GREENHOUSE AND FIELD TESTS FOR N_2 FIXATION IN FABA BEANS CV. DIANA INOCULATED BY DIFFERENT STRAINS OF *RHIZOBIUM LEGUMINOSARUM* FRANK.

A Thesis

Submitted to the Faculty

of

Graduate Studies

University of Manitoba

by

Banyong Toomsan

In Partial Fulfillment of the

Requirements for the degree

of

MASTERS OF SCIENCE

DEPARTMENT OF PLANT SCIENCE

May 1978

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ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. K. W. Clark under whose supervision this work was conducted, for his guidance during the investigation and preparation of this manuscript; Mr. J. R. Dean for his help all through the investigation and preparation of this manuscript.

The author also wishes to thank Dean L. H. Shebeski, Dr. S. B. Helgason and Dr. L. J. LaCroix for their interest in Khon Kaen University. Special thanks to Mrs. Ruth Durance, ex-CUSO volunteer, whose help enabled the author to come to Canada. Thanks are also due to L. Harrison, B. Cairns, C. Poersch, N. Ames, C. Bubar, R. TerHorst, and P. Becker for technical assistance, and to Ms. E. Menzies for helping correct the manuscript.

The author also wishes to thank his advisory committee, Dr. R. J. Soper, Dr. E. H. Stobbe and Dr. B. L. Dronzek.

This research was completed while the author was receiving a grant from Department of Plant Science, University of Manitoba. This support is greatfully acknowledged.

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ABSTRACT

Toomsan, Banyong. M.Sc. The University of Manitoba, May, 1978. Greenhouse and field tests for N_2 fixation in faba beans cv. Diana inoculated by different strains of Rhizobium leguminosarum Frank. Major professor: K. W. Clark.

Fifteen strains of faba beans rhizobia (Rhizobium leguminosarum Frank), originating from different countries, and the commercial inoculum (Q commercial) were tested in greenhouse conditions. The beans were grown in a soil mixture or growth pouches. Different strains performed differently in both media. Some strains were found to be effective in the soil mixture but not in the growth pouches, or vice versa. However, eight strains and the Q commercial were finally selected for further study in field conditions.

In the field test, one additional strain of Rhizobium from New Zealand was also included. The tests were conducted at two locations, representing low and high nitrogen soils. In the high nitrogen soil, seed yield was not increased by inoculation. There was no significant difference among strains. In low nitrogen soil, seed yield was increased by inoculation. Inoculation increased seed yield by increasing the number of pods per plant and 1,000 seed weight. Number of seeds per pod was not affected by inoculation. Number of seeds per plant was, of course, increased by inoculation.

Different strains performed differently in low nitrogen soil. Low soil nitrogen allowed the rhizobia to fully express their effectiveness. Two strains from Morocco (M_{11} and M_{12}) were found to show high acetylene reduction ability throughout the sampling periods. These two strains showed an increase in acetylene reduction at the

11 week harvest (pod filling stage). The high acetylene reduction at this stage resulted in higher seed nitrogen percentage, even though seed yields were not different from the other strains. These two strains might be suitable for dry areas or for a dry year. It is recommended that these two strains be added to the commercial inoculum in the same way as M_{21}, M_{22} and M_{23} are the components of the Q commercial inoculum.

The persistence of the rhizobia was followed in 1977. Approximately 90% of the plant examined in 1977 have nodules. This indicated that the rhizobia were still present, but their origin could not be clarified. Nodules were on the lateral roots. This indicated that the rhizobia were not in close proximity to the seeds and failed to nodulate early. It is recommended faba bean seed be inoculated before they are sown even though the history of the field shows that faba beans were grown previously.

FOREWARD

This thesis has been prepared in manuscript format. It consists of 4 sections. They are:

Section 1	Introduction
	Review of Literature
Section 2	Results of research in publication form, consisting of two unpublished papers, intended to be submitted to the Canadian Journal of Plant Science after some modifications.
Section 3	Discussion of entire research programme reported in section 2.
Section 4	Bibliography
	Appendices

SECTION 1

Introduction

Review of literature

INTRODUCTION

The small faba bean (Vicia faba L. var. minor) has been grown in Europe for centuries as a high protein crop (Presber 1972). China accounted for about 60% of the five million hectares planted to Vicia faba L. in 1965 (Dawson 1970). Soybeans (Glycine max (L.) Merr.), imported from the United States, are a major source of high protein feed supplement in Western Canada. However, with the rising price of soybean meal, Canada, which has a cool-climate unsuitable for growing soybeans, has become interested in small faba beans. The possibility of growing small faba beans in Manitoba and Saskatchewan was investigated in 1970 (Evans et al. 1972). Accumulated research data have shown good yields of the beans at various locations in the two provinces and protein content ranging from 23 to 32% (N x 6.25 dry basis) (Evans et al. 1972). Canada grew more than 6,000 hectares of faba beans in 1973 (Evans and Rogalsky 1975).

The rhizobium responsible for faba bean nodulation is Rhizobium leguminosarum Frank, the rhizobia which nodulate Lathyrus, Lens, Pisum and Vicia spp. (Buchanan and Gibbons 1974). Peas (Pisum sativum L.) have been grown in the prairies for many years, and many soils are expected to harbour the rhizobia forming a successful symbiosis with pea roots. Evans et al (1972) in their report on the testing of 15 cultivars of faba beans in Manitoba and Saskatchewan suggested that inoculation with pea inoculum might be beneficial. Nonetheless, strains of rhizobia highly effective on pea or vetch are often ineffective on faba beans (Burton 1976). In Saskatchewan, Johnson (1974) noticed that no inoculum and pea inoculum gave similar results and the stunted unhealthy

looking faba bean plants from such treatments were in sharp contrast to those where specific inoculum was used. The requirement of faba beans for inoculum was reported in a survey of farmers' fields in Manitoba (Candlish and Clark 1975).

N_2 fixation in faba beans varies with host cultivars (Burton 1976, El-Sherbeeny et al. 1977b) and strains of Rhizobium (El-Sherbeeny et al. 1977a). Burton (1976) reported variation in symbiotic N_2 fixation by 7 small-seeded cultivars of Vicia faba L. inoculated with an effective strains of Rhizobium leguminosarum, strain 175F1. The amount of nitrogen fixed varied and ranged from 30 to 45 mg per plant. El-Sherbeeny et al. (1977a) studied the effect of 20 different isolates of rhizobia on Vicia faba L. Plants in association with the individual Rhizobium isolates exhibited large differences in dry matter yield, nitrogen content, efficiency of nitrogen utilization and date of first flower.

The objective of this study was to select different strains of Rhizobium, originating from different countries, in greenhouse conditions and test them further in field conditions.

REVIEW OF LITERATURE

The growth, nitrogen fixation, and nodulation of legumes are influenced by many different factors. Three factors are reviewed as followed:

1) Effect of Moisture Stress

It is known that moisture stress affects growth, yield and quality of various legumes. Kilmer et al (1960) studied the mineral composition of eight forage species grown at four levels of soil moisture. They found that forage yield increased as moisture levels increased. The concentrations of nitrogen, sulfur, and boron in the plant were not significantly affected by variation in the soil moisture supply. However, the concentration of phosphorus in all eight species increased as soil moisture supply increased.

Bourget and Carson (1962) also found that the yield of oat and alfalfa decreased with increasing moisture stress obtained by depleting available moisture to 75, 50, 25 and nearly 0%.

Mack (1973) found that the cooking quality of field peas cv. Kapuskasing 3880-4 and Weitor 702 changed markedly when grown under variable soil temperature-moisture conditions in a field environment.

Mederski and Jeffers (1973) studied yield response of soy bean varieties grown at low and high moisture stress conditions. Under high moisture stress conditions, the yield of the most stress resistant varieties was reduced about 20%, while the yield of the least stress resistant varieties was reduced about 40%. The absolute reduction in yield for the most stress sensitive varieties was approximately 1000 Kg/ha, while the yield of the least stress-sensitive varieties was reduced about 200 to 400 Kg/ha.

Jones (1963) studied the effect of soil moisture gradients on the growth and development of broad beans (Vicia faba L.). The high water

table caused rotting of seed and restriction of growth while lowest water table resulted in poor emergence and loss of some plants through wilting. Maximum growth was obtained when the water table was 6 inches below the soil surface. The experiment suggests that broad bean are remarkedly tolerant of water logging, and drought in the early stages of growth is more liable to cause loss of yield than is excess water. Elston et al (1976) reported that water stress decreased absolute growth rate of Vicia faba but did not affect the duration of growth very much.

Moisture stress results in nodule shedding in Phaseolus vulgaris, peas, birds foot trefoil and soybean (Wilson 1939), 1942). Phaseolus vulgaris (Wilson 1931) shed nodules when soil moisture was reduced, a reduction from 20 to 12% of soil water resulting in about 36 per cent of nodules being shed.

Masefield (1952) found that there was a steady fall in the nodulation of P. vulgaris as the English summer became progressively drier. He also observed that nodulation was consistently higher at Samaru, Northern Nigeria than at most places in the south, attributing this to the heavier soils in the north, which were much wetter during the growing season. In Malaya (Masefield 1957), he observed more nodulation in soils with a higher water table, and vice versa, irrespective of the chemical and physical composition of the soils, although on roots overtaken by a rising water table nodules in contact with the water decayed prematurely, because of the anaerobic conditions in that region. These observations led him to conclude that, on the whole, nodulation in tropical soils was less than in temperate soils because tropical soils were more often drier.

Irrigation was found to increase nodulation of Vicia faba, P. vulgaris, Pisum sativum in temperate conditions (Masefield 1961) and Vigna unguiculata and Dolichos lablab in tropical condition (Doku 1970, Habish and Mahdi (1976).

The effect of moisture stress on N_2 fixation process has just been recently studied. Kuo and Boersma (1971) found that the amount of N_2 fixed by soybean, per unit of CO_2 absorbed decreased with increasing soil water suction, indicating that the N_2 fixation process was more sensitive than photosynthesis to water stress.

Sprent (1971a) studied the effect of water stress on nitrogen fixing ability of detached soy bean nodules. She found that reduction of water content down to 80% of the maximum nodule fresh weight, led to a proportionate slowing down of acetylene reduction, with a concomitant reduction in respiratory activity. She also noticed gross structural changes in the nodules and suggested that such nodules are probably shed by the plants.

The effect of water stress on the fine structures of soy bean nodules was studied in detail by Sprent (1972a). She found that water stress affected the outer cells of the nodule more quickly and more severely than the inner cells. In any region, vacuolated cells are more susceptible to stress than non-vacuolated cells. It was concluded from this study that the vacuolated cells of the cortex play an integral part in the nitrogen fixing activities of the whole nodules. Under normal conditions of 20% oxygen (it might be less in the soil), the outerpart of the nodule would be respiring most efficiently and reductant might be transported from there to the fixing sites. At higher pO_2 a greater proportion of non-infected cells would be contributing essential metabolites to the nitrogen fixing sites. The most logical route for this transport would be by plasmodesmata, since the metabolites would remain in the cytoplasm throughout. The supply of carbon skeletons for amino acid synthesis ultimately comes from photosynthesis.

Sprent (1972b) studied the effect of water stress on acetylene reduction (N_2 fixation) of Vicia faba and Glycine max. She found that acetylene reducing activity in both legumes was depressed by drought and the activity could be restored by irrigation. The maximum nitrogen fixation occurred at, about field capacity; above this, activity was reduced due to water logging. She noticed that wilting in the lower leaves was generally a good indication that nodules were functioning at sub-optimal rate. Sprent suggested that water stress affects nodule activity directly, but this effect may be aggravated by reduced supplies of photosynthates from wilted leaves.

Water stress has also been reported to reduce acetylene reduction in field grown soy bean (Mague and Burris 1972), Trifolium repens (Engin and Sprent 1973), Lupinus arboreus (Sprent 1973, Sprent and Silvester 1973) and Phaseolus vulgaris (Sprent 1975).

The necessity for a continuing supply of photosynthates to the nodules for the maximum nitrogenase activity has been shown in Pisum sativum (Virtanen et al 1955, Lawrie and Wheeler 1973), Vicia faba (Lawrie and Wheeler 1975), and Alnus glutinosa (Wheeler 1971).

Huang et al (1975a) designed an apparatus that permitted acetylene reduction (N_2 fixation) by root nodules to be measured in situ simultaneously with net photosynthesis, dark respiration, and transpiration of the shoot in soy bean plants. They found that as the soil desicated, acetylene reduction decreased, and the decrease was correlated with decreases in photosynthesis and transpiration. This decrease was studied further (Huang et al 1975b) to see whether it was the decrease in photosynthesis or transpiration that inhibits

acetylene reduction. It was found that the inhibition of acetylene reduction caused by low water potentials and their after effects could be reproduced by depriving shoots of atmospheric CO_2 even though the soil remained at a water potential that should have favoured rapid acetylene reduction. The inhibition of acetylene reduction at low water potentials could be partially overcome by exposing the shoots to high CO_2 concentrations. When transpiration was varied independently of photosynthesis and dark respiration in plants having high water potentials, no effects on acetylene reduction could be observed. There was no correlation between transpiration and acetylene reduction in the CO_2 experiments. They concluded that the inhibition of shoot photosynthesis, not transpiration, accounted for the inhibition of nodule acetylene reduction at low water potentials.

Beside reducing photosynthates to the nodules, water stress might affect the nodule activity directly. Sprent (1971a, 1972a) found that water stress changed the fine structure of the nodules and reduced the respiration rate. Pankhurst and Sprent (1975) found that 75 per cent of the N_2 fixing (measured as the reduction of C_2H_2) and 50 per cent of the respiratory activity of detached nodules was lost when the turgid nodules were changed to moderately stressed nodules. Severely stressed nodules showed almost complete loss of N_2 fixing activity and up to 80 per cent loss of respiratory activity. Increasing the oxygen partial pressure completely restored both N_2 fixation and respiration in moderately stressed nodules, but only partial recovery was possible in severely stressed nodules. They discussed the results in view of

the possible development of a physical barrier to gaseous diffusion and/or the possible altered affinity of the nodule leghaemoglobin for O_2 in the water-stressed nodules.

The effect of water logging on fixation has been studied. Sprent (1969) found that immersion of the detached nodules in water inhibit C_2H_2 reduction by reducing O_2 supply to the nodules. This effect could be overcome by increasing the partial oxygen pressure. This has also been confirmed by Schwinghammer et al (1970), Sprent (1971b), Mague and Burris (1972). Huang et al (1975a) found no acetylene reduction was detectable in flooded soil. Removing the soil eliminated the inhibitory effect of flooding suggesting that rates of gas exchange were restricted between the nodules and the soil atmosphere.

In Phaseolus vulgaris, Sprent (1975) reported that water logged conditions affected nodule number, size and water content. The nodules were small and ridged. The ratio of surface to volume was increased but there was no evidence that this equipped nodules to fix any more efficiently under water logged conditions. Acetylene reduction was reduced and different strains of Rhizobium performed differently under water logged condition.

Minchin and Summerfield (1976) found that under water logged conditions, cowpea (Vigna unguiculata (L.) Walp.) developed 'lenticels' type nodules and increased nodule cortication. These were regarded as adaptive anatomical responses which facilitate continued symbiotic fixation under such a condition.

Sprent and Gallacher (1976) studied the effect of water stress and water logging on soy bean nodules. They found that under both conditions the nodules showed lowered C_2H_2 reducing activity and O_2 uptake, but enhanced CO_2 and ethanol evolutions. The effects of stress could be partially or completely overcome by increasing the O_2 supply. It was concluded that both water deficiency and excess might depress nitrogenase activity by restricting supplies of intermediates from aerobic pathway. At the same time fermentative pathways were stimulated and could lead to inhibitory concentrations of ethanol being produced.

The effect of water stress on N_2 fixation in Vicia faba under the field conditions has been reported. Sprent et al (1977) studied seasonal growth patterns in Vicia faba as affected by population density, shading and its relationship with soil moisture from 1972-5. Soil moisture stress was found to retard the growth of Vicia faba. They suggested that water supply may be a more important factor controlling yield than either solar radiation or plant competition, with the period following pod setting being especially vital. At this time plant water requirement may often be in excess of supply. Maximum potential acetylene reducing activity per plant varied little from year to year (Sprent and Bradford 1977). At low population densities a maximum rate of activity was observed shortly after flowering. As density increased, moisture stress also increased and thus made the maximum potential reducing activity per plant decreased.

Sprent (1972b) found that the soil water content for maximum acetylene reducing activity in Vicia faba in the field was about

field capacity. The soil water content below or above this point reduce acetylene reducing activity. In pot experiment with sand, Tu and Hietkamp (1977) found the optimum water content for acetylene reduction by the nitrogenase of the soy bean root nodules was 60-80 per cent of the moisture holding capacity of the sand.

In pot experiment in the greenhouse, Hume et al (1976) reported that depletion the soil moisture around the soy bean root nodules did not affect acetylene reducing activity as long as the water was sufficiently supplied from the lower part of the root (below the nodule zone). Only the soil around nodules was very dry or near field capacity, did the acetylene reduction decrease. This experiment indicated that nodule moisture status was maintained if plant received adequate moisture from below the nodule zone.

In Vigna unguiculata (L.) Walp., it was found by Summerfield et al (1976) that repeated wilting prior to flowering markedly reduced yields compared to the unstressed control. This was mainly by decreasing subsequent pod production. Nodule weight and nitrogenase activity were also much reduced. Wilting after flowering did not reduce yield and nitrogenase activity was less affected.

2) Effect of Combined Nitrogen

The influence of available inorganic nitrogen on the nitrogen fixation processes in leguminous plants has been the subject of a number of investigations. The early work reviewed by Fred, Baldwin and McCoy (1932) demonstrated that nodulation, and thus fixation can be inhibited by concentrations of available inorganic nitrogen.

By using nitrogen isotope of mass 15, it was found that symbiotic nitrogen fixation in soy beans, peanuts, alfalfa, ladino-clover and birdsfoot trefoil, diminished with the presence of inorganic nitrogen fixation (Thornton 1946, Thornton and Broadbent 1948, Norman and Kram pitz 1945, and Allos and Bartholomew 1955).

More recently, with the advent of the acetylene reduction technique as a method for determining nitrogen fixation, it has been found that combined nitrogen reduces acetylene reduction (symbiotic nitrogen fixation) (For example Lawn and Brun (1974), Candlish and Clark 1975, and Gibson and Pagan 1977).

High nitrate concentration has been found to inhibit alfalfa nodulation to a greater extent than high ammonium concentration (Richardson et al 1957). Thornton (1936) observed that if the growth medium contained nitrate, lucerne plant had few root hairs, and fewer of the root hairs curled in response to inoculation with nodule bacteria. Thornton considered curlings to be a necessary prelude to infection by the bacteria. Curled root hairs outnumbered infections and nodules even at inhibiting concentrations of nitrate, but Thornton argued that reduced curling should reduce the chance of infection, and postulated that the reduction in number of curled root hairs was the reason for reduction in number of nodules.

This has been supported by Munns (1968) who found that when nitrate was maintained in continuous supply in solution culture at concentration 0.02 mM to 2 mM it reduced the number of curled root hairs and of nodules of lucerne. Nitrate inhibited formation of infection threads and augmented the proportion of arrested infection

threads, to such an extent that could be important in limiting nodule number. Nitrate was also found to delay nodulation. The delay occurred early after inoculation and corresponded with delay in the appearance of infection threads.

Combined nitrogen may have a local (external) effect in root hair infection. Tanner and Anderson (1963, 1964) proposed that nitrate may cause a destruction of indole-acetic acid (I.A.A.) which is important for nodulation process. Rhizobia reduced nitrate to nitrite. Nitrite was capable of catalytically destroying, indole-acetic acid. Ammonium, urea or glycine also reduced the formation of indole-acetic acid, but not through the formation of nitrite.

However, Gibson and Pagan (1977), by using nitrate-reductase deficient mutants of rhizobia, found that nitrate retarded initial nodulation by the mutant stains to an extent similar to that found with the parent strains. It is therefore unlikely that nitrite produced from nitrate by the rhizobia, plays a significant role in the inhibition of nodulation by nitrate.

Gibson and Nutman (1960) reported that combined nitrogen inhibited nodule initiation. High rates of combined nitrogen inhibited while lower rates enhanced the development of initiated nodules (Hinson 1975). In Hinson's experiment, roots of 17 day old soy bean plants were divided and transplanted into pairs of pots so that half of the root system received no N and the other half recieved N ranging from 0-240 ppm. Number of nodules in pots without N did not differ significantly, but nodule fresh weight was greater when the companion pot received 30-120 ppm N than when it received 0 or 240 ppm. This

experiment showed non-localised effect of combined nitrogen on nodule development.

Harper and Cooper (1971) reported that uniform incorporation of ammonium nitrate (dispersed throughout a 30.5 cm soil column) impaired nodule development of soy beans more than deep application (lower 10 cm of soil column) when ammonium nitrate was applied at a rate of 150 ppm in growth chamber studies. Nodule fresh weight and haemoglobin content were decreased by nitrogen application, while nodule number was not markedly affected, indicating an effect on nodule development rather than on root infection or nodule initiation.

A small amount of combined nitrogen has been found to promote nodulation. Richardson et al (1957) studied the effect of various concentration of sodium nitrate and ammonium chloride on the ability of Rhizobium meliloti to infect and fix nitrogen in three varieties of alfalfa. Nodulation was inhibited at high level of combined nitrogen. A small amount of combined nitrogen, however, appeared to promote nodulation.

Allos and Bartholomew (1959) studied the influence of increasing increments of available inorganic nitrogen on symbiotic nitrogen fixation of five legumes in greenhouse condition. All legumes responded in growth and nitrogen uptake to the addition of inorganic nitrogen. In some instances the increase in growth resulting from fertilization caused increases in fixation of nitrogen. When the applied nitrogen exceeded that necessary for growth increase, it tended to replace the fixation process. Fixation processes never supplied

sufficient nitrogen for maximum growth under the conditions of the experiment. Each species exhibited an apparent capacity to supply by fixation only about one-half to three-fourths of the total nitrogen which could be used by the plant.

Pate and Dart (1961) found that in barrel medic (Medicago tribuloides Desr.) and vetch (Vicia sativa L. and Vicia atropurpurea Desf) bacterial partnership of a host plant varied greatly in their nodulation responses to a range of amounts of nitrogen applied at sowing. Some bacterial strains exhibited varying degrees of stimulation of nodule number, growth and fixation by low or medium amounts of nitrogen. Higher levels of combined nitrogen depressed symbiosis. Other strain responses showed a severe restriction of symbiosis with any amount of added nitrogen.

Gibson (1965) studied subterranean clover inoculated with an effective strain, TA1, or less effective strain, CC10 of Rhizobium. Based on total plant nitrogen, strain CC10 was 70% as effective as strain TA1 without added nitrogen, but 87% as effective with 1 mgN supplementation.

Gibson (1975) reported marked stimulation of nitrogen fixation by early supplementation with combined nitrogen in soy beans and lupins. With soy beans, 7 mM nitrate nitrogen was included in the nutrient solution for 14 days after sowing, and the pots then eluted with nitrogen free nutrient. Nitrogen assimilation by these plants was comparable to that of inoculated plant supplied with 7 mM nitrate-nitrogen throughout, and nitrogen fixation was 3 times better than that of control plants not supplemented. A similar situation was found

with the lupins.

The effect of combined nitrogen on legumes in the field condition has been studied. Many workers reported failures of response to nitrogen fertilization. Welch et al (1973) studied the yield response of soy bean (Glycine max (L.) Merrill) to nitrogen added at different rates, times and methods of application, as direct and residual, and as inorganic or organic sources. Considering all the studies, yields were significantly increased in only 3 out of 133 instances and these occurred at high, uneconomical rates of N fertilizer. It was therefore concluded the N available to the plant was not the growth factor that limited soy bean yields in that study.

Pal and Saxena (1975) studied the response of nodulating and non-nodulating isolines of soy bean cv. Clark and Harosoy to N application. Nodulating lines did not respond to applied N when they were effectively inoculated or grown on land which had previously grown inoculated soy beans. On land growing soy bean for the first time, uninoculated Clark and Harosoy crops needed 300 and 235 KgN/ha, respectively, to give maximum seed yields. It was concluded that symbiosis was better than N application for nutrition of soy beans.

