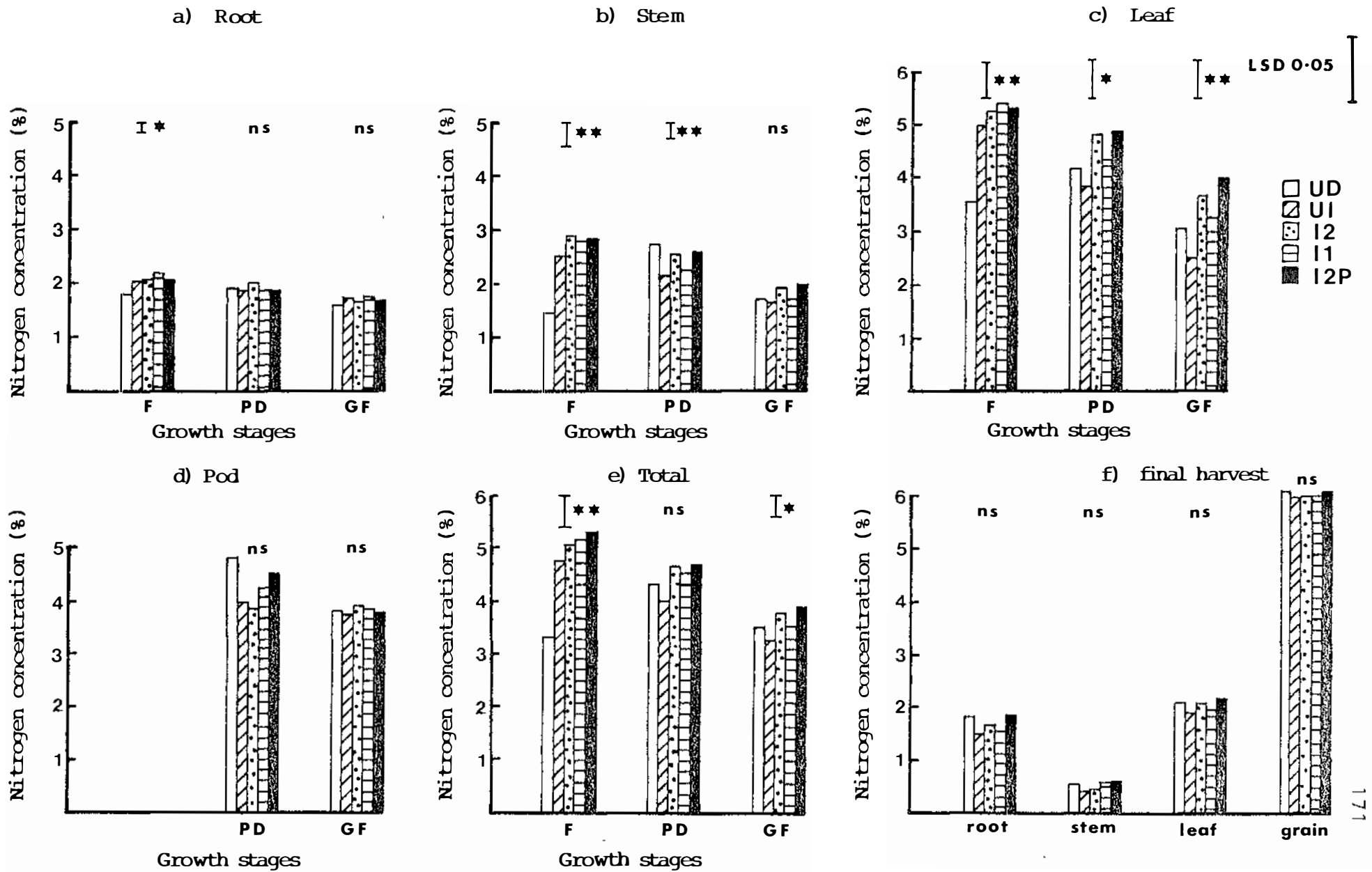


Figure 5.8 Nitrogen concentration of plant components for different treatments of soybeans. (Growth stages as for Figure 5.3).

the difference came mainly from the significantly lower nitrogen concentration in all components of the undefoliated determinate cultivar compared with the other four treatments of the indeterminate cultivar. At the pod development stage, the nitrogen concentration values in the stem of UI and I1 were significantly lower than those of the other treatments (Figure 5.8b). There were no differences among treatments for root and stem nitrogen concentration at the grain filling stage (Figure 5.8 a,b) but the leaf component in the 60% defoliation treatments and 30% defoliation (I2 and I2P, and I1, respectively) had significantly higher nitrogen concentrations than that of the undefoliated indeterminate cultivar (UI) (Figure 5.8c). Pod nitrogen concentration showed no difference among treatments at either the pod development or grain filling stages (Figure 5.8d). Total plant nitrogen concentration in UI was significantly lower than those of I2 and I2P at the grain filling stage (Figure 5.8e). Final nitrogen concentration values of root, stem, leaf and grain were very similar amongst all treatments (Figure 5.8f).

The pattern of nitrogen accumulation in each treatment was described by quadratic curves and presented in Figure 5.9 and Appendix 5.8. Defoliation treatments markedly reduced the nitrogen accumulated per plant over time (Table 5.14). At the final sampling, I2 and I2P (60% defoliation) had about 70% of the total nitrogen relative to the undefoliated indeterminate cultivar (UI), but these amounts were still much higher than that of the undefoliated determinate cultivar (UD), which had only 51% of the UI amount. However, by grain filling there was no significant difference between I2 and I1.

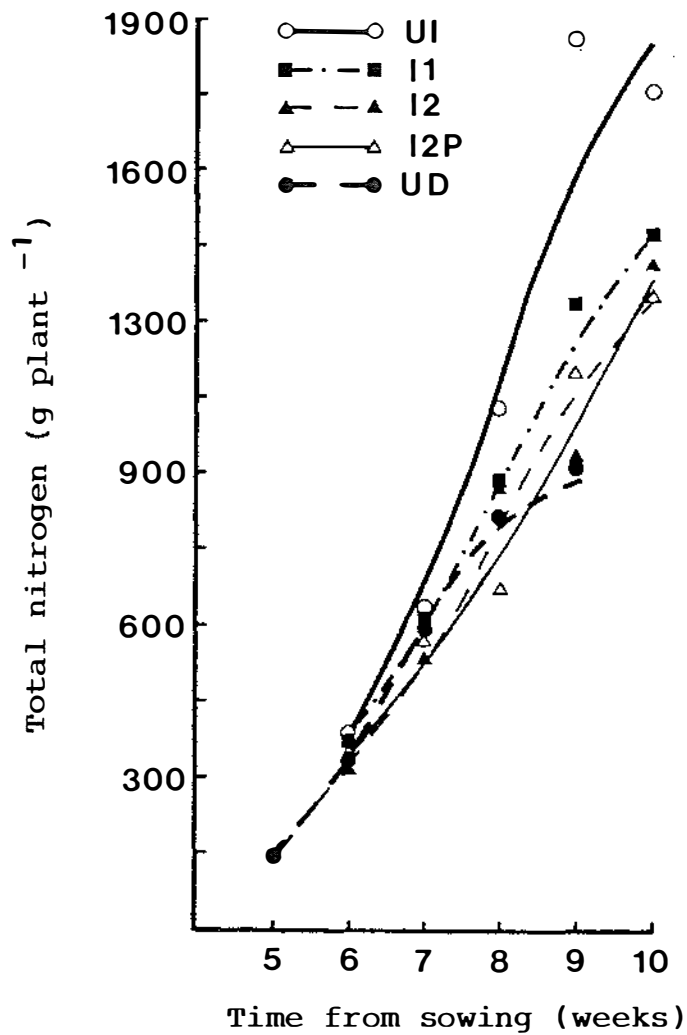


Figure 5.9 Time course of total plant nitrogen of different treatments of soybean (plotted values are means of 5 replicates. S.E. of regression coefficients are in Appendix 5.8).

Table 5.14 Comparison of total plant nitrogen (mg) among different treatments of soybean at three growth stages.

Treatment	Growth stage					
	Flowering		Pod Development		Grain Filling	
	(mg)	(%)	(mg)	(%)	(mg)	(%)
UD	143.4 ^b	(40)	584.6 ^d	(54)	884.5 ^c	(51)
UI	361.9 ^a	(100)	1082.4 ^a	(100)	1751.1 ^a	(100)
I2	315.7 ^a	(87)	782.0 ^{bc}	(72)	1242.1 ^b	(71)
I1	360.4 ^a	(100)	881.6 ^b	(81)	1382.4 ^b	(79)
I2P	346.5 ^a	(86)	744.9 ^c	(69)	1277.9 ^b	(73)

Means in the same column sharing the same letter are not significantly different compared by t-test ($p \leq 0.05$).

Numbers in brackets are % of UI values.

The leaf + stem nitrogen was also described by quadratic curves (Figure 5.10 and Appendix 5.9) which showed that the accumulation of nitrogen in the defoliation treatments started to decline by week 8, this was about one week before the decline shown for UI . In I2P, where 20% of the pods were removed, the leaf + stem nitrogen showed less of a decline than those of the other defoliation treatments (I2 and I1) i.e. at the final sampling of I2P plants had about 30 mg N less than that of the previous week, whereas in I2 and I1 plants the reductions were about 120 mg N.

Pod nitrogen for all treatments during the entire sampling period increased linearly with time (Figure 5.11, Appendix 5.10) but there was no significant difference between the different rates of accumulation under each treatment. The final seed nitrogen concentration was not affected by defoliation treatments either (as explained in 5.3.1) nor was the partitioning of nitrogen to seed. Consequently all treatments had very similar nitrogen harvest indices (Table 5.11).

5.3.4 The relative sensitivity of plant characters to defoliation:

The relative sensitivity of different plant characters to defoliation could provide information which may help to identify the relationship between leaf area and the other plant characters. The discriminant analysis technique was chosen to analyse six variables jointly (*viz.* root, stem, pod and nodule dry weight, acetylene reduction activity, and total plant nitrogen) to identify the original attributes which contributed most to distinguish between treatments

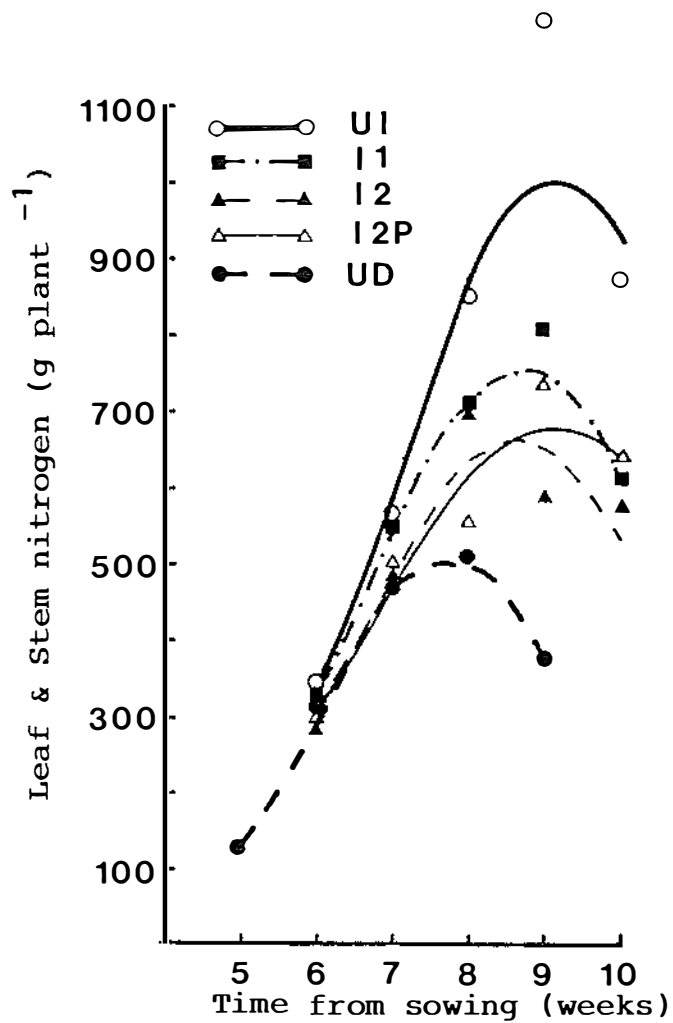


Figure 5.10 Time course of leaf and stem nitrogen of different treatments of soybean (plotted values are means of 5 replicates; S.E. of regression coefficients are in Appendix 5.9).

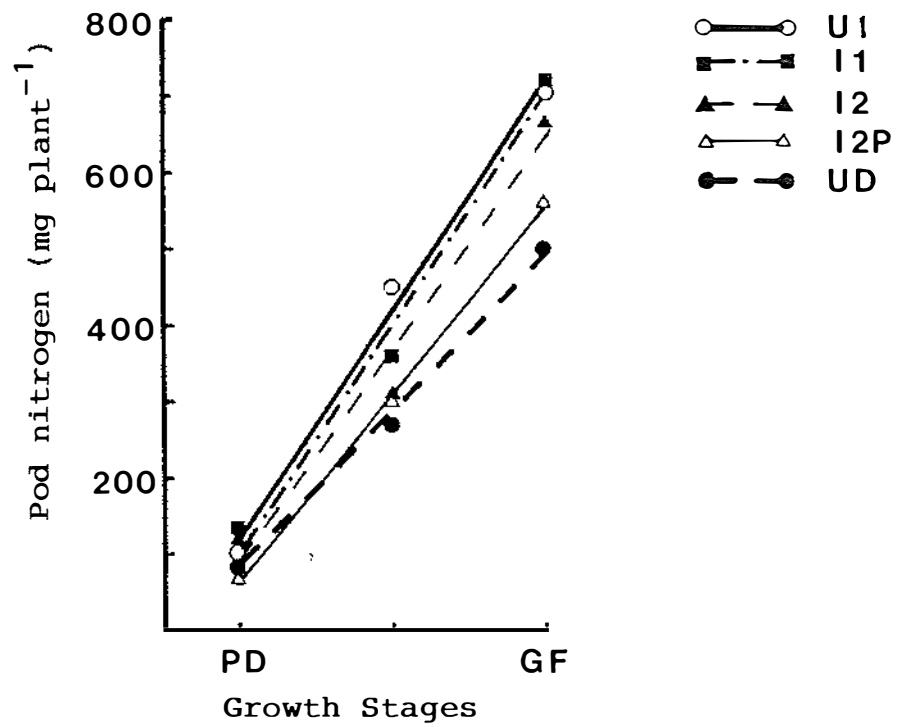


Figure 5.11 Time course of pod nitrogen from different treatments of soybean. (Plotted values are means of 5 replicates; growth stages as for Figure 5.2 and S.E of regression coefficients are in Appendix 5.10).

using the discriminant functions which take into account a large proportion of total dispersion of the original data. With these six variables as original attributes, the results showed that the first two discriminant functions were significant ($P < 0.01$) and they took into account 75% and 20% of the variance of the original attributes respectively (Table 5.15a). From the structure matrix, a ranking of the contribution of the original attributes to the particular discriminant function could be made. In the first function, the ranking of the original attributes was root dry weight, stem dry weight, nodule dry weight, total plant nitrogen, acetylene reduction activity and pod dry weight (Table 5.15b). In the second function, the ranking was slightly different from the first. Since the first function had taken into account 75% of the variance of the original attributes, it was probably reasonable to claim that the relative sensitivity of plant characters to defoliation in this experiment was as follows: root dry weight, stem dry weight, nodule dry weight, total plant nitrogen, acetylene reduction activity and pod dry weight. This indicates that the defoliation treatments which took place from flowering, had affected subsequent dry-matter accumulation to a greater extent than either acetylene reduction activity or nitrogen accumulation.

5.4 DISCUSSION

The yield process in grain crops involves the production of photosynthate by leaves and its utilization by developing seed to synthesize storage material (Heitholt and Egli, 1985). From the previous experiments (in Chapters 3 and 4), the greater total dry

Table 5.15 Information from multiple discriminant analysis (a) and structure matrix (b) for the four treatments of the indeterminate soybean cultivar.

a)							
Function	Eigenvalue	Percent of variance	Canonical correlation	After function	Chi-square	df	Significance
1	0.998	75.67	0.71	0	116.29	24	****
2	0.268	20.35	0.46	1	34.27	15	***

b) structure matrix

Variable	Function	Function
Root dry weight	0.79	0.30
Stem dry weight	0.54	0.34
C ₂ H ₂ activity	0.21	-0.01
Total plant nitrogen	0.39	0.23
Nodule dry weight	0.42	-0.09
Pod dry weight	0.10	0.08

matter production and higher yield of the indeterminate cultivar was mainly a reflection of the greater plant size and higher leaf area. One of the main objectives of this experiment was to compare therefore, plant growth and yield of the indeterminate cultivar when it was subjected to a range of defoliation treatments.

