SOME EFFECTS OF NITRATE AND AMMONIUM NITROGEN ON THE MINERAL COMPOSITION OF PASTURE GRASSES

By

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TABLE OF CONTENTS

LIST OF FIGURES, PLATES AND TABLES

FIGURES

PLATES

TABLES

SECTION I

INTRODUCTION

Nitrogen is unique among the major plant nutrients derived from soils in that it may be taken up by plants either as an anion or cation. Nitrate and ammonium comprise the pool of assimilable nitrogen, but their proportionate contribution varies considerably under differing climatic and soil conditions.

Bear (1950) formulated a general rule for a constant balance between the number of equivalents of cations and anions in the herbage of growing plants. From this it may be suggested that the uptake of cations and anions, other than ammonium and nitrate, will be markedly influenced by changes in the ionic form of nitrogen being absorbed by non-legumes. That uptake of ionic nitrogen normally exceeds that of any other ionic species, supports this suggestion.

Experiments undertaken in this investigation were designed to test the validity of the foregoing postulate, using pasture grasses, with a view to establishing whether changes in the nitrogen regime in the field could be of agronomic significance. An elucidation of the relationships between the form of mineral nitrogen available and certain physiological processes within the test plants, was also sought.

SECTION II

LITERATURE REVIEW

A SOIL NITROGEN

1 INTRODUCTION

The nitrogen cycle in grasslands has been reviewed in detail (C.A.B. Bulletin 46, 1962) and with particular reference to grass/legume associations under N.Z. conditions (Walker, 1956, 1962; Sears, 1956, 1960, 1963). This review is biassed towards the qualitative aspects of mineral nitrogen (N) availability to pasture grasses.

2 NITROGEN REGIME UNDER GRASSLANDS

(a) General

Grassland soils generally contain low or negligible amounts of nitrate (NO_3) in contradistinction to soils under crops or fallow (Martin, 1962; Robinson, 1963). It has been suggested that this is because grasses absorb ammonium (NH_4) too quickly for nitrification to commence, whether the NH_4 arises from mineralization of organic N (Martin, loc.cit.) or is added directly in fertilizer (Richardson, 1938). This may explain the continuously low level of mineral N recorded under European grasslands, as grasses and associated microflora remove N from soil very rapidly (Walker *et al.*, 1954). However, it does not satisfactorarily explain the apparent absence of nitrification, as pasture soils frequently contain the major part of their mineral N as NH_4, at higher levels than in arable soils (Martin, 1962; Robinson, 1963). A specific inhibition

of nitrification by exudates from grass roots has been postulated (Theron, 1951; Soulides and Clark, 1958) but it is not universally accepted (Harmsen and van Schreven, 1955; Russell, 1961). This writer feels that the relative levels of the two N forms present in soils at any time, as detected chemically, is not necessarily indicative of their proportionate uptake by plants. Ammonium, the substrate for NO_3 formation, is not free to diffuse towards the sites of assimilation, whether microbes or plants, because of its association with soil colloidal surfaces (Brown, 1963). Free diffusion of NO_3 may result in its absorption at a rate commensurate with formation, so that no accumulation is observable. Wiersum (1961, 1962a, 1962b) has drawn attention to the efficient utilisation of the soil volume by the root systems of grasses, in contrast to other species, which could account for differences in NO_3 levels between soils under pastures and arable soils. That NH_4 levels are generally higher under grasslands could be owing simply to a more rapid cycling of N, especially where the grazing animal is involved, resulting in higher "background" values for NH_4.

(b) Seasonal Rhythm in N Form

The part played by grazing ruminants in the N cycle has been reviewed by McDonald (1962). Of the N returned to soil by animals, some 80% is voided in urine. This is the major pathway for transformation of herbage organic N, including that fixed by clovers, to soil inorganic forms under N.Z. conditions (Sears, 1956; O'Connor, 1966). This process continues throughout the

year. There is no precise information as to whether the quantitative significance of the urinary cycle shows a seasonal pattern. Obviously, D.M. intake during the winter period diminishes, under most systems of farming, but herbage N content may be twice that encountered in summer, with improved pastures (Metson, in press). Doak (1952) has studied the chemical changes when urine is voided on to pasture. Hydrolysis of urea to ammonia was rapid giving a pronounced increase in pH. This resulted in some loss of N to the atmosphere and interference with nitrification. It was concluded that loss of elementary N to the air was not important, but that rainfall, with consequent leaching of urea, NO_2 and NO_3, played a major part in the fate of urinary N. The significant point arising is that if nitrification of urinary N is occurring throughout the year, NO_3 would be detectable in drainage waters at all times, as N is lost by leaching almost entirely as NO_3 (Martin and Skyring, 1962).

Butler and Hopewell (pers. comm., G. W. Butler) studied the loss of minerals in drainage waters from a high fertility catchment under a grazed grass/clover sward. With the appearance of drainage waters in autumn, NO_3 levels in solution were very high, of the order of 20 ppm. This level dropped quite rapidly with progressive leaching, until no NO_3 was detectable in drainage waters during winter and early spring. NO_3 reappeared in late spring, at comparatively low levels. A similar pattern was established by analysis of the drainage effluent from soils receiving ammonium fertilizers during observations on spaced plants (loc.cit.). Puke (1959) concluded that highest levels of NO_3

occurred in early summer and autumn, in soils under mixed swards. Simpson (1962) found that soils under improved pastures in New South Wales, accumulated NO_3 in substantial amounts during summer and autumn. It disappeared from the topsoil after the heavy rains of autumn and winter. Collier (1964) found that drainage waters from a clay soil studied with lysimeters, contained high NO_3 concentrations after a dry summer, whether the soil was cropped or fallow. Metson (pers.comm.) measured the NO_3 content of surface soils from a variety of sites in Hawke's Bay and Wairarapa. Measurements were made in February. Where growth had been inhibited by urine, NO_3 in the soil below these "burned" patches ranged from 30-190 ppm. Under new growth on old urine patches soil NO_3 ranged from 5-70 ppm. This supports the published data of Thompson and Coup (1943) for N.Z. conditions.

Indirect evidence for seasonal patterns in nitrification under improved pastures comes from studies on the chemical composition of grasses. Butler (1959) reported that grass with high inorganic NO_3 levels was most frequently observed during the autumn flush of grass growth. After an unusually dry winter followed by warm spring temperatures, a similar situation was once recorded in September. Metson found that the NO_3 content of rapidly growing grass, influenced by recent urination, was some 4 times that of herbage nearby, during autumn sampling.

From this evidence, it is concluded that there is a marked seasonal pattern in the form of N available to grasses in improved pastures. As a generalisation, NH_4 predominates during the normally cold, wet soil conditions of winter and early spring.

With increasing soil temperatures and aeration, the activity of nitrifying bacteria is enhanced and the relative contribution of NO_3 to assimilable N increases. With warm rains, followed by intermittent dry periods, NO_3 assumes increasing importance. If leaching is not extensive, NO_3 accumulates in the surface soil and attains major significance during the autumn flush of grass growth. With progressive rainfall, subsequent leaching and falling temperatures, the significance of NO_3 declines while NH_4 assumes predominance again in a repeating seasonal rhythm.

Reported N.Z. observations are difficult to reconcile with the introductory remark of Robinson (1963): "..... the general failure of grasslands to display nitrification"; and with the statement of Martin (1962): "..... : the present compromise is to assume that nitrification plays little or no part in the N nutrition of permanent pastures and that grasses absorb their N almost entirely as ammonium". Under high fertility conditions, when grass/clover associations are attaining grass dominance and soil organic N is near equilibrium (phases 3-4; Sears, 1960) field observations in N.Z. show that nitrification and subsequent NO_3 assimilation by grasses play a major part in N nutrition during some periods of the year. This serves as a warning against extrapolating European observations to local conditions, without N.Z. confirmation. Dutch workers have difficulty in giving credence to the high N contents reported for N.Z. winter and spring herbage (pers.comm. Metson). It may be that under the N.Z. grass/clover grazing system, all phases of

the urinary cycle operate at higher equilibrium levels than in Europe.

(c) Soil Factors

There is ample evidence that the generalisation above may be overridden by local soil factors.

(i) pH Below pH 6.5, nitrification becomes progressively retarded with increasing acidity to the point of irreversible impairment at pH 3.9 (Martin, 1962). He suggested that the apparent lack of nitrification observed in Europe under old, established grasslands may be because of low pH. Robinson (1963) concluded that the low population of nitrifiers in a virgin tussock-grassland soil in N.Z. was in part owing to its natural pH of 5.5 being below the optimum for nitrification - an observation in agreement with Ross (1960) and confirmed by O'Connor (1966) studying similar soils. Many workers have recorded a negative relationship between pH and nitrification; e.g. Moravec (1963) in Czechoslovakian soils under grassland associations and Ishizawa and Matsuguchi (1962) with Japanese soils.

Low pH, and subsequent lack of nitrification, does not necessarily imply low fertility conditions. Ammonification is far less sensitive to extremes of soil pH than nitrification, because the process involves many kinds of soil micro-organisms (Martin, 1962). On the basis of observations over several years, O'Connor (1966) has concluded that on the naturally acid tussock-grasslands, as long as legume mineral nutrition is safeguarded, pasture development can be carried forward under acid conditions

with negligible nitrification occurring. Similarly, very high production has been maintained at the Te Awa substation of Grasslands Division, where soil pH is 5.0 - 5.1 (pers.comm., C. Michie). In as far as data are available for N.Z., soils of low pH, such as that just cited, would be expected to have a low population of nitrifiers and low nitrifying ability. NH_4 would be expected to be the major N form assimilated by plants throughout the year.

(ii) Temperature, Moisture and Aeration are considered briefly together, as they are virtually inseparable in the field. Optimum soil temperature for the combined activities of Nitrosomonas and Nitrobacter is reportedly 25-27°C under ideal moisture conditions (Skyring and Callow, 1962). Nitrification can be detected in vitro, however, at temperatures approaching 0°C (Gerretsen, 1942; Schaefer, 1964). There has been a great deal of repetitive observation of increasing nitrification with increasing temperature to an optimum near 27°C (Parker and Larson, 1962; Anderson and Boswell, 1964, and others).

Heavy rain, besides its obvious effect of leaching NO_3, inhibits nitrification (Skyring and Callow, 1962). Nitrifiers are strictly aerobic. As moisture above field capacity and the O_2 content of soil air are inversely related (Russell, 1961) nitrification is progressively inhibited with increasing precipitation. Eventually, under anerobic conditions, denitrification occurs (Skyring and Callow, 1962; Parker and Larson, 1962). Russell (1961) discussed the high levels of CO_2 in the soil atmosphere under pastures and its marked increase in wetted soils. As nitrifiers are inhibited by high concentrations of CO_2, Russell suggested that

this may account for the apparent lack of nitrification under European grasslands.

Of considerable agricultural significance is that the appearance of NO_3 is markedly increased by alternate wetting and drying, as opposed to any steady state of moisture (Birch and Friend, 1956; Birch, 1958; Calder, 1954, 1957) and by fluctuations, rather than a steady state, in temperature (Frederick, 1956). This "Birch effect" may account for the report by Butler (1959) that under high fertility pastures, the NO_3 concentration increases to high levels when light, warm rains follow lengthy dry periods.

It is concluded that the relative significance of NH_4 is greatest with low soil temperatures and/or high water contents with attendant, poor aeration. This would include all waterlogged soils and soils which are badly drained or heavily poached so as to cause temporary retention of water above field capacity. Everything else equal, NO_3 assumes greater importance in warm well drained soils under aerobic conditions, especially with alternate wetting and drying.

These effects of temperature, moisture and aeration on N transformations are in general harmony with the observed seasonal rhythm of N form under high fertility conditions, suggesting that the recorded pattern is the result of climatic effects on nitrification.

(iii) Organic matter status may appreciably alter the generalised situation for high fertility conditions outlined in (II, A, 2, (b)). Under low fertility conditions on mineral soils organic N levels are

low, the C/N ratio is comparatively high and these characteristics are generally coupled with a naturally low pH (Robinson, 1963; O'Connor, 1966). The urine cycle is quantitatively much less significant owing to the extensive nature of the farming on such soils (O'Connor, 1966).

The importance of the C/N ratio in the course of N transformations has been reviewed (Harmsen and van Schreven, 1955). Because of the interdependence of these two elements in metabolic processes, mineral N is incorporated into microbial protein almost as rapidly as it is mineralized, in soils with a high C/N ratio. In high fertility soils, on the other hand, with a low C/N ratio, mineral N is released in excess of the requirements of the heterotrophic organisms metabolising the contained carbon compounds in the organic matter. This excess of mineral N is available to plants and the autotrophic nitrifiers, which rely on NH_4 as their energy source.

Studies have been made of nitrification in N.Z. soils under native tussock-grasslands. These soils showed little nitrifying potential in their virgin state, which was attributed to a combined lack of mineral N substrate to maintain a population of active nitrifiers and a naturally low pH (Ross, 1960; Robinson, 1963; O'Connor, 1966). Nitrifier population was stimulated by addition of urea, with more lasting effects when lime was also applied (Robinson, 1963; O'Connor, 1966). After reviewing recent evidence Martin (1962) concluded that plants are weak competitors for NH_4 compared with micro-organisms, and that heterotrophes compete more efficiently for a limited supply of NH_4 than do nitrifiers.

These views are supported by Ross (1960) who found that nitrifying activity in Taupo soils was negligible in the virgin state but increased after a period under improved pastures. He did not apportion the cause between better aeration, increased P availability or increased N substrate from clover growth, however.

It is concluded that under low fertility conditions, especially as these are generally associated with low pH, nitrification is not a significant pathway in N transformations. Because of the intense competition between resident non-legumes and soil organisms for mineralised NH_4, grasses assimilate virtually all their mineral N in the NH_4 form. A large area of soils subjected to extensive farming practice falls into this category in N.Z.

(iv) Cultivation promotes mineralization of organic N (Russell, 1961). Normally ammonification is slower than nitrification and is the rate-limiting step (Martin, 1962). Tillage of high fertility soils will therefore result in accumulation of NO_3, especially if cultivation precedes a period of dry fallow. Newly sown pasture is likely to have a high NO_3 content as a consequence (Butler, 1959).

O'Connor (1966) reported that mineralization in soil under tussock-grassland was negligible following cultivation from its natural state. However, after manuring and tillage, mineralization, as measured by N availability to subsequent crops, was greater even though total soil N had not changed measurably. This suggested that the rate of mineralization was affected by the quality (especially the C/N ratio) of recently added plant residues - a finding reported by others (Russell, 1961).

B THE UPTAKE, METABOLISM, AND ACCUMULATION OF MAJOR ELEMENTS IN PLANTS

1 INTRODUCTION

One aspect of the nutritional value of pasture herbage is its content of major elements derived from the soil (Whitehead, 1966). Many complex factors are responsible for determining and modifying the content of these major elements, as measured in herbage. An understanding of these interactions is a pre-requisite to any manipulation of the environment with a view to improving the nutritional value of herbage.

This section of the review deals with some aspects of these interactions, with particular reference to the situation during NH_4 and NO_3 assimilation.

2 NITROGEN METABOLISM IN NON-LEGUMES

(a) Introduction

Apart from water, N is probably the major factor limiting agricultural production. The quantitative significance of N in pasture grasses is large in relation to other soil-derived elements. To achieve their inherent capabilities, pasture grasses have to derive a greater equivalent quantity of N from soils than of any other element, except silicon in some cases (Dijkshoorn, 1957 *et seqq.*; Sutcliffe, 1962; Cunningham, 1964 *et seqq.*). The importance of N has maintained a central place in the interest of soil scientists and plant physiologists for more than a century. Early work has been reviewed by Miller (1938) and McKee (1962). With the advent of new techniques, in particular partition chromatography,

and isotopic techniques, there have been dramatic advances in the field of N metabolism in the past 15 years. The result has been a spate of reviews dealing with various aspects of the subject.

The organic N constituents of non-legumes arise principally from inorganic mineral N derived from soils. It is proposed to review N metabolism in plants only insofar as an understanding of the biological processes involved is necessary in the present study. Organic N compounds are indispensible as structural and functional components in all plant tissues, but any further elaboration of this is beyond the scope of the current review. For a full review of N metabolism in plants the reader is referred to the following: Ruhland (1958); S.E.B. Symposium No. 13 (1959), and McKee (1962).

(b) Sources of Nitrogen

It is generally agreed that inorganic N provides the major source of N for assimilation (Miller, 1938; McKee, 1962). It was concluded in a previous section (II, A) that, for grasses, mineral N may be available as NO_3 or NH_4 depending on environmental and soil conditions. The possible contribution of soil-derived nitrogenous organic compounds to the N nutrition of plants has been reviewed (Burris, 1959; Konnonova, 1961). Some of these compounds may enter the plant and be assimilated. It may be concluded that their quantitative contribution to total N uptake is insignificant by comparison with mineral N and they are not considered further in this review. Situations giving rise to appreciable quantities of nitrate or urea in soils, and their assimilation by plants, appear to be very specialised (Skyring and Callow, 1962; Martin, 1962) so that plant metabolism of exogenous nitrite and urea is not discussed.

(c) Assimilation of Mineral Nitrogen by Plants

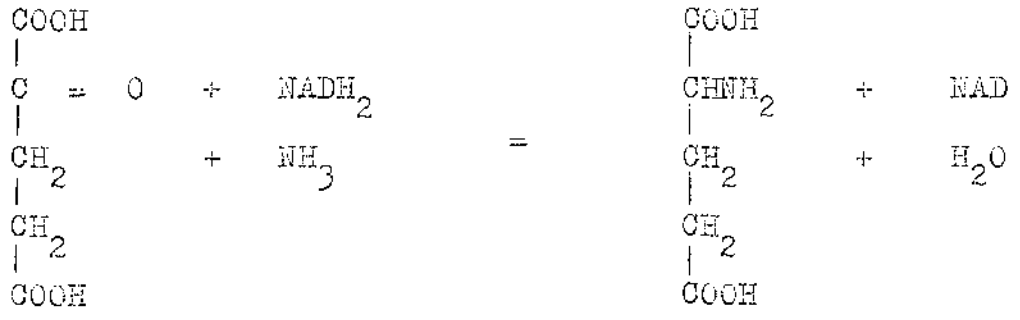
Both forms of mineral N are biologically incorporated as ammonia, which involves the prior reduction of NO_3. The enzyme systems involved have been reviewed recently (Kessler, 1964). After absorption, the reduction of NO_3 in the plant apparently occurs in the soluble part of the protoplasm. The first step has been clarified, and involves the reduction of NO_3 to NO_2 under the influence of the enzyme, nitrate reductase, a metallo-flavoprotein containing molybdenum. The hydrogen donor is the reduced form of either coenzyme, I or II.

$$NO_3^- + \begin{matrix} NADPH_2 \\ \text{or } NADH_2 \end{matrix} = NO_2^- + H_2O + \begin{matrix} NADP \\ \text{or } NAD \end{matrix}$$

Nitrate reductase is an adaptive enzyme. It is induced by the presence of NO_3 in a wide variety of tissues (Filner, 1966). Molybdenum is required for its synthesis which may be quite rapid. The steps in the further reduction of NO_2^- to NH_3 in higher plants are not clear. Iron, copper and manganese are required cofactors probably together with an unknown organic cofactor. Whether or not free intermediates are involved is argued (McKee, 1962; Kessler, 1964). In generalised form, the overall equation for NO_3 reduction can be written as:

$$HNO_3 + 8H = NH_3 + 3H_2O$$

The further assimilation of NH_3, whether arising from NO_3 or NH_4, requires that inorganic N is combined with a carbon skeleton, provided basically by photosynthesis. This is accomplished by amination of α-ketoglutaric acid (Davies *et al.*, 1964):



α-ketoglutaric acid L-glutamic acid

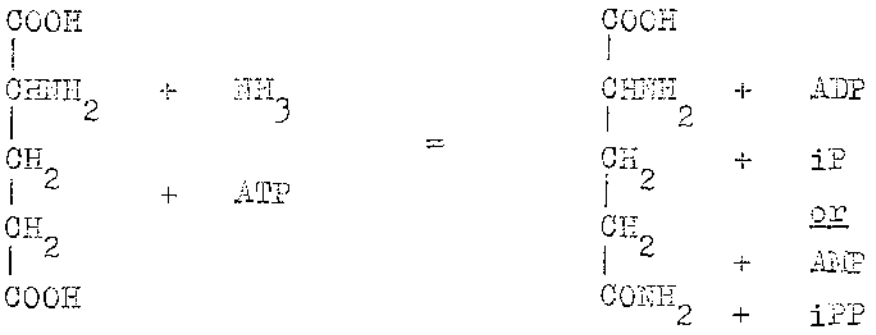
As the equilibrium for this reaction lies strongly towards the right, where free NH_3, α-ketoglutaric acid and reduced coenzyme are available inorganic N is rapidly incorporated. Glutamic acid is the immediate substrate for formation of other nitrogenous organic materials. The amino-group may be transferred to other keto-acid carbon skeletons, with subsequent formation of the corresponding amino-acids according to the general formula:

R_1 CH NH_2 COOH + R_2 CO COOH = R_1 CO COOH + R_2 CHNH_2 COOH

glutamic acid Keto-acid α-ketoglutaric acid amino-acid

Many of the amino-acids incorporated into plant protein arise directly in this way. Of particular interest in this discussion is that the dicarboxylic amino-acid, aspartic acid, arises by transamination of oxaloacetic acid from glutamic acid in the presence of the widespread enzyme glutamic-aspartic transaminase (Burris, 1953; Davies et al., 1964). Thus, by cyclic amination and transamination, limited amounts of α-ketoglutaric acid and glutamic acid may act as intermediates in the formation of a wide variety of amino-acids during the incorporation of mineral N into the organic N pool. The two amides, glutamine and asparagine, play

a central role in N interconversions. They serve as storage agents for NH_3, an otherwise toxic substance, and frequently comprise a large proportion on non-protein N (NPN). Amides are important in the translocation of organic N (for recent reviews see Loomis and Stumpf, 1958; McKee, 1962). Glutamine is synthesised by amidination of glutamic acid in the presence of either of two forms of the enzyme, glutamine synthetase:



glutamic acid — glutamine

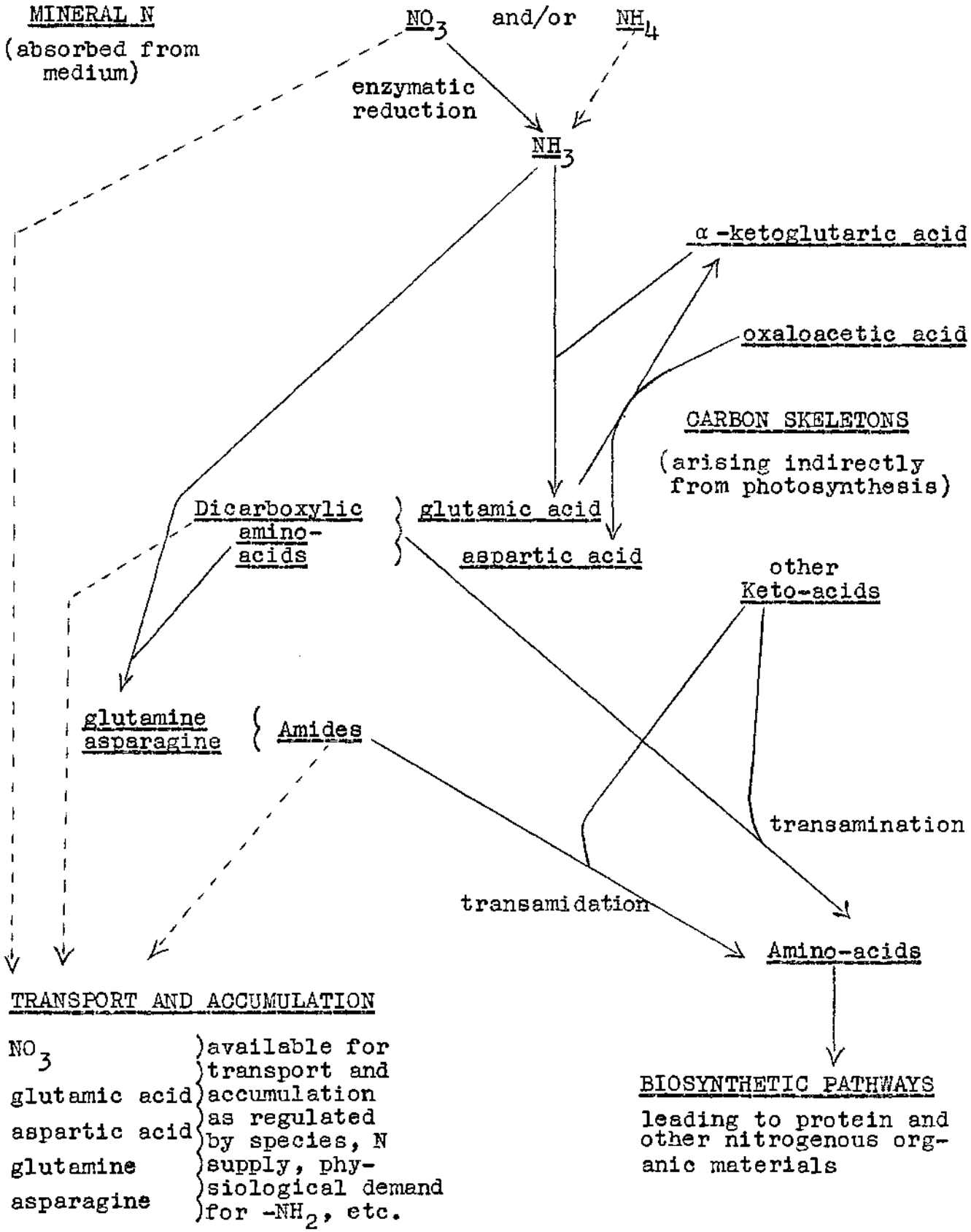
The equilibrium lies strongly towards NH_3 incorporation. Asparagine, the 4 C analogue of glutamine, is present in tissues in varying amounts. It is conventional to consider that it is formed by amidination of aspartic acid (Webster, 1959) in a manner similar to that of glutamine formation. As the precise reaction sequence is open to question (pers.comm., M. Lever) no equation is presented.

Glutamine, asparagine, glutamic acid and aspartic acid are quantitatively the major storage compounds for organic N, but many others exist (McKee, 1962). Their rate of synthesis, hence their levels in tissues, varies with species and stage of growth. Their synthesis is related to conditions favouring amino-acid biosynthesis including the availability of mineral N (Walker, 1962) especially in the NH_4 form (McKee, 1962).

Mineral N may be incorporated into organic forms in both roots and aerial tissues (Street, 1949; Burris, 1959). Russian work, reviewed by McKee (1962), has shown a rapid circulation of organic materials between different plant organs. Carbohydrates passed from the leaves to the roots where they were metabolised, presumably to keto-acids, to provide carbon skeletons for amino-acid synthesis. Amino-acids synthesised in the roots were largely transported to the shoots. The synthesis of a wide range of amino-acids in roots has been demonstrated for a variety of species, but roots are not the only seat of this process (loc. cit.). Bekmukhamedova (1961) found that inorganic N did not exceed 20% of total N in the bleeding sap of maize, whether plants were receiving NO_3 or NH_4. The concentrations of total and organic N were higher with NH_4 nutrition. The overall pattern developed from long term experiments is for carbohydrates to move towards the plant roots and for nitrogenous organic materials to move upwards from the roots (McKee, 1962). Roots are important sites for inorganic N assimilation into the organic pool, particularly if NH_4 is available (Kursanov, 1956; Bekmukhamedova, 1961). Ammonium never accumulates in healthy plant tissues, except in a few atypical "acid plants" (Street, 1949; McKee, 1962) and is reportedly toxic at comparatively low concentrations (loc. cit.). It is assumed, therefore, to be rapidly incorporated into organic forms in the roots directly after absorption.

The metabolism, transport and accumulation of N is summarised in Figure 1, which is entirely illustrative. Biochemical reactions are not shown in full. The term "transamidation" has been used loosely to cover the reaction sequence by which amide N is trans-

FIGURE 1 CONVERSION OF INORGANIC N TO ORGANIC FORMS, TRANSPORT AND STORAGE WITHIN THE PLANT (Original)



ferred to keto-acids for the formation of amino-acids, although the transfer may be much more complex than a simple transamidation (McKee, 1962).

3 NITRATE VERSUS AMMONIUM IN PLANT NUTRITION

Comparisons between these two forms of mineral N in plant nutrition have been the subject of several reviews (Miller, 1938; Street, 1949; Hewitt, 1952; Street and Sheat, 1958; Burris, 1959; McKee, 1962, and others). As will be shown in the following sections, the relative efficiency of NO_3 and NH_4 is influenced by a number of environmental and plant variables. An understanding of these effects is vital in the interpretation of experimental observations regarding the effects of N form on plant composition.

(a) Factors Affecting the Relative Efficiency of N Forms

(i) Species differences: Ammonium has been reported as the more effective sole N source for some species: potato, rice and some species of beet which were apparently unable to metabolise NO_3 (Street and Sheat, 1958); birch (Van Tuil, 1965); azalea (Burris, 1959); and some "acid plants" (e.g. Begonia spp) which will accumulate NH_4 salts of organic acids when fed NH_4, (McKee, 1962). Other plants, have been reported to grow better with NO_3 as the sole N source: cereals including barley, oats, rye and wheat (Street and Sheat, 1958); some beets, contradicting the observation above (McKee, 1962; Van Tuil, 1965) and poplar (Van Tuil, 1965). Grasses were reported to grow well with either N source (pers.comm., E. A. Kirkby). Wallace (1954) suggested that

specific differences in ability to utilise NO_3 may be related to genetic control over potential nitrate reductase activity.

Many plants can assimilate both N forms from culture solutions and have frequently been reported to develop most vigorously when supplied both forms simultaneously. Droineau and Blanc (1960) considered that increased yield of cocksfoot receiving $NO_3 + NH_4$, as opposed to NO_3 alone, was due to a better utilisation of the already reduced NH_4 in the synthesis of organic N compounds. Kirkby (pers.comm.) found that buckwheat died unless at least 15% of its N was supplied as NO_3, and suggested that the addition of NO_3 allowed a more normal organic acid synthesis. Grasmanis and Leeper (1965) found a marked stimulation in the growth of apple trees when only 1% of NO_3 was added to NH_4 cultures. They felt that the oxidising function of this trace of NO_3 may have had some important plant physiological implication. Street and Sheat (1958) attributed this superiority of the two forms in combination to the physiological stabilisation of pH and associated effects, such as iron availability, rather than to a direct effect of the two ions on metabolic balance.

As will be discussed subsequently, environment can be an overriding factor in the relative efficiency of either N form. As available data are generally based on single investigations with attendant variations in environment it is not possible to be dogmatic about the relative usefulness of either ion as the sole N source. An exposé of current confusion over the relative merits of NH_4 and NO_3 in the nutrition of rice is given by Karim and Vlamis (1962). However, in their review Street and Sheat (1958) concluded that some true specific differences do exist, and that the two forms in com-

bination frequently result in improved growth over either form separately.

(ii) Stage of development: Intense N assimilation is typical of young plants in general (McKee, 1962). Several workers have established for a variety of species, including cereals, that where NO_3 and NH_4 were both available, NH_4 absorption was greater during the early stages of development. At a later stage, the relative absorption rates were reversed and NO_3 uptake predominated (Street and Sheat, 1958). These authors suggested that the NO_3 reducing system develops only during or following germination.

(iii) Carbohydrate status: Assimilation of either N form involves the utilization of carbohydrates as the source of energy for NO_3 reduction, for the reductive amination of either N form, and to provide the carbon skeletons for amino-acid synthesis (II, B, 2, (c)). Under conditions of rapid N assimilation in the dark, carbohydrates are markedly depleted, but in light this condition may be more or less balanced by photosynthesis (McKee, 1962).

