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JOHNSTON WR

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BIOCHEMICAL AND PHYSIOLOGICAL STUDIES ON RACES OF
Festuca rubra L. FROM A SERPENTINE
AND NON-SERPENTINE SOIL.

A thesis submitted for the degree of

Doctor of Philosophy

in the

University of Stirling

by

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ABSTRACT

Soil solutions were extracted, using a centrifugation method, from a Scottish serpentine soil from Meikle Kilrannoch, Angus. Analyses showed that the soil was outstanding for its very high ratio of magnesium to calcium and it also contained relatively high levels of nickel.

The suitability of the centrifugation method of extracting soil solutions from a range of serpentine soils from Britain and Rhodesia was assessed. Whilst the technique seemed successful for the Meikle Kilrannoch soil, the validity of its application to other soils was not proven.

The levels of elements in Meikle Kilrannoch soil solutions were used as the basis for the composition of the solutions for water culture experiments. These experiments showed large differences between serpentine and non-serpentine Festuca rubra in response to a number of elements. Increasing solution nickel and magnesium reduced the dry weights of the non-serpentine plants and caused them to absorb large quantities of potentially toxic metals. However, increasing calcium supply appeared to ameliorate much of the toxic influences of nickel and magnesium in the non-serpentine race. By contrast, high calcium supply, particularly when micro-nutrient levels were high, depressed the growth of the serpentine race. In both races, increasing calcium supply promoted the absorption of magnesium; conversely,

increasing magnesium supply depressed calcium uptake. The serpentine race maintained a lower tissue Mg/Ca ratio than the non-serpentine race in all treatments. Non-serpentine plants seemed unable to maintain high tissue potassium levels in serpentine-simulated conditions.

Chromium was shown to have some effect in determining growth rates of both races in the experimental conditions but at concentrations which were not related to those in the soil solution.

Assays were carried out of the activity of root-surface acid phosphatase in different culture media. In the serpentine race, when micronutrients were absent or in short supply, nickel specifically enhanced the enzyme activity. This suggests that the serpentine race has evolved a use for, if not a strict requirement for, nickel. Increasing micronutrients removed the enzyme-activity enhancement of nickel and appeared to inhibit enzyme activity in the serpentine race. No root-surface phosphatase activity enhancement by nickel was noted for the non-serpentine race. The serpentine race also apparently required higher levels of magnesium for optimum enzyme activity.

Carbohydrates accumulated in the non-serpentine race when nickel and magnesium supply were high, indicating a generally unfavourable metabolic response to these conditions. This high-carbohydrate effect was dependent on

the supply of micronutrients and calcium. The serpentine race appeared able to synthesize and use carbohydrate in all the experimental treatments.

A short investigation was undertaken into the chemical form of nickel in MK1 and Rhodesian soil solutions.

Finally, the data derived from the water culture experiments were used to construct a computer model of the MK1 soil/Festuca rubra interrelationship.

CHAPTER 1

INTRODUCTION

Serpentine soils are rich in magnesium, have relatively high levels of nickel, chromium, cobalt and iron but are generally low in calcium and major nutrients. They often bear a distinctive vegetation and although there is a considerable body of work which relates the serpentine soils to their vegetation (reviewed in Proctor and Woodell, 1975) a number of problems remain to be solved.

One approach to understanding serpentines has been the chemical analysis of plants and soils and this has yielded some valuable information (e.g. Lyon et al. 1971; Proctor, Burrow and Craig 1979).

However, an experimental approach is necessary if the causal factors and mechanisms of tolerance in serpentines are to be elucidated. Unfortunately many of the experimental methods employed to date have not been entirely satisfactory.

Crop plants have often been used to study the effects of putatively important toxic factors. Oats (Avena sativa*) have been a favourite test plant (e.g. Hunter and Vergnano 1953, Soane and Saunder 1959, Spence and Millar 1963 and Proctor 1971a) since they produce well-documented characteristic toxicity symptoms. However,

*Plant names, unless the author is quoted, are taken from Clapham Tutin and Warburg (1962).

data from crop-plant experiments must necessarily be of limited relevance in explaining the response of native plants in serpentine soils.

Tolerance tests involving root growth measurements in single salt solutions have been used by Proctor (1971b) to demonstrate the apparent resistance of serpentine races to magnesium and nickel. Such tests are only of limited value for many reasons, including their failure to take into account the effects of interactions between soil ions (c.f. Proctor and McGowan, 1976).

The growth of serpentine and non-serpentine races of plants in complete nutrient water or sand culture (e.g. Madhok (1965), Main (1970), Marrs and Proctor (1976) has provided information concerning the ways in which races adapt to serpentine conditions. However, much of this culture media work has been restricted to a study of calcium and magnesium. Moreover, the composition of the nutrient media differed in important ways from the solutions which are likely to bathe roots in serpentine soils.

Johnston (1976) carried out a preliminary investigation of the adaptation of a race of Festuca rubra to the extremely toxic Scottish serpentine soil from Meikle Kilrannoch, Angus. (A full site description is given in Johnston 1976.) This work involved growing serpentine and non-serpentine races in soil from Meikle Kilrannoch, (henceforth called MK1). Although my results broadly

agreed with the conclusions of Proctor (1971a and b) about the key role of magnesium in this soil there was, it was felt, much scope for continuing this study using more refined water culture techniques.

With the exception of a few researches (notably those of Vergnano and co-workers and Willett (1975)) investigations of serpentine plants at the biochemical level have rarely been carried out. Accordingly, in the present study I conducted investigations into the activity of a root-surface enzyme and also changes in plant carbohydrates in response to solutions of different mineral composition.

In all the experiments described in this thesis two races of Festuca rubra were compared: the MK1 race (from Meikle Kilmarnoch) and the NS (non-serpentine) race (from an acid brown earth within the grounds of Stirling University). The soil at the site of collection of the NS race has been analysed and described by Johnston (1976). Voucher specimens of both races of F. rubra investigated in this study are stored in the herbarium, Biology Department, University of Stirling.

CHAPTER 2

THE EXTRACTION AND ANALYSIS OF SOIL SOLUTION FROM MK1 SOILS.

Introduction

Soils form complex and heterogenous media of organic and inorganic constituents which include an aqueous solution. The soil solution bathes the plant roots and is influenced by many factors. Microorganisms and roots withdraw water and nutrients; respiratory carbon dioxide and exudates of varying types can change the solution's composition. There is a constant ionic exchange between the soil solid phase and its surrounding solution.

Because of experimental difficulties with soils as growth media, workers in plant mineral nutrition have often used water cultures which enable precise control of the solution bathing the roots. When formulating a nutrient solution to simulate a particular soil, a knowledge of the concentration of ions in the soil solution is most useful. For example, Nielsen (1972) pointed out that the concentration of a particular ion in the solution at the active root surface is a prime factor in determining the rate of uptake of that ion. In a similar way, yield and plant metal content may be influenced by soil solution metal concentrations.

There have been various direct and indirect methods developed for isolating soil solutions (Nielsen, 1972). Direct methods estimate the chemical composition of the

soil solution after isolation by :

- (1) soil compaction
- (2) displacement with a) water b) methanol-acetone
c) liquid paraffin d) air (the pressure membrane
method)
- (3) centrifugation
- (4) suction through suction cups
- (5) absorption on porous material such as filter
paper or "ceramic points".

In the indirect methods the chemical composition of filtrates of soil suspensions in equilibrium with water or dilute salt solutions is determined.

Nielsen (1972) pointed out that there are drawbacks to most of the techniques although he favoured the suction-filter method since this enables removal or solution from soils without disturbing crops roots. Davies and Davies (1963), on the other hand, supported centrifugation in preference to displacement of soil solution by another liquid, which they judged might undesirably alter the soil, and to extrusion under high pressure, which they claimed was unsuitable for routine work. Soils analysed here were extracted using centrifugation in a similar way to that described by Anderson et al. (1973) who worked with an Australian serpentine soil.

The most commonly used soil extractants in routine analyses, acetic acid and ammonium acetate, often give very different estimates for heavy metals within the same

same soil (Proctor and Woodell 1975). Moreover both extractants give estimates of the relatively labile soil metals which may not be related in a simple way to their concentration in the soil solution. Much water culture work would suggest the soil solution concentration is the critical factor. In addition, for any understanding of nutrient interaction, the concentration of each mineral component bathing the plant roots should be known.

Materials and Methods

MK1 soils were sampled to a depth of 10 cm and eleven samples were used in subsequent analyses. Samples were air-dried, ground and then sieved through a 2 mm sieve.

Each sample was placed in a 7 cm plastic pot standing in a dish. Soils were watered with deionized water until a 2 mm depth of water collected in the dish. Samples were maintained in this way for three days by addition of deionized water to the dish. Samples were then centrifuged at 12,000 'G' for 20 min at 0°C. Anderson et al. (1973) pointed out that this removed 58% of the available water in soils derived from ultrabasic rocks which they had sampled. They calculated this figure (of 58% water displacement) after measuring the soils' -15 bar retentivity value to be 16.2%, using an acetate membrane in a pressure chamber. The necessary apparatus for determining retentivity values was not available in Stirling and it was decided simply to follow Anderson's technique of

spinning at 12,000 'G'. The experimental results in later sections indicate that this method does extract ecologically meaningful levels of elements in the MKI soil solution.

The solutions obtained were analysed for nickel, chromium, cobalt, magnesium, calcium, potassium, sodium and iron by atomic absorption spectrophotometry using a Perkin-Elmer 373 instrument. In addition the concentrations of nitrate, ammonium and phosphate ions were determined by colorimetric methods and sulphate ions by a turbidimetric technique as described by Allen et al. (1974).

Results

Means and standard errors are presented (Table 1) for soil solution analyses from MKI (the results of individual analyses are given in Appendix IV). Magnesium was in a large excess compared with calcium and appeared to be the dominant cation in these soils. It was notable that the predominant form of nitrogen was nitrate ions. Nickel was comparatively high although chromium and cobalt (metals often thought to be important in serpentine plant/soil interrelationships) were below the detection limit of 0.1 ppm.

Data in Table 1 indicate an imbalance between cations and anions. This may be explained since only a limited number of elements in soil solutions could be

Table 1. Mean and standard error (S.E.) of ion concentration (ppm) in MKI soil solutions.*

	Ni	Cr	Co	Fe	K	Na	Mg	Ca	Mg/Ca	NH ₄ ⁺	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻
Mean	0.6	<0.1	<0.1	0.2	5.3	14.8	182	10.8	16.5	0.6	188	4.4	26
S.E.	0.1			0.1	0.5	0.6	15	0.5	1.06	0.1	7	0.7	4

*Throughout this thesis, unless otherwise stated, ions are represented without regard for possible hydration states.

determined because of the small volumes of solutions derived from each sample. The imbalance (the analyses indicated a preponderance of positively charged ions) is probably corrected by chloride ions which may be recruited to the soil via rainfall.

Discussion

There are many references concerning "plant available" elements in serpentine soils, for example Proctor and Woodell (1975), Shewry and Peterson (1976) and Slingsby and Brown (1977). Extractants used include dilute acetic acid, neutral ammonium acetate and chelating agents such as 10% w/v disodium EDTA. It would be expected that metal levels extracted by the above reagents would be higher than concentrations found in soil solutions. Johnston (1976) found MK1 nickel levels to range from 5.5 ppm to 73 ppm in ammonium acetate extracts. These values greatly exceed the nickel levels in MK1 soil solutions.

There is a general shortage of soil solution data for serpentine soils. Anderson et al. (1973) found nickel to range between 0.13 ppm and 3.25 ppm in soil solutions when soils derived from ultrabasic rocks in New South Wales, Australia, were extracted by centrifugation. They also gave values for cobalt (0.03 - 0.14 ppm) and chromium (0.02 ppm) but magnesium and calcium were not determined. The highest nickel level Anderson et al. recorded was found in an area where oat plants showed severe nickel toxicity symptoms. Whilst the concentrations of nickel

noted in MK1 soils were generally lower than those found by Anderson et al., data presented in later chapters will illustrate that nickel may be ecologically important at MK1.

The high magnesium concentration in the MK1 soil solution confirms that this element is likely to be an important influence on plant growth at this site. Proctor (1971a) and Johnston (1976), on the basis of growth experiments in MK1 soil had concluded that high magnesium levels are the predominant toxic influence on non-serpentine races of plants.

The high nitrate levels presumably represent the major balancing ion for magnesium. There is evidence to suggest that many serpentine soils contain little or no nitrogen fixing bacteria (Proctor and Woodell, 1975) and leguminous plants are absent from Meikle Kilrannoch. It seems that the origin of the nitrate in MK1 soils is from precipitation. These soils are poorly drained which, in the absence of high soil organic matter, helps account for the accumulation of nitrate. Since nitrate analyses have rarely been attempted in serpentine soils comparisons with other situations are difficult.

The comparatively low phosphorus level in MK1 soil solutions represents the small soluble fraction of a fairly high total concentration of this element since Johnston (1976) found total phosphorus at this site ranged between 260 and 1200 ppm (mean 820 ppm).

CHAPTER 3

THE GROWTH OF MK1 AND NS RACES OF Festuca rubra IN WATER CULTURE

Introduction

The growing of different races of single species in contrasting soils gives information on plant tolerance to a range of edaphic conditions. However, with such an approach it is often impossible to study the effect of individual elements and their interactions with reference to the plant response. This is mainly because the chemical composition of soils is difficult to manipulate beyond simply adding fertilizers. Also because of the heterogeneity of soils and the complexity of the root/soil interface it is difficult to clearly define the mineral composition of the root environment.

Water culture techniques offer an alternative approach to the study of the plant response to its mineral environment. The mineral composition of solutions can be clearly defined, and interactions between constituents of the growth media followed. Water culture also permits the mineral content of roots to be analysed, a difficult procedure for soil-grown plants because of contamination of roots by soil particles.

The value of water culture experiments depends partly on the composition of solutions used and it is important to try to simulate the ionic environment of roots in the

field. In the investigation which follows, the differences in response of MK1 and NS races of Festuca rubra were studied in water culture. A culture medium devised from the soil solution (Table 1) was used. Because of the limited volume of samples available, iron was the only micronutrient determined in the soil solutions. Micro-nutrient concentrations used to prepare nutrient solutions were based on the measured iron levels as described in Table 3. Nutrient and nickel levels were varied in a series of experiments to study their importance individually and in interactions.

Materials and Methods

(a) Preparation of plant material

Tillers of MK1 and NS F. rubra were grown in John Innes No. 2 compost for eight weeks. They were then cut below the first node and rooted (figure 1) in a solution of 0.2 g l^{-1} calcium nitrate (which had been found to promote rooting) in a growth room maintained at 20°C with 16 h daylength for eight days.

(b) Nutrient solutions and culture media

The concentrations of stock nutrient and nickel solutions and the final concentration of each component in the culture media used are given in Tables 2-6. Culture media were prepared by mixing macronutrients, micronutrients and magnesium stock solutions in about two l of deionized water followed by the addition of deionized water until the final dilution was 100 times. The pH of

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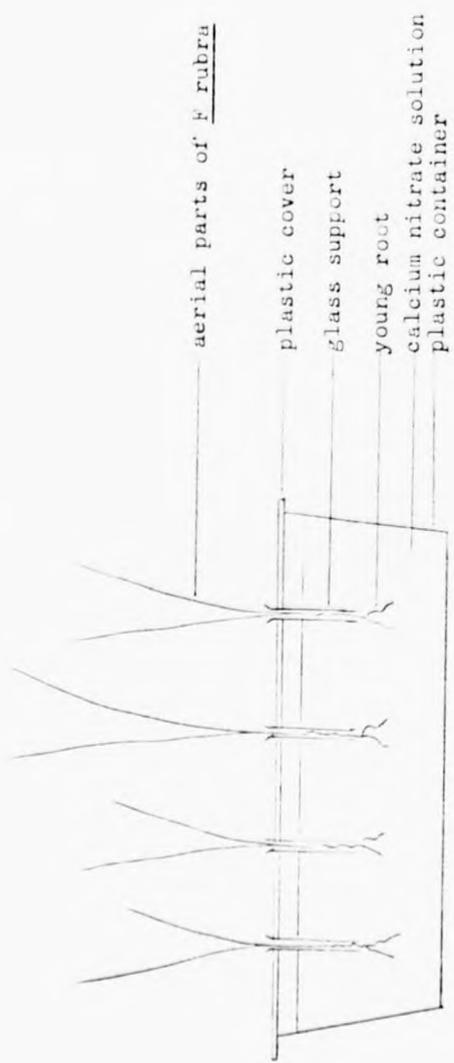


Figure 1 apparatus used to initiate rooting in F. rubra.

Table 2. Macronutrient solutions used to prepare media in which F. rubra was grown.

Reagent	Concentration (mM)		
	"low" calcium	stock "high" calcium	"low" calcium "high" calcium
$\text{NH}_4\text{H}_2\text{PO}_4$	10.0	10.0	0.1
KH_2PO_4	20.0	20.0	0.2
$\text{Ca}(\text{NO}_3)_2$	30.0	30.0	0.3
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0	90.0	0.9
NaCl	60.0	60.0	0.6
Na_2SO_4	3.0	3.0	0.03

In "high" calcium solutions additional calcium was added in equivalent amounts of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and not as $\text{Ca}(\text{NO}_3)_2$ in order to keep the nitrate concentration similar to that found in MKI soil solution.

Table 2. Macronutrient solutions used to prepare media in which *F. rubra* was grown.

Reagent	Concentration (mM)		
	"low" calcium	stock "high" calcium	"low" calcium "high" calcium culture media
$\text{NH}_4\text{H}_2\text{PO}_4$	10.0	10.0	0.1
K_2HPO_4	20.0	20.0	0.2
$\text{Ca}(\text{NO}_3)_2$	30.0	30.0	0.3
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0	90.0	0.9
NaCl	60.0	60.0	0.6
Na_2SO_4	3.0	3.0	0.03

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Table 3. Micronutrient solutions used to prepare media in which F. rubra was grown.

Reagent	Concentration (μM)			
	stock		culture media	
	"low"	"high"	"low"	"high"
NaFeEDTA	1000	1000	10.0	10.0
H_3BO_3	460	4600	4.6	46.0
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	91.0	910	0.91	9.1
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	7.6	76.0	0.076	0.76
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	3.2	32.0	0.032	0.32
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	5.2	52.0	0.052	0.52

Iron was the only micronutrient which was analysed for in MK1 soil solutions and the levels found were used in all culture media. The iron level was about 10% of that used in Hoagland and Arnon culture solutions. Consequently, all micronutrients (except iron which was used at the same concentration throughout) were provided in "high" micronutrient treatments (similar to Hoagland and Arnon levels) and reduced by a factor of 10 for "low" micronutrient treatments.

Table 4. Magnesium solutions used to prepare media in which F. rubra was grown.

stock magnesium concentration (M)	Reagent	Reagent concentration (M)	Magnesium concentration in culture media (mM)
0.75	Mg(NO ₃) ₂ ·6H ₂ O	0.75	7.5
	NaNO ₃	0	
0.40	Mg(NO ₃) ₂ ·6H ₂ O	0.40	4.0
	NaNO ₃	0.70	
0.15	Mg(NO ₃) ₂ ·6H ₂ O	0.15	1.5
	NaNO ₃	1.2	

NaNO₃ was added at the concentrations shown to maintain similar NO₃⁻ concentrations in magnesium treatments below 7.5 mM.

Table 5. Nickel solutions involved in the preparation of growth media.

Reagent	nickel concentration (ppm)	
	stock	in culture media
$\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	420	0.7
"	840	1.4
"	1260	2.1

Table 6. Composition of culture media in different treatments.

Experiment No.	Final concentration used in culture media			
	macronutrients	micronutrients	magnesium (mM)	nickel (ppm)
1	"low" calcium	"high"	1.5, 4.0, 7.5	0, 0.7, 1.4
2	"low" calcium	"low"	1.5, 4.0, 7.5	0, 0.7, 1.4
3	"high" calcium	"low"	1.5, 4.0, 7.5	0, 0.7, 1.4
4	"high" calcium	"high"	1.5, 4.0, 7.5	0, 0.7, 1.4

Macro- and micronutrient values refer to those given in Tables 2 and 3.

the media was then adjusted to 5.5 using hydrochloric acid or ammonia solution. The addition of nickel to treatments is described in the following section (c).

(c) Growth experiments

In the experiments summarised in Table 6, the interaction of three levels of magnesium (1.5, 4.0, 7.5 mM) with 0, 0.7, 1.4 ppm nickel was studied for two levels of calcium and two levels of micronutrients. A fourth level of nickel (2.1 ppm) was initially incorporated but proved lethal for NS plants, at higher magnesium levels, and was abandoned.

600 cm³ glass beakers covered with aluminium foil were used as containers. In treatments containing nickel, 1 cm³ of the appropriate stock nickel solution was placed in the beaker and made up to 600 cm³ with a culture medium of the appropriate magnesium concentration. In 0 ppm nickel treatments, 1 cm³ of water was added instead of nickel solution. Tillers in their glass supports were removed from the rooting solution, rinsed with deionized water and placed in pairs in the beakers of culture media, as shown in figure 2.

In all experiments, three replicates of each combination of nickel and magnesium were used. For each experiment, material was grown in a randomized block design in a growth room maintained at 20°C and 16 h daylength. Culture media in each beaker were topped up daily with deionized water and changed weekly. The experiments

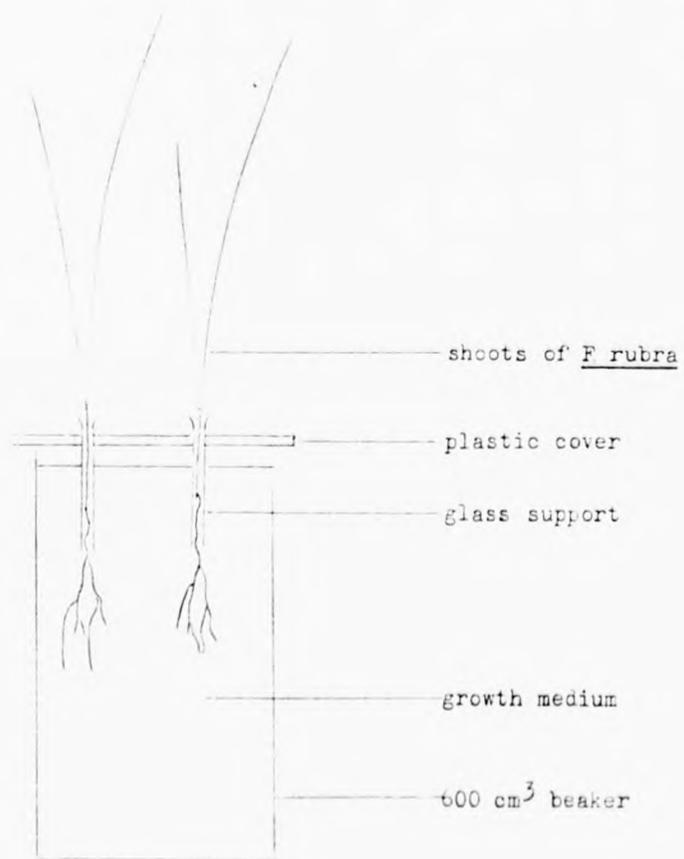


Figure 2 Apparatus used to conduct culture media experiments.

were harvested after eight weeks and the plants were then washed thoroughly with deionized water. Shoots were separated from roots using scissors, placed in separate paper bags, dried at 60°C for five days and then weighed. In the case of experiment No. 2 the fresh weight of MK1 and NS shoots were noted after four and eight weeks growth.

(d) Chemical analyses

Oven-dry shoot and root material were wet-ashed with concentrated nitric acid and subsequent analyses carried out using a Perkin-Elmer model 373 atomic absorption spectrophotometer.

Results

(Unless otherwise stated, data were subjected to analysis of variance. The analysis was designed to determine the variance ratio due to replicates, treatment combinations, race, nickel, magnesium, interactions between race x nickel, race x magnesium, nickel x magnesium and race x nickel x magnesium. The variance ratio data were used to indicate significant differences between treatments. When significant differences due to magnesium or nickel (treatments which consisted of three levels of the metal) were noted, the individual levels accounting for the significant differences were identified by comparing the standard errors of the means resulting from each treatment. This comparison was achieved in the following way. The standard error of a mean (or a set of means) was multiplied by the value of "t" corresponding

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to the 1% level of probability for $2n - 2$ (where n = number of data items constituting each mean) degrees of freedom. When the addition (or subtraction) of the standard error x "t" value to one of the means being compared caused the means to overlap, then the two means were judged to be not significantly different. Unless otherwise stated, statistical significance referred to in the text is at the 1% level.

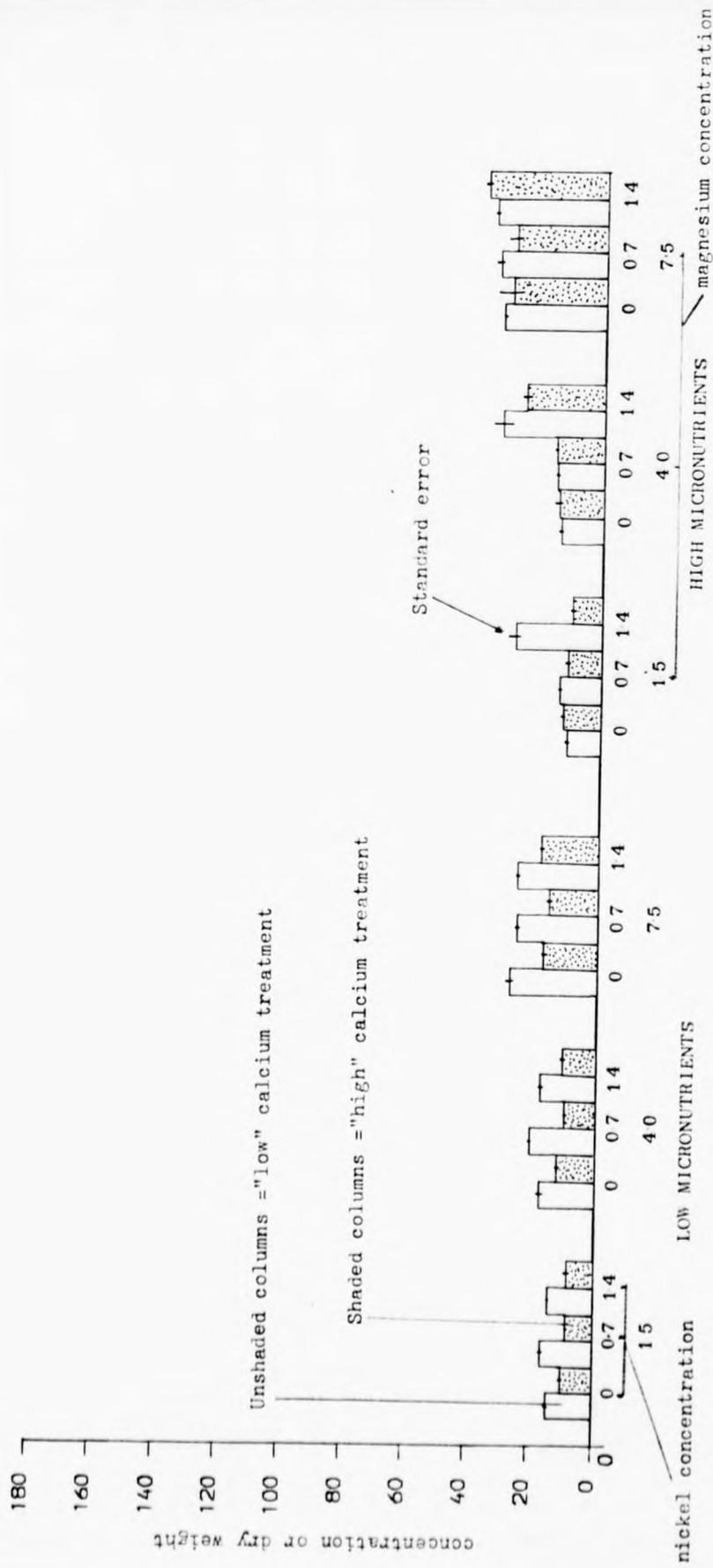
Throughout the text, the terms "high" or "low" micronutrients and "high" and "low" calcium refer to treatments, the composition of which, is defined in Tables 2 and 3 under "culture media".)

1) Dry Weights (figure 3A - 3D)

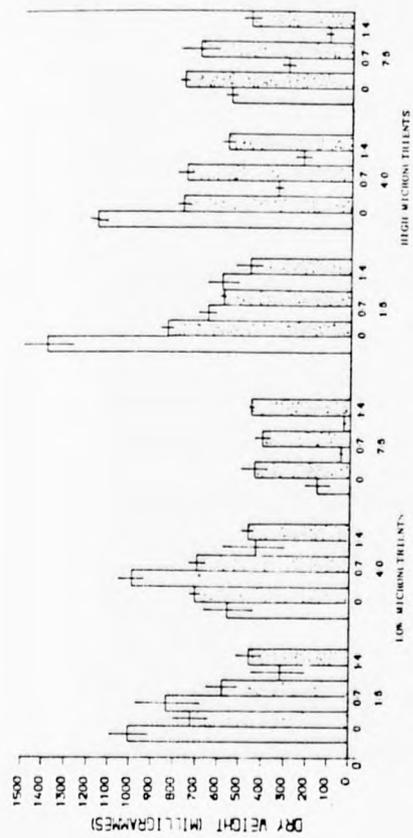
a) Shoots

Increasing nickel and magnesium appeared to have little effect on the shoot dry weight of MK1 plants. "High" calcium levels generally resulted in a lower shoot dry weight at a given nickel and magnesium level. "High" micronutrient treatments resulted in significantly lower dry weight, particularly at low magnesium and nickel supply, than "low" micronutrients. Shoot mean dry weight of MK1 race was restricted to a comparatively narrow range in all treatments (211.8 - 568.8 mg).

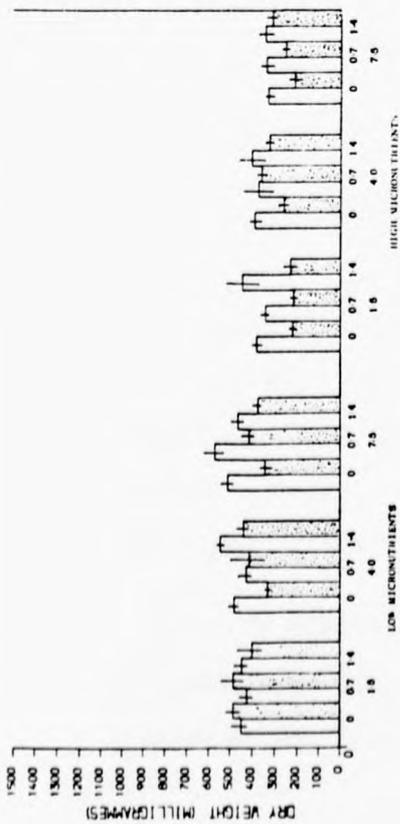
By contrast, NS dry weight fell significantly with increasing nickel and magnesium with a significant interaction between these two elements. "High" calcium levels resulted in higher dry weights consistently only



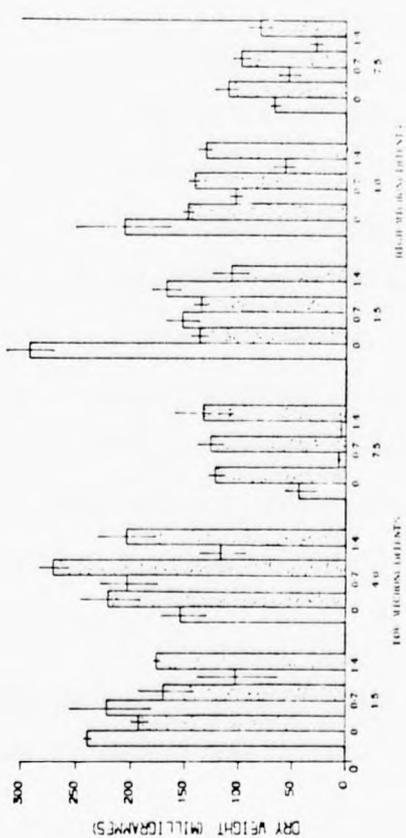
Key for figures 3 to 12 and 22 and 23.