5.4.1 Plant growth: Partial defoliation can increase the rate of photosynthesis of remaining leaves (Treharne, 1972). Some workers have also noted higher net assimilation rate (NAR) in defoliated plants (e.g. Pandey and Singh, 1981) which is in agreement with the present results. The results confirmed that more severe defoliation (I2 and I2P) led to higher NAR values (Table 5.3).

In peanut, Williams *et al.* (1976) found that defoliation decreased the absolute growth rate of the stems. Similarly Enyi (1975) found defoliation reduced the dry weight of stems in cowpea, soybean, green gram and peanut; defoliation had the greatest effect on the number of branches of soybean when the plants were defoliated at the vegetative stage. In the present experiment, stem dry weights of the defoliated indeterminate plants were significantly lower than that of the undefoliated indeterminate cultivar (UI) (Figure 5.2 and Table 5.4). However, the stem dry weights of the indeterminate cultivars even after defoliation were still much higher than that of the determinate cultivar at all stages.

Defoliation also adversely affected the number of branches (Table 5.5). The result suggested that competition for photosynthate between vegetative and reproductive growth was probably the main cause of the branch number reduction. For example, the final branch number per plant in I2 and I2P was significantly different (Table 5.5) even

though both received the same degree of defoliation but plants in I2P had 20% pod removal. With less competition for photosynthate from pods, I2P was still able to maintain a higher rate of vegetative growth (branching). Similarly, Heitholt and Egli (1985) reported no difference in the number of branches per plant between the control and deflowered soybean plants.

The competition for photosynthate also affected root growth to some extent i.e. root dry weight in I2 was significantly lower than that of UI from the first week after the commencement of defoliation; and root dry weight of I2P was significantly higher than that of I2 soon after pods were removed at the fourth sampling period (Figure 5.3). Brouwer (1962) observed plant growth patterns of several species such as tomato, bean and rape seed and suggested that shoot:root ratio was generally constant. He suggested that this was a result of 'functional equilibria'. Sanders and Brown (1976) also found that even though the physical shoot:root ratio was varied by grafting, the shoot:root ratio based on the dry weight of plant material remained constant at approximately 10:1. Results from the present experiment supported these findings, since there were no differences among the shoot:root ratios of the defoliated treatments and the undefoliated treatment of the indeterminate cultivar (Figure 5.4). In general the determinate cultivar had a higher shoot:root ratio than the other treatments from the pod development stage onwards. This was due to the shorter period of root growth in the determinate cultivar (UD) than that in the other treatments. A lower shoot:root ratio of the indeterminate cultivar than that of the determinate cultivar indicated that the indeterminate cultivar had a relatively

larger root surface area than the determinate cultivar. Thus, with this characteristic the indeterminate cultivar may be able to explore a greater soil volume especially in situations of water and mineral deficiency. The similarity of the rate of change in the shoot:root ratio (slope of the regression line) for all treatments indicates that the changes in shoot and root growth were closely related.

Defoliation has been reported to reduce nodule dry weight for example in white clover (Chu and Robertson, 1974) and in field bean (Igwilu, 1982). Chu and Robertson (1974) also indicated that nodule growth was reduced to a greater extent than root growth. In the present experiment nodule dry weight was affected by defoliation (Figure 5.5 and Table 5.7) but to a lesser extent than root growth - this resulted in a higher ratio of nodule:root in the defoliated treatments (Figure 5.6). This difference may be due in part to different species and in part to different levels of defoliation. In Chu and Robertson's experiment all of the leaves were removed.

5.4.2 Yield and yield components: Seed production in most grain legume crops, including soybean, appears to depend largely on photosynthate production after the onset of flowering (Pate and Minchin, 1980). The degree of reduction in grain yield due to defoliation had been shown to vary with crop species (Enyi, 1975) and to be influenced not only by the stage of plant development at which defoliation occurred but also by growth types (Goli and Weaver, 1986). There have been reports that 'mild' defoliation (20 to 50%) in several legumes had little adverse effect on seed yield (e.g. Ezedinma, 1973 for cowpea; Thomas *et al.*, 1974 for soybean; and Hammerton, 1975 for

pigeon pea). Results from the present experiment supported these reports; for example, there was no significant difference between yield of the undefoliated treatment (UI) and the 30% defoliation treatment (I1) (Table 5.8). In contrast, with severe defoliation (I2 and I2P) the reduction in yield was significant.

Seed yield reductions in the present experiment were mainly associated with yield reductions from decreases in branch number rather than from changes to the main stem. Seed yield and pod number on a per branch basis were similar (Table 5.10). Similar observations were also reported when soybean was grown at non-optimal planting dates (Board, 1985).

In I2 and I2P, which had the same level (60%) of leaf removal but with 20% pod removal in I2P, the difference in grain yield was not significant. This must have been due to a compensatory increase in the number of pods in I2P as no difference was found in final pod number between the two treatments (Table 5.8). A similar effect of pod removal in soybean has also been reported (Thomas *et al.*, 1974; Ham *et al.*, 1976). Ham *et al.* (1976) found that removal of 50% of the pods at the end of flowering reduced yield by only 9-10% because of a compensatory increase in seed size and in the number of pods set at the non-depoddled nodes.

Most studies have shown that the reduction in pod number per plant is the yield component most affected by defoliation and the factor primarily responsible for the reduction in overall yield (e.g. McEwen, 1972; Lawn and Brun, 1974a; Goli and Weaver, 1986). This is in agreement with the finding of the present experiment (Table 5.8).

Tanaka (1980) reported that in grain legumes, the source capacity (leaves) during *the stage of early* pod development is the key factor for pod setting. Clearly, reducing the leaf area in the indeterminate cultivar to the same level as that of the determinate cultivar (*viz* I2 and I2P vs UD) from pod development stage markedly reduced the yield of the indeterminate cultivar. However, even where leaf area and pod numbers were similar up to the early grain filling stage (I2P), the indeterminate cultivar was still able to produce significantly higher seed yield than the undefoliated determinate cultivar (UD). These data suggested that the differences in yield were probably in part a result of differences in photosynthetic capabilities of the plant. For example it has been reported that high yielding cultivars may obtain their yield advantage from increased rates of leaf photosynthesis (e.g. Dornhoff and Shibles, 1970, 1976; Bhagsari *et al.*, 1977). Furthermore, photosynthesis may also control yield via nitrogen fixation especially in the case of a plant which relies solely upon symbiotic nitrogen fixation. This may well suggest that the indeterminate cultivar is not only superior in seed yield but may also have a greater contribution from nitrogen fixation and nitrogen distribution.

5.4.3 Nitrogen fixation: The importance of photosynthetic products for symbiotic nitrogen fixation has long been recognized (e.g. Lindstrom *et al.*, 1952). The marked diurnal variation in nitrogen fixation also indicates that the process is quite sensitive to the supply of photosynthetic assimilates (e.g. in soybean, Lawn and Brun, 1974a; and in pea, Bethlenfalvay *et al.*, 1978b). Since leaves are the

major source of photosynthate for the nodulated root system, treatments which reduce photosynthate supply should reduce the development of nodule mass and/or its specific activity. In the present experiment, defoliation treatments did result in a lower nodule mass, but had no effect on the specific activity (Table 5.13). In other words, variation in nodule mass was the predominant factor regulating the acetylene reduction activity. This could also be supported by the similarity of the 'nitrogen fixing efficiency' (as expressed by the slope of the regression of total plant nitrogen and nodule dry weight) among the four indeterminate cultivar treatments (Figure 5.7). This result is in good agreement with reports for soybean (Lawn and Brun, 1974a) and other legume species (Bethlenfalvay *et al.*, 1978a for pea; Chen and Sung, 1982 for mungbean). The 'nitrogen fixing efficiency' of the determinate cultivar (UD) was significantly higher than those of the other treatments. This indicates that the determinate cultivar had higher specific activity than the indeterminate cultivar. A similar observation was made in the previous experiment (Chapter 4).

The depodding treatments, which reduce competition for photosynthate, have been reported to enhance nodule growth and nodule activity (Lawn and Brun, 1974; Chen and Sung, 1982). However, this was not the case in the present experiment where there was no difference between I2 and I2P (Figure 5.5 and Table 5.7). This may be due to a lower level of depodding (20%) compared with the higher levels (50 and 100%, respectively) in the experiments of Lawn and Brun (1974) and Chen and Sung (1982).

In general, all of the indeterminate cultivar treatments had higher total acetylene reduction activity than that of the undefoliated determinate cultivar (UD) after the pod development stage (2 weeks after first flowering). At the final sampling (grain filling stage) the determinate cultivar had only about half of the activity of the indeterminate cultivar irrespective of treatment (Table 5.12). The difference did not reach statistical significance due largely to the high variability of the data set (e.g. the mean and standard deviation of UD were 29.89 (9.84) and of UI were 58.37 (25.97) micro mol C₂H₄ h⁻¹).

5.4.4 Nitrogen distribution and re-distribution: Leaves play an important role in nitrogen metabolism. They are generally the most important organ in amino acid synthesis and in nitrogen storage because large amounts of nitrogen are required in leaf growth and because nitrogen deficiencies strongly limit leaf growth and photosynthesis (Novoa and Loomis, 1981). Defoliation thus affected leaf sink strength for nitrogen directly by decreasing its size. In the present experiment, nitrogen concentrations of the defoliated treatments in the leaf and stem components were increased (Figure 5.8). This suggested that the strength of the nitrogen source (nodules) did not decrease to the same extent as the main nitrogen sink (leaves). A similar observation has also been reported by Bayne *et al.* (1984).

The similarity in nitrogen concentration at plant maturity might indicate that there was a greater re-distribution of nitrogen to the pods in the defoliated plants. Soon after the completion of *the*

defoliation treatment, in the re-distribution process the sink (pods) was not affected to the same extent as was the source (leaves) hence more nitrogen had to be re-distributed from the vegetative plant parts to pods.

The reduction of total plant nitrogen by up to 30% in both I2 and I2P (60% defoliation) (Figure 5.9) was in close agreement with the results reported by Igwilo (1982) in field bean. He found that 50% defoliation reduced total plant nitrogen by 28%. It has also been reported that pod removal and partial defoliation varied the time at which maximum nitrogen content occurred in pea (Bethlenfalvay *et al.*, 1978b). The result from the present experiment did not support this finding (Figure 5.8e) as the maximum nitrogen content of the total plant in the indeterminate cultivar treatments was reached at the flowering stage. This could be due to the difference in the level of pod removal (20% in the present experiment compared with complete pod removal in Bethlenfalvay *et al.*'s study).

As discussed in section 5.4.3, acetylene reduction activity of the defoliated indeterminate cultivar was still greater than that of the determinate cultivar even where the leaf area was similar at pod development stage onwards. This was reflected in about 20% (relative to UI) more nitrogen being accumulation in I2 and I2P compared with that of the undefoliated determinate cultivar (UD) (Table 5.14).

5.4.5 Relative sensitivity of plant characters to defoliation: Under any given set of environmental conditions, the shoot:root balance of a species is remarkably stable (Wareing and Patrick, 1975). If this balance is disturbed, for example by partial defoliation, the

partitioning of assimilates over the following period of time changes so that a higher proportion of assimilate is incorporated into new leaf tissue at the expense of root growth. With partial defoliation, therefore, root growth was probably the first growth character affected. This would support the finding of the present experiment (Table 5.15b) that root dry weight was the most sensitive character to defoliation. In contrast, the least sensitive character was pod dry weight.

In soybean, it was shown that dry-matter production under equidistant spacing was a linear function of solar radiation interception (Shibles and Weber, 1966). It has been established from the previous experiment (Chapter 4) that leaf area was the key factor influencing the production of greater total dry-matter and subsequently more seed yield in the indeterminate cultivar. Therefore for the determinate cultivar, the source capacity may be limiting yield. However, even when the indeterminate cultivar was partly defoliated (I2) to a level similar to the leaf area of the determinate cultivar (UD) at pod development stage i.e. had similar source capacity which was reported as the key factor determining number of pod setting (Tanaka, 1980), I2 still out-produced the determinate cultivar in seed yield (Table 5.8). Other workers (Fehr *et al.*, 1977, 1981) also found that indeterminate cultivars of soybeans had less yield reduction than determinate cultivars when they were defoliated. In their experiments all the leaves were removed. This suggests that the indeterminate types may also have a greater sink capacity (reproductive sites) than the determinate types. In other words, the determinate cultivar may be sink-limited. A similar

conclusion was drawn by Constable and Hearn (1978) when they found that ^{the} determinate cultivar 'Bragg' had little requirement for storage carbohydrate from stem compared with that of the indeterminate cultivar 'Ruse'.

In the indeterminate cultivar when the source capacity (i.e. leaf area) was limited, pod dry-weight was the least sensitive parameter (Table 5.15b). This indicates that the reproductive sink (pod) may have a greater strength in drawing assimilates than the vegetative components (i.e. root and stem). The relative sensitivity of the root, stem and pod dry weight to partial defoliation supports this conclusion (Table 5.15b). It could also suggest that the indeterminate cultivar may have ^a photosynthetic potential exceeding the demand of assimilates by the reproductive sites. So a decrease in leaf area caused by defoliation would not have a serious impact on yield. This assumption could be supported by less yield reduction in the indeterminate cultivar compared with those of the determinate cultivars (Fehr *et al.*, 1977, 1981).

From the results of the present experiment it could be concluded that leaf area of the indeterminate cultivar certainly was the major factor in producing higher yield when compared with the determinate cultivar. It also suggested that the indeterminate cultivar not only produced more yield but also may be more suitable than the determinate cultivar for regions with less favourable environments such as those found in the tropical conditions.

CHAPTER 6

GENERAL DISCUSSION

The objectives of these studies were to identify the effects of different growth types (determinate and indeterminate) of selected grain legumes on their yield, nitrogen fixation, and nitrogen distribution and to identify the plant parameter that has the greatest influence on the difference obtained. In this chapter the results and conclusions from the various experiments are discussed in relation to these overall objectives.

6.1 DIFFERENCE BETWEEN DETERMINATE AND INDETERMINATE GROWTH TYPES.

6.1.1 Plant growth: All of the experiments in this study showed that there were differences in vegetative and reproductive growth pattern between the two growth types.

A. Vegetative growth:

i) Shoot growth:

With a longer period of vegetative growth, the indeterminate growth type had significantly higher total plant dry-matter than the determinate growth type when plants reached ^{the} pod development stage. This difference occurred in both bean (Table 3.1) and soybean (Tables 4.1 and 5.2). The difference was due, in part, to the indeterminate cultivars of both bean and soybean reaching the flowering stage later (1 week) than the determinate cultivars. It was also due to more leaf area being developed per plant in the indeterminate cultivars at every growth stage including the first

flowering stage (Tables 3.3, 4.1, and 5.1). Absolute growth rate at any time in the growing plant is the product of net assimilation rate (NAR) and the amount of leaf area present (Jackson, 1963). The higher NAR in the indeterminate cultivars after the pod development stage (Figures 3.4 and 4.4), and their larger leaf area, could therefore explain their higher dry-matter yield relative to that of ^{the} determinate cultivars. Earlier work reported that variation in dry-matter production between varieties of the same crop, was mainly brought about by variation in leaf area (Watson, 1952). This conclusion is supported by the present data (Figure 6.1) which showed that the dry-matter of both the determinate and indeterminate cultivars increased almost linearly with an increase in leaf area per plant.

One of the plant characters closely linked to the higher leaf area of the indeterminate soybean cultivar was branch number. Results from the defoliation treatments (Chapter 5) showed that the indeterminate cultivar continued to produce branches after the flowering stage, and that the partial defoliation (60% -I2) at this stage reduced the number of branches significantly (Table 5.4). Competition for photosynthate between vegetative and reproductive growth was probably the main cause of branch number reduction, since plants with the same degree of defoliation but with 20% pod removal (I2P) had a similar branch number to that of the undefoliated plants (UI). The implications of the branch number in field conditions will be discussed later.