William and Diatloff (1975) studied the response of urea application at different rates and different stages of growth of soy bean in Australia. Nodulated plants did not respond to applied nitrogen except in one year when abnormal climatic conditions of low rainfall and high temperature decreased nodulation. Applied nitrogen decreased nodulation, the most severe effect being with inoculated soy bean grown in rhizobia-free soil under conditions of high temperatures

and decreasing soil moisture.

In Brazil, Olsen et al (1975) reported applied nitrogen did not increase seed yields or nodulation rating on the Santa Maria soil and decreased seed yields, nodulation rating and plant height at a rate > 224,> 112 and 448 Kg N/ha, respectively in the São Pedro soil.

Criswell et al (1976) studied the effect of adding anhydrous ammonia and organic matter on components of nitrogen fixation and yield of soy beans. Yield did not increase by the treatments. Organic matter increased nodule weight per plant, but the larger nodules fixed less nitrogen per unit less than did those in the untreated plots. Added nitrogen reduced nitrogen fixation per hectare by 39-48% of that on the untreated plots, with nodule weight and efficiency both reduced.

Even though some workers reported failures to get a response from nitrogen fertilizer application in field conditions, some workers were able to get some responses. Harper (1974) reported results of field and outdoor hydroponic studies with soybeans. Seed yield of plants totally dependent on atmospheric nitrogen was less than one half of the yield of plant utilizing both nitrate and atmospheric nitrogen under hydroponic growth conditions. Plants grown on a low nitrate level had higher symbiotic N_2 (C_2H_2) fixation rates than those grown on no nitrate. Similarly, seed yield of plants grown in hydroponic on high nitrate levels, which inhibited symbiotic fixation, was less than yield of plants utilizing both nitrate and atmospheric nitrogen. Therefore, it was concluded that symbiotic nitrogen fixation and nitrate utilization appeared essential for maximum yield.

Ham et al (1975) studied the effect of different forms of nitrogen fertilizer on the yield of nodulating and non-nodulating soybean isolines. Added nitrogen increased seed yield and protein content, weight per seed and oil yield per hectare, but generally reduced seed oil content. All sources of fertilizer decreased nitrogen fixation, plant nodule weight, nodule number and weight per nodule. Increases in seed yield and/or seed protein percentage in the nodulating lines suggest nitrogen fixation failed to supply amount of nitrogen essential for maximum seed yield and/or protein percentage.

Bhangoo and Albritton (1976a, b) reported applied nitrogen fertilizer increased seed yield and root growth but decreased nodulation in soy bean. Symbiotic nitrogen fixation ceased with 448 KgN/ha, while with 56 KgN/ha symbiotic fixation was not inhibited. For greatest utilization and efficiency of fixed nitrogen, it was recommended that applied nitrogen should be in the range of 56-112 Kg/ha, with allowance for residual soil nitrogen.

Lawn and Brun (1974) studied the effect of various rates of ammonium nitrate applied at the end of flowering to Chippewa 64 and Clay soybeans. The application of nitrogen caused a decline in acetylene reduction activity for both varieties. Seed yield and seed protein content for Clay, and seed protein content and vegetative protein for Chippewa 64, were all increased by the application of nitrogen.

Edje et al (1975) reported Phaseolus vulgaris seed yield increased from 2.2.t/ha, when no nitrogen was applied, to 3.8t/ha when 200 KgN was applied.

Summerfield et al (1976) reported cowpea (Vigna unguiculata

(L.) Walp.) supplied with nitrogen fertilizer has more nitrogenase activity than the unsupplied ones.

McEven (1970) reported that applying large dressing of fertilizer nitrogen to seed bed lessened nodulation of Vicia faba by as much as 50%. Cut nodule surface appeared red in colour so that effective fixation was assumed, but the rate of fixation was not determined. The yield results suggest that there is no prospect for the economic use of nitrogen fertilizer in the seed bed for Vicia faba. Dean and Clark (1977) concluded from their experiments that faba beans (Vicia faba) are essentially self sufficient in nitrogen from symbiotic fixation in low nitrate soils.

3) Effect of Temperature

The effect of soil temperature on legume symbiosis was first studied by Jones and Tisdale (1921). Different legumes performed differently at high soil temperatures. Peas were dwarfed at 30°C, clover developed poorly at 36°C, while alfalfa and soybeans grew very well at 36°C. Maximum dry weight per soybean plant were obtained at 24°C. At 30° and 36°C, weight of tops was as great as at 24°C while the weight of nodules declined rapidly. The variability in nitrogen content was greatest in the shoots. The roots were uniform. There was a sudden increase in nitrogen at 24°C as compared with that at 18°C and a sudden fall at 33°C as compared to that at 30°C.

Meyer and Anderson (1959) studied the effect of two root temperatures on nitrogen fixation of Trifolium subterraneum L. by using agar tube culture in a glass house condition. The inoculated plants

were well noculated at both temperatures. Moderately high root temperature (30°C) exerted a specific inhibitory effect on symbiotic nitrogen fixation, while at lower temperature (20°C) plants inoculated with Rhizobium trifolii fixed nitrogen and grew normally. Uninoculated plants responded normally to nitrogen treatment at both temperatures.

Mes (1959a) reported that temperate legumes, Vicia sativa L., Pisum sativum L. and Lupinus luteus L., reacted in accordance with the results of Meyer and Anderson (1959). An increase in the day temperature from 18°, 19°, or 21°C to either 25°C or 27°C., generally decreased the nitrogen percentage and the total nitrogen content of the plants. An increase in the night temperature from 10°C to 21°C, generally also decreased the total nitrogen content although the actual nitrogen percentage often increased. For tropical or subtropical legumes, Arachis hypogoea, Glycine max (Mes 1959a) and Stizolobium deeringianum (Mes 1959b), it was found that a low night temperature was more harmful to the growth of the plant when they were dependent on the Rhizobium for their nitrogen nutrition than when nitrogen was supplied to the growth medium. Nitrogen fixation in these legumes was found to be poor below 18°C.

Dart and Mercer (1965) studied the nodulation and growth of cowpea plants (Vigna sinensis) under two light intensities, at six temperatures and by using different levels of ammonium nitrate. The optimum temperature for growth and nitrogen fixation was at 27°C. The optimum for primary root nodulation was at 24°C and for secondary root nodulation at 33°C.

Pate (1961, 1962) studied the symbiosis of Medicago tribuloides (barrel medic) and Vicia atropurpurea (purple vetch) over a range of constantly maintained day temperatures at different light

intensities and at low night temperatures. Total nitrogen fixation in both species showed a maximum at 24°C with the higher light intensities benefiting fixation in all temperature conditions. At high temperatures (above 27°C) there was a depression of symbiotic activity. The various symbiotic combinations differed in their relative abilities to engage symbiosis under different temperature conditions. Each strain responded in a different manner to the temperature.

High root temperature (30°C) has a specific effect on nitrogen fixation and induces nitrogen deficiency in subterranean clover while the same shoot temperature (30°C) did not affect nitrogen fixation in the same way (Possingham et al 1964). The harmful effect of high root temperatures on nitrogen fixation was similar, regardless of whether the temperature was constant or fluctuating. The decrease in nitrogen fixation was not due to a decrease in the number of nodules. The number of nodules was in fact greater at high root temperature, and could well have been a result of the temperature induced nitrogen deficiency. The growth was good when ammonium nitrate was given to the plants.

Philpotts (1967) reported that high soil temperature could cause nodulation failure of cowpeas (Vigna sinensis). Also nodule formation and development were reduced and fewer large nodules occurred and a higher proportion of nodules appeared ineffective.

Joffe et al (1961) found that symbiotic nitrogen fixation is a thermo-sensitive process in which effective fixation is confined to relatively narrow temperature limits. The differential response of two legumes was evident, low root temperatures inhibited nitrogen

fixation more strongly in the case of Arachis hypogoea L. than Trifolium pratense L.

Gibson (1963) studied the effect of root temperatures ranging from 5 to 30°C on the growth and symbiotic nitrogen fixation of four varieties of Trifolium subterraneum L., inoculated with each of two strains of Rhizobium trifolii. He found that symbiotic nitrogen fixation was reduced at root temperature 22°C, and at 5°C was only 10-17% of that achieved at 18°C. At 30°C there was a marked reduction in nitrogen fixation by some host strain combinations. Some symbiotic combinations achieved a level of growth similar to that made by plants receiving adequate combined nitrogen while the others were consistently less effective. For both dry weight and nitrogen fixation, there was a significant interaction between the varieties and bacterial strains throughout the temperature range, and above 18°C, the degree of this interaction was influenced by the root temperature. Earlier Gibson (1961) had found that root temperature influenced the relative effectiveness of the symbiotic combinations.

Nutman (1961) studied the symbiotic effectiveness of 15 varieties and of four tetraploid lines of subterranean clover in association with several strains of nodule bacteria. He found relatively small but significant differences in yield occurred between host varieties and the varietal order of response depended some what, but not enough to show any well-defined variety-strain specificity. He, therefore, concluded that the interaction between host varieties and bacterial strains was of minor importance.

Gibson (1965) studied nitrogen fixation by six varieties of

Trifolium subterraneum L., each inoculated with a number of strains of Rhizobium trifolii, under a range of temperatures. In order to minimize differences in the rate of nodule establishment and early nitrogen fixation between varieties and strains, he used relative nitrogen assimilation rates (R_N) and relative growth rates (R_W) to compare different symbiotic combinations. R_N was found to be a more satisfactory parameter than R_W for comparing the varietal and temperature treatments. This was particularly true for the higher root temperatures (25-30°C) which severely reduced nitrogen fixation by three of the eight strains tested. This did not seriously affect the R_W of the plants inoculated with these strains. The presence of strong statistical interactions in the analysis of the data suggest that the nitrogen fixing ability of the varieties depends on both the root temperature and the strain of nodule bacteria.

Gibson (1966) found that at the lower root temperatures (5° and 10°C), the translocation of nitrogen to the shoot was retarded in both nodulated and ammonium nitrate control plants, and nitrogen was retained in the roots. Up to 18°C there was a progressive increase in the proportion of the total nitrogen assimilated and translocated to the shoots. In the nodulated treatments, there were both host variety and bacterial strain effects on the distribution of fixed nitrogen. With an increase in root temperature from 8 to 18°C, the increase in the rate of shoot and root dry weight gain was similar (two varieties), or the rate of increase in the dry weight of the shoots increased more than that of the roots. Up to 18°C the percentage nitrogen level in the roots of the nodulated plants was a

function of a strain of nodule bacteria, where as the percentage nitrogen level in the shoots was a function of the host variety. The strain of nodule bacteria affected the morphology of the root system of the host plant. Above 20°C root temperature, changes in dry weight increase and its distribution between the roots and the shoots, were largely controlled by the effect of root temperature on symbiotic nitrogen fixation (root temperature x bacterial interaction) although varietal effects were also evident.

Gibson (1969) re-examined the effect of bacterial strain and root temperature on the retention of nitrogen in the root system of Trifolium subterraneum. The root systems of plants nodulated by the moderately effective Rhizobium trifolii strains NA30 possessed a higher percentage nitrogen than those nodulated by the fully effective strain TA1, although the number of nodules formed by each strain was similar. The difference was due to a greater weight of nodule tissue on the NA30 nodulated plants, and also to a higher percentage nitrogen in the NA30 nodules; this latter effect was due to a higher concentration of non-protein nitrogen. The overall effect of these differences was to reduce the amount of nitrogen translocated to the shoot of the NA30 plants, in both absolute terms and as a proportion of the total amount of nitrogen fixed. Another difference between the two strains was the rate of nitrogen fixed per unit (dry weight or leghaemoglobin content) of nodule tissue. At 8°C root temperature, a higher proportion of nitrogen fixed was retained in the nodule system compared with that for plants grown at 15 and 22°C. At this lower temperature, nodule dry weight and nodule nitrogen constituted a

higher proportion of total plant dry weight and total nitrogen than they did at the higher temperatures. It was therefore proposed that the best Rhizobium symbionts were those strains that not only maintain high rates of fixation per unit dry weight of nodule tissue but also released the highest proportion of fixed nitrogen for use in general plant growth.

Heinrichs and Nielsen (1966) grew alfalfa varieties of diverse genetic origin to determine the effect of soil temperature (5-27°C) on herbage and root growth. The air temperature varied between 15° and 32°C. Varieties of Medicago sativa generally yielded more herbage and roots than those of Medicago falcata. The growth of alfalfa was greatly affected by soil temperature; the most herbage was produced at 27°C and most root and nodular tissue at 12°C. Strain x variety interaction was not studied.

Kunelius (1970) studied the effect of various root temperature on nitrogen fixation of 3 varieties of birdsfoot trefoil (Lotus corniculatus L.), each being inoculated with 6 strains of Rhizobium or dependent on ammonium nitrate. The plants were grown in growth pouches. He found that when the plants were dependent on symbiotic nitrogen fixation the highest dry weights and nitrogen yields per plant were obtained at 18 or 24°C depending on symbiotic combination. At 9° and 12°C nitrogen fixation was depressed and the growth was poor. The dry weight of plants at 9°C were 19 to 45% of those at 24°C. At 30°C the growth and nitrogen fixation were generally depressed. If combined nitrogen as ammonium nitrate was given the growth was superior

to plants depending on symbiotic nitrogen fixation at all root temperatures. Significant interactions indicated that nitrogen fixing ability of varieties was dependent on both root temperatures and the strains of Lotus rhizobia.

The effect of temperature on the formation of rhizobial root nodules of beans was investigated by Barrios et al (1963) by using a method of isolated root culture under axenic conditions. Optimum nodulation was obtained at 20°C. At 12°C and 33°, the root growth was reduced and nodulation was nil. At 17° and 30°C the growth was as good as 25°C but nodulation was reduced an average of 70%. They concluded that decreased nodulation at 17°C and 30°C was therefore unrelated to root growth; neither could it be explained on the basis of size of the effective inoculum (number of rhizobia) and abundance of root hairs. Roots held at 30°C nodulated as much as roots held at 25°C, provided they were exposed to 25°C at least for three days after inoculation.

Lie (1971a, b) reported that pea cv. Iran failed to form nodules with Rhizobium strain PRE, when the plants were grown at 20°C, the optimum temperature for most pea plants, but its nodulation was normal at 26°C. Selection for Rhizobium strains capable of nodulating this cultivar at 20°C produced one strain (No. 310), which nodulated well both at 20° and 26°C. The majority of Rhizobium strains belonged to what was termed intermediate type i.e. where only a few nodules were formed at 20°C but large number at 26°C.

Nodulation of various tropical legumes is very sensitive to 'low' root temperature (Dart and Mercer 1965; Gibson 1971). Nodulation of tropical and subtropical legumes, i.e. Glycine wightii,

Desmodium uncinatum, Desmodium intortum, Stylosanthes humilis and Phaseolus artopurpureus; were found to be poor or absent at 18°C. Nodulation at 24°C was good for the last three species. At 30°C nodulation was good for all species. At 36°C, nodulation was poor.

Roughley et al (1970) studied the influence of root temperature on root hair infection of Trifolium subterraneum L. by Rhizobium trifolii Dang. They found that the infection of root hairs was markedly delayed at 7°C, but once started the infection proceeded for a longer period of time than at 19°C, the optimum temperature for this symbiosis. Ultimately the same number of infected root hairs was found at the two temperatures used. Surprisingly, the first nodule was observed 24 hours after finding the first infection thread at 7°C, whereas at 19°C a period of about 3 days elapsed between the first infection observed and the appearance of the nodules. From this experiment the conclusion was drawn that the infection was markedly delayed but the nodule initiation was accelerated at low temperatures.

However, Lie (1971a, 1974) considered that the results with pea plants favour the hypothesis that nodule initiation is particularly sensitive to low temperature. In the experiment a pea plant (cv. Iran) was used, which is unable to form nodules at 20°C or lower, but nodulation takes place at 26°C. The requirement for the higher temperature is only confined to the second and/or third day after inoculation. These results indicate that infection, growth of the nodules and nitrogen fixation proceed normally at 20°C, but nodule initiation requires a slightly higher temperature.

Roughley (1970) reported that the structure of nodule changed

when the temperature was lowered. Progressively less bacteroid tissue was formed with decreasing temperatures and at 7°C almost no bacteroid could be observed. The highest amount of bacteroid tissue was formed at 11°C but the efficiency of nitrogen fixation per unit of bacteroid tissue was the highest at 19°C, the optimum temperature for symbiosis.

A detailed study has been made by Pankhurst and Gibson (1973) on subterranean clover in symbiosis with Rhizobium strain NA30. At 22°C the symbiosis proceeded normally resulting in a well developed central bacteroid tissue. These plant cells were packed with strongly enlarged bacteroids (about 8-10 time the size of the vegetative bacterial cell) and each bacteroid was enclosed in a separate membrane envelope. When this symbiosis was kept at 30°C the nodules formed contain a large zone with cells, penetrated by infection threads. These infection threads were highly branched and had a distorted shape when compared with normal infection threads. The bacteroid zone was disorganized and the plant cells contained membrane envelopes, each containing 4-6 small bacteroids, usually not larger than normal vegetative bacteria. Some of the cells, especially in the degenerating zone, might be filled with a polysaccharide, presumably released from the infection threads. From the same study, it was found that the symbiosis between subterranean clover and Rhizobium strain TA1 was less affected by high temperature. These results suggest that degeneration of the bacteroid tissue is rapid at high temperatures and this may result in shortening of the period of nitrogen fixing activity of the nodule. It may presume that Rhizobium strains, capable of performing good symbiosis at high temperatures

delay nodule degeneration longer than other Rhizobium strains do.

Kuo and Boersma (1971) studied the effect of soil water suction and root temperature on rates of nitrogen fixation, transpiration, and net photosynthesis of soy beans (Glycine max (L.) Merr.). The range of root temperature and soil water suction considered was 10 to 37.8°C and 0.30 to 2.50 bars, respectively. With increasing root temperature rates of nitrogen fixation, transpiration and photosynthesis increased slowly at first and then rapidly until an optimum temperature was reached followed by a decrease at higher temperature. Optimum temperatures were 27°C, 30°C and 27°C for rates of nitrogen fixation, transpiration and net photosynthesis respectively. Rates of the three parameters decreased with increasing soil water suction. At 10 and 15°C, water stress had a more adverse effect than at 24° and 32°C, possibly related to the lower uptake by plant roots at lower temperatures.

Sandhu and Hodges (1971) studied the effects of photoperiod, light intensity and temperature on vegetative growth, flowering and seed production of chickpea (Cicer arietinum L.). They found that plants grown in high light intensity (28 Kilolux), 16-hour photoperiod, and at 22.5°C, produced more flowers and seed than other treatment combinations.

Dart et al (1975) reported the effects of soil temperature on nitrogen fixation in soybeans and chickpeas. Chippewa soybeans inoculated with 3 Rhizobium strains (CB 1809, Sm1b and CC 705) when grown at 21°, 27°, or 33°C day temperature, there was little difference in nitrogen fixation between strains at 21°C, but at 27°C and especially

33°c, Sm1b was the most effective. CC705, as effective as CB1809 at 27°C, at 33°C formed red nodules with some nitrogenase activity, but which fixed little nitrogen.

Chickpeas grew and fixed similar amounts of nitrogen with all strains at root temperatures from 15-25°C, but nodulation and nitrogen fixation at 30°C was dependent on Rhizobium strains, the best strain Ca-2 fixing more than 60% as much as at 23°C. No nodules were formed by any strains at root temperature greater than 32°C.

Minchin et al (1976) studied the effects of different soil temperature regimes on vegetative growth, symbiotic nitrogen fixation and seed yield of two cowpea cultivars (K2802 and Prima). Mean maximum soil temperatures above 32°C significantly reduced vegetative growth of both cultivars, through their effects on branch, peduncle and root dry weight per plant and, to a lesser extent, leaf production. The warmest temperature regime (35.4°C) also reduced nodule activity, especially in cv. Prima. Seed yields were adversely affected, due largely to change in the number of peduncles per plant, as mean maximum soil temperature increased from 25.8° to 35.4°C.

Munns et al (1977) studied the alfalfa nodule distribution and inhibition of nitrogen fixation by heat in both greenhouse and field conditions. In greenhouse solution cultures, acetylene reduction and nitrogen fixation by nodules were impaired by daily heating of the culture solutions to 32°C, and eliminated by repeated exposures to 36°C or a single exposure to 40°C. However in a field trial, alfalfa showed no signs of nitrogen deficiency and responded little to the application of 500 Kg NH_4NO_3/ha although soil temperatures above 32°C were recorded

for 5 to 6 hours each day during a 6 day hot spell following a late summer cut, with peak soil temperatures above 40°C at 2 cm depth. The lack of response in the field trial was explained by observation of temperature profiles and nodule distribution. Only the top most 5 cm of soil heated above 30°C, and this contained less than 10% of the nodules. Most of the nodules were at depth 10 to 30 cm, and remained at nearly optimal temperatures of 22° to 27°C.

SECTION 2

Results of research in publication form

RESULTS OF RESEARCH

1. The response of faba beans cv. Diana to different strains of *Rhizobium*: Greenhouse conditions.

ABSTRACT

Three separate but identical experiments using partially sterilized mixed soils and a growth pouch experiment were conducted in the greenhouse in the fall of 1975, to determine the effectiveness of 15 strains of *Rhizobium* with faba beans cv. Diana. In the soil experiments, the 5 week harvest showed differences in nodulation but not dry weight. At the 8 week harvest, nodulation, acetylene reduction, dry weight and % N content differed among strains. There were highly significant correlations (r) among nodulation score, C_2H_4 production and % nitrogen. At the 11 week harvest, results were similar to the 8 week harvest. Highly significant correlations (r) were found among nodulation score, C_2H_4 production and dry weight.

In the growth pouch experiment, different strains showed differences in nodulation and dry weight. Ethylene production in this experiment was low. This is thought to be due to a barrier that might prevent O_2 diffusion to the nodules or the nodules were too young to function properly.

From these 4 experiments, 8 strains were selected for further study in the field. They were M_4, M_{11}, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23}, and were compared with Q commercial inoculum.

INTRODUCTION

Rhizobia are generally screened for infectiveness and effectiveness in the greenhouse or growth room conditions. The greenhouse test is more valuable with a forage than with a grain producing legume as there is a direct relationship between dry weight and N-content of the forage legumes (Erdman and Means 1952), and the reproductive cycle does not dominate the plant so completely as it does with the grain legumes. Prudent use of the greenhouse and growth chamber can aid in eliminating poor or totally ineffective strains (Burton 1976). Many methods in the study of nitrogen fixation in the greenhouse or light room have been summarized (Vincent 1970). After the discovery that C_2H_2 inhibits nitrogen fixation in extracts of Clostridium pasteurianum (Schöllhorn and Burris 1966, Dilworth 1966), a method of acetylene-ethylene assay for N_2 fixation in laboratory and field conditions was developed (Hardy et al 1968). This technique proves to be useful in determining N_2 fixation effectiveness in different symbionts (eg. Hardy et al 1968, Schwinghammer et al 1970, Wacek and Brill 1976, Dean and Clark 1977).

The experiments were conducted in the greenhouse in the Fall of 1975 by using partially sterilized soil mixture and growth pouches.

The objective of the experiments was to evaluate the symbiotic relationships between different Rhizobium strains and faba beans cv. Diana under greenhouse conditions, and to select Rhizobium strains for further study in the field.

MATERIALS AND METHOD

Sixteen different strains of *Rhizobium* were obtained from different countries. They were:

Rhizobium strains	Country of origin
M_3	Rothamsted, England
M_4	Rothamsted, England
M_{11}	Morocco
M_{12}	Morocco
M_{13}	Sweden
M_{14}	Sweden
M_{15}	Sweden
M_{16}	Sweden
M_{17}	Sweden
M_{18}	Sweden
M_{19}	Sweden
M_{20}	Sweden
M_{21}	Nitragin Company, U.S.A.
M_{22}	Nitragin Company, U.S.A.
M_{23}	Nitragin Company, U.S.A.
Q commercial	Nitragin Company, U.S.A.

