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EFFECTS OF MYCORRHIZAL FUNGI ON GROWTH, NODULATION, AND NITROGEN FIXATION OF ALFALFA (MEDICAGO SATIVA L.) SELECTED FOR HIGH AND LOW NITROGENASE ACTIVITY

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EFFECTS OF MYCORRHIZAL FUNGI ON GROWTH, NODULATION, AND NITROGEN FIXATION OF ALFALFA (*MEDICAGO SATIVA* L.) SELECTED FOR HIGH AND LOW NITROGENASE ACTIVITY

by

Ali Sidahmed Mohmed Hassan

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Ali Sidahmed Mohmed Hassan

entitled Effects of mycorrhizal fungi on growth, nodulation and nitrogen fixation of alfalfa (Medicago sativa L.) selected for high and low nitrogenase activity.

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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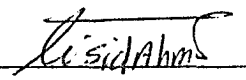
SIGNED: 

This piece of work is dedicated to

my Mother and Father and to

my Wife, Samia and my

Son, Mohammed

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ABSTRACT

Twelve F_1 families of alfalfa (Medicago sativa L.) plants having different potential for nitrogenase activity, and the two parental populations were tested for response to mycorrhizal inoculation in a low-phosphate soil mixture in the greenhouse. The purpose of this study was to: a) determine the effects of vesicular-arbuscular mycorrhizae on growth, nutrition and nodulation of these 14 populations, b) determine if differences existed between the populations with regard to several morphological characteristics, and c) determine if certain characteristics can be transmitted across generations.

The 14 populations were evaluated under four treatments: control no Mycorrhizae, no Rhizobium; Rhizobium alone; Mycorrhizae alone; and the combination of Mycorrhizae and Rhizobium. The growth parameters measured differed significantly among the treatments and among the 14 populations studied, and no significant interaction between the populations and the treatments were found. The dual Mycorrhizae and Rhizobium treatment significantly increased plant height at 30 days and 60 days after planting, leaf area per plant, and plant top-dry-weight at two harvest dates. Mycorrhizal inoculation, however, decreased specific-leaf-weight significantly.

Nitrogen fixation parameters such as nodule mass score, fibrous root score, and nitrogenase activity were increased significantly by the dual inoculation of mycorrhizae and rhizobium. The 14 populations

differed significantly in nodule mass score, fibrous root score, and nitrogenase activity. Mycorrhizal inoculation increased nitrogen fixation more than plant growth.

Correlation coefficients indicated that increased Nitrogenase activity is positively correlated with increased nodule mass, increased fibrous root mass, greater top-dry-weight, and leaf area.

A step wise multiple regression showed that 49% of the variation in nitrogenase activity can be explained by the variation due to nodule mass, fibrous root mass, top-dry-weight, and leaf area.

Several morphological characters showed a heritable response. Plants selected for high nitrogenase activity and high top dry weight transmitted these characteristics to their progenies.

CHAPTER 1

INTRODUCTION

The rising cost of agricultural fertilizers is a major expenditure for farmers, especially in developing countries. With high-energy costs and diminishing reserves of raw materials, there is a demand for development of a more sustainable agricultural system which requires lower fertilizer input.

Legumes are undoubtedly a special case because they can be supplied with the two major nutrients, nitrogen (N) and phosphorus (P), through naturally existing biological systems. Legume culture could be enhanced through the use of two microorganisms; namely nitrogen-fixing bacteria and vesicular-arbuscular (VA) mycorrhizal fungi. Mycorrhizal fungi will help increase the utilization of both current fertilizer applications and residual nutrients from previous fertilizer application (Cox and Robson, 1980). On the other hand, rhizobium bacteria help to provide an adequate nitrogen supply to legumes by fixing atmospheric nitrogen.

Alfalfa (Medicago sativa L.) is a widely cultivated crop in Arizona and soils in which this crop is grown are often low in available P. The scarcity of soluble P in the soil is a limiting factor in legume growth because it not only affects plant growth but also nodulation and nitrogen fixation (Gates and Wilson, 1974). Significant increases in

yield and quality of alfalfa forage can be achieved through the use of adapted symbionts; rhizobium bacteria, vesicular-arbuscular mycorrhizal fungi and adapted alfalfa cultivars.

The effects of VA mycorrhizal fungi in improving nodulation, nitrogen fixation, growth and nutrition of legumes is now well established (Azcon, Azcon and Barea, 1979; O'Bannon, Evans and Peadon, 1980). In soybean (Glycine max L.), French bean (Phaseolus vulgaris L.), alfalfa, and other legumes, mycorrhizae have been found to increase growth and yield, foliar nitrogen concentration, phosphorus concentration, nodule size, nodule number, and nitrogenase activity. All of these effects can be linked to an increased phosphorus supply to mycorrhizal plants (Rhodes, 1980).

The ability of legumes to fix more atmospheric nitrogen can be achieved by genetic modification of either the host plant or the bacterial symbiont. Most earlier investigations were concentrated only on the bacteria. However, the realization that the formation of a symbiotic relationship between root-nodule bacteria and a legume is determined by genetic factors in both the host plant and the bacteria came recently (Aughtry, 1948). Nutman, Mareckova, and Raicheva (1971) described nitrogen fixation as the phenotypic expression of two associated genotypes. Genetic variability for nitrogen fixation in alfalfa has been demonstrated and plant selection has actually resulted in some improvement of nitrogen fixation in alfalfa (Duhigg, Melton and Baltensperger, 1978; Viands, Barnes and Heichel, 1981).

The objectives of this study were:

(1) To evaluate the contribution of an indigenous strain of VA mycorrhizal fungi isolated from Arizona desert soils to growth and nutrition of alfalfa genotypes having different potential for nitrogen fixation.

(2) To evaluate the effects of VA mycorrhizal fungi on nodulation and nitrogen fixing ability in the selected genotypes.

(3) To determine genetic variability for characters associated with nitrogen fixation.

(4) To determine the degree of relationship among different plant characters which may aid in developing an alfalfa breeding program to enhance nitrogen fixation potential.

CHAPTER 2

LITERATURE REVIEW

Effects of Mycorrhizae on Mineral Nutrition of Legumes

Perhaps the most intensely studied interaction involving vesicular arbuscular (VA) mycorrhizal fungi is that between legumes, VA mycorrhizal fungi, and *Rhizobium* spp. This tripartite symbiosis offers the plant the benefit of both improved phosphorus nutrition through VA mycorrhizal activity and improved nitrogen supply by N_2-fixing bacteria.

The scarcity of soluble P in the soil is a limiting factor in legume growth, because it not only affects plant growth but also nodulation and nitrogen fixation (Gates and Wilson, 1974). Munns and Mosse (1980) found that mineral nutrients such as zinc, copper and molybdenum may limit rhizobial growth, nodulation, or symbiotic nitrogen fixation. Phosphorus and some of these minor elements may be supplied to plants by mycorrhizal infection (Rhodes and Gerdeman, 1980). Thus, the dual inoculation with suitable species of rhizobium and mycorrhizae not only enhances the nutrient content in the above-ground plant material but also may provide a nutrient supply that is well balanced.

Ross and Harper (1970) demonstrated that mycorrhizal inoculation of soybean increased the concentration and content of nitrogen in the plant's tops and induced 53% increase in nitrogen content of the seeds

produced by nodulated plants. Similar results were reported by Crush (1974) and Daft and El-Giahmi (1976). They found mycorrhizal and nodulated plants contained more nitrogen and phosphorus in their leaves and stems. Barea, Escudero, and Azcon-Aguilar (1980) found that inoculation of alfalfa with an introduced endophyte increased the total uptake of N and P in two phosphate-fixing soils. In field trials, Azcon-Aguilar and Barea (1981) demonstrated that alfalfa plants inoculated with the VA mycorrhizal Glomus mosseae alone and plants inoculated with both Glomus and Rhizobium possessed higher levels of N, P, and K than uninoculated control plants or those inoculated with Rhizobium alone. They explained the increase in percent N may be due to the mycorrhizal endophyte, which may have acted by enhancing nodulation or nitrogenase activity in legume nodules or by stimulating the number and activity of free-living N_2-fixers in the soil. Mosse, Powell and Hayman (1976) found that inoculation with VA endophytes increased phosphorus uptake by three legumes. They further found that the percentage of phosphorus in Stylosanthes and Centrosema nodules was twice as high as in the parent roots, irrespective of the absolute concentration in the parent roots.

Several greenhouse experiments showed that mycorrhizae markedly increased phosphorus uptake at low and intermediate rates of applied phosphorus fertilizers. At 80 ppm phosphorus, mycorrhizal inoculation increased P, Cu, Zn and Fe, but decreased Mg, Ca and had no effect on Mn and K concentrations in the tops of six alfalfa cultivars and clones (Lambert, Cole and Baker, 1980). This led them to conclude that

screening of alfalfa lines for phosphorus efficiency or for content of P, Cu, and Zn should be conducted under mycorrhizal conditions. The phosphorus and nitrogen content of soybeans, expressed as milligrams/plant, were greatly increased by mycorrhizal infection; but, in spite of this uptake increase, the percent P and percent N did not differ significantly from that of nonmycorrhizal plants (Asimi, Gianinazzi-Pearson and Gianinazzi, 1980). Similar results were reported by Steinberg (1982).

Differences in both infectivity and effectiveness of mycorrhizal fungi at increasing P uptake and plant growth were demonstrated by several researchers. Abbot and Robson (1981) found that five species of endomycorrhizal fungi differ in their ability to stimulate P uptake in subterranean clover (Trifolium subterraneum L.) when inoculated into two untreated field soils which were more phosphorus deficient. The differences in the effectiveness of fungi appear to be related to the rate and time of formation of mycorrhizae as well as to the amount of infection developed by each strain of these fungi.

Effects of Mycorrhizae on Legume Growth

Most researchers have demonstrated that the improved P uptake by mycorrhizal fungi and the improved nitrogen fixation by rhizobium was translated into improved growth of the legume host.

O'Bannon, Evans and Peaden (1980) noticed that VA mycorrhizal plants generally grew more rapidly and appeared healthier than nonmycorrhizal plants on soil of low fertility. Kucey and Paul (1982)

using labelled carbon determined the flow rate of carbon to VA mycorrhizal fungi and rhizobial symbionts of 4 to 5-week-old faba beans (Vicia faba L.). They found measured rates of CO_2 fixation were higher in symbiotic beans than for nonmycorrhizal beans.

VA mycorrhizae may influence the partitioning of photosynthates between shoots and roots and consequently mycorrhizal-nodulated plants have a lower root-to-shoot ratio than plants inoculated with either symbiont alone (Asimi et al., 1980). As pointed out by Smith (1980), this is a typical response to improved mineral nutrition.

Mosse et al. (1976) found an appreciable increase in dry matter of clover, Stylosanthes and Centrosema, only when the P concentration of the uninoculated plants was lower than 0.15%. Azcon et al. (1979) found that Glomus mosseae inoculation alone improved the yield of alfalfa; whereas rhizobium inoculation alone did not. The highest increase in dry matter production was found under the combined rhizobium and mycorrhizae treatment. Azcon and Barea (1981) subjected inoculated and noninoculated alfalfa to two harvests under field conditions. In the first harvest, all inoculation treatments significantly increased plant growth. The best treatment was Rhizobium plus Glomus. There were no significant differences between the combined Rhizobium and Glomus and the Rhizobium alone. However, in the second harvest the effects of the endophyte inoculation persisted and the dual inoculum was significantly more effective than any other treatment. This led them to conclude that Glomus mosseae and Rhizobium meliloti were efficient biological fertilizers that help legumes obtain their nutrients when grown in

phosphate-fixing soils. Islam and Ayanaba (1981) studied the effects of inoculation with Glomus mosseae on the performance of two cowpea (Vigna unguiculata L.) cultivars under field conditions. They also found that inoculation increased shoot dry matter of both cultivars. Similar results were reported by Daft and El-Giahmi (1976) who found that Glomus mosseae increased the yield of fruits and plant size of peanuts (Arachis hypogaea L.). Asimi et al. (1980) found differences in growth rate between mycorrhizal and nonmycorrhizal soybeans at about 58 days after planting and these differences increased until harvest. At harvest, fruits, shoots, roots and nodule yields of soybeans were significantly greater in mycorrhizal than nonmycorrhizal controls. Because shoot growth increased more than root growth, the root-to-shoot ratio decreased with mycorrhizal infection. However, when 80 ppm P were added, the growth of nonmycorrhizal plants was greater than mycorrhizal plants.

O'Bannon et al. (1980) studied four alfalfa cultivars differing in area of adaptation, dormancy, hardiness, and rapidity of recovery after harvest and found them to respond favorably to six out of seven mycorrhizal isolates. Plant vigor and shoot dry weights were increased by inoculation with mycorrhizae except when inoculated with G. monosporus. Two nondormant experimental alfalfa strains responded differently when inoculated with three mycorrhizal isolates, two of which were from Arizona desert soils. The Hayden PX-1 strain had significantly greater dry-stem-weights while the High Nodulating Lew strains had significantly less dry-stem-weights when inoculated with three mycorrhizal isolates (Steinberg, 1982). Lambert et al. (1980)

found six clones and cultivars of alfalfa different significantly in their response to mycorrhizal inoculation.

In some experiments, especially those in growth cabinets, mycorrhizal plants briefly appear smaller than the nonmycorrhizal controls during the first to second week after inoculation (Hayman, 1983). This early growth depression in plants, which later responded very favorably to mycorrhizae, has also been reported by Smith, Smith, and Nicholas (1981) who noticed a temporary growth depression in plants grown in autoclaved soil during the early stages of mycorrhizal infection, preceding the positive response.

Occasionally the host-endophyte balance may shift from mutual symbiosis to parasitism. This usually happened when plants were grown under poor light and temperature conditions (Hayman, 1974), or in soils containing more than adequate plant-available phosphates. The imbalance in symbiosis of a mycorrhizal plant growing in a low-P soil under poor light and temperature, is believed due to the reduced flow of phosphate rather than a carbon deficiency (Hayman, 1983). If the available soil phosphate is more than adequate for plants, then the fungus may act as a parasite. Unfavorable effects of some endophytes on certain pasture legumes at high levels of added superphosphates were reported by Crush (1976). At very high levels of added phosphorus fertilizers, the fungus may also be detrimental because its addition to the plant's intake of phosphate could lead to phosphate toxicity (Mosse, 1973). In contrast, O'Bannon et al. (1980) found that Glomus monosporus

significantly decreased plant vigor and shoot dry weight of four alfalfa cultivars grown in a low phosphate soil.

The Carbon Cost of Dual Symbiosis

a) Nitrogen Fixation Carbon Cost

The carbon cost of nitrogen fixation by Rhizobium spp. has been estimated at about 6.5 C/g fixed nitrogen (Phillips, 1980). The essential process is the transfer of a pair of electrons to a nitrogenase substrate, either dinitrogen or acetylene. In addition, there is the production and maintenance cost of the nodule and its contents (Tinker, 1984). From changes in CO_2 production associated with the reduction, the actual cost of N reduction can be measured. Witty, Minchin and Sheehy (1983) estimated the cost of N reduction to be in the range of 2 to 5 moles CO_2 per mole C_2H_2 reduced. For N, this equals 3.5 to 9 g C/g N depending upon the symbiotic host and Rhizobium strain. This may represent about 5 to 6% of the total photosynthates.

b) Carbon Cost of Mycorrhizal Association

Kucey and Paul (1982) used labelled carbon to determine the flow rate of carbon to VA mycorrhizal fungi and Rhizobium symbionts of 4 to 5-week-old faba beans. They reported that 4% of the total photosynthates were used by mycorrhizal fungi. On the other hand, nodules utilized about 6% of the carbon fixed by nonmycorrhizal hots and 12% of the carbon fixed by mycorrhizal hosts. However, Silsbury, Smith and Oliver (1983) were unable to detect any differences in the carbon economy of mycorrhizal and nonmycorrhizal subterranean clover. From the results of many researchers, it appeared that the

presence of the two symbionts in a host did not represent a significant drain on the host photosynthates and, as pointed out by Pang and Paul (1980), infected plants appeared to compensate for the drain in photosynthates by an increase in photosynthesis.

Effects of Mycorrhizae on Nitrogen Fixation

The roles of VA mycorrhizae in nodulation and nitrogen fixation of legumes have received much attention from scientists in diverse disciplines. The evidence of mycorrhizal stimulation of nitrogen fixation by the legume-Rhizobium system, as measured by the acetylene reduction technique, is well documented.

The experiment of Mosse et al. (1976) confirms and elaborates on the findings of many researchers that VA mycorrhizae can have important effects on nodulation and nitrogen fixation of legumes in phosphorus deficient soils. They found that only mycorrhizal plants of three legumes were able to nodulate in severely phosphorus deficient soils. Such synergistic interactions in which vesicular arbuscular phosphorus mycorrhizal fungi stimulated nodulation and nitrogen fixation, were reported by many authors. Daft and El-Giahmi (1974, 1976) found that several parameters directly associated with the nitrogen fixation process in species of Phaseolus, Arachis, and Medicago were enhanced by mycorrhizal inoculation. The rates of acetylene reduction, the amount of nodular tissue formed, nodule weight, and the concentration of leg-heamoglobin were greater in mycorrhizal and nodulated plants than in the controls which were nonmycorrhizal but nodulated. Similar results were reported by Islam and Ayanaba (1981) who noticed an increase in

nodule yield of two cowpea cultivars when inoculated with the endophyte Glomus mosseae. Asimi et al. (1980), using time-course studies, showed that nitrogenase activity of mycorrhizal soybean plants was already significantly higher than that of non-mycorrhizal plants 48 days after planting and it remained high up to harvest reaching a maximum after about 80 days. The overall nitrogenase activity was further stimulated by mycorrhizae when 0.25 and 0.5 g KH_2 PO_4/kg soil were added. However, with the addition of 1.0 g KH_2 PO_4, the pattern, and the amount of total nitrogenase activity, did not differ significantly between mycorrhizal and nonmycorrhizal soybeans. Kucey and Paul (1982) using the ^{15}N tracer technique found that mycorrhizal and nodulated faba beans fixed more nitrogen than those nodulated but not mycorrhizal. They attributed the increase in nitrogen fixation to an increase in nodule biomass rather than an increase in nodule weight.

Whether mycorrhizae enhance symbiotic nitrogen fixation only through the stimulation of host-plant mineral nutrition or whether they also have a more direct effect on nodulation and nitrogenase activity is a subject of controversy. A close relationship exists between host nutritional status and nodule formation. Mosse et al. (1976) found that nodulation and nitrogenase activity of Stylosanthes and Centrosema were negligible when plant phosphorus concentration was much below 0.2% and virtually no nodules formed on plants containing about 0.1% phosphorus. The findings of Abbot and Robson (1977) and the elaboration of Robson, O'Hara, and Abbot (1981) further support the idea that the effects of VA mycorrhizae on nodulation and nitrogen fixation in subterranean clover (Trifolium subterraneum L.) closely

parallels responses in growth and nutrition of the host plant. These authors suggested that mycorrhizal effects on nodulation and nitrogen fixation came as a consequence of the stimulation of host-plant nutrition and these positive effects occurred at the same time as the growth responses.