Good and Bell (1980) showed that total growth of a plant depends almost equally on the rate of photosynthesis and on the proportion of the photosynthetic products devoted to the synthesis of

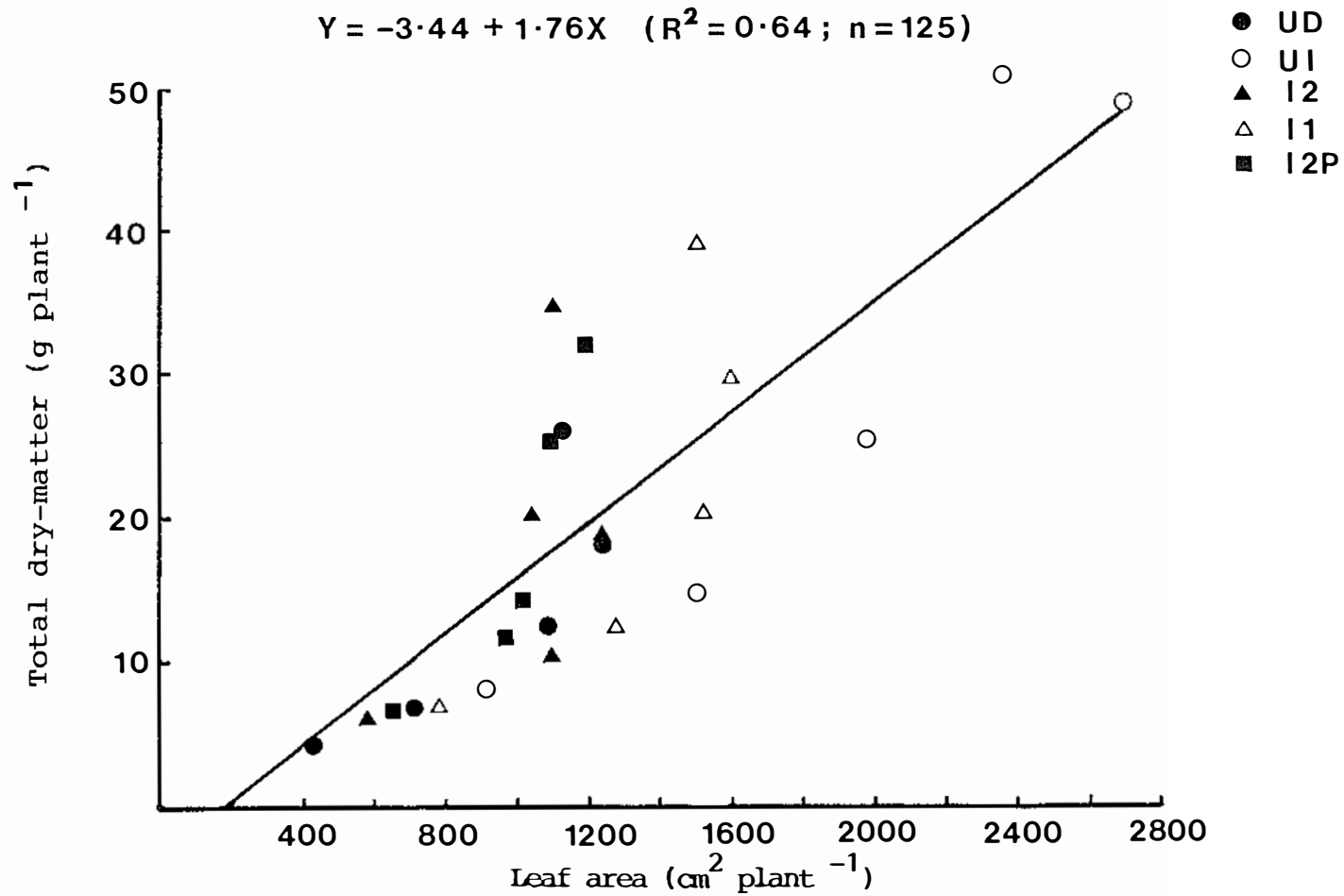


Figure 6.1 Relationship of total dry-matter (dependent variable) of partial defoliated indeterminate cultivar and undefoliated eteterminate cultivar regressed against leaf area per plant (independent variable).

new photosynthetic area. In this present study, it was shown that the ratio of dry-matter partitioned to the leaf of the indeterminate cultivar was significantly higher than that partitioned in the determinate cultivar at the same growth stage (Figure 4.6). This partitioning pattern, therefore, may also contribute to the difference between the two growth types.

These different growth patterns suggest that a continuation of vegetative growth during the reproductive stages in indeterminate cultivars helps them to build up a larger source capacity than that of determinate cultivars, a greater source capacity could also have significant influence on nitrogen fixation, nitrogen distribution and finally on seed yield as will be discussed in later sections.

It has been reported that many leguminous species are morphologically unstable particularly under some environmental conditions which have been shown to affect the form of growth of the two types. For example, some determinate cowpea cultivars can elongate and become indeterminate in form in warm (24°C) as compared with cool (19°C) night temperature conditions (Summerfield and Wein, 1980). In the present study there was a difference in the total plant dry-matter between the two growth types from the flowering stage onwards in the controlled environmental conditions (Table 4.1) whereas in glasshouse-grown soybean the difference occurred only from the late grain filling stage onwards (Table 3.1). This could have been due to the effects of environmental conditions such as temperature: the minimum night temperature in the glasshouse was recorded as 12°C , whereas under the controlled environment conditions it was constant at 18°C . Night temperature has also been shown to have a strong influence

on the accumulation and partitioning of dry matter in soybean plants during early reproductive growth (Summerfield, 1977; Thomas and Raper, 1978; Thomas *et al.*, 1981; and Seddigh and Jolliff, 1984). Seddigh and Jolliff (1984) found that higher night temperatures (16 and 24 °C compared with 10°C) enhanced early vegetative growth (i.e. increased leaf area and total dry matter accumulation). In spite of also advancing reproductive development. They also reported that high night temperature appeared to increase reproductive sink strength via a direct effect on the seed which, in turn, enhanced the allocation of dry-matter to the seeds at the expense of vegetative tissues and the pod wall. Furthermore, it has been suggested that differences in light quality can be a main cause of morphological variation (Kretchmer *et al.*, 1977). Care should be taken, therefore, when determinate and indeterminate growth types are compared under different temperature and light environment conditions.

ii) Root growth:

In most comparative studies of the growth of determinate and indeterminate types of legumes, the root component has been frequently neglected-possibly because of the inaccessibility of roots, especially in the field. In this present study, the results showed that the indeterminate cultivars had consistently more roots and a longer root growth phase than the determinate cultivar (Figures 3.5, 4.1 and 5.4). This might be due to a significantly higher proportion of photosynthate reaching the roots of the indeterminate cultivar as indicated by the ratio of root dry-weight to total plant dry-weight (Figure 4.6).

It was shown in this work that root growth was the most sensitive character affected by defoliation in the indeterminate cultivar (Section 5.3.4). It could be concluded, therefore, that the larger leaf area per plant in the indeterminate cultivar was undoubtedly important for its ability to support more root growth which, in turn would also have influenced nodule growth, nitrogen fixation and eventually seed yield.

The root also could influence the function of the shoot (Wardlaw, 1980). For example, Humphries and Thorne (1964) measured the effect of root formation on photosynthesis of attached dwarf bean leaves. They concluded that the root system determined the magnitude of the sink for accumulation of photosynthate and that it had a significant influence on the rate of photosynthesis.

The implication of photosynthate movement between growth types are quite important since roots are dependent on carbohydrate received from the shoot. Some of this carbohydrate is consumed in respiration by nodulated roots, some is used in the formation of root and nodule dry matter during growth, and some serves as the carbon source for the formation of the products of nitrogen fixation (Pate, 1979). The significantly higher movement of ^{14}C from ^{the} labelled leaf downward of the indeterminate cultivar than that of the determinate cultivar at ^{the} pod development stage (Table 4.4) could indirectly suggest that roots of the indeterminate cultivar received more carbohydrate from the shoot. Hence, it could maintain growth of root and nodule for longer period.

B. Reproductive growth:

It has been suggested that competition between vegetative and reproductive growth during flowering and pod set may reduce the number of pods set (Greer and Anderson, 1965). Thus the proportion of photosynthate allocated to reproductive growth during these stages could have a direct effect on pod and seed number and hence on yield. This led to the suggestion that cultivars with a determinate growth type may exhibit less competition during flowering and pod set and thus produce higher yield (Shibles *et al.*, 1975). One way to compare the competition between vegetative and reproductive components growth during flowering and pod set is to compare the dry-matter partitioning. The ratio of the dry-matter partitioned between vegetative and reproductive plant parts during flowering and fruit set has been called the 'partitioning coefficient' (fruit weight divided by total above-ground biomass x 100) (Egli *et al.*, 1985). Egli *et al.* (1985) found that in field grown soybeans, the coefficient was similar between the two growth types at the same growth stage. In contrast to their results, the controlled environment conditions-grown soybeans in the present study showed significant difference in the partitioning coefficients of the two growth types (Table 6.1). It was also noteworthy that the partitioning coefficients in the present study were higher than those reported by Egli *et al.* (1985) for the same growth stages (Table 6.1). The lower coefficient of the indeterminate cultivar probably indicates that there was more competition between vegetative and reproductive components than was the case for the determinate cultivar. However, with a larger source capacity, the indeterminate cultivars were able to produce higher numbers of pods,

Table 6.1 Partition coefficients of bean and soybean from different environmental conditions at two growth stages.

Environment	Species	Growth Type	Growth Stage		
			Pod Development	Grain Filling	
Glasshouse	Bean	D	18.6	47.5	
		I	7.2	48.0	
		F-test	*	ns	
	Soybean	D	14.7	45.0	
		I	10.1	31.3	
		F-test	*	*	
	Controlled Environment	Soybean	D	23.1	55.3
			I	14.5	39.0
			F-test	**	**
Field	Soybean	D	2.0	9.3	
		I	2.0	14.0	

(Egli et al., 1985)

i.e. higher numbers of final pods per plant (Tables 3.10, 4.2 and 5.7). This also suggests that the indeterminate cultivar sets pods at the later stages and that variation in pod set might indeed be closely related to growth types.

At the final harvest, the 'harvest index' (defined as ratio of grain yield:total above-ground biomass) or 'partitioning coefficient' at the final harvest of the determinate cultivar was significantly higher than that of the indeterminate cultivar in both of the experiments carried out under the controlled environment conditions (Tables 4.2 ; 5.10). However, no difference was found in the glasshouse-grown soybean (Table 3.13) possibly because of the lower harvest index of the determinate cultivar (52%) compared with in controlled environment conditions (65%). Sinclair *et al.* (1981) suggested that the harvest indices of indeterminate cultivars might be more stable due to plants being able to adjust the number of seeds which would be initiated over a long period of time. Although the results reported in the present study suggested that the determinate cultivar was more efficient in partitioning total biomass to grain (i.e. higher harvest index; Tables 4.2; 5.11), it did not however, result in any yield advantage (Tables 4.2; 5.8). This was because the indeterminate cultivar was approximately twice as large as the determinate cultivar which negated the more subtle influence of harvest index.

C. Seed yield:

Seed yield is comprised of a series of yield components (viz. pods per plant, seeds per pod, and weight per seed) which in

turn, are influenced by a combination of plant and environmental factors (Adams, 1975).

Amongst the three yield components, the number of pods per plant was the most important yield component character showing difference between the two growth types of soybean in this present study (Tables 3.12; 4.2 ; 5.8). This component was also reported as being the most important component in determining grain yield in bean (Adams, 1975), soybean (e.g. Shibles *et al.*, 1975; Laohasiriwong, 1985) and lupin (Withers, 1979). When yield and yield components were analysed separately, for those collected from the branches and those collected from the main stem, the results indicate that the difference between the two growth types was mainly due to differences coming from the branch components (Table 4.3). However, on a per branch basis there was no difference in yield or yield components (Table 5.10). Furthermore, partial defoliation of the indeterminate cultivar did reduce the number of branches (Table 5.5). These results suggest, therefore, that the longer period of vegetative growth in the indeterminate cultivar leads to a higher number of branches, higher number of pods per plant and finally to a higher seed yield.

6.1.2 Nitrogen fixation:

Legume species have two major sinks for photosynthates - their fruits (i.e. the protein-rich seeds) and their nodulated roots (Minchin *et al.*, 1981). Considerable evidence has been accumulated to emphasize a direct link between the rate of nitrogen fixation and the rate of photosynthesis (Hardy and Havelka, 1976). Thus nodule function might be limited by the supply of photosynthate from the host (Atkins, 1984).

In the present study, the acetylene reduction activity of the indeterminate soybean cultivar was consistently higher than that of the determinate cultivar in all of the experiments (Figures 3.6 ; 4.8 and Table 5.12). The results also indicated that the difference between the two growth types was primarily due to the difference in the mass of nodules (Figures 3.7 ; 4.7 and 5.5). Since nodule mass depended on an adequate supply of photosynthate from the shoot, this would suggest that the indeterminate cultivar supplied more photosynthate to the root component which, in turn, supported more nodules than occurred in the determinate cultivar. A significant difference in root growth between the two growth types, as discussed in the previous section in this chapter, plus a greater movement of ^{14}C downward from the labelled leaf in the indeterminate cultivar (Table 4.5), lend further support to this argument.

The increase in acetylene reduction activity associated with a rapid increase in the weight of nodules has been shown to involve young reproductive structures (i.e. flowers and young pods) (Peat *et al.*, 1981). They found that soybean plants which had been maintained in the vegetative stage, had a five-fold greater leaf area and more than twice the root dry weight than the (reproductive) control plants. However, nodule dry weight of the vegetative plants was only 63% that of the control plants. Hence they suggested that some factors (may be hormonal) associated with the presence of the reproductive structures, may promote nitrogen fixation during the early part of the reproductive period. Hence the indeterminate soybean cultivars, which had a relatively long flowering period, should have long period of nitrogen fixation. This could be supported

by the results from the present study which showed that the indeterminate cultivar had a prolonged phase of rapid nitrogen fixation (Figures 3.6; 4.8 and Table 5.12). Some early works were also in general agreement with these observations (Hardy *et al.*, 1971; Duke *et al.*, 1979).

The 'nitrogen fixing efficiency' (as expressed by ^{the} slope of the regression equation of total plant nitrogen (log scale) and nodule dry weight -Dobereiner, 1966) consistently showed that the determinate cultivar had higher fixing efficiency than that of the indeterminate cultivar in every experiment, in the present study (Figures 3.9, 4.10, 5.7). With the specific acetylene reduction activity changing quite drastically with time (Figure 4.8) the expression of 'nitrogen fixing efficiency' by the regression technique of Dobereiner (1966) may be more suitable as a means of comparing nodule efficiency under very low or nitrogen free growing conditions to minimize the effects of different[†] contributions of soil nitrogen.

Results from the defoliation experiment (Chapter 5) showed that partial defoliation affected acetylene reduction activity less than it affected root and nodule growth (Table 5.15). This observation indicates a different pattern of response by the nodule to photosynthate stress in comparison to other plant components. With 60% defoliation, nodule activity (i.e. specific acetylene reduction activity; Table 5.13) was maintained, while nodule development was reduced (i.e. lower nodule dry-weight at ^{the} grain filling stage; Table 5.7). Active nodule growth, as indicated by net nodule dry-weight increase, in the indeterminate cultivar was longer in duration than that of the determinate cultivar (Figures 4.7 and 5.5). Hence the

indeterminate cultivar might have some advantage over the determinate cultivar in fixing nitrogen especially under conditions where leaf area could not develop fully or was partly destroyed.

6.1.3 Nitrogen distribution:

6.1.3.1 Nitrogen accumulation:

The accumulation of total nitrogen in both bean and soybean plants followed a very similar pattern to that for dry matter accumulation (Figures 3.1 and 3.8; Figures 4.1 and 4.12). This was in general agreement with that reported for soybean by Hanway (1976). Furthermore, the results in Chapter 4 (Figure 4.12) indicated that the indeterminate cultivar accumulated nitrogen faster (higher regression coefficients) than the determinate cultivar.

Partitioning of nitrogen to different plant parts showed that ^{after flowering, relatively} more nitrogen was partitioned to the leaf and stem components of the indeterminate cultivar than to those of the determinate cultivar (Figure 4.14). In the indeterminate cultivar, therefore, more nitrogen was directed to leaf and stem growth, which in turn, provided photosynthate for increasing nitrogen fixation thus ensuring and extending the rapid nitrogen accumulation period. Withers (1979) suggested that an indeterminate lupin cultivar, which allowed nitrogen fixation to occur over a longer period, could have an advantage for nitrogen accumulation over determinate cultivars which might not be able to fully utilise the environmental resources available. Results in the present study also supported this suggestion. For example, at the grain filling stage the determinate soybean cultivar accumulated five times more nitrogen under controlled environment conditions when

compared with those in the glasshouse, whereas the relative difference for the indeterminate cultivar was eight-fold (Tables 3.7 and 4.6)

One way of improving the efficiency of nitrogen utilization would be to have a high ratio of nitrogen content in the seed relative to that of the above-ground portion of the plant (i.e. to have a high nitrogen harvest index). In all of the experiments reported in the present study there were no differences between the nitrogen harvest indices of the two growth types. In other words, the major difference between the two growth types was largely due to nitrogen accumulation rather than to a difference in the efficiency of nitrogen partitioning.

6.1.3.2 Nitrogen re-distribution:

In analyzing the photosynthate and nitrogen requirements of seeds, Sinclair and de Wit (1975) found that soybeans were unique among the 24 crop species studied. Soybeans have both a high protein and lipid content, and therefore require the highest rate of nitrogen supply to the seed. In contrast, their rate of biomass production is one of the lowest. They hypothesized that soybeans were 'self destructive' because the loss of nitrogen from the vegetative component to support seed development could cause a loss of physiological activity and ultimately lead to senescence.