Faba bean seeds cv. Diana were obtained from the Department of Plant Science, University of Manitoba.

The DiSPO growth pouches (16.5 x 17.5 cm) were developed by Northrup, King & Co., Minneapolis, Minnesota, U.S.A.

Experiments using soil and growth pouches were conducted in the greenhouse to determine the effectiveness of different strains of *Rhizobium*.

a) Soil experiments. Soil from the Carman Weed Research Station was mixed with sand and perlite in the volume ratio of 2:2:1,

respectively. The mixture was pasteurized at 82°C in a Dillon Automatic Soil Pasteurizer (Dillon Industries, Inc., Melrose, Mass., U.S.A.) for 6 hours on 3 consecutive days. The soils were then potted in 12 cm. diameter pots which had been immersed previously in 95% ethanol. The pots were then put in plastic bags with the plastic extending 4 inches above the top of the pots. Holes were cut in the bottom of the plastic bags to facilitate drainage of excess water from the pots.

Faba bean seeds cv. Diana were chosen to uniform size. The seeds were surface sterilized by immersing in 3% sodium hypochlorite for 5 minutes, rinsed with 95% ethanol and then washed 10 times with sterilized distilled water. Seeds were sown in the pots at the rate of 4 seeds per pot on October 5, 1975.

The pots were then divided into 18 different groups, each of which consisted of 6 pots. Each group was inoculated with 4 ml of 7 day old Rhizobium culture which had been grown in yeast mannitol broth (Vincent 1970). There were 16 Rhizobium strains and 2 uninoculated controls. The treatments were designated as follows: T_1, uninoculated control A; T_2, Q commercial; T_3 to T_{17} for M_3, M_4, M_{11}, M_{12}, M_{13}, M_{14}, M_{15}, M_{16}, M_{17}, M_{18}, M_{19}, M_{20}, M_{21}, M_{22} and M_{23} respectively; T_{18} for uninoculated control B. The pots were watered with distilled water for the first three weeks and later on with tap water as required.

The 6 pots of the 18 treatments were grouped in the greenhouse on 3 different benches and each group was completely randomized. The groups represented 3 different sampling dates (at 5,8 and 11 weeks).

The plants were thinned to 2 plants per pot at 2 weeks after planting. The plants were given $CaSO_4$, $NaH_2PO_4.H_2O$ and KCl at the rate

of 0.3, 0.6 and 0.5 g per pot respectively after thinning. The pots were rerandomized every week.

The plants were harvested according to the dates mentioned earlier. Plant tops were retained and oven-dried at 70°C for 48 hours and the dry weight was determined. Nitrogen content was determined by the Kjeldahl method. A visual assessment of nodulation was made on the basis of a 0-5 scale: 0 signifying no nodulation and 5 very abundant nodulation (Dean and Clark 1977). The roots were then subjected to an acetylene reduction technique and the gas samples analyzed in the laboratory following the procedure of Candlish and Clark (1975) with slight modifications; mason jars of 890 ml rather than 400 ml volume were used, and 20 ml rather than 15 ml acetylene was introduced into the jars.

b) Growth pouch experiment. Faba bean seeds cv. Diana were chosen to uniform size and surface sterilized as mentioned earlier. The surface sterilized seeds were then germinated in petridishes containing 1% sterilized agar for 3 days. Uniform seedlings were chosen and transfered to the growth pouches containing 30 ml of sterilized seedling nutrients (modified Candlish and Clark 1975). The tops of the pouches were covered with aluminum foil. Two holes were cut on each side of the pouch, one for the seedling and the other for a drinking straw. The drinking straws served as the holes for refilling the nutrient solution. The tops of the drinking straws were capped with aluminum foil caps. Paper clips were used to close the openings to prevent contamination.

The seedling nutrient solution (modified Candlish and Clark

1975) consisted of 0.348 g KH_2PO_4; 0.412 g $NaH_2PO_4.H_2O$; 0.118 g K_2SO_4; 0.522 g $CaSO_4.2H_2O$; 0.107 g $MgSO_4.7H_2O$; 0.336 g $MgCl_2.6H_2O$; 1 ml micronutrient solution and 0.1 ml iron solution in 1,000 ml of water. The micronutrient solution contained 4 g H_3BO_3; 1.4 g $MnSO_4\ H_2O$; 0.575 g $ZnSO_4.7H_2O$; 0.125 g $CuSO_4.5H_2O$ and 0.073 g $(NH_4)_6Mo_7O_{24}.4H_2O$. The iron solution was 5.4 g $Fecl_3.6H_2O$ and 0.657 g Na_2H_2 EDTA in 100 ml of water.

The plants were left in greenhouse conditions for 10 days. Then they were divided into 17 groups, each having 8 pouches. Each group representing one treatment with 8 replications.

One ml of 7 day old rhizobia culture grown on yeast mannitol broth (Vincent 1970) was used to inoculate the plant in the pouch. Treatment numbers were assigned as follows:

Treatment number	Rhizobium strain
1	uninoculated control
2	Q commercial
3	M_3
4	M_4
5	M_{11}
6	M_{12}
7	M_{13}
8	M_{14}
9	M_{15}
10	M_{16}
11	M_{17}
12	M_{18}
13	M_{19}

Treatment number	*Rhizobium* strain
14	M_{20}
15	M_{21}
16	M_{22}
17	M_{23}

The pouches were arranged in test tube baskets in randomized complete block design with 8 replications and left in the greenhouse. The light in the greenhouse was adjusted to a 16 hour day and an 8 hour night.

The plants were watered later with sterilized 1/4-strength seedling nutrient solution by using an automatic syringe.

The plants were harvested 5 weeks after inoculation. The tops were saved and oven-dried to determine dry weight. The roots were visually assessed for nodulation and then subjected to acetylene reduction as mentioned earlier.

RESULTS

a) Soil Experiments

The results of the 5 week harvest are summarized in Table 1. Only nodulation score and dry weight are reported in this table. Due to acetylene fault and loss of samples, ethylene production and nitrogen content are not reported.

Table 1. Nodulation, dry weight of faba beans inoculated with various strains of Rhizobium. The beans were grown in the greenhouse and harvested at 5 weeks after planting. 1/

Rhizobium strains	Nodulation* Score	Dry weight* (g/pot)
Uninoculated control A	o e	1.03 abcd
Q commercial	2.67a	1.15 abcd
M_3	0.50 cde	0.92 cd
M_4	3.17 a	0.94 cd
M_{11}	2.83 a	1.02 abcd
M_{12}	2.83 a	1.06 abcd
M_{13}	1.83 b	0.95 cd
M_{14}	2.83 a	1.25 ab
M_{15}	0.33 de	0.94 cd
M_{16}	3.50 a	1.26 a
M_{17}	1.17 bcd	1.01 abcd
M_{18}	1.33 bc	0.98 cd
M_{19}	1.33 bc	1.01 abcd
M_{20}	1.83 b	0.90 d
M_{21}	2.83 a	1.17 abc
M_{22}	3.00 a	1.00 bcd
M_{23}	2.67 a	1.24 ab
uninoculated control B	0.33 de	1.02 abcd

1/ For analysis of variance see appendices 1 and 2.
* Means of 2 plant/pot.
a-e Means within columns followed by same letter are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

At 5 week harvest, different strains show different degrees of nodulation. Uninoculated control A had no nodules but uninoculated control B had some contamination. Only one pot in uninoculated control B had been contaminated. In general, uninoculated treatments were poorly nodulated when compared to most inoculated treatments. Strains M_4, M_{11}, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23}, and Q commercial were well nodulated and not significantly different. The nodules formed on these treatments were round and pink, mostly concentrated on the tap roots which show that the nodules were effective in nitrogen fixation. The rest of the strains tested were considered to give poor nodulation. Nodules formed were small, whitish and mostly concentrated on the lateral roots which reflect that these nodules were formed by ineffective bacteria.

Dry weight results do not clearly show the difference between treatments. The inoculated treatments did not significantly differ from the uninoculated treatments. There are tendencies that some strains are better or worse than the uninoculated controls.

Table 2 shows the results of the 8 week harvest results. In this experiment, nodulation, acetylene reduction, dry weight and percentage of nitrogen content are reported. The uninoculated treatments show some degree of contamination, nodules were formed on the lateral roots. Strains M_4, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q commercial show good nodulation, while M_{11}, M_{13} show fair nodulation. The rest of the inoculated treatments were considered to be poorly nodulated. Acetylene reduction data shows that strains M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q commercial were highly effective. M_4 produced

Table 2. Nodulation, acetylene reduction, dry weight and nitrogen content of faba beans inoculated by different strains of Rhizobium and grown in the greenhouse for 8 weeks before harvest. 1/

Rhizobium strains	Nodulation score*	C_2H_4 production* (μ moles/pot/hr)	Dry weight* (g/pot)	% Nitrogen content
Uninoculated control A	1.17 cd	0.49 c	2.39 de	2.99 bc
Q commercial	3.33 a	5.45 ab	4.06 b	3.72 a
M_3	1.50 c	0.80 c	2.11 def	2.67 c
M_4	3.33 a	3.17 bc	3.16 c	3.66 a
M_{11}	2.50 b	0.29 c	3.12 c	3.59 a
M_{12}	3.17 a	7.01 a	4.23 ab	3.68 a
M_{13}	2.50 b	o c	2.53 d	2.70 c
M_{14}	3.50 a	6.22 ab	4.03 b	3.54 a
M_{15}	1.33 cd	o c	1.87 ef	2.82 c
M_{16}	2.83 ab	4.69 ab	3.24 c	3.50 a
M_{17}	1.17 cd	o c	1.86 ef	2.01 d
M_{18}	0.83 d	o c	1.87 ef	1.73 d
M_{19}	1.00 cd	o c	1.55 f	1.84 d
M_{20}	0.83 d	o c	2.06 def	1.73 d
M_{21}	3.17 a	4.78 ab	4.80 a	3.41 ab
M_{22}	3.33 a	4.51 ab	3.97 b	3.61 a
M_{23}	3.17 a	6.25 ab	4.24 ab	3.91 a
Uninoculated control B	0.67 d	o c	2.18 def	3.02 bc

1/ For analysis of variance see Appendices 3-6.
* Means of 2 plant/pot.
a-f Means within columns followed by same letter are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

ethylene at the rate of 3.17 μ moles/pot/hr. and may be considered to be fair even though it is not significantly different from the controls (0.49 and 0 μ moles/pot/hr. respectively). M_{13}, M_{15}, M_{17-20} do not show any acetylene reducing activity while M_3, M_{11} show some. Dry weight data shows that M_4, M_{11}, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q commercial are better than the uninoculated controls. M_3, M_{13}, M_{15}, M_{17-20} are not significantly different from the uninoculated controls. Percentage of nitrogen content follows the same trend as dry weight. M_4, M_{11}, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q commercial are not significantly different but higher than the uninoculated controls. The rest of the strains have the same percentage of nitrogen content or less than the uninoculated controls. This reflects that some of the strains even become the parasite instead of living as symbiotic relationship.

Table 3 shows the results of the harvest at 11 weeks after planting. Contamination in the uninoculated controls increased, the nodules formed were on lateral roots. M_4, M_{11}, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q commercial had excellent nodulation and the nodules were pink in colour and mostly concentrated on tap roots. The rest of the strains had poorer nodulation, sometimes even worse than the uninoculated controls. As before these nodules were usually whitish and formed on lateral roots. The nodules formed on the uninoculated controls varied. Some looked like they were formed by the effective strain (pink in colour), some looked small and whitish (ineffective strains). This reflects that contamination in the controls is random and could come from any strain. Some strains were more poorly nodulated

than the uninoculated controls. This reflects that contamination in the inoculated treatment afterward is unlikely to be a serious problem. This means that the nodules formed on the inoculated treatments are likely to come from the inoculated strains. The data for ethylene

Table 3. Nodulation, acetylene reduction, dry weight and nitrogen content of faba beans inoculated by different strains of Rhizobium and grown in the greenhouse for 11 weeks. 1/

Rhizobium strains	Nodulation score	C_2H_4 production* (μ moles/pot/hr)	Dry weight* (g/pot)	% Nitrogen content
Uninoculated control A	1.67 bc	1.40 b	2.70 cde	2.45 abcd
Q commercial	3.67 a	8.00 a	7.06 ab	2.55 abc
M_3	1.67 bc	2.82 b	3.53 cd	2.73 abc
M_4	4.00 a	9.66 a	6.42 b	2.34 abcd
M_{11}	3.17 a	6.91 a	6.26 b	2.64 abc
M_{12}	3.67a	9.46 a	7.75 ab	2.45 abcd
M_{13}	2.17 b	2.66 b	3.49 cd	2.14 cd
M_{14}	4.00 a	10.28 a	7.78 ab	2.92 ab
M_{15}	1.50 bcd	2.17 b	2.86 cde	2.99 a
M_{16}	4.00 a	10.09 a	7.22 ab	2.50 abc
M_{17}	1.00 cde	1.59 b	2.67 cde	2.23 bcd
M_{18}	0.83 cde	0.56 b	2.02 de	2.34 abcd
M_{19}	0.33 e	0 b	1.72 e	1.80 d
M_{20}	0.67 de	0.47 b	2.01 de	2.23 bcd
M_{21}	3.50 a	8.60 a	8.22 a	2.58 abc
M_{22}	3.50 a	7.96 a	7.47 ab	2.47 abcd
M_{23}	4.00 a	8.50 a	8.23 a	2.72 abc
Uninoculated control B	1.17 cde	3.35 b	3.74 c	2.43 abcd

1/ For analysis of variance see Appendices 7-10.
* 2 plants/pot.
a-e Means within columns followed by the same letter are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

production follows the same trend as nodulation score. M_4, M_{11}, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q commercial are fixing nitrogen effectively. The rest of the strains fix nitrogen poorly and are not different from the uninoculated controls. M_{19} does not fix nitrogen, it also has the lowest nodulation score. Dry weight data also confirms the previous data. The nine strains previously mentioned above are superior to the uninoculated controls. The rest of the strains have dry weights equal to or less than the uninoculated treatments. The data for the percentage of nitrogen content is less distinguishable. Most of the strains have the same percentage of nitrogen content. M_{15} is the highest (2.99%) while M_{19} is the lowest (1.80%).

b) Growth Pouch Experiment

The results of the growth pouch experiment are shown in Table 4. Only nodulation score, ethylene production and dry weight per plant are presented. Uninoculated controls did not form nodules. Nodulation of Q commercial was excellent while M_{11}, M_{12}, M_{13}, M_{14}, M_{21}, M_{22} and M_{23} were also good. M_{14} and M_{16} had fair nodulation. M_{17-20} had poor nodulation, while M_3 and M_{15} did not form any nodules at all. Ethylene production data shows that M_{11}, M_{12}, M_{13}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q commercial are fixing nitrogen. Surprisingly, M_4 which shows fair nodulation did not show nitrogenase activity. M_3, M_{15}, M_{17-20} and the uninoculated control did not show any nitrogenase activity. Dry weight data shows that M_{11}, M_{12}, M_{14}, M_{21}, M_{23} and Q commercial are highly effective. M_{13}, M_{16} and M_{22} are intermediate while the rest are considered to be ineffective. M_4 again shows poorest dry matter yield. This reflects that M_4 in this condition is living like a parasite.

Table 4. Nodulation, acetylene reduction and dry weight of faba beans inoculated by different strains of *Rhizobium* and grown in the growth pouches in the greenhouse for 5 weeks after inoculation. 1/

Rhizobium strains	Nodulation score	C_2H_4 production (μ moles/plant/hr)	Dry weight (g/plant)
Uninoculated control	0 f	0 c	0.24 ef
Q commercial	3.25 a	0.90 a	0.63 a
M_3	0 f	0 c	0.25 ef
M_4	2.00 d	0 c	0.18 f
M_{11}	2.75 abc	0.64 ab	0.50 abc
M_{12}	2.63 abcd	0.17 bc	0.54 ab
M_{13}	2.50 bcd	0.23 bc	0.33 de
M_{14}	3.13 ab	0.30 bc	0.49 abc
M_{15}	0 f	0 c	0.27 def
M_{16}	2.25 cd	0.19 bc	0.41 bcd
M_{17}	1.13 e	0 c	0.23 ef
M_{18}	1.00 e	0 c	0.25 ef
M_{19}	1.00 e	0 c	0.30 def
M_{20}	1.00 e	0 c	0.20 f
M_{21}	2.50 bcd	0.46 ab	0.50 abc
M_{22}	2.63 abcd	0.22 bc	0.37 cde
M_{23}	2.75 abc	0.31 bc	0.52 ab

1/ For analysis of variance see Appendices 11-13.
a-f Means within columns followed by the same letter are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

DISCUSSION

Carman soil was used in this study because it is low in nitrogen. The soil is fine sandy loam and is found to be low in $CaCO_3$, $SO_4^{=}$, P, NO_3^{-}, and K. The pH of the soil is 7.4. Therefore the extra nutrient was added when the plants were two weeks old.

At 5 week harvest, the uninoculated controls did not have nodules. M_4, M_{11}, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q commercial showed good nodulation. Q commercial consisted of 3 strains i.e. M_{21}, M_{22} and M_{23}. The rest of the strains were more poorly nodulated. Even though these nine strains were well nodulated, the dry matter yields were not significantly different from the controls. The explanation for this may be that at this stage of growth the plants were dependent on stored nitrogen in the seeds and nitrogen in the soil. Soil analysis showed that the soil mixture contained approximately 4 ppm available NO_3^{-}. The soil nitrate and seed nitrogen may be adequate for growth in this period. Atmospheric nitrogen fixation might have begun because nodules were pink in colour. But this may not contribute much nitrogen to the plant.

At the 8 week harvest, contamination in the controls increased. The finding that nodulation in the controls occurred mostly in lateral roots reflected that contamination occurred later. Nutman (1958) points out that the foci of infection are available for a limited time only and change position with the growing root tip. Nodulation of the previously mentioned strains and M_{13} were better than the rest of the strains. Nodulation of M_3, M_{15}, M_{17-20} were surprisingly poor and were not significantly different from the controls. Ethylene production data

shows that M_4, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q commercial were significantly different from the rest of the strains. M_{11} showed good nodulation but only produced ethylene at the rate of 0.29 μ moles/pot/hr. This is at variance with the dry weight and % nitrogen results in which M_{11} gave results comparable to the other good strains. Dry weight and % nitrogen agree reasonably well with nodulation score and C_2H_4 production. The correlation (r) between different characters are summarized in Table 5.

Table 5. Correlation(r) between nodulation score, acetylene reduction, dry weight and % nitrogen content of 8 week old faba beans (N = 18).

	C_2H_4 production	Dry weight	% Nitrogen
Nodulation score	0.8523**	0.9027**	0.8439**
C_2H_4 production	--	0.9064**	0.7596**
Dry weight	--	--	0.8330**

Values for r significant at $P \leq 0.01$** or $P \leq 0.05$*.

There is a good correlation between nodulation score and C_2H_4 production, nodulation score and dry weight, nodulation score and % nitrogen, C_2H_4 production and % nitrogen and dry weight and % nitrogen. These results agree with Dean and Clark (1977).

At 11 week harvest, nodulation scores of M_4, M_{11}, M_{12}, M_{14}, M_{16}, M_{21-23} and Q commercial were outstanding while M_{13} would be considered fair. Contamination in the controls increased and was reflected in the ethylene production which was 1.40 and 3.35 μ moles/pot/hr. in control A and B respectively. Ethylene production data showed a clear

cut difference between effective and ineffective strains. Ineffective strains showed no better ethylene production than contamination in the controls. M_{19} which showed poor nodulation did not show any acetylene reducing activity. It is likely that contamination plays a minor role in the expression of the effectiveness of strains. In other words, all the strains could fully express their potential without being masked by contamination. This was probably due to contamination occurring late in the experiment.

The % nitrogen content data is less obvious. There is no clear cut difference between treatments. The explanation for this may be that the effective strains fixed more nitrogen which led to vigorous growth. When the plants became bigger and older, the nitrogen content dropped. While the ineffective strains fixed less nitrogen, the plants had poorer growth and the nitrogen content did not change that much.

The correlation (r) between different characters is summarized in Table 6. There is a highly significant correlation between nodulation score and C_2H_4 production, nodulation score and dry weight, and C_2H_4 and dry weight. The correlations (r) between nodulation score and % nitrogen, C_2H_4 production and % nitrogen, and dry weight and % nitrogen, are not significant.

Table 6. Correlation (r) between nodulation score, acetylene reduction, dry weight and % nitrogen content of 11 week old faba beans (N = 18).

	C_2H_4 production	Dry weight	% Nitrogen
Nodulation score	0.9738^{**}	0.9598^{**}	0.4747
C_2H_4 production	--	0.9707^{**}	0.4519
Dry weight		--	0.4663

Values for r significant at $P \leq 0.01^{**}$ or $P \leq 0.05^{*}$.

For the growth pouch experiments, contamination was eliminated because everything was sterilized. The uninoculated control showed no nodulation. M_4, M_{11}, M_{12}, M_{13}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q showed good nodulation. M_{17-20} showed poor nodulation, while M_3 and M_{15} failed to nodulate completely. In the previous soil experiment these two strains had poor nodulation which might have been due to contamination. Ethylene production data shows poor results. All strains that have good nodulation, except M_4, produced ethylene. The amount of ethylene production is low and erratic (c.v. = 237%). This may be because the plants were harvested when they were too young or restricted aeration in the pouches may have prevented oxygen from reaching the nodules. Since the roots were immersed in nutrient solution in the pouches, there is a possibility that a thin film of water could have been developed around the root nodules and restricted oxygen diffusion. Thus N_2 fixation as measured by acetylene reduction is low and erratic. Sprent (1969) found that immersion of the detached nodules in water inhibited C_2H_2 reduction by reducing O_2 supply to the nodules. Schwinghammer et al. (1970) obtained only low rates of acetylene reduction with wet pea nodules. This effect of water logging on acetylene reduction has also been reported by Sprent (1971b), Mague and Burris (1972) and Huang et al (1975a). M_4 showed fair nodulation but no acetylene reducing activity and dry weight is the lowest.

In this water logged condition, M_4 may have been parasitic. Sprent (1975) noticed that different Rhizobium strains of Phaseolus vulgaris performed differently in water logged conditions. In this case, M_4 may not be able to do well in water logged conditions. The

other reason is that M_4 may be slow to become established and function. This can also be confirmed by the soil experiment when M_4 did not do well at the 5 week harvest. The correlations (r) between nodulation score, C_2H_4 production and dry weight are shown in Table 7.

Table 7. Correlation (r) between nodulation score, C_2H_4 production and dry weight of faba beans grown in the pouches (N = 17).

	C_2H_4 production	Dry weight
Nodulation score	0.3022	0.7886^{**}
C_2H_4 production	--	0.8557^{**}

Value for r significant at $P \leq 0.01^{**}$ or $P \leq 0.05^{*}$.

CONCLUSION

From soil and growth pouch experiments, it was found that nitrogen fixation in faba beans cv. Diana was affected by the strains of Rhizobium. Failure to form nodules resulted in no nitrogen fixation. The ability of a particular strain to form nodules is determined by the genetics of the host (Norris 1967). M_3 and M_{15} failed completely to form nodules in the growth pouch experimentand produced sparse nodulation in the soil experiment. This might have been due to contamination. Strains M_{17-20} were poorly nodulated in both soil and growth pouch experiments. M_4 treated plants were well nodulated throughout but nodule did not fix nitrogen in the growth pouch experiment. M_{13} treated plants were fairly well nodulated but failed to fix nitrogen in the soil. Therefore M_4, M_{11}, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23} and Q commercial were selected for further study in the field.

2. The response of faba beans cv. Diana to different strains of *Rhizobium*: Field tests.

ABSTRACT

The strains selected previously, and one additional strain of faba bean *Rhizobium*, were tested under field conditions at two locations representing low and high nitrogen soils in the summer of 1976. All the selected strains except one, M_{14}, showed good nodulation at both locations. In low nitrogen soil, different strains showed differences in effectiveness as measured by the acetylene reduction technique. Inoculation increased seed yield in low nitrogen soil. All of the strains, except M_{14}, were not significantly different in final seed yield. However, the strains from Morocco (M_{11}, M_{12}) gave significantly higher seed nitrogen than the rest of the strains. In high nitrogen soil, there were no significant differences among the treatments. Inoculation did not increase the yields. The poor performance of M_{14} was considered to be due to loss of effectiveness during storage.