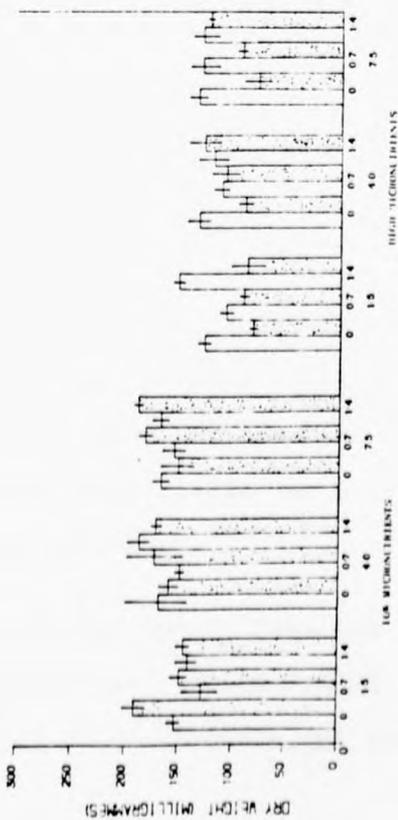
(a) MK1 ROOTS



(b) MK1 ROOTS



(c) NS ROOTS



(d) NS ROOTS

Figure 3. Dry weights of MK1 and NS plants.

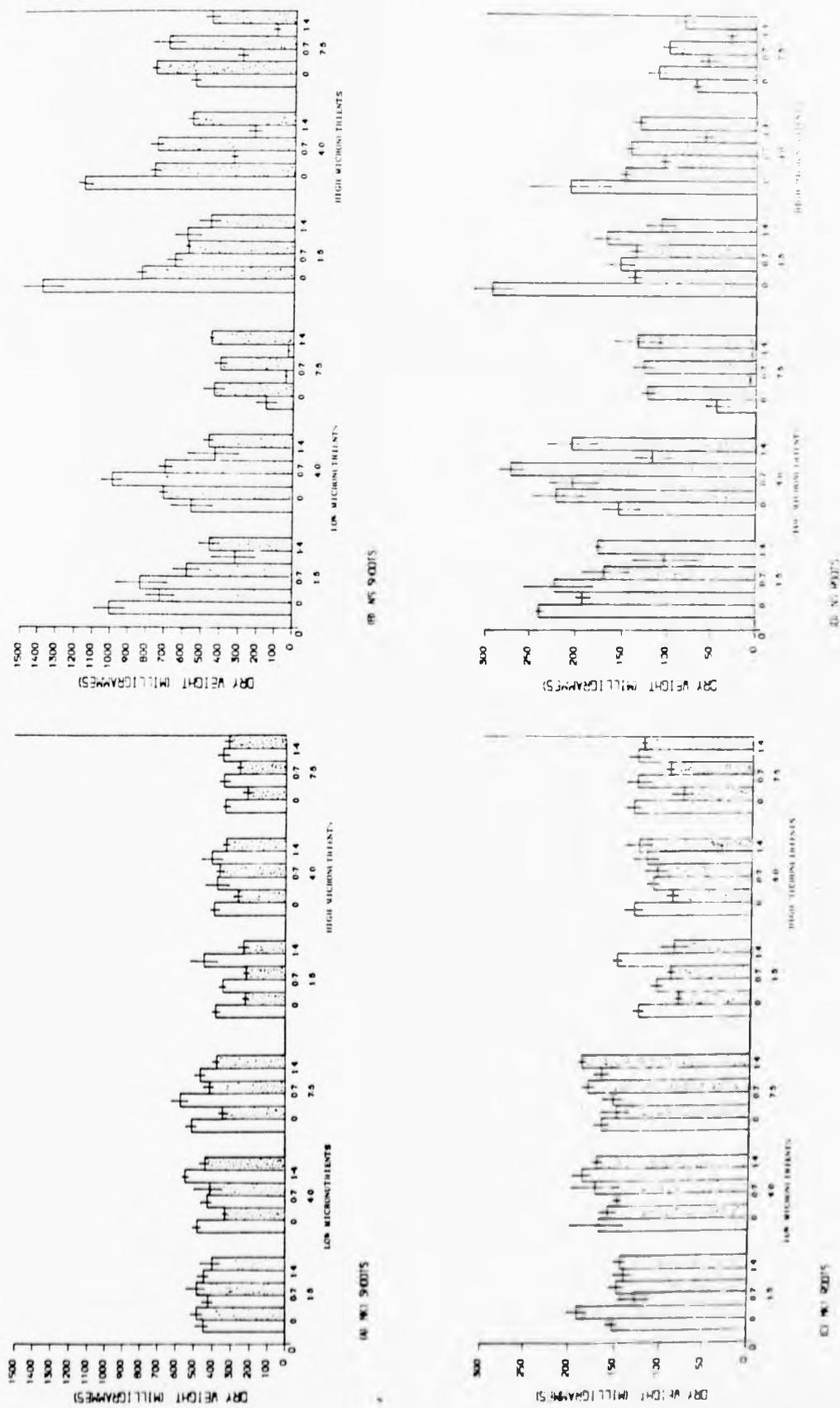


Figure 3. Dry weights of MK1 and NS plants.

in 7.5 mM magnesium treatments. "High" micronutrients resulted in higher dry weights particularly at 7.5 mM magnesium. The plants in the higher nickel and magnesium treatments with "low" calcium and micronutrients showed no growth during the experiment and were dark green with leaf blades with brown tips or occasionally the whole plants were entirely brown. With the exception of the 4 mM magnesium treatment with "low" calcium and micronutrients, NS shoot dry weight was highest in the absence of nickel.

NS shoot dry weights were generally higher than those of MK1 shoots except at 7.5 mM magnesium and "low" calcium.

b) Roots

In general, the dry weights of MK1 and NS roots paralleled that of shoots in the same treatments.

2) Nickel (figures 4A - 4D)

a) Shoots

A significant increase in MK1 shoot nickel concentration was noted with increasing nickel levels and was particularly pronounced at "high" micronutrient and "high" calcium supply. In these conditions, nickel was highest in shoots in the low magnesium treatment.

In NS shoots, increasing nickel supply resulted in a significant increase in shoot nickel. With 7.5 mM magnesium and "low" calcium and micronutrients, NS shoot

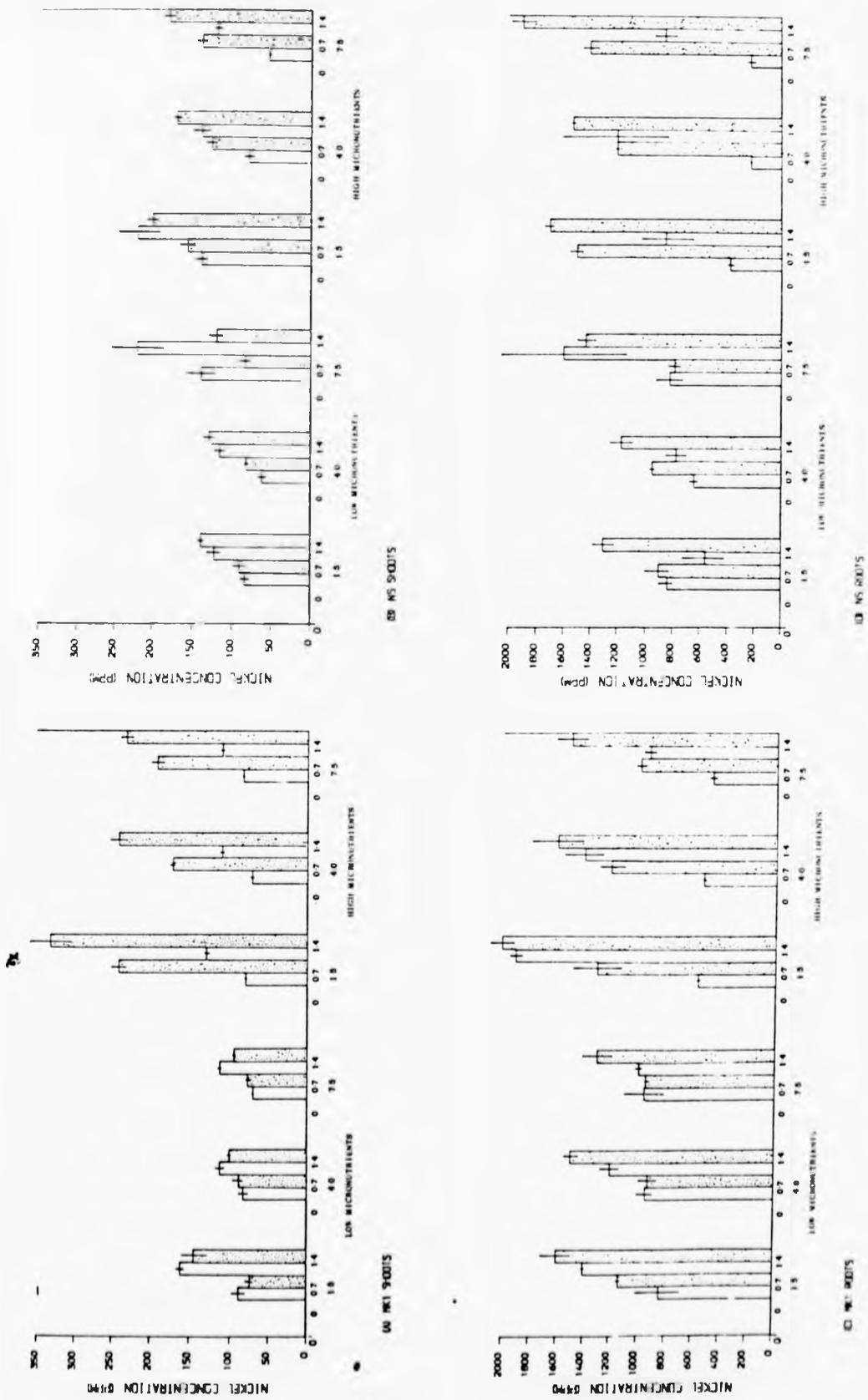


Figure 4. Nickel concentration in MK1 and NS plants.

nickel increased significantly compared with low magnesium treatments. By contrast, with "high" micronutrients with "low" calcium, increasing magnesium caused a decrease in NS shoot nickel.

The two races differed significantly in their response to "high" calcium and micronutrients since MK1 race took up more nickel than the NS race at each level of nickel and magnesium.

b) Roots

With the exception of the "low" calcium and micronutrients treatment there was evidence in the MK1 plants of a significant interaction between nickel and magnesium (increasing magnesium tended to reduce root nickel). This was opposite in effect to that observed in the shoots. "High" calcium treatments resulted in an increase in root nickel compared with "low" calcium treatments.

Magnesium had no consistent influence on root nickel in NS plants although "high" calcium levels caused an increase in root nickel concentration.

Races differed significantly in their root nickel when grown in "low" calcium treatments. Roots of both races contained much more nickel than their shoots.

3) Iron (figures 5a - 5D)

a) Shoots

With "high" micronutrients and calcium supply, MK1 shoot iron was significantly higher compared with

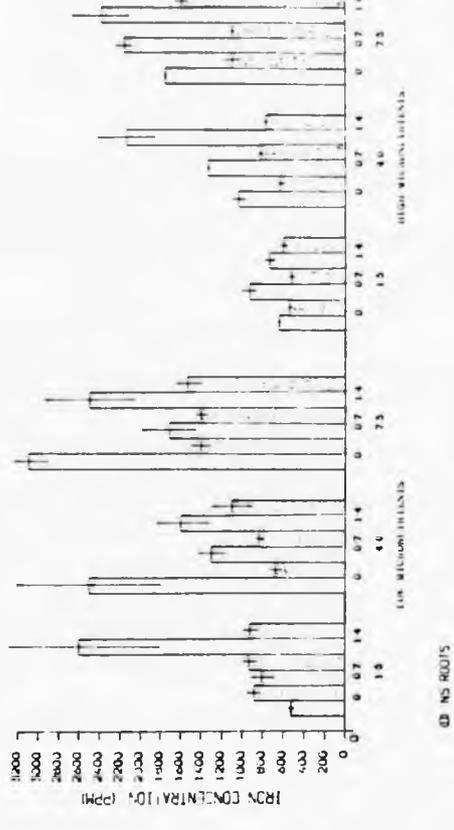
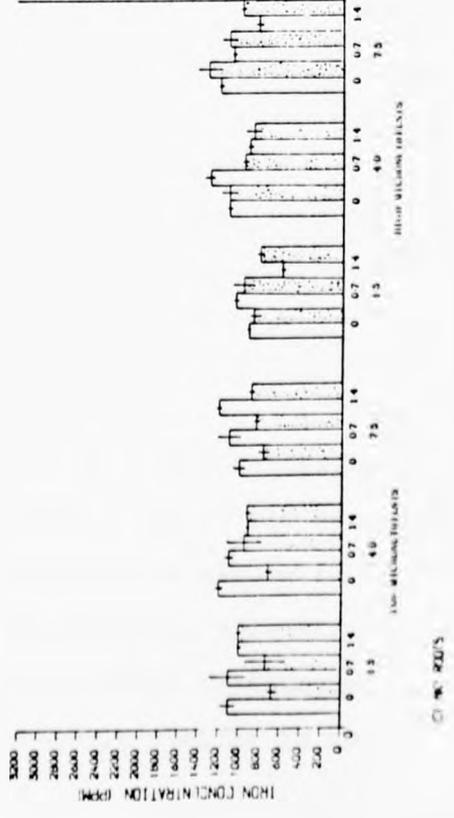
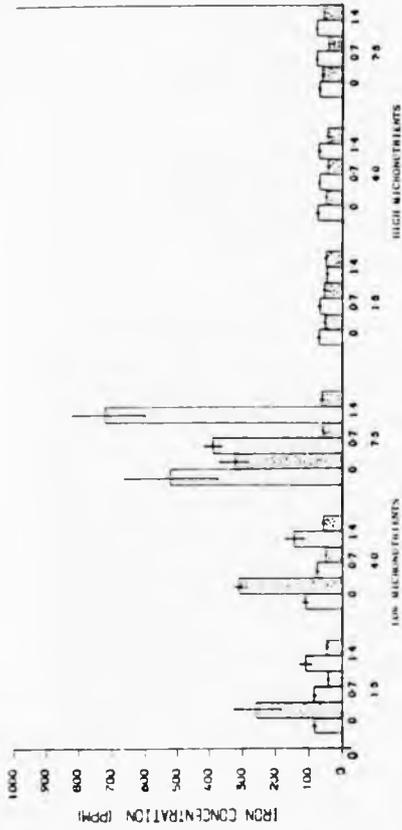
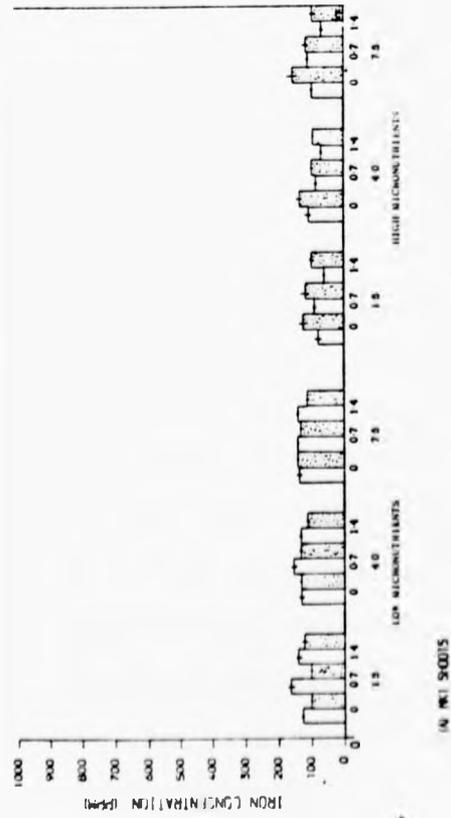


Figure 5. Iron concentration in MK1 and NS plants.

treatments where calcium was "low".

The situation is complex in NS shoots. All treatments which included "high" micronutrients gave rise to similar concentrations of iron. With "low" micronutrients and calcium, 7.5 mM magnesium resulted in a dramatic increase in iron concentrations in shoots. Increasing nickel alone had no clear-cut effect. With "low" micronutrients and "high" calcium, the presence of nickel significantly reduced NS shoot iron at all levels of magnesium. Another difference between races was the greater iron concentration in NS shoots in "low" micronutrient with "high" calcium treatments in the absence of nickel.

b) Roots

MK1 root iron was similar in all treatments. In NS roots, increasing magnesium induced a significant increase in iron uptake. Nickel alone caused a significant (at the 5% level) increase in NS root iron content in the 1.5 mM magnesium treatment with "low" micronutrients and calcium. "High" calcium treatments generally gave rise to lower NS root iron levels.

There were significant differences in the response of each race to nickel and magnesium. The levels of iron at 7.5 mM magnesium and "low" calcium were higher in NS compared with MK1 roots. However, with the exception of "low" micronutrients with 7.5 mM magnesium treatments, the iron levels in roots were generally similar in both

races when calcium was "high".

In both races, iron concentrations were much higher in roots than shoots.

4) Zinc (figures 6A - 6D)

a) Shoots

With "low" micronutrients and calcium, increasing nickel resulted in a general decrease in MK1 shoot zinc at the 7.5 mM magnesium level. In "high" micronutrient and calcium treatments, MK1 shoot zinc was significantly higher compared with shoots from "low" calcium treatments.

With "low" micronutrients and calcium levels, 1.4 ppm nickel caused a significant increase in NS shoot zinc.

In both races, the levels of shoot zinc increased in all "high" micronutrient treatments compared with "low" micronutrient treatments. With the exception of 1.5 mM magnesium treatments at "high" micronutrients with "low" calcium levels, there were significant differences between zinc concentration of races in response to nickel and magnesium. In "high" micronutrient treatments, shoot zinc was generally higher in the MK1 race.

b) Roots

With "high" micronutrients, MK1 root zinc concentrations increased significantly when calcium was "high". Increasing nickel caused a decrease in root zinc in the MK1 race. Levels of root zinc were significantly higher

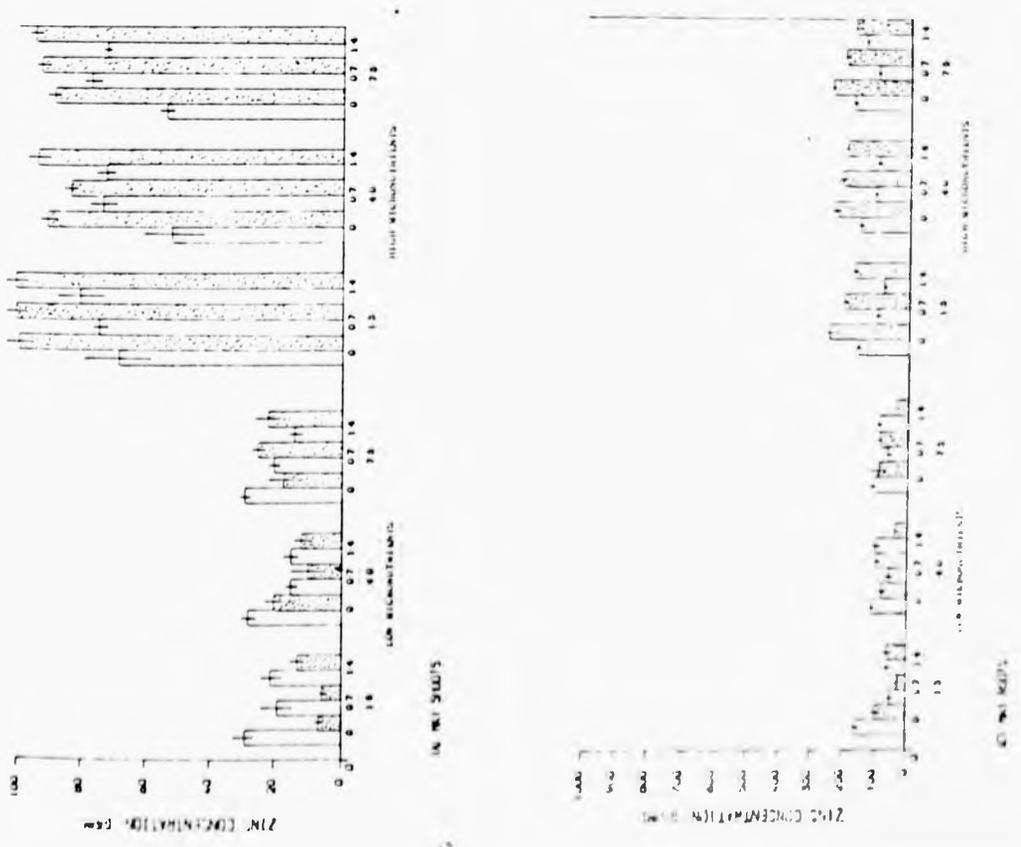
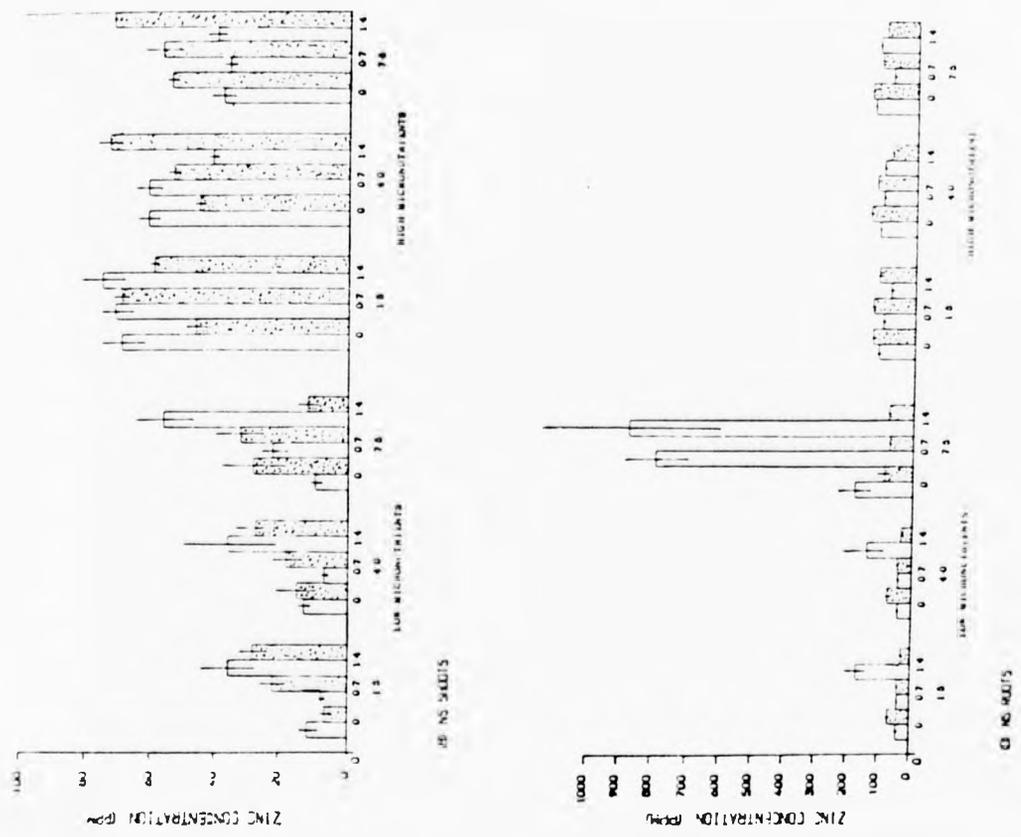


Figure 6. Zinc concentration in MK1 and NS plants.

when micronutrients were "high", especially when calcium was "high".

In NS roots, with "low" micronutrients, 1.4 ppm nickel caused an increase in root zinc particularly with 7.5 mM magnesium.

At "high" micronutrient levels, the WKL race took up significantly more zinc than the NS race when calcium was "high". With "low" micronutrients and calcium, zinc was higher in the NS race at 1.4 ppm nickel at each level of magnesium and at all levels of nickel with 7.5 mM magnesium.

5) Manganese (figures 7A - 7C)

a) Shoots

Increasing nickel usually caused a significant decrease in WKL shoot manganese when micronutrients and calcium were "low", in 1.5 and 4.0 mM magnesium treatments. WKL shoots generally contained more manganese when micronutrients were "high".

With NS shoots, increasing magnesium reduced shoot manganese levels in "high" micronutrient with "low" calcium treatments. Manganese in NS shoots was greater when micronutrients were "high".

In "high" micronutrients treatments WKL shoots contained much more manganese than NS shoots.

when micronutrients were "high", especially when calcium was "high".

In NS roots, with "low" micronutrients, 1.4 ppm nickel caused an increase in root zinc particularly with 7.5 mM magnesium.

At "high" micronutrient levels, the MK1 race took up significantly more zinc than the NS race when calcium was "high". With "low" micronutrients and calcium, zinc was higher in the NS race at 1.4 ppm nickel at each level of magnesium and at all levels of nickel with 7.5 mM magnesium.

5) Manganese (figures 7A - 7D)

a) Shoots

Increasing nickel usually caused a significant decrease in MK1 shoot manganese when micronutrients and calcium were "low", in 1.5 and 4.0 mM magnesium treatments. MK1 shoots generally contained more manganese when micronutrients were "high".

With NS shoots, increasing magnesium reduced shoot manganese levels in "high" micronutrient with "low" calcium treatments. Manganese in NS shoots was greater when micronutrients were "high".

In "high" micronutrients treatments MK1 shoots contained much more manganese than NS shoots.

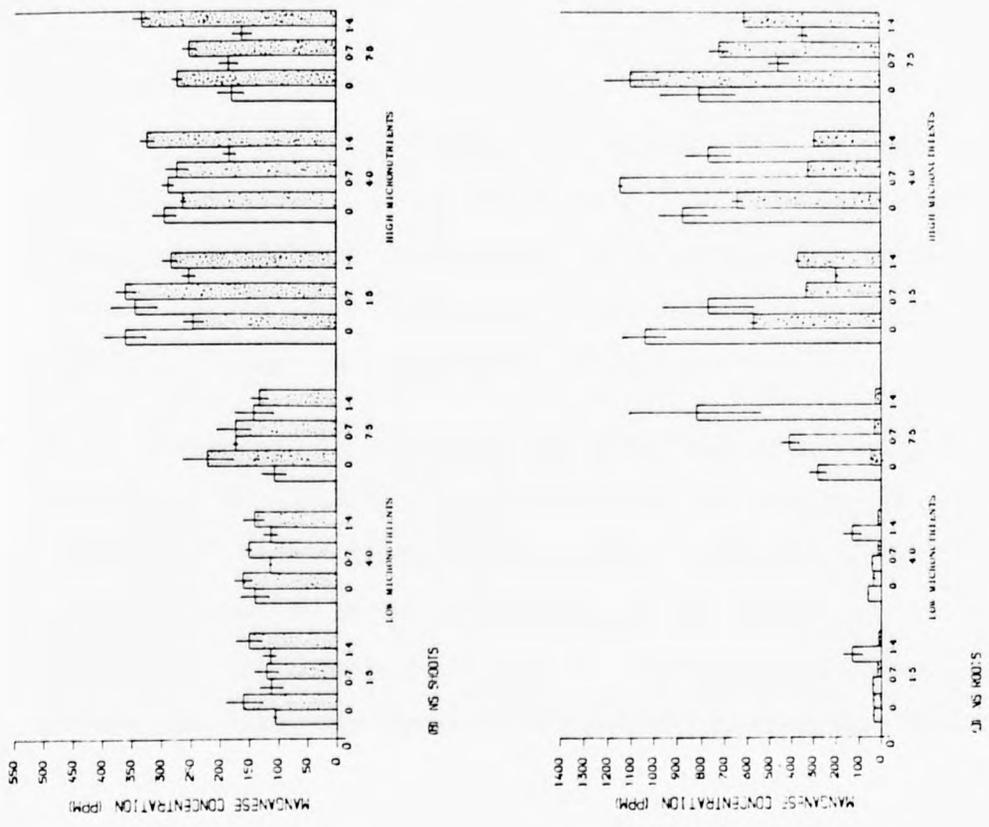


Figure 7. Manganese concentration in MK1 and NS plants.

b) Roots

Increasing nickel, caused a fall in MK1 root manganese in all treatments. When calcium was "low", roots contained more manganese than those grown in "high" calcium conditions. There was significantly more manganese in roots grown in "high" micronutrient treatments.

A decrease in manganese was noted with increasing nickel in NS roots in "high" micronutrient treatments. With "low" micronutrients and calcium, 1.4 ppm nickel caused a rise in NS root manganese at all levels of magnesium but particularly with 7.5 mM magnesium when there was a notable nickel x magnesium interaction. When micronutrients were "high", NS roots from "low" calcium treatments generally contained more manganese than "high" calcium ones, with the exception of the 7.5 mM magnesium treatment.

6) Copper (figures 8A - 8D)

a) Shoots

Magnesium and nickel interacted at "high" micronutrient levels when calcium was "low" at 7.5 mM magnesium when increasing nickel produced an increase in MK1 shoot copper. With "low" micronutrients the most notable effect was that roots in zero nickel treatments with "low" calcium contained significantly more copper than those from treatments containing nickel. MK1 shoots from zero nickel and "low" calcium treatments contained more copper when micronutrients were "low".

