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DESTA BEYENE

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**PHYSIOLOGY AND PERSISTENCE OF ALFALFA WITH CHANGES IN
SOIL CHEMISTRY**

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PHYSIOLOGY AND PERSISTENCE
OF ALFALFA WITH CHANGES
IN SOIL CHEMISTRY

by

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B.S., Alemaya Agricultural College, Ethiopia, 1970
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DISSERTATION

Submitted to the University of New Hampshire
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the Requirements for the Degree of

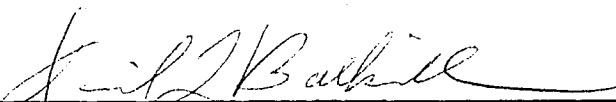
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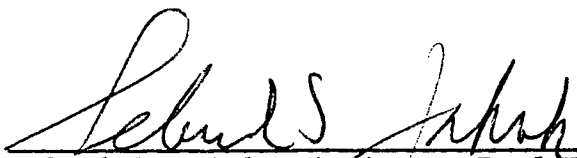
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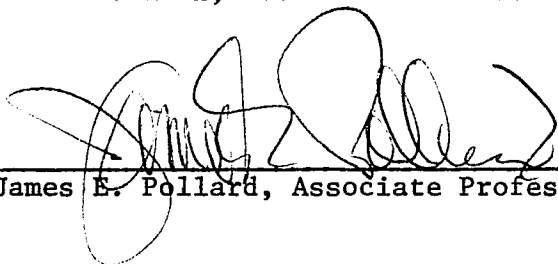
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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	ix
ABSTRACT	x
INTRODUCTION	1
REVIEW OF THE LITERATURE	2
Nature of Soil Acidity	2
Liming and Soil Reaction	3
Lime and Phosphorus Relationships	4
Soil Phosphorus-Aluminum Interactions	5
Legume Response to Soil Acidity	7
Aluminum Toxicity and Tolerance	7
Nodulation and Dinitrogen Fixation	10
Phosphorus Nutrition of Legumes	11
Metabolic Role of P	11
Role of P in Dinitrogen Fixation	12
MATERIALS AND METHODS	14
Greenhouse Investigations	14
Experiment 1: Response of Alfalfa to Lime and Superphosphate.	14
Experiment 2: Lime Requirement of Alfalfa.	15
Experiment 3: Influence of Lime on Nitrogen Fixation of Two Alfalfa Varieties.	16
Experiment 4: Influence of Lime on the Mobility of Phosphorus-32 in Alfalfa.	17
Analytical Procedures	22
Soil Chemical Analyses	22
Plant Chemical Analyses	23
Root Length and Nodulation Measurements	23
Nitrogen Fixation Assay	24
Field Investigations	25
Experiment 1: Influence of Lime on Al Saturation and Available P.	25
Experiment 2: Influence of Lime and Phosphorus on Alfalfa Growth.	26

Experiment 3: Influence of Lime on Chemical Changes in Soil Profile.	28
Statistical Analysis	29
RESULTS AND DISCUSSION	30
Greenhouse Investigations	30
Experiment 1: Response of Alfalfa to Lime and Superphosphate.	30
Experiment 2: Lime Requirement of Alfalfa.	36
Experiment 3: Influence of Lime on Nitrogen Fixation of Two Alfalfa Varieties.	51
Experiment 4: Influence of Lime on the Mobility of Phosphorus-32 in Alfalfa.	58
Field Investigations	65
Weather and Soil Data	65
Experiment 1: Influence of Lime on Al Saturation and Available P.	65
Experiment 2: Influence of Lime and Phosphorus on Alfalfa Growth.	99
Experiment 3: Influence of Lime on Chemical Changes in the Soil Profile.	103
SUMMARY AND CONCLUSIONS	109
LITERATURE CITED	111

LIST OF TABLES

1	The lime and phosphate treatments for the Charlton sandy loam at Demerit Farm.	27
2	Soil characteristics of the four soils used in the greenhouse experiments prior to treatment. . .	31
3	Yield of alfalfa, plant P concentration, and soil mineral analysis as influenced by dolomitic limestone in Experiment 1.	34
4	Yield of alfalfa, plant P concentration, and soil mineral analysis as influenced by phosphate fertilizer in Experiment 1.	35
5	Main effects of lime on soil pH, Ca, Mg, K, Al and P for Experiment 2.	37
6	Main effects of P fertilizer on soil pH, P, Al, Ca, Mg and K for Experiment 2.	38
7	The main effects of lime rate on dry matter yield of alfalfa herbage at harvest for Experiment 2.	42
8	The main effects of lime rate on nodulation, root length, nitrogen fixation and dry matter of alfalfa for Experiment 2.	43
9	Tissue composition of alfalfa from the 1980 harvests of the Paxton sandy loam for Experiment 2.	45
10	The main effects of P fertilizer on the dry matter yield of alfalfa herbage at harvest for Experiment 2.	47
11	The main effects of P fertilizer on nodulation, root length, nitrogen fixation and dry matter yield of alfalfa for Experiment 2.	48
12	Tissue composition of alfalfa from the 1980 harvests for Experiment 2.	49
13	Coefficients of determination (r^2), for the relationships among soil P and Al saturation, top and root dry weights (DW), root length, plant P and nitrogen fixation for alfalfa in Experiment 2.	50
14	Soil analysis for the Paxton soil in Experiment 3.	52
15	Dry matter yield of alfalfa varieties as influenced by lime and P treatments in Experiment 3.	53
16	Nodulation score of Iroquois as influenced by lime and P treatment in Experiment 3.	54
17	Effect of lime and P applications on nitrogen fixation in alfalfa for Experiment 3.	56
18	Effect of lime and P applications on the concentration of organic acids in alfalfa roots for Experiment 3.	57

19	The relative concentrations of Al, P and Ca in alfalfa as measured by Energy Dispersive Analysis by X-ray (EDS).	62
20	The effect of lime on the dry matter yield of four alfalfa cultivars for Experiment 4.	64
21	Weather data at Durham for 1976-1981 growing seasons.	66
22	Initial soil analysis of field experiments.	67
23	The effect of lime on the dry matter yield of Iroquois alfalfa for each harvest in field Experiment 1.	69
24	The effect of lime on total dry matter yields of alfalfa for each year.	70
25	Relative effectiveness (R.E.) of lime on alfalfa dry matter yield for field Experiment 1.	71
26	Regression analysis for alfalfa dry matter yield with lime rate (t/ha).	73
27	The effect of lime on alfalfa dry matter yield and the profits obtained from the application.	76
28	Simple correlations (r) for the relationships among yield, plant N, Ca, Mg, P, Al and Mn for 1980 and 1981 in field Experiment 1.	77
29	The effect of lime on tissue composition of alfalfa for the first harvest of 1980 and 1981 in field Experiment 1.	79
30	The effects of lime on the % N and yields of N and protein for the first harvest.	81
31	Soil analysis for the Charlton sandy loam in July 1980 for field Experiment 1.	84
32	Soil analysis for the Charlton sandy loam in July 1981 for field Experiment 1.	85
33	The influence of lime on P, Ca, Mg and K as determined by two different extracting solutions.	86
34	The influence of lime on soil organic matter, Al, H, Mn and Fe for field Experiment 1.	88
35	Relationships between lime rates (X) and soil-plant chemical properties and crop yields for 1981 in field Experiment 1.	90
36	Regression models relating alfalfa dry matter yields of 1981 to selected soil chemical factors in field Experiment 1.	91
37	Regression models relating alfalfa dry matter yield of 1981 to selected plant chemical factors in field Experiment 1.	92
38	Main effects of lime on dry matter yield and soil-plant characteristics of 1979 and 1981 for the Demerit Experiment.	100

39	Main effects of phosphate fertilizer on dry matter yield and soil-plant characteristics of 1979 and 1980 for the Demerit Experiment.	101
40	Soil analysis of the Woodward Experiment for July 1981.	104
41	The effect of lime on tissue composition of alfalfa for the Woodward Experiment in August 1981.	108

LIST OF FIGURES

1	Alfalfa plants in Rootainers for ³² P study. . . .	21
2	Changes in soil pH with liming during a 53-day period for greenhouse Experiment 1.	32
3	Mean Ca and P changes within the soil during a 53-day period for greenhouse Experiment 1. . . .	33
4	Differential response of alfalfa plants to lime and P treatments in greenhouse Experiment 2. . . .	39
5	Differential response of alfalfa seedlings to lime and P treatments in greenhouse Experiment 2.	40
6	Autoradiograph of alfalfa plants grown in soil at 0 t/ha of lime for greenhouse Experiment 4. . .	59
7	Autoradiograph of alfalfa plants grown in soil at 6.9 t/ha of lime for greenhouse Experiment 4. .	60
8	Main effects of lime on the dry matter yield of alfalfa for 1976-1981.	68
9	The influence of lime on alfalfa dry matter yield as shown through regression analysis. . . .	74
10	Relations between lime, plant P and Al during the period from 1976 to 1981 for field Experiment 1.	78
11	Changes in soil pH and exchangeable Al of a Charlton soil under three soil depths for field Experiment 1 in 1981.	82
12	Contour surface of alfalfa yield for 1981 as a function of plant P and Al. Viewing angle: azimuth 60°, elevation 30°.	94
13	Contour surface of alfalfa yield for 1981 as a function of plant N and Al. Viewing angle: azimuth 60°, elevation 30°.	96
14	Contour surface of alfalfa yield for 1981 as a function of plant N and P. Viewing angle: azimuth 60°, elevation 30°.	97
15	Contour surface of plant N for 1981 as a function of plant P and Al. Viewing angle: azimuth 60°, elevation 30°.	98
16	Relationships between lime rate, soil pH, and exchangeable Ca at three soil depths for 1981 in field Experiment 3.	105
17	Relationships between lime rate, soil Mg, and P at three soil depths for 1981 in field Experiment 1.	106

ABSTRACT

PHYSIOLOGY AND PERSISTENCE OF ALFALFA WITH CHANGES IN SOIL CHEMISTRY

by

DESTA BEYENE

University of New Hampshire, May 1982

The influence of liming on soil chemical changes and on nodulation, nitrogen fixation and yield of alfalfa was investigated.

In the field, increasing rates of lime improved alfalfa dry matter yield at the Kingman Farm. Optimum dry matter yield was obtained with 3.5 tons lime/ha. A quadratic response curve showed that liming accounted for about 70 percent of the yield variation in alfalfa. Herbage N, P and Mg increased in response to liming six years after establishment. The simple correlations between dry matter yield and plant N, plant P and plant Mg were positive and significant. Plant Al and Mn showed negative influences on plant P, N and Mg as well as on dry matter yield.

Total nitrogen accumulation and protein yields were significantly increased with liming. Nitrogen content was strongly associated with the yield of alfalfa. Depletion of soil N appeared to be the main cause for the improvement in the response of N fixation and yield to liming, especially in the last two years (1980-1981). This is in agreement with the general conclusion that legumes become more sensitive to soil acidity and thus respond to liming more readily if they depend on symbiotic N₂ fixation for the supply of N.

The relation between herbage composition and alfalfa yield was studied with regression analysis. In 1981 optimum

alfalfa dry matter yield of 14.9 tons/ha was obtained with a herbage composition of 0.33 % P, 1.0 % Ca, 0.33 % Mg, 4.7 % N and 2.4 % K. This optimum yield was 143 % of the control yield.

Analysis of soil cations indicated that levels of exchangeable Ca and Mg increased with liming. Soil pH increases from liming were found to cause decreases in exchangeable Al and soil P. The decreases in exchangeable Al and available P (Bray #1) could be associated with precipitation and adsorption (fixation) reactions, respectively. Low soil pH, low soil P and exchangeable Ca, and high exchangeable Al are the dominant stresses for alfalfa persistence. The neutralization of the acidity in the top 30 cm soil depth was adequate for maintaining a high productivity of alfalfa. Thus, the incorporation of the applied lime to the plow layer becomes an important consideration when maximum effectiveness of the lime is required.

The yield and analytical data from the Demerit and Woodward Farms clearly demonstrated that the effectiveness of lime in improving alfalfa yield was dependent on the maintenance of adequate P and K supplies and on favorable environmental conditions. Alfalfa crop that was poorly established did not receive any benefit from the applications of lime and/or P fertilizer.

In the greenhouse, exchangeable Al was found to be a better indicator of crop response to lime application than soil pH. The relationship of improved yield to reduced Al saturation was far more pronounced than the relationship between yield and soil pH. Top and root dry weights were shown to be more correlated to Al saturation than pH or available P. Neutralization of soil acidity equivalent to 150 percent of the exchangeable Al through liming gave the highest total dry matter yield and nitrogen fixation. Application of P fertilizer does not decrease exchangeable

Al to the same extent as does liming. The interaction between lime and P fertilizer was not significant.

Work with radioactive phosphorus in the soil showed that liming caused a precipitation of the applied ^{32}P . The P was reverted and so less ^{32}P was mobilized to plant roots when lime was applied to the soil. The ratio of Al/P in 'Iroquois' alfalfa roots was reduced through liming.

Liming did significantly decrease exchangeable Al and resulted in improved dry matter yields for alfalfa both under greenhouse and field conditions. Increases in soil pH from liming beyond the 5.8 level led to a decrease in the availability of soil P and showed no improvement in alfalfa dry matter yields. The main purpose of liming should, therefore, be to alleviate the stress from toxic levels of soil Al on the growth of alfalfa.

INTRODUCTION

Interest in legumes has increased tremendously in the past decade because of the high energy requirements to manufacture nitrogen fertilizer. The cost of nitrogen fertilizer (urea) has increased from about fifty dollars in 1970 to two hundred and thirty dollars per ton in 1980. As supplies of fossil fuel dwindle and world population increases, the resources available for producing plant protein become more and more expensive. Forage legumes, like alfalfa, which can meet their own nitrogen needs become increasingly important in our agricultural system. Alfalfa can fix about 150 kg N/ha per year.

Soil acidity poses a serious problem to intensive cropping of many soils in New England. This has led to a growing concern for the potential improvement of these soils for increased forage production. Lime has generally been used to correct soil acidity and increase the availability of certain plant nutrients. However, the critical lime rate, at which nutrient uptake, nitrogen fixation and alfalfa growth are optimized, is not well established.

The objectives of the present investigation were threefold; namely, a) to study the influence of lime on yield, N fixation and plant-soil characteristics, b) to evaluate soil aluminum as an index to lime requirements and c) to examine the relationship between yield and nutrient composition of alfalfa.

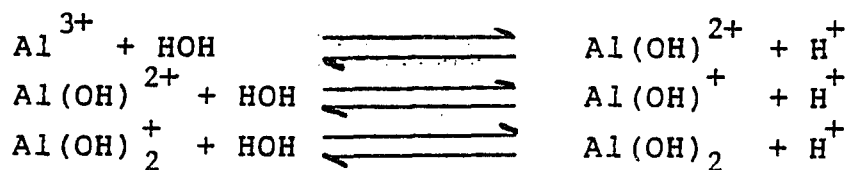
REVIEW OF THE LITERATURE

Nature of Soil Acidity

The soils of New Hampshire are acidic spodosols, highly weathered with illite as the major clay mineral (Gamble, 1954). The acidity is in part a function of the rain water leaching away exchangeable bases replacing them with hydrogen (H) and aluminum (Al) ions. According to Tisdale and Nelson (1975) acidity in soils has several sources: a) humus or organic matter, b) aluminosilicate clays, c) hydrous oxides of iron and aluminum, d) exchangeable aluminum, e) soluble salts and f) carbon dioxide. It is generally believed that the presence of exchangeable Al, along with the depletion of basic cations such as Ca, Mg, and K is responsible for the development of acid soils.

Soil acidity is a function of hydrogen and aluminum cations (Black, 1967; Brady, 1974; Russell, 1974). The H⁺ ions resulting from the hydrolysis of Al and Fe-compounds react with and dissolve or decompose soil minerals. The action by chelating agents from microbes on the parent material (Alexander, 1976) and the hydrolysis of inorganic acids (Jackson, 1963) have been postulated to be important sources for soil Al.

According to more recent concepts of the chemistry of soil acidity, the bulk of reserve acidity in soils exists largely in the form of Al, hydroxy-Al ions such as Al(OH)²⁺ and Al(OH)₂⁺ or as charged hydroxy-Al complexes (Coleman and Thomas, 1967; Russell, 1974; McLean, 1976; Bohn, et al., 1979). While the actual compounds and reactions are complex and not completely understood, the hydrolysis reactions are useful in understanding the role of Al in soil acidity:

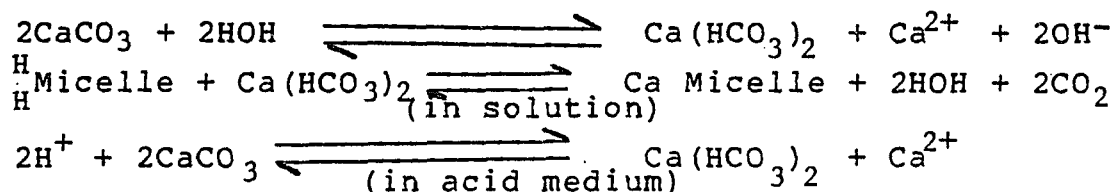


As the soil system becomes more acid, the H^+ concentration increases and the equilibrium moves toward the left. The hydroxy-Al may exist as charged polymers and as the system becomes acidic, the charge on these polymers increases. These increased positive charges satisfy the negatively charged exchange sites of soil colloids so the effective exchange capacity of the soil is reduced with increased levels of soluble Al.

Liming and Soil Reaction

The use of liming materials to amend acid soils is not a new practice for New England (Taylor, 1910). Liming a soil increases its exchangeable bases, reduces Al and Mn levels, decreases anion adsorption and releases them into solution, and improves conditions for microbial action.

All liming materials will react with acid soils, the calcium (Ca) and magnesium (Mg) replacing H on the colloidal complex (Coleman, et al., 1958; Coleman and Thomas, 1967). The adsorption of the Ca and Mg raises the percentage base saturation of the exchange complex and the pH of the soil solution is increased correspondingly. The mechanism controlling the reaction of liming materials with acid soils is complex, but the overall reaction can be depicted by the following equations (Brady, 1974):



It is generally believed that the liming reaction begins

with the neutralization of H^+ ions in the soil solution by OH^- ions furnished by the liming material (McLean, 1973). As long as sufficient H^+ ions are in the soil solution, Ca^{2+} and HCO_3^- ions will continue to go into solution.

Lime tends to move slowly in the soil profile and so the initial beneficial effects are mainly in the immediate vicinity of application. Thus surface application of lime without some degree of mixing in the soil is not effective in correcting subsoil acidity (Pearson, et al., 1973). For a given soil and climate, rates and final depths of lime penetration effects depend on the amount of lime applied and on the lapse of time (Brown, et al., 1956).

Strongly acid subsoils drastically reduce crop yields (Pearson, 1966; Doss and Lund, 1975). A high Al saturation of the exchange complex restricts exploitation of the subsoil by roots for moisture and nutrients. The problem of highly acid subsoils restricting plant root growth has long been recognized in the Southeastern U.S. soils. Metzger (1934) and Brown and Munsell (1938) reported that 10 to 14 years were required for surface-applied lime to increase the soil pH to a 15 cm depth.

Lime and Phosphorus Relationships

The beneficial effects of liming on phosphorus (P) availability, according to many soil scientists, are directly associated with the neutralization of the toxic levels of aluminum. In acid soils lime is mainly applied to precipitate the Al and ultimately increase the amount of available P. It is not possible to chemically reduce the soil level of Al, but its reactivity with P may be reduced somewhat by increasing the base saturation of acid soils (Brown, 1967). As the exchangeable Al is neutralized, the added lime (Ca) will react with the surface hydroxyl of hydrated oxides of Al and Fe (Yuan, 1970). This will tend

to reduce P fixation in the soil and result in increased availability of P to plants.

Soil P response to lime can vary considerably between soils (Lanyon, et al., 1977; Mengel and Kamprath, 1978). In their study to relate Al saturation with extractable P in soils, Sartain and Kamprath (1975) indicated that the level of Al saturation was markedly reduced and extractable P increased with lime applications. On the other hand, Reeve and Sumner (1970) showed with several Florida soils that the levels of exchangeable Al were practically eliminated by liming but there was no increase in extractable P. Munns (1965) also found that lime reduced Al saturation but had no influence on the release of P in the soil. Kamprath (1970) stated that the reduction of Al saturation should be the aim of liming.

Soil Phosphorus-Aluminum Interactions

Acid soils are generally associated with low levels of Ca and P and toxic levels of Al and Mn (Andrews, 1977). Many studies have examined the interactions of phosphorus and aluminum as influenced by soil pH. The reaction of P with exchangeable Al may be a precipitation or adsorption and follows the Langmuir isotherm (Woodruff and Kamprath, 1965). The Al^{3+} ion is predominant below pH 4.7, $Al(OH)_2^+$ between pH 4.7 and 6.5, $Al(OH)_3^0$ between pH 6.5 and 8.0 and $Al(OH)_4^-$ above pH 8.0 (Marion, et al., 1976).

The majority of the P in the soil is unavailable to plants due to precipitation and/or fixation by Al and iron (Fe) below pH 6.0 and precipitation by Ca above pH 7.0 (Brady, 1974). In general, Ca-phosphates are more soluble than Al-phosphates, and the latter are more soluble than Fe-phosphates (Chang and Jackson, 1957; McLean, 1976). With an increase in pH or OH ion activity, $AlPO_4$ and $FePO_4$ release PO_4^{3-} in soluble form; and the Al and Fe remain in

insoluble form as $\text{Al}(\text{OH})_3$ and $\text{Fe}(\text{OH})_3$. Conversely, with a decrease in pH or with an increase in PO_4^{3-} activity, a tendency exists for $\text{Al}(\text{OH})_3$ and $\text{Fe}(\text{OH})_3$ to react with PO_4^- to form AlPO_4 and FePO_4 . The OH^- anions are subject to replacement by PO_4^{3-} at low pH values but not at high pH values.

The amount of total P in soils is in the range of only 0.02 to 0.30 % P compared with 3 to 9 % for Al, and 1.4 to 4.0 % for Fe (Baker, 1976). A substantial amount of P is associated with soil organic matter (Williams, 1970) and in mineral soils the proportion of organic P comprises between 20 and 80 % of the total P (Mengel and Kirby, 1978). Olson and Englestand (1972) found that representative soils from the Midwestern United States average about 0.3 % total P in the topsoil; the more weathered soils of the Southeastern U.S. and tropical soils gave .05 % and .02 %, respectively.

Adsorbed phosphate exceeds the phosphate of the soil solution by a factor of 10^2 to 10^3 . The phosphate concentration of the soil solution itself is very dilute and in fertile arable soils is about 3 ppm P (Mengel, et al., 1969; Hossner, et al., 1973). The maintenance of a steady concentration of this solution P is of paramount importance to the sustained vigorous growth of crops.

The most important phosphorus ions in the soil solution for plant use are HPO_4^{2-} and H_2PO_4^- . The concentration of these two major orthophosphates in soil solution is intimately related to the pH of the soil (Black, 1967; Brady, 1974; Thomas, 1974). The H_2PO_4^- ion is favored in more acid soils whereas the HPO_4^{2-} ion is favored above pH 7.0. The H_2PO_4^- species is the most soluble form of P found in the soil and it occurs in the greatest amount between pH 4.0 and 5.0 where the concentration of soluble Al is the highest. Susuki, Lawton and Doll (1963) indicated that Ca-P and secondarily Al-P were important in supplying P to plants in 17 Michigan soils ranging in pH from 4.8 to 7.8. Bishop

and Barber (1958) found Ca-P, Al-P and Fe-P to be important in supplying P to plants growing on upland lake plain soils in Indiana, whereas Fe-P and to a lesser extent Al-P were important on central Indiana soils (Al-abbas and Barber, 1964). According to Vijayachandran and Harter (1975), the role of Fe in the fixation of P is considered less important than Al in temperate soils. Significant correlations were found between P fixation and exchangeable Al only.

Legume Response to Soil Acidity

Legume growth is severely limited by the effects of high Al saturation on growth, nodulation, and nutrient availability (Kamprath and Foy, 1971; Kamprath, 1970; Mengel and Kamprath, 1978). Roberts (1980) has fully reviewed the work on the effects of soil acidity on legume growth.

Aluminum Toxicity and Tolerance

There is some evidence that low levels of Al can have a beneficial effect on plant growth although the mechanism is not well understood (Foy, 1974). Higher plants have been reported to contain about 200 ppm Al in the dry matter (Hutchinson, 1945).

The direct toxicity of Al ions in solution is generally the major factor reducing growth and yield of legumes on acid soils (Foy, 1974; McLean, 1976; Olmas and Camargo, 1976; Silva, 1976). Aluminum toxicity is particularly severe below pH 5.0 but has been reported at soil pH values as high as 5.5 (Adams and Lund, 1966). Aluminum often accumulates in the roots leading to root damage and poor root growth (MacLeod and Jackson, 1965; Foy, et al., 1969; Kerridge, et al., 1971; Reid, et al., 1971; Konzak, et al., 1976).

Aluminum appears to reduce the solubility of P and restrict its uptake and utilization by plants. The upper plant parts of Al toxic plants are frequently low in P. The toxicity in the tops is therefore often characterized by symptoms similar to those of P deficiency such as dark green leaves, stunted plant growth, delayed maturity and purpling of the stems (Foy, 1974; Long and Foy, 1970). Soils classified as Al-toxic may differ widely in their content of exchangeable Ca; this may influence the degree of Al toxicity and kinds of plant symptoms produced at given levels of pH and exchangeable Al. Higher amounts of exchangeable Ca were found to produce less Al stress (Foy, et al., 1969). Therefore, in screening plants for Al tolerance using hydroponics or soils some of the important soil factors to consider are pH, levels of Al, P and Ca.

Various criteria have been used to determine the Al tolerance of different cultivars. The effect of Al on the dry weight of plant tops has been used as an index of Al tolerance (Armigh, et al., 1968) on the assumption that the dry weight of tops reflects the capacity of roots to take up nutrients and water from the soil. Since Al is known to inhibit cell division (Clarkson, 1965; Matsumoto and Morimura, 1980) various measurements of root growth, such as root dry weight (Reid, et al., 1971) and root length (Kerridge, et al., 1971) have also been used as criteria for evaluating Al tolerance of plants. Another index used is the ability of plants to absorb and utilize P in the presence of high Al levels without showing P deficiency (Jones, 1961; Foy and Brown, 1964). High external concentrations of Al in the root zone resulted in accumulations of P in roots and a decrease in tops (Wright and Donahue, 1953); and reduction in Ca uptake and translocation (Johnson and Jackson, 1964). Soybean cultivars tolerant of high Al levels had higher Ca concentrations in roots and tops than did sensitive

cultivars (Foy, et al., 1969). Foy, et al., (1967) suggested the use of root color and degree of branching as indicators for Al tolerance.

The content of Al in plant foliage was not found to be a good indicator of the degree of tolerance. There appears to be no strong correlation between Al content and the degree of tolerance (Jackson, 1967; Foy, et al., 1967).

There are a number of mechanisms that have been proposed to explain the differential Al tolerance of plant species. Jones (1961) and Grime-Hodgson (1969) suggested that Al tolerance results from a binding or chelating process. They propose that Al is chelated by organic acids (citric, oxalic or malic acid) and prevented from interfering with P metabolism. The organic acids are thought to be responsible for maintaining the high foliar concentrations of Al in the less sensitive species.

Turner (1969) indicated that tolerance to high foliar Al contents may depend on alteration of certain key enzymes which govern metabolic activity. The ability to selectively bind Al within the freespace of the root has also been reported to improve Al tolerance (Clarkson, 1969). The majority of plants growing in acid conditions have a mechanism that restricts the entry of Al into cell metabolism within the root (Lunt and Kafranek, 1970). Quellette and Dessureaux (1958) found that the more tolerant alfalfa clones retained more Al in their roots than in the tops.

Ali (1973) has more recently proposed that cell membranes are differentially permeable to Al. He suggested the site of exclusion to be the plasmalemma in roots, since varietal differences in Al tolerance in wheat resulted from the exclusion of Al from these cells.

Nodulation and Dinitrogen Fixation

The close involvement of photosynthesis in nodule development and function has been appreciated since 1930 (Allison, 1935; Gibson, 1977; Hardy and Havelka, 1976). It is generally accepted that improved overall photosynthesis results in improved nitrogen fixation (Gibson, 1977; Evans and Russell, 1971). This is associated with the increased supply in reducing power and energy for nitrogenase activity by pyruvate, which is an important substrate for ATP generation needed in nitrogen fixation.

The effects of environmental factors on legume growth and symbiotic nitrogen fixation have been studied extensively (Bethlenfulvay and Philips, 1977; Kitamura and Nishimura, 1977; Lie, 1974; Sprent, 1972; Waughmanu, 1977). Impairment of legume nodulation and nodule function by an environmental stress can induce N starvation and limit growth of the host plant. Conversely, impairment of the host plant growth can limit nodulation and N fixation. Aluminum limits top growth and root elongation in the host plant (Munns, 1977).