Kessler (1964) has reviewed the possible biochemical relationships between photosynthesis and NO_3 reduction. Apparently NO_3 and CO_2 compete for the photochemically produced hydrogen donors. Such an effect has been shown to be direct in vitro. Where NO_3 assimilation was occurring in green tissues it led to a decreased rate of CO_2 incorporation at low light intensities. However, at light saturation, where enzyme capacity was limiting the rate of CO_2 reduction, addition of NO_3 stimulated photosynthesis, possibly by providing oxidised forms of the hydrogen donors. The relationship

between NO_3 assimilation and photosynthesis <u>in vivo</u> is probably less direct, in view of the evidence for a considerable reduction of NO_3 in plant roots (II, B, 2, (c)). Ample production of carbohydrates may favour NO_3 assimilation by providing the necessary substrate (<u>loc.cit.</u>). Alberda (1961) found that defoliation of perennial ryegrass clones was followed by marked decrease in water-soluble carbohydrate, and a temporary increase in the inorganic NO_3 content of the tissues. This effect was more noticeable when defoliation coincided with an increased NO_3 supply, as opposed to defoliation a week before or after the increase in N supply. He concluded that increasing the N supply to intact ryegrass plants generally led to an increase in N content and a decrease in carbohydrate content in the foliage. Field observation by Bryant and Ulyatt (1965) have supported this finding under N.Z. conditions. They applied high and low rates of "nitrolime" to a short-rotation ryegrass sward. All N fractions were higher in the herbage at the high N application rates, and water-soluble carbohydrate content lower than for the low N application. The disappearance of these differences between treatments over a 2-3 week period was considered the result of a short-lived fertilizer effect. As NO_3 assimilation necessitates the expenditure of additional energy in its reduction to NH_3, it might be expected to deplete carbohydrates more severely than already reduced NH_4. Nitrate is, however, reported to be a suitable N source over a wide range of carbohydrate contents (Street and Sheat, 1958). Unlike NH_4, NO_3 can be accumulated in plant tissues if conditions are not conducive to N metabolism. Deficiencies of light, carbohydrates, or certain micronutrients are reported to

cause accumulation of NO_3 in tissues to high levels, with no apparent toxic effects on the plant (Spencer, 1958).

While assimilation of NO_3 is largely dependent on photosynthesis and therefore on concurrent carbohydrate status, NH_4, by contrast, may exert an effect on carbohydrate metabolism. Unlike NO_3, it is toxic if accumulated, so must be organically incorporated to form useful, or at least harmless, materials (McKee, 1962). It has been shown repeatedly that in the presence of adequate respirable carbohydrate, NH_4 assimilation proceeds so rapidly that no more than a trace of NH_4 is detectable in plant tissues, even at high levels of NH_4 availability (Street and Sheat, 1958). High levels of NH_4 at low light intensity have been shown to exhaust carbohydrate reserves with greater intensity than equivalent levels of NO_3. Several workers noted that "ammonium toxicity" symptoms have resulted from a continuous supply of NH_4 under winter lighting conditions, but not during summer, and that induced toxicity could be overcome by increased lighting (loc.cit).

The carbohydrate status, hence lighting conditions, must be considered in assessing the relative merits of either N form, therefore.

(iv) Aeration of the media: Street and Sheat (1958) concluded that to maintain optimal N metabolism there is a requirement for higher O_2 tensions when NO_3 is replaced by NH_4, with such species as tomatoes, oats and soyabeans. Plants such as rice, adapted to grow in soils of low O_2 tension and poor nitrification, are frequently active in NH_4 assimilation under these conditions, however (loc. cit.).

The physiological explanation for this observation is not clear, as it is also well documented that provision of NO_3 results in a higher rate of respiration as opposed to culture of the same tissues without N, or with NH_4 (McKee, 1962). A possible explanation, not suggested by these authors, may be that under the experimental conditions prevailing when these observations were made, NH_4 was of necessity assimilated in the roots, while much of the NO_3 was translocated prior to metabolism in aerial tissues. The response of NH_4- fed plants to increasing O_2 tension might then be explicable in terms of increased metabolic activity in the roots and an enhanced supply of energy substrate for amination and amidation. Metabolism of NO_3 in aerial tissues, to the contrary, may be unaffected by increasing O_2 tension above a threshold level required for absorption.

Whatever the explanation, the observation does point to the importance of aeration in any study of the relative efficiency of the two N forms.

(v) pH of the media: Any study of NH_4 and NO_3 nutrition is made more difficult by the problem of pH stabilisation. Owing to the magnitude of N uptake relative to other ions, in NO_3 solutions anion uptake greatly exceeds cation uptake and there is a drift towards alkalinity in the external medium; with NH_4, cation uptake is predominant and there is an acidification of the medium. These external alkaline and acidic effects have been reviewed (Walker, 1960; De Wit et al., 1963; Van Tuil, 1965) and are considered in more detail in a subsequent section. Unless they are controlled, the effects of N form are confounded with external pH. The most satis-

factory method for pH stabilisation is a flowing culture technique. Addition of soluble buffers may interfere with metabolism unless they are sparingly soluble salts, such as calcium carbonate and/or phosphates (Street and Sheat, 1958). A further problem involved in the study of NH_4 at alkaline pH is the possible volatile loss of NH_3 gas, especially from well aerated culture solutions (pers. comm., C. V. Fife).

Hewitt (1952) concluded that the optimum pH range for NH_4 absorption was 6.0 - 6.5 and for NO_3, 4.5 - 5.0; and that the rates of uptake of NH_4 and NO_3 were about equal at a pH near 6. This view is not accepted by Street and Sheat (1958). They concluded that over the normally encountered range of pH, assimilation of N rather than its entry and accumulation within cells, was the factor determining the effect of pH on utilization, and considered the question of pH optima for the two N forms as still open to question. Quoting from a review of this problem by Nightingale (1948) they state: "in a well buffered nutrient medium, provided there results no deficiency of essential elements, there is required no different pH value for NH_4 than for NO_3 nutrition." They considered that results observed for "pH optima" arose from interactions between N form, pH and the uptake of elements other than N, as discussed in the following section. Lycklama (1963) has investigated NO_3 and NH_4 absorption by perennial ryegrass seedlings. He found that NO_3 uptake at 25°C increased with increasing pH from 4.5 to 6.2 and decreased again with increasing alkalinity. Absorption of NH_4 was unaffected by increasing pH in the range 4.0 - 6.5, at low or high temperatures, but absorption increased markedly between pH 6.5 and

8.5. In the lower pH range, NH_4 uptake at 20°C was 3 times that from solutions at 35°C. With increasing pH this ratio fell and reached 1.3 at pH 8.5. Lycklama explained these observations for NH_4 in terms of absorption of the NH_4 ion below pH 6.5 with an increasing uptake of uncharged NH_4OH molecules as pH values increased above 6.5. This is a similar explanation to that given by other authors (loc. cit.). Uptake of NH_4 was measured by analysis of the nutrient solutions before and after absorption. No check for NH_3 volatalization was reported and may be expected to have occurred from the "vigorously aerated" solutions especially at the higher pH levels. The interrelationship observed between NH_4 "uptake", increasing pH above 6.5 and temperature may be explained in terms of the effect of a 15°C temperature difference on a chemical reaction. McKee (1962) has discussed the effects of pH on the ionic or molecular forms of NH_4-N in solutions. While NH_4 uptake has not differed substantially in the pH range 4.0 - 7.0 in Lycklama's work, nor in that of several other experimenters (McKee, 1962), the NH_4OH concentration would have increased several hundreds of times (loc. cit.). This has led McKee (1962) and Street and Sheat (1958) to the conclusion that absorption of NH_4OH molecules at higher pH values is not a valid reason for the preferential uptake of NH_4 under these conditions, as suggested by some authors.

The indirect effects of pH cannot be overlooked. Karim and Vlamis (1962) found that iron had to be replenished frequently in NO_3 cultures, owing to the external alkalinity during NO_3 uptake. It is quite conceivable that many of the studies designed to test the relative effectiveness of the two N forms at different pH values

have been confounded with indirect, external pH effects.

(vi) Ionic composition of the medium: Ammonium nutrition has been shown to lower the base content of a variety of plant tissues, especially the content of Ca and Mg (II, B, 3, (b)). Several experiments have shown that increasing the Ca content of NH_4 solutions improved growth and increased the pH range over which NH_4 supported good growth (Street and Sheat, 1958; McKee, 1962). Chouteau (1960) concluded that depression of tobacco yields with NH_4 nutrition was due to impaired cation uptake, especially that of Ca, rather than to acidification of the medium.

There are interactions between the form of N applied and the uptake of other essential elements, the causes for which are discussed later. Because of this, induced deficiencies of essential elements may determine relative efficiency, rather than the N sources *per se*.

(vii) Conclusions: The following general conclusions are made:

(a) both forms of N are readily absorbed;

(b) as a consequence of the differential absorption of anions and cations with NO_3 and NH_4 respectively, the uptake of other elements may be affected, either internally by metabolism or externally by differences in pH at the root surface;

(c) that the relative efficiency of the two N forms may differ according to internal factors including species, stage of growth and carbohydrate status;

(d) that the relative efficiency of either ion may differ according to external factors including pH, aeration, the ionic composition of the medium and the levels of N being studied, and

any environmental factor affecting the carbohydrate status of the plants; and

(e) that the results obtained from any experiment, as a consequence of the above, may be greatly affected by the experimental conditions.

The degree to which environmental conditions have been controlled or even reported, varies widely with experiments, so that results from different studies are not necessarily strictly comparable even where the same species have been used.

(b) The Effects of NO_3 and NH_4 on Plant Composition

(i) Non-protein nitrogen: Plants receiving NH_4, frequently have a higher amide content, a larger quantity of free amino-acids and amides and a higher proportion of total N in the non-elaborated organic N fraction; NO_3 fed plants, in contrast, often have a high inorganic N content, lower levels of free amino-acids and amides and a comparatively low non-elaborated organic N fraction (Street and Sheat, 1958). Accumulation of the dicarboxylic amino-acids and amides was related to conditions favouring amino-acid synthesis, especially N availability (Walker, 1962). McKee (1962) states that amides are formed in response to NH_4 nutrition to a greater extent than with equivalent NO_3. Margolis (1960) found that the amide content of tomato roots and shoots was higher in NH_4 fed plants. Grasmanis and Leeper (1965) reported that wheat plants receiving NO_3 had much lower glutamine levels but a similar content of arginine, aspartic acid and glutamic acid, in comparison with those receiving NH_4. By contrast, apple trees receiving NH_4 had a

much higher asparagine content (loc.cit.).

Attention has already been drawn to the observation that inorganic N accumulation frequently occurs with NO_3 but rarely with NH_4. Besides atypical "acid plants" (II, B, 2,(c)) high levels of NH_4 have been recorded in plant tissues (Delmas and Routchenko, 1962; Grasmanis and Leeper, 1965). As these plants were suffering from "ammonium toxicity" at the time of analysis, their physiology was not normal.

Lycklama (1963) studied the interactions among mineral N forms during absorption by intact perennial ryegrass seedlings. Experiments were conducted at 25°C in solutions maintained at pH 6.3, and absorption was studied over a sixty-minute period. Uptake of NH_4 was slightly reduced with increasing NO_3 concentration in the external medium. Nitrate uptake from a solution of equivalent concentration was progressively and markedly inhibited by increasing concentration of NH_4 in the solution. As accumulation of NO_3 by plant tissues was not affected by NH_4, its effect in depressing NO_3 uptake was ascribed to an inhibition of NO_3 reduction. In a parallell experiment, the absorption and reduction of nitrite proved to be completely independent of NH_4 concentration. This localised the NH_4 effect on NO_3 assimilation to an inhibition of the nitrate reductase reaction. The alternative explanation of energy substrate depletion with increasing NH_4 availability and assimilation was not likely, as some effect on nitrite reduction and metabolism would be expected. Filner (1966) has studied the regulation of the enzyme, nitrate reductase, with cultursd tobacco cells. Besides its induction by NO_3 (II, B, 2, (c)) it was repressed by several amino-

acids including asparagine, aspartic acid and glutamic acid. This could provide the biochemical basis for the observations of Lycklama. As discussed previously amides and dicarboxylic amino-acids accumulate during NH_4 assimilation which could lead to an inhibition of nitrate reductase. It could also explain the greater efficiency of NH_4 in the nutrition of seedlings (II, B, 3, (a), (ii)). The amides, particularly asparagine, can reach high levels in seedlings during the mobilisation of stored protein, which could lead to a repression of nitrate reductase activity. Both these suggestions require further investigation.

(ii) Carbohydrates: Plants fed NO_3 generally have a higher carbohydrate content than those provided with equivalent amounts of NH_4 (Street and Sheat, 1958). Rapid assimilation of NH_4 was observed to deplete carbohydrate reserves, in particular those of sugars and starch, during short-term observations, to a greater extent than was the case with NO_3. This situation is intensified by darkness or low light level (McKee, 1962). Several investigations covering a variety of crop plants have shown that plants continually supplied with NH_4 have lowered lignin and cellulose contents, reduced carbohydrate reserves and are dark green, soft and succulent, except in midsummer under ideal lighting conditions (Street and Sheat, 1958). Tokimasa and Suetomi (1958) found that NH_4 uptake by wheat and barley plants was retarded by shading or reduction of daylength, indicating this relationship between carbohydrates and NH_4 in cereals. Delmas and Routchenko (1962) found that maize receiving NH_4 accumulated large amounts of free NH_4 in the sap at an early stage of development and exhibited "ammonium toxicity" symptoms. Plants died unless conditions

conducive to rapid photosynthesis were provided, in which case the plants recovered and assimilated the free NH_4 in the sap. No metabolic disturbance was observed when maize plants under similar conditions received equivalent levels of NO_3, indicating that the diversion of carbohydrates for NH_4 assimilation eventually led to a physiological derangement of maize under low light intensities.

(iii) Organic acids: Levels have been repeatedly shown to be affected by the form of N nutrition. Plants receiving NO_3, relative to those receiving NH_4, have higher foliar contents of organic acids. This has been reported after numerous observations on many species, over a range of experimental conditions: cotton (Ergle and Eaton, 1949); various species (Kursanov, 1956); tobacco (Chouteau, 1960); maize and tomato (Ćoić et al., 1961, 1962a, 1962b); maize (Delmas and Routchenko, 1962); barley, ryegrass and cocksfoot (De Wit et al., 1963); sugar beet, poplar and birch (Van Tuil, 1965); tomato (Kirkby, 1966), and others. This general observation is supported in recent reviews: Thimann and Bonner (1950); Street and Sheat (1958); Burris (1959), and McKee (1962). Street and Sheat (1958) reported that malic, citric and oxalic acid contents in particular were higher in plants receiving NO_3, for a variety of species. Crombie (1960), while agreeing with the conclusion that total organic acids are higher in plants receiving NO_3, has provided experimental evidence for a variable relationship between N form and oxalic acid content. There was a slow rise or a slow decline in oxalic acid levels with NH_4 nutrition, depending on the species studied. She concluded that malic and citric acids in particular increased with NO_3 nutrition. The physiological basis for these observations is

discussed in a later section.

(iv) Inorganic salt content: The uptake and subsequent foliar content of inorganic salts have been reported to be affected by N form in a variety of studies covering a range of species: Cotton (Ergle and Eaton, 1949); wheat and perennial ryegrass (Scharrer and Jung, 1955); lemon and bean (Wallace and Ashcroft, 1956); lemon (Wander and Sites, 1956); soyabean (Bhan et al., 1960); tobacco (Chouteau, 1960); maize and tomato (Colïc et al., 1961, 1962a, 1962b); maize (Delmas and Routchenko, 1962); perennial ryegrass (Dijkshoorn, 1964); Italian ryegrass (Nielsen and Cunningham, 1964; Cunningham and Karim, 1965; Cunningham and Nielsen, 1965); wheat and apple trees (Grasmanis and Leeper, 1965); sugar beet, poplar and birch (Van Tuil, 1965); tomato (Kirkby, 1966), and others. Plants fed NH_4, relative to those receiving NO_3, have a reduced uptake of bases and an increased uptake of inorganic anions. Antagonism between NH_4 and metallic cations has frequently been reported to affect the divalent ionic species, especially Ca, more than K and Na. The synergism between NH_4 and inorganic anions is frequently reported as affecting S, then P, more than Cl. This may be due in part to the experimental technique (II, C) as the S supply of NH_4 cultures normally exceeds that in NO_3, while Cl is generally omitted. These interactions between N form and inorganic salt uptake are considered in more detail in a later section.

(v) Miscellaneous: Kursanov (1956) reported that NH_4 favoured the formation of reduced compounds in plants, such as rubber and ethereal oils, while NO_3 intensified the synthesis of organic acids. This report was based on the work of Vladimirov, part of which has appeared in an English text (Vladimirov, 1945a, 1945b).

4 IONIC BALANCE IN PLANT TISSUES

(a) Introduction

While electropotential differences exist across membranes in plant organelles (Lundegårdh, 1960; Briggs et al., 1961) it is axiomatic that the plant must maintain an overall electrostatic balance during the uptake and metabolism of minerals derived from the soil in ionic form. Cations and anions are rarely absorbed by plant tissues from soils or culture solutions in equivalent amounts (De Wit et al., 1963). This section is a discussion of the physiological mechanisms involved in the maintenance of electroneutrality with particular reference to the situation during NO_3 and NH_4 assimilation.

A brief clarification of terms is necessary. One may consider first the "ionic balance" in any tissue at any time. It represents the sum of positively charged ionic species equated by an equivalence of negatively charged ionic species in the tissues, as dictated by the requirements of electrical neutrality. The term "ionic balance" will be used subsequently in a general way to denote this balance. Consider next the "gross cation-anion balance" of tissues. From tissue analysis one may estimate the total uptake of cations and anions from the medium under prescribed conditions. This is not the "ionic balance" however, as subsequent metabolism of absorbed ions alters their charge, as discussed later. In the following discussion the interplay between ion uptake, accumulation and metabolism in plants is dealt with, because it is their interrelationships which determine the "ionic balance" at any time.

In this account all element concentrations are expressed in milli-equivalents/100 g D.M. (me. %) as concentrations on a weight-

percentage basis are not directly applicable.

(b) Determining Ionic Balance

(i) General: In the formulation of cation-anion balances in plant tissues it has been found satisfactory to confine attention to the macro-elements occurring at levels from 5-300 me. % (Dijkshoorn, 1957a) Cunningham (1964b) found that total Fe +Al + Mn + Cu comprised only 1-2% of total cation equivalents in the herbage of Italian ryegrass, and concluded that their exclusion from ionic balances caused no serious error. A similar conclusion was reached by Van Tuil (1965) for perennial ryegrass. The soil-derived metallic cations may therefore be calculated as the sum of K + Na + Mg + Ca in me. %. An insignificant proportion of these metallic cations is involved in metabolism in such a way that they cease to exert their cationic properties in the tissue as a whole (Dijkshoorn, 1963; Kirkby, 1966). A small amount of Mg is involved in complex formation and some Ca may be absorbed by structural configuration, for instance.

The major non-metallic elements derived from soils in ionic form are N, P, S and Cl. Silicon was included in the calculations of some earlier workers (Bear, 1950). Dijkshoorn (1957a) concluded that a satisfactory balance was obtained when Si was omitted from calculations, even though appreciable levels may be found in grass herbage. Cunningham (1964a) found a better relationship between uptake of cations and anions when Si was omitted from calculations for ryegrass. He concluded that "soluble silica" is present in soils as hydrated silicic acid ($Si(OH)_4$) at the prevailing pH, and that Si does not enter the plant as anionic silicate. Silicate ions would not appear below pH 9.0 (Dijkshoorn, 1963); Si can therefore be

ignored in cation-anion balances.

(ii) The gross cation-anion balance: In calculating the total uptake of cations, the metallic cations offer no difficulty. They are absorbed as monovalent (Na + K) or divalent (Ca + Mg) cations. Nitrogen presents the major difficulty. It may be absorbed by the plant as an anion and/or a cation, and the relative contribution of each to the nutrition of plants growing in soils is generally unknown. Under experimental conditions, however, this can be determined. In the compilation of gross cation-anion balances for uptake due respect must be paid to whether N entered the plant as NH_4 or NO_3; NH_4 uptake being included in total cations and NO_3 uptake in total anions (Scharrer and Jung, 1955; Dijkshoorn, 1964; Cunningham and Nielsen, 1965). Phosphorus is absorbed largely,if not entirely, in the monovalent orthophosphoric state (Sutcliffe, 1962). The monovalent form predominates in soils of acid or slightly acid reaction and at the prevailing tissue pH which is normally slightly acidic, and is therefore considered to enter the plant as the orthophosphate ion (Dijkshoorn, 1957 et seqq; Cunningham, 1964 et seqq.; Van Tuil, 1965). Sulphur enters the plant as divalent sulphate and chlorine as monovalent chloride, and present no difficulty.

Gross cation and anion uptake by plants is therefore calculated as:

total cation uptake = the me. sum of K + Na + Mg + Ca (+ total N in the case of NH_4 nutrition);

total anion uptake = the me. sum of total S + total P (calculated as monovalent) + Cl (+ total N in the case of NO_3 nutrition).

The calculation is generally made by tissue analysis for the content of these elements and the expression of these results in me. %.

(iii) Ionic Balance: The metallic cations are not metabolised after accumulation (De Wit et al., 1963). They are predominantly in their ionic form inside the plant, although some of the divalent cations, particularly Ca, may be precipitated as oxalate crystals or be rendered indiffusible as counterions to uronic acids (Kirkby, 1966). As all these fractions are measurable in the compilation of the ionic balance of tissues, total metallic cations are calculated as exhibiting their normal ionic valence. The other major cation, NH_4, does not accumulate in tissues as a free ion during normal metabolism (II, B, 2 (c)). The total cation content of plant material is therefore equal to the sum of the metallic cations.

The bulk of NO_3 assimilated by grasses is metabolised into organic form and ceases to exist as a free ion (Dijkshoorn, 1963). What is present in tissues as inorganic NO_3^- contributes to the inorganic anion content of tissues. Sulphur is similarly metabolised to a varying degree and only inorganic $SO_4^=$ contributes to the inorganic anion content of tissues (loc. cit.). Chloride remains almost entirely as a water-soluble anion in tissues (Johnson and Ulrich, 1959) so that total Cl^- contributes to the anion content of tissues. Phosphorus is involved in metabolism to a varying extent but with adequate P nutrition some 80% of total P was present in ryegrass herbage as free orthophosphate (Dijkshoorn and Lampe, 1961). The organic linkage involved in esterification does not affect its ionic state at the prevailing tissue pH (Dijkshoorn, 1963) and terminal phosphate esters still exert a single negative charge. Total

P in tissues is therefore calculated as orthophosphate in the ionic balance. De Wit et al. (1963) have shown conclusively that calculation of P as trivalent leads to nonsensical ionic balances. The proportion of total P involved in di- and triphosphate esters is too small to affect this calculation significantly (Dijkshoorn and Lampe, 1961).

The contribution of ions derived from the medium to the ionic balance of tissues may therefore be calculated as:

total cation content = the me. content of metallic cations

total anion content = the me. content of $SO_4^=$ + $H_2PO_4^-$ + Cl^- + NO_3^-

These are not generally equal in plant tissues and the mechanisms by which plants maintain electroneutrality is the subject of the following sections.

(c) Tissue pH

(i) The buffering capacity of plant tissues: Over the normally encountered pH range of 5-6 proteins play little or no part in the buffering capacity of plant tissues (Dijkshoorn, 1963; Kirkby, 1966). The bulk of the buffering capacity is due to phosphate (Dijkshoorn, 1963). Dijkshoorn and Lampe (1961) concluded that the buffering capacity of P in ryegrass herbage was such that only 2-8 me. of H^+ or OH^-/100 g D.M. was required to shift tissue pH one unit, depending on whether measurements were made between pH 5 and 6 or 6 and 7. They concluded that the chemical buffering capacity of plant tissues is comparatively low over the normally encountered pH range in tissues.

(ii) The pH of plant tissues: Wadleigh and Shive (1939) found that the pH of expressed corn leaf sap was 5.6 $\pm$ 0.2 for plants grown in media ranging in pH from 3.0 - 8.0 and provided with either NO_3 or NH_4.

Ulrich (1941) found that the pH of expressed sap from excised barley roots was 5.5 $\pm$ 0.3 after an 8 hour period of salt absorption from a wide variety of salt solutions. Hurd (1958) recorded the pH of macerated beet discs as 5.9 $\pm$ 0.3 after 6 days of immersion in water and salt solutions, both buffered at either pH 6.1 or 8.5. The tissue pH of pasture grasses is reported to be around 6.0 (De Wit et al., 1963) and to be between 5.0 and 6.0 irrespective of the form of N metabolised (Van Tuil, 1965). It may be concluded therefore that tissue pH does not vary outside fairly narrow margins. For small grain plants, including pasture grasses, tissue pH is slightly acid, between 5.0 and 6.0 (Dijkshoorn, 1962; De Wit et al., 1963; Van Tuil, 1965).

(iii) Tissue pH in relation to salt uptake: In the final analysis, irrespective of the mechanism of salt uptake, excess cation accumulation from the medium must be equated either by an equivalent extrusion of H^+ to the external solution or an equivalent uptake of OH^-/HCO_3^- into the plant, to maintain electroneutrality in both plant and medium (Dijkshoorn, 1963; Van Tuil, 1965). Conversely, excess anion accumulation must be equated by an equivalent loss of OH^-/HCO_3^- from the plant to the medium or an equivalent uptake of H^+. Dutch workers have termed this the "apparent alkalinity of excess cation uptake" and the "apparent acidity of excess anion uptake" and assume a theoretical model for cation-anion balance considerations, with an equivalent of OH^-/HCO_3^- accompanying an excess of cations into the tissues and an equivalent of H^+ accompanying excess anions (Van Tuil, 1965). The theoretical alkali or acid uptake involved in excess cation or anion absorption by plant tissues is frequently very large in relation to the buffering capacity of plant tissues. The data of

Ulrich (1941) serve to illustrate this. Excess cation uptake by barley roots immersed for 8 hours in a variety of salt solutions ranged from -20 to +50 me./l. of expressed sap, depending on the salt supplied. Although the buffering capacity of expressed sap was only 25 me./l. per pH unit in the pH range 5.0-6.0 (after Van Tuil, 1965), sap pH remained within the limits 5.5 $\pm$ 0.3, with no obvious relationship between sap pH and the numerical value for equivalents of cations minus equivalents of anions. This has led to the recognition of an effective "metabolic buffer" against pH movement within plant tissues involving the synthesis or degradation of organic anions according to whether salt uptake is "internally alkaline" or "internally acidic". Tissue pH is confined to a narrow range of variation, and excess cation uptake is not balanced by an equivalent increase of OH^-/HCO_3^- within tissues nor is excess anion accumulation balanced by an equivalent increase of H^+ in tissues. In this respect, the Dutch terminology "apparent alkalimity of excess cation accumulation" and "apparent acidity of excess anion accumulation" is rather misleading. The role of organic anions in the maintenance of ionic balance is discussed in the following sections.

(d) Organic Acids in Plant Tissues

For the detailed biochemistry of organic acid metabolism in plants, the reader is referred to Walker (1962) and Davies et al., (1964); and for the interrelationships between "dark CO_2 fixation" into organic acids and other plant metabolic processes, the above papers and those of Mazelis and Vennesland (1957); Davies (1959) and Tecl (1962) may be consulted. The interrelationships between

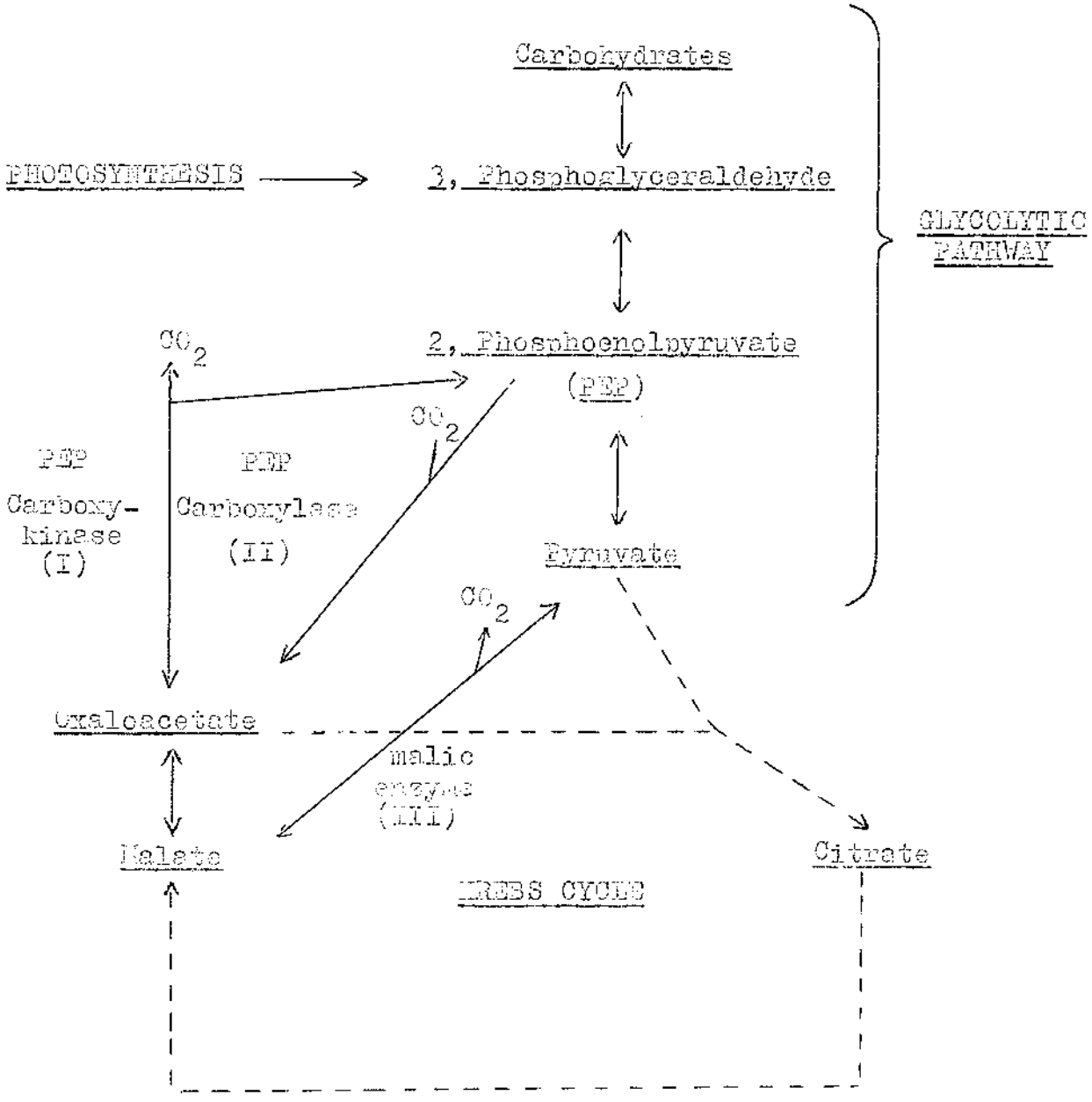
organic acid metabolism and other plant metabolic processes is covered in this review only to the extent that some knowledge of these reactions is required in subsequent discussion.

(i) Metabolism of organic acids: There are three β-carboxylase systems known in plants which will accomplish the "dark fixation of CO_2" into 3 C intermediates of the glycolytic pathway, to yield dicarboxylic acids (figure 2). The enzyme, phospoenolpyruvate carboxykinase (I) will catalyse the freely reversible carboxylation of PEP to yield the dicarboxylic organic acid, oxaloacetic acid. Another widespread plant enzyme, PEP carboxylase (II), catalyses the same reaction. Because of the large decreases in free energy and the high affinity of this enzyme for its substrate, it constitutes the most effective carboxylation system at present known (Walker, 1962). The reaction is considered to be irreversible, with a pH optimum of roughly 7.5 - 9.5 at equilibrium HCO_3^- concentration. Malic enzyme (III) catalyses the oxidative decarboxylation of malic acid to yield pyruvate. The same enzyme will catalyse the decarboxylation of oxaloacetate to pyruvate at an acid pH of about 5.0, which reaction is probably not reversible. Goodwin (1960) and Walker (1962) consider that the decarboxylation of malic acid would be favoured in tissues, and that the equilibrium would lie towards carboxylation only at CO_2 concentrations not likely to be encountered *in vivo*. Davies *et al.*, (1964) consider this reaction freely reversible and dependent on the availability of reduced coenzyme. The pH optimum for the carboxylation is reported as about 7.0. All these enzyme systems are at least partly non-particulate.

Enzyme systems I and II both yield oxaloacetic acid by carboxylation. This does not accumulate appreciably as the equilibrium

FIGURE 2 THE RELATIONSHIPS BETWEEN ORGANIC ACIDS AND 3C INTERMEDIATES IN CARBOHYDRATE METABOLISM

(Modified from Teel, 1962)



between oxaloacetic acid and malic acid favours the accumulation of the latter. Carboxylation reactions can, however, lead to the accumulation of any of the Krebs Cycle acids (or their associates) as they are all interconvertible.

