

THE UNIVERSITY OF MANITOBA

THE EFFECT OF NITROGEN AND MOISTURE AVAILABILITY  
ON GROWTH AND SYMBIOTIC NITROGEN FIXATION IN LENTILS  
(Lens culinaris)

by

Frances Lynne Walley

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SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE  
MASTER OF SCIENCE

Department of Soil Science

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FRANCES LYNNE WALLEY

A thesis submitted to the Faculty of Graduate Studies of  
the University of Manitoba in partial fulfillment of the requirements  
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## ABSTRACT

Field and growth chamber studies were conducted to evaluate the effect of various soil conditions, including nitrogen and moisture availability on yield and potential symbiotic nitrogen fixation of lentils. Application of fertilizer N at rates ranging from 0-200 kg N.ha<sup>-1</sup> and 0-360 ppm N in field and growth chamber experiments, respectively, resulted in dry matter and seed yield increases. Nitrogen stress, simulated by the addition of barley straw to the soil, limited lentil yields. Results evidence the fact that lentils are not capable of symbiotically fixing enough nitrogen to meet optimum plant growth requirements.

Moisture availability was also found to be an important factor in attaining high dry matter and seed yield of lentils. Yields were significantly reduced by the application of moisture stress and were notably influenced by the physiological stage at which stress was applied.

The effect of nitrogen availability on symbiotic nitrogen fixation was evaluated. Quantity of nitrogen symbiotically fixed in lentils was estimated using the 'A' Value method, the <sup>15</sup>N Assisted Difference method and the Classical Difference method. Nonnodulating soybeans served as the reference crop. Although these methods estimated similar quantities of nitrogen fixed under controlled conditions, the N balance techniques proved unreliable under field conditions.

Increasing increments of applied N were shown to reduce symbiotic N fixation. Nitrogen stress delayed the onset of symbiotic fixation and thus reduced the total quantity fixed.

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## TABLE OF CONTENTS

<u>Chapter</u>	<u>Page</u>
1. INTRODUCTION .....	1
2. LITERATURE REVIEW .....	2
2.1 Symbiotic Nitrogen Fixation and Yield of Lentils .....	2
2.1.1 Effect of Nitrogen Application .....	3
2.1.2 Effect of Water Stress .....	6
2.2 Methods of Measuring Symbiotic Nitrogen Fixation .....	10
3. MATERIAL AND METHODS .....	20
3.1 Growth Chamber Experiments .....	20
3.1.1 Growth Chamber Experiment A .....	20
3.1.2 Growth Chamber Experiment B .....	25
3.1.3 Growth Chamber Experiment C .....	29
3.2 Field Experiments .....	35
3.2.1 Field Experiment 1983 .....	35
3.2.2 Field Experiments 1984 .....	42
3.3 Analytical Procedures .....	51
3.3.1 Soil Analysis .....	51
3.3.2 Plant Analysis .....	53
3.3.3 Ethylene Analysis .....	56
4. RESULTS AND DISCUSSION .....	58
4.1 Growth Chamber Experiments .....	58
4.1.1 Growth Chamber Experiment A .....	58
4.1.2 Growth Chamber Experiment B .....	70
4.1.3 Growth Chamber Experiment C .....	77
4.2 Field Experiments .....	108
4.2.1 Field Experiments 1983 .....	108
4.2.2 Field Experiments 1984 .....	134
5. SUMMARY AND CONCLUSIONS .....	180
LITERATURE CITED .....	185

## LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Summary of physical and chemical characteristics of soil used in growth chamber experiments.	21
2. Treatments used in Growth Chamber Experiment A for lentils and nonnodulating soybeans.	22
3. Treatments used in Growth Chamber Experiment B for lentils.	26
4. Treatment descriptions for lentils and nonnodulating soybeans used in Growth Chamber Experiment C.	30
5. Date, days from seeding and developmental stage of lentils at each harvest taken in Growth Chamber Experiment C.	34
6. Summary of physical and chemical characteristics of soil used in Field Experiments 1983.	36
7. Treatment descriptions for lentils and nonnodulating soybeans used in Field Experiment 1983.	38
8. Date, days from seeding and developmental stage of lentils at each harvest taken in Field Experiment 1983.	41
9. Summary of physical and chemical characteristics of soils used in Field Experiments 1984.	43
10. Treatment descriptions for lentil and nonnodulating soybeans used in Field Experiments 1984.	45
11. Date, days from seeding and developmental stage of lentils at each harvest taken in Field Experiments 1984.	48
12. Gas Chromatograph run parameters.	57
13. Effect of nitrogen availability on dry matter yield of lentil and nonnodulating soybean shoots (Growth Chamber Experiment A).	59
14. Effect of nitrogen availability on nitrogen accumulation in lentil and nonnodulating soybean shoots (Growth Chamber Experiment A).	62

