

MANAGEMENT PRACTICES AFFECTING POTENTIAL NITROGEN
FIXATION IN GRAIN LEGUMES AND THE RESIDUAL
NITROGEN SUBSEQUENTLY LEFT FOR FOLLOWING CROPS

and

THE EFFECT OF SIX SUMMER GRAIN LEGUMES GROWN WITH
DIFFERENT DURATIONS OF IRRIGATION ON THE SUBSEQUENT
NITROGEN UP-TAKE AND YIELD OF COTTON THE FOLLOWING YEAR

By

Stephen H. Buster

Supervisors:

Dr. Donald A. MacLeod
Dr. Arthur S. Hodgson

A thesis submitted for partial fulfilment
of the requirements for the degree of
Bachelor of Rural Science (Honours) at the
University of New England, Armidale.
October, 1988

ACKNOWLEDGEMENTS

"Of making many books there is no end, and much study wearies the body.

*Now all has been heard;
here is the conclusion of the matter:
Fear God and keep his commandments,
for this is the whole duty of man."*

Ecclesiastes 12:12-14

In pursuit of knowledge and wisdom in this world created and sustained by God, I must thank Him first and foremost for the perspectives and lessons on life He has taught me. Without His insight and balance, life would not have been half as exciting and meaningful. So it is Him to whom I must thank in giving me the mental capacity and discipline to complete this degree.

To Dr. Donald MacLeod, my co-supervisor, who through this degree and project has stimulated my interest in soil management. Thank you for offering this project and for your helpful comments on the rough drafts of this thesis. Thank you for your friendship and encouragement in my time at University.

To Dr. Arthur Hodgson, my other co-supervisor: Thank you for allowing me to be part of your experimental project that is very practical to the real world. You have broadened my education with stimulating conversations concerning aspects of this project.

Thanks to Dr. Ian Davies for your help in running Genstat used for the statistical analysis of my results.

Chris Hartigan, thank you for your friendship and listening ear during our time at Narrabri. It was a comfort to have someone in the same situation.

To Greg Cone alias Coney, Ian Rochester and Karen Rose, thank you for your technical expertise in helping process the experimental material.

Finally last but not least, to my lovely wife Maria, thank you for your moral, spiritual and social concern and encouragement for my well being in our first year of marriage. God has truly blessed me with a loving, caring companion and friend.

TABLE OF CONTENTS

Acknowledgements.....	i
Table of Contents.....	ii
List of Tables.....	iv
List of Figures.....	v
A . Literature Review	
Summary.....	1
Chapter 1. Grain Legumes in Australia.....	2
Chapter 2. Symbiotic Nitrogen Fixation	
2.1 Introduction.....	7
2.2 <i>Rhizobium</i> , Infection and Nodulation	
2.2.1 Description of the bacterium.....	8
2.2.2 Infection and nodulation by <i>Rhizobium</i> ..	11
2.3 Controls of Fixation Affected by Management Decisions	
2.3.1 The energetics of fixation.....	12
2.3.2 Effects of combined nitrogen on fixation	15
2.4 Conclusions.....	18
Chapter 3. Management Factors Affecting Nitrogen Fixation and Residual Nitrogen of a Legume Crop.....	19
3.1 Pre-planting Practices	
3.1.1 Soil fertility.....	20
3.1.2 Land preparation.....	23
3.1.3 Pre-emergent pesticides.....	25
3.2 Planting Practices	
3.2.1 Inoculation practice.....	27
3.2.2 Planting density.....	30
3.2.3 Time of planting.....	31
3.3 Practices During Plant Growth.....	31
3.3.1 Post-emergent fertiliser treatment.....	32
3.3.2 Irrigation effect.....	33
3.3.3 Pesticide control practice.....	37
3.3.4 Cultivation practice.....	37
3.4 Harvest Practice.....	38
3.5 Crop Residue Practice.....	39
3.5.1 Duration of decomposition.....	40
3.5.2 Mineralisation and tillage.....	41
3.5.3 Decompostion and soil nitrogen.....	42
Conclusions and Future Research.....	43

B . Experimental Project

Abstract..... 45

Introduction..... 46

Materials and Methods..... 48

 Experiment rational..... 49

 Experimental design and treatments..... 49

 Crop agronomy..... 50

 Plant measurements..... 51

Results

 1. Treatment effect..... 54

 2. Irrigation time effect..... 55

 3. Species effect..... 60

 4. Nitrogen uptake and cotton yield..... 63

 5. Nitrogen uptake and removal by legumes..... 64

Discussion

 Irrigation time effect..... 66

 Species effect..... 70

 Regression of lint on nitrogen uptake..... 76

 Conclusions..... 78

References..... 80

Appendix 1. Effect of field slope and duration of furrow irrigation on grain yield of six grain legume crops..... 88

Appendix 2. Effect of field slope and duration of furrow irrigation on shoot nitrogen uptake of six grain legumes on 6 February 1987..... 89

Appendix 3. Effect of field slope and duration of furrow irrigation on shoot nitrogen uptake of two grain legume crops on 7 April 1987..... 90

LIST OF TABLES

LITERATURE REVIEW

Table 1.1 Area sown to grain legumes by states ('000 ha).....	4
Table 1.2 Area of grain legumes in Australia ('000 ha).....	4
Table 2.1 Some major characteristics of nodules of grain legumes.....	10
Table 3.1 Effect of combined nitrogen on growth, nodulation, and nitrogenase activity of pigeon pea, common bean and cowpea grown in nitrogen-free medium..	21
Table 3.2 Total plant nitrogen, seed nitrogen, residual nitrogen and nitrogen harvest index percentage (NHI) for three legume species.....	38
Table 3.3 Estimations of crop residue decomposed using ^{14}C and ^{15}N labelled material.....	41

EXPERIMENTAL PROJECT

Table 1. Monthly rainfall, maximum and minimum temperatures experienced during the 1987-88 cotton season.....	48
Table 2. Levels of significant differences for each treatment and measured parameter.....	54
Table 3. Apparent nitrogen usage by the legume species and cotton.....	65
Table 4. Estimated fertiliser equivalents for the six legume species.....	75

LIST OF FIGURES

LITERATURE REVIEW

Figure 3.1 Nitrogen content at 30 and 45 days and the amount of nitrogen fixed by 45 days for *Vigna mungo* plants grown in 10 % Kettering loam and supplied the equivalent of 0-100 kg N/ha as ammonium nitrate at sowing..... 23

Figure 3.2 As for Figure 3.2 but using *Vigna radiata*..... 23

EXPERIMENTAL PROJECT

Figure 1. Experimental design..... 50

Figure 2. Cotton petiole nitrate levels at three times of irrigation, half a day before the first irrigation..... 55

Figure 3. Cotton petiole nitrate levels as affected by three times of irrigation and six previous legume species four days after irrigation started..... 56

Figure 4. Cotton petiole nitrate levels as affected by three times of irrigation and six previous legume species eight days after irrigation started..... 57

Figure 5. Nitrogen content of the YML (4 DAI) and shoots (11 DAI) of cotton as affected by three irrigation times..... 58

Figure 6. Nitrogen up-take by cotton shoots 88 (11 DAI) and 133 days post plant as affected by three irrigation times..... 58

Figure 7. Lint yield of cotton as affected by three irrigation times..... 59

Figure 8. Cotton shoot dry-matter production 133 days after planting as affected by the legume species grown the prior year..... 60

Figure 9. Total nitrogen up-take by cotton 133 days after planting as affected by the legume species grown the prior year..... 61

Figure 10. Harvested seed cotton made up of seed, lint and trash as affected by the legume species grown the prior year..... 62

Figure 11. Regression of cotton yield components, seed cotton, seed and lint on nitrogen up-take at day 133..... 63

LITERATURE REVIEW

SUMMARY

The growth of grain legumes into Australian farming systems has been increasing in the 1980's. Part of their value in crop rotations is their ability to fix atmospheric nitrogen in symbiosis with *Rhizobium*. This may decrease the need for nitrogen fertiliser for crop following the legume crop. This is because some of the fixed nitrogen may be left in the legume crop residue after grain harvest and thus become available to the following crop. Consequently legumes have a reputation of maintaining or increasing soil nitrogen status.