Soil acidity has long been known to induce N deficiency in legumes by preventing normal nodulation. The range of pH and Ca concentration in which nodulation is affected varies with the species and Rhizobial strain. Acidity, Al toxicity, and Ca deficiency inhibit Rhizobium growth, root infection and nodule activity enough to account for symbiotic failure (Munns, 1977; Robson, 1979).

The exact effects of Al toxicity on nodulation or N fixation have not been identified in isolation from other variables that depend on soil pH. It is generally believed, however, that the major effect of Al in acidic soils is in the inhibition of Rhizobium growth (Dobereiner, 1966; Vincent, 1977). The response to soil acidity by a host

plant is usually related to the sensitivity of the specific Rhizobium. For example, Rhizobium meliloti is known to be the most sensitive species to acidic pH; this is a characteristic that is also shared by the host plant, alfalfa.

Several papers have reported increased nodulation in soil with reduced Al saturation (Moschler, et al., 1960; Rice, 1975; Rice, et al., 1977). Zakaria, et al. (1977) showed that soybean nodule number and weight increased with decreasing Al saturation. Sartain and Kamprath (1977) also found that soybean nodule number improved with reduced Al saturation. The weight and number of alfalfa nodules were found to significantly increase with a reduction in Al saturation (Mengel and Kamprath, 1978; Roberts, 1980).

Phosphorus Nutrition of Legumes

The importance of P in the early crop development and N fixation is well documented (Newton and Orme-Johnson, 1980; Thomas and Peaslee, 1974; Munns, 1977). Root growth is stimulated relative to shoot growth when adequate supply of P is available.

Metabolic Role of Phosphorus

The capability of plants to take up phosphate is fixed genetically but the rate of P uptake is pH dependent. Most of the available P is not close enough to the roots to reach them and so plant utilization of P applied to soils is usually less than 10 percent (Schenk and Barber, 1979). Differences in the ability of crop species to utilize soil P were partially explained through variations of root morphology (Atkinson, 1973; Barley, 1970) and root physiology (Barber, 1978). Root morphology may be described by root radius, root length, and root hair density. Root

physiology is important for P uptake. Besides morphological and physiological root parameters, chemical effects of roots on the soil environment are involved in P uptake processes from soils (McLachlan, 1976; Barber, 1978).

Phosphate is readily mobile in the plant and can be translocated in an upward or downward direction (Clarkson, et al., 1968; Morard, 1970). Organic as well as inorganic phosphates play a major role in phloem transport (Hall and Baker, 1972) .

Phosphates absorbed by plant cells rapidly become involved in metabolic processes. Phosphate in the plant occurs in inorganic form as orthophosphate and to a lesser extent as pyrophosphate (Mengel and Kirby, 1978). The organic forms of P (phosphorylated sugars and alcohols) are mainly intermediary compounds of metabolism. Jackson and Hagen (1960) reported that 80 % of the phosphate absorbed was incorporated into organic compounds which consisted mainly of hexose phosphates and uridine diphosphates. It appears that the unique function of phosphate in metabolism is its formation of pyrophosphate bonds (in ATP) and subsequent energy transfer (Evans and Russell, 1971).

Role of P in Dinitrogen Fixation

The importance of P in nodulation and nitrogen fixation has been demonstrated by various researchers (de Mooy and Pesek, 1966; Cassman, et al., 1981). Nodules are a strong sink for ^{32}P . Moustafa, et al., (1971) used foliar application of ^{32}P to show that P was translocated to the nodules in large amounts; the majority of the recovered ^{32}P was in AMP, ADP and ATP.

Nodulation is an energy requiring process (Gibson, 1977). The energy requirement for N fixation in vitro and in vivo is 12-15 and 5-30 moles ATP per mole of N_2 fixed, respectively. Levels of N fixation, nodule activity and the

carbohydrate in nodules were highly correlated with the supply of P (Graham and Rosas, 1979). The increase in N fixation as a result of a higher P supply could improve the carbohydrate supply to nodules according to these researchers.

Claims that phosphate improved nodulation appear in reports of greenhouse and field trials with several species (Heltz and Whiting, 1928; Gates, 1974). According to de Mooy and Pesek (1966), phosphate greatly increased the size and number of nodules on soybeans but only when both phosphate and potassium (K) were applied at heavy rates. Similar increases in number and weight of soybean nodules with added P were also found by Jones, et al., (1977).

The commonly observed increase in percentage nitrogen in plants when fertilized with P may indicate that maximum N fixation requires more P than maximum growth (Andrew and Robins, 1969). This would suggest that more P is needed for optimum nodule function than for the growth of the host plant.

MATERIALS AND METHODS

GREENHOUSE INVESTIGATIONS

Experiment 1: Response of Alfalfa to Lime and Superphosphate.

This experiment was designed to study the effects of lime and phosphorus application on soil chemical changes with time.

The soil for this study was a Paxton sandy loam (Woodbridge-Paxton) collected from Pittsfield, New Hampshire in 1978; the top 15 cm was removed and screened at the collection site. The initial soil pH was 5.3 with only 2 % Al saturation and a moderate supply of available P. In November 1979, dolomitic limestone (21 % Ca and 12 % Mg) was thoroughly blended with the soil at rates equivalent to 0.5, 1.0, 2.0 and 3.0 tons/hectare. The mixture was placed into 10 cm (4 in) pots and incubated under alternate wetting and drying sequence for 3 weeks to permit the lime reaction to attain equilibrium. Soil samples were collected for chemical analysis every two weeks.

In January 1980, 166 kg K and 2 kg B/ha were uniformly incorporated with the soil. Differential P rates of 0, 44, 88, 176 and 352 kg P/ha were applied by uniformly mixing the soil with triple superphosphate as the P source. The experimental design was a random complete block with three replications.

'Honeoye' alfalfa seed was inoculated by thoroughly mixing it with a Nitragin culture (Nitragin Co., Milwaukee, Wisconsin) and planted at a rate of 15 seeds per pot. Pots were placed on a greenhouse bench under 40 watt Sylvania (26 watts/m²) cool white fluorescent lights with a 16-hour daylength. The ambient temperature was 21 C (70 F) during the day and 15 C (60 F) in the night. Two weeks after

seeding, the plants were thinned to 10 plants per pot.

Soil samples taken during the period of incubation prior to planting and after final harvest were analyzed for pH, Ca, Mg and P according to the methods outlined in the section on Analytical Methods. Forty days after seeding, plants were harvested, oven dried at 70 C for 48 hours and weighed. Plants from similar treatments from the three replications were consolidated in order to obtain sufficient plant material for P assay using the Molybdate-vandate yellow method (Chapman and Pratt, 1961).

Experiment 2: Lime Requirement of Alfalfa.

The objective of Experiment 2 was to evaluate exchangeable aluminum (Al) as an index of lime requirement by alfalfa. A major problem of managing acid soils is to estimate the quantity of lime required to raise the soil pH to a certain level.

The soil was Paxton loamy sand (Woodbridge-Paxton) collected from Ridge Road, Northwood, New Hampshire in October 1979; the top 15 cm of the soil profile was sampled and screened at the site. The initial soil pH was 4.9 with about 50 % Al saturation and very low level of soil P.

Lime (CaCO_3) was added in May, 1980 at rates to theoretically neutralize 0, 75, 100, 150 and 200 % of the 1 N KCl exchangeable Al (McLean, et al., 1959). These rates are equivalent to 0, 3.6, 7.1, 10.7 and 14.3 tons per hectare of dolomitic limestone. The limed soil was then placed into 20 cm (8 in) pots and watered regularly to allow the lime to solubilize. In July 1980, 83 kg K and 2 kg B/ha were applied to all treatments and uniformly incorporated. Phosphorus was applied from triple superphosphate at rates equivalent to 0, 44, 88 and 132 kg P/ha and mixed uniformly with soil. The experimental design was a random complete block with a 5 x 4 factorial arrangement in three

replications.

Honeoye alfalfa was seeded at a rate of 20 seeds per pot immediately after the application of the P fertilizer. The pots were placed on a greenhouse bench under 40 watt Sylvania (26 watts/m²) cool white fluorescent lights with a 16-hour daylength. The ambient temperature in the greenhouse was the same as in Experiment 1. Two weeks after seeding, the plants were thinned to 15 plants per pot.

In August 1980, (6 weeks after sowing), five whole plants were carefully removed from the soil, washed with distilled water, and stored in the cold room at 9 C. Nodulation score, root length measurements and nitrogen fixation assays were carried out as described in the section on Analytical Procedures. At the same time, the tops of the remaining 10 plants were harvested, oven dried at 70 C for 48 hours and weighed. A second harvest was made six weeks later and the final harvest was made in November 1980. At final harvest, plant tops and roots from the 10 plants in each pot were separately oven dried at 70 C for 48 hours and weighed. The harvested tops from the 10 plants of each pot were ground in a Wiley Mill to pass a 40-mesh screen and ashed at 500 C for three hours in preparation for chemical analysis.

Following the above harvest, soil samples were collected from each pot and analyzed for the various elements as described earlier in Experiment 1.

Experiment 3: Influence of Lime on Nitrogen Fixation of Two Alfalfa Varieties.

The objective of Experiment 3 was to determine the interactions between per cent aluminum saturation of soil, varietal lime requirement and nitrogen fixation.

Soils from selected treatments of Experiment 2 were transferred into 10 cm (4 in) pots on April 29, 1981. The

treatments selected for further study consisted of two phosphorus rates (0 and 100 kg P/ha) and five lime rates (equivalent to neutralize 0, 75, 100, 150 and 200 % of 1 N KCl exchangeable Al) that were applied a year earlier. Two varieties of alfalfa were used in the experiment: 'Iroquois', a commercial variety and 'Spredor 2' received from Dr. Walter M. Larson, Northrup King Co., Minneapolis, Minnesota. 'Spredor 2' is considered to be more tolerant to acidic conditions and 'Iroquois' is a standard variety in the New England area. The experimental design for each alfalfa variety was a random complete block with 5x2 factorial arrangement in three replications.

The two alfalfa varieties were seeded at a rate of 20 seeds per pot in May 1981. The planted pots were placed on a greenhouse bench under 40 watt Sylvania (26 watts/m²) cool white fluorescent lights with a 16-hour daylength. The ambient temperature in the greenhouse was the same as in Experiment 1. Two weeks after seeding, the plants were thinned to 10 plants per pot.

Forty days after seeding the plants were removed from the pots and the roots washed with distilled water. Single plants from each pot were randomly picked, stored immediately in the cold room at 9 C and measurements were taken for nodule number and nitrogen fixation. Plant roots from each treatment were used for organic acid determination (Hiatt, 1967) in order to determine if an association existed between the level of organic acids and plant tolerance to Al toxicity. Whole plants were also oven dried at 70 C for 48 hours and weighed.

Experiment 4: Influence of Lime on the Mobility of Phosphorus-32 in Alfalfa.

The ability of plants to utilize P in the presence of Al in acidic soils is an indication of a tolerance to low pH

(Foy, et al., 1978). The objective of this experiment was to assess the movement of P in 4 alfalfa varieties as influenced by liming. Electron dispersion analysis via X-ray (EDS) and radioactive phosphorus (^{32}P) were used to evaluate the distribution and mobility of P in the legume.

The soil for this study was Charlton loamy sand collected from Kingman Farm, Madbury, New Hampshire in August 1980; the top 20 cm of the soil profile was sampled and screened at the site. The initial soil pH was 5.1 with a high level of available P.

Hydrated lime at rates equivalent to 0, 3.5 and 6.9 tons/ha was applied on January 12, 1981. Phosphorus and potassium at rates equivalent to 97 kg P/ha and 183 kg K/ha from triple superphosphate and muriate of potash, respectively, were added and mixed uniformly with the soil before seeding. The limed soil was placed into Rootainers (Spencer Lemaine Industries, Edmonton, Canada) and watered regularly until seeding. Four varieties of alfalfa were used in the study: 'Iroquois', a commercial variety; 'Spredor 2', received from Dr. Walter M. Larson, Northrup King Co., Minneapolis, Minnesota; 'Arc F.C. 45028', received from Dr. M.B. Tesar, Michigan State University, East Lansing, Michigan; and 'B13 A14', received from Dr. James H. Elgin, Beltsville Research Center, Beltsville, Maryland. The experimental design was a random complete block with three replications.

Seeds of the four alfalfa varieties were sown at a rate of 10 seeds per pot eight days after lime application; a week after emergence plants were thinned to five per pot. The Rootainers were placed on a greenhouse bench under 200 watt General Electric incandescent lamps (80 watts/m²) with a 16-hour daylength. The ambient temperature in the greenhouse was the same as in Experiments 1-3. Three weeks after planting, the Rootainers from two replications were transferred to a separate room in preparation for work with

^{32}P (Fig 1).

Soils were directly spiked using $\text{H}_3^{32}\text{PO}_4$ at levels of 5, 10 and 20 uCi per cell in a Rootrainer. In each cell five random holes at a depth of 2.5 cm (1 in) were made into which the ^{32}P was spiked using a 20 ul micropipette (VWR Scientific Inc., Boston, Massachusetts).

After 10 days, the whole plants were carefully removed from the soil and the roots washed with distilled water and rinsed with a solution of 30 ppm P from KH_2PO_4 . The plants were then carefully wrapped in Saran Wrap and placed immediately adjacent to X-ray film (Kodak, No-screen Film, NS-5T) in film cassettes. The film was developed within 40 to 48 hours using the procedure described by Roberts (1980).

Plants from the third replication were removed from the Rootrainer a month after sowing and partitioned into tops and roots; the tops and roots were weighed separately. Leaf, stem and root samples from plants treated with 0 and 6.9 t/ha lime were harvested and stored at 9 C for assay via EDS.

The samples for EDS analysis were prepared according to the procedure described by Tanaka and Yoshida (1974). The plant parts were sampled as follows: leaf, leaflets were randomly picked from the trifoliolate; stem, sections were cut one centimeter above the transition zone between the stem and roots; roots, sections were cut one centimeter below the transition zone. Plant sections were cut into 5 mm discs using a cork borer, and then embedded in Spurr's plastic resin (Ernest F. Fullam, Inc., Schenectady, New York). The plant sections in the epoxy resin were placed in BEEM capsules (Ernest F. Fullam, Inc., Schenectady, New York) and kept in a vacuum oven for 72 hours at a pressure of 1.40 kg/cm^2 (20 lbs/in^2) and a temperature of 60 C to harden.

The embedded samples were trimmed using a small hacksaw and a razor to create a smooth observing surface. Trimmed

plant slices were mounted on carbon stubs (SPI Supplies, Westchester, Pennsylvania) and oven dried at 70 C for about four hours. The sample on the carbon stub was scanned at a 1000 Å depth of penetration for Al and P against an appropriate standard. The EDS package (International Inc., Prairie View, Illinois) on the AMR 1000A Scanning Electron Microscope (AMR Corporation, Bedford, Massachusetts) is equipped with a ZAF correction which compares specimens to standards and corrects for atomic number (Z), absorption (A), and fluorescence (F).

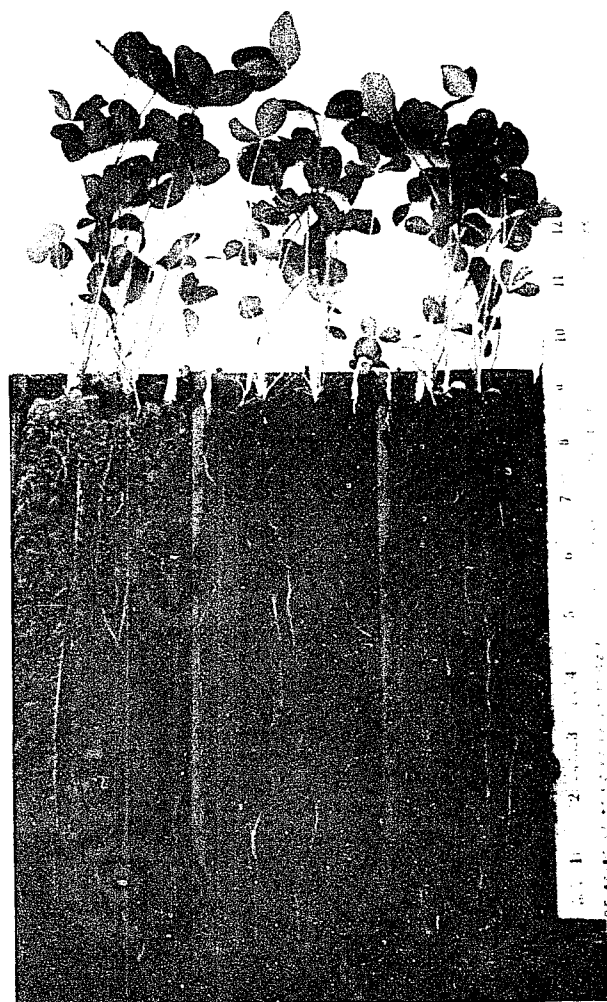
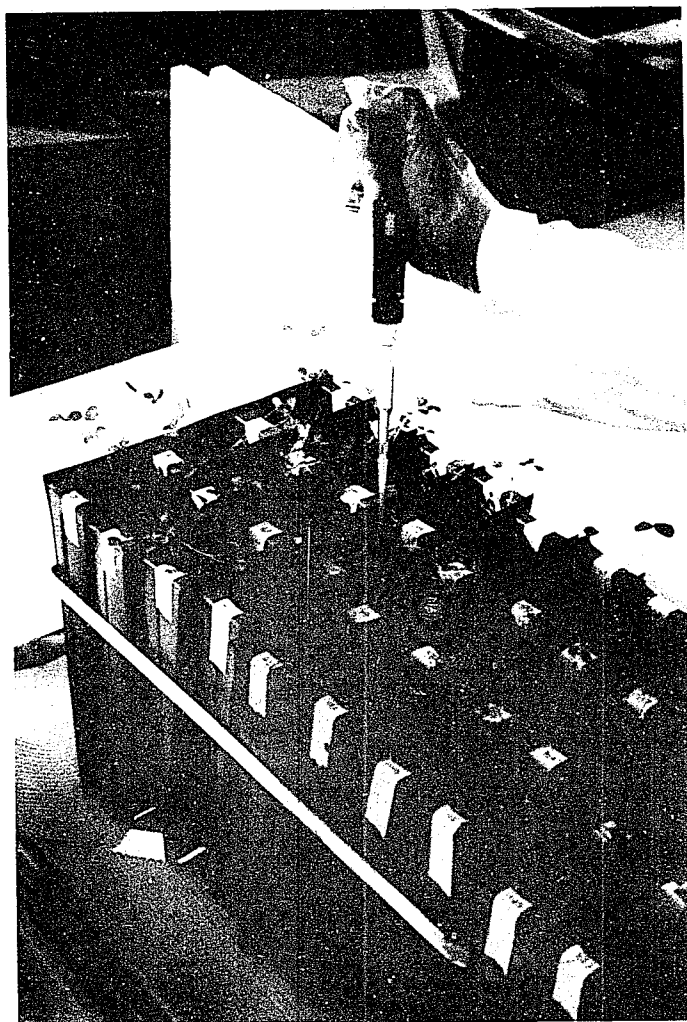


Figure 1. Alfalfa plants in Rootainers for ^{32}P study.

ANALYTICAL PROCEDURES

Soil Chemical Analysis

All soil samples were screened through a 2 mm or 10 mesh sieve to remove stones, gravel and coarse roots prior to analysis. The screened samples were air-dried at room temperature before testing.

Soil pH was measured on a 1:1 soil to water slurry with an Orion Digital pH meter. Total acidity (exchangeable hydrogen) was determined by 0.1N NaOH titration of the filtrate extracted using 0.5N barium acetate (Russel, 1950). Exchangeable Ca, Mg, and K were extracted with 1N, pH 7.0 ammonium acetate (Chapman, 1965) and determined by atomic absorption/flame emission spectrophotometry (Model 251 AA/FE, Instrumentation Laboratory, Wilmington, Massachusetts). Lanthanum chloride was added for the analysis of Ca and Mg at a final concentration of 0.1 % La to prevent the formation of phosphate compounds with these elements (Skoog and West, 1974). Exchangeable Al was extracted with 1.0N KCl and measured by atomic absorption using nitrous oxide as the oxidant (McLean, *et al.*, 1959). Extractable Fe and Mn were determined with the Morgan Soil Testing System by the Soil and Plant Testing Laboratory in Waltham, Massachusetts. The Morgan extractant was a 10 % solution of sodium acetate in 3 % acetic acid with a pH of 4.8. Available P was extracted with a Bray #1 solution (Bray and Kurtz, 1945) and measured colorimetrically according to the method of Olsen and Dean (1969). Organic matter was measured as loss of dry matter after ashing soil for 7 to 8 hours at 400 C; the mineral matter is assumed to be unchanged at this temperature (Jackson, 1958). The Walkley-Black method (Jackson, 1958) which involves the oxidation of organic matter by chromic acid was also used in the soils of field Experiment 1.

Plant Chemical Analysis

Plant samples to be chemically analyzed were oven dried at 70 C for 48 hours and ground in a Wiley Mill to pass a 20-mesh screen. Samples were then ashed at 500 C for three hours and digested in 0.1N HCl. Analysis of Ca, Mg and Al was done by atomic absorption as described earlier; Lanthanum chloride was added for Ca and Mg analysis; potassium chloride was added for Al analysis to prevent ionic interference. Potassium was analyzed with flame emission. Plant P was measured with molybdate-vandate yellow according to Chapman and Pratt (1961). Nitrogen was determined by the Kjeldahl method (Horwitz, 1975). Total organic acids were determined by the procedure described by Hiatt (1967).

Root Length and Nodulation Measurements

Root length of alfalfa plants was determined by the procedure of Tenant (1975), modified from the Newman method (1966). Test equipment consisted of a shallow white enamelled tray (25 x 40 cm), a 10x illuminated magnifying glass and a hand tally counter. Grid squares of 1 x 1 and 2 x 2 were prepared using acetate sheet. For length estimates the acetate sheet with grid squares was placed on the bottom of the tray. Water containing the roots was poured in and the root positioned over the grid. The length of the entire root system was calculated using the following formula:

$$\text{Root length (R)} = \text{No. of intercepts (N)} \times \text{Length conversion factor}$$

The conversion factors for the 1 and 2 cm grid squares were 0.79 and 1.57 respectively (Tenant, 1975).

Nodule number, size, position and color were assessed

based on arbitrarily assigned numerical values as proposed by Rice, et al. (1977). Roberts (1980) has adequately outlined the scoring system used to evaluate the nodules.

Nitrogen Fixation Assay

Nitrogen fixation was measured via acetylene reduction using the procedure of Hardy, et al. (1968). The analysis was performed with a Perkin-Elmer Model 990 gas chromatograph (GLC) equipped with flame ionization detectors and a stainless steel column that was 1.22 m long and 0.32 cm in diameter and packed with a Porapak N 80 to 100 mesh (Supelco, Inc.). The carrier was prepurified nitrogen at a flow rate of 40 ml/min. The flame was a hydrogen/air mixture provided by breathing air and ultra-high purity hydrogen (Airco, Inc.). Operating temperature for the injection block, column oven and manifold were 105 , 80 , and 110 C respectively. Retention time for ethylene (C_2H_4) was one minute, and concentration was determined by comparison of sample peak height with that of a known concentration of ethylene standard.

Roots from alfalfa plants stored for 24 hours in the cold room at 9 C were excised, placed in 250 ml Mason jars and immediately sealed. Fifty milliliters of air was removed from each jar and replaced with an equal volume of generated acetylene (C_2H_2) with a plastic syringe. The amount of acetylene used was generated by the reduction of calcium carbide with water. The jars were incubated at 25 C (77 F) for 30 minutes in a water bath. At the end of the incubation period, one ml gas samples were withdrawn and analyzed for C_2H_4 against the 1.3 ppm standards.

FIELD INVESTIGATIONS

Four field experiments were conducted to determine the long term effects of lime on Al saturation and yield of alfalfa on acidic soils.

Experiment 1: Influence of Lime on Al Saturation and Available P.

The objective of this experiment was to determine the effect of various degrees of Al saturation on performance and persistence of alfalfa over a six year period. Special attention was given to interactions between plant and soil nutrients because of the current interest in minimum tillage and surface liming.

The experiment was started in 1975 on a sandy loam soil (Hollis-Charlton) at the Kingman Farm, Madbury, New Hampshire. The soil was slightly acidic with a low level of P supply and a high degree of Al saturation. In August 1975, rates of 0, 3.5, 6.9 and 13.8 tons/ha of dolomitic limestone were plowed and disked into the soil and a basal dressing of 27.5 kg N and 145 kg P/ha applied. Boron was sprayed at 1.1 kg B/ha. The land was plowed and disked to obtain the desired seedbed; 'Iroquois' alfalfa was seeded with a Brillon cultipacker seeder one week after liming and fertilizer applications. The experimental design was a random complete block with four replications.

The first harvest for dry matter yields was taken in June 1976 with the Carter harvester (Carter Mfg. Co., Lafayette, Indiana). During the period from 1976 to 1981, three to four harvests were made annually. All plots were measured following harvest to accurately determine harvested area. Samples for dry matter determination were collected from the chopped legume while those for tissue analysis were hand collected to avoid any possibility of soil

contamination. The alfalfa plants (tops) from the first cut of each year were oven dried at 70 C for 48 hours, ground in a Wiley Mill and dispatched to the Research and Extension Analytical Laboratory in Wooster, Ohio for chemical analysis.

Each year after the first and final harvests a topdressing of 183 kg K and 2.2 kg B/ha was made, except in 1976 when 219 kg K/ha was applied after the first cut and 110 kg K/ha after the third cut. Crown counts were made after the second and third cuts in 1981. Soil samples were taken in July 1979 and 1980 and in June and August 1981 at 0-10, 10-20 and 20-30 cm depths in the soil profile, and analyzed for pH, Ca, Mg, K, Al and P as described in Analytical Procedures. In addition, the Modified Morgan Test (Lunt, et al., 1950) was used for chemical analysis of the soil samples collected in July 1980 and 1981.

Experiment 2: Influence of Lime and Phosphorus on Alfalfa Growth.

The objective of Experiment 2 was to examine the influence of dolomitic and hydrated limestone on the nodulation, composition, persistence and yield of alfalfa under minimum tillage.

The experiment was laid out in August 1977 on a Hinckley sandy loam (Hollis-Charlton) at the Demerit Livestock Farm, Lee, New Hampshire. The soil had an initial pH of 5.6 at the 0-10 cm depth and a low level of available P. The experimental design was a split block with P treatments applied across the four replications in one direction and lime in the opposite direction.

The lime and phosphorus treatments which were surface applied in May 1978 are presented in Table 1. Potassium was applied from muriate of potash at a rate equivalent to 100 kg K/ha uniformly to all plots. In August 1978, glyphosate

was applied to the plots at 2.24 kg/ha; and three days later, 'Iroquois' alfalfa was sown with the Zip seeder (Midland Mfg., Mississippi).

The first harvest was taken in June 1979 with the Carter harvester and samples for dry matter determination and tissue analysis were hand collected. One week after harvest, the plots were fertilized with 100 kg K/ha and 2.2 kg B/ha. The second harvest was taken in August and the third harvest on November 10. The percent legume was estimated by four independent observers and the plots measured following each harvest. Two harvests were made in 1980 and samples for dry matter and tissue analysis collected.

In early 1981, the alfalfa seeding was poor; the plants were stunted and showed signs of winterkill. The crop performance was so poor that no harvests could be made.

Soil samples for chemical analysis were collected at 0-2, 2-5, 5-10 and 10-20 cm depths in 1978 and 1979, but at a depth of 0-10 cm in 1980.

Table 1. The lime and phosphate treatments for the Charlton sandy loam at Demerit Farm.

Lime Rate	Applied P, kg/ha					
	0	53	97	106	194	
	t/ha	Method of Application*				
	0	+	+	-	+	-
Hydrated	1.2	+	+	-	+	-
	2.3	+	+	-	+	-
Dolomitic	6.9	+	+	-	+	-
	13.8	+	+	-	+	-

* + = P applied in bands
- = P broadcast

Experiment 3: Influence of Lime on Chemical Changes in the Soil Profile.

This experiment was established in November 1979 to study the effects of liming on subsoil acidity.

The soil was a Paxton fine sandy loam (Hollis-Charlton) located at the Woodward Farm, Durham, New Hampshire. It had an initial pH of 5.5 with a moderate supply of available P and high soil organic matter. In May 1980, dolomitic lime at rates of 0, 4.6, 9.2 and 18.4 tons/ha was surface applied along with a basal dressing of 55, 97 and 183 kg/ha of N, P and K respectively. The experimental design was a random complete block with four replications.

'Iroquois' alfalfa was seeded using the Zip seeder immediately after lime application. The crop growth was severely suppressed from competition by quackgrass (Agropyron repens L.), orchardgrass (Dactylis glomerata L.), curly dock (Rumex crispus L.), pigweed (Amaranthus retroflexus L.) and crabgrass (Digitaria sanguinalis L.). Paraquat was applied at 2.4 l/ha (1 qt/acre) and the whole experiment was mowed to control weeds in 1980 and 1981; but the operation was not successful. The alfalfa plant growth was so poor no useful harvest could be made, except for plant samples collected in August 1981 for tissue analysis.