In contrast, Smith and Daft (1977) reported that mycorrhizae induced increases in nitrogen fixation rates in alfalfa before any effect occurred on plant growth. Their findings suggested that nodules had first call on the phosphates. Asimi et al. (1980) also noticed a stimulation of nitrogenase activity by VA mycorrhizae before plant growth responses were evident. Waidyanatha, Yogaratnam, and Ariyartre (1979) found that nitrogenase activity of Pueraria sp. still increased when the growth-phosphate response curve became asymptotic. This suggested that nodule function may be preferentially stimulated by mycorrhizal infection, which makes phosphate directly available to the nodules. This is further confirmed and elaborated by the time course studies of Smith et al. (1981). They found that mycorrhizal effects on nodulation and nitrogenase activity, and nodule efficiency occurred before any positive growth response to VA mycorrhizae in a low nutrient soil. Mosse et al. (1976) found the percentage of phosphorus in Stylosanthes and Centrosema nodules was twice as high as in the parent roots irrespective of the absolute concentration of P in the parent roots. In contrast, Smith et al. (1981) did not find increases in phosphorus concentration of alfalfa nodules but found increases in phosphorus

concentration of roots and concluded that these phosphorus concentrations in roots stimulated the development of effective nodule symbiosis. Furthermore, increased phosphate additions first eliminated mycorrhizal effects on growth and then, progressively, those on nodulation and nitrogenase activity suggesting that plant growth and nodule functioning show differential responses to and demand for phosphorus (Asimi et al., 1980).

Mycorrhizae may have other beneficial effects besides improved phosphorus supply and these beneficial effects interact positively to increase nodulation and nitrogenase activity. Safin, Boyer and Gerdemann (1972) found that mycorrhizal soybean plants have lower resistance to water transport than nonmycorrhizal plants and the major effect of mycorrhizae appear to be the reduction of root resistance to water flow. The uptake of other ions of low mobility was also enhanced by mycorrhizal infection. The uptake of zinc (LaRue, McClellan and Peacock, 1975), sulphur (Gray and Gerdemann, 1973), Copper, and Cadmium were improved by mycorrhizae. However, mycorrhizae do not appear to increase the uptake of ions which are readily mobile in the soil like nitrate but they can increase nitrogen uptake only when ammonium, which is relatively immobile, is the source of nitrogen (Smith, 1980).

Nonnutritional Interactions of the Tripartite Symbiosis

Many nonnutritional interactions between VA mycorrhizae, Rhizobium spp., and the host plant have been suggested by some researchers.

Extracellular polysaccharides produced by Rhizobium meliloti enhanced VA mycorrhizal formation on alfalfa. These compounds may have improved VA mycorrhizal symbiosis through the establishment of entry points (Azcon, Barea and Oliveras, 1980). They also may help the development of the preinfection phase of the VA mycorrhizae by increasing root exudates (Oliveras, Montoya and Palomares, 1977). Many microorganisms, especially Rhizobium, are able to produce substances which have phytohormonal activity. VA mycorrhizal infection was increased in alfalfa by using cell-free supernatants of Rhizobium meliloti. The magnitude of the effect was similar to that of pure plant hormones (Azcon, Azcon-Aguilar and Barea, 1978). Furthermore, these interactions become complicated as VA mycorrhizae are able to produce plant hormones. Mosse (1972) found that VA mycorrhizae stimulated branching of infected roots and Allen, Moore, and Christensen (1980) demonstrated that VA mycorrhizae increased the cytokinin levels in host plants.

Inheritance of Nitrogen Fixation Trait

The formation of a symbiotic relationship between root nodule bacteria and a legume is determined by genetic factors in both the host plant and the bacteria. Initially, emphasis was given to the variation between strains of bacteria in their ability to nodulate specific legumes. However, Nutman (1948) and Aughtry (1948) showed that factors in the host were equally important in deciding whether or not nodules were formed.

The ability of rhizobia to induce nodulation is called infectiveness while the nitrogen fixing capacity is termed effectiveness (Burton, 1972). The plant host and the bacteria each possess factors which determine the level of effectiveness of nodulation. Erdman and Means (1953) found an interaction between host variety and bacterial strains in the effectiveness of nodulation of three alfalfa varieties. Similar results were reported by Burton and Wilson (1939) for the alfalfa varieties 'Hairy Peruvian', 'Ladak', and 'Grim' and a number of isolates of Rhizobium meliloti. Gibson (1962) reported differences among six alfalfa cultivars in their ability to form an effective symbiosis with an Australian strain of R. meliloti. He concluded that when yield was a character under selection, those plants which formed an effective symbiosis with indigenous populations of root-nodule bacteria had a selection advantage over those plants which lacked genes for successful symbiosis. Gershon (1961) suggested that several genes were involved in determining effectiveness of symbiosis with two Lotus spp. and two bacterial strains. El-Sherbeeny, Lawes and Mytton (1977) found significant genetic variation in both the effectiveness of the symbiosis with one strain of rhizobium and the efficiency of utilization of nitrogen among eight varieties of Vicia faba L. Two plant genes (sym_2 and sym_3) affecting the symbiotic process in fieldpeas (Pisum sativum L.), were described by Holl (1975). The sym_2 gene affected nodulation while sym_3 affected fixation. The two genes segregate independently as dominant Mendelian characters and effective symbiosis requires the presence of at least one dominant gene at each locus.

Ineffectiveness of nodules was studied by many researchers. Ineffectiveness of nodulation in red clover (Trifolium pratense L.) was controlled by two recessive genes (Nutman, 1957). Smith and Knight (1984) found that nonstrain specific ineffective nodulation in crimson clover (Trifolium incarnatum L.) was controlled by a single recessive gene pair with possible modifier genes. Viands, Vance, Heichel, and Barnes (1979) concluded that ineffectiveness in alfalfa was a heritable, host-conditioned trait. Peterson and Barnes (1981) suggested four different genetic systems produced host-determined ineffective nodules in alfalfa. All four types of ineffective nodules were simply inherited as recessives and were expressed in the presence of normally effective rhizobium strains.

Quantitative genetic variation for nitrogen fixation has been studied by some researchers. Tan (1981) found significant interactions of general and specific combining abilities for acetylene reduction rate of alfalfa genotypes with rhizobium strains suggesting differential expression of gene effects for alfalfa genotypes with different strains of rhizobium. However, Pinchbeck, Hardin, Cook and Kennedy (1980) found that quantitative genetic variation in overall fixation was due solely to general combining ability. Hobbs and Mahon (1982), working with peas (Pisum sativum L.), found heterosis in nitrogen fixation per plant. They attributed it to nonallelic interactions. Seetin and Barnes (1977) found that crosses between high acetylene reducing alfalfa clones produced high acetylene reducing progeny and low x low crosses produced low acetylene reducing progeny. High x low

crosses produced progeny with intermediate values. They concluded that the capacity of alfalfa - R. meliloti symbiosis to fix nitrogen was affected by the host plant's genotype. Duhigg et al. (1978) selected within an alfalfa variety for high and low rates of acetylene reduction. A heritable response was noted for acetylene reduction values, dry weight production, and total N content. Hoffman and Melton (1981) found some evidence which suggested intracultivar variability exceeded intercultivar variability for nitrogen fixation in alfalfa. Furthermore, selection for high levels of acetylene reduction and nodule mass in two broad-based alfalfa germplasm pools resulted in increased nitrogen fixation potential compared to the low selections in nil-nitrate studies conducted under greenhouse conditions (Viands et al., 1981).

Many workers have used nodule number, nodule mass and nodule color and size as indicators of nitrogen fixation. Seetin and Barnes (1977) found morphological traits such as number of fibrous roots, nodule numbers and high top and root weights were positively associated with acetylene reduction. Similar results were reported by Duhigg et al. (1978) and Iruthayath and Vlassak (1982). Hobbs and Mahon (1982) found significant positive correlations between nitrogen fixation per plant and total shoot, and between both characters and shoot weight in peas.

CHAPTER 3

MATERIALS AND METHODS

Soil

The soil mixture used in this study consisted of field soil, collected from the University of Arizona Campus Agricultural Center, sand, and vermiculite mixed in a 2:1:1 ratio. In 1983, the soil mixture was steam sterilized at about 100 C for 24 hours in an autoclave. In 1984, due to the large soil volume, the soil mixture was fumigated using methyl bromide gas. The soil mixture was then potted in 17.5 cm deep and 15.3 cm diameter pots. A detailed chemical analysis of the soil mixtures used is shown in Table 1.

Treatments

Four treatments were applied in both 1983 and 1984.

1) Control: No Rhizobium, no mycorrhizae.
2) Rhizobium treatment: Rhizobium inoculum alone.
3) Mycorrhizae treatment: Mycorrhizal inoculum alone.
4) Rhizobium + Mycorrhizae: Both Rhizobium and mycorrhizal inocula.

Rhizobium Inoculum

The Rhizobium inoculum used in both 1983 and 1984 was Alfalfa Special #I furnished by the Nitragin Company of Milwaukee,

Table 1. Chemical analysis of the soil mixture used in the study. 1984.

pH	Ece $X10^3$	Soluble salts PPM	Na meq/L	K meq/L	ESP	N PPM	P PPM
7.9	1.19	833	2.29	0.62	0.32	28.7	8.5

Wisconsin. This peat-base inoculum contained three Arizona Rhizobial strains: Strain #102F58, Strain #102 F59, and Strain #102 F81.

In 1983, about 2.27 grams (0.005 lb) of this inoculum were incorporated into the soil of each pot before planting. The seeds were also inoculated prior to planting and a small amount was applied as a slurry over the soil in the pots after planting.

In 1984, the inoculum was applied as a slurry on top of the soil in the pots at time of planting and also one week after seedling emergence. A total of 9.07 grams (0.02 lb) per pot was used.

A mixture of Rhizobium strains was used rather than a single strain to represent more closely the field situation.

Mycorrhizal Inoculum

Mycorrhizal inoculum used in this study was obtained from sorghum plants grown in pots of soil containing the mycorrhizal fungus Glomus intraradices. The inoculum was kindly provided by Dr. H.E. Bloss of the Department of Plant Pathology, University of Arizona. Glomus intraradices was grown in a sand culture on Sorghum vulgare var. Sudanese (Piper-Hitchc.) (Sudan grass) as hostplant for about 6 months prior to use. The inoculum used consisted of sand and rootlets of Sudangrass chopped finely and thoroughly mixed together. In 1983, about 226.9 grams (0.5 lb) of this inoculum were incorporated into the soil of each pot and thoroughly mixed before planting. In 1984, the pots receiving mycorrhizal treatments were half filled with soil and then about 113.4 gram (0.25 lb)/pot mycorrhizal inoculum was layered and the

remainder of each pot was filled with soil. The soil was tapped firmly, so that the soil surface was about 2.0 cm below the top rim of each pot. To prevent treatment contamination, each pot was placed on a plastic cup to raise it off of the bench surface.

Plant Material

In 1983, plant materials used were seeds of two bidirectional selections for high and low nodule mass score from the alfalfa cultivar 'Lew'. Before planting, seeds were surface sterilized for 10 minutes in 2.5% household bleach, washed three times with distilled water, and allowed to air dry before planting. Each pot was planted with four seeds at 2.0 cm depth. Later, the seedlings were thinned to one plant per pot. Plants were cut to 2.5 cm stubble height at first flowering. At first flowering of the regrowth, the entire plants were harvested. The experiment was initiated in mid June and the entire plants were harvested by the end of October. At final harvest in October, the two bidirectional selections were scored for nodule mass, fibrous root development, and nitrogenase activity. Two genotypes that had high nitrogenase activity and high top dry weight and two genotypes that had low nitrogenase activity and low top dry weight were selected.

Stem cuttings were made from these selected genotypes in January 1984. Cuttings were made approximately 5.0 cm long and had at least one leaf. A rooting compound containing IAA, IBA and a mild fungicide was used to promote rooting. All cuttings were rooted in vermiculite in flats 52.5 x 37.5 x 10.0 cm deep. When the

cuttings had rooted, they were transferred into 17.5 and 15.3 cm diameter pots containing the same soil mixture as previously described. Propagules of the four selected genotypes were then allowed to grow to maturity and bloom in the greenhouse. Plants were given a photoperiod treatment to induce flowering. The dark period was interrupted with one hour of light at midnight. Plants were crossed using the following scheme based on nitrogenase activity and top dry weight.

High X High

Low X Low

High X Low

Low X High

Reciprocal crosses were kept separate to detect any maternal influences. To facilitate crossing, the stems of growing plants were tied to stakes throughout the seed-setting period. Crossing was done when most of the flowers on a raceme were in bloom. The cross was then labelled with a plant tie band (around the peduncle of the raceme) containing the date and type of cross. All crossing was done by hand transfer of pollen, with emasculation using a machine with a small vacuum pump. To facilitate emasculation the raceme was first emersed gently in water. Then the standard petal was clipped and the flower was gently tripped by applying suction using a glass tubing drawn to a 1 mm tip and inserted in a rubber hose attached to a machine with a small vacuum pump (Kirk, 1930). Thoroughness of pollen removal was

determined with a low-powered binocular magnifier. Pollen was collected and transferred onto emasculated flowers using a boat-shaped hardpaper. About 12 different crosses were made during the spring and early summer of 1984. Seeds were allowed to mature and then harvested when the peduncle of the raceme was dry. The seeds were threshed with a small scrub-board type handthresher.

In August 1984, the seeds of the 12 F_1 families and the two original populations; High-Nodulating Lew and Low-Nodulating Lew were surface sterilized using 2.5% household bleach and washed three times with distilled water and allowed to air dry. Then the seeds were scarified with sandpaper and germinated on moist blotter paper in petri-dishes. When the radicles emerged, the seedlings were transferred into 17.5 cm deep and 15.3 cm diameter plastic pots filled with the same soil mixture as previously described. The experiment was initiated in August 1984 and the entire plants were harvested at the end of December. Plants were irrigated as needed with irrigation water that had 0.32 ppm N. Plants were sprayed as needed with kelthane and Malathion to control spider mites and insect pests.

Greenhouse Conditions

To enhance plant growth and flowering, additional illumination was provided with a bank of 300-watt bulbs in 1984 from August until the end of the experiment in December. Greenhouse temperature ranged from 29 C for the day's high to 18 C for the night's low.

1) Growth Parameters Measured

a) Plant Height

Plant height (cm) was measured from the soil surface to the growing tip. Plant height was measured at five different growth stages. Height (1) was taken when plants were 30 days old, Height (2) was taken when plants were 60 days old, Height (3) was taken when plants were 90 days old and at the first harvest. To measure the rate of recovery of the 14 alfalfa populations, Height (4) was taken 15 days after the first harvest. Height (5) was taken at the end of the experiment. Plants at this time were about 150 days old.

b) Dry Matter Production

Plants were cut to 2.5 cm stubble height at two harvest dates. Harvest I was taken when plants were at first bloom and about 90 days old. Harvest II was taken at the end of the experiment in December when the plants were at the first flower of the regrowth. At both harvests, shoots were dried to constant weight in an oven at 26.7 C and their weights were recorded in grams.

c) Specific Leaf Weight

A sample of nine leaves was taken from the main stem of each plant; three from the bottom, three from the middle, and three from the top of the stem. These nine leaves were run through a LI-3100 Area meter machine (manufactured by Li-Cor, Lincoln, Nebraska) which measured and recorded the leaf area. Then, the nine leaves were dried to constant weight in an oven at 26.7 C and their dry weight was determined. Specific Leaf Weight (SLW) was determined using the formula:

$$\text{Specific Leaf Weight (SLW)} = \frac{\text{Dry wt. of 9 leaves (mg)}}{\text{Leaf area of 9 leaves (cm}^2\text{)}}$$

$$= \text{mg/cm}^2\text{/plant}$$

d) Leaf Area

Leaf area/plant was determined from specific leaf weight. Leaves were separated from stems (petiole included with stems). These leaves were oven dried to constant weight at 26.7 C and then their dry weight was recorded.

Leaf area/plant was determined by the formula:

$$\text{Leaf area/plant (DM}^2\text{)} = \frac{\text{Total leaf dry wt./plant}}{\text{Specific leaf wt./plant x 1000}}$$

$$= \text{DM}^2\text{/plant}$$

2. Estimates of Nodulation and Nitrogen Fixation

a) Acetylene reduction assay

At first flower of the regrowth, top growth was removed, and roots of each plant were carefully removed from their container and carefully shaken to remove the soil. Then they were immediately placed in a wide-mouth 960 ml mason jar equipped with a serum stopper. To determine nitrogenase activity, an acetylene reduction technique, similar to the one described by Hardy, Holsten, Jackson and Burns (1968) was used. The lids on the mason jars containing the roots were closed tightly. Fifty ml of air were removed from the jars with a syringe and 50 ml of acetylene were added to each jar and mixed thoroughly. After an incubation period of 30 minutes, the gas phase was sampled and

about 10 ml of gas were transferred into 10 ml evacuated vactainer tubes (made by Becton-Dickinson Company, Rutherford, New Jersey). These samples were later injected into a gas chromatograph to measure the amount of ethylene produced. Standard samples were prepared the same way but without nodulated roots. A gas chromatograph machine model 5730A (manufactured by Hewlett-Packard Company, Palo Alto, California) equipped with a hydrogen flame ionization detector and a column 80% Poropack N and 20% Poropack Q was used for acetylene-ethylene (C_2H_2 - C_2H_4) assay to determine nitrogenase activity. Ethylene peak areas of samples from gas chromatographic analysis were compared with peak areas of the standards. The following equation was used to convert amounts of acetylene reduced. For the same volume injected for both standards and samples.

$$\text{Response factor (RF)} = \frac{\text{Standard (conc.)}}{\text{Area}} = \text{PPM/area}$$

For sample = sample area X RF = conc. in ppm (ethylene)

$$= \frac{\mu\text{L ethylene}}{\text{L gas}} \times \frac{1\ \mu\text{mole}}{24.05} \times 0.969 \times 2.0$$

= μ moles Ethylene/plant/hr.

b) Estimation of nodule mass and fibrous root mass

After taking gas samples for nitrogenase activity determination, roots were visually scored for both nodule mass and fibrous root mass. Because alfalfa produces many small nodules, the quantitative measure of nodule mass is very difficult. Nodulation was scored using a scale of 1 to 5 with 1 = few nodules and 5 = many nodules.

Fibrous root mass was also determined on a scale from 1 to 5 with 1 = few fibrous (tertiary) roots and 5 = many fibrous roots.

3. Tissue Chemical Analysis

Shoots of alfalfa plants were separated into stems and leaves, dried and ground to pass through a 40-mesh screen. Stem and leaf tissue of all replications were composited and representative samples were taken for chemical analysis for N and P using the autoanalyser Techincon machine.

First, representative samples were weighed; about 0.5 gm for stems and 0.2 gm for leaves. The weighed samples were put in 25 mm X 20 mm Pyrex test tubes along with 1.0 gm K_2SO_4, 0.1 gm Na_2 SeO_3 and 10 ml concentrated sulfuric acid. Samples were then digested at about 400 C on a block digester for 3 hours, or until they turned clear amber color. After cooling, the digestate was quantitatively transferred to a 100 ml volumetric flask and diluted with distilled water to 100 ml then transferred to polyethylene bottles. From these bottles, a 0.4 ml sample was run through the Techincon autoanalyser model sampler (manufactured by Techincon Industrial System, Terrytown, New York). The same digestate was used for both N and P determination.

Statistical Analysis

Data were analyzed as a split-plot design using the analysis of variance. Mean separations were made using the Least Significant Difference test (LSD) ($p \leq 0.05$ level). Also, simple correlations and step-wise regressions were determined.

CHAPTER 4

RESULTS AND DISCUSSION

Mycorrhizal plants grew faster and appeared healthier than non-mycorrhizal plants one month after inoculation (Table 2). Such plants also had more stems per plant than nonmycorrhizal plants.

The presence of mycorrhizae in the mycorrhizal treatments was confirmed by viewing some stained roots under the light microscope. However, no mycorrhizae were found in the roots of the control and Rhizobium plants. It appears there are some strains of rhizobia that can tolerate steam sterilization and soil fumigation by methyl bromide because some nodulation was found on the control and mycorrhizae treated plants. Also the contribution of air-borne rhizobia is a possibility.