In the present context, it is sufficient to consider the overall system as completely reversible, according to the following generalised equation:

$$RH + CO_2 = RCOOH$$

It may be seen that decarboxylation of dicarboxylic organic acids which are present in tissues as organic anions must yield an equivalent amount of internal alkalinity, as dictated by electrostatic considerations (Dijkshoorn, 1963):

$$RCOO^- + H_2O = RH + HCO_3^-$$

Conversely, the formation of organic acids by carboxylation yields an equivalent of internal acidity on dissociation of the acid formed:

$$RH + CO_2 = RCOO^- + H^+$$

These enzymes, working in concert, provide a ready pathway for variations in the organic acid content of tissues. They also provide for the replenishment of Krebs Cycle intermediates. It may be seen from figure 2, therefore, how N assimilation in general and NH_4 in particular, can lead to the depletion of carbohydrates (II, B, 3, (b), (ii)).

(ii) <u>Specific differences in organic acid content</u> have been reviewed by Thimann and Bonner (1950). <u>Total content</u> of organic acids in tissues were reported to be as high as 450 me. % in beet leaves. Van Tuil (1965) reported a normal organic acid content of 350 me. % for young beet leaves. Values between 300 and 350 me. % were found

by Pierce and Appleman (1943). The foliar content of organic acids in legumes was reported to range between 150 and 250 me. % (Pierce and Appleman, 1943; Thimann and Bonner, 1950). Ryegrass herbage has been reported to contain approximately 100 me. % of total organic acids, irrespective of age (De Wit, et al., 1963). They concluded that the foliage of small grain species in general, including grasses, contain 90-110 me. % of organic acids. Van Tuil (1965) measured the organic acid content of ryegrass herbage receiving different levels of NO_3 nutrition. Total organic acids ranged between 60 and 90 me. %, with lower levels associated with high Cl and low N levels in herbage. Individual organic acids are subject to large variations according to species (Pierce and Appleman, 1943; Thimann and Bonner, 1950) and vary with the stage of development (Thimann and Bonner, 1950), nutrition (Van Tuil, 1965) and form of N assimilated by plants (Kirkby, 1966). It is therefore not possible to give a spectrum of organic acids for the herbage of any particular species. Malic, citric and oxalic acids are generally considered to be quantitatively the most significant. Pierce and Appleman (1943) found that these three acids constituted about 30% of the total in the leaves of bluegrass and wheat, and 40-50% of those in the stems and leaves of legumes. Davies and Hughes (1954) found that malic and citric acids together constituted about 50% of total organic acids in grasses. Van Tuil's (1965) figures show that malic, citric and oxalic acids together constitute some 35-60% of total organic acids in ryegrass herbage, depending on nutrient supply.

(e) Experimental Observations of Ionic Balance

(i) Uptake by isolated tissues from single-salt solutions: Ulrich

(1941) was among the first to make a critical evaluation of the physiological processes involved during unequal cation and anion uptake by plants. Studying salt absorption by barley roots he concluded that excess cation accumulation was balanced by an equivalent increase in the organic anion content of the tissues, while excess anion accumulation from the medium was equated by a decline in organic anion content. As no changes in the sap content of free NH_4 or amide N were related to disunity in gross cation-anion uptake, he concluded that the organic anions did not arise from amino-acids. Rather, the observed increase or decrease in R.Q. with excess anion or cation absorption respectively, led Ulrich to the conclusion that organic anions were derived from the incomplete oxidation of some respiratory substrate. Burström (1945) substantiated Ulrich's work when studying salt absorption by wheat roots. He found that an increase or decrease in malate levels approximately equalled the excess or deficit of cations absorbed. Poel (1953) and Jacobson (1955) have studied the biological pathways invdved in organic anion synthesis and degradation in barley roots during differential ion uptake, using radio-labelled carbon. It was concluded that CO_2 was fixed into 3C sugar derivatives by roots in the dark. The magnitude of CO_2 fixation was largely determined by concurrent salt accumulation, being greatest during excess cation uptake, and depressed in relation to controls when excess anion accumulation occurred. Malate was the major organic anion affected.

Salt uptake from a variety of salt solutions was studied by Jacobson and Ordin (1954) using excised roots from several species. They concluded that during ion absorption, ionic balance in tissues

was maintained by the synthesis or disappearance of an equivalent amount of organic anions, mainly malate, according to whether cation/anion uptake exceeded or was less than unity, respectively. This was the major compensating mechanism in young roots. In older roots they concluded that exchange of pre-existing cations attained a greater importance in the overall balance of uptake. However they did not take into account exchange and adsorption reactions in "Donnan free space" (Briggs, et al., 1961) as opposed to true metabolic accumulation. Further supporting evidence of a similar nature has been given by other authors (Graf and Aranoff, 1955; Hurd, 1958; Jackson and Coleman, 1959; Bhau et al., 1960, and others).

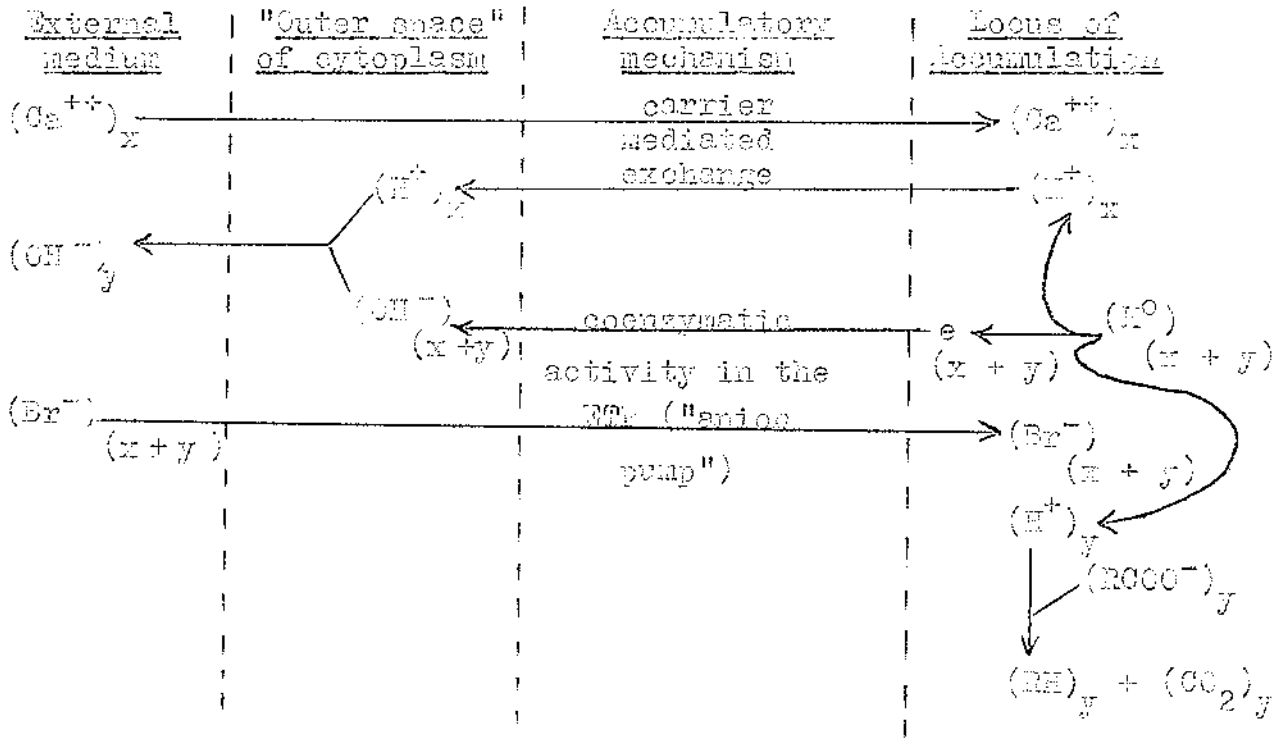
It is now proposed to drew up hypothetical balance sheets for unequal cation and anion accumulation by isolated tissues from neutral salt solutions. For detailed reviews of current concepts of ion accumulation the reader is referred to the following: Epstein (1956, 1962); Laties (1959); Lundegårdh (1960); Robertson (1960); Briggs et al. (1961); Butcliffe (1962); Jennings (1963); Brouwer (1965) and Lieberman and Baker (1965).

Consider, first, excess anion accumulation from a salt solution containing a readily absorbed anion and a relatively immobile cation ($CaBr_2$). The equivalent uptake of Br^- is (x + y), exceeding that of Ca^{++} (x) by (y) equivalents. To maintain electroneutrality in both the tissue and the medium three possibilities exist:

a. organic cations may accumulate in the tissues and organic anions be released to the medium in an amount (y) equivalent to excess anion accumulation. There is no evidence or known mechanism for this;

b. an equivalent amount (y) of H^+ may accompany the excess of Br^- into the tissues, leaving (y) equivalents of OH^-/HCO_3^- in the medium to balance the unpaired Ca^{++}. This does not happen as tissue pH is more or less constant in spite of low buffering capacity (II, B, 4, (c));

c. an equivalent amount (y) of organic anions may be decarboxylated and the released OH^-/HCO_3^- secreted into the medium, to balance the unpaired Ca^{++}. The evidence available shows that this is what happens. Physiological alkalisation of the external medium has been observed repeatedly during excess anion absorption (II, B, 3, (a),(v) and has been discussed by Wolken (1960), De Wit et al. (1963) and Van Tuil (1965). The situation is summarised in the following diagram:



Hydrogen (H°) from respiratory substrates provides the energy for accumulation. Electrons pass down the cytochrome system (ETP) to yield OH^{-} at the terminal oxidase. Owing to the coenzymatic activity of inorganic anions, during the passage of electrons outwards there is an equivalent flow of Br^{-} inwards and (x + y) equivalents of Br^{-} are accumulated, the ETP acting as an "anion pump" (Lundegårdh, 1960; Robertson, 1960). Because of the high affinity between Br^{-} and the anion pump, organic anions are not accumulated (loc. cit.). The restrained proton available for exchange uptake of Ca^{++} is equivalent to the passage of electrons (x + y). Owing to the immobility of Ca^{++}, only x equivalents are accumulated by carrier-mediated exchange for x equivalents of H^{+}. Thus y equivalents of H^{+} are restrained from movement across the permeability barrier and temporarily equate the excess Br^{-} accumulated. Behind the permeability barrier, y equivalents of organic anions are decarboxylated and the released alkalinity combines with the restrained H^{+} to form H_2O, and the cations which were previously balancing the organic anions now balance the excess Br^{-}. That acid pH values favour decarboxylation of organic anions (II, 3, 4, (d), (i)) may be the "triggering" mechanism for the decline in organic anion levels. In the medium there has been an extrusion of OH^{-}/HCO_3^{-}, which is y equivalents in excess of the passage of H^{+}. This balances the excess of Ca^{++} remaining in solution and gives the observed pH increase. This scheme appears the most probable to the author and covers all the requirements for excess anion uptake by tissues from a neutral salt solution:

a. internal pH remains static;

b. external pH increases;

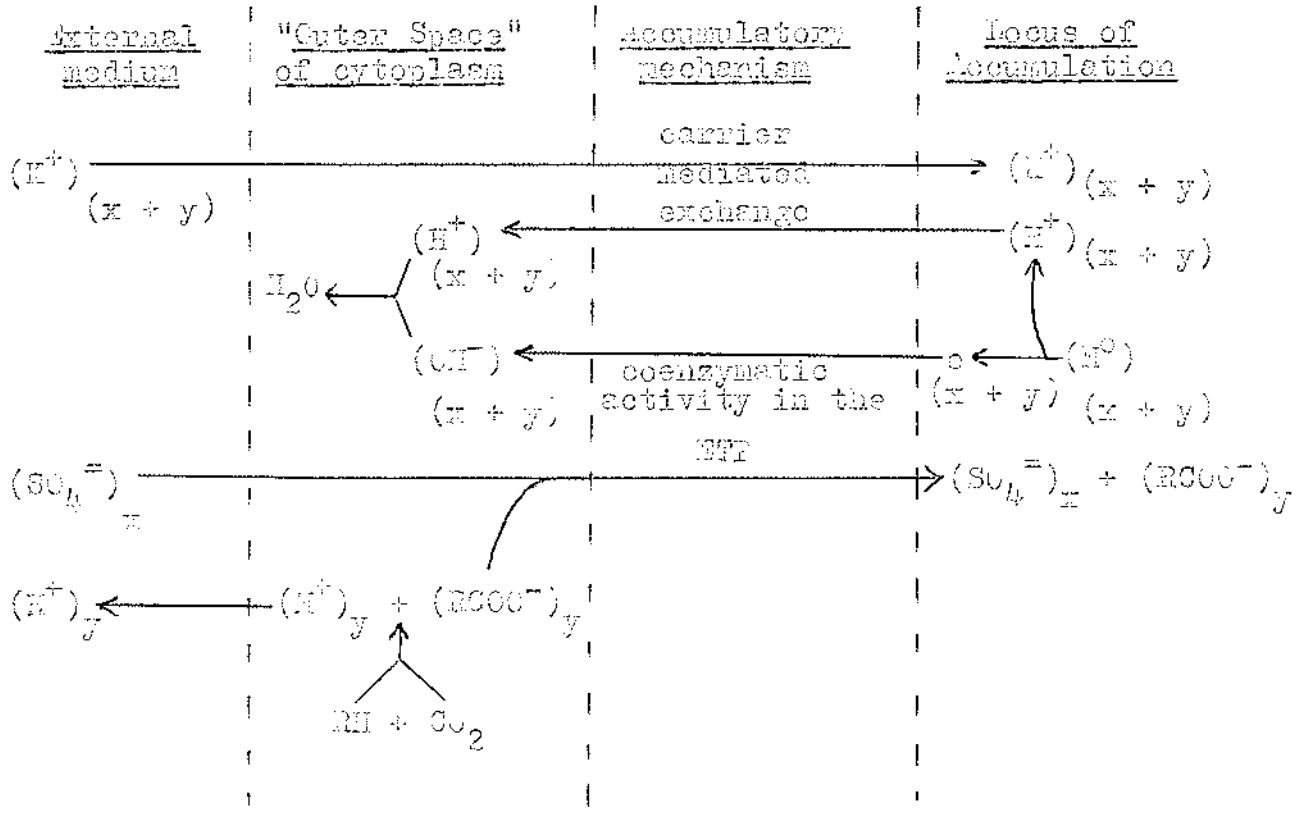
c. there is a decline in organic anions equivalent to the excess anion uptake;

d. the R.Q. value increases as a result of the decarboxylation of organic anions;

e. electroneutrality is maintained within the tissues and in the medium; and

f. the concept is in harmony with current evidence for the mechanisms of inorganic ion accumulation.

A similar illustrative example can be drawn up for excess cation accumulation from a neutral salt solution containing a relatively mobile cation and an immobile anion (K_2SO_4). Let the uptake of K^+ be $(x + y)$ equivalents and that of $SO_4^=$, x equivalents. Cation uptake exceeds anion uptake by y equivalents. The situation is summarised in the following diagram:



Again the separation of protons and electrons from respiratory H° provides the energy required for the accumulation. As (x + y) equivalents of electrons traverse the ETS outwards, an equivalent of anions is accumulated. Owing to the low affinity between $SO_4^=$ and the "anion pump", only x equivalents of $SO_4^=$ are accumulated together with y equivalents of organic anions, which share a greater proportion of total transport capacity in the absence of a highly mobile anion (Lundegårdh, 1960). Carriers mediate the exchange of (x + y) equivalents of H^+ for the same amount of M^+ from the solution, so that within the tissues, M^+ is balanced by $SO_4^=$ and organic anions. As there is an equal release of H^+ and OH^-/HCO_3^- during accumulation, this provides no overall effect on the external solution. However, H^+ is released to the external solution in an amount (y) equal to the uptake of organic anions, the M^+ arising from dissociation of the organic acids formed by carboxylation in the "outer space" of tissues (Lundegårdh, 1960). The H^+ released causes a physiological acidification of the medium. The widely held view that the synthesis of organic acids within the locus of accumulation supplies an equivalent of H^+ for exchange uptake of excess cations (Burström, 1951) is not shared by this author. Such a process shows no interrelationship with active transport systems as understood and entails the invocation of a separate (unknown) mechanism to "trigger" the synthesis of organic acids when tissues are placed in a salt solution whose anion has a low affinity for the anion transport system. This scheme satisfies all the requirements for excess cation absorption by isolated tissues from a neutral, single salt solution:

a. internal pH remains static;

b. external pH decreases;

c. there is an increase in organic anions in the tissues, equivalent to the excess cation accumulation;

d. the R.Q. value decreases as a result of carboxylation reactions to yield organic acids;

e. electroneutrality is maintained within the tissues and in the medium; and

f. the concept fits current evidence for salt transport and accumulation.

These schemes fit the Dutch concept that the "apparent internal acidity of excess anion uptake" is neutralized by an equivalent consumption of organic anions and the subsequent production of internal alkali; and that the "apparent internal alkalinity of excess cation uptake" is neutralized by the equivalent synthesis of organic acids and subsequent production of internal acidity (Dijkshoorn, 1963, and others).

(ii) Ionic balance in plants during nutrient uptake and metabolism

If salts were merely accumulated the considerations of the previous section would suggest that during NH_4 nutrition there would be large accumulations of organic anions, as cation uptake greatly exceeds that of anions. It is almost invariably found however, that substitution of NH_4 for NO_3 leads to a distinct decline in organic anion levels (II, B, 3, (b), (iii)). This observation can be explained in terms of the metabolism of N in particular, and S to a less extent.

In comparison with other soil-derived nutrients, plants

require large quantities of N (II, D, 2, (a)). During NO_3 nutrition the plant assimilates a large excess of anions, whilst with NH_4, a large excess of cations. The following data for wheat herbage from Scharrer and Jung (1955) illustrate this. (Gross cation-anion uptake has been calculated from tissue analysis data, with due respect to the form of N; the results from a "high nutrition" treatment have been rounded off).

GROSS CATION-ANION BALANCE

(all contents in me.%)

Treatment	Cations			Anions			Ratio
	ΣC	N	ΣC+N	ΣA	N	ΣA+N	Cations / Anions
NO_3-N	90	-	90	40	170	210	0.4
NH_4-N	75	250	325	130	-	130	2.4

In spite of the reversal of charge, N uptake per unit of tissue was of the same order of magnitude in both instances. In the NO_3-N series, total anion uptake was 210 me.%, the major contribution coming from N, and total cation uptake was 90 me.%. With the NH_4-N plants, total anion uptake has fallen to 130 me.%, but the uptake of anions other than N has increased three-fold. Total cation uptake has leaped to 325 me.%, in spite of some reduction in metallic cations, as total N entered the plant as a cation. The result has been a change in the gross cation-anion ratio from 0.4 with NO_3 to 2.4 with NH_4. Some consideration must now be given to how the same plant species can accumulate a large excess of anions

on the one hand and a large excess of cations on the other.

The explanation lies in the metabolism of N and S as first proposed by Dijkshoorn in (1958b) and expanded by the Dutch school since.

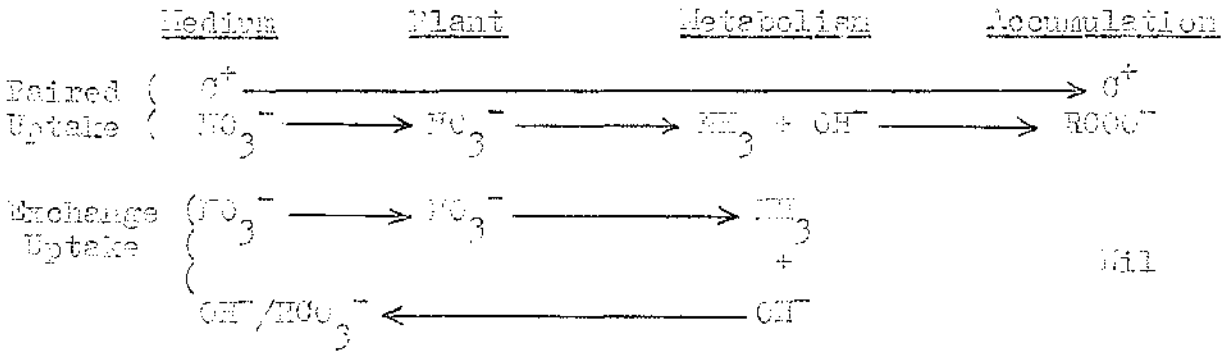
Nitrate and SO_4, once absorbed, undergo reductive assimilation to a varying degree according to conditions. By far the bulk of NO_3 is normally metabolised into non-ionic, organic forms. The overall equations may be written as: (Dijkshoorn, 1963).

$$NO_3^- + 8H = NH_3 + 2H_2O + OH^-$$

$$SO_4^{--} + 8H = SH_2 + 2H_2O + 2OH^-$$

Electrostatic considerations demand that the negative valences be released as anions, presumed by Dijkshoorn to be strongly basic OH^- groups, which will equilibrate with CO_2 in the formation of HCO_3^-. By analysis of the organic fractions of healthy perennial ryegrass leaves, Dijkshoorn et al. (1960) were able to calculate the ratio, me. NO_3 assimilated/me. SO_4 assimilated, which was an approximately constant value of 10.5. It may be seen, therefore, in the discussions following, that the contribution of NO_3 metabolism to the overall balance far outweighs that of SO_4. This reductive assimilation of NO_3 and SO_4 provides a continuous source of anions which may be converted to organic acids and/or returned to the external medium. As much of the NO_3 metabolism occurs in the roots (II, B, 2, (c)) the question of whether or not transfer of the OH^-/HCO_3^- produced to the external medium requires the intermediary participation of an organic anion, is largely academic. If a cation accompanies the anion during initial absorption (paired uptake), then on metabolism of the anion there will be the net re-

tention of an organic anion to balance the accompanying cation. If no cation is absorbed (exchange uptake) the net result is the exchange of an equivalent of OH^-/HCO_3^- from the plant for NO_3 or SO_4 from the medium. This is summarised in the following diagram:



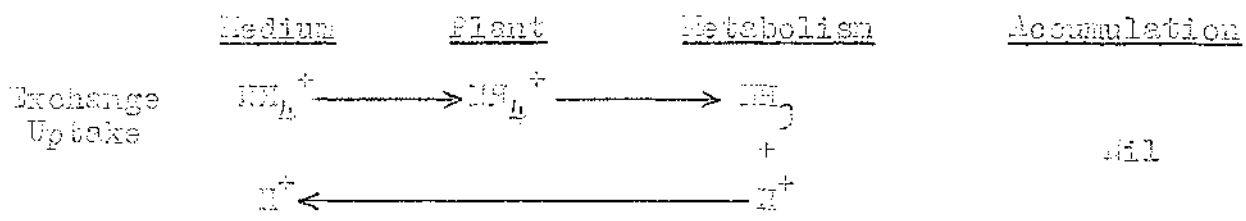
During paired uptake, the cation-anion ratio is 1. With exchange uptake, the entry of NO_3^- is effected without any net accumulation of ions. This means that with NO_3 nutrition, the upper limit to the cation-anion ratio is 1.0, without invoking any other mechanism, and there is virtually no lower limit as long as NO_3 is the principle anion being absorbed. The uptake and assimilation of NO_3 and SO_4 can continue to a large degree therefore, quite independently of the uptake of other ionic species from the medium. This explains how a large excess of anions can be accumulated by tissues receiving NO_3. Metallic cations accumulated by paired uptake with anions which are not subsequently metabolised (Cl, F, inorganic S) will remain in the tissues, balancing the charge of these inorganic anions.

A similar situation has been visualised by Dijkshoorn (1958b) for NH_4 nutrition. The assimilation of NH_4 involves an equivalent release of acidity according to the equation:

$$NH_4^+ = NH_3 + H^+$$

In a manner analogous to that during NO_3 assimilation, NH_4 assimilation provides a continuous source of H^+ which can be exchanged

for more NH_4^+ from the medium, as shown in the following diagram:



The metallic cations may be assimilated during paired uptake with inorganic anions (Cl, P, S). The only limitation placed on cation and anion absorption with NH_4^+ is that inorganic anion uptake cannot exceed metallic cation uptake without a considerable drop in tissue pH and/or the existence of free NH_4 salts in the tissues. In this respect, the use of the results of Scharrer and Jung (1955) has been entirely illustrative. The tissues from their NH_4 treatment contained inorganic anions at 130 me.% and inorganic cations at 75 me.%, giving an excess of inorganic anions of 55 me.%. If their analytical techniques were beyond question this indicates an abnormal metabolism, which may be the case, as a close perusal of their paper shows that Mg and all micro-elements were apparently omitted completely from their sand cultures.

It is concluded that much of the N, irrespective of form, can enter the plant and be metabolised in such a way that N uptake can continue largely independently of the uptake of metallic cations and inorganic anions. As long as NO_3 is the major anion absorbed, there is theoretically no lower limit to the gross cation-anion balance during absorption from a NO_3-N medium. Conversely, there is theoretically no upper limit to the gross cation-anion balance during absorption from an NH_4-N medium, as long as NH_4 is the major cation absorbed.

Pierce and Appleman (1943) grew 12 species of plants under uniform conditions and subsequently determined the foliar content of metallic cations, inorganic anions and ether-soluble, non-volatile organic acids. In all species, there was a considerable excess of metallic cations in the herbage. This excess (i.e. me. sum cations - me. sum inorganic anions) showed a virtual 1:1 relationship with the me. content of organic acids. From this they concluded that the excess of metallic cations in tissues is balanced by the anions of organic acids. Maksimovich and Bakhir (1951) observed that during the vegetative phase of growth of sugar beet, uptake of inorganic ions was in a ratio near unity. At this stage, the measured increase in total organic acids in tissues roughly mirrored the P content. They considered, therefore, that organic anions were replacing NO_3 and SO_4 as these inorganic anions were being metabolised. At a later stage of growth, uptake of NO_3 declined, and a considerable excess of cations was accumulated and equated by organic anion accumulation in the tissues. Their chemical analyses showed that the ratio;

$$\frac{\text{me. sum cations} - \text{me. sum inorganic anions}}{\text{me. organic anions}}$$

was approximately unity at all stages of maturity, for individual plant parts and for whole plants. Van Tuil (1965) found that the content of low molecular weight, non-volatile organic acids in the herbage of ryegrass receiving a variety of nutritional treatments, was equivalent to about 30% of the excess of metallic cations over inorganic anions in the tissues. Malate, citrate and oxalate comprised the major part of these organic anions. Kirkby (1966) assayed the organic acids in tomato receiving different forms of

mineral N. Irrespective of plant part (leaves, petioles, stems or roots), and irrespective of the form of N metabolised, total cations in tissues were virtually exactly equated by the content of inorganic anions + non-volatile organic acids + uronic acids. He concluded that the indiffusible anions of polyuronic acids also contributed to the ionic balance in tissues, a conclusion similarly reached by Van Tuil, (1965) for ryegrass, when he found that his calculations showed only about 90% of excess cations balanced by non-volatile organic acids. His analytical methods did not include uronic acids.

It is concluded that the ionic balance in any tissue at any time is given by the equation:

me. total cations (K + Na + Mg + Ca) = me. inorganic anions (Cl^- + $SO_4^{=}$ + $H_2PO_4^-$ + NO_3^-) + me. (total non-volatile organic acids + uronic acids).

The whole concept may be best summarised by the balance sheet for the uptake, utilization and accumulation of mineral elements in the herbage of perennial ryegrass, calculated by Higginbottom (1962). Foliage was analyzed after 28 days regrowth with adequate NO_3 nutrition and low Cl availability.

Balance-sheet of uptake and utilization

by *Lolium perenne* L.

(from Dijkshoorn, 1962)

Uptake		(me.%)
Cations (K + Na + Mg + Ca)		180
Anions (S + P + Cl + N)		320
"Apparent acidity of uptake" (H^+)		140
Utilization		
Cations		0
Anions	NO_3	240
	SO_4	10
	Cl	0
	P	0
Final State		
Metabolic alkali		250
Acidity of uptake		140
Excess internal alkalinity		110
Organic anions (malate, etc.)		110
Σ C - inorganic anions		110

The gross cation-anion balance for uptake gave an excess of 140 me.% of anions or an "apparent acidity of uptake" of 140 me.%. The reductive assimilation of NO_3 and SO_4 provided 250 me.% of internally released alkalinity, so that the excess of internal alkali was 110 me.%. The final balance in tissues was:

total cations = 180 me.% ;

inorganic anions = 320 - 250 = 70 me.%

the excess of cations was equivalent to the excess internal alkali released, 110 me.%. As tissue pH is constant, this alkalinity must have been transferred to organic anions, which equate the excess of cations. The numerical value for excess cations over inorganic anions was in quite good agreement with the known content of 90-100 me.% of non-volatile organic anions in ryegrass (loc.cit.; De Wit et al., 1963) although they were not measured in this particular study. The discrepancy in excess cations is almost certainly owing to the fact that Dijkshoorn overlooked the contribution of uronic acids (Van Tuil, 1965; Kirkby, 1966). The uronic acid content of ryegrass should be in the range 10-20 me.% (pers. comm., J. Dunlop).

(f) Ionic Balance in Tissues in Relation to N form

The effect of NH_4, relative to NO_3, in reducing cation content in herbage, especially that of the divalent cations, increasing inorganic anion content and the associated reduction in organic acid content has been discussed (II, B, 3, (b), (iii) and (iv)). The explanation for these observations is very simple. Owing to the synergism between NO_3 and metallic cations and its antagonism with other inorganic anions during uptake from the medium, plants receiving NO_3 have a larger excess of cations in their herbage, assuming that conditions are normal and the bulk of the accumulated NO_3 is metabolised. As shown in the previous section, excess cations are balanced by organic anions, so that these plants have a high organic anion content. The same species under identical

conditions but receiving NH_4, have a reduced cation content and an enhanced inorganic anion content owing to the antagonism between cationic N and metallic cations and its synergism with inorganic anions, as noted previously. Relative to plants of the NO_3 series, the gap between cation content and inorganic anion content has narrowed. As the difference is balanced by organic anions, the measured organic acid content falls.

This provides a simple, acceptable explanation for the differences observed. It remains to investigate the physiological basis for these interactions between N form and other inorganic ionic species, during uptake and accumulation.

(i) "Carrier" competition: Several authors have concluded that these interactions may be explained on the basis that NO_3 provides a large excess of rapidly absorbed anions, and NH_4, a large excess of cations, resulting in competition for "carriers" (Street and Sheat, 1958). Mulder (1956) considered that H^+ released within the cytoplasm of root tissues during NH_4 assimilation was the most probable explanation for the observed depression of Mg uptake by NH_4 fertilizers applied to acid soils. He concluded that H^+ was a strongly competitive ion with respect to cations such as Ca and Mg, while highly mobile cations such as K, were less affected by this competition. Competition for "carriers" in the conventional sense (Epstein, 1956, 1962) between NO_3 and other inorganic anions, and between NH_4 and metallic cations, has not been observed (loc. cit.). However, De Wit et al., (1963) showed a marked antagonism between NH_4 and K during accumulation by excised barley roots.

The writer finds "carrier competition" unsatisfactory as an explanation, in view of the above evidence and with due regard

to the fact that the conventional carrier theory provides no basis for the observed synergisms.

(ii) The relative sites of N metabolism: During NH_4 assimilation, metabolic incorporation of N occurs in the roots by necessity (II, 3, 2, (c)), possibly in the outer layer of root tissues (Mulder, 1956). The resulting organic-N compounds are largely translocated to the herbage and do not exhibit ionic properties in the sense that inorganic ions and organic anions do. From the point of view of ionic balance they may be considered as neutral compounds (Van Egmond, 1965). The uptake of metallic cations exceeds that of inorganic anions even during NH_4 assimilation (Cunningham and Nielsen, 1964; Kirkby, 1966). As these values for uptake have been estimated by tissue analysis, this means that there has been an excess of metallic cations transported to the tissues, and these are balanced in the tissues by organic anions. According to the Dutch concept this would involve an apparent uptake of HCO_3^- equivalent to the excess cations but at prevailing sap pH (II, 3, 4 (c)) the HCO_3^- ion would not exist in solution (Sutcliffe, 1962). Therefore the excess of cations must be balanced during translocation by organic anions formed in the root. It is significant that it is the divalent cations which are most affected, suggesting that they are more reliant on transport as the salts of organic acids than as free ions.