The persistence of the *Rhizobium* in low nitrogen soil was measured in the summer of 1977. Approximately 90% of the plants examined had nodules but mostly on lateral roots. At 5 week harvest, nodulation, acetylene reduction, plant dry weight and nitrogen percentage were not significantly different, suggesting cross-contamination between plots. There were differences in nodulation score but not acetylene reduction, plant dry weight or nitrogen percentage at the 9 week harvest, suggesting some strains might have poor surviving ability.

The Moroccan strains (M_{11} and M_{12}) showed good acetylene

reduction and gave significantly higher seed nitrogen content than the rest of the strains in the dry year of 1976 in the sandy loam soil. It is recommended that these two strains be added to the commercial inoculum in the same way as M_{21}, M_{22} and M_{23} are the components of the Q commercial inoculum. Faba bean seeds should be inoculated each year to ensure early and good nodulation, even though the previous history of the field shows that faba beans were grown before.

INTRODUCTION

Laboratory tests to compare effectiveness of *Rhizobium* strains on legumes do not always give a valid guide to their performance in the field. For example, Cloonan and Vincent (1967) using strains which had done well in controlled environments, obtained satisfactory nodulation of cowpea (*Vigna sinensis* (L.) Endl. ex. Hassk) and dolichos (*Dolichos lablab* L.) in only one of ten field trials sown in a clean cultivated seed bed and one of eight when seeded directly into a grass sward. Thus, although results have also been good with this approach, some of the strains selected have failed in the field because of their inability to establish or persist within the physical (Marshall et al. 1963), chemical (Munns 1965, Chatel and Parker 1972) and biological (Brockwell et al. 1968. Herridge and Roughley 1975, Roughley et al. 1976) environments of particular soils. Climatic conditions vary from year to year, one strain may be effective in one year and ineffective in another (Abel and Erdman 1964). With grain-producing legumes, field tests are mandatory as the true symbiotic capability of a strain of rhizobia is attained only with the N-stress of fully developed podding plants with normal photosynthesis.

From the previous greenhouse experiments, 8 strains of *Rhizobium leguminosarum* Frank and Q commercial inoculum were chosen for further study in field conditions. An additional strain from New Zealand, designated as M_{24}, was also included in the field experiments. The experiments were conducted in the summer of 1976 at two locations. To follow the persistence of inoculated rhizobia in the soil, uninoculated seed was sown at one location in the summer of 1977, in the plots which

carried the strain test in 1976.

An experiment was also conducted in the greenhouse using growth pouches to compare the results from the field and the greenhouse conditions. In this experiment, the root temperature was controlled at 14°C. The room temperature was 20°/15°C in a 16/8 hr. day.

MATERIALS AND METHODS

Nine Rhizobium strains plus Q commercial were used in the study. These strains were M_4, M_{11}, M_{12}, M_{14}, M_{16}, M_{21}, M_{22}, M_{23}, M_{24}, and Q commercial. Bacterial inoculants for field experiments were prepared as follows: bacteria were grown in 250 ml erlenmeyer flasks containing 150 ml of yeast mannitol broth (Vincent 1970). Each bacterial strain was grown for 7 days and then mixed with 160 g of finely ground peat (adjusted to pH 6.8 with $CaCO_3$). The bacterial concentration before adding to the peat was 10^8 cells/ml. The moisture content of the peat after mixing was 50%. The mixed peat was incubated at room temperature for two days before using. The same peat, adjusted to the same moisture content with sterilized distilled water and incubated for two days was used as the control.

Faba beans seeds cv. Diana were used in the studies. Four hundred seeds were packaged in a bag. Each bag was inoculated with 2 g of inoculum, except the Q commercial treated bags which received 1 g of inoculum per bag. This was to equalize the number of rhizobial cell in the treatments.

The experiments were conducted at 2 locations: the Carman Weed Research Station (location A) and the aboretum (location B). Location A was on very fine sandy loam 60 miles southwest of Winnipeg. Location B was on clay at the University of Manitoba campus in Winnipeg.

Soil sampling to 15 cm depth was carried out prior to seeding at each location in the spring. Location A had 1.8 ppm NO_3^-, 8 ppm available P, 153.3 ppm available K, 4.4 ppm $SO_4^=$ and the pH was 7.4. Location B had 30 ppm NO_3^-, 10.3 ppm available P, 455.8 ppm available K,

13.9 ppm $SO_4^=$ and the pH was 7.6. Fertilizer was applied at location A at the rate of 116 Kg K/ha broadcast pre-seeding, and 57 Kg P and 22 Kg S/ha broadcast following emergence. Location A was treated with the herbicide trifluralin at 1.12 Kg a.i./ha, and the herbicide was incorporated thoroughly prior to seeding. When necessary, weeds were removed at both locations by hand-hoeing.

Plot dimensions were 16.8 x 0.6 m at Location A with 0.31 m between plots, 10.7 x 0.6 m at location B with 0.31 m between plots. Each plot contained four rows 15.0 cm apart. Inoculum at the mentioned rate was added to the seed immediately prior to seeding. The four-row small plot seed drill metered seed at approximately one seed per 6 cm of rows.

Eleven treatments were randomized in six blocks at each location. Treatments were: plain peat (T_1 = control), Q commercial (T_2), $M_4(T_3)$, $M_{11}(T_4)$, $M_{12}(T_5)$, $M_{14}(T_6)$, $M_{16}(T_7)$, $M_{21}(T_8)$, $M_{22}(T_9)$, M_{23} (T_{10}) and $M_{24}(T_{11})$. Seeding was completed on May 27 and May 28, 1976 at location B and A respectively.

Plants were sampled at all locations for nodulation and acetylene reduction at 5, 8 and 11 weeks after planting. The plants were removed from the two centre rows of a 4.6 m section at one end of each plot. Four plants per plot were lifted at each sampling. This made a total sample of 24 plants per treatment.

A visual assessment of nodulation was made in the field on the basis of a 0-5 scale: 0 signifying no nodulation and 5 very abundant nodulation. Acetylene reduction of the nodules on the separate roots was carried out in the field and gas samples analyzed in the laboratory

following the procedure of Candlish and Clark (1975) with slight modifications; mason jars of 890 ml rather than 400 ml volume were used, and 20 ml rather than 15 ml acetylene was introduced into the jars. Plant tops were retained at all samplings and oven-dried at 70°C.

Precipitation was variable in both total amount and distribution among locations. Totals for May to August were 136 mm for location A and 289 for location B. June was the wettest month, with 54 and 63% of the total May to August precipitation falling in that month at location A and B respectively.

Plots were cut and stooked on September 3 and 4, 1976 for location B and A respectively. A 10.7 and 6.1 m length of the 4 rows was taken between a sampling area at one end and a 1.5 m uncut section at the other, at location A and B respectively. Ten plants were randomly sampled from the two centre rows and taken to the laboratory to determine yield components. Dried material was threshed through a static Hege plot combine. Nitrogen in all seed samples was determined by the Kjedahl method. One thousand seed weights were also determined.

To compare the results from the field and greenhouse conditions, a growth pouch experiment was conducted in the growth room. The plants were grown in the pouches as mentioned previously in the greenhouse experiment with slight modification. The plants were left in room conditions for 3 days, instead of 7 days in the greenhouse, before inoculation. After inoculation the plants were left in room conditions for another 3 days. Then they were randomly put into test tube baskets and later transferred to a water bath that had been adjusted to give a root temperature 14°C (Kunelius 1970). The beans were harvested

6 weeks after root temperature treatment. Plants in one basket formed a replicate. Experimental units consisted of 2 separated plants (2 pouches) which were pooled together for nodulation rating, acetylene reduction, dry weight and nitrogen determinations. There were 10 treatments with 8 replications. The treatments assigned were; uninoculated control (T_1), Q commercial (T_2), $M_4(T_3)$, $M_{11}(T_4)$, $M_{12}(T_5)$, $M_{14}(T_6)$, $M_{16}(T_7)$, $M_{21}(T_8)$, $M_{22}(T_9)$ and $M_{23}(T_{10})$.

The air temperature of the growth room was 20°/15°C in a 16/8 hour day.

To follow the persistence of inoculated rhizobia applied in 1976, an experiment was conducted on the same area at location A in the summer of 1977. Uninoculated faba bean seeds cv. Diana were drilled into the same plots on May 15, 1977. Plants from the centre rows were lifted, examined for nodulation, acetylene reduction, dry weight and nitrogen content at 5 and 9 weeks after planting. Four plants per plot were lifted at each sampling. This made a total sample of 24 plants per treatment.

RESULTS

Location A was low in available nitrate nitrogen (1.8 ppm) while location B was high (30 ppm) in the top 15 cm of soil at the beginning of the season. In the adjacent plots at both locations, it was reported that nitrate nitrogen increased over the growing season (Dean and Clark 1977).

Table 8 shows nodulation, acetylene reduction and dry weight of 5 week old faba beans at both locations. Nodulation at location B was found to be more abundant that at location A. Nodulation of the controls at both locations was significantly less than the others. Nodulation of the rest of the strains except M_{14}, at location B was not significantly different. Nodulation at location A was more interesting. M_{16}, M_{21}, M_{22}, M_{23} were found to be excellent. Q commercial, M_4, M_{11}, M_{12} were found to be fair. And M_{14} was found to be poorly nodulated and not significantly different from the controls. Nodules at this stage were still small but looked pink at both locations. Ethylene production data agrees reasonably well with nodulation score in location B. All strains in location B did not show significant differences in ethylene production but were significantly different from the controls. The highest ethylene production in location B was 5.78 μ moles/plant/hr (M_{12}), and the lowest was 2.16 μmoles/plant/hr (control). Ethylene production in location A varied among strains with M_{22} showing highest ethylene production (6.88 μmoles/plant/hr). The control treatment was the lowest in ethylene production (0.40 μ moles/plant/hr) but it was not significantly different from M_{14}. The rest of the strains showed reasonably good nitrogenase activity.

Table 8. Nodulation, Acetylene Reduction and Dry Weight of Faba Beans at Location A and B. The Beans were Harvested at 5 Weeks After Planting. 1/

Strains	Location A				Location B			
	Nodulation score	C_2H_4 production (µ moles/ plant/hr)	Dry weight (g/plant)	N-content %	Nodulation score	C_2H_4 production (µ moles/ plant/hr)	Dry weight (g/plant)	N-content %
Control	0.79 d	0.40 d	1.70 d	3.56 c	1.54 c	2.16 b	1.80 a	3.99 a
Q Commercial	2.33 bc	4.32 c	2.11 abc	4.33 a	3.04 a	4.19 a	2.00 a	4.43 a
M_4	2.29 c	6.33 abc	2.14 ab	4.37 a	3.25 a	5.31 a	1.93 a	4.14 a
M_{11}	2.29 c	4.80 bc	2.36 a	4.47 a	2.92 a	4.31 a	2.01 a	4.29 a
M_{12}	2.75 ab	5.24 abc	1.98 bcd	4.39 a	3.46 a	5.78 a	1.93 a	4.21 a
M_{14}	1.08 d	1.07 d	1.79 cd	3.93 b	2.21 b	3.12 a	1.91 a	4.30 a
M_{16}	3.17 a	5.41 abc	2.19 ab	4.46 a	3.29 a	4.27 a	1.82 a	4.01 a
M_{21}	3.13 a	6.38 ab	2.48 a	4.49 a	3.13 a	5.19 a	2.06 a	3.99 a
M_{22}	3.08 a	6.88 a	2.39 a	4.39 a	3.13 a	4.19 a	2.11 a	4.28 a
M_{23}	3.00 a	5.65 abc	2.28 ab	4.40 a	3.33 a	4.13 a	1.86 a	4.27 a
M_{24}	3.00 a	5.07 abc	2.25 ab	4.38 a	3.00 a	5.01 a	1.91 a	4.23 a

a-d Means within columns followed by same letter are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

1/ For analysis of variance see appendices 14-21.

Dry weight yields at location B were not significantly different. At location A, the strains showed differences in dry matter yields. M_{21} gave maximum dry matter yield and the control treatment was the lowest.

Nitrogen content was not significantly different between the strains in location B. Location A showed differences among treatments. The control treatment had the lowest N content (3.56%). M_{14} gave second poorest N content. The rest of the strains were not significantly different.

Table 9 shows the results of the 8 week harvest at both locations. Different strains were different in nodulation at both locations. The controls were consistently lower in nodulation than the rest of the strains. M_{14} was found to be poorer than the rest of the inoculated treatments and was not significantly different from the control treatments in location A. At location A, nodules were found to be large and pink while location B nodules were smaller and white. Ethylene production by different strains was not significantly different at location B. Ethylene production by the control at location A was lowest (0.75 μ moles/plant/hr) but not significantly different from M_{14} (2.35 μ moles/plant/hr). M_{11}, M_{12}, and M_{22} were outstanding. M_{11} produced highest ethylene (10.37 μ moles/plant/hr).

Dry weight and N content were not significantly different among strains at location B. At location A different strains showed differences in dry weight and N content. Dry weight of the control was lowest and significantly lower than M_{14}. M_{11} gave highest dry matter yield. N content in the control was also lowest at location A

Table 9. Nodulation, Acetylene Reduction and Dry Weight of Faba Beans at Location A and B. The Beans were Harvested at 8 Weeks after Planting. 1/

Strains	Location A				Location B			
	Nodulation score	C_2H_4 production (µ moles/ plant/hr)	Dry weight (g/plant)	N-content %	Nodulation score	C_2H_4 production (µ moles/ plant/hr)	Dry weight (g/plant)	N-content %
Control	0.67 d	0.75 c	5.98 c	2.48 d	1.92 d	2.00 a	7.02 a	3.79 a
Q commercial	3.21 c	6.95 ab	7.73 ab	3.22 bc	3.46 b	2.64 a	7.28 a	3.86 a
M_4	3.50 bc	9.51 ab	7.56 ab	3.31 ab	4.04 ab	2.92 a	6.88 a	3.75 a
M_{11}	3.50 bc	10.37 a	8.96 a	3.53 a	3.71 ab	3.04 a	8.02 a	3.89 a
M_{12}	3.96 ab	10.36 a	7.78 ab	3.48 a	4.25 a	3.66 a	7.96 a	3.84 a
M_{14}	1.25 d	2.35 c	6.88 bc	3.00 c	2.58 c	2.90 a	7.40 a	3.84 a
M_{16}	4.29 a	6.60 b	8.56 a	3.09 bc	4.13 ab	2.27 a	7.16 a	3.78 a
M_{21}	4.08 ab	7.48 ab	7.73 ab	3.18 bc	3.92 ab	2.67 a	7.33 a	3.89 a
M_{22}	4.21 a	10.24 a	7.59 ab	3.29 ab	3.88 ab	2.47 a	7.31 a	3.99 a
M_{23}	4.04 ab	6.50 b	7.63 ab	3.30 ab	3.92 ab	2.27 a	7.75 a	3.79 a
M_{24}	4.04 ab	7.62 ab	7.87 ab	3.18 bc	3.96 ab	2.19 a	7.59 a	3.72 a

a-d Means within columns followed by same letters are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

1/ For analysis of variance see appendices 22-29.

(2.48%) and significantly lower than M_{14}. M_{11} again had the highest N content (3.53%).

Tables 10 and 11 show the results of the 11 week harvest at locations A and B respectively. Plants were separated into pods and stem. The number of pods were counted. The yield data were presented on a per plant basis. At location B, the controls were poorest in nodulation. M_{14} was second poorest and the rest of the strains were good and not significantly different. Most of the nodules were white and some showed green colour at base of the nodules. Ethylene production was poor and no significant differences were found among treatments. Pod number, pod weight, and leaves and stem weight per plant were not significantly different. Nitrogen content in pods, and in leaves and stems, were not significantly different among treatments.

At location A, nodulation of the control plants was poorest. M_{14} treated plants were the next poorest treatment. M_{16} showed excellent nodulation. The nodules were larger and pinker when compared to the nodules found in location B. Ethylene production was found to be highest in M_{12} (11.65 μ moles/plant/hr) at location A and lowest in the control treated plants (0.69 μ moles/plant/hr). Dry weight per plant was found to be different amongst treatments. M_{22} showed the highest dry weight (23.57 g/plant) and the control was the lowest (7.78 g/plant). Pod number, pod weight and leaves and stem yields followed the same trend. N content in pods, and leaves and stem was also found to follow the same trend. The control was poorest when compared to the other treatments. The N content in leaves and stem was found to be lower than in the pods. Half of the total nitrogen in the top part of

Table 10. Nodulation, Acetylene Reduction, Yield of Faba Bean at Location A. The Beans were Harvested at 11 Weeks After Planting. 1/

Strains	Nodulation score	C_2H_4 production (μ moles/ plant/hr)	Yield/Plant				N Content (%)	
			Pod no.	Pod yield (g)	Leaves & stem (g)	Total dry weight (g)	Pods	Leaves & stem
Control	0.54 e	0.69 e	2.67 d	1.74 d	6.04 c	7.78	2.90 e	1.55 c
Q Commercial	3.50 bc	7.80 bc	7.33 ab	6.35 abc	13.91 ab	20.26	3.58 d	2.08 a
M_4	3.63 bc	8.70 abc	6.88 abc	6.70 abc	13.47 ab	20.17	3.92 abc	2.09 a
M_{11}	3.04 c	10.72 ab	8.21 a	7.83 a	15.46 a	23.29	4.03 a	2.18 a
M_{12}	3.63 bc	11.65 a	8.13 a	7.50 ab	15.15 a	22.65	4.02 a	2.27 a
M_{14}	1.33 d	3.41 de	5.29 c	5.09 c	11.52 b	16.61	3.58 d	2.00 ab
M_{16}	4.42 a	5.59 cd	7.29 ab	7.06 abc	13.75 ab	20.81	3.72 cd	2.01 a
M_{21}	3.50 bc	6.53 c	6.13 bc	5.64 bc	12.13 ab	17.77	3.96 ab	2.12 a
M_{22}	4.00 ab	8.58 bc	7.88 ab	8.12 a	15.45 a	23.57	3.75 cd	2.05 a
M_{23}	4.00 ab	6.82 c	7.92 a	7.45 ab	14.68 ab	22.13	3.85 abc	2.05 a
M_{24}	4.17 ab	6.73 c	7.33 ab	7.26 ab	14.30 ab	21.56	3.81 bc	1.94 b

a-e Means within columns followed by same letters are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

1/ For analysis of variance see appendices 30-33.

Table 11. Nodulation, Acetylene Reduction, Yield of Faba Beans at Location B. The Beans were Harvested at 11 Weeks After Planting. 1/

Strains	Nodulation score	C_2H_4 production (μ moles/ plant/hr)	Yield/Plant				N content	
			Pod no.	Pod Yield (g)	Leaves & stem (g)	Total dry weight (g)	Pods	Leaves & stem
Control	1.58 c	1.40 a	8.83 a	7.56 a	15.03 a	22.59 a	4.15 a	2.09 a
Q Commercial	3.54 a	2.50 a	9.08 a	7.48 a	15.37 a	22.85 a	4.29 a	2.31 a
M_4	3.71 a	2.63 a	9.13 a	6.78 a	14.55 a	21.33 a	4.40 a	2.20 a
M_{11}	3.71 a	3.89 a	8.79 a	7.55 a	14.99 a	22.54 a	4.34 a	2.22 a
M_{12}	3.83 a	3.44a	9.88 a	7.93 a	16.32 a	24.25 a	4.29 a	2.26 a
M_{14}	2.46 b	2.28 a	8.88 a	7.50 a	15.08 a	22.58 a	4.41 a	2.17 a
M_{16}	3.92 a	2.64 a	9.50 a	7.97 a	15.50 a	23.47 a	4.28 a	2.14 a
M_{21}	3.88 a	1.84 a	9.17 a	7.16 a	15.58 a	22.74 a	4.26 a	2.32 a
M_{22}	3.96 a	2.36 a	8.83 a	6.47 a	14.17 a	20.64 a	4.33 a	2.44 a
M_{23}	3.54 a	1.30 a	8.63 a	8.84 a	17.00 a	25.84 a	4.23 a	2.11 a
M_{24}	3.46 a	1.66 a	9.46 a	7.88 a	15.95 a	23.83 a	4.43 a	2.24 a

a-e Means within columns followed by same letters are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

1/ For analysis of variance see appendices 34-37.

the plant was found to be in the pods at this stage of growth.

Tables 12 and 13 show yield components, yields and nitrogen content of faba beans grown at locations A and B. At location B, there were no significant differences in yield components i.e. Pod no./plant, seed no./plant, seed/pod and 1,000 seed weight. There were no significant differences in seed yield/plot and nitrogen percentage. The highest seed yield was 1.09 Kg/plot (2941 Kg/ha) which gave a nitrogen yield equivalent to 149.73 Kg N/ha. At location A, pod no./plant, seed no./plant and 1,000 seed weight showed significant differences among treatments. M_{11} had highest pod no./plant, seed no./plant and 1,000 seed weight. The number of seeds per pod was not significantly different among treatments. The control was found to be lowest in all yield components. M_{14} was the next poorest treatment. All the inoculated treatments had significantly higher yields than the control. The yield of the control was 0.8 Kg/plot (1232 Kg/ha). M_{14} was the poorest of the inoculated treatments (1.00 Kg/plot or 1539 Kg/ha). The rest of the strains did not give significantly different results in final seed yield. M_{22} gave the highest seed yield/plot (2402 Kg/ha). The nitrogen percentage data shows interesting results. The Moroccan strains (M_{11} and M_{12}) were significantly higher in N content than the rest of the strains (5.29 and 5.21% respectively). The Swedish strains (M_{14} and M_{16}) were not significantly different and were lower in N content when compared to the Moroccan strains. The control was poorest in N content (3.94%). The estimated N yield/ha was found to be highest in the Moroccan strains, M_{11} (125.27 Kg/ha) and M_{12}(122.70 Kg/ha). The lowest N yield was from the control (48.54 Kg N/ha).

Table 12. Yield, Yield Components, and Nitrogen Content of Faba Beans Grown at Location A. 1/

Strains	Yield Per Plant			1,000 seed wt.	Seed Yield (Kg)		N-Yield	
	Pod no.	Seed no.	Seed/pod	(g)	Per plot	Per hectare (estimated)	% N	Per hectare (estimated)
Control	3.83 d	11.03 c	2.84 a	363 c	0.80 c	1232	3.94 e	48.54
Q Commercial	7.15 ab	20.05 a	2.80 a	394 b	1.43 a	2206	4.74 cd	104.56
M_4	6.42 bc	19.80 a	3.07 a	402 ab	1.40 a	2155	5.01 b	107.97
M_{11}	7.85 a	22.57 a	2.89 a	412 a	1.54 a	2368	5.29 a	125.27
M_{12}	7.47 ab	20.65 a	2.75 a	409 a	1.53 a	2355	5.21 a	122.70
M_{14}	5.50 c	15.28 b	2.77 a	395 b	1.00 b	1539	4.58 d	70.49
M_{16}	7.17 ab	21.43 a	2.98 a	398 ab	1.42 a	2179	4.64 d	101.11
M_{21}	7.07 ab	20.25 a	2.86 a	401 ab	1.47 a	2263	4.92 b	111.34
M_{22}	6.60 abc	20.00 a	3.02 a	401 ab	1.56 a	2402	4.83 c	116.02
M_{23}	6.78 abc	20.73 a	3.03 a	405 ab	1.40 a	2156	4.96 b	106.94
M_{24}	7.32 ab	20.93 a	2.86 a	403 ab	1.47 a	2253	4.90 bc	110.40

a-e Means within columns followed by same letters are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

1/ For analysis of variance see appendices 38-40.