Many management practices of the farmer will influence the amount of nitrogen fixed by the grain legumes crop and thus the amount left for the following crop. Practices like ground preparation, inoculation, fertiliser usage, irrigation and crop residue management all affect the amount of nitrogen fixed and left by the legume crop. The following review covers these and other management practices. As well, certain biological aspects of nitrogen fixation are discussed giving background to the effects of management practices.

CHAPTER 1

GRAIN LEGUMES IN AUSTRALIA

Grain legumes are a relatively new crop in Australia when compared to the traditional cereal crops that have, and continue, to dominate the Australian cropping scene. Grain legumes are characterized by high protein and/or oil content in their grain. Values range from 20.6 to 44.3 percent protein for chickpeas and lupins respectively with most of the other grain legumes in between (Sprent and Minchin, 1985). Soybeans and groundnuts, while having high protein, also have high oil content (23.5 and 49.0 percent respectively) (Norton *et al.*, 1985). Consequently there is considerable nutritional value to both man and stock when the grain is used for food. Grain legume are considered by some people to be part of the answer to the food problems of the world, particularly the under-developed countries where protein can be lacking in the people's diet (McWilliam and Dillon, 1987).

The value of legumes to any agricultural system lies in their ability to maintain a symbiotic relationship with the bacteria *Rhizobium*, which can 'fix' atmospheric dinitrogen gas, N_2 . This nitrogen (N) is then made available to the host plant to be incorporated into plant's cellular structure. (This process will be outlined in further detail in Chapter 2.) As N is often one of the most limiting nutrients to plant growth in agricultural farming systems, legumes are a valuable resource as they do not have the same reliance on soil N as other agricultural plants.

The contribution in the past and in the future to Australian agriculture by legumes should not be underestimated. Donald (1982) notes that, particularly in the 1950's and 1960's the progress of agriculture, and even the whole Australian economy, depended largely on the extension of 'pasture improvement' by the sowing of exotic legume species. Based on the relationship of superphosphate usage to N fixation, it was estimated that the annual contribution of N by legumes in Australia from 1971-2 to 1975-6, was about 1.5 Mt in fertilized permanent pastures and 0.5 Mt in pasture-cereal rotations (Donald, 1982). This is put into perspective when compared with the estimated use in 1984 of N fertilizer on pastures and forage crops of only 35kt (Myers and Henzell, 1985). The energy required to produce urea in equivalent amounts to the fixed N has been estimated to be approximately 3.6 Mt of oil (Donald, 1982).

The expansion of grain legume crops in Australia has been much slower than that of the pasture legumes, and interest in grain legumes is only now beginning to expand (Hamblin, 1987). This can be seen from the production trends shown in Table 1.1 and Table 1.2. The large increases in Victoria and Western Australia are primarily due to the use of peas and lupins in wheat-legume rotations (Hamblin, 1987). Other possible reasons for the inclusion of grain legumes into cropping rotations, besides economic returns from the grain include; soil N benefit, disease break, and soil structure improvement (Herridge, 1982a).

The prediction by White *et al.* (1978), that Australia was on the threshold of a major change in the traditional ley

pasture-cereal crop rotation system, is reflected in the exponential growth of area sown to grain legumes (Table 1.2). The growth is largely due to grain legumes being incorporated into the cereal cropping system. Delane (1987) comments that lupins (the main component of the increased growth), are not just a realistic complement to wheat but an essential element of wheat production in the northern wheat belt of Western Australia.

Table 1.1 Area sown to grain legumes by states ('000 ha)

State	Year			
	1971	1976	1981	1986
New South Wales	14	4	22	162
Queensland	13	14	17	74
South Australia	14	24	69	162
Tasmania	2	1	1	1
Victoria	6	6	60	256
Western Australia	27	96	98	674

Source: Hamblin, 1987.

Table 1.2 Area of grain legumes in Australia ('000 ha)

Legume	Year					
	1982/83	1983/84	1984/85	1985/86	1986/87	1987/88
Soybean	48.3	48.0	63.0	71.0	66.0	52.4
Lupin	256.9	378.9	593.8	587.6	746.0	1065.0
Field pea	114.1	92.5	140.5	209.0	310.0	552.0
Faba bean	2.2	4.2	8.0	19.0	37.0	45.7
Chick pea	-	3.1	6.4	26.0	71.0	52.9
Cowpea	11.2	7.9	6.4	8.0	13.0	25.0
Mung bean	15.9	17.1	23.9	34.0	38.0	40.1
Navy bean	5.8	5.9	6.5	7.0	8.0	15.0
Total	406.2	557.6	848.6	961.6	1289.0	1848.0

Source: Rees, 1988

Reports of residual N from prior legume crops benefiting the next crop are frequent. White *et al.* (1978) report that in Victoria wheat following lupins consistently had higher yield than wheat following wheat (a yield increase of 60 to 80 %). This increase was attributed mainly to the residual N from the lupins. Rowland *et al.* (1986) in Western Australia report wheat increases from lupin rotations of 30 to more than 100 % in comparison to wheat monoculture. Doughton and MacKenzie (1984), estimated the residual effect of mungbeans on sorghum was equal to an application of 68 kg N/ha in southern Queensland. Likewise soybeans are estimated to have supplied 76 kg N/ha for the following sorghum crop (Clegg, 1982).

This N benefit from legumes provides a means of decreasing fertilizer cost and maintaining soil fertility. A survey in 1974-75 of north-western New South Wales wheat soils by Doyle (1977), revealed that N deficiency is far more widespread and more severe on the relatively more fertile clay soil than has previously been recognized. This area was previously thought to be adequate in soil N for wheat, but 40 % of 141 sites surveyed gave economic responses to 30 kg N/ha. Ten years previously only 22 % of surveyed sites responded to 30 kg N/ha. Only sites with more than 30 ppm soil nitrate (measured in May) did not respond to fertilizer (Doyle, 1977). Since wheat protein content and yield may increase with increasing rates of N fertiliser (Barber and Jessop, 1987), grain legumes fixing atmospheric N will become more important in maintaining soil fertility and decreasing fertilizer cost.

Grain legumes will play an increasingly more important role in maintaining and improving soil fertility. Decisions made concerning the management of grain legume crops, will influence the amount of N fixed by the crop, and the amount left in the crop residue after harvest of the grain. The following review is concerned with the effects of crop management decisions on N fixation and the subsequent residual N left for the following crop. Firstly, certain aspects of the legume plant, the bacterium, infection and nodulation, and controls of fixation will be discussed to provide background information. Then, management decisions concerned with the production of the grain legume crop which influence N fixation and residual N will be reviewed.

CHAPTER 2

SYMBIOTIC NITROGEN FIXATION

2.1 Introduction

Early in the nineteenth century, Sir Humphry Davy first suspected the existence of symbiotic nitrogen (N) fixing systems, in which bacteria fix N in association with the roots of plants. It was confirmed later in 1886-8 by the German scientists H. Hellreigel and H. Wilfarth (Postgate, 1978). Fixation of N is now known to be the most important input to the N cycle from both ecological and agricultural viewpoints (Postgate, 1978). The following text will describe the main features of the legume plant, the symbiotic bacteria, aspects of nodulation and hence some of the controls of N fixation. Only those controls that are primarily affected by management decisions will be discussed.

Plants from the Leguminosae family can be found in all continents, occupying many diverse environments. Only grasses exploit greater ecological diversity than the legumes, although the grasses may be less diverse morphologically (Adams and Pipoly, 1980). Due to the large variation in natural habitats, the herbaceous legumes show large diversity of morphological and physiological form, which is often related to adaption to their environment.

