

EFFECT OF PHYSICAL AND NUTRITIONAL FACTORS
OF THE ENVIRONMENT ON NITROGEN FIXATION,
PLANT COMPOSITION, AND YIELD OF
DARK RED KIDNEY BEANS
(PHASEOLUS VULGARIS L.)

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ABSTRACT

EFFECT OF PHYSICAL AND NUTRITIONAL FACTORS OF THE ENVIRONMENT ON NITROGEN FIXATION, PLANT COMPOSITION, AND YIELD OF DARK RED KIDNEY BEANS (PHASEOLUS VULGARIS L.)

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Field experiments were established in Michigan in 1970 and 1971 to (1) determine the effect of soil temperature, plant age, and diurnal effects on nitrogen fixation, (2) to determine the total nitrogen fixed by dark red kidney beans during a complete growth cycle, and (3) to determine the effect of lime, sulfur, and molybdenum on nitrogen fixation, plant composition, and yield of dark red kidney beans.

In the first year, clear and black plastic mulch treatments were established to obtain differences in soil temperature in the field. Soil temperature was increased most by the clear plastic mulch treatments and only slightly by the black plastic mulch treatments. As a result of the increased soil temperature, plant growth was increased, potassium and phosphorus content of the plants were higher, and nodules developed earlier on the bean plants. Plants from treatments which had the lowest soil temperatures fixed the most nitrogen. Age of the dark red kidney beans had a profound effect on nitrogen fixation. Nitrogen fixation commenced ten days after the beans emerged, reached a maximum 39 days after emergence, and then declined as the beans matured. Twice as much nitrogen was fixed during the daylight hours as at night. Total nitrogen fixed by the dark red kidney beans during

the growing season was only 9.12 kg/ha. Yield was not significantly increased by any of the plastic mulch treatments.

In the second year of experiments, three rates of lime, two sulfur rates, and two molybdenum levels were established on sandy loam soil at two locations in Michigan. Lime and sulfur were applied broadcast and molybdenum as a seed treatment. Plant growth and color response to lime and sulfur was noted early in the growing season. Sulfur increased the nitrogen and sulfur content of the plants and increased nitrogen fixation. Yield was increased by both lime and sulfur. The yield increase by sulfur was mainly at the zero lime rate (increase of 336 kg/ha). When lime was applied the yield response to sulfur was less. Lime at the 2800 and 5600 kg/ha rates increased yield by 226 and 413 kg/ha, respectively. Molybdenum in nearly every case reduced yield. Lime and sulfur increased the yield of protein and sulfur increased the methionine content of the protein which is an indication of better protein quality.

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To Barbara

This thesis is affectionally dedicated to my wife for her
many sacrifices throughout the duration of this study.

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INTRODUCTION

Nitrogen fixed from the atmosphere by the Rhizobium-legume association of navy beans, red kidney beans, and in some cases soybeans has not been sufficient for maximum yield. Consequently, Michigan bean growers apply over 5000 tons of supplemental nitrogen to these crops each year. This results in a sizeable investment for the grower and also increases the potential for N loss by leaching and runoff. If more nitrogen were fixed symbiotically less fertilizer nitrogen would be needed and many of the problems associated with fertilizer nitrogen could be alleviated.

Some of the factors which influence the amount of nitrogen fixed by a leguminous crop are light, temperature, moisture, soil reaction, and presence of adequate nutrients other than nitrogen. If gains in nitrogen fixation are to be made, all of these factors must be optimized so that both partners of the symbiotic relationship - plant and nodule bacteria - can express fully their inherent capabilities. To set forth such a set of optimum conditions will require an understanding of the response of the host and bacteria in symbiosis to a variety of conditions.

One of the problems in the past has been the lack of a rapid, sensitive, and inexpensive assay for measuring nitrogen fixation so that the numerous factors which influence the nitrogen fixation reaction might be examined in the natural environment. The development of the new acetylene-ethylene assay by Hardy and Knight (1967), after Schollhorn and Burris (1966) and Dilworth (1966) independently discovered that the

enzyme nitrogenase which normally reduces gaseous N_2 to NH_3 also reduces acetylene to ethylene, has enhanced studies in this area. The method is reported by Hardy et al. (1968) to be 1000 times more sensitive than the ^{15}N procedure and to be adaptable to laboratory, greenhouse, and field study. This important development provided the stimulus for the research reported in this thesis.

The objectives of this study were:

- (1) To evaluate the effect of soil temperature on nitrogen fixation and determine if soil temperature is limiting nitrogen fixation and yield of dark red kidney beans in Michigan.
- (2) To determine the influence of plant age and diurnal changes in light and temperature on nitrogen fixation by dark red kidney beans.
- (3) To determine the total nitrogen fixed by dark red kidney beans during a complete growth cycle.
- (4) To determine the effect of lime, sulfur, and molybdenum on nitrogen fixation, plant composition, and yield of dark red kidney beans.

LITERATURE REVIEW

Symbiotic Nitrogen Fixation

The legumes are a relatively large group of plants which are capable, with the aid of bacteria of the genus Rhizobium, of reducing and utilizing atmospheric nitrogen. Nitrogen fixed by this symbiotic process is of tremendous agricultural importance. Burris (1965) reported that in the United States alone, leguminous crops fix an estimated 5.5 million tons of nitrogen per year. This roughly equals the amount of nitrogen now applied annually in the form of nitrogenous fertilizers (Harre, 1969).

Root Infection, Nodule Initiation, and Growth

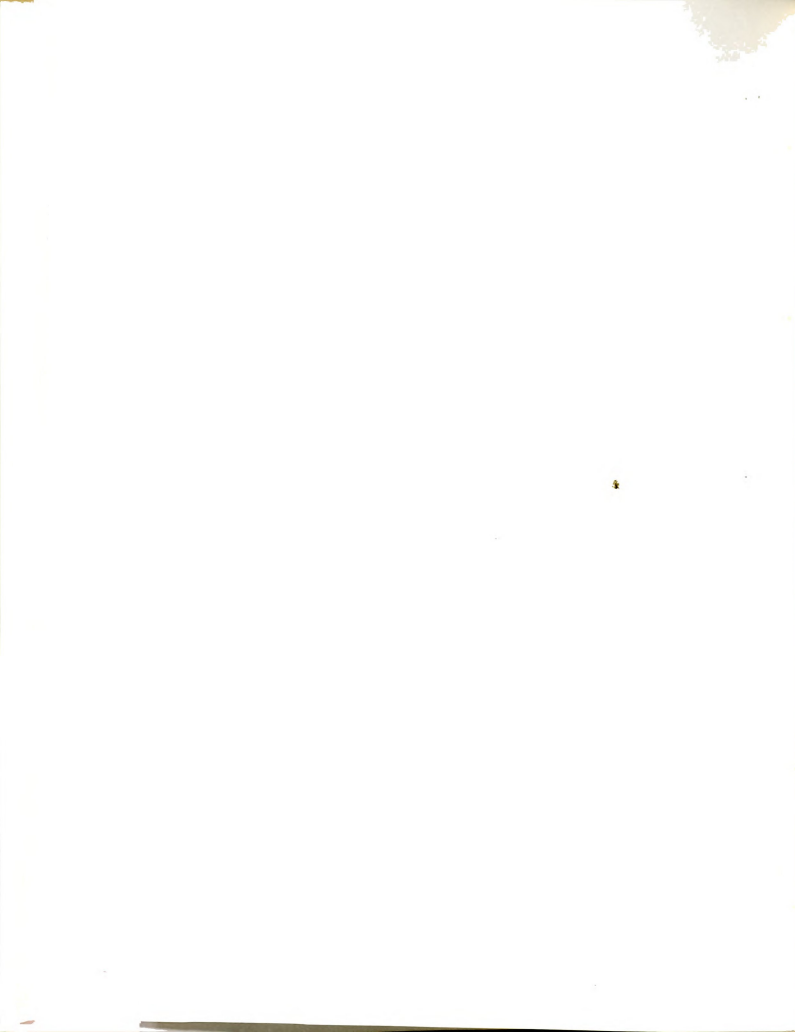
To fix nitrogen the Rhizobium must establish itself within the cells of the root tissue of the host plant. Events leading to infection and development of this symbiotic association have been studied extensively and are reported as follows: First, the legume secretes from its roots organic substances which stimulate the rhizosphere microflora. The bacteria respond by congregating in large numbers around the root in a thick layer of cells which is bound on the outside by a membrane-like substance (Dart and Mercer, 1964). Within this membrane-bound matrix, rhizobia in the microflora proliferate and build up to high populations. The rhizobia convert tryptophan, one of the substances secreted by the root, to the plant hormone, indoleacetic acid (Kefford, Brockwell, and Zwar, 1960). This hormone induces elongation of the root hairs and (possibly in conjunction with another Rhizobium product) causes the root hairs to deform and curl.

After curling, the root hairs become infected. Just how the rhizobia penetrate the root hair is not known. Two hypotheses have been suggested. One is that pectinase, an enzyme induced by the polysaccharide coat of the rhizobia and produced by the root, breaks down pectin compounds holding together the cellulose microfibrils of the root hair cell wall and allows the highly flagellated coccoid forms of rhizobia to pass through (Dart and Mercer, 1964). The other hypothesis is that a direct invagination of the root hair cell wall occurs (Nutman, 1956). Which ever way infection takes place, once inside, the rhizobia are surrounded by a hypha-like infection thread which is synthesized by the root. The infection thread extends inward and penetrates into the cortex. Here the rhizobia are released and infect the adjacent root cells. If the infected cell is a normal diploid cell, it may be destroyed by the infection. If it is a tetraploid cell it is stimulated to divide. The rhizobia multiply rapidly within the tetraploid cells and become surrounded singly or in small groups by portions of the host cell membrane. The rhizobia are then transformed into swollen, misshapen, and sometimes branched forms called "bacteroids". Concomitantly, the pigment leghemoglobin which is required for nitrogen fixation and which gives effective nodules their pink color, is developed and surrounds the bacteroids. Eventually, through progressive divisions of the tetraploid cells, a tumor-like nodule develops which has a highly organized structure and contains several well defined zones: an outer cortical region, a meristematic region, a vascular system with connections to the plant proper, and in the center of the nodule the bacteroid region where nitrogen fixation takes place.

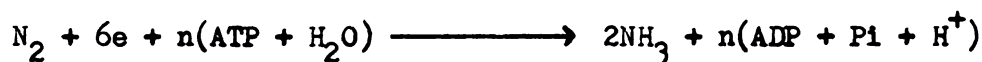
Biochemistry of Nitrogen Fixation by Legume Root Nodules

The enzyme that reduces atmospheric nitrogen to ammonia is called "nitrogenase" and is located within the bacteroids of the root nodule (Bergerson and Turner, 1967). The active portion of the enzyme is a metalloprotein complex consisting of Mo-Fe protein and Fe protein, neither of which alone can reduce nitrogen. The Mo-Fe protein is reported by Morris, Dalton, and Mortenson (1969) to have a molecular weight of 160,000 to 180,000 and to contain two Mo, 15-16 Fe, and 12 labile S atoms per molecule. The Fe protein is smaller and is reported by Jeng et al. (1969) to have a molecular weight of 39,000 and to contain two Fe, two labile S, and eight free SH-groups.

According to Hardy et al. (1971), nitrogenase performs two separate but essential functions in the reduction of N_2 . One function is the activation of electrons supplied by some external reducing agent. The other function is to bind and activate the N_2 molecule. Klucas and Evans (1968) have suggested that the source of electrons for nitrogen fixation is the respiratory electron transport chain with its associated oxidative phosphorylation. They have also shown that β -hydroxybutyrate, a compound that makes up almost 40% of the dry weight of the bacteroid, is capable of supplying electrons to nitrogenase if benzyl or methyl viologen is supplied as an electron carrier. Recently, Burton et al. (1970) discovered another possible pathway for transport of electrons to nitrogenase. They found a non-heme iron from bacteroids of soybeans that would function as a carrier in the light-dependent transfer of electrons from photosystem I of chloroplasts to nitrogenase.



The electron activation mechanism of the enzyme activates the electrons using energy supplied by hydrolysis of ATP. Upon addition of N_2 at the substrate activation and binding site, and hydrogen from the solvent, the activated and reduced metal enzyme complex carries out the reduction of N_2 to ammonia (Hardy et al., 1971). From studies of bi-metallic complexes, Chatt et al. (1969) determined that Fe was the probable binding site for N_2 and Mo which functions by reducing the strength of the dinitrogen bond was the terminus of the electron activation system. The over-all reaction for nitrogen fixation can be expressed in equation form as shown below:



Essential reactants include an electron donor, an electron acceptor, and ATP. Products include reduced and oxidized forms of the electron acceptor and donor, respectively, and the hydrolysis products of ATP: ADP, P_i , and H^+ . Ammonia produced by the reaction is rapidly transformed into α -amino compounds which become available to the host plant as a source of nitrogen (Aprison, Magee, and Burris, 1954).

In addition to the reduction of N_2 , nitrogenase has been shown to reduce a number of other triple bonded molecules such as acetylene, nitrous oxide, azide, and cyanide (Hardy and Burns, 1968). The reduction of these compounds require ATP and a strong electron donor exactly as does the reduction of N_2 . The reduction of acetylene to ethylene by nitrogenase has recently become widely used as an assay for nitrogen fixation activity.