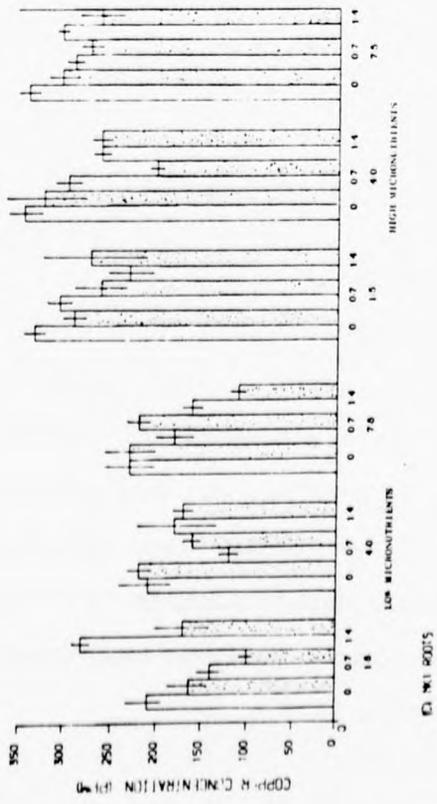
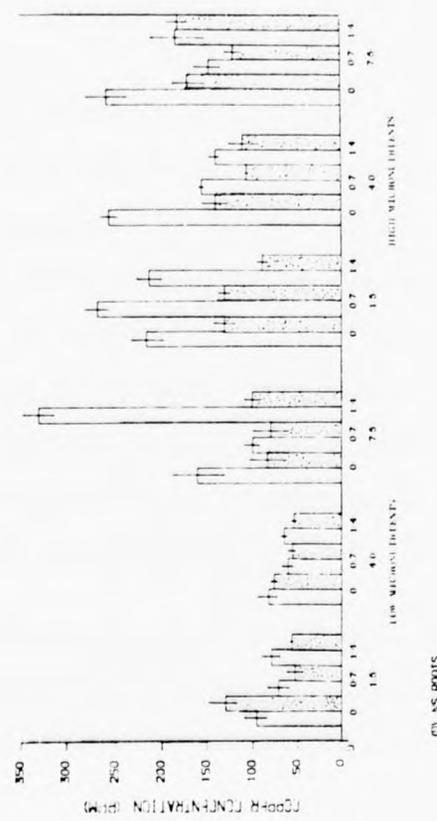
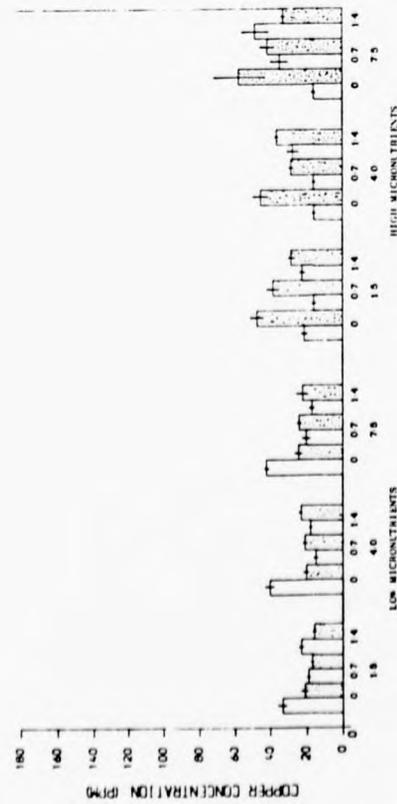
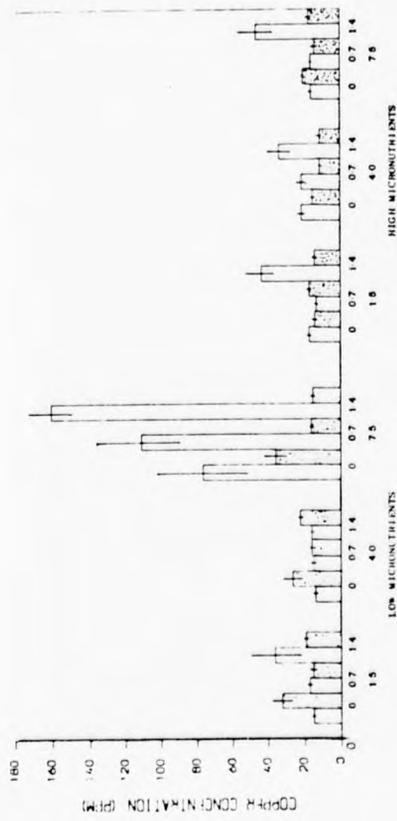


Figure 8. Copper concentration in MK1 and NS plants.

The 1.4 ppm nickel level in the "high" micronutrients with "low" calcium media produced NS shoots with significantly higher copper concentrations. In "low" micronutrients with "high" calcium conditions, the copper concentration in NS shoots from the zero nickel treatment was higher than in shoots grown in the presence of nickel. With "low" calcium and micronutrients, 7.5 mM magnesium caused a large increase in NS shoot copper which was enhanced by a 7.5 mM magnesium x nickel interaction.

The two races differed from each other at "high" micronutrient and calcium levels when the MK1 race contained more copper than the NS race. The NS race took up more copper than the MK1 race at "low" micronutrient and calcium levels in the presence of 7.5 mM magnesium.

b) Roots

MK1 roots from "high" micronutrient treatments contained significantly more copper than those from "low" micronutrient conditions when calcium was "high".

Magnesium alone did not influence NS root copper in any treatment at "high" micronutrient levels. With "low" micronutrients, there was an interaction between nickel and magnesium where 7.5 mM magnesium and 1.4 ppm nickel caused a large rise in NS root copper when calcium was "low". With "high" calcium and "low" micronutrients, in the absence of nickel, 1.5 mM magnesium resulted in an increase in root copper.

With the exception of the 7.5 mM magnesium, 1.4 ppm nickel treatments when micronutrients and calcium were "low", MK1 roots in all treatments contained significantly higher levels of copper than the NS race.

The levels of copper in MK1 and NS roots were higher than those found in their shoots.

7) Potassium (figures 9A - 9D)

a) Shoots

In "low" micronutrient treatments, MK1 shoot potassium was higher when calcium was "low". With "high" micronutrients, shoot potassium increased at "high" calcium levels.

With "low" micronutrients and calcium conditions, increasing nickel, at 1.5 mM magnesium, caused NS shoot potassium to increase. There were no clear changes in potassium concentration in NS shoots due to changes in nickel, magnesium, calcium or micronutrients, although potassium was low in NS shoots from 0.7 ppm nickel, 7.5 mM magnesium treatment with "low" micronutrients and calcium.

Potassium concentrations were higher in MK1 shoots compared with NS shoots from most treatments.

b) Roots

With increasing magnesium, MK1 root potassium generally increased in "high" micronutrient with "low"

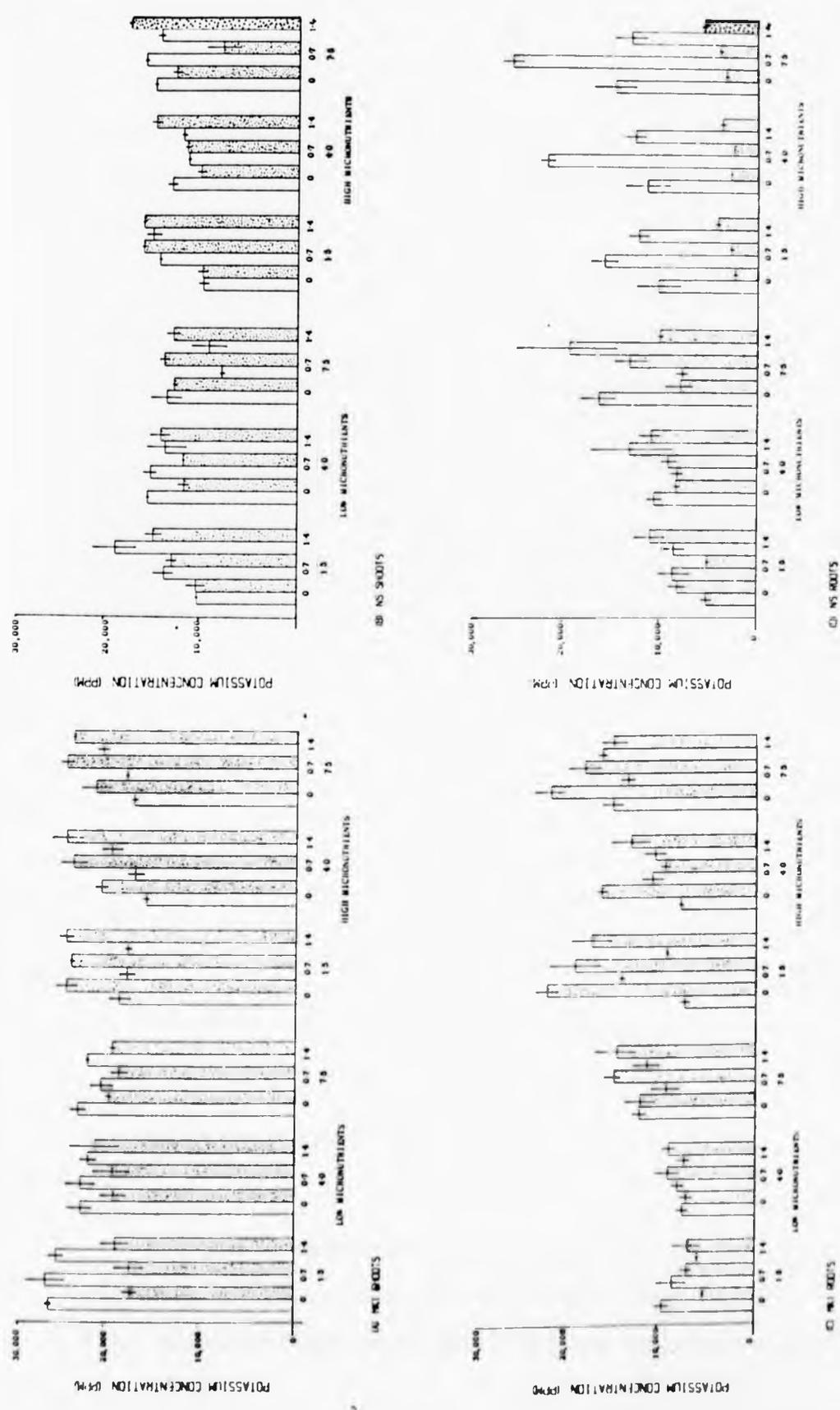


Figure 9. Potassium concentration in MK1 and NS plants.

calcium treatments. With "low" micronutrient increasing magnesium caused an increase in MK1 root potassium. With "high" calcium levels, root potassium was generally higher in "high" micronutrient treatments.

For NS roots there was an interaction between 0.7 ppm nickel and magnesium, in "high" micronutrient and "low" calcium conditions, which caused an increase in root potassium as magnesium increased. In "high" micronutrients root potassium was significantly lower when calcium was "high". With "low" micronutrients and calcium, increasing magnesium tended to increase NS root potassium with the exception of 0.7 ppm nickel, 7.5 mM magnesium treatment where potassium was comparatively low.

There were no significant differences between races when micronutrients were "low". However, with "high" micronutrients and calcium, MK1 root potassium was much higher than in the NS race. With "high" micronutrients and "low" calcium, the NS roots contained more potassium than MK1 roots.

Potassium was higher in the shoots of both races compared with their roots.

8) Magnesium (figures 10A - 10D)

a) Shoots

Increasing magnesium caused an increase in MK1 shoot magnesium when micronutrients and calcium were "high". With "high" micronutrients and "low" calcium, increasing

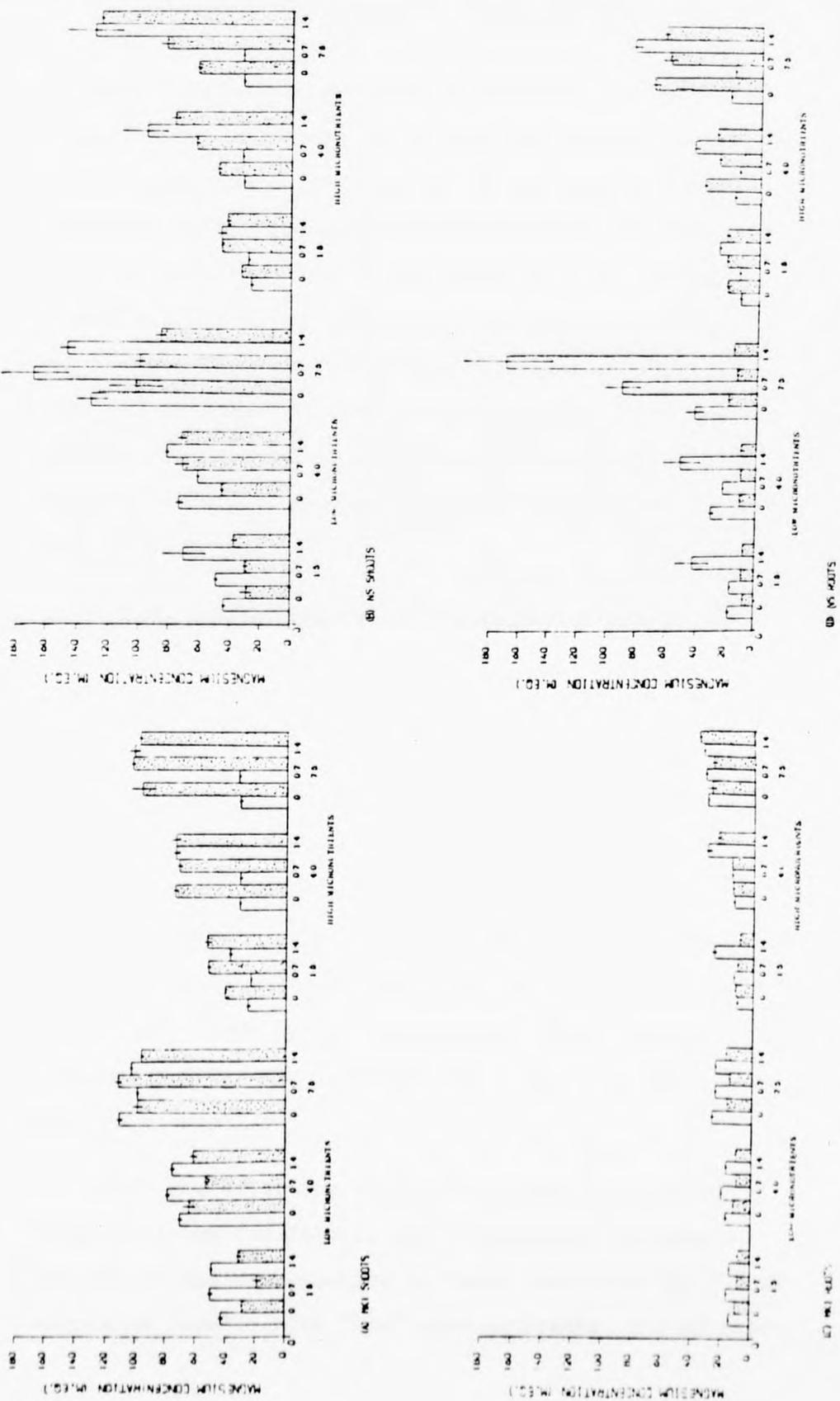


Figure 10. Magnesium concentration in MK1 and NS plants, (M.Eq. = mEq/100g oven dry material).

magnesium alone did not cause an increase in shoot magnesium but there was an interaction between magnesium and 1.4 ppm nickel resulting in an increase in shoot magnesium with increasing magnesium and nickel supply. With the exception of 1.4 ppm nickel at 4.0, 7.5 mM magnesium, MKI shoots contained less magnesium when calcium was "low" at "high" micronutrient supply. At 1.5, 4.0 mM magnesium levels MKI shoots in "low" calcium treatments contained significantly more magnesium than those from "high" calcium conditions when micronutrients were "low".

In NS shoots, magnesium concentration generally increased with increasing magnesium and nickel when micronutrients and calcium were "high". With "high" micronutrients and "low" calcium, increasing magnesium alone had no significant effect but there was a 1.4 ppm nickel x magnesium interaction when shoot magnesium increased significantly. With the exception of the 1.4 ppm nickel treatment, shoots from "low" calcium and "high" micronutrient conditions contained less magnesium than those from "low" micronutrient conditions. "Low" calcium treatments generally contained more magnesium when micronutrients were "low".

With the exception of "low" micronutrients with "high" calcium conditions, races contained different concentrations of magnesium in their shoots at the 7.5 mM magnesium level. With "low" micronutrients, the NS race

contained more magnesium than the MK1 race. With "high" micronutrients and calcium, MK1 shoots generally contained more magnesium than the NS race with the exception of the 7.5 mM magnesium and 1.4 ppm nickel treatment.

b) Roots

With "high" or "low" calcium, 7.5 mM magnesium caused a marked increase in MK1 root magnesium level when micronutrients were "high". At 1.5 and 4.0 mM magnesium with 1.4 ppm nickel there was a magnesium x nickel interaction resulting in a sharp rise in root magnesium when calcium and micronutrients were "low". In "low" micronutrient treatments root magnesium increased slightly with increasing magnesium. With "low" micronutrients, roots in "low" calcium conditions contained more magnesium than those in "high" calcium treatments.

NS root magnesium increased with increasing magnesium when micronutrients and calcium were "high". With "high" micronutrients and "low" calcium, there was an interaction between magnesium and 1.4 ppm nickel causing a significant rise in NS root magnesium. With the exception of 1.4 ppm nickel treatments, NS roots in "high" micronutrients contained more magnesium when calcium was "high". With "low" micronutrients, increasing magnesium caused an increase in root magnesium when calcium was "high". Root magnesium increased markedly at the 7.5 mM magnesium level when calcium was "low". Nickel interacted with magnesium here accounting for much of the increase in NS root magnesium.

9) Calcium (figures 11A - 11D)

a) Shoots

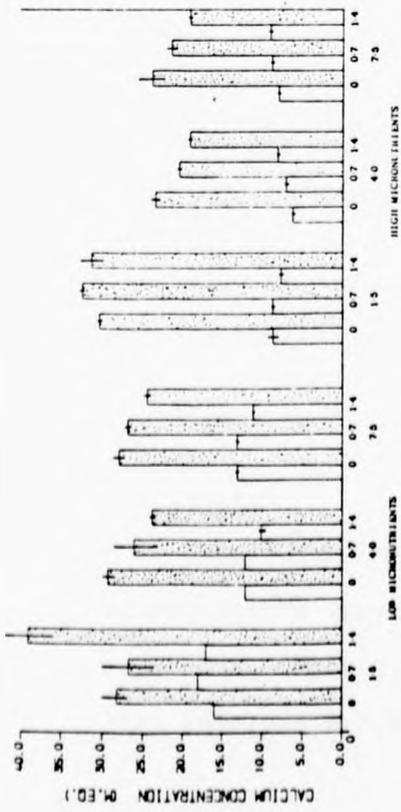
Nickel had little influence on MK1 shoot calcium whilst increasing magnesium caused a significant decrease in shoot calcium when micronutrients and calcium were "high". 4.0 and 7.5 mM magnesium also caused shoot calcium to fall with "low" micronutrients and calcium levels. Shoots in "high" calcium treatments contained more calcium. "High" micronutrients appeared to reduce shoot calcium compared with "low" micronutrient treatments when calcium was "low".

Nickel was not important in determining NS shoot calcium levels, although 7.5 mM magnesium and nickel interacted to give a rise in shoot calcium in "low" micronutrient and calcium conditions. With "low" micronutrient and "high" calcium, increasing magnesium reduced NS shoot calcium. With "low" calcium, shoots from 7.5 mM magnesium treatments with "high" micronutrients had significantly lower calcium concentrations than shoots from the corresponding "low" micronutrient treatments.

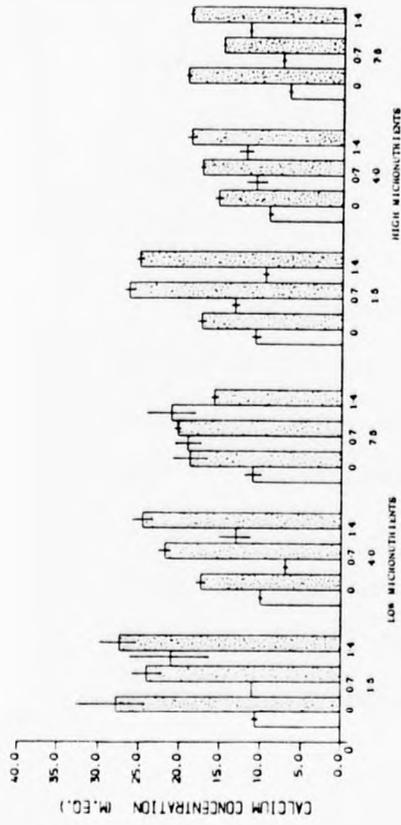
MK1 shoot calcium was significantly higher than levels in NS shoots in "high" calcium treatments. With "high" micronutrients and "low" calcium, NS shoot calcium was higher than in the MK1 race.

b) Roots

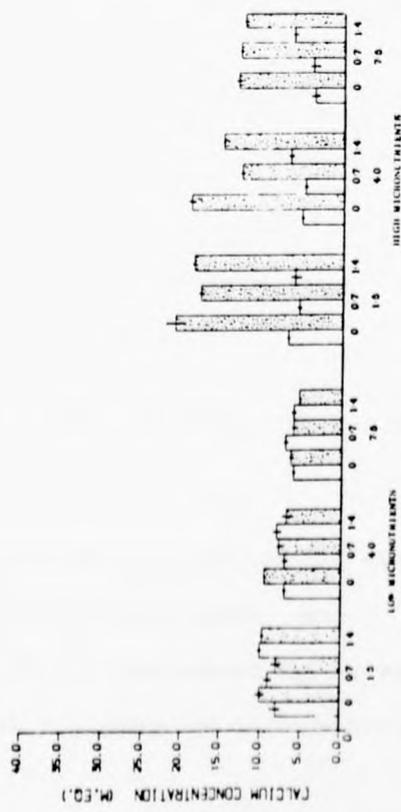
Increasing magnesium significantly reduced root



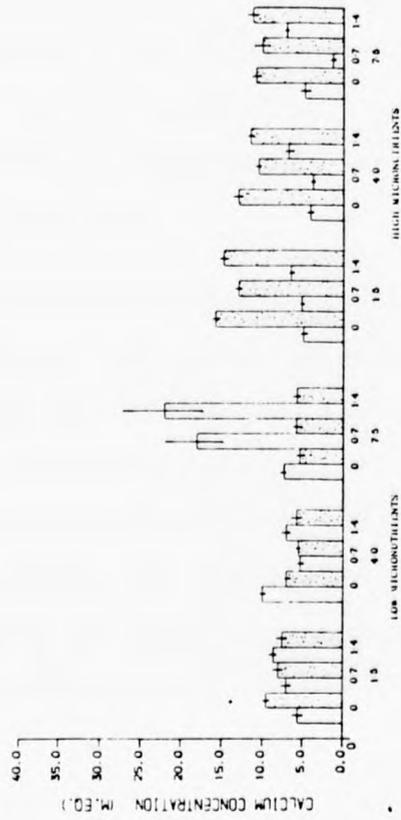
(a) MK1 SHOOTS



(b) NS SHOOTS



(c) MK1 ROOTS



(d) NS ROOTS

Figure 11. Calcium concentration in MK1 and NS plants, (M.Eq. = mEq./100g oven dry material).

calcium levels in all treatments except "high" micronutrients and "low" calcium conditions in MK1 roots. Roots from "high" calcium treatments contained significantly more calcium when micronutrients were "high".

NS root calcium fell significantly with increasing magnesium when calcium was "high". Root calcium rose sharply in "low" micronutrient and calcium conditions with 7.5 mM magnesium in the presence of nickel. With the exception of roots influenced by the nickel x magnesium interaction, NS root calcium was significantly higher in "high" micronutrients and calcium treatments compared with roots from other treatments.

MK1 root calcium was significantly higher in all "high" calcium treatments compared with NS roots grown in similar conditions. The levels of NS root calcium were much higher than in MK1 roots when grown in "low" micronutrient and calcium treatments with 7.5 mM magnesium in the presence of nickel.

Calcium was higher in the shoots of both races compared with levels found in their roots.

10) Magnesium/Calcium (Mg/Ca) ratio (figures 12A - 12D)

a) Shoots

With "high" micronutrients and calcium, Mg/Ca ratio of MK1 shoots increased with increasing nickel and magnesium. When calcium was "low", the shoot Mg/Ca ratio rise involved a 1.4 ppm nickel x magnesium interaction.

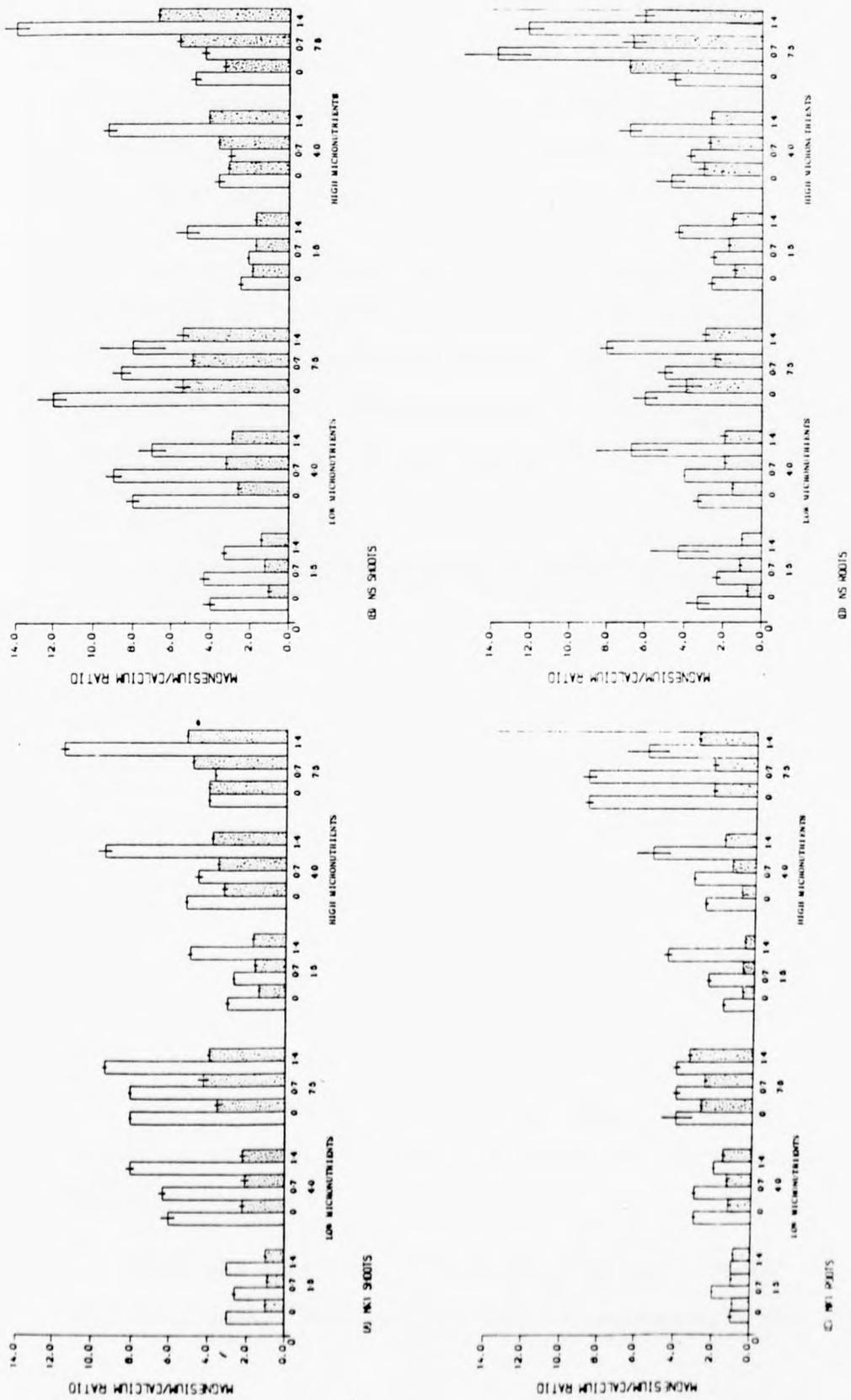


Figure 12. Magnesium/calcium ratio in MK1 and NS plants.

With "low" micronutrients, MK1 shoot Mg/Ca ratio seemed uninfluenced by nickel but increased with increasing magnesium concentration. In all treatments, Mg/Ca ratio was higher when calcium was "low".

The trends outlined above for MK1 shoots were paralleled by NS shoots. MK1 shoots maintained a significantly lower Mg/Ca ratio than NS shoots in all treatments except "high" micronutrients, "low" calcium, 1.5 mM magnesium with 0 and 0.7 ppm nickel.

b) Roots

With "high" micronutrients and "low" calcium, increasing nickel at 1.5, 4.0 mM magnesium levels increased the root Mg/Ca ratio in MK1 roots. At 7.5 mM magnesium, nickel had no significant effect. The major influence in the rise of the Mg/Ca ratio in "high" and "low" micronutrient treatments was the increasing magnesium level. Mg/Ca ratio in MK1 roots was higher in "low" calcium treatments, particularly when micronutrients were "high".

In NS roots, there was a significant interaction between magnesium and nickel, when micronutrients were "high" and calcium "low", which resulted in a large increase in the Mg/Ca ratio. In all other treatments, magnesium alone was the major influence in increasing the Mg/Ca ratio.

With the exception of a single treatment ("high" micronutrients, "low" calcium, 7.5 mM magnesium, 0 ppm

With "low" micronutrients, MK1 shoot Mg/Ca ratio seemed uninfluenced by nickel but increased with increasing magnesium concentration. In all treatments, Mg/Ca ratio was higher when calcium was "low".

The trends outlined above for MK1 shoots were paralleled by NS shoots. MK1 shoots maintained a significantly lower Mg/Ca ratio than NS shoots in all treatments except "high" micronutrients, "low" calcium, 1.5 mM magnesium with 0 and 0.7 ppm nickel.

b) Roots

With "high" micronutrients and "low" calcium, increasing nickel at 1.5, 4.0 mM magnesium levels increased the root Mg/Ca ratio in MK1 roots. At 7.5 mM magnesium, nickel had no significant effect. The major influence in the rise of the Mg/Ca ratio in "high" and "low" micronutrient treatments was the increasing magnesium level. Mg/Ca ratio in MK1 roots was higher in "low" calcium treatments, particularly when micronutrients were "high".

In NS roots, there was a significant interaction between magnesium and nickel, when micronutrients were "high" and calcium "low", which resulted in a large increase in the Mg/Ca ratio. In all other treatments, magnesium alone was the major influence in increasing the Mg/Ca ratio.

With the exception of a single treatment ("high" micronutrients, "low" calcium, 7.5 mM magnesium, 0 ppm

nickel), the MK1 race maintained a significantly lower Mg/Ca ratio than NS roots.

Discussion

General considerations

Much of the work on serpentines involving the growth of plants in nutrient solutions has involved the use of crops, particularly oats (Avena sativa), e.g. Hunter and Vergnano (1953), Proctor and McGowan (1976). Oats have been favoured since they are easily grown and give rise to specific nickel toxicity symptoms (which include white longitudinal stripes on leaves (Hunter and Vergnano, 1952)). However, there are obvious limitations in applying conclusions from crop plants to species native to serpentine soils. Work has been carried out using species commonly found on and off serpentines with serpentine endemics, e.g. Main (1970), Madhok and Walker (1969), Marrs and Proctor (1976), where calcium and magnesium interrelationships alone were the main interest. However, the solutions used to grow these plants were modified from Hoagland and Arnon (1950). No attempt was made to relate the culture solutions to the composition of the serpentine soil solution. In the present work the effects and interactions of soil ions were studied using culture solutions based on an analysis of MK1 soil solutions (Table 1).