Table 13. Yield, Yield Components, and Nitrogen Content of Faba Beans Grown at Location B. 1/

Strains	Yield Per Plant			1,000 seed wt.	Seed Yield (Kg)		N-Yield	
	Pod no.	Seed no.	Seed/pod	(g)	Per plot	Per hectare (estimated)	% N	Per hectare (estimated)
Control	8.38 a	23.73 a	2.83 a	371 a	1.00 a	2692	5.04 a	135.68
Q Commercial	8.83 a	25.28 a	2.86 a	362 a	1.00 a	2692	5.03 a	135.40
M_4	8.18 a	23.45 a	2.87 a	375 a	1.09 a	2941	5.06 a	148.81
M_{11}	8.35 a	23.15 a	2.78 a	373 a	1.07 a	2885	5.19 a	149.73
M_{12}	8.35 a	23.97 a	2.88 a	374 a	1.04 a	2795	5.14 a	143.66
M_{14}	8.77 a	24.55 a	2.80 a	372 a	1.07 a	2880	5.05 a	145.44
M_{16}	8.25 a	23.02 a	2.79 a	371 a	1.04 a	2789	5.14 a	143.35
M_{21}	7.20 a	20.92 a	2.88 a	372 a	1.04 a	2801	5.02 a	140.61
M_{22}	8.08 a	23.12 a	2.87 a	370 a	1.01 a	2712	5.13 a	139.13
M_{23}	8.78 a	24.42 a	2.79 a	370 a	1.06 a	2856	5.14 a	146.80
M_{24}	7.88 a	22.42 a	2.85 a	366 a	1.08 a	2920	5.04 a	147.17

a Means within columns followed by same letters are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

1/ For analysis of variance see appendices 41-43.

The results of the growth pouch experiment are shown in Table 14. In this experiment, the root temperature was controlled at 14°C. The air temperature was 20°/15°C in a 16/8 hour day. Uninoculated controls did not have any nodules. M_{14} showed poorer nodulation than the rest of the strains. M_{16} and M_{22} had excellent nodulation. Ethylene production data followed the same trend as nodulation score. The controls did not reduce acetylene. M_{16} gave maximum ethylene production and M_{14} the lowest. The rest of the strains showed no significant differences in ethylene production. Plant dry weight showed that the uninoculated controls were the poorest. M_4 and M_{14} treated plants had higher dry weight but were not significantly different from the controls. M_{22} gave maximum dry weight. The nitrogen content was low in the controls, M_4 and M_{14}. The rest of the strains had higher nitrogen percentages. Q commercial had the maximum percentage of nitrogen.

Table 15 shows the results of the 1977 experiment. The experiment was reconducted in the same area at location A. The uninoculated seeds were sown in the stubbles of the previous year's crop. The land was not prepared. The seeds were directly drilled into the stubbles by the four-row small seed drill. Treatment number were assigned according to the previous year.

At the 5 week harvest, the treatments were not significantly different in % nodulated plant, nodulation score, ethylene production, dry weight and percentage of nitrogen content. About 90% of the plants examined at this period had nodules, but nodulation was sparse and poor when compared to the plants of the previous year at the same stage. Nodules were found to be on the lateral roots.

Table 14. Nodulation, Acetylene Reduction, Dry Weight and Nitrogen Content of Faba Beans Grown in the Pouches. 1/

Rhizobium strains	Nodulation score*	C_2H_4 production* (μ moles/hr)	Dry weight* (g)	Nitrogen content (%)
Control	o d	o c	0.90 f	2.76 d
Q commercial	3.25 ab	1.59 ab	1.61 bcd	3.72 a
M_4	2.63 ab	1.50 ab	1.12 ef	2.99 cd
M_{11}	3.25 ab	1.69 ab	1.66 abc	3.60 ab
M_{12}	2.88 ab	1.53 ab	1.84 ab	3.50 ab
M_{14}	1.50 c	1.22 b	1.26 def	2.78 d
M_{16}	3.38 a	2.83 a	1.68 abc	3.64 ab
M_{21}	3.00 ab	1.60 ab	1.79 abc	3.22 bc
M_{22}	3.38 a	2.46 ab	2.03 a	3.41 abc
M_{23}	2.50 b	1.86 ab	1.43 cde	3.46 ab

* Figures are the means of 2 plants.

a-f Means within columns followed by same letters are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

1/ For analysis of variance see appendix 44.

Table 15. Percent Nodulated Plants, Nodulation Score, Acetylene Reduction, Dry Weight and % Nitrogen of Faba Beans at Location A. The Beans were Grown in the Area where 1976 Rhizobium Strains Test was Conducted in 1977. 1/

Rhizobium Strains	5 Week Harvest					9 Week Harvest				
	% Nodulated plant	Nodulation score	C_2H_4 production (µ moles/ plant/hr)	Dry weight (g/plant)	% N	% Nodulated plant	Nodulation score	C_2H_4 production (µ moles/ plant/hr)	Dry weight (g/plant)	% N
Control	75.00 a	1.58 a	2.55 a	2.30 a	3.72 a	95.83 a	2.33 bc	4.84 a	19.58 a	3.20 a
Q Commercial	87.50 a	1.67 a	2.03 a	1.78 a	4.02 a	95.83 a	2.46 bc	3.79 a	20.58 a	3.29 a
M_4	91.67 a	1.83 a	3.13 a	2.48 a	3.77 a	79.17 a	2.00 bc	1.20 a	19.96 a	3.15 a
M_{11}	95.83 a	2.38 a	6.61 a	2.59 a	4.08 a	91.67 a	2.58 abc	9.51 a	22.33 a	3.35 a
M_{12}	95.83 a	1.96 a	2.91 a	2.63 a	4.08 a	95.83 a	2.96 ab	4.12 a	19.04 a	3.31 a
M_{14}	83.33 a	1.54 a	3.26 a	1.90 a	3.88 a	95.83 a	2.92 ab	7.77 a	21.38 a	3.33 a
M_{16}	100.00 a	2.42 a	4.62 a	2.42 a	3.83 a	100.00 a	3.63 a	4.74 a	19.83 a	3.08 a
M_{21}	95.83 a	2.04 a	4.85 a	2.28 a	4.00 a	95.83 a	2.71 abc	2.88 a	18.54 a	3.26 a
M_{22}	83.33 a	1.96 a	4.81 a	2.71 a	3.90 a	95.83 a	3.00 a	3.10 a	21.25 a	3.52 a
M_{23}	91.67 a	2.00 a	3.36 a	2.33 a	4.03 a	83.33 a	1.75 c	0.82 a	19.46 a	3.26 a
M_{24}	95.83 a	2.71 a	5.76 a	2.55 a	3.87 a	100.00 a	3.58 a	7.48 a	21.38 a	3.23 a

a-c Means within columns followed by same letters are not significantly different by Duncan's new multiple range test at $P \leq 0.05$.

1/ For analysis of variance see appendices 45 and 46.

At the 9 week harvest, the treatments were also not significantly different in percentage of plants nodulated. About 94% of the examined plants were nodulated. Nodulation score was significantly different among treatments. M_{16} had the highest nodulation score while M_{23} was the poorest. C_2H_4 production, dry weight and percentage of nitrogen were not significantly different among treatments.

DISCUSSION

Poor nodulation of the controls at both locations A and B indicated the success of the non-sterilized peat inoculum. Non-sterilized peat had been used in Rhizobium strain tests for chick peas (Okon et al. 1972). Nodulation scores in the controls at location B were higher than in location A. High nodulation scores at location B were due to the higher scores in the controls of replications 3 and 4 (see Appendices 18, 26, and 34). The high scores in replications 3 and 4 may have been due to the low lie of the land. After seeding, continuous rainfall in June with some heavy rains caused flooding in the experimental area especially in these 2 blocks (see Appendix 48). The rain may have brought the rhizobia from the adjacent plots and contaminated the controls in these 2 replications. Another reason may be that the nodules in the controls in the two blocks might have been formed by the indigenous Rhizobium strain. The history of the field for the past 6 years was checked and there was no previous history of faba beans or peas in this area during the 6 year period. The area was put into summer fallow in 1970, 1971, 1973, and 1975. Rapeseed was grown in 1972 and 1974. It is likely that the high nodulation score in replication 3 and 4 was due to contamination from the adjacent inoculated treatments. However, the other reason cannot be completely neglected.

Nodulation at location B was found to be greater than location A at the 5 week harvest. This might be because the soil at location B was clay and therefore wetter than the sandy loam soil in location A. This might affect the survival of the rhizobia and thus nodulation. The high nitrate (30 ppm) in the top 15 cm did not seem to have any

retarding effect on nodulation. At the 8 and 11 week harvest, nodulation in location A increased, and nodules were bigger than in location B. It is likely that high nitrate in location B did not retard nodule formation but nodule development was retarded. Harper and Cooper (1971) found that high nitrogen fertilizer application did not reduce nodule number but did reduce nodule fresh weight and haemoglobin content, indicating an effect on nodule development rather than on root infection or nodule initiation. The results reported here seem to agree with them.

Nodulation of M_{14} treated plants was found to be poorer than the rest of the inoculated treatments at both locations throughout the experimental period. The previous experiments in the greenhouse using soils and growth pouches showed that M_{14} was a very effective strain in terms of nodulation and nitrogen fixation. The failure of this strain might have been to an inherent failure of the inoculum strain to colonize the immediate root zone and soil (Cloonan and Vincent 1967), or the strain had lost its effectiveness during storage. To check the effectiveness of this strain and the others, a growth pouch experiment with controlled root temperature was conducted and the results are presented in Table 14. The strains used to inoculate the pouches were from the same tubes of slant agar that were used to grow the rhizobia for making the peat inoculum. The results of the growth pouch experiment in Table 14 showed that M_{14} was poorer than the other inoculated treatments in nodulation, C_2H_4 production, dry matter yield and percentage of N content. It is likely that the poor performance of M_{14} in the field was due to the loss of effectiveness of this strain during storage rather

than the poor survival capability in the soils.

Location A was low in soil nitrate, with differences in nitrogen fixing abilities showing as early as 5 weeks after planting. C_2H_4 production, dry weight and percentage of nitrogen were different among treatments. In the greenhouse experiments using the soil mixture, dry weight of the beans at 5 weeks after planting was not significantly different. This was probably due to the soil nitrate at location A (1.8 ppm) being lower than in the soil mixture (4.0 ppm). At location B, ethylene production in the inoculated treatments was not significantly different but was significantly higher than the controls. The plants at both locations were fixing nitrogen at the same rate at this stage of growth. The average of C_2H_4 production were 4.68 and 4.33 μ moles/plant/hr at location A and B respectively. This reflected that the soil nitrogen (30 ppm NO_3^-) did not have an inhibiting effect on the nitrogenase activity of the nodules at location B at this stage of growth. During this first sampling, the soil was damp at both locations. This was due to moisture storage in the soil from previous rain in June (see Appendix 47 and 48). Water stress was unlikely to be the problem at this stage of growth. However, dry matter yield and percentage of nitrogen content were not significantly different among the inoculated treatments and the control at location B. Soil nitrogen might supply enough nitrogen for the demand of the control.

At the 8 and 11 week harvest, ethylene production by different strains at location A showed significant differences and was higher than the 5 week harvest. Ethylene production by different strains at location B was not significantly different. Ethylene production by different

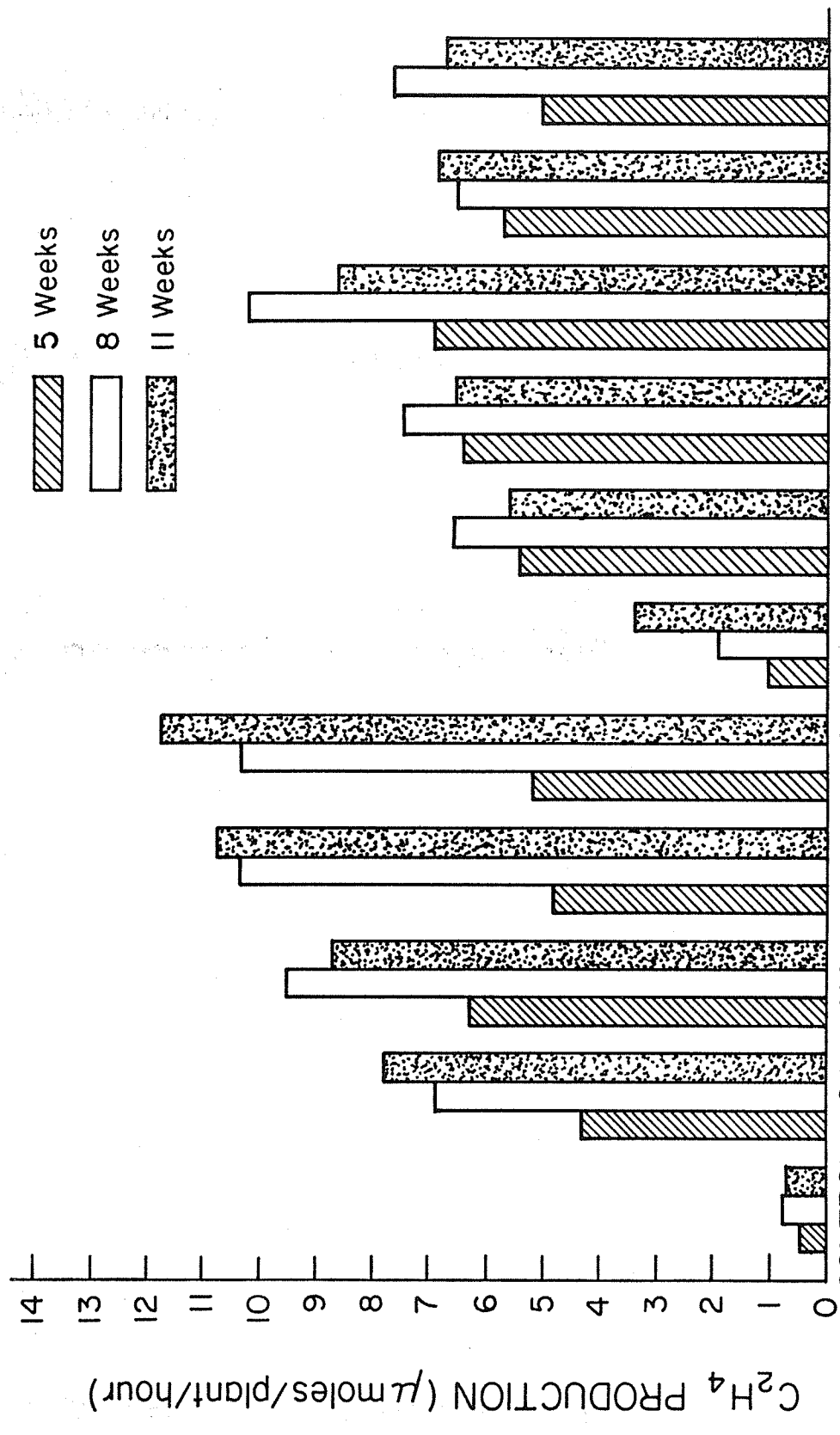

Figure 1. Ethylene production by faba beans inoculated with different strains of rhizobia. The beans were grown at location A and harvested at different ages. The figures are μ moles C_2H_4 produced/plant/hour.

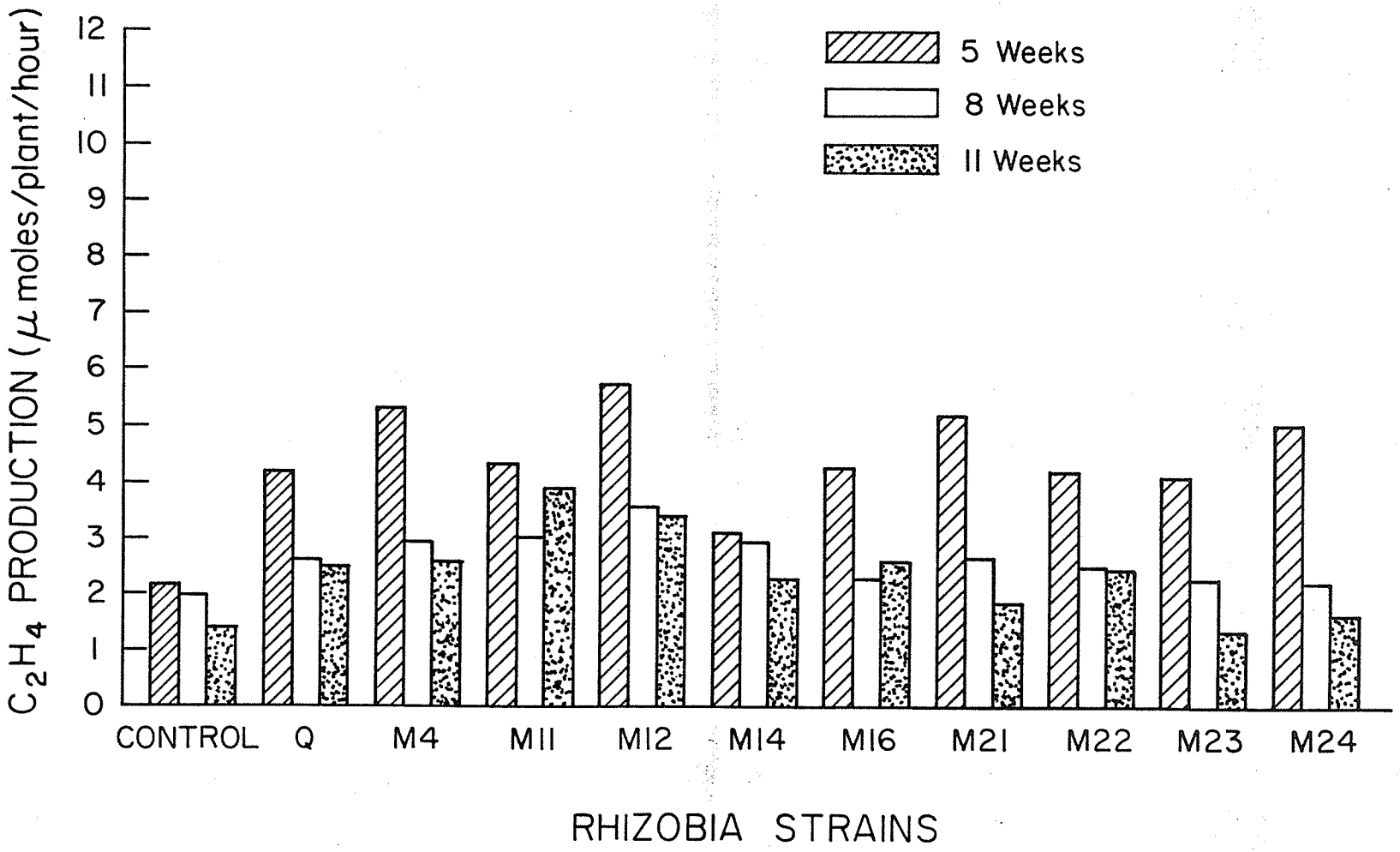

Figure 2. Ethylene production by faba benas inoculated with different strains of rhizobia. The beans were grown at location B and harvested at different ages. The figures are μ moles C_2H_4 produced/plant/hour.

strains with time are summarized in figures 1 and 2. Ethylene production at location B was less than location A at both the 8 and 11 week harvest. The mean ethylene production at the 8 week harvest was 7.16 and 2.64 μ moles/plant/hr at location A and B respectively. At the 11 week harvest, the mean ethylene production for location A and B was 7.02 and 2.36 μ moles/plant/hr respectively.

The reason for low ethylene production at location B might be due to the inhibiting effect of soil nitrogen. Soil nitrogen increases as summer progresses due to a mineralization process. Dean and Clark (1977) reported that soil nitrate nitrogen increases with the growing season under faba beans. This increase in soil nitrogen might inhibit the nodule activities. From visual observations, the nodules at the 8 week harvest were whitish and at 11 weeks the nodules were white and some were green. These characteristics suggest that the nodules were not functioning properly. Combined nitrogen has been reported to reduce symbiotic nitrogen fixation as measured by the acetylene reduction in soybeans (Lawn and Brun 1974), faba beans (Candlish and Clark (1975), Sirato and subterraneum clover (Gibson and Pagan 1977).

Soil moisture might also play an important role in the differences in C_2H_4 production at both locations at the last two harvests. As mentioned earlier, at the first harvest (July 5 for location B and July 6 for location A), there was no moisture stress. Therefore C_2H_4 production at both locations was similar. At the 8 and 11 week harvest (July 26 and August 18 respectively for location B) the plants were under water stress (see Appendix 48). Only 9.5 mm of rain were

recorded from the first harvest to the second harvest, but the soil was not too dry because of the moisture from the rain in the previous month. From the second harvest to the third harvest, 36.3 mm of rain were recorded but the soil was really dry. Thus moisture stress might play a significant role in reducing nitrogenase activity of the beans at location B. The beans at location A were not really under moisture stress at the last two harvests (see Appendix 47). At the 8 week harvest (July 27), the soil was moist due to heavy rain on July 20 (18.8 mm) and 5.8 mm on July 25. At the 11 week harvest (August 17), the beans had received about 13.5 mm of rain since August 8. It was noticed that in each treatment the top soil (nodule zone) was dry. Storage water from below the root zone might have contributed a significant amount of water to the beans because the beans did not show any wilting symptoms. The effect of water stress on nitrogen fixation in legumes is recognized (Sprent 1972b). Water stress may affect nodule activity by changing fine structure of nodules (Sprent 1972a) or may reduce supplies of photosynthates from wilted beans (Huang et al. 1975b). Hume et al. (1976) reported that soil moisture around the soybean root nodules did not affect acetylene reducing activity as long as the water was sufficiently supplied from below the nodule zone. The observation that the top soil at location A was always dry while acetylene reduction was still high supports Hume et al's results.

Dry matter yield increased with time at both locations (Table 8, 9, 10, and 11). At the 11 week harvests the plants were separated to pods and leaves plus stems. Since the soil at location A was low in nitrogen, different strains of Rhizobium could fully express their

symbiotic effectiveness. These were reflected in variation in dry weight yield among treatments. The inoculated treatments were always higher in dry matter yield than the controls throughout at location A. There were no significant differences among treatments at any harvest at location B. High soil nitrogen was probably responsible for these insignificant differences in dry matter yield.

Nitrogen percentage of different treatments showed significant differences at all harvests at location A. The controls were always low in nitrogen percentage. At location B, the percentage of nitrogen was not significantly different among treatments at all harvests, again attributable to high soil nitrogen.

Seed yields, yield components and seed nitrogen percentage are shown in Tables 12 and 13. Inoculation at location A significantly increased pod and seed number per plant. Seed number per pod was not affected. One thousand seed weight was increased by inoculated treatments. The final seed yield of the inoculated treatments was significantly different from the controls. M_{14} was the poorest and significantly lower than the rest of the strains. The final seed yield per plot agreed well with seed number per plant. Seed nitrogen percentage of M_{11} and M_{12} was significantly higher than the rest of the inoculated treatments. Figure 1 shows that these two strains were very effective in nitrogen fixation. These two strain showed an increase in acetylene reducing activity with time. At the 11 week harvest, these two strains had the highest nitrogenase activity, while most of the strains showed decreasing nitrogenase activity. The prolonged nitrogenase activity at the pod filling stage in these two strains resulted in a higher

percentage of nitrogen in the seed even though seed yields were not significantly different from the other strains, except M_{14} and the controls. These two strains originated from Morocco and showed the potentials to be better than M_{21}, M_{22}, and M_{23} which were the components of the Q commercial in these environmental conditions. These two strains might be the strains that could best tolerate stress and fix nitrogen under these conditions. Water stress and soil temperatures might be the prevailing climatic conditions in this location. Different strains of Rhizobium are different in their abilities to fix nitrogen at different temperatures in barrel medic and purple vetch (Pate 1961, 1962), Subterraneum clover (Gibson 1961, 1963, 1965, 1966, 1969) and birdsfoot trefoils (Kunelius 1970). Estimated seed nitrogen yield per hectare ranged from 48.54 Kg in the control to 125.27 Kg in M_{11}. The estimated nitrogen fixation ranged from 22 Kg/ha in M_{14} to 77 Kg/ha in M_{11}. These figures did not take into account some nitrogenase activity in the controls and nitrogen in the vegetative parts, which of course were higher in the inoculated treatments, that were not included. The figures might have been higher if these two factors were taken into account. Nodulation in the controls might have been due to (1) the presence of Rhizobium in non-sterilized peat, (2) indigenous soil Rhizobium and (3) Rhizobium from the adjacent plots.