Environmental Factors Affecting Nitrogen Fixation

Light:

The requirement that legumes be illuminated to actively fix nitrogen has been illustrated by a number of research workers (Bergersen, 1970; Lindstrom, Newton, and Wilson, 1952; Virtanen, Moisio, and Burris, 1955; and Hardy et al., 1968). Virtanen et al. (1955) reported that nitrogen fixation was reduced considerably when light grown pea plants were placed in the dark. They also observed that upon returning the plants to light, nitrogen fixation increased. Vincent (1965) indicated that light effects on nitrogen fixation were almost entirely related to photosynthesis. Increased photosynthesis resulted in increased activity of the nodules.

Bergersen (1970) found that fixation of nitrogen by soybeans was not only sensitive to light but to the intensity of the light. He observed that nodules assayed on days which were bright and sunny produced 6.7 μ moles of ethylene/hr/g while those assayed on days with more than 50% cloud cover produced only 3.0 μ moles of ethylene/hr/g.

Lindstrom et al. (1952) showed that predarkening clover plants for 24 hours completely stopped nitrogen fixation. Campbell and Lees (1967) reported that when plants with effective nodules are kept in the dark for a prolonged period, not only does nitrogen fixation cease, but the rhizobia in the nodules become plant pathogens which invade and eventually kill the nodule tissue.

Evidence of an additional action of light on the nitrogen fixation system was reported recently by Lie (1971). He found that the phytochrome system which governs many plant responses has some control over nodulation. Peas and broadbeans that were grown in red light produced

abundant nodules but those grown in blue light had only a few. Further experiments revealed that far-red light supplied to either the shoots or roots for only a few minutes inhibited nodulation, and that the inhibitory effect was counteracted by red light.

Temperature:

Since the early studies of Jones and Tisdale (1921), temperature has been recognized as an important factor in all stages of the nitrogen fixation process. It affects growth and survival of the root nodule bacteria, infection, nodule development, and nodule function.

Allison and Minor (1940) reported that temperature for growth of eight species of rhizobia was optimum between 27 and 31C. Growth rates of all species were depressed at 37C except that of Rhizobium meliloti. At 15C growth was only one-fourth to one-half that at 30C. Fred, Baldwin, and McCoy (1932) reported a somewhat lower temperature optimum for rhizobia, 20-28C.

Whiting and Schoonover (1920) indicated that temperature was an important factor in hastening nodulation. Gibson (1967a) found that subterranean clover plants nodulated most rapidly and with the highest rate at a root temperature of 30C. The maximum temperature at which nodules were formed was 33C and the minimum was 7C. Small et al. (1968) found that Pencil Podded Black Wax beans nodulated significantly better at 29C than at 25 and 27C. Barrios, Raggio, and Raggio (1963) using the same material found that nodulation was virtually nil at 12 and 33C. Lie (1971) reported that peas required a higher temperature (26C) for optimum nodulation only during the second and/or third day after inoculation. Later growth of the nodule and nitrogen fixation were reported to be far better at 20C.

Optimum temperature for fixation of nitrogen has been shown to vary with the host plant. Aprison, Maggee, and Burris (1954) indicated that for soybeans the optimum temperature was 25C. Small et al. (1968) reported an optimum at 21C for Pencil Podded Black Wax beans. Gibson (1963) reported that root temperatures between 22 and 26C were optimum for subterranean clover.

Meyer and Anderson (1959) found that increases in temperature above the optimum depressed nitrogen fixation to a greater extent than comparable decreases in temperature. They reported that nodules of subterranean clover fixed nitrogen actively at 20C, maximum at 25C, but hardly at all at 30C. This same effect was shown by Kuo and Boersma (1971) for soybeans. They found that the rate of fixation increased slightly over the temperature range of 10 to about 16C, rapidly over the range 16 to 24C, and decreased sharply at temperatures above 27C. Others that have reported a specific inhibitory effect of high temperature on nitrogen fixation are Mes (1959b), Pate (1961), and Hardy et al. (1968). Mes (1959b) reported that an increase in the day temperature from 18-21C to either 25 or 27C generally decreased the nitrogen content of peas and lupins. Pate (1961) observed a specific suppression of symbiotic fixation by purple vetch at growth temperatures above 27C. Hardy et al. (1968) reported that soybeans maintained in growth chambers for periods of 1 to 14 days at 30C had only 10 to 20% of the acetylene reducing activity of those at 20C.

It has also been shown that low temperatures depress nitrogen fixation. Mes (1959a) found that when velvet beans were dependent on rhizobia for their nitrogen nutrition, their growth and nitrogen

content were strongly depressed by lowering the night temperature from 18 to 10C. Gibson (1967b) reported that the rate of fixation by subterranean clover plants nodulated under favorable temperature conditions was reduced at temperatures below 23C. Hardy et al. (1968) reported that the acetylene reducing activity of soybeans was lower at 10 to 15C than at 20 to 30C. Kuo and Boersma (1971) suggested that limited translocation of carbohydrates to the root nodules at low soil temperatures can depress fixation.

Soil moisture:

Of all the environmental factors affecting nitrogen fixation, water supply has probably received the least attention. Only recently has the importance of this factor been realized. It is now known that moisture stress affects nodule formation, function, and longevity.

McKee (1961) observed that birdsfoot trefoil grown on soil low in moisture nodulated poorly with up to 55% of the plants still not nodulated 77 days after seeding. Plants on the same soil kept near field capacity were well nodulated at 37 days. Vincent (1965) reported that the failure of legumes to nodulate under dry conditions was the result of reduced rhizobial growth and survival. He also found that the suitability of the root hairs for invasion was adversely affected by drought.

Experiments by Wilson (1931) showed that up to a third of the nodules on red kidney bean plants were shed when soil moisture was substantially reduced. Masefield (1961) reported the same effect for French beans and peas as soil became progressively drier. Spent (1971) indicated that shedding of nodules resulted from the cytoplasmic continuum between infected and uninfected cells being destroyed.



Sprent (1971) categorized the effects of water stress on nodule function into two classes: (1) where the fresh weight of the nodules was between 80 and 100% of the maximum fresh weight, and (2) where it was below 80%. In the first case a submaximal rate of fixation resulted which was related to the water content. The effect was reversible. In the second case fixation ceased, the effect was irreversible, and the nodules degenerated.

Studies by Kuo and Boersma (1971) showed that as soil moisture suction increased from .35 to 2.5 bars the rate of nitrogen fixation decreased by approximately 50%. They attributed the decrease to a reduction in the translocation of carbohydrates to the root nodules.

Sprent (1971) studied the effect of waterlogging on nitrogen fixation. She found that nodules immersed in water reduced acetylene at only a very low level. She indicated that the low activity was due entirely to a lack of oxygen.

Soil reaction:

Soil reaction has long been recognized as a factor influencing the nitrogen fixation process. It affects growth and survival of the root nodule bacteria. It plays a role in the infection process and it indirectly influences fixation by causing irregularities in the nutrition of the nodule bacteria and the host plant.

Vincent (1965) reported that the inability of the root nodule bacteria to proliferate under acid conditions was the most important factor affecting the nodulation and growth of legumes on acid soils. Experiments by Bryan (1923) showed that soybean rhizobia were unable to survive in soil below pH 4.0, red clover rhizobia below pH 4.5, and alfalfa rhizobia below pH 5.0. Additional evidence of the sensitivity

of rhizobia to acid soil conditions have been reported by Loneragan and Dowling (1958), Peterson and Gooding (1941), Fred and Loomis (1917), and Albrecht (1933).

Munns (1968) found that nodulation of alfalfa was pH-dependent even though a large Rhizobium population was present. He observed that when nutrient solution was kept at pH 5.5, root hairs curled and became infected, but at pH 4.4 no curling or infection took place. He also noted that once the root hairs had curled, the nutrient solution could be lowered again to pH 4.4 without adverse effects on subsequent infection and nodule development. Later, Munns (1969) reported that the acid sensitive step was due to the inactivation of pectinase, an enzyme which breaks down pectin in the walls of the root hair and allows infection to occur. He postulated that the enzyme's pH-requirement might be closely correlated with that of nodulation.

Combined nitrogen:

The adverse effect of nitrogenous fertilizers on nodulation and nitrogen fixation in leguminous plants has been observed and studied by many workers. There is evidence that the inhibitory effect results from both a high total nitrogen content in the plant (Wilson, 1940; van Schreven, 1959; and Cartwright and Snow, 1962) and from a local and specific effect of nitrate on the early stages of nodule formation (Gibson and Nutman, 1960; Raggio, Raggio, and Torrey, 1965; and Tanner and Anderson, 1963).

Allison and Ludwig (1934) reported that the depressing effect of high nitrogen in the plant was largely overcome by promoting photosynthesis or by adding sucrose to the growth medium. Wilson (1940)



concluded from evidence obtained from a number of experiments that the degree of nodulation and nitrogen fixation was governed by the internal carbohydrate : nitrogen ratio of the plant and that deviation from some critical carbohydrate : nitrogen ratio would result in sub-optimal nodulation and nitrogen fixation. To verify Wilson's hypothesis, Cartwright and Snow (1962) and van Schreven (1959) conducted several experiments in which combined nitrogen and carbohydrates were applied to the roots, to parts of the root, and to the leaves of a number of legumes. They concluded as did Wilson that the effect of combined nitrogen was exerted internally.

Evidence of an external effect of combined nitrogen on nodulation was reported by Gaumann, Jaag, and Roth (1945). They used a divided root technique and found that nitrate inhibited nodulation only on the parts of the root in direct contact with nitrate-nitrogen. Tanner and Anderson (1963) showed later that nitrate inhibited the conversion by rhizobia of tryptophan to IAA, which is considered to be a prerequisite for root hair infection. It was also shown by Tonhazy and Pelczar (1954) that nitrite catalyzed the oxidation of IAA.

To further study the effects of external nitrate on nodulation, Raggio et al. (1965) developed a technique whereby excised roots of legumes could be fed nitrate separately through the base of the root or externally to the growth medium. They found that nitrate added to the growth medium significantly reduced nodule formation, while nitrate supplied via the base of the root had no inhibitory effect. They concluded that the effect of nitrate was completely an external effect. Cartwright (1967) was quick to disagree with their results. He repeated Raggio et al. experiment and found that nitrate was less readily

absorbed through the hypocotyl segment than through the root hairs. Cartwright indicated that the difference in nodulation observed by Raggio et al. was not due to an external effect of nitrate but was due to differences in uptake of nitrate. Cartwright maintained that the adverse effect of nitrate was from the accumulation of unsequestered nitrogen compounds and the depletion of carbohydrates in the plant.

Effect of Molybdenum on Nitrogen Fixation and Growth of Legumes

Molybdenum is now known to be a constituent of the nitrogen fixation enzyme, nitrogenase, (Klucas et al., 1968b) and to function in the fixation of atmospheric nitrogen by weakening the dinitrogen bond (Chatt et al., 1969). The involvement of molybdenum in nitrogenase explains many of the early observations that symbiosis dependent legumes required molybdenum.

Observations by Bortels (1937) gave the first indication that molybdenum was required by legumes. He observed that red clover, soybean, and pea plants grown in sand cultures contained more nitrogen and grew better when small amounts of molybdenum were applied. Experiments by Jensen and Betty (1943) showed that legume root nodules contained 5 to 15 times more molybdenum than other plant parts. Mulder (1948) reported that molybdenum deficient nodules of peas were smaller and more numerous than those on normal plants, and were not pink as usual but were yellow and brownish-gray. Virtanen and Laine (1946) had reported earlier that such nodules fixed little nitrogen.

Many workers have reported significant response to molybdenum on leguminous crops. Anderson (1948) found that subterranean clover plants that were molybdenum deficient were pale green and contained only 2.5% nitrogen as compared to 3.4% nitrogen in normal dark green plants

treated with 140 g of molybdenum per hectare. In addition where molybdenum was applied the yield of clover was increased from 928 kg to 1706 kg of dry matter per hectare. Hagstrom and Berger (1963) reported that Na-molybdate applications of 2.25 kg/ha on Wisconsin soils (containing 0.26-0.46 ppm total molybdenum) increased yields of red clover and yields, nodulation, and nitrogen content of soybeans and peas. In a three year study on alfalfa, Younge and Takahashi (1953) reported a highly significant response to molybdenum. The first full year of production after fertilization resulted in 146% increase in dry matter production and 163% increase in yield of protein. Others that have reported beneficial effects of molybdenum on legumes are True and Shrewsbury (1958), Reiseanaur (1957), Anderson and Spencer (1949), Parker and Harris (1962), and Gupta (1970).

Symptoms of molybdenum deficiency in legumes are most often the well known signs of nitrogen starvation - pale yellow foliage and stunted growth. This is due to the large requirement for molybdenum in the nitrogen fixation process. Symptoms of molybdenum excess in legumes have not been reported although additions of molybdenum have in several instances resulted in reduced yield. In Indiana, Foy and Barber (1959) reported that yield of alfalfa was reduced when molybdenum was applied with high rates of lime. In a similar situation in Iowa, deMooy (1970) observed a reduction in yield of soybeans when 230 g/ha of molybdenum was applied as a seed treatment on calcareous soil. Jackman (1956) found that dusting clover with molybdenum depressed growth but showed no abnormal visual symptoms. He suggested that the depression might be the result of micronutrient interaction. Molybdenum has been shown to interact with several elements. MacKay

Chipman, and Gupta (1966) observed a mutual antagonism between copper and molybdenum. Toxicities of one nutrient could be alleviated by application of the other; and deficiencies of one nutrient were aggravated by excess of the other. Davies (1956) reported that molybdenum and sulfate interact. Stout et al. (1951) attributed this effect to competition between similar sized and charged ions. Other elements known to interact with molybdenum are phosphate, manganese and potassium.