Plant Height

The results from adding VA mycorrhizal fungi and Rhizobium bacteria on plant height (cm), at five different growth stages are presented in Table 2. Height (1) was taken 30 days after planting. Plant height differed significantly among the four treatments used in this study. Treatment means ranged from 7.3 cm for the Rhizobium treatment to 11.2 cm for the combined mycorrhizae and Rhizobium treatment. The dual inoculation of mycorrhizae and Rhizobium produced a 47% increase in plant height over the control. Mycorrhizae alone produced

Table 2. Effects of addition of VA mycorrhizal fungi and Rhizobium bacteria on plant height of the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity, 1984.

Treatment	Height 1		Height 2		Height 3		Height 4		Height 5	
	Mean (cm)	% change over control	Mean (cm)	% change over control	Mean (cm)	% change over control	Mean (cm)	% change over control	Mean (cm)	% change over control
Control	7.6a	--	19.5ab	--	41.2a	--	23.9a	--	48.2a	--
Rhizobium	7.3a	-3.9	18.3a	-6.2	38.8a	-5.8	22.9a	-4.2	47.4a	-1.7
Mycorrhizae	9.7ab	27.6	24.7b	26.3	45.3a	10.0	27.8a	16.3	50.0a	3.7
Mycorrhizae + Rhizobium	11.2b	47.4	25.3b	29.6	45.0a	9.2	26.0a	8.8	49.8a	3.3

Means followed by the same letter within the same column are not significantly differnet at 0.05 level according to the least significant difference test.
LSD = 3.0, 5.9, 10.5, 12.1 and 8.8 for H1, H2, H3, H4 and H5, respectively.
Height 1 = taken when plants 30 days old.
Height 2 = taken when plants 60 days old.
Height 3 = taken when plants 90 days old and at first harvest.
Height 4 = taken when plants were 15 days after the first harvest.
Height 5 = taken at the end of the experiment and when plants were 150 days old.

about 28% increase in height over the control. However, in the Rhizobium alone treatment, plant height was less than the control by about 4.0%.

Differences in plant Height (1), as affected by the addition of VA mycorrhizae and Rhizobium, are presented in Fig. 1. Mycorrhizal inoculation increased plant height more than the control and Rhizobium treatments. Generally, plants selected for high nitrogenase activity and high top dry weight produced progenies that are taller under the control, Rhizobium and Mycorrhizae alone treatments. However, under the dual inoculation of Mycorrhizae and Rhizobium treatment, plants selected for low nitrogenase activity and low top dry weight produced progenies similar in plant height to the high X high progenies. The check population, High Nodulating Lew produced shorter plants than any other population studied under all four treatments.

Plant Height (2) was determined 60 days after planting (Table 2). Plant height differed significantly among the treatments. Treatment means ranged from 18.3 cm for the Rhizobium treatment to 25.3 cm for the combined Mycorrhizae and Rhizobium treatment. However, the control treatment was not significantly different from the mycorrhizae alone and the combined inoculation of Mycorrhizae and Rhizobium treatments. The dual inoculation of mycorrhizae and Rhizobium increased plant height by 29.6% over the control while the mycorrhizae alone had about 26.3% increase in plant height over the control. However, plants with the Rhizobium treatment were shorter than the control plants by 6.2%. Plant Height (2) as affected by the addition of VA mycorrhizae and

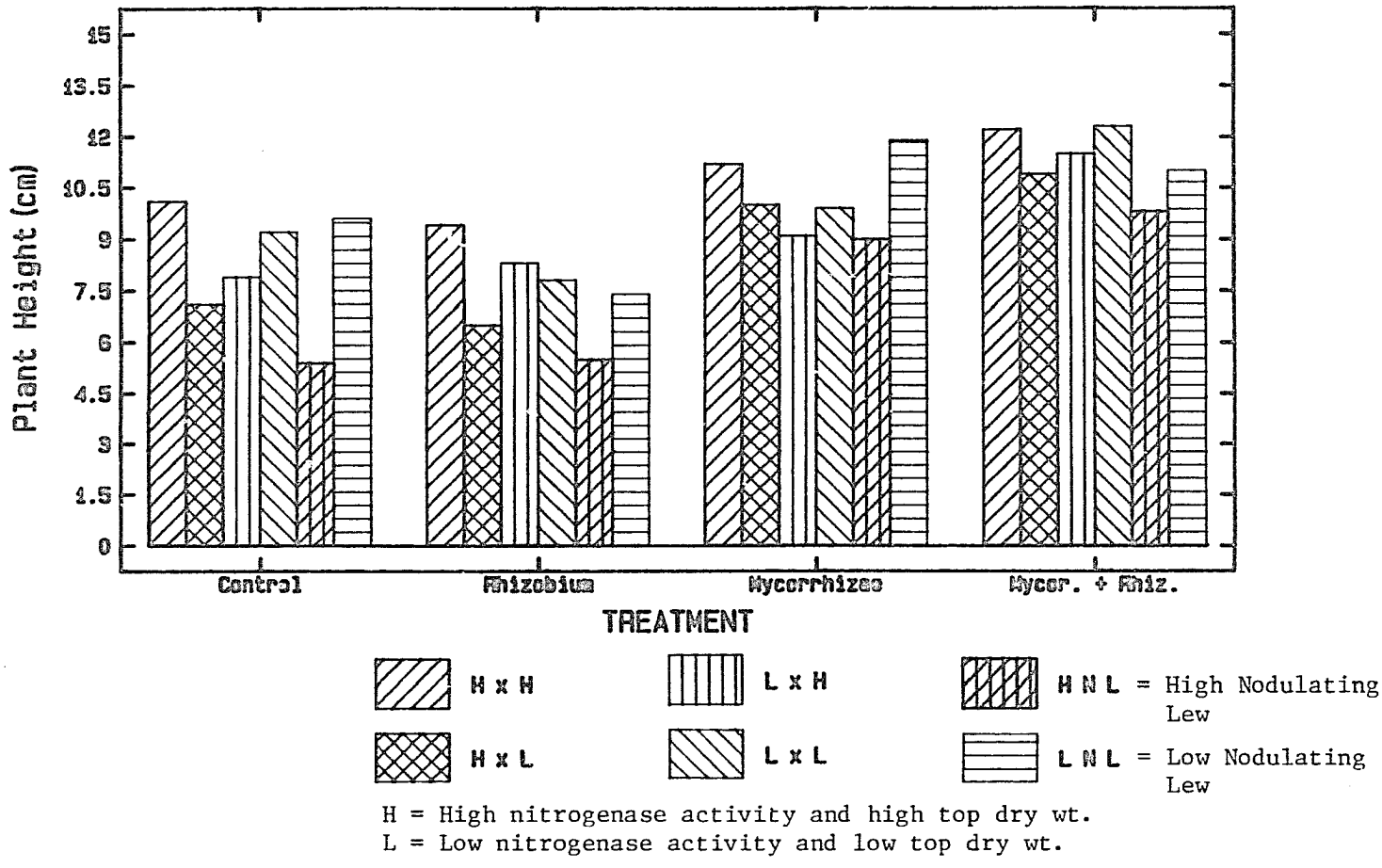


Fig. 1. Effects of addition of VA mycorrhizal fungi and Rhizobium on plant height of 30-day-old alfalfa plants.

Rhizobium is presented in Fig. 2. The same trend as in Height (1) existed. Generally, plants selected for high nitrogenase activity and high top dry weight produced taller progenies under the control, Rhizobium and Mycorrhizae alone treatments. Mycorrhizal inoculation seemed to enhance plant height of the check population, Low-Nodulating Lew more than the other populations 60 days after planting.

Height (3) was obtained 90 days after planting and at the first harvest (Table 2). By this time, there were no significant differences between the treatments in plant height. Treatment means ranged from 38.8 cm for the Rhizobium alone treatment to 45.3 cm for the mycorrhizae alone treatment. However, the combined inoculation of mycorrhizae and Rhizobium increased plant height 9.2% over the control. Mycorrhizae alone treatment increased plant height by 10.0% over the control, however, the Rhizobium treatment was shorter than the control by 5.8%. Plant Height (3) as affected by the addition of VA mycorrhizae and Rhizobium is shown in Fig. 3. Generally, plants selected for high nitrogenase activity and high top dry weight produced taller progenies under the control and Rhizobium treatments and they produced plants with similar heights to High X Low crosses and the check population, Low-Nodulating Lew, under the mycorrhizae alone treatment. However, under the combined mycorrhizae and Rhizobium treatment, High X High crosses and the two check populations, High-Nodulating Lew and Low-Nodulating Lew produced plants with similar heights.

To measure the rate of recovery after Harvest 1, Plant Height (4) was measured 15 days after cutting (Table 2). There were no significant

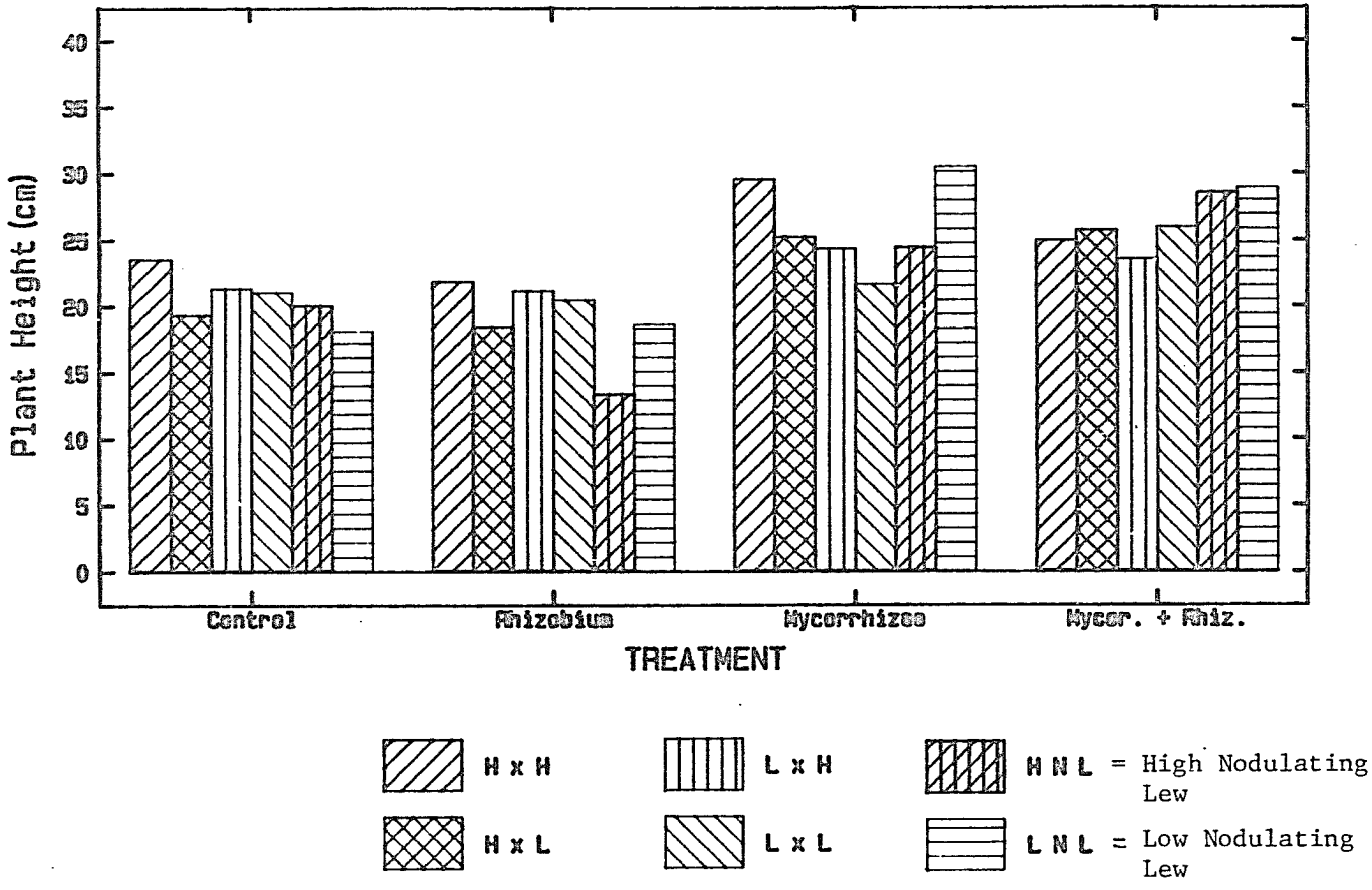
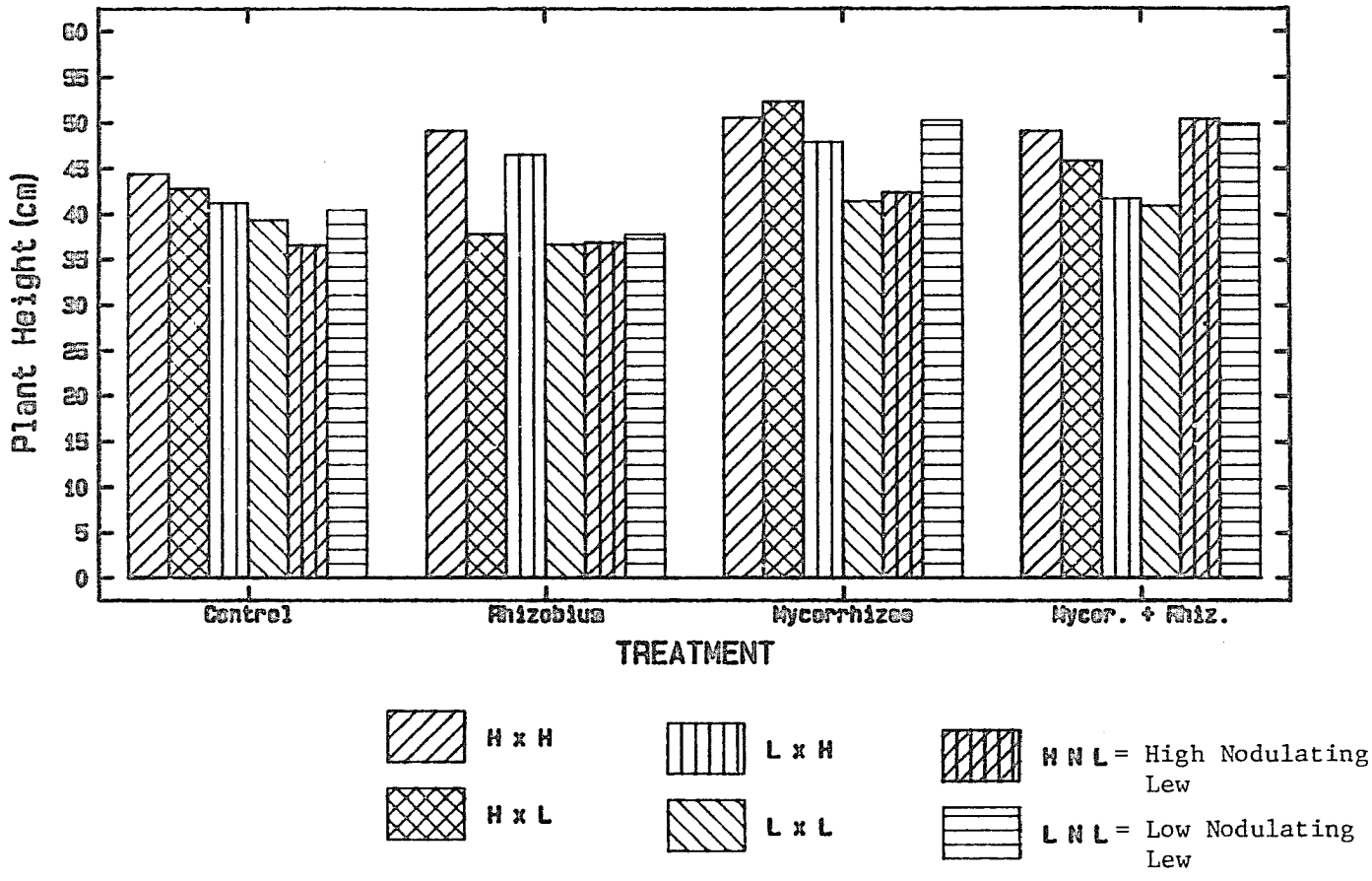


Fig. 2. Effects of addition of VA mycorrhizal fungi and Rhizobium on plant height of 60-day-old alfalfa plants.



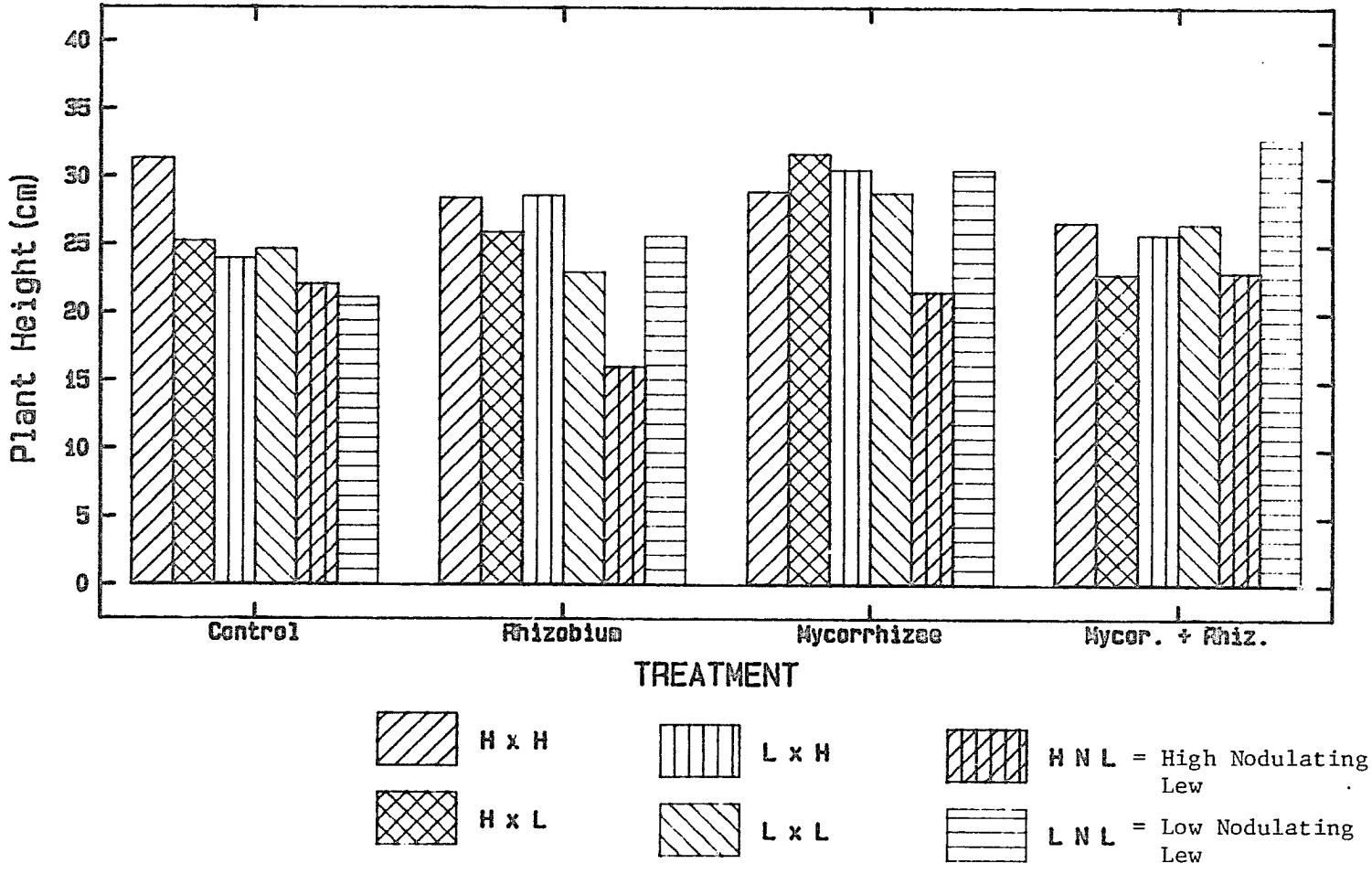
H = High nitrogenase activity and high top dry wt.
L = Low nitrogenase activity and low top dry wt.