Comparison of the present study with pot experiments using MK1 soils.

Preliminary experiments (not reported in detail) had investigated "high" micronutrients, "low" calcium with three levels of magnesium and nickel. It was believed that this culture medium composition was a good approximation to that of the soil solution. However, when dry weights of shoots from 7.5 mM magnesium with 0.7 ppm nickel treatments were compared with the data of Johnston (1976), where the same clones used in this study were grown in MK1 soils, discrepancies were noted. NS shoots from the water culture experiment had significantly higher dry weights than those grown in MK1 soils (Table 7). It seemed that some further factor was involved in producing the unexpectedly high dry weights.

When experiments were carried out with "low" micronutrient and "low" calcium, in the 7.5 mM magnesium and 0.7 ppm nickel treatment, NS shoot dry weights were similar to those recorded by Johnston (1976) for NS F. rubra grown in MK1 soil under identical growth room conditions. Although there were some discrepancies it was concluded that the soil solution in MK1 soils might best be represented by the "low" micronutrient, "low" calcium, 7.5 mM magnesium, 0.7 ppm nickel treatment (which will henceforth be referred to as the "serpentine condition"). Additionally, the levels of metals found in plants from the "serpentine condition" (which will be discussed later), were compared with those reported by

Johnston (1976) for plants grown for four weeks in MK1 soils in growth room experiments and in F. rubra occurring naturally at the MK1 site. In the present study, it was found that the fresh weight of MK1 shoots grown in the "serpentine condition" increased threefold from 1110 mg after four weeks growth to 3320 mg after eight weeks growth. The fresh weight of the NS race did not significantly change over this period. Thus the MK1 dry weight recorded from the pot experiments of Johnston (1976) were multiplied by a factor of three to make the earlier study and the present one more comparable. The dry weight data (Table 7) for pot experiments have already been transformed by a factor of three.

Table 7 indicates that metal levels in plants from water culture, pot experiments and the field were broadly similar in spite of the very different growing conditions. Notable exceptions were the levels of magnesium and calcium for which the levels in plants from water culture were higher than those from soils, although the Mg/Ca ratio in water culture and pot-grown plants was similar. Again, the dry weights of MK1 shoots were different in water culture and soil-grown plants. These differences may be accounted for in terms of factors present in MK1 soils, e.g. chromium and cobalt, which have not been included in water culture treatments because the levels of these elements could not be determined in soil solutions, although they are known to be taken up, by plants grown in MK1 soil (Johnston 1976). In addition, other factors

Table 7. Dry weights and metal concentrations in MKI and NS F. rubra shoots grown in the "serpentine condition" in water culture, pot experiments and in the field.

Race	Treatments	Origin of data	Mean dry weight (mg)	Mean metal concentration (ppm)									
				Ni	Fe	Zn	Mn	Cu	K	Mg	Ca	Mg/Ca	
MKI	Water culture	Present study	568	70	140	20	120	20	28,000	12,000	2,600	4.6	
	Pot experiments	Johnston (1976)	300**	100	185	nd	60	nd	28,000	6,800	1,400	4.9	
	Field	Johnston (1976)	nd*	63	158	12	13	5	42,000	7,200	450	16.0	
NS	Water culture	Present study	40	140	380	22	170	110	10,900	20,400	3,900	5.2	
	Pot experiments	Johnston (1976)	36	180	310	nd	100	nd	12,500	8,100	1,350	6.0	

* nd = not determined

** This value has been calculated from Johnstons' data, as described in the text.

such as physical problems of root penetration and the complexity of nutrient supply in soils were not considered in culture media experiments. Work reported later (Chapter 6) has suggested that chromium may have some effect on growth of MK1 and NS F. rubra. Future water culture studies would include at least trace amounts of these metals.

Dry weights of plants grown in varying mineral conditions in the water culture experiments.

The MK1 race showed a reduction in shoot and root dry weight in treatments containing higher levels of micronutrients than those believed to occur in MK1 soils. "High" experimental calcium levels also depressed dry weights. In spite of this, the MK1 race showed a capacity to maintain growth at a fairly constant rate when in solutions of greatly varying mineral composition. By contrast, the NS race was very sensitive to changes in mineral composition. At each level of nickel, increasing magnesium caused a considerable decrease in dry weight of the NS race when micronutrients were "high" and calcium "low". With the "low" micronutrient and "low" calcium treatment, magnesium had its most severe effect and 7.5 mM magnesium produced the lowest dry weights of any treatment. "High" calcium levels appeared to ameliorate much of the toxic influence of both nickel and magnesium.

The differences in shoot and root dry weight noted for each race in a given treatment are almost certainly

related to differences in adaptation to adaphic conditions. The NS race was generally intolerant of nickel and magnesium which reduced growth unless "high" calcium levels were supplied. Whilst this calcium amelioration tended to provide more favourable growth conditions for the NS race, "high" calcium per se did not result in increased weight in MK1 plants. The influence of calcium on the growth of NS plants is rather complex. In favourable growth conditions (low magnesium and nickel), "high" calcium supply reduced the dry weights of NS plants but, as mentioned earlier, promoted growth in unfavourable conditions (high magnesium and nickel). This apparent "toxicity" of calcium to NS plants at low magnesium and nickel levels, and its amelioratory effect in unfavourable conditions is puzzling and requires further investigation.

The slight increase in shoot dry weight of MK1 race with increasing magnesium in the "low" micronutrient with calcium treatment is perhaps indicative of a higher magnesium requirement in this race although the evidence is not so strong as for the serpentine plants investigated by Main (1970) and Marrs and Proctor (1976). Main (1970) also noted that the yield of a serpentine race of Agropyron spicatum increased sharply with increasing calcium up to 1 mM calcium although this was not noted with MK1 F. rubra.

Proctor and McGowan (1976) found that increasing magnesium (up to 5 mM) offset the toxic effect of 1.5 ppm nickel in oat plants. Conversely Minguzzi and Vergnano

(1948) postulated that in Alyssum bertolonii (a nickel accumulator) nickel counteracted high magnesium levels, a role normally fulfilled by calcium. In the present study, nickel and magnesium interacted differently from that noted by the previously mentioned workers. In "low" calcium treatments NS shoot and root dry weight decreased sharply when nickel and magnesium supply increased. When the calcium supply was increased the effect of the nickel x magnesium interaction was less severe. These differences make it clear that care must be taken when attempting extrapolation of a finding, within one system or species, to another.

Variations in tissue metal concentrations in plants from various culture media.

Nickel

The interaction between "high" micronutrients and "high" calcium causing a sharp rise in MK1 shoot (but not root) nickel content is difficult to interpret. In the "serpentine condition", the NS race took up high levels of nickel and this may account to some extent for the fall in dry weights of this race in this treatment. With the exception of the "serpentine condition" addition of "high" calcium to a treatment was associated with an increase in NS root nickel. "High" calcium conditions may ameliorate some of the toxicity associated with nickel enabling higher nickel levels to be tolerated without adverse effects as Crooke and Inkson (1955) demonstrated.

Iron

In most treatments MKI shoot and root iron concentrations were unaffected by changes in mineral composition of the culture media. However, MKI shoot iron levels were reduced in "high" micronutrients with "low" calcium conditions. The nature of this micronutrient x calcium interaction was unclear. Most of the iron taken up by MKI plants remained in the roots with approximately only 10% being transported to the shoots.

The work of Crooke, Hunter and Vergnano (1954) showed that in oat plants, nickel toxicity symptoms decreased when iron supply was high and that the nickel content of leaf blades fell with high iron concentrations in the culture media. Iron in leaf blades also fell when nickel supply was increased. A similar situation occurred in NS F. rubra here. With "low" micronutrients (it should be remembered that iron supply was the same with "high" or "low" micronutrient treatments) and "high" calcium, the addition of nickel to culture media caused a highly significant fall in iron concentration in NS shoots (this was not noted in NS roots or MKI plants). The fall in iron concentration in NS plants was not associated with any significant decrease in NS shoot dry weight. In "low" micronutrient and calcium treatments, significant increases in NS shoot iron were noted in 7.5 mM magnesium treatments. This increase in iron would not appear to be an attempt by NS shoots to maintain a lower nickel/iron ratio (Crooke Hunter and Vergnano (1954) suggested that chlorosis was reduced in

oat plants when the nickel/iron ratio was reduced) since with zero nickel in the culture solution (and therefore very low nickel in the shoots) at 7.5 mM magnesium, the iron levels were similar to NS shoots containing high levels of nickel. In hindsight another level of iron in culture media would have been useful. However, the important point arising from these data is the complex nature of the interaction between micronutrients, magnesium and calcium in the NS race which serves to influence metal uptake (iron in this case) and thereby possibly the growth of the plant.

As for shoots, the influence of magnesium supply on iron content in roots would merit further work. There does not appear to be any clear relationship between iron levels in roots with those in shoots. The supply of calcium was important in determining the levels of iron in NS roots. "High" calcium reduced the amount of iron in NS roots and greatly reduced the effect of magnesium (which stimulated iron uptake and retention in roots in low calcium treatments). This antagonism between magnesium and calcium supply was most pronounced in "high" micronutrient treatments. The interaction between magnesium and calcium with micronutrients makes the mechanism of iron uptake and retention in NS roots a most complex picture.

Zinc

All shoots had higher zinc concentrations in "high" micronutrient treatments (where the level of supply was

ten times higher than in the "low" micronutrient treatments). The MK1 shoots had the most pronounced response where zinc was four times higher in plants from "high" compared with "low" micronutrients. The difference of zinc in plants between treatments and its relationship to the growth of NS plants, in particular, is not clear. One feature which is striking is the antagonism between magnesium and calcium supply in determining zinc content of NS shoots. With "high" micronutrients, increasing magnesium supply reduced the level of NS shoot zinc when calcium was "low". However, "high" calcium supply maintained zinc at similar levels in shoots as magnesium increased.

Roots of both races contained more zinc when in "high" micronutrient conditions. In MK1 roots, "high" calcium stimulated zinc uptake with "high" micronutrient conditions. There was a large increase in zinc in NS roots, in the "serpentine condition", which is difficult to explain. It is unlikely to result from a passive uptake due to the breakdown in cell membrane integrity since the levels of other metals, e.g. iron and potassium, do not increase in NS roots in these conditions (unless the high magnesium and nickel make cell membranes more permeable to certain metals only). If the uptake is not passive, then high magnesium and nickel, at "low" micronutrient and calcium supply, possibly promote the selective active uptake of zinc. The fact that the addition of "high" calcium to NS roots in the "serpentine condition" prevents zinc from accumulating suggests the importance of cell membrane

integrity and the possible effects of magnesium and nickel upon it. Läuchli and Epstein (1970) demonstrated that the absence of calcium in solutions containing corn roots, resulted in potassium being lost from roots to the solution and rubidium (initially present in the solution) being taken up into roots, i.e. the tissue was "leaky" in the absence of calcium. This generally agreed with Wyn Jones and Lunt (1967) who stressed the importance of calcium in maintaining membrane integrity. It is therefore suggested that at "low" calcium and "low" micro-nutrient supply, high concentrations of magnesium and nickel cause some loss in membrane integrity making the tissue "leaky" to certain (but not all) metals. Membrane integrity is restored when calcium supply is "high".

Manganese

The high levels of manganese in MK1 shoots from "high" micronutrient treatments was most likely a direct response to the higher supply of manganese in these treatments. In similar treatments, NS shoots contained much less manganese than MK1 shoots. Relatively high manganese supply is a feature of the soil at the NS plant's site of origin (Johnston 1976) and NS plants possibly have a less avid uptake of manganese.

Nickel and magnesium supply had an interactive effect on MK1 root manganese levels. Increasing magnesium tended to increase the levels of MK1 root manganese whilst, at each level of magnesium, nickel tended to reduce the manganese concentration.

By way of contrast, for NS roots, with "high" micronutrients, both increasing magnesium and nickel generally tended to depress manganese levels when calcium was low. However, at "high" calcium levels NS response to manganese was similar to that outlined for MK1 roots. With "low" micronutrients and calcium, NS root manganese increased sharply at 7.5 mM magnesium levels in a similar way to that described for zinc. The suggestions made to rationalize zinc uptake in these conditions, may also apply to manganese.

Copper

Large differences in copper concentration were not noted in the shoots of both races from "high" and "low" micronutrient conditions contrasting with the plant response to zinc and manganese. This emphasizes that there is not a single, overall response to micronutrients.

Whilst zinc and manganese accumulated in NS roots in "low" micronutrient and calcium conditions with 7.5 mM magnesium, copper tended to accumulate in shoots from these treatments. Only the 1.4 ppm nickel treatment in NS roots from these conditions accumulated large amounts of copper. Clearly there were differences between responses to copper and those of other micronutrients although the large increases in copper may perhaps be explained in a similar way to that of zinc and manganese. Copper entering roots through "leaky" membranes may be transported to shoots actively.

Potassium

The results indicate that ability to maintain adequate potassium may be important in MK1 plants and to the survival of plants on serpentine soils in general. In MK1 plants, potassium was in high concentrations in all treatments. Johnston (1976) suggested that the maintenance of high potassium concentrations in MK1 shoots was a major factor in the improved growth of MK1 compared with NS F. rubra in MK1 soil. The NS race hardly grew at all in this soil and its shoots had a low potassium concentration (see Table 7) compared with the MK1 race. When grown in John Innes potting compost (where potassium supply was presumed to be higher than in MK1 soil) the potassium concentration in NS shoots was not significantly different from that of the MK1 race. Potassium has so many functions e.g. enzyme activation (potassium is required by 46 enzymes for maximum activity) and in photosynthesis where it apparently promotes the translocation of photosynthate from leaves (Epstein, 1972). However, Epstein (1972) does point out that high concentrations of potassium are required for enzyme activation since this element does not have a high affinity for organic ligands, including enzymes for which it is a cofactor. The most usual cofactors for enzymes are micronutrients and are required at extremely low concentrations whereas for maximal activity of enzymes using potassium as a cofactor, concentrations of potassium as high as 50 or 100 mM are common. It may be that the generally lower levels of potassium in NS

plants, particularly in the "serpentine condition" where NS shoots contained only one third that found in MK1 shoots, are too low for optimum enzyme activity in this race. Indeed the levels of potassium noted in NS shoots from this treatment are within the range considered to be deficiency levels of this element by Evans and Sorger (1966). There seems little doubt that the MK1 race is adapted to maintaining high shoot potassium levels in conditions of low potassium supply and in the presence of high magnesium since, for example, Epstein (1972) points out that there may be interference of potassium by high magnesium uptake. A similar situation was noted by Gigon and Rorison (1972) where they found that the ability of Deschampsia flexuosa to take up potassium in the presence of ammonium ions could be one factor explaining its tolerance of acidic soils.

Increasing magnesium appeared to stimulate potassium uptake and/or retention in MK1 roots in all treatments with the exception of the "high" micronutrients with "high" calcium conditions.

In NS roots grown in "high" micronutrient and calcium conditions the potassium levels were very low. The interaction of micronutrients and calcium producing this effect may relate to some competition between calcium and potassium for uptake by the roots. If this were the case, then the competition only operates at "high" micronutrient levels.

Magnesium

Of all elements, it was the content of magnesium which most strongly influenced the growth of the NS race. Increases in magnesium in shoots were always associated with a decrease in dry weight at given levels of calcium and micronutrients. The growth-reducing effect of magnesium in NS plants was always enhanced in the presence of nickel.

The minor changes in magnesium concentrations of MK1 roots in the various treatments perhaps illustrate the adaptation, in terms of regulation of uptake and distribution of this metal, shown by MK1 race. Magnesium concentration in MK1 shoots increases as magnesium supply increases in all treatments with the surprising exception of 0 and 0.7 ppm nickel treatments in "high" micronutrient with "low" calcium conditions. Why magnesium is not taken up, in substantial quantities, into shoots under these particular conditions is unknown.

NS root and shoot magnesium increased as magnesium supply increased. The magnesium concentrations were greatest in plants from the "serpentine condition". Levels were also high in shoots and roots from the "high" micronutrients with "high" calcium treatments. It was notable that as for MK1 roots in 0 and 0.7 ppm nickel conditions with "high" micronutrients and "low" calcium, the magnesium content of NS shoots and roots did not increase with increasing magnesium supply. Why this should occur in that particular treatment combination is unknown. It was interesting to note

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that with "high" micronutrients an increased calcium supply promoted magnesium uptake into NS roots and shoots and MK1 shoots. The acceleration of absorption of one element by another, usually calcium, is known as the "Viets effect". Epstein (1972) argued that the presence of calcium is the normal physiological condition and that the rate of ion absorption is diminished in the absence of calcium, not increased in its presence. However, when quite high concentrations of calcium (such as the 1.2 mM level used here) appear to accelerate the absorption of an ion (magnesium in this case) beyond the rate found at low and moderate concentrations of calcium then a true "Viets effect" may be inferred. Whilst 0.3 mM calcium (the concentration used in "low" calcium treatments) is low compared with the range of calcium concentrations often found in soil solutions (as given in Epstein, 1972), calcium could in no way be considered absent. Therefore the stimulation of magnesium uptake by high calcium supply is a clear "Viets effect". This contrasts with the work of Madhok (1965) when increasing the calcium supply (0.01, 0.1, 1.0, 10.0 mM calcium) caused the level of magnesium taken up by Helianthus annuus and H. bolanderi ssp. exilis (Heiser) to fall. The differences in these findings emphasise the dangers of generalising from experimental responses in one species.

Calcium

When calcium supply was "high", both MK1 and NS races responded by taking up greater quantities of calcium into

shoots and roots. The calcium status of NS shoots for the "serpentine condition" was similar to that in shoots from all treatments containing "high" calcium. This may be a response to the unfavourable conditions NS plants experience in the "serpentine condition", since calcium is a well known ameliorating influence in conditions of metal toxicity. This response was more pronounced in NS roots. It must be remembered, however, that many other metals increased in concentration in NS plants when in this treatment. It is possible, therefore, that the high calcium in NS plants from the "serpentine condition" is merely a passive entry through cell membranes damaged by high nickel and magnesium.

The fact that "high" calcium supply depressed NS dry weights when nickel and magnesium were low, and increased weights when nickel and magnesium were high, was puzzling. Some additional work involving determining changes in membrane permeability at "high" and "low" calcium supply at varying levels of magnesium and nickel would have been helpful here. Changes in membrane integrity at "high" calcium supply which may ameliorate toxic metal effects by preventing accumulations of high levels of various metals might also reduce the rate at which nutrients are taken into tissues during growth thereby limiting growth rates. This gains some support when it is remembered that "high" calcium supply prevented the occurrence of high levels of zinc, manganese, copper and magnesium in NS plants when micronutrient supply was low and magnesium and nickel were

high. The depression of dry weights with increasing calcium supply when magnesium was low was not noted in Helianthus annuus and H. bolanderi ssp. exilis by Grover (1960) where increasing calcium increased dry weights. However, Grover only used a range of calcium between 0 and 0.4 mM calcium.

It was noted that a high calcium status in shoots and roots, particularly when micronutrients supply was "high", tended to be associated with lower growth of MK1 plants. The unfavourable response to the high levels of these elements is possibly a consequence of an adaptation to soils where these elements are normally in short supply. However, the MK1 race does show some tolerance to these conditions and continues to grow, if at a slower rate. With "high" micronutrient supply MK1 shoot calcium levels increased four times when calcium supply increased four times (0.3 mM to 1.2 mM) at 1.4 mM magnesium levels. However, at 7.5 mM magnesium levels, MK1 shoot calcium only increased 1.5 times with "high" calcium supply. Apparently in the MK1 race (and in the NS race with the exception of roots and shoots from the "serpentine condition") increasing magnesium reduced the uptake of calcium. The depression of calcium uptake by increasing magnesium supply is consistent with the findings of Madhok (1965) when increasing magnesium (0.01 - 10 mM) at each level of calcium caused a fall in calcium levels of Helianthus annuus and H. bolanderi ssp. exilis.

Mg/Ca ratio

In both races, the Mg/Ca ratio decreased markedly when "high" calcium conditions were used. One of the most striking features was the ability of MK1 race to maintain a lower Mg/Ca ratio than NS plants in all treatments. This would imply that the MK1 race is more able to take up more calcium and/or reduce its magnesium uptake when magnesium supply is high than the NS race. The differences between races in this respect were small making it difficult to say how differences in Mg/Ca ratio came about. Madhok (1965) demonstrated that the serpentine endemic Helianthus bolanderi ssp. exilis (a serpentine race) maintained a lower Mg/Ca ratio than non-serpentine H. annuus by taking up less magnesium and marginally more calcium. However, the situation is complex and there are likely to be differences between species. Johnston (1974), for example, showed that Cerastium holostoides took up much more magnesium relative to calcium at a serpentine site in Scotland.

High Mg/Ca ratio alone did not account for the observed dry weights of NS plants. With "high" micro-nutrient and "high" calcium supply (and therefore high internal calcium concentrations) the increasing Mg/Ca ratio noted when magnesium supply was increased did not cause any large reduction in NS dry weights. However, when calcium supply (and therefore the internal calcium levels) was "low" an increasing Mg/Ca ratio was associated with a large decrease in NS dry weight. It is therefore not a

high Mg/Ca ratio per se which is important in limiting growth in NS plants but also the concentration of calcium in the plant tissue. This supports the findings of Proctor (1971b) who demonstrated that the presence of calcium ameliorated the toxicity of magnesium in non-serpentine Agrostis stolonifera and that magnesium toxicity is dependent on the calcium concentration.

General conclusions

Perhaps the most striking result of this work in water culture is the evidence of interactions between factors. Although magnesium was apparently more important than any other element in influencing the growth of plants in the water culture there were many important interactions of elements.

The very low weights of the NS race in the "serpentine condition" medium appear to be a consequence of a number of factors. In this medium, the NS race took up relatively large amounts of several metals, notably nickel, iron, copper, zinc, magnesium and calcium from the solution. High levels of iron, copper and magnesium were also noted in NS plants when grown in conditions of "high" micro-nutrient, "low" calcium, 7.5 mM magnesium and 1.4 ppm nickel, a treatment combination which also produced very low growth rates. It is not unreasonable to suggest, therefore, that the high levels of at least iron, copper and magnesium may in some way partly account for the low growth of NS plants in these treatments. The fact that increasing

the calcium supply prevented the unusual accumulation of the metals mentioned (and increased the growth of NS plants in these treatments) suggests that cell membrane integrity was adversely affected at low calcium supply by the high magnesium and nickel. Membrane integrity was apparently restored at "high" calcium supply thereby reducing the absorption of metals.

The inability of the NS race to take up and maintain high potassium levels in "serpentine conditions" was also a notable feature which contrasted with the MK1 race response. This was consistent with the work of Madhok (1965), who noted that in high magnesium treatments, potassium levels were higher in a serpentine endemic species of Helianthus than in a cultivated species.

The poorer growth of MK1 race when micronutrients and calcium were "high" may be accounted for in terms of the generally high metal levels noted in this race when in these conditions. "high" calcium supply apparently produces a "Viets effect" (as already pointed out for magnesium uptake) with micronutrient uptake thereby promoting the absorption of these elements which are known to be toxic in quite low concentrations to intolerant plants e.g. Gregory and Bradshaw (1965). The "Viets effect" was not noted for micronutrient absorption in the NS race. It is paradoxical that calcium, which is usually associated with reducing the toxic effects of metals, is in the MK1 race apparently intensifying the effects of micronutrients and

reducing growth. It appears to be the case that the MK1 race has adapted to serpentine soil conditions where micronutrients and calcium are usually low and that high levels of these elements are toxic to this race. A similar conclusion was reached by Jefferies and Willis (1964) who found that Juncus squarrosus and Nardus stricta only exist in soils of a narrow and low range of mineral nutrients, particularly with regard to calcium concentration.

Whilst it must be stressed that no single element considered in isolation from all others can account for the observed growth of plants in each treatment, it was magnesium which was most strongly associated with dry weights. Increases in magnesium content of NS plants were always associated with decreases in dry weight. Superimposed over this major influence was the effect of nickel supply, increases in which reduced dry weights. The effect of nickel increased with increasing magnesium concentration and the interaction of both was enhanced when micronutrients and calcium supply were reduced.

The MK1 race appears to be adapted to serpentine soils possibly through having a high requirement for magnesium, maintaining a low internal Mg/Ca ratio, being able to maintain a high potassium content and by having cell membranes which do not lose integrity when magnesium and nickel concentrations in the soil solution are high and calcium supply low. Somehow they prevent the absorption of high levels of potentially toxic metals. A

further point of interest was the response of the MK1 race when micronutrients and calcium were "low" and nickel was absent. Under these conditions certain metals (zinc, copper and manganese) were taken in greater amounts than when nickel was present in the culture medium. This may suggest that either a) in the absence of nickel the MK1 race takes up one or more of these micronutrients and uses it to substitute for a function normally fulfilled by nickel, or b) since nickel is not available in the culture medium there is less competition for a carrier system responsible for micronutrient (or nickel) uptake. Both a) and b) could operate simultaneously. The adaptation of the MK1 race to nickel in MK1 soils is also illustrated by the root surface enzyme data (Chapter 5) where nickel was shown to be biochemically active and MK1 race responded favourably to its presence.

CHAPTER 4

THE ROLE OF CHROMIUM

Introduction

The question of the role of chromium as a causal factor in serpentine ecology is particularly tantalising for the ecologist (Proctor and Woodell, 1975). The total quantities of chromium in serpentine soils are very variable. Whilst nickel is an integral part of serpentine minerals, chromium occurs as an accessory mineral, chromite, which is distributed erratically (Faust and Fahey, 1962). Total levels of chromium can be very high, contrasting with the low, often undetectable concentrations extracted by reagents used to estimate "plant available" cations. For example, Wild (1974) found very low (0.2 ppm) dilute acetic acid extractable chromium in some Rhodesian soils which contained up to 12.5% total chromium.

Species have been shown to exhibit marked differences in their response to chromium. Lyon et al (1971) working in New Zealand, found Hebe odora (Hook. f.), on a soil containing 8500 ppm chromium, had only 141 ppm in its ashed foliage, whilst Leptospermum scoparium (J.R. et G. Forst) in a soil containing 8750 ppm chromium had 1760 ppm in its ashed tissue. Chromium has a physiological role in animals (Schwarz and Mertz, 1961) but there is no evidence of any such function in plants. The physiology of accumulator plants such as Leptospermum scoparium and Sutera fodina

(Wild) would merit further investigation. Lyon et al (1969a, b) found that the chromium accumulator Leptospermum scoparium having taken up $^{51}\text{CrO}_4^{2-}$ ions from the surrounding medium, converted some of this into trisoxalatochromium (III) ions (accounting for 18% of the total ^{51}Cr in the soluble fraction from roots).

Species differences in plant chromium levels have been noted in plants growing in MK1 soils by Johnston (1976). For example, in Cochlearia officinalis the mean chromium level was 130 ppm whereas Armeria maritima was found to have only 61 ppm in its leaves. The concentration of chromium in Festuca rubra was 76 ppm. Since chromium was below the detection limit in MK1 soil solution, this metal was not included in the water culture experiments described in Chapter 3. However, in view of the fairly high plant chromium levels in the field, I undertook an investigation in water culture to study the influence of chromium (III). This oxidation state of chromium was used since Johnston (1976) demonstrated the greater toxicity of chromium (III) compared with chromium (VI) in single salt tolerance tests using MK1 and NS races of F. rubra.

In addition, an attempt was made to find a simple method of determining chromium complexes within the plant. This is described in Appendix III.

Materials and Methods

Experimental details of water culture techniques were

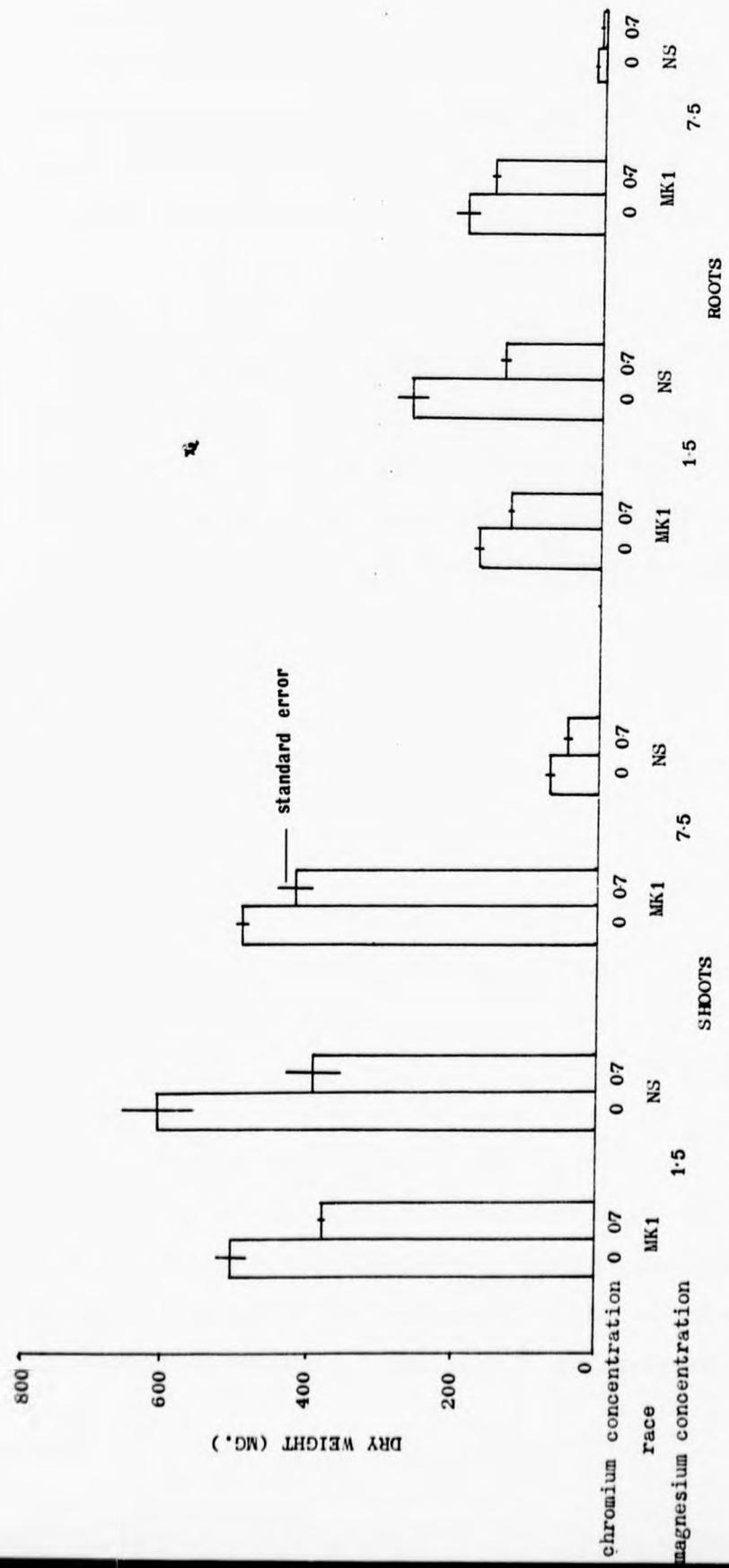


FIGURE 13 THE INFLUENCE OF CHROMIUM (III) ON THE DRY WEIGHTS OF MK1 AND NS PLANTS.

generally similar to those described in Chapter 3, except that only two levels of chromium, 0 and 0.7 ppm, were used and the 4.0 mM magnesium treatment was omitted. ^{Low micronutrients and calcium were used} Plant metal analyses were not carried out and the influence of chromium was judged from its effect on dry weight.