Effect of Sulfur on Nitrogen Fixation and Growth of Legumes

Although sulfur is a constituent of the nitrogen fixation enzyme, nitrogenase, it has not been shown to affect nitrogen fixation, directly. An indirect affect on nitrogen fixation was reported by Anderson and Spencer (1949). They found that sulfur deficiency in clover inhibited protein synthesis which in turn inhibited nitrogen fixation. Stewart and Porter (1969) found a large accumulation of non-protein N (nitrate, amides, and free amino acids) in sulfur deficient beans. Protein N accounted for less than 40% of the total N in the sulfur deficient plants as compared with 75% of the total N in plants adequately supplied with sulfur. Wooding, Paulsen, and Murphy (1970) reported that soybeans grew poorly when sulfur was deficient. They also found that nodule quantity and nitrogen fixation were reduced.

Sulfur fertilization has also been shown to have a pronounced effect on protein quality. Mertz, Singleton, and Garly (1952) analyzed sulfur deficient alfalfa and alfalfa adequately supplied with sulfur for nearly all the amino acids. Their study showed that 16 amino acids were 35 to 65% lower when sulfur was deficient. Sheldon, Blue, and Albrech (1957) reported that the methionine content of soybeans grown in nutrient solution containing 96 ppm S was twice as high as those

grown in solution containing 16 ppm S. Mertz and Matsumoto (1956) found that in sulfur deficient alfalfa the arginine content doubled, while the concentration of glutamic acid, histidine, and isoleucine decreased to one-half and methionine content to one-third that of normal plants.

A number of research workers have used total sulfur in plants as an indicator of the sulfur status. Pumphrey and Moore (1965) reported that for alfalfa the critical level was .22% S at the early bloom stage. Alway (1927) reported values ranging from .07 to .50% S and assigned .30% as the level indicative of adequate sulfur. Jordan and Bradley (1958) found that in field grown clover the critical level of sulfur was between .10 and .16%.

Total N to total S ratio has also been used as an index for determining sulfur response. Pumphrey and Moore (1965) reported that a N:S ratio of 11:1 was the critical level in diagnosing sulfur deficiency. Ratios lower than 11:1 indicated adequate sulfur. Higher ratios gave significant yield response to sulfur 96% of the time. Stewart and Porter (1969) concluded from a study on beans that when the N:S ratio of the plant was above 16:1 sulfur was limiting protein synthesis, and when the ratio was 20 or more, sulfur was severely deficient.

Growth response to sulfur by leguminous crops has been extensive. Conrad, Hall, and Chaugale (1948) reported a 3- to 4-fold increase in the growth of bur clover from application of sulfur in the Ojai Valley of California. On a sulfur deficient area of Minnesota, Caldwell, Seim, and Rehm (1969) found that sulfur tripled the yield of alfalfa. Results of an experiment conducted by Singh (1970) showed that application of



sulfur resulted in a highly significant increase in yield of peas, 832 kg/ha vs. 1816 kg/ha for sulfur treated peas. Wooding et al. (1970) reported that sulfur deficiency significantly decreased the growth of both nodulated and non-nodulated soybean seedlings. Others that have reported response to sulfur are Neller (1926), Harward, Chao, and Fang (1962), Stewart and Porter (1969), and Jones (1962).

Effect of Lime on Nitrogen Fixation and Growth of Legumes

The beneficial effects of lime on the growth of legumes have been recognized for centuries. Fred, Baldwin, and McCoy (1932) reviewed the subject and concluded that liming most soils (except those near pH 7.0 or higher) stimulated nodulation and nitrogen fixation. Many of the early investigators attributed the response to the neutralizing value of lime which improved conditions for rhizobial growth and survival. Buckman and Brady (1969) recently listed several other ways lime might benefit plant growth; (1) by correcting deficiencies of calcium and magnesium, (2) by neutralizing toxic compounds, organic and/or inorganic, (3) by retarding plant diseases, (4) by improving the soil physical condition, and (5) by increasing the chemical availability of plant nutrients.

In a study on the nodulation and growth of soybeans, Albrecht (1932) reported that the principal benefit of lime was calcium as a plant nutrient. Burton, Allen, and Berger (1961) showed that growth, pod yields, and nitrogen fixation of nodulated bean plants were greatly enhanced by high levels of calcium and magnesium. Plants furnished 300 ppm of calcium and 100 ppm of magnesium produced three times as many green pods and contained twice as much nitrogen as plants receiving only 100 ppm calcium and 25 ppm of magnesium. Loneragan (1959) reported



that the nitrogen fixation process was rarely affected by deficiencies of calcium in the root nodule, but was most likely influenced by a reduced supply of carbohydrate resulting from calcium deficiency in the host plant.

There have been no reports that magnesium specifically affects the nitrogen fixation process (van Schreven, 1958). Graham (1938) indicated that magnesium might play an indirect role in the production of nodules and in nitrogen fixation by rendering the supply of calcium more effective.

Lime can have a sizeable effect on nitrogen fixation by altering pH which influences the availability of a number of plant nutrients. One of the most pronounced is the effect of lime on the response of legumes to molybdenum. In many areas it has become a common practice to control molybdenum deficiencies entirely by liming the soil to pH 6.0 or 6.5. Evans, Purvis, and Barber (1951) reported that Nixon loam extracted with normal ammonium acetate over a range of pH 5.0 to 9.5 resulted in increased amounts of available molybdenum with increasing pH.

Sulfur is another element that is influenced by lime. Elkins and Ensminger (1971) reported that sulfate-S in soil solution of a Dothan Clay increased three-fold in going from pH 5.0 to 5.5 and nearly 17-fold when the pH was raised from 5.0 to 7.6.

MATERIALS AND METHODS

1970 Field Study

In 1970 a field experiment was established at the Montcalm Experimental Farm, Entrican, Michigan to evaluate the effects of soil temperature, plant age, and diurnal influences on nitrogen fixation by dark red kidney beans. The soil was a McBride sandy loam and had a pH of 6.8. On June 8 the experimental area was planted to Charlevoix dark red kidney beans. Normal cultural and tillage practices were followed except nitrogen fertilizer was not applied. Seeding rate was 140,850 plants/ha, basic fertilizer was 280 kg of 0-20-20/ha, and row spacing was 71 cm. The beans emerged on June 15. Three days after emergence the center two rows of the plots were covered with sheets of clear and black plastic mulch in order to establish differences in soil temperature. The sheets of plastic were pulled and stapled together in the row around the beans as shown in Plate 1 below. Treatments established were (1) check - no



Plate 1. Photograph showing the clear plastic mulch treatment.

plastic mulch, (2) black plastic mulch - 18 days then removed, (3) black plastic mulch - entire growing season, (4) clear plastic mulch - 18 days then removed, and (5) clear plastic mulch - entire growing season. The treatments were arranged in a randomized complete block and were replicated four times. Plot size was 4.3 x 15.2 meters.

To evaluate the effects of the plastic mulch treatments and at the same time determine the influence of plant age on nitrogen fixation, nitrogen fixation measurements were taken every 4-5 days until 67 days after plant emergence. The Acetylene-ethylene assay described by Hardy et al. (1968) was used to measure nitrogen fixation. The procedure was to harvest five bean plants from each plot, decapitate the tops, and place the roots and intact nodules in a half-pint fruit jar that was made air tight with a rubber jar ring and a zinc cap fitted with a rubber serum stopper. Atmospheric nitrogen was removed from the sealed jars by inserting a hypodermic needle through the serum stopper and evacuating and flushing three times with a gaseous mixture of 78% argon, 22% oxygen, and 0.04% CO₂ using a hand vacuum pump and the gas manifold apparatus shown in Plate 2. Samples for a complete replication of treatments were evacuated and prepared for assay at the same time to reduce variation. Pressure after flushing was adjusted to atmospheric with a mercury manometer. The jars were removed from the flushing apparatus and 23 ml of the gaseous mixture was withdrawn by hypodermic syringe and replaced with acetylene. The jars were then placed in the soil where the plants were dug so roots and nodules would incubate at their former growth temperature. Reduction of acetylene to ethylene by the nodules was stopped after one hour by injecting 11 ml of 6 N H₂SO₄ after removing 11 ml of gas.

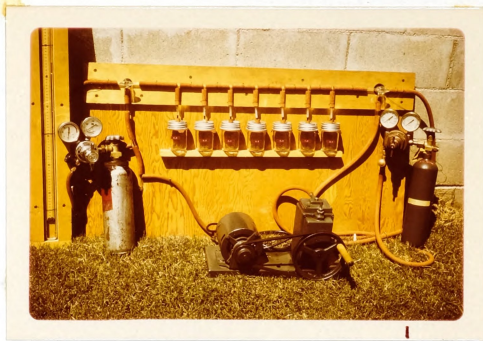


Plate 2. Photograph showing the hand vacuum pump and the gas manifold apparatus used in the acetylene-ethylene assay.

Ethylene formed was detected and measured by gas chromatography using a H-flame ionization detector and a stainless steel Porapak N (100-120 mesh) column (116 cm x 31 mm) operated at 80C. Carrier flow rate was 30 ml helium/min. Retention times for ethylene and acetylene peaks were 28 and 45 seconds, respectively. Ethylene concentration of samples was measured by comparing chromatograph peak heights of duplicate injections of 0.1 ml gas samples with a standard curve. Volume of the gaseous phase was determined by measuring the volume of water required to fill the jar with the roots and nodules inside. Production of ethylene was converted to nitrogen fixation activity using a theoretical conversion factor of one-third mole N_2 fixed for each mole of ethylene formed as shown in the following equation.

$$\begin{aligned} \text{Kg N} \\ \text{fixed} \\ \text{per ha} = \frac{1(\text{peak ht})(\text{slope std})}{3(22,400 \text{ ml C}_2\text{H}_4/\text{mole})} \left(\frac{\text{vol sample}}{\text{vol std}} \right) \left(\frac{140,850 \text{ plants/ha}}{5 \text{ plants/sample}} \right) \left(\frac{.028 \text{ kg N}}{\text{mole N}_2} \right) \\ \text{per day} \quad \times (\text{diurnal factor } .81)(24 \text{ hrs}) \end{aligned}$$

The diurnal factor, 0.81, was calculated from data collected on August 4-5, 1970 (Figure 2). This factor is required in order that nitrogen fixation measurements taken during the day light hours might be extrapolated to a 24 hour period.

Each time plants were harvested for nitrogen fixation measurements, soil temperature and soil moisture were recorded. Soil moisture was determined by oven-drying approximately 300 g of soil taken from the root zone where the plants were dug. Soil temperature was measured with a small probe-type thermometer (12.6 cm depth). At 35 and 44 days after plant emergence, whole plant samples (excluding the roots and nodules which were used for nitrogen fixation measurements) were collected and analyzed for nitrogen and other chemical elements. The plants were dried (65C), weighed and ground to pass a 40 mesh screen prior to analysis.

A diurnal sampling for nitrogen fixation was made fifty-three days after the beans emerged (Aug. 4-5). Samples were collected every two hours from guard rows of the check plots and were analyzed by the procedure described previously. Soil and air temperatures were also recorded. Grain yields were determined by harvesting 15.2 meters of row.

1971 Field Studies

Two sites were selected in the spring of 1971 for experiments to study the effect of lime, sulfur, and molybdenum on nitrogen fixation

and yield of dark red kidney beans. One site was located in Ionia County on the Robert Westbrook farm and the other in Montcalm County on the Theron Comden farm. Both soils were a sandy loam, had a pH of 5.9, and were on the borderline of molybdenum response according to ammonium oxalate extraction. Three rates of lime (0, 2800, and 5600 kg/ha), two sulfur rates (0 and 45 kg/ha), and two molybdenum levels (0 and 70 g/ha) were established at each location. The experimental design was a split-split plot replicated four times with lime rates as the main plots, sulfur rates as the subplots, and molybdenum levels as the sub-subplots. Plot size was 4.3 x 15.2 meters. Dolomitic, hydrated lime and sulfur (applied as K_2SO_4 with the 0 sulfur plots receiving equivalent amounts of potassium as KCl) were broadcast, disked and plowed under prior to planting. Molybdenum was applied as a seed treatment in the form of sodium molybdate powder. Charlevoix dark red kidney beans were planted on June 3 and June 4 at the Ionia and Montcalm locations, respectively. Planting time fertilizer consisted of 123 kg of 0-46-0/ha. Seeding rate was 140,850 plants/ha. Nitrogen fertilizer was not applied. The beans emerged on June 10 at the Montcalm location and on June 11 at the Ionia location. Nitrogen fixation measurements were taken twice at the Ionia location (61 and 75 days after plant emergence) and four times at the Montcalm location (32, 46, 60, and 74 days after plant emergence). The same procedure for measuring nitrogen fixation was used as described previously except that H_2SO_4 was not used to stop the reduction of acetylene to ethylene. Instead, a syringe of the gas after incubation was transferred to a pre-evacuated serum bottle. The reason for modifying the procedure was that H_2SO_4 reacted with materials in the jars and produced volatiles which caused pressure inside the jars to build up.