The mechanisms by which NH_4 could decrease organic anion transport from the root are open to speculation. Acidification of the external medium because of excess cation accumulation, and acidification of root tissues because of the acidity released

during NH_4 assimilation, could affect the carboxylation systems as discussed in the next section. Alternatively, the large demand for organic acids caused by NH_4 assimilation in the roots may be drawn from a limited supply of organic acids, in competition with cation transport. The data of Cunningham and Nielsen (1963) show that the ratio, me. Ca + Mg/me. Na + K transported to the herbage of Italian ryegrass declined a little with increasing NH_4 application to soil, but was quite independent of soil temperatures of 11, 19.5 and 28°C. From the data of Lycklama (1963) for full-grown perennial ryegrass plants assimilating NH_4, temperature changes over this range would be expected to bring about a much more rapid assimilation of NH_4 with each temperature increase. This in turn means a more intense acidification of both medium and tissues. No systematic variation in the ratio divalent/monovalent cations was observed in relation to temperature, but the ratio decreased with increasing NH_4 at any temperature. Therefore, under the conditions of that experiment the data support the contention that NH_4 assimilation was, in fact, in competition with divalent cation transport, for a limited supply of substrate. A temperature effect would be expected if acidification by NH_4 assimilation were the controlling mechanism. Substrate level could have been limited by the lighting conditions of the experiment. Having established that an antagonism between metallic cation transport and NH_4 assimilation does exist, the synergism with inorganic anions may be explained as due to the increased importance of paired uptake and transport of inorganic cations and anions in the absence of NO_3, and/or to the reduced availability of organic anions for excess cation accumulat-

ion.

When plants are receiving NO_3, a variable amount of the NO_3 absorbed may be metabolised in the roots, and the remainder translocated to the herbage as the anion. Accumulation of NO_3 in herbage is largely related to factors impeding its metabolism, particularly low carbohydrate supply, poor lighting and deficiencies of essential micronutrients (Spencer, 1958). It is difficult to assess the relative contribution of root tissues and aerial tissues to NO_3 assimilation, although it was concluded (II, 5, 2, (c)) that the roots are an important site for the bulk of this assimilation. As such, the synergism between NO_3 and metallic cations during uptake and accumulation may be explained in any of the following ways:

(a) There may be an increased paired transport to the leaves, of cations with ionic NO_3;

(b) nitrate assimilation in the roots may provide organic anions for the translocation of cations to the herbage;

(c) uptake and assimilation of NO_3 may provide an alkaline environment in the medium and the root tissues, thus indirectly favouring the provision of organic anions for the transport of metallic cations.

Cunningham and Nielsen (1963, 1965) have studied the mineral composition of Italian ryegrass herbage in relation to soil temperature and NO_3 availability. The foliar content of cations was directly related to soil temperature and to NO_3 availability; increasing as NO_3 availability increased at any soil temperature, and increasing with soil temperature at any level of NO_3 application to the soil. The content of divalent cations (Ca + Mg) relative

to that of monovalent cations (Na + K) in the herbage exhibited the same relationships to soil temperature and NO_3 availability. An increased assimilation of NO_3 over this soil temperature range (11, 19.5 and 28^{0}C) would be expected from the results of Williams (1962) and Lycklama (1963). Conditions which favoured the assimilation of NO_3, therefore, also favoured cation uptake, especially that of divalent cations. If the effect of increased soil temperature were considered to result in an increased metabolism of NO_3 in the roots (the foliage of all treatments was maintained in a uniform environment), then it may be concluded that the observed synergism between NO_3 and cations was the result of an increased translocation of Ca and Mg as the salts of organic anions, provided directly or indirectly by NO_3 assimilation. However, as the effect of higher soil temperatures is open to the alternative interpretation of an increased translocation of cations, especially Ca and Mg, as their NO_3 salts out of the roots to the herbage, the possible mechanisms underlying this synergism cannot be irrefutably separated.

The antagonism between NO_3 and other inorganic anions during uptake may be explained by the reduced share of (P + S + Cl) in total transport capacity, in the presence of adequate NO_3, owing to the reduced importance of paired uptake of cations with inorganic anions other than NO_3, under such conditions.

(iii) Tissue pH in relation to N form: Mulder (1956) reported that the pH of macerates of root and shoot tissues from peas which had been receiving NO_3 or NH_4, were higher where the plant was metabolising NO_3. The differences were not large, ranging between

0.1 and 0.3 pH units. Kirkby (1966) reported large differences in the pH of tissue macerates for tomato plants. Those receiving NO_3 had a pH value of 5.60 for roots while the corresponding value for NH_4 was 4.70. The difference showed a more or less gradation from roots through stems and petioles to leaves, where the values were 5.50 and 5.00. Kirkby felt that this difference could in part explain the reduced organic anion content of NH_4 tissues and the related reduction in cation content in relation to NO_3, as higher pH values favour the dark fixation of CO_2 and synthesis of organic acids (II, B, 4, (d), (i)).

This conclusion is not necessarily correct. It is not known whether the carboxylation enzymes are exposed to "tissue acidity" as measured by the pH of macerates. In fact it is known that a considerable proportion of the more important organic acids in plants are isolated from metabolic turnover pools, presumably stored in the vacuole (MacLennan, 1963). Furthermore, tissue pH is considered to be effectively buffered against change during NO_3 and NH_4 absorption (De Wit et al., 1963; Dijkshoorn, 1964; Van Tuil, 1965). In field studies on the growth of brocolli receiving NO_3 and NH_4 fertilizers, Sheart (1966) found no pH differences in the tissue macerates receiving either N form. There were larger measurable differences within N form between plantings, than between N forms within plantings.

The content of total P was higher in all plant parts, except roots, of the tomatoes receiving NH_4 in the study of Kirkby. Presumably, therefore, the buffering capacity was also higher (II, B, 4, (c), (i)). That lower pH values were found in stems, petioles

and leaves would suggest that free acids had accumulated in these tissues and consideration must be given to the question of whether metabolism in these tissues was normal.

(iv) External pH in relation to N form: It has been noted previously that NO_3 nutrition results in the uptake of a large excess of anions and a resultant increase in the pH of the medium, while the reverse is the case with NH_4 (II, B, 3, (a) (v)). This raises the possibility of an interaction between N form, external pH and ion uptake and accumulation.

Several experiments have shown that when isolated tissues are held in alkaline media, in relation to the same tissues held in acidic media, the uptake of inorganic cations is increased, accumulation of inorganic anions depressed, and that the excess cation accumulation is balanced by the synthesis of an equivalent amount of organic anions in the tissues (Jacobson and Ordin, 1954; Hurd, 1958; Lundegårdh, 1960; Sutcliffe, 1962; and others). The argument as to whether this "ph affect on cation uptake" is the result of HCO_3^- accumulation per se and subsequent organic anion synthesis (Hurd, 1958; Sutcliffe,1962) or the result of a shift in the equilibrium in favour of organic acid formation and dissociation (Lundegårdh, 1960) is academic and cannot be proven by experiment (Miller, 1960). Bhan et al., (1960) concluded that the commonly observed reduced uptake of cations by plants receiving NH_4 could be largely the result of low pH rather than NH_4. They grew soyabeans for two weeks in solution culture with NO_3 and NH_4, and maintained two series of solutions at acid and alkaline pH values. The me. sum of (K + Ca + Mg) was depressed by NH_4 in re-

lation to NO_3 at acid pH values. This was a true antagonism as yield also fell. However, where HCO_3^- salts were supplied to the culture solutions to give a pH of 8.5 in the media, the me. sum of cations was the same for the leaves from both N forms. The yield of the NH_4 plants at pH 8.5 was less than half that of the NO_3 plants, so that part of the increase in cations may have been related to a concentration effect resulting from the yield decrease. It is interesting to convert their data to the ratio, me. divalent cations/me. monovalent cations, and consider them in relation to pH and N form. Irrespective of N form the ratio was higher at pH 8.5 than at pH 5 to 6. For NH_4, the increase was from 0.74 at acid pH to 2.8 in the alkaline medium, almost all accounted for by a decline in K content and a fourfold increase in Ca content. If this were a true "pH affect on cation uptake" involving accumulation of organic anions and an equivalent of cations, it gives an independent verification of the suggestion made previously that the transport and accumulation of divalent cations could be more dependent on the movement of organic anions than on the accumulation of inorganic anions. This experimental observation must be weighed against the characteristics generally observed in the leaves of plants suffering from lime-induced chlorosis (see Bear, 1960):

a. a high Na + K/Ca + Mg ratio;

b. a high organic acid content.

This physiological disease is caused by a natural or lime-induced alkaline soil reaction. The general observations suggest that an alkaline soil environment, relative to an acid soil, causes an excess of accumulation of cations balanced by organic anions, but

that the uptake of monovalent cations rather than Ca and Mg, is enhanced.

Unfortunately, little is known of the pH characteristics in the immediate environment of the root during differential ion accumulation. Walker (1960) has discussed the movement of cations from the soil solution to the soil colloidal complex, resulting in an equivalent displacement of H^+ to neutralize the OH^-/HCO_3^- released to the medium by the plant during excess anion accumulation. What data are available, however, suggest that this equilibrium may not be established at the root surface during active ion uptake, at least not in poorly buffered media. Experiments cited by Mulder (1956) and Street and Sheat (1958) established a steep pH gradient near the root surface, even where the pH of the bulk of the medium was rigidly controlled. Sand cultures supplying NH_4 with a bulk pH of 6.0, had a pH of 4.0 - 4.5 at the root surface, and as low as pH 2.8 - 3.0 with high levels of NH_4. Sand cultures supplying NO_3, with a bulk pH of 4.5, had a pH at the root surface of 5.6. Lundegårdh (quoted from Street and Sheat, 1958) measured the pH at the surface of wheat roots as 3.0, which was very acid in relation to the medium. Jones (1961) measured the pH at the root surface of plants growing in fly ash of pH 8.5, and found it to be 6.0. Obviously then, the pH at the root surface does not have to be the same as that in the bulk solution or soil.

All that can be concluded is that pH gradients possibly exist around the roots of plants which are actively absorbing nutrients. Because of the external alkaline effect of NO_3 uptake, such a gradient would be alkaline, and with NH_4, acidic. In view

of experimental evidence for a pH affect on cation uptake, and associated organic acid accumulation this is a possible contributing factor to the observed reduction of cation uptake and associated reduction in organic anion content with NH_4 nutrition, in relation to NO_3.

(g) Root CEC and N Metabolism

As some of the previous considerations in this section may be related to current concepts about root CEC it is relevant to make some brief observations in this section.

It has been found that NH_4, relative to NO_3, reduces root CEC (Wander and Sites, 1956, and others) and that increasing NO_3 availability increases root CEC (Heintze, 1961, and others). Mouat and Dunlop (pers.comm., M.C.H. Mouat) found that increasing NO_3 availability resulted in a marked increase in the CEC of wheat roots, but only at very low N levels, a maximum being reached at a little more than 1% N in leaves. Wallace et al., (1958) found that pretreatment of excised roots from several species with NH_4 decreased subsequent Na uptake, relative to NO_3 pretreatment. Increasing levels of NO_3 resulted in greater subsequent Na uptake. Bhan et al. (1960) reported that pretreatment of plants with NO_3 resulted in an enhanced accumulation of K and Ca, relative to NH_4 pretreatment. Pretreatment of excised roots with HCO_3^- at alkaline pH also resulted in an enhanced cation accumulation (loc.cit.). This bicarbonate effect has been observed by others as discussed in the previous section.

Both forms of pretreatment are known to result in organic anion accumulation in tissues and the above evidence indicates that

preformed organic anions are important in subsequent cation accumulation.

If one accepts the view that organic acid anions are restrained from movement from the tissues into the medium, but that their counter-ions are subject to exchange reactions with cations in the external medium (Burström, 1951; Lundegårdh, 1960) then some of the organic acid content of the roots may be included in measurements of root CEC, whether by isotopic exchange, or electrodialysis followed by the determination of exchangeable H^+ in the roots.

Kirkby (1966) found that the content of polyuronic acids was not affected by the form of N available to tomato plants, and concluded that the primary structures and cell walls of tomato roots were formed quite independently of N form. The organic acid content of the roots receiving NO_3 was, however, seven times that of roots from the NH_4 series.

Reported variation in root CEC in relation to form and level of N needs re-examination, therefore, in the light of these considerations. Cunningham and Nielsen (1963) reported root CEC of Italian ryegrass as independent of N form, N level and soil temperature. It was virtually constant at ca. 15 me.%. They did not report their technique of measurement.

(h) Conclusions:

During ion accumulation by isolated tissues any excess or deficit of cation accumulation is balanced by the synthesis or catabolism of organic anions. Irrespective of form, mineral N can be assimilated by intact plants in such a way that it is largely

independent of the uptake of other anions and cations from the medium. Plants receiving NO_3 absorb a large excess of anions; those fed NH_4, a large excess of cations. While this uptake of N is largely independent of the uptake of other ions, there is an interaction between the form of N and the uptake of cations and anions from the medium. Herbage from plants receiving NO_3 has a high metallic cation content and a lower inorganic anion content, in comparison with NH_4-fed tissues. Because the excess of cations over inorganic anions is balanced by organic anions, the NO_3-fed plants have a higher organic anion content also. It is not clear whether the greater content of cations in tissues receiving NO_3 is the result of an increased paired transport of metals as the salts of NO_3, which on subsequent metabolism forms organic anions to equate the excess cations in the tissues; or whether it is an indirect effect of NO_3 metabolism on the movement of organic anion salts from the roots.

5 INTERIONIC RELATIONSHIPS IN RYEGRASS HERBAGE

(a) Introduction

In studying interactions between elements during foliar accumulation it is sometimes difficult to separate "real" interactions from "apparent" interactions (Cunningham, 1963). The latter type may arise, for instance, where a nutritional treatment grossly affects yield. A high-yielding plant may have a low content of a particular element, not as the result of a true interaction, but simply due to growth dilution. In terms of the nutri-

tional value of herbage it may be of little moment whether the reduced content of a particular element is the result of growth dilution, but in any attempt to elucidate the physiological basis for interactions, it is essential that real and apparent interactions are separated. For this reason, the use of ratios between the contents of elements has become popular, on the assumption that any growth dilution equally affects both the numerator and denominator of the ratio. The ratio between the foliar content of total metals and total non-metals has been used in studies on pasture grasses. This has, unfortunately, become known as the "cation-anion ratio" (Cunningham, 1964a *et seqq.*) which is incorrect, as much of the mineral N in herbage may enter the plant as a cation (*loc.cit.*). The abbreviation, R-value, will subsequently be used to denote the metal/non-metal ratio in herbage:

$$R \text{ value} = \frac{\text{me.sum of } (K + Na + Mg + Ca)}{\text{me.sum of } (S + P + Cl + N)}$$

(b) *R-values as a Constant*

Bear (1950) concluded from a number of previous studies that R values were an approximate constant for the tissues of plants grown at any given pH. When plants were grown in a uniform environment there was a tendency for the foliar content of total cations to be constant and a related tendency for total non-metals (including Si) to be constant. The application of fertilizers resulted in a change in foliar content of cations and a related change in total non-metals, such that the R value did not change appreciably. Between environments there were large differences in the contents of metallic cations and total non-metals owing to

dilution by carbohydrates, but these differences were relative and did not affect P values.

Opposed to an earlier report by Van Stellie (1930) that the total cation content of ryegrass herbage was constant, Dijkhuizen (1957a) found that the content of metallic cations in perennial ryegrass herbage increased markedly with increasing application of the NO_3 salts of K, Na, Mg or Ca to the soil medium. There was a related increase in the foliar content of non-metals, most of which was the result of increased N uptake. The increased uptake of cations and anions was related, such that irrespective of the accompanying cation and irrespective of the rate of application of the NO_3 salts, P-value was a constant of approximately 1.5. This observation was confirmed by a later experiment (Dijkhuizen, 1957b) with perennial ryegrass growing in soil. Fertilizers were applied to give a constant dressing of NPK with NO_3 progressively replaced by Cl, S or P. While considerable variation in metallic cation content and the content of non-metals in herbage was observed, the variation was related in such a way as to maintain P values at the approximately constant value of 1.5.

As discussed in the following section, subsequent evidence has shown that P values are not constant in ryegrass herbage.

(c) Factors affecting P-values

(i) Stage of growth. Dijkhuizen (1958b) studied the relationships between P values, levels of fertilizer N application, and the stage of regrowth of perennial ryegrass in pot cultures. A clay soil of pH 7.0 was fertilized with K_2HPO_4 to ensure adequate K and P levels. Varying amounts of NH_4, NO_3 were added to the soil, and under these

conditions nitrification should have been quite rapid (II, 1, 2, (c), (i)). Part of the data are presented in figure 3, for low levels of N addition (a) and high levels of N addition (b). Considering the R values first (i.e. $\frac{C}{N+K}$), it may be seen that at both levels of N application, R values increased from 0.45 to 0.55 over the first 27 days of the experiment. Richhoomn interpreted this as a region of metabolic control over R-values. With increasing age some "internal factor" caused a decline in N content, owing to a decreased ability of the plants to assimilate NO_3 into organic forms, per unit of D.M. The decline in total N, metallic cations and inorganic anions over this period was partly owing to increased carbohydrate formation, but the rate of decline in total non-metals was relatively faster than that of metals, resulting in an increased R-value. The significance of exchange uptake of NO_3, therefore, was declining with increasing maturity, and paired uptake assumed a greater relative importance (II, 3, 4, (c), (ii)). Shortly after 20 days' growth, the NO_3 content of herbage in the low N series showed a steep decline, indicative of exhaustion of N in the medium. The "critical yield" was reached in the low N series (a) after about 27 days, and following this there was a rapid increase in R-value, from 0.55 to 0.70 over a period of about 10 days. The content of metallic cations dropped from 185 to 150 me./% over this period, but the decline in non-metal content was relatively greater, from 330 to 200 me./%. This apparent decline in content was largely owing to increased carbohydrate deposition. The depletion of mineral N in the soil gave a situation where exchange uptake of N could no longer occur, so that paired uptake assumed predominance.

It is not possible, with the data given, to work out what the actual ratio was between cations and anions absorbed after the "critical yield". However, to have changed the R-value for the whole tissue of the plants from 0.55 to 0.70 it was obviously much greater than 0.70 over this period. The cation-anion ratio during uptake after the critical yield may well have exceeded unity, as found by [illegible] and [illegible] (1951) in the more mature stages of growth for sugar beet, with the excess cations transported as the salts of organic anions. Figure 9 shows that in the high N series (b) this effect of "critical yield" was not noticeable as the external supply of NO_3 was not exceeded, unless perhaps near the end of the experimental period, as indicated by a falling NO_3 content in the leaves. R-value increased at the same rate as was observed during the period of metabolic control, again showing a decreased importance of exchange uptake, and an increased importance for paired uptake as maturity progressed.

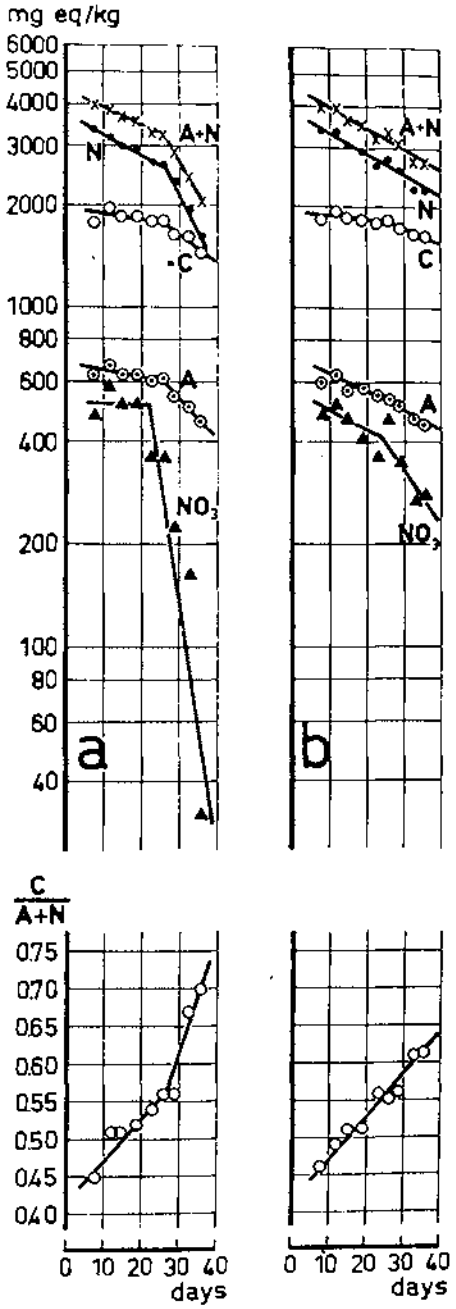
It is concluded that the R-value determined in perennial ryegrass at any time is subject to large variation with the maturity of the plant. It may also be greatly modified by the availability of NO_3 in the external medium.

(ii) <u>Form and level of N.</u> Cunningham (1964a, 1964b) has measured the metal/non-metal ratios in the herbage of Italian ryegrass for more than 100 samples from field sites in Britain. He established a significant negative correlation between R-value and N content in the herbage. Large R-values were associated with low N contents and small R-values with a high N content. He concluded: "Although soil, fertiliser treatments, climate, age of plant, season and

FIGURE 3

MINERAL COMPOSITION OF PERENNIAL RYEGRASS HERBAGE IN RELATION TO STAGE OF GROWTH AND N AVAILABILITY

(from Dijkshoorn, 1958b)



IONIC CONCENTRATIONS IN THE YIELD IN MG EQUIVALENTS PER KG OF DRY MATTER PRODUCED. NITROGEN (N), CATIONS C (= K + Na + Mg + Ca) ANIONS A (= Cl + P + S), TOTAL ANIONS (A + N) AND NITRATE (NO_3), PLOTTED AGAINST THE TIME OF REGROWTH.

The lower graphs show the cation-anion ratio : C/(A + N).

a: fertilized with 140 mg N per pot.
b: fertilized with 560 mg N per pot.

strain can affect the concentration of the individual elements in Italian ryegrass, these factors influenced the R-value of Italian ryegrass less than did the P supply". The negative correlation between R-value and P content is spurious, as the x ordinate (P content) comprised 7o% of the denominator of the y ordinate (R-value). It simply shows that R-value is not a constant, disproving the theory of Lees (1950) and the view previously held by Dijkhooorn (1957a, 1957b, 1958a). R-values ranged from 0.3 to 0.9 in the samples. At any P content, R values were lower for samples which had received most of their N as NH_4, as opposed to NO_3. This is presumably a reflection of the established synergism between NO_3 and metallic cations and NH_4 and inorganic anions, and antagonisms between NO_3 and inorganic anions and NH_4 and metallic cations (II, 3, 4, (2)). Without more knowledge of the growing conditions of these samples, little can be made of the other interactions cited, as it is not known to what extent yield was effected.

The negative relationship between R-values and P content in herbage have been reaffirmed by subsequent experiments (Cunningham and Nielsen, 1965). Plants with a low P content as the result of low P availability, had high R-values irrespective of the form of N available. This is in agreement with the data of Dijkhooorn (1958b) shown in figure 3 for NO_3 nutrition of perennial ryegrass. Because of P depletion in the medium the significance of exchange uptake was reduced at low levels of P availability, sooner than was the case with ample P available. As a result, R-values increased more rapidly after P depletion. The data of Cunningham and Nielsen (loc.cit) indicate that the same situation exists whether

NO_3 or NH_4 are supplied to ryegrass.

(iii) Temperature. The data of Dijkshoorn and 't Hart (1957) for the effects of different temperatures on the mineral composition of perennial ryegrass have been recalculated by [illegible] (1959) according to the present concept. Plants maintained at 10 or 20°C over the 31 day period showed a progressive increase in L-value with time, similar to that resulting from increasing maturity (Figure 3). Plants at 20°C had higher L-values than those at 10°C, at any stage of regrowth. As the temperature may have affected yields also, one cannot make any precise interpretation as to the relative significance of mineral and exchange uptake in relation to temperature. Plants transferred from 10 to 20°C on day 10 showed a normal increase in L-value after the transfer. Cation content of the herbage increased, which was almost entirely due to greater K uptake, while the non-metal content of the herbage decreased. L-values remained virtually constant after plants were transferred from 20 to 10°C.

Cunningham and Nielsen (1965) found that the L-values of Italian ryegrass herbage also increased with increasing soil temperature. Unlike the previous authors, they sampled only after 56 days' growth and maintained the tops of the plants in a uniform environment. Where plants were receiving NO_3, exchange uptake of N must have been relatively more important at low soil temperatures than at higher soil temperatures, as their results were not systematically related to yield. There was no obvious effect of soil temperature on L-values for plants receiving NH_4.

(iv) <u>Lighting</u>. Cunningham and Nielsen (1965) measured the R-values in Italian ryegrass herbage after 25 days regrowth with 100%, 66% or 44% of glasshouse "daylight". For plants receiving NO_3, R-values were inversely related to light intensity, being highest at 44% of daylight. As both yield and temperature increased with increased lighting, this may be interpreted as an enhanced assimilation of NO_3 and resultant increased importance of exchange uptake of HCO_3 with greater light intensity. Such an effect could have been exerted by an increase in carbohydrate substrate for NO_3 assimilation at greater light intensities (II, 2, 3, (a), (iii)) under the conditions of this experiment.

There was no clear relationship between R-values and lighting for plants receiving NH_4.

(v) <u>Maxima Recorded for R-values</u>

Cunningham (1964b) and Cunningham and Nielsen (1965) have recorded R-values in excess of unity for Italian ryegrass grown in a glasshouse. Values ranged as high as 1.4, and those in excess of unity were associated with N deficiency (1-1.5% N in herbage).

This material was harvested after 56 days' growth. Extrapolation of the data in figure 3 for the low N series (a), suggests that the R-value would have been near or even in excess of unity 56 days after the initial defoliation of perennial ryegrass. The observation is interesting in that the presence of more metals than non-metals in the herbage means that the excess of cations must have been translocated as their organic anion salts, even if all the N moved from the roots up to foliage as inorganic NO_3 (II, 2, 4, (f)).

(b) Interactions between Elements During Uptake

(i) Antagonisms: Van Itallie (1938) studied the mineral composition of Italian ryegrass which had been grown in a potted soil, to which Ca, Mg, Na and K had been added as their carbonates. These minerals were applied at constant total rates in a widely varying cation replacement series. He concluded that the "replacing power" of cations in the herbage of ryegrass was in the following descending order: K, Na, Mg, Ca. The total cation content of herbage was remarkably constant at 200 me. %, which may have been due to the fact that all plants had a common N supply and were harvested at the same stage of growth. Dijkshoorn (1957c) grew perennial ryegrass in soil with K, Na, Mg or Ca added in increasing amounts as their NO_3 salts. In the K and Na series, increased cation uptake with increased NO_3 supply was almost entirely owing to a greater uptake of the cation supplied. In the Mg and Ca series, total cation content increased with greater NO_3 application also, but much of this increase was the result of an increased Na and K uptake from the soil. It was concluded that the replacing power of the major cations was Na = K which were greater than Mg = Ca. This observation was supported by Reid (1955) for perennial ryegrass. De Wit *et al.* (1963) differentiated between a two-ion (Na and K) competitive system and a four-ion (Na, K, Ca and Mg) competitive system during cation uptake by perennial ryegrass. They concluded that the two-ion system is selective towards K. Cunningham (1963) reported the following "real" antagonisms during the accumulation of cations by perennial ryegrass.

a. Mg vs L and Ca;

b. L vs Mg, Ca, Mg;

c. NH_4 vs L.

The antagonism between NH_4 and L only was not apparent in later work; instead, an antagonism between NH_4 and total cation content (K + Na + Mg + Ca) was established (Cunningham and Nielsen, 1965).

Dijkshoorn (1957b) studied the effects of associated anions of L fertilizers on the mineral composition of perennial ryegrass herbage. Plants were grown in a potted soil to which a constant amount of K was added. The associated NO_3 was replaced by H_2PO_4, SO_4 or Cl, in a replacement series. Chloride was strongly antagonistic with NO_3, and SO_4 replaced NO_3 to a greater extent than did H_2PO_4. This NO_3 vs Cl antagonism was supported by later work (Dijkshoorn, 1958a). Using culture solutions, Dijkshoorn (1959) concluded that the relative replacing power of anions other than NO_3, was in the decreasing order Cl, H_2PO_4 then SO_4. The reversal of order for the last two anions, P and L, was considered to be the result of immobilisation of added P by soils in the previous experiment comparing these anions. Antagonism between NO_3 and Cl, has been shown in the experiments of De Wit et al. (1963) using perennial ryegrass. The replacing power of anions other than NO_3 was Cl, H_2PO_4 and SO_4 in decreasing order (loc.cit.).

(ii) *Synergism*. Dijkshoorn (1957a) showed that an increase in NO_3 supply resulted in an increased content of metallic cations for perennial ryegrass grown in soil. This was a true synergism as yield and total cation content increased over the experimental period of 28 days. The monovalent cations were most affected by

varying the level of NO_3. In a similar experiment, Dijkshoorn (1957b) found a marked synergism of both NO_3 and Cl with total cations. This effect was not noticeable with H_2PO_4 or SO_4 under the experimental conditions. The interaction between NO_3 and monovalent cations should be regarded as a mutual synergism. In the first experiment (Dijkshoorn, 1957a) where the Ca and Mg salts of NO_3 were applied, the synergism with cations was mainly the result of increased Ca and Mg uptake. Presumably, therefore, the synergism would not have occurred to the same extent if the medium had provided a low availability of monovalent cations.

Cunningham (1964a, 1964b) established a positive correlation between total cation content and N content in Italian ryegrass samples, irrespective of which form of N had been applied in fertilizer. As these samples were taken from divergent field sites throughout Britain, they doubtless varied in maturity and N supply. It has been clearly shown in figure 9 that increasing maturity results in a decline of both N content and total cation content in perennial ryegrass, by progressive growth dilution. The data of Cunningham (loc.cit.) may be explained in part, therefore, as an effect of growth dilution. An earlier publication (Cunningham and Nielsen, 1963) showed that under experimental conditions there was, in fact, no increase in the total cation content of Italian ryegrass with increasing NH_4 availability in soil. There was, however, a marked synergism between NO_3 and total cations, in the same experiment. Cunningham (1963) reported a synergism between Cl and K with Italian ryegrass.

C METHODS WHICH CAN BE USED FOR THE PROVISION OF NO_3 OR NH_4 NUTRIENT TREATMENTS

I [illegible]

(a) pH Stabilization

The external similar and specific effects of NO_3 and NH_4 nutrition have been discussed (II, A, C, (a), (v)). The relative efficiency of either N form may be altered by pH (*loc. cit.*) variation of which may, in part, be the cause of the observed differences in mineral composition between plants receiving either N form (II, B, 4, (f), (iv)). To prevent N form from being confounded with external pH, the latter must be regulated.

(b) Associated Ions

To supply NO_3 and NH_4, whether as fertilizers to soil or as salts in a culture solution, at least one other ionic species must be altered. As this problem is insurmountable, it becomes a case of selecting the variable(s) which are least likely to affect the results.

(c) Element Availability

Uptake of an element is not a linear function of concentration (Epstein, 1956). With increasing external concentration a limiting rate of absorption is reached. Therefore, if one knew what these concentrations were, any ionic species could be varied providing that osmotic effects did not interfere. In the static culture solutions of [illegible] (1959), 5 me./l. of Na or K and 6 me./l. of Ca and Mg did not appear to provide for maximum absorption by

perennial ryegrass under the conditions of that experiment. The data of De Wit *et al.* (1963) show that under the conditions of their experiments, i.e. 12 days' regrowth in static culture solutions, 9 me./l. of K, Na, Cl or NO_3 apparently did not provide for maximum absorption by perennial ryegrass. However, variation in foliar K content was less than that of Na, both of which varied far less than Cl or N contents over the range of concentrations used. Sulphur content increased some 20-25% as SO_4 increased from 1.0 - 9.0 me./l. in solutions. As the data are simply not available for grasses, to show at what point uptake of major elements ceases to be limited by external concentration, under any prescribed set of experimental conditions, it seems advisable to make as few changes as possible in the "availability" of elements between treatments.

A further problem is the fact that equal additions of NO_3 and NH_4 to soils do not necessarily imply equal availability. While NH_4 is readily assimilated by plants in general, it is held in exchangeable form on the soil colloids, but NO_3 is free to move towards the roots of plants by mass flow and diffusion. This may be more a problem in the interpretation of the physiological basis for results, rather than in the design of the experiment. The interaction of NH_4 with soil colloids is an inseparable factor in NH_4 assimilation by plants growing in soils. As such, it is additive to any effect of N form in determining the mineral composition of herbage.