Although nitrogen re-distribution from the vegetative parts to the grain (Chapter 4, Table 4.7) was proportionally similar between the two growth types, the absolute values were significantly different - the indeterminate cultivar re-distributed about twice as much nitrogen to the grain than the determinate cultivar. This high re-distribution could, in part, contribute to the higher seed yield in

the indeterminate cultivar since increasing the quantity of nitrogen available for grain development in soybean could be accomplished by increasing the whole plant nitrogen accumulation and/or by increasing the proportion of vegetative nitrogen translocated to the developing grain (Jeppson *et al.*, 1978). Furthermore, Loberg *et al.* (1984) concluded that yield differences among soybean cultivars were, in part, dependent upon nutrient re-distribution. Amongst the nutrients studied (N, P and K), nitrogen was identified as being the most important.

Although the difference between percentage nitrogen re-distributed from the leaf component between the two types was not significant, there was an indication that the percentage nitrogen re-distributed from the leaves of the indeterminate cultivar was lower (Table 4.7). In general, leaves appeared to be more important as a source of mobilised nitrogen than the supporting stem tissues (Pate and Minchin, 1980). The results from the present study also supported this observation (Table 4.7). Pate and Minchin (1980) suggested that it might be desirable to develop cultivars which draw on stem and pod wall nitrogen in preference to leaf nitrogen during seed-fill. This would allow the leaves to senesce slowly thus maintaining a flow of assimilates to the nodules during seed development. Results from the present study indicated that ^{as} significantly higher percentage nitrogen was re-distributed from the stem of the indeterminate cultivar than that of the determinate cultivar. In contrast, lower proportions were re-distributed from leaves and pod wall of the indeterminate cultivar, although the difference was not significant statistically ($P > 0.05$) (Table 4.7). This trend, coupled with the higher acetylene reduction

activity of the indeterminate cultivar at the grain filling stage (Figures 3.6 and 4.8) could support the hypothesis that nitrogen re-distributed from the stem was important. This character might be worth looking for when developing new cultivars (as suggested by Pate and Minchin, 1980).

6.2 THE KEY FACTOR DETERMINING THE DIFFERENCE BETWEEN THE TWO GROWTH TYPES.

From the discussion presented in the previous sections, leaf area per plant was identified as the parameter that had the greatest influence on plant growth, nitrogen fixation and nitrogen distribution between the two growth types. This observation, coupled with the discriminant analysis presented in Chapter 4 (Table 4.7), would suggest that leaf area was the key factor determining the difference between the two growth types. Although, there has not been any work that the author is aware of using the multivariate approach to determine the difference between the two growth types. The importance of leaf area has certainly been highlighted in this present study (Figures 4.15, 4.16; Table 4.8).

Tanaka (1980) reported that in grain legume the source capacity (leaves) during *the stage of early* pod development was the key factor influencing pod setting. Furthermore, photosynthate supply from the leaves to the nodules had been recognized as one of the major limiting factors influencing nitrogen fixation in legumes (e.g. Lawn and Brun, 1974a; Bethlenfalvay *et al.*, 1978b; Chen and Sung, 1982). Leaves were also reported as the major source of nitrogen which was *transferred* to keep normal seed growth during which the demand for

nitrogen for seed formation was high (Withers and Forde, 1979; Warembourg and Fernandez, 1985). So it could be concluded that with greater leaf area per plant, the indeterminate cultivar would have a greater photosynthetic capacity which would be important for plant growth and nitrogen fixation and finally leading to higher seed yield.

However, caution must be used in extrapolating these results to field conditions since, in controlled environment, plants were well spaced and total photosynthesis was likely to be much higher than under a more light limiting situation within a well-grown crop in the field. At field densities shading can be expected to decrease the advantage of higher leaf area, especially during the later stages of growth. High density could also affect branch number. However, research with determinate isolines (Hicks *et al.*, 1969), with randomly selected semi-determinate genotypes (Green *et al.*, 1977) and determinate cultivars of two maturity groups (Beaver and Johnson, 1981) had found no yield advantage over indeterminate cultivars in narrow row widths. Lawn *et al.* (1977) found that the indeterminate cultivars showed no yield differences for a wide range of populations under full irrigation which indicated that these cultivars can adjust to wide differences in plant density within the row under conditions favourable to growth. It has been shown that on the debranched soybean plants, there was a large increase in number of pods set per node and bigger seed size than that of the control plants (Beuerlein *et al.*, 1971). Furthermore, the defoliation experiment in the present study indicated that with about 60% defoliation, seed yield of the indeterminate cultivar was reduced only 17%, whereas ^{the} yield advantage of the undefoliated indeterminate cultivar over the undefoliated

determinate cultivar was about 50% (Table 5.8). So, the advantage of the the indeterminate cultivar may still *persist in part* under field conditions.

Although in this experiment, leaf area per plant has been identified as the most important plant parameter influencing growth, seed yield, nitrogen fixation and nitrogen distribution, as pointed out earlier (p. 206) caution must be used in extrapolating the results to field conditions.

Under a closed canopy situation in the field, crop growth rate is determined more by the total intercepted radiation than by the absolute amounts of leaf area per plant (Monteith, 1977).

However results obtained from the present experiment will still be of relevance because there are situations in the tropics where spaced legume plants are used in a relay cropping system. Further research to differentiate the significance of the contribution of leaf area per plant as a factor influencing crop growth between spaced and closed canopy plants is important.

CHAPTER 7CONCLUSIONS AND IMPLICATION FOR FUTURE RESEARCH7.1 CONCLUSIONS:1. The differences between the two growth types.

1.1 Growth pattern: There were differences in growth pattern between the determinate and indeterminate growth types of both crops studied (bean (*Phaseolus vulgaris*) and soybean (*Glycine max*)). The differences could be concluded as follows:

i. The indeterminate type produced two to two and a half times more total dry-matter than the determinate type depending on whether it was grown in the glasshouse or in the controlled environment conditions. This advantage was mainly brought about by greater leaf area and root dry weight per plant in the indeterminate cultivars of both species. This was the result of longer vegetative growth in the indeterminate cultivars.

1.2 The magnitude of difference between the two growth types varied under the different environmental conditions, for example, vegetative components (dry-weights of leaves, stems and roots) were about two to four times across all components depending on whether it was grown in the glasshouse or the controlled environment conditions.

1.3 Seed yield: There were differences in the seed yield and yield components between the two growth types.

i. Total seed yield of the indeterminate type was about two to three times higher than that of the determinate type. The higher harvest index of the determinate cultivar did not result in any yield advantage because the indeterminate cultivar was approximately twice as large as the determinate cultivar.

ii. For both growth types, pod number was the most important yield component in determining yield. In soybean, the difference in pod number between the two types was primarily the result of a higher number of branches in the indeterminate cultivar.

1.4 Nitrogen fixation: There were differences in nitrogen fixation activity between the two growth types.

i. Acetylene reduction activity per plant of the indeterminate soybean cultivar was higher than that of the determinate cultivar from pod development stage. This was because of higher nodule mass and longer duration of nodule growth in the indeterminate cultivar.

ii. However, the specific acetylene reduction activity data showed that the determinate cultivar had higher activity per unit nodule dry weight than that of the indeterminate cultivar. A similar conclusion also was drawn from the 'nitrogen fixing efficiency' technique as expressed by the slope of the regression equation between total plant nitrogen (log scale) and nodule dry weight.

1.5 Nitrogen distribution:

i. The difference between the two growth types was largely due to higher amount of nitrogen accumulated by the indeterminate type rather than ⁱⁿ efficiency in nitrogen partitioning, since final nitrogen distribution to the grain as represented by the nitrogen harvest index' for both growth types was similar.

ii. For both growth types, the proportion of nitrogen re-distributed^d from vegetative parts to grain was similar. However, with ^a larger source of nitrogen accumulated, the absolute value of nitrogen re-distributed was about twice as much as in the indeterminate cultivar.

iii. It was concluded that both growth types needed to transfer nitrogen from vegetative parts to support high grain nitrogen requirement. The amount of nitrogen re-distributed would depend on the ability of plants to accumulate nitrogen before the re-distribution occurred.

iv. Leaves were the major source of nitrogen re-distributed from the vegetative components to the grain for both growth types.

v. However, The proportion of nitrogen re-distributed from the stem of the indeterminate cultivar was significantly higher than that of the determinate cultivar. It was suggested that the high nitrogen re-distribution from ^{the} stem might be the character worth looking for when developing new cultivars, since it could mean that plant which draw on stem and pod-wall nitrogen in preference to leaf nitrogen would allow the leaves to senesce slowly.

2. Key factor influencing the differences between growth types.

2.1 Leaf area per plant was identified as the key factor influencing the difference between the determinate and indeterminate growth types.

2.2 The extent to which leaf area was important in contributing towards the advantage of the indeterminate cultivar could be concluded as follows:

i. The higher leaf area in the indeterminate cultivar resulted in a longer period of vegetative growth which led to higher number of branches, higher number of pods per plant and finally to higher seed yield.

ii. Defoliation had a greater effect on the root and nodule growth than acetylene reduction activity.

iii. It was concluded that the indeterminate cultivar may be more suitable to the harsh environmental conditions of the tropics, especially under conditions where leaf area could not develop fully or was partly destroyed.

7.2 POSSIBLE IMPLICATIONS FOR TROPICAL AGRICULTURE.

Many tropical soils are limited in their ability to achieve high crop productivity due to severe deficiency in soil nitrogen (Gibson *et al.*, 1982). Nitrogen losses in tropical regions are also much higher than comparable temperate regions (Dobereiner and Campelo, 1977). Furthermore, subsistence farmers in the tropics are unlikely to invest in mineral nitrogen fertilizers. Therefore, legumes will continue to be an

important component in the cropping systems of the tropics (Gomez and Zandstra, 1977). In addition to their grain yielding ability, legumes should also be selected based upon their ability to contribute nitrogen towards the soil nitrogen pool hence benefiting the companion or following crops. For example, those legumes with a high proportion of their nitrogen retained in the non-harvested residues or with a high percentage nitrogen in the residual tissues may be more appropriate as nitrogen suppliers for the low input tropical cropping systems. Given this situation, the priority frequently given to legume breeding programmes, such as to improve the harvest index (maximizing the fraction of plant production that is removed as grain) may not be the most appropriate criterion.

The role of the determinate and indeterminate growth types should be assessed carefully relative to their possible contribution in a cropping system. There is a trend amongst modern legume breeders in the developed countries to favour the determinate plant type, which is more compatible with mechanical harvesting (Khan, 1977). Although, farmers in the tropics will normally use the indeterminate type legumes in their intercropping system, there is, however a tendency to follow the developed countries in adopting the determinate growth type as the new cultivars for these regions. Evidence from the present study indicated that the indeterminate cultivar was superior in nitrogen fixation, nitrogen distribution and seed yield. The superiority of the indeterminate cultivar over that of the determinate cultivar had also been reported by others. For

example, higher nitrogen fixation in beans (Graham, 1981), less affected by defoliation in soybean yield (Fehr *et al.*, 1977; 1981) and producing higher yield under dry land conditions in cowpea (Chaturvedi *et al.*, 1980). These reports together with that highlighted in the present study suggested that under the harsh and less mechanised environment of the tropics it might be worth looking at the indeterminate legumes more closely.

7.2.1 Possible role of leaf area as a selection criterion for cultivars superior in nitrogen-fixation

To select cultivars superior in nitrogen-fixation - legume breeders require a rapid, non-destructive analysis, amenable to large population of plants (Rennie and Kemp, 1981).

Of the plant parameters considered, nodule dry-weight per plant was found to be most closely correlated to acetylene reduction activity both in this present study (Chapter 4) and work reported by others (e.g. Denison *et al.*, 1985). However, assessment on the basis of nodular mass or number is laborious and time consuming (Rennie and Kemp, 1981) and is unlikely to be suitable as a selection technique.

Growing legumes in nitrogen-free medium forces total dependence by the plant on seed and symbiotic nitrogen, ^{and} thus might provide a convenient screening procedure for nitrogen-fixing ability (Graham, 1981). In the present study leaf area per plant was closely related to total plant nitrogen (Figure 4.15), thus leaf area per plant *per se* might be suitable as an

indicator for the absolute amount of total nitrogen fixed. So, comparison on the basis of relative size of the leaf area developed over a common time frame may provide a suitable non-destructive method of assessing nitrogen fixing abilities. This must be conducted in a soil medium with very low nitrogen or be nitrogen-free.

7.2.2 Future research:

Before the advantage of the different growth types of legumes can be exploited to the full, we need to identify the relative contributions of the determinate and indeterminate growth types both in terms of producing high seed yield and supplying nitrogen to a cropping system. The following are some suggestions:-

1. To investigate the extent of nitrogen fixation, nitrogen distribution and seed yield difference between the two growth types across a large germplasm collection preferably under field conditions.

2. To compare the ability of different growth types of legumes to enrich the soil or to transfer fixed nitrogen to companion or following crops under different environmental and management conditions.

APPENDIX AStatistical symbols

Throughout this thesis, unless otherwise stated, the following symbols were used:

ns = non significant at $P = 0.05$

Significance levels

*	$0.05 = P > 0.01$
**	$0.01 = P > 0.005$
***	$0.005 = P > 0.001$
****	$P > 0.001$

Where the term "statistical significance" is used, unless otherwise specified, refers to 5% level of probability.

Appendix BAcetylene Reduction Technique

Whole roots and nodules were carefully removed from the pots, the detached roots and nodules were then held at ambient temperature in sealed 1 litre preserving jars containing 100 cc of commercial grade acetylene. After an hour, gas samples were withdrawn into 5 ml Vacutainer tubes. The total gas phase value of each plant-incubation vessel unit was determined by water displacement.

Gas samples from all experiments were analysed on a Pye unicam 204 gas chromatograph, at Grasslands Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand. Ethylene was measured with a flame ionisation detector and a glass column 900 mm x 4 mm I.D., packed with 80/100 mesh Poropak T, and operated at 125°C.

At the time the incubation vessels were sampled, the gas chromatograph was calibrated for each analytical run with ethylene standards drawn into 5 ml Vacutainer tubes.

Acetylene peak heights were also checked for evidence of faulty incubation jars.

The total nitrogen fixation and specific nitrogen fixation were then calculated, using the Basic computer program from the Department of Scientific and Industrial Research, Palmerston North, New Zealand. The program had been transferred to the Massey University computer system by the author. The calculation procedure was as follow:

First from values of the ethylene standard

$$D = (A*B)*0.1$$

where A = scale

B = peak height in cm

From blank sample

$$X = (((C/H)*(A*B)*0.1)*Z)/D$$

From samples

$$Y = (((C/H)*(A*B)*0.1)*Z)/D$$

where C = gas incubation volume in cm³

H = injection volume in cm³

Z = nmoles/injection volume of ethylene standard

The nitrogen fixation micro mole ethylene/hour is

$$N = (((60/P)*Y)-X)*.001$$

where P = incubation length in minute

Appendix CKjeldahl nitrogen content determinationPlant material

Plant samples were placed loosely in aluminium drying tins, in a forced-draught oven which was operated at 80°C for at least 24 hours. They were then ground in a cutter mill to pass through a 1 mm screen.

The samples to be analysed were then dried over night at 80°C and stored in a glass container.

The Kjeltec Auto System I by Tecator was used to determine the total nitrogen content.

Digestion procedure

Place 0.2 gm of sample in the digestion tube, add 1 tablet of Kjeltab Auto (containing 1.5 gm K_2SO_4 and 7.5 mg Se) and 5 ml of nitrogen free H_2SO_4 conc. Place digestion stand with prepared samples and exhaust manifold, in the preheated digester (420°C). Digest for 3-5 minutes with maximum air flow through the exhaust manifold. Then adjust the flow until fumes are just contained. Continue digestion to a total time of 30 minutes. Remove stand with tubes and exhaust manifold and place the entire assembly in the cooling stand beside the digester. When the sample solutions have cooled sufficiently they are diluted and mixed with 30 ml of de-ionized water.

Distillation and Titration

The semi-micro Kjeldahl method was used, using 40% NaOH in the steam distillation and 0.2 M HCl in the titration with 1% Boric

acid and methyl red/bromocresol green indicator as a receiver solution.

A blank determination and a known standard sample are always used at least once a day, or whenever a new batch of any of the chemicals has been changed.

Put the sample tubes to the Kjeltac Auto 1030 Analyzer. Distillation, titration and calculation of percentage of nitrogen content are performed automatically.

Appendix D

Controlled environment room environmental conditions

The controlled environment rooms at the Climate Laboratory, Plant Physiology Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand (Anon, 1981) were used with the following conditions:

Temperature	day	25° C (± 0.5° C)
	night	18° C (± 0.5° C)
Relative Humidity	day	65% (± 5%)
	night	85% (± 5%)
Photoperiod	14	h

Lighting was supplied to each room by 4x1000W Sylvania 'Metalarc' high pressure discharge lamps, and 4x1000W Philips tungsten halogen lamps.

-The mean photosynthetic photon flux density at plant height was 690 micro mol s⁻¹m⁻² as measured by Licor LI 185 meter with an LI 190S quantum sensor

-The corresponding mean photosynthetic irradiance was 147 Wm⁻² as measured with an Licor LI 185 meter with LI 190SE flat response photosynthetic irradiance sensor.