Plant samples were collected at the early bloom stage (32-33 days after emergence) and were dried, weighed, and ground prior to analyzing for nitrogen, sulfur, and other chemical elements. Soil samples were taken from all zero molybdenum plots on August 5 for $\text{SO}_4\text{-S}$ and pH determinations. Grain yields were measured by harvesting 41.1 meters of row. A small sample of grain from each plot was dried, ground, and used for determination of moisture, nitrogen, sulfur, and methionine. All grain yields were adjusted to 18% moisture.

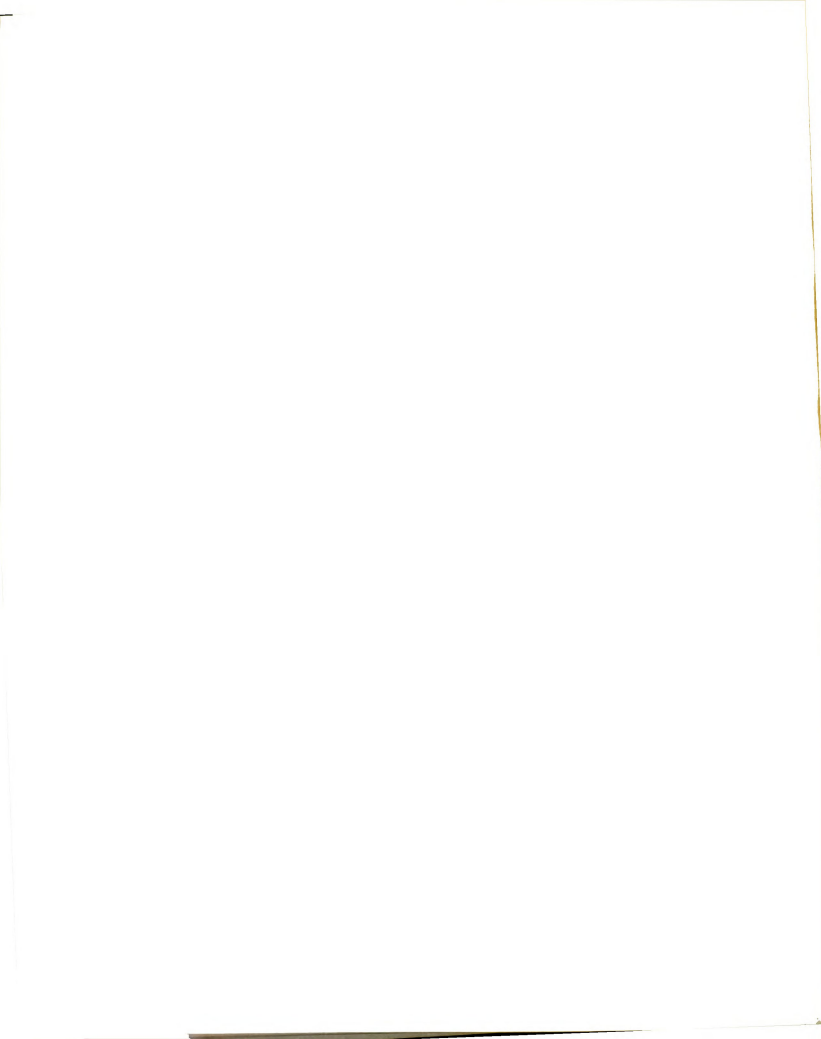
Analytical Procedures

Soil Analysis

Soil pH was measured with a Beckman glass electrode using a 1:1, soil:water ratio. The soil-water suspension was stirred, allowed to stand for 15 minutes and restirred prior to reading.

Available molybdenum was determined by shaking 25 g of soil overnight with 250 ml of 0.34 N ammonium oxalate solution buffered at pH 3.3 with oxalic acid. The resulting suspension was centrifuged (2500 rpm for ten minutes) and a 150 ml aliquot of the supernatant solution was evaporated to dryness. The residue was placed in a muffle furnace at 400C for four hours and then dissolved in 20 ml of 50% HCl. The resulting solution representing 15 g of soil was used for determination of molybdenum by the thiocyanate-stannous chloride procedure (Reisenaur, 1965).

Sulfate sulfur was determined at the University of Wisconsin Soil Testing Laboratory, Madison, Wisconsin. Twenty g of oven dry soil was shaken with 50 ml of 500 ppm P as Ca $(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ in 2 N HOAc solution for 30 minutes. One-tenth g of activated charcoal was added and the solution was shaken for another 15 minutes. The suspension



was filtered through a No. 2 then No. 42 filter paper. Ten ml of the filtrate were combined with 10 ml of 6.25% HNO_3 containing 2 ppm S as K_2SO_4 (seed solution) and 5 ml of 8.7 N HOAc containing 0.5% gum acacia. Barium chloride (0.5 g) was then mixed with the sample and after a ten minute waiting period the samples were read on a Turner model 111 Fluorometer-Nephelometer with a 436 mμ primary filter and a 415 mμ secondary filter.

Plant Analysis

Total nitrogen in plant and grain samples was determined by the micro-Kjeldahl procedure (Bremner, 1960). Fifty mg of sample was weighed on a cigarette paper, folded in, and transferred quantitatively to a 50 ml Kjeldahl flask. Approximately 1.33 g of K_2SO_4 -catalyst was added and washed down with 2 ml of deionized water. Fifteen minutes later 2 ml of concentrated H_2SO_4 was added. The samples were then placed on a digestion rack and digested for one-half hour past the point of clearing. After cooling and after adding 10 ml of deionized water, the samples were distilled with NaOH into a H_3BO_3 -indicator solution. The amount of nitrogen in the distillate was determined by titrating with 0.0227 N H_2SO_4 .

Total Sulfur in plant and grain samples was determined at the International Mineral and Chemical Corporation, Growth Sciences Center, Libertyville, Illinois 60048. Fifty mg of sample was burned in a stream of oxygen with a Leco Rapid Sulfur Determinator. The SO_2 evolved was collected and titrated by the iodometric method.

P, K, Ca, Zn, Mn, Fe, and Cu in plant tissue was determined by x-ray emission spectrography at the International Mineral and Chemical Corporation, Growth Sciences Center, Libertyville, Illinois 60048.



Methionine was determined microbiologically by assay with Leuconostoc mesenteroides P-60 using dehydrated Bacto-Methionine Assay Medium (Difco Laboratories, Inc., Detroit, Mich.). Samples were prepared for assay by hydrolyzing 0.5 g of sample with 10 ml of 20% HCl in the autoclave for 30 minutes at 1.0 kg/cm^2 pressure (Evans, Bandemer, and Bauer, 1962). The samples were cooled, adjusted to pH 7.0 with 12 and 4% NaOH, filtered, and brought to a volume of 100 ml with distilled water. Duplicate portions (0.8 ml) of each sample were pipetted into equal sized test tubes. Five ml of Bacto-Methionine Assay Medium was added and final volume brought to 10 ml with distilled water. The tubes were capped, sterilized in the autoclave for 10 minutes (1.0 kg/cm^2 pressure), cooled to 37C, and inoculated with one drop of L. mesenteroides P-60 culture (Prepared by inoculating Bacto Micro Inocula Broth, Difco Laboratories, Inc., Detroit, Mich. directly from a stock culture, incubating overnight at 37C and diluting 1:10 with 0.85% NaCl solution). The samples were then placed in an incubator for 24 hours at 37C. Following incubation, the tubes were placed in flowing steam for 10 minutes to stop organism growth. Optical density (700 mμ) of the cultures was measured and compared with a standard curve to determine methionine.

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RESULTS AND DISCUSSION

Effect of Plastic Mulch Treatments on Nitrogen Fixation, Plant Composition, and Yield of Dark Red Kidney Beans

Clear and black plastic mulch was used in the field to establish differences in soil temperature during the nodulation period and during the entire growth cycle of kidney beans. The purpose was to determine whether soil temperature during these periods was in any way limiting nitrogen fixation. Soil temperature was increased most by the plastic mulch treatments early in the season when the beans were small (Table 1). As the season progressed the plastic mulch treatments had less effect. This trend is undoubtedly the result of increased soil shading by the growing bean plants. During the period (3-18 days after plant emergence) when nodulation occurred, soil temperature under clear plastic was on an average 4.8C warmer than bare soil and 4.0C warmer than soil covered with black plastic. On a season basis, clear plastic mulch increased soil temperature 1.5C while black plastic mulch had little or no effect. Soil temperature of all treatments was maximum 15 days after plant emergence (avg. 32.1C) and minimum 35 days after emergence (avg. 15.2C). The day to day variation in soil temperature coincided closely with changes in air temperature.

Since there was some concern about the plastic mulch treatments influencing soil moisture, moisture content of the soil was monitored. As shown in Table 2, the plastic mulch treatments had little effect on soil moisture. On only one occasion (21 days after plant emergence) was soil moisture differences among treatments significant. Soil moisture was maximum for all treatments 30 days after plant emergence

Table 1. Effect of plastic mulch treatments on soil temperature at various stages after emergence of dark red kidney beans

Plastic mulch treatments	Days after plant emergence						
	5	10	15	21	25	30	35
	Soil temperature (C)						
No plastic	19.6	22.8	31.1	27.0	21.0	22.8	14.9
Black 18 days	21.0	24.5	30.8	25.5	21.0	21.5	14.8
Black entire	20.8	24.5	30.4	30.9	21.8	21.9	15.6
Clear 18 days	25.2	28.4	34.0	26.3	21.1	21.8	15.0
Clear entire	25.2	28.9	34.2	29.9	22.2	22.6	16.0
LSD (0.05)	1.8	1.3	1.4	2.0	0.6	0.5	0.3

Plastic mulch treatments	Days after plant emergence						
	39	44	49	53	58	67	Avg. 5-67
	Soil temperature (C)						
No plastic	18.5	23.1	22.4	25.9	23.2	21.5	22.6
Black 18 days	18.5	22.2	21.2	26.0	22.9	22.0	22.5
Black entire	18.5	22.4	21.2	24.4	22.2	21.9	22.8
Clear 18 days	18.5	22.8	22.1	26.0	23.6	22.4	23.6
Clear entire	19.0	22.6	21.9	25.2	23.4	22.6	24.1
LSD (0.05)	0.4	0.5	0.7	ns	0.9	ns	0.7



Table 2. Effect of plastic mulch treatments on soil moisture at various stages after emergence of dark red kidney beans

Plastic mulch treatments	Days after plant emergence						
	5	10	15	21	25	30	35
	Soil moisture (%)						
No plastic	-	-	10.2	7.2	12.2	15.5	12.5
Black 18 days	-	-	12.9	9.4	13.4	17.0	13.9
Black entire	-	-	13.3	10.3	13.6	15.0	12.6
Clear 18 days	-	-	13.9	9.4	13.2	16.6	11.8
Clear entire	-	-	13.6	11.6	13.2	15.7	14.9
LSD (0.05)	-	-	ns	1.9	ns	ns	ns

Plastic mulch treatments	Days after plant emergence						
	39	44	49	53	58	67	Avg. 15-67
	Soil moisture (%)						
No plastic	12.8	9.7	6.2	4.5	10.7	15.0	10.6
Black 18 days	14.0	10.1	6.5	4.4	11.6	15.4	11.7
Black entire	13.0	11.2	7.1	5.1	11.7	15.8	11.7
Clear 18 days	13.6	9.8	6.8	5.0	11.2	15.9	11.6
Clear entire	12.4	10.8	6.8	4.8	11.6	16.0	12.0
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns

(avg. 16.0%) and minimum 53 days after emergence (avg. 4.8%). Soil moisture at the 53 day sampling was very near the wilting moisture percentage of the soil. Wilting of some plants was observed.

Plant growth differences among treatments were noted early in the season. Plants grown on soil covered with clear plastic were larger than plants grown on soil covered with black plastic which in turn were larger than plants grown on bare soil. These differences in growth were still evident 44 days after plant emergence as shown by the plant dry weights (Table 5).

When plants were harvested for nitrogen fixation measurements ten days after plant emergence, a difference in nodulation was observed. Plants grown on bare soil had not yet formed nodules while plants grown on soil covered with either black or clear plastic had several nodules per plant. This difference in nodulation is reflected in the 10 day nitrogen fixation data (Table 3). The no plastic treatment did not show any nitrogen fixation. When nitrogen fixation was measured five days later, nodules were found on all plants. These findings are in agreement with those reported by Gibson (1967a). He found that root temperature markedly affected the amount of time it took for the first nodule to appear. Nodules appeared first at 30C and 5 and 8 days later at 17 and 12C, respectively.

