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Effect of Method of Inoculation and Lime
Placement on the Establishment and Yield
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David E. Huset

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EFFECT OF METHOD OF INOCULATION AND LIME PLACEMENT
ON THE ESTABLISHMENT AND YIELD OF ALFALFA
(MEDICAGO SATIVA L.) ON ACID
SOILS IN MICHIGAN

By

David E. Huset

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ABSTRACT

EFFECT OF METHOD OF INOCULATION AND LIME PLACEMENT ON THE ESTABLISHMENT AND YIELD OF ALFALFA (MEDICAGO SATIVA L.) ON ACID SOILS IN MICHIGAN

By

David E. Huset

Alfalfa is the primary forage crop in Michigan due to its high productivity on soils with a high pH, but on acid soils it is difficult to establish and yields are generally poor. The objectives of this study were to determine if lime-pelleted or Pelinoc-treated seeds and/or broadcasting lime on the surface would improve the establishment and yield of alfalfa on acid soils, pH 4.8 to 5.5, over conventional inoculation. Investigations were carried out in two years at four field locations. The results indicated that lime-pelleted and Pelinoc-treated seeds did not improve the establishment or first-year yields, but there was a trend toward greater yields in the second year. Surface-broadcast lime was nearly as effective in improving yields as incorporating the lime to a depth of 20 cm.

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INTRODUCTION

There is an extensive acreage in northern Michigan in permanent grass pasture. These areas are generally unproductive, often very hilly, with sandy and droughty soils with acid root zones.

The incorporation of a deep-rooted legume into these areas would greatly increase their productivity. Legumes have the capacity of fixing atmospheric N which would both increase yields and improve the quality of the forage.

Presently, there are no legumes which can survive acid, sandy soil conditions and produce a significant amount of forage for several years. Red clover can tolerate acid soils and is easily established, when compared to other legumes. It is a short-lived perennial, however, which would survive only two to three years under most management systems. Birdsfoot trefoil is a long-lived, perennial legume which yields well on acid soils, but it is difficult to establish and is not drought tolerant. Alfalfa is a long-lived high-producing legume which can tolerate drought, but it is sensitive to acid soils. If this problem could be overcome, it would be the legume of choice because of its high yield potential and drought tolerance.

It is recommended in Michigan that the pH of soils be increased to 6.8 prior to seeding alfalfa by incorporating the lime into the plow layer (Warncke et al., 1976). Christenson and Doll (1973) described several benefits of liming acid soils in Michigan, including (1) improving yields, (2) reducing harmful concentrations of Al and Mn, (3) increasing the availability of many essential nutrients, (4) supplying a Ca directly and Mg if dolomitic limestone is used, (5) promoting favorable microbial activity, and (6) increasing longevity of legume stands which have a high Ca requirement.

The slope of many of the acid soils, which need a deep-rooted legume to improve production, does not allow for conventional tillage without a risk of erosion. This prevents the incorporation of lime as recommended. Broadcasting lime on the surface has not been recommended because of the slow downward movement of lime into the root zone. Applying a small amount of lime with the seed at planting has been attempted in research trials, but this method would not be feasible on a farm scale.

In Australia, lime pelleting of clover seed has been successful in establishing clover on acid soils (Loneragan, 1955). Many of these soils have never been seeded to clover before and have very low populations of rhizobia bacteria. This practice has prompted some seed companies in the United States to coat alfalfa seeds with finely pulverized lime. The practice of lime coating is an attempt to protect the

rhizobia and provide a greater number of viable rhizobia on the seed for nodulation. Various other methods, including the Pelinoc process by Nitragin Co., have been developed to increase the number of viable rhizobia on the seed for nodulation.

The objectives of this study were (1) to determine if lime pelleting or the Pelinoc treatment could improve the establishment and subsequent yield of alfalfa on acid soils over conventional moist inoculation and (2) to determine if broadcasting lime on the surface could be effective in establishing alfalfa on acid soils.

LITERATURE REVIEW

It has been accepted for many years that liming acid soils will improve their productivity as well as improving the general tilth of the soil. Liming soils to a pH of 6.5 to 6.8 has been recommended for alfalfa production for several years in the mid-west (Woodruff, 1967). The benefits of liming have been studied extensively by many researchers including Fried and Peech (1946), Schmehl et al. (1950), York et al. (1954), Pearson (1958), Moschler et al. (1960), Foy (1964), and Munns (1965).

Pearson (1958), in a review, listed the benefits of liming acid soils as (1) supplying Ca and Mg, (2) maintaining availability of applied nutrients, (3) improving availability of native soil nutrients, (4) enhancing desirable types of microbial activity, (5) improving plant root development, and (6) reducing toxic effects of Al and Mn. The relative importance of these factors varies from soil to soil.

The effect that liming has on the availability of K is not clearly understood. Kamprath and Foy (1971), in discussing the effect of lime on K, found that conflicting data exist. On some acid soils, liming reduces the amount of K leached and thereby increases the amount of K in the

root zone. On certain soils which contain low amounts of exchangeable K, liming may induce a K deficiency through ion competition effects. In support of this, York et al. (1954) applied lime to a Mardin silt loam with an original pH of 5.0 in the greenhouse. They found that when no K was added to the pots, liming did not increase alfalfa yields and actually decreased them at high lime levels. When K was added, however, liming increased yields very significantly. They attributed these results to an interaction between Ca and K.

The effect that liming has on P availability is closely correlated to its effect on Al solubility. Increasing the pH to 5.5 reduces Al solubility significantly and also increases the availability of P. Very insoluble Al-P precipitates form, inside and outside the root, when the pH is less than 5.5 (Kamprath and Foy, 1971).

Research conducted by Stewart and Pearson and reported by Pearson (1958) showed that liming increased the amount of P taken up by crimson clover but decreased the amount of P that was taken up from the applied P which was labeled. This indicated that lime increased the availability of native P but decreased the availability of applied P possibly by the formation of more basic forms of phosphate having lower water solubilities.

Foy (1964) reported that the symptoms of Al toxicity, which occur on many acid soils in the eastern United States, are very similar to P deficiency and result in stunted

plants. The roots are short and thick and the plants can eventually wilt due to lack of moisture. It is caused in part by Al precipitating P inside and outside the roots. On two of seventeen acid soils tested, yield increases of alfalfa due to liming correlated better with a reduction in exchangeable Al than with any other factor. Several other reports indicate that increased alfalfa yields due to liming were the result of simultaneous increases in P uptake and reductions in exchangeable Al in the soil. Munns (1965), working in Australia, found that on acid soils with a pH of less than 5.5, liming increased alfalfa yields. On these same soils, however, heavy applications of P eliminated the response of alfalfa to lime. He attributed the effect of lime as reducing exchangeable Al which was blocking the uptake of P at low pH values. Moschler et al. (1960), working on an acid soil with a pH of 4.9, found that increasing the pH to 5.5 was sufficient to achieve nearly maximum yields of alfalfa. They found that the yield increase correlated very closely with reductions in exchangeable Al in the soil.

Since most liming materials contain Ca, liming increases the abundance of Ca in the soil. Kamprath and Foy (1971) reported that Ca deficiency rarely occurs in the field. Walsh (1973) reported that Wisconsin soils normally contain from 1000 to 10,000 pounds of exchangeable Ca per acre, and that Ca deficiency is observed only on very acid sandy soils. On certain soils, over-liming has been

reported. Pierre and Browning (1935) indicated that liming an acid soil to a pH of 7.0 caused a reduction in first year alfalfa yields by an average of 46%. They attributed the yield reductions to a disturbed phosphate nutrition. Although Ca deficiency is generally not considered the reason for poor alfalfa yields on acid soils, Foy (1964) indicated that on one acid soil Ca deficiency was probably the reason for increased yields after liming. Dawson (1958) increased alfalfa yields by the addition of a Ca solution to an isolated part of the root. He attributed the increased yield to an antagonistic relationship between Ca and Mn. Schmehl et al. (1950) also determined that one advantage of liming is that of providing a better Ca:Mn ratio in the soil and in the plant. When the Ca:Mn ratio in the plant was less than 66:1, alfalfa yields were reduced. Fried and Peech (1946) also concluded that Mn may interfere with the uptake of Ca. Foy (1964) reported that high levels of Ca in the soil can prevent Mn from accumulating in the plant and thus prevent toxicity. He determined that on nine of seventeen acid soils, alfalfa yield increased due to liming were the result of a reduced level of Mn in the plant. When the level of Mn in the plant was reduced, yields reached their peak. Kamprath and Foy (1971) indicate that increasing the soil pH to 5.5 reduces the amount of Mn in the Mn^{+2} state--the state which is most available for plant uptake--and thereby reduces the toxicity problem.

The availability of Mo increases as the pH increases. Evans et al. (1951) found that liming an acid soil increased alfalfa yields up to a pH of 6.5. When Mo was added, however, liming had no beneficial effects on yield. They attributed the yield increases due to liming to an increase in Mo availability. Foy and Barber (1959) obtained a yield increase in alfalfa due to applications of Mo. Lime applications reduced the need for Mo. Gupta (1969) found that on some soils alfalfa responded to Mo but was unresponsive to Mo on others. Research done in Michigan by Powles and Tesar (1976) showed that on a soil with a pH of 5.5 alfalfa did not respond to applications of Mo but did respond to additions of lime. This indicated that the response of alfalfa to lime was not related to a Mo deficiency. Ahlrichs et al. (1963), working on a soil with a pH of 5.5, found that Mo did not increase yields, but both lime and Mo increased the concentration of Mo in the alfalfa tissue.

Munns (1965), working in Australia, indicated that the major cause of poor alfalfa yields on many acid soils is that the population of symbiotic, nitrogen-fixing bacteria is very low which results in poor nodulation and very little nitrogen-fixation. In another report, Munns (1968) determined that at a pH of 4.4 the root hairs did not curl and become infected. Raising the pH to 5.4 allowed for normal infection. He attributed this to H-ion toxicity, a problem which does not normally affect the host plant. Kamprath and Foy (1971) report that the reasons for a reduction in

nodulation and nitrogen fixation on acid soils are similar to the reasons for reduced yields of alfalfa. Aluminum toxicity reduces the root length and thereby reduces the number of sites available for nodulation. Nodule formation is very sensitive to deficiencies of Mo and Ca. When Ca and Mo levels are increased, nodulation occurs more readily. Jones et al. (1978) report that, on soils which contain high populations of ineffective strains of rhizobia, the nodulation of effective strains is reduced.