Differences in day, night, and average seasonal temperatures are the main environmental factors affecting the distribution of legumes. Temperature effects are exhibited by different species having different critical temperature requirements for seed germination, vegetative growth, flowering, and seed set (Adams and Pipoly, 1980). Legumes also display specific responses to daylength, and may require short or long days in which to initiate flowering (Adams and Pipoly, 1980).

In the Leguminosae family there are three sub-families: the Mimosoideae, Caesalpinioideae, and the Papilionoideae. The most important agriculturally of these three is the Papilionoideae sub-family, as the grain and pasture legumes are part of this group (Langer and Hill, 1982). It is this group that is able to be exploited by agriculture due to the groups potential to fix N.

2.2 *Rhizobium*, Infection and Nodulation

2.2.1 Description of the Bacterium

Rhizobium are free living bacteria occurring in most soils but also combining symbiotically with plants from the Leguminosae family. They contain the enzyme nitrogenase which is responsible for reducing molecules of dinitrogen (N_2) to ammonium (NH_4^+). *Rhizobia* are classified as short to medium Gram-negative rods, usually occurring singularly or in pairs. Being aerobic chemorganotrophs their energy requirements are met by organic substrates, and they grow best at 25 - 30°C on complex media.

There are two main types of *Rhizobium*: the fast growers that have a mean generation time of 2-4 hours, and the slow growers with a mean generation time of 6-8 hours. The fast growers include *R.phaseoli*, *R.leguminosarum* and *R.lupini*, which nodulate common beans, peas and lupins respectively. The slow growers are now classified under the new genus *Bradyrhizobium*, and include *R.japonicum* and the cowpea type which nodulate soybeans and *Vigna* spp. respectively (Vincent, 1977; Sprent and Minchin, 1985). The fast growing species use many sugars and organic acids, whereas the slow growers are more specialized and commonly prefer pentoses as their carbon source. Attributes of some important agricultural *Rhizobia* and their host are shown in Table 2.1.

A 'strain' of *Rhizobium* refers to any culture of *Rhizobium* that is not known to have had a common clonal history with another culture of *Rhizobium*. *Rhizobium* 'type' refers to any number of strains grouped together by a convenient characteristic, for example a similar serological reaction (Vincent, 1977).

The nitrogenase enzyme is very susceptible to oxygen gas. The presence of free oxygen near the enzyme not only stops the reduction of N_2 to NH_4^+ but the enzyme's proteins are also irreversibly destroyed (Postgate, 1978). As fixation is expensive in terms of energy (see section 2.3.1), a continuous flux of oxygen to the bacteroids (N fixing *Rhizobium* enclosed in the host's nodule), is needed. Oxygen is used in the main energy releasing biochemical reaction, oxidative phosphorylation, to make adenosine triphosphate (ATP), (the biological energy currency). Therefore oxygen

Table 2.1 Some major characteristics of nodules of grain legumes

Tribe of legume host		<i>Rhizobium</i>			Nodule growth	Principal product exported
Genus	Species	Species	Growth*	Mode of infection		
PHASEOLEAE						
<i>Cajanus</i>	<i>cajan</i>	promiscuous	slow	root hair	determinate when young	ureides
<i>Glycine</i>	<i>max</i>	<i>japonicum</i>	slow/fast**	root hair	determinate	ureides
<i>Phaseolus</i>	<i>lunatus</i>	cowpea type	slow	root hair	determinate	ureides
<i>Phaseolus</i>	<i>vulgaris</i>	<i>phaseoli</i>	fast	root hair	determinate	ureides
<i>Psophocarpus</i>	<i>tetragonolobus</i>	slow growing	slow	root hair	determinate when young	ureides
<i>Vigna</i>	<i>mungo</i>	cowpea type	slow	root hair	determinate	ureides
<i>Vigna</i>	<i>radiata</i>	cowpea type	slow	root hair	determinate	ureides
<i>Vigna</i>	<i>unquiculata</i>	cowpea type	slow	root hair	determinate	ureides
VICIEAE						
<i>Lens</i>	<i>culinaris</i>	<i>leguminosarum</i>	fast	root hair	indeterminate	amides
<i>Pisum</i>	<i>sativum</i>	<i>leguminosarum</i>	fast	root hair	indeterminate	amides
<i>Vicia</i>	<i>faba</i>	<i>leguminosarum</i>	fast	root hair	indeterminate	amides
CICEREAE						
<i>Cicer</i>	<i>arietinum</i>	sp.	fast	root hair	indeterminate	amides, ureides
AESCHYNOMENEAE						
<i>Arachis</i>	<i>hypogaea</i>	various	fast/slow	lateral root junctions	indeterminate	ureides, amides
GENISTEAE						
<i>Lupinus</i>	spp.	<i>lupini</i>	fast/slow	root hair	indeterminate, collar-shaped	amides

* On defined media; ** fast-growing strains have recently been isolated in China

Source: Sprent & Minchin, 1985

concentration around the bacteroids must be kept low to avoid inactivating the nitrogenase enzyme, but at the same time it must be high enough for ATP production. This oxygen 'protection/regulation' constraint is provided by the host plant through keeping the bacteroids in a nodule with a low but continuous supply of oxygen (Witty *et al.*, 1984).

This 'oxygen' constraint on the nodule operates in three ways. First, gaseous exchange occurs through a regulated permeable membrane between the outer cortex and inner cells of the nodule. Second, respiration by the inner cells helps to keep oxygen tensions low, and third, leghaemoglobin buffers the cytosol and transports oxygen to the bacteroids (Sheehy *et al.*, 1983; Witty *et al.*, 1984). Due to the oxygen constraint *Rhizobium* normally do not fix N except when they are contained within a host's nodule (Postgate, 1978).

2.2.2 Infection and Nodulation by *Rhizobium*

Much has been written on the infection and nodulation process between *Rhizobium* and their host legumes (Dart, 1977; Dazzo, 1980). It is sufficient for this review to give a brief overview.

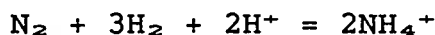
Although nearly all soils contain *Rhizobia*, their distribution and numbers developing in the legume root rhizosphere will influence infection (Dart, 1977). There is mutual host-symbiont recognition of the *Rhizobia* on the legume rhizoplane. The rhizosphere and mucigel layer are rich in growth-stimulating substances, which may facilitate

the attraction of *Rhizobia* to the plant root surface from the surrounding soil environment (Rolfe and Richardson, 1987). Most of the *Rhizobium* that have come into contact with their specific host remain on the root surface. Some, however, interact with newly emerging root hairs and initiate a pronounced curling of the growing hair cells. Infection proceeds as *Rhizobia*, entrapped within the curled root hair begin to induce an infection thread, which penetrates the plant tissue and continues to grow and ramify in the root cortex. From the infection thread the bacteria are released into the dividing root cortex cells and are packaged within the plant membrane thus resulting in the formation of a nodule. The *Rhizobium* continue to grow until ultimately they differentiate into bacteroids which are capable of fixing N (Rolfe and Richardson, 1987; Dart, 1977).

2.3 Controls of Fixation Affected by Management Decisions

2.3.1 The Energetics of Fixation

The fertilizer industry can fix atmospheric N at intense pressures and temperatures with a catalyst. The reaction:



is exothermic but requires large inputs of energy mostly for the reduction of H₂O (steam) to H₂. This reaction is accomplished biologically by the multicomponent enzyme nitrogenase, which not only reduces N₂ but also protons and acetylene (Schubert and Ryle, 1980).

As in the industrial process, the energy required in the biological system is large, the energy being needed to overcome the high activation point for the reaction. Under optimal conditions the reaction takes the following form:



where MgATP and MgADP are magnesium complexes of adenosine-triphosphate (ATP) and adenosine-diphosphate (ADP).

As the above reaction shows, the relatively high values of 12 ATP indicate the cost of N_2 fixation to the plant is quite large. This is a conservative estimate calculated by assuming that at least two ATP are consumed for each electron transferred (Schubert and Ryle, 1980). Therefore the above cost of 12 ATP is about 0.75 glucose equivalents. This does not include the additional costs of nodule growth and maintenance, nor the carbon skeletons, ATP, and reductant required for the assimilation of the NH_4^+ . Most of the energetic evaluations have been done *in vitro* and are not easily extrapolated to the field.

