

AN ABSTRACT OF THE THESIS OF

Patti L. Schuttler for the degree of Master of Science in Horticulture
presented on December 14, 1987.

Title: EARLY MACRONUTRIENT UPTAKE AND PARTITIONING IN GLYCINE
MAX L. Merr.

Abstract approved:

Timothy L. Righetti

Soybeans were grown in hydroponic solutions and in perlite to devise procedures to measure plant mineral accumulation and solution depletion over time. Electron emission spectrometry and micro-Kjeldahl-autoanalyzer analytic techniques were used to monitor mineral content of individual plants and their corresponding solutions. An automated computer assisted system of converting raw data on either plant tissue or hydroponic solutions into amounts of uptake for individual macronutrients was devised. Uptake calculated from depletion measurements was similar to uptake

determined from plant mineral accumulation during the uptake periods that were evaluated. Both analytic procedures are sensitive enough to measure small increments in mineral uptake.

MES buffer (2-N morpholinoethanesulphonic acid) at 5 mM adequately maintained pH in 50 ml volumes of aerated solutions used for nutrient uptake experiments. MES at 1.5 mM was ineffective. At 5 mM, MES did not affect growth or uptake of most macro-nutrients. Potassium uptake, however, was enhanced by the MES buffer in non-nodulated seedlings.

When seedlings are grown in the absence of fixed nitrogen, nodulation causes seedlings to proportion more of the seed nitrogen reserve to the roots. Smaller nodulated root systems initially result in lower mineral uptake and less plant biomass production. Eventually, nodulated plants reach a similar size to their non-nodulated counterparts, but they do not show a growth advantage until nitrogen fixation has been active for some time. Even though total absolute amounts of nitrogen in the nodulated plants are higher than those in non-nodulated seedlings, the nitrogen increase is not reflected immediately in increased growth.

Early Macronutrient Uptake and Partitioning
in Glycine Max L. Merr.

by

Patti L. Schuttler

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed December 14, 1987

Commencement June 1988

APPROVED:


Associate Professor of Horticulture in charge of major


Head of the Department of Horticulture


Dean of the Graduate School

Date thesis is presented December 14, 1987

Typed by Patti L. Schuttler

ACKNOWLEDGEMENTS

My sincere appreciation goes to the following people:

- Dr. Tim Rhigetti, for advice and encouragement. He was energetic, enthusiastic, and always positive.
- Drs. P. J. Bottomley, K. Cromack, H. Lagerstadt and L. Boersma for serving as members of my committee.
- Jim Werntz, Fred Dixon and Kris Wilder of the Plant Analysis Laboratory in the department of Horticulture, for prompt analysis of samples , computer assistance, and friendship.
- A horticulture-related network of friends. . . Nancy, Fred, Lori, Loretta, Linda, Jim, Cynthia, Ruth and others. We all encouraged each other, and this helped a lot.
- Finally, thanks and love to Rich Wittrup, who provided computer and graphics assistance and a very special motivation for completing this degree.

TABLE OF CONTENTS

CHAPTER

I.	INTRODUCTION	1
II.	LITERATURE REVIEW	5
	Solution pH and related consequences in solution culture systems	5
	Nutritional aspects of nodulation and nitrogen fixation	20
III.	USE OF ELECTRON EMISSION SPECTROMETRY AND MICRO-KJELDAHL- AUTO ANALYZER PROCEDURES TO MEASURE MINERAL ACCUMULATION BY MONITORING PLANT CONTENT AND SOLUTION CONCENTRATIONS	29
	Summary	29
	Introduction	30
	Experimental Materials and Methods	32
	Experiment 1	34
	Experiment 2	37
	Experiment 3	38
	Results and Discussion	41
IV.	EVALUATION OF MES BUFFER AS PH CONTROL IN SHORT TERM NITROGEN FREE NUTRIENT SOLUTION EXPERIMENTS USING SOYBEAN SEEDLINGS	48
	Summary	48
	Introduction	49
	Materials and Methods	51

TABLE OF CONTENTS cont.

	Experiment 1	51
	Experiment 2	53
	Seedling Evaluation	54
	Results and Discussion	56
V.	EARLY MACRONUTRIENT UPTAKE AND PARTITIONING IN <u>GLYCINE MAX L.</u>	62
	Summary	62
	Introduction	63
	Experimental Materials and Methods	66
	Results and Discussion	67
	25 Day Analysis	70
	45 Day Analysis	73
VI.	BIBLIOGRAPHY	83
VII.	APPENDIX	88

LIST OF FIGURES

Figure

- | | | |
|----|---|----|
| 1. | Schematic representation of an example of a single flowing culture unit. | 19 |
| 2. | Experiment 3 - Comparison of nitrogen content for A. seed and plants at 25 days
B. seed and plants at 45 days. | 44 |
| 3. | Experiment 2 - Solution pH's with and without MES buffer at 5mM. | 59 |

LIST OF TABLES

Table

1.	Concentrations of nutrient elements in three standard culture solutions (μM).	18
2.	Micromolar concentrations of elements in three dilute nutrient solutions used in flowing culture experiments with several plant species.	45
3.	Concentrations of nutrient elements in full strength Schüttler - Righetti N-free solution.	46
4.	Experiment 1 - measured depletion from solution versus measured accumulation in plant tissue (Δ milligrams per tube or plant).	47
5.	Experiment 2 - measured depletion from solution versus measured accumulation in plant tissue (Δ milligrams per tube or plant).	47
6.	Experiment 1 - comparison of solution treatments on growth and mineral accumulation in nodulated seedlings at 1.5 mM MES.	60
7.	Experiment 2 - comparison of solution treatments on growth and mineral accumulation in nodulated seedlings at 5 mM MES.	60
8.	Experiment 2 - comparison of solution treatments on growth and mineral accumulation in non-nodulated seedlings at 5 mM MES.	60
9.	Physical properties of buffers (Good et al. 1966).	61
10.	Comparison of experimental conditions for soybean seedlings using two concentrations of MES buffer.	77

LIST OF TABLES cont.

Table

11.	Experiment 3 - plant part weights and nitrogen content of soybean seedlings (milligrams and percent).	78
12.	Experiment 3 - Mean amounts of macroelements in seed and soybean seedlings (milligrams per seed or plant part).	79
13.	Experiment 2 - Biomasses, accumulated dry weights (mg) and ratios comparing nodulated and non-nodulated seedlings at transplant and harvest.	80
14.	Experiment 2 - Plant part weight ratios for nodulated and non-nodulated seedlings at harvest.	81
15.	Experiment 2 - Partitioning of accumulated macro-nutrients in nodulated and non-nodulated seedlings at harvest.	82

CHAPTER I

INTRODUCTION

Foliar and fruit mineral analyses have been used since the early 1940's, and are valuable tools that aid in the fertilizer management of many horticultural and agricultural crops (Chapman 1941, Thomas 1945). Deficient, optimal, and toxic foliage and fruit concentrations for mineral elements (generally presented as percent or ppm of oven dried tissue), have been established for various crops and ornamentals in many parts of the world. Examples include crops such as rice, (Ishizuka 1978), grapes, (Christensen 1984), treefruits, nuts, small fruits and holly, (PAL publication, OSU 1987), blueberries, (Doughty et al. 1984), and fruit trees, (Boynton and Compton 1945). Methods commonly used to determine mineral composition of wet or dry ashed plant tissue include atomic absorption or electron emission spectrometry for most mineral elements and the standard micro-Kjeldahl digestion/ autoanalyzer procedure for determining total nitrogen.

Plasma emission spectrometry has recently emerged as the analytic tool of choice for many plant tissue applications involving

multielement determination, because it has some advantages over atomic absorption and spark emission spectroscopy (Isaac and Johnson 1985). Greater flexibility in the range of concentrations of various elements that can be handled compared with the atomic absorption method is one advantage. Both low and high concentrations of the same element can be determined using plasma emission spectrometry because of its dynamic linear range, which gives it high sensitivity and eliminates the need for the multiple dilutions commonly required with other methods. The plasma emission spectrometer can also be adapted to more varied sample types and solution matrices than atomic absorption machines. Multielement analyses on a single plant sample have become routine, and it is now possible to conduct experiments in mineral nutrition that were logistically and economically unfeasible in the past.

The first objective of this thesis was to develop procedures to monitor tissue and solution concentration in plant experiments that would allow the efficient study of short term mineral uptake by young seedlings. The feasibility of growing seedlings in a hydroponic situation and using inductively coupled argon plasma spectrometry and the standard micro-Kjeldahl determination of N to

measure seedling mineral uptake and mineral partitioning was evaluated.

A second objective of this thesis research was to investigate, using ICP spectrometry, the effects of the dipolar organic buffer 2-(N-morpholino) ethanesulfonic acid (MES) on the growth, mineral uptake and mineral partitioning of plants grown in hydroponic solutions. My goal was to develop a means of pH control in mineral solutions that did not alter normal plant mineral uptake and growth in short term experiments. By combining extensive monitoring with adequate pH control, much definitive research becomes possible.

The third objective of the thesis work became the development of computer methods for converting data as it is recorded by the spectrometer (percent or ppm dry matter or solution) into computed amounts of elements present in individual roots, shoots and plants and/or in mineral solutions at the times they were sampled or evaluated. An automated system that could convert raw data on either plant tissue or hydroponic solution into computed amounts of uptake for individual plants was desired.

In the course of the study, information on early macronutrient uptake and partitioning in *Glycine max* L. Merr was also obtained.

Very young soybean seedlings were used to determine whether or not statistically significant differences in uptake and partitioning of the N, P, K, Ca, Mg, and S could be detected between nodulated and non-nodulated seedlings grown without fixed nitrogen both in perlite and in solution culture with MES buffer to control solution pH.

CHAPTER II

LITERATURE REVIEW

Although hydroponics systems have advantages in the study of plant mineral uptake and nutrition, the control of solution pH and dealing with related nutritional consequences are major problems in using these systems for research into plant physiology, biochemistry and the uptake and partitioning of mineral elements. Monitoring uptake rates from nutrient solutions is difficult, especially if pH control and the use of dilute solutions are desired. This review highlights past and present approaches to solving these difficulties and explains why the use of MES buffered nutrient solution was selected to minimize these limitations. Nutritional aspects of nodulation and nitrogen fixation with emphasis on relationships between pH and N source are also discussed.

Solution pH and related consequences in solution culture systems

The effects of NO_3^- -ion and NH_4^- -ion on the pH of nutrient cultures has been well documented. In general, if either NO_3^- -ion or NH_4^- -ion are supplied to higher plants as a single source of nitrogen in solution culture, the solution rapidly becomes more basic or

acidic, respectively, unless the solution is strongly buffered. Researchers using legumes have additional problems. Nodulated legumes growing in nitrogen-free medium can drastically decrease the pH of the solutions (Bond 1950, Ahmed and Evans 1960, Israel and Jackson 1978).

In many solutions, high concentrations of PO_4 -ion are used as the buffering agent. This procedure has limitations, however, because as plants decrease phosphate concentration in a solution, they also lower the buffering capacity. Growth rates of many plant species also can be slowed by very high phosphorus concentrations in the root zone. Loneragan and Asher (1967) found reduced growth in clover and other species that was associated with high uptake rates of phosphate. Although this is referred to as phosphate toxicity, reduced growth is probably caused by phosphate induced reduction in the uptake and/or translocation of micronutrients including Zn, Cu, and/or Fe (Mengel and Kirkby 1982). Even moderate phosphate concentrations of around 0.5 mM with pH's greater than 5 can induce iron chlorosis in some species.

It is possible to grow soybeans in solutions which use nitrate

as the sole source of fixed nitrogen and phosphate at around 1 mM concentration as the buffering agent (Lahave, Harber and Hagman 1976). Some soybean varieties, however, are especially sensitive to high concentrations of phosphate in solution, and require a balance between nitrogen and phosphorus levels to obtain satisfactory plant growth (Howell R W 1964).