Hastings and Drake (1960) and Loneragan et al. (1955), in New Zealand and Australia, report that lime pelleting--covering the seed, coated with rhizobia, with a layer of finely ground limestone--seems to be effective in establishing legumes on acid soils. Burton (1975) suggests that a lime coating can protect the rhizobia in situations where they would otherwise be killed.

Baker et al. (1968) seeded lime pelleted alfalfa into three soils and obtained significant yield increases on two of the soils but not the third. The two that gave yield increases had not been seeded to alfalfa for several years. The one that showed no yield increase had recently been seeded to alfalfa. They concluded that lime pelleting could be advantageous on soils which had a very low population of symbiotic, nitrogen-fixing bacteria. Jones et al. (1978) reported that on soils with low populations of rhizobia, it is necessary to have a large number of viable rhizobia on the seed to assure effective nodulation. The

use of a peat-base inoculum with some type of sticker, Pelgel-Pelinoc or gum arabic, was superior to other methods of inoculation. Olsen and Elkins (1977), working on a soil with a pH of 4.7, found that lime pelleted alfalfa did not increase yield or stand density over moist inoculation with a peat-base inoculum. Conversely, Kunelius and Gupta (1975) obtained yield increases due to lime pelleting on a soil with a pH of 5.3.

Soil acidity affects both the alfalfa and rhizobia bacteria; the pH at which maximum yields occur varies from soil to soil. Foy (1964) concluded that, because of the number of growth-limiting factors occurring on acid soils, the pH of the soil is not a good criterion for determining lime requirement. Work conducted in Michigan by Ross et al. (1964) supports Foy's conclusion. Nine different soils with pH values around 5.5 were limed at several rates and seeded to alfalfa. Three of the soils yielded well with no lime additions and did not respond to lime; two soils responded to the lowest increment of lime, and yields did not increase at higher lime levels. On the other four soils, yields gradually increased with increasing lime applications. They concluded that soil pH was not a good indicator of lime requirement.

The effect that the placement of lime has on yield, root development, and nodulation has been studied by several researchers. In an early study, Watenpaugh (1936) treated the surface and sublayers of a soil with varying amounts of

limestone in the greenhouse. He concluded that root growth and yield were correlated with pH. At a pH of 4.8 root growth was nearly stopped, but when the pH was raised to 5.0 root growth returned to normal.

Pohlman (1946) applied lime at three rates to a soil with an original pH of 5.6 in the greenhouse. He limed three different depths--0 to 8", 8 to 16", and 16 to 24"--in all possible combinations of no lime, low lime and high lime. Liming the 8 to 16"-layer at the low rate or the 16 or 24"-layer at the high rate increased yields by three-fold even when the surface was not limed. Yield increases due to liming the surface were dependent upon the pH of the sublayers. When the sublayers were not limed, the addition of lime to the surface had a greater effect than when the sublayers were limed. Root growth and nodule development were concentrated in areas that received lime.

Woodhouse (1956), working in the field with soils that had pH values of 4.8 to 5.6, applied lime at three rates in three different methods. The lime was either broadcast on the surface, mixed with the top four inches, or plowed under to a depth of four inches. The results indicated that mixing the lime into the surface four inches is superior to broadcasting it on the surface or plowing it under. Three times as much lime was required when surface broadcasting to obtain the same yield as when mixing it into the upper four inches. These results are in agreement with those obtained by Longenecker and Markle (1952). Working

with crimson clover, they found that mixing the lime into the root zone was far superior to liming the surface in both yield and root growth. The roots were concentrated in areas that received lime.

Hourigan et al. (1961), working on a soil with an initial pH of 5.0, found that, if the surface was adequately limed, the pH of the subsoil was not important in obtaining maximum yields. They attributed this to a reduction in exchangeable Al in the soil. Lathwell and Peech (1964) reported that, incorporating small quantities of lime into the first few inches of the soil had a decided yield advantage to placing it at any other depth.

MATERIALS AND METHODS

Investigations were carried out in 1977 and 1978 at four field locations and in one greenhouse trial. The pH of the unlimed soils ranged from 4.8 to 5.5.

Field Studies

Experiment I--East Lansing, sod-seeded, pH 5.5, three methods of inoculation and seven lime treatments, two years--1977 and 1978.

Experiment I was established on the Michigan State University farm at East Lansing in 1977. The soil was a Miami sandy loam, a member of the fine-loamy, mixed, mesic family of Typic Hapludalfs with a 4% slope. Soil tests prior to initiating the experiment indicated a pH of 5.5, 80 kg of P, and 180 kg of K/ha.

Glyphosate was sprayed at 2.2 kg/ha four weeks before seeding to kill the quackgrass sod. Vernal alfalfa was seeded at 17.9 kg/ha on 20 May. The seed on all sod-seeded plots (lime treatments 1 to 6) was drilled 1.25 cm deep in contact with 123 kg of 0-46-0 fertilizer per hectare using a modified fertilizer-grain-legume drill with 11 openings spaced 17.78 cm apart. The same drill, adapted as a band seeder to place the seed in a band 4 cm above the

fertilizer, was used on lime treatment seven. This treatment was similar to that used normally in Michigan when band seeding alfalfa on a prepared seedbed (Tesar, 1978).

Three methods of inoculation were included in the experiment. Moist inoculation was achieved by moistening the seed with water and mixing the moistened seeds with a peat-base inoculum. The Pelinoc treatment, a proprietary system (Nitragin Co., Milwaukee, WI), consists of mixing the seeds with a sticker supplement called Pelgel and covering the moistened seeds with a peat-base inoculum, Pelinoc. Lime pelleting is a procedure where a peat-base inoculum is mixed with a gum arabic solution. The seeds are wetted with the slurry and rolled in finely pulverized CaCO_3 until they are well coated. The ratio of seed to CaCO_3 was approximately 2:1 and was determined by the manufacturer (Celpril Industries) of the product used in this experiment.

Since lime-pelleted seeds and Pelinoc-treated seeds weighed more than moist inoculated seeds, adjustments were made in the seeding rate to insure that 17.9 kg of raw seed was seeded per hectare. The seed was moist inoculated and inoculated with Pelinoc the day before seeding and kept cool until seeding.

The experiment was established as a split-plot, randomized, complete block with three replications. The plots were 2.13 by 9.14 m. The variables were as follows:

Lime treatment (main plots):

1. No lime
2. 2.8 metric tons/ha surface broadcast one week before seeding
3. 5.6 metric tons/ha surface broadcast one week before seeding
4. 11.2 metric tons/ha surface broadcast one week before seeding
5. 224 kg/ha in the row, drilled at seeding in contact with the seed
6. 448 kg/ha in the row, drilled at seeding in contact with the seed
7. 5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm one week prior to seeding

Methods of inoculation (split plots):

1. Moist inoculation--Nitragin Co., Milwaukee, WI
2. Pelinoc--Nitragin Co., Milwaukee, WI
3. Lime pellet--Celpril Industries, Manteca, CA

The plots were irrigated with 3.5 cm of water after seeding and with 10 cm of water during August. The seeding was sprayed with 1.1 kg of dalapon and 1.1 kg of 2,4-DB/ha for control of grassy and broadleaf weeds. Ten days later 2.2 kg of dalapon/ha was applied for additional grassy weed control. Sevin was sprayed as needed for leafhopper control. The alfalfa weevil was controlled by spraying a malathion-methoxychlor tank mix. In the spring of 1978 the plots were fertilized with 0-14-42 at 800 Kg/ha.

The plots were harvested twice in 1977 on 27 July and 6 October. Three harvests were made in 1978 on 23 June, 2 August and 11 October. Yields were determined by cutting an area 0.91 by 8.23 m using a Carter, self-propelled harvester. A 1000 gram fresh weight sample was taken from

each plot, dried with forced-air at 65 C for 48 hours, and weighed to determine percentage dry matter.

Growth ratings were made on 13 July. Stand density was determined after the last cutting in 1977 by digging three rows, 91 cm long, and counting the crowns. These plants were cut off at the crown, washed, cut to a length of 12.5 cm, dried with forced-air at 65 C, and weighed for root weight determinations.

Following the last harvest in 1977, two root cores were taken from each plot. The cores were 10 cm in diameter and 20 cm deep. The root cores were washed in a specially designed root washer, Figure 1. Nodules were removed with tweezers and separated into segments based on depth. The depth ranges were 0 to 2.5, 2.5 to 5, 5 to 10 and 10 to 20 cm. The nodules were dried with forced-air at 65 C and weighed to determine dry nodule weight by depth. Only a few nodules were present in the 10 to 20 cm depth so they were included in the 5 to 10 cm depth.

Soil samples were taken in late August of 1977 and in mid-October of 1978 for pH analyses. The soil cores were separated into five segments based on depth, Figure 2. The depth ranges were 0 to 2.5, 2.5 to 5, 5 to 10, 10 to 20, and 20 to 30 cm. Ten cores were composited to make one sample. Three samples were taken from each lime treatment in 1977. In 1978, one sample was taken from each lime treatment.

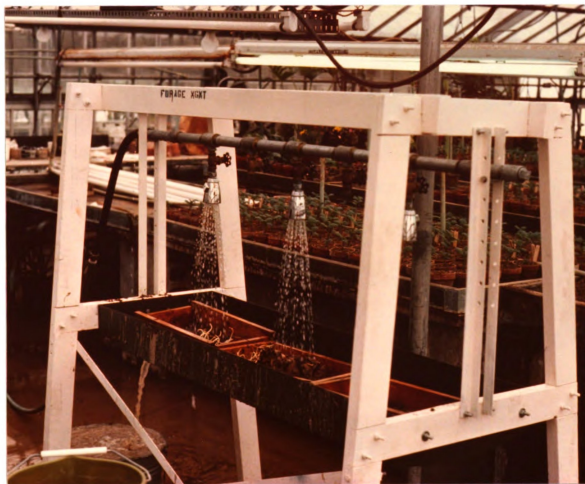


Figure 1. Root washer (above) and one root core being washed (right).





Figure 2. Soil core separated into segments based on depth.

Samples were dried with forced-air at 50 C and ground. A 1:1 (by weight) soil to water slurry was prepared and stirred for 30 minutes before testing. A Beckman model 3560 pH meter, using the automatic reading feature set at the 1% accuracy level, was used for the analyses.

Experiment II--East Lansing, clear seeded on prepared seed-bed, pH 5.1 to 5.5, three methods of inoculation and six lime treatments, two years--1977 and 1978.

Experiment II was established on the Michigan State University farm in East Lansing in 1977. The soil was a Hillsdale sandy loam, a member of the coarse-loamy, mixed, mesic family of Typic Hapludalfs with a 6% slope. Soil tests prior to beginning the experiment indicated a range in pH values from 5.1 to 5.5, 50 Kg of P, 135 Kg of K, and 1400 Kg of Ca/ha.