2 SOIL AND [illegible]

(a) Fertilizers

Numerous experiments have assessed the relative effectiveness of commonly used NO_3 and NH_4 fertilizers, and the practical significance of these field trials cannot be denied. But in view of evidence that a small amount of nitrification may greatly enhance the value of NH_4 sources of N (III, 3, 3, (c), (ii)), such an approach is not satisfactory in any incisive investigation into the relative effects of the two N forms.

(b) "N-Serve" (2-chloro-6-(trichloromethyl) pyridine)

This chemical, on volatilization in soil, destroys the Nitrosomonas bacteria responsible for the biological oxidation of NH_4 to nitrate (Goring, 1962a; Dow Chemical Bulletin, 1962). The rate of application required to effect complete control over nitrification for a period of 6 weeks was found to vary with soil type, and ranged from 0.05 to 20 ppm. The rate of reinfestation of treated soils by Nitrosomonas varied with soil type. Higher levels of application generally caused a longer delay in nitrification. Concentrations of "N-Serve" ranging from 12.5 to 25 ppm. had no noticeable effect on the growth rate of a wide variety of seedling plants (loc.cit.). The chemical has been made available for experimental purposes, by the Dow Chemical Company.

There have been several reports of its effectiveness in varying degree, with field application of NH_4 fertilizers: Goring (1962b), simulated field conditions; Gunner et al. (1962), field application; Bremner and Gunner (1962), cotton, corn and sugarbeet; Gunner and Nilson (1964), cotton; Volpe et al. (1965), sugar cane, and others. A laboratory study by Hattori (1963) showed that this chemical was apparently specific to the nitrifiers among the chemo-

autotrophic microorganisms, and no harmful effects were observed on a number of heterotrophic microorganisms. This confirmed the earlier report of Loria (1962a). The compatibility of "N-Serve" with inoculated legume was tested by Odell and Mulley (1963) in a glasshouse study. They found a reduction in seedling growth, which was severe at 20 ppm. and slight at 1 ppm. The chemical caused changes in nodule morphology and deformation of root tips (loc.cit.), which may have been related to its effects on growth.

Redemann et al. (1964) reported that when "N-Serve" was added as an aqueous mixture to soil, it was subsequently lost by the combined processes of volatilization, and degradation to 6-chloropicolinic acid. This hydrolysis product, but not the parent compound, was detected as residues in the leaves of lettuce, carrots, oats and tomatoes, when grown on soils treated with "N-Serve" (Redemann et al., 1965).

"N-Serve" has been used to prevent nitrification in soils during experiments designed to compare the two NH_4 forms (Nielsen and Cunningham, 1963, et seq.; Van Cleef, 1964). There have been no reports of adverse effects on growth during either of these experiments, in which 10 ppm. of the chemical was used as an aqueous mixture with soil. Cunningham and Nielsen (loc.cit.) using a soil of pH 6.4, found that nitrification of added NH_4 was not completely prevented over the first 10 days of the experiment. "N-Serve" was completely effective for the remainder of the 3 week period, at three soil temperatures, and levels of NH_4 in soil ranging from 200 to 1,000 ppm. Van Cleef and Kemp (1964) studied nitrification of added NH_4 fertilizer in a soil whose initial pH

of 3.1 was raised progressively up to 7.3 by increments of $CaCO_3$. Soils were incubated in a glasshouse at 18°C and NO_3 was determined 1, 3, 2$\frac{1}{2}$, 4$\frac{1}{2}$, 7$\frac{1}{2}$ and 30 days after the imposition of treatments. Without "Observe" nitrification was evident at pH 6.6 after 21 days, and was more intense at higher pH values. Nitrification was virtually prevented at all pH values and all levels of N_2 application over the entire 30 days, where 11 p.p.m. of "Observe" had been added to the soil.

"Observe" therefore appears to be effective in the prevention of nitrification under experimental conditions, where its application can be rigidly controlled. Repeated field trials in Australia failed however, to show any effective control over nitrification under those conditions (pers.comm., D. L. Baby).

(c) <u>pH Stabilization</u>

In soil experiments, this problem is less significant than in solution cultures, providing that the soil is well buffered, although little is known of the pH at the root surface during experiments comparing NH_4 and NO_3 nutrition (II, 3, 4, (f), (iv)). The problem of external pH stabilization may be over-rated in soil experiments. From a practical viewpoint it is immaterial whether the observed changes in mineral composition of herbage arise from the ion <u>per se</u>, or its associated effects on plant physiology, through changes in tissue pH and/or external pH (II, 3, 4, (f)). Thus changes in external pH may cause indirect effects on mineral composition by altering the availability of elements in the soil, such as Mn or metallic cations (Mulder, 1956) or by altering the equilibrium content of HCO_3^- in the solution phase (Burd, 1956).

However, inasfar as that these effects are an inherent characteristic of the assimilation of either N form under field conditions, very effective pH stabilisation could mask true treatment effects. On the other hand, if the experimenter were trying to separate N effects from pH effects, pH stabilisation would be of paramount importance.

(c) Associated Ions

(i) Cations with NO_3. It would be inadvisable to supply NO_3 as the salt of a cation which is normally present in soils as an ion in very small amounts. Of the major cations, Ca normally exceeds in equivalents the total of all other cations, both in the solution phase and on the exchange complex (Walker, 1960; Russell, 1961). Calcium also causes less interaction with other elements than do Na or K, during uptake by perennial ryegrass (II, 2, 5, (c)). Its choice as an accompanying cation seems the most acceptable. Addition of the Mg salt on the other hand would lead to a much greater proportionate change in availability. Cunningham and Nielsen (1965) and Van Suil (1965) used Ca $(NO_3)_2$ as a source of NO_3 in their soil experiments.

(ii) Ions with NH_4. Ammonium carbonate/bicarbonate solutions have been used to apply NH_4 to soils. Application would result in at least a temporary increase in pH and could cause physical damage to plants (Pore, 1960). Owing to the marked synergism between Cl and metallic cations during uptake by ryegrass (II, 2, 5, (c), (ii)), NH_4Cl should not be used. Ammonium dihydrogen phosphate (+ "K-Serve") was used by Cunningham and Nielsen (1965). To avoid effects arising from increased P availability with higher

levels of NH_4 application, a constant amount of N was applied and Ca was diminished as NH_4 was increased, in a replacement series. Sulphate causes less interaction during the uptake of other elements by perennial ryegrass, than do any of the alternative anions (II, D, 5, (e)). Therefore the use of $(NH_4)_2SO_4$ would appear to be the most acceptable of the alternatives, unless S metabolism or response to S were being studied. Van Dijk (1965) used $(NH_4)_2SO_4$ (+ "N-Serve") to supply NH_4 in soil experiments.

3 <u>SOLUTION CULTURE</u>

(a) <u>Sand Cultures</u>

Salts in solution may be added to an inert, supporting medium, such as sand or vermiculite. Solutions may be periodically replaced by leaching the containers with fresh solution, or they may be static, with water added to equate evapotranspiration. The latter method was used by Schenker and Kuhn (1955) who reported: "estimations performed on the H ion concentration in the quartz sand showed no differences worth mentioning between the individual experimental series". This is surprising in view of the fact that they also reported plant cation/anion ratios of 0.4 with NO_3 and 2.4 with NH_4, which one might have expected to result in a marked pH difference between treatments (II, D, 4, (e), (ii)).

Exchange resins have been used in sand cultures to supply NO_3 and NH_4 ions, the other elements being supplied in a common, N-free culture solution (Hewitt, 1952). This technique was used by White and Warren (1962). The presence of an anionite in the NO_3 cultures and a cationite in the NH_4 series could offset movement in external pH. However, the two N forms are subject to exchange

with other ions in solution, especially those high in the lyotropic series, altering the availability of the other elements. Wolte and Momer (loc.cit.) encountered Ca and Mg deficiencies in the NH_4 treatment of their experiment (pers.comm., D. A. Hinkly).

(b) Flowing Cultures

This technique is probably the best available for an experiment of this type (Street and Sheat, 1958). A method has been described by Andrew and Listers (1962). It obviates the problems of external pH movement and depletion of the culture solution, but an enormous supply of suitable water is required, and there is a large expense on chemicals for the preparation of solutions.

(c) Static or Periodically-renewed Culture Solutions

These are most commonly used in experiments comparing N forms in plant nutrition. The following are some examples of the formulae which have been used. For uniformity, the concentration of major elements is expressed in me./l. as recommended by Hewitt (1952). Phosphate has been considered as trivalent and the associated cation and H tabulated separately, to illustrate the use of buffers.

Lucher et al. (1947)

NO_3-N

	NO_3	PO_4	SO_4	Cl	Total
K	2.1	1.0			3.1
Na					
Ca	4.5				4.5
Mg	8.6		2.8		11.4
NH_4					
H		2.0			2.0
Total	15.2	3.0	2.8		21.0

NH_4-N

	NO_3	PO_4	SO_4	Cl	Total
K		3.1			3.1
Na					
Ca			4.5		4.5
Mg			2.8		2.8
NH_4			15.2		15.2
H		6.2			6.2
Total		9.3	22.5		31.8

magnesium, P and S were varied between solutions, and Mn and Cl were omitted completely. In view of the interactions established for ryegrass, variation in Mg and P would be unacceptable in any study of the effects of N form on the uptake of individual ions. Solutions were changed daily.

Wander and Sites (1956)

NO_3-N

	NO_3	H_2PO_4	SO_4	Cl	Total
K		0.33	1.80		2.13
Na	14.30				14.30
Ca			10.00		10.00
Mg			5.70		5.70
NH_4					
H		0.67			0.67
Total	14.30	1.00	17.50		32.80

NH_4-N

	NO_3	H_2PO_4	SO_4	Cl	Total
K		0.33	1.80		2.13
Na					
Ca			10.00		10.00
Mg			5.70		5.70
NH_4			14.30		14.30
H		0.67			0.67
Total		1.00	31.80		32.80

Sulphate was varied, Cl completely excluded and a large addition of Na was made to the NO_3 series only. When required, pH was adjusted with H_2SO_4 and $Ca(OH)_2$, and solutions were periodically replaced. The solubility product of $CaSO_4$ was exceeded by more than 90% in the NH_4 solutions, accentuated by the use of $Ca(OH)_2$ for pH control. For this reason, and because of the large variation in Na between treatments, the formulae are far from ideal for any study of nutrient uptake.

[illegible] (196?)

NO_3-N

	NO_3	PO_4	SO_4	Cl	Total
K		4.00		0.50	4.50
Na		1.00			1.00
Ca	10.00				10.00
Mg			3.75		3.75
NH_4					
H		3.00 +2.00			[illegible]
Total	10.00	15.00	3.75	0.50	29.25

NH_4-N

	NO_3	PO_4	SO_4	Cl	Total
K		4.00		0.50	4.50
Na		1.00			1.00
Ca			10.00		10.00
Mg			3.75		3.75
NH_4			10.00		10.00
H		3.00 +2.00			10.00
Total		15.00	23.75	0.50	39.25

Only SO_4 was varied between treatments. Solutions were periodically renewed and pH stabilised by titration with $Ca(OH)_2$ and H_2SO_4. The content of Ca and SO_4 in the NH_4 solution was such that the solubility product of $CaSO_4$ was almost exceeded, which may have been accentuated by the use of $Ca(OH)_2$ for titration. This basic technique has been used by others ([illegible], 1963; [illegible] and Leeper, 1965; [illegible], 1966, and others) but the low level of Na and Cl have not always been included.

Cain *et al.* (1962)

NO_3-N

	NO_3	PO_4	SO_4	Cl	Total
K	0.33	0.50 +0.50			1.33
Na				0.05	0.05
Ca	1.00		2.00	1.00	4.00
Mg	0.66		2.00		2.66
NH_4					
H		1.00 +0.25			1.25
Total	2.00	2.25	4.00	1.05	9.30

NH_4-N

	NO_3	PO_4	SO_4	Cl	Total
K		0.17 +0.50			0.67
Na				0.05	0.05
Ca			1.33	0.66	2.00
Mg			1.33		1.33
NH_4		0.33	1.33	0.33	2.00
H		0.25 +0.66 +0.33			1.25
Total		2.25	4.00	1.05	7.30

All major cations, except Na, have been halved in the NH_4 solution. Mono- and dibasic phosphates have been used to impart some buffering capacity to solutions. Doubling the ratio of Na to other cations in the NO_3 series seems unwise in view of the marked antagonism between Na and other cations, and its synergism with NO_3 (II, D, 5, (c)). As the concentration of major cations in the NO_3 series is below that required to provide for maximum absorption by ryegrass in static cultures (II, C, 1, (c)), the fact that it is halved for all cations, except Na, in the NH_4 solution could result in a reduced cation uptake, due simply to reduced availability.

(d) Discussion

The variation of NH_4 only between treatments would appear to be least objectionable of the many changes which can be made to supply either form of N in culture solutions. Its low order of interaction during the uptake of other elements and its sluggish increase in foliar content with increasing external concentration, have been discussed in respect to ryegrass (II, D, 5, (e) and II, C, 1, (c)).

The upper limit to the concentration of elements in static culture solutions, with due respect to the solubility products of Ca salts, is probably set by osmotic pressure. Hewitt (1952) concluded that osmotic effects may interfere above 1.0 atmospheres. All the examples cited lie within this limit; the NH_4 series of Thornton (1960) had an osmotic pressure of ca. 0.7 atms. Obviously then, culture solutions cannot be formulated to provide all elements in excess of the concentrations known to be insufficient to satisfy

ammonium uptake by perennial ryegrass in static culture (II, 3, I, (c)).

Whether to include Cl in culture solutions is problematical. If included, it has to be supplied at equal rates to both solutions because of its marked synergism with metallic cations during uptake by ryegrass (II, 1, 5, (c), (iii)). If included in small amounts in both series (Clouston, 1960) any real treatment effect may be masked by its low availability, uptake being regulated by supply rather than ionic interaction. If high levels are included, an already unnatural medium becomes less natural, as high levels of Cl would not be expected under field conditions, except in saline soils. In view of this, and because Cl has been shown to be highly antagonistic with NO_3 while its relationship with NH_4 is unknown, it is best omitted except for trace quantities to satisfy plant physiological requirements.

Attention to stabilise pH becomes much more important in solution culture. As the media are generally only very slightly buffered, the pH moves rapidly and becomes confounded with N form. Solutions are normally titrated to some nominated pH, so that the NO_3 series is generally alkaline overall and the NH_4 series acidic, in relation to the nominated pH, as illustrated by the following data from Lindy (1966). The NO_3 solutions were titrated periodically from pH ca. 6.5 back to 5.5 with H_2SO_4. The NH_4 series were titrated at intervals from pH ca. 4.0 back up to pH 5.5. There was probably more than one pH unit difference between the solutions, for most of the experimental period. Insofar as that external pH may affect the results obtained (II, 2, 4, (f), (iv)) the effects of N form and pH were not separated.

Next to using flowing cultures, the nearest that the experimenter can get to completely eliminating this variable, is to allow both series of solutions to fluctuate between limits of pH. This technique is described in a later section (III, 7). The use of high concentrations of soluble buffers to prevent any pH movement may adversely affect plants (Street and Sheat, 1958).

4 CONCLUSIONS

Ideally, in the type of work under consideration, an experiment should be designed to include the many possible variables at different levels, and by suitable statistical treatment they should either be eliminated or a measure of their contribution to the results obtained. Such an approach is probably beyond the ability of a single experimenter in view of the volume of work involved. The compromise is to conduct more than one experiment under differing conditions, and to minimise the undesirable aspects of the technique. By comparison of the overall pattern of results in the light of other published work, systematic effects should be evident.

CHAPTER III

EXPERIMENTS

1 GLASSHOUSE TRIALS

(a) Introduction

Three glasshouse experiments were undertaken in this study. Of these, two were conducted in a soil medium and are referred to as experiment I and experiment II. Experiment III was carried out using solution cultures.

Preliminary discussions raised the question as to whether it would be better to pursue this work out-of-doors, because:

(i) the results would be more directly applicable to field conditions (personal comm., I. J. Mitchell); and

(ii) there was insufficient control over temperature and light in the available glasshouse.

These disadvantages were weighed against the problems involved with working in the field. The major objection to outside experimentation was the extreme likelihood of extensive but unknown losses of NO_3 by leaching in one of the treatments, which would have led to problems of interpretation. Even had arrangements been made to have experimental pots under cover during rain, the results would still not have been directly applicable to field conditions (Butler et al., 1962). It was decided to confine the experiments to the glasshouse because greater control could be exerted over the treatments, although it was realised that this approach would limit extrapolation of the results obtained.

The experimental design, which was basically the same for all experiments, is summarised in the following diagram.

Treatment \ Block	I	II	. . . . .	N
Ryegrass (W_3)	A	B	. . . . .	C
Ryegrass (W_4)	A	B	. . . . .	C
Sweet vernal (V_3)	a	b	. . . . .	c
Sweet vernal (V_4)	a	b	. . . . .	c

A ..... J = clonal material from 10 ryegrass plants

a ..... j = clonal material from 10 sweet vernal plants

Vegetative material from a single ryegrass and sweet vernal plant was used within each block to reduce within-block variance. Different plants were used between blocks to increase the range of applicability of results. The same 10 genotypes of each species were utilized for the 10 replications in all three experiments.

This layout allowed subsequent statistical analyses to test the significance of:

(i) nitrogen form effects;

(ii) species differences; and

(iii) interaction between these;

for each experiment. The three experiments could be considered as three replications of 10 ryegrass genotypes (A - J) and 10 sweet vernal genotypes (a - j), receiving either NO_3 or NH_4. This allowed subsequent statistical tests among the plants used for genetic differences in the characters studied.

(b) Experiment I

Plants were grown in soil with or without addition of the nitrification inhibitor, "N-Serve". This was a 2 x 2 factorial experiment with 10 replications, comparing the two N forms over both species.

(c) Experiment II

The design was exactly the same as that for experiment I, except that additional mineral N was supplied with fertilisers. "N-Serve" was included in the NH_4 treatment.

(d) Experiment III

Nutrient solutions were formulated to supply equal amounts of N:

(i) wholly as NO_3;

(ii) half as NO_3 and half as NH_4; and

(iii) wholly as NH_4.

The imposition of a further N form treatment gave a 3 x 2 factorial experiment replicated 10 times. The two N forms were compared singly, and in conjunction, over both species. "N-Serve" was included in all treatments.

2 GRASSES

'Grasslands Ariki' ryegrass (Lolium (multiflorum x perenne) perenne Corkill, 1964) and sweet vernal (Anthoxanthum odoratum L.) were chosen, as indicators of high and low fertility conditions respectively ([illegible], 196[illegible]). Ryegrass tillers were taken at random from the F_1 generation following an open pollination of 8 parent plants in a glasshouse at Grasslands Division, Palmerston

North. Sweet vernal tillers were taken from plants growing in divergent sites nearby.

Single tillers of both species were propagated in a sand-frost bed, with an electronic leaf giving automatic mist control. Sweet vernal tillers were severely infected with rust, but this condition was successfully controlled by the use of "[illegible]" ([illegible] 11-22).

When rooted, tillers were transferred to flats containing clean sand. With a full nutrient solution periodically supplied, these plants grew well. Single tillers were placed in the propagating pit on 11 March, 1964 and the first experiment was planted on 6 April. By this time young plants were apparently rooting and growing vigorously with 5-10 tillers each.

3 Soil

Topsoil from an experimental area at the Massey University sheepfarm was used for the pot experiments where soil was required in the growth medium (experiments I and II). The area had been under grazed pasture with a long history (25 years) of heavy fertilizer application, as follows:

superphosphate	at	4 cwt/ac./an. ;
lime	at	5 cwt/ac./an. ; and
muriate of potash	at	1 cwt/ac./an.

The soil is a yellow-grey earth (Tokomaru silt loam) and a comprehensive chemical analysis was carried out on soil from this site in [illegible] ([illegible], unpublished). The following data for

the 0-3" horizon have been taken from these figures.

cation exchange capacity (CEC)	13.3 m.e.% ;
total exchangeable bases (TEB)	14.2 m.e.% ;
exchangeable calcium	11.1 m.e.% ;
calcium as a proportion of CEC	78% ;
carbon	4.0% ;
total nitrogen	0.31% ;
carbon/nitrogen ratio (C/N)	13 :
organic matter (carbon x 1.724)	6.9% ; and
pH	6.2

The area had been subjected to the same management practices subsequent to the above determinations. It is unlikely that the values had changed substantially by the time of sampling in 1974.

An area was trenched to about 3" depth to provide the required amount of soil, which was air dried and screened. Superphosphate in amount equivalent to 3 cwt/3" ac. was applied and mixed evenly into the sifted soil. Perlite was used as a diluent to prevent the development of undesirable physical conditions in the potted soil. Equal volumes of soil and perlite were mixed by stirring and the mixture passed through a soil divider several times for adequate blending.

In the following content, the mixture of soil and perlite is generally referred to as soil.

4. PREPARATION OF SOIL AND FILTERED SOLUTIONS

(a) General

Experimental problems associated with reversal of the ionic

charge of mineral N have been discussed (II, C). The major issues are differences between treatments in:

(i) pH ; and

(ii) the supply of nutrients, other than mineral nitrogen.

It was concluded that these variables cannot be completely eliminated, as they are an integral part of a study of this nature. In the following experiments the writer endeavoured to minimise, as far as possible, any effects which could arise from these confounded features.

(b) Experiment I

Analytical data for the soil used have been given (III, 3). It had a high organic matter content, low C/N ratio and a pH of 6.3 Under the experimental conditions of temperature, moisture and aeration a comparatively rapid mineralization of organic N was expected. In this experiment, reliance was placed on the natural processes of mineralization and subsequent nitrification of released NH_4 to NO_3, to provide the NO_3 treatment. In the NH_4 treatment "N-Serve" was added to prevent the biological oxidation of released ammonium nitrogen (II, C, 2, (b)).

"N-Serve" was added to the soil at 10 ppm. (w/w for the soil and perlite mixture) in the NH_4 treatment. It was dissolved in acetone and applied with an atomiser to the moistened soil in a clean concrete mixer. A control amount of acetone only was similarly applied to the soil of the NO_3 treatment. Bulk soils, after thorough mixing, were held in airtight containers until potting and planting the next day.

The foregoing experiment may be considered as representative

of field conditions covering a wide range in the activity of nitrifying organisms. At best, the N forms can only be described as "predominantly NO_3" and "predominantly NH_4". During the release of organic N, an unknown amount could be taken up as NH_4, prior to its oxidation, by plants of the NO_3 series. Similarly, it cannot be said with certainty that over the experimental period (78 days) nitrification was completely inhibited by the addition of "N-Serve" in the NH_4 series. However, by conducting two further experiments of a similar nature, the writer envisaged an indirect measure of the effectiveness of the N treatments, by comparison of the pattern of results obtained among the experiments as a whole.

The soil used had a comparatively high CEC and was considered likely to provide effective buffering against pH shift in the external medium. As no mineral N was added, this experiment was not complicated by the inclusion of accompanying anions and cations, which must inevitably be added with NH_4 and NO_3 respectively. It was felt that the confounded influences listed at the beginning of this section had been minimised.

(c) Experiment II

In contrast to the previous experiment mineral N was supplied to the soil as NH_4 and NO_3 salts. This allowed plants to be established in pots prior to the imposition of N treatments. Herbage was cut and discarded several times to induce a low N status both in the soils and the plants, to lessen the significance of any contribution to mineral N from mineralisation of organic matter, over the experimental period.

When treatments were imposed, plants of the NO_3 series received a dressing of $Ca(NO_3)_2$, equivalent to 3 cwt/3" ac. for the *in situ* soil (180 mg. N/pot). The NH_4 series received an equivalent amount of N as $(NH_4)_2SO_4$. Fertilizers were dissolved in water prior to application. "N-Serve" was dissolved in acetone and added to the $(NH_4)_2SO_4$ solution to give 10 ppm. by weight of the potted soil mix or 7.8% by weight of added fertilizer N. It was found that addition of a small quantity of a commercial wetting agent facilitated the dispersion of "N-Serve", for at this high concentration its maximum solubility in water was greatly exceeded. Control amounts of acetone and wetting agent were included in the $Ca(NO_3)_2$ solution. These solutions were injected into the plastic pots using an automatic hypodermic syringe. With a 1" needle, 5 ml. portions were injected into the soil through the mid-point of each of the 4 sides of every pot. Besides placing the fertilizers right into the root zone, this technique prevented loss of the added "N-Serve" by volatalisation. Solutions were applied on 15 August 1964 and again on 27 September. Eight control pots received identical experimental treatments, but contained no plants. Mineral N analyses were to be done on these soils at the end of the experiment to obtain a picture of the N regime, as a measure of the effectiveness of the experimental technique.

As in experiment I, the natural buffering capacity of the soil used was relied on to minimise any movement in external pH.

The use of fertilizers raised the problem of selecting a suitable cation and anion to accompany NO_3 and NH_4 respectively. For the NO_3 treatment, $Ca(NO_3)_2$ was the logical choice. Foliar

Ca content in ryegrass is less subject to change with changes in external concentration, than is the content of any other major metallic cation (II, C, 1, (c)). Further, Ca comprised almost 80% of TEB in this soil and its addition would have resulted in a much smaller proportionate change in external concentration than would have been the case with addition of any of the alternative accompanying cations. In the NH_4 series, $(NH_4)_2SO_4$ was used. The sluggish participation of the sulphate ion in uptake and metabolism by ryegrass has been reviewed (II, B, 5, (e) and II, C, 1, (c)). No serious interaction with soil chemical or biological processes could be foreseen for $(NH_4)_2SO_4$, especially as nitrification was to be inhibited. The heavy application of superphosphate used (III, 3) reduced the proportional difference in S availability between the two treatments. No subsequent leaching occurred during the experiment so that S status should have been high in all cases.

(d) Solution Culture: Experiment III

This technique was used in a final experiment because it offered several advantages over the two previous experiments. It allowed the provision of N wholly in either form and in combination. The absence of colloidal properties in the medium meant that when the two forms were applied at equal rates, they were equally available. "N-Serve" could also be included in all treatments, whereas previously it had been confounded with NH_4.

The formulation of culture solutions to supply NO_3 and NH_4 has been discussed and criticised (II, C, 3). It was concluded that variation in sulphate content among treatments was more desirable than the numerous alternatives, as facilities for con-

tinually replaced solution cultures were not available.

The following formulae were used (all concentrations in me./l.).

$\underline{NO_3\text{-}N}$

	NO_3	PO_4	SO_4	Cl	Total
K		0.9 +0.2	2.0		3.1
Na					-
Ca	3.0				3.0
Mg	2.0				2.0
NH_4					-
H		1.8 +0.1			1.9
Total	5.0	3.0	2.0	-	10.0

Osmotic pressure, ca.0.28 atmos.

$\underline{NH_4\text{-}N}$

	NO_3	PO_4	SO_4	Cl	Total
K		0.9 +0.2	2.0		3.1
Na					-
Ca			3.0		3.0
Mg			2.0		2.0
NH_4			5.0		5.0
H		1.8 +0.1			1.9
Total	-	3.0	12.0	-	15.0

Osmotic pressure, ca.0.39 atmos.

$\underline{NH_4NO_3\text{-}N}$

	NO_3	PO_4	SO_4	Cl	Total
K		0.9 +0.2	2.0		3.1
Na					-
Ca			3.0		3.0
Mg			2.0		2.0
NH_4	2.5				2.5
H		1.8 +0.1			1.9
Total	2.5	3.0	7.0	-	12.5

Osmotic pressure, ca.0.34 atmos.

Micronutrients

Boron	0.5 ppm.	as	H_3BO_3
Manganese	0.5 ppm.	as	$MnCl_2.4H_2O$
Zinc	0.05 ppm.	as	$ZnSO_4.7H_2O$
Copper	0.02 ppm.	as	$CuSO_4.5H_2O$
Molybdenum	0.02 ppm.	as	$NaMoO_4.2H_2O$
Ferric iron	5.0 ppm.		
"N-Serve"	0.5 ppm. on alternate days		

All macronutrient salts, except $CaSO_4$, were prepared as single salt, stock solutions of high concentration. All micronutrients, except iron which was prepared separately, were compounded into a single stock solution. Stock solutions were stored in amber glass winchesters, with a few drops of toluene added to each. There was no indication of any microbial growth in these storage containers. Separate siphons and measuring cylinders were used for each salt solution during preparation of culture solutions. Because of the low solubility of $CaSO_4$, there was little point in its preparation as a stock solution.

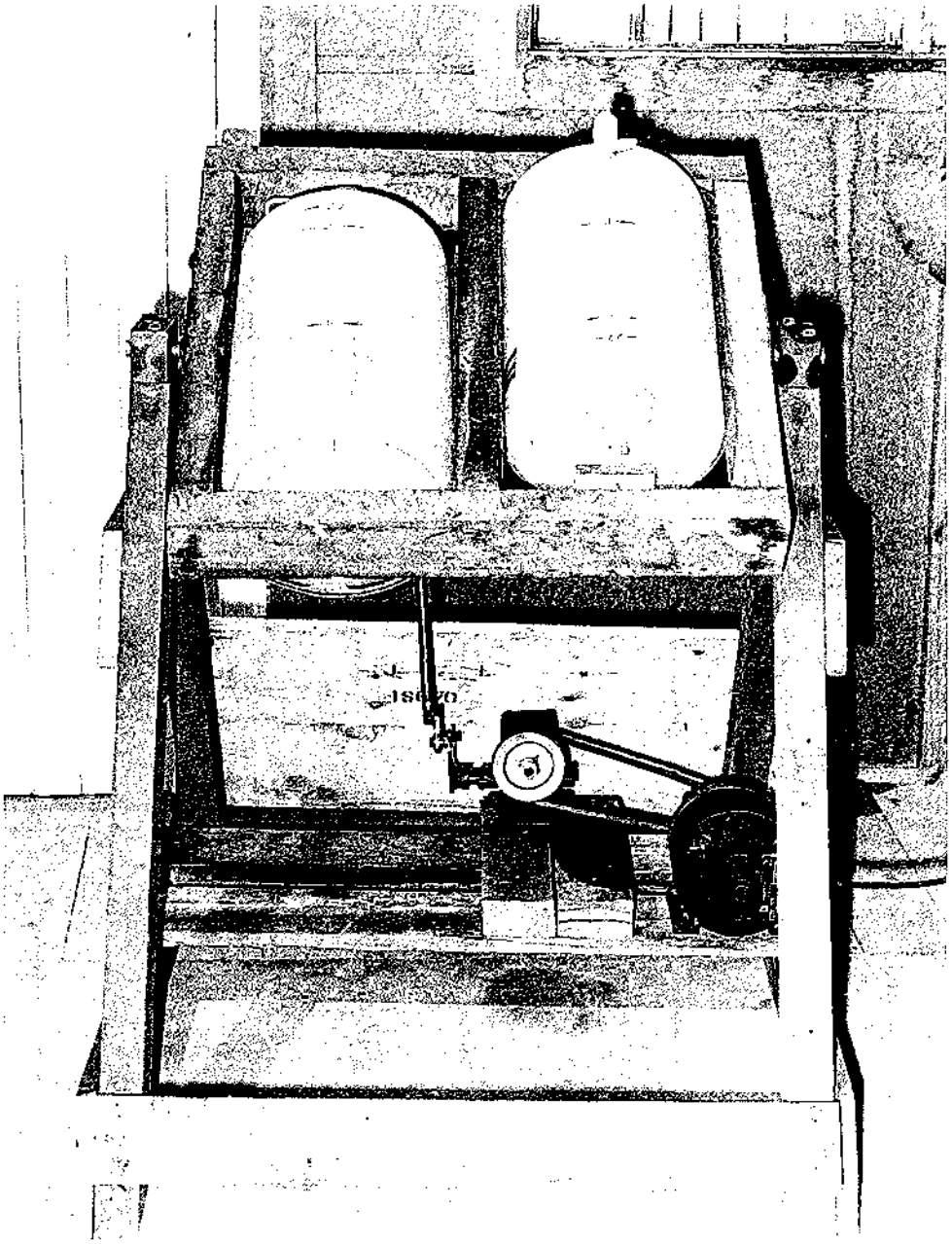
Plate 1 shows the shaker, designed and built by the author, to facilitate the preparation of culture solutions. The required amounts of $CaSO_4.2H_2O$ and "N-Serve" were added to 20 l. of water in 25 l. aspirators, containing a few pebbles. With a continual rocking action provided by the shaker, these chemicals dissolved overnight. This was considered preferable to the alternative addition of $Ca(OH)_2$ and H_2SO_4 to water. After addition of the other nutrient salts to these solutions the aspirators were returned to the shaker for thorough mixing prior to application.

