THE UNIVERSITY OF MANITOBA

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

by

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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 $N.ha^{-1}$ 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 ^{15}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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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^{-1} (Slinkard, 1982) to 2,242 kg ha^{-1} (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^{-1} (Slinkard, 1982) to 2,242 kg ha^{-1} (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^{-1} may take up approximately 100 kg N ha^{-1}.

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^{-1}. 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^{-1}. 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 NH_4^+ and 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^{-1} 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