Six weeks prior to seeding, the field was sprayed with glyphosate at 2.2 kg/ha to kill existing vegetation. Prior to seeding, 0-14-42 was broadcast at 1200 kg/ha with a commercial fertilizer spreader and incorporated with a disk. Eptam was sprayed at 4.0 kg/ha and incorporated immediately for weed control.

Vernal alfalfa was seeded on 23 July at 9.0 kg/ha using a 5-row, nursery seeder in plots 0.91 by 7.62 m. Seeding rate adjustments and seed treatment practices were as in experiment I.

The experiment was established as a split-plot, randomized, complete block with four replications. The variables were as follows:

Lime treatments (main plots):

1. No lime
2. 2.8 metric tons/ha incorporated to a depth of 10 cm one month before seeding
3. 5.6 metric tons/ha incorporated to a depth of 10 cm one month before seeding
4. 11.2 metric tons/ha incorporated to a depth of 10 cm one month before seeding
5. 5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm one month before seeding
6. 11.2 metric tons/ha before plowing one month before seeding

Methods of inoculation (splot plots):

1. Moist inoculation--Nitragin Co., Milwaukee, WI
2. Pelinoc--Nitragin Co., Milwaukee, WI
3. Lime pellet--Celpril Industries, Manteca, CA

The plots were irrigated with 1 cm of water per day for eight days following seeding to help insure uniform emergence. The seeding was sprayed post-emergence with 2,4-DB at 1.1 Kg/ha for broadleaf weed control. Sevin was sprayed for leafhopper control as needed. The alfalfa weevil was controlled with a malathion-methoxychlor tank mix.

The plots were harvested once in 1977 on 14 October and four times in 1978 on 9 June, 12 July, 29 August, and 18 October. An area 0.91 by 6.70 m was harvested and dry matter yields determined as in experiment I. Growth ratings were made on 23 September.

Manganese toxicity symptoms were observed in September of 1977. Samples were taken of whole plant tops which were

cut off just above the crown. These samples were dried with forced-air at 65 C, ground and analyzed by ARL Emission Spectrography for Mn, Al and Fe by International Minerals and Chemicals, Terre Haute, Indiana.

Stand density and root yield were determined as in experiment I. Soil samples were taken after the final harvest in 1977 and in 1978. One sample comprising 10 cores was taken from each lime treatment and analyzed as in experiment I.

Experiment III--Kellogg farm, clear seeded on prepared seed-bed, pH 4.8, four methods of inoculation and four lime treatments, 1978.

Experiment III was established on the Kellogg farm near Battle Creek in 1978. The soil was a Kalamazoo sandy loam, a member of the fine-loamy over sandy or sandy skeletal mixed, mesic family of Typic Hapludalfs with a 4% slope. Soil tests prior to initiating the experiment indicated a pH of 4.8, 80 Kg of P, 350 Kg of K, and 700 Kg of Ca/ha.

Glyphosate was sprayed at 2.2 Kg/ha three weeks before seeding to kill existing vegetation. On the day of seeding, 0-46-0 was broadcast at 224 kg/ha and incorporated with a disk. Eptam was sprayed at 4.0 Kg/ha and incorporated immediately for weed control.

Vernal alfalfa was seeded on 16 May at 9.0 kg/ha using a 5-row, nursery seeder in plots 0.91 by 7.60 m. Seeding rate adjustments and seed treatments were made as in experiment I.

The experiment was established as a split-plot, randomized, complete block with four replications. The variables were as follows:

Lime treatments (main plots):

1. No lime
2. 2.8 metric tons/ha surface broadcast after seeding
3. 11.2 metric tons/ha surface broadcast after seeding
4. 5.6 metric tons/ha before plowing + 5.6 metric tons/ha surface incorporated one week before seeding

Methods of inoculation (split plots):

1. No inoculation
2. Moist inoculation--Nitragin Co., Milwaukee, WI
3. Pelinoc--Nitragin Co., Milwaukee, WI
4. Lime pellet--Celpril Industries, Manteca, CA

The plots were harvested twice in 1978 on 27 July and 24 October. An area 0.91 by 6.70 m was harvested, and yields were determined as in experiment I. Seedling counts were made on 29 June. Four directed subplots, each 30 x 45 cm, were counted per plot. Growth ratings were made on 18 August.

Soil samples were taken in late September for pH analyses. One sample comprising 10 cores was taken per lime treatment and analyzed as in experiment I.

Experiment IV--East Lansing, clear seeded on prepared seed-bed, pH 5.3, four methods of inoculation and four lime treatments, 1978.

Experiment IV was established on the Michigan State University farm at East Lansing in 1978. The soil was a Miami sandy loam, a member of the fine-loamy, mixed, mesic family of Typic Hapludalfs with a 6% slope. Soil tests

prior to initiating the experiment indicated a pH of 5.3, 40 Kg of P, and 90 Kg of K/ha.

Three weeks before seeding, glyphosate was sprayed at 2.2 Kg/ha to kill existing vegetation. Fertilizer was broadcast at a rate of 224 Kg of 0-46-0 and 224 Kg of 0-0-60/ha and incorporated with a disk. Eptam at 4.0 Kg/ha was sprayed and incorporated immediately for weed control.

Vernal alfalfa was seeded on 19 June at 9.0 Kg/ha using a 5-row, nursery seeder in plots 0.91 by 7.60 m. Seeding rate adjustments and seed treatments were made as in experiment I.

The experiment was established as a split-plot, randomized, complete block with four replications. The variables were as follows:

Lime treatments (main plots):

1. No lime
2. 2.8 metric tons/ha surface broadcast after seeding
3. 11.2 metric tons/ha surface broadcast after seeding
4. 5.6 metric tons/ha before plowing + 5.6 metric tons/ha surface incorporated two weeks before seeding

Methods of inoculation (split plots):

1. No inoculation
2. Moist inoculation--Nitragin Co., Milwaukee, WI
3. Pelinoc--Nitragin Co., Milwaukee, WI
4. Lime pellet--Celpril Industries, Manteca, CA

Leafhoppers were controlled with sevin as necessary. The plots were harvested once in 1978 on 2 October. Yields were determined by cutting an area 0.91 by 7.60 m. Growth ratings were made on 22 September. Stand density was determined as in experiment I.

Soil samples were taken in mid-October for pH analyses. One sample comprising 10 cores was taken from each lime treatment and analyzed as in experiment I.

The monthly precipitation data for 1977 and 1978 at East Lansing and for 1978 at the Kellogg farm are presented in Table 1.

Table 1.--Monthly precipitation (mm) for 1977 and 1978 at East Lansing and for 1978 at the Kellogg farm.

Month	East Lansing		Kellogg farm
	1977	1978	1978
January	23.6	67.6	104.4
February	15.7	9.7	8.6
March	67.3	55.1	29.7
April	94.0	37.3	71.6
May	10.2	58.9	74.9
June	93.5	57.4	165.9
July	86.6	38.4	62.0
August	52.3	71.4	43.2
September	133.9	96.3	148.3
October	36.8	56.1	83.1
November	46.2		
December	59.2		

Greenhouse Experiment

The greenhouse experiment was established at East Lansing during the winter of 1978. The soil was from the upper 15 cm of the same Hillsdale sandy loam used in experiment II. It was removed from the field in November and stored in the greenhouse for three months and was not sterilized.

The soil was sieved to remove stones and clods. The 16 pots which received no lime were filled first to avoid contamination. The remaining 16 pots were filled, four at a time, with soil that had been limed. The lime was thoroughly mixed with the soil in a cement mixer at a rate of 70.0 g reagent grade CaCO_3 /pot. Each clay pot held four liters of soil and was 15 cm in diameter.

Vernal alfalfa was seeded 0.5 cm deep at a rate of 50 seeds per pot on 18 February. The seeds which received the Pelinoc treatment were treated on the day before seeding. Moist inoculation was done just prior to seeding. The seeds which received no inoculation were seeded first to avoid contamination followed by lime pelleted, moist inoculated, and Pelinoc-treated seeds in that order.

The experiment was established as a randomized, complete block with four replications. The pots within each replication were re-randomized every two days to minimize light and temperature gradients. The variables were as follows:

Lime treatments:

1. No lime--pH 5.1 before experiment and 6.2 at termination of experiment
2. Lime--70.0 g/pot--pH 7.8

Methods of inoculation:

1. No inoculation
2. Moist inoculation--Nitragin Co., Milwaukee, WI
3. Pelinoc--Nitragin Co., Milwaukee, WI
4. Lime pellet--Celpril Industries, Manteca, CA

An equal amount of distilled water was applied to each pot each time the soil appeared dry. On 20 March the plants were thinned to provide the maximum number of plants per pot per replication since stands were not uniform. Stands were thinned to 10 plants per pot in one replication, 15 plants each in two replications, and 20 plants in one replication. On 24 March one gram of KH_2PO_4 was added to each pot in the form of a solution to provide 90 kg of P and 112 kg of K/ha.

The experiment was terminated on 25 April. The plant tops were removed, dried, and weighed. The roots were washed in a root washer, Figure 1. Plants were rated on a scale of 1 to 5 based on the number of effective nodules per root. The number of nodulated roots per pot was recorded, the nodules were removed, and the nodules and roots were dried and weighed.

RESULTS AND DISCUSSION

Field Studies

Experiment I--East Lansing, sod-seeded, pH 5.5, three methods of inoculation, seven lime treatments, two years--1977 and 1978.

Yields and pH changes

Seeding year.--Seeding-year yields averaged 4.82 metric tons/ha and, these data are presented in Table 2. Second-cutting yields were 0.96 metric tons/ha greater than first-cutting yields due in part to 10 cm of irrigation water applied during August. There were no significant yield differences between methods of inoculation or due to amount or placement of lime. The average pH of the upper 10 cm of the unlimed plots was 5.49 which was probably not low enough for either Al or Mn to become toxic, Table 3. Seeding-year yields were not correlated with the pH of the upper 2.5 cm of the soil, Table 4. In the first year, liming the surface increased the pH of the surface 2.5 cm of the soil but had no effect below that depth. In the second year, the high rates of lime tended to increase the pH of the 2.5 to 5 cm depth. Drilling a small amount of lime with the seed increased the pH of the 0 to 2.5 cm depth

Table 2.--Seeding-year dry matter yields in metric tons/ha of sod-seeded alfalfa^a as affected by three methods of inoculation and seven lime treatments. Exp. I, East Lansing. 1977.