Location B which was high in nitrate nitrogen showed no significant differences among the strains. Seed inoculation did not increase seed yields or yield components. C_2H_4 production in this location, except in the 5 week harvest, was less than half of the C_2H_4

production in location A. Faba beans were self-sufficient in nitrogen from symbiotic fixation in low nitrate soils in Manitoba (Dean and Clark 1977), and nitrogen fertilizer applications did not significantly increase seed yields in England (McEven 1970).

The finding that nitrogenase activities in location B were lower than at location A, suggested that the plants depended mostly on soil nitrogen. Soil analysis showed that location B was high in nitrate nitrogen. Nitrate nitrogen may lower nitrogenase activity of the nodules by nitrite inhibition. Virtanen (1950) suggested that nitrites form an NO-compound with leghaemoglobin, thus destroying its function as an O_2--supplier to the bacteroids. Nitrite is also a potent inhibitor of nitrogenase activity, in bacteroids extracted from soybean nodules (Rigaud et al. 1973), in N_2--fixing cultures of rhizobia (Pagan et al. 1977) and of the nitrogenase enzyme in vitro (Kennedy et al. 1975). Oghoghorie and Pate (1971) suggested that the reduction of nitrogenase activity by nitrate nitrogen was due to photosynthate deprivation. Gibson and Pagan (1977) suggested that the lowered nitrogenase activity in the nodules on plants supplied with nitrate is due to factors other than nitrite inhibition. Their results offered some support for the "photosynthate deprivation" hypothesis. They also suggested that if photosynthesis deprivation was the principal factor responsible for a decline in nitrogenase activity following the application of nitrate to nodulated plants, the search for strains of Rhizobium able to withstand the effects of combined nitrogen would appear to stand little chance of success. At an adjacent plot at location B, Dean and Clark (1977) found that inoculum treatment did not increase seed yields. Seed yields

eventually decreased with inoculum treatments. They suggested the more abundant nodules diverted a significant amount of carbohydrates away from the developing pods. However, it has been found that a strain of soybean Rhizobium was able to fix nitrogen even at a high level of nitrogen fertilizer application (Burton 1976).

The results of sowing uninoculated faba bean seeds on the stubble of the previous crop at location A are shown in Table 15. About 90% of the plants examined were nodulated at both harvests. However, nodulation was not good when compared to the previous year's nodulation results on plants of similar age. Nodules were mostly on lateral roots. This shows that nodulation occurred late due to the lack of the Rhizobium in the rhizosphere when the root first emerged. There were no differences in nodulation score among plots in the 5 week harvest. The plots which had been controls in 1976 showed the same degree of nodulation as the inoculated treatments. This indicates that the Rhizobium could have been cross-contaminated between plots or the indigenous Rhizobium in the control plots multiplied and increased in number. C_2H_4 production, plant dry weight and nitrogen percentage at this point showed no significant differences. This seemed to support the hypothesis that contamination was likely to have occurred.

However, nodulation score at the 9 week harvest showed some differences. M_{16} showed best nodulation while M_{23} was the poorest. The results were contradictory to the 5 week harvest which showed no differences in nodulation scores. This might have been due to M_{23} having poor surviving ability, or due to poor sampling technique. M_{14} which had been shown to lose effectiveness in the previous year showed good

nodulation and good acetylene reduction. This might have been due to cross-contamination among plots or the increase in the number of M_{14} itself. If the later case occurred, it could be considered undesirable because M_{14} had been ineffective before. Since, we were unable to distinguish the differences between the Rhizobium spp. used, we were unable to conclude that the contradictory results for nodulation in the 5 and 9 week harvest score were due to the poor survival of some particular strains or whether or not cross-contamination did occur.

Since the plots where the experiment was conducted, were the same plots as the previous year, there was no soil preparation or chemical treatments for weed control. The seeds were directly drilled to the stubbles. Wild oats (Avena fatua L.), common milkweed (Asclepias syriaca L.), canada thistle (Cirsium arvense (L.) Scop.), and especially russian thistle (Salsola pestifer Nels.) were heavily infested. The plots were sprayed once with dinoseb at the rate of 1.4 Kg a.i./ha when the beans were in 2-4 leaf stage to control russian thistle. The chemical spray failed to control the weeds, therefore the plots were hand-hoed. At the 5 week harvest, weeds were under control. However, after that the weeds were not controlled properly and they outgrew the beans.

C_2H_4 production, dry weight and nitrogen percentage did not show any significant differences at the 9 week harvest. Ethylene production data (Appendix 46) showed wide variations (c.v. = 114%). Competition from weed for water and light might have the effect on these variation in acetylene reduction. Photosynthates are known to be important for supplying energy for nitrogenase activity in Vicia faba

(Lawrie and Wheeler 1975). Water stress may affect the activity of the nodules directly (Sprent 1972a) or may reduce supplies of photosynthates from wilted leaves (Huang et al. 1975b). However, M_{11} showed the highest ethylene production (9.51 μ moles/plant/hr). M_{23} did not show significant different in dry weight and percentage of protein even though it showed poor nodulation. This reflected that the plant depended on soil nitrogen from the previous year.

CONCLUSION

The experiments at both locations indicated that M_{14} was only weakly effective in nodulating faba beans. The growth pouch experiment with controlled root temperature showed that M_{14} deteriorated in effectiveness. Location A, which was low in nitrogen, was ideal for testing the effectiveness of the Rhizobium strains. Low soil nitrogen permitted the rhizobia to express their full potential. High nitrogen soil masked the expression of the symbiotic effect. Seed yields were not significantly different among the inoculated treatments, except M_{14} which gave significantly lower yields than the rest. However, M_{11} and M_{12}, which showed good nitrogen fixing ability as measured by acetylene reduction technique, gave significantly higher seed nitrogen than the rest of the strains. This suggested that these two strains from Morocco performed well in the dry year or dry area.

The finding that approximately 90% of the plants examined in the 1977 persistence test had nodules, showed that rhizobia were still present, but their origin could not be clarified. The nodules found on lateral roots also showed that the rhizobia were not in close proximity to the seeds and failed to nodulate early. To ensure early and good nodulation, faba beans should be inoculated before they are sown even though the previous history of the field shows that faba beans were grown before.

The excellent performance of two Moroccan strains in ethylene production and seed nitrogen yield in the dry year at location A, suggested that these two strains should be added in the commercial inoculum.

This is to ensure good fixation and nitrogen yield in a dry year or dry area. The present Q commercial inoculum and its composit strains (M_{21}, M_{22} and M_{23}) was found to be inferior to the two Moroccan strains at location A.

SECTION 3

Discussion of entire research programme reported in Section 2

DISCUSSION AND CONCLUSION

From soil and growth pouch experiments, it was found that nitrogen fixation in faba beans cv. Diana was affected by the strains of Rhizobium. Failure to form nodules resulted in no nitrogen fixation. The ability of a particular strain to form nodules is determined by the genetics of the host (Norris 1967). M_3 and M_{15} failed completely to form nodules in the growth pouch experiment and produced sparse nodulation in the soil experiment. This might have been due to contamination. Strains M_{17-23} were poorly nodulated in both the soil and growth pouch experiments. M_4 treated plants were well nodulated throughout but nodules did not fix nitrogen in the growth pouch experiment. This may have been due to slow establishment of the nodules formed by this strain or it may not function well in water logged conditions. Sprent (1975) also noticed variations in the performance of different Phaseolus vulgaris rhizobia under water logged conditions. M_{13} treated plants were fairly well nodulated but failed to fix nitrogen in the soil. Therefore this strain was not chosen for further study in the field.

In the soil experiment the 5 week harvest did not show clear differences between effective and ineffective strains due to the soil and seed nitrogen. If the soil mixture had 4 ppm available nitrate, differences in dry weight would not yet be expected at the 5 week harvest. However, differences among treatments could be detected later, provided that contamination was kept to a minimum in the first 3 weeks. Contamination that occurred later did not mask the expression of the inoculated strains. This can be confirmed by the results of the 8 and 11 week

harvests. The method of screening the Rhizobium in the greenhouse using a partially sterilized soil mixture is valuable only when the Rhizobium strains to be tested are very different from one another like the ones reported here. Its usefulness in comparing closely effective strains is doubtful. Therefore the growth pouch experiment was conducted. Many methods have been developed to study the effectiveness of Rhizobium strains (Vincent 1970). Recently, Wacek and Brill (1976) have developed a rapid method for determining the Rhizobium effectiveness in soy beans. The effectiveness assay is completed within 2 weeks after seedlings have germinated and several thousand seeds can be readily tested in one month.

However, field testing is the crucial method to determine the Rhizobium effectiveness. The strains that perform well in greenhouse conditions may fail in field conditions (Cloonan and Vincent 1967). The performance of the strains vary with the year (Abel and Erdman 1964).

Field tests at both locations A and B indicated that M_{14} was only moderately effective in nodulating faba beans. The growth pouch experiment with controlled root temperature showed M_{14} deteriorated in effectiveness. In the greenhouse tests M_{11} and M_{12} were always found to be inferior to M_{21}, M_{22} and M_{23}. However, in the field conditions in 1976 at location A, the two Moroccan strains proved to be superior to any other strain being tested at the same time. The seed yields by these two strains were not significant different from the others. Ethylene production data indicated that these two strains showed increasing ethylene production at 11 weeks after planting (pod filling

stage) while most of the strains showed decreasing ethylene production. This suggested that these two strains performed well in a dry year. They may be able to stand the moisture stress or high temperature better than other effective strains. The effects of soil moisture stress and soil temperature on nitrogen fixation by these two strains are worth looking at. Moisture stress is known to affect nitrogen directly by changing the fine structure of nodules (Sprent 1972a) or indirectly by reducing the supplies of photosynthates from the leaves (Huang et al 1975b). Pankhurst and Gibson (1973) suggest that Rhizobium strains capable of performing good symbiosis at high temperatures delay nodule degeneration longer than other strains do. Either water stress or high soil temperature tolerance by these two strains led to their increased effectiveness at location A in 1976. This needs to be clarified.

The excellent performance of the two Moroccan strains in ethylene production and seed nitrogen yield in the dry year at location A, suggested that these two strains should be added in the commercial inoculum. This is to ensure good fixation and nitrogen yield in a dry year or dry area. The present Q commercial inoculum and its composit strains (M_{21}, M_{22}, and M_{23}) were found to be inferior to the two Moroccan strains at location A.

Failure to get response from different strains at location A is very discouraging. Burton (1976) reported one strain of soy beans Rhizobium increased seed yield even at high rates of nitrogen fertilizer application. This is not the case in faba beans. McEwen (1970) reports that there is no prospect for the economic use of nitrogen fertilizer in the seed bed for faba beans. Dean and Clark (1977) found that inoculation

did not increase seed yields in high nitrogen soils, seed yields even decreased in one location. They suggested that the abundance of nodules diverted the photosynthates from the filling pods to the nodules. They also suggested that faba beans were self-sufficient in N_2 fixation in low nitrogen soils.

The finding that approximately 90% of the plants examined in the 1977 persistence test had nodules showed that the rhizobia were still present, but their origin could not be clarified. The nodules found on lateral roots also indicated that the rhizobia were not in close proximity to the seeds and failed to nodulate early. To ensure early and good nodulation, faba beans should be inoculated before they are sown even though the history of the field shows that faba beans were grown previously. If different strains of Rhizobium could be identified, we would be able to know which strains survived better than the others in a dry year and what the degree of cross contamination was among plots. This is one of the areas that need to be studied.

Weeds were the problem in the 1977 persistence experiment. Since soil disturbance was not desirable, seeds were directly drilled into the stubbles. However, weeds turned out to be the major problem and seemed to obscure the results. The chemical recommended by the weed specialists failed to control the weeds. Therefore weed control in faba beans is also one of the areas that needs to be studied.

SECTION 4

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Appendices

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APPENDIX 1

Nodulation Score of Faba Beans Inoculated with Different Strains of *Rhizobium* and Grown in the Greenhouse. The Beans Were 5 Week Old at Harvest

Treatment	Repetition						Mean
	1	2	3	4	5	6	
1	0	0	0	0	0	0	0
2	3	2	3	3	2	3	2.67
3	1	0	1	0	0	1	0.50
4	4	4	3	2	2	4	3.17
5	3	3	3	2	3	3	2.83
6	2	4	3	3	2	3	2.83
7	1	3	2	0	2	3	1.83
8	3	3	3	4	3	1	2.83
9	2	0	0	0	0	0	0.33
10	4	3	3	4	4	3	3.50
11	2	0	2	1	1	1	1.17
12	2	1	2	1	1	1	1.33
13	1	1	1	1	2	2	1.33
14	2	1	2	2	2	2	1.83
15	3	3	3	2	2	4	2.83
16	4	3	3	2	3	3	3.00
17	3	3	2	2	3	3	2.67
18	2	0	0	0	0	0	0.33

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	17	130.0000	7.6471	15.7607	1%
Error	90	43.6667	0.4852		c.v. = 31.88%
Total	107	173.6667			

APPENDIX 2

Dry Weight of Faba Beans (g/pot)* Inoculated with Different Strains of Rhizobium and Grown in the Greenhouse. The Beans Were 5 Weeks Old at Harvest

Treatment	Repetition						Mean
	1	2	3	4	5	6	
1	0.92	1.30	0.99	1.16	0.75	1.06	1.03
2	1.13	1.16	0.88	1.39	1.19	1.16	1.15
3	1.08	0.72	1.11	0.86	0.96	0.80	0.92
4	0.95	1.01	1.00	0.87	0.62	1.16	0.94
5	0.94	1.12	1.00	0.98	1.01	1.08	1.02
6	0.49	1.42	1.26	0.94	0.90	1.36	1.06
7	0.95	1.28	0.85	0.68	0.90	1.05	0.95
8	1.30	1.25	1.32	1.39	1.29	0.92	1.25
9	0.89	1.01	0.94	1.00	0.94	0.83	0.94
10	1.34	1.21	1.40	1.32	1.27	1.02	1.26
11	1.15	0.93	0.97	0.96	1.09	0.97	1.01
12	0.91	0.83	1.26	0.91	0.99	0.95	0.98
13	0.68	0.88	1.15	1.10	1.09	1.13	1.01
14	0.91	1.21	0.73	0.87	0.95	0.71	0.90
15	0.93	1.46	1.19	0.91	0.98	1.54	1.17
16	1.36	0.64	0.93	0.81	1.20	1.04	1.00
17	1.12	1.08	1.19	1.22	1.32	1.50	1.24
18	1.22	1.03	0.96	1.07	0.92	0.93	1.02

* 1 pot consisted of 2 plants.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	17	1.3910	0.0818	2.3779	1%
Error	90	3.0958	0.0344		c.v. = 17.74%
Total	107	4.4868			

APPENDIX 3

Nodulation Score of Faba Beans Inoculated with Different Strains of Rhizobium and Grown in the Greenhouse. The Beans were 8 Week Old at Harvest

Treatment	Repetition 1	2	3	4	5	6	Mean
1	1	1	1	1	2	1	1.17
2	4	3	3	4	3	3	3.33
3	1	2	1	2	2	1	1.50
4	4	3	3	4	3	3	3.33
5	2	3	2	3	3	2	2.50
6	3	3	3	3	4	3	3.17
7	2	3	3	3	2	2	2.50
8	4	4	4	3	3	3	3.50
9	1	2	2	2	0	1	1.33
10	3	3	3	2	3	3	2.83
11	1	2	1	1	1	1	1.17
12	1	1	1	1	1	0	0.83
13	1	1	1	1	1	1	1.00
14	1	1	1	1	1	0	0.83
15	3	3	3	4	3	3	3.17
16	3	4	4	3	3	3	3.33
17	4	4	3	3	2	3	3.17
18	1	1	0	1	1	0	0.67

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	17	118.9630	6.9978	26.9873	1%
Error	90	23.3333	0.2593		c.v. = 23.30%
Total	107	142.2963			

APPENDIX 4

C_2H_4 Production (μ Moles/pot/hr.) of Faba Beans Inoculated with Different Strains of Rhizobium and Grown in the Greenhouse. The Beans were 8 Week Old at Harvest

Treatment	Repetition 1	2	3	4	5	6	Mean
1	0	0	0	0	2.93	0	0.49
2	7.84	6.55	4.17	0	8.37	5.78	5.45
3	0	4.80	0	0	0	0	0.80
4	0	0	0	10.16	4.51	4.35	3.17
5	0	0	0	0	0	1.75	0.29
6	0	10.59	5.90	11.10	6.49	10.95	7.51
7	0	0	0	0	0	0	0
8	8.12	7.28	9.13	7.97	0	4.84	6.22
9	0	0	0	0	0	0	0
10	0	8.66	6.15	0	6.77	6.57	4.69
11	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0
15	0	6.41	0	7.28	10.19	4.81	4.78
16	6.45	0	7.25	5.41	6.79	1.17	4.51
17	7.95	10.92	7.48	4.59	1.80	4.75	6.25
18	0	0	0	0	0	0	0

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	17	790.2320	46.4842	7.4374	1%
Error	90	562.2101	6.2501		c.v. = 101.89%
Total	107	1352.4421			

APPENDIX 5

Dry Weight of Faba Beans (g/pot)* Inoculated with Different Strains of Rhizobium and Grown in the Greenhouse. The Beans were 8 Weeks Old at Harvest

Treatment	Repetition						Mean
	1	2	3	4	5	6	
1	2.33	3.04	2.23	1.98	2.54	2.21	2.39
2	4.98	3.94	3.70	3.83	3.60	4.33	4.06
3	2.30	3.12	1.41	2.24	1.71	1.85	2.11
4	2.93	3.07	2.51	3.84	3.47	3.15	3.16
5	2.77	3.31	3.35	2.09	3.81	3.41	3.12
6	3.80	5.00	3.94	4.41	4.50	3.73	4.23
7	2.02	3.01	2.67	3.07	2.21	2.22	2.53
8	4.80	3.65	4.88	3.42	3.76	3.65	4.03
9	1.87	2.03	2.05	2.09	1.41	1.76	1.87
10	2.98	3.90	3.50	1.95	3.99	3.13	3.24
11	1.75	2.40	1.99	1.59	1.88	1.54	1.86
12	2.31	2.01	1.48	1.40	1.91	2.10	1.87
13	1.26	1.73	1.35	2.08	1.32	1.54	1.55
14	2.40	1.88	2.15	1.79	1.95	2.19	2.06
15	4.23	4.98	4.43	5.28	5.09	4.76	4.80
16	3.40	4.03	4.71	3.90	3.85	3.90	3.97
17	5.08	5.33	4.59	3.66	3.31	3.46	4.24
18	2.54	2.78	1.49	2.03	2.28	1.95	2.18

* 1 pot consisted of 2 plants.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	17	110.2894	6.4876	25.8986	1%
Error	90	22.5462	0.2505		c.v. = 16.92%
Total	107	132.8356			

APPENDIX 6

Percentage of Nitrogen Content of Faba Beans Inoculated with Different Strains of Rhizobium and Grown in the Greenhouse. The Beans were 8 Week Old at Harvest

Treatment	Repetition						Mean
	1	2	3	4	5	6	
1	2.85	3.23	3.36	2.14	3.36	3.01	2.99
2	3.58	3.55	3.58	3.65	4.26	3.71	3.72
3	2.40	3.10	2.66	3.36	2.82	1.66	2.67
4	3.42	3.78	3.68	3.71	3.87	3.52	3.66
5	3.46	3.46	3.62	3.62	3.65	3.71	3.59
6	4.00	3.68	3.94	3.87	2.88	3.68	3.68
7	3.14	2.69	2.62	2.88	2.50	2.37	2.70
8	3.20	3.33	3.33	4.16	3.62	3.58	3.54
9	2.34	3.33	3.07	3.87	2.50	1.79	2.82
10	3.20	3.74	3.65	4.06	3.20	3.17	3.50
11	1.82	2.50	1.79	2.11	2.02	1.83	2.01
12	1.47	1.82	1.76	1.98	1.66	1.70	1.73
13	1.92	1.50	1.95	1.70	1.89	2.05	1.84
14	1.66	1.73	1.66	1.57	1.60	2.14	1.73
15	3.14	3.55	3.58	3.20	3.33	3.68	3.41
16	3.81	3.58	4.16	2.94	3.84	3.30	3.61
17	4.35	4.00	3.14	3.62	4.00	4.35	3.91
18	2.72	3.42	2.72	3.23	3.14	2.88	3.02

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	17	56.7926	3.3407	24.3491	1%
Error	90	12.3487	0.1372		c.v. = 12.32%
Total	107	69.1413			

APPENDIX 7

Nodulation Score of Faba Bean Inoculated with Different Strains of Rhizobium and Grown in the Greenhouse. The Beans were 11 Weeks Old at Harvest

Treatment	Repetition						Mean
	1	2	3	4	5	6	
1	1	1	2	1	3	2	1.67
2	5	4	4	3	3	3	3.67
3	1	1	2	0	1	2	1.67
4	4	4	4	4	4	4	4.00
5	3	4	3	3	2	4	3.17
6	4	4	3	4	3	4	3.67
7	2	3	1	2	4	1	2.17
8	4	4	4	4	4	4	4.00
9	2	2	1	1	1	2	1.50
10	3	5	4	4	4	4	4.00
11	2	0	1	1	1	1	1.00
12	0	1	0	1	2	1	0.83
13	0	0	0	0	1	1	0.33
14	0	1	0	1	1	1	0.67
15	3	4	3	4	3	4	0.50
16	3	3	4	4	3	4	0.50
17	4	3	5	4	4	4	4.00
18	1	2	1	0	2	1	1.17

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	17	195.3333	11.4902	26.2933	1%
Error	90	39.3333	0.4370		c.v. = 27.05%
Total	107	234.6667			

APPENDIX 8

C_2H_4 Production (μ Moles/pot/hr.)* of Faba Beans Inoculated with Different Strains of Rhizobium and Grown in the Greenhouse. The Beans were 11 Week Old at Harvest

Treatment	Repetition						Mean
	1	2	3	4	5	6	
1	0.26	0.81	1.00	1.38	0	4.97	1.40
2	13.02	8.01	8.94	7.19	3.04	7.77	8.00
3	1.13	2.19	5.56	0	2.35	5.71	2.82
4	12.80	8.11	3.47	11.66	10.58	11.36	9.66
5	8.80	5.82	10.80	4.79	4.61	6.64	6.91
6	15.11	8.31	3.90	13.70	5.86	9.87	9.46
7	2.68	4.69	0	1.30	6.00	1.28	2.66
8	8.09	9.77	15.13	8.42	10.38	9.86	10.28
9	2.83	2.31	0	2.90	0	4.98	2.17
10	17.35	12.49	7.07	8.05	8.46	7.10	10.09
11	7.82	0	1.73	0	0	0	1.59
12	0	0	0	0	3.35	0	0.56
13	0	0	0	0	0	0	0
14	0	0	0	1.34	1.46	0	0.47
15	11.64	13.02	7.26	6.84	5.63	7.22	8.60
16	7.06	3.92	13.09	7.15	13.13	3.40	7.96
17	9.58	7.62	4.92	12.18	6.42	10.27	8.50
18	2.48	8.42	0	0	8.24	0.96	3.35

* 1 pot consisted of 2 plants.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	17	1509.4748	88.7926	10.6589	1%
Error	90	749.7324	8.3304		c.v. = 54.99%
Total	107	2259.2072			

APPENDIX 9

Dry Weight of Faba Beans (g/pot)* Inoculated with Different Strains of Rhizobium and Grown in the Greenhouse. The Beans were 11 Weeks Old at Harvest