Fig. 3. Effects of addition of VA mycorrhizal fungi and Rhizobium on plant height of 90-day-old alfalfa plants.

differences between the treatments at this particular plant-growth stage. Treatment means ranged from 22.9 cm for the Rhizobium treatment to 27.8 cm for the mycorrhizae alone treatment. The dual inoculation of mycorrhizae and Rhizobium increased plant height 8.8% over the control. Mycorrhizae alone increased plant height 16.3% over the control and the Rhizobium treatment was still less than the control plants by about 4.2%.

Plant Height (4) as affected by the addition of VA mycorrhizae and Rhizobium is presented in Fig. 4. The 14 populations studied did not behave similarly under the four different treatments. Plants selected for high nitrogenase activity and high top-dry-weight produced taller progenies under the control treatment but not under the other treatments. The check population, High-Nodulating Lew, had the shortest height under the Rhizobium and mycorrhizae treatments. Under the combined mycorrhizae and Rhizobium treatment, the check population, Low-Nodulating Lew, produced taller plants.

Plant Height (5) was determined at final harvest (Table 2). At this time plants were 150 days old. There were no significant differences between treatments in plant height. Treatment means ranged from 47.4 cm for the Rhizobium treatment to 50.0 cm for the mycorrhizae alone treatment. The dual inoculation of mycorrhizae and Rhizobium increased plant height 3.3% over the control while mycorrhizae alone had 3.7% increase over the control. Plants receiving the Rhizobium treatment were shorter than the control plants by 1.7%. Plant Height (5) as affected by the addition of VA mycorrhizae and Rhizobium is presented in



H = High nitrogenase activity and high top dry wt.
L = Low nitrogenase activity and low top dry wt.

Fig. 4. Effects of addition of VA mycorrhizal fungi and Rhizobium on plant height taken 15 days after the first harvest.

Fig. 5. At this stage of plant development all F_1 crosses produced, on the average, taller plants than the two check populations; High-Nodulating Lew and Low-Nodulating Lew under both the control and Rhizobium treatments but not under mycorrhizal inoculation.

Two general points can be inferred from these results:
(1) Mycorrhizal inoculation enhanced plant height during early plant development (30 days after planting) and this effect seemed to diminish in magnitude as the nonmycorrhizal plants seemed to catch up later. At the third month after planting, either plants had become pot-bound for the two mycorrhizal treatments or else plants under mycorrhizal treatments reached their maximum height earlier than plants in the control and Rhizobium treatments.
(2) The added Rhizobium inoculum did not enhance plant height at early plant development as plants in the Rhizobium alone treatment were consistently shorter than those in the control treatment. The added rhizobia may be competing with other plant functions for photosynthates at early plant development. However, this negative effect seemed to disappear progressively as the season advanced.

These results were in agreement with those reported by Daft and El-Giahmi (1974) who found small increases in plant height in the Rhizobium treatment at final harvest; whereas, in the combined mycorrhizae and Rhizobium treatments, maximum height was reached after 49 days. Asimi et al. (1980) reported differences in plant height of mycorrhizal and nonmycorrhizal soybeans 58 days after planting.

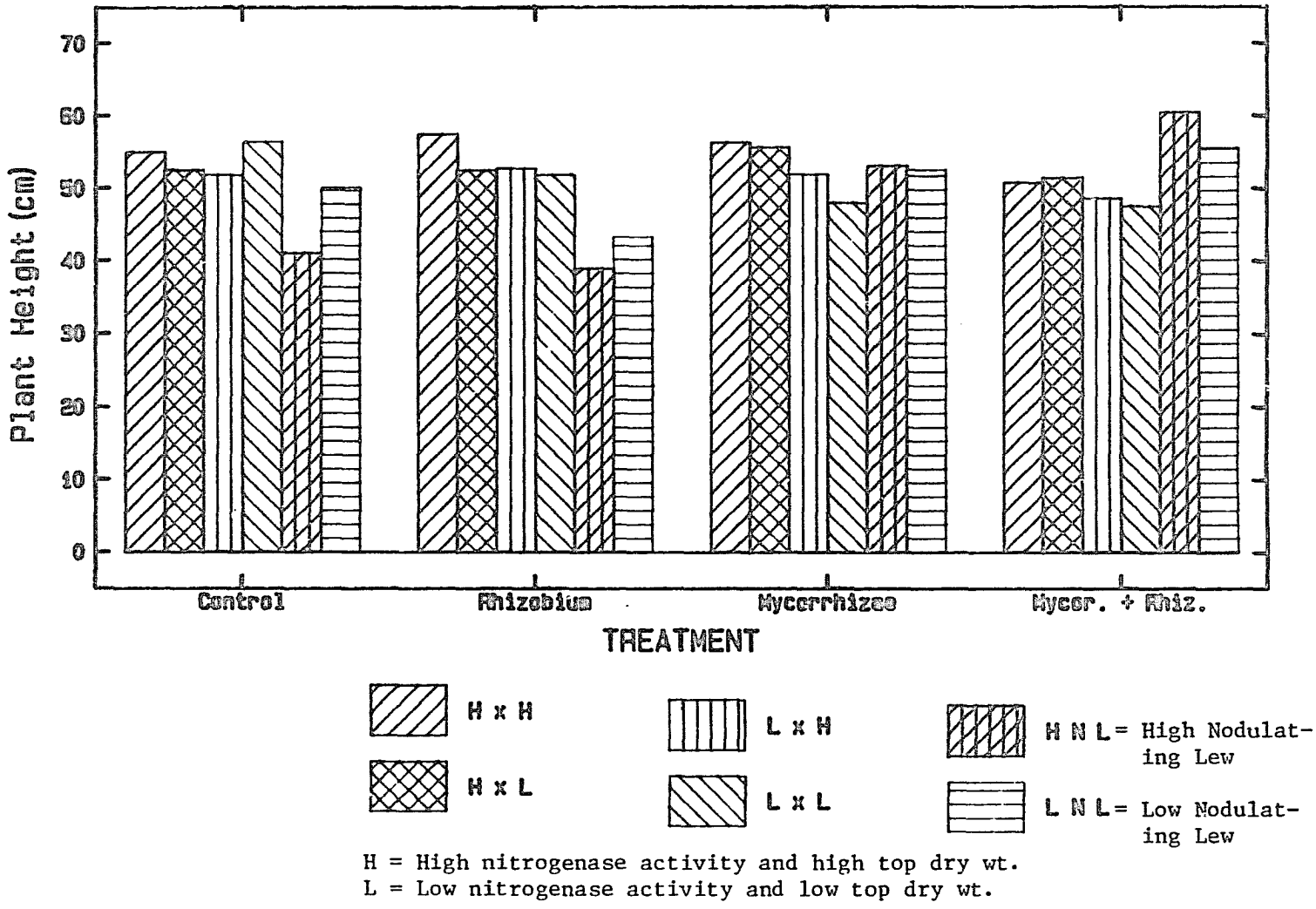


Fig. 5. Effects of addition of VA mycorrhizal fungi and Rhizobium on plant height of 150-day-old alfalfa plants.

The average height of plants determined 30 days after planting differed significantly among the 14 populations studied (Table 3). The progenies of high X low cross #8 had the lowest mean height of 7.1 cm while the progenies of high X high cross #1 were the tallest of all entries and had mean height of 11.5 cm. On the average, plants selected for high nitrogenase activity and high top dry weight produced numerically taller progenies ($\bar{x}$ = 10.7 cm), plants selected for low nitrogenase activity and low top dry weight produced numerically shorter progenies than the high X high progenies ($\bar{x}$ = 9.8 cm) but numerically taller plants than both the high X low ($\bar{x}$ = 8.6 cm) and the low X high ($\bar{x}$ = 9.2 cm) crosses.

The average plant height determined 60 days after planting differed significantly among the 14 populations studied (Table 4). The progenies of high X low cross #8 had the shortest mean height of 19.5 cm, while progenies of the high X high reciprocal cross #2 were the tallest of all entries and had mean height of 25.4 cm. When comparing the F_1 crosses with each other, on the average, high X high crosses produced numerically taller progenies ($\bar{x}$ = 24.9 cm) while the low X low crosses ($\bar{x}$ = 22.2 cm), high X low crosses ($\bar{x}$ = 22.2 cm) and low X high crosses ($\bar{x}$ = 22.5 cm) produced progenies with numerically similar height. At this stage of growth, all F_1 hybrids were not significantly different from the two check populations, High-Nodulating Lew and Low-Nodulating Lew.

The average height of plants determined at first harvest and when plants were 90 days old, differed significantly among the 14

Table 3. Mean comparison for plant height (cm/plant) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 30 days after planting, 1984.

Entry	Entry Mean (cm/plant)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	11.5 d		
2	9.9 abcd		
	$\bar{x}$=10.7	127.4	107.0
Low X Low			
3	10.9 cd		
4	8.7 abcd		
	$\bar{x}$=9.8	116.7	98.0
High X Low			
5	8.8 abcd		
6	8.3 abcd		
7	10.4 cd		
8	7.1 a		
	$\bar{x}$=8.7	103.6	87.0
Low X High			
9	10.2 bcd		
10	11.0 d		
11	8.2 abc		
12	7.5 ab		
	$\bar{x}$=9.2	109.5	92.0
Checks			
High Nod. Lew	8.4 abcd	100.0	
Low Nod. Lew	10.0 bcd		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test LSD = 2.8.

Table 4. Mean comparison for plant height (cm/plant) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 60 days after planting, 1984.

Entry	Entry Mean (cm/plant)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	24.4 bcd		
2	25.4 cd		
	$\bar{x}$=24.9	115.8	103.8
Low X Low			
3	22.8 abcd		
4	21.6 abcd		
	$\bar{x}$=22.2	103.3	92.5
High X Low			
5	21.1 abc		
6	22.3 abcd		
7	25.7 d		
8	19.5 a		
	$\bar{x}$=22.2	103.3	92.5
Low X High			
9	24.7 bcd		
10	23.3 abcd		
11	21.8 abcd		
12	20.4 ab		
	$\bar{x}$=22.6	105.1	94.2
Checks			
High Nod. Lew	21.5 abcd	100.0	
Low Nod. Lew	24.0 abcd		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test LSD = 4.5.

populations studied (Table 5). At this particular stage of plant growth and development, progeny of the low X low cross #3 had the lowest mean height of 38.4 cm while progeny of high X high cross #1 were the tallest of all entries and had a mean height of 49.3 cm. On the average, high X high crosses produced numerically taller progenies ($\bar{x}$ = 48.3 cm) and low X low crosses produced progenies that were numerically shorter ($\bar{x}$ = 39.6 cm). The high X low ($\bar{x}$ = 44.7 cm) and low X high ($\bar{x}$ = 44.3) produced progenies with intermediate heights. Only high X high cross #1 and low X high cross #11 produced significantly taller plants than the check population, High-Nodulating Lew.

To measure the rate of recovery of the 14 populations studied, plant height was taken 15 days after the first harvest. The average height of plants differed significantly among the 14 populations studied (Table 6). At this time, progeny of the high X low cross #7 were the tallest of all entries studied and had a mean height of 31.3 cm while the check population High-Nodulating Lew had the lowest mean height of 20.6 cm.

On the average, plants selected for high nitrogenase activity and high top-dry-weight produced numerically taller progenies ($\bar{x}$ = 28.8 cm) and plants selected for low nitrogenase activity and low top dry weight produced progenies that were numerically shorter in height ($\bar{x}$ = 25.7 cm). The high X low ($\bar{x}$ = 27.8 cm) and the low X high ($\bar{x}$ = 27.2 cm) crosses produced plants with intermediate numerical values.

Mean comparison of plant height (cm) determined at final harvest, when plants were 150 days old, is presented in Table 7. The average

Table 5. Mean comparison for plant height (cm/plant) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 90 days after planting, 1984.

Entry	Entry Mean (cm/plant)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	49.3 d		
2	47.2 bcd		
	$\bar{x}$=48.3	116.1	108.3
Low X Low			
3	38.4 a		
4	40.8 ab		
	$\bar{x}$=39.6	95.2	88.8
High X Low			
5	44.9 abcd		
6	46.5 bcd		
7	47.3 bcd		
8	39.9 ab		
	$\bar{x}$=44.7	107.5	100.2
Low X High			
9	43.3 abcd		
10	42.3 abcd		
11	49.0 cd		
12	42.7 abcd		
	$\bar{x}$=44.3	106.5	99.3
Checks			
High Nod. Lew	41.6 abc	100.0	
Low Nod. Lew	44.6 abcd		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test LSD = 7.4.

Table 6. Mean comparison for plant height (cm/plant) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 15 days after first harvest, 1984.

Entry	Entry Mean (cm/plant)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	31.0 de		
2	26.5 bcde		
	$\bar{x}$=28.8	139.8	104.7
Low X Low			
3	25.5 abc		
4	25.9 abcde		
	$\bar{x}$=25.7	124.8	93.5
High X Low			
5	28.4 bcde		
6	28.0 bcde		
7	31.3 e		
8	23.4 ab		
	$\bar{x}$=27.8	135.0	101.1
Low X High			
9	29.4 cde		
10	23.3 ab		
11	30.5 cde		
12	25.6 abcd		
	$\bar{x}$=27.2	132.0	98.9
Checks			
High Nod. Lew	20.6 a	100.0	
Low Nod. Lew	27.5 bcde		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test LSD = 5.4.

Table 7. Mean comparison of plant height (cm/plant) for the check populations; High Nodulating Lew, Low Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 150 days after planting, 1984.

Entry	Entry Mean (cm/plant)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	53.6 abc		
2	56.1 bc		
	$\bar{x}$=54.9	113.7	108.9
Low X Low			
3	49.8 ab		
4	51.9 ab		
	$\bar{x}$=50.9	105.4	100.9
High X Low			
5	51.8 ab		
6	52.8 abc		
7	59.4 c		
8	48.2 a		
	$\bar{x}$=53.1	109.9	105.4
Low X High			
9	54.3 abc		
10	49.4 ab		
11	52.9 abc		
12	48.5 a		
	$\bar{x}$=51.3	106.2	101.8
Checks			
High Nod. Lew	48.3 a	100.0	
Low Nod. Lew	50.4 ab		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test LSD = 7.0.

height of plants differed significantly among the 14 populations studied. Progenies of the high X low cross #8 had the lowest mean height of 48.2 cm while progenies of the high X low cross #7 were the tallest of all entries studied and had a mean height of 59.4 cm. On the average, plants selected for high nitrogenase activity and high top dry weight produced taller progenies ($\bar{x}$ = 54.9 cm). Plants selected for low nitrogenase activity and low top-dry-weight produced numerically shorter progenies ($\bar{x}$ = 50.9 cm) and high X low ($\bar{x}$ = 53.1 cm) and low X high ($\bar{x}$ = 51.3 cm) produced progenies that were intermediate in height. At all five stages of plant development, the interactions between treatments and populations were not significantly different.

Reciprocal crosses at all five stages of plant development, were not significantly different in plant height indicating that maternal influences on plant height were negligible (Tables 3, 4, 5, 6, and 7).

Leaf Area

The effects of adding VA mycorrhizae and Rhizobium on leaf area (Dm^2/plant) determined at first harvest is presented in Table 8 and Fig. 6. There were significant differences between the treatments in leaf area (Dm^2/plant). Treatment means ranged from the highest mean leaf area/plant of 3.74 Dm^2/plant for the mycorrhizae treatment to the lowest mean leaf area of 2.02 Dm^2/plant for the Rhizobium treatment. The combined inoculation of mycorrhizae and Rhizobium increased leaf area by 53.7% over the control while mycorrhizae alone

Table 8. Effects of addition of VA mycorrhizae and Rhizobium on leaf area (dm^2/plant) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity, 1984.

Treatment	Mean Leaf Area (Dm^2/plant)	% Change over Control
Control	2.29 ab	
Rhizobium	2.02 a	-11.8
Mycorrhizae	3.74 b	63.3
Mycorrhizae + Rhizobium	3.52 b	53.7

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 1.49.

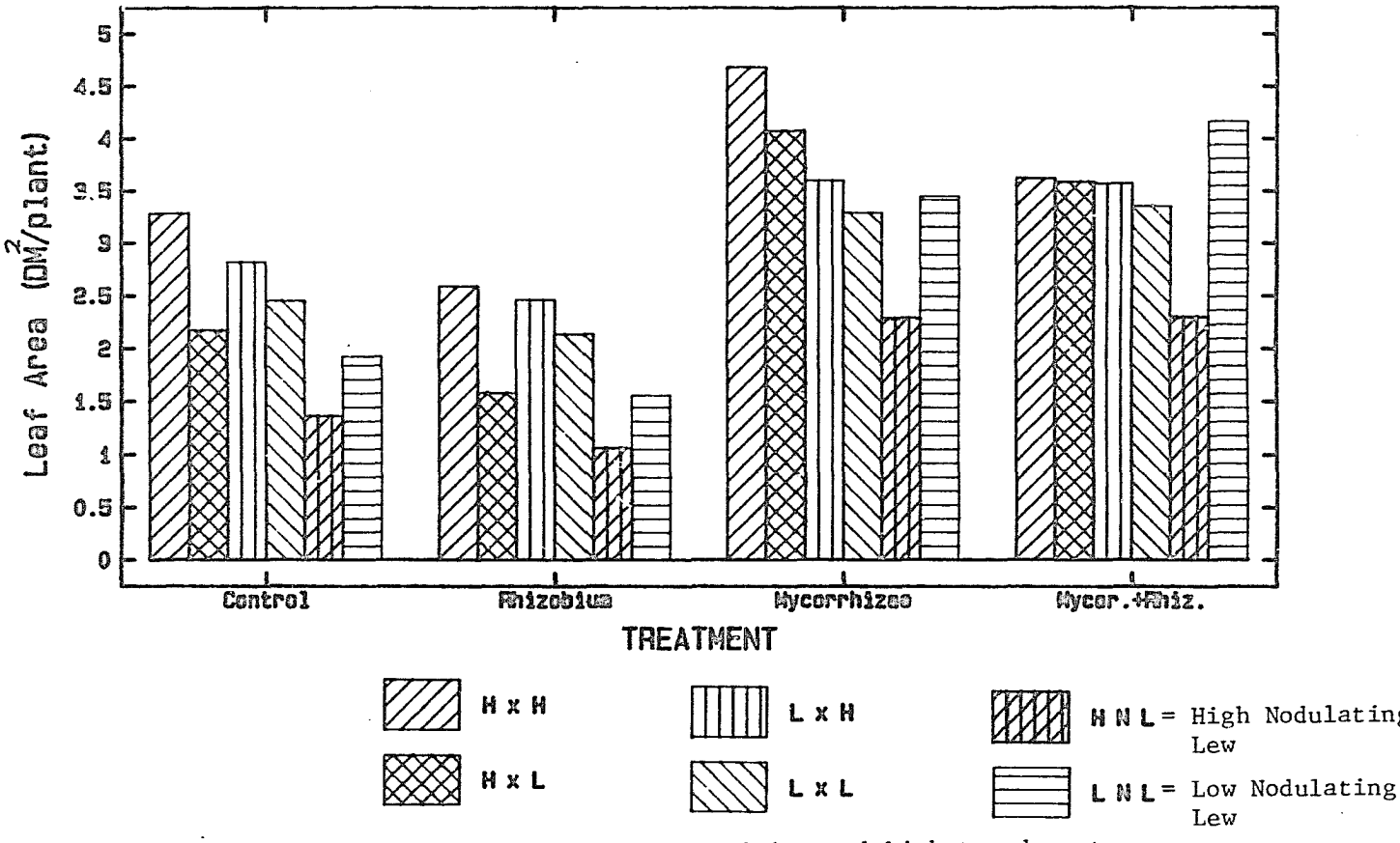


Fig. 6. Effects of addition of VA mycorrhizal fungi and Rhizobium on leaf area of 90-day-old alfalfa plants.

increased leaf area by 63.3% over the control. However, the Rhizobium treatment produced insignificantly less leaf area than the control by about 11.8%. The added Rhizobium inoculum may be competing for photosynthates that otherwise might have been used by plants to produce more leaves. The increase in total leaf area of mycorrhizal plants may be due to the fact that mycorrhizal plants had larger leaflets while plants receiving the Rhizobium and control treatments had smaller leaflets. The production of more stems/plant by mycorrhizal inoculation may also have contributed to higher total leaf area of mycorrhizal plants. Generally, plants selected for high nitrogenase activity and high top-dry-weight produced progenies with higher leaf area under the control, Rhizobium and mycorrhizae alone treatments (Fig. 6). However, under the combined mycorrhizae and Rhizobium treatment, the check population Low-Nodulating Lew exceeded all populations in total leaf area production. The check population High-Nodulating Lew produced less leaf area under the four treatments. Variation in leaf area/plant between the F_1 families is more pronounced under the control, Rhizobium and mycorrhizae alone treatments. Under the dual inoculation of mycorrhizae and Rhizobium, the F_1 families produced similar leaf areas.