A further complication to the fixation cost is the ATP-dependant evolution of H_2 . This is catalysed by nitrogenase and can account for between 25 and 50 % of the total nitrogenase-catalysed substrate reactions (Minchin *et al.*, 1981). For some strains of *Rhizobium* not all the nodules formed exhibit net H_2 evolution, but they recycle the H_2 gas. Hence some are able to couple it to oxidative phosphorylation to regain extra ATP (Schubert and Ryle, 1980; Minchin *et al.*, 1981).

It has been estimated in a review by Minchin *et al.* (1981) that the cost of nodular N_2 fixation per mole is between 25.5 and 49 ATP equivalents. Minchin *et al.* (1981) also indicate that the cost depends on the proportion of electrons involved in H_2 production, the presence or absence of an uptake hydrogenase, and the type of organic nitrogenous compounds exported. Hardy *et al.* (1980) have estimated that to fix 28g of N_2 in soybeans, requires 60 ATP equivalents. Fifty percent of these are involved in the actual N_2 and H^+ reduction and the other 50 % are used for synthesis and maintenance of the system as well as import and export of nodular substrates and products. These values are put into perspective by Schubert and Ryle (1980), who estimate that between 10 and 30 % of the total net photosynthate produced by the plant may be utilized by N fixation in the legume nodule.

Much attention has been devoted to what limits N fixation from a metabolic viewpoint (Sprent and Minchin, 1985). Hardy *et al.* (1980) proposed that fixation is limited by the amount of carbohydrate that is exported to the roots and nodules. Through concentrating CO_2 in the atmosphere they were able to decrease photorespiration, thereby increase cropped soybean yields by 98 %. On the other hand, oxygen supplied to the bacteroids for use in respiration may be the factor limiting fixation (Bergerson, 1977).

These two positions may be reconciled as a result of recent findings. Work carried out by Sheehy *et al.* (1983) and Witty *et al.* (1984) indicates there are two types of nodules: those that can adjust rapidly within ten minutes to

increased O₂ pressures, and those that can only adjust slowly. Nodules associated with white clover, lucerne and pea are in the first category, those associated with soybean and sainfoin are in the second category. Other work by Sheehy *et al.* (1980) and Williams *et al.* (1982) showed that with increasing shoot photosynthesis, fixation activity in lucerne increased linearly. For soybeans however, a maximum fixation level was reached thus indicating oxygen might be limiting fixation. These results show that whether it is carbohydrate or oxygen that limits N fixation, will depend on the species in question. Thus, soybeans appear to be limited by oxygen whereas lucerne would be limited by carbohydrate.

2.3.2 The Effects Combined Nitrogen on Fixation

Depending on the level in the soil, combined N (particularly nitrate and ammonium) can either stimulate or inhibit N fixation. Generally, high levels of nitrate inhibit nodulation and fixation, whereas low levels stimulate these processes. At intermediate levels it is to be assumed that combined N has no effect, though this level has not been well documented.

Pate and Dart (1961), Gibson (1976) and Eaglesham *et al.* (1983) have shown that small amounts of combined N can stimulate nodulation and N fixation in different legume species by *Rhizobium* strains. This stimulation is thought to occur in young plants when the plant is dependent on seed reserves for N supply. The strength of the competition between shoots, roots and developing nodules for free amino-

acids depends on the environmental conditions, such as photoperiod, moisture conditions, soil oxygen levels and soil temperature levels (Fiiter and Hay, 1981). Supplementation with combined N should alleviate this N stress, but the timing and level of supplementation is important. The later the application and larger the level applied will tend to reduce instead of stimulate nodulation and fixation (Gibson, 1976).

The inhibiting effect on nodulation by high levels of soil nitrate was thought to arise from the reduction of nitrate to nitrite by the *Rhizobia* (Gibson *et al.*, 1977). Nitrite then acted as a catalyst to destroy indolelactic acid (IAA), which is needed for infection and nodule initiation (Tanner and Anderson, 1964). This theory was questioned by Gibson and Pagan (1977), who showed that nodulation by *Rhizobium* deficient in nitrate-reductase was also delayed by the presence of nitrate. Hence, if nitrite does inhibit nodulation, it must be produced by the plant. Gibson and Pagan (1977) then proposed that initiation is more likely to be retarded by a nitrate-induced carbohydrate insufficiency in the roots.

Similarly, N fixation in the nodule is thought to be reduced by nitrate either directly through the production of nitrite in the nodule thus inhibiting the nitrogenase enzyme, or indirectly through limiting carbohydrate supply to the nodule (Gibson *et al.*, 1977; Sprent and Minchin, 1985). Bacterioids were thought to contain the nitrate-reductase enzyme, but as stated above this was supposedly disproven by Gibson and Pagan (1977). However, nitrate-reductase activity

has since been found in the cytosol of nodule cells (Streeter, 1982), hence nitrite could still be responsible for reduced N fixation. Later work by Streeter (1985a) showed that the concentration of nitrite in nodules was unrelated to nodule weight per plant or to specific acetylene reduction activity. Yet nodule growth and nitrogenase activity was still inhibited up to 83 % by nitrate, regardless of the *Rhizobium* strain involved.

The carbohydrate deficiency theory is based on observations that less carbon flows to nodules after plants are given nitrate (Sprent and Minchin, 1985). However, work by Streeter (1981, 1985b), throws doubt on the validity of this theory. He showed that nitrate-induced decline in fixation by soybean nodules was not accompanied by any significant changes in the carbohydrate pool concentrations within the nodule.

Recent genetic research on the induction of nodulation and N fixation has shown that legume plants release stimulatory hydroxyflavones and isoflavones in the root hair zone to initiate nodulation. Similarly, inhibitory compounds are released along the root as well. It has been proposed that it is the stimulator:inhibitor ratio that determines the sites of nodule initiation (Rolfe and Richardson, 1987). If these or similar substances also control nitrogenase activity, it would make sense that the release of these substances are somehow controlled by the N status of the plant. Hence, if the plant has adequate N in the form of nitrate, it produces more inhibitory compounds thus reducing nodule initiation and nitrogenase rate. If on the other

hand, it is deficient in N it releases stimulatory compounds having the opposite effect. These biochemical effects would also be controlled by oxygen levels and photosynthate levels as discussed earlier. If this mechanism is correct, then nitrate would indirectly control nodulation and fixation.

2.4 Conclusions

The use of legume crops by agricultural systems is very dependant on the symbiotic relationship between the host plant and the bacterium. An understanding of the relationships between the bacterium and the host plant is required to best manipulate them for agronomic purposes. For this reason the legume plant and the bacterium have been briefly discussd. Controls of fixation mainly affected by agronomic management decisions were also discussed to give background information for the following chapter.

CHAPTER 3

MANAGEMENT FACTORS AFFECTING NITROGEN FIXATION AND RESIDUAL NITROGEN OF A LEGUME CROP.

Different management practices affect nitrogen (N) fixation and the subsequent residual N left in the soil available for following crop after grain legume harvest. These management factors include: decisions on species to grow, time of planting, crop densities, and cultivation practices, as well as decisions on application of pesticides, fertilisers, irrigation, and crop residue practices. The more important effects of these management practices are discussed in relation to five time periods: pre-planting, planting, crop growth, harvest and post harvest

3.1 PRE-PLANTING PRACTICES

Three main aspects of management affect potential N fixation before planting: soil fertility, land preparation, and pesticide practices.

3.1.1 Soil Fertility

The aim of fertiliser application is to reduce limitations on legume growth and nutrient deficiency. As responses to fertilisers differ for legume species, it is beyond the scope of this review to discuss each species and fertiliser and the reader is referred to Doyle's (1987) review of current fertiliser use with grain legumes in Australia. The present discussion is restricted to soil acidity and soil N, both of which can place important constraints on N fixation in Australia.