The effect of the plastic mulch treatments on rate of nitrogen fixation by the dark red kidney beans is shown in Table 3. Out of the 13 samplings for nitrogen fixation, differences among treatments were significant only three times. In general, plants from treatments which had the lowest soil temperatures (No plastic, Black 18 days, and Black entire) fixed the most nitrogen. The least nitrogen was



Table 3. Effect of plastic mulch treatments on rate of nitrogen fixation by dark red kidney beans at various stages after plant emergence

Plastic mulch treatments	Days after plant emergence						
	5	10	15	21	25	30	35
	N ₂ Fixed (kg N/ha/day)						
No plastic	.000	.000	.006	.056	.216	.143	.223
Black 18 days	.000	.013	.001	.029	.137	.177	.286
Black entire	.000	.014	.012	.018	.112	.281	.382
Clear 18 days	.000	.014	.015	.019	.155	.227	.335
Clear entire	.000	.016	.005	.016	.071	.254	.274
LSD (0.05)	-	ns	ns	.027	.105	ns	ns

Plastic mulch treatments	Days after plant emergence						
	39	44	49	53	58	67	Avg. 10-67
	N ₂ Fixed (kg N/ha/day)						
No plastic	.327	.274	.285	.041	.167	.080	.151
Black 18 days	.411	.229	.320	.040	.174	.062	.156
Black entire	.325	.275	.258	.040	.178	.065	.163
Clear 18 days	.273	.313	.153	.016	.095	.082	.142
Clear entire	.271	.208	.121	.030	.060	.073	.116
LSD (0.05)	ns	ns	.115	ns	ns	ns	ns

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fixed by plants grown on soil covered the entire season with clear plastic. This treatment had the highest soil temperatures. The plastic mulch treatments that were removed at 18 days did not improve nitrogen fixation even though it was thought that these treatments would provide the best conditions for fixation of nitrogen. According to previous literature, high soil temperatures early would promote nodulation and low soil temperatures later would benefit fixation of nitrogen. Nitrogen fixation by plants grown on bare soil was as good as any of these treatments. These results indicate that nitrogen fixation in the field is not limited by too low a soil temperature. If anything, the soil temperatures may be too high for optimum nitrogen fixation.

The effect of the plastic mulch treatments on chemical composition of the bean plants, 35 and 44 days after emergence, is shown in Table 4. At the 35 day sampling phosphorus was significantly higher in plants from plots on which clear and black plastic remained throughout the growing season. Potassium was significantly higher in plants from all plots on which plastic was applied. These differences in uptake are apparently a reflection of the differences in soil temperature resulting from the plastic mulch treatments. Robinson, Sprague and Cross (1959) found that phosphorus uptake by red clover was determined to a large extent by the temperature of the soil. Phosphorus uptake increased markedly with increased soil temperature. Worley, Blaser, and Thomas (1963) reported that potassium uptake by soybeans increased 8-fold between soil temperatures of 15 and 30C. All differences in plant composition that were significant at the 35 day sampling were no longer significant at the 44 day sampling. The beans were apparently

Table 4. Effect of plastic mulch treatments on chemical composition of dark red kidney bean plant tissue - 35 and 44 days after plant emergence

Plastic mulch treatments	35 days after plant emergence								
	N	P	K	Ca	Mg	Zn	Mn	Fe	Cu
	%					ppm			
No plastic	4.0	.32	2.65	2.00	.56	34	115	933	13
Black 18 days	3.9	.34	3.24	1.85	.51	37	110	920	14
Black entire	4.0	.36	3.22	1.84	.54	42	95	774	14
Clear 18 days	3.8	.34	3.16	1.74	.48	36	101	808	13
Clear entire	3.8	.36	3.00	1.84	.51	40	105	661	14
LS D (0.05)	ns	.03	.31	ns	ns	ns	ns	ns	ns

[illegible]



able to overcome, with time, differences in uptake caused by soil temperature in early growth.

Yield of the dark red kidney beans was not significantly increased by the plastic mulch treatments (Table 5). An effect of the plastic mulch treatments is indicated since all plastic treatments resulted in slightly higher yield than the no plastic treatment. This difference in yield would have to be ascribed to improved conditions for growth rather than to increased nitrogen fixation.

Table 5. Effect of plastic mulch treatments on plant dry weight and yield of dark red kidney beans

Plastic mulch treatments	44 day* plant dry wt. (g)	58 day* plant dry wt. (g)	Grain yield (kg/ha)
No plastic	91	134	2457
Black 18 days	107	118	2823
Black entire	107	122	2791
Clear 18 days	108	117	2750
Clear entire	138	160	2750
LSD (0.05)	18	ns	ns

* Weight of five plants.

Seasonal and Diurnal Variation in Nitrogen Fixation
by Dark Red Kidney Beans

The rate at which nitrogen is fixed in relation to age of the dark red kidney bean plant is shown in Figure 1. Fixation of nitrogen was first observed ten days after emergence. The initial rate of fixation for the first 20 days was .010 kg N fixed/ha/day. Thereafter, nitrogen fixation increased at a rate of .017 kg N fixed/ha/day until the maximum rate was reached 39 days after emergence. The high rate of fixation during this period is apparently the result of (1) the increased demand for nitrogen by the developing bean pods and (2) the increased photosynthetic activity and synthesis of carbohydrates. During the period 40 to 65 days after emergence, nitrogen fixation declined at a rate of .009 kg N/ha/day. The sudden drop in fixation 54 days after emergence was due to moisture stress (Table 2).

Summation of daily fixation rates in Figure 1 indicate that only 9.12 kg of nitrogen was fixed during the growing season. The total nitrogen fixed by the dark red kidney beans is substantially below the average Kjeldahl fixation values for fieldbeans reported by Lyon and Bizzell, 1934 (64 kg N fixed/ha/season) and Erdman, 1959 (45 kg N fixed/ha/season). Kjeldahl estimates of nitrogen fixation generally have a tendency to overestimate the amount of nitrogen fixed because the values are based on increases in the soil or plants or both over a period of time and often include sources of nitrogen other than that fixed by the rhizobium-legume association. The 9.12 kg of nitrogen fixed by the dark red kidney beans is also substantially below the 70 kg of N fixed/ha/season measured by the acetylene-ethylene procedure for soybeans by Hardy et al. (1968). Comparison of data collected by Hardy et al. and that collected in



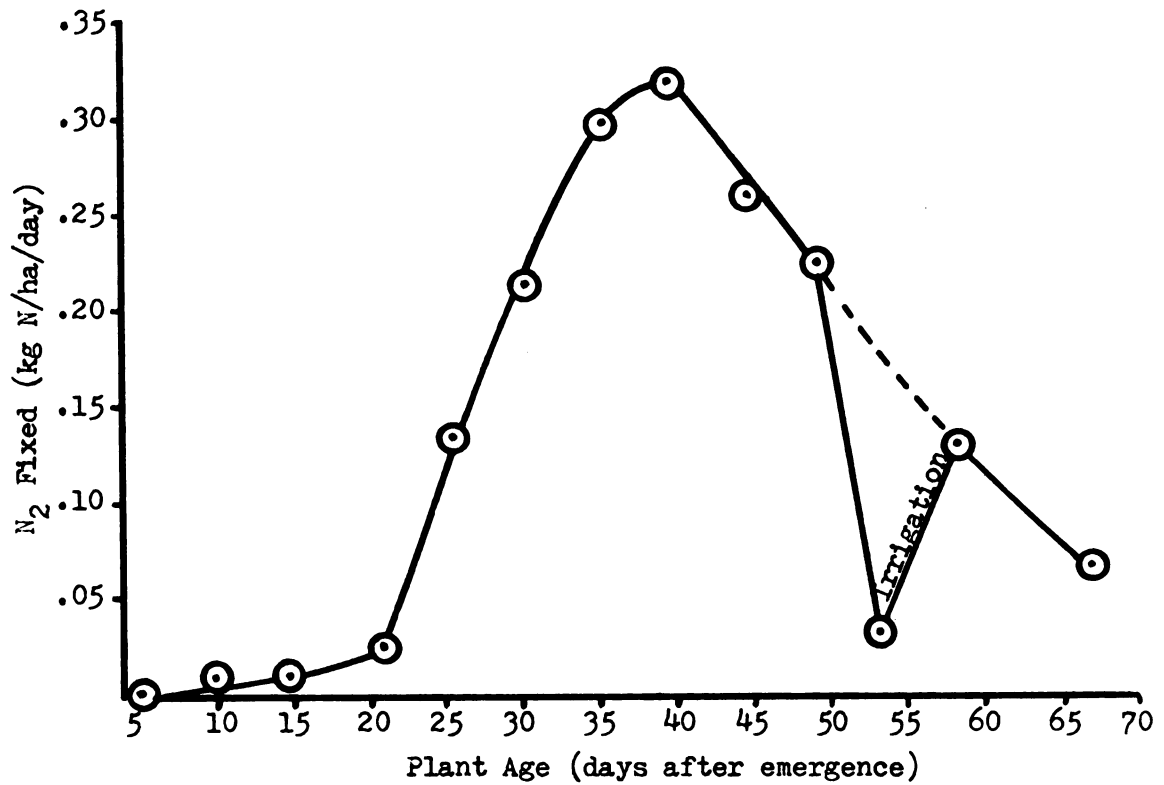


Figure 1. Rate of nitrogen fixation in relation to age of the dark red kidney bean plant. Each point represents 100 plants and is the mean fixation rate for all plastic mulch treatments.



this experiment indicate that the difference in nitrogen fixed is primarily the result of three factors, (1) the rate of fixation per plant is twice as high for soybeans, (2) the soybean fixation period is twice as long, and (3) the number of plants per hectare is substantially higher for soybeans (363,000 plants/ha) than for dark red kidney beans (140,850 plants/ha).

Figure 2 shows the diurnal pattern of nitrogen fixation by dark red kidney beans. Nitrogen fixed during the 24-hour period (11:AM Aug. 4 - 11:AM Aug. 5) was twice as high during the daylight hours as it was at night. This same relationship between light and rate of nitrogen fixation was observed by Hardy et al. (1968). Campbell and Lees (1967) reported that Rhizobium in the nodules required some photosynthetic product(s) to fix nitrogen and only fixed appreciable amounts when the plants were actively photosynthesizing.

A noticeable parallelism between air temperature and nitrogen fixation during the diurnal cycle is evident (Figure 2). This is probably only coincidental since sunlight and air temperature tend to vary together. If air temperature is involved, its most likely effect would be on the translocation of photosynthetic products from the plant tops to the root nodules. Studies by Swanson and Bohney (1951) showed that transport of sucrose from bean leaves was maximum when petiole temperatures were between 20 and 30C, but at petiole temperatures of 5 to 7.5C, rate of transport was reduced by as much as 50%.

Soil temperature during the 24 hour cycle appeared to have no effect on nitrogen fixation. This is not surprising since soil