These results were in agreement with those reported by Pacovsky, Paul and Bethlenfalvay (1986). They found that with no P added, dual inoculation of mycorrhizae and Rhizobium increased leaf area significantly in soybeans. Daft and El-Giahmi (1976) found that mycorrhizal peanut plants had more leaves than nonmycorrhizal controls.

The average leaf area of plants differed significantly among the 14 populations studied (Table 9). The check population

Table 9. Mean comparison for leaf area (dm^2/plant) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 90 days after planting, 1984.

Entry	Entry Mean (cm/plant)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	3.72 d		
2	3.36 bcd		
	$\bar{x}$=3.54	202.3	127.3
Low X Low			
3	2.77 bc		
4	2.84 bcd		
	$\bar{x}$=2.81	160.6	102.2
High X Low			
5	2.51 ab		
6	3.07 bcd		
7	3.10 bcd		
8	2.75 bc		
	$\bar{x}$=2.86	163.4	104.0
Low X High			
9	3.61 cd		
10	2.51 ab		
11	3.43 bcd		
12	2.90 bcd		
	$\bar{x}$=3.11	177.7	111.9
Checks			
High Nod. Lew	1.75 a	100.0	
Low Nod. Lew	2.78 bc		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test LSD = 0.92.

High-Nodulating Lew had the lowest leaf area of 1.75 Dm^2/plant while the high X high cross #1 produced progenies with the highest leaf area of 3.72 Dm^2/plant. The check population High-Nodulating Lew produced significantly less leaf area than all other populations except high X low cross #5 and low X high cross #10. The reason for the lower leaf area produced by the High-Nodulating Lew strain is difficult to explain and further research may be needed to come up with an answer. Comparing the F_1 families with each other, plants selected for high nitrogenase activity and high top-dry-weight produced progenies which averaged numerically more leaf area ($\bar{x}$ = 3.54 Dm^2/plant) and plants selected for low nitrogenase activity and low top-dry-weight produced progenies with numerically lower leaf area ($\bar{x}$ = 2.81 Dm^2/plant). The high X low ($\bar{x}$ = 2.86 Dm^2/plant) and the low X high ($\bar{x}$ = 3.11 Dm^2/plant) crosses produced progenies with numerically intermediate values. Since the high X low crosses and low X high crosses were not closer to the high X high crosses, major dominant genes may not be involved in controlling the leaf area characteristics.

Except for high X low cross #5 and its reciprocal low X high cross #9, all other reciprocal crosses were not significantly different in leaf area indicating that maternal influences were very small in affecting the leaf area characteristic.

Specific Leaf Weight

Specific leaf weight (mg/cm^2/plant), as affected by the addition of VA mycorrhizae and Rhizobium, is presented in Table 10. There

Table 10. Effects of addition of VA mycorrhizae and Rhizobium on specific leaf weight (mg/cm^2/plant) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity, 1984.

Treatment	Mean Specific Leaf Weight (mg/cm^2)	% Change over Control
Control	3.35 b	
Rhizobium	3.32 b	-0.9
Mycorrhizae	2.53 a	-24.5
Mycorrhizae + Rhizobium	2.60 a	-22.4

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.30.

were significant differences between the treatments. Treatment means ranged from a high of 3.35 mg/cm^2/plant for plants in the control treatment to a low of 2.53 mg/cm^2/plant for plants in the mycorrhizae treatment. The dual inoculation of mycorrhizae and Rhizobium decreased specific leaf weight significantly by 22.4% when compared to the control. The mycorrhizae treatment decreased specific leaf weight significantly by 24.5% compared to the control. It was observed that mycorrhizal plants had larger and broader leaflets while plants in the control and Rhizobium treatments had smaller leaflets. Daleney and Dobrenz (1974) found that alfalfa plants with small leaves had the greatest specific leaf weight, palisade tissue thickness, and leaf thickness while plants with the largest area per leaflet had thinner leaves and were lower in specific leaf weight.

Mean specific leaf weight of plants differed significantly among the 14 populations studied (Table 11). Low X low cross #3 and low X high cross #9 produced progenies with the lowest mean specific leaf weight of 2.80 mg/cm^2/plant. The low X high cross #11 produced progenies with the highest specific leaf weight of 3.12 mg/cm^2/plant. On the average, plants selected for high nitrogenase activity and high top-dry-weight produced progenies with higher specific leaf weight ($\bar{x}$ = 2.98 mg/cm^2). Plants selected for low nitrogenase activity and low top-dry-weight produced progenies with numerically lower specific leaf weight ($\bar{x}$ = 2.87 mg/cm^2). The high X low ($\bar{x}$ = 2.97 mg/cm^2) and the low X high ($\bar{x}$ = 2.95 mg/cm^2) produced progenies with specific leaf weights closer to the high X high crosses indicating that major dominant genes may be involved in controlling the specific leaf weight

Table 11. Mean comparison for specific leaf weight (mg/cm^2/plant) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 90 days after planting, 1984.

Entry	Entry Mean (cm/plant)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	2.95 abcd		
2	3.00 abcd		
	$\bar{x}$=2.98	95.8	104.2
Low X Low			
3	2.80 a		
4	2.94 abcd		
	$\bar{x}$=2.87	92.3	100.3
High X Low			
5	2.83 ab		
6	3.08 cd		
7	2.90 abcd		
8	3.05 bcd		
	$\bar{x}$=2.97	95.5	103.8
Low X High			
9	2.80 a		
10	2.91 abcd		
11	3.12 d		
12	2.97 abcd		
	$\bar{x}$=2.95	94.9	103.1
Checks			
High Nod. Lew	3.11 d	100.0	
Low Nod. Lew	2.86 abc		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.24.

trait. Reciprocal crosses were not significantly different in specific leaf weight indicating that maternal influences were not important. Barnes et al. (1969) found significant variation in specific leaf weight both within and among alfalfa varieties studied. They attributed the difference in specific leaf weight to both genetic as well as environmental factors. It is interesting to note that high X high crosses had the highest leaf area (Table 9) and also had the highest specific leaf weight (Table 11). Both leaf area and specific leaf weight contribute to final yield, and it seems possible to combine both high leaf area and high specific leaf weight in one cultivar. Barnes et al. (1969) reported that specific leaf weight and leaf area/plant of alfalfa were genetically independent traits.

Dry Matter Production

Dry matter production was determined at two harvest dates. Harvest-1 was taken when alfalfa plants were 90 days old and Harvest-2 was taken at the end of the experiment when plants were 150 days old.

Dry matter production at Harvest-1, as affected by the addition of VA mycorrhizae and Rhizobium, is presented in Table 12 and Fig. 7. Dry matter production differed significantly among the four treatments. The control and Rhizobium treatment produced the lowest mean dry weight of 1.19 gm/plant. The dual inoculation of mycorrhizae and Rhizobium produced the highest mean dry weight of 1.91 gm/plant. Dry matter production was increased significantly by 60.5% over the control by inoculation with both endophytes. The endophyte mycorrhizae alone increased dry weight by 46.2% over the control, however, this

Table 12. Effects of addition of VA mycorrhizae and Rhizobium on mean dry weight of the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity at first harvest, 1984.

Treatment	Mean dry weight (gm)	% Change over Control
Control	1.19 a	-
Rhizobium	1.19 a	0.0
Mycorrhizae	1.74 ab	46.2
Mycorrhizae + Rhizobium	1.91 b	60.5

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.59.

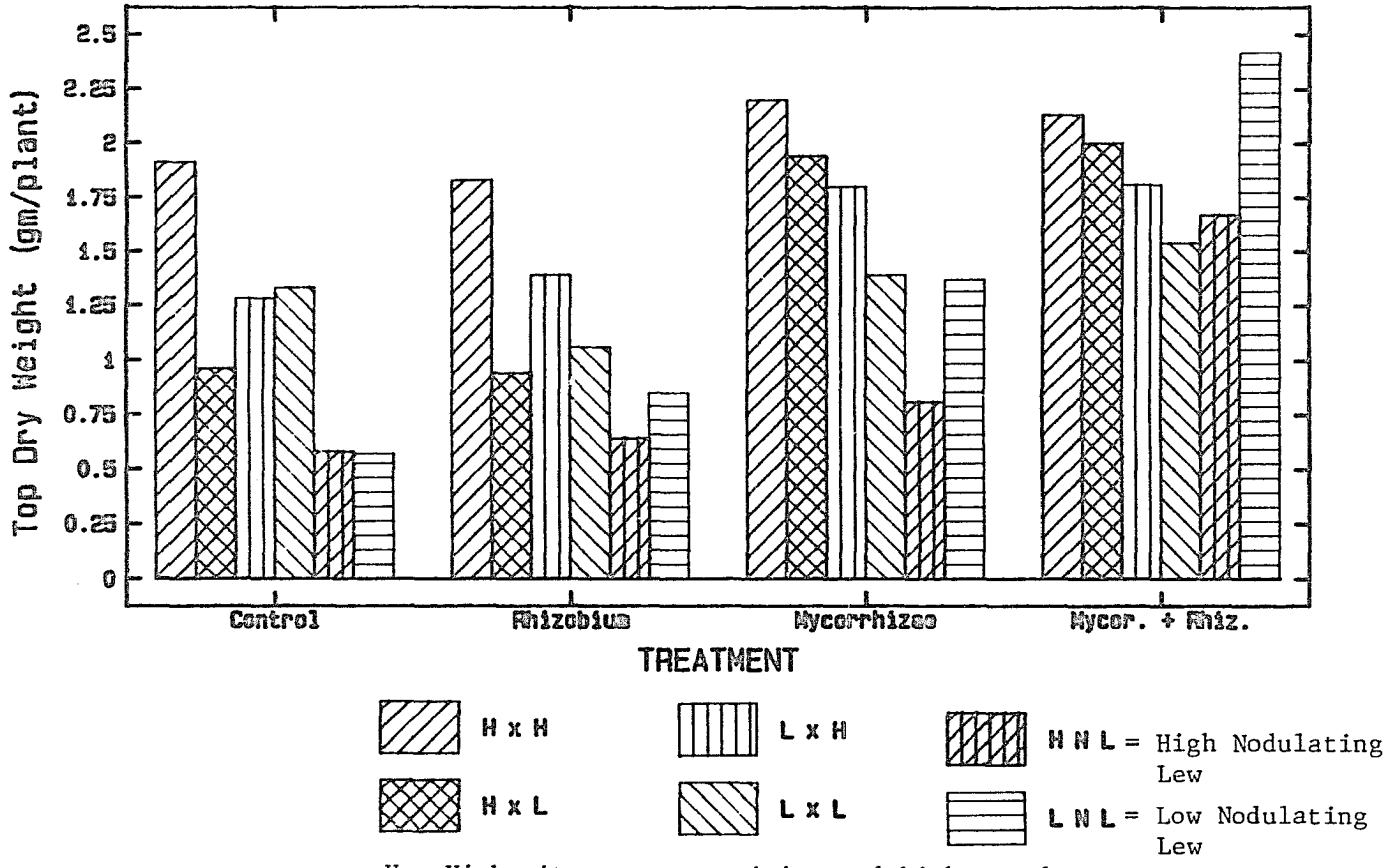


Fig. 7. Effects of addition of VA mycorrhizal fungi and Rhizobium on plant dry weight of 90-day-old alfalfa plants.

increase was not significant. Plants selected for high nitrogenase activity and high top-dry-weight produced progenies with higher dry matter production under the control, Rhizobium and mycorrhizae alone treatments but not under the dual inoculation of mycorrhizae and Rhizobium (Fig. 7). Mycorrhizal inoculation increased dry matter of all populations under study. The High-Nodulating Lew strain produced less forage under all treatments.

Dry matter production at Harvest-2 differed significantly among the four treatments (Table 13). The control treatment had the lowest mean dry weight of 1.75 gm/plant while the dual inoculation of mycorrhizae and Rhizobium had the highest mean dry weight of 2.77 gm/plant. The dual inoculation of mycorrhizae and Rhizobium persisted and extended its beneficial effects into the second harvest. Dry matter production was significantly increased 58.3% by the two endophytes. The mycorrhizae alone treatment increased dry weight by 53.7% over the control while the Rhizobium treatment increased dry weight by 15.4% over the control but both treatments were not significantly different from control.

Plants selected for high nitrogenase activity and high top-dry-weight produced progenies with more dry weight under the control, Rhizobium, and mycorrhizae alone treatments but not under the dual inoculation of mycorrhizae and Rhizobium (Fig. 8). The check population, High-Nodulating Lew, produced less dry matter under all treatments. The reason for this is not known and more research may be

Table 13. Effects of addition of VA mycorrhizae and Rhizobium on mean dry weight (gm/plant) of the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity at final harvest, 1984.

Treatment	Mean dry weight (gm)	% Change over control
Control	1.75 a	-
Rhizobium	2.02 ab	15.4
Mycorrhizae	2.69 ab	53.7
Mycorrhizae + Rhizobium	2.77 b	58.3

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.95.

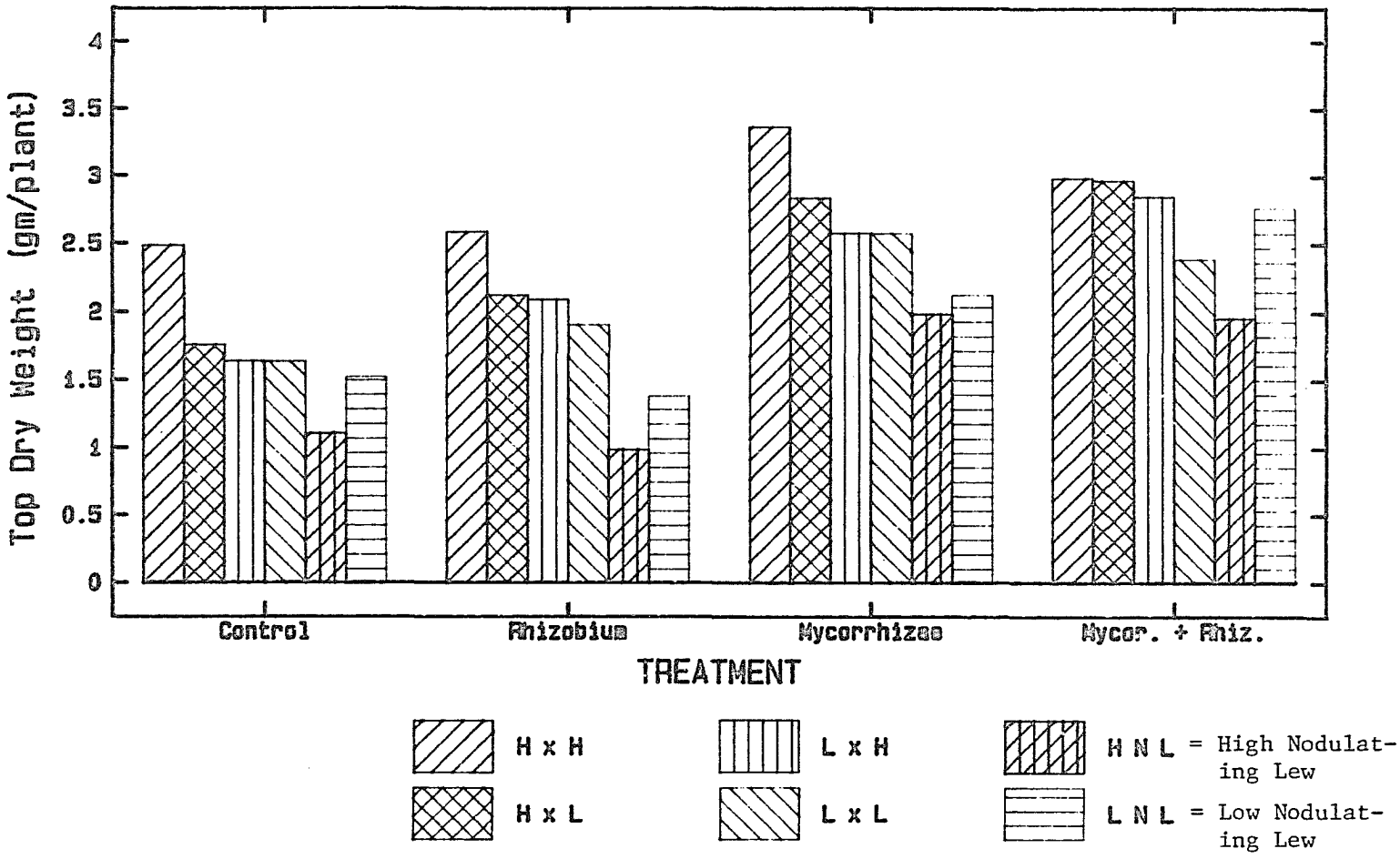


Fig. 8. Effects of addition of VA mycorrhizal fungi and Rhizobium on plant dry weight of 150-day-old alfalfa plants.

needed to find an answer. In a similar study, Steinberg (1982) found that the Hayden-PX-1 alfalfa strain produced significantly greater dry weight than the High-Nodulating-Lew strain under all treatments.

Mycorrhizal inoculation increased dry matter production at both harvests. The uninoculated control and mycorrhizae alone treatment showed some nodulation by indigenous rhizobium strains that were able to withstand soil fumigation with methyl bromide. However, in dual inoculated plants, the introduction of Rhizobium was effective and plant dry weight was increased by 60.5% and 37.1% for Harvest-1 and Harvest-2, respectively (Mycorrhizae + Rhizobium treatment compared with the Rhizobium alone treatment).

Similar results were reported by several other researchers. O'Bannon et al. (1980) found that mycorrhizal inoculation increased dry weight of four alfalfa cultivars. Smith and Daft (1977) found that dual inoculation of alfalfa with mycorrhizae and Rhizobium produced significantly more dry matter than Rhizobium treated control plants. Similar results were reported by Islam and Ayanaba (1981). Bloss and Pfeiffer (1981) reported a five-fold increase in total dry weight of 30-day-old mycorrhizal guayule plants (Parthenium argentatum Gray) grown in sterile soil without additional fertilizer.