Results

Chromium caused a reduction in dry weight of shoots and roots in both races at each magnesium level (figure 13). The reduction in dry weight was most pronounced in the NS race. In the MK1 race, grown in 0.7 ppm chromium, the high magnesium treatment produced slightly (not statistically significant) higher dry weights of shoots and roots compared with those in 1.5 mM magnesium. 7.5 mM magnesium greatly reduced the dry weight of the NS race in the presence and absence of chromium.

Discussion

Cr (III) when applied to culture media at a concentration of 0.7 ppm reduces growth in both races, the retardation being greater in the NS race. Whilst there appeared to be an interaction between Cr (III) and magnesium in MK1 plants, the results were not statistically significant. However, further work using a range of Cr (III) concentrations may give some useful information concerning this interaction. Such a relationship between chromium and magnesium, should it be confirmed, may be an important adaptation of MK1 race. Whilst 0.7 ppm chromium was found

to influence dry weights of MKI plants (and 0.7 ppm nickel was not, as shown in Chapter 3), the level used does not match that in MKI soils and further experimentation at much lower chromium levels may merit consideration to determine the importance of this metal.

CHAPTER 5

ROOT-SURFACE ACID PHOSPHATASE ACTIVITY IN

MK1 AND NS PLANTS

Introduction

Serpentine soils are prime examples of unusual soil chemistry determining special vegetation. Woolhouse (1969) pointed out that at the root surface structural and catalytic proteins are in direct contact with the chemical constituents of the soil solution. It might be expected therefore that the root-surface enzymes of serpentine-adapted plants should have special properties.

In most soils only a small proportion of total phosphorus is readily available for uptake by plants. Most of the phosphorus is present as insoluble phosphates of calcium, iron and aluminium or as organo-phosphorus compounds. Inorganic phosphates may be hydrolysed by bacteria and organo-phosphorus compounds by both bacteria and fungi. Woolhouse (1969) pointed out that several workers have shown that higher plants when grown in sterile media can utilize insoluble phosphorus sources, probably through the mediation of root-surface phosphatases. Under field conditions, however, the contribution of these enzymes to phosphorus mobilisation is uncertain.

Whatever the importance of root-surface enzymes to the survival of plants found in serpentine soils, these enzymes

CHAPTER 5

ROOT-SURFACE ACID PHOSPHATASE ACTIVITY IN

MK1 AND NS PLANTS

Introduction

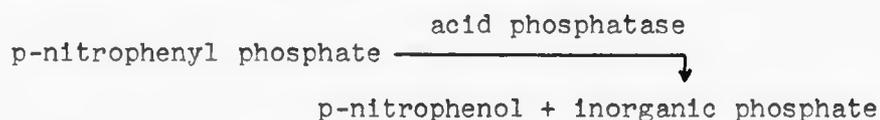
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Whatever the importance of root-surface enzymes to the survival of plants found in serpentine soils, these enzymes

may be useful in studying the plant response to the soil conditions. Accordingly, the influence of putatively ecologically important metals in serpentine soils on root-surface phosphatase activity was studied by incubating roots in solutions of differing composition.

Root surface acid phosphatase activity may be easily determined since the enzyme shows a high affinity for p-nitrophenyl phosphate, catalysing the reaction



In the presence of sodium hydroxide, p-nitrophenol forms a yellow phenolate ion. The reaction rate can be determined by measuring the increase in concentration, in unit time, of the phenolate ion, using a spectrophotometer.

An initial investigation was undertaken to determine the pH optima for acid phosphatases from MK1 and NS races of Festuca rubra. The enzyme activity could then be experimentally determined at optimum pH when roots were incubated in solutions of varying mineral composition.

Materials and Methods

a) Determination of pH optima for acid phosphatase.

Acetate buffers (pH 3.5, 4.2, 4.9, 5.6) were prepared by adding glacial acetic acid to 1M sodium acetate. The buffers were diluted to 0.1 M acetate. A 0.1 M solution of tris buffer was prepared and adjusted to pH 7.4. The

preparation of the roots and the determination of acid phosphatase activity is described later. The incubation medium used is given in Table 9.

b) Time courses

To ensure the concentration of substrate (100 mg p-nitrophenyl phosphate in 25 cm³ water) used in cell enzyme assays was not rate-limiting, plots of phosphatase activity with respect to time (time courses) were made for both races of F. rubra. Enzyme activity was determined at optimum pH, after 15, 30, 45, 60, 75 and 90 min as described under "enzyme assay" and "calculation of enzyme activity" in c).

c) Acid phosphatase activity in solutions of varying mineral composition

1) Preparation of root material

Tillers of MK1 and NS F. rubra were removed from pots containing John Innes No. 2 compost and cut below the first node. Tillers were rooted in two nutrient regimes (Table 8), using the apparatus shown in figure 1, for 11 days at 20°C and 16 h daylength.

11) Preparation of incubation media

The surface acid phosphatase activity was determined in roots, grown in one of the two rooting media described in Table 8, when they were incubated in media of varying mineral composition (Table 9). The volume of each component of the incubation mixture is given in Table 10 which refers to volume of stock solutions shown in Tables 2 and 3.

Table 8. Culture media chemical combinations used for rooting tillers of F. rubra.

Rooting medium No.	Final concentration			
	*macronutrients	+ micronutrients	magnesium	nickel
1	"low" Ca	"low"	7.5 mM	0 ppm
2	"low" Ca	"low"	1.5 mM	0 ppm

* refers to "low" Ca levels of nutrients given under "culture media" in Table 2, Chapter 3.

+ refers to "low" levels of micronutrients given under "culture media" in Table 3, Chapter 3.

12 mM sodium nitrate was added to rooting medium No. 2 to maintain a similar nitrate concentration to that found in rooting medium No. 1.

Table 9. The composition of the mineral components in incubation media used to study the interactions of metals in relation to root-surface acid phosphatase activity.

Rooting medium No.	macronutrients	micronutrients	magnesium (mM)	nickel (ppm)
1	"low" Ca	absent	7.5	0
1	"low" Ca	absent	7.5	0.7
1*	"low" Ca	"low"	7.5	0
1	"low" Ca	"low"	7.5	0.7
1	"low" Ca	"high"	7.5	0
1	"low" Ca	"high"	7.5	0.7
1	"high" Ca	absent	7.5	0
1	"high" Ca	absent	7.5	0.7
1	"high" Ca	"low"	7.5	0
1	"high" Ca	"low"	7.5	0.7
1	"high" Ca	"high"	7.5	0
1	"high" Ca	"high"	7.5	0.7
2	"low" Ca	"low"	1.5	0
2	"low" Ca	"low"	1.5	0.7
2	"low" Ca	"low"	4.0	0
2	"low" Ca	"low"	4.0	0.7

* This treatment combination was also used to determine the pH optima and time course for acid phosphatase.

The reference to "low" or "high" Ca under macronutrients and "low" or "high" under micronutrients in Table 9 refers to concentrations of nutrients given in Tables 2 and 3.

Table 10. Volume of each component in the incubation media used to determine acid phosphatase activity.

Component	Volume (cm ³)
buffer	10
macronutrients	0.12
micronutrients*	0.12
magnesium**	0.12
nickel+	0.50
water	0.14
substrate	1.00

* 0.12 cm³ water was added when micronutrients were absent in treatments.

** refers to 0.12 cm³ of 0.15 or 0.40 or 0.75 M magnesium stock solution.

+ 0.5 cm³ water was added in 0 ppm nickel treatments. In treatments which included nickel a 16.8 ppm nickel solution was used giving a final concentration of 0.7 ppm.

iii) Calibration curve

A calibration curve of p-nitrophenolate against optical density was prepared in order to estimate the concentration of p-nitrophenol formed by the action of the root-surface enzyme on the substrate.

Solutions of p-nitrophenol ranging between 0 and 50 g cm⁻³ were prepared. One cm³ of each solution was added to 2 cm³ of 0.5 M sodium hydroxide and mixed with 5 cm³ of deionized water. The optical density of the resulting yellow solutions was measured at 405 nm using a Cecil CE 343 u.v./visible spectrophotometer.

iv) Enzyme assay

Assays were carried out in triplicate for each incubation medium shown in Table 2. Roots were gently washed in deionized water and quickly dried on tissue paper. The first 3 cm of a root tip (including any laterals) was cut, with scissors, from the root system and the fresh weight recorded. Individual root tips were transferred to test tubes containing all components of the incubation media (Table 10) with the exception of the substrate. The test tubes were shaken and placed in a water bath at 20°C. After 10 min the reaction was started by the addition of 1 cm³ of substrate (100 mg of p-nitrophenyl phosphate dissolved in 25 cm³ deionized water). After shaking, the optical density at 405nm was determined after 20, 40 and 60 min by removing 1 cm³ incubation solution and mixing it with 2 cm³ 0.5 M sodium hydroxide and 5 cm³ deionized water.

The concentration of p-nitrophenol was determined from the calibration curve. When the assay was completed, the root tips were dried at 60°C for five days, then weighed.

v) Calculation of enzyme activity

The concentration of p-nitrophenol read off from the calibration curve, as described in iv), represents the number of μg in each 1 cm^3 aliquot removed from incubation solution after each time interval. In order to reduce any sampling error, graphs were plotted of $\mu\text{g cm}^{-3}$ p-nitrophenol against sampling time (i.e. 20, 40 and 60 min) and a best-fit line drawn. The concentration of p-nitrophenol at each time interval was read off from these graphs and used in subsequent calculations. Initial studies of the time course of phosphatase activity (figures 16 and 17) indicated the reaction was linear up to 90 min. Willett (1975) showed a similar linearity over this time interval for phosphatase from serpentine and non-serpentine grasses.

Prior to the 60 min sampling interval, 2 cm^3 of solution had been withdrawn (1 cm^3 at 20 min and 1 cm^3 at 40 min) from each incubation mixture, leaving 10 cm^3 in each tube. The number of $\mu\text{g cm}^{-3}$ of p-nitrophenol at 60 min was multiplied by 10 giving the total concentration remaining in each tube. The number of $\mu\text{g cm}^{-3}$ found at 20 and 40 min was added to this value giving the overall concentration of p-nitrophenol found after 60 min. The enzyme activity was expressed as $\mu\text{g p-nitrophenol g}^{-1}\text{ root}$

dry weight h^{-1} . Other workers e.g. Woolhouse (1969) and Willett (1975), expressed activity in terms of root surface area. I found this impossible to measure since F. rubra roots often have extensive lateral development in the first few cm from the tip. In addition, in view of the quite large number of assays undertaken, the use of dry weights provided a simple routine method enabling accurate comparisons to be made within this study.

Results

a) pH optima for root-surface acid phosphatase

The curves of enzyme activity against pH for NS and MK1 roots (figures 14 and 15) show both races of F. rubra have similar pH optima of 4.9 for acid phosphatase activity. The NS race roots showed a sharper activity peak at optimum pH compared with those of the MK1 race. All subsequent enzyme assays were carried out at pH 4.9.

b) Time courses

Plots of phosphatase activity with respect to time for MK1 (figure 16) and NS (figure 17) races of F. rubra at pH 4.9 showed the reaction was linear up to 90 min. This indicated that substrate concentration was not rate limiting for the assays which were completed in less than 90 min and it was decided to assay for acid phosphatase over a 60 min period.

c) Enzyme assays

(The term "absence of micronutrients" will be used to

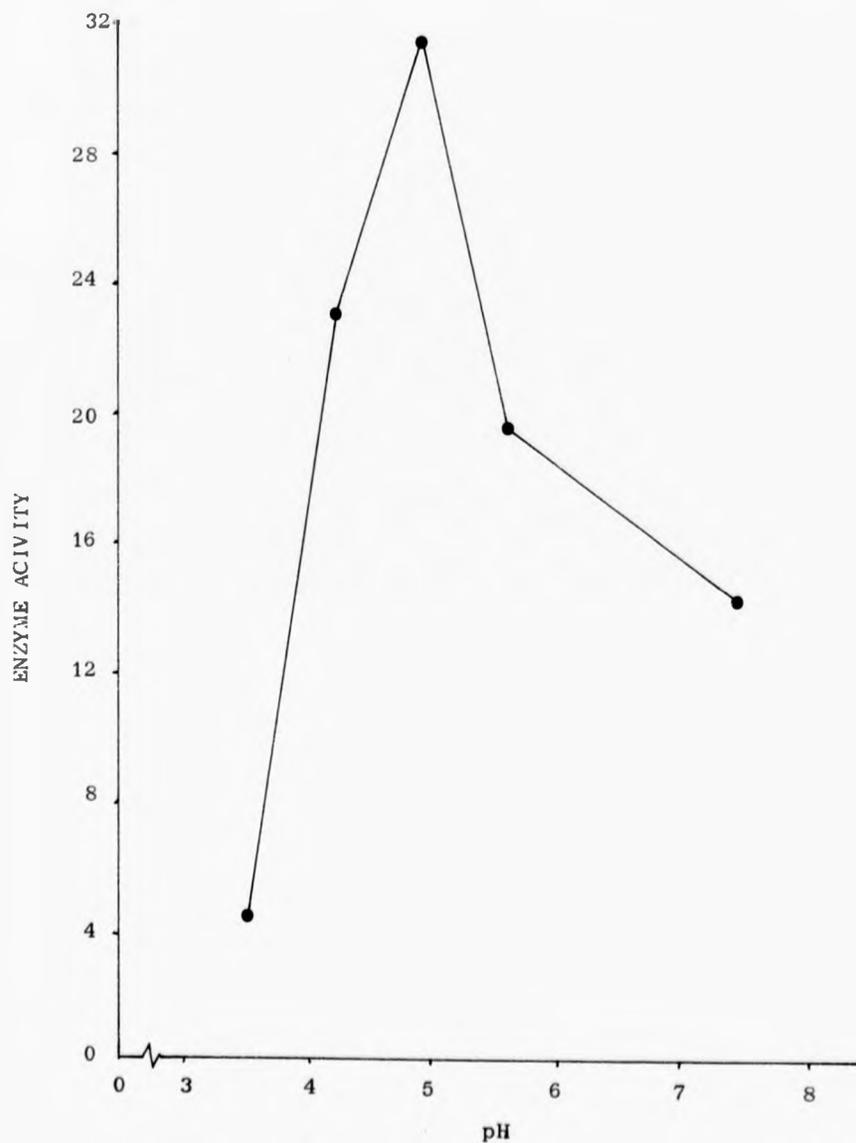


FIGURE 14 PLOT OF pH AGAINST ENZYME ACTIVITY ($\mu\text{g. P-NITRO PHENOL/g. ROOT DRY WEIGHT/HOUR}$) OF ACID PHOSPHATASE FROM NS PLANTS.

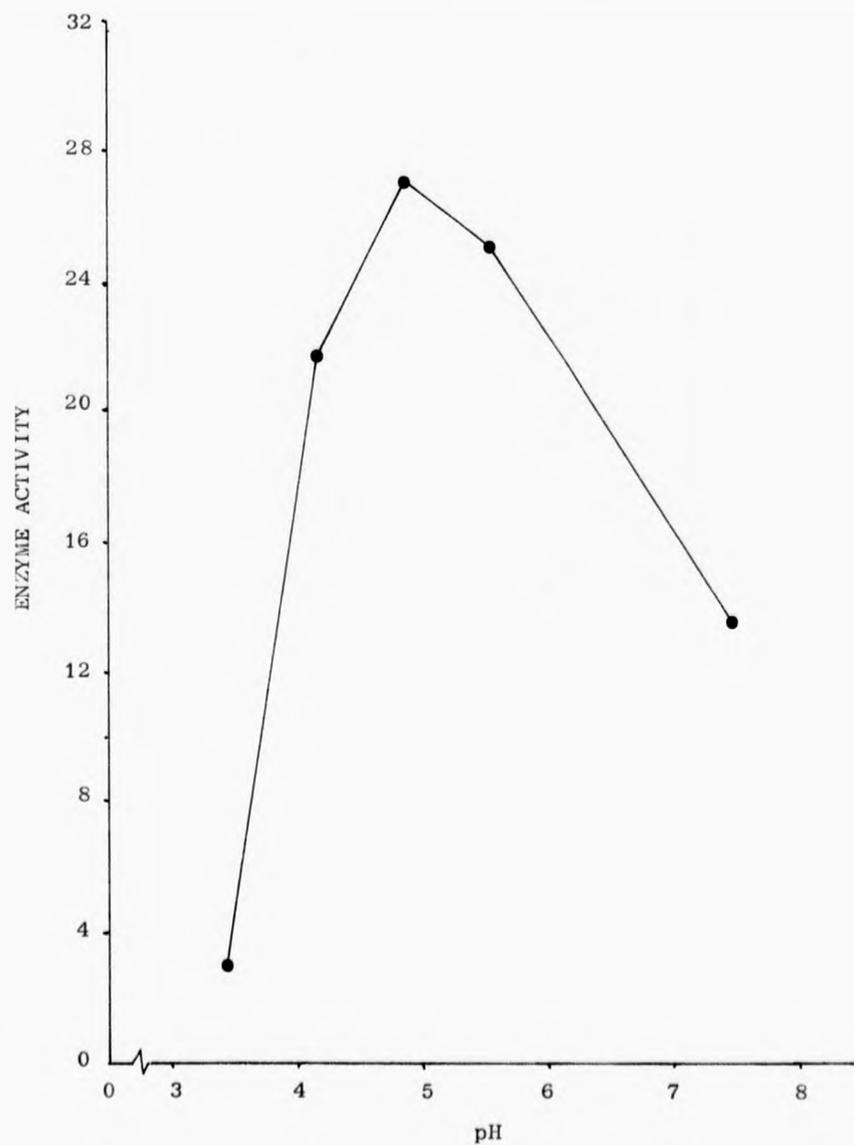


FIGURE 15 PLOT OF pH AGAINST ENZYME ACTIVITY ($\mu\text{g. P-NITRO PHENOL/g. ROOT DRY WEIGHT/HOUR}$) OF ACID PHOSPHATASE FROM MK1 PLANTS.

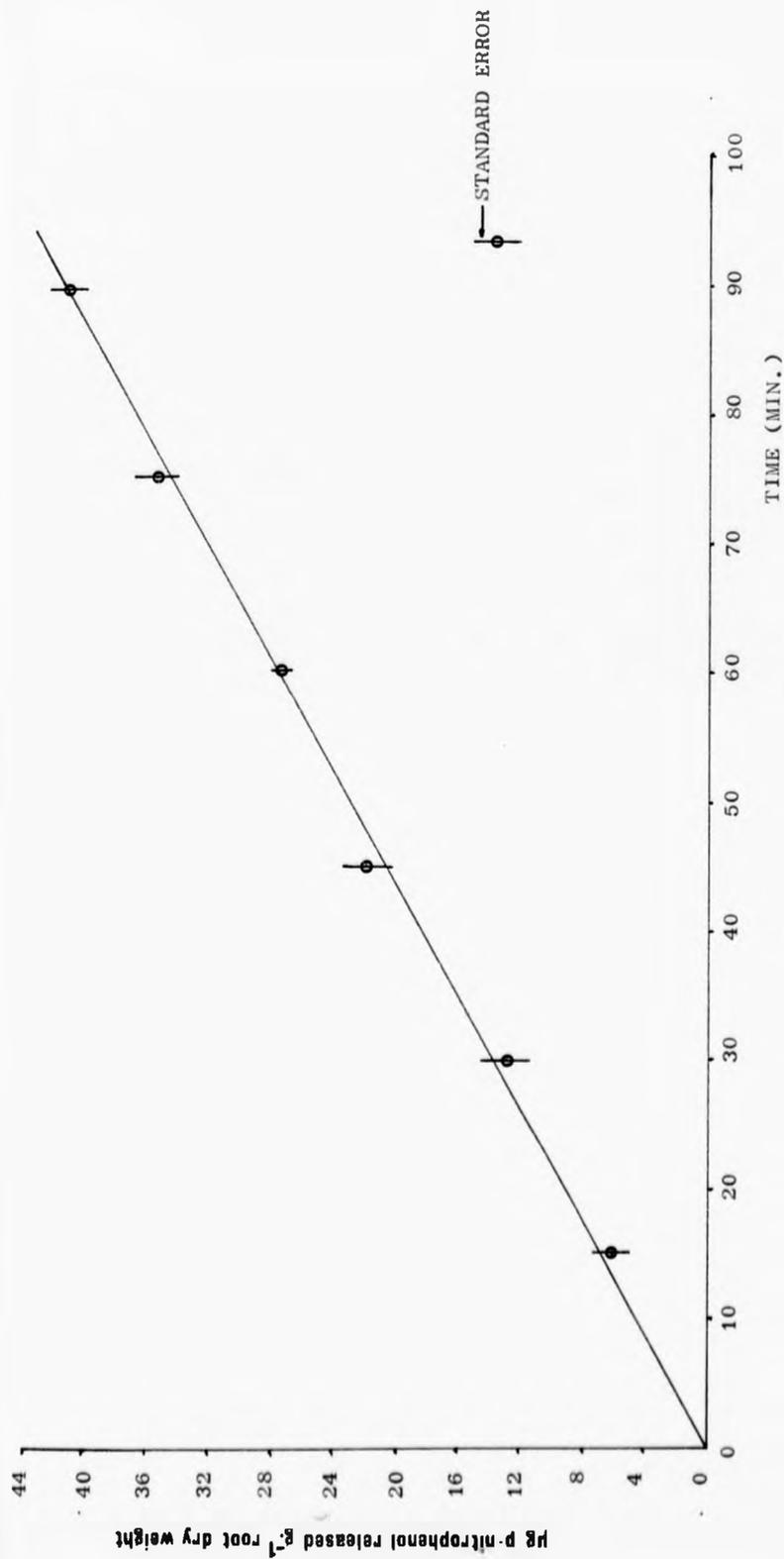


FIGURE 16 TIME COURSE FOR ACID PHOSPHATASE ACTIVITY (μg . P-NITROPHENOL/ g . ROOT DRY WEIGHT/HOUR) FROM MK1 ROOTS.

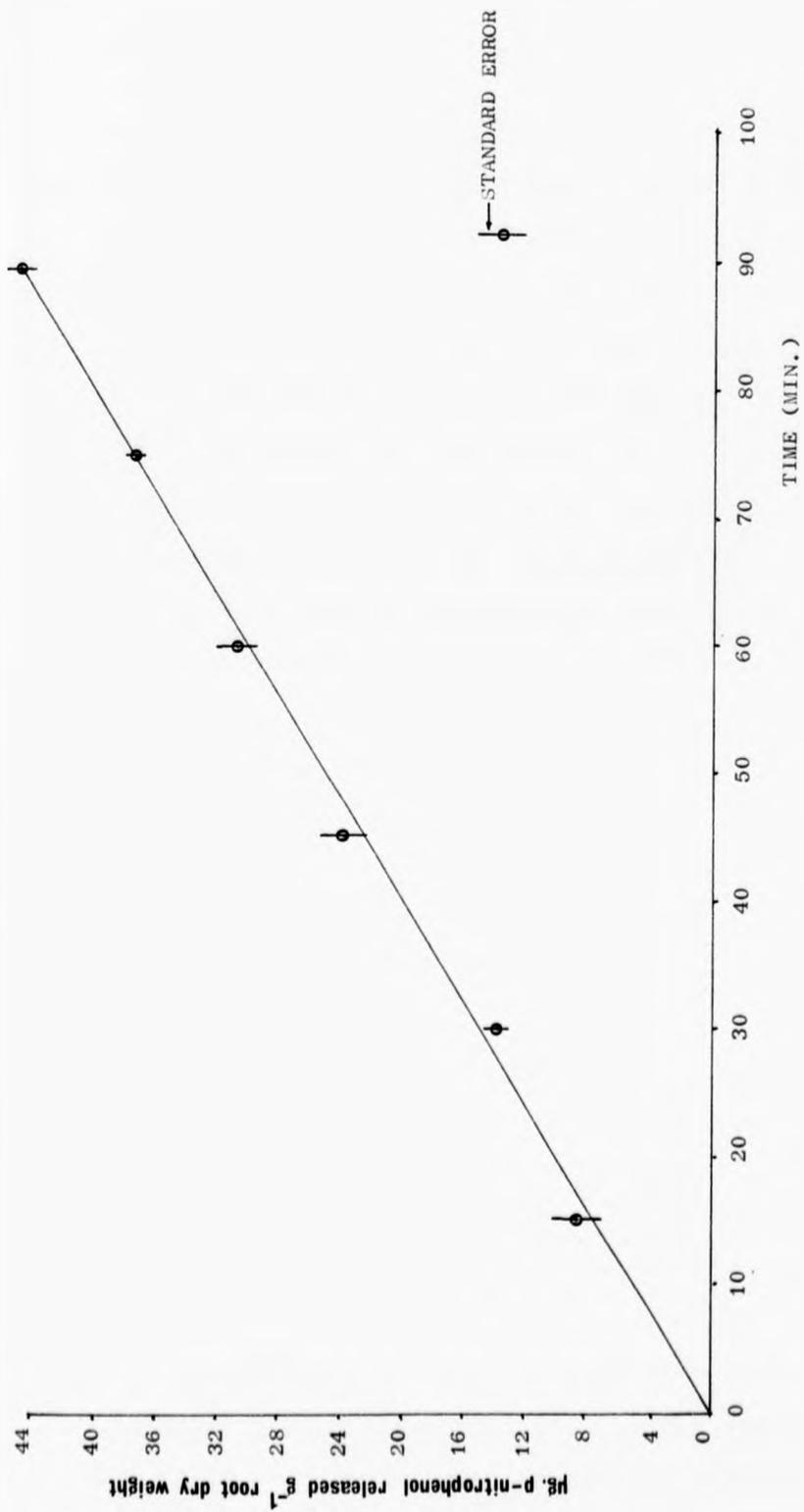


FIGURE 17 TIME COURSE FOR ACID PHOSPHATASE ACTIVITY ($\mu\text{g. P-NITROPHENOL/g. ROOT DRY WEIGHT/HOUR}$) FROM NS ROOTS.

describe treatments in which micronutrients were not added.)

i) MK1 plants

Figure 18 shows enzyme activity was invariably higher in roots incubated in the "absence of micronutrients". A striking feature was the increase in enzyme activity (significant $p < 0.01$), when 0.7 ppm nickel was added to the incubation media, in the absence of micronutrients at "low" and "high" calcium levels. A less pronounced activity increase ($p < 0.05$) due to nickel was noted in the "low" micronutrients with "low" calcium treatment. It was noted that in the "low" micronutrients with "high" calcium treatment the addition of nickel caused a decrease in enzyme activity but the difference was not significant. In the absence of nickel and at "low" micronutrient levels, the enzyme activity increased ($p < 0.02$) from "low" to "high" calcium. No significant differences were found in enzyme activity between treatments which included "high" micronutrients.

The addition of lead or chromium (III) (figure 20) to the incubation medium did not cause any increase in enzyme activity. However, when both nickel and either lead or chromium (III) were present the activity increase associated with nickel was lost.

In experiments where the magnesium concentration was progressively increased (figure 21) the enzyme activity was found to increase with increasing magnesium concentration. Activity differences were statistically significant,

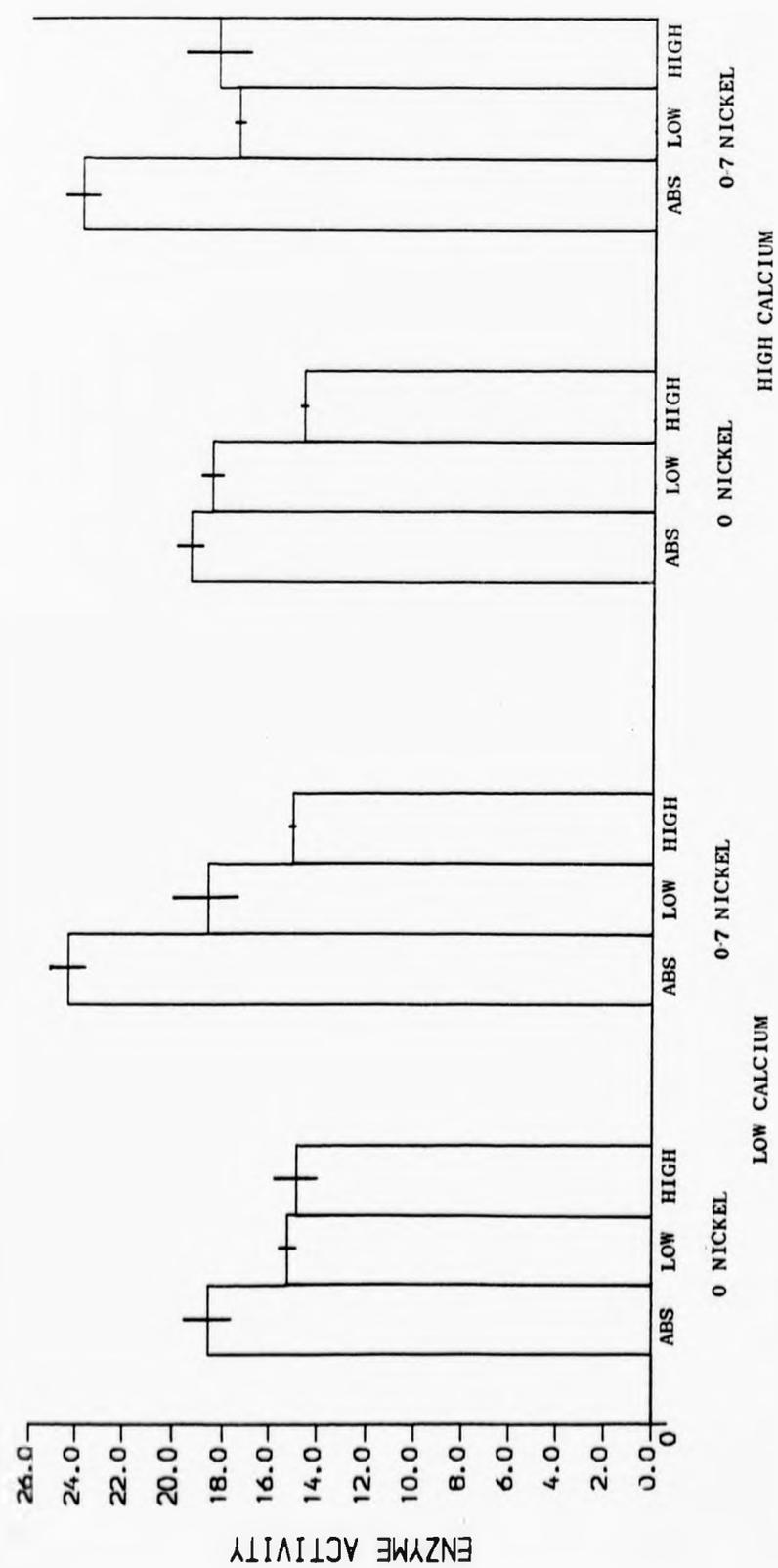


FIGURE 18. ACID PHOSPHATASE ACTIVITY (µGRAM. P-NITROPHENOL / GRAM. ROOT DRY WEIGHT/HOUR) IN MK1 PLANTS.