In July 1981, soil samples were collected at 0-10, 10-20 and 20-30 cm depths into the soil profile and analyzed for pH, Ca, Mg, K, Al and P as described in the Analytical Procedures.

Statistical Analysis

Analysis of variance on all measured responses was carried out using Duncan's New Multiple Range Test. Multiple regression analysis was used to determine the relations between soil-plant analysis and alfalfa yields.

Regression analysis was also used to compare the Bray #1 versus the Modified Morgan for the extraction of P using soil samples from field Experiment 1. Correlation tests were made to compare the $1 \text{ N } \text{NH}_4\text{OAC}$ against the Modified Morgan for determining cations on the same soil samples. For purposes of comparing methods of organic matter analysis, correlation tests were conducted on the ignition and dichromic acid determinations using soil samples from field Experiment 1.

Contour surface analysis of alfalfa yield was performed using the applications library programs SYMAP and SYMVU. The independent variables for the three-dimensional surface were soil and plant analytical data from field Experiment 1.

RESULTS AND DISCUSSION

Greenhouse Investigations

Experiment 1: Response of Alfalfa to Lime and Superphosphate.

The initial soil chemical properties for Experiment 1 and all subsequent greenhouse studies are included in Table 2.

Soil pH increases from lime were observed consistently throughout the eight week period (Figure 2). Soil pH started to decline 28 days after sowing, suggesting that liming was most effective during the first month of application.

Lime rates higher than 0.5 t/ha showed a decrease in the availability of P (Table 3). The decrease in available P, especially 28 days after sowing, appeared to be associated with an increase in soil Ca (Figure 3). Soil Mg was significantly increased with lime rates of 2.0 t/ha and above, while exchangeable Ca was markedly increased at the highest lime rate only. The initially high Ca level could be attributed to the immediate reaction of the lime with soil colloids. According to Racz and Soper (1967) soils at higher lime rates were found to be saturated with Ca and Mg, which in turn tended to form insoluble forms of P upon reaction. Roberts (1980) had also reported that application of dolomitic limestone did not increase the availability of P in a Paxton soil.

Application of phosphate fertilizer significantly increased exchangeable Ca but had no effect on either pH or Mg (Table 4). Results on available P were inconsistent, although there were significant differences between the treatments. Maximum soil P (62 ppm) was found at the highest P rate.

Table 2. Soil characteristics of the four soils used in the greenhouse experiments prior to treatment.

Soil	Experiment	pH	Ca	Mg	K	Al	CEC*	Al Saturation**	P
			----- meq/100 g -----					%	ppm
Paxton	1	5.3	4.5	1.50	0.33	0.10	6.43	1.6	50
Paxton	2	4.9	0.9	0.13	0.45	1.44	2.92	49.3	10
Paxton	3	4.7	1.1	0.19	0.46	1.07	2.17	38.6	13
Charlton	4	5.1	3.4	0.78	0.49	0.44	5.11	8.6	91

* CEC = Sum of Al, Ca, Mg and K.

** Al Saturation = $Al / (Ca + Mg + K + Al) \times 100$.

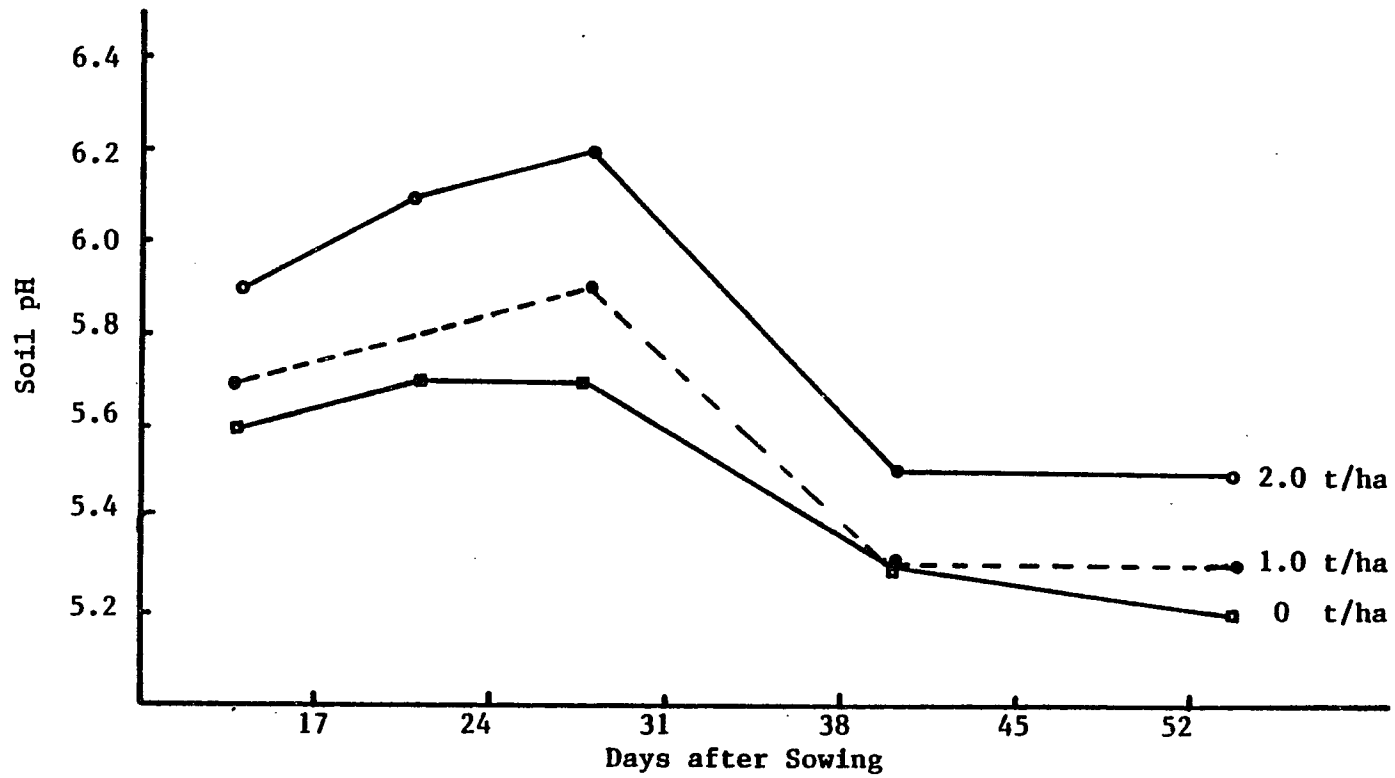


Figure 2. Changes in soil pH with liming during a 53-day period for greenhouse Experiment 1.

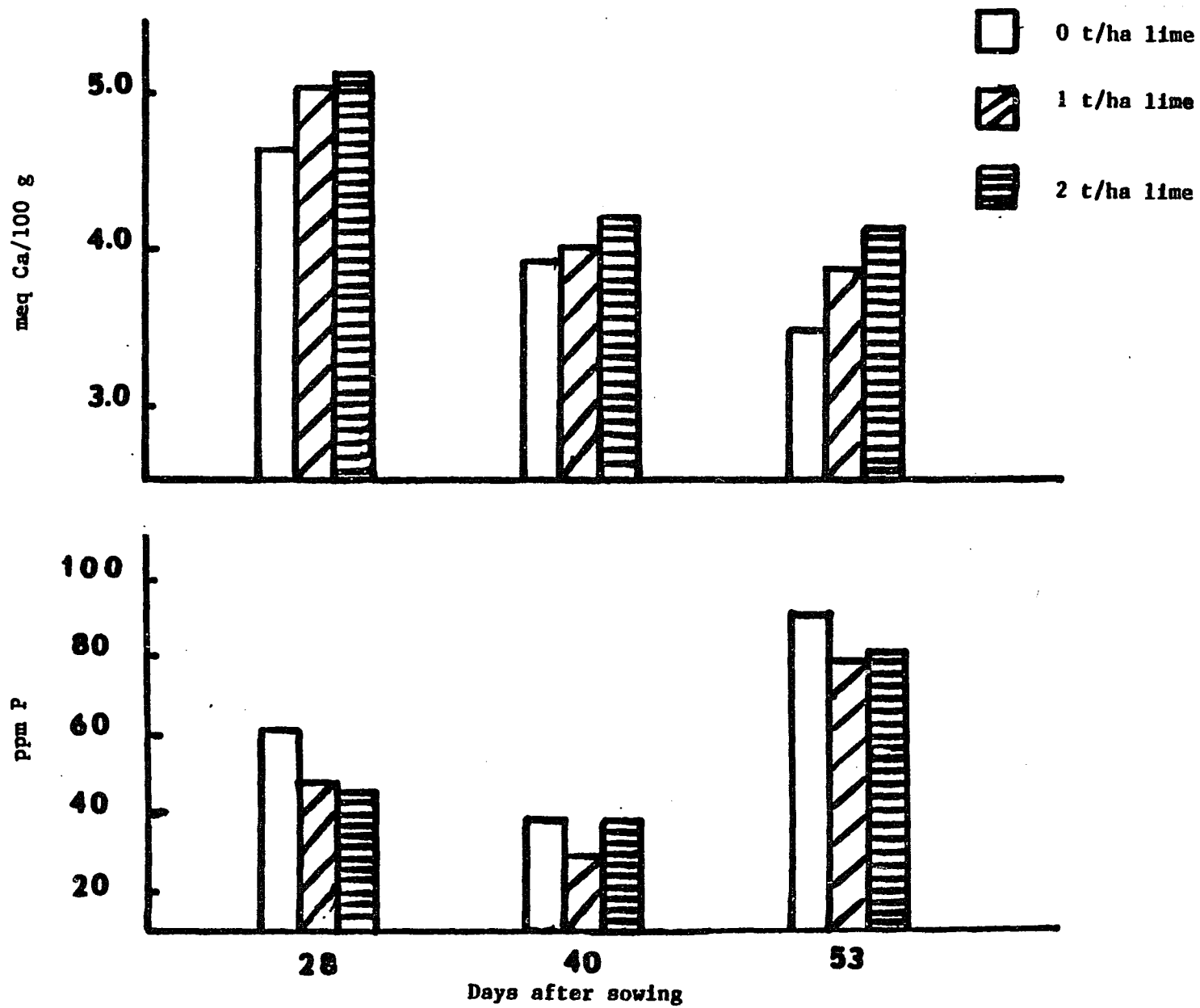


Figure 3. Mean Ca and P changes within the soil during a 53-day period for greenhouse Experiment 1.

Table 3. Yield of alfalfa, plant P concentration, and soil mineral analysis as influenced by dolomitic limestone in Experiment 1.* Each value is the mean of three replications.

Lime Rate	Yield	Soil				Plant P
		pH	Ca	Mg	P	
t/ha	mg/pot		--- meq/100 g ---		ppm	%
0	128 a ¹	5.7 a	4.6 a	1.4 a	60 b	0.41 c
0.5	140 a	5.9 b	4.9 a	1.5 a	63 b	0.12 a
1.0	146 a	5.9 b	5.0 a	1.3 a	46 a	0.10 a
2.0	132 a	6.2 c	5.2 a	1.9 b	44 a	0.16 a
3.0	149 a	6.3 c	6.0 b	2.3 c	47 a	0.23 b

* The soil was sampled 28 days after sowing.

¹ Means within a column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

Table 4. Yield of alfalfa, plant P concentration, and soil mineral analysis as influenced by phosphate fertilizer in Experiment 1.* Each value is the mean of three replications.

Phosphorus Rate	Yield	Soil				Plant P
		pH	Ca	Mg	P	
kg P/ha	mg/pot		--- meq/100 g ---		ppm	%
0	128 a ¹	5.7 a	4.6 a	1.4 a	60 c	0.41 b
44	144 a	5.8 a	4.5 a	1.6 a	32 a	0.22 a
88	137 a	5.9 a	5.0 ab	1.6 a	44 b	0.23 a
176	153 a	5.9 a	5.4 bc	2.0 a	57 c	0.22 a
352	157 a	5.8 a	5.9 c	1.6 a	62 c	0.27 a

* The soil was sampled 28 days after sowing.

¹ Means within a column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

Alfalfa yields were not significantly influenced by either the application of lime or superphosphate (Tables 3 and 4). This lack of response could be partly due to the reduced P uptake which may have resulted from P fixation by Ca. The initially low exchangeable Al (0.1 meq/100 g) and moderate level of P (50 ppm) for this particular soil may have contributed to the low yield response to liming. There was no significant interaction between lime and P fertilizer.

Experiment 2: The Lime Requirement of Alfalfa.

The role played by aluminum in the fixation of soil phosphorus and its detrimental effect on crop yield has been recognized by many researchers (Hsu, 1964; Harter, 1969). Therefore, the purpose of this study was to evaluate the use of exchangeable Al as an index for determining the lime requirement by alfalfa. For this study, a soil with high exchangeable Al (1.44 meq/100 g) and low available P (10 ppm) was selected (Table 2). The lime rates used based on exchangeable Al were 0, 0.75, 1.50, 2.25 and 3.0 meq CaCO_3 /100 g.

The increase in soil Ca and pH from liming was accompanied by a significant reduction in exchangeable Al with Al saturation being reduced from 25 % to 4 % at the lowest and highest lime rates, respectively (Table 5). Similar results have been reported by various researchers (Reeve and Sumner, 1970; Munns, 1965). An increase in available P was observed only with the first increment of lime. The reduction in available P with lime rates greater than 0.75 meq/100 g was probably due to P fixation as a result of a precipitation reaction. Application of P resulted in an increase of soil P and a slight decrease in exchangeable Al (Table 6). It is possible that the applied P could in part contribute to the labile fraction of P and

Table 5. Main effects of lime on soil pH, Ca, Mg, K, Al and P for Experiment 2. Each value is the mean of three replications at four P rates.

Lime Rate	pH	Ca	Mg	K	Al	CEC	Al Saturation	P
meq CaCO ₃ /100 g		----- meq/100 g -----					%	ppm
0	4.7 a ¹	1.4 a	0.19 a	0.46 c	0.67 c	2.72 a	24.6 c	13 a
0.75	4.7 a	1.6 a	0.17 a	0.44 c	0.34 b	2.55 a	13.3 b	18 b
1.50	5.0 b	2.2 b	0.16 a	0.35 b	0.18 a	2.89 ab	6.2 a	14 a
2.25	5.1 bc	2.2 b	0.14 a	0.25 a	0.12 a	2.71 a	4.4 a	14 a
3.00	5.3 c	2.7 c	0.15 a	0.33 b	0.12 a	3.30 b	3.6 a	11 a

¹ Means within a column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

Table 6. Main effects of P fertilizer on soil pH, P, Al, Ca, Mg and K for Experiment 2. Each value is the mean of three replications at five lime rates.

Phosphorus Rate	pH	Ca	Mg	K	Al	CEC	Al Saturation	P
kg P/ha		----- meq/100 g -----					%	ppm
0	4.6 a ¹	1.9 a	0.16 a	0.40 a	0.25 a	2.71 a	9.2 a	9.0 a
44	4.7 a	2.0 a	0.15 a	0.38 a	0.26 a	2.79 a	9.3 a	14.1 b
88	4.9 a	2.0 a	0.17 a	0.34 a	0.24 a	2.75 a	8.7 a	14.5 b
132	5.1 a	2.1 a	0.16 a	0.34 a	0.23 a	2.83 a	8.1 a	18.2 c

¹ Means within a column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

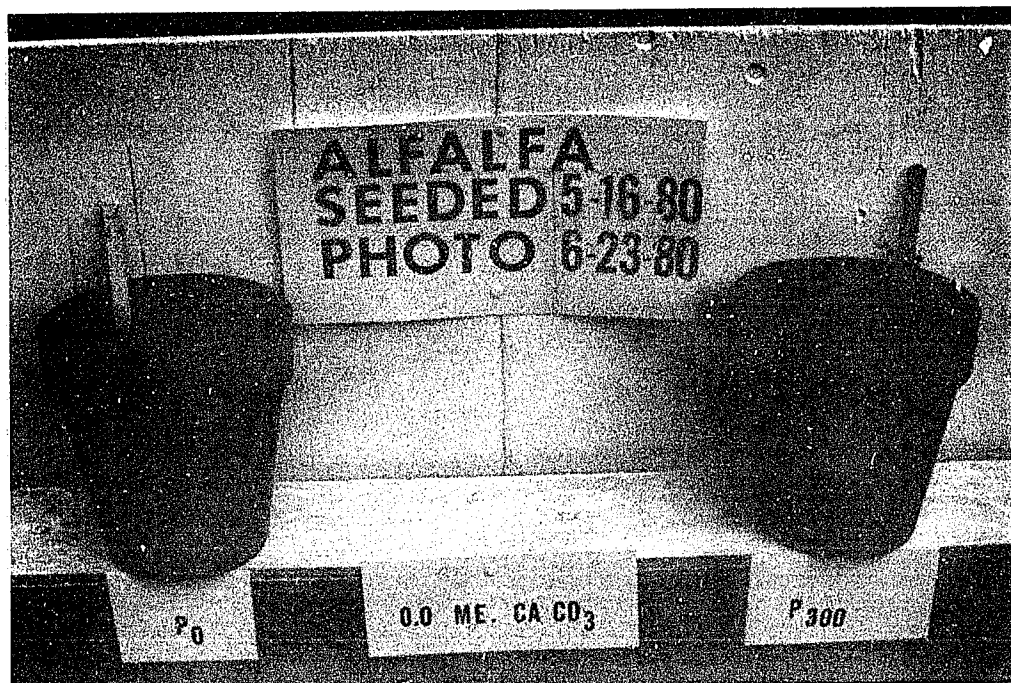


Figure 4. Differential response of alfalfa plants to lime and P treatments in greenhouse Experiment 2.

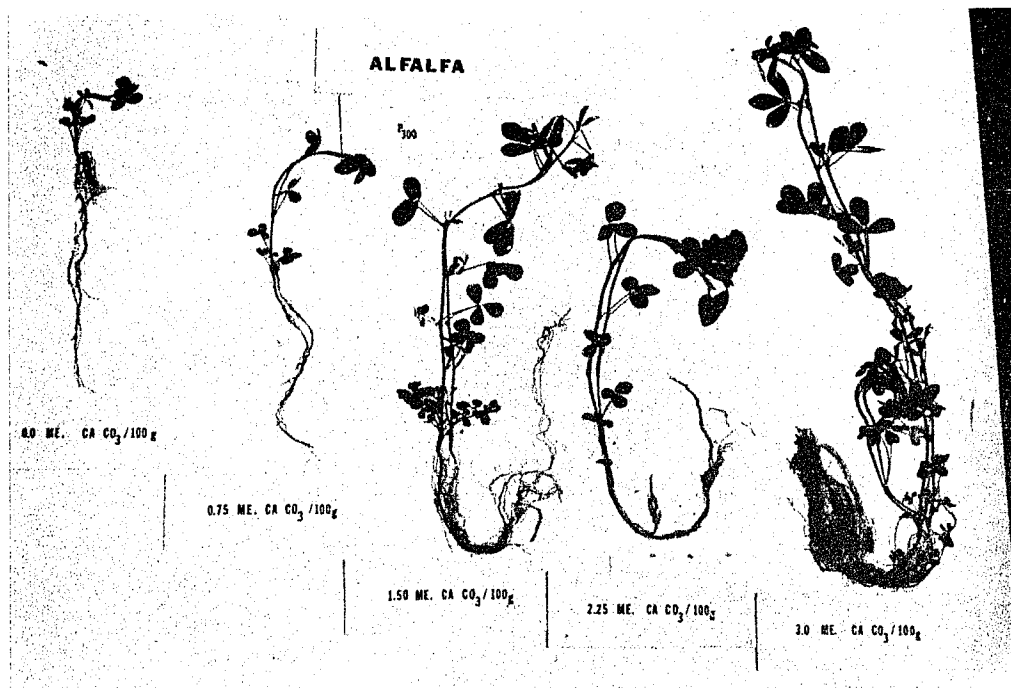
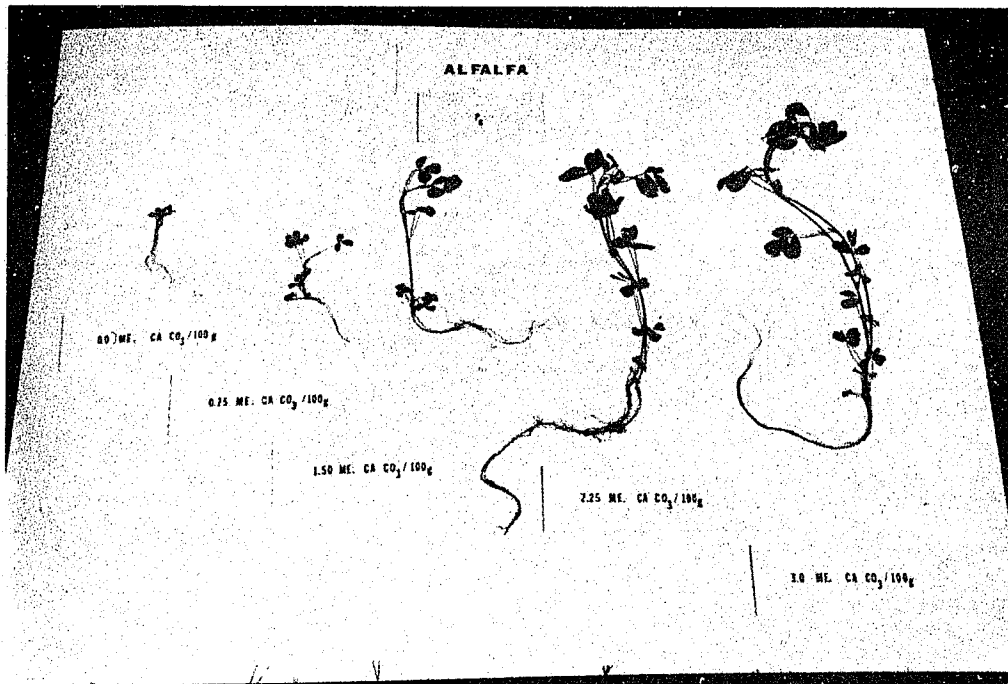


Figure 5. Differential response of alfalfa seedlings to lime and P treatments in greenhouse Experiment 2.

at the same time react with some of the free Al in the exchange complex. This is a clear indication of the fact that application of P fertilizer does not decrease exchangeable Al to the same extent as does liming.

Both lime and P applications benefitted plant growth (Figure 4). Application of lime and P also improved root development and growth (Figure 5). The improved crop growth from liming was remarkable in contrast to P application. The fact that simultaneous application of lime and P appeared to improve plant growth and root development suggests that there was an interaction between the two treatments. The added lime would neutralize the excess exchangeable Al and the superphosphate increase the supply of P available to the plant. High levels of Al are generally believed to induce stunting, depress root elongation and inhibit lateral and root hair development (Munns, 1965; Andrews and Johnson, 1976). Therefore, the reduction of exchangeable Al through liming could lead to the improvement in top and root growth when there is an adequate supply of P.

Top, root and total dry matter yields were significantly increased with lime rates higher than 0.75 meq $\text{CaCO}_3/100$ g (Tables 7 and 8). Such dramatic yield improvement with liming implies a sufficiency of soil P for good seedling growth.

Nitrogen fixation was not significantly affected by liming although nodulation was markedly improved. This was probably due to the relatively high soil organic matter (4 % - dichromate method) which may have served as a source for N supply required by the crop. The relatively high energy requirement for nitrogen fixation may govern the above response. At the higher levels of lime the amount of carbohydrate translocated to the nodules was not large enough to cause any improvement in the nitrogenase activity. For example, it has been reported recently (Hardy and

Table 7. The main effects of lime rate on dry matter yield of alfalfa herbage at harvest for Experiment 2. Each value represents the mean of three replications at four phosphate levels.

Lime Rate	H a r v e s t D a t e				Total
	7/23	9/18	11/8	12/22	
meq CaCO ₃ /100 g	----- Dry matter, g/pot -----				
0	0.17 a ¹	0.04 a	0.10 a	0.38 a	0.69 a
0.75	0.36 a	0.18 a	0.13 a	0.57 a	1.24 a
1.50	1.59 b	1.54 b	0.95 b	2.59 bc	6.67 b
2.25	2.05 c	1.82 c	1.24 c	3.21 c	8.32 b
3.00	1.96 c	1.98 c	0.86 b	2.11 b	6.91 b

¹ Means within a column followed by the same letter(s) do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

Table 8. The main effects of lime rate on nodulation, root length, nitrogen fixation and dry matter yield of alfalfa for Experiment 2. Each value represents the mean of three replications at four phosphate levels.

Lime Rate	Nodulation Score	Root length	Nitrogen fixation	Dry matter yield	
				Top	Root
meq CaCO ₃ /100 g		cm	uM C ₂ H ₄ /hr/plant	g/pot	
0	2.0 a ¹	26.7 a	44.5 a	0.05 a	0.03 a
0.75	3.0 a	15.7 a	43.9 a	0.13 a	0.04 a
1.50	6.0 b	59.6 b	43.7 a	0.50 b	0.12 b
2.25	7.0 b	93.8 c	45.0 a	0.77 c	0.20 c
3.00	5.0 b	103.1 c	44.6 a	0.79 c	0.22 c

¹ Means within a column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

Havella, 1976; Lawn and Brun, 1974) that legumes require high levels of carbohydrate supply to meet their energy requirements for N fixation. It is interesting to note that the nodulation score and root length were significantly increased with liming. Apparently, the improved root penetration into the soil profile helped to increase the dry matter yield but failed to substantially increase the level of N_2 fixation. With time, however, it is possible that the depletion of soil N could lead to an improvement in N_2 fixation.

There was a small increase in plant P and Ca with liming especially at the early stages of growth (Table 9). Plant Al for the highest lime rate was generally lower than the control. This reduction in the uptake of Al could be related to a decrease in exchangeable Al due to liming. The response pattern for plant Mg and K was less definite.

There was a general tendency for top, root and total dry matter yields to increase with P application (Tables 10 and 11). Again, there was no significant increase in N_2 fixation from P application, although nodulation and root length were markedly increased; there was no significant interaction between liming and application of P. This lack of response in N_2 fixation is expected to be transient and, with time, the positive effect of added P on N fixation could become more obvious. Application of P fertilizer did not improve the uptake of P while generally decreasing plant Al (Table 12). This may suggest that 0.22 % P is sufficient for good plant growth at low Al saturation levels. The critical level of P for alfalfa may vary from 0.20 to 0.25 % according to Martin and Matocha (1974).

Table 9. Tissue composition of alfalfa from the 1980 harvests of the Paxton sandy loam for Experiment 2. Each value is the mean of four P levels. Replications were combined in order to provide sufficient material for analysis.

Date of Sampling	Lime Rate	P l a n t N u t r i e n t s				
		P	Ca	Mg	K	Al
	meq CaCO ₃ /100 g	----- % -----				ppm
7/23	0.00	-- *	--	--	--	--
	0.75	0.22	1.81	0.35	2.15	36
	1.50	0.24	1.40	0.30	2.01	21
	2.25	0.24	1.64	0.30	1.78	12
	3.00	0.25	2.03	0.31	2.05	6
9/18	0.00	0.19	1.86	0.29	1.82	97
	0.75	0.18	1.46	0.28	1.91	215
	1.50	0.21	2.35	0.36	1.70	135
	2.25	0.21	2.49	0.32	1.67	108
	3.00	0.21	3.01	0.35	1.60	90
11/8	0.00	0.24	1.30	0.22	1.45	200
	0.75	0.25	1.38	0.30	1.42	163
	1.50	0.23	1.52	0.37	1.65	102
	2.25	0.22	1.62	0.35	1.64	78
	3.00	0.24	1.58	0.33	1.53	58

* Not enough plant sample for analysis.

The relationships between dry matter yield and root length and between Al saturation and top and root dry weights were significant (Table 13). This supports the earlier finding where liming significantly reduced exchangeable Al and resulted in increased dry matter yields (Table 8). The variability in Al saturation and soil P accounted for only 1 and 28 %, respectively, of the variation in N₂ fixation. This may indicate that soil P was more critical than Al saturation for N₂ fixation on a short term basis although neither were significant parameters in the regression. In the long run, however, the reduction in Al saturation from liming may cause increases in soil P and plant P and ultimately improve the level of N₂ fixation. Plant P content was not significantly associated with dry weights of tops or roots or acetylene reduction activity of alfalfa. This may suggest that most of the plant P must have been used for both growth and storage instead of being translocated to the nodules. Obviously during this time the alfalfa plant was using primarily soil N as its major source for N supply. Under conditions of greater N depletion in soil, N fixation would increase and the role of P in the fixation process could assume greater importance. Recently, Seetin and Barnes (1977) and Duhigg, et al., (1978) found that top dry weight, root fresh weight and nodule score of alfalfa were positively correlated with rate of acetylene reduction in N-free conditions.