Lime, amount and method of application	Method of inoculation	Cut 1	Cut 2	Total
0	Moist	1.99	3.12	5.11
	Pelinoc	1.86	2.86	4.72
	Lime pellet	2.15	2.76	4.91
	Average	2.00	2.91	4.91
2.8 mt/ha broadcast	Moist	2.07	2.93	5.00
	Pelinoc	1.91	2.84	4.75
	Lime pellet	1.95	2.91	4.86
	Average	1.98	2.89	4.87
5.6 mt/ha broadcast	Moist	1.86	2.99	4.85
	Pelinoc	1.89	2.74	4.63
	Lime pellet	2.03	2.84	4.87
	Average	1.93	2.86	4.79
11.2 mt/ha broadcast	Moist	2.22	3.09	5.31
	Pelinoc	1.82	2.88	4.70
	Lime pellet	1.90	2.82	4.72
	Average	1.98	2.93	4.91
224 Kg/ha in the row	Moist	1.40	2.92	4.32
	Pelinoc	1.84	2.94	4.78
	Lime pellet	1.64	2.83	4.47
	Average	1.62	2.90	4.52
448 Kg/ha	Moist	1.95	3.01	4.96
	Pelinoc	2.09	2.86	4.95
	Lime pellet	2.14	2.79	4.93
	Average	2.06	2.88	4.94
11.2 split ^b	Moist	1.63	2.84	4.47
Average	Moist	1.91	3.01	4.92
	Pelinoc	1.90	2.85	4.75
	Lime pellet	1.97	2.82	4.79
	Average	1.93	2.89	4.82
LSD 0.05				NS
C.V.%: lime				17.9
inoculant				8.8

^aAll sod-seeded except the split application treatment which was band seeded on a prepared seedbed.

^b5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm prior to seeding.

Table 3.--Soil pH analysis by depth of Miami sandy loam, sampled in the fall of 1977 and 1978, as affected by seven lime treatments made in May 1977. Exp. I, East Lansing.

Lime, amount and method of application	Year	Depth, cm				
		0-2.5	2.5-5	5-10	10-20	20-30
0	77	5.72	5.51	5.36	5.37	5.60
	78	5.67	5.48	5.47	5.39	5.47
2.8 mt/ha broadcast	77	6.47	5.57	5.17	5.09	5.28
	78	6.23	5.63	5.36	5.05	5.22
5.6 mt/ha broadcast	77	6.59	5.60	5.41	5.34	5.40
	78	6.64	5.82	5.58	5.19	5.19
11.2 mt/ha broadcast	77	6.84	5.59	5.21	5.08	5.24
	78	7.00	6.17	5.57	5.05	4.91
224 Kg/ha in the row ^a	77	6.32	5.66	5.29	5.31	5.42
	78	5.61	5.41	5.23	5.02	5.09
448 Kg/ha in the row ^a	77	6.51	5.80	5.55	5.36	5.39
	78	5.90	5.63	5.27	5.14	5.13
11.2 mt/ha ^b	77	6.80	6.58	6.09	5.84	5.63
	78	6.76	6.90	6.46	6.05	5.25

^aLime drilled in the row and soil samples were taken in the row.

^b5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm.

Table 4.--Correlation coefficients between the pH of the upper 2.5 cm of the soil and 1977 yields, 1978 yields, vigor, and stand density of sod-seeded alfalfa as affected by seven lime treatments. Exp. I, East Lansing.

	1977 yield	1978 yield	Vigor	Stand density
pH	0.22	0.20	-0.01	0.11

No significance at the 0.05 level (55 df).

during the first year, but the increase had largely disappeared in the second year. Application of lime in a split application, with one half of the lime plowed under and the remainder incorporated into the upper 10 cm, increased the pH to a depth of 20 cm.

Second year.--Three harvests were made in the second year, and total yields averaged 10.27 metric tons/ha, Table 5. There were no significant yield differences between methods of inoculation. There appeared to be a slight trend toward greater yields on lime pellet- and Pelinoc-treated plots when no lime was applied, but these differences were not significant.

Yields tended to increase as the rate of lime application increased but did not become significant until 11.2 metric tons/ha were applied. Yields were not correlated with the pH of the upper 2.5 cm of the soil, Table 4. There was no significant difference between surface broadcasting the lime or applying it in a split application with one half plowed under and the remainder incorporated to a depth of 10 cm. The banding of a small amount of lime in the row did not significantly increase yields. This was expected, because the small amount of lime (224 or 448 kg/ha) was not adequate to correct the soil pH in the root zone.

The increased yields due to liming may have been the result of a greater utilization of nutrients. The lime may have increased the utilization of P or K, which were

Table 5.--Second-year dry matter yields in metric tons/ha of sod-seeded alfalfa^a as affected by three methods of inoculation and seven lime treatments. Exp. I, East Lansing, 1978.

Lime, amount and method of application	Method of inoculation	Cut 1	Cut 2	Cut 2	Total
0	Moist	4.73	2.19	2.27	9.19
	Pelinoc	5.10	2.44	2.34	9.88
	Lime pellet	5.39	2.27	2.39	10.05
	Average	5.07	2.30	2.33	9.70
2.8 mt/ha broadcast	Moist	5.01	2.34	2.73	10.08
	Pelinoc	5.06	2.47	2.71	10.24
	Lime pellet	5.26	2.14	2.64	10.04
	Average	5.11	2.32	2.69	10.12
5.6 mt/ha broadcast	Moist	5.15	2.34	2.48	9.97
	Pelinoc	5.51	2.64	2.45	10.60
	Lime pellet	6.14	2.26	2.41	10.81
	Average	5.60	2.41	2.45	10.46
11.2 mt/ha broadcast	Moist	6.05	2.65	2.60	11.30
	Pelinoc	5.81	2.62	2.83	11.26
	Lime pellet	5.26	2.35	2.60	10.21
	Average	5.71	2.54	2.67	10.92
224 Kg/ha in the row	Moist	5.26	2.05	2.67	9.98
	Pelinoc	5.11	1.98	2.62	9.71
	Lime pellet	5.83	1.93	2.62	10.38
	Average	5.40	1.99	2.64	10.03
448 Kg/ha	Moist	5.47	2.40	2.83	10.70
	Pelinoc	4.95	2.32	2.61	9.88
	Lime pellet	5.80	2.18	2.50	10.48
	Average	5.41	2.30	2.65	10.35
11.2 split ^b	Moist	6.00	2.44	2.80	11.24
Average	Moist	5.28	2.33	2.60	10.21
	Pelinoc	5.26	2.41	2.59	10.26
	Lime pellet	5.62	2.19	2.52	10.33
	Average	5.39	2.31	2.57	10.27
LSD lime 0.05					0.96
0.01					1.37
inoculant 0.05					NS
C.V.%: lime					8.9
inoculant					7.0

^aAll sod-seeded except the split application treatment which was band seeded on a prepared seedbed.

^b5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm prior to seeding.

applied in the spring of the second year. The lime may also have increased the availability of native soil nutrients.

Liming may also have increased the amount of nitrogen fixation. This may have been an important factor since both lime pellet- and Pelinoc-treated plots tended to show yield increases on unlimed plots. The purpose of these treatments was to increase the number of viable rhizobia available for nodulation. When the pH was low, the increased number of rhizobia could have resulted in a greater amount of nitrogen fixation. When the pH was increased, the lower number of rhizobia on the moist inoculated seeds could have been sufficient since more of them could have survived.

Vigor, stand density and root yield

The results of a vigor rating, stand density determinations, and root yields are presented in Table 6. There were no significant differences in vigor between methods of inoculation or lime treatments.

There were no increases in stand density due to either methods of inoculation or increased amounts of lime. There was a significant increase in stand density, however, caused by placement of the lime. When the lime was incorporated or drilled with the seed, stand density increased significantly. One explanation is that the lime, when drilled with the seed, neutralized the acidifying effect of the phosphate, starter fertilizer. This acidifying effect

Table 6.--Seeding-year vigor rating,^a stand density (plants/m²), and dry matter root yield (g/m², 0 to 12.5 cm deep) of sod-seeded alfalfa^b as affected by three methods of inoculation and seven lime treatments. Exp. I, East Lansing, 1977.

Lime, amount and method of application	Method of inoculation	Vigor rating	Stand density	Root weight
0	Moist	6.73	224.4	129.7
	Pelinoc	7.03	182.6	107.5
	Lime pellet	6.57	209.3	105.3
	Average	6.78	205.4	114.2
2.8 mt/ha broadcast	Moist	6.27	166.9	88.6
	Pelinoc	7.00	255.1	107.8
	Lime pellet	5.97	166.2	105.3
	Average	6.41	196.1	100.6
5.6 mt/ha broadcast	Moist	6.83	181.3	97.1
	Pelinoc	6.63	262.0	114.3
	Lime pellet	6.80	264.0	96.6
	Average	6.75	235.8	102.7
11.2 mt/ha broadcast	Moist	6.90	206.6	100.5
	Pelinoc	6.53	175.8	114.9
	Lime pellet	6.93	229.1	118.8
	Average	6.79	203.8	111.4
224 kg/ha in the row	Moist	6.93	348.2	106.7
	Pelinoc	7.27	275.0	108.8
	Lime pellet	6.77	230.5	107.9
	Average	6.99	284.6	107.8
448 kg/ha in the row	Moist	7.87	316.0	108.8
	Pelinoc	7.60	346.1	108.8
	Lime pellet	7.63	359.8	102.0
	Average	7.70	340.6	106.5
11.2 split ^c	Moist	6.79	317.4	105.5
Average	Moist	6.92	240.6	105.2
	Pelinoc	7.01	249.4	110.4
	Lime pellet	6.78	243.2	106.0
	Average	6.90	244.4	107.2
LSD lime 0.05		NS	60.9	NS
0.01			86.6	
inoculant 0.05		NS	NS	NS
C.V.%: lime		24.9	23.7	21.4
inoculant		11.0	19.1	14.7

^aRating scale: 0 = least vigor and 10 = greatest vigor.

^bAll sod-seeded except the split application treatment which was band seeded on a prepared seedbed.

^c5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm prior to seeding.

could have prevented the germination of some of the seeds when the lime was not incorporated. Increasing the amount of lime drilled with the seed from 224 to 448 Kg/ha increased stand density to a greater extent. When the lime was incorporated, as in the split-application, the seed was band-seeded on the surface of a prepared seedbed and was not in contact with the fertilizer.

There were no differences in root yield as affected by methods of inoculation or lime treatments. This would indicate that there were probably no Al toxicity problems which would have caused a reduction in root growth.