LIST OF TABLES (continued)

<u>Table</u>	<u>Page</u>
15. Effect of nitrogen availability on seed yield, nitrogen accumulation in seed, percent protein of seed and harvest index (Growth Chamber Experiment A).	65
16. Effect of nitrogen availability on the accumulation of symbiotically fixed nitrogen, as determined by the Classical Difference Method (Growth Chamber Experiment A).	67
17. Effect of nitrogen availability on percent nitrogen derived from symbiotic nitrogen fixation in lentil shoots (Growth Chamber Experiment A).	69
18. Effect of water stress applied at different physiological stages on dry matter and nitrogen accumulation in lentil shoots (Growth Chamber Experiment B).	73
19. Effect of water stress applied at different physiological stages on seed yield, nitrogen accumulation in seed, percent protein of seed and harvest index (Growth Chamber Experiment B).	76
20. Effect of nitrogen availability on dry matter accumulated by lentil shoots, lentil roots and nonnodulating soybean shoots, determined at successive harvest dates (Growth Chamber Experiment C).	79
22. Effect of nitrogen availability on nitrogen accumulation in lentil shoots, lentil roots and nonnodulating soybean shoots, determined at successive harvest dates (Growth Chamber Experiment C).	84
23. Effect of nitrogen application on percent nitrogen derived from fertilizer (Ndff) in lentil shoots, lentil roots and nonnodulating soybean shoots, determined at successive harvest dates (Growth Chamber Experiment C).	87
24. Effect of nitrogen application on fertilizer nitrogen accumulation in lentil shoots, lentil roots and nonnodulating soybean shoots, determined at successive harvest dates (Growth Chamber Experiment C).	92
25. Effect of nitrogen application on percent utilization of fertilizer nitrogen by lentil and nonnodulating soybean shoots, determined at successive harvest dates (Growth Chamber Experiment C).	94



LIST OF TABLES (continued)

<u>Table</u>	<u>Page</u>
25A. Level of significance associated with differences between percent utilization of fertilizer nitrogen by lentils and nonnodulating soybeans (Growth Chamber Experiment C).	94
26. Effect of nitrogen application on lentil and non-nodulating soybean 'A' values, determined at successive harvest dates (Growth Chamber Experiment C).	97
26A. Level of significance associated with differences between lentil and nonnodulating soybean 'A' values (Growth Chamber Experiment C).	97
27. Effect of nitrogen application on the accumulation of symbiotically fixed nitrogen in lentils as calculated using the 'A' value method (Growth Chamber Experiment C).	100
28. Effect of nitrogen availability on the accumulation of symbiotically fixed nitrogen in lentils as calculated using the <sup>15</sup> N Assisted Difference method (Growth Chamber Experiment C).	101
29. Effect of nitrogen availability on the accumulation of symbiotically fixed nitrogen in lentils as calculated using the Classical Difference method (Growth Chamber Experiment C).	102
30. Effect of nitrogen application on seed yield, percent protein of seed and harvest index of lentils (Growth Chamber Experiment C).	107
31. Dry matter and nitrogen accumulation in lentils and nonnodulating soybeans determined throughout the growing season (Field Experiments 1983).	111
32. Percent nitrogen derived from fertilizer (Ndff), fertilizer nitrogen accumulation and percent utilization of fertilizer nitrogen in lentils and nonnodulating soybeans determined throughout the growing season (Field Experiments 1983).	115
33. 'A' values for lentils and nonnodulating soybeans determined throughout the growing season (Field Experiments 1983).	119