CO₂ level was monitored, but was uncontrolled remaining within 310-370 micro l CO₂/l air.

Air flow down to plants was 0.3-0.5 m sec⁻¹ as measured with an Alnor Instruments' thermoanemometer.

Appendix 3.1 Information from growth curves of total plant dry weight (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $[\ln(Y) = B_0 + B_1 x + B_2 x^2]$

Species	Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
Bean	D	-3.700	(0.611)	0.884	(0.122)	-0.033	(0.006)	0.88	30
	I	-1.898	(0.464)	0.506	(0.089)	-0.011	(0.004)	0.94	33
Soybean	D	-3.301	(0.496)	0.607	(0.091)	-0.018	(0.004)	0.89	36
	I	-1.823	(0.492)	0.299	(0.091)	-0.001	(0.004)	0.94	36

Appendix 3.2 Information from growth curves of leaf and stem dry weight (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $[\ln (Y) = B_0 + B_1 x + B_2 x^2]$.

Species	Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
Bean	D	-3.126	(0.860)	0.773	(0.174)	-0.035	(0.008)	0.46	30
	I	-3.409	(0.568)	0.831	(0.109)	-0.033	(0.005)	0.77	33
Soybean	D	-4.139	(0.626)	0.762	(0.115)	-0.028	(0.005)	0.71	36
	I	-3.194	(0.648)	0.534	(0.119)	-0.014	(0.005)	0.84	36

Appendix 3.3 Information from growth curves of leaf area per plant (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $[\ln(Y) = B_0 + B_1 x + B_2 x^2]$.

Species	Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
Bean	D	-1.102	(1.550)	1.334	(0.313)	-0.061	(0.015)	0.46	30
	I	1.086	(0.681)	0.936	(0.131)	-0.039	(0.006)	0.68	33
Soybean	D	1.232	(0.703)	0.638	(0.129)	-0.024	(0.006)	0.55	36
	I	1.556	(0.702)	0.529	(0.129)	-0.015	(0.006)	0.78	36

Appendix 3.4 Information from growth curves of root dry weight (dependent variable, of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $[\ln (Y) = B_0 + B_1 x + B_2 x^2]$.

Species	Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
Bean	D	-3.715	(0.606)	0.649	(0.121)	-0.029	(0.006)	0.53	30
	I	-2.330	(0.639)	0.414	(0.123)	-0.015	(0.005)	0.56	33
Soybean	D	-4.999	(0.420)	0.714	(0.077)	-0.026	(0.003)	0.84	36
	I	-4.009	(0.482)	0.551	(0.089)	-0.016	(0.004)	0.89	36

Appendix 3.5 Information from curves of acetylene reduction activity (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form ($\ln(Y) = B_0 + B_1 x + B_2 x^2$)

Species	Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
Bean	D	-29.339	(4.813)	5.771	(0.908)	-0.261	(0.041)	0.63	27
	I	-11.474	(2.365)	2.412	(0.407)	-0.108	(0.017)	0.73	27
Soybean	D	-22.062	(3.112)	3.807	(0.492)	-0.146	(0.019)	0.71	27
	I	-28.051	(4.322)	4.789	(0.683)	-0.181	(0.026)	0.67	27

Appendix 3.6 Information from growth curves of nodule dry weight (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form ($Y = B_0 + B_1 x + B_2 x^2$).

Species	Growth type		(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
Bean	D	-27.005	(3.925)	4.484	(0.741)	-0.193	(0.033)	0.65	30
		-16.145	(1.656)	2.546	(0.318)	-0.102	(0.014)	0.78	33
Soybean	D	- 9.818	(1.137)	0.948	(0.209)	-0.026	(0.009)	0.83	36
	I	-10.051	(0.735)	0.978	(0.135)	-0.022	(0.006)	0.95	36

Appendix 3.7 Information from growth curves of total plant nitrogen (dependent variable), of determinate and indeterminate beans and soybeans, regressed against time (independent variable) using quadratic equation form $[\ln (Y) = B_0 + B_1 x + B_2 x^2]$.

Species	Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
Bean	D	-1.590	(0.068)	1.053	(0.183)	-0.039	(0.008)	0.85	27
	I	-0.902	(0.859)	0.866	(0.148)	-0.025	(0.006)	0.95	27
Soybean	D	2.067	(1.630)	0.258	(0.258)	-0.004	(0.009)	0.69	27
	I	2.834	(1.612)	0.044	(0.255)	-0.010	(0.009)	0.89	27

Appendix 3.8 Information from linear regression of total plant nitrogen (dependent variable) regressed against nodule dry weight (independent variable) with the equation form $\ln(Y) = B_0 + B_1 X$.

Species	Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	R^2	n
Bean	D	4.079	(0.087)	3.826	(0.328)	0.88	21
	I	4.603	(0.221)	1.934	(0.416)	0.53	21
Soybean	D	3.989	(0.065)	4.906	(0.409)	0.88	21
	I	4.134	(0.077)	2.533	(0.201)	0.89	21

Appendix 4.1 Information from growth curves of plant dry weights (dependent variable), of determinate and indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($\ln(Y) = B_0 + B_1 x + B_2 x^2$) or linear equation form ($Y = B_0 + B_1 x$).

Plant Character	Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
Leaf	D	-6.116	(0.946)	1.921	(0.278)	-0.118	(0.020)	0.88	25
	I	-5.046	(1.080)	1.534	(0.276)	-0.076	(0.017)	0.92	25
Stem	D	-7.155	(0.999)	2.032	(0.294)	-0.123	(0.021)	0.90	25
	I	-8.118	(1.190)	2.183	(0.304)	-0.113	(0.019)	0.93	25
Root	D	-3.443	(1.273)	0.823	(0.375)	-0.050	(0.027)	0.47	25
	I	-6.563	(1.801)	1.688	(0.460)	-0.088	(0.029)	0.78	25
Pod	D	-21.155	(1.594)	3.480	(0.210)			0.94	20
	I	-40.533	(3.658)	5.632	(0.427)			0.91	20
Total	D	-4.881	(0.915)	1.616	(0.269)	-0.083	(0.019)	0.95	25
	I	-4.459	(0.798)	1.479	(0.204)	-0.064	(0.013)	0.98	25

Appendix 4.2 Information from growth curves of leaf area per plant (dependent variable), of determinate and indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($\ln(Y) = B_0 + B_1 x + B_2 x^2$).

Growth type		(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
D	-1.032	(0.869)	1.999	(0.256)	-0.124	(0.018)	0.90	25
I	0.653	(1.421)	1.563	(0.363)	-0.084	(0.023)	0.78	25

Appendix 4.3 Information from growth curves of nodule dry weight (dependent variable), of determinate and indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($\text{Ln}(Y) = B_0 + B_1 x + B_2 x^2$).

Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
D	-6.002	(1.043)	1.438	(0.307)	-0.092	(0.022)	0.71	25
I	0.666	(1.214)	-0.436	(0.310)	0.043	(0.019)	0.85	25

Appendix 4.4 Information from curves of integrated acetylene reduction activity (dependent variable) of determinate and indeterminate soybeans, regressed against time (independent variable), using quadratic equation form ($\ln(Y) = B_0 + B_1 x + B_2 x^2$).

Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
D	-11.316	(1.760)	3.237	(0.477)	-0.180	(0.032)	0.96	20
I	- 5.610	(2.627)	1.319	(0.626)	-0.039	(0.037)	0.96	20

Appendix 4.5 Information from linear regression of total plant nitrogen (dependent variable) regressed against integrated acetylene reduction activity (independent variable) with equation form $Y = B_0 + B_1 x$.

Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	R^2	n
D	228.32	(48.07)	16.33	(2.68)	0.67	20
I	505.89	(56.07)	21.11	(2.23)	0.83	20

Appendix 4.6 Information from linear regression of total plant nitrogen (dependent variable) regressed against nodule dry weight (independent variable) with the equation form $\text{Ln}(Y) = B_0 + B_1 x$.

Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	R^2	n
D	3.83	(0.30)	3.57	(0.52)	0.68	25
I	5.55	(0.15)	0.93	(0.12)	0.70	25

Appendix 4.7 Information from growth curves of plant nitrogen (dependent variable), of determinate and indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($\ln(Y) = B_0 + B_1 x + B_2 x^2$), or linear equation form ($Y = B_0 + B_1 x$).

Plant Character	Growth type	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
Leaf	D	-4.372	(1.025)	2.658	(0.301)	-0.180	(0.021)	0.82	25
	I	-0.546	(1.332)	1.517	(0.340)	-0.086	(0.021)	0.67	25
Stem	D	-9.406	(1.225)	3.884	(0.360)	-0.273	(0.026)	0.84	25
	I	-5.112	(1.988)	2.243	(0.508)	-0.121	(0.032)	0.79	25
Pod	D	-891.834	(92.862)	148.871	(12.246)			0.89	20
	I	-1586.686	(146.408)	221.070	(17.077)			0.90	20
Total	D	3.201	(0.984)	2.241	(0.289)	-0.130	(0.021)	0.94	25
	I	1.023	(0.953)	1.050	(0.244)	-0.043	(0.015)	0.95	25

Appendix 5.1 Information from growth curves of total plant dry weight (dependent variable), of determinate and four treatments of indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($\ln(Y) = B_0 + B_1 x + B_2 x^2$).

Treatment	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
UD	-3.201	(0.955)	1.193	(0.281)	-0.054	(0.020)	0.94	25
UI	-5.281	(1.297)	1.655	(0.332)	-0.073	(0.021)	0.95	25
I2	-2.657	(1.614)	0.949	(0.413)	-0.034	(0.026)	0.89	25
I1	-3.492	(1.471)	1.191	(0.376)	-0.048	(0.023)	0.92	25
I2P	-1.326	(1.112)	0.638	(0.284)	-0.016	(0.018)	0.94	25

Appendix 5.2 Information from growth curves of leaf area per plant (dependent variable), of determinate and four treatments of indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($\ln(Y) = B_0 + B_1 x + B_2 x^2$).

Treatment	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
UD	-0.016	(0.895)	1.746	(0.263)	-0.107	(0.019)	0.88	25
UI	-0.114	(1.448)	1.692	(0.370)	-0.090	(0.023)	0.81	25
I2	-0.903	(1.479)	1.882	(0.378)	-0.110	(0.023)	0.64	25
I1	0.219	(1.369)	1.626	(0.350)	-0.092	(0.022)	0.70	25
I2P	3.116	(1.263)	0.819	(0.323)	-0.042	(0.020)	0.63	25

Appendix 5.3 Information from growth curves of stem dry weight (dependent variable), of determinate and indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($\text{Ln}(Y) = B_0 + B_1x + B_2x^2$).

Treatment	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
UD	-5.801	(1.059)	1.674	(0.311)	-0.096	(0.022)	0.89	25
UI	-9.044	(1.545)	2.390	(0.395)	-0.123	(0.025)	0.91	25
I2	-8.240	(1.996)	2.177	(0.510)	-0.116	(0.032)	0.79	25
I1	-8.839	(1.975)	2.365	(0.505)	-0.128	(0.031)	0.81	25
I2P	-8.058	(1.237)	2.120	(0.316)	-0.111	(0.020)	0.92	25

Appendix 5.4 Information from growth curves of root dry weight (dependent variable), of determinate and four treatments of indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($\text{Ln}(Y) = B_0 x + B_2 x^2$).

Treatment	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
UD	-1.213	(1.545)	0.361	(0.455)	-0.020	(0.032)	0.10	25
UI	-6.718	(1.417)	0.797	(0.362)	-0.097	(0.022)	0.82	25
I2	-0.521	(2.172)	0.159	(0.566)	-0.001	(0.035)	0.44	25
I1	-5.511	(1.701)	1.486	(0.435)	-0.082	(0.027)	0.62	25
I2P	-3.013	(2.275)	0.911	(0.582)	-0.050	(0.036)	0.27	25

Appendix 5.5 Information from linear regression of shoot:root ratio (dependent variable), of determinate and four treatments of indeterminate soybeans, regressed against time (independent variable) using linear equation form ($Y = B_0 + B_1x$)

Treatment	B_0	(S.E. B_0)	B_1	(S.E. B_1)	R^2	n
UD	-14.668	(2.351)	3.327	(0.329)	0.82	25
UI	- 8.336	(1.900)	1.846	(0.234)	0.73	25
I2	- 6.774	(1.530)	1.632	(0.188)	0.76	25
I1	- 9.789	(2.393)	2.066	(0.295)	0.68	25
I2P	- 9.698	(2.255)	1.917	(0.277)	0.67	25

Appendix 5.6 Information from growth curves of nodule dry weight (dependent variable) of determinate and four treatments of indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($\ln(Y) = B_0 + B_1 x + B_2 x^2$).

Treatment	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
UD	-5.020	(1.348)	1.112	(0.397)	-0.065	(0.028)	0.66	25
UI	-1.817	(1.587)	0.140	(0.406)	0.009	(0.025)	0.81	25
I2	-3.572	(1.456)	0.666	(0.372)	-0.026	(0.023)	0.78	25
I1	-2.737	(1.186)	0.461	(0.303)	-0.016	(0.019)	0.80	25
I2P	-0.916	(1.037)	-0.048	(0.265)	0.017	(0.016)	0.86	25

Appendix 5.7 Information from linear regression of total plant nitrogen (dependent variable) of determinate and four treatments of indeterminate soybeans, regressed against nodule dry weight (independent variable) using linear equation form ($\ln(Y) = B_0 + B_1 x$).

Treatment	B_0	(S.E. B_0)	B_1	(S.E. B_1)	R^2	n
UD	4.190	(0.224)	3.152	(0.349)	0.78	25
UI	5.560	(0.139)	1.234	(0.124)	0.81	25
I2	5.184	(0.103)	1.527	(0.108)	0.90	25
I1	5.150	(0.122)	1.564	(0.119)	0.88	25
I2P	5.288	(0.109)	1.382	(0.112)	0.87	25

Appendix 5.8 Information from growth curves of total plant nitrogen (dependent variable), of determinate and four treatments of indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($Y = B_0 + B_1 + B_2 x^2$).

Treatment	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
UD	-2.886	(0.928)	2.190	(0.273)	-0.124	(0.019)	0.95	25
UI	-1.081	(1.587)	1.623	(0.406)	-0.077	(0.025)	0.89	25
I2	0.368	(1.397)	1.231	(0.357)	-0.056	(0.022)	0.89	25
I1	0.535	(1.329)	1.226	(0.340)	-0.056	(0.021)	0.89	25
I2P	2.199	(0.951)	0.777	(0.243)	-0.028	(0.015)	0.94	25

Appendix 5.9 Information from growth curves of leaf and stem nitrogen (dependent variable), of determinate and four treatments of indeterminate soybeans, regressed against time (independent variable) using quadratic equation form ($\ln(Y) = B_0 + B_1 x + B_2 x^2$).

Treatment	B_0	(S.E. B_0)	B_1	(S.E. B_1)	B_2	(S.E. B_2)	R^2	n
UD	-5.610	(1.054)	3.092	(0.310)	-0.201	(0.022)	0.90	25
UI	-2.974	(1.867)	2.179	(0.477)	-0.120	(0.030)	0.76	25
I2	-2.609	(1.403)	2.105	(0.359)	-0.122	(0.022)	0.75	25
I1	-2.335	(1.487)	2.070	(0.380)	-0.119	(0.024)	0.72	25
I2P	-0.157	(0.970)	1.458	(0.248)	-0.079	(0.015)	0.85	25

Appendix 5.10 Information from linear regression of pod nitrogen (dependent variable) of determinate, and four treatments of indeterminate soybeans, regressed against time (independent variable) using linear equation form ($Y = B_0 + B_1 x$).