Adjusting nitrate-ammonium ratios is another method of controlling solution pH. Imsande (1986) investigated the nitrate-ammonium ratio required for pH homeostasis in hydroponically grown soybean. Soybean plants (*Glycine max* (L.) Merr.) were grown hydroponically on medium with 3.0 mM nitrogen supplied as various combinations of KNO_3 and NH_4NO_3 . All media was supplemented with 1.0 mol m^{-3} MES, pH 6.4, and changed once a week, when plants were weighed. In flasks supplemented with approximately 1.8 mmoles of KNO_3 plus 0.6 mmoles of NH_4NO_3 , the pH of the medium remained unchanged. It was shown that plants receiving all of their nitrogen from nitrate must excrete or neutralize at least 0.8 mmole of hydroxyl ion d^{-1} , whereas plants deriving all their nitrogen from ammonium must excrete or neutralize approximately 2.1 mmoles of hydrogen ion d^{-1} . Although nitrate-ammonium ratios can be used to

minimize pH changes in solutions, constant maintenance of a specific ratio is difficult in non-flowing systems. Furthermore, researchers investigating physiological processes that are associated with nodulation, NO_3^- -ion uptake, induction of in vivo nitrate reductase activity and nitrite reductase activity often require legumes grown with N sources other than nitrate. Nodulated soybean seedlings grown under N-free conditions may have some advantages as test plants, since confounding factors of NO_3^- -ion or NH_4^- -ion uptake and the respective associated H-ion or OH-ion generation are reduced.

In the late 1950's and early 1960's researchers working with corn sought methods of producing healthy, uniform young corn plants for biochemical experimentation using a hydroponic system (Hageman, Flesher et al, 1961). Rapid nitrate absorption by corn plants grown in nutrient solutions can quickly raise solution pH. This can induce iron chlorosis by decreasing iron availability at pH's of 5.5 or higher, and previous work had indicated that this is often the major reason for a poor growth response (Franco and Loomis 1947). Phosphate (PO_4^- -ion) buffered systems can be inappropriate because high phosphate concentration alone may induce an iron

chlorosis. The major innovation in the early corn studies (Hageman et al 1961) was the use of the carboxyl cation exchange resin IRC-50 (in a 10/1 ratio of the H-form to the C- form at 35 g/l) to maintain the solution pH within the range of 4.2 to 4.8. Use of the resin allowed production of healthy and uniform one to five week old corn plants for biochemical experimentation using hydroponic procedures. Advantages included the use of smaller containers (two quart), less nutrient solution and less space per plant than previous approaches.

Bugbee and Salisbury (1985) tested the binding affinities of Amberlite IRC-50 resin for essential elements found in mineral solutions. When standard quantities of IRC-50 resin were equilibrated with solutions, large amounts of magnesium and manganese were removed from solution. Zinc, Cu and K increased in solution, indicating probable contamination from the resin. Calcium concentrations increased when the calcium loaded resin was used and decreased with the use of the H-form of the resin, but the intermediate forms had relatively small net effect on total calcium in solution. The authors conclude that overcoming some of the

problems associated with the use of the IRC-50 resin is complex.

Lahav, Harper and Hageman (1976) devised a system of growing non-nodulated soybeans in relatively small volumes of nutrient solution with a single N source other than NO_3^- -ion. These studies involved nitrate uptake in initially nitrate-deprived plants and the induction of nitrate reductase activity. Because their objectives did not include attempts to measure depletion and nutrient uptake, the use of a cation exchange resin was appropriate. They also investigated use of the carboxy resin (Amberlite IRC-50) to determine if it might provide the buffering needed to maintain solution pH within a range conducive to vigorous plant growth.

Urea, amides and five different ammonium salts were tested as single sources of reduced nitrogen for soybeans. Control of pH in the solutions containing both ammonium salts and the carboxy resin was not very effective. In unbuffered solutions seedlings grew less when cultured with urea than when provided with nitrate. Rapid decline in the solution pH was thought responsible for the limited plant growth with urea, but adjusting the pH daily did not alter the injurious effects. The Ca form of the carboxy resin at 50 g/liter when added to the urea nutrient medium stabilized the pH, and

growth was equivalent to that of nitrate supplied seedlings. Although this system was successful with soybean, peas (*Pisium sativum*) showed less growth when grown with urea plus the resin than they did with nitrate as their only nitrogen source.

Based on this study, the authors recommended 6 mM urea as a single nitrogen source plus 50 g/liter of the Ca-IRC resin for hydroponically grown soybeans. The IRC resin has sufficient buffering capacity to maintain pH within a range suitable for growth of vigorous soybean plants (pH of about 5).

Harper and Nicholas (1976) wanted to determine the optimum pH levels for soybean nodule development and function. They developed a rather sophisticated recirculating ion exchange system to control pH within desired limits. Urea was used as a nitrogen source and Amberlite IRC-50 in a resin column was used to control pH. A pH level of 9 could be established and maintained using 100% of the Ca-ion form of the resin and a pH level of 3.7 could be achieved using 100% of the H-ion form. Intermediate H levels could be achieved by varying the ratio of the Ca-form to the H-form.

The system maintained pH within 0.5 to 0.9 units of the initial pH for a 14 day growth period and allowed rather precise

manipulation of desired pH. It provided a means of separating the effects of pH from the effects of N-source on soybean nodulation. The authors report the optimum pH range for nodule mass and N_2 -fixation at 5.2 to 7.0 with urea nutrition. Urea was the recommended N source as it was less inhibitory to nodulation than either NO_3^- -ion or NH_4^- -ion.

Unfortunately, it is not possible to determine depletion from the solution when exchange resins are used. Relatively large volumes of solution must be used, and the magnitude of the differences in solution concentrations in the continuous flow through systems before and after plant exposure is difficult to experimentally measure. The complex recirculating system often required when using Amberlite IRC resins to control pH and the ability of the resins to bind trace metal ions has made their widespread use impractical (Figure 1).

Asher and Edwards (1978) reviewed the relevance of dilute solution culture studies to the problems of growing legumes in low fertility tropical soil. Very large volume continuously flowing solution cultures are one means of controlling the chemical environments of plant roots precisely and for extended periods

(Asher et al 1965). However, there are considerable problems involved in creating nutrient solution systems that realistically mimic soil solution that plant roots normally encounter. The facilities needed are rather complex and sophisticated as shown in Figure 1, and creating them is expensive. Since the solutions used are dilute, nutrient concentrations may approach limits of detection by normal techniques of solution analysis.

An auto-titrator system was devised to study ion uptake by intact plants in flowing solution culture by Asher et al (1965) and Clement et al (1974). Hatch and Canaway (1984) used a microcomputer to obtain greater flexibility. They grew intact perennial ryegrass plants (*Lolium perenne* L.) in nutrient solutions with pH controlled at 5.0, 5.5, 6.0 and 7.0. Earlier work done by Glass et al. (1983) demonstrated the capability of microcomputers to monitor ion activities during depletion of stirred nutrient solutions by intact plants over a period of 15 minutes. Hatch and Canaway concluded that their sampling and monitoring assembly could maintain the prescribed pH values within set limits of ± 0.1 pH units.

Computer monitored systems can maintain precise pH control of hydroponic media, but they require constant addition of acids or bases. Counterions added with H^+ or OH^- introduce variability between treatments and make monitoring depletion of the solution difficult. Although a computerized system could theoretically maintain levels of several nutrients in solution, the counterion problem (any added salt consists of both anion and cations) makes them complex and expensive to create. For these reasons, monitoring short term depletion of smaller volumes of dilute solutions is attractive. Solutions can be changed before substantial depletion occurs, but differences are large enough to be accurately measured. If solution pH could be maintained in small volumes of dilute solution, this would be an ideal system.

In 1966, a biochemistry paper presented the results of testing twelve new hydrogen ion buffers for general biological research (Good et al 1966). The pK_a 's of the buffers ranged from 6.15 - 8.35 (Table 9). Ten of the buffers tested were bipolar amino acids, including the nitrogen-substituted taurine 2-(N-MORPHOLINO) ETHANESULFONIC ACID (trivial name MES) which had the lowest pK_a (6.15) of all the buffers tested. MES, along with the other bipolar

buffers tested, was superior to previously used conventional buffers in buffering the Hill reaction and the phosphorylation-coupled oxidation of succinate by bean mitochondria. The relatively low pK_a of MES falls within the pH ranges desired for many plant nutrient solution systems. However, MES was not evaluated as a means of pH control in hydroponic systems until many years after it was introduced as a feasible hydrogen ion buffer for biochemical research.

Rys and Phung (1985) investigated the effects of a range of dipolar organic buffers on the pH control of the rooting medium, plant growth and symbiotic development in the *Trifolium repens*-*Rhizobium trifolii* symbiosis under different nitrogen nutrition regimens. They evaluated five buffers; MES, ADA, ACES, BES, and MOPS (Table 9) at a concentration of 2.0 mM for their effects on pH of nutrient solution in which nodulated *Trifolium repens* L. were grown. The solution volume was 37 cm³. Solutions were not stirred for the entire 26 - 28 days of the experiments. Nutrient solution used contained 7.13 mol m⁻³ nitrogen as (NH₄)₂SO₄ and initial pH's were adjusted to each respective buffer pK.

The buffers ADA and ACES completely inhibited plant growth.

The other 3 buffers did not control the solution pH's at 2.0 mM over the 28 day period of the experiment; however, plant dry matter was higher but nodule number was lower in the presence of these buffers. MES and MOPS were tested further. They were supplied at five different concentrations ranging between 0 and 12 mol m⁻³ to solutions with and without (NH₄)₂SO₄. MES at 9 and 12 mol m⁻³ reduced the growth of plants dependent on fixing dinitrogen. All other concentrations of MES significantly increased plant yield and reduced nodule number for the plants provided with NH₄-ion. MOPS had no effect on plant yield or nodule number.

Imsande and Ralston (1981) devised a simple, reproducible and rapid assay to measure the rate of dinitrogen fixation in soybeans throughout the lifetime of the plants. They combined hydroponic growth with the sensitive acetylene reduction technique and successfully used MES buffer (pK_a of 6.1) at 1.0 to 2.0 mM to buffer hydroponic growth medium. Volumes of solution used were 1 liter, and solution was totally replaced at 3 to 4 day intervals. Hydroponic portions of the experiments lasted 4 to 6 weeks.

Bugbee and Salisbury (1985) also tested MES at concentrations of 1 and 10 mM with beans (non-nodulated), corn, lettuce, tomatoes,

and wheat. Pregerminated, uniform seedlings were transplanted into 2 liter volumes of solution with and without the MES buffer and grown for three to four weeks. All treatments in all trials were routinely monitored and adjusted to maintain $\text{pH } 5.8 \pm 0.5$ pH units if required. The nitrogen source in the nutrient solution was a combination of NO_3^- -ion at 8 mM and NH_4^- -ion at 1.5 mM. The relative growth rates among controls and MES treatments were nearly identical for each species during the trial period. The pH was stabilized by 1 mM MES under the conditions used. They also tested the resistance of the MES buffer to microbial decomposition using titration tests.

MES appears to be biologically inert and does not interact significantly with the other solution ions. MES has several characteristics that make it useful in nutrient solution studies: a pKa of 6.15, high solubility, low binding constants with essential ions, and resistance to microbial breakdown. It is readily available in a pure form and has a relatively high molecular mass (195), which makes it fairly impermeable to membranes; (Good et al 1966) and (Bugbee and Salisbury 1985). A combination of MES buffered

hydroponic systems with sophisticated analytical procedures to study nodulated and non-nodulated legume seedlings could be used to obtain valuable information regarding the absorption and partitioning of nitrogen and other nutrients.

Table 1. Concentrations of nutrient elements in three standard culture solutions (μM).

Element	Crone's -N solution*	Hoagland's No. 1 solution+	Long Ashton solution++
Nitrogen (NO_3^- -ion)	---	15000	12000
(NH_4^- -ion)	---	---	---
Potassium	10060	6000	4000
Calcium	5320	5000	4000
Sulphur	4930	2000	1510
Magnesium	2030	2000	1500
Phosphorus	2610	1000	1300
Iron	1495	25**	100
Boron	46	46	50
Manganese	9	9	10
Zinc	0.8	0.8	1
Copper	0.3	0.3	1
Molybdenum	0.1	0.1	0.5
Cobalt	---	---	0.2

Table adapted from Asher and Edwards 1978.

* As modified by Bond (1951), concentrations calculated on the assumption of complete solubility of the component salts. This assumption not fully met according to Bond and Mackintosh (1975).

** Renewed twice weekly.

+ Ref. Hoagland and Arnon (1950).

++ Ref. Hewitt (1966).