Nodulation

There were no differences in nodule weight between either methods of inoculation or lime treatments, Table 7. Nodule weights were low with some cores being almost completely barren of nodules. This may have occurred, because the cores were removed after the alfalfa had been cut. It has been suggested that nodules shed from the root after each cutting (Burton, 1972).

The high variability in the nodule determinations (C.V.: 79.9%) was probably due to (1) a poor technique for removing the cores from the field, (2) the lack of numbers sampled, and (3) the possible shedding of some nodules either before or after the core was removed.

Table 7.--Dry nodule weight (mg/core) by depth of alfalfa roots, sampled in the fall of the seeding year, as affected by three methods of inoculation and seven lime treatments. Exp. I, East Lansing, 1977.

Lime, amount and method of application	Method of inoculation	Depth, cm			
		0-2.5	2.5-5	5-10	Total
0	Moist	72.5	95.2	35.8	203.5
	Pelinoc	64.8	116.2	87.2	268.2
	Lime pellet	93.3	132.8	33.3	259.4
	Average	<u>76.9</u>	<u>114.7</u>	<u>52.1</u>	<u>243.7</u>
2.8 mt/ha broadcast	Moist	142.2	96.5	40.7	279.4
	Pelinoc	110.2	89.2	44.0	243.4
	Lime pellet	65.3	112.0	21.3	198.6
	Average	<u>105.9</u>	<u>99.2</u>	<u>35.3</u>	<u>240.4</u>
5.6 mt/ha broadcast	Moist	96.8	27.8	34.3	158.9
	Pelinoc	62.3	85.2	62.7	210.2
	Lime pellet	69.2	81.0	21.3	171.5
	Average	<u>76.1</u>	<u>64.7</u>	<u>39.4</u>	<u>180.2</u>
11.2 mt/ha	Moist	85.2	76.8	24.3	186.3
	Pelinoc	99.2	80.0	55.5	234.7
	Lime pellet	99.3	96.5	32.7	228.5
	Average	<u>94.6</u>	<u>71.2</u>	<u>37.5</u>	<u>216.5</u>
224 kg/ha in the row	Moist	103.5	56.8	15.5	175.8
	Pelinoc	97.8	85.3	50.2	233.3
	Lime pellet	98.0	71.5	13.8	183.3
	Average	<u>99.8</u>	<u>71.2</u>	<u>26.5</u>	<u>197.5</u>
448 kg/ha in the row	Moist	125.8	77.7	17.7	221.2
	Pelinoc	148.7	70.7	20.8	240.2
	Lime pellet	131.5	87.3	38.0	256.8
	Average	<u>135.3</u>	<u>78.6</u>	<u>25.5</u>	<u>239.4</u>
11.2 mt/ha ^a	Moist	81.6	70.8	19.0	171.4
Average	Moist	104.3	71.8	28.1	204.2
	Pelinoc	97.2	87.8	53.4	238.4
	Lime pellet	92.8	96.9	26.8	216.5
	Average	<u>98.1</u>	<u>85.5</u>	<u>36.1</u>	<u>219.7</u>
LSD 0.05		NS	NS	NS	NS
C.V.%: lime					79.9
inoculant					46.9

^a5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm prior to seeding.

Experiment II--East Lansing, clear-seeded on a prepared seedbed, pH 5.1 to 5.5, three methods of inoculation, six lime treatments, two years--1977 and 1978.

Yields and pH changes

Seeding year.--Seeding year yields were low, averaging 1.38 metric tons/ha, due to the late seeding on 23 July, Table 8. There were no significant yield differences due to methods of inoculation. When no lime was applied, however, the lime pellet- and Pelinoc-treated plots tended to outyield the conventionally moist-inoculated plots, but this increase was not significant.

There were significant yield increases due to liming. The application of 11.2 metric tons/ha before plowing did not increase yields significantly, but applications of lime, ranging from 2.8 to 11.2 metric tons/ha, incorporated into the upper 10 cm of the soil or 11.2 metric tons/ha applied in a split application increased yields significantly. The highest yields occurred when 11.2 metric tons/ha were applied in a split application with one half of the lime plowed under and the remainder incorporated to a depth of 10 cm. Yields in the seeding year were highly correlated with the pH of the upper 2.5 cm of the soil ($r = 0.61$), Table 9. The average pH of the upper 10 cm of the unlimed soil was 5.26, Table 10, which is low enough for toxicities to occur. Incorporation of lime to a depth of 10 cm increased the pH to that depth, but it did not

Table 8.--Seeding-year dry matter yields in metric tons/ha of summer-seeded alfalfa as affected by three methods of inoculation and six lime treatments. Exp. II, East Lansing, 1977.

Lime, mt/ha and method of application	Moist	Pelinoc	Lime pellet	Average
0	1.04	1.18	1.17	1.13
2.8 incorporated	1.45	1.44	1.37	1.42
5.6 incorporated	1.29	1.52	1.48	1.43
11.2 incorporated	1.48	1.40	1.53	1.47
11.2 split applic. ^a	1.72	1.67	1.51	1.63
11.2 before plowing	1.12	1.27	1.22	1.20
Average	1.35	1.41	1.38	1.38 NS
LSD 0.05				0.21
0.01				0.29

C.V.%: lime plots = 17.7; inoculant plots = 7.9

^a5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm prior to seeding.

Table 9.--Correlation coefficients between the pH of the upper 2.5 cm of the soil and 1977 yields, 1978 yields, vigor, and stand density of summer-seeded alfalfa as affected by six lime treatments. Exp. II, East Lansing.

	1977 yield	1978 yield	Vigor	Stand density
pH	0.61**	0.23	0.71**	0.26*

**,* significant at the 0.01 and 0.05 level (70 df), respectively.

Table 10.--Soil pH analyses by depth of Hillsdale sandy loam, sampled in the fall of 1977 and 1978, as affected by six lime treatments made in June 1977. Exp. II, East Lansing.

Lime, mt/ha and method of application	Year	Depth, cm				
		0-2.5	2.5-5	5-10	10-20	20-30
0	77	5.56	5.23	5.12	5.15	5.28
	78	5.45	5.12	5.01	5.10	5.34
2.8 surface incorporated	77	6.06	5.98	5.89	5.14	5.07
	78	6.06	5.86	5.68	5.28	5.37
5.6 surface incorporated	77	6.41	6.40	6.17	5.22	5.08
	78	6.38	6.34	6.24	5.38	5.34
11.2 surface incorporated	77	6.53	6.56	6.49	5.14	4.98
	78	6.56	6.50	6.54	5.33	5.09
11.2 split application ^a	77	6.54	6.51	6.26	5.56	5.06
	78	6.58	6.50	6.42	5.68	5.41
11.2 before plowing	77	6.03	5.86	5.91	5.78	5.13
	78	6.24	5.87	5.83	5.79	5.42

^a5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm.

affect the pH at greater depths. The split application of lime increased the pH of the upper 20 cm. When 11.2 metric tons/ha were plowed under, the pH was increased to a depth of 20 cm, but the increase in the upper 10 cm was only as large as when 2.8 metric tons/ha were incorporated into the upper 10 cm.

Second year.--Total second-year yields averaged 11.89 metric tons/ha and are presented in Table 11. There were no significant yield differences between methods of inoculation. There was a trend toward greater yields for lime pellet- and Pelinoc-treated plots when no lime was applied, but this increase was not significant. Prior to the first cutting, a slight color difference of the foliage was observed between methods of inoculation when the pH was below 5.3, but no color differences occurred when the pH was greater than 5.3, Figure 3. The alfalfa on plots which received the lime pellet or Pelinoc treatment was darker in color, possibly because of greater nitrogen fixation. The increased number of rhizobia applied per seed with lime pellet- and Pelinoc-treated seeds could have increased nitrogen fixation when the pH was below 5.3. At higher pH values, the lower number of rhizobia applied with conventional moist inoculation could have been sufficient, because of a better environment for fixation.

There were significant yield increases due to lime treatments, Table 11. The yield increases were not correlated

Table 11.--Second-year dry matter yields in metric tons/ha of summer-seeded alfalfa as affected by three methods of inoculation and six lime treatments. Exp. II, East Lansing, 1978.

Lime, mt/ha and method of application	Method of inoculation	Cut 1	Cut 2	Cut 3	Cut 4	Total
0	Moist	3.51	2.17	1.63	1.71	9.02
	Pelinoc	3.54	2.73	1.78	1.95	10.00
	Lime pellet	<u>3.71</u>	<u>2.83</u>	<u>1.70</u>	<u>1.88</u>	<u>10.12</u>
	Average	3.58	2.58	1.70	1.85	9.71
2.8 surface incorporated	Moist	4.04	3.05	1.86	2.20	11.15
	Pelinoc	4.43	3.23	2.01	2.35	12.02
	Lime pellet	<u>3.98</u>	<u>3.05</u>	<u>1.69</u>	<u>2.15</u>	<u>10.87</u>
	Average	4.15	3.11	1.85	2.23	11.34
5.6 surface incorporated	Moist	4.23	3.81	2.08	2.43	12.55
	Pelinoc	4.05	3.51	1.83	2.46	11.85
	Lime pellet	<u>4.12</u>	<u>3.61</u>	<u>1.98</u>	<u>2.49</u>	<u>12.20</u>
	Average	4.13	3.64	1.96	2.46	12.21
11.2 surface incorporated	Moist	4.44	3.74	1.90	2.64	12.72
	Pelinoc	4.49	3.66	1.93	2.54	12.62
	Lime pellet	<u>4.59</u>	<u>3.43</u>	<u>1.96</u>	<u>2.45</u>	<u>12.43</u>
	Average	4.51	3.61	1.93	2.54	12.59
11.2 split application ^a	Moist	4.56	4.55	2.21	3.06	14.38
	Pelinoc	4.57	3.89	2.46	2.73	13.64
	Lime pellet	<u>4.50</u>	<u>3.71</u>	<u>1.89</u>	<u>2.60</u>	<u>12.70</u>
	Average	4.54	4.05	2.18	2.80	13.57
11.2 before plowing	Moist	3.85	3.43	1.82	2.37	11.47
	Pelinoc	4.13	3.52	1.68	2.64	11.97
	Lime pellet	<u>4.24</u>	<u>3.72</u>	<u>1.77</u>	<u>2.53</u>	<u>12.26</u>
	Average	4.07	3.56	1.76	2.51	11.90
Average	Moist	4.11	3.46	1.92	2.40	11.89
	Pelinoc	4.20	3.42	1.95	2.44	12.01
	Lime pellet	<u>4.19</u>	<u>3.39</u>	<u>1.83</u>	<u>2.35</u>	<u>11.76</u>
	Average	4.17	3.42	1.90	2.40	11.89
LSD lime 0.05						2.20
0.01						3.04
inoculant 0.05						NS
C.V.%: lime						21.2
inoculant						9.1

^a5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm prior to seeding.