Treatment	B_0	(S.E. B_0)	B_1	(S.E. B_1)	R^2	n
UD	-1396.33	(192.11)	210.09	(23.89)	0.85	15
UI	-2308.14	(225.81)	302.84	(24.99)	0.92	15
I2	-2172.21	(245.21)	282.31	(27.13)	0.89	15
I1	-2318.72	(225.90)	301.39	(24.99)	0.92	15
I2P	-1923.26	(133.20)	248.03	(14.74)	0.96	15

REFERENCES

- Adams, M.W. (1975).
 Plant architecture and yield in grain legumes. Report of the T.A.C. working group on the biology of yield of grain legume.
 Rome, FAO, 16p.
- Alberda, T. and J.M. Witlage-de Boer (1983).
 Distribution of dry matter and nitrogen between the different plant parts in intact and depodded soya bean plants after flowering.
Netherland Journal of Agriculatural Science 31:171-179
- Allen, E.K. and O.N. Allen (1961).
 Nitrogen fixation. The scope of nodulation in leguminosae.
Recent Advances in Botany 1:585-588
- Alvey, N.G. et al. (1977).
Genstat statistic program.
 Rothamsted Experimental Station.
- Anderson, J.A.D. (pers. comm.) Crop Research Division, DSIR, Pukekohe, New Zealand.
- Anderson, L.R. and B.L. Vasilas (1985).
 Effects of planting date on seasonal patterns of nitrogen assimilation and partitioning by two soybean cultivars.
Journal of Plant Nutrition 8:657-677
- Andrew, C.S. (1977).
 Nutritional restraints on (forage) legume symbiosis pp 253-274 in Vincent, J.M., A.S. Whitney and J. Bose (eds) Exploiting the legume-Rhizobium symbiosis in tropical agriculture.
 College of Tropical Agriculture, University of Hawaii Misc. Pub. #145
- Andrew, C.S. and M.F. Robins (1969).
 The effect of phosphorus on the growth and chemical composition of some tropical pasture legumes in growth and critical percentage of phosphorus.
Australian Journal of Agricultural Research 20:665-674
- Anon. (1981).
 The DSIR Climate Laboratory. Alpha: DSIR Extension information # 14.
- Atkins, C.A. (1984).
 Efficiencies and inefficiencies in the legume/rhizobium symbiosis -A review.
Plant and Soil 82:273-284

- Atkins, C.A., D.F. Herridge and J.S. Pate (1978).
The economy of carbon and nitrogen in nitrogen-fixing annual legumes. pp 211-242 in Isotopes in biological dinitrogen fixation.
Vienna, International Atomic Energy Agency.
- Ayanaba, A. (1980).
The potential contribution of nitrogen from rhizobia -A review.
Soil Bulletin 43:211-219
- Baker, D.A., G.P. Chapman, M. Standish and M. Bailey (1983).
Assimilate partitioning in a determinate variety of field bean pp 191-199 in Jones, D.G. and D.R. Davies (eds) Temperate legumes: physiology genetics and nodulation.
London, Pitman Advance Publishing Program.
- Bayne, H.G., M.S. Brown and G.J. Bethlenfalvay (1984).
Defoliation effects on mycorrhizal colonization, nitrogen fixation and photosynthesis in the Glycine-Glomus-Rhizobium symbiosis.
Physiologia Plantarum 62:576-580
- Beaver, J.S. and R.R. Johnson (1981).
Yield stability of determinate and indeterminate soybeans adapted to the northern United States.
Crop Science 21:449-454
- Beaver, J.S., R.L. Cooper and R.J. Martin (1985).
Dry matter accumulation and seed yield of determinate and indeterminate soybeans.
Agronomy Journal 77:765-769
- Bell, F. and P.S. Nutman (1971).
Experiments on nitrogen fixation by nodulated lucerne.
Plant and Soil special volume:231-261
- Bello, A.B., W.A. Ceron-Diaz, C.D. Nickell, E.O. El Sherif and L.C. Davis (1980).
Influence of cultivar, between-row spacing and plant population of fixation of soybeans.
Crop Science 20:751-755
- Bergersen, F.J. (1970).
The quantitative relationship between nitrogen fixation and the acetylene reduction assay.
Australian Journal of Biological Sciences 23:1015-1025
- Bergersen, F.J. (1975).
Factors controlling N fixation in Rhizobium pp 152-165 in Ayanaba, A. et al. (eds) Biological nitrogen fixation in farming systems of the tropics.
New York, John Wiley and sons.

- Bergersen, F.T. (1977).
Physiological chemistry of dinitrogen fixation by legumes pp 519-556 in Hardy, R.W.F. and W.S. Silver (eds) A treatise on dinitrogen fixation Section III. Biology. New York, Wiley Interscience.
- Bergersen, F.J. (1980).
(ed) Methods of evaluating biological nitrogen fixation. Chichester, John Wiley and Sons, 702p.
- Bergersen, F.J. (1982).
Root nodules of legumes: structure and functions . Chichester, Research Studies Press, 250p.
- Bergersen, F.J. and D.J. Goodchild (1973).
Cellular location and concentration of leghaemoglobin in soybean root nodules.
Australian Journal of Biological Science 26:741-756
- Beringer, J.E. (1984).
The significance of symbiotic nitrogen fixation in plant production.
CRC Critical Reviews in Plant Sciences 1:269-286
- Bernard, R.L. (1972).
Two genes affecting stem termination in soybeans.
Crop Science 12:235-239
- Bethlenfalvay, G.J. and D.A. Phillips (1977a).
Ontogenetic interactions between photosynthate and symbiotic nitrogen fixation in legumes.
Plant Physiology 60:419-421
- Bethlenfalvay, G.J. and D.A. Phillips (1977b).
Effect of light intensity on efficiency of carbon dioxide and nitrogen reduction in Pisum sativum L.
Plant Physiology 60:868-871
- Bethlenfalvay, G.J., S.S. Abu-Shakra, and D.A. Phillips (1978a).
Interdependent of nitrogen nutrition and photosynthesis in Pisum sativum L.
Plant Physiology 62:127-130
- Bethlenfalvay, G.J. ,S.S. Abu-Shakra, K. Fishbeck and D.A. Phillips (1978b).
The effect of source-sink manipulations on nitrogen fixation in peas.
Physiologia Plantarum 43:31-34

- Beuerlein, J.E., J.W. Pendleton, M.E. Bauer and S.R. Ghorashy (1971).
Effect of branch removal and plant populations at equidistant spacings on yield and light use efficiency of soybean canopies.
Agronomy Journal 63:317-319
- Bhagsari, A.S., D.A. Ashley, R.H. Brown and H.R. Boerma (1977).
Leaf photosynthetic characteristics of determinate soybean cultivars.
Crop Science 17:929-932
- Board, J.E. (1985).
Yield components associated with soybean yield reductions at nonoptimal planting dates.
Agronomy Journal 77:135-140
- Bremner, J.M. and C.S. Mulvaney (1982).
Nitrogen-total pp 595-624 in Page, A.L., R.H. Miller and D.R. Keeney (eds) Method of soil analysis part 2 Chemical and microbial properties 2nd editions.
Wisconsin, American Society of Agronomy.
- Brockwell, J.(1971).
An appraisal of an IBP experiment on nitrogen fixation by nodulated legumes.
Plant and Soil special volume:265-272
- Broughton, W.J. (1981).
(ed) Nitrogen fixation Vol. I Ecology.
New York, Clarendon Press, 306p.
- Broughton, W.J. (1982).
(ed) Nitrogen fixation Vol. II. Rhizobium.
New York, Clarendon Press, 353p.
- Broughton, W.J. (1983).
(ed) Nitrogen fixation Vol. III. Legumes.
New York, Clarendon Press, 339p.
- Brouwer, R. (1962).
Nutritive influences on the distribution of dry matter in the plant.
Netherland Journal of Agricultural Science 10:399-408

- Brun, W.A. (1978).
Assimilation pp 45-76 in Norman, A.G. (ed) Soybean physiology agronomy and utilization.
New York, Academic Press.
- Bushby, H.V.A. (1982).
Ecology pp 35-75 in Broughton, W.J. (ed.) Nitrogen fixation
vol. 2 Rhizobium.
Oxford, Clarendon Press.
- Buttery, B.R. (1969).
Analysis of the growth of soybeans as affected by plant
population and fertilizer.
Canadian Journal of Plant Science 49:675-684
- Carlson, J.B. (1973).
Morphology p 17-95 in Caldwell, B.E. (ed) Soybean
improvement, production and uses.
Wisconsin, American Society of Agronomy.
- Chaturvedi, G.S., P.K. Aggarwal and S.K. Sinha (1980).
Growth and yield of determinate and indeterminate cowpeas
in dryland agriculture.
Journal of Agricultural Science 94:137-144
- Chen, C.L. and F.J.M. Sung (1982).
Effect of source-sink manipulation on nitrogen fixation in
mungbeans.
Field Crop Research 5:225-231
- Chen, H.K. and H.G. Thornton (1940).
The structure of 'ineffective' nodules and its influence on
nitrogen fixation.
Proceedings of Royal Society 129B:208-229
- Chu, A.C.P. and A.G. Robertson (1974).
The effects of shading and defoliation on nodulation and
nitrogen fixation by white clover.
Plant and Soil 41:509-519
- Constable, G.A. and A.B. Hearn (1978).
Agronomic and physiological responses of soybean and sorghum
crops to water deficits. I. Growth, development and yield.
Australian Journal of Plant Physiology 5:159-167
- Cooley, W.W. and P.R. Lohnes (1971).
Multivariate data analysis.
New York, John Wiley and Sons, 364p.

- Dart, P. (1977).
Infection and development of leguminous nodules. pp 367-472
in Hardy, R.W.F. (ed) A treatise on dinitrogen fixation
section iii. Biology.
New York, John Wiley & sons.
- Dart, R.A. and J. Halliday (1980).
Relationship between Rhizobium and tropical forage legumes
pp 597-601 in Summerfield, R.J. and A.H. Bunting (eds)
Advances in legume Science.
Kew, Royal Botanical Garden.
- Denison, R.F., P.R. Weisz and T.R. Sinclair (1985).
Variability among plants in dinitrogen fixation (Acetylene
reduction) rates by field-grown soybean.
Agronomy Journal 77:947-950
- deMooy, C.T., J. Pesek and E. Spaldon (1973).
Mineral nutrition pp 267-362 in Caldwell, B.E. (ed)
Soybeans: improvement, production and uses.
Wisconsin, American Society of Agronomy.
- Derman, B.D., D.C. Rupp and L.D. Nooden (1978).
Mineral distribution in relation to fruit development and
monocarpic senescence in Anoka soybeans.
American Journal of Botany 65:205-213
- Dilworth, M. (1966).
Acetylene reduction by nitrogen fixing preparations of
Clostridium pasteurianum
Biochimica et Biophysica Acta 127:285-294
- Dilworth, M.J. , A.D. Robson and D.L. Chatel (1979).
Cobalt and nitrogen fixation in Lupinus angustifolius L.
II. nodule formation and function.
New Phytologist 83:63-79
- Dobereiner, J. (1966).
Evaluation of nitrogen fixation in legumes by the regression
of total plant nitrogen with nodule weight.
Nature 210: 850-851
- Dobereiner, J. and A.B. Campelo (1977).
Importance of legumes and their contribution to tropical
agriculture pp 191-220 in Hardy R.W.F. and A.H. Gibson
(eds) A treatise on dinitrogen fixation section iv. agronomy
and ecology
New York, John Wiley & sons.
- Dornhoff, G.M. and R.M. Shibles (1970).
Varietal differences in net photosynthesis of soybean
leaves.
Crop Science 10:42-45

- Dornhoff, G.M. and R.M. Shibles (1976).
 Leaf morphology and anatomy in relation to C_2O_2 exchange rate of soybean leaves.
Crop Science 16:377-381
- Draper, N. and H. Smith (1966).
Applied regression analysis.
 New York, John Wiley and Sons, 407p.
- Duke, S.H., L.E. Schrader, C.A. Henson, J.C. Servaites, R.D. Vogelz and J.W. Pendleton (1979).
 Low root temperature effects on soybean nitrogen metabolism and photosynthesis.
Plant physiology 63:956-962
- Dunphy, E.J., J.J. Hanway and D.E. Green (1979).
 Soybean yields in relation to days between specific developmental stages.
Agronomy Journal 71:917-920
- Eaglesham, A.R.J., F.R. Minchin, R.J. Summerfield, P.J. Dart, P.A. Huxley and J.M. Day (1977).
 Nitrogen nutrition of cowpea (Vigna unguiculata) III. distribution of nitrogen within effectively nodulated plants.
Experimental Agriculture 13:369-380
- Edwards, D.G. (1975).
 Nutritional factors limiting nitrogen fixed by Rhizobia pp 189-294 in Ayanaba, A. et al. (eds) Biological nitrogen fixation in farming systems of the tropics.
 New York, John Wiley and Sons.
- Egli, D.B. and J.E. Leggett (1973).
 Dry matter accumulation patterns in determinate and indeterminate soybeans.
Crop Science 13:220-222
- Egli, D.B., E. Leggett and W.G. Duncan (1978a).
 Influence of N stress on leaf senescence and N redistribution in soybean.
Agronomy Journal 70:43-47
- Egli, D.B., J.E. Leggett and J.M. Wood (1978b).
 Influence of soybean seed size and position on the rate and duration of filling.
Agronomy Journal 70:127-130
- Egli, D.B., R.D. Guffy and J.E. Leggett (1985).
 Partitioning of assimilate between vegetative and reproductive growth in soybean.
Agronomy Journal 77:917-922

- Elton, J. and A.H. Bunting (1980).
Water relations of legume crops pp 37-42 in Summerfield,
R.J. and A.H. Bunting (eds) Advances in legume Science.
Kew, Royal Botanical Garden.
- Enyi, B.A.C. (1975).
Effects of defoliation on growth and yield in groundnut
(Arachis hypogea), cowpea (Vigna unguiculata), soybean
(Glycine max) and green gram (Vigna aureus).
Annals of Applied Biology 79:55-66
- Ezedinma, F.O.C. (1973).
Effects of defoliation and topping on semi-upright cowpea
(Vigna unguiculata (L.) Walp.) in a humid tropical
environment.
Experimental Agriculture 11:177-182
- Fehr, W.R., B.K. Lawrence and T.A. Thompson (1981).
Critical stages of development for defoliation of soybean.
Crop Science 21:259-262
- Fehr, W.R., C.E. Caviness and J.J. Vorst (1977).
Response of indeterminate and determinate soybean cultivars
to defoliation and half-plant cut-off.
Crop Science 17:913-917
- Flinn, A.M., C.A. Atkins and J.S. Pate (1977).
Significance of photosynthetic and respiratory exchanges in
the carbon economy of the developing pea fruit.
Plant Physiology 60:412-418
- Gallacher, A.E. and J.I. Sprent (1978).
The effect of different water regimes on growth and nodule
development of greenhouse grown V. faba.
Journal of Experimental Botany 27:1233-1241
- Gate, C.T. (1974).
Nodule and plant development in Stylosanthes humilis H.B.K.
symbiotic response to phosphorus and sulphur.
Australian Journal of Biology 22:45-55
- Gibson, A.H. (1965).
Physical environment and symbiotic nitrogen fixation II.
root temperature effects on the relative nitrogen
assimilation rate.
Australian Journal of Biological Science 18:295-310
- Gibson, A.H. (1971).
Factors in the physical and biological environment affecting
nodulation and nitrogen fixation by legume.
Plant and Soil special volume:139-152

- Gibson, A.H. (1974).
Consideration of the growing legume as a symbiotic association.
Indian National Science Academy Proceedings 40B:741-767
- Gibson, A.H. (1976).
Recovery and compensation by nodulated legumes to environmental stress. pp 385-403 in Nutman, P.S. (ed) Symbiotic nitrogen fixation in plant.
New York, Cambridge University Press.
- Gibson, A.H. (1977).
The influence of environment and managerial practices on the legume-rhizobium symbiosis . pp 393-450 in Hardy R.W.F. and A.H. Gibson (eds) A treatise on dinitrogen fixation Section iv. agronomy and ecology.
New York, John Wiley and Sons.
- Gibson, A.H. (1980).
Host determinants in nodulation and nitrogen fixation in Summerfield, R.J. and A.H. Bunting (eds) Advances in legume science.
Kew, Royal Botanical Gardens.
- Gibson, A.H., B.L. Dreyfus and Y.R. Dommergues (1982).
Nitrogen fixation by legumes in the tropics pp 37-73 in Dommergues, Y.R. and H.G. Diem (eds) Microbiology of tropical soils and plant productivity.
The Hague, Martinus Nijhoff/Dr W. Junk Publishers.
- Gifford, R.M. and L.T. Evans (1981).
Photosynthesis, carbon partitioning and yield.
Annual Review of Plant Physiology 32:485-509
- Goli, A. and D.B. Weaver (1986).
Defoliation responses of determinate and indeterminate late-planted soybeans.
Crop Science 26:156-159
- Gomez, A.A. and H.G. Zanstra (1977).
An analysis of the role of legumes in multiple cropping systems pp 81-95 in Vincent, J.M., A.S. Whitney and J. Bose (eds) Exploiting the legume-rhizobium symbiosis in tropical agriculture. College of Tropical Agriculture University of Hawaii Misc. Pub # 145 .
- Good, N.E. and D.H. Bell (1980).
Photosynthesis plant productivity and crop yield pp 3-51 in Carlson, P.S. (ed) The biology of crop productivity.
New York, Academic Press.
- Gordon, I.L. (pers. comm.) Agronomy Department, Massey University,
Palmerston North, New Zealand.