Treatment	Repetition						Mean
	1	2	3	4	5	6	
1	1.89	2.70	3.25	2.86	1.52	3.99	2.70
2	8.29	6.59	6.58	6.07	6.98	7.83	7.06
3	3.50	2.81	5.93	1.79	2.80	4.37	3.53
4	7.80	7.34	5.13	6.87	6.22	5.15	6.42
5	7.08	5.75	6.74	6.26	4.57	7.18	6.26
6	10.40	7.47	7.75	8.22	4.50	8.15	7.75
7	2.88	4.87	2.52	2.78	4.43	3.43	3.49
8	9.90	6.17	9.19	7.15	7.59	6.67	7.78
9	4.38	1.75	2.19	3.43	1.33	4.07	2.86
10	8.99	8.68	6.10	6.67	6.39	6.48	7.22
11	6.43	1.03	2.36	1.71	2.17	2.31	2.67
12	1.48	1.63	1.63	2.00	3.47	1.90	2.02
13	2.15	1.30	2.02	1.56	1.41	1.89	1.72
14	1.57	1.96	1.70	2.65	2.06	2.13	2.01
15	9.97	8.34	7.35	7.89	8.98	6.78	8.22
16	6.83	6.66	9.18	6.72	7.55	7.89	7.47
17	9.11	7.42	7.70	8.70	8.29	8.14	8.23
18	4.37	6.58	2.28	1.43	5.54	2.23	3.74

* 1 pot consisted of 2 plants.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	17	628.4956	36.9703	24.4982	1%
Error	90	135.8182	1.5091		c.v. = 24.26%
Total	107	764.3137			

APPENDIX 10

Percentage of Nitrogen Content of Faba Beans Inoculated with Different Strains of Rhizobium and Grown in the Greenhouse. The Beans were 11 Weeks Old at Harvest

Treatment	Repetition						Mean
	1	2	3	4	5	6	
1	2.75	2.40	2.21	2.62	2.46	2.27	2.45
2	2.05	2.54	2.11	3.36	3.07	3.14	2.55
3	3.14	3.20	2.43	2.43	2.98	2.21	2.73
4	2.56	2.40	2.08	2.30	2.05	2.62	2.34
5	2.14	2.24	1.95	3.49	3.62	2.40	2.64
6	2.66	2.46	2.43	2.18	2.88	2.08	2.45
7	2.27	1.98	2.14	2.59	1.79	2.05	2.14
8	2.75	2.24	2.30	3.01	3.07	4.16	2.92
9	3.17	2.50	2.21	2.98	3.23	3.87	2.99
10	2.59	3.23	3.23	2.05	2.02	1.86	2.50
11	2.66	2.14	2.53	2.34	2.11	1.57	2.23
12	1.66	2.59	2.11	2.66	3.14	1.89	2.34
13	1.22	2.18	1.70	2.21	2.08	1.41	1.80
14	2.50	1.47	1.63	2.82	2.66	2.30	2.23
15	2.82	2.18	2.05	3.49	2.30	2.62	2.58
16	2.82	2.14	2.18	2.37	2.78	2.53	2.47
17	1.76	2.50	3.94	3.58	2.30	2.24	2.72
18	3.14	2.50	2.43	2.11	2.18	2.21	2.43

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	17	8.2217	0.4836	1.8672	5%
Error	90	23.3139	0.2590		c.v. = 20.59%
Total	107	31.5356			

APPENDIX 11

Nodulation Score of Faba Beans Inoculated with Different Strains of *Rhizobium* and Grown in Pouches. The Beans were Harvested at 5 Weeks After Inoculation

Treatment	Replication								Total	Mean
	1	2	3	4	5	6	7	8		
1	0	0	0	0	0	0	0	0	0	0
2	3	5	4	3	3	3	3	2	26	3.25
3	0	0	0	0	0	0	0	0	0	0
4	2	2	1	3	3	2	1	2	16	2.00
5	3	4	3	4	3	2	1	2	22	2.75
6	3	2	4	3	2	2	2	3	21	2.63
7	3	3	3	2	2	3	1	3	20	2.50
8	4	3	3	3	3	3	2	4	25	3.13
9	0	0	0	0	0	0	0	0	0	0
10	2	1	3	3	2	2	2	3	18	2.25
11	1	1	2	1	1	1	1	1	9	1.13
12	0	1	1	1	1	1	1	2	8	1.00
13	2	1	1	1	1	0	1	1	8	1.00
14	1	1	0	1	2	1	1	1	8	1.00
15	3	3	3	2	1	2	3	3	20	2.50
16	3	3	4	2	2	2	3	2	21	2.63
17	4	3	2	2	2	3	3	3	22	2.75
Total	34	33	34	31	28	27	25	32	244	

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	16	163.7353	10.1710	25.4976	1%
Replications	7	4.8235	0.6891	1.7275	N.S.
Error	112	44.6765	0.3989		c.v. = 35.20%
Total	135	213.2353	1.5795		

APPENDIX 12

C_2H_4 Production (μ Moles/plant/hr.) of Faba Beans Inoculated with Different Strains of Rhizobium and Grown in Pouches. The Beans were Harvested 5 Weeks After Inoculation

Treatment	Replication 1	2	3	4	5	6	7	8	Total	Mean
1	0	0	0	0	0	0	0	0	0	0
2	1.87	3.25	1.38	0	0.67	0	0	0	7.17	0.90
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0.62	0.11	1.00	3.08	0	0.29	0	0	5.10	0.64
6	0	0	0.79	0.53	0	0	0	0	1.32	0.17
7	1.13	0.74	0	0	0	0	0	0	1.87	0.23
8	1.12	0	1.25	0	0	0	0	0	2.37	0.30
9	0	0	0	0	0	0	0	0	0	0
10	0	0	0.33	1.15	0	0	0	0	1.48	0.19
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
15	0.33	2.24	0.41	0	0.66	0	0	0	3.64	0.46
16	0.62	0	1.16	0	0	0	0	0	1.78	0.22
17	0.59	1.06	0.86	0	0	0	0	0	2.51	0.31
Total	6.28	7.40	7.18	4.76	1.33	0.29	0	0	27.24	

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	16	8.6919	0.5432	2.4025	1%
Replication	7	4.5594	0.6513	2.8806	1%
Error	112	25.3217	0.2261		c.v. = 237.39%
Total	135	38.5730	0.2857		

APPENDIX 13

Dry Weight of Faba Beans (g/plant) Inoculated with Different Strains of *Rhizobium* and Grown in Pouches. The Beans were Harvested at 5 Weeks After Inoculation

Treatment	Replication 1	2	3	4	5	6	7	8	Total	Mean
1	0.56	0.14	0.16	0.25	0.22	0.12	0.17	0.26	1.88	0.24
2	0.64	0.83	0.55	0.39	0.82	0.64	0.68	0.46	5.01	0.63
3	0.26	0.30	0.28	0.24	0.30	0.18	0.22	0.24	2.02	0.25
4	0.16	0.13	0.23	0.27	0.22	0.15	0.14	0.10	1.40	0.18
5	0.63	0.60	0.61	0.88	0.27	0.56	0.24	0.22	4.01	0.50
6	0.49	0.32	0.84	0.79	0.56	0.37	0.40	0.55	4.32	0.54
7	0.37	0.53	0.25	0.38	0.34	0.33	0.12	0.31	2.63	0.33
8	0.62	0.30	0.59	0.36	0.57	0.60	0.44	0.45	3.49	0.49
9	0.36	0.24	0.19	0.30	0.24	0.31	0.36	0.18	2.18	0.27
10	0.26	0.22	0.44	0.71	0.54	0.45	0.33	0.29	3.24	0.41
11	0.16	0.26	0.39	0.17	0.15	0.15	0.28	0.26	1.82	0.23
12	0.11	0.30	0.32	0.28	0.27	0.20	0.25	0.23	1.96	0.25
13	0.43	0.22	0.24	0.37	0.32	0.28	0.27	0.29	2.42	0.30
14	0.11	0.21	0.22	0.12	0.21	0.30	0.29	0.13	1.59	0.20
15	0.72	0.70	0.41	0.32	0.50	0.62	0.36	0.36	3.99	0.50
16	0.50	0.24	0.68	0.26	0.23	0.27	0.41	0.34	2.93	0.37
17	0.54	0.82	0.57	0.50	0.34	0.44	0.59	0.38	4.18	0.52
Total	6.92	6.36	6.97	6.59	6.10	5.97	5.55	5.05	49.51	

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	16	2.5443	0.1590	9.4083	1%
Replications	7	0.1821	0.0260	1.5385	N.S.
Error	112	1.8903	0.0169		c.v. = 35.71%
Total	135	4.6167	0.0342		

APPENDIX 14

Nodulation Score* of Faba Beans Grown at Location A and Harvested 5 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	0.75	0.50	1.00	1.00	0.50	1.00	0.79
2	2.00	3.00	2.75	2.75	2.00	1.50	2.33
3	2.00	2.25	2.50	2.00	2.25	2.75	2.29
4	2.00	2.50	1.75	2.50	2.25	2.75	2.29
5	2.75	2.25	3.50	2.75	3.25	2.00	2.75
6	1.00	1.00	1.75	0.75	1.00	1.00	1.08
7	3.25	3.00	3.75	3.25	2.75	3.00	3.17
8	3.25	3.25	3.25	3.00	3.00	3.00	3.13
9	3.00	3.25	3.00	3.25	3.00	3.00	3.08
10	3.25	3.25	2.75	3.00	2.75	3.00	3.00
11	2.75	3.00	2.50	3.00	3.25	3.50	3.00

*Each figure represents the mean of 4 plants.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	40.4811	4.0481	30.9487	1%
Replications	5	0.4267	0.0833	0.6369	N.S.
Error	50	6.5417	0.1308		c.v. = 14.78%
Total	65	47.4394	0.7298		

APPENDIX 15

C_2H_4 Production (μ Moles/plant/hr.) of Faba Beans Grown at Location A and Harvested 5 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	0.80	0	1.47	0	0	0.11	0.40
2	3.10	7.04	5.35	4.86	3.92	1.63	4.32
3	5.00	4.68	5.16	6.20	9.81	7.12	6.33
4	5.94	6.59	4.76	3.97	3.70	3.83	4.80
5	5.50	4.92	7.62	3.56	7.03	2.80	5.24
6	1.09	1.54	1.95	0	1.37	0.49	1.07
7	8.53	5.07	5.60	5.30	5.11	2.83	5.41
8	6.73	8.73	6.38	5.25	4.59	6.62	6.38
9	8.96	4.68	7.97	5.85	6.65	7.14	6.88
10	7.12	4.34	3.66	6.46	7.99	4.33	5.65
11	4.53	5.60	4.07	2.76	8.10	5.33	5.07

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	263.1962	26.3796	11.4051	1%
Replications	5	20.6909	4.1382	1.7932	N.S.
Error	50	115.3835	2.3077		c.v. = 32.43%
Total	65	399.2706	6.1426		

APPENDIX 16

Dry Weight of Faba Beans (g/plant) Grown at Location A and Harvested 5 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	1.60	1.88	1.10	1.53	2.08	2.03	1.70
2	1.95	2.23	2.28	1.65	2.18	2.40	2.11
3	1.98	2.45	1.80	1.65	2.65	2.33	2.14
4	1.95	2.30	2.40	2.25	2.83	2.40	2.36
5	1.53	1.93	1.83	1.78	2.73	2.13	1.98
6	1.63	1.60	1.50	1.63	2.28	2.10	1.79
7	2.83	2.15	2.10	2.18	1.83	2.08	2.19
8	2.68	2.80	1.93	2.05	2.90	2.50	2.48
9	2.35	2.73	2.25	2.13	2.45	2.45	2.39
10	2.80	2.53	1.78	1.90	2.88	1.80	2.28
11	2.93	2.00	1.95	2.10	2.78	1.73	2.25

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	3.5788	0.3579	3.7713	1%
Replications	5	2.9078	0.5816	6.1286	1%
Error	50	4.7465	0.0949		c.v. = 14.32%
Total	65	2.7651			

APPENDIX 17

N Percentage of Faba Beans Grown in Location A and Harvested 5 Weeks After Planting

Treatment	Replication 1	2	3	4	5	6	Mean
1	3.55	4.00	3.78	3.33	3.00	3.68	3.56
2	4.42	4.48	4.61	4.64	4.19	3.65	4.33
3	4.35	4.54	4.48	4.67	3.94	4.22	4.37
4	4.80	4.64	4.54	4.32	4.19	4.32	4.47
5	4.61	4.64	4.42	4.26	4.06	4.32	4.39
6	4.45	3.52	3.65	4.03	3.78	4.16	3.93
7	4.38	4.54	4.45	4.38	4.51	4.48	4.46
8	4.77	4.54	4.58	4.77	3.84	4.42	4.49
9	4.48	4.19	4.67	4.38	4.29	4.32	4.39
10	4.64	4.32	4.58	4.48	4.26	4.13	4.40
11	4.32	4.29	4.83	4.42	4.29	4.10	4.38

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	4.8617	0.4862	9.1563	1%
Replications	5	1.3585	0.2717	5.1167	1%
Error	50	2.6567	0.0531		c.v. = 5.38%
Total	65	8.8770	0.1366		

APPENDIX 18

Nodulation Score* of Faba Beans Grown at Location B and Harvested 5 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	1.25	0.50	2.50	3.00	1.00	1.00	1.54
2	3.00	2.25	4.00	4.00	3.00	2.00	3.04
3	4.25	2.50	3.50	3.75	2.50	3.00	3.25
4	3.25	2.50	3.00	2.75	2.75	3.25	2.92
5	4.00	2.75	3.00	4.00	3.75	3.25	3.46
6	2.00	2.25	2.50	3.50	1.25	1.75	2.21
7	3.75	2.75	3.25	3.50	3.25	3.25	3.29
8	3.25	3.00	3.50	3.00	3.75	2.25	3.13
9	3.75	3.00	2.75	3.50	3.00	2.75	3.13
10	3.50	3.25	3.75	3.75	3.00	2.75	3.33
11	2.75	3.00	2.75	3.50	3.00	3.00	3.00

*Each figure represents the mean of 4 plants.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	19.3146	1.9315	8.2861	1%
Replications	5	7.9773	1.5955	6.8447	1%
Error	50	11.6553	0.2331		c.v. = 16.45%
Total	65	38.9406	0.5991		

APPENDIX 19

C_2H_4 Production (μ Moles/plant/hr.) of Faba Beans Grown at Location B and Harvested 5 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	0.59	0.74	6.24	4.04	0.34	0.98	2.16
2	4.68	2.93	7.44	4.79	2.93	2.36	4.19
3	7.70	2.26	8.65	8.99	2.79	1.47	5.31
4	5.31	2.96	5.33	5.12	3.72	3.39	4.31
5	7.33	3.33	7.44	7.94	6.74	1.88	5.78
6	2.64	2.99	7.48	4.35	1.27	0	3.12
7	3.62	3.97	6.27	5.75	2.67	3.34	4.27
8	3.74	4.98	9.19	6.25	6.03	0.97	5.19
9	5.17	3.97	6.37	4.23	3.05	2.36	4.19
10	4.90	5.28	6.12	3.89	3.12	1.49	4.13
11	4.10	5.07	8.31	7.12	3.46	2.01	5.01

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	63.2160	6.3276	3.8320	1%
Replications	5	196.7977	39.3595	23.8586	1%
Error	50	82.4843	1.6497		c.v. = 46.94%
Total	65	342.4980	5.2692		

APPENDIX 20

Dry Weight of Faba Beans (g/plant) Grown at Location B and Harvested 5 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	1.43	1.80	1.55	1.23	1.98	2.80	1.80
2	2.15	1.85	1.58	1.70	2.68	2.05	2.00
3	1.98	2.03	1.25	1.70	2.10	2.50	1.93
4	2.03	1.90	1.63	1.13	2.65	2.70	2.01
5	2.23	1.45	1.60	1.78	1.98	2.55	1.93
6	1.93	1.95	1.40	1.50	2.60	2.10	1.91
7	1.50	1.65	0.88	1.48	2.73	2.70	1.82
8	2.05	2.30	1.95	1.55	2.20	2.28	2.06
9	1.85	2.68	1.80	1.88	1.98	2.50	2.11
10	1.95	1.88	1.55	1.50	2.15	2.15	1.86
11	2.05	1.68	1.50	1.83	2.05	2.38	1.91

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	0.5600	0.0560	0.6715	N.S.
Replications	5	7.3865	1.4773	17.7134	1%
Error	50	4.1686	0.0834		c.v. = 14.89%
Total	65	12.1152	0.1864		

APPENDIX 21

N Percentage of Faba Beans Grown at Location B and Harvested 5 Weeks After Planting

Treatment	Replication 1	2	3	4	5	6	Mean
1	3.94	4.74	3.62	4.03	3.68	3.94	3.99
2	4.29	5.06	3.71	3.94	4.74	4.86	4.43
3	4.19	4.80	3.87	3.62	4.35	4.00	4.14
4	4.67	4.77	3.65	3.81	4.86	3.97	4.29
5	4.58	4.58	4.03	3.94	4.13	3.97	4.21
6	4.29	4.96	3.94	3.62	4.83	4.16	4.30
7	4.13	4.48	3.62	3.23	4.61	4.00	4.01
8	4.13	4.06	3.52	3.71	3.90	4.61	3.99
9	4.42	3.87	3.55	4.54	4.38	4.90	4.28
10	4.67	4.48	3.46	4.13	4.32	4.54	4.27
11	4.38	4.74	3.68	3.55	4.70	4.32	4.23

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	1.2624	0.1262	1.2633	N.S.
Replications	5	6.8099	1.3620	13.6336	1%
Error	50	4.9928	0.0999		c.v. = 7.54%
Total	65	13.0651	0.2010		

APPENDIX 22

Nodulation Score* of Faba Beans Grown at Location A and Harvested 8 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	0.75	0.50	0.75	0.50	0.50	1.00	0.67
2	1.25	3.75	3.25	3.50	4.00	3.50	3.21
3	3.25	3.50	2.75	4.00	3.25	4.25	3.50
4	4.00	3.25	3.75	4.00	2.25	3.75	3.50
5	3.50	4.50	4.50	3.25	4.00	4.00	3.96
6	1.00	1.00	1.00	1.00	1.75	1.75	1.25
7	4.50	4.75	4.00	4.50	3.50	4.50	4.29
8	4.25	4.25	4.25	3.75	3.75	4.25	4.08
9	3.75	4.25	4.25	3.25	5.00	4.75	4.21
10	3.50	4.50	4.50	4.25	3.50	4.00	4.04
11	3.50	4.25	4.50	3.75	4.50	3.75	4.04

*Each figure represents the mean of 4 plants.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	90.9754	9.0975	30.9334	1%
Replications	5	2.2727	0.4545	1.5454	N.S.
Error	50	14.7064	0.2941		c.v. = 16.23%
Total	65	107.9545	1.6608		

APPENDIX 23

C_2H_4 Production (μ Moles/plant/hr.) of Faba Beans Grown at Location A and Harvested 8 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	0	0	0.65	0	1.13	2.72	0.75
2	2.54	6.34	6.02	5.49	15.19	6.09	6.95
3	9.15	9.14	6.63	8.97	8.67	14.47	9.51
4	14.95	9.47	13.37	9.86	5.56	8.98	10.37
5	11.27	11.77	12.73	6.12	11.97	8.30	10.96
6	2.97	0.76	1.66	3.18	3.49	2.05	2.35
7	6.53	8.37	3.41	7.85	8.59	4.82	6.60
8	10.82	7.94	10.08	4.22	3.44	8.38	7.48
9	8.73	10.33	7.70	7.00	12.51	15.16	10.24
10	5.95	10.97	7.36	5.07	5.18	4.44	6.50
11	6.51	9.62	7.64	6.46	10.65	4.86	7.62

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	604.9192	60.4919	8.2433	1%
Replications	5	28.1689	5.6338	0.7677	N.S.
Error	50	366.9133	7.3383		c.v. = 37.86%
Total	65	1000.0014	15.3846		

APPENDIX 24

Dry Weight of Faba Beans (g/plant) Grown at Location A and Harvested 8 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	6.15	6.85	4.40	3.58	7.13	7.78	5.98
2	6.73	8.45	6.60	6.43	10.55	7.65	7.73
3	6.05	7.93	6.28	7.90	8.38	8.83	7.56
4	8.58	8.13	9.45	8.50	10.68	8.40	8.96
5	6.80	10.28	6.65	7.10	8.10	7.78	7.78
6	8.43	6.78	5.35	4.93	7.58	8.25	6.88
7	10.50	9.13	6.93	7.10	8.88	8.85	8.56
8	7.50	8.03	7.00	6.85	7.50	9.53	7.73
9	7.15	5.23	8.30	5.68	9.50	9.68	7.59
10	5.65	9.25	7.43	6.95	8.95	7.58	7.63
11	8.55	7.63	6.90	7.83	10.78	5.53	7.87

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	36.0165	3.6016	2.4918	1%
Replications	5	40.6828	8.1366	5.6293	1%
Error	50	72.2722	1.4454		c.v. = 15.69%
Total	65	148.9715	2.2919		

APPENDIX 25

N Percentage of Faba Beans Grown at Location A and Harvested 8 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	2.50	2.40	2.82	2.46	2.24	2.46	2.48
2	2.94	3.26	3.36	3.42	3.23	3.10	3.22
3	3.10	3.33	3.20	3.36	3.36	3.49	3.31
4	3.49	3.71	3.39	3.58	3.52	3.46	3.53
5	3.52	3.42	3.62	3.55	3.36	3.39	3.48
6	3.46	2.88	3.36	2.72	3.14	2.46	3.00
7	2.88	3.14	2.85	3.20	3.30	3.14	3.09
8	3.49	3.04	3.30	3.39	2.72	3.14	3.18
9	3.33	3.52	3.17	3.36	3.07	3.26	3.29
10	3.42	3.26	3.42	3.33	3.17	3.17	3.30
11	3.36	3.20	3.26	3.20	2.88	3.20	3.18

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	4.6724	0.4672	12.2304	1%
Replications	5	0.2452	0.0490	1.2827	N.S.
Error	50	1.9098	0.0382		c.v. = 6.13%
Total	65	6.8274	0.1050		

APPENDIX 26

Nodulation Score* of Faba Beans Grown at Location B and Harvested 8 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	0.75	0.75	3.25	3.50	2.50	0.75	1.92
2	3.00	3.25	4.25	4.25	3.00	3.00	3.46
3	4.00	3.50	4.25	4.25	3.75	4.50	4.04
4	3.00	3.50	4.00	4.00	3.50	4.25	3.71
5	4.00	3.50	4.50	4.25	4.75	4.50	4.25
6	2.00	1.75	4.00	3.50	2.25	2.00	2.58
7	4.25	3.75	4.50	4.25	4.25	3.75	4.13
8	4.25	3.25	4.50	3.75	4.00	3.75	3.92
9	4.50	3.25	4.00	4.00	4.00	3.50	3.88
10	3.00	3.75	3.75	4.50	3.75	4.75	3.92
11	3.25	3.75	5.00	3.50	3.50	4.75	3.96

*Each figure represents the mean of 4 plants.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	31.1686	3.1169	10.1132	1%
Replications	5	9.3182	1.8636	6.0467	1%
Error	50	15.4110	0.3082		c.v. = 15.36%
Total	65	55.8977	0.8600		

APPENDIX 27

C_2H_4 Production (μ Moles/plant/hr.) of Faba Beans Grown at Location B and Harvested 8 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	0.91	0.60	4.29	3.28	2.93	0	2.00
2	2.74	2.87	3.06	5.87	1.11	0.20	2.64
3	4.53	0.10	6.73	4.60	1.56	0	2.92
4	3.10	2.17	6.08	5.21	1.06	0.60	3.04
5	3.32	0.95	6.63	4.26	6.05	0.77	3.66
6	2.14	3.55	4.47	5.93	0.62	0.71	2.90
7	5.04	0.46	3.26	3.88	0.15	0.80	2.27
8	4.38	1.95	5.86	2.42	1.23	0.15	2.67
9	3.49	0.30	5.28	2.95	2.79	0	2.47
10	1.92	2.32	3.94	3.66	1.17	0.59	2.27
11	1.60	1.15	6.32	3.32	0.75	0	2.19

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	13.6340	1.3634	0.8888	N.S.
Replications	5	172.2220	34.4444	22.4540	1%
Error	50	76.7011	1.5340		c.v. = 46.94%
Total	65	262.5571	4.0393		