Kamprath (1970) indicated that lime rates based on the relationship, $\text{meq Al}/100 \text{ g} \times 2.0 = \text{meq CaCO}_3/100 \text{ g}$, will give an exchangeable Al saturation of 15 % or less (0.2 meq Al/100 g). This amount of lime was approximately one-sixth that necessary to raise soil pH to 6.5 and indicates a significant economic advantage. Thus, the use of exchangeable Al as an index to lime requirement could be of practical use.

Table 10. The main effects of P fertilizer on the dry matter yield of alfalfa herbage at harvest for Experiment 2. Each value represents the mean of three replications at five lime rates.

Phosphorus Rate	H a r v e s t D a t e				Total dry matter
	7/23	9/18	11/8	12/22	
kg P/ha	----- g/pot -----				
0	0.93 a ¹	0.91 a	0.42 a	1.29 a	3.55 a
44	1.22 a	1.13 a	0.68 ab	1.85 ab	4.88 b
88	1.38 a	1.30 b	0.81 b	2.10 b	5.59 b
132	1.37 a	1.11 b	0.72 b	1.84 ab	5.04 b

1 Means within a column followed by the same letter(s) do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

In the present study, it was found that the Al saturation could be reduced to 6.2 % (0.18 meq Al/100 g) with a lime rate equivalent to 1 x meq Al/100 g. Alfalfa yields were increased 967 % over the control (Table 7) when exchangeable Al was reduced from 0.67 to 0.18 meq/100 g; the relationship of improved yield to reduced Al saturation was far more pronounced than the relationship between yield and soil pH.

Table 11. The main effects of P fertilizer on nodulation, root length, nitrogen fixation and dry matter yield of alfalfa for Experiment 2. Each value represents the mean of three replications at five lime rates.

Phosphorus Rate	Nodulation Score	Root length	Nitrogen fixation	Dry matter yield	
				Top	Root
kg P/ha		cm	$\mu\text{M C}_2\text{H}_4/\text{hr}/\text{plant}$	-----	g/pot ----
0	1.7 a ¹	49.2 a	43.3 a	0.30 a	0.07 a
44	4.1 b	54.6 a	44.6 a	0.41 ab	0.11 b
88	5.4 c	68.7 a	44.1 a	0.47 bc	0.12 b
132	5.9 c	70.4 b	45.3 b	0.61 c	0.17 c

1 Means within a column followed by the same letter(s) do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

Table 12. Tissue composition of alfalfa from the 1980 harvests for Experiment 2. Each value is the mean of five lime rates. Replications were combined in order to provide sufficient material for analysis.

Date of Sampling	Phosphorus Rate	P	Ca	Mg	K	Al
	kg P/ha	----- % -----				ppm
7/23	0	0.24	1.75	0.32	2.19	10
	44	0.24	1.60	0.31	1.95	14
	88	0.22	1.76	0.31	1.93	27
	132	0.26	1.70	0.31	1.88	12
9/18	0	0.20	2.22	0.32	1.70	155
	44	0.20	2.35	0.33	1.74	126
	88	0.21	2.43	0.35	1.73	116
	132	0.21	2.45	0.33	1.75	137
11/8	0	0.25	1.42	0.32	1.58	142
	44	0.21	1.52	0.32	1.55	130
	88	0.24	1.48	0.31	1.50	114
	132	0.24	1.50	0.31	1.52	94

Table 13. Coefficients of determination (r^2) for the relationships among soil P and Al saturation, top and root dry weights (DW), root length, plant P and nitrogen fixation for alfalfa in Experiment 2.

	Top DW	Root DW	Root length	Nitrogen fixation
			cm	$\mu\text{M C}_2\text{H}_4/\text{hr/plant}$
Root DW (g)	0.98 **	-----	0.98 **	0.53
Al saturation (%)	0.81 *	0.74 *	0.64	0.01
Soil P (ppm)	0.28	0.36	0.49	0.28
Plant P (%)	0.41	0.35	0.40	0.21
Nitrogen fixation ($\mu\text{M C}_2\text{H}_4/\text{hr/plant}$)	0.22	0.28	0.34	-----

*,** Significant at the 0.05 and 0.01 levels, respectively.

Experiment 3: Influence of Lime on Nitrogen Fixation of Two Alfalfa Varieties.

In this experiment, the main objective was to determine if a difference in lime requirement existed between two alfalfa varieties. The lime treatments 0, 0.75, 1.50, 2.25 and 3.0 meq $\text{CaCO}_3/100$ g were the same ones that were applied and used in Experiment 2.

The soil analytical data on Table 14 were results obtained a year after lime application. The soil pH was generally low and exchangeable Al and available P high compared to those for Experiment 2 (Table 5). Under most circumstances, effectiveness of lime on soil chemical changes decreases as a function of time elapsed after lime is applied. There are two basic reasons for this. The first involves a fast initial reaction between Ca or Mg and soil, with the rate of reaction continuously decreasing until it reaches equilibrium. Equilibrium rates are sensitive to degree and frequency of mixing and also to temperature (Gardner and Jones, 1973). A second factor in the residual influence of lime is crop withdrawal of the added Ca. Tisdale and Nelson (1975), for example, showed that soil pH sharply increased 6 to 12 months after the application of dolomitic limestone and tended to decrease thereafter.

Liming significantly reduced the degree of Al saturation from about 40 to less than 10 % (Table 14). The most striking difference between the two P rates was the level of P and exchangeable Al. At the higher P rate, with 3.00 meq $\text{CaCO}_3/100$ g, the levels of P and Al were 38 ppm and 0.30 meq/100 g, respectively. It is possible that the relatively low level of soil P could be related to P fixation by the soil Al on the exchange complex. The value of exchangeable Al is slightly higher than the critical value of 0.2 meq Al/100 g, which is considered to be detrimental to legumes (Moschler, et. al., 1980).

Table 14. Soil analysis for the Paxton soil in Experiment 3. Each value represents the mean of three replications.

Phosphorus Rate	Lime Rate	pH	P	Ca	Mg	K	Al	CEC	Al Saturation
kg P/ha	meq CaCO ₃ /100 g		----- meq/100 g -----						%
0	0	4.5 a ¹	23 a	1.6 a	0.40 b	0.46 c	1.69 b	4.15 c	40.7 d
	0.75	4.5 a	29 b	1.7 a	0.38 b	0.49 c	1.38 b	3.95 bc	34.9 d
	1.50	4.8 b	27 b	2.5 c	0.40 b	0.41 bc	1.40 b	4.71 c	29.7 cd
	2.25	4.8 b	29 b	2.3 bc	0.21 a	0.26 a	0.57 a	3.34 a	17.1 bc
	3.00	4.9 b	23 a	2.0 b	0.20 a	0.38 b	0.60 a	3.18 a	18.9 bc
44	0	4.3 a	34 c	1.6 a	0.41 b	0.44 c	1.78 b	4.23 c	42.1 d
	0.75	4.6 b	34 c	1.9 a	0.40 b	0.46 c	1.32 b	4.08 c	32.4 d
	1.50	4.7 b	42 d	2.4 c	0.40 b	0.36 b	0.83 a	3.99 bc	20.8 c
	2.25	4.8 bc	34 c	2.2 bc	0.18 a	0.21 a	0.57 a	3.16 a	18.0 bc
	3.00	5.1 c	38 cd	2.6 c	0.23 a	0.41 bc	0.30 a	3.54 a	8.5 a

1 Means within a column followed by the same letter(s) do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

The data on Table 15 show that there was no significant interaction between lime rate and levels of P fertilizer. For 'Iroquois' the highest dry matter yield was obtained with 3.00 meq CaCO₃/100 g and no P fertilizer. In the case of 'Spredor 2', however, maximum yield was found with 1.50 meq CaCO₃/100 g and 44 kg P/ha. Looking at the lime means, dry matter yields increased with liming but differences were not significant. Similarly, the main effect from the application of P fertilizer was not significant.

Table 15. Dry matter yield of alfalfa varieties as influenced by lime and P treatments in Experiment 3. Each value is the mean of three replications.

Variety	Phosphorus Rate	Lime rate, meq CaCO ₃ /100 g				
		0	0.75	1.50	2.25	3.00
	kg P/ha	----- mg/pot -----				
<u>Iroquois</u>	0	301 ns	332 ns	587 ns	441 ns	675 ns
	44	371	613	486	579	491
<u>Spredor 2</u>	0	280	607	500	545	522
	44	502	584	714	563	653

ns = Not significant

Nodulation score for 'Iroquois' was not significantly influenced by liming or fertilizer P (Table 16). There was no significant interaction between the lime rate and the level of P fertilizer. The nodules of 'Spredor 2' were very small and so no score was recorded for this variety.

Table 16. Nodulation score of Iroquois as influenced by lime and P treatment in Experiment 3. Each value is the mean of three replications.

Phosphorus Rate	Lime Rate	Nodulation Score			
		Position	Number	Size	Total
kg P/ha	meq CaCO ₃ /100 g	units			
0	0	2.5	2.3	0.9	5.7 ns
	0.75	1.7	4.0	0.6	6.3
	1.50	1.9	4.5	0.4	6.8
	2.25	1.0	4.2	0.1	5.3
	3.00	1.4	3.9	0.1	5.4
44	0	2.6	3.9	0.9	7.4
	0.75	2.5	4.2	0.3	7.0
	1.50	1.9	4.5	0.1	6.5
	2.25	1.4	4.5	0.4	6.3
	3.00	1.9	4.1	0.4	6.4

ns = Not significant

Nitrogen fixation was significantly increased through liming only in the case of 'Iroquois' (Table 17). There was a significant interaction between the lime rate and the level of P fertilizer for variety 'Iroquois' only. This suggests that the two varieties responded differently to the applications of lime and fertilizer P. There was considerable variation between the measurements for the control and the other treatments. But, this appears to be associated with the large variability that was observed on the alfalfa dry matter yields on Table 15. Maximum N_2 fixation was obtained with 3.00 meq $CaCO_3/100$ g and no P fertilizer for both varieties. The difference of response in N_2 fixation between the two varieties is probably due to the interaction of several factors, i.e., actual concentration of Al, number of effective nodules per plant, photosynthate supply and/or translocation, etc.

Organic acid content of excised plant roots increases when cations are accumulated in excess of anions (Hiatt, 1967). These organic acids are thought to be involved in chelating the excess Al and prevent it from interfering with the metabolism of P (Jones, 1961). In the present study, the level of organic acids increased with liming but differences were not significant (Table 18). 'Spredor 2' had a higher level of organic acid than 'Iroquois'. The extent to which the Al organic acid complexes produced by the alfalfa varieties are influenced through liming cannot be determined from this study. Neither can it be ascertained if the relatively higher levels of organic acid in 'Spredor 2' indicate a greater tolerance to Al. Because of the difficulty involved in controlling and measuring complex soil properties, much of the work with Al tolerance has been conducted by using nutrient solutions rather than the soil as used in this study. Further research to clarify the differences in N_2 fixation and organic acid level between the two cultivars is warranted since 'Spredor 2' is

Table 17. Effect of lime and P applications on nitrogen fixation in alfalfa for Experiment 3.

Variety	Phosphorus Rate	Lime rate, meq CaCO ₃ /100 g		
		0	1,50	3,00
	kg P/ha	----- uM C ₂ H ₄ /hr/plant -----		
<u>Iroquois</u>	0	4.88 a ¹	24.64 cd	30.00 d
	44	20.36 c	20.89 c	17.44 b
<u>Spredor 2</u>	0	21.43 a	23.39 a	28.93 a
	44	9.29 a	26.01 a	22.68 a

1 For each variety, means within a column followed by the same letter(s) do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

Table 18. Effect of lime and P applications on the concentration of organic acids in alfalfa roots for Experiment 3. Replications were combined in order to provide sufficient material for analysis.

Variety	Phosphorus Rate	Lime rate, meq CaCO ₃ /100 g					Mean
		0	0.75	1.50	2.25	3.00	
	kg P/ha	----- organic acid, ueq/g -----					
<u>Iroquois</u>	0	40	40	32	56	48	43
	44	48	40	64	48	40	48
<u>Spredor 2</u>	0	40	48	56	56	64	53
	44	32	80	48	96	56	62
	Mean	40	52	50	64	52	

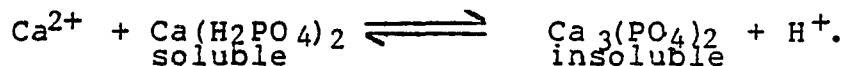
regarded as somewhat acid tolerant.

It is clear from these results that maximum alfalfa growth occurs when a certain amount of the exchangeable Al has been effectively neutralized. No yield benefits were obtained when liming did not reduce exchangeable Al below 0.30 meq/100 g (Table 14).

Experiment 4: Influence of Lime on the Mobility of Phosphorus-32 in Alfalfa.

The ability of a legume plant to tolerate high levels of acidity is closely associated to the utilization of P (Foy and Brown, 1964). In the present study, the movement and distribution of P in alfalfa were examined.

The autoradiographs of the alfalfa cultivars clearly showed that P-32 was more mobile in the absence of lime (Figures 6 and 7). Although this general tendency was observed on all the cultivars, the autoradiographs for 'Iroquois' and 'B 13 A 14' were selected for showing a more consistent relationship. The alfalfa plants receiving no lime treatment were completely saturated with P-32; the darker areas indicate the presence of radioactive phosphorus. Since the isotope ^{32}P was placed in the soil at exact distances and depths, the amount of radioactivity taken up by the plant is a measure of the intensity of root activity and the spread of the root system. The reduced P-32 uptake in the presence of lime could be explained in terms of reversion by the following equation:



McLachlan (1976) suggested that lime could revert soil applied P thus reducing its availability to plants. In this study, there was strong evidence to show that the efficiency of P-32 uptake decreased with liming. The hydrated lime

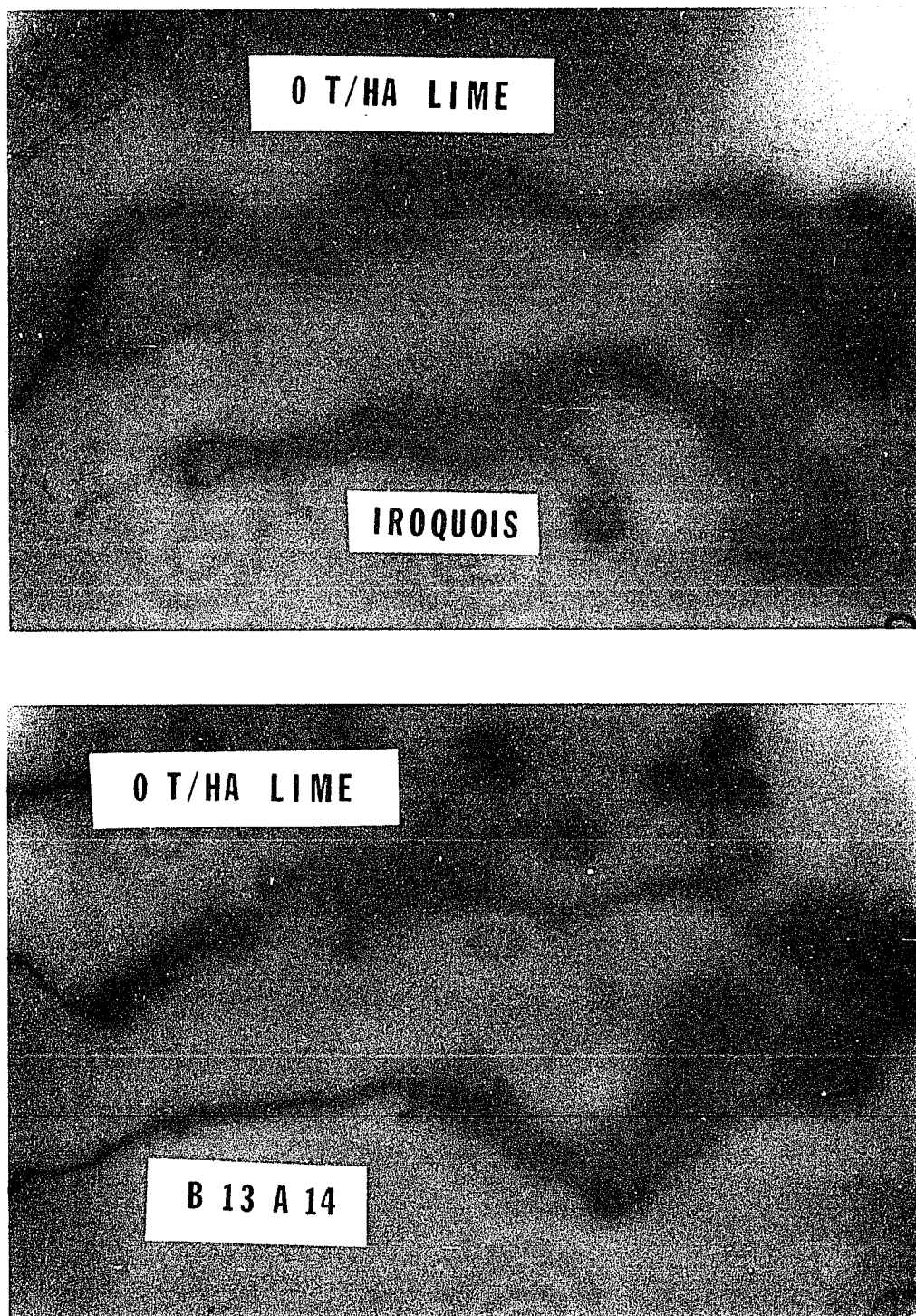


Figure 6. Autoradiograph of alfalfa plants grown in soil at 0 t/ha of lime for greenhouse Experiment 4.

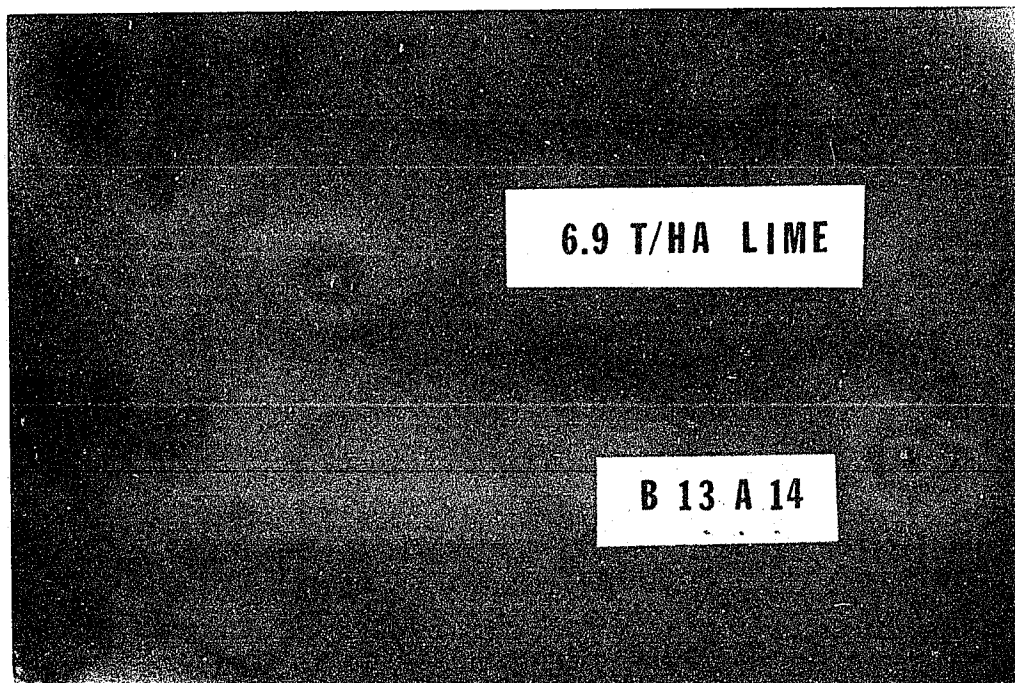
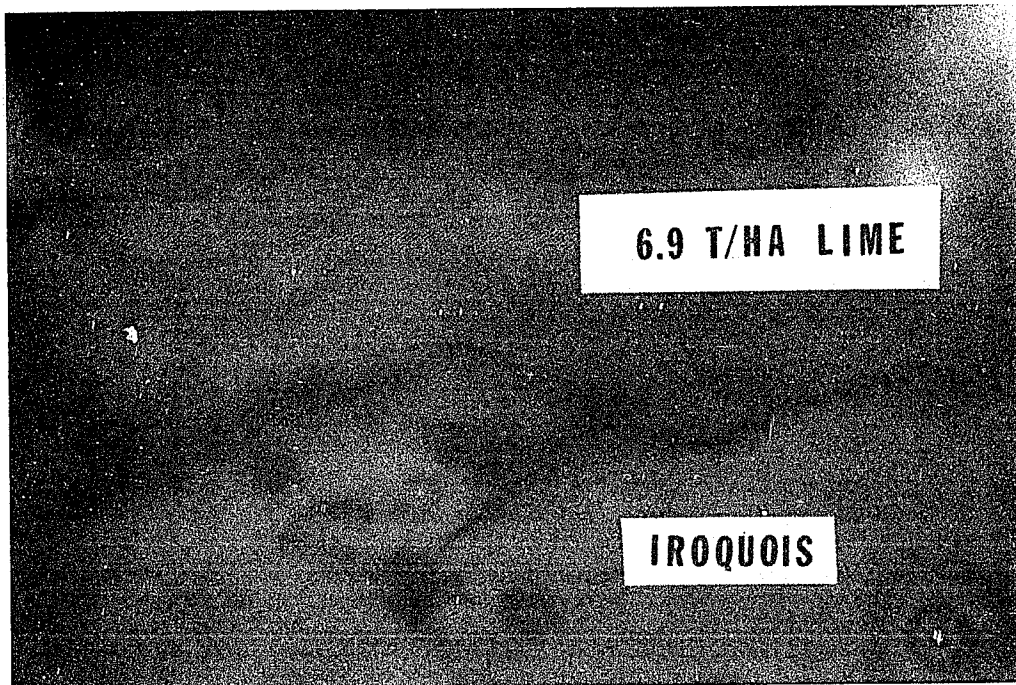


Figure 7. Autoradiograph of alfalfa plants grown in soil at 6.9 t/ha of lime for greenhouse Experiment 4.

[Ca(OH)₂] used in this experiment had a neutralizing value of 130 %; this meant that neutralization of acidity was rapidly effected. Since this lime is a highly soluble material, it would readily react with the spiked P-32 forming an insoluble form of phosphate complex which is unavailable to the plant.

The microdistribution of mineral elements in legumes has not been extensively studied. There are very few reports of investigations on the distribution of elements in grain crops (Tanaka, et al. 1974; Chino and Baba, 1976). This may be due to a lack of appropriate techniques for measuring the amounts of various elements in microscopic regions. Energy dispersive analysis by X-ray (EDS), however, permits the simultaneous determination of many elements in plant parts.

The microanalysis of alfalfa leaves and roots by EDS showed a clear difference between the lime treatments. Some of the results were erratic and so only the data from two cultivars were selected for presentation. There are difficulties involved with certain artifacts from sample preparation and beam damage. It must be realized that in EDS analysis, the X-rays are excited not only from the sample but also from the coating material and specimen stub. Elemental loss can result from beam damage and/or contamination of the specimen surface (Echlin, 1978); and hence the readings must be used with caution.

The relative concentration of Ca in the root appeared to increase with liming while showing a slight decrease in the leaf (Table 19). The relative concentration of Al and the Al/P ratio in the root decreased with liming in the case of 'Iroquois' but not with 'Arc F.C. 45028'. In the leaf, however, Al concentration increased slightly with liming for both cultivars. This is contrary to an earlier finding on Table 9 where the concentration of Al in alfalfa plants was shown to decrease with liming. The elemental counts for P

Table 19. The relative concentrations of Al, P and Ca in alfalfa as measured by Energy Dispersive Analysis by X-ray (EDAX).

Cultivar	Lime rate	Plant part	Al	P	Ca	Al/P
	t/ha		----- % -----			
<u>Iroquois</u>	0	root	12.6	1.9	8.5	6.6
		leaf	2.1	3.1	39.5	0.7
	6.9	root	1.8	8.6	26.4	0.2
		leaf	7.5	5.2	15.0	1.4
<u>Arc F.C. 45028</u>	0	root	5.4	5.0	1.5	1.1
		leaf	0.8	1.9	23.3	0.4
	6.9	root	42.4	3.9	5.1	10.9
		leaf	2.4	0.8	22.7	3.0

were less consistent. The decrease in the Al/P ratios from 6.6 to 0.2 for 'Iroquois' root would indicate a marked reduction in the uptake of Al through liming. This is indeed consistent with soil analytical data which showed a pH increase from 5.1 to 6.3 resulting in a decrease in exchangeable Al from 0.44 to 0.22 meq/100 g through liming. With this acid soil, liming should increase P solubility since a trivalent ion (Al^{3+}) is being replaced by a divalent one (Ca^{2+}). The increased uptake of P and decrease in Al absorption from liming would result in lower Al/P ratios. It is not clear why there was a higher Al uptake by Arc F.C. 45028 plants receiving lime treatment. Such anomalies illustrate why analytical results from EDS must be used with caution.

The Al/P ratio for the leaf was generally lower than that of the root. This may suggest the existence of a mechanism that reduces the translocation of Al to the leaf. McCormick and Borden (1974) had shown that a strong interaction between Al^{3+} and PO_4^{3-} occurred in the cell wall of barley roots. On the basis of the results obtained here, precipitation in the root system is a reasonable speculation for the fate of a fraction of the Al absorbed.

With the use of proper techniques of sample preparation and appropriate standards, EDS can be a useful tool in studying the distribution patterns of the various elements. Further micro-analytical studies would be required to assess the effectiveness of the method and attempt to localize Al and P on an inter- and intra-cellular basis.

Dry matter yield data of the four cultivars is shown in Table 20. For each lime treatment five plants were harvested from a single replication. Only 'Arc F.C. 45028' and 'B 13A 14' showed a yield increase over the control with 3.5 t/ha lime. These results are inconclusive but provide some information on the general response pattern for the different cultivars. More definite yield response could be

obtained by conducting simple experimental designs. However, the major emphasis of the present investigation was to assess the mobility of P as influenced by lime application.

Table 20. The effect of lime on the dry matter yield of four alfalfa cultivars for Experiment 4.

Cultivar	Lime rate, t/ha		
	0	3.5	6.9
			mg/pot
Iroquois	590	390	467
Spredor 2	425	341	429
Arc F.C. 45028	548	979	708
B 13 A 14	470	649	350

FIELD INVESTIGATIONS

Weather and Soil Data

Rainfall at the research site for the 1976-1981 period along with the 45-year average is shown in Table 21. The annual rainfall for the last three years (1979-1981) was generally higher than the 45-year average. There was unusually high rainfall for August 1979 and 1980 and July 1981. These conditions may have influenced the soil chemical reactions, thus confounding the effects of lime on the availability of nutrients, especially in regard to continuity of results over the 6-year period.

The initial soil chemical characteristics for all the field experiments are shown in Table 22. All three soils had a pH of less than 5.7 with low to moderate supply of P. The organic matter content of all soils was quite high probably due to having been untilled for many years. The level of exchangeable Al was very high for the Charlton soil used in Experiment 1.

Experiment 1: Influence of Lime on Alfalfa Dry Matter Yield as Related to Al Saturation and Available P.

All lime treatments significantly increased the mean dry matter yield compared with the control; yield differences between the lime treatments themselves were not significant (Figure 8). The dry matter yield from individual harvests was not significantly affected by liming for the first four years (Table 23). However, there was a significant yield increase from liming in the first harvest and all four harvests for 1980 and 1981, respectively. When results for each year were statistically analyzed, significant yield benefits from lime treatments occurred only in 1981 (Table 24). There was a general tendency for

Table 21. Weather data at Durham for 1976-1981 growing seasons.

	April	May	June	July	Aug.	Sept.	Oct.	<u>Total</u>
<u>Precipitation, mm.</u>								
*45 yr.	95	85	82	84	76	88	82	592
1976	-	85	21	124	94	47	-	371
1977	-	60	103	41	57	129	-	390
1978	69	133	85	25	78	7	89	486
1979	84	130	26	59	177	78	134	688
1980	134	31	76	66	104	53	122	586
1981	83	57	100	157	52	99	107	655
<u>Temperature, °C</u>								
<u>Daily Max.</u>								
*45 yr.	13.8	20.5	25.6	28.5	27.5	23.3	17.4	
1978	12.6	21.2	26.2	29.1	27.7	22.2	17.3	
1979	12.6	20.7	25.4	29.3	26.0	23.6	15.9	
1980	14.6	20.7	24.4	29.3	27.2	24.5	14.7	
1981	16.6	21.9	26.5	27.9	26.9	21.2	15.0	
<u>Daily Min.</u>								
*45 yr.	0.5	5.6	11.0	13.9	12.8	8.7	3.1	
1978	-0.5	3.8	9.5	11.4	12.7	5.3	1.5	
1979	-0.2	7.4	9.4	14.2	12.2	6.6	2.0	
1980	0.7	4.7	8.8	13.5	12.5	8.5	0.5	
1981	1.2	5.3	10.1	13.2	10.4	8.0	1.2	

*45 year average precipitation, daily maximum and minimum temperatures from Byers and Goodrich (1977).