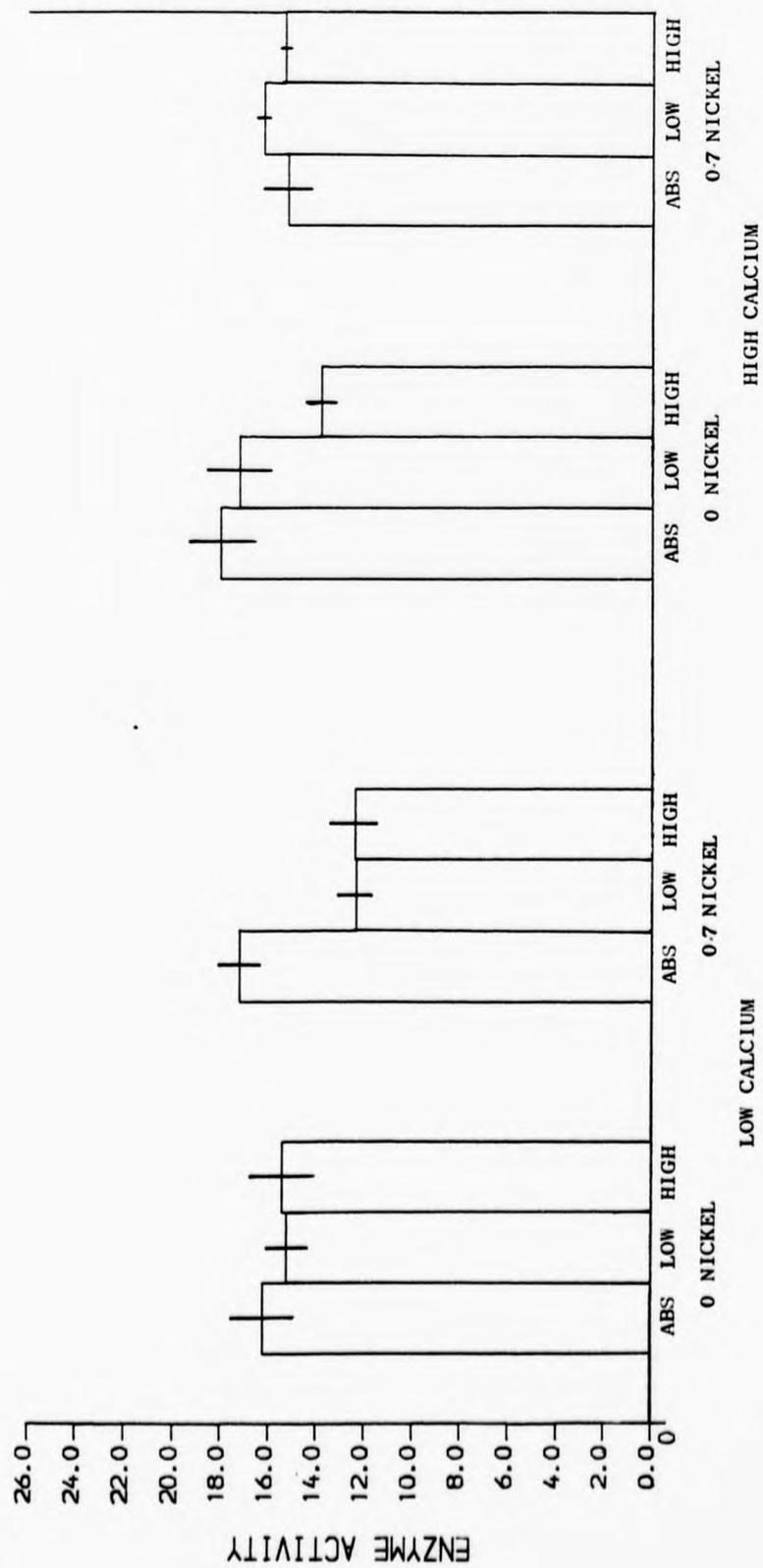


FIGURE 19 ACID PHOSPHATASE ACTIVITY (µGRAM. P-NITROPHENOL/ GRAM. ROOT DRY WEIGHT/HOUR) IN NS PLANTS

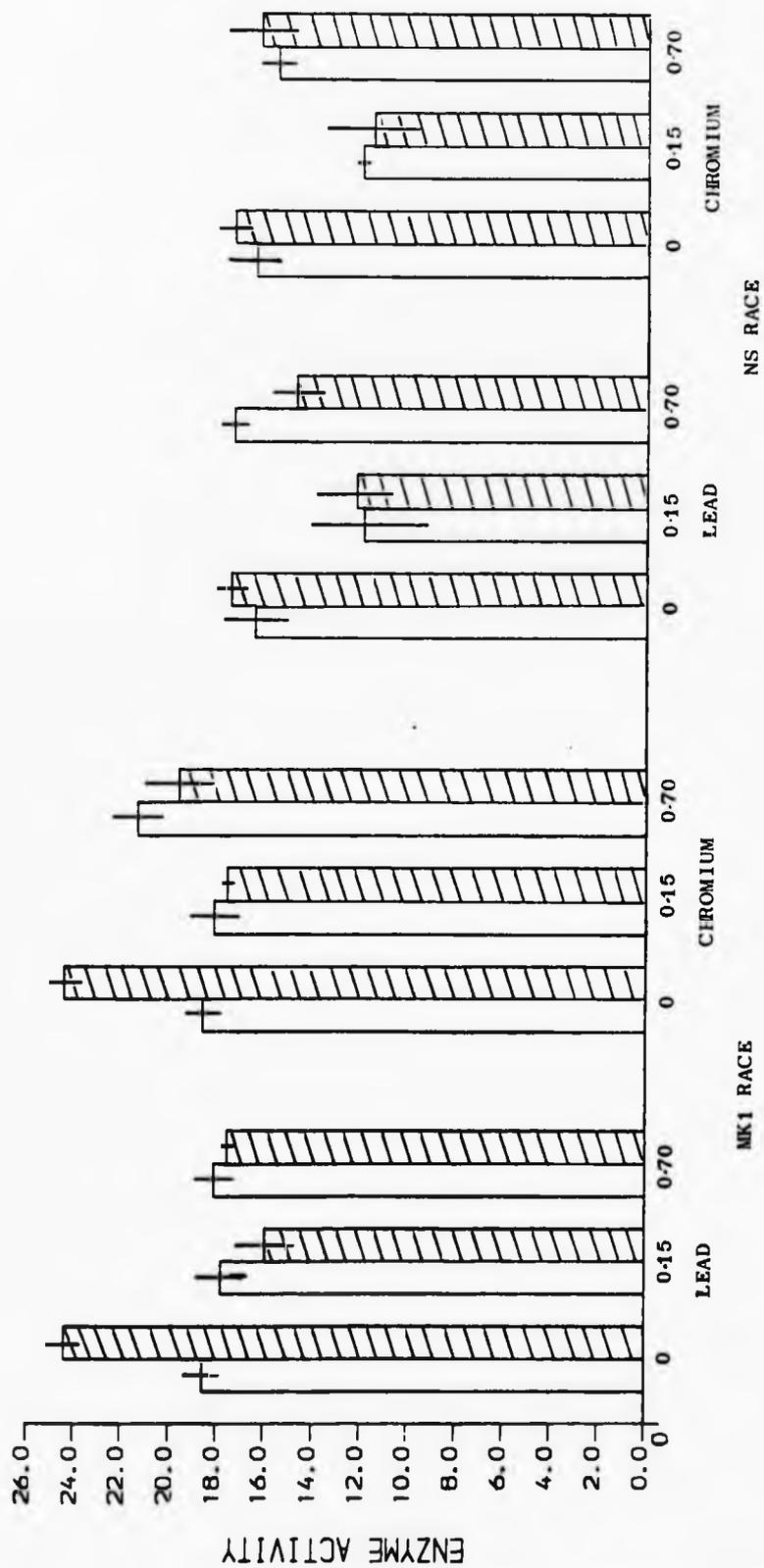


FIGURE 20 ACID PHOSPHATASE ACTIVITY (UGRAM. P-NITROPHENOL/GRAM. ROOT DRY WEIGHT/HOUR) WHEN CHROMIUM AND LEAD WERE PRESENT IN THE INCUBATION MEDIA

Unshaded columns = activity when nickel was absent from incubation media
 Cross-hatched columns = activity when 0.7ppm nickel was present in the incubation media

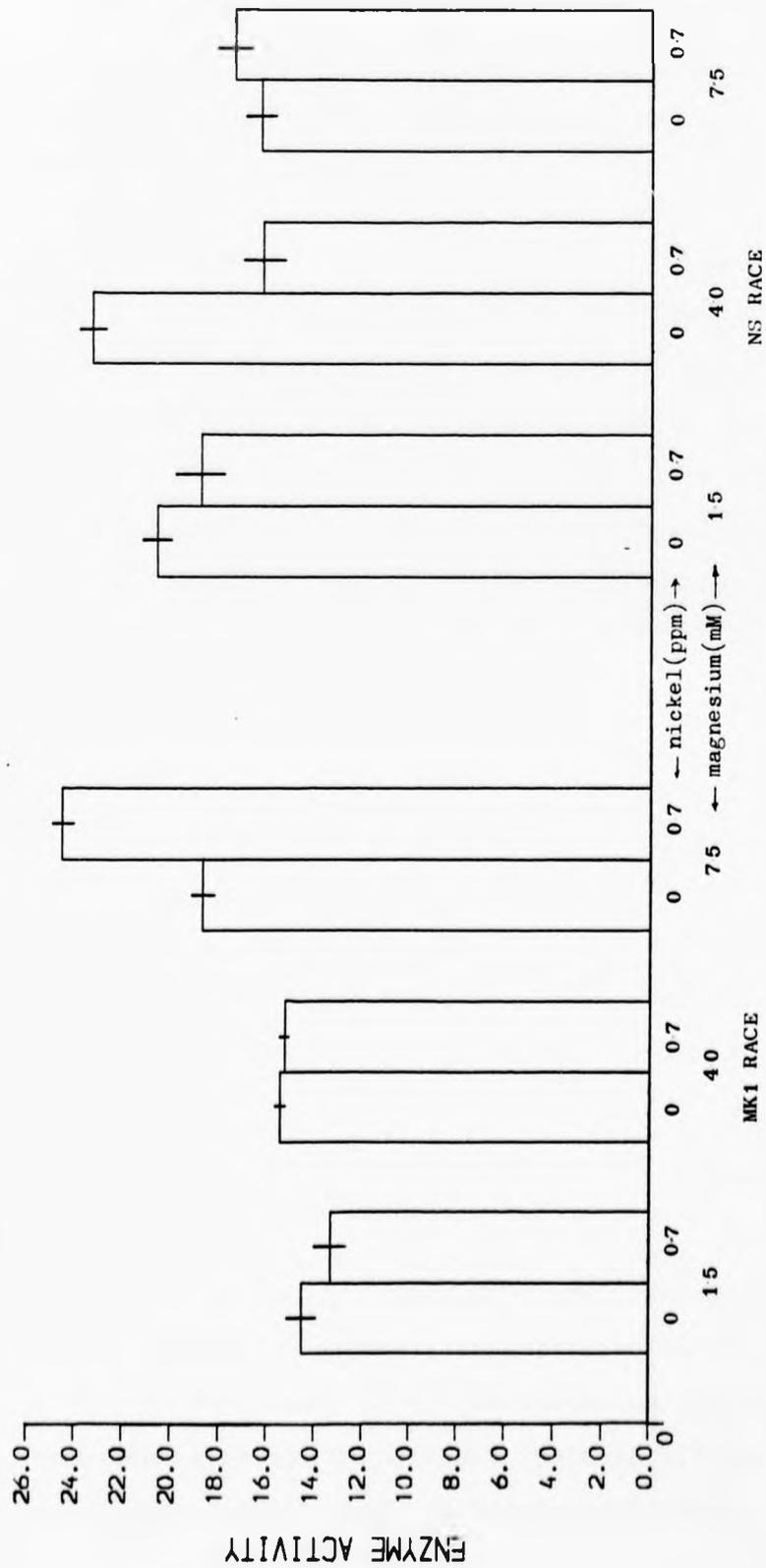


FIGURE 21 ACID PHOSPHATASE ACTIVITY (µGRAM, P-NITROPHENOL/GRAM, ROOT DRY WEIGHT/HOUR) IN MK1 AND NS PLANTS WITH INCREASING MAGNESIUM SUPPLY.

in the presence of 0.7 ppm nickel, at each level of magnesium. However, in the absence of nickel, no significant difference in activity was noted between the 1.5 and 4.0 mM magnesium treatments.

ii) NS plants

As for MKI plants, the enzyme activity in NS plants (figure 19) decreased with increasing micronutrient concentration. In most treatments, enzyme activity was lower in NS compared with MKI plants. This difference was statistically significant ($P < 0.01$) when nickel was present in the incubation media. There was no enhancement in activity when nickel was added to a treatment. In the presence of nickel and both levels of micronutrients enzyme activity increased moving from "low" to "high" calcium treatments (significant $P < 0.01$).

With the addition of 0.15 ppm lead or chromium (III) (figure 20) a depression in activity was noted in NS plants. When 0.7 ppm of either metal was added the enzyme activity appeared to rise to levels similar to those noted when lead and chromium (III) were absent. The addition of nickel had no effect on the enzyme activity in the presence of lead or chromium (III).

With increasing magnesium concentration (figure 21) in the absence of nickel, the enzyme activity was highest at 4.0 mM and lowest at 7.5 mM magnesium. These differences were statistically significant (between 1.5 and 4.0 mM treatments $P < 0.10$; between 4.0 and 7.5 mM treatments

$P < 0.01$). In the presence of nickel, there was a trend (although not statistically significant) towards lower enzyme activity with increasing magnesium concentration.

Discussion

The similarity in pH optima for both races was surprising in view of the differing pH found in the native soil of each race. Johnston (1976) found the average pH of soils, taken from MK1 was 6.9 whilst the acid brown earth soil, from which the NS race was collected, had a pH of 5.2. The NS root-surface acid phosphatase pH optimum of 4.9 was hence very similar to the pH of its native soil.

Data presented here suggest there are a number of interactions which influence the activity of root-surface acid phosphatase in F. rubra. The nickel enhancement of enzyme activity, in MK1 plants in the absence of micro-nutrients is the most striking feature. A search of acid phosphatase literature (most of which refers to animal acid phosphatases) revealed no mention of nickel acting as an activator. Indeed, the weight of evidence suggested nickel is an inhibitor of activity e.g. in red blood cell acid phosphatase (Hollander, 1971). Willett (1975) concluded that nickel produced no significant difference in the activity of root-surface acid phosphatase from serpentine tolerant and non-tolerant species of grass. Unfortunately, comparisons between Willett's work and this study are difficult since the techniques employed differed

in many respects e.g. Willett used relatively high levels of nutrients and high (37°C) incubation temperatures. It is believed that the enzyme incubation conditions employed in this study represent the chemical conditions root-surface acid phosphatases are more likely to encounter in serpentine soils since the composition of incubation solutions was based on soil solution data obtained earlier. Whilst it is realised that the incubation temperature (20°C) used in the present study is likely to be higher than normally encountered in MK1 soils, it has the merit of being the same as the temperature at which the water culture experiments (Chapter 3) were carried out.

The nickel-induced increase in MK1 enzyme activity suggests this race has evolved an enzyme system activated by nickel in the absence of micronutrients. Edaphic conditions would favour such selection since two of the classical "serpentine conditions" are low micronutrient levels and relatively high nickel concentrations. This is apparently not a general feature of F. rubra since the NS race acid phosphatase did not respond to nickel in the same way. Micronutrients, which are often associated with the role of enzyme activation, were notable in this study for their suppression of enzyme activity when in concentrations greater than those due to contamination i.e. in treatments to which micronutrients were not added. This was true for both races but the differences in the NS race were usually insignificant. The greater influence of micronutrients on the MK1 race, where differences in activity due to their

presence or absence were in most cases statistically significant, may represent a response of this race to metals usually at very low concentrations in serpentine soils. The loss of the nickel activation in the MK1 race, on the application of micronutrients, seems likely to be an inhibition of an enzyme system unadapted to relatively high levels of these nutrients.

More evidence, that the response of MK1 plants to nickel is specific, is gained when the lead and chromium (III) treatment data is considered. Neither of these metals produced the increase in activity noted with nickel. The MK1 race was generally unaffected by the presence of lead or chromium (III), the enzyme activity remaining similar to that found in roots incubated in the absence of these metals. However, the loss of activity enhancement due to nickel, when lead or chromium (III) were present, suggests some competition for association with the enzyme. The fall in activity when 0.15 ppm lead or chromium (III) was added to NS roots (in the presence or absence of nickel) suggests some direct inactivation of the enzyme by these metals.

Calcium has been reported to be an inhibitor of various acid phosphatases (Hollander, 1971). By contrast, Willett (1975) found that the acid phosphatase activity of serpentine intolerant grasses showed some increase with the addition of calcium although he did not report on the statistical significance of these data. The serpentine

race of the grass which he studied showed no response to calcium. This is in general agreement with data presented in this study. In the presence of nickel, NS root enzyme activity fell with the application of micronutrients at "low" calcium levels. Calcium in general appeared to have a beneficial effect on activity in NS plants. MK1 plants were largely unaffected by this element although some increase in activity was noted at "low" micronutrient levels when calcium was increased in the absence of nickel. The general lack of response of MK1 plants to calcium possibly reflects an adaptation to the low soil levels of this element found at the MK1 site. Further studies would merit pursuit where the levels of magnesium would be adjusted according to the calcium concentration such that the same magnesium/calcium ratio was maintained in treatments. This would give valuable information as to whether it is calcium per se or differences of magnesium/calcium ratio which affect the NS plants.

Magnesium has been reported to be both an activator and inhibitor of different phosphatases (e.g. Fernley (1971), Hollander (1971)). Willett (1975) reported that increasing magnesium concentration had no significant effect on acid phosphatase from both serpentine and non-serpentine species of grass. This contrasts with data presented here where magnesium asserted a definite influence over enzyme activity. In MK1 roots, increasing magnesium concentration increased enzyme activity in the presence or absence of nickel. The MK1 root enzyme appeared to have a

high requirement for magnesium which would reflect an adaptation to serpentine soils. This activation by high magnesium levels was enhanced in the presence of nickel. The combination of 7.5 mM magnesium and 0.7 ppm nickel (the concentrations in which these elements occurred in MK1 soil solutions) produced almost double the enzyme activity found with 1.5 mM magnesium in the absence of nickel. The NS race enzyme was activated by increasing magnesium from 1.5 to 4.0 mM and then inhibited when magnesium was increased to 7.5 mM in the absence of nickel. The activation at 4.0 mM magnesium did not occur in the presence of nickel. The NS race apparently has a root surface enzyme system adapted to operate most efficiently at magnesium and nickel levels usually lower than those associated with serpentine soils.

The increase in activity in NS plant enzyme when, for example, 0.7 ppm chromium (III) was added to treatments (figure 20) may be an artefact similar to that mentioned by Woolhouse (1969). He noted, whilst working with Agrostis tenuis, that the initial decrease in enzyme activity with increasing metal ion (aluminium and lead) concentration was followed by a secondary rise in activity. This apparent stimulation of the enzyme can be attributed to breakdown of cell membrane integrity resulting in substrate molecules penetrating freely into the cells and coming into contact with internal phosphatases associated with mitochondria and other cell components. The data presented in figure 20 indicate that MK1 roots are not subject to this secondary increase in activity and may have membranes capable of

maintaining integrity in extreme ionic environments.

This breakdown in membrane integrity in NS roots could make the differences in activity between treatments less pronounced. In treatments which are known to be unfavourable for NS plants (e.g. the "serpentine condition" of Chapter 3) the root-surface enzymes of this race, whilst demonstrating some loss in activity compared with more favourable treatments, usually managed to maintain relatively high activities. Some further studies to determine the concentrations of substrate reaching internal phosphatases in each treatment would be useful. These would enable the activity of internal phosphatases to be subtracted from the overall enzyme activity and would greatly increase the value of the technique.

CHAPTER 6

CARBOHYDRATES IN MK1 AND NS PLANTS

Introduction

Rozema (1978) commented on the possible role of carbohydrate in maintaining osmotic relations of root material with the external environment. He worked with species sensitive and insensitive to high salinity (150 to 300 mM sodium chloride). Such an osmotic role for carbohydrates in plants under serpentine soil conditions was thought unlikely since high osmotic stress conditions do not occur in the MK1 soils. The sum concentration of cations measured in MK1 soil solutions was approximately 8.5 mM. (It is interesting to note that the reasons for the frequent association of maritime plants with serpentine soils (e.g. Proctor and Woodell, 1975) are therefore probably not connected with the water potential per se.)

Rozema (1978) found that there was an accumulation of carbohydrates in some species when they were subjected to conditions of salinity stress. It was felt that there might be a general principle of carbohydrate accumulation in response to environmental stress. In view of this, an investigation was undertaken to determine changes in carbohydrate concentration in plant tissues grown in solutions of differing mineral composition. Changes in cellular chemicals when plant roots are bathed in different solutions may give some indication of the plant's capacity

to continue growth by maintaining normal biochemical pathways. This inner stability may differ between races adapted to different edaphic environments.

Materials and Methods

a) Growth conditions

Plant material, grown as described in Chapter 3, was used for carbohydrate analyses.

b) Quantitative determinations of soluble carbohydrate using the anthrone reagent method.

Plants were harvested after eight weeks growth. Shoots and roots were separated using scissors, and washed thoroughly; in tap water and then in deionized water. Shoots and roots were placed in separate paper bags and stored in a deep freeze for periods up to 10 days. (This was necessary because of shortage of oven space.) When oven space was available, shoots and roots were dried at 60°C for seven days.

Known weights of plant material (c. 0.05 g) were placed in conical flasks, fitted with air-cooled reflux heads, and boiled in 75 cm³ of 80% ethanol for 45 min. The supernatant was then decanted and made up to 100 cm³ with 80% ethanol. Two cm³ of extract were mixed with 0.5 cm³ anthrone reagent (2% anthrone in ethyl acetate) in large test tubes. The tubes were cooled in ice and 5 cm³ of cold concentrated sulphuric acid were carefully layered

above the solution. When all samples in a particular run had been prepared to this stage, the contents of the tubes were quickly and thoroughly mixed and the tubes placed in boiling water for exactly 10 min. An 80% ethanol blank was prepared in a similar way. Tubes were then cooled rapidly and the optical density of the solutions recorded at 625 nm, against the blank, using a Cecil CE 343 spectrophotometer. Optical density was recorded within 30 min of the final cooling.

D-glucose standards were prepared and treated as above with each run of samples. The standards, ranging between 0 and 40 $\mu\text{g cm}^{-3}$ D-glucose, were freshly prepared in 80% ethanol prior to each run.

c) Gas-liquid phase chromatography (G.L.C.)

This technique was used to determine which sugars were present in the soluble carbohydrate fraction from tissue grown in certain culture media. During the extraction procedure, organic acids were separated from sugars and both fractions were analysed. Organic acid results proved rather inconclusive and are only mentioned briefly in this chapter but are given in full in Appendix II. However, the methods of preparation and analyses of both sugars and acids are given here.

Between 0.2 and 0.5 g of deep-frozen root or shoot were boiled for 30 min in approximately 50 cm^3 80% ethanol, contained in conical flasks fitted with air-cooled reflux heads. The supernatant was then passed through a cation-

exchange resin (Dowex -50W, 100-200 mesh, H⁺ form), to convert all acids to the free form, and then through an anion-exchange resin (Dowex -1, 200-400 mesh, formate form). Acids were held on the latter resin whilst neutral compounds, including the sugars, passed through and were collected. 50 cm³ distilled water were passed through the column to ensure all sugars were washed from the anion resin. Acids were collected in a separate container by passing 25 cm³ 6N formic acid followed by 50 cm³ distilled water through the anion resin. Sugars and acids were then evaporated to dryness in pear-shaped flasks by rotary evaporation and stored below 0°C.

Organic acids and sugars decompose at the normal operating temperatures of G.L.C. equipment. It is therefore necessary to synthesize stable, volatile derivatives of these compounds which can then be analysed. Trimethylsilyl (TMS) ethers of sugars and acids are the usual stable derivatives to be prepared. For sugars, 500-800 μ l anhydrous pyridine were added to the dry samples which were then shaken vigorously for 1 min. 100 μ l hexamethyldisilane (HMDS) followed by 50 μ l trimethylchlorosilane (TMCS) were then added. After shaking for 1 min the mixture was allowed to stand for at least 20 min before analysis. Analyses could then be carried out since Sweeley et al. (1963), found TMS sugar derivatives were formed rapidly at room temperatures.

For organic acids, more stable TMS derivatives were

formed when silylated with N, O- bis-(trimethylsilyl) acetamide (BSA). To the dry acid samples were added 200 μ l anhydrous pyridine followed by shaking for about 10 min. 200 μ l BSA were added with shaking and the mixture left to stand for a further 30 min. Standard sugar and acid samples were treated in a similar way to the experimental root and shoot samples.

TMS derivatives of sugars and acids are stable for at least 56 h (Phillips and Jennings, 1976), and all sample analyses were carried within this period.

Aliquots (2-5 μ l) of the TMS sugar or acid derivatives were injected into a Pye Unicam (series 104) gas-liquid chromatograph with a wide-range amplifier and dual flame ionization detector connected to an automatic digital integrator (Infortronics, model CRS 204) and chart recorder. The injection port temperature was 200°C and the carrier gas was nitrogen at a flow rate of 60 cm³ min⁻¹. Standard twin coiled glass columns 2.1 m long were used (internal diameter 0.6 cm) containing 2% SE 52 on 80-100 mesh Gas Chrom Q. The oven temperature was under programme control. Bottom and top temperatures were held for 1 and 10 min respectively. For acids, bottom temperature was 100°C rising by 10°C min⁻¹ to top temperature of 200°C. For sugars, bottom temperature was 150°C rising by 5°C min⁻¹ to top temperature of 250°C.

Peaks were identified by co-chromatography. Root or shoot sugar and acid derivatives were run through the

system and component peak distribution and area noted along with the retention time of each component. On following runs a small volume (1 μ l) of one standard sugar or acid TMS derivative was injected into the system at the same instant as the root or shoot derivative sample. The peak (or occasionally peaks) which increased in area was assigned the identity of the particular standard included with the root or shoot sample.

When certain standard sugar derivatives were prepared care was taken to ensure that equilibrium was reached between the compounds' anomeric forms (e.g. α and β glucose). This was achieved by dissolving the sugar in water and allowing it to stand for a few minutes. The sugar was then taken to dryness and silylated as described previously.

All reagents, glassware and samples were kept dry since the presence of water results in an equilibrium between the silylated and unsilylated forms of the sugars or organic acids.

An initial attempt was made to use G.L.C. quantitatively, and the details are given in Appendix I. Whilst this worked very well, the large number of samples which would be analysed during the study and the long extraction, preparation and run-time procedures involved, made this method impractical. Instead, total soluble carbohydrate was estimated using the much simpler anthrone method described in the previous section.

Results

a) Qualitative analyses and relative distribution changes of individual components.

i) Organic acids

G.L.C. data indicated five acids were normally present in tissues, of which only malic and citric acid were identified. The distribution of each acid in plants grown in the varying treatments were difficult to interpret; I have, however, included them in Appendix II.

ii) Carbohydrates

In tissues grown in solutions containing "high" micro-nutrients, "low" calcium at three levels of magnesium and 0, 0.7 ppm nickel, six major peaks were found in gas-liquid chromatograms. Four peaks were identified by co-chromatography as fructose (α , β anomers remaining unresolved) α and β glucose and myoinositol. Two peaks ("C" and "D"), which could not be identified, occurred at the end of the chromatograms in a region normally associated with disaccharides. Whilst it was not possible to compare absolute concentrations of individual sugars between treatments, relative amounts of each sugar were compared with glucose within each treatment. Sugars were expressed as percentages of β -glucose (figures 22A - 22D) within each treatment thus permitting differences between treatments to be compared.

In the majority of samples, glucose (α and β anomers)

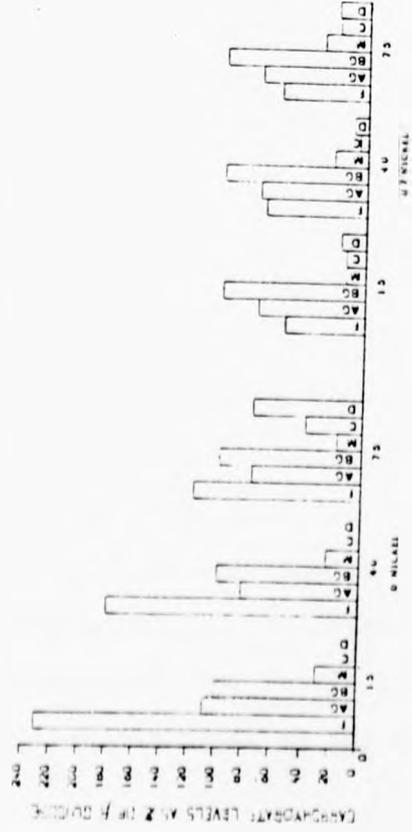
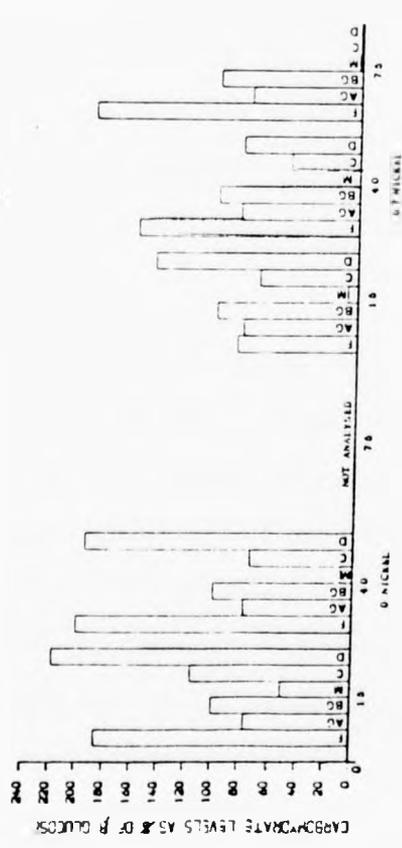
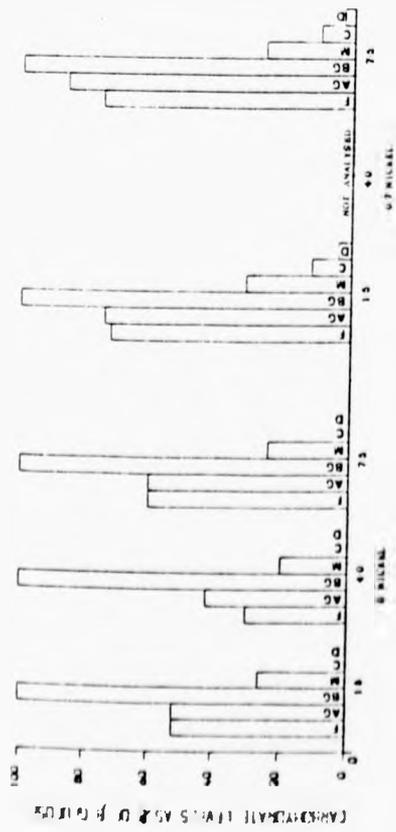
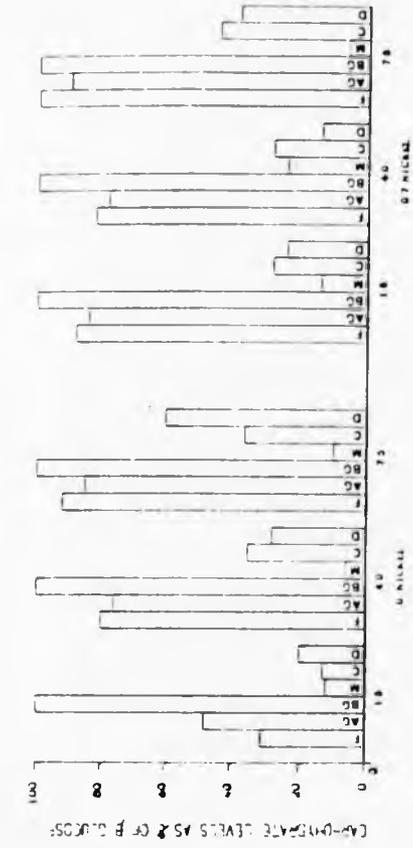


Figure 22. Qualitative analyses of carbohydrate from MK1 and NS plants. F=fructose; AG= α -glucose; BG= β -glucose; M=myoinositol; C and D=unidentified sugars.

was the predominant sugar. This supported the use of glucose standards for the quantitative estimation of soluble carbohydrates, the results of which are given later.