## LIST OF TABLES (continued)

<u>Table</u>	<u>Page</u>
34. Accumulation of symbiotically fixed nitrogen in lentils as calculated using the 'A' value method, the $^{15}\text{N}$ Assisted Difference method and the Classical Difference method (Field Experiments 1983).	120
35. Effect of nitrogen application on various yield components of lentils (Field Experiments 1983).	123
36. Effect of nitrogen application on various yield components of nonnodulating soybeans (Field Experiments 1983).	126
37. Effect of nitrogen application on various nitrogen uptake components by lentils and nonnodulating soybeans (Field Experiments 1983).	128
38. Effect of nitrogen application on 'A' values of lentils and nonnodulating soybeans (Field Experiments 1983).	131
39. Effect of nitrogen application on the accumulation of symbiotically fixed nitrogen as calculated using the 'A' value method, the $^{15}\text{N}$ Assisted Difference method and the Classical Difference method (Field Experiments 1983).	132
40. Effect of nitrogen application on dry matter yield of lentils determined throughout the growing season (Field Experiments 1984).	138
41. Effect of nitrogen application on dry matter yield of nonnodulating soybeans determined throughout the growing season (Field Experiments 1984).	139
42. Effect of nitrogen application on nitrogen accumulation in lentils determined throughout the growing season (Field Experiments 1984).	143
43. Effect of nitrogen application on nitrogen accumulation in nonnodulating soybeans determined throughout the growing season (Field Experiments 1984).	145
44. Effect of nitrogen application on seed yield, seed nitrogen accumulation, percent protein and harvest index of lentils (Field Experiments 1984).	148

LIST OF TABLES (continued)

<u>Table</u>	<u>Page</u>
45. Effect of nitrogen application on percent nitrogen derived from fertilizer in lentils and nonnodulating soybeans determined throughout the growing season (Field Experiments 1984).	150
46. Effect of nitrogen application on fertilizer nitrogen accumulation in lentils and nonnodulating soybeans determined throughout the growing season (Field Experiments 1984).	152
47. Effect of nitrogen application on percent utilization of fertilizer nitrogen by lentils and nonnodulating soybeans, determined throughout the growing season (Field Experiments 1984).	154
48. Effect of nitrogen application on 'A' values for lentils and nonnodulating soybeans, determined throughout the growing season (Field Experiments 1984).	156
48A. Level of significance associated with differences between lentil and nonnodulating soybean 'A' values determined by the T-test procedure (Field Experiments 1984).	158
49. Effect of nitrogen application on the accumulation of symbiotically fixed nitrogen as calculated using the 'A' value method, determined throughout the growing season (Field Experiments 1984).	159
50. Effect of nitrogen application on the accumulation of symbiotically fixed nitrogen as calculated using the <sup>15</sup> N Assisted Difference method throughout the growing season (Field Experiments 1984).	160
51. Effect of nitrogen application on the accumulation of symbiotically fixed nitrogen, as calculated using the Classical Difference method throughout the growing season (Field Experiments 1984).	161
52. Effect of nitrogen application on symbiotic nitrogen fixation (C <sub>2</sub> H <sub>2</sub> reduction) at different developmental stages of lentils (Field Experiments 1984).	163
53. Dry matter and nitrogen accumulation in the shoots and roots of lentils and nonnodulating soybeans, determined throughout the growing season (Field Experiments 1984).	166

LIST OF TABLES (continued)

<u>Table</u>	<u>Page</u>
54. Contribution of shoots to total dry matter and nitrogen yield of lentils and nonnodulating soybeans (Field Experiments 1984).	168
55. Percent nitrogen derived from fertilizer in shoots and roots of lentils and nonnodulating soybeans (Field Experiments 1984).	171
56. Fertilizer nitrogen accumulation in shoots and roots of lentils and nonnodulating soybeans (Field Experiments 1984).	172
57. Percent utilization of fertilizer nitrogen by shoots and roots of lentils and nonnodulating soybeans (Field Experiments 1984).	174
58. 'A' values for lentils and nonnodulating soybeans determined throughout the growing season, calculated using shoot and total plant sample (Field Experiments 1984).	175
58A. Level of significance associated with differences between lentil and nonnodulating soybean 'A' values determined by the T-test procedure (Field Experiments 1984).	177
59. Accumulation of symbiotically fixed nitrogen in lentils determined using the 'A' value method, the <sup>15</sup> N Assisted Difference method and the Classical Difference method; calculated using shoot and total plant samples (Field Experiments 1984).	178

## LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1.	Effect of nitrogen availability on the accumulation of symbiotically fixed nitrogen by lentils.	104
2.	Dry matter accumulated by lentils and nonnodulating soybeans over the growing season (Field Experiments 1983).	112
3.	Nitrogen accumulated by lentils and nonnodulating soybeans over the growing season (Field Experiments 1983).	113
4.	Fertilizer nitrogen accumulation in lentils and non-nodulating soybeans throughout the growing season (Field Experiments 1983).	117

## 1. INTRODUCTION

Lentils (Lens culinaris) are a high protein seed crop used primarily for human consumption. Recently introduced into western Canada, conclusive information regarding the production of lentils is limited. Seed yield estimates ranging from 900 kg ha<sup>-1</sup> (Slinkard, 1982) to 2,242 kg ha<sup>-1</sup> (Manitoba Agriculture, 1980) have been proposed.