Table 22. Initial soil analysis of field experiments. Results are from samples collected from the 0-10 cm soil depth.

Analysis	1	<u>Experiments</u> 2	3
pH	5.1	5.6	5.5
P, ppm	35	16	60
<u>Exchangeable cations</u>			
<u>NH₄OAc, pH 7.0</u>			
Ca, meq/100 g	1.07	2.0	3.25
Mg, meq/100 g	0.59	1.0	1.33
K, meq/100 g	0.79	1.0	0.38
<u>IN K Cl</u>			
Al, meq/100 g	0.90	0.20	0.06
<u>Organic Matter, %</u>			
Ignition	5.1	6.50	7.63

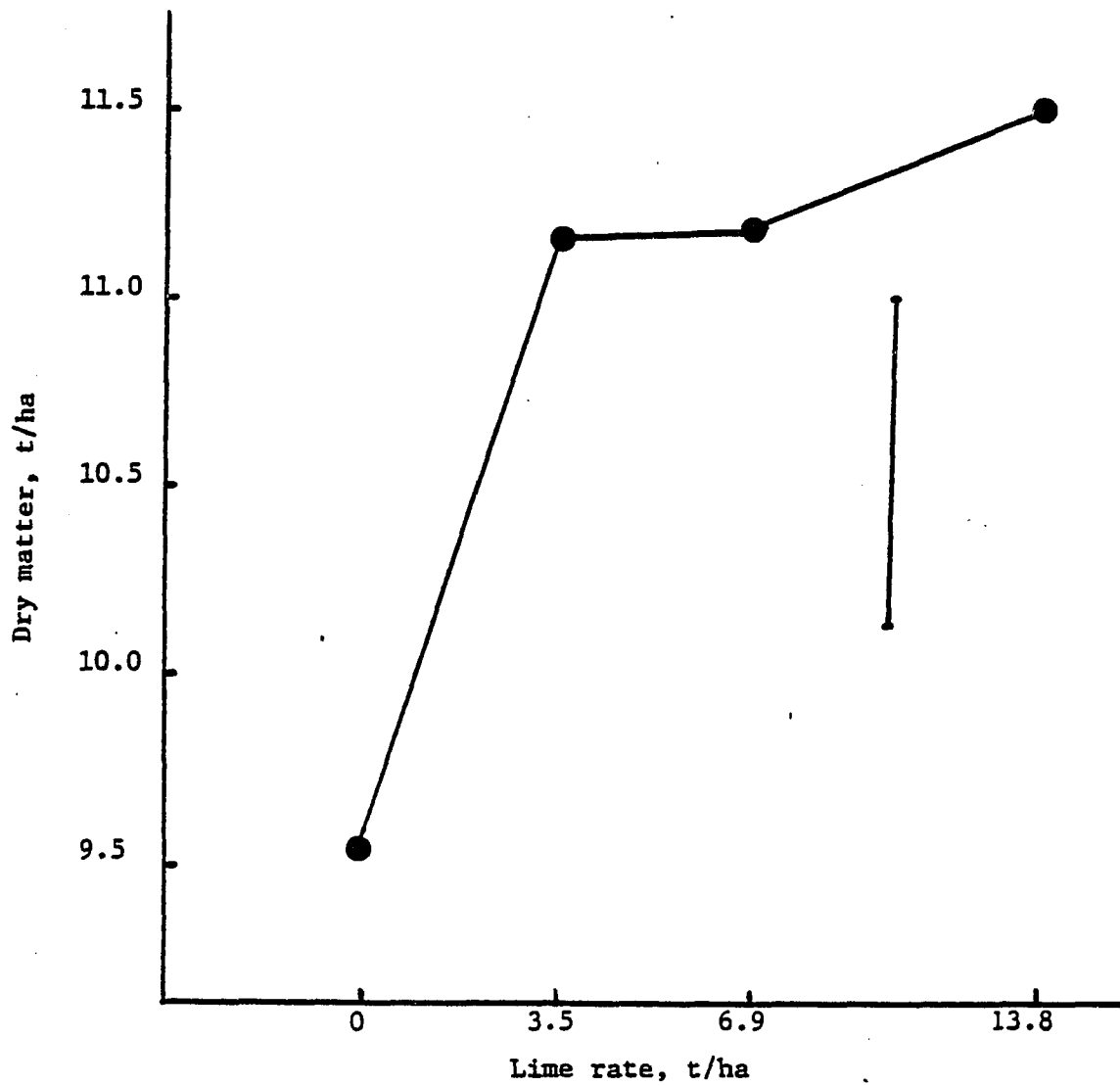


Figure 8. Main effects of lime on the dry matter yield of alfalfa for 1976-1981. Each data point represents an average of 24 values. LSD at 5% level shown as vertical line.

Table 23. The effect of lime on the dry matter yield of Iroquois alfalfa for each harvest in field Experiment 1. Each value is the mean of four replications.

Year	Harvest	Lime rate, t/ha			
		0	3.5	6.9	13.8
		mt/ha			
1976	1	3.43 a ¹	3.82 a	3.82 a	3.59 a
	2	2.12 a	2.32 a	2.44 a	2.46 a
	3	2.55 a	2.85 a	2.76 a	2.76 a
1977	1	4.19 a	5.01 a	3.96 a	4.74 a
	2	2.30 a	2.37 a	2.32 a	2.37 a
	3	2.12 a	2.51 a	2.71 a	2.71 a
1978	1	3.52 a	3.54 a	3.43 a	3.73 a
	2	3.29 a	2.97 a	2.90 a	3.45 a
	3	1.89 a	2.05 a	1.96 a	2.16 a
1979	1	3.75 a	4.53 a	4.74 a	4.69 a
	2	4.14 a	4.44 a	4.35 a	4.19 a
	3	3.04 a	3.08 a	3.40 a	3.24 a
	4	1.66 a	2.02 a	2.09 a	1.96 a
1980	1	3.22 a	4.07 b	4.28 b	4.74 b
	2	3.57 a	4.32 a	4.35 a	4.39 a
	3	2.53 a	2.53 a	2.65 a	2.88 a
1981	1	3.16 a	5.18 b	5.23 b	5.28 b
	2	2.89 a	4.40 b	4.30 b	4.07 b
	3	2.77 a	3.14 b	3.36 b	3.13 b
	4	1.59 a	2.14 b	2.20 b	2.04 b

1 Means within each row followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

the total dry matter yield to increase during the last three years. The relatively higher dry matter yields for 1979 and 1981 are partly due to the greater number of harvests. The combined analysis of variance showed an F ratio of 78 and 20 for year and lime, respectively, indicating that time had indeed a significant influence on the alfalfa yield increments.

Table 24. The effect of lime on total dry matter yields of alfalfa for each year. Each value is a mean of four replications.

Lime rate	Year					
	1976	1977	1978	1979	1980	1981
t/ha	mt/ha					
0	7.62 aA ¹	8.58 aA	8.68 aA	12.91 aC	9.31 aB	10.40 bB
3.5	8.63 aA	9.91 aB	8.55 aA	14.08 aC	10.90 aB	14.86 bC
6.9	8.97 aA	8.98 aA	8.28 aA	14.53 aC	11.25 aB	15.10 bC
13.8	9.14 aA	9.92 aA	9.34 aA	14.08 aC	12.01 aB	14.53 bC

1 Within each year, means in a column followed by the same small letter and means in a row followed by the same capital letter do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

The relative effectiveness (RE) of lime on the yield of alfalfa is shown in Table 25. The highest percentages of lime effectiveness were those for 1980 and 1981. There was a general tendency for the effectiveness to increase sharply at the initial lime rate and then level off at the higher rates. This would suggest that additional lime beyond the 3.5 t/ha may not result in any substantial yield advantage, possibly due to P reversion as indicated previously under a

Table 25. Relative effectiveness (R.E.) of lime on alfalfa dry matter yield for field Experiment 1.

Year	Lime rate, t/ha		
	3.5	6.9	13.8
	*Relative effectiveness, %		
1976	13.3	17.8	19.9
1977	15.5	4.8	15.5
1978	- 1.6	- 4.8	7.4
1979	7.0	10.6	7.8
1980	17.0	20.7	28.9
1981	42.9	45.2	39.7

$$* \text{ R.E. (\%)} = \frac{\text{Dry matter yield from lime treatment} - \text{Control}}{\text{Control}} \times 100$$

greenhouse condition.

Regression analysis on the total dry matter yields for the 6-year period showed that lime accounted for a significant portion of the yield variation in 1976, 1980 and 1981 (Table 26). The best fit to the relationship between the 6-year average dry matter yield and the rate of lime was described by a quadratic function of the form $Y = a + bX + cX^2$, where Y = dry matter yield; X = rate of lime; a = the intercept and b and c are the constants which determine the rate of yield increase per unit of lime. The response curve for the combined analysis showed that lime rate accounted for 70 % of the yield variation in alfalfa (Figure 9). Crown counts after the second and third harvests of 1981 showed poor correlation ($r < 0.20$) with dry matter yield. Although there was an increase in the number of crowns/m² with liming, i.e. from 35 to 50 for the 0 and 13.8 t/ha lime respectively, it was not an important contributor to the significant yield response from liming. Possibly there was a compensatory increase in leaf area with the fewer plants since the crowns for the control appeared to be larger.

An economic analysis of the quadratic response curve revealed that the optimal lime rate corresponding to the maximum yield was 13.8 t/ha (Table 27). But, profit would appear to be maximized at 3.5 t/ha. Based on the annual net return of \$4.00 for the economic optimal, the total profit from alfalfa yield for the 6 years would be \$24.00. Increased lime application beyond the economic optimal led to a loss in net returns.

Table 26. Regression analysis for alfalfa dry matter yield with lime rate (t/ha). The data represent four replications per lime rate for field Experiment 1.

Year	Equation	r ²
1976	Y = 7.98 + 0.10 (Lime Rate)	0.74 *
1977	Y = 8.92 + 0.07 (Lime Rate)	0.38 ns
1978	Y = 8.42 + 0.05 (Lime Rate)	0.42 ns
1979	Y = 13.46 + 0.07 (Lime Rate)	0.37 ns
1980	Y = 9.79 + 0.18 (Lime Rate)	0.85 *
1981	Y = 12.29 + 0.24 (Lime Rate)	0.39 *

* Significant at 5% level.

ns Not significant.

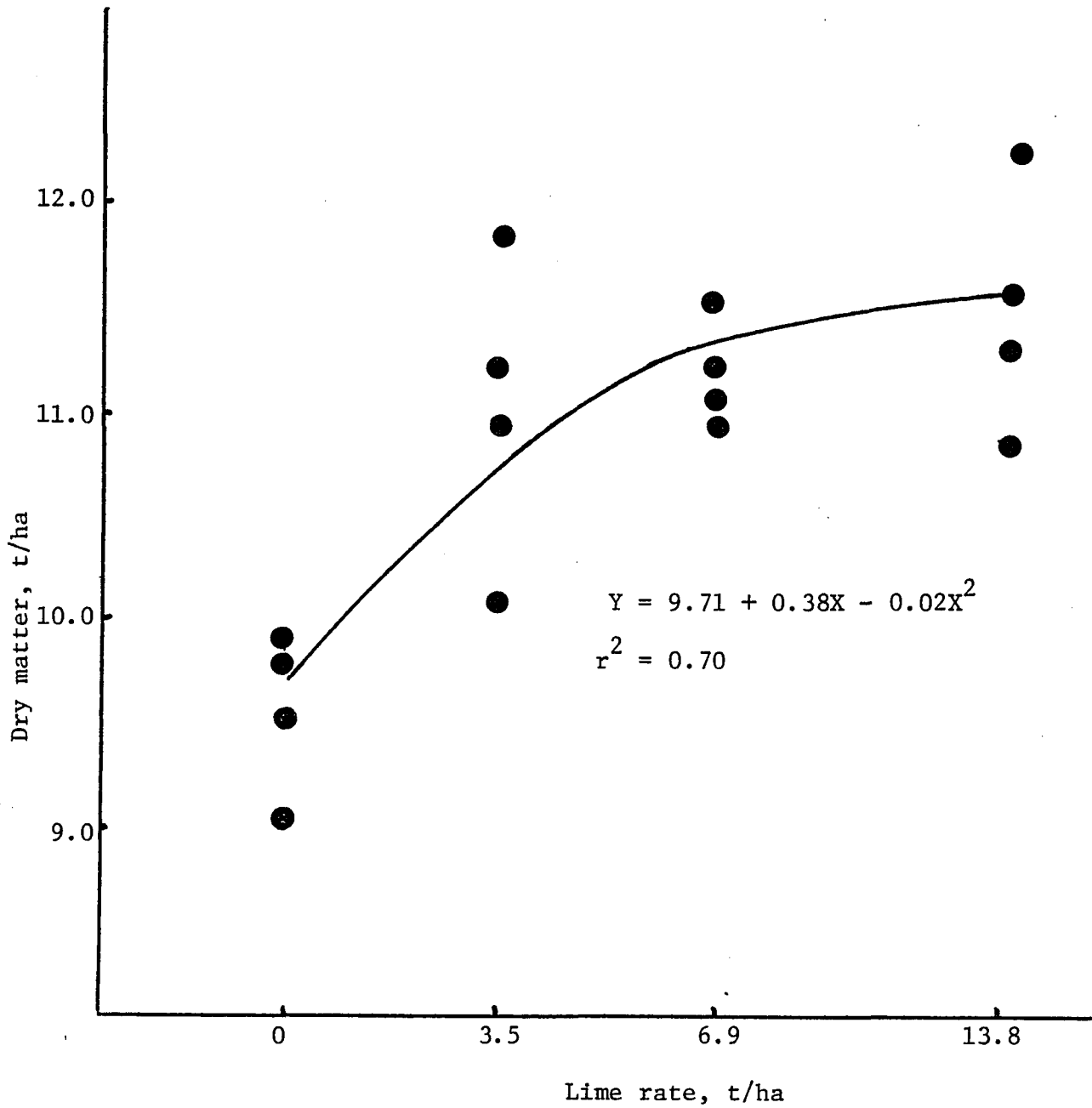


Figure 9. The influence of lime on alfalfa dry matter yield as shown through regression analysis. Each data point represents an average of 24 values.

The correlation values in Table 28 show that the 1980-81 alfalfa dry matter yield was strongly associated with plant N, P, Mg, Al and Mn. Significant negative correlations existed between yield and Al and Mn. The correlations between plant Al and N, Mg and P were also negative for both years, indicating that high levels of Al could suppress the uptake of these elements. In particular, the inverse relationship between plant Al and P is clearly shown in Figure 10. During the 6-year period it appeared that soil Al was being hydrolyzed and ultimately precipitated as $\text{Al}(\text{OH})_3$, which in turn led to the decline in exchangeable Al with time. More available P was being released for plant uptake when the soluble Al was converted into an insoluble form; this tendency was more evident in the presence of lime. Furthermore, the low levels of plant Al in the final year (1981) indicated that indeed there was more free P for metabolic use by the plant. The P content of plants treated with 3.5 tons/ha limestone, for example, was 28 kg P/ha for 1976 in contrast to 49 kg P/ha for 1981. This indicates that the improvement in P uptake resulting from Al precipitation was more pronounced when P content was considered instead of P concentration. It has been reported by Fletcher and Kurtz (1964) that high levels of plant Al can restrict, through precipitation, the plant P available to metabolism and thus decrease N_2 fixation and growth. Clarkson (1965) also showed that a large portion of the P in barley roots of plants grown in high Al levels made no contribution to the P incorporated into intermediates in metabolism.

Plant P and Mg generally increased while Al and Mn were reduced markedly through liming (Table 29). Apparently there is a general tendency for Mg and P uptake to increase with a decrease in Al or Mn. Similar results were found by Ouellette and Dessureaux (1958) and Sartain and Kamprath (1977) working on alfalfa and soybeans, respectively. Godo and Reisenauer (1980) had shown that increased microbial activity can increase the amount of available Mn and its uptake. Thus, the increased Mn uptake observed in the present study could be a result of the increase in microbial activity due to liming. There was an increase in the uptake of Fe for 1981, and a significant reduction of plant Zn through liming in 1980 (Table 29).

Table 27. The effect of lime on alfalfa dry matter yield and the profits obtained from the application.

Rate of lime	Response to lime, dry matter yield	Value of response ⁺	Cost of lime applied ⁺	Profit from lime
t/ha	t/ha	t/ha	\$/ha	\$/ha
3.5	1.09	109	105	4
6.9	1.69	169	207	-38
13.8	1.79	179	414	-235

⁺ The following prices are assumed: lime, \$30/ton; alfalfa hay, \$100/ton. No other establishment costs have been included.

Table 28. Simple correlations (r) for the relationships among yield, plant N, Ca, Mg, P, Al and Mn for 1980 and 1981 in field Experiment 1. Values in parentheses are those for 1980.

	Yield t/ha	Al ppm	Mn ppm	P %
Yield (t/ha)		-0.89 ** (-0.72 **)	-0.90 ** (-0.66 **)	0.76 ** (0.56 *)
N (%)	0.84 * (0.52 *)	-0.85 ** (-0.68 **)	-0.76 ** (-0.45)	0.89 ** (0.90 **)
Ca (%)	-0.36 (-0.09)	0.30 (0.20)	0.40 (0.20)	-0.15 (0.29)
Mg (%)	0.75 ** (0.58 *)	-0.81 ** (-0.82 **)	-0.87 ** (-0.81 **)	0.81 ** (0.59 *)
P (%)	0.76 ** (0.56 *)	-0.87 ** (-0.73 **)	-0.75 ** (-0.52 *)	

*,** Significant at 5 and 1% levels, respectively.

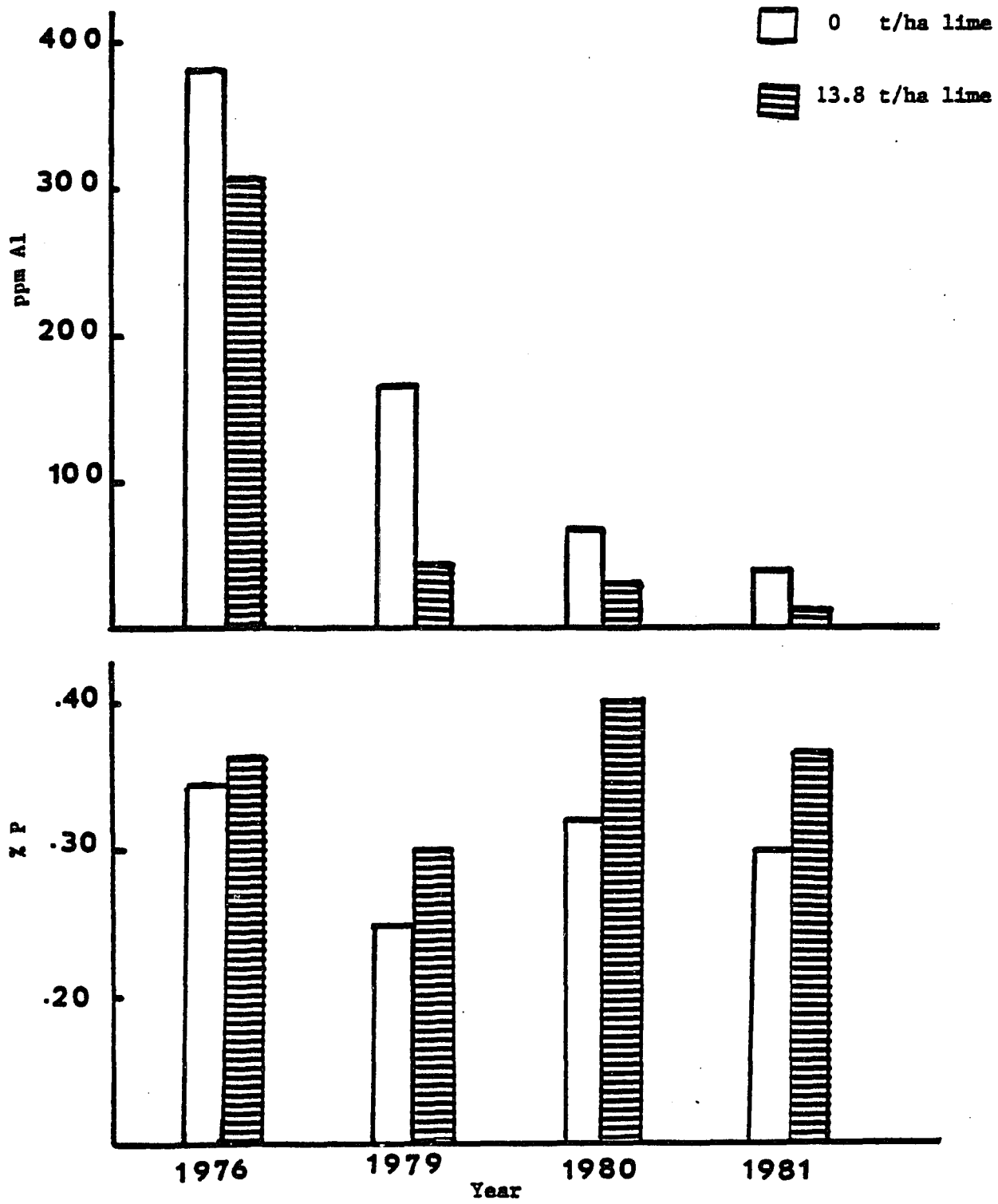


Figure 10. Relations between lime, plant P and Al during the period from 1976 to 1981 for field Experiment 1.

Table 29. The effect of lime on tissue composition of alfalfa for the first harvest of 1980 and 1981 in field Experiment 1. Each value is an average of four replications.

Plant nutrient	Lime rate, t/ha							
	1980				1981			
	0	3.5	6.9	13.8	0	3.5	6.9	13.8
P %	0.32 a ¹	0.40 b	0.35 a	0.40 b	0.30 a	0.33 b	0.37 c	0.36 c
Ca %	1.18 a	1.14 a	1.10 a	1.20 a	1.17 a	0.96 a	1.08 a	1.01 a
Mg %	0.29 a	0.38 b	0.40 b	0.43 b	0.28 a	0.33 b	0.36 b	0.37 b
K %	2.62 a	2.67 a	2.76 a	2.70 a	2.39 a	2.40 a	2.43 a	2.29 a
Al ppm	73.5 b	27.0 a	31.0 a	26.3 a	44.5 b	20.5 a	17.5 a	12.8 a
Mn ppm	61.8 b	36.8 a	32.8 a	28.5 a	61.1 b	36.5 a	32.0 a	27.2 a
B ppm	39.8 a	38.5 a	33.5 a	34.8 a	33.7 a	31.8 a	32.7 a	28.8 a
Fe ppm	80.3 a	85.3 a	94.8 a	97.0 a	65.8 a	69.2 b	74.0 c	73.8 c
Cu ppm	6.5 a	6.5 a	5.5 a	5.4 a	5.6 a	5.4 a	5.2 a	4.7 a
Zn ppm	32.5 b	29.3 b	24.0 a	25.3 a	45.3 a	36.7 a	34.1 a	32.3 a

¹ Means within each plant nutrient followed by the same letter for a given year do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

Nitrogen content was strongly associated with the yield of alfalfa; the total yield of N and crude protein were markedly increased with liming for 1980 and 1981 (Table 30). The increase in the amount of accumulated N is likely from a higher level of dinitrogen fixation, although no measurements were made of N_2 fixation. This means that dinitrogen fixed by nodules must have been used for both growth and storage. Nitrogen fixation as well as nitrification are known to be enhanced by liming to a pH 5.5 or 6.5 (Tisdale and Nelson, 1975). With legumes, the growth of the plant is increased because of the greater amount of N_2 fixed. Nitrogen fixation is an expensive process for the cell and so it is controlled to avoid wasting energy when the micro-organisms have other sources of combined N. The synthesis and activity of nitrogenase is repressed when there is ammonium (NH_4^+) or nitrate (NO_3^-) present in the soil. Legume fixation of N is at a maximum only when the level of available soil N is at a minimum. It is reasonable to assume, therefore, that by the end of the five growing seasons, total N in the soil could be depleted, thereby forcing the alfalfa plant to use atmospheric N and N_2 fixation to meet its needs. Under optimum conditions alfalfa can obtain as much as 90 % of its N from atmospheric sources (Munns, 1965). Obviously, an increased N fixation will lead to an increase in N accumulation and growth. Major, et al., (1979) showed that alfalfa shoot dry weight was a good estimator for nodule dry weight and nodule activity in N-free rooting medium.

The above mentioned results may suggest that the yield increases due to liming are partly a result of the increases in plant N, P and Mg as well as decreases in Al and/or Mn.

The positive yield response to liming could further be explained in terms of the effect of lime on soil chemical changes. Soil pH was substantially increased with the application of lime at all depths (Figure 11). This increase in pH could have contributed to the reduction in the level of exchangeable Al from 0.45 to less than 0.10 meq/100 g, i.e. from 30 to 1 % Al saturation. There was a sharp increase in pH as well as a big drop in exchangeable Al at the initial lime rate. At higher pH levels Al is readily precipitated into insoluble form (Munns, 1965). There was a significant negative correlation between soil pH and Al ($r = -0.79$) as well as between soil pH and soil P ($r = -0.63$) in 1981.

Table 30. The effects of lime on the % N and yields of N and protein for the first harvest. Each value is the mean of four replications.

Year	Assay	Lime rate, t/ha			
		0	3.5	6.9	13.8
1978	% N	3.2 a ¹	3.3 a	3.4 a	3.3 a
	N, kg/ha	111 a	116 a	115 a	122 a
	Protein, kg/ha	695 a	726 a	720 a	761 a
1980	% N	4.1 a	4.8 a	4.4 a	4.7 a
	N, kg/ha	125 a	184 b	177 b	213 b
	Protein, kg/ha	782 a	1152 b	1105 b	1334 c
1981	% N	4.4 a	4.8 b	4.8 b	4.8 b
	N, kg/ha	127 a	247 b	252 b	254 b
	Protein, kg/ha	862 a	1541 b	1577 b	1563 b

1 For each assay, means within a row followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

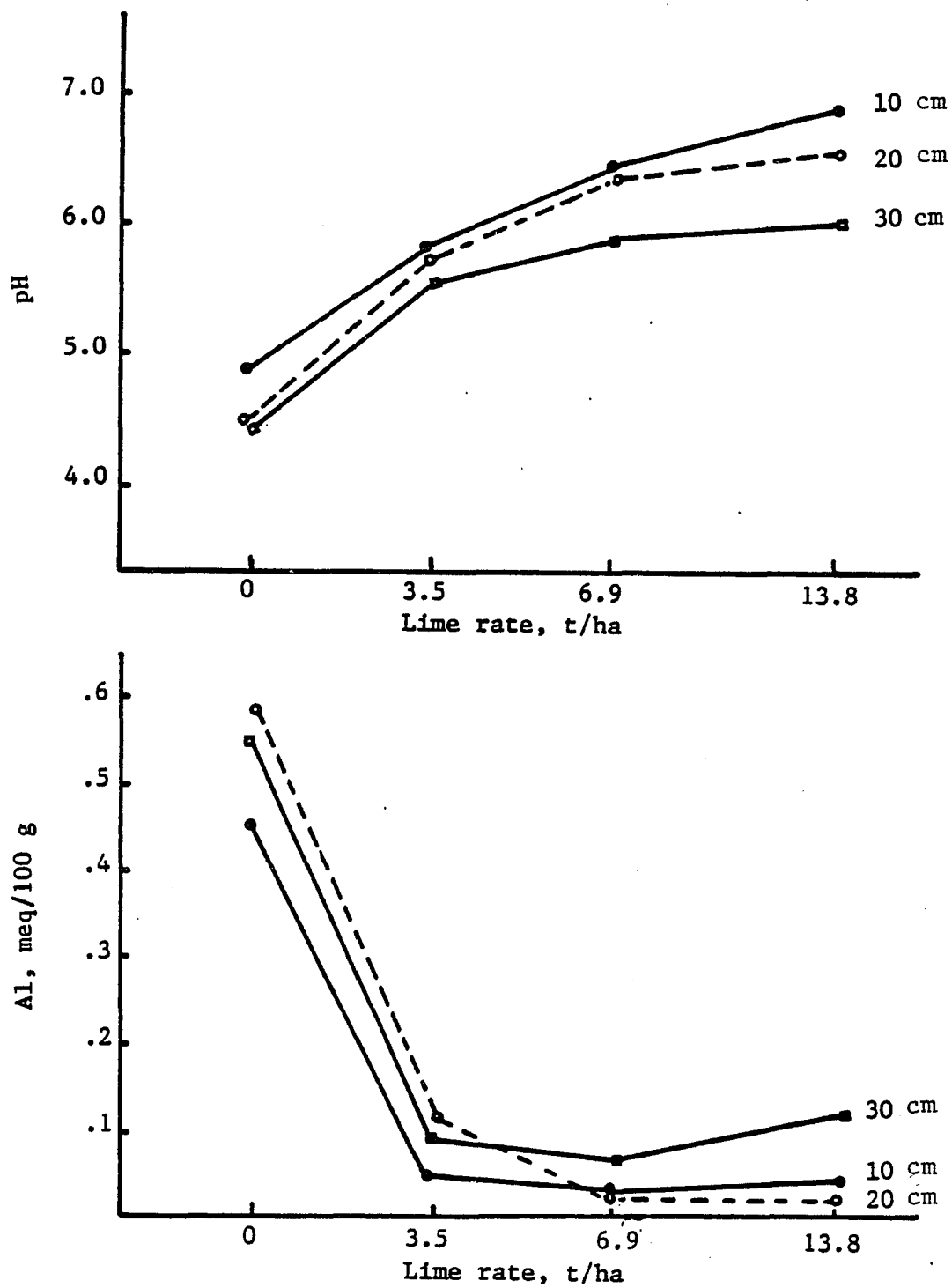


Figure 11. Changes in soil pH and exchangeable Al of a Charlton soil under three soil depths for field Experiment 1 in 1981. Each data point represents an average of four values.