In serpentine shoots, the relative amounts of each sugar remained similar within each treatment. It was noted, however, that fructose and α -glucose increased slightly relative to β -glucose at each magnesium level when nickel was present. In roots, fructose was more abundant than β -glucose when nickel was absent but the amount fell with each increment of magnesium. There was an increase in sugars "C" and "D" compared with β -glucose at 7.5 mM magnesium in the absence of nickel. Myoinositol and α -glucose remained similar at each level of magnesium. In the presence of nickel, the sugar distribution was similar for each concentration of magnesium, paralleling the distribution in shoots closely.

In NS shoots, in the absence of nickel, the relative amount of each sugar (with the exception of myoinositol) increased, compared with β -glucose, as the magnesium level increased. In the presence of nickel, shoot sugars had a similar distribution at each magnesium level. This was similar to the distribution at the highest magnesium level in the absence of nickel. In roots there was usually more fructose than β -glucose, the level appearing to increase with increasing magnesium concentration. The levels of sugars "C" and "D" were greater than β -glucose at the lower

was the predominant sugar. This supported the use of glucose standards for the quantitative estimation of soluble carbohydrates, the results of which are given later.

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In NS shoots, in the absence of nickel, the relative amount of each sugar (with the exception of myoinositol) increased, compared with β -glucose, as the magnesium level increased. In the presence of nickel, shoot sugars had a similar distribution at each magnesium level. This was similar to the distribution at the highest magnesium level in the absence of nickel. In roots there was usually more fructose than β -glucose, the level appearing to increase with increasing magnesium concentration. The levels of sugars "C" and "D" were greater than β -glucose at the lower

magnesium concentration but appeared to fall slightly with increasing magnesium concentration, the level of "C" falling below that of β -glucose. α -glucose and myoinositol remained similar at 1.5 and 4.0 mM magnesium. In the presence of nickel, fructose rose, as magnesium increased, to a level exceeding that of β -glucose.

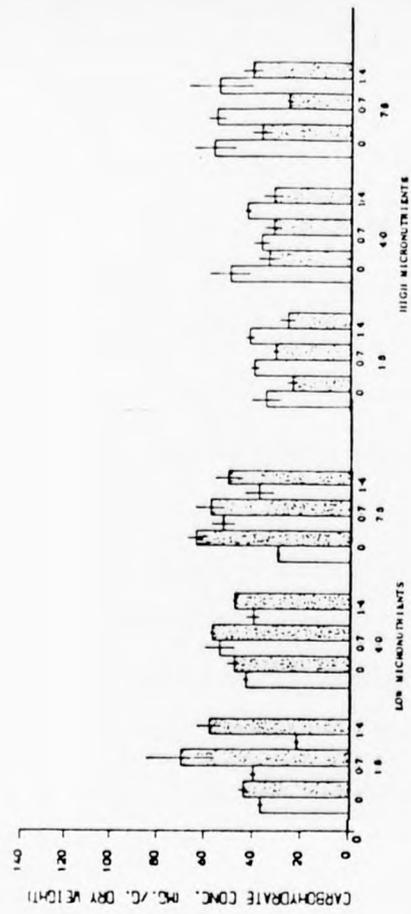
b) Quantitative analyses

Statistical significance of differences between treatments was evaluated by the use of 't' tests; all significant differences reported in this chapter are at the 1% level.

1) MK1 plants

In MK1 shoots, the only significant difference in carbohydrate concentrations was noted between "low" and "high" calcium treatments with "high" micronutrients at 1.5 mM magnesium and 0, 0.7 ppm nickel (figure 23A).

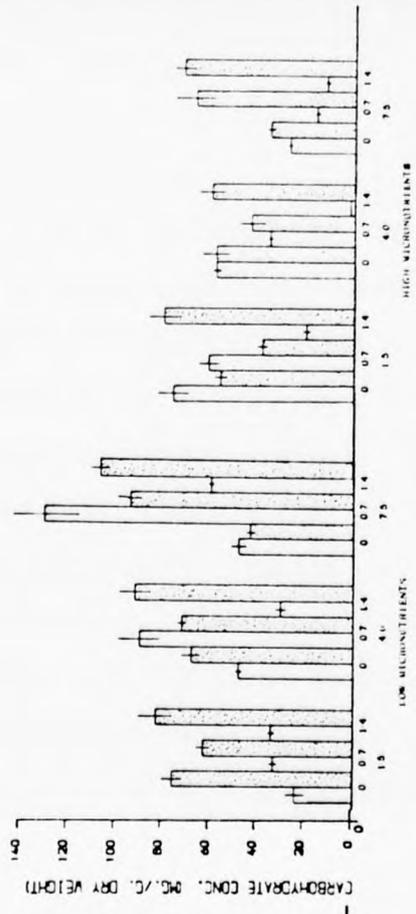
In solutions containing "high" micronutrients, no significant differences were noted between carbohydrate levels in roots in all treatments with the exception of that between the 1.5 mM magnesium, 1.4 ppm nickel and 4.0 mM magnesium, 0.7 ppm nickel at "low" calcium treatments (figure 23C). At "low" micronutrient levels, carbohydrates were significantly higher in all treatments which included "high" calcium. As for shoots, increasing magnesium or nickel appeared to have little consistent influence on carbohydrate levels in roots.



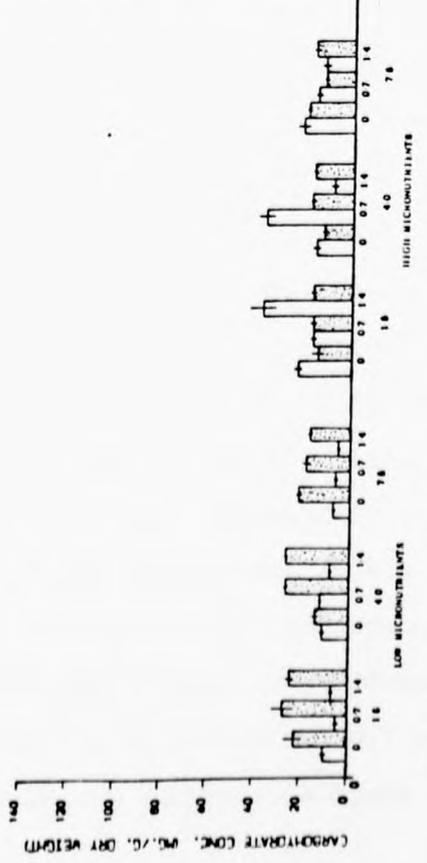
MK1 SHOOTS



MK1 ROOTS



NS SHOOTS



NS ROOTS

Figure 23. Carbohydrate concentration in MK1 and NS plants.

ii) NS plants

With "high" micronutrients, shoot carbohydrate was higher at "low" calcium levels although the differences were only significant at 1.5 mM magnesium (figure 23B). At each calcium level, there were no significant differences in carbohydrate concentrations as magnesium or nickel increased. Figure 23B does indicate, however, that there was a trend for carbohydrate to accumulate slightly as magnesium increased. With "low" micronutrients, no clear trends due to magnesium or nickel were noted and there were no significant differences between treatments with the exception of shoots from the "low" calcium, 1.5 mM magnesium, 1.4 ppm nickel treatment where carbohydrate concentrations were lower than in shoots from other treatments. It was noted, however, that "high" calcium treatments were associated with higher carbohydrate concentrations in shoots than were "low" calcium treatments.

In NS roots (figure 23D) some significant differences were noted between treatments. At "high" micronutrient levels, with "low" calcium, carbohydrate concentration fell as nickel increased at each level of magnesium. This fall in carbohydrate was gradually reversed at "high" calcium levels as nickel and magnesium increased. These differences (with "high" calcium) were not significant at 4.0 mM magnesium where the transition to increased carbohydrate levels occurred. With "low" micronutrients, carbohydrate tended to accumulate with increasing magnesium and 0.7 ppm nickel. At "high" calcium levels, in contrast to those of

ii) NS plants

With "high" micronutrients, shoot carbohydrate was higher at "low" calcium levels although the differences were only significant at 1.5 mM magnesium (figure 23B). At each calcium level, there were no significant differences in carbohydrate concentrations as magnesium or nickel increased. Figure 23B does indicate, however, that there was a trend for carbohydrate to accumulate slightly as magnesium increased. With "low" micronutrients, no clear trends due to magnesium or nickel were noted and there were no significant differences between treatments with the exception of shoots from the "low" calcium, 1.5 mM magnesium, 1.4 ppm nickel treatment where carbohydrate concentrations were lower than in shoots from other treatments. It was noted, however, that "high" calcium treatments were associated with higher carbohydrate concentrations in shoots than were "low" calcium treatments.

In NS roots (figure 23D) some significant differences were noted between treatments. At "high" micronutrient levels, with "low" calcium, carbohydrate concentration fell as nickel increased at each level of magnesium. This fall in carbohydrate was gradually reversed at "high" calcium levels as nickel and magnesium increased. These differences (with "high" calcium) were not significant at 4.0 mM magnesium where the transition to increased carbohydrate levels occurred. With "low" micronutrients, carbohydrate tended to accumulate with increasing magnesium and 0.7 ppm nickel. At "high" calcium levels, in contrast to those of

"low" calcium, there was no significant difference between magnesium treatments and higher nickel generally resulted in an increase in carbohydrate concentration.

Discussion

Qualitative changes in carbohydrates

The qualitative changes in carbohydrates (figure 22), if difficult to interpret precisely, illustrate the influence of external mineral composition on carbohydrate metabolism. It was notable that nickel apparently conferred stability to sugar-type distribution in MK1 plants, particularly in roots. The absence of nickel resulted in the fluctuation in the composition of the sugars. The nature of this apparent stability imparted to MK1 plants by nickel merits further investigation. No similar stability was noted in NS plants although fluctuations were only small in shoots in the presence of nickel. Root sugar composition in NS plants varied greatly in the presence or absence of nickel. The differences in root sugar composition between MK1 and NS plants may reflect differences in carbohydrate moved from aerial parts or could represent differences in biochemical processes in root tissue.

Quantitative carbohydrate analyses

The similar concentrations of soluble carbohydrate (figure 23) in MK1 shoots, under a variety of mineral compositions, indicate perhaps a general tolerance of mineral extremes. The significant differences between

carbohydrate concentrations in MK1 roots grown at "low" and "high" calcium levels with "low" micronutrients might reflect a response to an element normally low in serpentine soils. This suggestion gains support when it is noted that when MK1 plants were grown in conditions which best approximate to MK1 soil conditions ("low" micronutrients; "low" calcium; 7.5 mM magnesium; 0.7 or 1.4 ppm nickel), root carbohydrate concentrations were maintained at very low levels.

By way of contrast, NS plants showed marked fluctuations in carbohydrate concentration, particularly in roots, with changes in external mineral composition. With "high" micronutrients, carbohydrate levels fell with increasing mineral stress (i.e. increasing magnesium and nickel concentration). However, at "low" micronutrient levels, soluble carbohydrates increased as mineral stress increased. It would appear that the carbohydrate levels result from several interactions. Possible explanations for these observed trends are :

a) The most severe mineral stress conditions ("low" micronutrients, "low" calcium and increasing magnesium and nickel), represent the least favourable growth conditions for NS plants (see Chapter 3). Carbohydrates might be expected to have accumulated since tissue metabolism was adversely affected and energy utilisation processes proceeded slowly.

b) The fall in carbohydrate levels in NS roots under slightly more favourable conditions (i.e. "high" micro-

nutrients) might be caused by more growth but with an inefficient use of energy sources.

c) Calcium might offset much of the toxicity due to magnesium and nickel in NS roots, allowing metabolic processes to proceed whilst making more efficient use of available carbohydrate energy sources. This could lead to an apparent increase in carbohydrate levels in roots.

The work of Rozema (1978) supported the view that species sensitive to ionic stress tend to accumulate carbohydrate when in unfavourable mineral conditions. He worked with Juncus and Agrostis species and Festuca rubra ssp. littoralis grown in increasing salinity levels. He found an increase in the sugar content of all species at increased salinity levels and noted that this increase in sugar content was most pronounced in relatively salt-sensitive Juncus species. Rozema suggested that the accumulation of sugars in salt-sensitive species may reflect a blocked glycolytic pathway and that growth and carbohydrate metabolism of salt resistant species was the least affected by increased salinity. A blocked glycolytic pathway might manifest itself in ways similar to those outlined in a) and b) above. The removal of such a blockage, as the supply of nutrients became more favourable to the plant, might result in the situation described in c).

Rozema (loc. cit.) also suggested root carbohydrate may serve some osmotic adjustment role in the plants he studied. A similar role for carbohydrate in plants studied

here seems unlikely in view of the comparatively low osmotic stress experienced by MK1 and NS roots.

Conclusions

Whilst the carbohydrate analyses have presented some interesting points - mainly that carbohydrates accumulated in the NS race when magnesium and nickel supply were high - the data should be considered as a first step for further research. The present study has identified the mineral conditions which appear to produce changes in carbohydrate metabolism. Further work might investigate how these changes are brought about by magnesium and nickel.

CHAPTER 7

COMPUTER MODEL OF MK1 SOILS

General principle of model

The model is based on the use of linear equations to generate data corresponding to the growth and absorption of ions by plants growing in solutions of known mineral composition. Initially, dry weights and metal contents of plants grown in each treatment combination used in the water culture experiments (Chapter 3) were regressed against the levels of magnesium used in each treatment combination. This was carried out using a Diehl desk-top computer. The linear regression constants obtained were incorporated into the computer model programme.

As the programme runs, the level of nickel, magnesium and calcium in the soil solution being studied, are entered. The model firstly uses the magnesium input to generate the dry weight and metal content of plants that would result from the presence of the input level of magnesium when in solutions containing reference* concentrations of nickel and calcium. The computer does this by using the regression constants mentioned previously. The new data generated are then used to calculate new linear regression constants when when evaluated with the input levels of soil solution

*The reference concentrations of nickel and calcium were those used in the water culture experiments i.e. 0, 0.7, 1.4 ppm nickel and 0.3, 1.2 mM calcium.

nickel, produce dry weights and metal contents which would result from the presence of the input levels of magnesium and nickel. Finally, these data are evaluated with reference to the input levels of calcium occurring in the soil solution. Consequently, the model will predict the dry weight and metal content of plants which would theoretically result from eight weeks growth in solutions containing nickel, magnesium and calcium levels found in the particular soil solution which is being studied. The model asks the user if predicted plant responses are required at "high" or "low" micronutrient levels and makes the appropriate modifications to the equations at each stage of evaluation. The predicted data for MK1 and NS F. rubra are then output and, having completed this step, the programme loops back to its beginning and asks if the user wishes to continue using the model.

The use of linear regression equations

If two variates are related by a rectilinear function, this relationship can be most generally expressed by

$$y = M_{yx}x + C_y \quad (1)$$

where M_{yx} and C_y are the y intercept and the slope, respectively, of the straight line relating y to x .

Providing M_{yx} and C_y are known, values of y may be predicted from a knowledge of x . If points of x and y lie exactly on a straight line then there is no problem in determining the parameters M_{yx} and C_y . In real situations, however,

the points are never exactly colinear but are scattered over an area around the theoretical line. To estimate the unknown parameters M_{yx} and C_y the variation due to x and y can be taken into consideration using the equations (2) and (3)

$$M_{yx} = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sum(x - \bar{x})^2} \quad (2)$$

$$C_y = \bar{y} - M_{yx}\bar{x} \quad (3)$$

where \bar{x} and \bar{y} are the mean values of the x and y variables. Similarly, if the values of M_{xy} or C_x are required, then (4) and (5) may be used.

$$M_{xy} = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sum(y - \bar{y})^2} \quad (4)$$

$$C_x = \bar{x} - M_{xy}\bar{y} \quad (5)$$

The computer programme

The programme, as it is presented here, was written to run on a ICL 4130 computer. The programme was too large to run interactively in the workspace usually available to users of the 4130 system. The model was, therefore, written into a BASIC LIBRARY programme which enabled it to be run interactively. However, this imposed limitations on improvement and expansion of the programme since editing of LIBRARY programmes is not possible within

The computer programme

```
10 GOTO 80
20 LET X=0
30 PRINT "IF YOU WISH TO CONTINUE, INPUT YES"
40 INPUT X$
50 IF X$="YES" THEN 70
60 STOP
70 RESTORE
80 LET A=0
90 LET F=0
100 LET Q=0
110 PRINT "SERPENTINE SOIL MODEL BEGINS"
120 PRINT "ENTER CONCENTRATION (PPM) OF NICKEL, MAGNESIUM, CALCIUM"
130 INPUT A, F, Q
140 PRINT
150 PRINT "DO YOU WISH TO SEE PROJECTIONS FOR HIGH OR LOW SOIL"
160 PRINT "MICRONUTRIENT CONDITIONS? INPUT HIGH OR LOW"
170 INPUT F7$
175 PRINT
178 PRINT
180 LET E=F/24.312
190 LET C7=Q/400.00
200 DATA 10,434,-26.06,480,0,0,0,0,1.51,124,6.42,95,0,02,28,1.5
202 DATA 6.15,-5.09,237,6.42,175,1.44,32,0.54,19,32,-663.3
204 DATA 35474,478,23524,1261,3412,1497,2,611,93,-72.48,3000,7
206 DATA -3.67,5682.57,2.01,153.71,-6.57,194.9,0,0,0,0,-19.27
208 DATA 1183.49,14.9,648,-7.8,160,-0.2111,91,3.67
210 DATA 97.4,1.65,75,8.3,49,201,10,63,158,474,7877,1134,3415
212 DATA 254,1332,258,401,-66,1786,-120,2254,-140.3,1178,-49.7
214 DATA 835,0,0,0,0,75.6,-90.27,9.54,255,-0.37,13.26,3.5,1.44
216 DATA -0.62,120,10,46,134.6,10.65,-11,0.63,28.2,621,15475,524
218 DATA 13892,1766,2413,1497,611,21.1,2075,-292,5534,36.4,119.7
220 DATA -12.74,233,0,0,0,413,240,94,573,24,-17.2,3,30,61,42.4
222 DATA -59.08,1,23,32,11,4,63,2,7,04,127,1868,2001,1134,3415
224 DATA 493,1595,258,401,42,1373,-130,2031,-8.78,405,-2.13,239
226 DATA 0,0,0,0,2.6,81,5,04,114,-2.23,67,-1.75,99,-4.22,413,-6.23
228 DATA 540,-0.81,21,1.78,42,-278,24675,-640,32700,109,3093,1070
230 DATA 3024,-0.25,1535,-202,6011,0.9991,126,-0.9046,06,0,0,0,0
232 DATA 44,874,72,773,2.55,150,-1.376,245,41,1005,35.8,557
234 DATA 0.844,333,1.66,295,1317,4457,33,19756,430,368,360.637
236 DATA -93.85,1416,-254,4567,-141.7,1643,-11.832,0,0,0,0
238 DATA -0.2477,0,1.47,44.9,-5.25,70,7,1.41,42,2,-30,406,4.39
240 DATA 238,-0.2477,19,1.03,11.88,1137,12503,691,12135,111,3152
242 DATA 575,3307,-133,2313,59.6,3208,-37.6,351,-4.777,152,0,0,0,0
244 DATA 187,334,95,340,3.29,103,1.55,129.9,-37.11,1067,91,375,6.6
246 DATA 214,6.78,117,779,8954,149,2185,180,1283,1028,910,-2.11
248 DATA 942,-163,3374,25.8,359,1.4,472,-11,482,-5.28,425,-3.00
250 DATA 93,-7.33,160,0,42,77,-7.91,147,-3.02,160,0,10,136,4.60,100
252 DATA -1.56,120,0,25,17,-1.11,21,1,21,13,1.59,9,-7.41,183,11.71
254 DATA 85,9.54,155,1.74,165,0.25,17,-0.95,23,1.15,16,0.92,16
256 DATA -1300,37270,-715,34500,217,24257,16.51,26920,966,5013
258 DATA 1058,4578,1372,2119,935,3744,-169,3668,-183,3420,1.83
260 DATA 5258,-230,6331,4.17,125,3.62,148,5.19,145,6.97,137,18.89
262 DATA 834,-66.05,1486,-21.83,971,25,1191,0,1100,36.51,070,13.39
264 DATA 778,-19.44,1017,1.63,49,4.51,64,9.3,30,-4.20,63,1.44
266 DATA 65,4,03,20,-2.02,60,-0.33,20,7.7,114.0,-19.00,209,19.81
268 DATA 74,-10.45,195,131,8198,915,4399,1322,4734,1288,4718
270 DATA 182,1742,203,1417,102,888,178,610,-46.8,1802,-114,2130
272 DATA -67.80,1727,-130,2031,-140,1230,-52.2,483,-32.5,697,-0.800
274 DATA 459,10.32,50,17.79,-1.07,89,-3.3,144,52.99,-48,105,-122
276 DATA 2.48,32,2,25,43,2.45,1.70,3.40,27,1.01,16,-0.77,3,11.1
```

```

300 DATA 88.4,72.181,0.16,111,-3.3,154,16.24,-23.87,21.98,-27
302 DATA 0.156,15,-0.75,22,-1464,23345,-2189,28722,221,16875
304 DATA -469,21368,2464,588,1595,5153,1372,2119,935,3744,311,1152
306 DATA 27.53,3588,-138,4931,-487,6265,-37.19,385,-17.49,158,-9.3
308 DATA 229,-7.89,285,0.8873,762,178,281,-21.8,971,25,1191,148,628
310 DATA 0.917,2229,83,698,183,737,131,-278,122,-137,5.5,27,7.93
312 DATA 11.64,-118,128,-161,2.15,9.7,2.36,5.7,5.88,55,43,-31,4.65
314 DATA 43.155,38,-149,9814,1813,8848,1322,4733,1288,4718
316 DATA 1538,-1366,2681,-787,182,888,178,618,398,486,476,536,-68
318 DATA 1562,-58,1511,-1.32,356,-16,467,3.48,261,12.58,233
320 DATA 0.79,74,-3.11,138,-7.43,232,-15.77,335,3.57,77,1.87,58
322 DATA -0.156,189,-0.431,96,0.457,77.8,-1.24,88,-1.88,96,-0.92,99
324 DATA -0.43,368,23.22,287,-1,487,-2.93,588,0.13,8.4,44.13,8.7
326 DATA 32.6,0.55,29.8,37,23883.585,23638,127,31888,-174
328 DATA 32422,157,2849,1244,3289,1821,4675,877,5195,22,1533,58
330 DATA 1414,-323,6336,-374,6222,3.91,99,-3.146,0.88716,96,5.44
332 DATA 89,-16,591,-159.9,2183,-58.45,1385,-79.8,2845,-1.39,1133
334 DATA 34.615,24,896,26.5,753,-0.458,183.9,74.61,-0.183,284
336 DATA -0.477,181,24,739,-8.53,447,-7.79,488,-4.31,362,-2.77
338 DATA 386,11.65,212,2.84,231,-1.56,278,-61.46,13133,1178,6924
340 DATA 0.15588,-325,16577,414.6,669,145,3867,386,628,559,488
342 DATA -58.51,1181,-1.83,1241,-155.96,3589,-211.92,3918
344 DATA -55.79,667,-76.81,636,16.94,598,-1.88,497,-8.25,114,-15.96
346 DATA 229,-9.39,171,-2.93,196,1.55,62,4.94,41,-2.88,53,1.31,41.9
348 DATA -5.92,81,-5.47,75,-1.79,67,2.81,59,-26.68,385,-14.4
350 DATA 259,-17,366,7.98,275,0.37,15,0.786,37.6,-0.412,15.7
352 DATA 0.578,11.5,458.8,17228,-79,19445,-1822,24266,378,28595,77.9
354 DATA 3391,1891,3435,747,4528,1888,2529,-182,2857,-13.76,2859
356 DATA -367,5127,-189,4969,-16,173,-22,180,-6.6,153,-5.14,128
358 DATA -28,297,-5.79,998,-11.9,1418,38.1545,288,575,264,617
360 DATA 95,399,171,248,-4.88,189,6.69,68,-3.3,134,-2.15,184
362 DATA -61,1858,14.76,372,60,161,43,235,-19,271,-3.88,195,-1.3
364 DATA 124,15.47,59,1565,14484,132,12327,197,2211,385,3276,128
366 DATA 1345,1163,1367,839,962,888,918,-138,1314,15.59,1299,-95
368 DATA 2646,-126,3848
1100 LET C1=0
1105 LET L1=0
1120 IF A=P THEN 1140
1130 GOTO 1220
1140 IF P%="LOW" THEN 1320
1150 LET K=0
1160 LET L=0
1170 READ M1
1180 LET K=K+M1
1190 LET L=L+1
1200 IF L=144 THEN 1320
1210 GOTO 1170
1220 LET K=0
1230 LET L=0
1240 READ M1
1250 LET K=K+M1
1260 LET L=L+1
1270 IF P%="HIGH" THEN 1300
1280 IF L=288 THEN 1320
1290 GOTO 1240
1300 IF L=576 THEN 1320
1310 GOTO 1240
1320 GOSUB 1400
1330 GOTO 1570
1400 IF A=P THEN 1990

```

```

1405 LET Z=0
1410 LET Z=Z+1
1420 READ M1
1430 ON Z GOTO 1440,1460,1480,1500
1440 LET A7=M1+E
1450 GOTO 1410
1460 LET F1=A7+M1
1470 GOTO 1410
1480 LET A7=M1+E
1490 GOTO 1410
1500 LET F2=A7+M1
1510 LET A1=F1-((F1+F2)/2)
1520 LET A2=F2-((F1+F2)/2)
1530 LET M=((A1+(-0.45))+(A2+0.45))/0.405
1540 LET C=((F1+F2)/2)-(M+0.75)
1550 LET W=M+C*7+C
1560 RETURN
1570 IF L1=1 THEN 1590
1580 PRINT "SERPENTINE FESTUCA RUBRA RESPONSE"
1590 PRINT
1600 PRINT "SHOOT DRY WEIGHT AFTER 8 WEEKS GROWTH =";W,"MILLIGRAMMES"
1602 PRINT
1605 PRINT "METAL CONCENTRATION IN SHOOTS FOLLOW"
1610 GOSUB 1400
1620 PRINT "  NI =";W,"PPM"
1630 GOSUB 1400
1640 PRINT "  SF =";W,"PPM"
1650 GOSUB 1400
1660 PRINT "  ZR =";W,"PPM"
1670 GOSUB 1400
1680 PRINT "  MN =";W,"PPM"
1690 GOSUB 1400
1700 PRINT "  CU =";W,"PPM"
1710 GOSUB 1400
1720 PRINT "  K =";W,"PPM"
1730 GOSUB 1400
1740 PRINT "  MG =";W,"PPM"
1750 GOSUB 1400
1760 PRINT "  CA =";W,"PPM"
1770 IF C1=1 THEN 1800
1780 PRINT "END OF SHOOT METAL DATA"
1790 GOTO 1820
1800 PRINT "END OF ROOT METAL DATA"
1810 GOTO 1910
1820 PRINT
1830 GOSUB 1400
1840 PRINT
1850 PRINT "ROOT DRY WEIGHT AFTER 8 WEEKS GROWTH =";W,"MILLIGRAMMES"
1860 PRINT
1870 PRINT "METAL CONCENTRATION IN ROOTS FOLLOW"
1880 LET C1=1
1890 GOSUB 1400
1900 GOTO 1620
1910 PRINT
1920 PRINT
1930 LET L1=L1+1
1940 IF L1=2 THEN 200
1950 PRINT "SERPENTINE FESTUCA RUBRA RESPONSE"

```

```
1960 LET C1=P
1970 GOTO 1320
1990 LET J=2
2000 LET Z=0
2010 LET Z=Z+1
2020 READ M1
2030 ON Z GOTO 2040,2060,2080,2100
2040 LET A7=M1*B
2050 GOTO 2010
2060 LET N1=A7+M1
2070 GOTO 2010
2080 LET A7=M1*B
2090 GOTO 2010
2100 LET N2=A7+M1
2110 LET A1=N1-(N1+N2)/2
2120 LET A2=N2-(N1+N2)/2
2130 LET M=((A1*(-0.35))+(A2*0.35))/0.245
2140 LET C=((N1+N2)/2)-(M*1.25)
2150 LET J=J+1
2160 IF J=2 THEN 2190
2170 LET R1=M*A+C
2180 GOTO 2000
2190 LET R2=M*A+C
2195 GOTO 1510
```

the 4130 system using the EDITDS subsystem offered to users at Stirling. For these reasons, the model is now run on the University of Aberdeen Honeywell 66/80 dual processor system. This will enable the model to be expanded and modified with greater ease.

Some notes on the programme

The first part of the programme (lines 10 - 190) requests information concerning the levels of nickel, magnesium and calcium occurring in the soil solution being examined and if predictions are to be made for low or high micronutrient conditions. The DATA statements (lines 220 - 366) contain the regression constants calculated from the results of the water culture experiments. The computer is instructed to take the input magnesium concentration and search the DATA statements until the appropriate set of regression constants are found, the location of which is determined by the input of micronutrient conditions and factors such as the race being analysed. The dry weight and metal content which would result if reference concentrations of nickel and calcium were present at that particular magnesium level are then calculated. Having done this, the computer uses these values to generate new regression constants using the equations outlined previously, at the levels at which nickel and calcium were input, and then calculates the overall theoretical dry weight and metal content of plants grown in soil solutions containing the levels of nickel, magnesium and calcium specified by the user at the beginning of the programme run.

the 4130 system using the EDITDS subsystem offered to users at Stirling. For these reasons, the model is now run on the University of Aberdeen Honeywell 66/80 dual processor system. This will enable the model to be expanded and modified with greater ease.