APPENDIX 28

Dry Weight of Faba Beans (g/plant) Grown at Location B and Harvested 8 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	10.03	7.18	4.53	6.55	6.48	7.35	7.02
2	6.40	8.30	5.53	8.68	6.15	8.65	7.28
3	8.25	6.33	5.53	6.18	7.00	8.08	6.88
4	8.10	10.20	6.05	5.35	8.60	9.48	8.02
5	9.03	5.40	6.20	6.90	9.03	11.18	7.96
6	7.48	7.80	6.23	5.73	7.88	9.33	7.40
7	7.05	7.63	5.50	4.83	7.60	10.35	7.16
8	9.08	9.05	6.25	4.35	6.30	8.93	7.33
9	8.33	5.95	6.10	6.10	9.40	7.95	7.31
10	6.15	6.70	6.33	8.33	8.88	10.13	7.75
11	7.78	8.48	6.53	4.48	9.33	8.95	7.59

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	8.0065	0.8007	0.5003	N.S.
Replications	5	81.8698	16.3740	10.2318	1%
Error	50	80.0140	1.6003		c.v. = 17.03%
Total	65	169.8904	2.6137		

APPENDIX 29

N Percentage of Faba Beans Grown at Location B and Harvested 8 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	3.68	3.74	4.10	3.74	3.81	3.68	3.79
2	3.78	3.94	3.84	3.84	3.97	3.81	3.86
3	3.65	4.26	3.97	3.74	3.04	3.84	3.75
4	3.78	4.00	4.06	3.87	3.90	3.74	3.89
5	3.78	3.87	4.00	4.00	3.62	3.78	3.84
6	3.90	3.78	3.90	3.87	3.68	3.90	3.84
7	3.58	3.84	3.81	3.94	3.94	3.55	3.78
8	3.84	3.90	4.03	3.78	3.87	3.94	3.89
9	4.03	4.00	3.84	4.19	3.84	4.03	3.99
10	3.94	3.90	3.74	3.46	3.81	3.90	3.79
11	3.74	3.87	3.74	3.71	3.81	3.46	3.72

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	0.3485	0.0349	1.1791	N.S.
Replications	5	0.2627	0.0525	1.7736	N.S.
Error	50	1.4781	0.0296		c.v. = 4.48%
Total	65	2.0893	0.0321		

APPENDIX 30

Nodulation Score* of Faba Beans Grown at Location A and Harvested 11 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	1.25	0.25	0.25	0.25	0.25	1.00	0.54
2	2.75	3.50	4.50	3.75	3.75	2.75	3.50
3	3.00	4.25	4.25	3.00	4.25	3.00	3.63
4	2.25	3.75	3.00	2.25	3.75	3.25	3.04
5	2.75	4.00	4.75	3.75	3.25	3.25	3.63
6	1.75	1.25	0.50	2.00	1.50	1.00	1.33
7	5.00	4.00	4.25	4.75	4.50	4.00	4.42
8	3.00	4.00	3.00	3.75	3.75	3.50	3.50
9	4.00	3.75	4.00	4.50	3.50	4.25	4.00
10	4.00	4.25	3.00	4.25	4.25	4.25	4.00
11	4.50	4.25	4.25	3.25	4.75	4.00	4.17

*Each figure represents the mean of 4 plants.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	88.7083	8.8708	27.9044	1%
Replications	5	0.8977	0.1795	0.5646	N.S.
Error	50	15.8939	0.3179		c.v. = 17.35%
Total	65	105.5000	1.6231		

APPENDIX 31

C_2H_4 Production (μ Moles/plant/hr.) of Faba Beans Grown at Location A and Harvested 11 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	0.10	0.19	0	0.44	0.97	2.45	0.69
2	3.66	10.76	10.36	7.65	9.04	5.35	7.80
3	5.93	11.04	10.97	7.78	10.99	5.48	8.70
4	7.34	14.01	9.08	12.15	12.18	9.55	10.72
5	10.02	10.23	20.32	9.41	12.87	7.07	11.65
6	6.32	2.42	0.83	8.17	2.37	0.35	3.41
7	7.76	3.66	3.66	6.59	9.21	2.67	5.59
8	5.47	9.16	6.50	5.21	7.06	5.77	6.53
9	7.84	9.85	9.91	7.64	6.42	9.82	8.58
10	6.78	8.70	7.39	6.93	6.22	4.89	6.82
11	5.22	5.20	7.63	7.00	9.00	6.32	6.73

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	578.9818	57.8982	10.2947	1%
Replications	5	60.1307	12.0261	2.1383	N.S.
Error	50	281.2057	5.6241		c.v. = 33.78%
Total	65	920.3183	14.1587		

APPENDIX 32

Analysis of Variance for Yield Components of Faba Beans Grown at Location A. The Beans were Harvested 11 Weeks After Planting

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
*1/					
Treatments	10	160.6061	16.0606	8.9107	1%
Replications	5	20.1184	4.0237	2.2324	N.S.
Error	50	90.1212	1.8024		c.v. = 19.68%
Total	65	270.8456			
2/					
Treatments	10	195.7145	19.5715	7.4552	1%
Replications	5	11.7307	2.3461	0.8937	N.S.
Error	50	131.2612	2.6252		c.v. = 25.20%
Total	65	338.7064	5.2109		
3/					
Treatments	10	440.4972	44.0497	7.1626	1%
Replications	5	25.6652	5.1330	0.8346	N.S.
Error	50	307.5023	6.1500		c.v. = 18.70%
Total	65	773.6647	11.9025		

*1/ Pod number per plant.

2/ Pod weight per plant.

3/ Leaves and stem weight per plant.

APPENDIX 33

Analysis of Variance for Nitrogen Percentage in Pods or Stems and Leaves of Faba Beans Grown at Location A. The Beans were Harvested 11 Weeks After Planting

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
*1/					
Treatments	10	6.1343	0.6134	23.4122	1%
Replications	5	0.5975	0.1195	4.5611	1%
Error	50	1.3094	0.0262		c.v. = 4.33%
Total	65	8.0411	0.1237		
2/					
Treatments	10	2.0068	0.2007	5.0682	1%
Replications	5	0.5659	0.1132	2.8586	5%
Error	50	1.9784	0.0396		c.v. = 9.80%
Total	65	4.5511	0.0700		

*1/ N percentage in pods.

2/ N percentage in leaves and stems.

APPENDIX 34

Nodulation Score* of Faba Beans Grown at Location B and Harvested 11 Weeks After Planting

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	1.00	0.75	3.25	2.75	1.25	0.50	1.58
2	3.00	2.75	4.25	3.75	3.75	3.75	3.54
3	3.00	3.25	4.00	4.00	4.25	3.75	3.71
4	3.25	4.75	4.25	3.75	2.75	3.50	3.71
5	3.25	3.50	4.25	4.00	4.50	3.50	3.83
6	3.00	1.75	2.50	3.00	2.75	1.75	2.46
7	4.00	3.50	4.75	4.25	3.25	3.75	3.92
8	3.75	4.00	4.25	4.00	4.50	2.75	3.88
9	4.50	4.25	4.50	4.25	4.00	2.25	3.96
10	2.75	4.25	3.75	3.00	4.50	3.00	3.54
11	3.00	3.00	4.00	4.50	2.50	3.75	3.46

*Each figure represents the mean of 4 plants.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	32.4583	3.2458	8.7088	1%
Replications	5	8.4508	1.6902	4.5350	1%
Error	50	18.6326	0.3727		c.v. = 17.87%
Total	65	59.5417	0.9160		

APPENDIX 35

C_2H_4 Production (μ Moles/plant/hr.) of Faba Beans Grown at Location B and Harvested 11 Weeks After Planting

Treatment	Replication 1	2	3	4	5	6	Mean
1	0.34	0.48	3.21	2.88	1.03	0.43	1.40
2	1.16	1.49	3.97	2.19	3.49	2.72	2.50
3	1.87	1.35	4.51	4.09	3.14	0.79	2.63
4	2.72	3.52	8.45	5.99	1.12	1.52	3.89
5	2.25	1.64	3.07	3.58	7.26	2.82	3.44
6	2.79	3.63	1.76	2.97	2.05	0.50	2.28
7	2.50	6.42	2.83	3.43	0.64	0	2.64
8	1.11	2.03	1.50	2.92	3.04	0.46	1.84
9	4.05	0.56	4.36	2.70	2.15	0.34	2.36
10	0.95	1.82	2.64	0.91	1.11	0.37	1.30
11	2.72	0.74	2.08	3.44	0.72	0.27	1.66

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	38.8349	3.8835	1.8835	N.S.
Replication	5	45.7301	9.1460	4.4357	1%
Error	50	103.0929	2.0619		c.v. = 60.91%
Total	65	187.6579	2.8870		

APPENDIX 36

Analysis of Varience for Yield Components of Faba Beans Grown at Location B. The Beans were Harvested 11 Weeks After Planting

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
*1/					
Treatments	10	8.4451	0.8445	0.3372	N.S.
Replications	5	19.9508	3.9902	1.5931	N.S.
Error	50	125.2367	2.5047		c.v. = 17.38%
Total	65	153.6326	2.3636		
2/					
Treatments	10	24.0470	2.4047	0.7578	N.S.
Replications	5	50.8760	10.1752	3.2065	5%
Error	50	158.6662	3.1733		c.v. = 23.58%
Total	65	233.5892	3.5937		
3/					
Treatments	10	38.4189	3.8419	0.6443	N.S.
Replications	5	57.1865	11.4373	1.9182	N.S.
Error	50	298.1231	5.9625		c.v. = 15.84%
Total	65	393.7285	6.0574		

*1/ Pod number per plant.

2/ Pod weight per plant.

3/ Leaves and stem weight per plant.

APPENDIX 37

Analysis of Varience for Nitrogen Percentage in Pods or Stem and Leaves of Faba Beans Grown at Location B. The Beans were Harvested 11 Weeks After Planting

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
*1/					
Treatments	10	0.4161	0.0416	1.4394	N.S.
Replications	5	0.5524	0.1105	3.8235	1%
Error	50	1.4435	0.0289		c.v. = 3.94%
Total	65	2.4120	0.0371		
2/					
Treatments	10	0.6074	0.0607	1.4556	N.S.
Replications	5	2.5248	0.5050	12.1103	1%
Error	50	2.0874	0.0417		c.v. = 9.16%
Total	65	5.2196	0.0803		

*1/ N percentage in pods.

2/ N percentage in leaves and stems.

APPENDIX 38

Analysis of Variance for Final Yield Components of Faba Beans Grown at Location A

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
*1/					
Treatments	10	75.4367	7.5437	6.9240	1%
Replications	5	15.5541	3.1108	2.8553	5%
Error	50	54.4742	1.0895		c.v. = 15.70%
Total	65	145.4650	2.2379		
2/					
Treatments	10	650.5576	65.0558	4.9349	1%
Replications	5	248.9576	49.7915	3.7770	1%
Error	50	659.1424	13.1828		c.v. = 18.77%
Total	65	1558.6576	23.9793		
3/					
Treatments	10	0.7397	0.0749	1.0777	N.S.
Replications	5	1.1997	0.2399	3.4518	1%
Error	50	3.4733	0.0695		c.v. = 9.10%
Total	65	5.4126	0.0833		
4/					
Treatments	10	9945.3856	994.5386	9.3969	1%
Replications	5	4621.8145	924.3629	8.7339	1%
Error	50	5291.8328	105.8367		c.v. = 2.58%
Total	65	19859.0329	305.5236		

*1/ Pod number per plant.

2/ Seed number per plant.

3/ Number of seeds per pod.

4/ 1,000 seed weight.

APPENDIX 39

Seed Yield of Faba Beans (Kg/plot)* Grown at Location A

Treatment	Replication 1	2	3	4	5	6	Mean
1	0.790	0.950	0.627	0.964	0.694	0.782	0.8012
2	1.568	1.459	1.154	1.407	1.578	1.441	1.4345
3	1.382	1.649	1.172	1.269	1.506	1.431	1.4015
4	1.506	1.733	1.225	1.685	1.762	1.328	1.5398
5	1.311	1.205	1.327	1.806	1.781	1.758	1.5313
6	1.277	1.107	0.774	0.799	1.049	1.000	1.0010
7	1.125	1.514	1.170	1.491	1.600	1.603	1.4172
8	1.109	1.731	1.025	1.555	1.937	1.474	1.4718
9	1.362	1.696	1.322	1.476	1.760	1.757	1.5620
10	1.399	1.603	1.206	1.356	1.595	1.255	1.4023
11	1.215	1.491	1.549	1.524	1.497	1.515	1.4652

*Plot size = 10.7 x 0.6 m^2.

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	3.4753	0.3475	12.4552	1%
Replications	5	1.0477	0.2095	7.5090	1%
Error	50	1.3946	0.0279		c.v. = 12.22%
Total	65	5.9176	0.0910		

APPENDIX 40

Seed Nitrogen Percentage of Faba Beans Grown at Location A

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	3.90	4.48	3.78	3.87	4.10	3.52	3.94
2	4.61	4.83	4.70	4.93	4.61	4.74	4.74
3	4.96	4.96	5.09	5.06	5.06	4.93	5.01
4	5.22	5.28	5.28	5.34	5.18	5.41	5.29
5	5.22	5.25	5.15	5.22	5.12	5.31	5.21
6	4.80	4.64	4.45	4.51	4.58	4.51	4.58
7	4.64	4.58	4.48	4.86	4.74	4.54	4.64
8	4.83	4.86	5.09	5.06	4.70	4.96	4.92
9	4.83	4.96	4.86	4.90	4.74	4.67	4.83
10	4.99	4.96	5.06	5.06	4.74	4.93	4.96
11	4.83	5.06	4.83	4.96	4.74	4.96	4.90

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	7.8436	0.7844	40.4330	1%
Replications	5	0.1964	0.0393	2.0258	N.S.
Error	50	0.9700	0.0194		c.v. = 2.89%
Total	65	9.0100	0.1386		

APPENDIX 41

Analysis of Variance for Final Yield Components of Faba Beans Grown at Location B

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
*1/					
Treatments	10	13.1370	1.3137	1.3949	N.S.
Replications	5	18.0848	3.6170	3.8405	1%
Error	50	47.0885	0.9418		c.v. = 11.72%
Total	65	78.3103	1.2048		
2/					
Treatments	10	82.3609	8.2361	0.9998	N.S.
Replications	5	166.7153	33.3431	4.0474	1%
Error	50	411.9064	8.2381		c.v. = 12.24%
Total	65	660.9826	10.1690		
3/					
Treatments	10	0.1001	0.0100	0.5464	N.S.
Replications	5	0.2883	0.0577	3.1530	5%
Error	50	0.9147	0.0183		c.v. = 4.77%
Total	65	1.3030	0.0200		
4/					
Treatments	10	829.4704	82.9470	0.8142	N.S.
Replications	5	2506.5747	501.3149	4.9207	1%
Error	50	5093.9004	101.8780		c.v. = 2.73%
Total	65	8429.9454	129.6915		

*1/ Pod number per plant.

2/ Seed number per plant.

3/ Number of seeds per pod.

4/ 1,000 seed weight.

APPENDIX 42

Seed Yield of Faba Beans (Kg/plot)* Grown at Location B

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	0.890	0.875	1.109	0.995	1.041	1.093	1.0005
2	0.920	1.033	1.105	0.899	0.893	1.152	1.0003
3	1.186	0.873	1.260	1.114	1.126	0.999	1.0930
4	1.135	0.875	1.249	1.015	1.019	1.139	1.0720
5	0.995	1.036	1.240	1.105	0.895	0.961	1.0387
6	1.033	1.067	1.212	0.965	1.155	0.989	1.0702
7	0.930	0.880	1.305	0.957	1.077	1.069	1.0363
8	1.006	1.071	1.081	1.061	0.889	1.138	1.0410
9	0.970	0.852	1.143	0.927	1.073	1.083	1.0080
10	1.089	1.095	1.173	0.887	1.041	1.082	1.0612
11	1.069	0.980	1.241	1.085	1.066	1.069	1.0850

Plot size = 6.1 x 0.6 m^2

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	0.0660	0.0066	0.9041	N.S.
Replications	5	0.3459	0.0692	9.4795	1%
Error	50	0.3630	0.0073		c.v. = 8.16%
Total	65	0.7749	0.0119		

APPENDIX 43

Seed Nitrogen Percentage of Faba Beans Grown at Location B

Treatment	Replication						Mean
	1	2	3	4	5	6	
1	4.90	5.15	4.93	5.28	4.86	5.09	5.04
2	4.83	5.25	4.93	5.34	4.93	4.90	5.03
3	4.83	5.41	4.86	4.96	4.99	5.31	5.06
4	5.09	5.31	5.25	5.25	4.99	5.25	5.19
5	5.12	5.34	4.86	5.18	5.09	5.22	5.14
6	4.90	5.02	4.80	5.34	4.99	5.22	5.05
7	4.80	5.31	4.93	5.44	4.93	5.41	5.14
8	5.06	5.18	4.80	5.18	4.74	5.15	5.02
9	5.06	5.41	5.18	5.28	4.90	4.96	5.13
10	4.99	5.38	5.06	5.41	4.90	5.09	5.14
11	4.90	5.31	4.86	5.12	4.93	5.09	5.04

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
Treatments	10	0.2152	0.0215	1.3782	N.S.
Replications	5	1.4238	0.2848	18.2564	1%
Error	50	0.7806	0.0156		c.v. = 2.46%
Total	65	2.4196	0.0372		

APPENDIX 44

Analysis of Variances of Different Characteristics of Faba Beans Grown in Pouches with Controlled Root Temperature at 14°C. The Beans were Harvested 6 Weeks After Root Temperature Treatment

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
*1/					
Treatments	9	82.0500	9.1167	23.2036	1%
Replications	7	10.7500	1.5357	3.9086	1%
Error	63	24.7500	0.3929		c.v. = 24.34%
Total	79	117.5500			
2/					
Treatments	9	40.4099	4.4900	3.3366	1%
Replications	7	13.5110	1.9301	1.4343	N.S.
Error	63	84.7807	1.3457		c.v. = 71.23%
Total	79	138.7016	1.7557		
3/					
Treatments	9	8.7817	0.9757	7.7314	1%
Replications	7	2.4393	0.3485	2.7615	5%
Error	63	7.9482	0.1262		c.v. = 23.17%
Total	79	19.1692	0.2426		
4/					
Treatments	9	9.0189	1.0021	5.8705	1%
Replications	7	4.9427	0.7061	4.1365	1%
Error	63	10.7563	0.1707		c.v. = 12.50%
Total	79	24.7179	0.3129		

*1/ Nodulation score.

2/ Ethylene production.

3/ Dry weight.

4/ Nitrogen percentage.

APPENDIX 45

Analysis of Variance of Different Characteristics of Faba Beans Grown at Location A in 1977. The Beans were 5 Weeks Old at Harvest

Source of variation	D.F.	S.S	M.S.	F. ratio	Level of significance
*1/					
Treatments	10	3352.2727	335.2273	1.7052	N.S.
Replications	5	274.6212	54.9242	0.2794	1%
Error	50	9829.5455	196.5909		c.v. = 15.49%
Total	65	13456.4394	207.0221		
2/					
Treatments	10	8.0587	0.8059	1.4304	N.S.
Replications	5	1.6439	0.3288	0.5836	N.S.
Error	50	28.1686	0.5634		c.v. = 37.39%
Total	65	37.8742	0.5826		
3/					
Treatments	10	123.2313	12.3231	1.4170	N.S.
Replications	5	161.8764	32.3753	3.7227	1%
Error	50	434.8330	8.6967		c.v. = 73.93%
Total	65	719.9407	11.0760		
4/					
Treatments	10	5.2109	0.5211	1.7238	N.S.
Replications	5	12.9273	2.5855	8.5528	1%
Error	50	15.1140	0.3023		c.v. = 23.30%
Total	65	33.2521	0.5116		
5/					
Treatments	10	0.9144	0.0914	1.3893	N.S.
Replications	5	4.1746	0.8349	12.6884	1%
Error	50	3.2909	0.0658		c.v. = 6.53%
Total	65	8.3798	0.1283		

*1/ Percentage nodulated plant.

2/ Nodulation score.

3/ C_2H_4 production.

4/ Dry weight per plant.

5/ Percentage nitrogen.

APPENDIX 46

Analysis of Variance of Different Characteristics of Faba Beans Grown at Location A in 1977. The Beans were 9 Weeks Old at Harvest

Source of variation	D.F.	S.S.	M.S.	F. ratio	Level of significance
*1/					
Treatments	10	2575.7576	257.5758	1.9101	N.S.
Replications	5	2320.0758	464.0152	3.4410	1%
Error	50	6742.4242	134.8485		c.v. = 12.41%
Total	65	11638.2576	179.0501		
2/					
Treatments	10	20.6061	2.0606	3.2394	1%
Replications	5	1.5303	0.3061	0.4812	N.S.
Error	50	31.8030	0.6361		c.v. = 29.33%
Total	65	53.9394	0.8298		
3/					
Treatments	10	446.9142	44.6914	1.6454	N.S.
Replications	5	90.7014	18.1403	0.6679	N.S.
Error	50	1358.0712	27.1614		c.v. = 114.12%
Total	65	1895.6868	29.1644		
4/					
Treatments	10	81.9602	8.1960	0.9852	N.S.
Replications	5	282.7576	56.5515	6.7975	N.S.
Error	50	415.9716	8.3194		v.c. = 14.21%
Total	65	780.6894	12.0106		
5/					
Treatments	10	0.4543	0.0454	1.8838	N.S.
Replications	5	1.0723	0.2145	8.9004	1%
Error	50	1.2068	0.0241		c.v. = 4.76%
Total	65	2.7334	0.0421		

*1/ Percentage nodulated plant.

2/ Nodulation score.

3/ C_2H_4 production.

4/ Dry weight per plant.

5/ Percentage nitrogen.

APPENDIX 47

Daily Rainfall (mm) From May to August 1976 at Carman Weed Research Station (location A)

Date	MONTH			
	May	June	July	August
1	-	1.8	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	3.6	**	-
7	-	3.3	-	-
8	-	0.8	-	7.9
9	-	4.6	5.1	1.3
10	-	0.3	-	-
11	-	-	-	-
12	-	22.1	-	4.3
13	3.6	2.3	-	-
14	-	1.5	-	-
15	5.3	-	-	-
16	-	-	-	-
17	-	27.7	-	****
18	-	-	-	-
19	-	-	0.3	-
20	-	-	18.8	6.9
21	-	1.3	-	-
22	-	-	-	-
23	-	-	-	-
24	-	-	-	-
25	-	5.8	5.8	-
26	-	1.0	-	-
27	1.0	-	***	4.3
28	*	-	0.5	-
29	-	11.4	-	-
30	-	-	-	-
31	-	-	-	-
Total	9.9	87.5	30.5	24.7

*Planting date.

**First sampling date.

***Second sampling date.

****Third sampling date.

APPENDIX 48

Daily Rainfall (mm) From May to August 1976 at the University of Manitoba (location B)

Date	MONTH			
	May	June	July	August
1	-	-	-	-
2	-	4.3	-	-
3	-	-	-	-
4	Trace	0.5	-	Trace
5	-	0.8	**	-
6	-	27.2	-	-
7	-	1.0	-	-
8	-	3.6	0.8	Trace
9	Trace	22.1	Trace	0.5
10	-	-	Trace	1.0
11	-	3.6	-	7.4
12	Trace	24.6	2.0	Trace
13	2.3	10.7	Trace	Trace
14	5.3	1.5	-	-
15	1.8	Trace	1.8	-
16	-	24.9	-	-
17	-	2.3	-	-
18	-	-	Trace	0.5****
19	-	-	3.6	0.5
20	-	-	-	14.0
21	-	1.5	-	-
22	-	Trace	Trace	-
23	-	-	-	-
24	-	14.0	-	-
25	-	28.7	1.3	Trace
26	1.0	3.8	***	24.9
27	*	-	1.3	10.2
28	-	7.4	-	-
29	-	-	24.6	-
30	-	-	1.5	-
31	Trace	-	-	-
Total	10.4	182.5	36.9	59.0

*Planting date.

**First sampling date.

***Second sampling date.

****Third sampling date.