Figure 3. Moist inoculated alfalfa in the center plot is lighter in color than the alfalfa in the lime pellet- and Pelinoc-treated plots at a pH of 5.0 (above); no color differences occurred between inoculants at a pH of 5.5 (right).



with the pH of the upper 2.5 cm of the soil, Table 9. The greatest yields occurred, as in the seeding year, when 11.2 metric tons/ha were applied in a split application. Yields increased significantly when 11.2 metric tons/ha were plowed under, but the increase was less than when an equal amount was applied in a split application. When the lime was incorporated into the upper 10 cm of the soil, yields increased gradually as liming rate increased, but the increases were not significant until 5.6 metric tons/ha were incorporated.

Prior to the first cutting, it was observed that alfalfa on the unlimed plots was not as dark green as that on the limed plots, Figure 4. The lighter green occurred in three replications, but it did not occur in the fourth which had a pH of 5.37 in the upper 10 cm. The color differences were not observed on subsequent growth. The darker color of the alfalfa on the limed plots was probably the result of a greater amount of nitrogen fixation.

Vigor, stand density, and root yield

There were no differences observed in vigor between methods of inoculation, Table 12. As with yield, the greatest vigor occurred when 11.2 metric tons/ha of lime was applied in a split application with one half of the lime plowed under and half incorporated to a depth of 10 cm. This treatment resulted in significantly more vigor than when the entire 11.2 metric tons/ha were plowed under to a



Figure 4. The alfalfa on plots which received no lime (pH 5.2) is not as dark green as alfalfa on plots which were limed.

Table 12.--Vigor rating,^a stand density (plants/m²), and dry matter root yield (g/m², 0 to 12.5 cm deep) of summer-seeded alfalfa as affected by three methods of inoculation and six lime treatments. Exp. II, East Lansing, 1977.

Lime, mt/ha and method of application	Method of inoculation	Vigor rating	Stand density	Root yield
0	Moist	5.00	222.6	49.20
	Pelinoc	5.62	237.0	51.82
	Lime pellet	5.50	272.0	58.02
	Average	5.38	244.2	53.02
2.8 surface incorporated	Moist	6.62	259.7	64.78
	Pelinoc	7.25	262.7	56.50
	Lime pellet	6.50	242.4	49.65
	Average	6.79	255.0	56.98
5.6 surface incorporated	Moist	6.38	238.8	52.82
	Pelinoc	6.75	239.4	57.38
	Lime pellet	6.62	250.8	60.62
	Average	6.58	243.0	56.94
11.2 surface incorporated	Moist	7.12	239.4	54.50
	Pelinoc	6.88	232.8	57.15
	Lime pellet	7.25	275.3	63.60
	Average	7.08	249.2	58.42
11.2 split application ^b	Moist	7.62	240.0	59.20
	Pelinoc	7.25	252.0	55.32
	Lime pellet	7.25	247.2	55.18
	Average	7.38	246.4	56.57
11.2 before plowing	Moist	6.12	229.2	49.20
	Pelinoc	7.00	258.0	53.48
	Lime pellet	6.50	253.8	52.02
	Average	6.54	247.0	51.57
Average	Moist	6.48	238.3	54.95
	Pelinoc	6.79	247.0	55.28
	Lime pellet	6.60	257.1	56.52
	Average	6.62	247.4	55.58
LSD lime 0.05		0.81	NS	NS
0.01		1.12		
inoculant 0.05		NS	NS	NS
C.V. %: lime		14.0	24.8	29.2
inoculant		8.2	13.5	22.2

^aRating scale: 0 = least vigor and 10 = greatest vigor.

^b5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm prior to seeding.

depth of 20 cm. All lime treatments significantly increased vigor when compared to the no lime treatment.

There were no differences in stand density or root yield due to either method of inoculation or lime treatment. Apparently no toxic factor prevented either establishment or root development on the unlimed plots.

Manganese toxicity and chemical composition

Approximately one month after emergence, some plants had leaflets that were cup-shaped with chlorotic areas around the margin of the leaflets, Figure 5. The problem was intensified in the areas where no lime had been applied and in low areas of the field where the soil had been temporarily waterlogged after seeding due to irrigation and subsequent rainfall. The moisture retention of the soil after irrigation is exemplified in Figure 6. Foy (1964) and also Schmehl et al. (1950) had identified these symptoms as Mn toxicity. A sample of the injured plants indicated a Mn concentration of 652 ppm in the alfalfa tops. Ouellette and Dessureaux (1958) developed a scale of severity of Mn toxicity on alfalfa related to the concentration of Mn in the plant tops. Their rating scale is as follows:

<u>Mn (ppm)</u>	<u>Severity of Mn toxicity</u>
0-175	None
175-250	Very light (chlorosis)
250-325	Light (chlorosis and cupping)
325-400	Medium (crinkling of some leaves)
400-	Severe (crinkling of most leaves)



Figure 5. Manganese toxicity symptoms on alfalfa in the field (above) and a comparison between a leaf from a plant which showed Mn toxicity symptoms and a leaf from a healthy plant (right).





Figure 6. View of experiment II showing greater water retention in replications I and II where the pH was lowest and Mn toxicity was a greater problem.

Using this rating scale it is probable that the injured plants were suffering from Mn toxicity, although Al toxicity could also have been involved. Foy et al. (1978) found that the Mn content of the plant tops correlates fairly well with yield reductions, but it could mask Al toxicity. Aluminum accumulates in the root, and concentrations of Al in the plant tops do not give a good indication of the damage caused by Al.

The results of a Mn toxicity rating are presented in Table 13. The toxicity was most pronounced in plots which were not limed. The toxicity was significantly reduced with all applications of lime. The problem was almost completely eliminated when 11.2 metric tons/ha were incorporated to a depth of 10 cm or applied in a split application with one half of the lime plowed under and the remainder incorporated to a depth of 10 cm. The problem was not completely alleviated when 11.2 metric tons/ha were applied before plowing.

The results of tissue analyses for Mn, Al, and Fe are presented in Table 14. No significant differences occurred between methods of inoculation for any of the elements, although the moist inoculation treatment tended to have a higher Mn concentration at a low pH. The analyses were performed for only three lime treatments. The results indicate that as little as 2.8 metric tons/ha incorporated into the upper 10 cm significantly reduced the Mn content of the plant tops, and 11.2 metric tons/ha applied in a

Table 13.--Manganese toxicity rating^a of summer-seeded alfalfa as affected by three methods of inoculation and six lime treatments. Exp. II, East Lansing, 1977.

Lime, mt/ha and method of application	Method of inoculation			
	Moist	Pelinoc	Lime pellet	Average
0	2.4	1.6	2.2	2.1
2.8 incorporated	0.5	0.4	0.6	0.5
5.6 incorporated	0.2	0.4	0.2	0.3
11.2 incorporated	0.0	0.1	0.1	0.1
11.2 split applic. ^b	0.1	0.0	0.0	0.0
11.2 before plowing	0.9	0.4	0.6	0.6
Average	0.7	0.5	0.6	0.6 NS
LSD 0.05				0.5
0.01				0.6

^aRating scale: 0 = no toxicity and 10 = greatest toxicity.

^b5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm.

C.V.%: lime plots = 53.6; inoculant plots = 24.5.

Table 14.--Chemical composition of alfalfa tissue as affected by three methods of inoculation and three lime treatments. Exp. II, East Lansing, 1977.

Lime, mt/ha and method of application	Method of inoculation	Mn (ppm)	Al (ppm)	Fe (ppm)
0	Moist	371	250	269
	Pelinoc	240	174	204
	Lime pellet	255	160	213
	Average	289	195	229
2.8 surface incorporated	Moist	127	149	206
	Pelinoc	138	134	187
	Lime pellet	174	154	214
	Average	146	146	202
11.2 split application ^a	Moist	91	170	210
	Pelinoc	128	165	217
	Lime pellet	104	174	218
	Average	108	170	215
Average	Moist	196	190	228
	Pelinoc	169	158	203
	Lime pellet	178	163	215
	Average	181	170	215
LSD lime 0.05 inoculant 0.05		142	NS	NS
		NS	NS	NS
C.V. %: lime inoculant		78	53	44
		44	28	22

^a5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm prior to seeding.

split application further reduced the Mn concentration. The results also indicate that lime reduced the Al content of the plant tops although the differences were not significant. The Fe content of the plants was not greatly affected by lime applications.

The results indicate that yields were highly correlated with pH, Mn content, Al content, and Mn toxicity, Table 15. Manganese toxicity and the Mn content of the plants were highly correlated with the pH of the upper 2.5 cm of the soil, but Al content of the plants was not correlated with pH.

Table 15.--Correlation coefficients among pH,^a tissue Mn content, tissue Al content, Mn toxicity rating, and yield of summer-seeded alfalfa as affected by three lime treatments. Exp. II, East Lansing, 1977.

	pH	Tissue Mn	Tissue Al	Mn toxicity	Yield
pH	--	-0.63**	-0.12	-0.81**	0.67**
Tissue Mn		--	0.52**	0.73**	-0.73**
Tissue Al			--	0.34*	-0.48**
Mn toxicity				--	-0.73**

^apH of the upper 2.5 cm of the soil.

**, * significant at the 0.01 and 0.05 level (34 df), respectively.

Prior to liming, the Mn content of the soil ranged from 35 ppm in the low wet areas to 18 ppm in the better drained areas. The temporary waterlogging, which occurred after seeding, could have reduced manganese oxides to the divalent state which is more readily taken up by plants causing the toxicity. During the second year, no waterlogging occurred, and no Mn toxicity symptoms were observed.

Experiment III--Kellogg farm, clear-seeded on a prepared seedbed, pH 4.8, four methods of inoculation, four lime treatments, 1978.

Yields and pH changes

Seeding-year yields were very low, averaging only 2.52 metric tons/ha for two cuttings, Table 16. The low yields were probably due to the very low pH and the droughtiness of the soil. There were no yield differences between methods of inoculation. The treatment which received no inoculation yielded as much as the treatments which received inoculation. This could indicate that (1) there was sufficient residual nitrogen in the soil to provide an adequate supply of N for the first year's growth, (2) there were sufficient numbers of viable rhizobia already present in the soil to inoculate the plants which received no inoculation, or (3) the rhizobia that were supplied on the inoculated seeds were killed by some toxic factor in the soil.