L.R. grade chemicals were used in the preparation of stock solutions of the macronutrients. The micronutrient solution was prepared from A.R. chemicals, except that the iron source was a commerical iron chelate containing 5% iron, all in the ferric form. All solutions, including the diluted culture solutions, were prepared with distilled water. The final pH of the dilute solutions was 6.2.

No satisfactory equipment for the aeration of culture

PLATE 1 THE SHAKER USED IN

THE PREPARATION OF CULTURE SOLUTIONS



solutions was available. It was therefore decided to use a "sand" culture technique with vermiculite as the supporting medium. Solutions were applied daily at 1 l./plant.

In selecting this "three-salt" solution (Hewitt, 1952) the following points were considered. Chloride was omitted as high Cl levels are not normally encountered in the field. Its inclusion in the minor elements and as an impurity in the L.R. grade macronutrients would have provided adequately for its participation in physiological processes. There appeared no more justification for the inclusion of sodium than for that of any other non-essential element. To compensate for its exclusion, a comparatively high K/Ca ratio was used (loc.cit.). Phosphate was used at a high level in all solutions. The mono- and dibasic potassium phosphates employed as the phosphate source imparted some slight buffering capacity to these media. Osmotic pressures were well below the limit of 1.0 atmos., above which it has been suggested that osmotic effects may interfere (loc.cit.).

For all treatments the concentration of every element, other than S, was kept constant. Depletion of the external solution had to be considered. There was sufficient distilled water to supply prepared solutions at the rate of 1 l./pot daily. From previous work, the author was able to calculate the probable daily uptake for each major element. At the lowest S concentration (NO_3-N) it was estimated that 3-5% of the S provided would be utilized. Data for ryegrass grown in solution culture, presented by De Wit et al., (1963), showed a maximum increase of 20% in foliar S content corresponding to a ninefold increase in external SO_4 concentration. It was considered that depletion should not have markedly affected S

uptake at the lowest concentration selected (2 me./l.). As the concentration of other elements was constant among treatments the question of depletion was less important. If there were large differences between treatments in the uptake of any elements, then any limitation to absorption caused by such depletion would simply mitigate against the experimental findings. It would not result in qualitatively false conclusions unless uptake were so depressed as to cause deficiency and abnormal metabolism. The only element for which such a situation has been reported is Ca during NH_4 absorption (II, B, 3, (v)). Data were not available to show at what point external concentration ceased to limit uptake by ryegrass. For this reason levels were chosen which would supply all elements at rates far in excess of estimated daily requirements; thus, for example, Ca was provided at a concentration such that estimated uptake would have been about 2.5% of the Ca available.

As the aim of this experiment was to obtain information on the plant physiological responses to different N forms it was essential to avoid any interfering effect arising from a limited supply of any nutrient. Under the conditions of the experiment all nutrients were supplied at luxury levels.

5 PLANTING

The arrangement of genotypes within and among experiments has been discussed (III, 1).

(a) Experiments I and II

For both soil experiments, "Ace" 5" plastic pots were used. Filled to about 0.5 cm. from the top, their capacity was 1.3 l.

(0.87 Kg. of the soil/perlite mixture on an oven dry basis). Because of the similarity between these experiments, they are considered together.

Prior to filling, a splayed, glass wool wick was inserted in the drainage hole at the bottom of each pot. Pots were then filled with soil which was shaken down firmly.

Plants were removed from the flats (III, 2) and the tops and roots were washed thoroughly and trimmed. They were immediately planted singly into the potted soils. Care was taken to ensure uniformity in tiller number and size of plants, as far as was reasonably possible.

After planting, about 0.5 cm. of coarse, washed sand was layered on to the surface of each pot to prevent soil contamination of foliage.

(b) Experiment III

Liver tins of 4.5 l. capacity were used. These were bituminised on the inner surface with drainage provided by holes at the bottom. Vermiculite was pressed firmly into the tins over a 1" layer of gravel, to ensure free drainage.

By this stage, the originally propagated plants had been maintained in the flats for 10 months. They lacked vigour and were not very uniform. Rather than use them, plants were taken from experiment II which had concluded. Because of the possibility of pretreatment effects on subsequent N metabolism (Lycklama, 1963) material from only one N form treatment was used. Each of the 10 ryegrass and sweet vernal plants was broken into three fairly uniform parts of about 20 tillers. After thorough washing, roots and tops were trimmed. These clonal subdivisions were planted singly

into the vermiculite. A surface layer of about 1 cm. of coarse, washed sand followed by 2 cm. of pea metal was added after planting, to prevent the vermiculite from floating when solutions were applied.

Fresh vermiculite normally confers an alkaline reaction on water, but the free bases involved are readily removed by leaching with water (pers.comm., C. V. Fife). Accordingly pots were leached soon after planting and were then maintained under an electronic leaf for three weeks. Every pot received a common maintenance dressing of 50 ml. of NH_4NO_3 nutrient solution every second day. When removed to the glasshouse the plants were growing vigorously. Both species were tillering, ryegrass more prolifically than sweet vernal, which produced a considerable regrowth from cut stems.

An attempt was made to establish uniform microbial populations in all pots to remove the risk of any effects which could arise from qualitative differences in micropopulations (Humphreys Jones and Waid, 1963). Leachate (100 ml.) was collected from each of the 60 containers. Several fresh soil cores from pasture sites were blended with water and added to the bulked leachate. The whole was thoroughly mixed and made up to 60 l. "N-Serve" was added at 5 ppm. in solution. The inoculum was then applied at the rate of 1 l./pot to all pots, prior to the start of the experimental treatments.

6 CONDUCT OF EXPERIMENTS

(a) Diary

The following is a calendar of significant dates for the three experiments.

		Time (days) total	Time (days) from last cut
Experiment I			
6/3/64	treatments started	0	0
20/3/64	first cut discarded	14	14
12/4/64	second cut harvested	37	23
27/4/64	third cut harvested	52	15
23/5/64	fourth cut harvested	78	26
Experiment II			
15/8/64	treatments started	0	0
29/8/64	first cut discarded	14	14
22/9/64	second cut harvested	38	24
31/10/64	third cut harvested	77	39
Experiment III			
1/12/64	treatments started	0	0
15/12/64	first cut discarded	14	14
6/1/65	second cut harvested	36	22
14/1/65	third cut harvested	44	8

(b) Experiments I and II

Because of their similarity, they are again considered together. Plate 2 shows experiment II in progress.

A sand bench was used for sub-irrigation in both soil experiments. Washed river sand, 2" deep, was placed over sheet plastic to form a level bed. Tap water was applied daily to the sand bench through a rubber hose, into which had been inserted hypodermic needles of standard gauge, to give a slow, even distribution of water. Water application varied with conditions. The

PLATE 2 EXPERIMENT II IN PROGRESS

sand bed was kept moist, but below saturation. Contact between the sand and potted soil was facilitated by the presence of a glass wool wick inserted in each drainage hole.

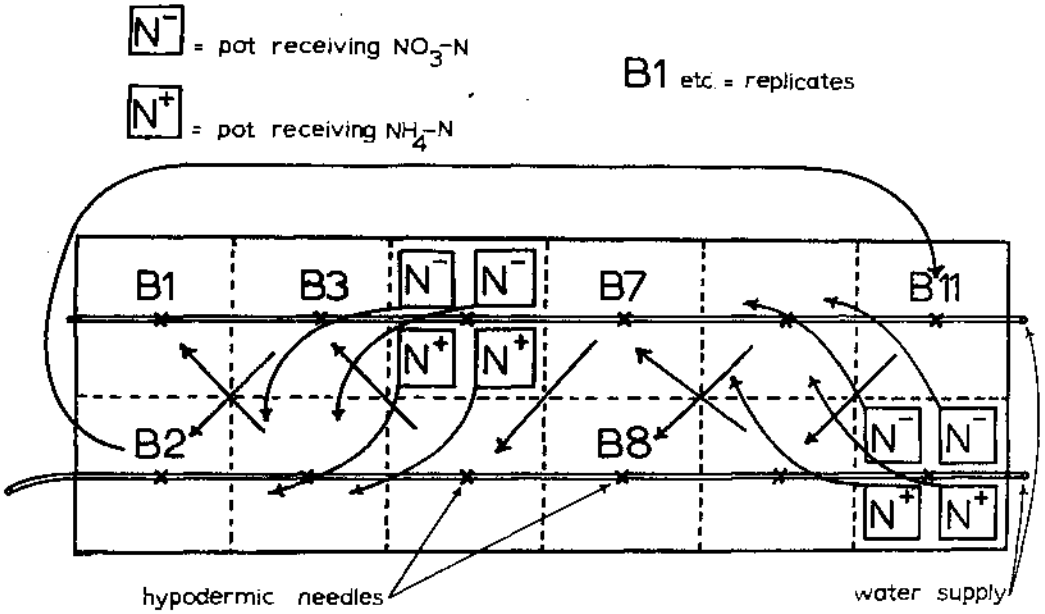
Pots were placed on the sand bench in blocks and moved every day. The pattern of movement was designed to minimise any lack of uniformity in the environment, both along and across the bench. Water application was arranged for water to move directly to each pot. Blocks were rotated systematically so that any pot was always placed in a position vacated by one of the same treatment. In this way, movement of mineral nitrogen between NO_3 and NH_4 pots was precluded and the repopulation of "N-Serve" treated soils with nitrifiers minimised. Watering arrangements and the pattern of rotation of blocks are illustrated in plate 3 and figure 4.

It was realised that the continual upward movement of moisture with this irrigation technique would result in the accumulation of mobile salts at the soil surface. To overcome this, tap water was judiciously applied to the surface of the potted soils. It was found that 50 ml./pot could be applied every two days without any apparent leaching. Water was applied less frequently when conditions were cool, and always after the sand bed had been irrigated, to reduce the likelihood of moisture movement from the pots into the sand. Control pots were divided into two series, one receiving this treatment, the other not. Results are reported on differences in the vertical distribution of mineral N between these two sub-treatments.

Sprays were applied periodically throughout these experi-

PLATE 3 IRRIGATION IN THE SOIL EXPERIMENTS

FIGURE 4 THE SYSTEMATIC MOVEMENT OF POTS IN THE SOIL EXPERIMENTS



ments. As a preventive measure against rust, "Maneb" was applied a day or two after each defoliation and only a trace of rust was noticed. The intermittent appearance of insect pests necessitated the use of nicotine sulphate as a successful eradicant.

No attempt was made to control lighting conditions. However, heating was supplied during cold periods encountered in the later stages of experiment I and during experiment II. An electric heater, with a fan which circulated warmed air, was centrally placed in the glasshouse. It was thermostatically controlled at 60°F, but frequently did not maintain this temperature. On sunny days, the problem was to keep temperatures down. As much ventilation as possible was provided, and the glasshouse was hosed inside two or three times during the hotter period of the day.

(c) Experiment III

Formulation and preparation of the culture solutions has been discussed (III, 4, (d)). A limit to the volume of solution which could be applied to each plant was imposed by the supply of distilled water available.

Vermiculite had been packed firmly into the tins to give a slow and even percolation of added nutrient solutions. One l. of the relevant solution was poured rapidly on to the top of each pot every morning. The surface layer of sand and pea metal above the vermiculite acted as a "reservoir" during the slower movement of the added solutions through the medium. After overnight drainage, unplanted tins contained an average 880 ml. of solution; most of the solution remaining from the previous day should therefore have been displaced on addition of the 1,000 ml. of fresh

solution every morning.

Plates 4 and 5 show aspects of this experiment. The first photograph was taken shortly after the plants had been defoliated. Pots in the foreground are standing on plastic buckets which were used to collect displaced solutions. These leachates were analysed immediately after collection, in an adjoining laboratory. Measurements were made on pH, and tests conducted for any nitrification in the NH_4 solutions. The same experiment, immediately prior to harvest, is shown in the second photograph.

Pots were arranged in blocks along a corrugated iron bench. As in the other experiments, pots were systematically moved daily to reduce any possible environmental effects. Blocks were rotated along, and treatments within blocks across, the bench. The spraying procedure was the same as that described for the two soil experiments.

High glasshouse temperatures were encountered during the experimental period (1 December 1964 - 14 January 1965). As much ventilation as possible was provided and the interior of the glasshouse was hosed two or three times on every bright day.

7 STATIC CULTURE EXPERIMENT

A static culture experiment was attempted following the conclusion of experiment II, but prior to the start of experiment III. The technique is reported in some detail because it embraced an original method to overcome confounding the effects of N form with pH shifts in the external medium, during static culture experiments. To avoid any confusion with the three experiments

PLATE 4 EXPERIMENT III IN PROGRESS

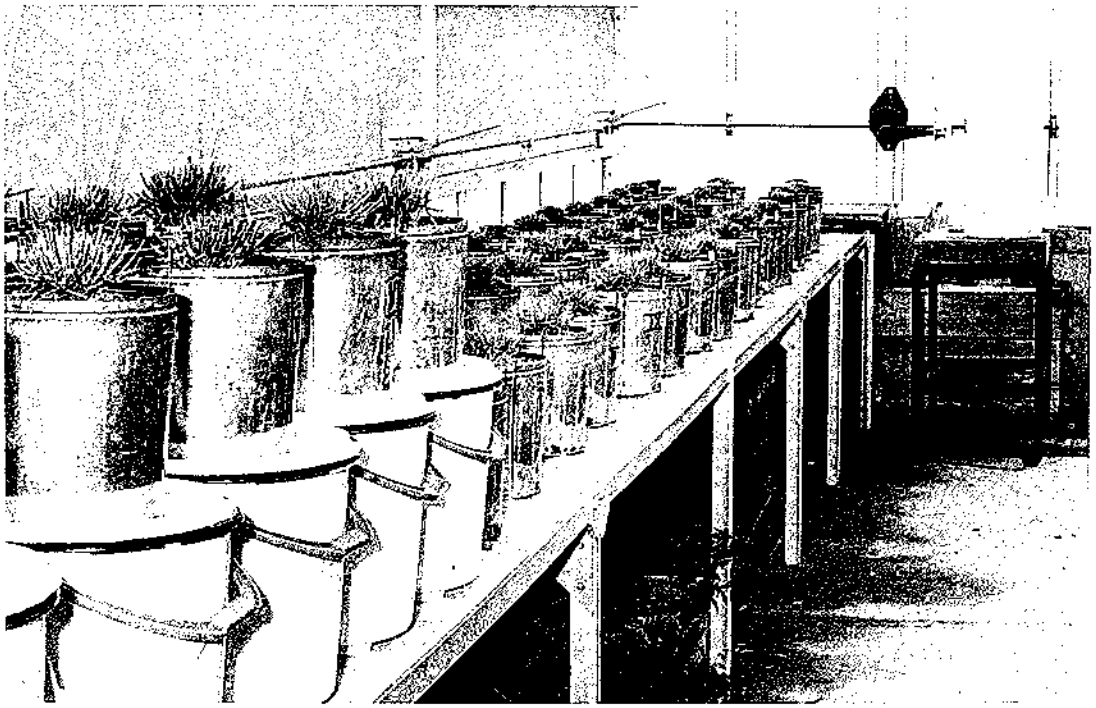
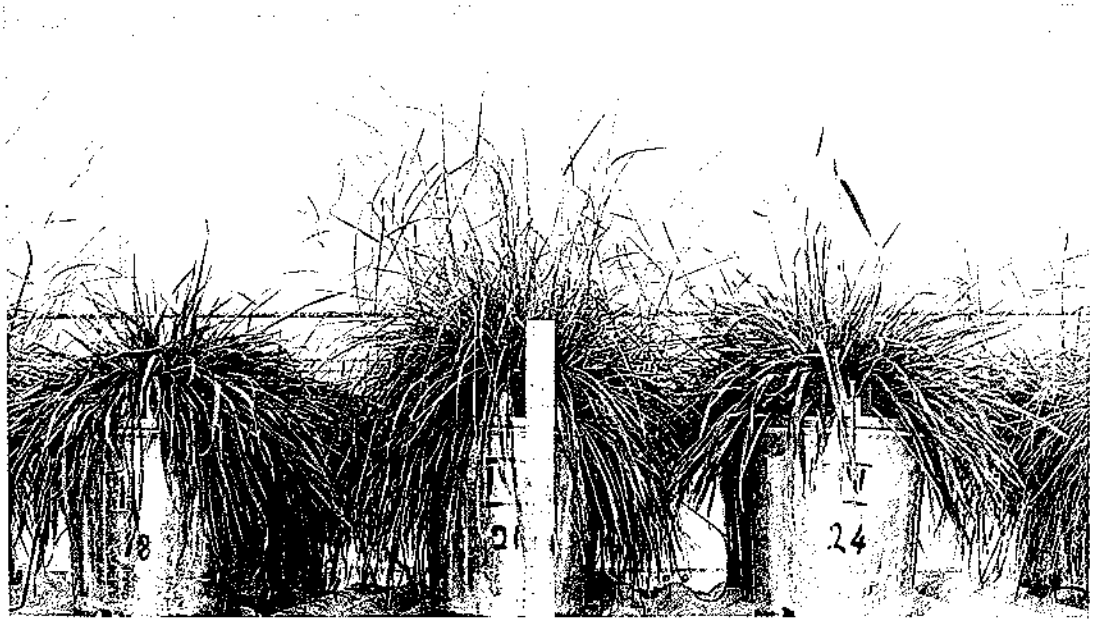


PLATE 5 PLANTS PRIOR TO HARVEST



which were concluded, this experiment is considered separately. It must be stated quite clearly that no analytical results of herbage arose from this experiment, which had to be abandoned owing to circumstances beyond the author's control.

The composition of nutrient solutions was the same as that described for experiment III, except that "N-Serve" was added at 1 ppm. when the solutions were prepared. Hewitt (1952) records the extensive use of tap water to prepare culture solutions in a variety of studies. An adequate supply of distilled water was not available at that stage and tap water was used in the preparation of these solutions. As the study concerned major elements and any salts in the tap water were common to all treatments, no serious objection to its use was visualised.

The experiment was conducted using 10 l. plastic buckets bituminised on the inner surface, each containing 9 l. of solution. Ryegrass and sweet vernal clones were "planted" singly through a hole of about 1" diameter in the centre of each of the inverted plastic lids and secured with non-absorbent cotton wool. Roots were completely immersed in the solutions. The experimental design was the same as that described for the other experiments, and containers were moved systematically to minimise any possible environmental variation. Continuous aeration was supplied by a pump which delivered a constant head of air to a pressure tubing supply line. This was tapped for each container by insertion of a hypodermic needle into the supply hose. Air was piped to each bucket through a standardised capillary tube and a stream of fine bubbles was obtained with a fish bowl aerator located at the bottom

of each container.

Plates 6 and 7 show aspects of this experiment. In plate 6 one plant has been removed and placed temporarily over an empty container. Electrodes from the portable pH meter have been inserted into the solution prior to titration. The apparatus for titration is located, with the pH meter, on a trolley. Plate 7 shows an established ryegrass plant. Growth was vigorous and healthy. Exposed to view is one of the fish bowl aerators.

Culture solutions were prepared with an original pH of 6.4 (found by experiment). Equivalent amounts of HNO_3 or NH_4OH were added to bring the pH of NO_3 solutions to 6.0 and NH_4 solutions to pH 7.0. The external alkaline effect in the NO_3 series should have resulted in a pH movement from the initial 6.0 up to 7.0, when it was proposed to titrate the solutions back to 6.0 with HNO_3. Conversely, with NH_4, the external acidic effect should have lowered the pH from its initial 7.0 to 6.0, at which point it would have been titrated back to 7.0 using NH_4OH. In this way, pH shift was to be confined to the limits 6.0 - 7.0 in both treatments.

This technique had previously been used by the author for an experiment with perennial ryegras, prior to commencement of the present study. It was found that very similar amounts of HNO_3 and NH_4OH were required to maintain pH within these limits. The following data are the mean of two replicates for observations made over a 16 day period:

$\underline{NO_3\text{-}N}$	$\underline{NH_4\text{-}N}$
0.27 me. HNO_3/day/l.	0.30 me. NH_4OH/day/l.

It was proposed to completely replace the 9 l. of culture solution

PLATE 6 TITRATION OF SOLUTIONS IN THE STATIC CULTURE EXPERIMENT

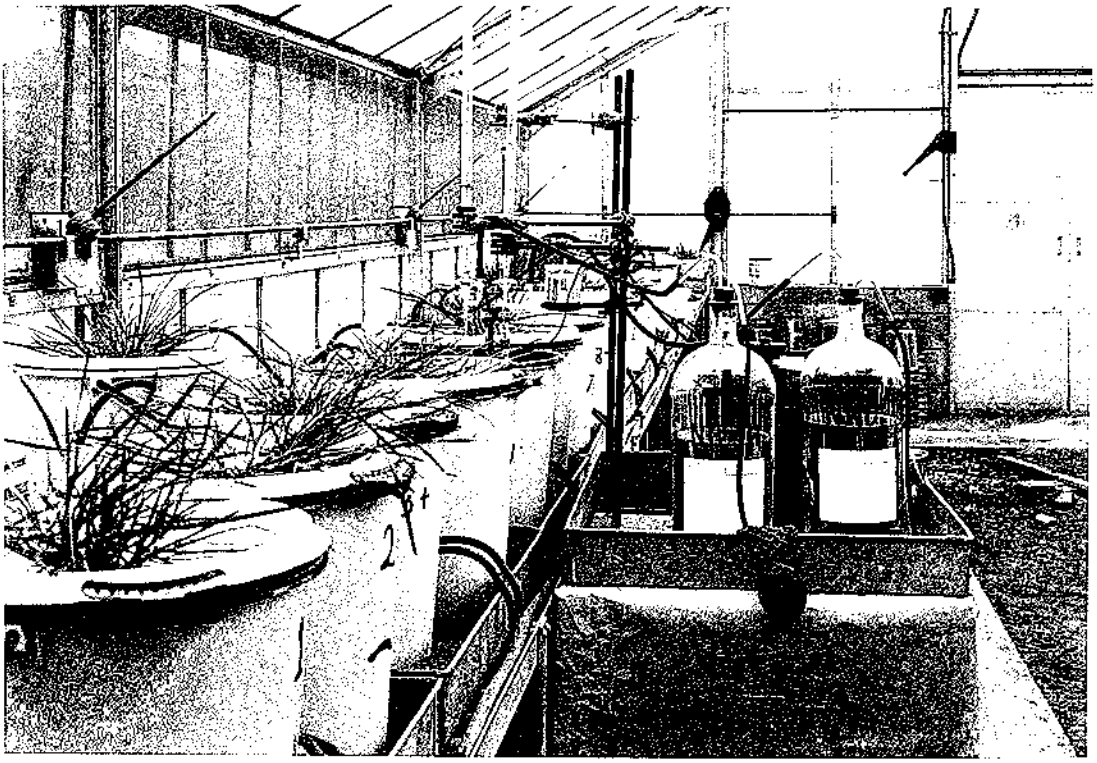


PLATE 7 AN ESTABLISHED RYEGRASS PLANT

every ninth day. Based on the above figures the difference in N addition between treatments would have been only ± 0.14 me. N/l. over 9 days, or ± 2.8% of the 5 me. N/l. originally added. The use of HNO_3 and NH_4OH for titration would have decreased the extent of N depletion and overcome the problem of introducing other elements, as has been the frequent case where $Ca(OH)_2$ has been used to titrate NH_4 solutions (II, C, 3, (d)).

A large volume of solution with some buffering capacity was provided for each plant, which should have resulted in a comparatively slow pH movement. The unpublished study cited previously had shown that with only 1 l. of solution/plant, pH in the NH_4 series could move from 7.0 to 3.0 in two days even when nitrification was prevented. Solutions were made to volume daily, prior to pH determinations. Measurements of pH were made at least once every day. Records were to have been kept on water usage and the amount of N added by titration, for each container.

Instead of the anticipated increase or decline in solution pH with NO_3 and NH_4 respectively, it was found that the pH of all solutions increased slowly, reaching an equilibrium after about three days. The pH of the NO_3 solutions rose from the initial 6.0 to approximately 7.0; NH_4 solutions from 7.0 to ca. 7.8.

At first it was considered that the culture solutions were being contaminated by entry of atmospheric ammonia in the air stream employed to aerate the solutions; a possible source of such contamination lay in the urea which was being applied daily as a foliar spray in an adjacent glasshouse. However, the same results were observed when a sulphuric acid "scrubber" was placed in series

with the air supply line.

That the pH of solutions reached an equilibrium after about three days of aeration suggested some sort of chemical equilibrium. The next step, therefore, was to conduct the experiment in the presence or absence of a number of variables which may have been responsible for the observed effects, including:

(i) Plants ;

(ii) the bituminous paint used on the inner surface of containers; and

(iii) the fish bowl aerators, which were made of porous ceramic material.

Irrespective of the sub-treatment the same results were observed in this experiment: all solutions became alkaline in relation to their initial pH, over a period of approximately three days. This caused a change in attitude, from considering the possibility of a gain in alkali by the solutions to considering a possible loss of acidity from the solutions. It was considered that if the tap water contained appreciable levels of carbonate/bicarbonate, the observations could have resulted from the establishment of an equilibrium between these ions and atmospheric CO_2. Solutions were prepared with tap water and distilled water, their initial pH being 6.9 and 5.8, respectively. Air was vigorously bubbled through 500 ml. portions of each solution overnight, resulting in an increase in the pH of the solutions prepared from tap water, to 8.0. There was virtually no change in the pH of solutions prepared with distilled water. It was concluded that the observed interference with the experimental technique was, in fact, the result of equili-

bration between the HCO_3 ion in the solutions prepared from tap water and atmospheric CO_2. Subsequent investigation of the Massey University water supply confirmed that it did have a high carbonate/bicarbonate content.

Sufficient distilled water was obtained to conduct the experiment as originally designed, in duplicate. The anticipated results were confirmed over a period of 7 days, after which the observations had to be discontinued because of mechanical failure of the pump supplying air to the solutions. When sufficient distilled water became available to conduct a fully replicated solution culture experiment there was no satisfactory equipment available for the aeration of culture solutions. This technique was abandoned therefore and a "sand" culture method used for experiment III (III, 4, (d)).

8 PREPARATION OF MATERIALS FOR ANALYSES

(a) Plant Material

(i) Harvesting: All harvesting was done in the evening. Experiments were cut by blocks, rather than treatments, to avoid any systematic errors. Plants were always cut to a height of about 1". This left more green material in the sweet vernal stubble than was left on the ryegrass plants.

All plants were trimmed prior to initiation of treatments in each experiment. After 14 days, the regrowth was cut and discarded, to reduce any pretreatment effects. When plants reached an approximate grazing height they were harvested. The material was placed immediately into a forced-air drying oven, where it was

held at 70°C for 16 hours. It was then weighed and placed into plastic bags.

Two or three harvests were taken during the course of each experiment (III, 6, (a)).

(ii) Preparation of samples: The dried material from all cuts of each plant within each experiment was bulked and ground in a Casella Seed Mill over a No. 28 sieve (.021"). Ground material was passed three times through a 2 mm. sieve, and thoroughly mixed. It was allowed to equilibrate with atmospheric moisture before storage in glass bottles.

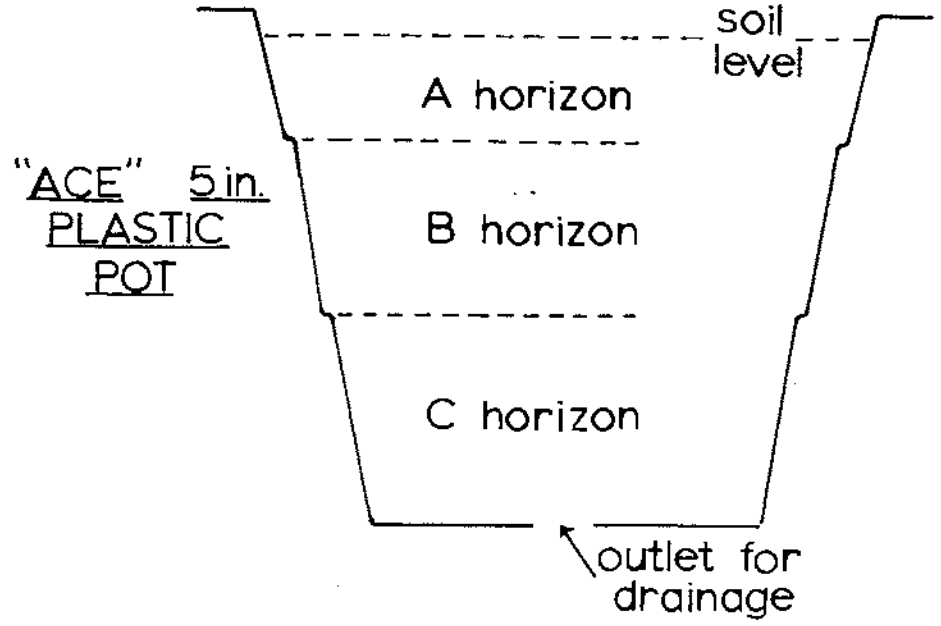
(b) Soil Samples

Control pots were taken directly from the glasshouse to the laboratory for mineral N analyses.

As information was desired not only on the amount and form of mineral N, but also on its vertical distribution, the pots were partitioned into arbitary "horizons", and determinations carried out separately on each. Indentations on the side of the pots provided convenient divisions. Soil from each horizon was mixed thoroughly by sieving and quartering; sub-samples were then taken for N and moisture determinations. Samples were analysed immediately after preparation.

The arbitary division of pots into "horizons" is illustrated in figure 5, which also gives weight and volume determinations. With this data, mineral N content could be calculated on both a ppm. N and mg. N/horizon basis.

FIGURE 5 THE ARBITARY DIVISION OF CONTROL POTS INTO "HORIZONS" FOR SOIL MINERAL NITROGEN ANALYSES



Horizon	Depth (cm.)	Volume		Soil Content (g.)
		Total (c.c.)	Proportion (%)	
A	2.5	380	29	250
B	4.0	530	40	350
C	4.8	415	31	274
Total	11.3	1325	100	874

(mean values for determination on 3 pots)

9 CHEMICAL ANALYSES

(a) General

All 140 herbage samples were analysed for K, Na, Mg, Ca, total N, total P, total S and Cl. Reference plant material, with analytical results from both Rukahia and Grasslands Division, was used to check the analytical methods adopted. Duplicate determinations were carried out. Where results exceeded a tolerance of $\pm$ 2%, a further two analyses were done. All results were expressed as me. % for the total content of elements, on an oven dry basis.

(b) Plant Material

(i) Potassium and Sodium were determined by flame photometry.

(ii) Calcium and Magnesium were determined by atomic absorption.

(iii) Total Nitrogen and Total Phosphorus were determined by the method of Cavell (1954) which allowed estimation of both elements on aliquots of the same Kjeldahl digest. A 0.14 g. sample was used.

Kjeldahl N is alleged to give unsatisfactory results, as variable amounts of free NO_3 are reduced and an unknown amount distilled off as nitric acid (Paech and Tracey, 1956). Cavell's method was compared with a Kjeldahl procedure, modified for NO_3 reduction, over a range of samples. Samples giving a higher yield of total N with the modified method invariably contained free NO_3 at levels exceeding 1,000 ppm. The method of Cavell was therefore used only for those samples containing lower levels of free NO_3.

For samples exceeding 1,000 ppm. NO_3 (all material from the NO_3 and NH_4NO_3 treatments of experiment III and one sample receiving NO_3 in experiment II) total N was determined using a Kjeldahl digest modified for NO_3 reduction with salicylic acid and sodium

thiosulphate (Paech and Tracey, 1956). This modified procedure interferred with total P determinations, which were carried out on separate digests using the vanado-molybdate method of Cavell (1954).

(iv) Chloride was determined using the Mohr method. Measurements must be made between pH 6.5 and 9.0 to avoid interference from chromic acid (Vogel, 1961). The writer modified the method of Johnson and Ulrich (1959) by using a chromate-dichromate indicator to buffer at pH 7.0. After checking, extraction periods were extended from the 10 minutes recommended (loc.cit) to 30 minutes.

Samples from the two soil experiments only were analysed. Tests on random samples and a bulked sample of material from experiment III revealed only trace amounts of chloride.