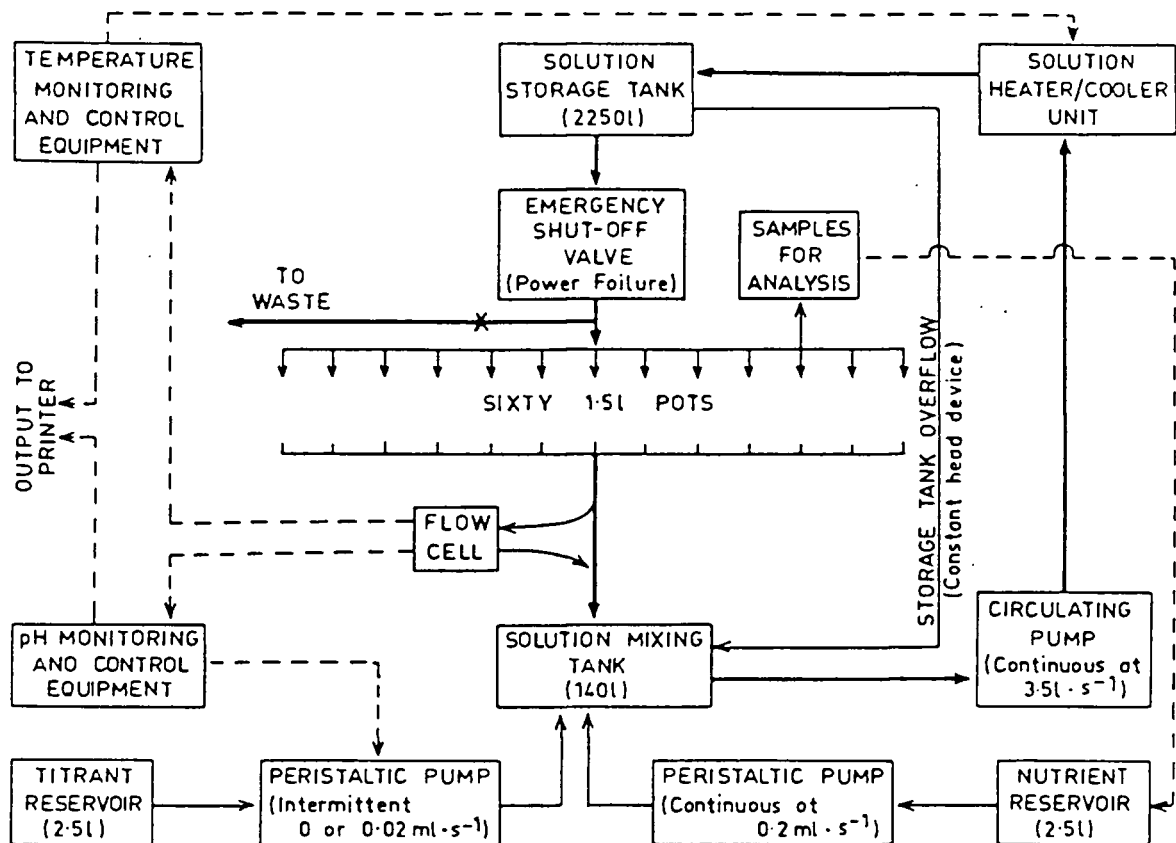


Figure 1. Schematic representation of an example of a single flowing culture unit (Asher and Edwards 1978). Solution flows indicated by solid arrows. Information flows indicated by dashed arrows.

Nutritional aspects of nodulation and nitrogen fixation

Early work with lucerne investigated the effects of several forms of nitrogen sources on initial nodulation; NaNO_3 , KNO_3 , $(\text{NH}_4)_2\text{SO}_4$, and NH_4NO_3 all delayed initiation of nodulation while urea, tested over a broad concentration range, did not (Subba Rao et al. 1974).

Dart and Wildon (1970) demonstrated less inhibition of nodulation in cowpea (*Vigna sinensis* Endl. ex Hassk.) and in purple vetch (*Vicia atropurpurea* Desf.) if the fixed-nitrogen source was urea than if it was either NO_3 -ion or NH_4 -ion. Vigue (1976) demonstrated that urea-supplied soybeans exhibited extensive nodulation compared with soybeans using nitrate as a single N source.

Rys and Mytton (1985) and Mytton and Rys (1985) in two related papers, investigated the potential for breeding white clover (*Trifolium repens* L.) with improved nodulation and nitrogen fixation when grown with combined nitrogen. The first paper focused on the effects of different amounts of nitrate nitrogen on phenotypic variation, and the second paper was an assessment of

genetic variation in *Trifolium repens* L. in regards to nodule number and acetylene reduction activity.

Plants were selected from a variable population for high, low and zero nodule numbers when they were supplied with abundant nitrate nitrogen under an aseptic tube culture technique. Plants within groups were intercrossed and progeny used to establish high, low and zero nodule families. These were subsequently tested for nodulation and acetylene reduction activity in the presence and absence of combined nitrogen. Under adequate nitrate nutrition, the unselected control group averaged 3.05 nodules per plant, the high group averaged 4 nodules per plant, and the low line averaged 1 nodule per plant ($P < 0.05$). Nodule numbers were strongly correlated with acetylene reduction activity, ($r = +0.92$), but C_2H_4 production was generally low, averaging, respectively, 4.9 and 3.1 nmol/plant per hour in the high and the low lines.

When the plants were grown without nitrate, nodule numbers increased to an average of 50 per plant and the average acetylene reduction rate rose to 175.5 nmol/plant per hour. There was no correlation between ability of selection lines to nodulate in the presence and absence of nitrate.

Results demonstrate that heritable genetic factors that control nodulation and nitrogenase activity exist. However, the phenotypic expression of these characters is so strongly inhibited by nitrate that increases in nitrogen fixation in the presence of fixed nitrogen which are likely to be of agricultural significance would require much greater genetic improvements than those demonstrated in this research. The authors also caution that this experiment tested only a very limited amount of the great genetic diversity that exists within and among species in the *Trifolium* genus.

Israel and Jackson (1978) reviewed research on the influence that nitrogen nutrition has on the uptake and translocation of other mineral ions in legumes. Nodulated legumes growing in nitrogen-free medium clearly decrease the pH of the solutions (Bond 1950, Ahmed and Evans 1960, Israel and Jackson 1978). In the nodulated legume that is fixing N_2 under nitrogen-free or low nitrogen conditions, considerable internal organic acid synthesis and H^+ -ion extrusion is predicted as a result of the excess cation uptake which occurs in the absence of NO_3^- -ion in the external solution. Evidence presented by Israel and Jackson confirms that substantial quantities of organic acid anions and/or negatively charged amino acids do

counter excess inorganic cations in xylem sap of nodulated *Glycine max* L. Merr. and a H^+ -ion extrusion pump operates in the root cell plasmalemma.

Rhizosphere acidity generated by excess cation uptake may affect nodulation. Munns (1968), working with lucerne, (*Medicago sativa* L.), showed that pH levels of 5.5 or lower reduced nodule numbers. Nodulation was almost totally prevented if the pH of the solution was 4.5 or lower. He found that pH primarily affects the infection process rather than later stages of nodular development. Rhizosphere acidity may also influence availability of P, Al and other micronutrients. Israel and Jackson also suggest that differences in generation of rhizosphere acidity within and among leguminous species may have significance for their adaptation to acid soils. Increases in rhizosphere acidity may influence the Ca requirement for nodulation by reducing interaction with infection sites. Unbuffered hydroponic systems would seem to offer an attractive method of screening genetic material for species and genotypes (or isolines) that produce less rhizosphere acidity under low N conditions and thereby are better adapted to tropical and

subtropical acid soils.

Few studies have concentrated on the relationship between mineral uptake and nitrogen fixation in young legumes. Franco and Munns (1982) worked with beans (*Phaseolus vulgaris* L.). They showed that acid growth medium ($\text{pH} < 5.4$) inhibited nodule initiation and affected uptake of other nutrients. They also found buildup of Cl-ion to toxic levels in the bean plants when grown in nitrate-free media, even when solution concentrations of Cl-ion were as low as 0.4 mM. Adding 0.5 to 1.0 mg N (as NH_4NO_3) to each plant delayed initial nodule growth and activity slightly, but it increased plant growth and solved the problem of severe N-deficiency that is common in nitrogen-deprived beans before the onset of N_2 -fixation.

Van Beusichem (1981) compared nutrient absorption by effectively nodulated pea plants during dinitrogen fixation with that of ineffectively nodulated pea plants provided with nitrate as a nitrogen source. In order to obtain morphologically comparable plants between the two nodulation conditions, it was necessary to maintain a low growth chamber temperature of 13 degrees Centigrade. The pH was kept constant at 5.5 using a pH meter with pH stat equipment operating an automatic burette. A large amount

of aerated liquid medium was required; 30 liters for each treatment of 40 plants. Two harvests were made, one at 21 days and one at 42 days.

At the 21 day evaluation by van Beusichem, both the root and the shoot biomasses of the dinitrogen fixing plants were lower by about 15% than those of the nitrate-furnished plants. By 42 days, the roots of the N_2 -fixing plants were slightly larger than the roots of the nitrate-fed plants, while the ratio of the shoot biomasses between the two nodulation states remained about the same as for the first harvest. The root and shoot tissue was dried, weighed and analyzed for total N and for K, Na, Ca, Mg, H_2PO_4 -ion, Cl, NO_3 -ion and SO_4 -ion. Chemical composition was reported in meq/kg DM. Van Beusichem concluded that nitrate supplied plants accumulate much more potassium.

Datta (1985) used ion-selective electrodes to make simultaneous and intermittent measurement of K, Ca, and NO_3 -ion by intact *Phaseolus vulgaris* L. plants under both aerobic and anaerobic conditions. Regulated proportions of air and nitrogen were passed through the solution to maintain desired oxygen tensions. Very small volumes of solution were used (50 ml) and samples were

drawn at various time. Solutions were completely changed in the tubes at 30 minute intervals. Anaerobiosis had a more direct and pronounced effect on K-ion absorption rather than NO_3 -ion absorption. Significant ion efflux (particularly of K ion) occurred under anaerobic conditions. This was explained on the basis of direct effect of ATP-ase on cation absorption by induction of an electrochemical and pH gradient. No effect was found on the rate of Ca ion absorption due to the low oxygen treatments.

Non-nutritional factors also affect nodulation in solution culture systems. Franco and Munns (1982) developed conditions and techniques for achieving good nodulation of *Phaseolus vulgaris* L. in continuously aerated solution. Bean plants were grown for these experiments under a variety of conditions, but all seedlings were initially germinated in vermiculite rinsed with 0.5 mM CaCl_2 and inoculated with *Rhizobium phaseoli* grown in yeast mannitol agar. Hydroponic growth situations ranged from small volumes (1 liter/plant) to large volumes (3 plants in a 19 liter volume) and pH's were controlled using KOH or H_2SO_4 . For plants with pre-established nodules, growth and activity of nodules and plant growth in solution culture paralleled that of plants grown in gravel culture.

Plants inoculated while growing in relatively small volumes of solution (1 liter) formed extensive nodules within 10 days, while plants growing in large volumes (3 plants/19 liters of solution) nodulated at this rate only if inoculated immediately at transfer into hydroponic solution. Nodulation was restricted to roots that had already formed at the time of transfer from gravel or vermiculite. Nodulation was both sparse and delayed if inoculation was done 2 or more days after transfer into the large volume solutions. This nodulation delay and inhibition could not be explained by failure of bacteria to colonize the roots or by lack of root hair development.

Delves et al. (1986) looked at the regulation of the soybean-Rhizobium symbiosis by shoot and root factors by using soybean mutants with altered symbiotic properties for grafting material. Grafts between the mutants and the wild types showed that supernodulation as well as hypernodulation is shoot controlled in two mutants, and most likely belong to two separate complementation groups. It is strongly suggested that plant organs other than the roots can affect the expression of the nodulation

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The extent to which uptake and transport of non-nitrogenous elements in nodulated legumes is moderated by the legume-rhizobium symbiosis has not been examined thoroughly. Devising a system whereby nodulated legume mineral uptake and partitioning could be precisely determined by measuring plant tissue content or nutrient solution depletion over time could help evaluate the effects of the legume-rhizobium symbiosis on accumulation and partitioning of mineral elements.