Mean dry weight per plant for Harvest-1 differed significantly among the 14 populations studied (Table 14). The check population, High-Nodulating Lew, had the lowest mean dry weight of 0.93 gm/plant while the high X high cross #1 progenies had the highest mean dry weight of 2.19 gm/plant. On the average, plants selected for high

Table 14. Mean comparison for dry weight (gm/plant) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 90 days after planting, 1984.

Entry	Entry Mean (cm/plant)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	2.19 f		
2	1.85 def		
	$\bar{x}$=2.02	217.2	155.4
Low X Low			
3	1.35 abcde		
4	1.32 abcd		
	$\bar{x}$=1.34	144.1	103.1
High X Low			
5	1.25 ab		
6	1.79 cdef		
7	1.52 bcde		
8	1.34 abcd		
	$\bar{x}$=1.48	159.1	113.8
Low X High			
9	1.88 ef		
10	1.26 abc		
11	1.71 bcdef		
12	1.45 abcde		
	$\bar{x}$=1.58	169.9	121.5
Checks			
High Nod. Lew	0.93 a	100.0	
Low Nod. Lew	1.30 abc		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.53.

nitrogenase activity and high top-dry-weight produced progenies with numerically higher top-dry-weight ($\bar{x}$ = 2.02 gm/plant). Plants selected for low nitrogenase activity and low top-dry-weight produced progenies with numerically lower top-dry-weight ($\bar{x}$ = 1.34 gm/plant). The high X low ($\bar{x}$ = 1.48 gm/plant) and the low X high ($\bar{x}$ = 1.58 gm/plant) produced progenies with numerical intermediate values.

Mean comparison of dry matter production at Harvest-2 is presented in Table 15. Mean dry weight differed significantly among the 14 populations studied. The check population High-Nodulating Lew had the lowest mean dry weight of 1.49 gm/plant while the progenies of high X high cross #1 had the highest mean dry weight of 3.03 gm/plant. The same trend as in the first harvest persisted in the second harvest. Generally, plants selected for high nitrogenase activity and high-top-dry-weight produced progenies with numerically higher top-dry-weight ($\bar{x}$ = 2.87 gm/plant) while plants selected for low nitrogenase activity and low top-dry-wegith produced progenies with numerically lower top-dry-weight ($\bar{x}$ = 2.13 gm/plant). The high X low ($\bar{x}$ = 2.42 gm/plant) and low X high ($\bar{x}$ = 2.30 gm/plant) produced progenies with numerical intermediate values. Plants selected for high nitrogenase activity and high top-dry-weight produced progenies that had significantly more forage than the two check populations; High-Nodulating Lew and Low-Nodulating Lew at both harvests (Table 14 and 15). It is difficult to compare cultivars with hybrid material because in alfalfa, although it is a cross-pollinated species, still a substantial amount of selfing occurs. Miller (1970) found that the

Table 15. Mean comparison for dry weight (gm/plant) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 150 days after planting, 1984.

Entry	Entry Mean (cm/plant)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	3.03 f		
2	2.70 def		
	$\bar{x}$=2.87	192.6	146.4
Low X Low			
3	1.97 ab		
4	2.29 bcde		
	$\bar{x}$=2.13	143.0	108.7
High X Low			
5	2.21 bcd		
6	2.79 ef		
7	2.64 cdef		
8	2.04 ab		
	$\bar{x}$=2.42	162.4	123.5
Low X High			
9	2.50 bcdef		
10	2.10 bc		
11	2.51 bcdef		
12	2.07 b		
	$\bar{x}$=2.30	154.4	117.3
Checks			
High Nod. Lew	1.49 a	100.0	
Low Nod. Lew	1.96 ab		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.56.

percentage of outcrossing with honeybees did not exceed 52%. Such a high amount of selfing may have lowered the forage production of these two check populations when compared with the F_1 hybrids. In New Mexico, Duhigg et al. (1978) found that plants selected for high acetylene reduction rates produced progenies significantly higher in top-dry-weight than the original 'Mesilla' alfalfa population.

Tissue Chemical Analysis

Chemical analyses of stems and leaves of alfalfa plants for N and P were determined using the Techincon autoanalyser model sampler machine. Nitrogen and phosphorus content of stems and leaves of alfalfa plants as affected by the addition of VA mycorrhizae and Rhizobium is presented in Table 16. The dual inoculation of mycorrhizae and Rhizobium increased N content of the leaves by 10.0% over the control. The mycorrhizae alone treatment increased N content in leaves by 19.0% while the Rhizobium treatment increased N content by 3.5% over the control. There was a very slight increase in N content of the stems. Generally, leaves contained almost twice as much N as did the stems. Similar results were reported by Hassan (1981).

There was a marked increase in phosphorus content in the leaves of mycorrhizal alfalfa plants (Table 16). The mycorrhizae alone treatment increased P content of the leaves 24.9% over the control while the dual inoculation of mycorrhizae and Rhizobium increased P content by 18.7% over the control. Similar results were reported by Crush (1974) and Daft and El-Giahmi (1974, 1976). They found that nitrogen and phosphorus content of plants were greater in the combined mycorrhizae

Table 16. Effects of addition of VA mycorrhizal fungi and Rhizobium bacteria on nitrogen and phosphorus content of the leaves and stems of the check populations; High Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity, 1984.

Treatment	N (µg/g)		P (µg/g)	
	Leaves	Stems	Leaves	Stems
Control	43096.0	23226.7	1980.0	1292.9
Rhizobium	44603.6	22410.7	1895.4	1235.9
Mycorrhizae	51283.2	22816.9	2473.9	1337.0
Mycorrhizae + Rhizobium	47398.9	23307.0	2350.7	1408.7

and Rhizobium treated plants than in nonmycorrhizal but Rhizobium treated controls. Phosphorus is an essential constituent of all living cells and it is involved in the conservation and transfer of energy in the metabolic reactions of living cells. Phosphorus distribution throughout the plant is governed by physiological needs. Many physiological processes, such as photosynthesis and respiration, take place in the leaves and more phosphorus is found in the leaves than in the stems (Table 16).

Nodulation and Nitrogen Fixation

Three parameters of nitrogen fixation were determined at final harvest; nodule mass score, fibrous root score, and nitrogenase activity. Apparently, there were some strains of indigenous rhizobia that withstood methyl bromide fumigation; the control and mycorrhizae alone treatment had some nodulation.

Nodule mass score, as affected by the addition of VA mycorrhizae and Rhizobium, is presented in Table 17 and Fig. 9. Mean nodule mass score differed significantly among the four treatments. The control treatment had the lowest mean nodule mass score of 1.16 while the dual inoculation of mycorrhizae and Rhizobium had the highest mean nodule mass score of 2.05. The dual inoculation of mycorrhizae and Rhizobium significantly increased nodule mass by 76.7% over the control. The Mycorrhizae alone treatment increased nodule mass by 44.8% over the control while the Rhizobium treatment increased nodule mass by 22.4% over the control, however, this difference was not significant.

Plants selected for high nitrogenase activity and high top-dry-weight produced progenies with the greatest nodule mass under all four

Table 17. Effects of addition of VA mycorrhizae and Rhizobium on nodule mass score of the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity, 1984.

Treatment	Mean Nodule Mass Score	% Change over Control
Control	1.16 a	
Rhizobium	1.42 ab	22.4
Mycorrhizae	1.68 bc	44.8
Mycorrhizae + Rhizobium	2.05 c	76.7

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.51.

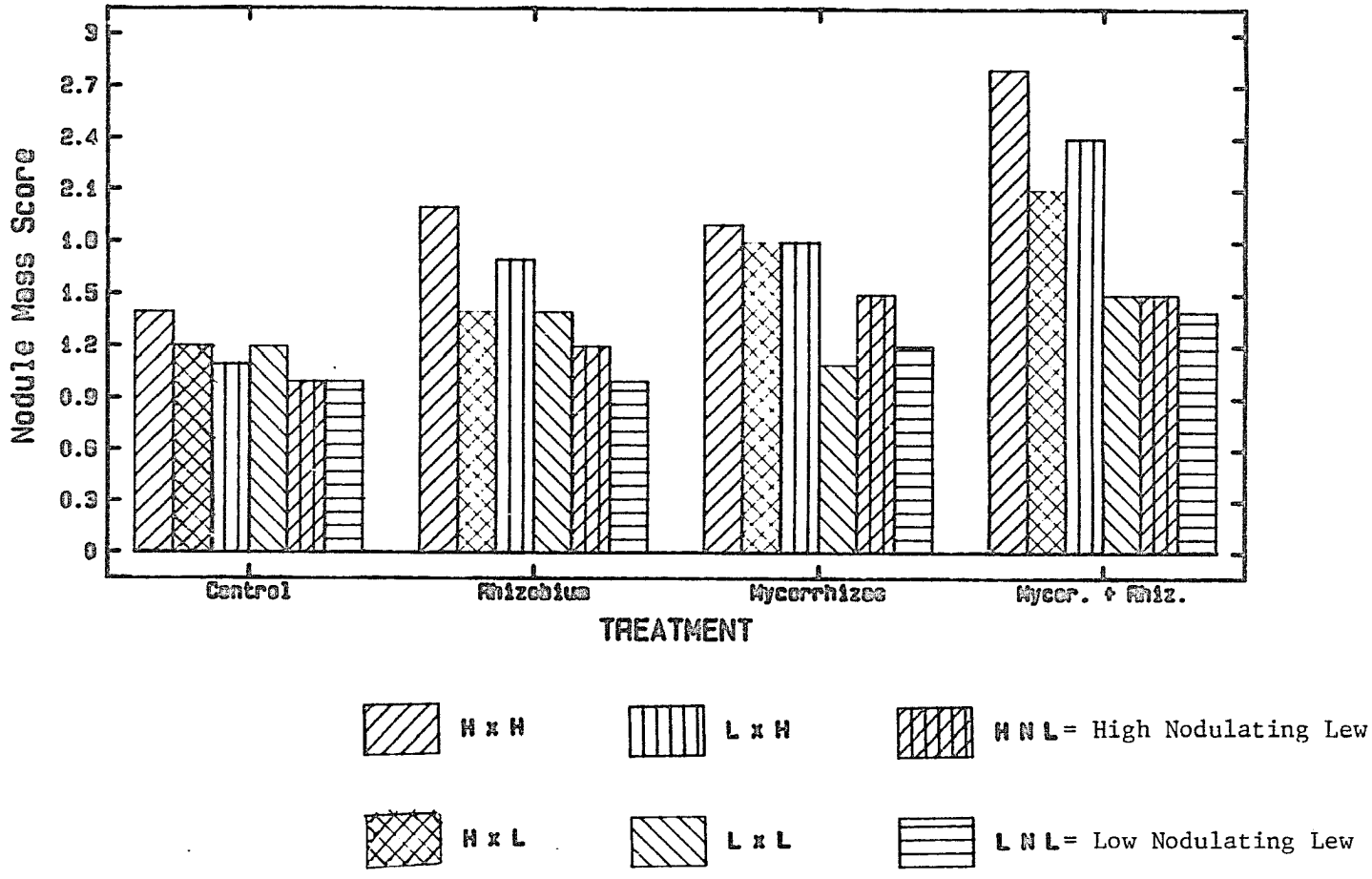


Fig. 9. Effects of addition of VA mycorrhizae and Rhizobium on nodule mass score of 150-day-old alfalfa plants.

treatments. The dual inoculation of mycorrhizae and Rhizobium increased nodule mass significantly more than the other treatments (Fig. 9). Plants selected for low nitrogenase activity and low top-dry-weight produced progenies with nodule mass score similar to that of the two check populations, High-Nodulating Lew and Low-Nodulating Lew. Greater nodule mass under mycorrhizal inoculation was essentially due to both an increased nodule number and nodule size. These results were in agreement with those of many researchers. Kucey and Paul (1982) found that dually infected plants had larger and more numerous nodules. However, Asimi et al. (1980) found that mycorrhizal inoculation increased nodule number rather than nodule size. Daft and El-Giahmi (1976) found that dual inoculation of peanut plants with mycorrhizae and Rhizobium increased nodule number by a factor of four when compared to nodulated but not mycorrhizal treated plants. Similar results were reported by Crush (1974) and Daft and El-Giahmi (1974).

Mean nodule mass score differed significantly among the 14 populations studied (Table 18). Low-Nodulating Lew strain had the lowest mean nodule mass score of 1.25 while the progenies of high X high cross #1 had the highest mean nodule mass score of 2.06. On the average, plants selected for high nitrogenase activity and high top-dry-weight produced progenies with numerically higher nodule mass score ($\bar{x}$ = 2.00), while plants selected for low nitrogenase activity and low top-dry-weight produced progenies with numerically lower nodule mass ($\bar{x}$ = 1.51). The high X low crosses ($\bar{x}$ = 1.61) and low X high crosses ($\bar{x}$ = 1.62) produced progenies with numerical intermediate values. Plants selected for high nitrogenase activity and high top-dry-weight produced progenies

Table 18. Mean comparison for nodule mass score for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 150 days after planting, 1984.

Entry	Entry Mean	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	2.06 e		
2	1.94 de		
	$\bar{x}$=2.00	114.3	160.0
Low X Low			
3	1.63 abcde		
4	1.38 abc		
	$\bar{x}$=1.51	86.3	120.8
High X Low			
5	1.69 abcde		
6	1.44 abc		
7	1.81 cde		
8	1.50 abcd		
	$\bar{x}$=1.61	92.0	128.8
Low X High			
9	1.94 de		
10	1.56 abcd		
11	1.29 ab		
12	1.69 abcde		
	$\bar{x}$=1.62	92.5	129.6
Checks			
High Nod. Lew	1.75 bcde	100.0	
Low Nod. Lew	1.25 a		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.48.

with nodule mass score that were significantly different from the check population Low-Nodulating Lew but they were not significantly different from the check population High-Nodulating Lew. Duhigg et al. (1978) found that plants selected for high acetylene reduction rates (high nitrogenase activity), produced progenies with significantly higher nodulation score than either the low selection or the original population. Jones and Burrows (1968) working with white clover (Trifolium repens L.), found that by selecting for a large amount of nodular tissue, the mean number of nodules/plant increased from 7.1 nodules per plant in the check to 10.7 in the F_1 and 11.3 in the F_2. Both the F_1 and F_2 means were significantly higher than the check. Except for high X low cross #7 and its reciprocal low X high cross #11, all other reciprocal crosses were not significantly different in nodule mass score indicating that maternal influences were very small. However, Seetin and Barnes (1977) found all reciprocal crosses were not significantly different and they also ruled out any maternal influences for this trait.

Fibrous Root Mass Score

Mean fibrous root score differed significantly among the four treatments (Table 19). The control treatment had the lowest fibrous root score of 1.79 while the dual inoculation of mycorrhizae and Rhizobium had the highest mean fibrous root score of 3.00. The dual inoculation of mycorrhizae and Rhizobium increased fibrous root mass significantly by 67.6% while the mycorrhizae alone treatment increased

Table 19. Effects of addition of VA mycorrhizae and Rhizobium on fibrous root score of the check populations; High-nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity, 1984.

Treatments	Mean fibrous root score	% Change over control
Control	1.79 a	
Rhizobium	2.20 ab	22.9
Mycorrhizae	2.63 bc	46.9
Mycorrhizae + Rhizobium	3.00 c	67.6

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.74.

fibrous root mass by 46.9% over the control. The Rhizobium treatment increased fibrous root mass by 22.9% over the control, however, this difference was not significant.

Plants selected for high nitrogenase activity and high top-dry-weight produced progenies with higher fibrous root score under the control, mycorrhizae alone, and the combined mycorrhizae and Rhizobium treatments (Fig. 10). Under the Rhizobium treatment, the high X control, mycorrhizae alone and the combined mycorrhizae and Rhizobium treatments (Figure 10). Under the Rhizobium treatment, the high X high and low X high crosses had similar fibrous root scores. The two check populations had a fibrous root score similar to the low X low progenies except under the combined mycorrhizae and Rhizobium treatment.

It was observed that mycorrhizal plants possessed larger and more profuse root systems with many fibrous roots while nonmycorrhizal control and Rhizobium inoculated plants had a tap root with very few or no branches. As suggested by Daft and El-Giahmi (1974), mycorrhizal infection produced a more actively growing root system. The enhancement of the root system of mycorrhizal plants may be due to an improved nutrition of mycorrhizal plants (Table 16) or it may be due to a hormonal effect since VA mycorrhizae increase hormone levels in host plants (Allen et al., 1982). Mosse (1962) found that VA mycorrhizae stimulated branching of infected roots.

Mean fibrous root score differed significantly among the 14 populations studied (Table 20). Progenies of plants selected for low nitrogenase activity and low top-dry-weight had the lowest mean fibrous

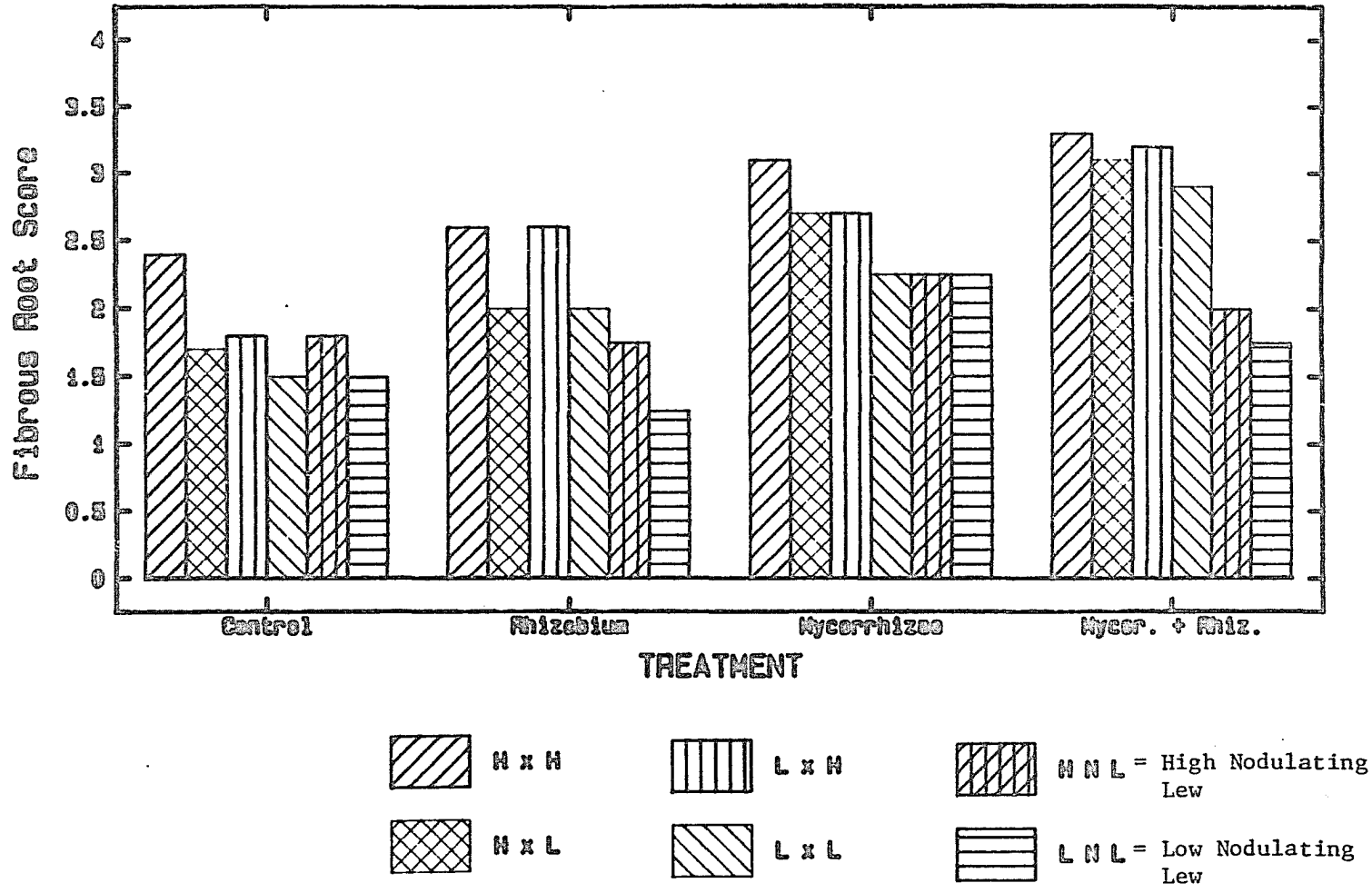


Fig. 10. Effects of addition of VA mycorrhizae and Rhizobium on fibrous root score of 150-day-old alfalfa plants.