Soil Acidity

Probably the largest restraint on N fixation in terms of soil fertility in Australia is that of acid soils. The effects of soil acidity on nodulation has been reviewed by Munns (1978). *Rhizobium* survival in the soil is related to pH, calcium and aluminium/manganese levels. Tolerance to these different factors varies greatly between strains of *Rhizobia* with some slow growers tolerating pH as low as 4.5 (Munns and Mosse, 1980). Infection is sensitive to pH and to low calcium, whereas nodule function and N fixation appears to be affected by high aluminium and manganese levels (Munns, 1978; Helyar, 1981). Generally growth of the legume host and their specific bacteria have similar tolerances to pH.

Due to differences in pH tolerance in both the *Rhizobium* and their host plant, selecting the appropriate species can help overcome soil acidity problems. Example of this strategy are the use of lupins in the acid soils of Western Australia and chickpeas for more alkaline soils. If soil pH is too low

or aluminium saturation is too high for the chosen crop species the soil can be limed to raise the pH and introduce calcium into the soil profile. Liming of an acid soil from pH 4.55 to 5.9 decreased aluminium saturation from 81 to 4 % and increased nodule number per plant on soybeans from 21 to 77 (Sartin and Kamprath, 1975). Nodule number was highly correlated with calcium in the primary root.

Soil nitrogen

The biological effects of soil combined N have been discussed in Section 2.3.2. Both stimulation and depression of nodulation and N fixation in glass house conditions can be seen from the data of Kumar Rao *et al.* (1981) in Table 3.1 At 25 ppm N both nodule weight and nitrogenase activity for cowpea increased but were depressed at 50 ppm N. Nodulation and fixation in pigeon pea, however, was depressed at 25 ppm N.

Table 3.1 Effect of combined nitrogen on growth, nodulation, and nitrogenase activity of pigeon pea, common bean and cowpea grown in nitrogen-free medium.

	Irrigated with solution containing (ppm N)				
	0	25	50	75	100
	Pigeon pea				
Top dry wt (g/plant)	2.65	3.23	3.47	3.84	3.62
Nodule dry wt (mg/plant)	170	128	41	29	15
Nitrogenase ($\mu\text{molC}_2\text{H}_4/\text{plant/hr}$)	11.8	8.4	3.2	2.1	0.6
	Common bean				
Top dry wt (g/plant)	3.63	5.38	6.52	6.52	6.64
Nodule dry wt (mg/plant)	188	113	67	33	7
Nitrogenase ($\mu\text{molC}_2\text{H}_4/\text{plant/hr}$)	12.8	18.5	13.2	3.41	1.01
	Cowpea				
Top dry wt (g/plant)	4.29	5.81	7.05	8.29	8.61
Nodule dry wt (mg/plant)	156	190	110	93	27
Nitrogenase ($\mu\text{molC}_2\text{H}_4/\text{plant/hr}$)	10.9	11.5	6.6	4.6	2.1

Source: Kumar Rao *et al.*, (1981)

Dart *et al.* (1976) showed differences in the field between black and green gram mung bean species (*Vigna mungo* and *Vigna radiata*) grown on a sandy loam with applied ammonia nitrate at rates up to 100 kg N/ha. *V. mungo* was slightly stimulated by 10 kg N/ha, whereas *V. radiata* was stimulated by additions up to 30 kg N/ha (Figures 3.1. and 3.2.). Nitrogen fixation for both species was reduced by a greater supply of combined N, with virtually no fixation at 80 or 100 kg N/ha.

Declines in N fixation in the field with applied N fertiliser have also been reported for pigeon peas and cowpeas (Kumar Rao *et al.*, 1981; Beverly and Jarrell, 1984). Even though yield may increase, the amount of fixed N decreased as the plants utilise the available mineral N. Therefore the overall effect of combined soil N will depend on the cultivar and species (Buttery and Dirks, 1987), the soil N concentration (Dart *et al.* 1976; Kumar Rao *et al.*, 1981) and the type of N fertiliser (Eaglesham *et al.*, 1983).

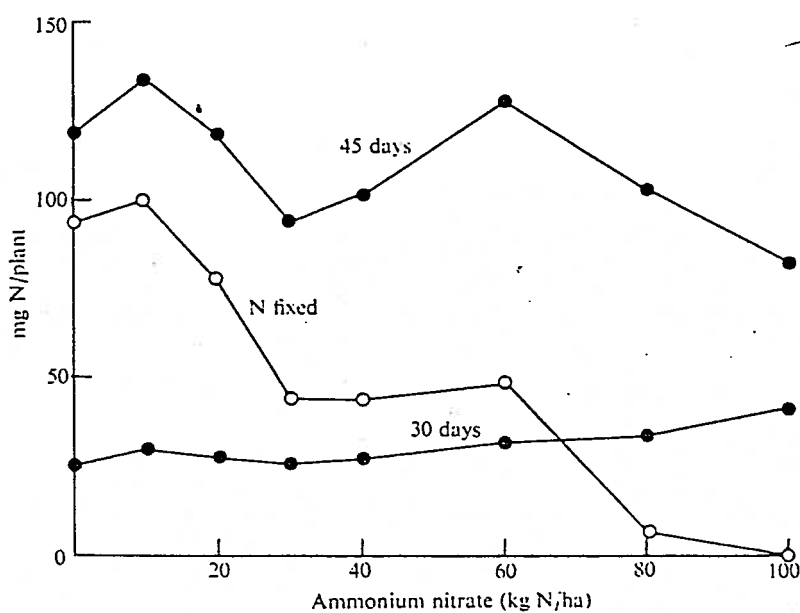


Fig. 3.1 Nitrogen content at 30 and 45 days and the amount of nitrogen fixed by 45 days for *Vigna mungo* plants grown in 10% Kettering loam and supplied the equivalent of 0-100 kg N/ha as ammonium nitrate at sowing.

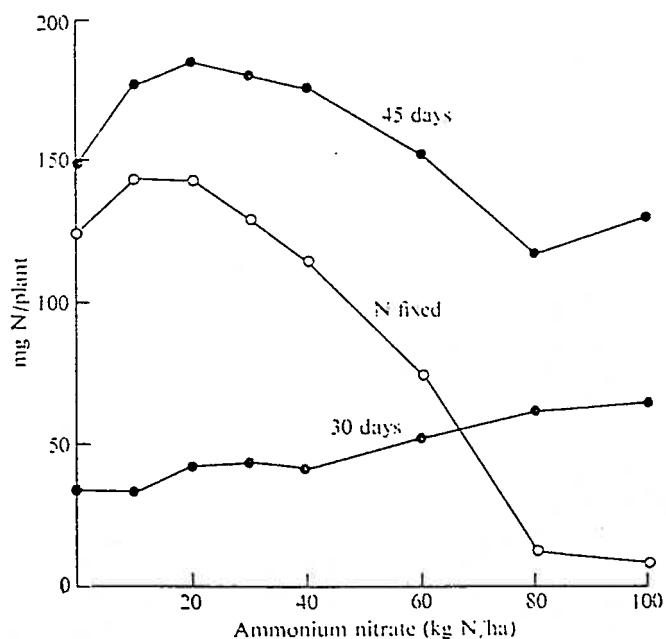


Fig. 3.2 Experimental details as for Fig. 3.1 but using *V. radiata*.
Source: Dart et al. 1976

3.1.2 Land Preparation

Minimum tillage farming systems are being advocated to reduce soil erosion, conserve moisture and avoid compaction of soil. Little work has been done on minimum tillage's effect on N fixation in grain legumes. The main effect on fixation from work that is available, is the mineralisation of N through cultivation.

Unpublished data by D. Herridge and J. Holland (pers. comm.) in the short term have shown that nodulation and fixation by some legumes is increased by minimum tillage. Soybeans (var. Forrest) showed a large increase in nodulation index (nodule mass as a percentage of shoot mass) and N fixation with min-till, as compared to cultivated treatments. However, cowpeas, mung beans and pigeon peas showed no significant differences over the treatments.