CHAPTER III

TITLE - USE OF ELECTRON EMISSION SPECTROMETRY AND MICRO-
KJELDAHL-AUTOANALYZER PROCEDURES TO MEASURE PLANT MINERAL
ACCUMULATION AND SOLUTION DEPLETION

Key words - *Glycine max* L., Electron emission spectrometry, Macronutrients, Micro-Kjeldahl procedure, Nitrogen fixation, Nodulation, Nutrient solution, Rhizobium, Soybean

Summary

Some soybean seedlings were grown in small volumes of hydroponic solutions and some in perlite in order to devise procedures to measure plant mineral accumulation and solution depletion over time. Electron emission spectrometry and microKjeldahl-autoanalyzer techniques were used to monitor mineral content of experiments solutions and plant material over time. An automated computer assisted system to convert raw data on either plant tissue or hydroponic solutions into amounts of uptake or depletion for individual micronutrients was devised. Initial attempts made with very dilute solutions that approximated soil conditions were unsuccessful, thus solution concentrations were

increased to lessen the analytical problems of detecting concentration changes at the limits of instrument sensitivity. Uptake calculated from plant mineral accumulation for the measured time periods was similar to the uptake determined from mineral depletion from the solutions. Measurements of depletion from solutions and macronutrient accumulation in plant tissue are both sensitive enough to measure small variations in mineral uptake in the soybean seedlings.

Introduction

Electron emission spectrometry and micro-Kjeldahl-autoanalyzer procedures are widely used to determine mineral concentrations in foliage or fruit, and fertilizer recommendations can be made for agricultural plants using these values. The use of these techniques in research on basic plant nutrition, however, has not been well explored. Combining nutrient solution plant growth systems and mineral measurement procedures would seem to be an excellent approach to precisely measure plant mineral uptake and partitioning. However, approaching realistic soil concentrations in experimental nutrient solutions without encountering either depletion or measurement problems has not yet been achieved.

Additional difficulties encountered include inadequate control of pH and related buffering problems.

Attempts to counter these problems have included use of inorganic buffers, ion exchange resins, continuous flow through systems, and computer monitoring with correction of pH and element concentration in solution. Each of these approaches has disadvantages when attempting to precisely measure macronutrient uptake and partitioning in plants growing in a hydroponic system. Although the equilibrium between a resin and ions in solution maintains a more constant concentration, it is not possible to measure depletion from a solution when a resin is used. Since flowing solutions are very dilute (Table 2), nutrient concentrations can approach limits of detection, and differences between incoming and outgoing concentrations can be too small to measure. Computer monitoring and automatically altering solutions are theoretically possible, but since every added salt contains both anions and cations, keeping all elements constant in a multielement system requires sophisticated and expensive facilities (Fig. 1). For these reasons a simple system that measures depletion of moderately

dilute solutions that are changed often enough to ensure that concentration of individual elements remains within a relatively constant range is attractive. Control of pH in these solutions will be discussed later.

A major question on the uses of micro-Kjeldahl-autoanalyzer procedures and electron emission spectrometry to measure macronutrient uptake in plants concerns the precision of each analytic procedure. How small are the incremental changes in either solution concentration or tissue content that can be measured with enough precision to ascertain statistically significant differences in nutrient depletion or accumulation between treatments? This report addresses this question of analytical precision. Portions of these experiments were also used to evaluate MES buffer (Chapter IV) and macronutrient partitioning (Chapter V). These aspects of this research will be discussed later.

Experimental Materials and Methods

In preliminary experiments, two week old seedlings (nodulated and non-nodulated) that had been propagated in perlite with an initial application of dilute CaSO_4 (600 ppm Ca) were transplanted

into individual aerated tubes containing 50 ml. of dilute solution with concentrations similar to those shown in Table 2. Stems were supported with plugs made of rayon batting. During the time plants were grown in growth chambers, solutions were changed at 24 or 48 hour intervals with three sampling times between changes to fresh solutions. Three ml. samples were removed from tubes using individual plastic syringes for each tube. Tubes were always brought back to 50 ml volume with distilled water both before and after sampling. BASIC computer programs were written to monitor depletion from solution for the five macronutrients. These programs converted raw data collected from the spectrometer to a cumulative amount of depletion, (in mg./tube) by accounting for differences in solution concentrations and the 6% decline in concentration caused by the removal of each sample.

Because of the low solution concentrations used and the further dilution caused by each sampling procedure, this approach quickly led to depletion of elements in solution to below detection limits of the ICAP spectrometer, and seedlings also showed signs of micronutrient deficiencies. Eventually, shorter time periods between solution changes and improvements in analytical procedures

could lead to improved data collection using this approach, but further refinement would be required. As a first step, the following alternative procedures were devised using higher concentrations of hydroponic solutions in order to obtain measurements of depletion and estimates of macronutrient uptake.

Experiment 1 – Nutrient Solution with MES at 1.5 mM

Three flats (50 seed/flat) of inoculated soybeans and three flats of noninoculated soybeans were germinated in perlite with an initial application of CaSO_4 (600 ppm Ca) at day 2 and applications of 10% nutrient solution (Table 3) on days 21, 23, and 25. The pH of the nutrient solution applied was initially adjusted to 6.6 with KOH. Both germination and the hydroponic portion of the experiment took place in growth chambers. The light-dark cycle was 16 hours of light and eight hours of darkness, and the light/dark temperatures were 24 degrees Centigrade in the light and 18 degrees Centigrade in the dark. Light intensity during light periods was measured at approximately $210 \text{ microeinsteins M}^{-2} \text{ sec}^{-1} \times 0.1 \text{ Watts/m}^2 \times 10 \text{ Lux}$.

Inoculated and noninoculated seeds were treated respectively before sowing with a distilled water slurry of Rhizobium peat and sterilized (autoclaved) Rhizobium peat. On day 26, 16 uniform seedlings of each nodulation state were weighed fresh and transplanted into 50 ml volume tubes of full strength nutrient solution (Table 3). Seedlings at transplant were chlorotic and showing other deficiency symptoms, and average seedling dry weight at transplant was calculated at 380 milligrams. About 50 uniform seedlings of each nodulation state which were not transplanted were also weighed fresh, separated into root and shoot, dried, reweighed and composited within treatments to be ground for ICAP mineral analysis. Root/shoot ratios, percent dry weight in the roots and shoots and macronutrient concentrations (all derived from the reference seedlings) were used along with individual fresh weights taken at time of transfer, to estimate nutrient amounts initially present in each individual seedlings before transfer into the nutrient solution.

Nodulated and non-nodulated seedlings were transplanted into aerated control and 1.5 mM MES (2-N-morpholinoethanesulfonic acid) tubes containing the nutrient solution as shown in Table 3.

Seedlings were harvested ten days later. MES is an organic buffer which has been investigated as a means of controlling solution pH's in some recent studies (Imsande and Ralston 1981, Rys and Phung 1985). Details of pH control will be discussed in Chapter IV.

At harvest, roots were rinsed twice in distilled water and blotted dry. Seedlings were separated into root and shoot, and respective fresh weights were taken. Plant material was oven dried at 70⁰ Centigrade for at least 48 hours, individually reweighed, composited within plant parts and treatments and ground for mineral analysis.

Standard procedures for mineral analysis by ICAP spectrometer were followed after dry ashing the plant material at 500⁰ C for four hours and dissolving the ash in 10% HNO₃. Both root and shoot tissue were analyzed for macronutrients. Single roots and shoots could not be analyzed for their mineral concentration because they did not weigh enough to make up the 250 mg. sample size required for a single spectrometer sample; thus they were composited within treatments to obtain concentration values of P, K, S, Mg, and Ca that were used, along with their respective individual root and shoot dry weights, to calculate the amounts of macronutrients present in the

seedlings at the time of harvest from the ten day uptake period.

Programs using dBase III software were written to convert the data from tissue concentration to actual amounts in milligrams of each element that was accumulated by the plants during growth in the mineral solutions. These values could then be compared with the amount of elements computed to be depleted from the solution.

Experiment 2 -Nutrient Solution with MES at 5.0 mM

Germination and inoculation procedures were similar to those described above, (Experiment 1), but seedlings were in perlite only 14 days with dilute mineral solution applications on days 12 and 13. Plants were green at time of transplant, with the first pair of true leaves fully expanded, and the computed mean seedling dry weight at transplant was 180 mg.

On day 14, 16 uniform seedlings of each nodulation state were weighed fresh and transplanted into 50 ml volume tubes of full strength nutrient solution (Table 3) that was buffered with 2-N (morpholino) ethanesulfonic acid (MES) at 5 mM. About 50 reference seedlings were also treated as described for Experiment 1. Harvest evaluations were similar for both solution culture experiments.

Another experiment was done to reveal more information on macroelement uptake and partitioning and to determine if the micro-Kjeldahl-autoanalyzer procedure of measuring nitrogen percentages was sensitive enough to detect differences in nitrogen accumulation and partitioning in soybean seedlings grown in nitrogen-free medium.

Experiment 3 - Materials and Methods

Nodulated and non-nodulated soybean seedlings were grown under nitrogen-free growth conditions for short term (25 day) and long term (45 day) periods in perlite medium. Because of space constraints, the two treatments were begun at different times. Attempts were made to keep conditions for the first 25 days of the 45 day treatment identical to those of the first treatment.

Twenty five days was selected as the early harvest, because plants were expected to be nodulated, but only minimally fixing nitrogen. By forty five days, nitrogen fixation was expected to be fully operative. For the twenty five day evaluation, six flats of presterilized perlite were planted with soybean seed that had been

soaked in distilled water for 12 hours.

Seedlings were germinated in growth chambers under conditions described for experiments 1 and 2. Autoclavable, polypropylene flats were placed on wire racks inside the chambers. The volume of perlite used per flat was $.03 \text{ m}^3$. Small holes allowed drainage of the distilled water and mineral solutions through the bottom of the flats. Each flat was planted with fifty seed. The seed planted in three of the flats was treated with a presterilized (autoclaved) Rhizobium peat inoculum. These flats were planted first to avoid rhizobial contamination of the non-nodulated seedlings. The other three flats were planted with seed that had been treated with effective Rhizobium peat inoculum. All flats received 500 milliliters of CaSO_4 solution per flat (at 600 ppm Ca) on day two and day four after planting, and were subsequently watered with distilled water as needed, until day 25, when they were harvested.

For the forty five day harvest, six other flats were treated as described above for treatment one until day 25. From day 25 until day 45 they then received alternate day applications of 500 milliliters/flat of 10% -N nutrient solution (10% of full strength solution as given in Table 3) and distilled water as needed.

Seedlings were harvested at day 45. No fixed nitrogen was introduced into the system with either treatment.

At both harvests about 35 uniform seedlings per flat were selected for analysis. Ten per flat were selected for ICAP spectrometer analysis and about 20 were chosen for the micro-Kjeldahl nitrogen analysis procedure. Seedlings were separated into shoot and root portions and individual fresh weights were taken. After oven drying at 70⁰ C for at least 24 hours, individual shoot and root dry weights were measured. Composited samples of shoots and roots were processed and analyzed for K, Ca, Mg, P, and S as previously described for experiments 1 and 2. The average concentrations of macroelements in the composited tissue sample were used along with individual dry weights to estimate and absolute amount (in milligrams) for K, Ca, Mg, P, and S present in each individual root. Oven dried shoots were treated similarly. Seedling roots and shoots from the 45-day treatment were generally dried, ground, and treated as described above. Nitrogen was determined in the ground root and shoot samples by standard micro-Kjeldahl-autoanalyzer procedures. Concentrations of macroelements and nitrogen were determined for oven-dried seed by ICAP

spectrometry and the micro-Kjeldahl-autoanalyzer procedure respectively, and average amounts of macronutrients and nitrogen were determined for an average soybean seed in the lot used by the procedures described above.

Concentrations of macroelements and nitrogen were also determined for oven-dried seed using similar procedures. Mean dry weight per seed for air dried, oven dried and redried after soaking were not significantly different.

Results and Discussion

Macronutrient accumulation and depletion results are presented for the two solution experiments, (Table 4 and Table 5).

Through the use of dry weight and depletion data and computer manipulation of measured values, uptake can be quickly evaluated. Both analytic procedures are sensitive enough to measure small amounts of mineral uptake in seedlings. Results obtained through the use of the two methods are fairly close. This gives confidence in the methods used and indicates that the use of ICAP spectrometry to measure mineral depletion from solution in a nondestructive

manner is possible. Future work could be done by investigating increasingly more dilute solutions in the attempt to come as close as possible to imitating realistic soil solutions while remaining within the detection limits of the spectrometer.

Comparisons of amounts of calculated nitrogen in seeds and at two different times of evaluation are presented for the third experiment (Figure 2). Seed nitrogen was about 11 milligrams. The 25 day evaluation for nitrogen shows that nodulated seedlings are not yet receiving a net measurable nitrogen increase via dinitrogen fixation, where by the time of the 45 day evaluation the nodulated plants clearly contain more nitrogen. The additional nitrogen, however, is not associated with significant differences between plant weights.