Lentils are a member of the Leguminosae family and, in association with the appropriate Rhizobium, are capable of meeting some of their nitrogen requirements through symbiotic nitrogen fixation. Unfortunately, very little information is available regarding lentil-Rhizobium symbiosis and, of that available, little is applicable to the western Canadian agro-climate.

In light of the growing interest in alternative cropping systems, it is likely that lentil production will continue to increase in Manitoba. Consequently, there is a need to obtain information on lentil production and nutrition. It may be desirable to retain as much nitrogen fixation as possible when producing lentils. Thus there is an additional need to determine the quantity of nitrogen which lentils are capable of fixing and identify factors which may affect fixation.

The objectives of this study were: 1) to determine the effect of various soil conditions including nitrogen and moisture availability on nitrogen fixation and yield of lentils, and 2) to evaluate methods of estimating symbiotic nitrogen fixation in lentils.

## 2. LITERATURE REVIEW

### 2.1 SYMBIOTIC NITROGEN FIXATION AND YIELD OF LENTILS

Lentils (Lens culinaris) are a recently introduced crop in western Canada and presently little conclusive information is available regarding production potential. Seed yield estimates ranging from 900 kg ha<sup>-1</sup> (Slinkard, 1982) to 2,242 kg ha<sup>-1</sup> (Manitoba Agriculture, 1980) have been proposed.

Summerfield (1981) reported that lentils are capable of achieving a mean seed protein content of twenty-five percent. Thus, it can be expected that lentils will exert a large demand on available nitrogen sources. Saxena (1981) suggested that a lentil crop yielding 2,000 kg ha<sup>-1</sup> may take up approximately 100 kg N ha<sup>-1</sup>.

Lentils are a member of the Leguminosae family and, in association with the appropriate Rhizobium, are capable of meeting some of their nitrogen requirements through symbiotic nitrogen fixation. Unfortunately, very little information is available regarding lentil-Rhizobium symbiosis and, of that available, little is applicable to the western Canadian agro-climate. Studies of nitrogen nutrition of lentils grown in Egypt indicated that more than eighty-five percent of the total nitrogen requirement of the crop may be met through nitrogen fixation (Rizk, 1966). Rennie (1984) reported that lentils grown in southern Alberta are capable of fixing 150 kg N ha<sup>-1</sup>. Unfortunately, total nitrogen uptake and seed yields were not reported. Summerfield (1981) reported estimates of nitrogen fixation ranging between 35 and

75 kg N ha<sup>-1</sup>. Again, corresponding total nitrogen uptake and seed yields were not reported. In light of the paucity of information available regarding the nitrogen nutritional status of lentils, it is difficult to estimate the contribution nitrogen fixation makes to total N.

Many authors have studied the effect of various environmental factors on symbiotic nitrogen fixation in grain legumes but few have reported on lentil-Rhizobium symbiosis. As Islam (1981) noted, this is due simply to a lack of information. However, much can be elucidated from data pertaining to similar legume-Rhizobium symbiotic relationships.

#### 2.1.1 Effect of Nitrogen Application

In non-leguminous crops, application of nitrogen fertilizer generally leads to an increase in dry matter yields. This effect is complicated in leguminous crops capable of fixing atmospheric nitrogen by the effect of inorganic N on the nitrogen fixing system. Allos and Bartholomew (1955) reported studies with soybeans, peanuts, alfalfa, lespedeza, Ladino clover and birdsfoot trefoil. In all cases the presence of available inorganic nitrogen diminished symbiotic fixation. The specific influence on fixation of various levels of inorganic nitrogen varied among the legumes studied. McAuliffe et al. (1958) found that nitrogen fixation in both Ladino clover and alfalfa was reduced by nitrogen application, with the degree of reduction being definitely related to the amount of nitrogen applied. Richards and Soper (1979) reported that in fababeans, the reduction in nitrogen fixation



associated with nitrogen application was described by a significant linear inverse relationship, indicating that fababeans preferentially feed from combined inorganic nitrogen rather than symbiotically fix their needs.