The effect on the Forrest variety of soybeans was considered to be related to increased mineral N levels in the soil through cultivation (in that season) which then depressed fixation in soybeans. This variety of soybeans appears to be more sensitive to nitrate levels than the other species. The cultivation effect is unlikely to be solely caused by nitrate, as pigeon peas are generally very sensitive to increased nitrate levels (Kumar Rao *et al.*, 1981). As well, plant dry-matter generally increases although N fixation decreases when nitrate levels increase. Yet all species decreased total dry-matter with cultivation which had more mineral N present.

In spite of whether increased nitrate through cultivation depressed nodulation and N fixation, the total N/ha that would have been fixed would have been greater as dry-matter production increased with minimum tillage.

This increase in N due to fixation resulting from minimum tillage is supported by Ellington (1987), who compared direct drilled to cultivated plots in a six year wheat-lupin rotation. It was found that direct drilled plots had higher

levels of total N and mineral N. Nitrogen fixation was not measured directly, but this increase in soil N was thought to occur through reduction in leaching of nitrate or by reducing denitrification over many years. Hence whether more N is fixed or simply conserved, minimum tillage appears to result in more N returned to the soil in the long term.

3.1.3 Pre-emergent Pesticides

The pre-emergent pesticides used in grain legume production include fungicides used in the storage of seed, herbicides to control weeds before or at planting, and insecticides used to control pest at planting. N fixation can be affected by pesticides in any one or more of four ways: a) the survival of rhizobia; b) the infection process; c) nodule formation and development; and d) N fixation in the nodule (Diatloff, 1986).

Differences exist in the various fungicides in their effects on N fixation. In the past, the most efficient fungicides have been the most damaging to *Rhizobia* and are typified by the organo-mercurial fungicides (Diatloff, 1986). Thiram and carboxin have been shown to be compatible with *R. japonicum*, whereas captan and PCNB decreased nodulation (Curley and Burton, 1975). In spite of this, thiram and captan are not as effective fungicides as the organo-mercurial fungicides (Diatloff, 1986). The new specific systemic fungicides are being hailed as a significant breakthrough in general, as they allow root diseases to be corrected through the foliage without affecting N fixation (Diatloff, 1986).

Two systemic fungicides (metalaxyl and benalaxyl), which allow more effective control of root rots do not affect any of the above four controls on N fixation by *R. japonicum* in soybeans.

Reports on pre-emergent herbicides triflurin, linuron atrazine and simazine generally indicate that they do not affect nodulation and fixation at suggested levels of use (Roughley and Brockwell, 1987). If nodulation and fixation have been decreased, it may be due to an effect on the legume rather than on the *Rhizobia* (Kumar *et al.*, 1981). Besides interaction between herbicides and *Rhizobium* species and strains, and between herbicides and legume species, there is also an interaction with soil organic carbon and clay content due to adsorption. Low levels of both organic carbon and clay appear to accentuate any toxicity which depresses nodulation and fixation (Dunigan *et al.*, 1972). Hence maintaining organic levels in the soil may decrease toxicity effects of herbicides.

Insecticides also vary in their effects on *Rhizobia* and subsequent nodulation and fixation. The effects of carbamates and organophosphates were found to differ between *Rhizobium* species (Lin *et al.*, 1972). Trinick (1982a) concluded that, as *Rhizobium* strains vary within and between species in the degree of their sensitivity to pesticides, no generalization could be made to differentiate between species of *Rhizobium*.

3.2 PLANTING PRACTICES

Management decisions that affect N fixation of a crop include; inoculation practice, planting density and time of planting as affected by the environmental conditions.

3.2.1 Inoculation Practice

As most soils contain varying levels of *Rhizobium* it has often been argued against the need for inoculation. Besides varying *Rhizobium* levels two factors to support inoculation are: (a) Symbiosis between *Rhizobium* and their host legume are relatively specific, and (b) specific *Rhizobium* are either effective or ineffective in their N fixing capacity (Trinick 1982b). (Effective *Rhizobium* are able to nodulate and fix N in the host plant whereas, ineffective *Rhizobium* can only nodulate the host plant (Dixon and Cannon, 1976)). Therefore inoculation is necessary to introduce a specific, effective strain of *Rhizobium* for a particular host.

Allen and Allen (1961) (cited in Brockwell, 1982) have listed four conditions which warrant the treatment of legume seed with inoculant preparations:

- a) the absence of the same or a symbiotically-related legume in the immediate past history of the land.
- b) poor nodulation when the same crop was grown on the land previously.
- c) a legume follows a non-legume crop in a rotation.
- d) in land reclamation work.

Inoculation methods have been reviewed by Brockwell (1982) and Roughley and Brockwell (1987). The methods are either seed or seed bed inoculation. Inoculation of the seed can be achieved by dusting the seed, slurry or pasteing the seed by a water solution, or seed pelleting with gum substance. Seed bed inoculation can be carried out by applying inoculant through irrigation water, by spraying inoculant below the seed, or by inoculating the seed of the prior crop when it is planted.

Of these methods the most common are seed coating in a slurry or by spraying inoculant below the seed. As the slurry method concentrates inoculant around the seed, more nodules are initially produced in the first six weeks (as root growth is close to the seed position). Compared to this, spray inoculation which distributes inoculant evenly along the row and encourages movement down the profile showed greater nodulation at 16 weeks (Roughley and Brockwell 1987). This was because the root had to grow down to the *Rhizobium*.

Nodulation failure during the first year of land being used for legume crops has been thought to occur through inadequate establishment of *Rhizobium* numbers at the time of sowing (Herridge *et al.*, 1987). Sowing and inoculating in hot, dry conditions in new land has resulted in N deficient plants. This was partly overcome by increasing inoculation levels up to 100 times the normal level. However, even at these levels the amounts of fixed N were less than when the crop was sown at the same time on soil previously colonized by *Rhizobium* (Herridge *et al.*, 1987).

Numbers of an inoculating strain of *Rhizobium* are often affected by the levels of competing *Rhizobium* in the soil, as well as by the soil environmental conditions. Competition for nodulation will come from both effective and ineffective strains in the soil. Hence the inoculating strain must be able to outcompete native *Rhizobium* strains (Trinick, 1982b). Brockwell *et al.* (1987) found that competition for nodulation could be decreased with increased rates of inoculum. Inoculated *Rhizobium* also gained a competitive advantage through specific placement in the zone of soil where infection first took place.

Although increased inoculation numbers can increase nodulation and hence N fixation, the full benefits are unlikely to be achieved unless levels of 10 to 50 times higher than normal are applied (Ralph, 1987). At present there are problems in applying amounts greater than 2 or 3 times the normal level in the field, as the amount of peat culture that can bind to the seed (that is the medium that *Rhizobium* are presented on) is limited, and further additions are wasted. Increases in *Rhizobium* numbers per gram of peat are held back by present inoculant culture practices, as further population expansion is restricted by waste products of bacterial metabolism (Ralph, 1987).

3.2.2 Planting Density

Under optimal conditions N fixation was shown to be limited by either the amount of photosynthate produced in the shoots, or by oxygen available to the bacteroids for biological energy production (Section 2.3.1). Photosynthate production is a function of the amount and intensity of light received by the plant (Gibson, 1976). Some plants are not limited by photosynthate under optimal conditions. However, if the conditions change so that light becomes limiting, these plants will be affected by reduced photosynthate. Regulating plant density is the main way in which management can affect the light received by the legume crop.

Redden *et al.* (1987) found a positive correlation between grain yield and ground cover in navy beans. Nelson and Weaver (1980) found that increasing soybean plant density from 50,000 plants to 200,000 plants/ha increased shoot dry matter production but there was a trend for grain yield to decrease. Hence more N would be returned to the soil with higher planting densities.