Table 31. Soil analysis for the Charlton sandy loam in July 1980 for field Experiment 1. Each value is a mean of four replications.

Soil depth	Lime rate	pH	P	Ca	Mg	K	Al	CEC	Al Saturation
cm	t/ha		ppm	meq/100 g					%
10	0	4.8 a ¹	69 a	0.31 a	0.13 a	0.52 a	0.45 b	1.41 a	31.9 b
	3.5	5.6 b	41 a	1.00 b	0.58 b	0.53 a	0.06 a	2.17 b	2.8 a
	6.9	6.4 c	48 a	1.59 b	0.91 bc	0.59 a	0.04 a	3.13 c	1.3 a
	13.8	6.7 c	48 a	3.18 c	1.24 c	0.58 a	0.03 a	5.03 d	0.5 a
20	0	4.9 a	47 a	0.38 a	0.10 a	0.47 a	0.47 b	1.42 a	33.1 b
	3.5	5.7 b	36 a	0.89 b	0.71 b	0.31 a	0.06 a	1.97 b	3.0 a
	6.9	6.2 b	50 a	1.49 c	1.04 b	0.33 a	0.03 a	2.89 c	1.0 a
	13.8	6.2 b	50 a	2.55 d	1.25 b	0.36 a	0.05 a	4.21 d	1.2 a
30	0	4.9 a	31 a	0.38 a	0.11 a	0.38 a	0.40 b	1.27 a	31.5 b
	3.5	5.5 ab	22 a	0.58 a	0.53 b	0.19 a	0.10 a	1.40 a	7.1 a
	6.9	5.8 b	21 a	0.97 b	0.69 b	0.23 a	0.04 a	1.93 ab	2.1 a
	13.8	5.7 b	24 a	1.33 c	0.77 b	0.21 a	0.09 a	2.40 b	3.8 a

¹ Means for each soil depth within a column followed by the same letter(s) do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

Table 32. Soil analysis for the Charlton sandy loam in July 1981 for field Experiment 1. Each value is a mean of four replications.

Soil depth	Lime rate	pH	P	Ca	Mg	K	Al	CEC	Al Saturation
cm	t/ha		ppm	----- meq/100 g -----					%
10	0	4.8 a ¹	143 a	0.63 a	0.16 a	1.18 a	0.45 b	2.42 a	18.6 b
	3.5	5.7 b	118 a	1.19 b	0.42 a	1.25 a	0.08 a	2.94 b	2.7 a
	6.9	6.4 c	97 a	1.69 bc	0.81 b	1.28 a	0.04 a	3.82 c	1.0 a
	13.8	6.8 c	60 a	2.06 c	1.04 b	1.31 a	0.06 a	4.47 d	1.3 a
20	0	4.6 a	92 a	0.63 a	0.16 a	1.09 a	0.58 b	2.46 a	23.6 b
	3.5	5.7 b	69 a	0.94 a	0.38 a	1.00 a	0.11 a	2.43 a	4.5 a
	6.9	6.4 c	51 a	1.81 b	0.97 b	0.97 a	0.04 a	3.79 b	1.1 a
	13.8	6.5 c	50 a	1.94 b	1.10 b	1.09 a	0.03 a	4.16 b	0.7 a
30	0	4.6 a	46 a	0.69 a	0.15 a	1.09 a	0.54 b	2.47 a	21.9 b
	3.5	5.4 b	41 a	0.88 ab	0.40 a	1.09 a	0.10 a	2.47 a	4.0 a
	6.9	5.9 c	45 a	1.25 b	0.70 b	1.03 a	0.07 a	3.05 b	2.3 a
	13.8	6.0 c	36 a	1.31 b	0.68 b	1.09 a	0.12 a	3.20 b	3.8 a

1 Means for each soil depth within a column followed by the same letter(s) do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

Table 33. The influence of lime on P, Ca, Mg and K as determined by two different extracting solutions for Experiment 1 in 1981. Each value is a mean of four replications.

Soil depth	Lime rate	Modified Morgan				Ammonium Acetate			Bray No 1 P	
		P	Ca	Mg	K	Ca	Mg	K		
cm	t/ha	ppm	----- meq/100 g -----							ppm
10	0	5.5 a ¹	1.04 a	0.28 a	1.03 a	0.63 a	0.16 a	1.18 a	143 a	
	3.5	4.8 a	2.59 b	0.83 b	0.94 a	1.19 b	0.42 a	1.25 a	118 a	
	6.9	4.2 a	4.24 c	1.66 c	0.94 a	1.69 bc	1.81 b	1.28 a	97 a	
	13.8	4.8 a	5.13 c	1.67 c	0.90 a	2.06 c	1.04 b	1.31 a	60 a	
20	0	6.5 a	1.03 a	0.22 a	0.54 a	0.63 a	1.16 a	1.09 a	92 a	
	3.5	4.0 a	2.08 b	0.82 b	0.43 a	0.94 a	0.38 a	1.00 a	69 a	
	6.9	5.5 a	3.30 c	1.65 c	0.24 a	1.81 b	0.97 b	0.97 a	51 a	
	13.8	4.8 a	4.25 d	1.67 c	0.38 a	1.94 b	1.10 b	1.09 a	50 a	
30	0	3.8 a	0.89 a	0.20 a	0.35 a	0.69 a	0.15 a	1.09 a	46 a	
	3.5	3.0 a	1.70 b	0.78 b	0.24 a	0.88 ab	0.40 a	1.09 a	41 a	
	6.9	3.2 a	2.11 bc	1.44 c	0.16 a	1.25 b	0.70 b	1.03 a	46 a	
	13.8	3.2 a	2.60 c	1.50 c	0.21 a	1.31 b	0.68 b	1.09 a	36 a	

¹ Means for each soil depth within a column followed by the same letter(s) do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

exchangeable Ca and Mg. The poor correlation for soil K may be due to the effect from the soil reserve. Slowly available K is generally considered to be unextractable by these procedures (Tisdale and Nelson, 1975). It becomes available to plants slowly and over longer periods of time.

In terms of relating the nutrient levels to crop response, soil Ca and Mg accounted for 52 and 61 % of the yield variation according to the Modified Morgan; in the case of the NH_4OAc extraction 50 and 43 % of the yield variation was accountable by soil Ca and Mg, respectively. Soil K accounted for only 30 % of the yield variation with both methods. Bray P accounted for only 41 % of the yield variation while the Modified Morgan P was able to explain 82 % of the yield variation; the correlation coefficient (r) between Bray #1 and Modified Morgan was only 0.34. This would suggest that the two methods have differing extractability for soil P and that the Modified Morgan P was a better predictor of crop response. According to Baker and Hall (1967) the ranges in Bray #1 P for Pennsylvania soils were: very high, 80 ppm; high, 50-80 ppm; medium, 30-50 ppm; low, 15-30 ppm; very low, less than 15 ppm.

Lime significantly reduced extractable Al as well as exchangeable Al and H (Table 34). Extractable Mn and Fe were also markedly reduced with liming. At high pH values, precipitation and unavailability of Mn and Fe is a common occurrence (MacKay, 1980). There was a negative correlation ($r = -0.60$) between pH and extractable Al. This is consistent with an earlier finding in this section wherein a strong negative correlation ($r = -0.79$) between pH and exchangeable Al was shown (Figure 10). The correlations between exchangeable and extractable Al ($r = 0.83$) and between exchangeable Al and H ($r = 0.82$) were also highly significant. This would suggest that the solubility of Al in the soil solution is a function of pH and that at a higher pH most of the extractable Al remains in a

Table 34. The influence of lime on soil organic matter, Al, H, Mn and Fe for field Experiment 1.
Each value is a mean of four replications. Data are from samples collected from the
0-10 cm soil depth in August 1981.

Lime rate	Organic matter		Extract- able	Exchange- able	Non- Exchange- able	Exchange- able acidity	Extractable	
	Dichromate+	Ignition					Mn	Fe
t/ha	-----	% -----	-----	meq Al/100 g	-----	meq H/100 g	-----	ppm -----
0	4.09 a ¹	5.9 a	1.50 b	0.54 b	0.96 a	5.12 c	4.0 b	10.6 b
3.5	3.89 a	5.8 a	1.08 a	0.08 a	1.00 a	3.54 b	2.4 a	5.0 a
6.9	3.90 a	5.9 a	0.88 a	0.07 a	0.81 a	2.43 a	2.3 a	5.5 a
13.8	4.12 a	5.9 a	0.91 a	0.05 a	0.86 a	1.95 a	1.9 a	5.4 a

¹ Means within each column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

$$+ Y (\text{Dichromate}) = -0.25 + 0.73 (\text{Ignition})$$

non-exchangeable form (Jackson, 1963). The greatest reduction in exchangeable as well as extractable Al occurred at the initial lime rate of 3.5 t/ha. Organic matter content in the soil did not change with liming; this lack of response to liming was clearly shown by both the dichromate and ignition methods with the simple correlation between the two methods being 0.78. Thomas (1975) had shown that small increases in organic matter can cause substantial decreases in the amount of exchangeable Al at a given pH. This implies that organic matter may be important in preventing Al toxicity to plants by lowering the amount of Al in soil solution. Under the present investigation the possible effect of organic matter on the neutralization of soluble Al appears to be masked by that of liming.

It is clear from Table 35 that lime accounted for a significant variation in the availability of P, Ca, Mg, Al and in soil pH. The negative slope values for soil P, exchangeable Al and H are indicative of the tendency for the concentration of these nutrients to decrease with liming. An increase in pH will definitely reduce exchangeable Al, lower active acidity and partly immobilize soil P. Soil P and exchangeable Ca and Mg showed relatively high values for r^2 . Lime also played an important role in the uptake of the various elements. The high negative slope values for plant Al and Mn show that the concentration of these two elements decreased markedly with liming. This is to be expected since increased pH from liming will decrease the availability and uptake of these two elements. Except for plant Ca, lime accounted for a significant portion of the variability in the concentration of the nutrients.

Plant-soil relations to dry matter yields are shown by the regression equations in Tables 36 and 37. A significant portion of the yield variation could be related to soil pH and exchangeable Al, H, Ca and Mg. These data indicate that increases in soil pH, Ca and Mg as well as the decrease in

Table 35. Relationships between lime rates (X) and soil-plant chemical properties and crop yields for 1981 in field Experiment 1.

Dependent variable Y	Equation	r^2
<u>Soil analysis</u>		
Available P	$Y = 140.1 - 5.95 X$	0.99 **
Exchangeable Ca	$Y = 0.77 + 0.10 X$	0.93 **
Exchangeable Mg	$Y = 0.22 + 0.06 X$	0.94 **
Exchangeable Al	$Y = 0.30 - 0.02 X$	0.50 **
Exchangeable H	$Y = 4.59 - 0.22 X$	0.84 **
pH	$Y = 5.07 + 0.14 X$	0.88 **
<u>Plant analysis</u>		
N %	$Y = 4.51 + 0.029 X$	0.55 **
P %	$Y = 0.31 + 0.004 X$	0.65 **
Ca %	$Y = 1.10 - 0.007 X$	0.21
Mg %	$Y = 0.29 + 0.006 X$	0.78 **
Al ppm	$Y = 35.3 - 1.77 X$	0.60 **
Mn ppm	$Y = 52.3 - 2.17 X$	0.71 **
Yield t/ha	$Y = 12.29 + 0.24 X$	0.39 **

** Significant at the 1% level of probability.

Table 36. Regression models relating alfalfa dry matter yields of 1981 to selected soil chemical factors in field Experiment 1.

	<u>R²</u>
Yield = 15.28 - 13.65 Exchangeable Al	0.48 **
Yield = 18.51 - 4.85 Extractable Al	0.27
Yield = -4.29 + 2.95 pH	0.45 **
Yield = 9.70 + 5.70 Exchangeable Mg	0.32 **
Yield = 8.20 + 3.56 Exchangeable Ca	0.50 *
Yield = 19.08 - 1.82 Exchangeable H	0.50 **
Yield = 17.77 - 0.04 Available P	0.52
Yield = 7.26 + 1.24 pH - 9.35 Exchangeable Al	0.51 **
Yield = 16.73 - 0.02 Available P - 11.35 Exchangeable Al	0.51
Yield = 17.66 - 7.08 Exchangeable Al - 1.04 Exchangeable H	0.53 *
Yield = 17.15 + 3.15 Extractable Al - 2.30 Exchangeable H	0.52 *

*,** Significant at 5 and 1% levels of probability, respectively.

Table 37. Regression models relating alfalfa dry matter yield of 1981 to selected plant chemical factors in field Experiment 1.

	<u>R²</u>
Yield = - 2.81 + 48.98 Plant P	0.79 **
Yield = 0.35 + 40.21 Plant Mg	0.63 **
Yield = 17.07 - 0.14 Plant Al	0.80 **
Yield = 19.12 - 0.14 Plant Mn	0.80 **
Yield = -22.32 + 7.70 Plant N	0.75 **
Yield = 18.13 - 2.74 Plant P - 0.14 Plant Al	0.80 **
Yield = 13.60 + 13.54 Plant P - 0.11 Plant Mn	0.82 **
Yield = 18.40 - 0.07 Plant Al - 0.08 Plant Mn	0.85 **
Yield = 15.49 + 4.06 Plant Mg - 0.13 Plant Al	0.80 **
Yield = 21.98 - 6.67 Plant Mg - 0.15 Plant Mn	0.81 **
Yield = 3.51 - 0.10 Plant Al + 2.69 Plant N	0.82 **

** Significant at the 1% level of probability.

exchangeable Al contributed to the significant yield improvement. The highest coefficient of determination (53 %) was found when yield was related to exchangeable Al and H. The relatively high negative slope value for exchangeable Al is indicative of the detrimental effects of this element on alfalfa plant growth. Increases in exchangeable Mg and Ca in the soil have beneficial influences on crop yield as shown by the large positive slope values. According to Alley (1980) regression models including pH or exchangeable Al, Ca and Mg accounted for 82 % of the variability in alfalfa yields. Based on the results from this experiment, incorporating lime to the 30 cm depth is sufficient to obtain a satisfactory root system for high crop yields of alfalfa.

The high slope value for plant P indicates the significant influence of this nutrient on crop growth; plant P alone accounted for 79 % of the yield variation. Plant Mg and N were also important contributors to the yield variation. There was only a small gain in R^2 when multiple regression equations were used. This may suggest that the interaction between any two nutrients, of those considered in Table 37, was not large enough to contribute to an improvement in the ability of the regression equations to predict crop response. The highest coefficient of determination (85 %) was found when yield was related to both plant Al and Mn. Unlike Al, Mn in soils exists in at least three states of oxidation and its availability to plants is governed by a host of factors. Hence, plant uptake of soil Mn is neither well understood nor readily predicted (Godo and Reisenauer, 1980).

Yield of alfalfa for 1981 was predicted as a contour surface (regression) on selected plant variables. The contour plot in Figure 12 gives a clear picture of the relationship between plant P, plant Al and dry matter yield. It shows that optimum alfalfa yields were obtained at low

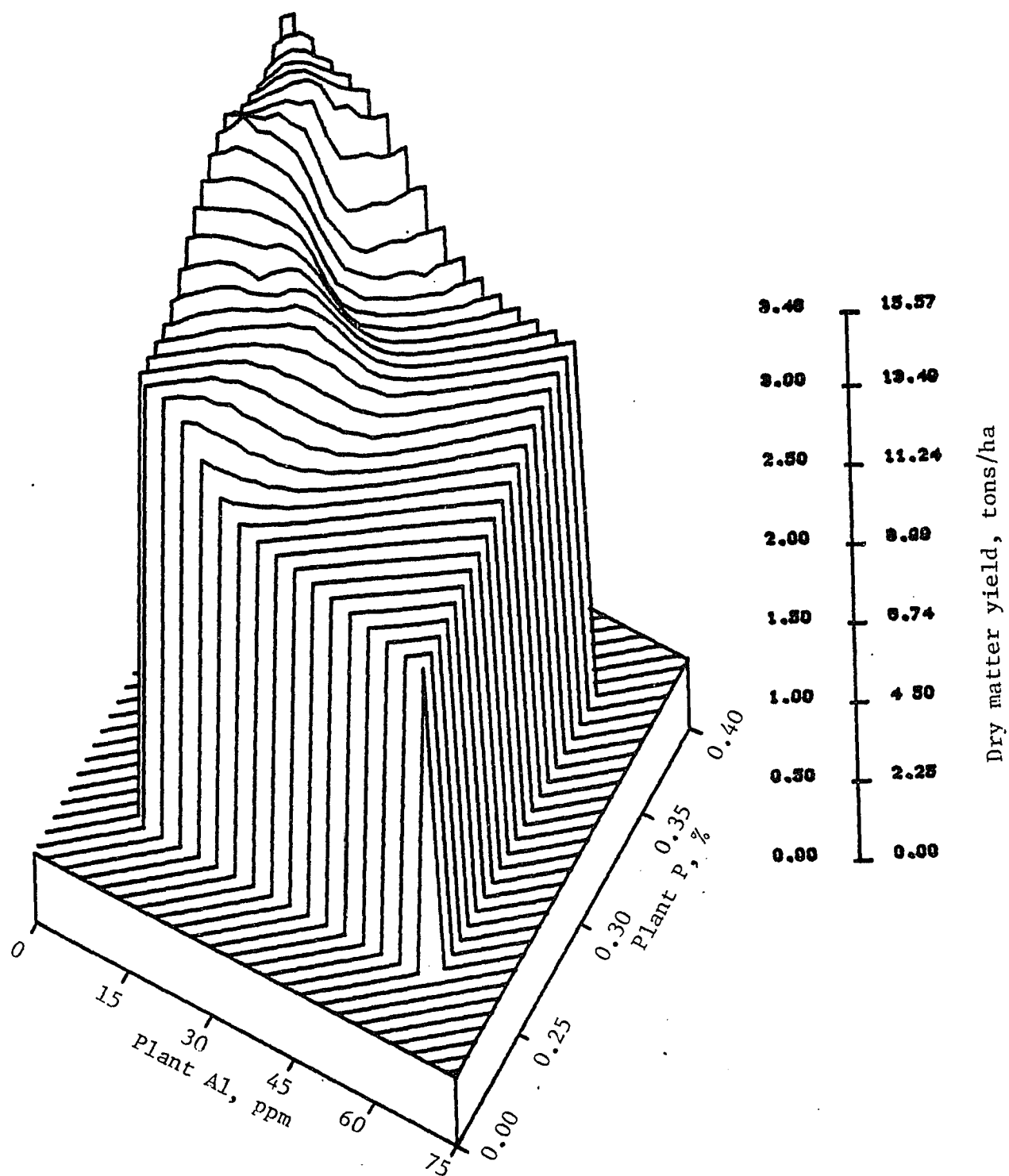


Figure 12. Contour surface of alfalfa yield for 1981 as a function of plant P and Al. Viewing angle: azimuth 60° , elevation 30° .

plant Al (less than 25 ppm) and high plant P (greater than 0.33 %). This is to be expected since increased pH from liming causes precipitation of Al which in turn enhances the uptake of P, resulting in higher dry matter yields. A similar relationship was found when plant Mn instead of Al was related to P and yield. It would appear that plant Mn levels greater than 40 ppm were detrimental to alfalfa yield.

The contour surface in Figure 13 shows that plant Al had a negative influence on alfalfa yield especially at low levels of plant N. Again, maximum yield was obtained when plant Al was less than 25 ppm and plant N greater than 4.7 %. The irregular plateau indicates that the relationship between yield and plant Al and N did not show a definite pattern. Maximum yield could be realized at high levels of plant P and N (Figure 14). However, high yields were also observed at low levels of plant N when the supply of plant P was greater than 0.30 %. The increased dry matter accumulation due to a higher uptake of P would mean a greater substrate supply for N_2 fixation. This is related to an increased supply in reducing power and energy for nitrogenase activity by the pyruvate (Gibson, 1977). Increase in percentage N in alfalfa as a result of improved N_2 fixation due to a higher uptake of P was reported by Andrew and Robins (1969). The relationship between plant P, plant Al and plant N is shown in Figure 15. The contour surface clearly shows that N concentration was maximized at low and high levels of plant Al and P, respectively.

The results from the contour surface clearly demonstrate the significance of nutrient status in alfalfa plants. But, in order to effectively use these regressions to predict crop response, it is important to consider the content as well as the total concentration of plant nutrients. This is necessary in view of the effect of nutrient dilution from an increase in dry matter

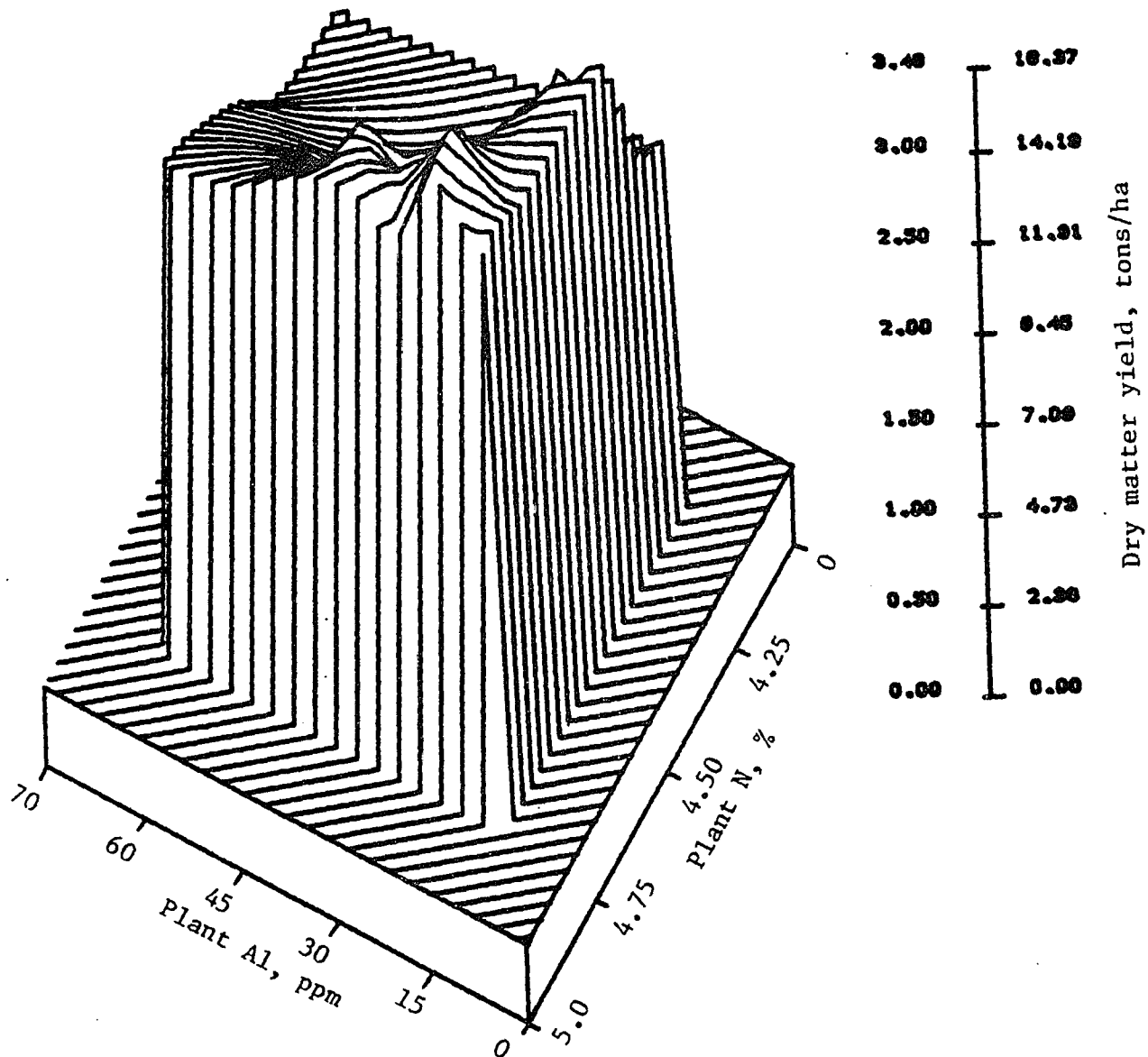


Figure 13. Contour surface of alfalfa yield for 1981 as a function of plant N and Al. Viewing angle: azimuth 60° , elevation 30° .

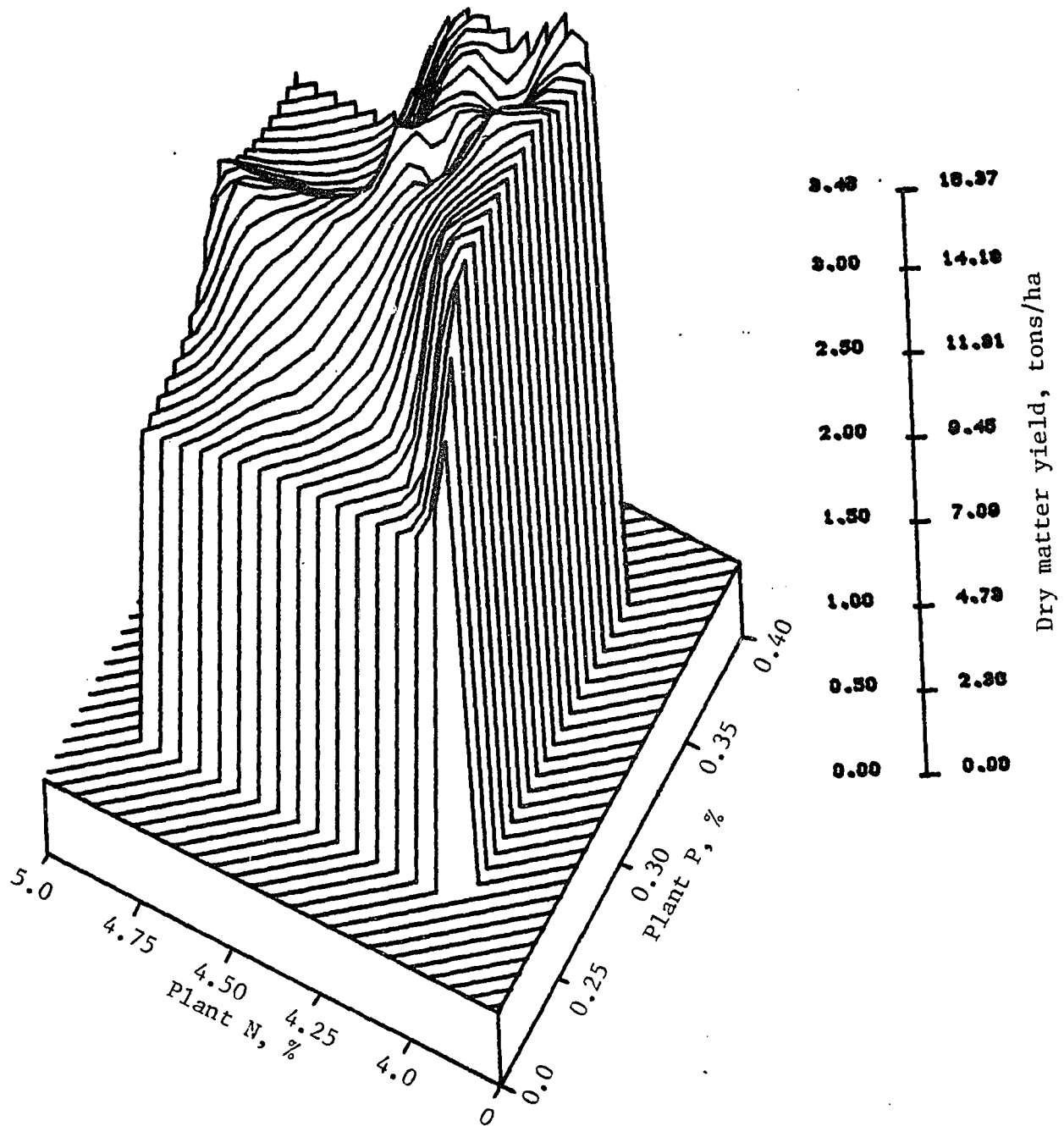


Figure 14. Contour surface of alfalfa yield for 1981 as a function of plant P and N. Viewing angle: azimuth 60° , elevation 30° .