There was a highly significant increase in yield due to liming. Yields were highest when 11.2 metric tons/ha

Table 16.--Dry matter yields (metric tons/ha), vigor rating,^a and stand density (plants/m²) of spring, clear-seeded alfalfa as affected by four methods of inoculation and four lime treatments. Exp. III, Kellogg farm, 1978.

Lime, mt/ha and method of application	Method of inoculation	Yield			Vigor rating	Stand density
		Cut 1	Cut 2	Total		
0	None	0.99	0.80	1.79	1.75	154.9
	Moist	0.88	0.74	1.62	2.00	171.5
	Pelinoc	1.05	0.68	1.73	1.88	157.2
	Lime pellet	<u>0.98</u>	<u>0.84</u>	<u>1.82</u>	<u>1.62</u>	<u>165.7</u>
	Average	0.98	0.76	1.74	1.81	162.3
2.8 surface broadcast	None	1.56	0.92	2.48	2.75	146.8
	Moist	1.36	0.78	2.14	2.75	155.4
	Pelinoc	1.43	0.97	2.40	2.75	185.0
	Lime pellet	<u>1.11</u>	<u>0.93</u>	<u>2.04</u>	<u>3.25</u>	<u>186.8</u>
	Average	1.37	0.90	2.27	2.88	168.5
11.2 surface broadcast	None	1.84	1.00	2.84	4.50	194.0
	Moist	1.80	0.98	2.78	4.38	177.8
	Pelinoc	1.85	0.98	2.83	3.88	188.1
	Lime pellet	<u>1.54</u>	<u>0.99</u>	<u>2.75</u>	<u>3.88</u>	<u>196.2</u>
	Average	1.76	0.99	2.75	4.16	189.0
11.2 split application ^b	None	2.35	1.00	3.35	5.12	210.1
	Moist	2.12	1.20	3.32	5.00	219.6
	Pelinoc	2.21	1.23	3.44	5.12	209.7
	Lime pellet	<u>2.09</u>	<u>1.12</u>	<u>3.21</u>	<u>4.88</u>	<u>192.2</u>
	Average	2.19	1.14	3.33	5.03	207.9
Average	None	1.69	0.93	2.62	3.53	176.5
	Moist	1.54	0.92	2.46	3.53	181.1
	Pelinoc	1.63	0.97	2.60	3.41	185.0
	Lime pellet	<u>1.43</u>	<u>0.97</u>	<u>2.40</u>	<u>3.41</u>	<u>185.2</u>
	Average	1.57	0.95	2.52	3.47	181.9
LSD lime 0.05				0.52	1.50	24.8
0.01				0.75	2.16	35.6
inoculant 0.05				NS	NS	NS
C.V.%: lime				25.8	54.2	17.0
inoculant				14.6	14.5	12.4

^aRating scale: 0 = least vigor and 10 = greatest vigor.

^b5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm prior to seeding.

were applied in a split application with one half of the lime plowed under and the remainder incorporated to a depth of 10 cm. The application of only 2.8 metric tons/ha on the surface increased yields significantly over the unlimed plots. The yield increase from 2.8 to 11.2 metric tons/ha on the surface approached significance. When 11.2 metric tons/ha were applied in a split application, however, yields were significantly higher than all other lime treatments. The soil pH of the upper 2.5 cm was highly correlated with yield, Table 17. With the very low pH, 4.70 in the upper 10 cm (Table 18), it is possible that increased yields due to liming could have been the result of a reduction of a toxicity. No Mn toxicity symptoms were observed, but Al or H-ion toxicity could have caused problems on the unlimed plots.

Surface broadcasting lime at a rate of 2.8 metric tons/ha altered the pH of the surface 2.5 cm only slightly--from 4.85 to 4.98, Table 18. This could indicate that the soil had a high Al content which prevented the pH from making a big change. It may also have been due to one heavy rainfall (approx. 125 mm) during June. This heavy rainfall could have washed the lime into depressions in the plots which were caused by tractor tires during field preparations. These depressions were not sampled. Surface broadcasting lime at 11.2 metric tons/ha significantly increased the pH of the upper 2.5 cm but had no effect below that depth.

Table 17.--Correlation coefficients between the pH of the upper 2.5 cm of the soil and yields, vigor, and stand density of spring, clear-seeded alfalfa as affected by four lime treatments. Exp. III, Kellogg farm, 1978.

	Yield	Vigor	Stand density
pH	0.63**	0.66**	0.59**

**significant at the 0.01 level (62 df).

Table 18.--Soil pH analyses by depth of Kalamazoo sandy loam, sampled in September 1978, as affected by four lime treatments made in May 1978. Exp. III, Kellogg farm, 1978.

Lime, mt/ha and method of application	Depth, cm				
	0-2.5	2.5-5	5-10	10-20	20-30
0	4.85	4.70	4.62	4.60	4.77
2.8 broadcast	4.98	4.58	4.46	4.58	4.65
11.2 broadcast	6.11	4.90	4.66	4.71	4.71
11.2 split ^a	6.62	6.41	5.26	4.98	4.99

^a5.6 metric tons/ha before plowing and 5.6 metric tons/ha incorporated to a depth of 10 cm.

The split application of lime increased the pH of the upper 10 cm but had little effect below that depth.

Vigor and stand density

No differences in vigor were observed between methods of inoculation, Table 16. As with yields, vigor was also correlated with the soil pH in the upper 2.5 cm, Table 17. The vigor was generally very poor on all plots, but as the rate of lime application increased the vigor also increased. The increase was not significant when 2.8 metric tons/ha were applied but became significant when 11.2 metric tons/ha were applied either on the surface or in a split application with one half of the lime plowed under and the remainder incorporated to a depth of 10 cm. The greatest vigor occurred with the split application, but this was not significantly greater than the surface application.

There were no significant differences in stand density between methods of inoculation, but significant differences did occur between lime treatments, Table 16. Stand density increased with increasing amounts of lime and correlated with the soil pH of the upper 2.5 cm, Table 17. The increases did not become significant until 11.2 metric tons/ha were applied. As with yield and vigor, the greatest stand density occurred when 11.2 metric tons/ha were applied in a split application. The increase in stand density due to liming could also indicate that a toxicity occurred on

the unlimed plots resulting in poor germination or seedling development.

Experiment IV--East Lansing, clear-seeded on a prepared seedbed, pH 5.2, four methods of inoculation, four lime treatments, 1978.

Yields and pH changes

Seeding-year yields averaged 2.37 metric tons/ha and are presented in Table 19. No differences occurred between methods of inoculation. The uninoculated plots yielded as well as inoculated plots. This could indicate that there were sufficient rhizobia already in the soil or that sufficient residual nitrogen was available for the first year's growth. The pH in the upper 10 cm of the soil was 5.14, Table 20. This was probably not low enough to kill all of the rhizobia or prevent nodulation from occurring. Surface broadcasting of lime increased the pH of the upper 2.5 cm from 5.25 to 6.19 but had no effect below that depth. The application of 11.2 metric tons/ha increased the pH of the upper 2.5 cm to 6.62. Lime applied in a split application with one half of the lime plowed under and the remainder incorporated to a depth of 10 cm increased the pH of the upper 10 cm but had very little effect below that depth.

Yields were not significantly affected by either amount or placement of lime, Table 19. At the high lime application rates, increases approached significance but were not significant. Yields were not correlated with the

Table 19.--Dry matter yields (metric tons/ha), vigor rating,^a and stand density (plants/m²) of summer-seeded alfalfa as affected by four methods of inoculation and four lime treatments. Exp. IV, East Lansing, 1978.

Lime, mt/ha and method of application	Method of inoculation	Yield	Vigor rating	Stand density
0	None	1.91	6.38	290.9
	Moist	1.98	6.25	332.0
	Pelinoc	2.12	6.50	302.2
	Lime pellet	1.84	6.38	340.5
	Average	1.96	6.38	313.9
2.8 surface broadcast	None	2.14	6.62	311.2
	Moist	2.08	6.50	304.0
	Pelinoc	2.32	6.75	273.5
	Lime pellet	2.23	6.75	311.8
	Average	2.19	6.66	300.1
11.2 surface broadcast	None	2.57	7.38	275.3
	Moist	2.64	7.38	290.3
	Pelinoc	2.60	7.38	342.3
	Lime pellet	2.78	7.38	296.9
	Average	2.65	7.38	301.2
11.2 split application ^b	None	2.62	7.50	313.0
	Moist	2.63	7.50	263.9
	Pelinoc	2.52	7.50	285.5
	Lime pellet	2.91	7.50	272.3
	Average	2.67	7.50	283.7
Average	None	2.31	6.96	297.6
	Moist	2.33	6.91	295.1
	Pelinoc	2.39	7.03	300.9
	Lime pellet	2.44	7.00	305.4
	Average	2.37	6.98	299.7
LSD lime 0.05		NS	0.73	NS
inoculant 0.05		NS	NS	NS
C.V.%: lime		46.1	13.1	18.3
inoculant		8.8	2.4	14.1

^aRating scale: 0 = least vigor and 10 = greatest vigor.

^b5.6 metric tons/ha before plowing plus 5.6 metric tons/ha incorporated to a depth of 10 cm prior to seeding.

soil pH, Table 21. No Mn toxicity symptoms were observed during the seeding year.

Vigor and stand density

The results of a vigor rating indicate that there were no differences in vigor between methods of inoculation, Table 19. There were differences in vigor between lime treatments. When 11.2 metric tons/hawere applied either on the surface or in a split application vigor increased significantly over the unlimed plots.

Stand density determinations showed no significant differences between methods of inoculation or lime treatments, Table 19.

Greenhouse Experiment

Top and root yields

Yields of tops and roots are presented in Table 22. Growth at the beginning of the experiment was slow due to damping-off (Pythium spp.), but once the plants became established growth was very rapid. There were no yield differences between methods of inoculation. The uninoculated treatments yielded as well as the inoculated treatments indicating that either adequate rhizobia were present in the soil or that nitrogen fixation was not related to yield, because sufficient nitrogen was available for growth.

Liming to a pH of 7.8 almost doubled both top and root yield. The increase in top growth due to liming is

Table 20.--Soil pH analyses by depth of Miami sandy loam, sampled in October 1978, as affected by four lime treatments made in June 1978. Exp. IV, East Lansing.