Table 20. Mean comparison for fibrous root score for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 150 days after planting, 1984.

Entry	Entry Mean (cm/plant)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	2.75 abc		
2	2.94 c		
	$\bar{x}$=2.85	126.7	126.7
Low X Low			
3	2.13 a		
4	2.19 a		
	$\bar{x}$=2.16	96.0	96.0
High X Low			
5	2.44 abc		
6	2.25 a		
7	2.50 abc		
8	2.31 ab		
	$\bar{x}$=2.38	105.8	105.8
Low X High			
9	2.75 abc		
10	2.38 abc		
11	2.50 abc		
12	2.88 bc		
	$\bar{x}$=2.63	116.9	116.9
Checks			
High Nod. Lew	2.25 a	100.0	
Low Nod. Lew	2.25 a		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test LSD = 0.62.

root mass of 2.13 while progenies of plants selected for high nitrogenase activity and high top-dry-weight had the highest mean fibrous root mass of 2.94. On the average, plants selected for high nitrogenase activity and high top-dry-weight produced progenies with numerically higher fibrous root score ($\bar{x}$ = 2.85) while plants selected for low nitrogenase activity and low top-dry-weight produced progenies with numerically lower fibrous root score ($\bar{x}$ = 2.16). The high X low ($\bar{x}$ = 2.38) and low X high ($\bar{x}$ = 2.63) produced progenies with intermediate values. Similar results were reported by Seetin and Barnes (1977). The high X high cross #2 produced progenies with significantly higher fibrous root score than the low X low progenies. This result is in agreement with that of Duhigg et al. (1978). They found that the progeny of alfalfa plants selected for low nitrogenase activity had significantly lower fibrous root score than the progeny of alfalfa plants selected for high nitrogenase activity.

Nitrogenase Activity

The acetylene reduction technique used in this study is an indirect measurement of the nodule ability to fix atmospheric nitrogen. Mean nitrogenase activity, as measured by the acetylene reduction technique, differed significantly among the four treatments (Table 21). The nodules of plants in the control treatment had the lowest mean nitrogenase activity of 0.5949 μ moles/plant/hr. while the dual inoculation of mycorrhizae and Rhizobium had the highest mean nitrogenase activity of 1.3819 μ moles/plant/hr. The dual inoculation of mycorrhizae and Rhizobium increased nitrogenase activity by 132.3% over the

Table 21. Effects of addition of VA mycorrhizae and Rhizobium on nitrogenase activity (μ moles/plant/hr.) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity, 1984.

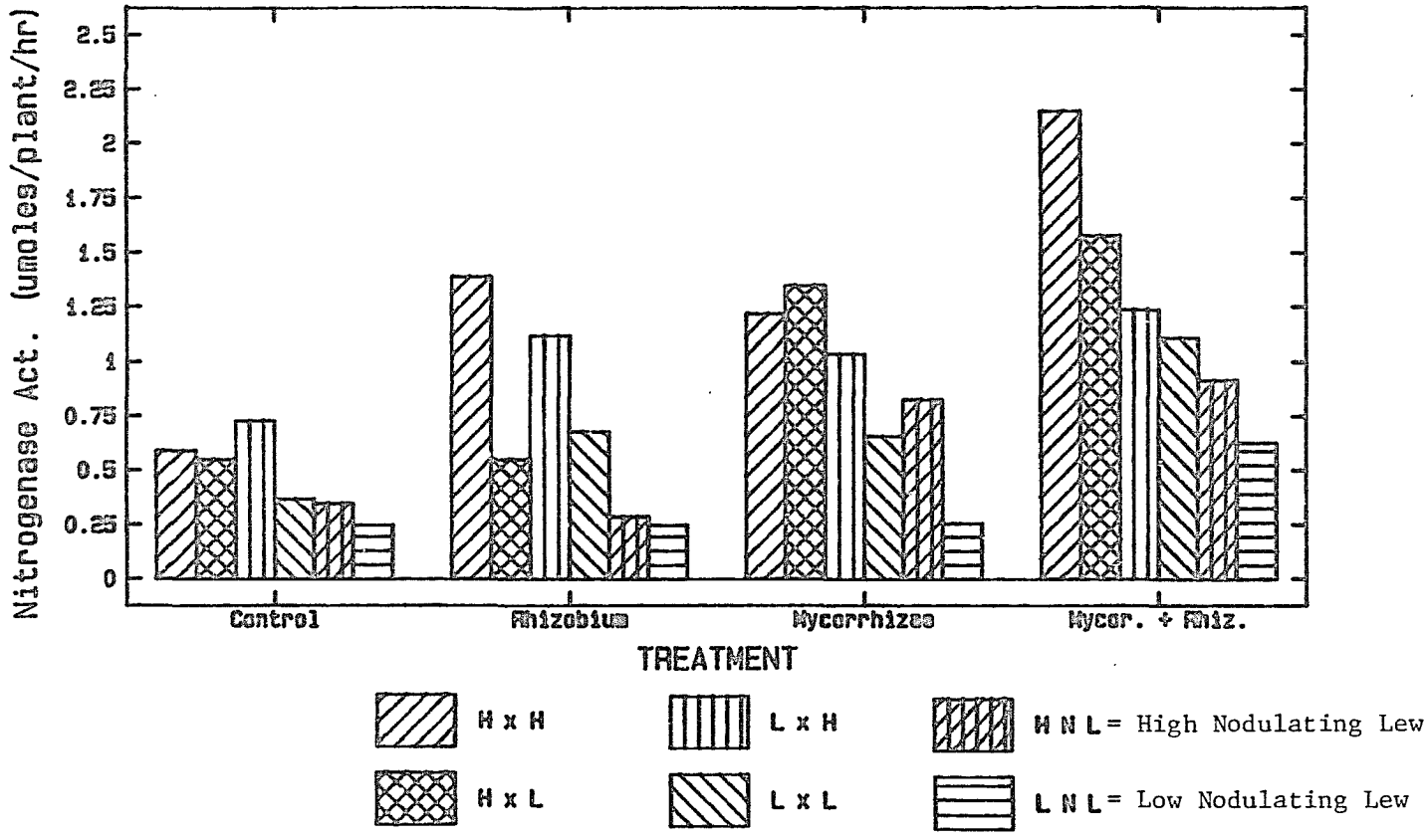
Treatment	Mean Nitrogenase Activity (μ moles/plant/hr.)	% Change over Control
Control	0.5949 a	-
Rhizobium	0.766 a	28.8
Mycorrhizae	1.0314 ab	73.4
Mycorrhizae + Rhizobium	1.3819 b	132.3

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.505.

control and by 80% over the Rhizobium treatment. The mycorrhizae alone treatment increased nitrogenase activity by 73.4% over the control while the Rhizobium treatment increased nitrogenase activity by 28.8% over the control, however, these increases were not significant.

The high X high crosses produced progeny with higher nitrogenase activity in the Rhizobium and the dual mycorrhizae and Rhizobium treatments (Fig. 11). The check population Low-Nodulating Lew produced lower nitrogenase activity under all four treatments. These results were in agreement with the results of several researchers. Daft and El-Giahmi (1974, 1976) found that the rates of acetylene reduction (nitrogenase activity) were greater in mycorrhizal and Rhizobium inoculated plants than in the nonmycorrhizal but nodulated controls. Asimi et al. (1981) found that mycorrhizal infection stimulated (140 to 177%) total nitrogenase activity per plant of nodulated soybeans growing in unamended soil. This indicated considerable nitrogen fixation by these plants. Daft and El-Giahmi (1976) reported that the rate of acetylene reduction was increased by a factor of four when peanut plants were mycorrhizal. The magnitude of increase in nitrogenase activity by mycorrhizal inoculation reported in this study were lower than those reported by some researchers. This may be due to the relatively high nitrogen content of the soil which may have depressed nodulation (Table 1). Also, nitrogenase activity determinations were done during late December when temperatures were lower. It is known that low temperatures can decrease nitrogenase activity (Gibson, 1962).

These results show that VA mycorrhizae can have important effects on nodulation and nitrogen fixation of legumes in a low P-soil



H = High nitrogenase activity and high top dry wt.
L = Low nitrogenase activity and low top dry wt.

Fig. 11. Effects of addition of VA mycorrhizal fungi and Rhizobium on nitrogenase activity of 150-day-old alfalfa plants.

and without additional fertilizers. Phosphorus is known to be an essential element for N_2-fixation, and as it was a limiting factor in this study, nitrogenase activity in the Rhizobium treated plants were significantly lower than the combined mycorrhizae and Rhizobium treatment (Table 21). Whereas plant growth was increased 60.5 and 58.0% for Harvest-1 and 2, respectively by mycorrhizal inoculation (Tables 12 and 13); nodule mass, fibrous root mass, and nitrogenase activity were increased 76.7, 67.6, and 132.3%, respectively (Tables 17, 19, and 21). It appeared that inoculation with VA mycorrhizae stimulated nodulation and nitrogen fixation more than plant growth. Thus, mycorrhizal inoculation may be more important for nitrogen fixation in legumes than for plant growth per se. This may be due to a greater requirement of phosphate for nodule activity than for plant growth and in fact mycorrhizal plants contained more phosphorus than the non-mycorrhizal but nodulated ones (Table 16). Unfortunately, phosphorus content in the nodules was not estimated in this study. However, Mosse et al. (1976) found that in Stylosanthes and Centrosema, nodules had twice as much phosphorus as did the roots. It is not known if the enhanced uptake of nutrients by mycorrhizae directly affects the bacteroids, if the bacteroids affect the mycorrhizae, or if they both interact by means of the nutrition of the host. Direct phosphate availability to nodules and also indirect effects of improved mineral nutrition on the carbohydrate supply to the nodules may also be important. This is further confirmed by Kucey and Paul (1982) who found that mycorrhizal and nodulated plants fixed more CO_2 than nonmycorrhizal but nodulated plants.

Table 22. Mean comparison for nitrogenase activity (μ moles/plant per hour) for the check populations; High-Nodulating Lew, Low-Nodulating Lew alfalfa and the intercross progenies of plants selected for high and low nitrogenase activity 150 days after planting, 1984.

Entry	Entry Mean (μmoles/plant/hr.)	% of the checks	
		High Nod. Lew	Low Nod. Lew
High X High			
1	1.22 cd		
2	1.54 d		
	$\bar{x}$=1.38	242.1	363.2
Low X Low			
3	0.85 abc		
4	0.56 ab		
	$\bar{x}$=0.71	124.6	186.8
High X Low			
5	1.00 abcd		
6	0.80 abc		
7	1.14 bcd		
8	1.05 bcd		
	$\bar{x}$=1.00	175.1	262.6
Low X High			
9	1.19 bcd		
10	0.93 abcd		
11	0.83 abc		
12	1.18 bcd		
	$\bar{x}$=1.03	181.2	271.8
Checks			
High Nod. Lew	0.57 ab	100.0	
Low Nod. Lew	0.38 a		100.0

Means followed by the same letter are not significantly different at 0.05 level according to the Least Significant Difference test. LSD = 0.64.

Mean nitrogenase activity/plant differed significantly among the 14 populations studied (Table 22). The check population Low-Nodulating Lew had the lowest mean nitrogenase activity of 0.38 μ moles/plant/hr., while the progenies of high X high reciprocal cross #2 had the highest mean nitrogenase activity of 1.54 μ moles/plant/hr. On the average, plants selected for high nitrogenase activity and high top-dry-weight produced progenies with numerically higher nitrogenase activity ($\bar{x}$ = 1.38 μ moles/plant/hr.), while plants selected for low nitrogenase activity and low top-dry-weight produced progenies with numerically lower nitrogenase activity ($\bar{x}$ = 0.71 μ moles/plant/hr.). The high X low crosses ($\bar{x}$ = 0.998 moles/plant/hr.) and the low X high crosses ($\bar{x}$ = 1.033 μ moles/plant/hr.) produced progenies with numerical intermediate values. These results were in agreement with those reported by Seetin and Barnes (1977) and Duhigg et al. (1978).

All reciprocal crosses were not significantly different in the expression of the nitrogenase activity trait and so maternal influences on this character were negligible. A similar conclusion was reached by Seetin and Barnes (1977).

Plants selected for high nitrogenase activity and high top-dry-weight produced progenies that had nitrogenase activity more than twice that produced by the check populations from which these selections were made. Previous studies also indicated that clones selected for high rates of acetylene reduction (nitrogenase activity) produced progenies with average acetylene reduction rates almost double the parental cultivars (Seetin and Barnes, 1977; and

Duhigg et al., 1978). Hobbs and Mahon (1982) found heterosis in nitrogen fixation per plant. The F_1's exceeded their higher parent in each cross.

Despite possible confounding effects with strains of Rhizobium, these results have established a heritable response to selection in nondormant alfalfa for nitrogenase activity. A point that needs to be emphasized is the performance of the two nondormant check populations, High-Nodulating Lew and Low-Nodulating Lew used in this study, the performance of 'Mesilla' a semi-dormant cultivar (Duhigg et al., 1978), and the performance of six dormant cultivars (Seetin and Barnes, 1977). All these cultivars had nitrogenase activity levels near that of the low X low selections. These results would indicate that these cultivars were relatively inefficient in factors related to nitrogen fixation, and strongly suggests that a hybrid cultivar would be more desirable.

The Pearson correlation procedure was applied to the various characters studied. Most of the characters exhibited highly significant positive correlations. Specific leaf weight was an exception. It had a negative correlation with all other characters measured (Table 23). Correlation coefficients in Table 23 indicated that increases in nitrogenase activity were significantly correlated with increased nodule mass ($r = 0.666$), increased number of fibrous roots ($r = 0.5793$), greater top weight ($r = 0.3559$ and $r = 0.3644$ for top-dry-weight at Harvests 1 and 2 respectively), and greater leaf area ($r = 0.3922$). However, nitrogenase activity/plant was negatively correlated ($r = -0.331$) with specific leaf weight.

Table 23. Pearson correlation coefficients for 12 characteristics studied, 1984.

	Nodule Mass Score NMS	Fibrous Root Score FRS	Nitrogenase Activity NA	Dry Weight (1) DW(1)	Dry Weight (2) DW(2)	Height (1) H(1)	Height (2) H(2)	Height (3) H(3)	Height (4) H(4)	Height (5) H(5)	Specific Leaf Weight SLW	Leaf Area LA
NMS	-											
FRS	.6175**	-										
NA	.6660**	.5793**	-									
DW(1)	.3942**	.3153**	.3559**	-								
DW(2)	.3789**	.4375**	.3644**	.5600**	-							
H(1)	.2643**	.2655**	.3312**	.5110**	.3835**	-						
H(2)	.3509**	.3598**	.3366**	.6070**	.4532**	.6884**	-					
H(3)	.3062**	.2276**	.2559**	.5312**	.4267**	.3801**	.6348**	-				
H(4)	.2947**	.2385**	.1632*	.5027**	.4448**	.3760**	.4678**	.5840**	-			
H(5)	.1820NS	.0872NS	.1270NS	.4011**	.3922**	.3195**	.5082**	.6060**	.5642**	-		
SLW	-.2742**	-.3434**	-.3310**	-.1895NS	-.3314**	-.3153**	-.3074**	-.2274**	-.0727NS	-.0320NS	-	
LA	.4125**	.4649**	.3922**	.7313**	.5797**	.5772**	.7545**	.5904**	.5102**	.4229**	-.4136**	-

NS = Not significant * Significant at 0.025 level ** Significant at 0.001 level

The significant positive correlation between dry matter production and nitrogenase activity indicated that nitrogenase activity could be increased without decreasing dry matter production. The cause and effect in such correlations is unknown. Plant size may be a direct function of available nitrogen and phosphorus which were provided through fixation and mycorrhizal inoculation, but total nitrogen fixed may also be a direct function of plant size. In the latter case, the larger the plant, the greater the leaf area and therefore the greater the amount of photosynthates available to the nitrogen fixation process. Alternatively, the larger the plant, the larger the root area available for nodulation. This interconnection of characters measured on a per plant basis may mean that the examination of one or two characters, e.g., nitrogenase activity and top-dry-weight, is not an investigation of those characters alone, but is complicated by interactions among several traits.

Multiple regressions determined for nitrogenase activity (μ moles/plant/hr.) are shown in Table 24. Regressions were determined in a stepwise manner, incorporating variables into the equation in order of their power to increase the explained variation. Nodule mass score accounted for 0.4449 of the variation, fibrous root score accounted for 0.047 of the variation, both dry weights added about 0.007% to the variation while leaf area was least in the equation and accounted for 0.00006% of the variation in nitrogenase activity. All the variables together explained about 49% of the variation in nitrogenase activity. The remaining 51% was assumed to be due to heritable plant X Rhizobium strain interactions (Barnes, 1978).

Table 24. Multiple regression: Nitrogenase activity/plant.

Independent Variables	Multiple r	r^2	r^2 Change	B	S.E.B.	Significance
Nodule Mass Score	.66702	.44492	.44492	.64218	.04814	.000
Fibrous Root Score	.70139	.49195	.04703	.21257	.04699	.000
Dry Weight (1)	.70552	.49776	.00581	.070609	.04425	.112
Dry Weight (2)	.70639	.49899	.00122	.035641	.04872	.465
Leaf Area	.70643	.49904	.00006	-.005634	.03597	.876
Constant				-.44389	.11095	.000

r = correlation coefficient
B = slope of regression line
S.E.B. = standard error of B
Sig. = significance of F for the variable indicated.

The most important variables in the regression equation were nodule mass score, fibrous root score and plant dry weight.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Two genotypes that had high nitrogenase activity and high top-dry-weight and two genotypes that had low nitrogenase activity and low top dry weight were selected from the two bidirectional selections, High-Nodulating Lew and Low-Nodulating Lew during 1983. Propagules of the four selected genotypes were crossed in all possible combinations in the greenhouse during the spring of 1984. Reciprocal crosses were kept separate to detect whether maternal influences existed. The resulting 12 F_1 families and the two original populations were grown in a low-phosphate soil mixture in the greenhouse from August until December 1984. The 12 F_1 families and the two original populations were evaluated for different plant morphological characteristics under four treatments.

It was observed that mycorrhizal plants grew faster, appeared healthier and had more stems per plant than nonmycorrhizal ones. Growth differences in height between mycorrhizal and nonmycorrhizal plants were evident 30 days after planting and extended until 60 days after planting. After this, growth differences in height were not significant indicating that mycorrhizae enhanced plant height early in plant development and this effect decreased with time as the mycorrhizal plants became pot bound or else plants reached their maximum height earlier.