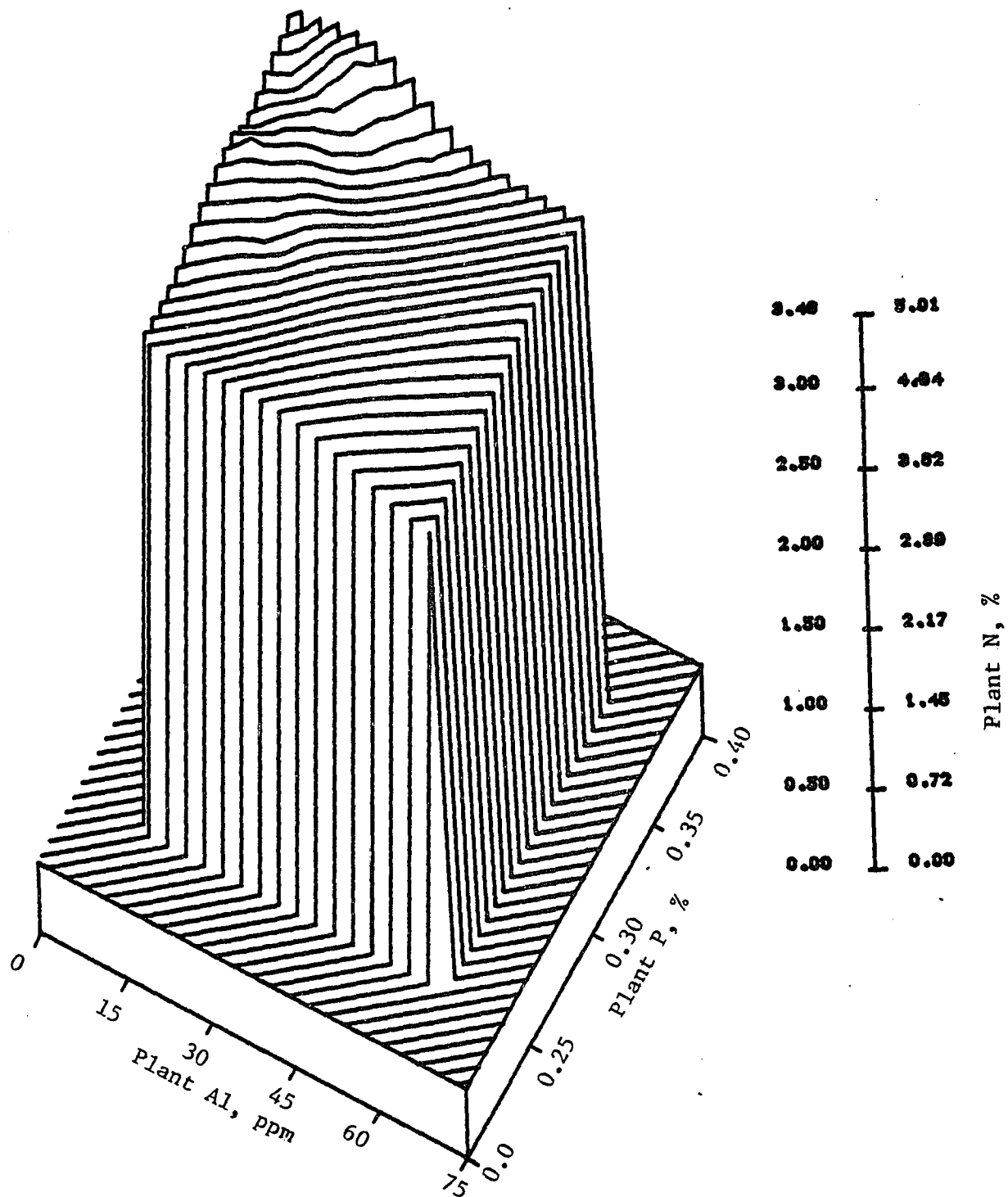


Figure 15. Contour surface of plant N for 1981 as a function of plant P and Al. Viewing angle: azimuth 60° , elevation 30° .

accumulation.

Experiment 2: Influence of Lime and Phosphorus on Alfalfa Growth:

This experiment was designed to study the effects of lime and phosphate applications on alfalfa persistence.

Data on dry matter yields as well as on nitrogen fixation, soil and plant analysis for 1979 have been reported by Roberts (1980). Based on results from a single year, he concluded that lime application did not significantly increase yield or N fixation and that addition of banded P eliminated the lime response in alfalfa. Application of 106 kg P/ha banded produced significantly higher yields than the addition of 194 kg P/ha broadcast in the unlimed and hydrated plots.

There was no significant increase in dry matter yield from liming in 1980. The dry matter yields were generally low with the highest yield of 2.75 t/ha being obtained with an addition of 2.3 t/ha hydrated lime; the yield for the control was 2.3 t/ha. There was also no yield benefit obtained from application of P although the highest yield of 3.0 t/ha was found when both banded and broadcast P were applied at 53 and 97 kg/ha, respectively.

In 1981, the crop growth was so poor no harvests were made. Most of the experimental plots had a ground cover of only 40 % alfalfa; the remaining percentage consisted of 20 % grassy weeds and 40 % bare areas.

The information in Tables 38 and 39 has been extracted systematically from the general data of 1979 and 1980 in order to show the relative effectiveness of lime and P applications with time. There was a slight yield increase over the control with the application of lime in 1979. In contrast to the yields of 1979, the yields of 1980 were extremely low. The sharp yield decline for 1980 may be

Table 38. Main effects of lime on dry matter yield and soil-plant characteristics of 1979 and 1981 for the Demerit Experiment.

Year	Lime treatment	Yield	Soil						Plant				
			pH	P	Al	Ca	Mg	K	P	Ca	Mg	K	Al
	t/ha	t/ha	ppm		-----	meq/100 g	-----		-----	%	-----	ppm	
<u>1979</u>	0	5.8	5.6	14	0.14	2.0	1.0	1.0	0.32	1.04	0.22	2.4	112
	Hydrated 2.3	6.2	5.8	16	0.09	2.5	0.8	1.1	0.30	1.49	0.22	2.5	101
	Dolomitic 13.8	6.8	6.0	14	0.05	2.6	1.2	1.1	0.32	1.03	0.26	2.5	101
<u>1980</u>	0	2.3	5.5	32	0.02	2.0	1.1	0.5	0.28	0.87	0.26	1.7	167
	Hydrated 2.3	2.8	5.7	34	0.01	2.6	1.4	0.5	0.28	1.08	0.28	1.9	283
	Dolomitic 13.8	2.3	6.1	32	0.02	2.0	1.2	0.5	0.27	0.89	0.24	1.7	267

Table 39. Main effects of phosphate fertilizer on dry matter yield and soil-plant characteristics of 1979 and 1980 for the Demerit Experiment.

Year	Phosphate treatment	Yield	Soil					Plant				
			pH	P	Al	Ca	Mg	P	Ca	Mg	K	Al
		t/ha		ppm	-- meq/100 g --		----- % -----			ppm		
<u>1979</u>												
	Banded, 106 kg P/ha	6.3	5.8	59	0.06	3.4	2.0	0.28	1.2	0.23	2.5	92
	Broadcast, 194 kg P/ha	5.6	6.0	94	0.04	3.0	1.2	0.31	1.2	0.23	2.5	106
	Banded broadcast 106 kg P/ha 194 kg P/ha	6.7	6.3	76	0.02	4.2	1.4	0.32	1.2	0.24	2.5	115
<u>1980</u>												
	Banded, 106 kg P/ha	2.5	6.1	29	0.02	2.1	1.1	0.28	0.92	0.24	1.6	250
	Broadcast, 194 kg P/ha	2.0	6.1	37	0.02	2.2	1.3	0.27	0.99	0.29	1.8	229
	Banded broadcast 106 kg P/ha 194 kg P/ha	2.3	6.2	31	0.02	2.3	1.3	0.27	0.93	0.24	1.8	238

partly related to the relatively lower plant P and higher plant Al concentrations than in 1979. The possible interference of Al in the metabolism of P cannot be ruled out. No obvious explanation of the increased uptake of Al with liming can be given based on the existing data. Jackson (1967) has cited instances wherein the Al content in the foliage was actually increased with the addition of lime or P, but did not discuss the cause for this occurrence.

The low level of exchangeable Al (less than 0.15 meq/100 g throughout the 30 cm soil profile) in the soil suggests that any advantage from liming must be related to increased availability of P or Ca rather than to the neutralization of acidity. Total exchangeable Al was only 0.76 meq/100 g; these values are very low compared to those reported for field Experiment 1. Particularly noteworthy is the fact that the uptake of Ca and P was not significantly increased with the application of lime or P. The average levels of Ca, P and K for the alfalfa plants in 1980 were much lower than those of 1979 and those plants in field Experiment 1. This may suggest that the supplies of Ca, P and K need to be improved substantially in order for the crop to sustain higher yields. The level of soil P for 1980 was less than 40 ppm while exchangeable K was below 1.0 meq/100 g.

The results from this experiment do not rule out the possibility that the low availability of nutrients caused part of the yield decline, nor do they exclude the possibility that other non-nutritional factors may have caused the poor crop persistence. This uncertainty arises because of the lack of information on factors such as soil aeration, soil temperature and soil water availability for soil organisms and plants. Poor root penetration into the soil due to unfavorable soil environment could limit the productivity of alfalfa. Also winter kill and desiccation may have partly contributed to the crop failure.

Experiment 3: Influence of Lime on Chemical Changes in the Soil Profile.

The purpose of this experiment was to examine the effects of lime on nutrient changes in the plow layer.

The data in Table 40 show that there was a significant increase in soil pH, Ca, Mg and CEC one year after lime application. Soil P was markedly reduced through liming while exchangeable K and Al remained unchanged. The decrease in soil P due to liming is in agreement with an earlier finding in greenhouse Experiment 4.

The relations between lime and the various soil characteristics as shown in Figures 16 and 17 demonstrate clearly that surface applied lime is most effective in raising soil pH in the top 10 cm of the soil profile. The greatest increase in soil pH and Mg at the 10 cm depth was observed with the initial lime rate. For tilled soils, it has been generally reported that surface applied lime decreased acidity of soils in the plow layer. However, with no-till culture, Brown and Munsell (1938) showed that 10 to 14 years were required for surface applied lime to increase the soil pH to a 15 cm depth. In the present study, soil pH increases from liming were most noticeable at the 0-10 cm depth one year after liming. Soil Ca and Mg increases from liming were evident within a 20 cm depth only; decreases in soil P were observed to the 30 cm depth. The penetrative effects of lime will, of course, vary with the exchange capacity of soils, type and particle size of lime, and also with the temperature and rainfall.

Since no satisfactory yield harvest was possible, the tissue composition of alfalfa plants was used as a biological assay to determine reasons for the poor crop response to liming. Application of lime improved the uptake of Na and decreased that of K but did not influence the uptake of the other plant nutrients (Table 41). The failure

Table 40. Soil analysis of the Woodward Experiment for July 1981. Each value is the mean of three replications.

Soil depth	Lime rate	pH	P	Ca	Mg	K	Al	CEC
cm	t/ha		ppm	----- meq/100 g -----				
10	0	5.7 a ¹	74 b	3.92 a	1.36 a	0.26 a	0.04 a	5.58 a
	4.6	6.5 b	47 a	4.42 b	2.00 b	0.23 a	0.03 a	6.68 b
	9.2	6.7 b	46 a	4.59 b	2.11 b	0.34 a	0.03 a	7.07 b
	18.4	6.7 b	31 a	5.25 c	2.53 b	0.51 a	0.02 a	8.31 c
20	0	5.5 a	56 a	3.00 a	1.00 a	0.38 a	0.03 a	4.41 a
	4.6	6.2 b	46 a	2.92 a	1.00 a	0.56 a	0.04 a	4.52 a
	9.2	6.2 b	42 a	3.75 b	1.39 b	0.38 a	0.05 a	5.19 b
	18.4	6.3 b	34 a	4.17 c	1.75 b	0.56 a	0.07 a	6.55 c
30	0	5.4 a	87 c	3.25 a	0.86 a	0.47 a	0.03 a	4.61 a
	4.6	6.2 b	64 b	3.34 a	0.94 a	0.34 a	0.03 a	4.65 a
	9.2	6.1 b	66 b	3.34 a	0.92 a	0.51 a	0.03 a	4.80 a
	18.4	6.1 b	45 a	3.00 a	0.94 a	0.51 a	0.02 a	4.47 a

¹ Means for each soil depth within a column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

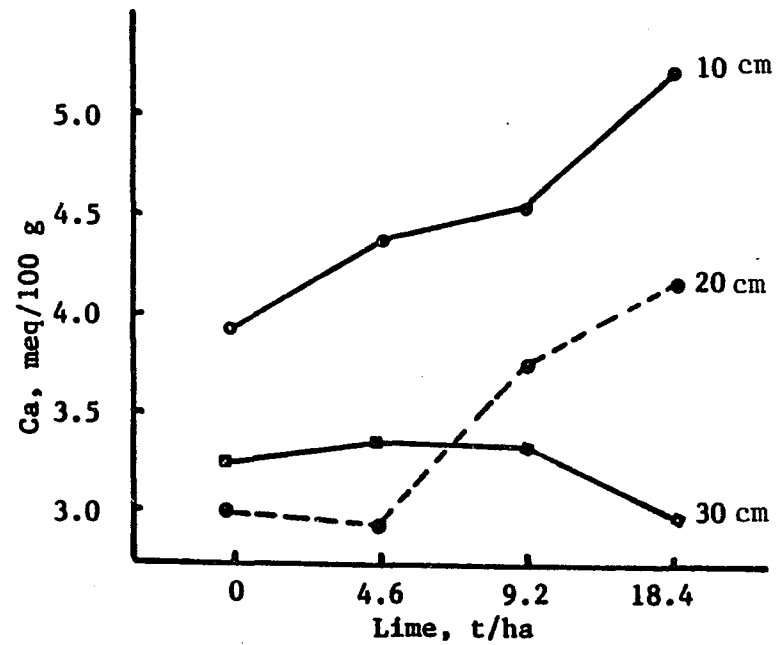
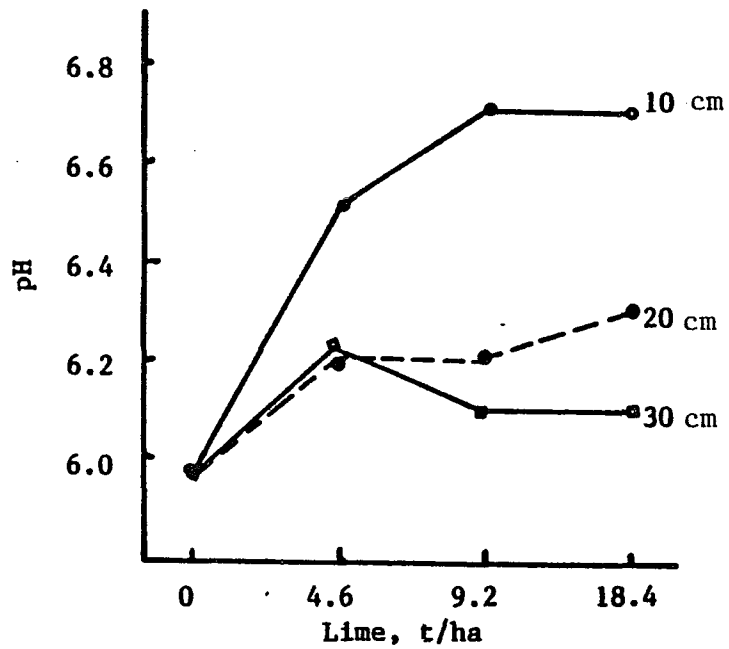


Figure 16. Relationships between lime rate, soil pH, and exchangeable Ca at three soil depths for 1981 in field Experiment 3. Each data point represents an average of three values.

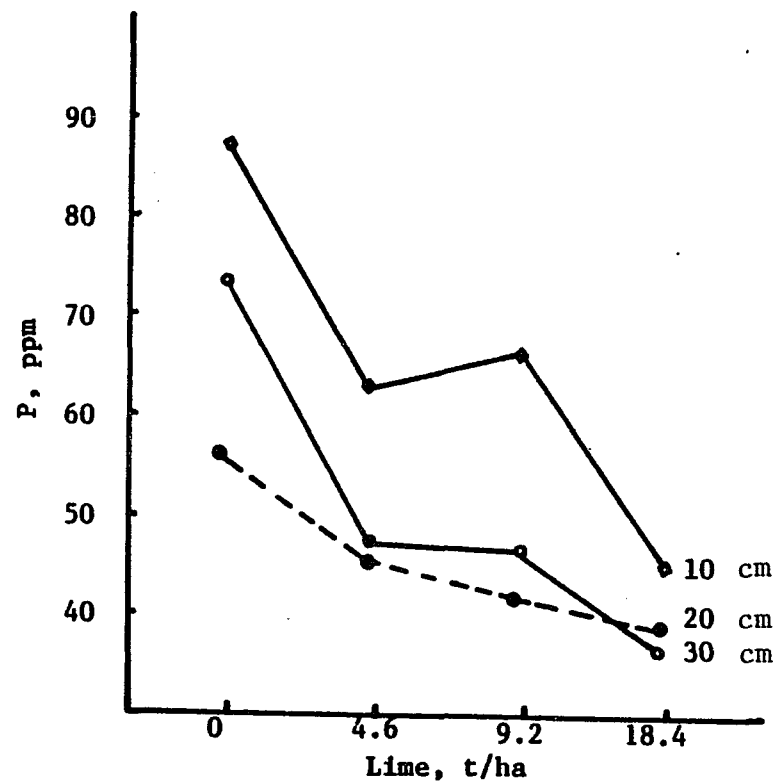
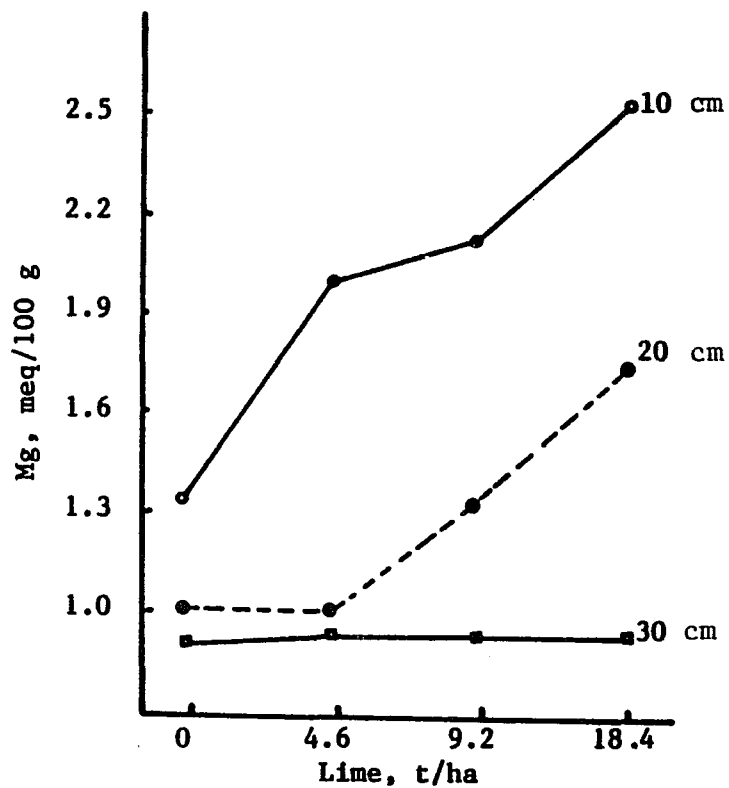


Figure 17. Relationships between lime rate, soil Mg, and P at three soil depths for 1981 in field Experiment 3. Each data point represents an average of three values.

of lime to increase the uptake of Ca is probably due to the initially high plant Ca (1.40 %) for the control plots. It is interesting to note that many researchers have already shown significant interactions between plant K and Na. Rain (1972) and Osmond (1968) observed a higher affinity for Na than for K in soil solutions. Sodium is one of the most loosely held of the metallic ions and is readily absorbed as Na^+ ion by plants. This may suggest that K^+ in solution is replaced by the Na^+ from soil colloids as dictated by the lyotropic series (Bohn, et al., 1979). Under the present investigation, presumably, Na was absorbed selectively in preference to K. According to La Haye and Epstein (1971), plants with poor root systems, as a result of inadequate Ca, transfer large quantities of Na into the tops. DeKock (1963) had also indicated that a high K/Ca ratio is characteristic of metal toxicity in alfalfa. This is unlikely to be the case in the present study since the Ca levels (1.40 %) in the plants were adequate.

The results reported in this section confirm that liming has significant influence on the availability and uptake of plant nutrients. These effects were still evident in the top 30 cm of the soil profile as well as on herbage composition one year after the application of lime. Particularly noteworthy is the fact that liming enhanced the interference of plant Na in the uptake of K.

Table 41. The effect of lime on tissue composition of alfalfa for the Woodward Experiment in August 1981. Each value is a mean of three replications.

Plant nutrient	Lime rate, t/ha			
	0	4.6	9.2	18.4
N %	3.74 a ¹	3.76 a	3.65 a	3.62 a
P %	0.29 a	0.30 a	0.29 a	0.30 a
Ca %	1.40 a	1.37 a	1.39 a	1.42 a
Mg %	0.29 a	0.32 a	0.30 a	0.36 a
K %	1.43 b	1.42 b	1.18 a	1.08 a
Al ppm	9 a	7 a	6 a	8 a
Mn ppm	39 a	30 a	26 a	30 a
Fe ppm	59 a	57 a	53 a	56 a
Zn ppm	25 a	24 a	23 a	25 a
B ppm	39 a	36 a	31 a	32 a
Cu ppm	7 a	7 a	8 a	8 a
Na ppm	149 a	167 b	154 ab	185 c

¹ Means within each plant nutrient followed by the same letter(s) do not differ significantly at the 5% level of probability according to Duncan's New Multiple Range Test.

SUMMARY AND CONCLUSIONS

Greenhouse and field studies were conducted to establish the relationship between soil chemical changes and performance of alfalfa; emphasis was on soil pH, available P and exchangeable Al.

Aluminum in the exchangeable form is a major source of acidity in the mineral soils having pH values of less than about 5.5. Results from the greenhouse studies showed that measurable quantities of exchangeable Al were not detected in any soil having a pH value greater than 5.8. Conversely, large amounts of exchangeable Al were present in soils having pH values less than 5.0. Increases in soil pH and Ca due to liming were usually accompanied by a significant reduction in the content and percent saturation of soil Al.

From the standpoint of P availability, there seems to be no advantage to liming in excess of about pH 5.8. High rates of lime appear to cause P fixation, thus reducing its availability to plants. It is reasonable to conclude, therefore, that exchangeable Al was a better indicator of P availability and crop response to lime application than soil pH. Differential pH changes due to liming are useful in explaining the differences in the degree of neutralization of soil Al by liming. For practical purposes, soils should be limed when they contain higher than 10 % Al saturation or greater than 0.30 meq/100 g of exchangeable Al. Lime rates higher than the equivalent amount required to neutralize 150 % of the exchangeable Al showed only small yield increases over that required to neutralize 100 % of the exchangeable Al in the soil complex.

The relative concentrations of plant Al and P as measured by EDS indicated that liming tended to decrease the Al/P ratio within plant tissues. This relationship was not, however, consistent with all the cultivars tested.

Exchangeable Al and soil pH accounted for 48 and 45 %, respectively,

respectively, of the yield variations in field Experiment 1. A regression model including both exchangeable Al and soil pH explained 51 % of the yield variation in alfalfa. For alfalfa, the acidity in the top 10 cm soil surface appeared to be more important than that of the subsoils once the crop is well established.

Simple regression models including plant P, plant Al or plant Mn were able to account for about 80 % of the yield variation in alfalfa. Plant Al or plant Mn per se are not, generally, considered good predictors of crop response. They are, however, important parameters to consider since both Al and Mn can interfere in the metabolic use of P by plants. Significant correlations were obtained between yield and plant N, plant Mg and plant P.

Lime accounted for a significant portion of the alfalfa yield variations in 1976, 1980 and 1981. A quadratic response curve gave the best fit for the relation between lime and the average yield for six years. The optimum alfalfa dry matter yield was realized with a herbage composition of 0.33 % P, 1.0 % Ca, 0.33 % Mg and 2.4 % K at the lime rate of 3.5 t/ha.

The crop failure at the Demerit and Woodward farms was attributed mainly to unfavorable environmental conditions. Because of the poor alfalfa establishment at these two sites, the effect of lime application was less evident.

In conclusion, it can be stated that the persistence of alfalfa is dependent on the maintenance of an adequate supply of soil P as well as a low level of exchangeable Al. Soil pH of 5.7 and Al saturation of less than 5 % were found to enhance the uptake of P and thus improve nitrogen fixation and yield of alfalfa.

LITERATURE CITED

- Adams, F., and Z.F. Lund. 1966. Effect of chemical activity of soil solution aluminum on cotton root penetration of acid subsoils. *Soil Sci.* 101:193-198.
- Alexander, M. 1976. Introduction to soil microbiology. 2nd ed. John Wiley and Sons, New York.
- Ali, S.M.E. 1973. Influence of cations on aluminum toxicity in wheat. Ph.D. Thesis, Oregon State University, Corvallis.
- Ali-abbas, H.A. and S.A. Barber. 1964. A soil test for phosphorus based upon fractionation of soil P. *Soil Sci. Soc. Amer. Proc.* 28:218-221.
- Alley, M.M. 1980. Initial soil chemical and crop yield responses to lime application. Virginia Polytechnic Institute and State University. *Agronomy Abstract.* 1980. p. 164.
- Allison, F.E. 1935. Carbohydrate supply as a primary factor in legume symbiosis. *Soil Sci.* 39:123-143.
- Andrews, C.S. 1977. Legumes and soil acidity. pp. 135-160. In J. Dobereiner, et al (ed.) *Limitations and potentials for biological nitrogen fixation in the tropics.* Plenum Press, New York.
- , and B. Johnson. 1976. Effect of calcium, pH and nitrogen on the growth and chemical composition of some tropical and temperate legumes. *Aust. J. Agric. Res.* 27:345-356.
- , and M.F. Robins. 1969. The effect of phosphorus on the growth and chemical composition of some tropical pasture legumes. *Aust. J. Agric. Res.* 20:665-674.
- Armiger, W.H., C.D. Foy, A.L. Fleming, and B.E. Caldwell. 1968. Differential tolerance of soybean varieties to acid high in exchangeable aluminum. *Agron. J.* 60:67-70.
- Atkinson, D. 1973. Some general effects of P deficiency on growth and development. *Phytol.* 72:101-111.
- Baker, D.E. 1964. A study of isotopic dilution as a method of relating phosphorus retention to availability of phosphorus in widely different soils. *Soil Sci. Soc. Am. Proc.* 28:511-517.
- . 1976. Acid soils. pp. 127-150. In M.J. Wright (ed.) *Plant adaptation to mineral stress in problem soils.*
- , and J.K. Hall. 1967. Measurement of P availability in acid soils of Pennsylvania. *Soil Sci. Soc. Amer. Proc.* 31:662-667.
- , and C.M. Woodruff. 1963. Influence of volume of soil per plant upon growth and uptake of phosphorus by corn from soil treated with different amounts of phosphorus. *Soil Sci.* 94:409-412.