In the two relatively short term hydroponics experiments discussed here, high P concentration was provided in order to avoid P deficiency and to test its capacity to control pH in the absence of MES buffer. This was done even though P concentrations as low as 1 mM have been reported to depress growth in some soybean varieties when grown under conditions of nitrogen deprivation. Howell (1964), Lahav, Harper and Hageman, (1976), Imsande and Ralston

(1981). It is possible that slow seedling growth observed could be an indication that the P concentration provided was too high. For future experiments, I would attempt to determine the minimum phosphate concentration required to both obtain good growth and avoid solution depletion. Factors to consider would include length of time between solution changes, length of hydroponic growth portion of the experiments, volumes of solutions used, and size and ages of seedlings. Although additional improvements can likely be made my approach was to try to get agreement between the two methods of uptake determination. Future experiments would use progressively more dilute solution concentrations with the goal of more closely mimicking realistic soil solutions while at the same time avoiding problems of deficiency (element depletion) and instrument sensitivity.

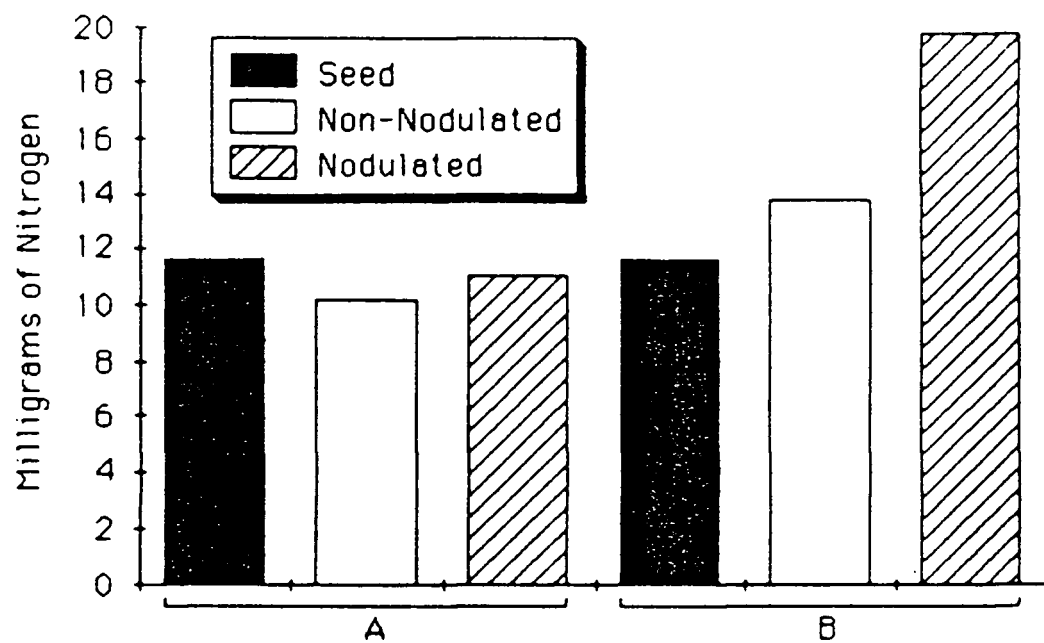


Figure 2. Experiment 3 - Comparison of nitrogen content for
A. seed and 25 day evaluation and
B. seed and 45 day evaluation.

Table 2. Micromolar concentrations of elements in three dilute nutrient solutions used in flowing culture experiments with several plant species.

Element or ion	Asher & Loneragan(1976)	Cox & Reisinger(1973)	Wild et al. (1974)
Nitrogen (NO ₃ -ion)	750	---	700
(NH ₄ -ion)	100	0-100	---
Potassium	250	110	1-33
Calcium	250	100	420-470
Sulphur	100	170	100
Magnesium	100	25	100
Phosphorus	.04-25	5	50
Iron	2*	2*	5 ⁺
Boron	3	1	2.5
Manganese	1	0.1	0.5
Zinc	0.5	0.01	0.05
Copper	0.1	0.01	0.02
Molybdenum	0.02	0.005	0.005
Cobalt	0.04	---	---

Table adapted from Asher and Edwards 1978.

* As Fe EDTA

⁺ Iron source not stated.

Table 3. Concentrations of nutrient elements in full strength Schuttler - Righetti N-free solution

Element or ion	μM	ppm
Nitrogen (NO_3^- -ion)	---	---
(NH_4^+ -ion)	---	---
Potassium	3730	147.0
Calcium	3740	150.0
Sulphur	3770	121.0
Magnesium	3780	92.0
Phosphorus	1550	48.0
Iron	26.9*	1.50*
Boron	44.4	0.48
Manganese	53.3	2.93
Zinc	26.5	1.71
Copper	.0881	.0056
Molybdenum	1.10	.106
Cobalt	.0916	.0054

* As Fe EDTA

Table 4. Experiment 1 ^a - Measured depletion from solution versus measured accumulation in plant tissue. (Δ milligrams per tube or plant)

	NO BUFFER				MES 1.5 mM			
	+ NOD		- NOD		+ NOD		- NOD	
	ACC	DEP	ACC	DEP	ACC	DEP	ACC	DEP
P	2.241	2.293	3.712	2.299	2.143	1.934	2.424	2.259
K	6.257	6.1	9.659	6.255	7.084	6.825	7.841	7.725
S	2.963	3.076	4.533	3.775	3.696	3.465	4.46	3.863
Ca	2.12	2.019	3.456	2.532	2.384	2.516	2.809	2.642
Mg	1.618	2.324	2.34	2.566	1.664	2.072	1.749	2.144

^a 26 days in perlite, MES = 1.5 mM for 10 days, mean dry weight at transplant = 380 mg.

Table 5. Experiment 2 ^a - Measured depletion from solution versus measured accumulation in plant tissue. (Δ milligrams per tube or plant)

	NO BUFFER				MES 5 mM			
	+ NOD		- NOD		+ NOD		- NOD	
	ACC	DEP	ACC	DEP	ACC	DEP	ACC	DEP
P	1.929	1.601	1.97	1.479	1.805	1.233	1.457	1.051
K	4.98	5.6	4.22	5.231	5.961	5.844	5.185	4.663
S	2.121	2.23	1.841	1.77	2.297	2.736	3.083	2.216
Ca	1.539	1.485	1.26	1.235	1.397	2.011	1.645	1.925
Mg	0.817	1.733	0.69	1.244	0.788	1.115	0.84	0.771

^a 14 days in perlite, MES = 5 mM for 8 days, mean dry weight at transplant = 180 mg.

CHAPTER IV

EVALUATION OF MES BUFFER AS PH CONTROL IN SHORT TERM
NITROGEN FREE NUTRIENT SOLUTION EXPERIMENTS USING SOYBEAN
SEEDLING

Key words - *Glycine max* L., MES, 2-N morpholinoethanesulfonic acid, Nitrogen fixation, nodulation, Rhizobium

Summary

MES buffer (2-N morpholinoethanesulphonic acid) was evaluated as to its ability to buffer small volumes of solution used for nutrient uptake experiments with nodulated and non-nodulated soybean seedlings. Solutions containing buffer and control solutions lacking buffer were regularly changed to separate direct effects of MES on macronutrient uptake from effects associated with pH differences between the buffered and the unbuffered treatments. MES was an effective buffer in unchanged solutions at 5 mM and did not have a large effect on shoot, root or nodule dry weights. The 1.5 mM concentration of MES was ineffective. Uptake of most macronutrients was similar regardless of the presence of MES, but potassium uptake was increased. Potassium uptake in non-nodulated

seedlings was most affected by the MES buffer.

Introduction

MES buffer (2-N morpholinoethanesulphonic acid) was introduced for use in biological research in 1966 (Good et al.), but only recently has interest developed in the use of MES for pH control of nutrient solutions used to grow legumes.

Bugbee and Salisbury (1985) found that MES could stabilize pH, appeared to be biologically inert, and did not interact significantly with the other solution ions in nutrient solutions. Growth rates among controls and 1 mM MES treatments for the solution volumes and time periods used were nearly identical for each species they investigated. The authors state that MES buffer warrants further consideration in nutrient research. MES has the following characteristics that make it useful in nutrient solution studies: a pKa of 6.15, high solubility, low binding constants with essential ions, and resistance to microbial breakdown. It is readily available in a pure form and has a relatively high molecular mass (195), which makes it fairly impermeable to membranes (Good et al 1966) and (Bugbee and Salisbury 1985).

Rys and Phung (1985) investigated the effects of several dipolar organic buffers on the pH control of the rooting medium, plant growth and symbiotic development in the *Trifolium repens*-*Rhizobium trifolii* symbiosis under different nitrogen nutrition regimens. They evaluated five buffers; MES, ADA, ACES, BES, and MOPS (Figure 5) at a concentration of 2.0 mM for their effects on pH. MES showed the most promise, and thus it was evaluated further at concentrations ranging from 0 - 12 mM for its effect on growth and pH in small volumes (37 ml) of unstirred solutions supporting the growth of young clover plants. The higher concentrations of MES (9 and 12 mol m⁻³) reduced the growth of the plants dependent nitrogen fixation. All concentrations of MES significantly increased plant yield and reduced nodule number for the plants provided with NH₄-ion. The authors conclude that pH control was responsible for the improved growth. However, it is important to separate direct versus indirect effects that MES may have on plant growth and mineral uptake. Both the pH and MES itself could have possible effects.

Very young soybean seedlings were used to determine whether or not statistically significant differences in shoot and root

weights or the uptake and partitioning of K, Ca, Mg, P and S could be detected between nodulated and non-nodulated soybeans grown in small volumes of nitrogen-free solution culture under two different concentrations of MES buffer. By regularly changing MES buffered and non buffered solutions it was possible to evaluate the effects of MES in systems where MES treated solutions have similar pH to the unbuffered solutions. The effects of MES on the pH's of the buffered versus the unbuffered solutions was also measured.

Materials and Methods

Two separate experiments were conducted. One used 1.5 mM MES and the other used 5.0 mM MES.

Experiment 1 - MES at 1.5 mM

Three flats (50 seed/flat) of inoculated soybeans and three flats of noninoculated soybeans were germinated in perlite with an initial application of CaSO_4 (600 ppm Ca) at day 2 and applications of 10% nutrient solution (Table 3) on days 21, 23, and 25. The pH of the nutrient solution applied was initially adjusted to 6.6 with KOH.

Both germination and the hydroponic portion of the experiment took place in growth chambers. Inoculated and noninoculated seeds were treated respectively before sowing with a distilled water slurry of *Rhizobium* peat and sterilized (autoclaved) *Rhizobium* peat. On day 26, 16 uniform seedlings of each nodulation state were weighed fresh and transplanted into 50 ml volume tubes of full strength nutrient solution (Table 3). Seedlings at transplant were chlorotic and showing other deficiency symptoms, and average seedling dry weight at transplant was calculated at 380 milligrams. About 50 uniform seedlings of each nodulation state which were not transplanted were also weighed fresh, separated into root and shoot, dried, reweighed and composited within treatments and ground for ICAP mineral analysis. These reference seedlings were used to estimate nutrient amounts initially present in the individual seedlings that were transferred into nutrient solution.

There were four solution treatments during the 10 day uptake period. PH's of all treatments were initially brought to 6.6 using KOH. Half of the seedlings were were grown in unbuffered nutrient solution (Table 3) and half were in solution buffered with MES at 1.5 concentration. Within the 2 buffer treatments half of the solutions

were changed for fresh solution at 48 hour intervals for fresh solutions, while the other half were left unchanged through the hydroponics portion of the experiment.

PH was measured for the first three 48 hour measuring periods and quickly fell to below 4.5 in all the solutions. A 1.5 mM concentration of MES was not adequate even in those solutions with substantial buffering already present due to high phosphate concentrations. Even though most of the growth period was while pH's were below 4.5, the seedlings still continued to grow and were harvested at 10 days.

These results suggested repeating the experiment using higher MES concentrations and younger seedlings at the time of transplant into solution.

Experiment 2 - MES at 5.0 mM

Germination and inoculation procedures were similar to those described above, (Experiment 1), but seedlings were in perlite only 14 days with dilute mineral solution applications on days 12 and 13. Plants were green at time of transplant, with the first pair of true leaves fully expanded, and the computed mean seedling dry weight at

transplant was 180 mg. Table 10 compares procedures and conditions for experiment 1 and 2.