- Graham, P.H. (1981).
Some problems of nodulation and symbiotic nitrogen fixation
in Phaseolus vulgaris L: A review.
Field Crop Research 4:93-112
- Graham, P.H. (1982).
Plant factors affecting symbiotic nitrogen fixation in
legumes pp 27-37 in Graham, P.H. and S.C. Harris (eds)
Biological nitrogen fixation technology for tropical
agriculture. Colombia, CIAT
- Graham, P.H. and J. Halliday (1977).
Inoculation and nitrogen fixation in the genus Phaseolus
pp 313-334 in Vincent, J.M., A.S. Whitney and J. Bose (eds)
Exploiting the legume-Rhizobium symbiosis in tropical
agriculture.
College of Tropical Agriculture, University of Hawaii Misc
Publication#145
- Graham, R.A. and T. Setter (1983).
Correlations of plant parameters with nitrogen fixation in
cowpea.
Biological Agriculture and Horticulture 1:335-338
- Graham, R.A. and T.W. Scott (1983).
Varietal characteristics and nitrogen fixation in cowpea.
Tropical Agriculture 60:269-271
- Green, D.E., P.F. Burlamaqui and R.M. Shibles (1977).
Performance of randomly selected soybean lines with
semideterminate and indeterminate growth habits.
Crop Science 17:335-339
- Greer, H.A.L. and I.C. Anderson (1965).
Response of soybeans to triodobenzoic acid under field
conditions.
Crop Science 5:229-232
- Hackett, C. and H.M. Raqson (1974).
An exploration of the carbon economy of the tobacco plant
II. Patterns of leaf growth and dry matter partitioning.
Australian Journal of Plant Physiology 1:271-281
- Ham, G.E., I.E. Liener, S.D. Evans, R.D. Frazier and W.W. Nelson
(1975).
Yield and composition of soybean as affected by N and S
fertilization.
Agonomy Journal 67:293-297

- Ham, G.E., R.J. Lawn and W.A. Brun (1976).
Influence of inoculation, nitrogen fertilizers and
photosynthetic source-sink manipulations on field-grown
soybeans pp 239-253 in Nutman, P.S. (ed) Symbiotic nitrogen
fixation in plants
London, Cambridge University Press.
- Hammerton, J.L. (1975).
Effects of defoliation on pigeon peas (*Cajanus cajan*).
Experimental Agriculture 11:177-182
- Hanway, J.J. (1976).
Interrelated developmental and biochemical processes in the
growth of soybean plants. pp 5-15 in Hill, L.D. (ed) World
Soybean Research.
Illinois, Interstate Printers and Publishers.
- Hanway, J.J. and C.R. Weber (1971a).
Dry matter accumulation in soybean (*Glycine max* (L)) plants
influenced by N, P, and K fertilization.
Agronomy Journal 63:263-266
- Hanway, J.J. and C.R. Weber (1971b).
N, P, and K percentages in soybeans (*Glycine max* (L))
plants.
Agronomy Journal 63:286-290
- Hanway, J.J. and C.R. Weber (1971c).
Accumulation of N, P, and K by soybeans (*Glycine max* (L))
plants.
Agronomy Journal 63:406-408
- Hanway, J.J. and C.R. Weber (1971d).
Dry matter accumulation in eight soybean varieties.
Agronomy Journal 63:227-230
- Hardy, R.W., R.C. Burns, R.R. Herbert, R.D. Holsten and E.K.
Jackson (1971).
Biological nitrogen fixation: a key to world protein.
Plant and soil special volume: 561-590
- Hardy, R.W.F. and U.D. Halvelka (1975).
Nitrogen fixation research: a key to world food.
Science 188:633-643
- Hardy, R.W.F. and U.D. Havelka (1976).
Photosynthate as a major limiting nitrogen fixation by
field-grown legumes with emphasis on soybeans. pp 421-439 in
Nutman, P.S. (ed) Symbiotic nitrogen fixation in plant.
London, Cambridge University Press.

- Hardy, R.W.F., J.G. Creswell and U.D. Havelka (1977).
Investigations of possible limitations of nitrogen fixation by legumes: 1. methodology, 2. identification and 3. Assessment of significance pp 451-468 in Newton, W., J.R. Postgate and D. Rodriguez-Barrueco (eds) Recent developments in nitrogen fixation.
London, Academic Press.
- Hardy, R.W.F., R.C. Burns and P.D. Holsten (1973).
Applications of the acetylene-ethylene assay for measurement of nitrogen fixation.
Soil Biology Biochemistry 5:47-81
- Hardy, R.W.F., U.D. Harvelka and P.G. Heytler (1980).
Nitrogen input with emphasis on nitrogen fixation. pp in Corbin, F.T. (ed) World Soybean Research Conference II Proceedings.
Cororado, Westview Press.
- Harper, J.E. and R.H. Hageman (1972).
Canopy and seasonal profiles of nitrate reductase in soybeans (Glycine max L. Merr).
Plant Physiology 49:146-154
- Havelka, U.D., M.G. Boyle, and R.W.F. Hardy (1982).
Biological nitrogen fixation pp 364-422 in Stevenson F.J. (ed) Nitrogen in agricultural soils. Agronomy Monograph #22
Wisconsin, American Society of Agronomy.
- Heitholt, J.J. and D.B. Egli (1985).
Influence of deflowering on dry matter production of soybeans.
Field Crop Research 12:169-173
- Herridge, D.F. and J.S. Pate (1977).
Utilization of net photosynthate for nitrogen fixation and protein production in an annaul legume.
Plant Physiology 60:759-764
- Hicks, D.R. (1978).
Growth and development. pp 17-44 in Norman A.G. (ed) Soybean physiology, agronomy and utilization.
New York, Academic Press.
- Hicks, D.R. and J.W. Pendleton (1969).
Effect of floral bud removal on performance of soybeans.
Crop Science 9:435-437
- Hicks, D.R., J.W. Pendleton, R.L. Bernard and T.J. Johnston (1969).
Response of soybean plant types to planting patterns.
Agronomy Journal 61:290-293

- Hinson, K. and E.E. Hartwig (1977).
Soybean production in the tropics.
Rome, F.A.O., 92p.
- Hume, L.J. (1981).
An investigation into the efficiency of nitrogen fixation in
sainfoin (Onobrychis viciifolia Scop.) M.Sc Thesis
Massey University, Palmerston North, New Zealand.
- Humphries, E.C. and G.N. Thorne (1964).
The effects of root formation on photosynthesis of detached
leaves.
Annals of Botany 28:391-400
- Hunt, R. (1982).
Plant growth curves the functional approach to plant growth
analysis.
London, Edward Arnold, 248p.
- Hunt, R. and I.T. Parsons (1974).
A computer program for deriving growth-functions in plant
growth-analysis.
Journal of Applied Ecology 11:297-307
- Hunter, M.N., P.L.M. de Fabrun and D.E. Byth (1980).
Response of nine soybean lines to soil moisture conditions
close to saturation.
Australian Journal of Experimental Agriculture and Animal
Husbandry 20: 339-345
- Igwilo, N. (1982).
Nodulation and nitrogen accumulation in field beans (Vicia
faba L.)
Journal of Agricultural Science 98:269-288
- Jackson, J.E. (1963).
Relationship of relative leaf growth rate to net
assimilation rate and its relevance to the physiological
analysis of plant yield.
Nature 197:909
- Jeppson, R.G., R.R. Johnson and H.H. Hadley (1978).
Variation in mobilization of plant nitrogen to the grain in
nodulating and non nodulating soybean genotypes.
Crop Science 18:1058-1062
- Johnson, D. and J. Dunphy (1983).
How soybean grow understanding and managing their
development.
Crop and Soil Magazine April-May :11-15

- Johnson, H.S. and D.J. Hume (1973).
Comparisons of nitrogen fixation estimates in soybeans by
nodule weight, leghemoglobin content, and acetylene
reduction.
Canadian Journal of Microbiology 19:1165-1168
- Jones, R.K., P.J. Robinson, K.P. Haydock and R.G. Megarrity
(1971).
Sulphur-nitrogen relationships in the tropical legume
Stylosanthes humilis.
Australian Journal of Agricultural Research 22:885-894
- Kato, Y. (1980).
Studies on nitrogen metabolism of soybean plants 5. The
effect of partial defoliation on the turn-over rate of total
protein in leaves.
Japanese Journal of Crop Science 49:405-411
- Khan, T.N. (1977).
Yield potential for tropical legumes from a geneticist's
point of view. pp 21-37 in Vincent, J.M., A.S.W. Whitney and
J. Bose (eds) Exploiting the legume-rhizobium symbiosis in
tropical agriculture.
College of Tropical Agriculture, University of Hawaii
Misc Publication #145
- King, P.P. (1983).
Nitrogen from the atmosphere to the crop.
Outlook on Agriculture 12:90-97
- Klecka, W.R. (1975).
Discriminant analysis pp 434-467 in Nie, N.H. et al. (eds)
SPSS statistical package for the social sciences second
edition.
New York, McGraw-Hill Inc.
- Ko, M.P., K.R. Barker and J.S. Huang (1984).
Nodulation of soybeans as affected by half-root infection
with Heterodera glycines.
Journal of Nematology 16:97-105
- Koller, H.R., W.E. Nyquist and I.S. Chorush (1970).
Growth analysis of soybean community.
Crop Science 10:407-412
- Kollman, G.E., J.G. Streeter, D.L. Jeffers and R.B. Curry (1974).
Accumulation and distribution of mineral nutrients,
carbohydrate and dry matter in soybean plants as influenced
by reproductive sink size.
Agronomy Journal 66:549-554

- Kretchmer, P.J., J.L. Ozbun, S.L. Kaplan, D.R. Laing and D.H. Wallace (1977).
Red and far-red light effects on climbing in Phaseolus vulgaris L.
Crop Science 17:797-799
- Kuo, T. and L. Boersma (1971).
Soil water suction and root temperature effects on nitrogen fixation in soybean.
Agronomy Journal 63:901-904
- Laohasiriwong, S. (1982).
Effects of duration of water stress at different growth stages on growth and yield of soybeans (Glycine max (L.) Merrill). M.Agr.Sc Thesis Massey University, Palmerston North, New Zealand.
- Laohasiriwong, S. (1985).
Yield response of selected soybean cultivars to water stress during different reproductive growth periods pp 383-386 in Shanmugasundaram, S. and E.W. Sulzberger (eds) Soybean in tropical and subtropical cropping system. Taiwan, AVRDC.
- LaRue, T.A. and T.G. Patterson (1981).
How much nitrogen do legume fix?
Advance in Agronomy 34:15-39
- LaRue, T.A.G. and W.G.W Kurz (1973).
Estimation of nitrogenase in intact legumes.
Canadian Journal of Microbiology 19:304-305
- Latimore, J. Jr., J. Giddens and D.A. Ashley (1977).
Effect of ammonium and nitrate N upon photosynthate supply and N fixation by soybeans.
Crop Science 17:399-404
- Lawn, R.J. and W.A. Brun (1974a).
Symbiotic nitrogen fixation in soybeans I. Effect of photosynthetic source-sink manipulations.
Crop Science 14:11-16
- Lawn, R.J. and W.A. Brun (1974b).
Symbiotic nitrogen fixation in soybeans. III. Effect of supplement nitrogen and intervarietal grafting.
Crop Science 14:22-25
- Lawn, R.J. and H.V.A. Bushby (1982).
Effect of root, shoot and Rhizobium strain on nitrogen fixation in four Asiatic Vigna species.
New Phytologist 92:425-434

- Lawn, R.J. and D.E. Byth (1973).
Response of soya beans to planting date in south-eastern Queensland I. Influence of photoperiod and temperature on phasic developmental patterns.
Australian Journal of Agricultural Research 24:67-80
- Lawn, R.J. and D.E. Byth (1974).
Response of soya beans to planting date in south-eastern Queensland II. Vegetative and reproductive development.
Australian Journal of Agricultural Research 25:723-737
- Lawn, R.J. and D.E. Byth (1979).
Soybeans pp 198-231 in Lazenby, A. and J.V. Lovett (eds)
Australian field crops II.
Sydney, Angus and Robertson.
- Lawn, R.J., D.E. Byth and V.E. Mungomery (1977).
Response of soybeans to planting date in South-eastern Queensland. III. Agronomic and physiological response of cultivars to planting arrangements.
Australian Journal of Agricultural Research 28:63-79
- Lawn, R.J., K.S. Fischer and W.A. Brun (1974).
Symbiotic nitrogen fixation in soybeans II.
Interrelationship between carbon and nitrogen assimilation.
Crop Science 14:17-22
- Li, C.C. (1975).
Path analysis -a primer.
California, The Boxwood Press, 347p.
- Lie, T.A. (1981).
Environmental physiology of the legume-Rhizobium symbiosis pp 105-134 in Broughton, W.J. (ed.) Nitrogen fixation vol. I Ecology.
Oxford, Clarendon Press.
- Lindstrom, E.S., J.W. Newton, and P.W. Wilson (1952).
The relationship between photosynthesis and nitrogen fixation.
Proceedings of the National Academy of Science, U.S.A. 38:392-396
- Loberg, G.L., R. Shibles, D.E. Green and J.J. Hanway (1984).
Nutrient mobilization and yield of soybean genotypes.
Journal of Plant Nutrition 7:1311-1327
- Loomis, R.S., R. Rabbinge and E. Ng (1979)
Explanatory models in crop physiology.
Annual Review of Plant Physiology 30:339-367

- Lowther, W.L. and J.F. Loneragan (1968).
Calcium and nodulation in subterranean clover (Trifolium subterraneum L.)
Plant Physiology 43:1362-1366
- Mague, T.H. and R.H. Burris (1972).
Reduction of acetylene and nitrogen by field-grown soybeans.
New Phytologist 71:275-286
- Mahon, J.D. (1977).
Respiration and the energy requirement for nitrogen fixation in nodulated pea roots.
Plant Physiology 60:817-821
- Malik, N.S.A. (1983).
Grafting experiments on the nature of the decline in N₂ fixation during fruit development in soybean.
Physiologia Plantarum 57:561-564
- Manhart, J.S. and P.P. Wong (1980).
Nitrate effect on nitrogen fixation (acetylene reduction)
Plant Physiology 65:502-505
- McClure, P.R. and D.W. Israel (1979).
Transport of nitrogen in the xylem of soybean plants.
Plant Physiology 64:411-416
- McEwen, J. (1972).
Effects of defoliating different zones on the plant in field beans (Vicia faba L.)
Journal of Agricultural Science 78:487-490
- Minchin, F.R. and J.S. Pate (1973).
The carbon balance of a legume and the functional economy of its root nodules.
Journal of Experimental Botany 24:259-271
- Minchin, F.R. and J.S. Pate (1974).
Diurnal functioning of the legume root nodule.
Journal of Experimental Botany 25:295-308
- Minchin, F.R., R.J. Summerfield, P. Hadley, E.H. Roberts and S. Rawsthorne (1981).
Carbon and nitrogen nutrition of nodulated roots of grain legumes.
Plant cell and environment 4:5-26
- Mitchell, R.L. and W.J. Russell (1971).
Root development and rooting patterns of soybean (Glycine max (L) Merrill) evaluated under field condition.
Agronomy Journal 63:313-316

- Mitchell, R.L. and W.J. Russell (1971).
Root development and rooting patterns of soybean
(Glycine max L. Merrill) evaluated under field conditions
Agronomy Journal 63:313-316
- Monteith, J.L. (1977).
Climate and the efficiency of crop production in Britain.
Philosophy Transaction of Royal Society (London) 281B:277-294
- Munns, D.N. (1970).
Nodulation of Medicago sativa in solution culture V. Calcium
and pH requirements during infection.
Plant and Soil 32:90-102
- Munns, D.N. (1977).
Mineral nutrition and legume symbiosis pp 353-391 in Hardy
R.W.F. and A.H. Gibson (eds) A treatise on dinitrogen
fixation section iv Agronomy and Ecology.
New York, John Wiley and Sons
- Munns, D.N. (1980).
Mineral nutrition and nodulation pp 47-56 in Corbin, F.T.
(ed) World Soybean Research Conference II proceedings.
Colorado, Westview Press
- Nelson, A.N. and R.W. Weaver (1980).
Seasonal nitrogen accumulation and fixation by soybeans
grown at different densities.
Agronomy Journal 72:613-616
- Neves, M.C.P., R.J. Summerfield and F.R. Minchin (1982).
Effects of complete leaf shading during the late
reproductive period on carbon and nitrogen distribution and
seed production by nodule -dependent cowpea (Vigna
unguiculata)
Tropical Agriculture 59:248-253
- Nicholas, J.C., J.E. Harper and R.H. Hageman (1976).
Nitrate reductase activity in soybean (Glycine max L. Merr)
I. Effect of light and temperature.
Plant Physiology 58:731-735
- Nie, N.H., C.H. Hull, J.G. Jenkins, K. Slembrenner and D.H. Bent
(1975).
SPSS: statistical package for the social sciences, second
edition.
New York, McGraw-Hill 675p.
- Nooden, L.D. (1984).
Integration of soybean pod development and monocarpic
senescence.
Physiologia Plantarum 62:273-284
- Norman, M.J.T., C.J. Pearson and P.G.E. Searle (1984).
The ecology of tropical food crops.
London, Cambridge University Press, 369p.