- Barber, S.A. 1974. Influence of plant roots on ion movement in soil. pp. 525-564. In E.W. Carson (ed.). The plant root and its environment. Univ. of Va. Press, Charlottesville.
- . 1980. Nutrient flux at the soil-root interface. 3:45-60. 11th Congress Int. Soc. Soil Sci., Edmonton, Canada.
- . 1980. Soil-plant interactions in the P nutrition of plants. In F.E. Khasawneh and E.C. Sample (ed.). The role of P in agriculture. Am. Soc. of Agron., Madison, WI.
- Barley, K.P. 1970. The configuration of the root system in relation to nutrient uptake. Adv. Agron. 22:159-210.
- Bethlenfalvay, G.B. and D. Phillips. 1977. Effect of light intensity on efficiency of CO₂ and N₂ reduction in Pisum sativum. Plant Physiol. 60:86-871.
- Beyers, G.I. and D.L. Goodrich. 1977. Selected Climates of New Hampshire. Research report number 60. October 1977. New Hampshire Agric. Exp. Sta., Durham, New Hampshire.
- Bishop, W.D. and S.A. Barber. 1958. The effect of soil phosphorus compounds on soil test correlations. Soil Sci. Soc. Amer. Proc. 22:435-439.
- Black, C.A. 1967. Soil-Plant Relationships. 2nd edition. John Wiley and Sons, New York.
- Bohn, H., B. McNeal and G. O'Connor. 1979. Soil Chemistry. John Wiley and Sons, New York.
- Brady, N.C. 1974. The Nature and Properties of Soils. 8th edition. MacMillan Pub. Co. Inc., New York.
- Bray, R.H. and L.T. Kurtz. 1945. Determination of total organic and available forms of phosphorus in soils. Soil Sci. Soc. Amer. Proc. 59:39-45.
- Brown, B.A. and R.I. Munsell. 1938. Soil acidity at various depths as influenced by time since application, placement, and amount of limestone. Soil Sci. Soc. Am. Proc. 3:217-221.
- , R.F. Holt, and A.V. King. 1956. Soil reactions at various depths as influenced by time since application and amount of limestone. Soil Sci. Soc. Amer. Proc. 20:518-522.
- Brown, J.C. 1967. Differential uptake of Fe and Ca by two corn genotypes. Soil Sci. 103:331-338.
- Burton, J.C. 1972. Nodulation and symbiotic nitrogen fixation. In C.H. Hanson (ed.). Alfalfa Science and Technology. Agronomy. 15:229-246. Am. Soc. of Agron., Madison, WI.
- Buss, G.R., J.A. Lutz and G.W. Hawkins. 1975. Yield response of alfalfa cultivars and clones to several pH levels in Tatum Subsoil. Agron. J. 67:331-334.
- Cassman, K.G., D.N. Munns, and D. Beck. 1981. Growth of Rhizobium strains at low concentrations of P. Soil Sci. Soc. Am. J. 45:520-523.

- Chang, S.C. and M.L. Jackson. 1957. Fractionation of Soil phosphorus. *Soil Sci.* 84:133-144.
- Chapman, H.D., and P.F. Pratl. 1961. Methods of soil, plant and water analysis. University of California Div. of Agric. Sci.
- Chapman, M.D. 1965. Foliar sampling for determining the nutrient status of crops. *World Crops.* 16:36-46.
- Chino, M. and A. Baba. 1976. Electron microprobe analysis of zinc and other elements within and around rice root growth in flooded soil. *Soil Sci. Plant Nutr.* 22(4):445-457.
- Clarkson, D.T. 1965. The effect of aluminum and some trivalent metal cations on cell division in root apices of Allium cepa. *Ann. Bot.* 29:309-315.
- _____. 1967. Interactions between Al and phosphorus on root surfaces and wall material. *Plant and Soil.* 27(3):346-356.
- _____. 1968. Metabolic aspects of aluminum toxicity and some possible mechanisms for resistance. *Brit. Ecol. Soc. Symp.* 9:381-397.
- _____, J. Sanderson and R.S. Russell. 1968. Ion uptake and root age. *Nature.* 220:805-806.
- Cole, C.V., and S.R. Olsen. 1959. Phosphorus solubility in calcareous soils. *Soil Sci. Soc. Amer. Proc.* 23:119-121.
- Coleman, N.T., E.G. Kamprath and S.B. Weed. 1958. Liming. *Advances in Agronomy.* 10:475-522.
- _____, and G.W. Thomas. 1967. The basic chemistry of soil acidity. pp. 1-41. In R.W. Pearson and F. Adams (ed.). Soil Acidity and Liming. Agronomy. No. 12.
- Dazzo, F.B., C. Napoli and D.N. Hubbel. 1976. Adsorption of bacteria to roots as related to host specificity in Rhizobium-clover symbiosis. *Appl. Environ. Microbiol.* 32:166-171.
- De Kock, P.C. 1963. The physiological significance of the K/Ca relationship in plant growth. *Outlook on Agr.* 4:93-98.
- De Mooy, C.J. and J. Pesek. 1966. Nodulation responses of soybeans to added P, K and Ca salts. *Agron. J.* 58: 275-280.
- _____. 1965. Ph.D. Thesis, Iowa State University.
- Dessureaux, L. 1969. Effect of Al on alfalfa seedlings. *Plant and soil* 30:93-97.
- Devine, T.E., C.D. Foy, A.L. Fleming, C.H. Hanson, T.A. Campbell, J.E. McMurtrey III and J.W. Schwartz. 1976. Development of alfalfa strains with differential tolerance to aluminum toxicity. *Plant and Soil.* 44:73-79.
- Dobereiner, J. 1966. Manganese toxicity effects on nodulation and N fixation of beans in acid soils. *Plant and Soil.* 24:153-166.

- Doss, B.D., and Z.F. Lund. 1975. Subsoil pH effects on growth and yield of cotton. *Agron. J.* 67:193-196.
- Dukigg, P., B. Melton and A. Baltensperger. 1978. Selection for acetylene reduction rates in alfalfa. *Crop Sci.* 18:813-816.
- Ecklin, P. and A.J. Saubermann. 1977. Preparation of biological specimens for X-ray microanalysis, SEM/1977/I, IIT Research Institute, Chicago, Il. 60616, 621-637.
- Epstein, E. 1972. Mineral Nutrition of Plants. Principles and Perspectives. John Wiley and Sons, Inc. New York.
- Evans, H.J. and S.A. Russell. 1971. Physiological chemistry of symbiotic nitrogen fixation by legumes. pp. 191-244. In J.R. Postgate (ed.). The chemistry and biochemistry of nitrogen fixation. Plenum Publishing Corporation, New York.
- Fletcher, M.F. and L.T. Kurtz. 1964. Differential effects of phosphorus fertility on soybean varieties. *Soil Sci. Soc. Amer. Proc.* 28:225-258.
- Fox, R.H. 1979. Soil pH, aluminum saturation and corn grain yield. *Soil Sci.* 127:330-334.
- Fox, R.L. 1979. Comparative responses of field grown crops to P concentrations in soil solutions. pp. 81-106. In H. Mussell and R.C. Stapels (ed.). Stress physiology in crop plants.
- Foy, C.D. 1974. Effect of aluminum on plant growth. pp. 601-642. In E.W. Carson (ed). The plant root and its environment. Univ. Press of Virginia, Charlottesville.
- _____, and J.C. Brown. 1964. Toxic factors in acid soils. *Soil Sci. Soc. Am. Proc.* 28:27-32.
- _____, R.L. Chaney and M.C. White. 1978. The physiology of metal toxicity in plants. *Ann. Rev. Plant Physiol.* 29:511-566.
- _____, A.L. Fleming, and W.H. Armiger. 1969. Aluminum tolerance of soybean varieties in relation to calcium nutrition. *Agron. J.* 61:505-511.
- _____, A.L. Fleming, G.R. Burns, and W.H. Armiger. 1967. Characterization of differential aluminum tolerance among varieties of wheat and barley. *Agron. J.* 31:513-521.
- Franklin, W.T. and H.M. Reisenhauer. 1960. Chemical characteristics of soil related to phosphorus fixation and availability. *Soil Sci.* 90:192-200.
- Gamble, J.F. 1954. An introductory study of the clay minerals in New Hampshire soils. M.S. Thesis, University of New Hampshire, Durham, New Hampshire.
- Gardner, B.R. and P.J. Jones. 1973. Effects of temperature on phosphate sorption isotherms and phosphate desorption. *Comm. Soil Sci. Plant Anal.* 4(2):83-93.
- Gates, C.T. 1974. Nodule and plant development in *S. humilis*. Symbiotic response to P and S. *Aust. J. Bot.* 22:45-55.

- Ghani, M.O. and M.A. Islam. 1946. Phosphate fixation in acid soils and its mechanism. *Soil Sci.* 62:293-306.
- Gibson, A.H. 1962. Genetic variation in the effectiveness of nodulation of lucerne varieties. *Aust. J. Agric. Res.* 19:388-399.
- . 1967. Carbon dioxide limitations of plant growth in tube culture, with special reference to legume-nodulation studies. *Aust. J. Biol. Sci.* 20:837-842.
- . 1977. The influence of the environmental and managerial practices on the legume - Rhizobium symbiosis. pp. 393-450. In R.W.F. Hardy and A.H. Gibson (ed.). *Treatise on dinitrogen fixation. Section IV Ecology and Agronomy.* John Wiley and Sons, New York.
- Gladstone, J. and J.F. Loneragan. 1970. Nutrient elements in herbage plants in relation to soil adaptation and animal nutrition. *In Agron. J.* 73(4):629-634.
- Godó, G.H. and H.M. Reisenauer. 1980. Plant effects on soil manganese availability. *Soil Sci. Soc. Am. J.* 44(5):993-995.
- Graham, P.M. and Rosas, J.C. 1979. Phosphorus fertilization and symbiotic nitrogen fixation in common bean. *Agron. J.* 71(6):925-926.
- Grigg, J.L. 1968. Prediction of plant response to fertilizers by means of soil tests. *N.Z.J. Agr. Res.* 11:345-355.
- Grime, J.P. and Hodgson, J.G. 1969. An investigation of the ecological significance of lime-chlorosis by means of large-scale comparative experiments. pp. 67-99. In I.H. Rorison, ed. *Ecological Aspects of the Mineral Nutrition of Plants.* Symp. British Eco. Soc. Sheffield.
- Hall, S.M. and D.A. Baker. 1972. The chemical composition of *Ricinus* phloem exudate. *Planta.* 106:131-140.
- Hardy, R.W.F., and V.D. Havelka. 1976. Photosynthate as a major factor limiting nitrogen fixation by field grown legumes with special emphasis on soybeans. pp. 421-437. In R.S. Nutman (ed). *Symbiotic nitrogen fixation in plants.* Cambridge University Press.
- , R. D. Holsten, E. K. Jackson and R. C. Burns. 1968. The acetylene-ethylene assay for N fixation: Laboratory and field evaluation. *Plant Physiol.* 43:1185-1207.
- Harter, R.D. 1969. Phosphorus adsorption sites in soils. *Soil Sci. Soc. Am. Proc.* 33:630-631.
- Heltz, G.E. and A.L. Whiting. 1928. Effect of fertilizer treatment on the formation of nodules in soybean. *J. Am. Soc. Agron.* 20:975-981.
- Hiat, A.J. 1967. Relationship of cell sap pH to organic acid change during ion uptake. *Plant Physiol.* 42(2):294-298.
- Horwitz, W. 1975. *Methods of Analysis of the Association of Official Analytical Chemists.* 12th edition. George Banta Company, Inc., Menasha, Wisconsin.

- Hossner, L.R., I.A. Freeouf and B.L. Polsom. 1973. Solution phosphorus concentration and growth of rice in flooded soils. *Proc. Soil Sci. Soc. Amer.* 37:405-408.
- Hsu, P.H. 1964. Adsorption of phosphate by aluminum and iron in soils. *Soil Sci. Soc. Am. Proc.* 28:474-478.
- Hutchinson, G.E. 1945. Aluminum in soils, plants and animals. *Soil Sci.* 60:29-40.
- Jackson, M.L. 1958. *Soil Chemical Analysis*. Prentice-Hall, Englewood Cliffs, N.J.
- . 1963. Aluminum bonding in soils. *Soil Sci. Soc. Amer. Proc.* 27:1-10.
- Jackson, W.A. 1967. Physiological effects of soil acidity. pp. 43-124. In R.W. Pearson and F. Adams: *Soil acidity and liming*. *Am. Soc. Agron. No. 12*.
- Jackson, P.C. and C.E. Hagen. 1960. Products of orthophosphate absorption by barley roots. *Plant Physiol.* 35:326-332.
- John, M.K., A.L. van Ryswyk and J.L. Mason. 1967. Effect of soil order, pH, texture, and organic matter on the correlation between phosphorus in alfalfa and soil test values. *J. Can. Soil Sci.* 47:157-161.
- Johnson, R.E. and W.A. Jackson. 1964. Calcium uptake and transport by wheat seedlings as affected by aluminum. *Soil Sci. Soc. Am. Proc.* 28:381-386.
- Jones, C.D., J.A. Lutz, and T.J. Smith. 1977. Effects of phosphorus and potassium on soybean nodules and seed yield. *Agron. J.* 69:1003-1007.
- Jones, L.H. 1961. Aluminum uptake and toxicity in plants. *Plant and Soil.* 13:297-310.
- Kamprath, E.J. 1970. Exchangeable Al as a criterion for liming leached mineral soils. *Soil Sci. Soc. Am. Proc.* 34:252-254.
- . 1973. Soil acidity and liming. pp. 126-128. In P.A. Sanchez (ed.) *A Review of soils Research in Tropical Latin America*. North Carolina Agri. Exp. Sta. Tech. Bull. 219.
- , and C.D. Foy. 1971. Lime-fertilizer-plant interactions in acid soils. pp. 105-151. In R.A. Olson. *Fertilizer Technology and Use*. 2nd ed. Soil Sci. Soc. Am., Madison.
- Keng, J.C.W. and G. Uehara. 1974. Chemistry, mineralogy and taxonomy of Oxisols and Ultisols. *Soil Crop. Sci. Soc. Fla. Prac.* 33:119-126.
- Kerridge, P.C., M.C. Dawson and D.P. Moore. 1971. Separation of degrees of Al tolerance in wheat. *Agron. J.* 63(4):586-591.
- Kitamura, Y. and S. Nishimura. 1977. Studies on mixed cultivation of tropical grass and legume. *J. Japan Grassl. Sci.* 23:152-158.
- Konzak, C.F., E. Polle and J.A. Kittrick. 1976. Screening several crops for aluminum tolerance. pp. 311-326. M.J. Wright. *Plant Adaptation to Mineral Stress in Problem Soils*. Cornell University, Ithaca, New York.

- La Haye, P.A. and E. Epstein. 1971. Calcium and salt tolerance by bean plants. *Physiol. Plant.* 25:213-218.
- Lanyon, L.E., B. Naghshineh-Pour, and E.D. McLean. 1977. Effects of pH level on yields and composition of pearl millet and alfalfa in soils with different degrees of weathering. *Soil Sci. Soc. Amer. Proc.* 41:389-394.
- Lawn, R.J. and W.A. Brun. 1974. Symbiotic nitrogen fixation in soybean. *Crop Sci.* 14:11-16.
- Lie, T.A. 1974. Environmental effects on nodulation and symbiotic N fixation. pp. 555-582. In A. Quispel (ed.) *The biology of N fixation.* North Holland Publishing Co., Amsterdam.
- Long, F.L. and C.D. Foy. 1970. Plant varieties as indicators of aluminum toxicity in the A horizon of a Norfolk soil. *Agron. J.* 62:679-681.
- Lunt, H.A., C.L.W. Swanson and H.G.M. Jacobson. 1950. The Morgan Soil Testing System. Bull. 541. Conn. Agric. Exp. Sta., New Haven, Connecticut.
- Lunt, O.R. and A.M. Kofranek. 1970. Manganese and aluminum tolerance of Azalea. pp. 559-573. In R.M. Samish (ed.) *Recent Advances in Plant Nutrition.* New York, Gordon and Breach.
- MacLeod, L.B. and L.P. Jackson. 1965. Effect of concentration of the Al ion on root development and establishment of legume seedlings. *Can. J. Soil Sci.* 45:221-234.
- . 1967. Aluminum tolerance of two barley varieties in nutrient solution, peat, and soil culture. *Agron. J.* 59:359-363.
- Major, D.J., M.R. Hanna, S. Smoliak, and R. Grant. 1979. Estimating nodule activity of alfalfa seedlings. *Agron. J.* 11(6):983-985.
- Marion, G.M., D.M. Hendricks, G.R. Dutt and W.M. Fuller. 1976. Aluminum and silica solubility in soils. *Soil Sci.* 121(2):76-86.
- Martena, D.C., J.A. Lutz and G.D. Jones. 1969. Form and availability of phosphorus in selected Virginia soils as related to available phosphorus tests. *Agron. J.* 61:616-621.
- Martin, W.E. and J.E. Matocha. 1974. Plant analysis as an aid in fertilization of forage crops. pp. 393-426. In L.M. Walsh and J.D. Beaton. *Soil Testing and Plant Analysis.* Soil Sci. Soc. of America, Inc., Madison, Wisconsin.
- Matsumoto, H. and S. Morimura. 1980. Repressed template activity of chromation of pea roots created by aluminum. *Plant and Cell Physiol.* 21(6):951-959.
- McCormick, L.H. and F.Y. Borden. 1974. The occurrence of Al-PO₄ precipitate in plant roots. *Soil Sci. Soc. Amer. Proc.* 38:931-934.
- McLachlan, K.D. 1976. Comparative P responses in plants to a range of available P situations. *Aust. J. Agric. Res.* 27:323-341.

- McLean, E.O. 1973. Testing soils for pH and lime requirement. pp. 77-95. In L.M. Walsh and J.D. Beaton (ed.) Soil testing and plant analysis.
- . 1976. Chemistry of soil aluminum. Commun. in Soil science and Plant Analysis. 7(7):619-636.
- , and H. Ssali. 1977. Effects of Phosphate rate, lime and gypsum on yields of millet and alfalfa from highly weathered soils. Soil Science. 123(3):155-164.
- , M.R. Heddleson and G.I. Post. 1959. Aluminum in soils. III. A comparison of extraction methods for soils and clays. Soil Sci. Soc. Am. Proc. 23:289-293.
- Mendez, J. and E.J. Kamprath. 1978. Liming of Latosols and the effect on P response. Soil Sci. Soc. Amer. Proc. 42(1):86-88.
- Mengel, D.B. and E.J. Kamprath. 1978. Effect of soil pH and liming on growth and nodulation of soybeans in Histosols. Agron. J. 70:959-964.
- Mengel, K. and E.A. Kirby. 1978. Principles of Plant Nutrition. International Potash Institute, Switzerland.
- , H. Grimme and K. Nemeth. 1969. Potential and actual availability of plant nutrients in soils. Lander, Forsch. 23/I. Sondesh., 70-91.
- Metzzer, W.H. 1934. Distribution of fertilizer residues in the soil after fourteen years of a fertilizer experiment with alfalfa. J. Am. Soc. Agron. 26:620-625.
- Morard, P. 1970. Distribution of P, studied with the radioactive isotope and with colorimetry in buckwheat grown in nutrient solution. C. R. Acad. Sci. Ser. D. 270:2075-2077.
- Morgan, A.J., T.W. Davies, and D.A. Erasmus. 1975. Changes in the concentration and distribution of elements during electron microscope preparation procedures. Micron. 6:11-23.
- Morris, H.D. and W.H. Pierre. 1949. Minimum concentration of Mn necessary for injury to various legumes in culture solutions. Agron. J. 41:107-112.
- Moschler, W.W., G.D. Jones, and G.W. Thomas. 1960. Lime and soil acidity effects on alfalfa growth in a red yellow podzolic soil. Soil Sci. Soc. Amer. Proc. 24:507-509.
- Moustafa, E., R.M. Greenwood and M. Boland. 1971. Transport of phosphorus from leaves to legume root nodules. Plant and Soil. 35:651-653.
- Munns, D.N. 1965. Soil acidity and growth of a legume. II. Reactions of aluminum and phosphorus in solution and effects of aluminum, phosphate calcium and pH on Medicago Sativa L. and Trifolium Subterraneum L. in solution culture. Aust. J. Agri. Res. 16:743-755.
- . 1977. Mineral nutrition and the legume symbiosis. pp. 354-383. In R.W.F. Hardy and A.H. Gibson (ed.) Treatise on dinitrogen fixation. IV. Agronomy and Ecology. Wiley, N.Y.

- _____. 1979. Soil acidity and nodulation. pp. 247-264. In C.S. Andrew and E.J. Kamprath (ed.) Mineral nutrition of legumes in tropical and subtropical soils. CSIRO. Melbourne.
- _____, and R.L. Fox. 1976. Depression of legume growth by liming. Plant Soil. 45:701-705.
- Murrmann, R.P. and M. Peech. 1969. Relative significance of labile and crystalline phosphates in soil. Soil Sci. 107:249-255.
- Newman, E.I. 1966. Methods of estimating the total length of root in a sample. J. Appl. Ecol. 3:139-145.
- Newton, W.E., and W.H. Orme-Johnson. 1980. Nitrogen Fixation. Vol. 1 and 2. University Park Press. Baltimore.
- Olmos, I.L.J. and M.N. Camargo. 1976. Incidence of aluminum toxicity in Brazilian soils; its characteristics and distribution. Ciencia e cultura. 28:171-180.
- Olsen, S.R. and F.S. Watanabe. 1966. Effective volume of soil around plant roots determined from phosphorus diffusion. Soil Sci. Soc. Amer. Proc. 30:598-602.
- _____, and L.A. Dean. 1969. Phosphorus. pp. 1035-1048. In C.A. Black (ed.). Methods of soil analysis, Part II. Agronomy Monograph 9. Amer. Soc. Agron., Madison, Wisconsin.
- Olson, R.A. and O.P. Englestad. 1972. Soil phosphorus and Sulphur. pp. 82-101. In Soils of the Humid Tropics. National Academy of Sciences, Washington.
- Osmond, C.B. 1968. Ion absorption in Atriplex leaf tissue. Aust. J. Biol. Sci. 21:1119-11130.
- Ouellette, G.L. and L. Dessureaux. 1958. Chemical composition of alfalfa as related to degree of tolerance to Mn and Al. Can. J. Plant Sci. 38:206-214.
- Pearson, R.W. 1966. Soil environment and root development. pp. 95-126. In W.H. Pierre, D. Kerkham, J. Pesek, and R. Shaw (eds.). Plant environment and efficient water use. Am. Soc. of Agronomy, Madison, WI.
- _____, J. Childs and Z.F. Lund. 1973. Uniformity of limestone mixing in acid subsoil as a factor in cotton root penetration. Soil Sci. Soc. Am. Proc. 37:727-732.
- Pratt, P.F. and M.J. Garber. 1964. Correlation of phosphorus availability by chemical tests with inorganic phosphorus fractions. Soil Sci. Soc. Amer. Proc. 28:23-26.
- Racz, G.J. and R.J. Soper. 1967. Reaction product of orthophosphates in soils containing varying amounts of calcium and magnesium. Can. J. Soil Sci. 47(3): 223-230.
- Rains, D.W. 1972. Salt transport by plants in relation to salinity. Ann. Rev. Plant Physiol. 23:367-388.
- Reeve, N.G. and M.E. Sumner. 1970. Lime requirements of Natal Oxisols based on exchangeable aluminum. Soil Sci. Soc. Amer. Proc. 34:595-598.

- Reid, D.A., A.L. Fleming and C.D. Foy. 1971. A method for determining aluminum response of barley in nutrient solution in comparison to response in Al-toxic soil. *Agron. J.* 63:600-603.
- Rhue, R.D. 1979. Differential aluminum tolerance in crop plants. pp. 61-80. In H. Mussell and R.C. Staples (ed.) Stress Physiology in Crop Plants.
- Rice, W.A. 1975. Effects of calcite and inoculum levels on nodulation and growth of alfalfa in an acid soil. *Can. J. Soil Sci.* 55:245-250.
- _____, D.C. Penny and M. Nyborg. 1977. Effects of soil acidity on Rhizobium numbers, nodulation and nitrogen fixation of alfalfa and red clover. *Can. J. Soil Sci.* 57:197-203.
- Robson, A.D. 1979. Mineral nutrients limiting nitrogen fixation in legumes. pp. 277-294. In C.S. Andrew and E.J. Kamprath (ed.). Mineral nutrition of legumes in tropical and subtropical soils. CSIRO Melbourne.
- Roberts, D.S.T. 1980. Growth, nodulation and nitrogen fixation of alfalfa and red clover as influenced by soil acidity and liming. Ph.D. Thesis, University of New Hampshire, Durham, New New Hampshire.
- Russel, D.A. 1950. Determination of exchangeable hydrogen pp. 21-23. In A Laboratory Manual for Soil Fertility Students. Wm.C. Brown Company, Dubuque, Iowa.
- Russell, E.W. 1974. Soil Conditions and Plant Growth. 10th Edition. William Clowes and Sons, Ltd., London.
- Sallee, W.R., A. Ulrich, W.E. Martin and B.A. Krantz. 1959. High phosphorus for alfalfa. *Calif. Agr.* 13(8):7-13.
- Sartain, J.B. and E.J. Kamprath. 1975. Effect of liming a highly Al-saturated soil on the top and root growth and soybean nodulation. *Agron. J.* 67(4):507-510.
- _____, and _____. 1977. Effect of soil Al saturation on nutrient concentration of soybean tops, roots and nodules. *Agron. J.* 69:843-845.
- Schenk, M.K. and S.A. Barber. 1979. Root characteristics of corn genotypes as related to P uptake. *Agron. J.* 71(6):921-924.
- Seetin, M.W. and D.K. Barnes. 1977. Variation among alfalfa genotypes for rate of acetylene reduction. *Crop Sci.* 17:783-787.
- Silva, A.R. da. 1976. Breeding for tolerance to aluminum and manganese toxicity in Brazil. *Ciencia e Cultura*, 28:147-149.
- Silver, W.S. and R.W.F. Hardy. 1976. Biological Dinitrogen fixation in Forage Production. pp. 1-34. In C.S. Hoveland (ed.) Biological N Fixation in forage-livestock systems. ASA special publication No. 28.
- Simpson, J.R., A. Pinkerton and J. Lazdouskis. 1977. Effects of subsoil Ca on the root growth of some lucerne genotypes in acidic soil profiles. *Aust. J. Agric. Res.* 28:629-638.
- Skoog, D.A. and D.M. West. 1974. Analytical chemistry. 2nd ed. Holt, Rinehart and Winston Co., New York.

- Sprent, J.I. 1972. The effects of water stress on nitrogen fixing root nodules. *New Phytol.* 71:603-611.
- Susuki, A., K. Lawton, and E.C. Doll. 1963. Phosphorus uptake and soil tests as related to forms of phosphorus in some Michigan soils. *Soil Sci. Soc. Amer. Proc.* 27:401-403.
- Tanaka, K., T. Yoskida and Z. Kasai. 1974. Distribution of mineral elements in the outer layer of rice and wheat grains using electron microbe X-ray analysis. *Soil Sci. Plant Nutr.* 20(1):87-91.
- Taylor, F.W. 1910. Alfalfa in New Hampshire. *New Hampshire Experimental Station Circular.* 10:1-8.
- Tenant, D. 1975. A test of a modified line intersect method of estimating root length. *J. Ecol.* 63:995-1001.
- Thomas, G.W. 1974. Chemical reactions controlling soil solution electrolyte concentration. p. 441-483. In E.W. Carson (ed.) The Plant Root and Its Environment. University of Virginia.
- _____. 1975. The relationship between organic matter content and exchangeable aluminum in acid soil. *Soil Sci. Soc. Am. Proc.* 39:591.
- _____, and D. E. Peaslee. 1973. Testing soils for P. pp. 115-132. In L.M. Walsh and J.D. Beaton (ed.) Soil Testing and Plant Analysis. Soil Sci. Soc. of America, Inc. Madison, WI.
- Tisdale, S.L. and W.L. Nelson. 1975. Soil Flexibility and Fertilizers. 3rd ed. MacMillan Pub. Co. Inc., New York.
- Turner, R.G. 1969. Heavy metal tolerance in plants. pp. 399-410. In I.H. Rorison. Ecological Aspects of the Mineral Nutrition of Plants. Symp. British Ecol. Soc., Sheffield.
- Ulrich, A. 1956. Plant analysis as a guide to the mineral nutrition of alfalfa. *Proc. VII Int. Grassland Congr.* 8:3-11.
- Vijayachandran, P.K. and R.D. Harter. 1975. Evaluation of phosphorus adsorption by a cross section of soil types. *Soil Sci.* 199:119-126.
- Vincent, J.M. 1977. Rhizobium General Microbiology. pp. 277-366. In R.W.F. Hardy and W.S. Silver (ed.). Treatise on Dinitrogen Fixation III. John Wiley & Sons. New York.
- Waughman, G.J. 1977. The effect of temperature on nitrogenase activity. *J. Exp. Bot.* 28:949-960.
- White, R.E. 1976. Studies on mineral ion absorption by plants. III. The interaction of Al, PO and pH on the growth of Medicago sativa. *Plant and Soil.* 46:195-208.
- Williams, E.G. 1970. Factors affecting the availability of soil phosphate and efficiency of phosphate fertilizers. Anglo-Soviet Symposium on Agrochemical Research on the use of Mineral Fertilizers. Moscow.

- Wilson, A.T. 1968. The chemistry underlying the phosphate problem in agriculture. *Aust. J. Sci.* 31:55-61.
- Woodruff, J.R. and E.J. Kamprath. 1965. Phosphorus adsorption maximum as measured by the Langmuir Isotherm and its relationship to phosphorus availability. *Soil Sci. Soc. Amer. Proc.* 29:148-150.
- Wright, K.E. and B.A. Donahue. 1953. Aluminum toxicity studies with radioactive phosphorus. *Plant Physiol.* 28:674-680.
- Yuan, T.L. 1970. Interpretation of soil pH in liming practices. *Soil and Crop Sci. Soc. Fla. Proc.* 30:200-210.
- Zakaria, Z.Z., V.N. Schroder, and K.J. Boote. 1977. Soybean responses to calcium and phosphorus under aluminum saturation. *Soil and Crop Sci. Soc. Fla.* 36:178-181.