On day 14, 16 uniform seedlings of each nodulation state were weighed fresh and transplanted into 50 ml volume tubes of full strength nutrient solution (Table 3). The younger seedlings used for the this experiment were more vigorous than those used in experiment 1, and were not showing signs of nitrogen or micronutrient deficiency. About 50 reference seedlings were also harvested and treated as described for Experiment 1. The four solution treatments were as described for Experiment 1 except the MES concentration was higher, at 5 mM., and plants were harvested at eight days. The solution pH's were recorded for this experiment at approximately 12 hour intervals throughout the entire 8 day period (Figure 2).

Seedling Evaluation

Harvest evaluation procedures were similar for both solution culture experiments. Roots were rinsed twice in distilled water and blotted dry. Seedlings were separated into root and shoot, and

respective fresh weights were taken. They were oven dried at 70⁰ Centigrade for at least 48 hours, reweighed, composited within treatments and ground for mineral analysis.

Standard procedures for mineral analysis by ICAP spectrometer were followed after dry ashing the plant material at 500⁰C. for four hours and dissolving the ash in 10% HNO₃. Both root and shoot tissue were analyzed for macronutrients. Single roots and shoots could not be analyzed for their mineral concentration because they did not weigh enough to make up the 250 mg. sample size required for a single spectrometer sample; thus they were composited within treatments to obtain concentration values of P, K, S, Mg, and Ca that were used, along with their respective individual root and shoot dry weights, to calculate the amounts of macronutrients present in the seedlings at the time of harvest.

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Results and Discussion (Experiments 1 and 2)

Results are shown in Tables 6, 7, and 8 for treatments effects on macronutrient accumulation and in Figure 3 for effects of MES at 5 mM on the pH of the solutions. MES at 1.5 mM did not have an effect on dry matter or macronutrient accumulation in the nodulated plants (Table 6). The non-nodulated plants showed a similar trend at this concentration. There was also little difference in treatment affects on growth and accumulation in the nodulated plants with a

MES concentration of 5 mM (Table 7). In the non-nodulated plants, however, the dry matter accumulation in the unchanged solutions buffered with 5 mM MES approached the dry matter accumulation for the unbuffered solutions which were changed at 24 hour intervals. It was also greater than the dry matter accumulation for the unbuffered solutions which were changed at 24 hour intervals. Since the pH was always slightly lower in the unchanged 5 mM MES treatment than it was in the unbuffered but changed treatments, the enhanced growth in the unchanged 5 mM MES treatment is not due to pH control. MES may have a small enhancement of growth that is not pH related.

There was a trend toward higher macronutrient accumulation in the buffered solution compared to the unbuffered solution for both the changed and the unchanged treatments; (Table 8) with the exception of phosphorus in the unchanged solutions. Another trend that is shown by all three tables, (especially for MES at 5 mM concentration in the non-nodulated seedlings) is that MES does seem to enhance potassium accumulation. Although nodule weight was not statistically compared, there were no obvious differences between the control and the MES treatments.

Figure 3 shows that MES is capable of controlling pH , keeping it between 6.6 and 5.9 over the eight days monitored. The control tubes (no buffer) which were not changed had a sharp decline in pH over the eight day accumulation period to around 4.4. These results indicate that MES merits further testing in nutrient uptake experiments to further evaluate its use as a means of pH control for nutrition experiments using nodulated and non-nodulated legumes. A small enhancement of growth by MES that is not pH related may prove to be of minor importance, but potassium differences could complicate future interpretation.

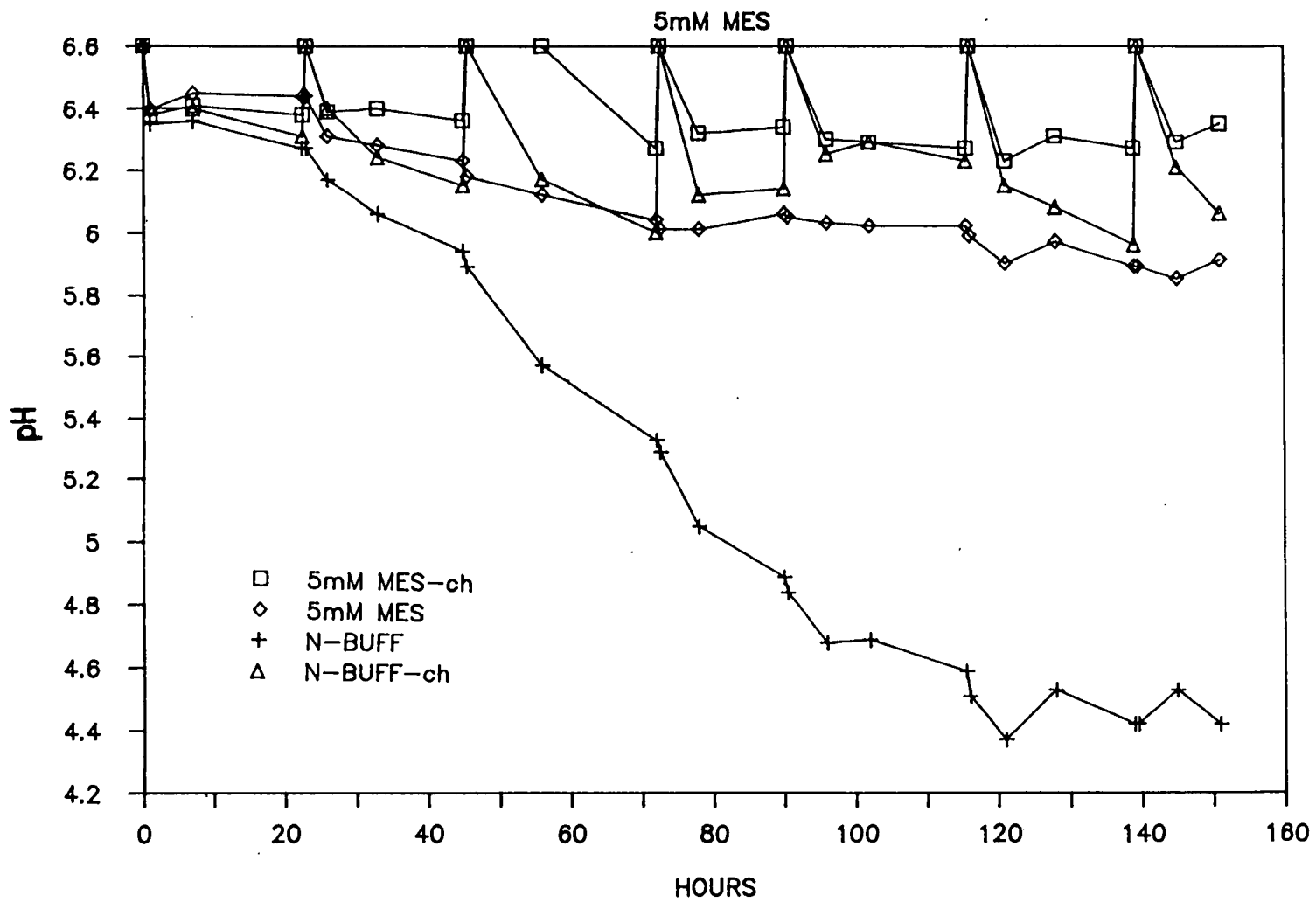


Figure 3. Experiment 2 - pH

Table 6. Experiment 1 - Comparison of solution treatments on growth and mineral accumulation in nodulated seedlings at 1.5 mM MES

	Changed		Unchanged	
	1.5 mM MES	Unbuffered	1.5 mM MES	Unbuffered
P	4.2	4.3	2.1	2.2
K	13.6	13	7	6.2
S	4.7	4.8	3.6	2.9
Ca	2.7	3.8	2.2	2.1
Mg	2.5	2.4	1.6	1.6
DM	310	290	245	210

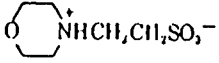
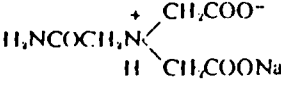
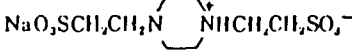
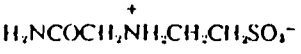

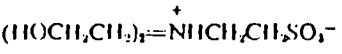
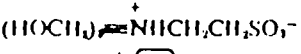

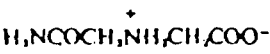
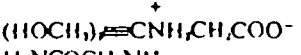
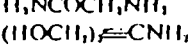
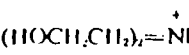


Table 7. Experiment 2 - Comparison of solution treatments on growth and mineral accumulation in nodulated seedlings at 5 mM MES

	Changed		Unchanged	
	5 mM MES	Unbuffered	5 mM MES	Unbuffered
P	2.1	1.9	1.8	1.9
K	7.2	6	5.9	5
S	2.7	2.4	2.3	2.1
Ca	1.8	1.9	1.3	1.5
Mg	1	0.9	0.7	0.8
DM	14	126	116	107

Table 8. Experiment 2 - Comparison of solution treatments on growth and mineral accumulation in non-nodulated seedlings at 5 mM MES

	Changed		Unchanged	
	5 mM MES	Unbuffered	5 mM MES	Unbuffered
P	2.4	1.1	1.4	1.9
K	6.4	3.4	5.2	4.2
S	2.5	1.5	3.1	1.8
Ca	2.5	1.3	1.6	1.2
Mg	0.9	0.7	0.6	0.4
DM	78	55	73	58

Table 9. Physical properties of buffers (Good et al. 1966).

Structure	Proposed Name	pK _a at 20°	ΔpK _a /°C	Saturated Solution at 0° (M)	Metal-Buffer Binding Constants Log K _M			
					Mg ²⁺	Cu ²⁺	Mn ²⁺	Cu ²⁺
	MES	6.15	-0.014	0.65	0.8	0.7	0.7	Negl
	ADA	6.6	-0.011	—	2.5	4.0	4.9	9.7
	PIPES	6.8	-0.0085	—	Negl	Negl	Negl	Negl
	ACES	6.9	-0.020	0.22	0.4	0.4	Negl	4.6
	Cholanine chloride	7.1	-0.027	4.2	Negl	Negl	Negl	Negl
	BES	7.15	-0.016	3.2	Negl	Negl	Negl	3.5
	TES	7.5	-0.020	2.6	Negl	Negl	Negl	3.2
	HEPES	7.55	-0.014	2.25	Negl	Negl	Negl	Negl
	Acetamidoglycine	7.7?	—	Very large	—	—	—	—
	Tricine	8.15	-0.021	0.8	1.2	2.4	2.7	7.3
	Glycinamide	8.2	-0.029	4.6	—	—	—	—
	Tris	8.3	-0.031	2.4	Negl	Negl	Negl	—
	Bicine	8.35	-0.018	1.1	1.5	2.8	3.1	8.1
	Glycylglycine	8.4	-0.028	1.1	0.8	0.8	1.7	5.8

CHAPTER V

EARLY GROWTH, MACRONUTRIENT UPTAKE AND PARTITIONING IN

GLYCINE MAX L.

Key words - Accumulation, *Glycine max* L., Macronutrients, Nitrogen, Nodulation, Partitioning

Summary

Consequences of nodulation on growth and early mineral uptake and mineral partitioning in nodulated and non-nodulated soybean seedlings grown under conditions of nitrogen deprivation were investigated in the course of studies designed to evaluate analytical procedures. Results from a solution experiment and a perlite experiment are discussed. During very early growth stages, nodulated seedlings have smaller biomasses than their non-nodulated counterparts. Dry weights of the nodulated seedlings, however, quickly overtake those of the non-nodulated seedlings until both plant types reach a similar level. Biomass increases first in the shoots, and then in roots. There is a period of time when

seedlings of the two nodulation states remain at similar dry weights. Nitrogen concentration in the nodulated seedlings increases over that in the non-nodulated seedlings during this period. This increase in concentration is seen initially in the roots, and later in the shoots.

The only significant differences found for macronutrient accumulation was for potassium and sulfur. Nodulated seedlings accumulated significantly more potassium over the 8 day uptake period than did their non-nodulated counterparts. Sulfur was also accumulated at a significantly higher rate in the roots of the nodulated seedlings.

Introduction

Few studies have concentrated on the relationship between early mineral uptake and nitrogen fixation in young legumes. Differences between nodulated and non-nodulated seedlings probably occur during early growth periods and could have important consequences if nodule establishment and early nodule function are affected.