The inhibitory effect of inorganic nitrogen on legume-Rhizobium symbiosis has been attributed to many mechanisms. Munns (1968) observed that if the growth medium contained nitrate, Medicago sativa had fewer root hairs, and fewer root hairs curled in response to inoculation with Rhizobium. Munns considered root hair production and curling to be a necessary prelude to infection and nodule development. Dart (1977) suggested that all stages of infection are sensitive to inorganic nitrogen, including thread growth. He found that fewer threads were formed, more were aborted and they appeared disorganized when R. meliloti was exposed to nitrate. Semu and Hume (1979) found that high levels of soil N availability, as well as applications of fertilizer N, decreased fixation in soybeans, (Glycine max L.) mainly by decreasing nodule numbers and size. Similarly, Hill-Cottingham and Lloyd-Jones (1980) reported that nodule weight in fababeans (Vicia faba (L.)) was depressed by nitrate and that there was a corresponding decrease in atmospheric nitrogen fixed. Chen and Phillips (1977) found that N fixation in Pisum sativum L. was reduced by  $\text{NH}_4^+$  and  $\text{NO}_3^-$  ions, and ascribed this reduction to earlier nodule senescence.

Wong (1980) suggested that nitrate has many effects on the development and nitrogen fixing activity of lentil root nodules. He found that lentils grown in nitrogen free nutrient solution had six times as many nodules per plant and that nodules weighed more than three times as

much as nodules of lentils grown in a nitrate containing solution. Furthermore, it was found that nitrate also inhibited N fixing ( $C_2H_2$  reduction) activity of lentil nodules. Wong observed that the inhibitory effects of nitrate were reduced by adding sugars to the growth medium, suggesting that the internal carbohydrate to nitrogen ratio governs nodule formation and nitrogen fixation.

Although the addition of combined nitrogen at high levels commonly inhibits development and function of legume root nodules, exceptions exist, suggesting the use of nitrogen fertilizers to stimulate nitrogen fixation. Dart and Wilson (1970) reported that urea applied at seeding at a rate of 112 ppm stimulated fixation in cowpeas (Vigna unguiculata L.) by twenty-nine percent above controls receiving no nitrogen. Agboola (1978) also studied cowpeas and concluded that increased fixation was related to concomitant increases in nodule numbers. Conversely Eaglesham et al. (1983) attributed increased fixation in cowpeas to increases in both nodule weights and nitrogenase activity. It has been postulated that the positive effects of applied nitrogen on cowpea symbiosis are explained in terms of an alleviation of the "N hunger" stage of growth during early development subsequent to cotyledon consumption and prior to the onset of significant nodule fixation (Pate and Dart, 1961). According to Allos and Bartholomew (1959), total fixation is closely related to the amount of growth. Thus, if alleviation of the N hunger stage leads to larger, more vigorous plant, there may be a tendency for plants to fix more nitrogen.

Hatfield et al. (1974) reported similar findings when studying soybeans. They found that nodule development was greatest when soybeans

received nitrogen for two weeks as compared to plants receiving no nitrogen. They concluded that a time lag in development of nodules for N fixation resulted in reduced growth and may have been responsible for limiting maximum nitrogen fixation and yield potential.

Yield responses of nitrogen fixing legumes to N fertilizer applied at planting are usually taken to indicate that N fixation is less than optimal. Rizk (1966), observing that lentils could fix up to eighty-five percent of their N requirements, suggested that little or no increase in yield can be anticipated with N fertilizer addition. He found that soil and symbiotically fixed N sources were generally sufficient to meet lentil nitrogen requirements. This contention was supported by Chowdhury et al. (1974), Sekhon et al. (1977) and Ojha et al. (1977), all of whom independently reported that the addition of 25 kg N ha<sup>-1</sup> did not lead to increases in lentil yield. However, although yield increases are generally taken to indicate insufficient N fixation, it does not follow that lack of response necessarily indicates that fixation is optimal for lentil N requirements. Small dressings of fertilizer nitrogen may lessen fixation of atmospheric N so that there is no net gain in the nitrogen available to the plant. McEwan (1970) has suggested that in order to determine whether yield is limited by fixation, nitrogen must be applied in amounts exceeding those usually fixed.

#### 2.1.2 Effect of Water Stress

Lentils have an indeterminate flowering habit and it has been suggested that some drought stress is required in the latter part of the