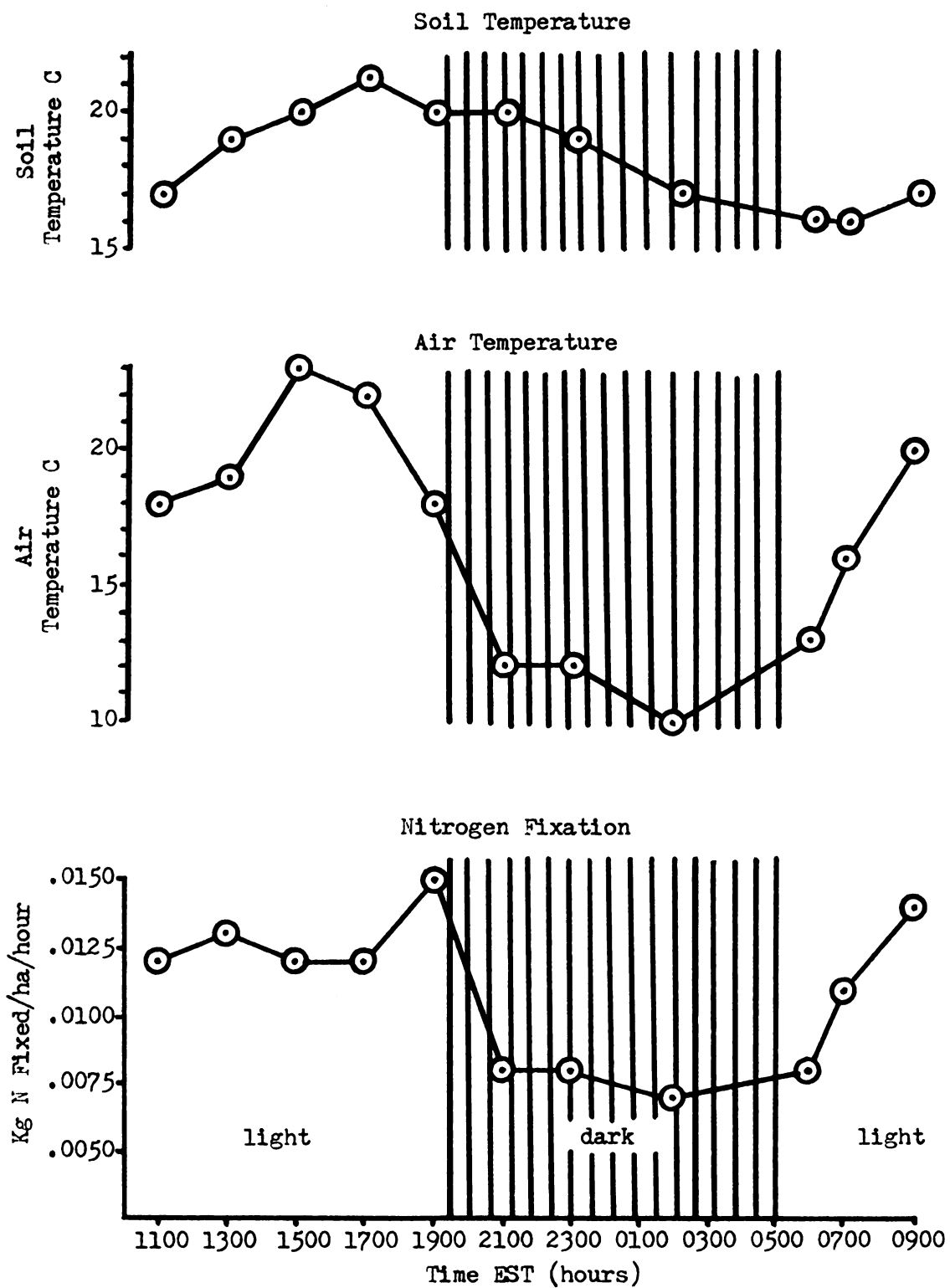
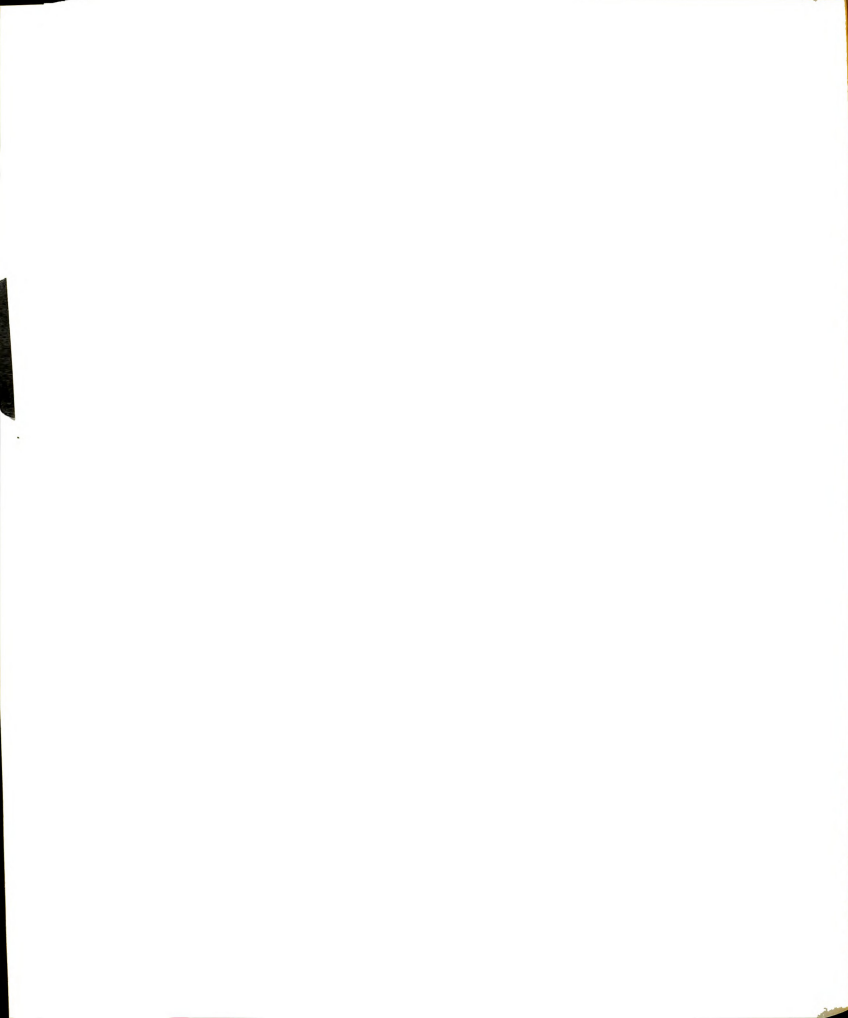


Figure 2. Diurnal variation in soil temperature, air temperature, and rate of nitrogen fixation by dark red kidney beans during the 24 hour period, 11:AM August 4 - 11:AM August 5.



temperatures only varied between 16 and 21C. Soil temperature has mainly been shown to influence nitrogen fixation at the extremes of the soil temperature range (below 10C and above 30C). Results similar to that observed here were reported by Roughly and Dart (1969). They found that 24 hour exposures of subterranean clover plants to soil temperatures ranging from 19 to 7C had little effect on nitrogen fixation.

Effect of Lime, Sulfur, and Molybdenum on Nitrogen Fixation, Plant Composition, and Yield of Dark Red Kidney Beans

Treatment effects on nitrogen fixation, plant composition, and yield of dark red kidney beans at the Montcalm and Ionia locations were almost identical. Data from both locations were combined and analyzed as a unit whenever possible. The criteria for combining data depended on logical grouping as well as homogeneity of error variance.

Growth and color differences among treatments were observed early in the growing season (14 to 21 days after the beans emerged). Plants which received neither lime nor sulfur (Plate 3) were light green, slightly stunted, and appeared nitrogen deficient in comparison with plants treated with sulfur (Plate 4) or lime. Since plants at this stage had not yet developed nodules the response was not from increased nitrogen fixation. Apparently, lime and sulfur had improved growth by supplying or making available some needed plant nutrient. Insight as to which element or elements might be involved is given by the chemical composition of the bean plants at the early bloom stage (Table 6). Chemical composition of plants for individual locations are presented in Tables 9 and 10 of the Appendix. Lime significantly increased the

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Plate 3. Photograph showing growth and color characteristics of plants from the lime 0, molybdenum 70 g/ha treatment without sulfur.



Plate 4. Photograph showing growth and color characteristics of plants from the lime 0, molybdenum 70 g/ha treatment when 45 kg sulfur/ha was applied.



Table 6. Effect of lime, sulfur, and molybdenum on chemical composition of dark red kidney bean plant tissue at the early bloom stage - Montcalm and Ionia locations combined

Treatments		%						ppm	
Lime	0	3.87	.32	1.73	2.93	.73	.17	34	238
Lime	2800 kg/ha	3.98	.30	1.51*	2.73*	1.05*	.18	30*	206
Lime	5600 kg/ha	3.97	.29*	1.36*	2.73*	1.21*	.15	27*	193*
Sulfur		3.85	.30	1.44	2.90	.99	.14	30	231
Sulfur	45 kg/ha	4.03*	.30	1.62*	2.69	1.01	.19*	32	194*
Molybdenum		3.94	.30	1.48	2.79	.97	.17	32	211
Molybdenum	70 g/ha	3.94	.30	1.58*	2.81	1.03	.16	29*	214

*Indicates significant difference from the zero treatment at the 0.05 level of significance.

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Mg content of the plants and decreased P, K, Ca, Zn, and Mn. The reduction in P, Zn, and Mn by lime most likely resulted from increased soil pH. The increase in uptake of Mg from the dolomitic liming material probably caused the decrease in Ca and K. It has been shown that plants tend to take up a constant amount of cations. If one cation in the plant is increased significantly, others tend to decrease and vice versa (Tisdale and Nelson, 1966). Levels of all these elements appear to be neither deficient nor toxic. Manganese is slightly higher than normal but not excessive. White (1970) reported that manganese in Charlevoix red kidney beans did not become excessive until manganese levels in the plant at the early bloom stage exceeded 800 ppm. Although not significantly, lime increased the nitrogen content of the plants. This difference in composition, 3.87 to 3.97% N, could possibly account for the improved growth and color of the limed plants. The additional nitrogen may have come from increased mineralization of soil organic nitrogen. The optimum pH for mineralization of organic substances in soil has been shown to be slightly on the alkaline side. Schachtschabel (1953) reported that mineralization of nitrogen increased from 100 to 1500 kg/ha when acid sandy soils high in humus were limed.

Sulfur significantly increased the N, K, and S contents of the plant and decreased Mn. Plants treated with sulfur contained 4.03% N as compared with 3.85% N in non-sulfur plants. Oke (1969) reported that sulfur deficiency leads to chlorosis and inability of the plant to utilize nitrogen. A closer look at the effect of individual treatments on nitrogen content of the plants showed that the increase in nitrogen by sulfur was mainly at the zero lime rate (Figure 3). A trend of this sort can be expected when both lime and sulfur are

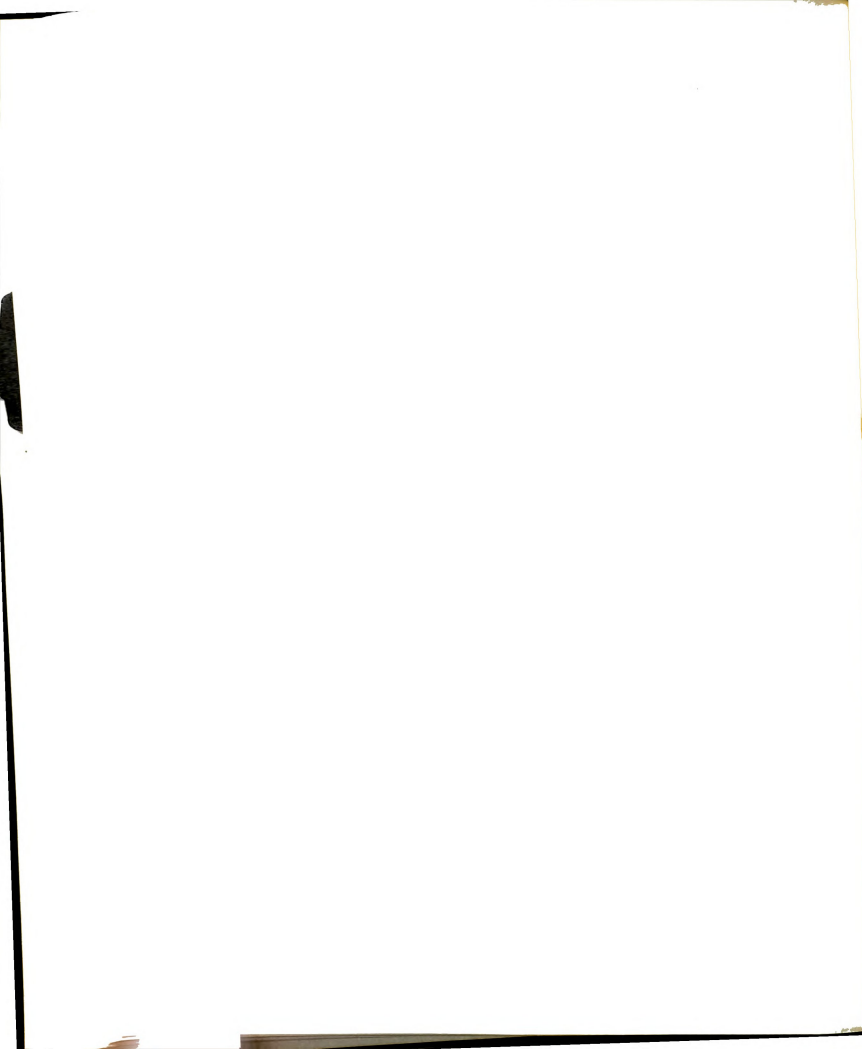


increasing the nitrogen content of the plant. When applied together, each will tend to offset the effect of the other. The sulfur treatment increased sulfur content in the plants from .14 to .19%. It is difficult to determine whether these levels are adequate since data on critical sulfur levels in dark red kidney beans are not available. If one compares the values with the critical level reported for soybeans (.15 - .20% S)¹ it would appear that sulfur is deficient in the non-sulfur plants. Another technique used to determine whether sulfur is sufficient is the total N : total S ratio of the plant. The N:S ratio in the non-sulfur plants is 28:1 and in plants treated with sulfur the ratio is 21:1. Stewart and Porter (1969) concluded from a study on pinto beans that when the N:S ratio of the plant was above 16:1 sulfur was limiting protein synthesis and when the ratio was 20 or more sulfur was severely deficient. These data indicate that sulfur might even be deficient in the sulfur treated plants.

The beans in these experiments nodulated much later than those of the previous year. At the Montcalm location, plants had only a few nodules 32 days after emergence and plants at Ionia did not develop significant numbers of nodules until 61 days after emergence. The delay in nodulation could have resulted from the extreme dry weather. It was noticed soon after the plots were irrigated that many small nodules developed.

Nitrogen fixation data for each location are presented in Figures 4 and 5. Nitrogen fixation data for individual samplings at each location are presented in Tables 11, 12, 13, 14, 15, and 16 of the

¹ Soil and Plant Analysis Laboratory, Soils Department, University of Wisconsin, Madison, Wisconsin 53706.



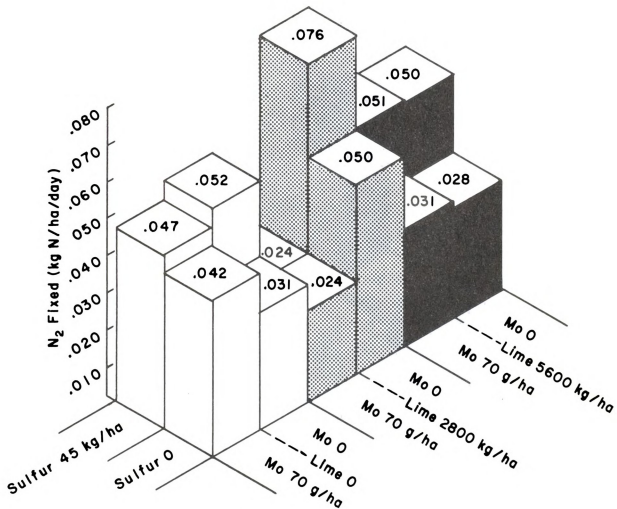


Figure 4. Effect of lime, sulfur, and molybdenum on rate of nitrogen fixation by dark red kidney beans at the Montcalm location - four sampling dates combined.