Similarly, Blumenthal *et al.* (1988) working with soybeans at four plant densities ranging from 50,000 to 400,000 plants/ha, found that greatest yield and dry matter production occurred at 200,000 plants/ha. There was a trend for residual N to increase with increasing plant density. Residues of the 50, 100, 200 and 400 thousand plants/ha had contributed 21, 22, 28, and 42 kg N/ha, respectively. The high level of residual N at the highest density was probably due to increased leaf drop (through shading) and plant death

before harvest. Less N being transported to the grain causing a reduced yield would mean more foliar N which is returned to the soil.

The above results show that planting density for maximum grain production is lower than that for maximum residual N.

3.2.3 Time of Planting

Moisture status and temperature of the soil at planting have an effect on N fixation mainly through their influence on germination of the seed and survival of the *Rhizobium* (see Section 3.2.1). Germination of the seed at a particular time will depend on the species and cultivar tolerances. Before planting the management needs to ensure the soil is within the correct temperature range for the chosen species and has adequate moisture for germination.

3.3 PRACTICES DURING PLANT GROWTH

This period in the context of this review can be defined as the period extending from germination of the seedlings, through the vegetative growth phase and fruit set to the time of fruit maturity. Therefore the cultural practices of applying post-emergent fertiliser, irrigation, cultivation of weeds, the application of post-emergent herbicides, the use of pesticides will be examined in relation to their effects on N fixation and residual N.

3.3.1 Post-emergent Fertiliser Treatment

The application of post-emergent N fertiliser has been shown to increase seed yields for some species of grain legumes (Afza *et al.*, 1987; Elowad and Hall, 1987). In Section 3.1.1 the effect of applying high levels of N before sowing has been discussed. It was concluded that high levels of applied N before sowing decreases N fixation, even though yield may be increased. The effects of N fertiliser on N fixation depends on the time when the fertiliser is applied to the growing crop.

Elowad and Hall (1987) applied 60 kg N/ha either in the soil or as a foliar spray to cowpeas at early flowering. They found that this reduced N fixation substantially, although seed weight was only increased by 11 %. In contrast, application of foliar N at 60 kg N/ha at pod-fill did not significantly affect N fixation as overall levels were low during this stage of plant development. Seed yield from this treatment was however, increased significantly by 20 %. Beverly and Jarrell (1984) using the same cultivar as Elowad and Hall (1987) also found that N fixation decreased with all applications of fertiliser after flowering until pod-fill. Afza *et al.* (1987), applying N to soybeans at pod-fill found that N fixation was not affected at 40 kg N/ha but was at 80 kg N/ha. Seed yield was increased by approximately 40 % at these two levels.

The usefulness of applying N at flowering is questioned as some commercial crops of cowpeas have not always shown yield increases (Elowad and Hall, 1987). Even if yield did

increase, the increase may not be worth the cost of fertiliser as well as the decreased N fixation. The application of fertiliser at pod-fill also poses practical problems. As plants cover the ground the use of tractors would damage the foliage and the use of large quantities water to avoid leaf burn may preclude the use of an aircraft (Elowad and Hall, 1987).

3.3.2 Irrigation Effect

Nitrogen fixation may be reduced by a deficit or an excess of moisture. Irrigation can alleviate the former but it may create waterlogging, particularly on clay soils. The effects of waterlogging on N fixation are discussed below.

Since irrigation saturates the soil profile (except for drip irrigation), the main chemical effect is a large reduction in the oxygen concentrations in the soil (Ponnamperuma, 1972). Various reports reviewed by Ponnamperuma suggest that the concentration of oxygen is decreased to one-hundredth of its initial value within 75 minutes of saturation, and no oxygen is detectable within 1 day of saturation. This arises as gas exchange between soil and air is reduced by some 10,000 times upon saturation. Hence any available oxygen is quickly consumed by soil organisms or plant roots (Ponnamperuma, 1972).

In Section 2.3.1 it was seen that under ideal conditions N fixation was restricted by either carbohydrate levels or by the oxygen flux to the bacteroids in the nodule. Minchin

(1973) (cited by Pate, 1976) using *Pisum sativum* has shown that N fixation is greatest when the soil is at field capacity. At this point the ambient oxygen level was 0.21 atmospheres. Chriswell *et al.* (1976) showed that nitrogenase activity in soybean nodules was initially reduced by 37 - 45 % when rhizosphere oxygen levels were reduced from 0.21 to 0.06 atm. These nodules recovered their original activity in 4 to 24 hours at the same low oxygen level. Continuous exposure at 0.02 atm initially reduced nitrogenase activity by 72 % and it only partially recovered after 95 hours. Sheehy *et al.* (1983) demonstrated that white clover nodules increased N fixation measured by acetylene reduction seven times as oxygen levels were increased from 0.05 to 0.30 atm. Hence regardless of the type of nodule (either fast or slow adaptation), low oxygen levels decrease N fixation.

There is a possible role of ethylene produced under waterlogged conditions affecting N fixation (Jackson and Drew, 1984; Smith, 1987). Ethylene may be produced endogenously within the legume's root to stimulate the formation of aerenchyma (Jackson and Drew, 1984). Grobbelaar *et al.* (1971) found that when *Phaseolus vulgaris* roots were exposed to air containing 10 ppm ethylene, N fixation declined by 90 % compared to control plants.

Various waterlogging experiments have shown the type of adaptation that occur in the nodule. Minchin and Summerfield (1976), upon waterlogging cowpeas up to 32 days, found increased nodule cortication and lenticel-type protuberances on nodules which were formed during waterlogging as compared with unstressed controls. Dry weight of nodules was reduced

by 60 % after 8 days of waterlogging. Further, Hong *et al.* (1977) found that after 8 days of waterlogging cowpeas total nitrogenase activity per plant was reduced by 84 %. Similarly, plants developed adaptive features like stimulating adventitious root formation and increasing nodule cortication. Another response that has been observed in bacteroid cultures of *R. japonicum* and cowpea *Rhizobium* is their ability to reduce nitrate in the absence of oxygen. Nitrate is used as an alternative electron acceptor to oxygen for ATP production thus nitrate reduced to nitrous oxide and N₂ gas (Jackson and Drew, 1984). This may partly explain why soybean and cowpea are more tolerant to waterlogging than some other species (Smith, 1987).

Unfortunately much of the research on the effects of waterlogging has been carried out under laboratory conditions over much longer times of saturation than normally occurs under irrigation. It is these experiments that have revealed the various mechanisms of nodule adaptation. However, some experiments have been carried out with similar durations of waterlogging as in the field. Cowpeas waterlogged continuously for four days in every ten days before flowering, had vegetative growth reduced by 50 % and N fixation reduced by 43 % compared to the non-waterlogged controls (Minchin *et al.*, 1978). For plants waterlogged one day in ten during the same period, vegetative production was reduced by only 25 %. Unfortunately the N fixation for this treatment is not given. Trung *et al.* (1985) showed that mung beans when waterlogged at first flowering stage for one or two days, decreased the rate of N fixation by about 73 and 92 % respectively compared to the non-waterlogged control.

The effects of waterlogging seem to be more acute when plants are stressed at early flowering. Minchin *et al.* (1978) found the effects of waterlogging was much more severe on cowpeas just before and during first flower than later in the season. Trung *et al.* (1985) showed that waterlogging was most detrimental to the growth and seed yield of mung beans at flowering, whereas preflowering treatments and post-flowering treatments were not as severely affected. The main effect seems to be in the reduced flower production hence pod set by the plant.

Differences exist between legume species as demonstrated by Hodgson *et al.* (1988). Six species (soybeans, mung beans, navy beans, adzuki beans, pigeon peas and cowpeas) were irrigated for 4, 16, and 32 hours for each of the four irrigations. All species showed reduced growth with increasing waterlogging at the first irrigation of the season, but soybeans, mung beans and cowpeas became less affected as they matured. This suggests that they are more tolerant than the other three species.