(v) Total sulphur: Because of low yields in experiment I, there was insufficient sample to carry out duplicate analyses for total S by gravimetric methods. Rather than bulk plant material within treatments, the writer sought a method requiring a small sample. A nitric-perchloric acid digestion procedure was selected (Blanchar et al., 1965) followed by turbidimetric determination of $BaSO_4$ (Lachica Garrido, 1964). Readings were made on a Technicon "AutoAnalyser" at 520 mμ. Sample size ranged from 0.05 - 0.2 g., depending on S content. Thirty-six determinations could be made daily.

(vi) Nitrate nitrogen was determined with the diphenylamine spot test (Johnson and Ulrich, 1959) to establish threshold levels in conjunction with total N determinations (III, 9, (b), (iii)).

(c) Soil Nitrogen

Ammonium and nitrate were determined using Richardson's

modification of Olsen's method (Piper, 1942) but the ammonia was collected in boric acid.

(d) Nitrate in Culture Solutions

Displaced solutions from the NH_4 treatment of experiment III were tested for the presence of NO_3. The brucine method of Peech and English (1944) was used. Colour development was checked against NO_3 standards and freshly prepared NH_4 solutions.

SECTION IV

RESULTS AND DISCUSSION

A THE EFFICACY OF "N-SERVE"

1 IN SOIL

In conjunction with experiment II, control pots which did not contain plants received the experimental treatments (III, 6, (b)). On conclusion of the experiment, their contents were analysed for form and level of mineral N and its distribution in the pots (III, 8,(b)). The results are presented in Table I.

The total recovery of N was similar for all treatments, approximately 145 mg. N/pot, suggesting that mineral N was not leached out of the soil by the overhead watering which was periodically carried out in the two soil experiments. The obvious difference between the watered pots (a) and the unwatered pots (b) was in the vertical distribution of NO_3 in that series. Where pots received no overhead watering (b) the bulk of N was recovered as NO_3 from the "A horizon", while in the watered series (a) there was more mineral N present as NO_3 in the "B horizon". Distribution of NH_4 was not affected by watering. This confirms that it was a correct decision to surface water the experimental pots periodically (III, 6, (b)). Without this treatment much of the NO_3 may have accumulated at the surface of the soil and become positionally unavailable to the plants.

There was only a trace of NH_4 recovered from pots which

TABLE I THE FORM, LEVEL AND DISTRIBUTION OF MINERAL N IN CONTROL POTS FROM EXPERIMENT II

(a) Pots which received overhead watering and sub-irrigation

Treatment		Mineral N Content	
		NO_3-N (mg.N)	NH_4-N (mg. N)
NO_3 series	A horizon	47.6	0.9
	B horizon	72.0	0.8
	C horizon	21.6	0.5
	Totals	141.2	2.2
		143.6	
NH_4 series	A horizon	13.3	52.2
	B horizon	6.8	50.1
	C horizon	4.6	18.1
	Totals	24.7	120.4
		145.1	

(b) Pots which received sub-irrigation only

		NO_3-N (mg.N)	NH_4-N (mg.N)
NO_3 series	A horizon	107.8	1.0
	B horizon	23.6	0.4
	C horizon	7.5	1.3
	Totals	138.9	2.7
		141.6	
NH_4 series	A horizon	9.3	59.6
	B horizon	5.1	50.5
	C horizon	3.4	22.3
	Totals	17.8	132.4
		150.2	

(each value is the mean from two pots in each series)

received NO_3 fertilizer, but about 15% of mineral N was recovered as NO_3 from the NH_4 series. It would appear that some of the added NH_4 was nitrified over the course of the experiment. This figure may have over-estimated the NO_3 content in the planted pots, however. The latter were subjected to a period of pre-treatment, with plants growing in them to induce a mineral N deficiency prior to the application of fertilizers and "N-Serve". Any mineralization and nitrification of organic N which occurred in the unplanted, control pots during this period would have allowed NO_3 to accumulate in the soil, and may have led to a greater "apparent nitrification" of added NH_4.

2 IN CULTURE SOLUTIONS

A colorimetric test for NO_3 was made daily on the NH_4 series of culture solutions in experiment III. The leachates were tested for NO_3 by comparison with NO_3 standards and freshly prepared NH_4 solutions (III, 9, (d)). There was no indication of any nitrification. If it did occur it must have been at the root surface, and been accompanied by a rapid absorption of the NO_3.

3 FREE NO_3 LEVELS IN HERBAGE: AN INDIRECT TEST FOR NITRIFICATION

In conjunction with total N determinations, all herbage was subjected to a "spot-test" for free NO_3 (III, 9, (b),(vi)). No material from plants which had received NH_4 in any of the experiments gave a positive reaction to the test. Herbage from plants which had received NO_3 in experiments I and II had free NO_3 contents ranging from a trace to a few hundred ppm., with

one sample containing over 1,000 ppm. Material from the NO_3 and NH_4NO_3 treatments of experiment III had free NO_3 contents in excess of 1,000 ppm.

4 CONCLUSIONS

It is concluded that "N-Serve" was quite successful in controlling nitrification in all experiments. Doubtless some nitrification occurred in the soil experiments, but tissue analyses suggest that NO_3 was not the major form of N entering the plants in the NH_4 treatments. As discussed in the following section, the overall pattern of results was similar in all experiments, lending support to this conclusion. The assimilation of N has been regarded as "predominantly NO_3" and "predominantly NH_4" in the two soil experiments (III, 4), while in the culture solutions all evidence suggests that it was "wholly NO_3" and "wholly NH_4" in these two treatments.

B THE EFFECTS OF N FORM ON THE YIELD AND MINERAL COMPOSITION OF RYEGRASS AND SWEET VERNAL

1 PRESENTATION OF RESULTS

The analyses of variance and tables of means and their estimated standard errors have been included in appendices 1-44. The results from all three experiments are summarised as follows:

(i) Appendix 45 is a summary of the means for the two N forms, averaged over both species. In general, these results will be referred to, unless the statistical analysis shows a significant interaction between N form and species, in which case the results

for the individual species will be discussed;

(ii) Appendix 46 is a summary of the effects of N form on ryegrass for all experiments;

(iii) Appendix 47 lists the results for sweet vernal; and

(iv) Appendix 48 is a summary of species differences, averaged over N forms, for all experiments. This will be referred to where inter-specific differences are discussed.

For convenience, these summaries unfold so that they may be lifted out and referred to during the discussions which follow. Limitations of space precluded the tabulation of standard errors, which are included with the table of means for each statistic in appendices 1-44. In the summaries of results, differences are indicated as either not significant (N.S.) or significant at the 5% (*) or 1% (**) levels of probability.

On the left hand side of appendices 45-48 are shown the results for three ratios. These ratios are:

(i) $\frac{\sum C}{\sum A} = \frac{\text{me. sum of (K + Na + Mg + Ca)\%}}{\text{me. sum of (S + P + Cl)\%}}$;

(ii) R-value, which has been defined previously (II, B, 5, (a)), and comprises the total metallic cation content over the total non-metal content; and

(iii) $\frac{\text{Cation uptake}}{\text{Anion uptake}}$, or the "gross cation-anion ratio" which has been defined (II, B, 4, (a) and II, B, 4, (b)) and comprises total metallic cation content (plus foliar N with NH_4 nutrition) over total inorganic anion content (plus foliar N with NO_3 nutrition). The gross cation-anion ratio is calculated on the assumption that all the N entered the plant as either NH_4 or NO_3 ions in the respective treatments.

An arc-sine transformation of the data for the first two ratios prior to statistical analyses was considered unnecessary. The data did not vary over a wide range and there was no obvious frequency imbalance. No significant species x N form interactions were detected.

Inspection of the data for the gross cation-anion ratio showed that the means were very obviously different between N forms, the values for NO_3 being about 10% of those for NH_4. To avoid inflation of the standard errors for the NO_3 means, and a reduction of those for NH_4, the analyses of variance were separated, and species compared within each N form. While R-value and gross cation-anion ratio are synonymous for plants receiving NO_3, the data for the NO_3 series was processed again to estimate the standard errors in the absence of the NH_4 series data.

One plot was lost in experiment II (sweet vernal receiving NO_3). This has been allowed for by conventional statistical methods, and as a result of this, there is an apparent discrepancy between R-value and gross cation-anion ratio in some cases.

2 YIELD

Yields of DM. within experiments did not differ significantly between plants receiving NO_3 or NH_4, except in the case of sweet vernal in experiment II only. No reason for the superiority of NH_4 in this one instance was apparent. There were large differences in yields between experiments; yields being of the order of 2 g. per plant in the first experiment, 8 g. in the second and 16 g. in the third. This was largely a reflection

of growing conditions and the size of the plants at the beginning of each experiment. For instance, the high yields associated with experiment III comprised two harvests over a period of 30 days, while the low yields in experiment I were obtained from three harvests over a period of 64 days. A diary of events has been presented (III, 6, (a)).

The similarity in yield between the two N forms, with the one exception noted, simplifies the interpretation of results. As discussed, yield may affect the mineral composition of plants by growth dilution (II, B, 5, (a), and figure 3, p. 75). The similarity in yield with either N form also indicated that the experimental treatments did no interfere with normal growth under conditions prevailing in any particular experiment. This is of particular interest in the case of "N-Serve", which was confounded with NH_4 in experiments I and II, as there has been a reported reduction of growth by legumes at lower levels of "N-Serve" than were used in the current work (II, C, 2, (b)). In experiment III, where 1 ppm. of "N-Serve" was included in all culture solutions, the highest yields were obtained with no indication of growth abnormality. As discussed in the following sections, the pattern of results for the effects of N form on the mineral composition of grasses varied quantitatively, rather than qualitatively, between experiments. On this basis it is concluded that "N-Serve" showed no apparent effects on either plant growth or physiological processes.

There was no yield difference between the species in experiment I, but in the two following experiments ryegrass out-

yielded sweet vernal by 3-4 g. per plant. Because of the possible relationships between yield and mineral composition this complicates the interpretation of inter-specific differences in mineral composition.

Only in experiment III were NO_3 and NH_4 provided simultaneously to plants, by design. This resulted in a yield increment over plants receiving either N form separately, which is in agreement with observations by other authors for a variety of plant species (II, B, 3, (a), (i)).

3 THE CONTENT OF METALLIC CATIONS

(a) Experiment I

In the first soil experiment plants which received NH_4, in relation to those receiving NO_3, had a reduced foliar content of bases, but this reduction from 157 to 148 me.% was comparatively small. The content of Ca and Mg was lower with NH_4, but these differences failed to reach statistical significance at the conventional level of probability ($P < .15$ and $< .10$ respectively).

There was no significant difference between the two species in total cation content. However, sweet vernal had a higher content of K and a lower content of Mg and Ca, than ryegrass. As there was no difference in yield between the two grasses, this suggests a "preference" by ryegrass for divalent cations, and by sweet vernal for K.

(b) Experiment II

Where NO_3 and NH_4 were applied as fertilizers, plants receiving NO_3 again had a higher base content (135 me.%) than

those receiving NH_4 (119 me.%). In both species, the levels of Na, Mg, and Ca were reduced with NH_4. To what degree the increased yield of sweet vernal contributed to this effect is not known. The level of K increased with NH_4 in the case of ryegrass, but was not different between treatments with sweet vernal. The explanation for this result is not clear, but it may have arisen because of different effects of the two N forms in the soil medium. For instance, with the NH_4 fertilizer the external acidic effect of NH_4 assimilation (II, 3, (a),(v)) probably coupled with a low level of nitrification (IV, A, I) may have led to more acid conditions than with NO_3, at least in the immediate environment of the root (II, B, 4, (f), (iv), and under these conditions there may have been a greater displacement of K from the soil colloids with NH_4 fertilizer. This increase in K availability may then have resulted in the observed greater uptake of K by the grasses. That the synergism between NH_4 and K content was not observed with sweet vernal may have been due to the 35% yield increment also associated with NH_4 in this experiment. Clearly, total K uptake by both species was greater with NH_4.

The possibility of a true synergism between NH_4 and K during uptake and translocation would appear less likely. This is the only experiment in the current work where NH_4 has resulted in an increase in K uptake. Many workers have found the uptake of Ca and Mg to be more clearly affected by N form than that of K (II, B, 3, (b), (iv) and Mulder, 1956). However, this writer has found no report of a synergism between NH_4 and K, which could

be ascribed to a true interaction between these two ions during uptake.

As in experiment I there was no difference between the two species in total cation content. Ryegrass had a higher level of Ca than sweet vernal; the reverse was the case for K. There was no inter-specific difference in Na or Mg in the herbage, under the conditions of this experiment.

(c) Experiment III

When these grasses received NO_3 in culture solutions their total base content was 174 me.%, which was considerably higher than that in the herbage of plants receiving NH_4 (144 me.%). The contents of Na, Mg and Ca were all diminished with NH_4. Plants supplied with a combination of both N forms together in the culture solutions were intermediate in every respect, except in their K content. With ryegrass, the only significant difference in K content was between plants receiving NH_4 which contained less than those grown with NH_4NO_3. Plants of the NO_3 series were intermediate, and not significantly different from either. The situation with sweet vernal was that plants receiving NO_3 or NH_4NO_3 had a significantly higher K content than plants receiving NH_4, but there was no difference between the NO_3 and NH_4NO_3 plants. No explanation for this result is apparent. However, the differences in ryegrass were small, the means varying between 107 and 116 me. K% only.

As in both soil experiments, there was no inter-specific difference in the level of total metallic cations in the herbage. While ryegrass contained more Mg and Ca than sweet vernal, the latter had a higher content of K and Na.

(d) Discussion

(i) N form effects: In all three experiments, plants receiving NH_4 had a reduced foliar content of bases in relation to those receiving NO_3. The divalent cations, Ca and Mg, were invariably depressed with NH_4 but there was more variability in the results for Na and K. The low magnitude of interactions in experiment I may have arisen either because of the comparatively restricted N regime (III, 4, (b)) or because the control over nitrification in the NH_4 series was not completely effective. That the differences between this and later experiments were basically quantitative suggests that the plants in the NH_4 treatment did, however, assimilate much of their N as NH_4.

(ii) Species differences: Within each experiment there was no difference between ryegrass and sweet vernal in total base content. Ryegrass always contained more Ca than sweet vernal, while the reverse was true for K. Where there were inter-specific differences in Mg and Na, ryegrass again had the higher content of divalent Mg and sweet vernal, of monovalent Na. It is concluded that ryegrass showed a preference for divalent cations, and sweet vernal a preference for monovalent cations, under the conditions of these experiments. That this was a true selective uptake is shown by the higher monovalent cation levels in sweet vernal, and divalent cations in ryegrass, in experiment I where there was no yield difference between the species. In the latter two experiments, ryegrass did outyield sweet vernal. But for the lower yield of sweet vernal to have masked a true selective uptake of monovalent cations, it would have to be conceded that greater D.M. production by this species would result in an in-

creased Ca and Mg content and a decreased Na and K content with no effect on total cation content; there is no evidence for this (III, B, 5, (c), (i)).

4 THE CONTENT OF NON-METALS

(a) Experiment I

Grasses which received NH_4 had a higher content of inorganic anions (S + P + Cl) than those receiving NO_3. The former contained 91 me.% and the latter 85 me.%, so that the difference was comparatively small. There was no significant effect of N form on the N content of the grasses, and the treatment difference in total non-metal content (S + P + Cl + N) failed to reach significance. The content of S was 1.4 me.% higher in grasses receiving NH_4, and that of P, 3.7 me.% higher, but there was no effect of N form on Cl. As there was no difference in N content, the observed change in S must have been the result of a true ionic interaction, not simply the result of a greater protein content in one treatment, as material from the NO_3 series in this experiment had free NO_3 levels which were a negligible proportion of total N (III, 9, (b), (iii)).

Ryegrass had a higher content of S and total inorganic anions (S + P + Cl) than sweet vernal. The greater content of P and Cl in ryegrass failed to reach statistical significance. There were no inter-specific differences in N or total non-metal content.

(b) Experiment II

Where grasses received NH_4 they again had a higher content of inorganic anions (70 me.%) than those receiving NO_3 (56 me.%).

There was no difference in the N content of ryegrass receiving either form of mineral N, but sweet vernal contained less N in the NH_4 series. As a definite relationship between yield and N content has been established for ryegrass (II, B, 5, (c), (i)) this effect probably arose from the greater yield of sweet vernal with NH_4. These differences between the two species in N content was reflected in the total content of non-metals (S + P + Cl + N) which was higher with NO_3 in the case of ryegrass, but was not significantly greater for sweet vernal. Ryegrass receiving NH_4 had a significantly higher content of both S and P, but there was no treatment effect on its Cl content. Sweet vernal, on the other hand, had a significantly higher content of P and Cl with NH_4, in spite of the yield increase, but the greater content of S failed to reach significance. It cannot be stated unequivocally that the observed synergism between NH_4 and SO_4 during uptake in this experiment was not the result of the application of $(NH_4)_2SO_4$ as a fertilizer in this treatment. However, in view of a similar interaction established in the previous experiment, where no mineral N was added to the soil in fertilizer form, it would seem that at least part of this interaction was a true synergism.

The only inter-specific differences which can be clearly separated from yield effects in this experiment are a higher content of S in ryegrass herbage and, as a result of this, a higher value for inorganic anions. Sweet vernal, in spite of lower yields, contained less S and a lower quantity of (S + P + Cl) than ryegrass.

(c) Experiment III

As in the two previous experiments, the herbage from both grasses contained more inorganic anions when the plants were assimilating NH_4. The content (67 me.%) was considerably greater than that in herbage receiving NO_3 (48 me.%). The NH_4 plants also contained appreciably more N. As there was no difference in yield this may well have been the result of the synthesis of organic N compounds, resulting in "nitrogen-rich" plants, during luxury consumption of NH_4 from the culture solutions. Such an effect has been reported for a number of species (II, B, 3, (a), (iii) and II, B, 3, (b), (ii)). These differences in both inorganic anions and N are reflected in the treatment differences in total non-metal content, the values for which were 345 me.% with NO_3 and 391 me.% with NH_4. In this experiment, Cl was omitted from the culture solutions for reasons discussed elsewhere (II, C, 3, (d)). Provision of NH_4 resulted in a large increase in S content from 27 me.% with NO_3 to 45 me.%. As P increased by only 1 me.% with NH_4, which was not significant, most of the difference between NO_3 and NH_4 in the level of inorganic anions (P + S) is a reflection of the effect of N form on S uptake. On the basis of the data from experiment I, this would be expected to be at least in part the result of a true positive interaction between NH_4 and SO_4. Herbage from the NH_4NO_3 series was intermediate in every respect, except that it contained more P than herbage from plants which received either N form separately. The differences involved were small, the mean P content varying only between 21 and 24 me.%.

This apparently anomolous result with P content, in view of the interactions between P and N form established in the two soil experiments, is most probably an artefact of the experimental conditions in experiment III. The content of SO_4, the only ion varied in the culture solutions besides N, was 2, 7 and 12 me./l. in the NO_3, NH_4NO_3 and NH_4 solutions respectively. The synergism between NH_4 and P may have been overriden by the antagonism between SO_4 and H_2PO_4, during uptake by the grasses in the NH_4 solutions. While the S/P ratio in the NO_3 solution was 2, in the NH_4 solution it was 12. An antagonism between S and P during uptake by perennial ryegrass has been established (II, B, 5, (e)).

Ryegrass in spite of a greater yield, contained more S and P than sweet vernal when the results were averaged over all N treatments. This resulted in a higher inorganic anion content (68 me.%) in the herbage of ryegrass, compared with 49 me.% in sweet vernal herbage. Sweet vernal contained more N than ryegrass, which may have been a reflection of its lower yield. As a result, there was no inter-specific difference in the content of total non-metals.

(d) Discussion

(i) N form effects: In all experiments, plants receiving NH_4 had an enhanced content of inorganic anions in relation to those which received NO_3. Ammonium nutrition resulted in a greater uptake of S in every case, but the increased S content of sweet vernal in experiment II was possibly masked by an increase in yield, and was not significant. In both soil experiments, NH_4 led to an increase in P content. In experiment III, the observed

increase with NH_4, as opposed to NO_3, was not statistically significant, which may have been an artefact of the experimental technique. The effects of N form on Cl content were variable, but generally NH_4 caused an increase in foliar content, which was not significant. This may have been a reflection of the experimental conditions. Presumably the bulk of the Cl assimilated by plants in the two soil experiments was supplied in the tap water used to irrigate the pots, as no salts containing Cl were applied as fertilizers. Uptake of Cl may therefore have been regulated by its rate of arrival of the roots rather than by true interaction. This interpretation is supported by the results for sweet vernal in experiment II, where a greater yield was associated with an increased Cl content. A yield increase would involve a greater total transpiration by the plants, hence a facilitated arrival of Cl at the roots by mass flow. The similarity in total N content between treatments in the two soil experiments would suggest that either N form was readily available to these grasses. The immobilisation of NH_4 by soil colloids (II, C, 1, (c)) led to no apparent inferiority of NH_4 under the conditions of these experiments. While the N content of sweet vernal was some 12% lower in plants which received NH_4 in experiment II, this was related to a 35% DM. increase in the same treatment, so that total N uptake was considerably greater with NH_4. The luxury supply of NH_4 in culture solutions resulted in a greater N content of plants.

These data show the large proportionate contribution of N to the total non-metal content of grass herbage. Equivalents

of N translocated to the leaves were from 3 to 6 times as great as the equivalent sum of other non-metals (S + P + Cl).

(ii) Species differences: The outstanding difference between the two species was the greater S content of ryegrass in every experiment when the results were averaged over N forms. This applied even where there was a greater yield by ryegrass. Largely as a result of its higher S content, ryegrass herbage also had a larger concentration of inorganic anions (S + P + Cl), than did sweet vernal. There were inter-specific differences in N content, ryegrass containing less than sweet vernal in experiments II and III, but these were directly related to higher yields by ryegrass in both experiments. While these differences in N content were observed under the experimental conditions where plants were harvested after the same period of regrowth, this does not necessarily imply that the same differences would be observed if plants were harvested at the same DM. yield, as carbohydrate formation can greatly influence N levels in herbage (II, B, 5, (c), (ii)).

5 CONCLUSIONS

(a) Yield

Under the conditions of these experiments, both forms of mineral N were equally effective as sources of N for the growth of ryegrass and sweet vernal. Ryegrass outyielded sweet vernal in the latter two of the three experiments.

(b) The Effects of N Form on Herbage Mineral Composition

(i) Metallic cations: Grasses which received NH_4 invariably

had a lower content of bases in their leaves than those fed NO_3. The reduction ranged from 8 to 30 me.% depending on the experimental conditions. The content of divalent cations was always lower with NH_4, but the results for K and Na were more variable.

These results confirm established interactions between the two N forms and metallic cations (II, B, 3, (b), (iv)).

(ii) Inorganic anions: Ammonium nutrition resulted in an increase in the foliar content of (S + P + Cl), in relation to NO_3. This increase ranged from 6 to 19 me.% depending on the experimental conditions. Plants which received NH_4 contained more S, but the results for P and Cl were variable, and probably related to the experimental conditions.

The data from these experiments confirm established interactions between the two N forms and inorganic anions (loc.cit).

(c) Inter-specific Differences in Mineral Composition

(i) Base content: There was no difference between ryegrass and sweet vernal in the total metallic cation content of herbage, under these experimental conditions. Ryegrass showed a "preference" for divalent cations, particularly Ca, and sweet vernal, for monovalent cations, especially K.

(ii) Non-metal content: Ryegrass invariably contained more S than sweet vernal. In the latter two experiments, sweet vernal contained more N than ryegrasss, but this difference between the two species could not be clearly separated from yield effects. Largely as a result of its higher S content, ryegrass contained more inorganic anions than sweet vernal.

C SOME PLANT PHYSIOLOGICAL ASPECTS OF NO_3 AND NH_4 ASSIMILATION BY GRASSES

1 IONIC BALANCE DURING N ASSIMILATION

(a) The Gross Cation-anion Ratio

Data for the gross uptake of cations and anions by the experimental plants have been brought together in table 2. Total N has been assumed to have entered the plant entirely as the anion or cation in the NO_3 and NH_4 treatments respectively. In experiment I, plants receiving NO_3 contained 157 me.% of metallic cations. With NH_4, this value was depressed to 148 me.%, but the uptake of 251 me.% of cationic NH_4 increased total cation uptake to 399 me.%. The content of inorganic anions was 91 me.% with NH_4, but with NO_3 this value was depressed to 85 me.%. However, the additional uptake of 249 me.% of anionic NO_3 increased the total anion uptake to 334 me.%. It may be argued that as some nitrification probably did occur in the two soil experiments, these data do not give a clear picture of cation and anion assimilation. It was concluded that only a negligible amount of nitrification could have occurred in the NH_4 culture solutions (IV, A, 2). Nevertheless, the greatest differences were found in that experiment. The base content of herbage which had received NO_3 was 174 me.%. With NH_4 the value was reduced to 144 me.%, but the assimilation of 324 me.% of cationic NH_4 increased total cation uptake to 468 me.%. In the NH_4 series inorganic anions totalled 67 me.%, and this was depressed with NO_3 to 48 me.%, but the attendant uptake of 297 me.% of anionic NO_3 gave a total anion uptake of 345 me.%.

TABLE 2 TOTAL CATION AND ANION UPTAKE WITH NO_3 AND NH_4 NUTRITION

(Results for N forms averaged over both species)

	Cations (me.%)			Anions (me.%)		
Experiment I	(K+Na+Mg+Ca)	NH_4	Total	(S+P+Cl)	NO_3	Total
NO_3-N	157	-	157	85	249	334
NH_4-N	148	251	399	91	-	91
Experiment II						
NO_3-N	135	-	135	56	211	267
NH_4-N	119	199	318	70	-	70
Experiment III						
NO_3-N	174	-	174	48	297	345
NH_4-N	144	324	468	67	-	67

These differences are shown in the gross cation-anion ratio in appendices 45-48. In all experiments, where plants received NO_3 they absorbed approximately two equivalents of anions for every one equivalent of cations. In the two soil experiments, when grasses received NH_4, cation assimilation exceeded that of anions by a factor of approximately 4.5. Under the same N treatment in experiment III, these grasses assimilated more than 7 equivalents of cations for every one equivalent of anions.

As shown in table 2, there was a small compensatory change in the uptake of other cations and anions when the ionic charge of N was reversed, but this effect was far from stoichiometric. The observations, therefore, rule out any concept of their being a constant ratio between the amount of cations and anions translocated to the herbage of these experimental plants. The data illustrate the effectiveness of "exchange uptake" of NO_3 for the anions released during its metabolism, and of NH_4 for the H ions released during its assimilation (II, B, 4, (e), (ii)). The data also show that N assimilation, irrespective of N form, proceeded in a manner largely independent of the uptake of other ionic species from the medium (loc.cit).

(b) The Relationships Between Metallic Cations and Inorganic Anions

The extent of interactions between the two forms of N and other ionic species has been shown in table 2. The induced changes in mineral composition are reflected in a significant reduction in the ratio for metallic cations/inorganic anions from 1.86 with NO_3 to 1.64 with NH_4 in the first soil experiment. The

corresponding values in experiment II were 2.45 and 1.67, and 3.72 and 2.23 in the final experiment. In every experiment, there was an antagonism between NO_3 and other inorganic anions, and a synergism between NO_3 and metallic cations, when the results are compared with those from the NH_4 series. Conversely, there was an antagonism between NH_4 and metallic cations, and a synergism between NH_4 and inorganic anions. These results confirm observations on many species (II, B, 3, (b),(iv)) including ryegrass (II, B, 5, (e)).

Without exception the value of this ratic exceeded unity. Alternatively, the uptake of metallic cations always exceeded that of inorganic anions, even with NH_4 nutrition. This was one of the stipulations laid down for normal metabolism by plants (II, B, 4, (e), (ii)). Values less than unity mean the accumulation of NH_4 and/or H ions in tissues to balance the excess inorganic anions (loc.cit.). Such a situation normally occurs to an appreciable extent only under conditions of ammonium toxicity (II, B, 3, (b), (i)).

Possible reasons for the interactions between the two N forms and other ionic species during uptake and translocation have been discussed (II, B, 4, (f)). The accumulation of cations in tissues must involve an equivalent accumulation of anions, both inorganic and organic, to maintain ionic balance (II, B, 4, (e), (ii)). During NH_4 nutrition, the translocation of an excess of metallic cations to the herbage must involve an equivalent movement of organic anions to equate the deficit of inorganic anions (II, B, 4, (f)). On this basis, the synergism

between NH_4 and inorganic anions (P + S + Cl) may be explained. Because of either the lower mobility of organic anions in comparison with inorganic anions, or because of a limitation to their "availability" during NH_4 nutrition (II, B, 4, (f), (ii)) the inorganic anions assume a greater role as counter-ions for the paired movement of metallic cations out of the root. On the other hand, with NO_4 nutrition much of the metallic cations may enter the root tissues as NO_3 salts. To what extent the cations are translocated as such, or as the salts of organic anions formed by NO_3 metabolism in the root, is not clear. Whichever is correct, NO_3 nutrition leads to a reduced role of other inorganic anions as counter-ions for the movement of cations to the herbage. The observed result is an antagonism between NO_3 and (P + S + Cl) when the mineral composition of herbage is compared with that which received NH_4.

It has been concluded that the assimilation of N, irrespective of form, apparently occurs by an exchange process in a manner which makes N uptake largely independent of the uptake of other ionic species (loc.cit.). This has been confirmed in the present work (IV,C,1,(a)). However, the interactions between NO_3 and NH_4 and other ionic species, found during these experiments (IV, B, 3 and IV, B, 4) show that there is not a complete independence, and are in agreement with the findings of other workers (II, B, 3, (b), (iv). What is surprising, however, is the small magnitude of the changes in the levels of other minerals when the ionic charge of N is reversed. In the present series of experiments, N uptake greatly exceeded that of total cations, and that

of inorganic anions on an equivalent basis, irrespective of N form (table 2, p. 148). Obviously then, ionic N had a low order of interference with other elements during uptake and translocation. A possible explanation for the observed effects of N form on the final mineral composition of herbage within each species can be offered on the basis of osmotic considerations. Irrespective of the precise physiological explanations NO_3 nutrition, in relation to NH_4, results in herbage which may be characterised as having:

a. a higher content of metallic cations, especially divalent cations (II, B, 3, (b), (iv)). This has been confirmed in the present work, and is discussed presently (IV, C, 2, (d)) ;

b. a lower content of inorganic anions other than NO_3 (II, B, 3, (b), (iv)). This has also been observed in all the present experiments (table 2); and

c. a higher content of organic anions (II, B, 3, (b), (iii)). This has been found in the current work, and is discussed in the next section (IV, C, 2).

It is well established that "low salt" flaccid tissues will absorb mineral salts rapidly, and that eventually a limiting salt content is reached (Sutcliffe, 1962). This limitation may be imposed by the osmotic pressure in the plant sap. In tissues which have received NO_3, the presence of a higher proportion of multivalent organic anions in the assemblage of total anions would result in a lower osmotic pressure on the basis of particle number alone, in comparison to NH_4 tissues where an equivalent amount of total anions would contain relatively more inorganic anions. Organic acid salts may also have lower activity coefficients, as evi-

denced by the precipitation of Ca and Mg salts of organic acids in tissues (Pierce and Appleman, 1943; Kirkby, 1966); which effect would be additive to that of particle number in causing a greater reduction of the osmotic pressure in NO_3 tissues than in NH_4 tissues. Further, the enhanced metallic cation content of tissues receiving NO_3 is associated with a greater proportionate increase in divalent cations than in monovalent cations, which would also result in a decreased osmotic pressure per equivalent amount of cations on the basis of particle number, when compared with an equivalent of cations in NH_4 tissues with a higher proportion of monovalent cations. Thus many plants, including those in the current experiments, can largely overcome any effects which may be expected to arise in their mineral composition, from the assimilation of a large excess of cations with NH_4 nutrition and anions with NO_3 nutrition. Because NO_3 nutrition, whether directly or indirectly, gives rise to tissues with the characteristics listed above, the differences between them and NH_4 tissues may be viewed as the result of interactions among mineral elements other than N, within the limitation of a maximum osmotic pressure in the plant sap. This concept is speculative and provides the basis for further investigation rather than statement of fact. High levels of Cl in ryegrass herbage result in a reduced content of multi-valent organic anions (Dijkshoorn, 1963; Van Tuil, 1965). In field samples of Italian ryegrass Cunningham (1964a) found that herbage which contained much Cl also had a lower total base content at any level of total "anions" (N + P + S + Cl) than did samples containing little

Cl. In this respect, the samples were similar to those which received much NH_4 fertilizer. It was also found that samples containing much Na had a lower total base content at any level of total "anions" than tissues containing little Na. Further, the high Na tissues tended to a maximum base content which was considerably lower than when Na levels were comparatively low. These observations suggest that osmotic effects may have limited salt absorption, resulting in a lower total mineral salt content in tissues which contained high levels of monovalent ions.