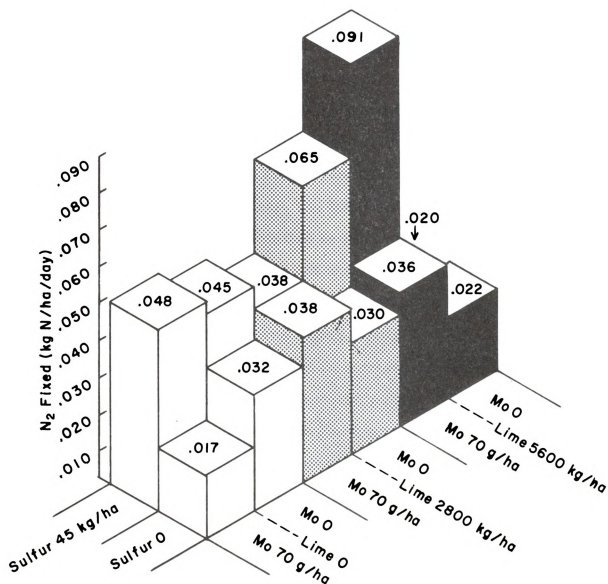


Figure 5. Effect of lime, sulfur, and molybdenum on rate of nitrogen fixation by dark red kidney beans at the Ionia location - two sampling dates combined.

Appendix. Although there was a great deal of variability in nitrogen fixation measured, some definite trends were observed. Sulfur in nearly every instance increased nitrogen fixation. Without sulfur, the average rate of fixation for all samplings at the Montcalm and Ionia locations was .034 and .029 kg N fixed/ha/day, respectively. When sulfur was applied, .050 and .051 kg N/ha/day was fixed. Although this is approximately a 60% increase in nitrogen fixation, it was not significant at the 0.05 level of probability. The extreme variation in nitrogen fixation within treatments appeared to be caused by variation in nodulation. To document this visual observation nodules were removed from the plants after nitrogen fixation measurements were taken 75 days after emergence at the Ionia location. The average variance in fresh weight of nodules between replicates within treatments was found to range between .18 and 2.53 g. Correlation analysis for nitrogen fixation and fresh weight of nodules showed a simple correlation of $r = .83$ which was highly significant. The interpretation is that 83% of the variability in nitrogen fixation measured was relatable to the variation in nodule fresh weight. The lime and molybdenum treatments did not influence the rate of nitrogen fixation nearly as much as the sulfur treatment and were far from significant.

Yield data for the combined locations are presented in Figure 6. Tables 17 and 18 of the Appendix give yields for individual locations. Differences in yield among treatments were much greater before moisture content of the grain was taken into account. Beans in the no lime, no sulfur treatments (Plate 5) matured early and were much dryer (14% moisture) at harvest than those treated with lime and sulfur (18% moisture). After adjusting all yield data to 18% moisture, statistical



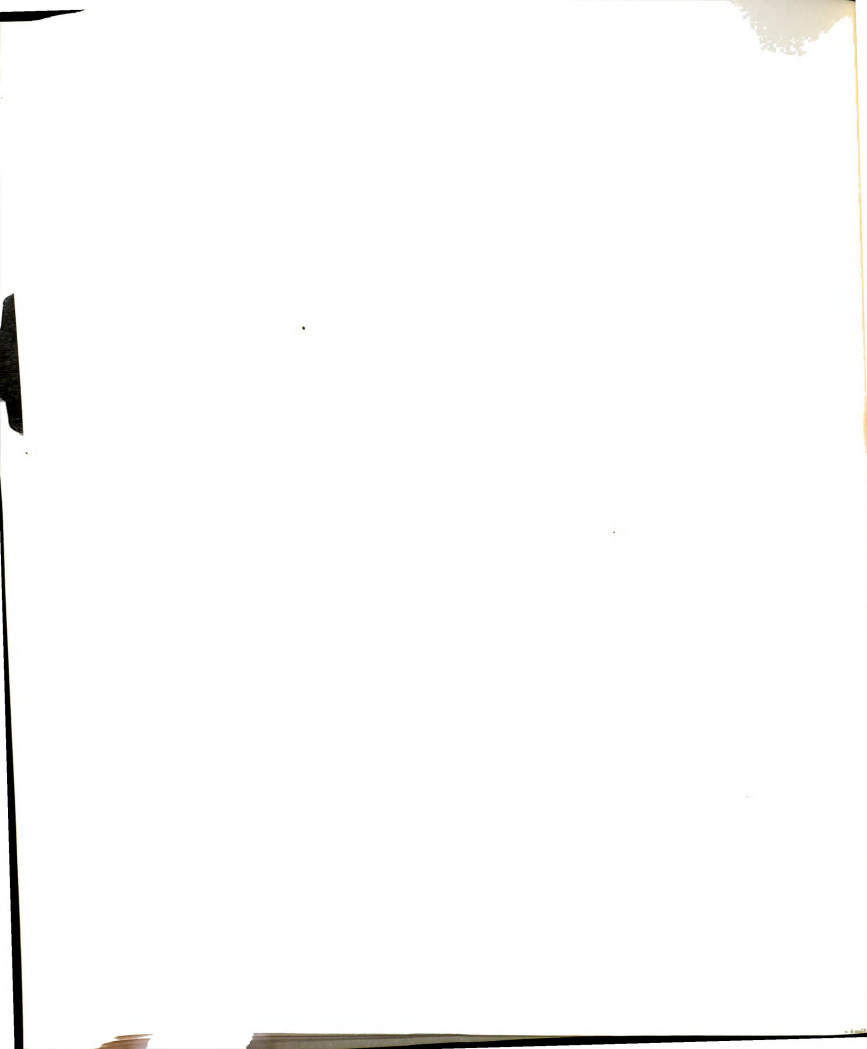




Plate 5. Photograph showing the difference in maturity of the lime 0, sulfur 0, molybdenum 70 g/ha treatment (foreground) and the lime 5600 kg/ha, sulfur 45 kg/ha, molybdenum 70 g/ha treatment (background).

analysis of the combined data showed that all main treatments still produced significant (0.05) yield differences.

Lime at the 2800 and 5600 kg/ha rates increased yield by 226 and 413 kg/ha, respectively. The yield increase by the first increment of lime was not significantly higher than the zero lime rate. Sulfur increased yield most (336 kg/ha) when lime was not applied. One explanation for the decreased sulfur response at the higher lime rates can be offered by the sulfur soil test data (Table 7). Lime increased $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extractable $\text{SO}_4\text{-S}$ in the soil from 8 to 10 ppm at the Montcalm location and from 5 to 7 ppm at the Ionia location. Correlation studies by Fox et al. (1964) showed that soils having less than 6 ppm $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extractable $\text{SO}_4\text{-S}$ would almost assuredly respond to sulfur. In the 6 to 10 ppm range, a response was possible and above 10 ppm a response was unlikely. It seems probable that lime could have eliminated the sulfur deficiency. The increase in available sulfur with application of lime could have come from a sulfur contaminant in the liming material or from increased mineralization of soil organic sulfur, but most likely was caused by increased availability due to higher soil pH. Mehlich (1964) found that sorbed sulfate in the soil not readily available to plants became increasingly available as pH of the soil increased. At the Montcalm location, lime increased soil pH from 5.6 to 6.9 and at the Ionia location from 5.9 to 7.1. L. M. Wash, University of Wisconsin, (personal communication) stated that soil pH was a most important factor in determining response to sulfur. He indicated that sulfur recommendations at the University of Wisconsin are now being made with respect to soil pH and sulfur soil test results. Only a few soils in Wisconsin have responded to sulfur when soil pH is above 6.0.

Table 7. Effect of lime and sulfur on $\text{SO}_4\text{-S}$ content and pH of the soil at the Montcalm and Ionia locations

Montcalm Location						
Sulfur rates (kg S/ha)	Lime rates (kg/ha)			Lime rates (kg/ha)		
	0	2800	5600	0	2800	5600
	$\text{SO}_4\text{-S}$ (ppm)			pH		
0	8	8	10	5.6	6.4	7.0
45	22	26	31	5.7	6.2	6.8
Lime means	15	17	21	5.6	6.3*	6.9*
Sulfur means	OS = 9 45S = 26*			OS = 6.3 45S = 6.2		

Ionia Location						
Sulfur rates (kg S/ha)	Lime rates (kg/ha)			Lime rates (kg/ha)		
	0	2800	5600	0	2800	5600
	$\text{SO}_4\text{-S}$ (ppm)			pH		
0	5	6	7	5.9	6.6	7.0
45	15	13	13	5.8	6.6	7.2
Lime means	10	9	10	5.9	6.6*	7.1*
Sulfur means	OS = 6 45S = 14*			OS = 6.5 45S = 6.5		

* Indicates significant difference from the zero treatment at the 0.05 level of significance.

The reduction in sulfur response at the higher lime rates might also be explained with respect to nitrogen metabolized. Since nitrogen fertilizer was not applied, nitrogen available to the plant was limited. More efficient use of nitrogen when sulfur was applied could account for much of the response to sulfur. Stewart and Porter (1969) found that lack of sulfur reduced the efficiency of available nitrogen by about 50%. Since lime also increased uptake of nitrogen, any nitrogen related sulfur response would tend to be diminished when lime was applied. This interpretation would also explain an observation made at the Ionia location. Outside of the plot area the farmer applied 77 kg/ha of fertilizer nitrogen. A replicated yield check in this area showed that yields were equivalent to those in the experiment where lime and sulfur were applied. This suggests that nitrogen was important and if applied would reduce the chance of getting a lime or sulfur response.

A significant reduction in yield resulted from the molybdenum seed treatment. The yield reduction did not appear to be caused by reduced germination, nitrogen fixation, or plant growth. Others reporting yield reductions from molybdenum have also noted no visual symptoms of molybdenum excess. DeMooy (1970) reported that 230 g/ha of molybdenum, as a seed treatment, reduced yield of soybeans by 249 kg/ha. Jackman (1956) suggested that reduced yield from applied molybdenum might be caused by micronutrient interaction.

Integration of bean yield data with nitrogen and sulfur content of the grain permits calculation of the total nitrogen and sulfur harvested in the crop. Results of these calculations are presented in Table 8. Lime and sulfur significantly increased nitrogen taken up

Table 8. Effect of lime, sulfur, and molybdenum on chemical composition and uptake of nitrogen and sulfur in the grain of dark red kidney beans - Montcalm and Ionia locations combined

Treatments	N	S	Methionine ^{1/}	N-uptake	S-uptake
	%			Kg/ha	
Lime 0	2.84	.19	.98	66.6	4.3
Lime 2800 kg/ha	2.87	.19	.96	73.5*	5.0
Lime 5600 kg/ha	2.89	.18	.97	79.7*	4.8
Sulfur 0	2.86	.18	.94	70.8	4.4
Sulfur 45 kg/ha	2.88	.19*	1.00*	75.8*	5.0*
Molybdenum 0	2.86	.19	.99	75.1	4.8
Molybdenum 70 g/ha	2.88	.19	.94	71.5*	4.6

*Indicates significant difference from the zero treatment at the 0.05 level of significance.

^{1/} Percent of protein.

in the grain and molybdenum reduced N-uptake. Sulfur content of the grain and uptake of sulfur were higher when sulfur was applied. If one uses the conversion, total N x 6.25 = total protein, yield of protein was also increased by both lime and sulfur. Equally important to amount of protein produced is quality of the protein. Coleman (1966) stated that methionine and cystine were good indicators of protein quality. The methionine content of the dark red kidney beans was significantly increased by the sulfur treatment (Table 8). These results agree with those reported by Sheldon, Blue, and Albrecht (1957) who found that sulfur increased the methionine content of soybeans. Steward et al. (1951) indicated that most of the sulfur taken up by the plant is elaborated into methionine and cystine, and so the importance of sulfur cannot be over emphasized. The level of methionine in the dark red kidney beans (.94-1.00% of the protein) is equivalent to that reported for phaseolin of kidney beans (1.10%) by Spector (1965) and that for soybeans (1.01%) reported by Evans, Bandemer, and Bauer (1962). The lime and molybdenum treatments did not influence significantly the methionine content of the grain. These results indicate that if sulfur deficiencies in Michigan are corrected both the quantity and quality of protein produced by dark red kidney beans can be improved.

SUMMARY AND CONCLUSIONS

Field experiments were established in 1970 and 1971 to (1) determine the effect of soil temperature on nitrogen fixation and yield of dark red kidney beans, (2) to determine the influence of plant age and diurnal effects on nitrogen fixation, (3) to determine the total nitrogen fixed by dark red kidney beans during a complete growth cycle, and (4) to determine the effect of lime, sulfur, and molybdenum on nitrogen fixation, plant composition, and yield of dark red kidney beans.