- Novoa, R. and R.S. Loomis (1981).
Nitrogen and plant production.
Plant and Soil 58:177-204
- Oke, O.L. (1969).
Sulphur nutrition of legumes.
Experimental Agriculture 5:111-116
- Okigbo, B.N. (1978).
Grain legumes in the agriculture of the tropics pp 1-11 in
Singh, S.R., H.F. Van Emden and T.A. Taylor (eds) Pest of
grain legumes :ecology and control.
London, Academic Press.
- Pandey, R.K. and V.B. Singh (1981).
Influence of partial defoliation on dry-matter production
and seed yield of urd bean (Vigna mango L. Hepper).
Journal of Agricultural Science 97:437-443
- Pankhurst, C.E. and J.I. Sprent (1976).
Effects of temperature and oxygen tension on the nitrogenase
and respiratory activities of turgid and water-stressed
soybean and french bean root nodules.
Journal of Experimental Botany 27:1-9
- Pate, J.S. (1976).
Physiology of the reaction of nodulated legumes to
environment pp 335-359 in Nutman, P.S. (ed) Symbiotic
nitrogen fixation in plants.
London, Cambridge University Press.
- Pate, J.S. (1977).
Functional biology of dinitrogen fixation by legumes. pp
473-517 in Hardy, R.W.F. and W.S. Silver (eds) A treatise
on dinitrogen fixation Section III: Biology.
New York, Wiley-Interscience.
- Pate, J.S. (1979)
Plant physiologist amongst the grain legumes.
Journal of the Royal Society of Western Australia 62:83-94
- Pate, J.S. (1980).
Transport and partitioning of nitrogen solutes.
Annual Review of Plant Physiology 31:313-340
- Pate, J.S. and D.B. Layzell (1981).
Carbon and nitrogen partitioning in the whole plant -A
thesis based on empirical modeling pp 94-134 in Bewley, J.D.
(ed) Nitrogen and carbon metabolism.
The Hague, Dr W. Junk Publishers.

- Pate, J.S. and D.F. Herridge (1978).
Partitioning and utilization of net photosynthate for
nitrogen fixation and protein production in an annual
legume.
Plant Journal of Experimental Botany 29:401-412
- Pate, J.S. and F.R. Minchin (1980).
Comparative studies of carbon and nitrogen nutrition of
selected grain legumes. pp 105-114 in Summerfield, R.J. and
A.H. Bunting (eds) Advances in legume Science.
Kew, Royal Botanical Gardens.
- Pate, J.S. and C.A. Atkins (1983).
Nitrogen uptake, transport, and utilization pp 245-298 in
Broughton, W.J. (ed) Nitrogen fixation volume 3:legumes
London, Oxford University Press.
- Patterson, T.G. and T.A. LaRue (1983).
Nitrogen fixation by soybeans: seasonal and cultivar effects
and comparison of estimates.
Crop Science 23:488-492
- Peat, J.R., F.R. Minchin, B. Jeffcoat and R.J. Summerfield
(1981).
Young reproductive structures promote nitrogen fixation in
soybean.
Annals Botany 48:177-182
- Phillips, D.A. (1980).
Efficiency of symbiotic nitrogen fixation in legume.
Annual Review of Plant Physiology 31:29-49
- Postgate, J. (1978).
Nitrogen fixation.
London, Edward Arnold, 68p.
- Postgate, J.R. (1982).
Fundamentals of nitrogen fixation.
Cambridge, Cambridge University Press, 252p.
- Rabie, R.K. (1981).
Nitrogen nutrition of legumes with special concern to seed
production.
Journal of Plant Nutrition 4:175-194
- Rabie, R.K. and K. Kumazawa (1979).
Effect of nitrate application and shade treatment on the
nitrogen fixation and yield of soybean plants.
Soil Science and Plant Nutrition 25:467-476
- Rabie, R.K., Y. Arima and K. Kumazawa (1979).
Growth, nodule activity and yield of soybeans as effected
by the form and application method of combined nitrogen.
Soil Science and Plant Nutrition 25:417-427

- Raper, C.D. Jr. and S.A. Barber (1970).
Rooting system of soybean II Physiological effectiveness as
nutrient absorption surfaces.
Agronomy Journal 62:585-588
- Rennie, R.J. and G.A. Kemp (1981).
Dinitrogen fixation in pea beans (Phaseolus vulgaris) as
effected by growth stage and temperature regime.
Canadian Journal of Botany 59:1181-1188
- Robinson, P.E. (1961).
Root-knot nematodes and legume nodules.
Nature 189:506-507
- Robson, A.D. (1978).
Mineral nutrients limiting nitrogen fixation in legumes
pp 277-293 in Andrew, C.S. and E.J. Kamprath (eds) Mineral
nutrition of legumes in tropical and sub tropical soils.
Melbourne, CSIRO
- Robson, A.D. (1983).
Mineral nutrition pp 36-55 in Broughton, W.J. (ed) Nitrogen
fixation volume 3 Legumes.
Oxford, Clarendon Press
- Roughley, R.J., J.I. Sprent and J.M. Day (1983).
Nitrogen fixation pp 233-260 in Hebblethwaite, P.D. (ed)
The Faba bean (Vicia faba L.): A basis for improvement.
London, Butterworths.
- Ryle, G.J.A., C.E. Powell and A.J. Gordon (1979).
The respiratory costs of nitrogen fixation in soybean cowpea
and white clover II. Comparisons of the cost of nitrogen
fixation and utilization of combined nitrogen.
Journal of Experimental Botany 30:145-153
- Sanders, J.L. and D.A. Brown (1976).
Effect of variations in the shoot:root ratio upon the
chemical composition and growth of soybean.
Agronomy Journal 68:713-717
- Schapaugh, W.T.Jr. and J.R. Wilcox (1980).
Relationship between harvest indices and other plant
characteristics in soybeans.
Crop Science 20:529-533
- Schollhorn, R. and R.H. Burris (1967).
Acetylene as a competitive inhibitor of N₂ fixation.
Proceeding National Academy of Science U.S.A. 58:213-216

- Schubert, K.R and G.J.A. Ryle (1980).
The energy requirements for nitrogen fixation in nodulated legumes pp 85-96 in Summerfield, R.J. and A.H. Bunting (eds) Advances in legume science.
Kew, Royal Botanical Gardens.
- Seddigh, M. and G.D. Jolliff (1984).
Effects of night temperature on dry matter partitioning and seed growth of indeterminate field-grown soybean.
Crop Science 24:704-710
- Sesay, A. and R. Shibles (1980).
Mineral depletion and leaf senescence in soybean as influenced by foliar nutrient application during seed filling.
Annals Botany 45:47-55
- Shama, A.K., B.B. Singh and S.P. Singh (1982).
Relationships among net assimilation rate, leaf area index and yield in soybean (Glycine max (L) Merrill) genotypes.
Photosynthetica 16:115-118
- Shibles, R.M. and C.R. Weber (1966).
Interception of solar radiation and dry matter production by various soybean planting patterns.
Crop Science 6:55-59
- Shimshi, D. (1969).
A rapid field method for measuring photosynthesis with labelled carbon dioxide.
Journal of Experimental Botany 20:381-401
- Shibles, R. (1980).
Adaptation of soybeans to different seasonal durations pp 279-288 in Summerfield, R.J. and A.H. Bunting (eds) Advances in legume science.
Kew, Royal Botanical Gardens.
- Shibles, R., I.C. Anderson and A.H. Gibson (1975).
Soybean pp 151-189 in Evans, L.T. (ed) Crop physiology some cases histories.
New York, Cambridge University Press.
- Silberbush, M. and S.A. Barber (1984).
Phosphorus and potassium uptake of field-grown soybean cultivars predicted by a simulation model.
Soil Science Society of America Journal 48:592-596
- Sims, A.P., B.f. Folkes, D.J. Barber and D. Walls (1983).
The relative importance of nitrogen assimilation and dinitrogen fixation in the economy of the pea crop. pp 159-174 in Jones, D.G. and D.R. Davies (eds) Temperate legumes physiology, genetics and nodulation
London, Pitman Books Limited.

- Sinclair, T.R. and C.T. de Wit (1975).
Photosynthate and nitrogen requirements for seed production
by various crop.
Science 189:565-567
- Sinclair, T.R. and C.T. de Wit (1976).
Analysis of the carbon and nitrogen limitations to soybean
yield.
Agronomy Journal 68:319-324
- Sinclair, T.R., S.C. Speath and J.S. Vendeland (1981).
Microclimate limitations to crop yield pp 3-27 in Miller,
M.H., D.M. Brown and E.G. Beauchamp (eds) Breaking the
soil/climate barriers to crop yield.
Canada, University of Guelph.
- Sivakumar, M.V.K. and R.H. Shaw (1978).
Methods of growth analysis in field-grown soya beans
(Glycine max (L) Merrill).
Annals Botany 42:213-222
- Sivakumar, M.V.K., H.M. Taylor and R.H. Shaw (1977).
Top and root relations of field-grown soybeans.
Agronomy Journal 69:470-473
- Skrdleta, V., V. Nasinec, A. Hyndrakova and M. Nemcova (1978)
Dinitrogen fixation- acetylene reduction in soybeans during
the reproductive growth period.
Biologia Plantarum 20:210-216
- Smartt, J. (1976).
Tropical Pulses.
London, Longman, 348p.
- Smith, F.M. (1982).
Mineral nutrition of legumes pp 155-172 in Vincent, J.M.
(ed) Nitrogen fixation in legumes.
Australia, Academic Press.
- Speath, S.C. and T.R. Sinclair (1983).
Variation in nitrogen accumulation and distribution among
soybean cultivars.
Field Crop Research 7:1-12
- Speath, S.C., H.C. Randall, T.R. Sinclair and J.S. Vendeland
(1984).
Stability of soybean harvest index.
Agronomy Journal 76:482-486
- Spencer, K. (1959).
Growth and chemical composition of white clover as effected
by sulphur suuply.
Australian Journal of Agricultural Research 10:500-509

- Sprent, J.I. (1976).
 Nitrogen fixation by legumes subjected to water and light stresses pp 405-420 in Nutman, P.S. (ed.) Symbiotic nitrogen fixation in plants.
 New York, Cambridge University Press.
- Sprent, J.I. (1979).
The biology of nitrogen fixing-organisms.
 London, McGraw Hill. 196p.
- Sprent, J.I. (1986).
 Nitrogen fixation in a sustainable agriculture.
Biological Agriculture and Horticulture 3:153-165
- Stanley, C.D., T.C. Kaspar and H.M. Taylor (1980).
 Soybean top and root response to temporary water tables imposed at three different stages of growth.
Agronomy Journal 72:341-346
- Stephenson, R.A. and G.L. Wilson (1977a).
 Pattern of assimilate distribution in soybeans at maturity. I. The influence of reproductive developmental stage and leaf position.
Australian Journal of Agricultural Research 28:203-209
- Stephenson, R.A. and G.L. Wilson (1977b).
 Pattern of assimilate distribution in soybeans at maturity. II. The time course of changes in ¹⁴C distribution in pods and stem sections.
Australian Journal of Agricultural Research 28:395-400
- Streeter, J.G. (1978).
 Effect of N starvation of soybean plants at various stages of growth on seed yield and N concentration of plant at maturity.
Agronomy Journal 70:74-76
- Summerfield, R.J. (1977).
 Vegetative growth, reproductive ontogeny and seed yield of selected tropical grain legumes. pp 251-271 in McFarlane, N.R. (ed) Crop production agent:- their biological evaluation.
 London, Academic Press.
- Summerfield, R.J., P.J. Dart, P.A. Huxley, A.R. Eaglesham, F.R. Minchin and J.M. Day (1977).
 Nitrogen nutrition of cowpea (Vigna unguiculata) I. Effects of applied nitrogen and symbiotic nitrogen fixation on growth and seed yield.
Experimental Agriculture 13:129-142

- Summerfield, R.J. and H.C. Wein (1980).
Effects of photoperiod and air temperature on growth and yield of economic legumes pp 17-36 in Summerfield R.J. and A. H. Bunting (eds) Advances in legume science.
Kew, Royal Botanical Gardens.
- Sutton, W.D. (1983).
Nodule development and senescence. pp 144-212 in Broughton W.J. (ed) Nitrogen fixation volume 3:legumes.
London, Oxford University Press.
- Taha, A.H.Y. and D.J. Raski (1969).
Interrelationships between root-nodule bacteria, plant-parasitic nematodes and their leguminous host.
Journal of Nematology 1:201-211
- Tanaka, A. (1980).
Source and sink relationship in crop production. Technical Bulletin No. 52
Taiwan, Food and Fertilizer Technology Center.
- Tanner, J.W. and S. Ahmed (1974).
Growth analysis of soybean treated with TIBA
Crop Science 14:371-374
- Thomas, G.D., C.M. Ignoffo, K.D. Biever and D.B. Smith (1974)
Influence of defoliation and depodding on yield of soybeans.
Journal of Economic Entomology 67:683-685
- Thomas, J.F. and C.D. Raper, Jr. (1978).
Effect of day and night temperatures during floral induction on morphology of soybeans.
Agronomy Journal 70:893-898
- Thomas, J.F., C.D. Raper, Jr., and W.W. Weeks (1981).
Day and night temperature effects on nitrogen and soluble carbohydrate allocation during early reproductive growth in soybeans.
Agronomy Journal 73:577-582
- Trang, K.M. and J. Giddens (1980).
Shading and temperature as environmental factors affecting growth, nodulation and symbiotic nitrogen fixation by soybeans.
Agronomy Journal 72:305-308
- Treharne, K.J. (1972).
Biochemical limitations to photosynthetic rates pp 285-303 in Rees, A.R. et al. (eds) Crop processes in controlled environments.
London, Academic Press.

- Troedson, R.J., R.J. Lawn, D.E. Byth and G.L. Wilson (1985).
Saturated soil culture -An innovative water management option for soybean in the tropics and subtropics pp 171-180 in Shanmugasundaram, S. and E.W. Sulzberger (eds) Soybean in tropical and subtropical cropping systems. Taiwan, AVRDC.
- Tu, J.C. (1978).
Protection of soybean from severe Phytophthora root rot by Rhizobium.
Physiological Plant Pathology 12:233-240
- Turner, N.C. and J.E. Begg (1981).
Plant-water relations and adaptation to stress.
Plant and Soil 58:97-131
- Turnispeed, S.G. (1973).
Insects pp 542-572 in Caldwell, B.E. (ed.) Soybeans: improvement, production and uses.
Wisconsin, American Society of Agronomy.
- Vigue, J.T., J.E. Harper, R.H. Hageman and D.B. Peters (1977).
Nodulation of soybeans grown hydroponically on urea.
Crop Science 17:169-172
- Villalobos-Rodriguez, E. and R. Shibles (1985).
Response of determinate and indeterminate tropical soybean cultivars to water stress
Field Crops Research 10: 269-281
- Wardlaw, I.F. (1980).
Translocation and source-sink relationships pp 297-339 in Carlson, P.S. (ed) The biology of crop productivity.
New York, Academic Press.
- Wareing, P.F. and J. Patrick (1975).
Source-sink relations and partition of assimilates in the plant pp 481-499 in Cooper, J.P. (ed) Photosynthesis and productivity in different environments.
Cambridge, Cambridge University Press.
- Warembourg, F.R. and M.P. Fernandez (1985).
Distribution and remobilization of symbiotically fixed nitrogen in soybean (Glycine max).
Physiologia plantarum 65:281-286
- Waters, L.J., P.J. Breen and H.J. Mack (1980).
Translocation of ¹⁴C- photosynthate, carbohydrate content, and nitrogen fixation in Phaseolus vulgaris L. during reproductive development.
Journal of the American Society of Horticultural Science 105:424-427

- Watson, D.J. (1952).
The physiological basis of variation in yield.
Advance in Agronomy 4:101-145
- Wein, H.C. and E.E. Ackah (1978).
Pod development period in cowpeas: varietal differences as related to seed characters and environmental effects.
Crop Science 18:791-794
- Williams, J.H., J.H.H. Wilson and G.C. Bate (1976).
The influence of defoliation and pod removal on growth and dry matter distribution in groundnuts (Arachis hypogaeae L. cv. Makulu Red).
Rhodesian Journal of Agricultural Research 14:111-117
- Wilson, K.G. and R.E. Stinner (1984).
A potential influence of Rhizobium activity on the availability of nitrogen to legume herbivores.
Oecologia 61:337-345
- Wilson, R.F., J.W. Burton, J.A. Buck and C.A. Brim (1978).
Studies on genetic male-sterile soybeans I. distribution of plant carbohydrate and nitrogen during development.
Plant Physiology 61:838-841
- Withers, N.J. (1979).
An evaluation of lupins (Lupinus spp) for seed protein production. Ph.D thesis. Massey University, Palmerston North, New Zealand.
- Withers, N.J. and B.J. Forde (1979).
Effects of water stress on Lupinus albus III. Response of seed yield and vegetative growth to water stress imposed during two or three growth stages.
New Zealand Journal of Agricultural Research 22:463-474
- Worrall, V.S. and R.J. Roughley (1976).
The effect of moisture stress on infection of Trifolium subterraneum L. by Rhizobium trifolii Dang.
Journal of Experimental Botany 27:1233-1241
- Zeiher, C., D.B. Egli, J.E. Leggett and D.A. Reicosky (1982).
Cultivar differences in N redistribution in soybeans.
Agronomy Journal 74:375-379