Therefore waterlogging decreases N fixation through reduced oxygen levels. The amount by which fixation is decreased depends on the species, the time of irrigation and the length of waterlogging. Species selection and short periods of irrigation are the main ways to decrease the adverse affects of waterlogging on fixation.

3.3.3 Pesticide Control Practices

As stated in Section 3.1.3, pesticides vary in their effects between *Rhizobium* species and legume crops. Hence it is unwise to generalize on their absolute effects on N fixation. Roughley and Brockwell (1987) note however, that the use of recommended herbicides at their correct concentrations will not affect fixation. This would also apply to most insecticides (Trinick, 1982a). The need for insect control is exemplified by Layton and Boethel (1987), who showed that the N fixation ability of soybeans decreased linearly in response to increased levels of defoliation by the soybean looper.

3.3.4 Cultivation Practice

Little work has been done on the effects of cultivation during crop growth on N fixation. If weeds are allowed to grow and are not properly controlled they will compete with the crop for nutrients and moisture, thereby stressing the crop. If this stress is severe enough, then the N fixing capacity of the crop will be reduced. If cultivation takes place too late in the season, damage may occur to the foliage thereby reducing the photosynthate capacity of the plant, which may in turn reduce N fixation.

3.4 HARVEST PRACTICES

At harvest N fixation has stopped due to lack of photosynthate moving from the leaves to the roots. The main factors determining how much residual N is left for decomposition are: the amount of N translocated to the seed from other plant parts, and how much N is taken off in the seed. These factors vary with species.

An example of the effect of species is given in Table 3.2 from data obtained by Chapman and Myers (1987). Soybeans removed a higher percentage of N than either mung beans or sesbania (*Sesbania cannabina*), but as they had more total plant N uptake than the other two species they returned slightly more N to the soil than mung beans but less than sesbania.

Table 3.2 Total plant nitrogen, seed nitrogen, residual nitrogen and nitrogen harvest index percentage (NHI) for three legume species.

Season	Soybean				Mung bean				Sesbania			
	Plant N ^a (kg N/ha)	Seed N ^b ()	Res N ^c ()	NHI (%)	Plant N ^a (kg N/ha)	Seed N ^b ()	Res N ^c ()	NHI (%)	Plant N ^a (kg N/ha)	Seed N ^b ()	Res N ^c ()	NHI (%)
1981-82	404	296	108	73	177	89	88	50	202	61	141	30
1982-83	329	262	67	80	111	52	59	47	36	35	101	26

^a Total nitrogen uptake in the plant.

^b Total nitrogen taken off in the seed.

^c Total nitrogen left in the crop residual after harvest.

^d NHI = seed N / total plant N * 100, ie. the amount of nitrogen removed by the seed compared to the total amount in the crop.

Source: Chapman and Myers, (1987).

Chapman and Muchow (1985) compared the N partitioning of several different grain legumes at different moisture regimes. They showed that under well watered conditions lablab bean and black gram left the most residual N (118 and 75 kg N/ha), pigeon pea, cowpea and green gram left similar amounts (49, 49, and 48 kg N/ha) with soybean leaving the least (22 - 24 kg N/ha). When moisture stress was induced during the growing season all species reduced N uptake and consequently the residual N left. Soybean and cowpea were least affected by moisture stress compared to the other species. Therefore for a given moisture regime, species could be selected for their particular residual value, though market forces and farm management practices will play a larger role in deciding the choice of crop.

Soybean had the highest N harvest index (NHI) (seed N / total shoot N) as in Chapman and Myers (1987) experiment, but it did not leave as much residual N as the total plant N uptake was lower than Chapman and Myers experiment. Hence residual N depends on total N uptake as well as the NHI, as the above two experiment show.

3.5 CROP RESIDUE PRACTICES

The availability of the residual crop N to the following crop is related to the rate of decomposition of plant matter to soil organic matter. For the N to become available to the next crop mineralisation of the nitrogenous compounds must occur. Under normal conditions 2 - 3 % of N in soil organic

matter is mineralised each year (Brady, 1984). Factors influencing decomposition and hence mineralisation rate include the carbon:nitrogen (C:N) ratio of the residue, soil water status, soil temperature, soil type, cultivation practice, inherent soil N, and the duration of the period of decomposition (El-Harris *et al.*, 1983; Ladd *et al.*, 1983; Myers and Wood, 1987). The main factors that can be manipulated by management are the last three; cultivation practice, soil N, and duration of decomposition.

3.5.1 Duration of Decomposition

Ladd *et al.* (1983) and Amato *et al.* (1987) observed decomposition rates of ^{14}C and ^{15}N labelled legume material in a sandy loam soil at field conditions. As Table 3.3 shows, initial decomposition is quick (4 weeks) but then slows as material becomes more resistant to decomposition. The much slower rate of decomposition of wheat straw observed by Amato *et al.* (1987) resulted from wheat straw's high C:N ratio of 73:1. This is compared to the legume residues C:N ratio of 13:1.

Net mineralisation of ^{15}N occurred with all plant residues at all times, though mineralisation was more extensive for the legume crops (Amato *et al.*, 1987). Immobilisation is thought to have occurred in the wheat straw at four weeks, though at ten weeks net mineralisation had occurred (10 % of input and 18 % at 31 weeks). These data show the importance of time in decomposition of residues. If cropping were to occur within the next year between 25 and 45 % of the

residual N should be available to the crop under normal field conditions.

Table 3.3 Estimations of crop residue decomposed using ^{14}C and ^{15}N labelled material

Material decomposed (%)	Time (weeks)			Source
	4	52	208	
<u>M.littoralis</u> ^{14}C	35	65	82	Ladd <u>et al.</u> , (1983)
	^{15}N	17	40	
<u>M.littoralis</u> ^{14}C	36	68		Amato <u>et al.</u> , (1987)
	tops ^{15}N	35	45	
<u>M.littoralis</u> ^{14}C	34	48		"
	roots ^{15}N	22	36	
Wheat straw ^{14}C	15	52		"
	^{15}N	7	23	

3.5.2 Mineralisation and Tillage

Minimum tillage has an important effect on the speed of mineralisation. Powlson (1980) showed that cultivation increased mineralisation of organic N. Differences between cultivated and non-cultivated plots increased with time over periods of two to six weeks. Other work by El-Harris et al. (1983) and Doran (1980) showed that mineralisation rate was higher in the top 5cm of the minimum tillage plots but the cultivated plots had more mineral N below this level. Both plots had similar levels of total N but cultivated plots had more mineral N. Since minimum tillage increased the level of facultative anaerobes and denitrifying soil micro-organisms, the potential rates of mineralisation and nitrification are higher for cultivation, while the potential for denitrification is higher for minimum tillage (Doran, 1980).

3.5.3 Decomposition and Soil Nitrogen

As the level of soil N can determine how fast decomposition hence mineralisation occurs, increasing the soil mineral N could increase the rate of mineralisation. This effect on mineralisation rate is mainly exhibited in the first four weeks after the addition of fertiliser. Beyond this time mineralised N begins to cycle and hence the overall effect of high levels of N becomes less important (El-Harris *et al.*, 1983). It is doubtful whether adding N fertiliser to increase the mineralisation of crop residues is economical, even in soil with very low mineral N levels.

CONCLUSION AND FUTURE DIRECTION

Grain legumes will become more important to Australian agriculture due to their ability to help maintain soil fertility. The above review has shown the importance of how management decisions and practices affect the amount of N fixed and subsequently left by a legume crop for the following crop. Much of the research has been centered around various factors concerning either N fixation by the legume crop or the residual N left from legume crop. From a farmers viewpoint, future research needs also to consider how factors affect both N fixation and the residual N benefit to the subsequent crop. For example, the effects of N fertiliser on grain legumes have been considered, but N fertiliser needs also to be considered with reference to the amount of N left for the following crop. Similarly, waterlogging is known to reduce N fixation but it is not known quantitatively to what extent waterlogging of legumes will affect the amount of residual N left for the following crop. Information of this type is necessary if farmers are to alter their management practices to derive the maximum benefit from their legumes crops.