Van Beusichem (1981) compared nutrient absorption by

effectively nodulated pea plants during dinitrogen fixation with that of ineffectively nodulated pea plants provided with nitrate as a nitrogen source. Plants were prenodulated before testing for differences in nutrient absorption between nodulation states. In order to obtain morphologically comparable plants between the two nodulation conditions, it was necessary to maintain a low growth chamber temperature of 13 degrees Centigrade. The pH was kept constant at 5.5 using a pH meter with pH stat equipment operating an automatic burette. A large amount of aerated liquid medium was required; 30 liters for each treatment of 40 plants. Two harvests were made, one at 21 days and one at 42 days.

At the 21 day evaluation by van Beusichem, both the root and the shoot biomasses of the dinitrogen fixing plants were lower by about 18% than those of the nitrate-furnished plants. By 42 days, the roots of the N_2 -fixing plants were slightly larger than the roots of the nitrate-fed plants, while the ratio of the shoot biomasses between the two nodulation states remained about the same as in the first harvest. The root and shoot tissue was dried, weighed and analyzed for total N and for K, Na, Ca, Mg, H_2PO_4 -ion, Cl, NO_3 -ion and SO_4 -ion. Chemical composition was reported in meq/kg DM.

Even though growth chamber temperatures were kept rather low to reduce initial growth of nitrate-supplied seedlings (13° C), the nitrogen fixing plants at the first harvest were about 82% of the biomass of the nitrate-supplied seedlings. In spite of considerable differences in dry weight between nodulation states, the author considers the plants in the two nodulation states to be "morphologically equivalent" and makes comparisons between nodulation states on a dry matter basis. He also concludes that nitrate-supplied plants for both treatments accumulated much more K than the dinitrogen fixing plants. These assumptions could be challenged in that they fail to take individual plant dry weights (biomasses) into account when comparing nutrient uptake between treatments.

Datta (1985) used ion selective electrodes to make simultaneous and intermittent measurement of K, Ca, and NO₃-ion by intact *Phaseolus vulgaris* L. plants under both aerobic and anaerobic conditions. Regulated proportions of air and nitrogen were passed through the solution to maintain desired oxygen tension. Very small volumes of solution were used (50 ml) and samples were

drawn at various times before solutions were completely changed in the tubes at 30 minute intervals. Anaerobiosis had a more direct and pronounced effect on K-ion absorption rather than NO_3 -ion. Significant ion efflux (particularly of K ion) occurred under anaerobic conditions. This was explained on the basis of direct effect of ATP-ase on cation absorption by inducing an electrochemical and pH gradient. No effect was found on the rate of Ca ion absorption due to anaerobiosis.

Perhaps studies such as the one described above could be attempted using a less complex setup if MES could be used to control pH instead of completely draining and refilling tubes with fresh medium after each half hour.

Experimental Materials and Methods

In the course of a series of experiments to develop monitoring techniques and study pH control, treatments were included to evaluate the consequences of nodulation on growth and mineral uptake and partitioning in very young soybean seedlings grown under conditions of nitrogen deprivation. Experimental procedures were discussed in Chapter III and Chapter IV. Experiments discussed

include the following.

1. A solution experiment looking at the organic buffer MES at 5 mM concentration as a means of pH control. (Experiment 2; Chapter IV.) Nodulated and non-nodulated seedlings were evaluated for biomass increase (roots, shoots and plants) and macronutrient mineral accumulation after an 8 day uptake period.
2. A flat experiment in which nodulated and non-nodulated soybean seedlings were grown in perlite for 25 day and 45 day evaluation periods. (Experiment 3, Chapter III).

Results and Discussion

Experiment 2 - MES at 5 mM Summary of results

At the time of transplant into hydroponic solution, after 14 days in perlite, the initial root, shoot and plant biomasses were all significantly higher for the non-nodulated plants. Initial dry weights for nodulated seedlings at the time of transplant were about 75% of the dry weights of the non-nodulated seedlings (Table 13). At harvest, after eight days in nutrient solution, there were no significant dry weight differences for roots, shoots or plants. Table

13 shows that during the 8 day period in hydroponics, the nodulated seedlings accumulated almost twice as much dry weight as the non-nodulated seedlings did, and most of this accumulated biomass was in the shoots. Although non-nodulated plants have greater initial biomass, both plant types reach a similar level until the lag phase for nitrogen fixation ends and nodulated plants are expected to have a clear advantage.

An analysis of variance was done comparing fresh weight and dry weight partitioning differences for the nodulated and non-nodulated seedlings at harvest. Table 14 summarizes ratios that were significantly different at the .01 level. Nodulated seedlings at the time of harvest had partitioned a higher percentage of their total available dry weight to the roots than had their non-nodulated counterparts. Comparison of dry weights at harvest between intact nodulated and the non-nodulated seedlings were not significantly different, but shoot dry weight in the nodulated plants at harvest made up a significantly lower percent of the total dry weight than did shoots of the non-nodulated plants. Both nodulated shoots and intact seedlings at harvest also had a lower percentage of dry weight, (or a higher percentage of water), than was found for the

non-nodulated seedlings. This could imply different rates of transpiration, and/or dry matter production) in the young seedlings that could be associated with nodulation under these conditions. It would be interesting to investigate whether this is related to hydroponic and nutrient solution situations, or whether this would occur in plants grown in a low nitrogen soil or sand.

Results in table 14 that deal with fresh weight and water content show that the percentage of dry weight in both shoots and intact plants is higher for non-nodulated seedlings than it is for the nodulated seedlings.

The non-nodulated roots at transplant had significantly higher amounts of total P, K, S, Ca, and Mg (all of the macronutrients) than the nodulated roots did (data not shown). This generally followed the biomass trend. The non-nodulated shoots at transplant also had significantly higher amounts of K and Mg than were found in the nodulated shoots. Non-nodulated seedlings at transplant had significantly higher amounts of P, K, and Mg than the nodulated plants had.

Table 15 shows that nodulated roots accumulated significantly

higher amounts of sulfur ($P < .01$) than did the non-nodulated roots, while shoots accumulated slightly higher amounts ($P < .05$) of potassium. For the intact seedlings, however, the only significant difference in accumulation was for potassium ($P < .01$). Nodulated seedlings accumulated significantly more potassium over the 8 day uptake period than did their non-nodulated counterparts.

Experiment 3 - Flat experiment

The average oven-dry weight for one of the soybean seeds after a twelve hour soak in distilled water is 169 milligrams, of which about 11 milligrams (6.5%) is nitrogen. Seed N = 6.46 to 6.85% or 10.92 to 11.58 mg./seed.

Table 11 compares root, shoot, and plant weights and computed nitrogen content for nodulated and non-nodulated soybean seedlings grown under the two different treatments.

25 day analysis

Biomass relationships at the 25 day evaluation were similar to those described for the 5 mM MES solution experiment, but the moisture content differences were not significant. Although non-nodulated seedlings were generally larger, only the roots showed

significant dry weight differences between the nodule states at the 25 day evaluation. There were no statistically significant differences in fresh weight or dry weight between the nodulated and non-nodulated shoots or whole plants. This indicates that soybean nodulation, at least under these conditions where exogenous fixed nitrogen was not supplied to the germinating seedlings, has a slight cost in biomass to the germinating seedling. The non-nodulated seedlings are slightly larger, (have higher dry weights and fresh weights) but the size difference is not statistically significant at this stage in germination. What is significantly different is the way in which this biomass is partitioned, or the percent of its biomass that the plant invests in the root. Nodulated seedlings have significantly smaller root systems at this stage and the morphology of the nodulated and non-nodulated roots is very different. The non-nodulated root is fuller and more finely branched than the nodulated root, which has fewer, but thicker, rootlets.

Nitrogen concentrations and total content of the roots, shoots, and seedlings are presented in Table 11. Nodulated roots, shoots, and seedlings were consistently significantly higher than non-nodulated roots, shoots, and plants. The total amount of nitrogen in

shoots and in intact seedlings was not statistically different between nodulated and non-nodulated plants. Furthermore, total amounts of nitrogen in both nodulated and non-nodulated seedlings were not significantly different from the original seed reserve of nitrogen, which was approximately 11 milligrams. This indicates that the nodulated seedlings are not yet receiving measurable amounts of fixed nitrogen via dinitrogen fixation. Although nodulated seedlings generally have higher nitrogen content, total shoot nitrogen and total plant nitrogen do not differ significantly between nodulation states. However total root nitrogen is significantly higher for the nodulated seedlings at the 1% level of probability. Although differences in total N content are small, the partitioning of nitrogen in seedlings for the two nodulation states does not simply follow the biomass distribution pattern. There is a significantly higher amount (milligrams) of nitrogen present in the roots of the nodulated seedlings, even though the dry weights and fresh weights of the nodulated roots are significantly lower than those of the non-nodulated roots. Nodulated seedlings put a larger proportion of their reserve seed nitrogen into their smaller,

nodulated root systems.

No significant differences in macronutrient content at the 25 day harvest were apparent for shoots or intact seedlings, but there are significantly higher amounts of P, Ca and Mg in the non-nodulated roots (Table 12). These differences are associated with biomass differences. In spite of the significant difference in root biomass between nodulation states at the 25 day harvest, the potassium content (milligrams present) is essentially the same in nodulated and non-nodulated roots. Total S content is significantly lower in the non-nodulated roots. For P, K, and Mg the seedlings were forced to use only their seed reserves until day 25. Initial CaSO_4 application resulted in fourfold more Ca and fivefold more S in seedlings than were present in seed reserves.

45 day analysis - weights

There were also no significant weight differences at harvest between the nodulation states for the forty five day evaluation. Nodulated seedlings were generally larger, though not significantly. Root biomass (dry weight) for nodulated roots had essentially caught up with that of the non-nodulated roots, even though root fresh

weights remained slightly higher for the non-nodulated roots. Although biomass differences were not apparent, evidence of nitrogen fixation is clearly reflected in the nitrogen content of nodulated seedlings (Table 11). Root percent nitrogen for both nodulation states is lower than that of the comparable roots from the earlier harvest, but there is a significant difference at the 1% level between the nodulation states. This also is true of the percent nitrogen for shoots and plants of the 45 day nodulated seedlings. The total nitrogen in the nodulated seedling at the 45 day harvest is also almost twice that of the nitrogen in the seed reserve, which indicates that by this time in the growth process, seedlings are receiving some nitrogen from the nodules. Partitioning differences caused by the original investment of seed nitrogen required to establish the nodules are no longer apparent in the seedlings at the 45 day analysis. Both nodulated and non-nodulated plants have approximately the same proportion of total N in the root tissue.

Differences found between nodulated and non-nodulated roots in the amount of P and Mg at the 25 day harvest have disappeared by the 45 day harvest. Potassium and sulfur contents in non-nodulated roots at the 45 day harvest are much higher than would be the case

if it were a simple relationship with biomass. This indicates that accumulated K and S are partitioned differently between root and shoot in the nodulated seedling than in the non-nodulated seedling. Sulfur partitioning was markedly different for the 25 day and the 45 day harvests.

Root and shoot contents were generally similar with the exception of Mg which was higher in the nodulated shoots.

Conclusions

Consequences of nodulation on growth and early mineral uptake and mineral partitioning in nodulated and non-nodulated soybean seedlings grown under conditions of nitrogen deprivation were investigated in the course of studies designed to evaluate analytical procedures. Results from a solution experiment and a perlite experiment are discussed. During very early growth stages, nodulated seedlings have smaller biomasses than their non-nodulated counterparts. Dry weights of the nodulated seedlings, however, quickly overtake those of the non-nodulated seedlings until both plant types reach a similar level. Biomass increases first in the shoots, and then in roots. There is a period of time when

seedlings of the two nodulation states remain at similar dry weights. Nitrogen concentration in the nodulated seedlings increases over that in the non-nodulated seedlings during this period. This increase in concentration is seen initially in the roots, and later in the shoots.

The only significant differences found for macronutrient accumulation was for potassium and sulfur. Nodulated seedlings accumulated significantly more potassium over the 8 day uptake period than did their non-nodulated counterparts. Sulfur was also accumulated at a significantly higher rate in the roots of the nodulated seedlings.

Table 10: Comparison of experimental conditions for soybean seedlings using two concentrations of MES buffer.