Lime, mt/ha and method of application	Depth, cm				
	0-2.5	2.5-5	5-10	10-20	20-30
0	5.25	5.08	5.13	5.00	4.94
2.8 broadcast	6.19	5.03	5.03	4.97	4.93
11.2 broadcast	6.62	5.07	5.01	4.93	4.91
11.2 split ^a	6.53	6.30	5.78	5.35	5.49

^a5.6 metric tons/ha before plowing and 5.6 metric tons/ha incorporated into the upper 10 cm.

Table 21.--Correlation coefficients between the pH of the upper 2.5 cm of the soil and yields, vigor, and stand density of summer-seeded alfalfa as affected by four lime treatments. Exp. IV, East Lansing, 1978.

	Yield	Vigor	Stand density
pH	0.14	0.39**	0.10

**Significant at the 0.01 level (62 df).

Table 22.--Dry-matter top and root yield, nodule weight, percent nodulation, percent effective nodulation, and nodule rating^a of alfalfa as affected by four methods of inoculation and two lime levels in the greenhouse; 1978.

pH	Method of inoculation	Top yield (g/pot)	Root yield (g/pot)	Nodule wt. (mg/pot)	% Nod.	% Effect. Nod.	Nodule rating
5.1	None	0.98	1.08	6.75	80.0	36.7	2.25
	to Moist	0.78	0.86	17.25	90.0	58.3	2.69
6.2	Pelinoc	0.75	0.78	19.25	86.7	56.7	2.53
	Lime pellet	<u>0.75</u>	<u>0.85</u>	<u>13.00</u>	<u>90.0</u>	<u>53.3</u>	<u>2.59</u>
	Average	0.81	0.89	14.06	86.7	51.2	2.51
7.3	None	1.75	1.68	4.75	81.7	20.0	2.06
	to Moist	1.62	1.78	2.75	96.7	11.7	2.09
7.8	Pelinoc	1.50	1.81	3.00	96.7	23.3	2.22
	Lime pellet	<u>1.39</u>	<u>1.36</u>	<u>3.75</u>	<u>96.7</u>	<u>15.0</u>	<u>2.14</u>
	Average	1.57	1.66	3.56	92.9	17.5	2.13
Ave.	None	1.37	1.38	5.75	80.8	28.3	2.16
	Moist	1.20	1.32	10.00	93.3	35.0	2.39
	Pelinoc	1.13	1.30	11.12	91.7	40.0	2.38
	Lime pellet	<u>1.07</u>	<u>1.11</u>	<u>8.38</u>	<u>93.3</u>	<u>34.2</u>	<u>2.36</u>
	Average	1.19	1.28	8.81	89.9	34.4	2.32
LSD	0.05	0.36	0.59	9.19	NS	3.5	0.37
	0.01	0.49	0.81	12.50		4.7	0.50
C.V.%		20.7	31.5	70.9	11.2	45.6	10.8

^aNodulation of individual plants scored on a 1 to 5 scale: 1 = no nodules, 2 = only small white (nonfunctional) nodules, 3 = one cluster of pink (functional) nodules, 4 = two or four clusters of functional nodules, and 5 = five or more clusters of functional nodules.

shown in Figure 7. This response is probably related to a greater utilization of nutrients or a reduction in toxicities. Nitrogen fixation was probably not related to yield since liming was negative correlated with nodule weight, Table 23.

Nodulation

The percentage of plants nodulated was not affected by the method of inoculation, Table 22. A nodule rating scale was used to score plants on their nitrogen-fixing ability. Seetin and Barnes (1977) used a similar scale and determined that their rating was highly correlated with acetylene reduction or nitrogen fixation. The scale is based on the number of effective nodules that are present on the root. An effectively nodulated plant had at least one cluster of effective nodules. The uninoculated plants were nodulated as often as the inoculated plants, but the nodules on the uninoculated plants were small and probably ineffective. The nodules on the inoculated plants which received no lime were clustered and effective. This resulted in a significantly higher nodule weight for inoculated plants which were not limed than uninoculated plants, Table 22. The nodule rating indicated that moist inoculated plants had a greater nitrogen-fixing ability than uninoculated plants when they were not limed. The lime pellet- and Pelinoc-treated plants had a higher nodule rating than the uninoculated plants, but the increase was not significant.



Figure 7. The beneficial effect of lime on top growth of alfalfa grown in the greenhouse is similar for each of all four methods of inoculation (above and right).



Table 23.--Correlation coefficients between lime application and percent nodulation, percent effective nodulation, nodule weight, nodule rating, top yield, and root yield of alfalfa grown in the greenhouse. 1978.

	% Nod.	% Effect. Nod.	Nodule Wt.	Nodule rating	Top yield	Root yield
Lime	0.13	-0.63**	-0.61**	-0.57**	0.60**	0.70**

**Significant at the 1% level (30 df).

The increase in effective nodulation on plants which received inoculation was not related to yield since there were no yield differences between methods of inoculation.

The percentage of nodulated plants were not significantly affected by liming, Table 22. When the soil was limed to a pH of 7.8, however, most of the nodules were small and probably ineffective. In contrast, when the soil was not limed, the nodules were clustered and effective. The percentage of plants effectively nodulated increased significantly on the unlimed treatments. This resulted in unlimed treatments having a higher nodule weight than limed treatments. The nodule rating indicated that liming the soil significantly reduced the nitrogen-fixing ability of the plants. A possible explanation is that the lime created a better environment for nitrification, and this inhibited the formation of nodules since the plants could get their nitrogen directly from the soil. The decrease in effective

nodulation did not affect yields which were highly correlated with lime, Table 23.

SUMMARY AND CONCLUSIONS

Yields were not significantly affected by the method of inoculation. During the seeding year, nitrogen fixation did not appear to be a significant factor. Apparently there was sufficient residual nitrogen in the soil to reduce the need for nitrogen fixation. This was demonstrated in the greenhouse where increased nodulation and nitrogen-fixing ability did not affect yield. In the field, plots that received no inoculation yielded as well as plots that received inoculation indicating that either nodulation was not related to yield in the seeding year, or sufficient rhizobia were already present in the soil.

During the second year, there was an indication that differences in yield were developing between methods of inoculation, but the differences were not significant. The alfalfa on plots that received the lime pellet or Pelinoc treatment were darker in color at a pH of 5.2 than the moist inoculated plants and were beginning to show a slight, although not significant, yield advantage over the moist inoculated plots. This could indicate that a greater amount of nitrogen fixation may occur on lime pellet- and Pelinoc-treated plots when the soil was not limed. As the need for

increased nitrogen from nitrogen fixation increased in the second year, there was an indication that the greater number of rhizobia applied on lime pellet- and Pelinoc-treated plots may be reflected in greater yields.

Seeding year yields varied in their response to increased applications of lime from soil to soil. In experiments I and IV, there were no seeding year responses due to liming. On experiments II and III, there were seeding year yield increases due to liming. The increased yields in experiments II and III were highly correlated with the pH of the upper 2.5 cm of the soil; values were $r = 0.61$ and 0.63 for experiments II and III, respectively.

Incorporating the lime into the plow layer was superior to plowing the entire amount of lime under to a depth of 20 cm. The highest seeding year yields occurred when the lime was applied in a split application, with one half of the lime plowed under and the remainder incorporated into the upper 10 cm. Broadcasting high rates of lime on the surface was nearly as effective in increasing seeding year yields as incorporating an equal amount of lime into the plow layer.

Second year yields increased significantly as the rate of lime application increased. The increased yields were not correlated with the pH of the upper 2.5 cm of the soil; values were $r = 0.20$ and 0.23 for experiments I and II, respectively.

The split application of lime provided the greatest yields in the second year, as in the seeding year, with yields of 16 and 40% greater than unlimed plots for experiments I and II, respectively. Broadcasting the lime on the surface was nearly as effective as incorporating an equal amount of lime into the plow layer in experiment I. In experiment II, the incorporation of as little as 5.6 metric tons/ha into the upper 10 cm of the soil was sufficient to provide a significant yield increase over the unlimed plots.

There were probably several factors involved in yield increases on limed soils. The factors varied in importance from experiment to experiment. One factor was likely an increased rate of nitrogen fixation on limed soils. This did not appear to be important in the seeding year, because there was probably sufficient residual nitrogen available for seeding year growth. This is supported by the results of the greenhouse study in which yields increased on limed soils while nodulation decreased. In the second year, increased nitrogen fixation was probably an important factor in improving yields on limed plots. On experiment II, alfalfa on the unlimed plots yellowed prior to the first cutting, apparently due to a nitrogen deficiency.

A second factor causing greater yields on limed soils was a reduction of Mn and possibly Al toxicity. In experiment II, Mn toxicity developed during the seeding year. Lime applications of as little as 2.8 metric tons/ha were sufficient to reduce the toxicity. Manganese toxicity

symptoms did not appear on the other experiments or in the second year on experiment II. Aluminum toxicity may have been involved in the poor yields on experiment III, but it was not positively identified.

Another factor causing greater yields on limed soils may have been an increased utilization of nutrients. This was probably the most important factor for increased yields on the limed soils in the greenhouse. In the field studies, it was difficult to determine the importance of increased nutrient availability on increased yields on limed soils. In the absence of toxicities, any increase in yield not attributed to increased nitrogen fixation was probably the result of greater nutrient utilization.

Another factor to be considered in causing greater yields on limed plots may have been an increased supply of calcium. This was probably not an important factor in these studies, because the Ca supply was not extremely low (700 to 1400 kg/ha) on any of the soils, and all of the soils had subsoils which were calcaerous.

Stand density was not affected by the method of inoculation. An increased number of rhizobia on the seed apparently does not affect alfalfa establishment on acid soils.

There was a significant increase in stand density due to liming on two of the four experiments. Incorporation of a small amount of lime with the seed increased stand density when the seed was drilled in contact with fertilizer

as in experiment I. This was probably due to a neutralization of the acidifying effect of the fertilizer which may have hindered germination and development. In experiment III, stand density was highly correlated with the pH of the upper 2.5 cm of the soil ($r = 0.59$). The increase in stand density due to increased pH could indicate that the pH was low enough to cause toxicity problems related to germination and seedling development. No stand density increases occurred with increased applications of lime on experiments II and IV, probably because there were no toxicities preventing germination.

The results of these studies indicate that lime pelleted seeds and the Pelinoc treatment do not aid in the establishment or yields of alfalfa in the seeding year. In the second year, a trend toward greater yields was observed for lime pellet- and Pelinoc-treated plots over moist inoculated plots, although this increase was not significant.

The results also indicate that broadcasting lime on the surface was nearly as effective in the establishment and yield of alfalfa as the incorporation of an equal amount of lime into the plow layer. The application of lime on the surface of the soil may enhance the productivity of sod-seeded alfalfa on acid soils.

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