In the first year of experiments, clear and black plastic mulch treatments were used to establish differences in soil temperature in the field during the nodulation period and during the entire growth cycle of dark red kidney beans. Soil temperature was increased most by the clear plastic mulch treatments and only slightly by the black plastic mulch treatments. As a result of the increased soil temperature, plant growth was increased, potassium and phosphorus content of the plants were higher, and nodules developed earlier on the bean roots. Plants from treatments which had the lowest soil temperatures (No plastic, Black plastic 18 days, and Black plastic entire) fixed the most nitrogen. The plastic mulch treatments that were removed after 18 days promoted early nodulation but did not cause any more nitrogen to be fixed than the no plastic treatment. These results indicate that nitrogen fixation in the field is not limited by too low a soil temperature. If anything, the soil temperatures may be too high for optimum nitrogen fixation. Yield of the dark red kidney beans was not significantly increased by any of the plastic mulch treatments.

Age of the dark red kidney bean plants had a profound effect on nitrogen fixation. Fixation of nitrogen was first observed ten days after the beans emerged. The rate of fixation was low during the first 20 days, increased seven-fold during the next 15 days, reached a maximum 39 days after emergence, and then declined as the plants matured. The importance of soil moisture stress was shown 54 days after emergence when it caused a sharp reduction in nitrogen fixation.

Only 9.12 kg of nitrogen was fixed by the dark red kidney beans during the entire growing season. This is substantially below fixation values reported for fieldbeans and soybeans.

The diurnal sampling for nitrogen fixation showed that twice as much nitrogen was fixed during the daylight hours as at night. Changes in soil temperature during the 24 hour cycle did not appear to have any affect on the rate of nitrogen fixation.

In the second year of experiments, three rates of lime, two sulfur rates, and two molybdenum levels were established on acid sandy loam soils at two locations in Michigan. Lime and sulfur were applied broadcast and molybdenum as a seed treatment. Response in growth and color to lime and sulfur was noted early in the growing season. Lime increased the Mg content of the plants and decreased P, K, Ca, Zn, and Mn. Sulfur increased the N, K, and S contents of the plants and decreased Mn. Sulfur in nearly every instance increased nitrogen fixation. Without sulfur, the average rate of fixation for all samplings at the Montcalm and Ionia locations was .034 and .029 kg N fixed/ha/day, respectively. When sulfur was applied, .050 and .051 kg N/ha/day was fixed. The lime and molybdenum treatments did not influence the rate of nitrogen fixation. Lime at the 2800 and 5600 kg/ha rates increased yield by 226 and 413



kg/ha, respectively. Sulfur, without lime, increased yield by 336 kg/ha. When lime was applied, the yield response to sulfur was less. Molybdenum in nearly every instance reduced yield. The lime and sulfur treatments increased the amount of protein produced (N content of the grain $\times 6.25$) and sulfur increased the methionine content of the protein.

Response to lime and sulfur on similar soil types is expected. Correcting sulfur deficiencies may allow bean growers to reduce the amount of nitrogen in their fertilizer programs because of increased nitrogen fixation and better utilization of existing soil nitrogen.

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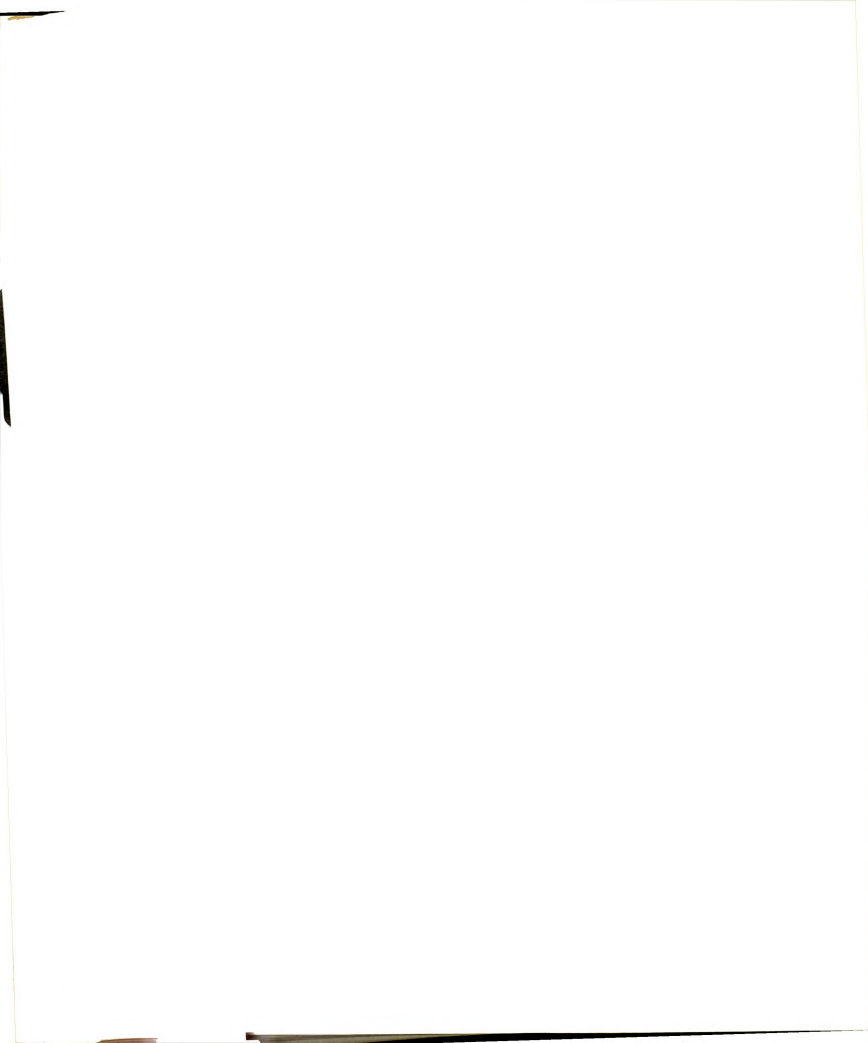


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APPENDIX

Table 9. Effect of lime, sulfur, and molybdenum on chemical composition of dark red kidney bean plant tissue at the Montcalm location - 32 days after plant emergence

Treatments	N	P	K	Ca	Mg	S	Zn	Mn
	%					ppm		
Lime 0	3.81	.30	1.69	2.58	.81	.16	32	306
Lime 2800 kg/ha	3.87	.28	1.51	2.49	1.14*	.16	29	290
Lime 5600 kg/ha	3.85	.26*	1.42	2.37	1.20*	.14	26	258
Sulfur 0	3.72	.28	1.44	2.61	1.05	.13	28	318
Sulfur 45 kg/ha	3.98*	.28	1.64*	2.35*	1.05	.17*	30*	251*
Molybdenum 0	3.79	.28	1.43	2.52	1.04	.15	30	280
Molybdenum 70 g/ha	3.90*	.28	1.65*	2.44	1.06	.15	28	289

*Indicates significant difference from the zero treatment at the 0.05 level of significance.

Table 10. Effect of lime, sulfur, and molybdenum on chemical composition of dark red kidney bean plant tissue at the Ionia location - 33 days after plant emergence

Treatments	N	P	K	Ca	Mg	S	Zn	Mn
	%					ppm		
Lime 0	3.93	.34	1.78	3.28	.67	.18	36	171
Lime 2800 kg/ha	4.09	.33	1.50*	2.97	.96*	.20	32*	121
Lime 5600 kg/ha	4.08	.31*	1.30*	3.10	1.21*	.16	28*	128
Sulfur 0	3.98	.33	1.43	3.19	.92	.15	32	143
Sulfur 45 kg/ha	4.08*	.33	1.61*	3.04	.97	.21*	33	137
Molybdenum 0	4.08	.33	1.53	3.06	.90	.18	34	141
Molybdenum 70 g/ha	3.98	.33	1.51	3.17	.99	.18	31*	139

*Indicates significant difference from the zero treatment at the 0.05 level of significance.

Table 11. Effect of lime, sulfur, and molybdenum on rate of nitrogen fixation by dark red kidney beans at the Montcalm location - 32 days after plant emergence

	Lime rates (kg/ha)						
Sulfur rates (kg S/ha)	0		2800		5600		Molybdenum means
	OS	45S	OS	45S	OS	45S	
Molybdenum rates (g Mo/ha)	N ₂ fixed (kg N/ha/day)						
0	.0005	.0042	.0016	.0047	.0025	.0016	.0025
70	.0023	.0097	.0007	.0054	.0004	.0026	.0035
Lime means	.0042		.0031		.0018		
Sulfur means	OS = .0013		45S = .0047*				

*Indicates significant difference from the zero treatment at the 0.05 level of significance.

Table 12. Effect of lime, sulfur, and molybdenum on rate of nitrogen fixation by dark red kidney beans at the Montcalm location - 46 days after plant emergence

	Lime rates (kg/ha)						
Sulfur rates (kg S/ha)	0		2800		5600		Molybdenum means
	OS	45S	OS	45S	OS	45S	
Molybdenum rates (g Mo/ha)	N ₂ fixed (kg N/ha/day)						
0	.0109	.0285	.0278	.0147	.0073	.0282	.0196
70	.0031	.0060	.0063	.0043	.0092	.0062	.0058
Lime means	.0121		.0133		.0127		
Sulfur means	OS = .0108		45S = .0146				

*Indicates significant difference from the zero treatment at the 0.05 level of significance.



Table 13. Effect of lime, sulfur, and molybdenum on rate of nitrogen fixation by dark red kidney beans at the Montcalm location - 60 days after plant emergence

		Lime rates (kg/ha)						
Sulfur rates (kg S/ha)	0		2800		5600		Molybdenum means	
	OS	45S	OS	45S	OS	45S		
Molybdenum rates (g Mo/ha)	N ₂ fixed (kg N/ha/day)							
0	.0919	.1498	.1629	.2804	.0692	.1544	.1514	
70	.1342	.1453	.0771	.0788	.0978	.1712	.1174	
Lime means	.1303		.1498		.1231			
Sulfur means	OS = .1055		45S = .1633					

*Indicates significant difference from the zero treatment at the 0.05 level of significance.

Table 14. Effect of lime, sulfur, and molybdenum on rate of nitrogen fixation by dark red kidney beans at the Montcalm location - 74 days after plant emergence.

		Lime rates (kg/ha)						
Sulfur rates (kg S/ha)	0		2800		5600		Molybdenum means	
	OS	45S	OS	45S	OS	45S		
Molybdenum rates (g Mo/ha)	N ₂ fixed (kg N/ha/day)							
0	.0227	.0266	.0072	.0025	.0317	.0172	.0180	
70	.0227	.0255	.0128	.0080	.0169	.0232	.0190	
Lime means	.0256		.0076		.0222			
Sulfur means	OS = .0198		45S = .0172					

*Indicates significant difference from the zero treatment at the 0.05 level of significance.



Table 15. Effect of lime, sulfur, and molybdenum on rate of nitrogen fixation by dark red kidney beans at the Ionia location - 61 days after plant emergence

		Lime rates (kg/ha)						
		0		2800		5600		Molybdenum
Sulfur	rates (kg S/ha)	OS	45S	OS	45S	OS	45S	means
Molybdenum	rates							
(g Mo/ha)		N ₂ fixed (kg N/ha/day)						
0		.0158	.0319	.0198	.0373	.0255	.0284	.0264
70		.0103	.0480	.0228	.0112	.0198	.0566	.0281

*Indicates significant difference from the zero treatment at the 0.05 level of significance.

Table 16. Effect of lime, sulfur, and molybdenum on rate of nitrogen fixation by dark red kidney beans at the Ionia location - 75 days after plant emergence

		Lime rates (kg/ha)						
		0		2800		5600		Molybdenum
Sulfur	rates (kg S/ha)	OS	45S	OS	45S	OS	45S	means
Molybdenum rates (g Mo/ha)		N ₂ fixed (kg N/ha/day)						
0		.0484	.0577	.0407	.0924	.0193	.0124	.0451
70		.0243	.0487	.0522	.0645	.0527	.1259	.0614
Lime means		.0448		.0625		.0526		
Sulfur means		OS = .0396		45S = .0669				

*Indicates significant difference from the zero treatment at the 0.05 level of significance.

Table 17. Effect of lime, sulfur, and molybdenum on yield of dark red kidney beans at the Montcalm location

	Lime rates (kg/ha)						
Sulfur rates (kg S/ha)	0		2800		5600		Molybdenum means
	OS	45S	OS	45S	OS	45S	
Molybdenum rates (g Mo/ha)	Yield (kg/ha)						
0	2676	3031	2885	2888	3390	3371	3040
70	2350	2827	2872	2926	2870	3114	2826*
Lime means	2721		2893		3186		
Sulfur means	OS = 2841		45S = 3026				

*Indicates significant difference from the zero treatment at the 0.05 level of significance.

Table 18. Effect of lime, sulfur, and molybdenum on yield of dark red kidney beans at the Ionia location

		Lime rates (kg/ha)						
Sulfur rates (kg S/ha)	0		2800		5600		Molybdenum means	
	OS	45S	OS	45S	OS	45S		
Molybdenum rates (g Mo/ha)			Yield (kg/ha)					
0	1844	2181	2115	2404	2521	2278	2224	
70	1853	2027	2054	2459	2332	2221	2158	
Lime means	1976		2258*		2338*			
Sulfur means	OS = 2120		45S = 2262					

*Indicates significant difference from the zero treatment at the 0.05 level of significance.



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