	Experiment 1	Experiment 2
Seed Inoculation	Rhizobium peat and "sterilized" Rhizobium peat	Rhizobium peat and "sterilized" Rhizobium peat
Days in Perlite	26	14
Number of days treated with 10% minus N mineral solution before transplanting	3; (Days 21, 23, 25)	2; (Days 12 and 13)
Plant condition at transplant	Plants chlorotic and showing other deficiency symptoms, first pair of true leaves and first trifoliolate fully expanded	Plants green, first pair of true leaves fully expanded
Computed mean plant dry weight at transplant	380 mg.	180 mg.
Nutrient solution volume per plant	50 mls.	50 mls.
Nutrient solution Initial pH	6.6	6.6
MES concentration	1.5 mM	5 mM
Days in nutrient solution	10	8

Table 11. Experiment 3 - Plant part weights and nitrogen content of soybean seedlings
(milligrams and percent)

25 day evaluation			45 day evaluation		
	+ Nod	- Nod		+ Nod	- Nod
R_DW **	71.5	82.7	R_DW	217.1	215
R_FW **	912.8	1109.4	R_FW	1587.7	1817.9
SH_DW	213.6	214	SH_DW	627.7	529.6
SH_FW	1222.7	1156.8	SH_FW	2126.9	1813.5
P_DW	285.1	296.8	P_DW	844.8	744.6
P_FW	2135.6	2266.2	P_FW	3714.6	3631.3
R_N **	3.004	2.208	R_N **	2.332	1.726
SH_N **	4.164	3.902	SH_N **	2.353	1.899
P_N **	3.874	3.428	P_N **	2.348	1.853
T_R_N **	2.14	1.82	T_RT_N **	5.05	3.695
T_SH_N	8.888	8.37	T_SH_N **	14.67	10.03
T_P_N	11.03	10.19	T_PL_N **	19.72	13.73

R=root
SH=shoot
P=plant

DW=dry weight (mg)
FW=fresh weight (mg)
N=Nitrogen

* significant at the .05 level
** significant at the .01 level
T=Total (mg)

Table 12. Experiment 3 - Mean amounts of macroelements in seed and soybean seedlings.
(milligrams per seed or plant part)

25 day evaluation

Seed			Roots		Shoots			Plants		
			+	-		+	-		+	-
DW	169	DW **	71	86	DW	215.9	212.5	DW	286.9	299.2
P	1.156	P **	0.0268	0.354	P	0.846	0.814	P	1.114	1.168
K	3.084	K	0.756	0.754	K	2.055	1.957	K	2.811	2.711
S	0.348	S *	0.74	0.624	S	0.871	0.803	S	1.611	1.427
Ca	0.518	Ca *	0.345	0.466	Ca	1.548	1.453	Ca	1.893	1.919
Mg	0.412	Mg *	0.061	0.09	Mg	0.338	0.332	Mg	0.399	0.422

45 day evaluation

Seed			Roots		Shoots			Plants		
			+	-		+	-		+	-
DW	169	DW	222.6	231	DW	645.6	580.1	DW	868.2	811.2
P	1.156	P	0.455	0.441	P	1.079	0.939	P	1.534	1.38
K	3.084	K **	1.099	1.623	K	4.792	4.448	K	5.891	6.07
S	0.348	S **	1.062	1.553	S	2.118	2.21	S	3.179	3.762
Ca	0.518	Ca **	0.727	0.562	Ca	2.907	2.964	Ca	3.634	3.527
Mg	0.412	Mg	0.289	0.311	Mg *	0.71	0.597	Mg	0.999	0.908

* significant at the .05 level

** significant at the .01 level

Table 13. Experiment 2 - Biomasses, accumulated dry weights (mg) and ratios comparing nodulated and non-nodulated seedlings at transplant and harvest.

	nodulated	non-nodulated	+/-
IDW-R**	22.3	33.5	.67
S**	127.5	166.1	.77
P**	149.8	198.6	.75
EDW-R	69.1	73.1	.95
S	196.4	192.2	1.02
P	265.5	258.4	1.02
ADW-R	46.9	39.6	1.18
S	68.9	26.1	2.64
P	115.7	59.8	1.93

IDW = Initial dry weight, at time of transplant

EDW = End dry weight, at time of harvest

ADW = Accumulated dry weight over the 8 day uptake period

** significant at $p < .01$

Table 14. Experiment 2 – Plant part weight ratios for nodulated and non-nodulated seedlings at harvest.

	nodulated	non-nodulated
R/P-DW**	.259	.216
S/P-DW**	.740	.784
R/S-DW**	.352	.283
S-DW/FW**	.152	.199
P-DW/FW**	.129	.164
S-H ₂ O/FW**	.848	.800
P-H ₂ O/FW**	.870	.836

R/P-DW = percent of seedling dry weight that is root

S/P-DW = percent of seedling dry weight that is shoot

R/S-DW = ratio of root dry weight to shoot dry weight

S-DW/FW = percent of fresh shoot that is shoot dry weight

P-DW/FW = percent of seedling that is dry weight

S-H₂O/FW = percent of shoot that is water

P-H₂O/FW = percent of seedling that is water

** significant at the .01 level

Table 15. Experiment 2 - Partitioning of accumulated macronutrients in nodulated and non-nodulated seedlings at harvest.

Roots (mg. of elements accumulated)

Element	Nodulated	Non-nodulated
P	1.13	1.19
K	2.98	2.76
S**	0.96	0.84
Ca	0.26	0.26
Mg	0.26	0.27

Shoots (mg. of elements accumulated)

Element	Nodulated	Non-nodulated
P	0.81	0.69
K*	3.02	2.37
S	1.36	1.49
Ca	1.32	1.22
Mg	0.62	0.52

Plants (mg. of elements accumulated)

Element	Nodulated	Non-nodulated
P	1.93	1.88
K**	6.00	5.13
S	2.32	2.33
Ca	1.59	1.48
Mg	0.88	0.79

* significant at $p < 0.05$

** significant at $p < 0.01$

CHAPTER VI

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APPENDIX

CHAPTER VII

APPENDIX

DISCUSSION OF ANALYTICAL AND COMPUTER METHODS USED

Comparing treatment differences in tissue element concentration by percentage fails to consider differences between plant weights and root/shoot biomass distribution. One research goal was to develop computer assisted procedures to quickly convert raw data obtained from ICAP spectrometry and the microKjeldahl-autoanalyzer procedure into absolute amounts of micronutrient uptake, either from measurements of plant tissue concentration or depletion from nutrient solutions.

Programs using dBase III software were written to convert data from tissue concentration expressions into milligrams of each element (in both roots and the shoots) that were accumulated between transplant and harvest. To determine the initial macronutrient contents of the seedlings before the measured nutrient uptake period, reference seedlings were used in the solution experiments. These were uniform seedlings harvested and analyzed at the same time that the experimental seedlings were transplanted into solution. Fresh and dry weights were taken for the individual

roots and shoots of the reference seedlings. Element concentration data was then determined for the reference roots and shoots using micro-Kjeldahl-autoanalyzer assay for nitrogen and the ICAP spectrometer for the macronutrients.

Fresh plant weights were obtained for individual experimental seedlings at the beginning of the uptake periods. These fresh weight values, along with root/shoot ratios, dry weight percentages and element concentration data obtained from the reference seedlings, were used in the computer programs to determine initial amounts of macronutrients present in the individual experimental seedlings at the beginning of the uptake period. When the seedlings were harvested at the end of the solution uptake period, both fresh and dry weights for roots and shoots were obtained. Roots and shoots were analyzed for final macronutrient concentration. Data was then processed through a series of additional computer programs. Net amounts for each macroelement accumulated over the time of uptake was computed by subtracting initial amount present from the amount present at harvest.

Developing similar methods of determining macroelement depletion from mineral solutions over the time of mineral

accumulation by the seedlings was a related objective. This was accomplished by sampling and evaluating nutrient solutions for macronutrients at the beginning and the end of the plant uptake period. Volumes of solution were kept constant at 50 cm³ by addition of distilled water. Concentration data was converted to absolute amount present in the solution for each element at each evaluation, and depletion was determined by subtraction.

Printouts of programs used for experiment 3 follow.

```
USE 7SPEC
COPY STRU TO FILE1
COPY STRU TO FILE2
A=1
DO WHILE A<55
    N=1
    DO WHILE N<6
        COPY NEXT 1 TO TEMP
        USE FILE1
        APPEND FROM TEMP
        N=N+1
    ENDDO
USE 7SPEC
SKIP
A=A+1
    N=1
    DO WHILE N<6
        COPY NEXT 1 TO TEMP
        USE FILE1
        APPEND FROM TEMP
        N=N+1
    ENDDO
USE 7SPEC
STORE 2 TO NSKIP
SKIP NSKIP
A=A+2
    N=1
    DO WHILE N<6
        COPY NEXT 1 TO TEMP
        USE FILE1
        APPEND FROM TEMP
        N=N+1
    ENDDO
USE 7SPEC
STORE 3 TO NSKIP
SKIP NSKIP
A=A+3
    N=1
    DO WHILE N<5
        COPY NEXT 1 TO TEMP
        USE FILE1
        APPEND FROM TEMP
        N=N+1
    ENDDO
```

```
USE 7SPEC
STORE 4 TO NSKIP
SKIP NSKIP
A=A+4
    N=1
    DO WHILE N<6
    COPY NEXT 1 TO TEMP
    USE FILE1
    APPEND FROM TEMP
    N=N+1
    ENDDO
USE 7SPEC
STORE 5 TO NSKIP
SKIP NSKIP
A=A+5
    N=1
    DO WHILE N<3
    COPY NEXT 1 TO TEMP
    USE FILE2
    APPEND FROM TEMP
    N=N+1
    ENDDO
USE 7SPEC
STORE 6 TO NSKIP
SKIP NSKIP
A=A+6
    N=1
    DO WHILE N<3
    COPY NEXT 1 TO TEMP
    USE FILE2
    APPEND FROM TEMP
    N=N+1
    ENDDO
USE 7SPEC
RNO=8
DO WHILE RNO<15
GOTO RNO
    N=1
    DO WHILE N<3
    COPY NEXT 1 TO TEMP
    USE FILE2
    APPEND FROM TEMP
    N=N+1
    ENDDO
USE 7SPEC
RNO=RNO+1
ENDDO
```

```
USE 7SPEC
RND=15
  DO WHILE RND<16
    GOTO RND
    N=1
    DO WHILE N<7
      COPY NEXT 1 TO TEMP
      USE FILE2
      APPEND FROM TEMP
      N=N+1
    ENDDO
  DO PLAY
  DO PLAY2
  DO PLAY3
```


*THIS IS REPINIT1.PRG-

```
USE 7NITPER
COPY STRU TO TEMP1
RNO=1
DO WHILE RNO<6
GOTO RNO
N=1
    DO WHILE N<9
    COPY NEXT 1 TO TRANSIT
    USE TEMP1
    APPEND FROM TRANSIT
    N=N+1
    ENDDO
USE 7NITPER
RNO=RNO+1
ENDDO
DO REPINIT2
ENDDO
CLEAR ALL
DO REPINIT3
ENDDO
CLEAR ALL
DO REPINIT3
ENDDO
CLEAR ALL
DO REPINIT4
ENDDO
CLEAR ALL
DO REPINIT5
ENDDO
CLEAR ALL
DO REPINIT6
ENDDO
CLEAR ALL
DO REPINIT7
ENDDO
CLEAR ALL
DO REPINIT8
ENDDO
```

```
USE 7SPEC
COPY STRUCTURE TO FILE3
COPY STRUCTURE TO FILE4
RNO=16
DO WHILE RNO<26
GOTO RNO
N=1
    DO WHILE N<7
    COPY NEXT 1 TO TEMP
    IF RNO<21
        USE FILE3
    ELSE
        USE FILE4
    ENDIF
    APPEND FROM TEMP
    N=N+1
    ENDDO
USE 7SPEC
RNO=RNO+1
GOTO RNO
IF RNO=20 .OR. RNO=25
N=1
    DO WHILE N<8
    COPY NEXT 1 TO TEMP
    IF RNO<21
        USE FILE3
    ELSE
        USE FILE4
    ENDIF
    APPEND FROM TEMP
    N=N+1
    ENDDO
    RNO=RNO+1
ELSE
ENDIF
ENDDO
```

REPBIT7 PRG

```
*THIS REPLICATES 6RECORDS 5X EACH
*___+NOD__TR2__ROOTS
USE 7NITPER
COPY STRU TO TEMP3
RNO=16
DO WHILE RNO<22
GOTO RNO
N=1
    DO WHILE N<6
    COPY NEXT 1 TO TRANSIT
    USE TEMP3
    APPEND FROM TRANSIT
    N=N+1
    ENDDO
USE 7NITPER
RNO=RNO+1
ENDDO

ENDDO
CLEAR ALL
DO REPBIT7
ENDDO
CLEAR ALL
DO REPBIT8
ENDDO
```