The added Rhizobium, in the Rhizobium treatment, had produced consistently lower plant height at all 5 stages of plant growth. This indicated that the added Rhizobium inoculum may be competing for photosynthates at early stages of plant development; however, this effect decreased progressively as the season advanced. There were significant differences between the 14 populations studied in plant height at all five stages of plant growth. Reciprocal crosses were not significantly different indicating that maternal influences on plant height were not important.

The dual inoculation of mycorrhizae and Rhizobium increased leaf area significantly more than the nonmycorrhizal treatments. Variation in leaf area among the 12 F_1 families was more pronounced under the control, Rhizobium and mycorrhizae alone treatments but not under the combined mycorrhizae and Rhizobium treatment.

The 14 populations studied differed significantly in leaf area production. Generally, plants selected for high nitrogenase activity and high top-dry-weight produced progenies with higher leaf area than other populations. Except for one cross, all reciprocal crosses were not significantly different in leaf area production indicating that maternal influences were very small.

The specific leaf weight data showed that the dual inoculation of mycorrhizae and Rhizobium and mycorrhizae alone treatments had significantly lower specific leaf weight than the nonmycorrhizal control and Rhizobium treatments. This is due to that mycorrhizal plants had larger and broader leaves while nonmycorrhizal plants had smaller leaves.

The 14 populations studied differed significantly in specific leaf weight. Generally, plants selected for high nitrogenase activity and high-top-dry weight produced progenies with higher specific leaf weight. Plants selected for low nitrogenase activity and low top-dry-weight produced progenies with lower specific leaf weight. Because high X low crosses produced progenies with specific leaf weights similar to the high X high crosses, major dominant genes may be involved in controlling the specific leaf weight trait. Reciprocal crosses were not significantly different indicating that maternal influences on specific leaf weight were not involved. Leaf area and specific leaf weight data indicated that it may be possible to combine high leaf area and high specific leaf weight in one cultivar.

Dry matter production at two harvest dates was significantly increased by dual inoculation of mycorrhizae and Rhizobium. The higher leaf area and the presence of more stems per plant in mycorrhizal plants may have contributed to this increase in dry weight of mycorrhizal plants compared to the nonmycorrhizal ones. At both harvests, the 14 populations studied differed significantly in top dry weight. On the average, plants selected for high nitrogenase activity and high top-dry-weight produced progenies with higher top-dry-weights. Plants selected for low nitrogenase activity and low top-dry-weight produced progenies with lower top-dry-weights. The high X low and low X high crosses produced progenies with intermediate values. Except for one cross in each harvest date, all reciprocal crosses were not significantly different in top-dry-weight indicating that maternal

influences were not important. Thus, plants selected for high top-dry-weight transmitted this character to their progenies.

A chemical analysis of tissue showed that there was only a slight increase in nitrogen content in leaves and stems of mycorrhizal plants. However, there was a marked increase in phosphorus content of the leaves of mycorrhizal plants compared to nonmycorrhizal ones. It is generally assumed that the nutritive value of leaves of forage crops is much superior to that of stems. The superiority of the leaves is reflected in their mineral content. The amount of N and P in the leaves found in this study was more than twice that found in the stems.

Three aspects of nitrogen fixation were examined in this study; nodule mass, fibrous root mass, and nitrogenase activity. All these parameters were increased significantly by dual inoculation with mycorrhizae and Rhizobium. The increase in nodule mass was essentially due to both an increase in nodule number and in size. Mycorrhizal inoculation produced more profuse and actively growing root systems. Mycorrhizal inoculation increased nodulation and nitrogen fixation more than plant growth. Thus, mycorrhizal inoculation may be of more importance for nitrogen fixation than for plant growth. This may be due to the high requirement of phosphate for nodule activity than for plant growth.

The 14 populations studied differed significantly in nodule mass score, fibrous root score, and nitrogenase activity. Generally, plants selected for high nitrogenase activity and high top-dry-weight

produced progenies with higher nodule mass, higher fibrous root mass, and higher nitrogenase activity. Plants selected for low nitrogenase activity and low-top-dry weight produced progenies with low nodule mass, low fibrous root mass, and low nitrogenase activity. The high X low and low X high crosses produced progenies with intermediate values. These results have indicated a heritable response to selection in nondormant alfalfa which can be transmitted across generations. The two check populations had nitrogenase activity similar to the progeny of low X low crosses indicating that these two populations were inefficient in factors related to nitrogen fixation. Reciprocal crosses for the three parameters of nitrogen fixation were not significantly different. This indicated that maternal influences on nitrogen fixation were negligible.

Correlation coefficients indicated that increases in nitrogenase activity were significantly correlated with increased nodule mass, increased number of fibrous roots, greater top-dry-weight and greater leaf area.

Nodule mass score, fibrous root score, top dry weight, and leaf area explained 49% of the variation in nitrogenase activity. The remaining 51% of the variation is unexplained.

Based on these results, it is recommended that evaluation of alfalfa germplasm for indices of nitrogen fixation and yield be done under the presence of mycorrhizae.

Breeding alfalfa for increased nitrogen fixation can be achieved by screening a large population for both high nodule mass and high

top-dry-weight. The selected plants can be further screened for nitrogenase activity. The results of this study indicate all research of this type should be done under the presence of mycorrhizae.

LITERATURE CITED

Abbot, L. K., and A. D. Robson. 1977. Growth stimulation of Subterranean clover with vesicular-arbuscular mycorrhizas. Aust. J. Agric. Res. 28: 639-649.

Abbot, L. K., and A. D. Robson. 1981. Infectivity and effectiveness of five endomycorrhizal fungi: competition with indigenous fungi in field soils. Aust. J. Agric. Res. 32: 621-630.

Allen, M. F., T. S. Moore, and M. Christensen. 1980. Phytohormone changes in Bouteloua gracilis L. infected by vesicular-arbuscular mycorrhizae. I. Cytokinin increases in the host plant. Can. J. Bot. 58: 371-374.

Asimi, S. V., Gianinazzi-Pearson, and S. Gianinazzi. 1980. Influence of increasing soil phosphorus levels on interactions between vesicular-arbuscular mycorrhizae and rhizobium in soybeans. Can. J. Bot. 58: 2200-2205.

Aughtry, J. D. 1948. Effect of genetic factors in Medicago on symbiosis with Rhizobium. Cornell Univ. Agric. Expt. Stn. Memo 280.

Azcon, R., Azcon-GD Aguilar, and J. M. Barea. 1978. Effects of plant hormones present in bacterial cultures on the formation and responses of VA mycorrhiza. New Phytol. 80: 359-364.

Azcon-Aguilar, C., R. Azcon, and J. M. Barea. 1979. Endomycorrhizal fungi and Rhizobium as biological fertilizers for Medicago sativa L. in normal cultivation. Nature (London) 279: 325-327.

Azcon-Aguilar, C., and J. M. Barea. 1981. Field inoculation of Medicago with VA-mycorrhiza and Rhizobium in phosphate-fixing agricultural soil. Soil Biol. Biochem. 13: 19-22.

Azcon-Aguilar, C., J. M. Barea, and J. Olivares. 1980. Effects of Rhizobium polysaccharides on VA mycorrhizal formation. 2nd International Symp. of Microbial Ecol., University of Warwick, Coventry, U.K. Abst # 187.

Barea, J. M., J. L. Escudero, and C. Azcon-Aguilar. 1980. Effects of introduced and indigenous VA-mycorrhizal fungi on nodulation, growth and nutrition of Medicago sativa L. in phosphate-fixing soils as affected by P-fertilizers. Plant and Soil 54: 283-296.

Barnes, D. K. 1978. Summary of Proceedings. P. 16-18. In Selecting and breeding legumes for enhanced nitrogen fixation. T. A. LaRue (ed.) Recommendations for research and proceedings of a workshop. Boyce Thompson Institute at Cornell, Ithaca, N.Y.

Barnes, D. K., R. B. Pearce, G. E. Carlson, R. H. Hart, and C. H. Hanson. 1969. Specific leaf weight differences in alfalfa associated with variety and plant age. Crop Sci. 9: 421-426.

Bloss, H. E., and C. M. Pfeiffer. 1981. Growth and nutrition of guayule plants. Ann. Appl. Biol. 99: 267-274.

Burton, J. C. 1972. Nodulation and symbiotic nitrogen fixation. In C. H. Hanson (ed.). Alfalfa Science and Technology. Agron. 15: 229-246. Am. Soc. Agron. Madison, Wis.

Burton, J. C., and P. W. Wilson. 1939. Host plant specificity among the Medicago in association with root nodule bacteria. Soil Sci. 47: 293-303.

Cox, W. J., and A. D. Robson. 1980. Optimization of plant nutrition-improving the efficiency of fertilizer utilization. Proc. Aust. Agron. Conf. pp. 157-176.

Crush, J. R. 1974. Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Growth and nodulation of some herbage legumes. New Phytol. 73: 743-749.

Crush, J. R. 1976. Endomycorrhizas and legume growth in some soils of the Mackenzie Basin, Canterbury, New Zealand. N.Z. Agric. Res. 19: 473-476.

Daft, M. J., and A. A. El-Giahmi. 1974. Effect of Endogone mycorrhiza on plant growth. VII. Influence of infection on the growth and nodulation in French bean (Phaseolus vulgaris L.). New Phytol. 73: 1139-1147.

Daft, M. J., and A. A. El-Giahmi. 1976. Studies on nodulated and mycorrhizal peanuts. Ann. Appl. Biol. 83: 273-276.

Delaney, R. H., and A. K. Dobrenz. 1974. Morphological and anatomical features of alfalfa leaves as related to CO_2 exchange. Crop Sci. 14: 444-447.

Duhigg, P., B. Melton, and A. Baltensperger. 1978. Selection for acetylene reduction rates in 'Mesilla' alfalfa. Crop Sci. 18: 813-816.

El-Sherbeeny, M. H., D. A. Lawes, and L. R. Mytton. 1977. Symbiotic variability in Vicia faba L. 2-Genetic variation in Vicia faba L. Euphytica 26: 377-383.

Erdman, L. W., and U. M. Means. 1953. Strain variation of Rhizobium meliloti on three varieties of Medicago sativa L. Agron. J. 45: 625-629.

Gates, C. T., and J. R. Wilson. 1974. The interaction of nitrogen and phosphorus on the growth, nutrient status and nodulation of Stylosanthes humilis H.B.K. (Townsville Stylo.) Plant and Soil. 41: 325-333.

Gershon, D. 1961. Genetic studies for effective nodulation in Lotus spp. Can. J. Microbiol. 7: 961-963.

Gibson, A. H. 1962a. Genetic variation in the effectiveness of nodulation of Lucerne varieties. Aust. J. of Agric. Res. 13: 388-399.

Gibson, A. H. 1962b. Physical environment and symbiotic nitrogen fixation. I. The effect of root temperature on recently nodulated Trifolium subterraneum L. plants. Aust. J. Biol. Sci. 16(1): 28-42.

Gray, L. E., and J. W. Gerdeman. 1973. Uptake of sulphur-35 by vesicular-arbuscular mycorrhizae. Plant and Soil 39: 687-689.

Hardy, W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns. 1968. The acetylene-ethylene assay for N_2-fixation: Laboratory and field evaluation. Plant Physiol. 43: 1185-1207.

Hassan, A. S. M. 1981. Leaflet to stem-petiole ratio and protein content in alfalfa (Medicago sativa L.) as affected by cultivar. M.S. Thesis, University of Arizona.

Hayman, D. S. 1974. Plant growth responses to vesicular-arbuscular mycorrhiza. VI. Effect of light and temperature. New Phytol. 73: 71-80.

Hayman, D. S. 1983. The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Can. J. Bot. 61: 944-963.

Hobbs, S. L. A., and J. D. Mahon. 1982. Heritability of N_2 (C_2H_2) fixation rates and related characters in peas (Pisum sativum L.). Can. J. Plant Sci. 62: 265-276.

Hoffman, D., and B. Melton. 1981. Variation among alfalfa cultivars for indices of nitrogen fixation. Crop Sci. 21: 8-10.

Holl, F. B. 1975. Host plant control of the inheritance of dinitrogen fixation in Pisum-Rhizobium symbiosis. Euphytica 24: 767-770.

Iruthayatha, E. E., and K. Vlassak. 1982. Symbiotic specificity in nodulation and nitrogen fixation between winged bean and Rhizobium. Scientia Hortic. 16: 313-322.

Islam, R., and A. Ayanaba. 1981. Effect of seed inoculation and preinfecting cowpea (Vigna unguiculata L.) with Glomus mosseae on growth and seed yield of the plants under field conditions. Plant and Soil 61: 341-350.

Jones, D. G., and A. C. Burrows. 1968. Breeding for increased nodule tissue in White clover (Trifolium repens L.). J. Agric. Sci., Camb. 71: 73-79.

Kirk, L. E. 1930. Abnormal seed development in sweet clover species crosses - A new technique for emasculating sweet clover flowers. Sci. Agric. 10: 321-327.

Kucey, R. M. N., and E. A. Paul. 1982. Carbon flow, photosynthesis and N_2 fixation in mycorrhizal and nodulated Faba beans (Vicia faba L.). Soil Biol. Biochem. 14: 407-412.

Lambert, D. H., H. Cole, Jr., and D. E. Baker. 1980. Variation in the response of alfalfa clones and cultivars to mycorrhizae and phosphorus. Crop Sci. 20: 615-618.

La Rue, J. H., W. D. McClellan, and W. L. Peacock. 1975. Mycorrhizal fungi and peach nursery nutrition. Calif. Agric. 29: 6-7.

Miller, M. K. 1970. Identification of alfalfa (Medicago sativa L. and M. Falcata L.) clones and cultivars and differentiation of hybrid from selfed progeny. Ph.D. Dissertation, University of Arizona.

Mosse, B. 1972. The influence of soil type and endogone strain on the growth of mycorrhizal plants in phosphate deficient soils. Rev. Ecol. Biol. Sol. 9: 529-537.

Mosse, B. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. IV. In soil given additional phosphate. New Phytol. 72: 127-136.

Mosse, B., C. L. Powell, and D. S. Hayman. 1976. Plant growth responses to vesicular-arbuscular mycorrhiza. VI. Interactions between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. New Phytol. 76: 331-342.

Munns, D. N., and B. Mosse. 1980. Mineral nutrition of legume crops. In Advances in Legume Science. R. J. Summerfield and A. H. Bunting (eds.) HMSO, London. pp. 115-125.

Nutman, P. S. 1948. Genetical factors concerned in the symbiosis of clover and nodule bacteria. Nature 157: 463-465.

Nutman, P. S. 1957. Symbiotic effectiveness in nodulated Red clover. III. Further studies on inheritance of ineffectiveness in the host. Heredity 2: 157-173.

Nutman, P. S., H. Mareckova, and L. Raicheva. 1971. Selection for increased nitrogen fixation in Red clover. In Lei, T. A. and E. G. Mudler (eds.). Biological nitrogen fixation in natural and agricultural habitats. Plant and soil special volume. Martinus Nijhoff, the Hague.

O'Bannon, J. H., D. W. Evans, and R. N. Peaden. 1980. Alfalfa varietal response to seven isolates of vesicular-arbuscular mycorrhizal fungi. Can. J. Plant Sci. 60: 859-863.

Olivares, J., E. Montoya, and A. Palomares. 1977. Some effects derived from the presence of extrachromosomal DNA in Rhizobium meliloti. In Recent Developments in Nitrogen Fixation. Newton, W., J. R. Postgate, and C. Rodriguez-Barrueco (eds.). pp. 375-386. Academic Press, New York and London.

Pacovsky, R. S., E. A. Paul, and G. J. Bethlenfalvay. 1986. Response of mycorrhizal and P-fertilized soybeans to nodulation by Brady rhizobium or ammonium nitrate. Crop Sci. 26: 145-150.

Pang, P. C., and E. A. Paul. 1980. Effects of vesicular-arbuscular mycorrhiza on C^{14} and N^{15} distribution in nodulated faba beans. Can. J. Soil Sci. 60: 241-250.

Peterson, M. A., and D. K. Barnes. 1981. Inheritance of ineffective nodulation and non-nodulation traits in alfalfa. Crop Sci. 21: 611-616.

Phillips, D. A. 1980. Efficiency of symbiotic nitrogen fixation in legumes. Ann. Rev. Plant Physiol. 31: 29-49.

Pinchbeck, B. R., R. T. Hardin, F. D. Cook, and I. R. Kennedy. 1980. Genetic studies of symbiotic nitrogen fixation in Spanish clover. Can. J. Plant Sci. 60: 509-518.

Rhodes, L. H. 1980. The use of mycorrhizae in crop production systems. Outlook on agriculture 10: 275-281.

Rhodes, L. H., and J. W. Gerdemann. 1980. Nutrient translocation in vesicular-arbuscular mycorrhizae. In cellular interactions in symbiosis and parasitism. Cook, C. B., P. W. Pappas, and E. D. Rudolp (eds.). The Ohio State University Press, Columbus, OH. pp. 173-195.

Robson, A. D., G. W. O'Hara, and L. K. Abbot. 1981. Involvement of phosphorus in nitrogen fixation by Subterranean clover (Trifolium subterraneum L.). Aust. J. Plant Physiol. 8: 427-436.

Ross, J. P., and J. A. Harper. 1970. Effect of endogone mycorrhizae on soybean yields. Phytopathol. 60: 1552-1556.

Safir, G. R., J. S. Boyer, and J. W. Gerdemann. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. Plant Physiol. 49: 700-703.

Seetin, N. W., and D. K. Barnes. 1977. Variation among alfalfa genotypes for rates of acetylene reduction. Crop Sci. 17: 783-787.

Silsbury, J. H., S. E. Smith, and A. G. Oliver. 1983. A comparison of growth efficiency and specific rate of check respiration of uninfected and vesicular-arbuscular mycorrhizal plants of Trifolium subterraneum L. New Phytol. 93: 555-566.

Smith, G. R., and W. E. Knight. 1984. Inheritance of ineffective nodulation in Crimson clover. Crop Sci. 24: 601-604.

Smith, S. E. 1980. Mycorrhizas of autotrophic higher plants. Biol. Rev. 55: 475-510.

Smith, S. E., and M. J. Daft. 1977. Interactions between growth, phosphate content and nitrogen fixation in mycorrhizal and nonmycorrhizal Medicago sativa L. Aust. J. Plant Physiol. 4: 403-413.

Smith, S. E., F. A. Smith, and D. J. D. Nicholas. 1981. Effects of endomycorrhizal infection on phosphate and cation uptake by Trifolium subterraneum L. Plant and Soil. 63: 57-64.

Steinberg, M. D. 1982. Influence of vesicular-arbuscular mycorrhizal fungi on alfalfa grown in Arizona. Ph.D. Dissertation, University of Arizona.

Tan, G. Y. 1981. Genetic variation for acetylene reduction rate and other characters in alfalfa. Crop Sci. 21: 485-488.

Tinker, P. B. 1984. The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. Plant and Soil. 76: 77-91.

Viands, D. R., D. K. Barnes, and G. H. Heichel. 1981. Nitrogen fixation in alfalfa - Responses to bidirectional selection for associated characteristics. USDA Tech. Bull. 1643, 24 P. U.S. Government Printing Office, Washington, D.C.

Viands, D. R., C. P. Vance, G. H. Heichel, and D. K. Barnes. 1979. An ineffective nitrogen fixation trait in alfalfa. Crop Sci. 19: 905-908.

Waidyanatha, U. P., N. Yogaratnam, and W. A. Ariyartre. 1979. Effects of mycorrhizal infection on growth and nitrogen fixation of Pueraria and Styosanthes and uptake of phosphorus from two rock phosphates. New Phytol. 82: 147-152.

Witty, J. F., F. R. Minchin, and J. E. Sheehy. 1983. Carbon costs of nitrogenase activity in legume root nodules determined using acetylene and oxygen. J. Expt. Bot. 34(145): 951-963.