Some notes on the programme

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Limitations and applications of the model

The programme was written to describe the results of water culture experiments which were believed to simulate MKI soil conditions. Using the model it has been possible to study the plant response to variations in the levels of nickel, magnesium, calcium and micronutrients supplied to them. The predicted plant response, which was output from the programme, was generally within 5% of the observed response. Occasionally, dry weight predictions deviated by slightly greater extents since equations relating growth rates to mineral composition of growth media are often curvilinear rather than linear.

Whilst the model is thought to work quite well there are many improvements which could be made. For example, variations in plant response due to changes in nitrogen, phosphorus and potassium could be written into the programme. The effect of the total absence of micronutrients might be a useful inclusion. Also, the influence of chromium and cobalt, changes in climatic factors and endogenous growth variations might all be useful additions to the model. In certain respects, particularly when predicting growth rates etc., the use of curvilinear equations rather than linear equations would probably be better and the model could be modified to accommodate this.

Further work on other serpentines would enable the model to be applied to a range of soil types. Deviations of the predicted from the observed plant response to the

soils being studied would suggest the importance of factors which have not been included in the model. Factors could be introduced into the programme until satisfactory agreement between observed and predicted results was achieved. Plants other than F. rubra could also be studied and written into the model thereby increasing its application. This might eventually permit a universal picture to be built up for serpentines of widely different chemical composition and with differing vegetations.

CHAPTER 8

THE VALUE OF SOIL SOLUTION MEASUREMENTS

IN SERPENTINE SOILS

Introduction

It was decided to extract and analyse soil solutions of soils from a number of serpentine areas. Comparisons of the levels of elements found in each soil and how they relate with the findings of other investigations, such as plant response to the particular soil, would enable the general suitability of the extraction procedure to be evaluated.

Soils were analysed from the following contrasting serpentine areas: 1. the southern outcrop of serpentine at Meikle Kilrannoch (site MK2), 2. the calcareous serpentine at Lime Hill (site LH) (sites MK2 and LH are described in more detail by Johnston, 1976), 3. the four main heath types (Coombe and Frost (1956)) from the serpentine rocks of the Lizard Peninsula, Cornwall, 4. several Rhodesian serpentine soils discussed by Proctor, Burrow and Craig (1979).

Materials and Methods

Soil solution extraction procedures were similar to those described in Chapter 2.

Results

Table 11 contains the means and standard errors for soil solution analyses from the range of serpentine soils. For some soil samples only limited volumes of solution were obtained after centrifugation and some elements were not analysed in these samples. In all British serpentines magnesium was in excess compared with calcium although in the case of LH the excess of magnesium was very slight. The Rhodesian soils analysed were similar to LH in this respect. The magnesium concentration was higher in MK1 soil compared with those given in Table 11. It was notable that the Cornish soils contained the lowest levels of calcium. Nickel varied considerably between soil types but mean values remained below 1.6 ppm in all cases. Nitrate levels in LH soils were 20 to 30 times higher than those from the Cornish soils but were lower than the levels found in MK1 soil solutions. Potassium was generally higher in the Rhodesian soils. Iron and sodium were usually higher in the Cornish soils compared with the Scottish soils. Chromium was detectable in all Cornish soils.

Discussion

In many respects the soil solution data are puzzling. For example, bioassay plants grown in the Rhodesian soils are known to show nickel toxicity symptoms (Proctor, Burrow and Craig 1979), and there is a strong body of evidence which demonstrates the importance of nickel in at

Table 11. Mean and standard error (SE) of element concentration (ppm) in the soil solution extracted from various serpentine soils.

Origin	Soil	Number of Replicates	Ni	Cr	Co	Fe	K	Na	Mg	Ca	Mg/Ca	NH ₄ ⁺	NO ₃ ⁻	PO ₄ ³⁻	SC ₄ ²⁻
Scottish	MK2	Mean	0.4	<0.1	<0.1	0.4	5.3	16.0	65	9.6	6.0	nd*	nd	nd	nd
		SE	0.1			0.2	1.6	2.3	32	2.2	1.6				
Scottish	IH	Mean	1.6	<0.1	<0.1	<0.1	2.8	41.3	54.0	49.3	1.1	<0.1	77	1.7	8.7
		SE	0.1				0.1	1.8	1.5	1.8	0.1		3	0.2	2.0
Cornish	Short Heath	Mean	0.1	0.1	<0.1	9.6	3.2	24.3	6.3	0.4	15.0	0.8	3.6	3.0	23
		SE	0	0.1		1.3	0.3	1.8	1.2	0.1	1.0	0.1	0.2	0.4	2
	Rock Heath	Mean	0.1	0.1	<0.1	5.4	11.3	112	15.6	3.8	4.2	0.1	3.2	2.2	11
		SE	0.1	0		0.3	0.4	3	0.7	0.1	0.2	0.1	0.3	0.2	2
	Tall Heath	Mean	1.1	0.3	<0.1	63.3	6.3	79.3	27.6	0.2	138	0.6	2.4	5.1	20
		SE	0.1	0.1		3.4	0.2	2.3	1.4	0.1	3.0	0.1	0.3	0.2	3
Mixed Heath	Mean	1.3	0.3	<0.1	46.0	8.0	85.3	25.3	0.3	84.8	0.8	2.3	4.1	29	
	SE	0.1	0.1		2.1	0.3	2.9	1.3	0.1	2.8	0.1	0.3	0.3	2	
Kingston Hill	Mean	0.6	nd	nd	0.9	20	nd	28	18	1.5	nd	nd	nd	nd	
	SE	0.1			0.4	3		6	3	0.1					
Tipperary Claims	Mean	0.7	nd	nd	0.9	19	nd	38	23	1.8	nd	nd	nd	nd	
	SE	0.1			0.4	7		13	8	0.3					
Rhodesian Umvuma	Mean	0.3	nd	nd	0.8	7	nd	19	15	1.2	nd	nd	nd	nd	
	SE	0			0.3	0.5		6	4	0.1					
Selikwe	Mean	0.1	nd	nd	2.3	18	nd	28	19	1.5	nd	nd	nd	nd	
	SE														
Noro	Mean	0.3	nd	nd	1.1	10	nd	23	9	1.2	nd	nd	nd	nd	
	SE														

*nd = not determined

least some of these sites (e.g. Craig 1977). However, the levels of nickel in these soil solutions were similar to those found in MK1 soils in which nickel toxicity symptoms in bioassay plants have not been demonstrated (Proctor, 1971b). Moreover, the high soil solution nickel levels found in "Tall" and "Mixed Heaths" from Cornwall would suggest nickel may be important in the plant response to these soils. Nickel toxicity symptoms in plants have never been demonstrated from these soils and the analyses of Lizard Heath plants by Marrs and Proctor (1978) revealed only small quantities of nickel.

Further problems arise with the interpretation of soil solution magnesium and calcium analyses. High Mg/Ca ratio is widely accepted as a major influence in many serpentine soils yet the average Mg/Ca ratio in the relatively innocuous Cornish "Tall" and "Mixed Heaths" was in excess of 100, whereas in the highly toxic MK1 soils it was only 16. However, it would be argued it is the absolute levels of elements which are important and not merely the ratio as Proctor (1971b) has indicated in MK1 soils.

Whilst experimental evidence has been presented during this study that the centrifugation method described was suitable for estimating plant available elements in MK1 soil solutions, the above data suggest this method may not be suitable for all soils and more work is required to establish the general applicability of this technique.

However, in spite of the confusing results for heavy metals and magnesium some of the soil solution data obtained are readily interpretable. Some of the Cornish serpentines are subject to mineral recruitment from sea spray (Malloch, 1971). "Rock Heath" soils are found on exposed cliffs (Coombe and Frost, 1956). Soils from this location had the highest sodium levels, although all Cornish soils were relatively rich in this element. "Short Heaths" are apparently least influenced in this respect and are found in more inland locations.

Iron in Cornish soil solutions was at higher concentrations than the exchangeable levels reported by Marrs and Proctor (1978). High iron levels are often associated with waterlogging. The highest iron level was found in "Tall Heath" soils which are often found in depressions and must be seasonally waterlogged, (Marrs and Proctor, 1978). On waterlogging, anaerobic respiration by soil bacteria results in a fall in redox potential causing elements to be reduced, Fe^{3+} going to Fe^{2+} for example. Since Fe^{2+} and its compounds are much more soluble than Fe^{3+} analogues, iron becomes much more abundant in the soil solution.

CHAPTER 9

ELECTROPHORESIS OF SOIL SOLUTIONS FROM MK1 AND RHODESIAN SOILS

Introduction

Since little is known about the chemical form in which nickel occurs in serpentine soil solutions, a preliminary investigation was undertaken to determine if nickel occurred simply as $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$ ions or as a metal chelate. A paper electrophoresis technique was employed to distinguish between ionic and complexed forms of nickel.

Materials and Methods

Soil solutions, extracted as described previously, from MK1 and Rhodesian soils were spotted onto the mid-line of Whatman 3MM chromatography paper. Two nickel spots (using a nickel standard of 0.7 ppm, made up from nickel nitrate) were also established on this line. All spots were constructed from 20 μl of solution and several papers were prepared in this way. On later papers an additional spot consisting of a mixture of soil solution and nickel standard was established.

The chromatograph was placed in a "Shandon" type low voltage electrophoresis tank with the mid-line of the paper overlying the central partition of the tank. Each side of the tank was filled with a pyridine/acetic acid/water buffer (100 cm^3 pyridine, 32 cm^3 glacial acetic acid made up to 10 l with deionized water) at pH 5.3. The

paper was moistened with this solution using a Pasteur pipette, taking care not to disturb the spots. The system was then connected to a "Volkam" power supply and run at a constant 250 volts with variable current for 15 min. When a second paper was to be run without changing the buffer solution in the tank, the polarity of the system was changed to reverse the hydrogen ion flux and maintain pH balance on both sides of the tank.

After the above treatment papers were dried in a fume cupboard and then sprayed with 0.1% dithioamide in 60% ethanol using a "Shandon" fine mist spray gun to reveal the new position of nickel from each spot.

Results

A typical electrophoretogram obtained is shown diagrammatically in figure 24. (Attempts were made to photograph electrophoretograms but there was insufficient contrast to give satisfactory reproductions.) The position of nickel from each spot was revealed as a blue area after spraying. Nickel was found to move approximately the same distance from both standard and sample spots but the intensity of coloration from the latter was considerably lower. The Rhodesian soil gave a darker colour than the MK1 sample, but was still slightly paler than the standard nickel spot. The mixture of soil solution and nickel standard gave a coloration similar in intensity to the standard above.

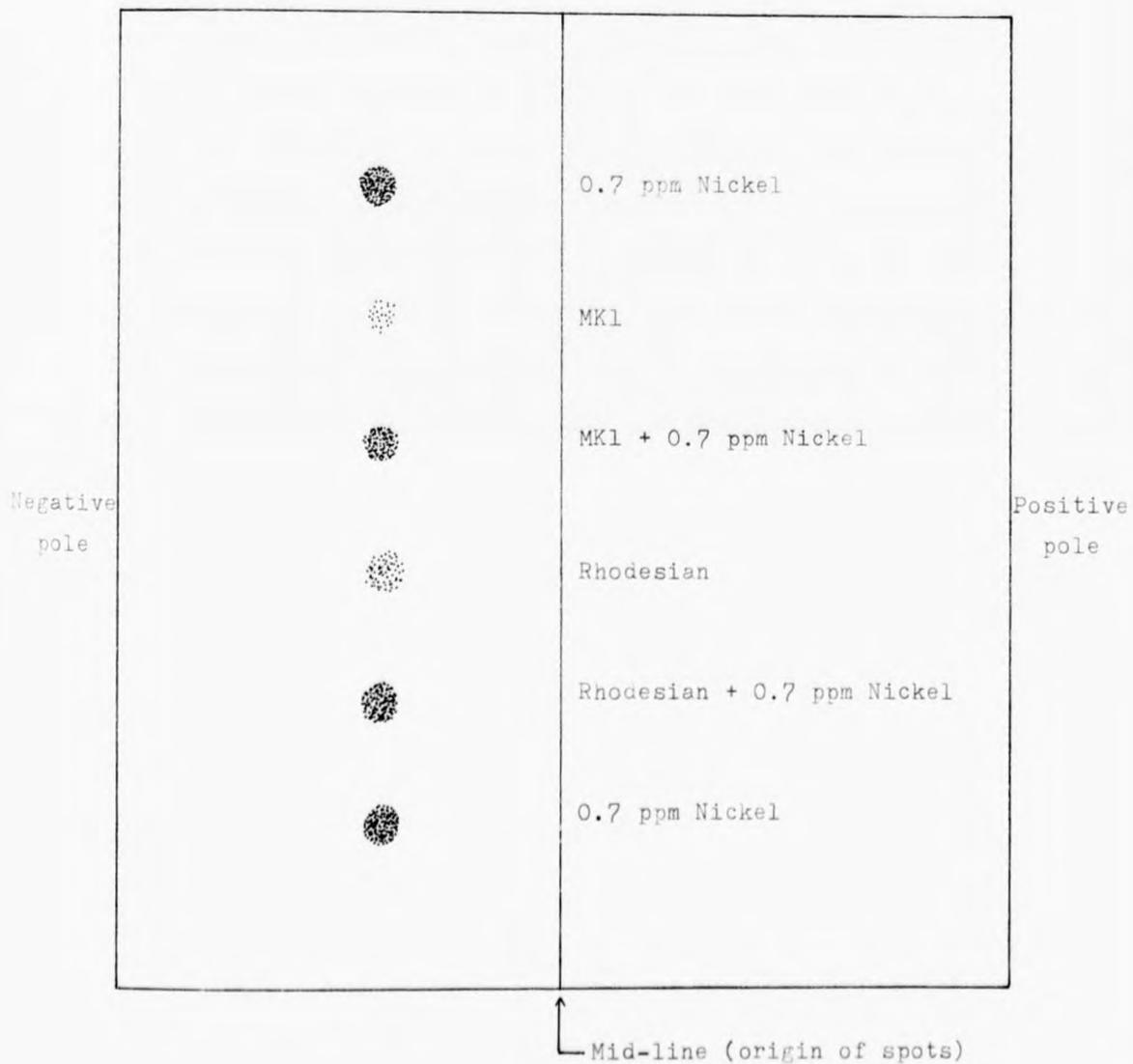


Figure 24 Electrophoretogram showing position and relative intensity of nickel from each source spot.

Discussion

Dithioamide gives a blue reaction with ionic nickel. The intensity of the developed spots suggests that a comparatively small part of the soluble nickel in MK1 soil solution occurs as ionic nickel. A larger fraction of ionic nickel appears to exist in the Rhodesian soil solution. Since the mixture of soil solution and nickel standard gave a similar colour intensity to the standard alone it seems unlikely that any component of the MK1 and Rhodesian soil solutions interferes with colour development. This may suggest that at least some of the nickel in both soils occurs as a soluble chelate and would agree with the little that is known about the reactions of nickel in soils.

McLean (1966) investigated the behaviour of nickel ions when added to a range of non-serpentine soils. He concluded that the reactions between nickel and soil organic matter were complex and involved a variety of processes including co-ordinating and chelating functional groups. Slingsby and Brown (1977) claimed that in the Scottish serpentine soils they investigated, short-lived accumulations of organic matter (humus) probably did not change the availability of nickel.

Whatever the form of nickel this metal is readily taken up by plants from both soils. Johnston (1976) found nickel to range between 18 and 260 ppm in several species taken from MK1 while Proctor, Burrow and Craig

(1979) found between 7.5 and 880 ppm nickel in several species from Rhodesia.

The effect on toxicity of the form in which nickel is made available to plants in water culture experiments is a topic worthy of further consideration. When the water culture study described in Chapter 3 was undertaken it was assumed that the nickel was most likely to be in an ionic form. In view of the electrophoresis work discussed here it would have been interesting to compare the response of plants to nickel when supplied as the ionic form and as a chelate e.g. complexed with EDTA or an organic acid.

CHAPTER 10

GENERAL CONCLUSIONS

(A) Important chemical factors in MK1 soil

Of all the soils studied here, MK1 was remarkable for the very high level of magnesium in its soil solution. Magnesium was almost three times higher in MK1 soils than MK2, which contained the next highest magnesium concentration to MK1. The exceptionally high magnesium in MK1 probably represents the most significant single factor which influences plants on this soil (as suggested by Proctor (1971b) and Johnston (1976)). In addition it would appear that plants on MK1 soils have also to be tolerant of relatively high levels of nickel and to be capable of growth at low levels of most essential nutrients. It is of interest that nitrogen would not appear to be a limiting factor on MK1 since nitrate levels were relatively high and this ion seems to be the major balancing anion for the very high soil magnesium levels.

The electrophoresis data indicated that nickel may be in a soluble (positively charged) complexed form in MK1 soil solutions. The significance of this in terms of the toxicity of this element to plants grown in MK1 soils was not investigated. However, comparisons of the levels of nickel taken up from water culture experiments (when nickel was supplied as Ni^{2+}) with the data of Johnston (1976), where plants were grown in MK1 soils, shows that broadly

similar concentrations of nickel were taken up into plants irrespective of possible differences in its form. Nevertheless it is appreciated that the form of nickel supply might influence the effect of nickel within the plant and further research is needed on this topic.

(B) The use of water culture experiments

The use of nutrient solutions, the compositions of which were based on soil solution analyses of MK1 soils, to study the plant response to particular soil conditions seems to have been justified. When MK1 and NS F. rubra were grown in culture media of mineral composition similar to that of MK1 soil solution (i.e. "low" micronutrients, "low" calcium, 7.5 mM magnesium and 0.7 ppm nickel - the "serpentine condition" as described in Chapter 3) they were found to have broadly similar dry weights and levels of metals as MK1 and NS F. rubra grown in MK1 soils by Johnston (1976) under similar light and temperature conditions.

One of the striking features of the experimental results from culture media (and enzymic activity) was the great complexity of the interactions between micronutrients, calcium, magnesium and nickel. They emphasize that any study involving plant/soil interrelationships must consider the response of plants to a given factor with reference to the status of many other factors.

Whilst chromium was below the detection limit of 0.1

ppm in MK1 soils, the culture media experiments using Cr (III) indicated that this element might influence growth of NS and MK races of F. rubra and that it may have interaction with magnesium which adds to the toxicity of the MK1 soil. Further investigation using lower levels of Cr (III) (and possibly Cr (VI)) would be of interest.

(C) Plant adaptations to the "serpentine condition"

The MK1 race is well-adapted to life in serpentine soils in many ways and may have a requirement for high magnesium. Increasing magnesium levels in culture solutions stimulated growth in the MK1 race, although dry weight differences between magnesium treatments fell short of significance. The MK1 race was not adversely affected by the presence of nickel in the soil solution. Moreover, the MK1 race seemed capable of taking up and maintaining high levels of potassium from serpentine soils where available potassium is low. This race was also shown to maintain comparatively low Mg/Ca ratios in shoots and roots in the "serpentine condition" of water culture treatments.

Other differences noted between MK1, which made good growth in "serpentine conditions", and the NS race which hardly grew at all in this treatment, were the large amounts of certain metals taken up by the NS race. This may be a consequence of some damage to cell membranes due to the extreme mineral environment. "High" calcium supply possibly restores membrane integrity to NS plants.

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"High" calcium and "high" micronutrient supply were generally advantageous to the NS race but resulted in poorer growth in the MK1 race. These components are normally low in serpentine soils and are possibly toxic to MK1 plants when in high concentrations. It was not possible to single out a micronutrient responsible for the differences in response of MK1 and NS plants. It is possible that all the micronutrients interact to produce the observed differences. Further work is required to elucidate this point.

The MK1 race was apparently able to synthesize and use carbohydrates normally in "serpentine conditions", and in other environments of extreme mineral composition. Serpentine conditions may inhibit normal glycolytic pathways to some extent in NS plants resulting, in part, in the poor growth of this race in these conditions.

There was some evidence that MK1 plants had a use for (if not a strict requirement for) nickel. It was noted that in "low" micronutrient with "low" calcium conditions in the absence of nickel, MK1 race took up higher levels of certain micronutrients (zinc, copper, manganese) compared with similar conditions when nickel was present. It is suggested that in "low" micronutrient conditions in the presence of nickel the MK1 race used nickel to fulfil some functions of micronutrients in NS race. In "low" micronutrient conditions when nickel was not present, the MK1 race took up higher levels of micronutrients perhaps to compensate for the lack of nickel.

There is further evidence that nickel may be used by MK1 plants. In the incubation medium which corresponded in mineral composition to the "serpentine condition" (except that micronutrients were completely absent), nickel stimulated the activity of root-surface acid phosphatase compared with similar treatments where nickel was absent. The presence of "low" micronutrients reduced the extent of stimulation due to nickel, and "high" micronutrients removed nickel stimulation altogether. This stimulation due to nickel was not noted in the NS race. It is suggested, therefore, that MK1 F. rubra uses nickel when micronutrients are absent or low to stimulate surface enzyme activity. Vergnano (pers. comm.) has found that the germination of seeds of Alyssum bertolonii (Desv.) (a nickel accumulator) is stimulated by the application of nickel. The response is specific to nickel.

Root-surface enzymes were also stimulated by high magnesium concentrations in the MK1 race. It has become apparent that magnesium participates in reactions involving group transfer, namely those in which phosphate participates, by serving as the intermediate carrier. Magnesium plays a predominant role in promoting the formation of the enzyme-substrate complex and the resulting intermediate of the reaction (Madhok, 1965). The concentration at which magnesium serves these roles, in an optimum capacity, is significantly higher in the Mk race compared with the NS race. The apparently optimum enzyme activity at high magnesium levels supports the view that the MK1 race

requires high magnesium. Marrs and Proctor (1976) demonstrated a high magnesium requirement in a serpentine race of Agrostis stolonifera.

(D) The use of computer models

The use of the computer model could be helpful in many ways. By measuring a few soil factors i.e. nickel, magnesium and calcium concentrations in soil solutions, it would be possible to predict the growth and mineral composition of F. rubra (both serpentine and non-serpentine races) grown in a particular soil. However, the findings given in Chapter 8 suggest care must be taken when interpreting soil solution data. The model is based on MK1 soil solutions and factors such as phosphorus, nitrogen and potassium are assigned particular concentrations. The model in its present form could not account for changes in plant response due to changes in the supply of these elements in a soil. Any significant variation of the observed from the predicted data might indicate that other factors (such as phosphorus and nitrogen) are also important in the particular soil being examined. Since the model can operate at "high" or "low" micronutrient input, it could suggest the status of these soil solution constituents which are difficult to measure.

APPENDIX I Quantitative determinations of organic acids
and sugars using G.L.C.

Many workers have used G.L.C. for the quantitative estimation of organic acids and sugars from plant tissues, for example, Pallas and Wright (1972), and Rutter et al. (1977). In the present study an attempt was made to use G.L.C. quantitatively in the study of carbohydrates and organic acids in MK1 and NS plants.

Carbohydrates and organic acids were extracted from plant tissues as described in Chapter 6. Internal standards of known concentration were added to the tissue extract prior to ion exchange chromatography. This ensured that quantitative losses of sample during the various processes involved in running the G.L.C., were experienced by the internal standards. Various standards were tried until ones were found which gave a peak in a blank region of the sample chromatograph i.e. an area not occupied by a component peak of the sample. For acids, p-hydroxybenzoic acid and for sugars, sorbitol TMS derivatives were found to be suitable internal standards. One mg of free acid or sugar were added to the sample extract and the mixture treated as described in Chapter 6. Integrated areas of components of the sample were compared with the 1 mg standard peak enabling the concentration of each component to be calculated.

Attempts were made to streamline the G.L.C. technique by omitting ion exchange and running sugars and acids

simultaneously. However, it was found the complex peak patterns arose which the digital integrator found difficult to resolve. This reduced the accuracy of quantitative determinations. In addition it was often found that citrate and fructose peaks could not be resolved from each other.

APPENDIX II Qualitative analyses of organic acids

The organic acids extracted from both races of F. rubra are shown in Table 12. Each acid is expressed as a percentage of citric acid.

Table 12. Organic acids in MK1 and NS plants expressed as a percentage of citric acid. Plants were grown in "high" micronutrient and "low" calcium conditions with two levels of nickel and three levels of magnesium.

		nickel(ppm)			0.7			
		magnesium(mM)			4.0 7.5			
		1.5	4.0	7.5	1.5	4.0	7.5	
		Organic acid						
MK1		A	56	75	110	359	nd*	73
		B	277	383	274	15	nd	3
	Shoot	malate	183	162	230	131	nd	96
		H	348	147	316	278	nd	83
		citrate	100	100	100	100	nd	100
		A	105	221	134	174	196	282
		B	233	410	321	+	+	+
	Root	malate	15	12	13	+	+	+
		H	+	+	+	+	+	+
		citrate	100	100	100	100	100	100
NS		A	40	17	26	12	41	4
		B	27	10	22	3	+	6
	Shoot	malate	64	112	308	36	59	19
		H	60	53	250	164	292	88
		citrate	100	100	100	100	100	100
		A	377	1000	nd	72	17	+
		B	+	+	nd	2	6	+
	Root	malate	+	+	nd	+	+	+
		H	+	+	nd	+	+	+
		citrate	100	100	nd	100	100	100

+ indicates the acid was present but in very low levels.

*nd = not determined.

APPENDIX III A colour test for a chromium complex.

Introduction

Lyon et al. (1969a, b) reported the existence of a tris-oxalatochromium complex in Leptospermum scoparium. A simple colour test is described here which could be used to identify this complex.

Materials and Methods

a) Preparation of plant material

Dry root and shoot material from the chromium water culture experiment (Chapter 4) was ground in an ice-cold mortar containing 10 cm³ of 50 mM tris buffer pH 7.5 and 1 cm³ of 5 mM dithiothreitol. The resulting solution was filtered under vacuum through a 25 m nylon net. The solution was kept in ice.

b) Staining for a chromium complex

A chromium complex (reported by Lyon et al. 1969a, b, in the roots of a chromium accumulator) was synthesized in order to test its behaviour in a low voltage electrophoresis system to act as a reference for any complexes that might be in the plant material and also for use in an attempt to find a dye reagent capable of revealing chromium in a complexed form. Tris-oxalatochromium (III) was prepared following the method of Palmer (1965). Spots of an aqueous solution of this complex were subject to electrophoresis as described in Chapter 9.

Table 13 Reagents used to locate chromium complex on chromatography paper.

Reagent	Solvent	Concentration	Coloration of chromium-complex spot
Potassium thiocyanate	water	1%	None
8-Hydroxyquinoline	60% EtOH	1%	"
Dithiooxamide	60% EtOH	1%	"
Acid alizarin	water	1%	"
*Basic alizarin	water	1%	"
+Bromocresol green	water	1%	"
Acetylacetone + Potassium thiocyanate			"
" 8-Hydroxyquinoline			"
" Dithiooxamide			"
" Acid alizarin			"
" Basic alizarin			"
" Bromocresol green			"
**Stannous chloride + Potassium thiocyanate			Pale yellow (unstable)
" 8-Hydroxyquinoline			Yellow spot on a blue background
" Dithiooxamide			None
" Acid alizarin			"
" Basic alizarin			"
" Bromocresol green			"
Acetylacetone + Bromocresol green + Acid alizarin			Pale yellow (unstable)
" " " "			Green/yellow spot on a purple background

- * Alizarin made basic with KOH, or acid with HCl.
- + Bromocresol green solution was made basic with KOH.
- ** Stannous chloride was a 1% aqueous solution.

Several dye reagents were used individually and in combinations, in an attempt to find a system which would reveal the position of the chromium complex on the chromatographic paper. After electrophoresis the papers were dried overnight and then sprayed with reagents as indicated in Table 13. In treatments including acetylactone or stannous chloride. Five minutes were allowed before the application of the second reagent. Acid or basic alizarin reagents were added 30 seconds after the bromocresol green in the last two treatments.

Plant samples were then run through the system using the chromium complex as a reference.

Results

When quite high concentrations of the chromium complex were spotted onto the chromatography paper, the natural greyish coloration of the complex could be observed allowing its progress to be followed when the system was running. The low voltage electrophoresis system caused the complex to move approximately 5 cm from the origin after 10 minutes.

Two reagent systems produced a stable colour reaction with the complex: (a) acetylactone/bromocresol green/basic alizarin, (b) acetylactone/bromocresol green. The colour spots given in Table 1 developed after about 15 min. When the plant material spots were sprayed with systems (a) or (b), no colour reaction was noted in the

area 5 cm from the origin after 10 minutes of electrophoresis, indicating that the chromium complex was either not present or in very low concentrations in F. rubra.

Discussion

Low voltage electrophoresis appears to be adequate to move the complex over some distance. However, its behaviour in the presence of other chromium complexes from plants (which have been noted by Lyon et al. 1969a, b) was not examined - mainly because the structure of these compounds do not appear to have been elucidated. It is possible that they all might move at the same rate in this system.

The failure of most reagents to give a colour reaction with the complex was probably due to its stability. The successful dye system employed acetylacetone which was thought to convert some of the complex to the more labile acetylacetone-chromium derivative. The stable yellow colour which developed was not very intense from low concentrations of the complex. The overall procedure may be of considerable use in investigations of chromium-accumulating plants. Perhaps a more sensitive method could be developed from this ground work if the complex could be converted to a more labile form on the chromatographic paper after separation.

APPENDIX IV Individual analyses of MKI soil samples

Replicates	Elemental concentration (ppm)											
	Ni	Cr	Co	K	Na	Fe	Ca	Mg	N ⁺ (NH ₄)	N ⁻ (NO ₃)	P ⁻ (PO ₄)	S ⁻ (SO ₄)
1	0.9	<0.1	<0.1	6.6	17.4	0.5	13.6	320	0.5	210	5.0	50
2	0.7	<0.1	<0.1	9.2	17.4	<0.1	10.8	166	1.0	175	10.0	25
3	0.6	<0.1	<0.1	6.1	15.4	0.2	10.4	150	0.8	167	2.5	10
4	0.7	<0.1	<0.1	5.0	12.4	0.1	11.7	170	0.5	200	7.5	25
5	0.7	<0.1	<0.1	3.0	15.4	<0.1	10.6	178	0.6	220	2.0	30
6	0.6	<0.1	<0.1	3.6	11.5	<0.1	9.0	156	0.3	150	2.5	35
7	0.5	<0.1	<0.1	5.2	13.1	<0.1	9.0	134	0.2	183	3.0	20
8	0.7	<0.1	<0.1	4.2	15.8	<0.1	11.2	174	0.8	170	3.0	15
9	0.6	<0.1	<0.1	6.0	12.5	0.2	11.0	170	0.7	200	5.0	40
10	0.6	<0.1	<0.1	6.0	14.8	<0.1	14.0	200	0.6	173	4.0	15
11	0.7	<0.1	<0.1	4.0	17.3	0.1	8.0	190	0.2	220	3.5	25

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