(c) R-value

R-values were significantly lowered by NH_4 nutrition in all experiments. As there was no overall effect of N form on N content in experiments I and II, this effect was caused by the established interactions between the two N forms and other ionic species during uptake and translocation. In experiment III, NH_4 also resulted in an increase in foliar N content so that this additive effect was also responsible for the reduction in R-values.

The R-values for individual ryegrass samples were plotted against their N content, as has been done for Italian ryegrass by Cunningham (1964a *et seq.*). The same negative relationship between R-value and N content was observed with these data, as that shown by Cunningham (*loc.cit.*). The variation within any treatment of any experiment more or less followed the mathematical model for the plot of:

$\frac{\sum C}{\sum A + N}$ against N, where

$\frac{\Sigma C}{\Sigma A + N}$ = R-value

ΣC = me. (K + Na + Mg + Ca)% which was the mean for that particular treatment and experiment (a constant)

ΣA = me. (S + P + Cl)% as above

N = me. N%

The negative relationship virtually followed the mathematical prediction, with some scattering which could be associated with genetic control over mineral composition (IV, C, 4, (b), (iv)). Visual assessment of these graphs simply served to show that N uptake by ryegrass was largely independent of the uptake of other ionic species under the conditions of these experiments, and that R-values were not constant. At any N content, R-values for plants receiving NH_4 were lower than those for NO_3, which is simply a reflection of the established interactions between the two N forms and other ions during uptake. These data could not be interpreted as support for the suggestion by Cunningham and Nielsen (1965) "that the proportions of cations and anions taken up from the soil by intact grass plants, as measured by R-value, are regulated by some plant mechanism linked to nitrogen metabolism."

(d) Discussion

Bear (1950) and Dijkshoorn (1957a, 1957b, 1958a) concluded that the "cation-anion" ratio in herbage was a constant and that cations and "anions" were transported to herbage in related amounts, such that changes in total content did not effect changes in this ratio. Allowing for the fact that these authors were

considering the metal/non-metal ratio (the gross cation-anion ratio has varied widely in the present work) this study has not supported their general rule. R-values have been significantly reduced by NH_4 in all cases. Further, significant differences have occurred between experiments for the same species receiving the same N form. For instance, the mean R-value for ryegrass receiving NO_3 in experiment I (0.485) was significantly lower than that for ryegras receiving NO_3 in experiment II (0.577; $P < .001$). The values for individual ryegrass samples ranged from 0.35 to 0.65 in the present study, which is in agreement with other evidence that R-value is not a constant (II, B, 5, (c)).

It is very difficult to assess whether grasses are assimilating NO_3 or NH_4 at any particular time, under field conditions (II, A, 2). Data for the mineral composition of grasses was therefore studied to see whether any sort of diagnostic technique could be devised by which one could determine the form of N which grasses were assimilating during these experiments, and which may have been applicable to field conditions. No clear-cut separation between NO_3 and NH_4 plants emerged. This may be demonstrated by the fact that the mean value for the ratio, metallic cations/inorganic anions, calculated for both species receiving NO_3 in experiment II (2.45) was not significantly different from the mean value of 2.23 calculated for both species receiving NH_4 in experiment III ($P > .50$).

While this writer does not attempt to define those factors of the environment which have caused these large variations in mineral composition between experiments, it is evident that they had a greater affect on the mineral composition of herbage than

did N form. That R-value does vary so greatly with environmental factors throws its usefulness into question. It appears to have no particular merit in terms of plant physiology, nor in the assessment of the nutritional value of herbage.

2 ORGANIC ANIONS

(a) Estimation

It was concluded previously (II, B, 4, (e)), that the ionic balance in plant tissues may be given the following equation:

me. $(K + Na + Mg + Ca)\%$ = me. $(SO_4^{=} + H_2PO_4^{-} + Cl^{-} + NO_3^{-})\%$ + me. organic anions %

In the present work it was therefore possible to calculate the content of organic anions in all tissues which received NH_4, and in the herbage of plants which received NO_3 in experiments I and II, where total N was virtually all in organic form (1,000 ppm. NO_3 in herbage is approximately equal to 1.5 me.%). The data are considered as an approximation. The amount of total S involved in organic combination was calculated from the N content, according to the equation of Dijkshoorn et al. (1960):

me. organic N x .054 = me. organic S

Inorganic NO_3 was ignored, but no estimations of organic anions were made for plants of the NO_3 and NH_4NO_3 series of experiment III because their free NO_3 content exceeded 1,000 ppm. by an unknown abount (III, 9, (b), (iii)).

(b) Organic Anion Content and N Form

The following data are the calculated organic anion values for both N forms averaged over the two species, showing the mean

organic anion contents and their estimated standard errors:

Experiment I	me. organic anions %
NO_3-N	86 ± 2.7
NH_4-N	71 ± 2.7
Experiment II	
NO_3-N	90 ± 3.2
NH_4-N	59 ± 3.2
Experiment III	
NO_3-N	Not calculated
NH_4-N	94 ± 2.4

Ammonium caused a reduction in the organic anion content in both soil experiments. This was the result of the established interactions between each of the two N forms and other elements during uptake. In the NO_3 series of experiment III, the excess of metallic cations over inorganic anions was 142 me.%. This may have given an organic anion content in excess of that for NH_4. Some 2.5% by weight of free NO_3 would be required in these tissues to prevent the difference from being significant. However, any difference awaits verification.

Numerous experiments embracing a variety of species including grasses have shown that NH_4, in relation to NO_3, has resulted in a reduction of the measured organic acid content of leaves (II, B, 3, (b), (iv)).

(c) Organic Anion Content and Yield

De Wit et al. (1963) concluded that a "normal" 100 me.% excess of metallic cations over inorganic anions is a pre-requisite for "good growth" of grasses in general, including ryegrass.

This view has been reiterated by Dijkshoorn (1963, 1964) and Van Tuil (1965). These authors also conclude that the reduced growth frequently associated with NH_4 nutrition is due to a "stress" on the normal organic anion content, which is reduced with this N form.

In the present study, DM. yield was not adversely affected with NH_4 nutrition, and this N form was superior in one experiment with sweet vernal. The organic anion content was depressed by NH_4, at least in the case of the two soil experiments, which does not support the Dutch theory that the yield of grasses is causally related to organic anion content. These results suggest that the inferiority of NH_4 which has frequently been observed during experiments comparing both N forms may, in fact, have arisen from the experimental conditions, such as insufficient lighting or lack of satisfactory pH control in the media, as suggested by Street and Sheat (1958) and discussed elsewhere (II, B, 3, (a)).

(d) Organic Anions and Metallic Cations

The ratio of divalent/monovalent cations in herbage was subjected to statistical analysis, and no significant species x N form interaction was detected. The following data are the means and their estimated standard errors for each N form averaged over both species:

Experiment I	$\frac{\text{me. (Mg + Ca)}\%}{\text{me. (K + Na)}\%}$
NO_3-N	0.52 ± 0.02
NH_4-N	0.49 ± 0.02

Experiment II	$\frac{\text{me. (Mg + Ca) \%}}{\text{me. (K + Na) \%}}$
NO_3-N	0.58 ± 0.03
NH_4-N	0.46 ± 0.03
Experiment III	
NO_3-N	0.43 ± 0.01
NH_4-N	0.31 ± 0.01

In all experiments, NH_4 was associated with a reduction in this ratio, but the difference was not significant in experiment I. The antagonism between NH_4 and metallic cations therefore affected Ca and Mg more than Na and K. This observation agrees with those of other workers (II, B, 3, (b), (iv)).

This reduced accumulation of divalent cations was associated with a reduced accumulation of organic anions when plants received NH_4, at least in the two soil experiments. On the basis of current evidence that accumulation of anions is the rate-limiting, energy dependent step in salt accumulation by plant tissues, and that cation absorption is a resultant carrier-mediated exchange (Lundegårdh, 1960; Robertson, 1960; Briggs et al., 1961), these data may be considered as supporting the suggestion made previously (II, B, 4, (f), (ii)) that the differential effects of NO_3 and NH_4 on cation absorption may in part be explained by their effects on organic anion accumulation. It was not clear whether the possible effects of NH_4 were direct (for instance by "competition" with divalent cations for a limited supply of organic anions), or indirect, through changes in tissue and/or external pH with attendant changes in organic anion "availability" (loc.cit.).

The alternatives cannot be separated with the information from the present study. Nothing was known of the pH at the root surface in the two soil experiments (II, B, 4, (f), (iv)), and the design of the solution culture experiment was such that external pH and N form were confounded. The pH of leachates from all pots was measured over a period of 8 days. The following are the mean values for each N form, averaged over both species, with their estimated standard errors:

	pH
NO_3-N	7.6 $\pm$ 0.03
NH_4-N	5.4 $\pm$ 0.03

The initial pH of all prepared solutions was 6.2. As both treatments were presumably near the same pH when pots were leached with fresh solutions each day, the average pH difference between the two treatments was something less than the difference between the values above.

A possible explanation for this relationship between organic anion accumulation and the relative participation of divalent cations in total cation accumulation may be envisaged. Exchange reactions are known to be involved in the upward movement of divalent cations, although to what extent such exchange phenomena are responsible for the determination of herbage mineral composition, is not clear (Butler and Bollard, 1966). Said (1959) concluded that regulative forces in the relative translocation of cations to the herbage of grasses arose from differences among the cations in their affinity for the charged sites present on plant colloids. Said characterised perennial

ryegrass as having a marked selective ability towards the alkali cations. The comparative discrimination against the alkali earth cations he attributed to their reduced mobility, resulting from their preferential adsorption on the bio-colloids (loc.cit.). Divalent cations may be chelated as the salts of organic acids. As such, their lack of ionic properties would preclude their participation in exchange reactions. Insofar as these exchange reactions may regulate the upward movement of Ca and Mg, any factor which caused an increase in the movement of organic anions from root to shoot could be expected to have a greater proportionate effect on the movement of divalent cations than on the movement of monovalent cations, because available evidence suggests that the former have a greater affinity for the exchange sites. Irrespective of the precise physiological basis for the greater organic anion accumulation with NO_3 nutrition, as opposed to NH_4, the synergism of NO_3 with Ca and Mg may be the result of an increased transport of the chelated salts. Jones (1961) found that aluminium was complexed with malic and citric acids prior to translocation in plants. He concluded that chelation maintained the Al in a "physiologically soluble" form, as it would otherwise have been expected to precipitate.

3 GENETIC CONTROL OVER THE MINERAL COMPOSITION OF RYEGRASS HERBAGE

(a) Statistical Methods and Presentation of Results

The design of the experiments has been discussed (III, I). Clonal material from each of the same 10 ryegrass and 10 sweet

vernal genotypes was used for the 10 replications in all three experiments. After removing variance resulting from differences among the experiments, a statistical test was made for significant differences among the genotypes for the characters studied in the present work.

Because of its focal importance in New Zealand agriculture and pasture plant breeding, the data for ryegrass only were processed. The 10 ryegrass plants used in this experiment were randomly selected from the F_1 generation following an open pollination of 8 parent plants (loc.cit.). As such, they may have comprised any of the possible 28 parental combinations, and the same parental combination may have been included more than once. The actual parents of any particular plant were not known. The 10 genotypes have been nominated A....J for tabulation of the results.

The data for plants receiving NO_3 and NH_4 were separated for statistical analyses, as it was not known to what extent there may have been a genotype x N form interaction during the uptake of other elements. Appendix 49 is a summary of the statistical analyses, and includes the coefficients of variation and estimated standard errors. The results are summarised in table 3. Genotypes have been ranked from lowest to highest down each column of the table. Differences among the genotypes have been indicated as either not significant (N.S.) or significant at the 5% (*) or 1% (**) levels of probability. In the case of significant results, the critical differences ($d_{.05}$ and $d_{.01}$) have been presented.

YIELD (g. D.M. /plant)		METALS (me. %)									
		K		Na		Mg		Ca		me. sum of metals Σ C	
NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
I	I	C	F	B	B	E	E	H	H	E	H
8.7	8.7	72.4	76.0	7.8	5.8	17.2	14.0	26.3	17.8	145	126
B	B	F	C	A	A	B	B	J	I	H	E
9.2	9.4	73.2	76.9	9.7	8.3	19.2	15.4	28.1	20.6	146	129
C	C	D	D	I	E	H	H	I	J	B	I
10.0	9.6	74.2	80.0	13.3	10.0	21.9	16.4	28.9	22.0	147	133
G	G	H	J	E	H	G	C	E	E	C	B
10.0	9.8	80.9	81.1	15.2	10.0	22.2	16.7	29.9	23.1	150	136
E	E	E	E	H	I	J	J	G	D	I	C
10.3	9.9	82.1	81.3	16.6	11.0	22.2	17.8	31.3	24.6	151	136
A	D	I	G	G	G	C	D	C	C	A	J
10.5	10.0	83.8	81.4	17.4	15.1	23.3	18.4	32.9	27.8	155	136
D	A	G	I	J	J	A	G	A	B	G	A
10.5	10.7	84.1	81.5	18.7	15.3	24.7	19.2	33.4	28.1	155	143
J	J	B	H	C	C	D	I	B	A	J	D
10.6	10.8	86.3	82.1	21.7	17.8	24.7	19.5	33.4	28.8	155	144
H	F	J	A	D	D	I	A	D	G	D	G
10.7	11.0	86.4	86.3	25.2	21.0	24.9	19.7	33.4	29.4	158	145
F	H	A	B	F	F	F	F	F	F	F	F
11.1	12.6	86.6	86.4	26.1	21.9	28.8	25.8	57.7	46.8	186	170
Result	N.S.	N.S.	*	N.S.	**	**	*	*	**	**	**	**
$d_{.05}$	-	-	9.5	-	8.4	7.7	5.3	5.3	10.9	8.6	15.4	14.5
$d_{.01}$	-	-	-	-	11.5	10.5	-	-	14.9	11.8	21.1	19.8

TABLE 3 GENOTYPIC RANKING OF RYEGRASS RESULTS

NON-METALS (me. %)												RATIO	
S		P		Cl		me. sum of anions ∑A		N		me. sum of non-metals ∑A + N		R Value	
NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
J	A	H	H	C	D	C	E	H	F	F	F	B	I
21.1	32.5	15.9	17.6	28.2	31.1	60.6	74.0	208	223	277	309	0.450	0.366
C	J	F	E	G	G	J	J	F	A	H	H	I	E
23.3	32.5	16.8	20.6	29.6	32.0	61.6	78.6	214	231	278	311	0.456	0.395
E	C	E	I	B	C	F	C	D	H	C	A	E	B
23.3	33.7	18.1	20.7	32.9	32.9	63.1	79.1	215	231	284	312	0.483	0.398
F	E	I	J	F	E	B	H	C	D	D	J	G	H
24.2	34.3	18.1	20.8	33.1	33.7	66.1	79.8	224	235	288	323	0.504	0.412
I	G	J	F	J	H	G	A	A	J	J	C	H	C
24.7	35.2	18.3	20.9	33.4	33.9	66.6	81.1	225	245	295	328	0.527	0.420
G	B	B	B	D	F	E	B	J	C	E	D	J	J
25.2	35.9	18.5	22.1	35.9	34.6	67.0	81.1	233	249	303	330	0.528	0.424
B	H	C	C	I	B	I	G	E	E	G	E	A	G
25.7	39.6	18.6	23.4	36.1	34.7	67.2	84.1	236	254	310	332	0.531	0.432
A	I	D	A	E	A	H	F	G	G	A	G	C	D
25.8	39.6	20.9	24.5	38.4	36.2	70.4	85.5	244	257	328	342	0.539	0.448
D	F	A	D	A	J	A	I	I	B	B	B	D	A
28.2	41.5	21.0	25.5	38.9	38.1	72.7	86.9	264	270	332	351	0.552	0.468
H	D	G	G	H	I	D	D	B	I	I	I	F	F
28.5	48.9	21.6	27.5	39.0	39.9	73.0	95.1	266	279	332	366	0.684	0.563
N.S.	N.S.	**	**	N.S.	N.S.	N.S.	N.S.	**	**	**	*	**	**
-	-	2.4	4.0	-	-	-	-	23	27	27	33	0.067	0.050
-	-	3.2	5.5	-	-	-	-	31	37	37	-	0.092	0.069

(b) Genetic Control Over Yield and Mineral Composition

(i) Yield: Differences among the genotypes in yield were not significant, irrespective of N form. The ranked order of genotypes was virtually the same whether plants had received NO_3 or NH_4, without the slightest indication of a genotype x N form interaction in determining yield.

(ii) Metallic cations: There were differences in the levels of Na, Mg, Ca and total bases, and these characters were under genetic control, whether plants received NO_3 or NH_4. A difference in K content, which was significant when plants received NO_3, failed to attain significance with NH_4. The range in Na content was almost fourfold, for Ca two and a half, and Mg almost twofold. Variation in K level was considerably smaller.

(iii) Non-metals: There were significant differences among the genotypes in the levels of total P and total N, when plants received either form of N. Largely as a result of differences in N content, there were related differences among the genotypes in total non-metal content, which were significant with either N form. The range of differences in the levels of these non-metals was considerably less than that encountered in the metallic cation contents. Differences in the levels of total S, Cl and total inorganic anions (P + S + Cl) were not significant. As Cl was not included in the media of experiment III, the data were reduced to only two observations on each genotype, receiving either NO_3 or NH_4, and this may have affected the result.

(iv) R-value: This proved to be under genetic control and was largely a reflection of differences in N and total base contents.

One genotype (F) had a significantly higher R-value than that of any other plant whether receiving NO_3 or NH_4 ($P < .01$). It also had a higher total base content than any other plants ($P < .01$) and the lowest total non-metal content of all plants, irrespective of N form.

(v) Discussion: The results confirm those of Butler et al., (1962). These authors studied 7 plants derived from crosses of perennial ryegrass and short rotation ryegrass (Lolium perenne x L. multiflorum). The plants were cloned and the clones grown in a fertile soil. Of the 12 mineral constituents analysed, 10 were under genetic control and most showed significant heritabilities. Of interest to the present study, these authors found significant differences in the contents of Na, Ca, free NO_3 and acid-soluble P, among the clones. No significant difference in K content was observed. In the present work, a significant difference in K content was found only where plants received NO_3. Of the metallic cations, Na displayed the largest range in levels in both the present study and that of Butler et al., (loc.cit.).

A singularly interesting point which has arisen in the current work is that there was no indication of a genotype x N form interaction in determining the yield or mineral composition of the test plants. Inspection of the data in table 3 shows that plants tended to rank in the same order of merit irrespective of the N form which they received. There was some variation in the results; significant differences between particular genotypes being lost with a change from one N form to the other, in some cases. But in no case was there a reversal of significance; a

plant was never significantly higher in the content of a particular element when receiving one N form and significantly lower when receiving the other. These results show, that under the experimental conditions, genetic control over mineral composition was exerted quite independently of the form of N which the plants were assimilating.

The apparent lack of any genotype x N form interaction suggests that the results for both N forms could probably be pooled for a single analysis of variance, with an attendant reduction in the variation of individual observations. On this basis significant differences in N content could be detected between genotypes A and C, and in total S content between genotypes D and J, as these plants ranked similarly when receiving NO_3 or NH_4.

(c) Correlations Among the Characters Studied

Spearman's rank correlations were used to test for possible relationships among the genotypes in the characters studied. The correlation coefficients were calculated for NO_3 and NH_4 separately using the relevant 10 paired characters.

No significant correlations between yield and the levels of individual elements was found, except in the case of N. Yield and N content were negatively related, irrespective of N form, under the experimental conditions:

NO_3-N $\quad r_s = -0.812 \quad (P < .01)$

NH_4-N $\quad r_s = -0.894 \quad (P < .01)$

Owing to the established negative relationship between yield and N content of ryegrass herbage (figure 3, p.75) the writer has

some reserve in considering the genetic control over N content as a true genetic control over N uptake. The results may have been partly a reflection of growth differences among the genotypes. The lack of any systematic relationship between yield and the foliar content of the other elements would suggest a true genetic control over absorption in the case of the metallic cations and P. If increased yield had resulted in decreased content by growth dilution, a negative correlation would have been expected as was the case with N. Conversely, if decreased content had caused a reduction in yield by sub-clinical deficiency, a positive correlation would have been expected.

Yield and R-value were positively correlated:

NO_3-N $\quad r_s = + 0.702 \quad (P < .05)$

NH_4-N $\quad r_s = + 0.564 \quad (P < .10)$

However, this was largely a reflection of the decreased N content with increasing yield, as there was no relationship between yield and total base content, nor between yield and inorganic anion content (S + P + Cl).

The value for the correlation between N content and total base content was small, negative and not significant:

NO_3-N $\quad r_s = -0.262 \quad (N.S.)$

NH_4-N $\quad r_s = -0.281 \quad (N.S.)$

Both N content and total metallic cation content were under genetic control in the test plants. Whatever the control mechanisms, they were apparently exerted in an independent manner. This lends no support to the suggestion of Cunningham and Nielsen (1965) that N metabolism and metallic cation accumulation in ryegrass

are causally linked. The observed antagonism between NH_4 and metallic cations in the current work (IV, B, 3) was apparently not the result of "carrier competition"(II, B, 4, (f), (i)). If the NH_4 ion were in competition for limited total transport of cations, a marked negative relationship between N content and total cation content would have been expected in the NH_4 series. One plant (F) contained more total cations than all the other plants whether receiving NO_3 or NH_4 ($P < .01$). Similarly, the observed antagonism between NO_3 and inorganic anions (IV, B, 4) was apparently not the result of carrier competition. There was no significant relationship between N and total inorganic anion contents, the values for the correlations being:

NO_3-N	- 0.152 (N.S.)
NH_4-N	+ 0.003 (N.S.)

Two plants (B and I) had a significantly higher N content than 7 others in the NO_3 series, and 4 others in the NH_4 series ($P < .05$). The same sort of parallellism among the genotypes and their content of individual elements held in all cases, with little change in ranking between N forms (table 3). These data therefore support the conclusion reached earlier, that the assimilation of NO_3 and NH_4 by grasses in the current work was largely independent of the uptake of other ionic species from the medium (IV, C, 1, (a)). It would appear that the observed differences in mineral composition caused by NO_3 and NH_4 were, in fact, the result of associated changes in the environment rather than the ionic form of N per se.; a suggestion which has been discussed (II, B, 4, (f)).

During NH_4 nutrition the movement of an excess of metallic cations over inorganic anions to the herbage necessitates an equivalent translocation of organic anions to maintain electrostatic balance (II, B, 4, (f), (ii)). For plants receiving NH_4, the organic anion content of tissues could be calculated (IV, C, 2, (a)). It was therefore decided to investigate the relationship between organic anion levels and cation content. No attempt was made to give a quantitative estimate for the correlation between organic anions and total cations. As the former have been calculated as the excess of the latter, any errors in the estimation of total cations would automatically increase the significance of the correlation. As the levels of each metallic cation had been determined independently, use of the divalent/monovalent cation ratio was acceptable. The values for these two characters are given in table 4, in ranked order. Both were under genetic control, the range in values being amost two-fold in each case. The two characters were also positively correlated:

$$NH_4\text{-}N \qquad r_s = +\ 0.821 \quad (P < .01)$$

This indicates that the genetic control over the relative uptake of divalent cations was linked to the genetic control over organic anion accumulation in these plants. On the basis of previous considerations to the effect that cation accumulation is dependent on anion accumulation in plants (IV, C, 2, (d)), this relationship suggests that the movement of divalent cations to the herbage was more dependent on organic anion translocation, than was the movement of monovalent cations. Neither of these

TABLE 4 GENOTYPIC RANKING OF THE ORGANIC ANION CONTENTS AND THE VALUES FOR THE DIVALENT/MONOVALENT CATION RATIO, FOR RYEGRASS PLANTS RECEIVING NH_4

Organic anions (me. %)	me. (Ca + Mg) % / me. (Na + K) %
H 59	H 0.37
I 60	E 0.41
D 62	J 0.41
E 64	D 0.43
B 69	I 0.44
J 70	C 0.48
C 71	B 0.50
A 74	G 0.51
G 75	A 0.52
F 97	F 0.75
Result **	**
$d_{.05}$ 12.9	0.12
$d_{.01}$ 17.7	0.16

two characteristics was related to N content, yield or total N uptake. There was, therefore, no indication that this genetic control was due to any indirect effect of NH_4 assimilation on tissue pH or external pH (II, B, 4, (f), (iii) and (iv)), as the intensity of NH_4 assimilation would be expected to be the major factor affecting them, under these conditions.

(d) Discussion

One of the principal aims in breeding pasture grasses should be to develop improved varieties which are not only productive, in terms of annual and seasonal yield,but which are also of desirable nutritional value to farm animals. Pasture herbage is not an agricultural end-product, but a means towards their production. All the elements investigated in the current work are required by animals, and must be present in their diet in reasonably balanced proportions (Whitehead, 1966). A further objective in pasture plant breeding, as currently practised in New Zealand, is to select improved varieties which will perform well over a wide range of environmental conditions, including differences in N regime.

It should be stressed that the results obtained in the present work apply strictly to the conditions of these experiments, and that the sample size of 10 plants limits the generality of the results.

In the present work, differences have been found in the levels of several major elements in the herbage of ryegrass, and these differences have been shown to be under genetic control. No estimates of heritability were made, as the estimated Error

Mean Squares included an unknown component of variance which may have arisen from genotype x experimental interactions. The recognition of these relatively large genetic differences in mineral content suggests that further detailed investigation should be made of the population to establish:

a. the range of these differences, and their heritabilities; and

b. their possible interrelationships with other characters of agronomic significance.

The above information would be a pre-requisite to any breeding programme aimed at the improvement of herbage mineral composition.

The absence of any apparent genotype x N form interaction in the determination of yield and mineral composition suggests that ryegrass selected under any particular qualitative N regime may be expected to perform in relatively the same manner, in terms of the characters studied, where the qualitative aspects of the N regime are different. This may be of particular significance in New Zealand, as much of the land area devoted to extensive farming practice may be continually under a "predominantly NH_4" N regime (II, A, 2, (c), (i)), while pasture plants may be selected under soil N conditions which are quite different. This must not be interpreted, however, as indicative that ryegrass plants selected under any particular quantitative N regime will necessarily perform in a similar manner when the quantitative characteristics of soil N are different. Interactions between genotypes and N levels have not been investigated in the present work.

There was a lack of any relationship between yield and

the levels of several important major elements in the present study, which illustrates that considerable differences in mineral composition of herbage can occur without any observable effect on yield. Similarly, Butler et al., (1962) found no significant genetic correlations between growth and mineral differences in ryegrass clones. This points to the danger inherent in plant breeding where DM. yield is the major criterion of selection. As an example, the suggested critical content of Mg in ryegrass herbage at which Mg deficiency may be expected to affect yield during vegetative growth is approximately 0.07% of DM. (De Wit et al., 1963). The suggested "safe level" for herbage Mg in relation to the occurrence of hypomagnesaemia in dairy cows is 0.2% of DM. (Kemp, 1960; Whitehead, 1966) or approximately three times the above figure. Pasture grass breeding with an associated screening for mineral content (whether direct, by chemical analyses, or indirect, by grazing trials) always allows the possibility of selecting parent plants of undesirable mineral composition, for instance of low Mg content. In this respect, New Zealand may be particularly vulnerable as major reliance has been placed on a very few species of pasture plants.

The relationship established between the content of organic anions and total metallic cations, and between organic anions and the relative uptake of divalent and monovalent metallic cations, requires verification under field conditions, and differing N regimes. If this relationship holds true under field conditions, grasses may be readily screened for total cations

and relative divalent cation content by the estimation of organic anions, using the rapid "nitrate corrected ash alkalinity" method of Van Tuil et al., (1964).

Comparison of the data for the effects of N form on the mineral composition of ryegrass (appendix 46) with those for the genetic differences in mineral content of ryegrass (table 3) shows that the latter effect was quantitatively much greater, especially for the metallic cations, under the conditions of these experiments.

4 CONCLUSIONS

Under the conditions of these experiments there was no indication that cations and anions are absorbed by grasses in constant proportions. With NO_3, the assimilation of a large excess of anions occurred, with NH_4, a large excess of cations. This agrees with the findings of other workers (II, B, 4, (e), (ii)). In the present study, there was no definite proportion of metals and non-metals in the herbage of ryegrass and sweet vernal, which agrees with recent evidence that R-value is not a constant (II, B, 5, (c)). It is concluded that N assimilation, irrespective of N form, occurred in ryegrass and sweet vernal in such a way that it was largely independent of the uptake of other cations and anions from the external medium. There were, however, some small compensatory changes in mineral composition. Nitrate nutrition led to higher organic anion contents in herbage, and was associated with a relatively greater uptake of divalent cations in both species, suggesting a causal relationship between

organic anion and divalent cation accumulation. Investigation of the analytical data for ryegrass has led to the recognition of large genetic differences in the levels of several important major elements, which would appear to warrant further investigation. There was no evidence to suggest that genetic control over the mineral composition of herbage was modified by the form of N assimilated by ryegrass, for the characters studied in this series of experiments. Where ryegrass received NH_4, the accumulation of organic anions was under genetic control, as was the relative uptake of divalent and monovalent cations, and these two characters were positively correlated. Under the conditions of these experiments, genetic differences in the mineral composition of ryegrass plants were greater than the differences caused by the two N forms, especially in the levels of metallic cations. Factors which caused a reduction in the organic anion content of ryegrass under the conditions of the experiments(NH_4 and genetic control), caused a relatively greater reduction in the levels of divalent cations than in the content of monovalent cations. This leads to the tentative suggestion that divalent cations are more reliant on chelation as the salts of organic acids for transport into the herbage of ryegrass, than are the monovalent cations. This theory may help to explain some of the established interactions between NO_3 and NH_4 and the uptake of metallic cations, on an intra-specific basis. The chelation of divalent cations as the salts of organic acids, by virtue of their non-polarity, may allow for a more rapid and extensive translocation in the plant.

SECTION V

SOME ASPECTS OF THE AGRONOMIC SIGNIFICANCE OF SEASONAL VARIATIONS IN THE SOIL N REGIME

1 INTRODUCTION

It has been concluded previously (II, A, 2, (b)) that under the high fertility soil conditions associated with the New Zealand grass-clover-grazing animal system of farming, there is a marked seasonal rhythm in the soil N regime. Ammonium predominates in the N nutrition of grasses in the winter and early spring periods, while the importance of NO_3 increases from spring through summer, and attains greatest significance during the "autumn flush" of grass growth. It is proposed to consider briefly some of the possible relationships which may exist between the soil N regime and the chemical composition of herbage and hence its nutritional value.

2 SEASONAL VARIATION IN THE MINERAL COMPOSITION OF PASTURE GRASSES

Accompanying seasonal variations in the N regime, there are also variations in major environmental factors including light, temperature and moisture, and changes in the botanical composition of a mixed sward. There may also be changes in the maturity of grasses if utilisation is less efficient during periods of maximum growth. It is, therefore, difficult to separate out any effects specifically arising from changes in the soil N regime.

Metson et al., (1966b) recently studied seasonal variations in the mineral composition of herbage from several field sites in the lower North Island. They found that the content of K, total N and total P in pasture grasses was lowest during summer and reached a maximum during winter. These relatively high values were maintained from March - April through to September - October, under the conditions prevailing during these observations. As grasses constituted 80% or more of the DM. at most samplings, the pattern was fairly representative of the mineral composition of the swards as a whole, which were under heavy stocking. Insofar as the present series of experiments has been representative of field conditions, this writer would expect an increase in the total P content of winter herbage, on the basis of the synergism between NH_4 and P which has been established in both soil experiments in the present work (IV, B, 4, (d), (i)). It is not clear to what extent the field observations of Metson et al., (loc.cit.) were also the result of varying herbage maturity. Whitehead (1966) reports no consistent, or regular seasonal variation in the content of N, P or K in grasses when the effects of advancing maturity are avoided by frequent defoliation. Metson et al., (loc.cit) also found an increase in Mg and Ca levels in grasses during late spring, summer and autumn. This increase in the content of divalent cations roughly coincided with the decline in the foliar levels of N, P and K. While growth dilution may have been partly responsible for the decrease in levels of the latter, this explanation cannot be applied to the simultaneous increase in the levels of the former. The current series of

experiments would suggest that the observed seasonal increase in Ca and Mg levels was the result of the increasing availability of soil NO_3, as a synergism between NO_3 and metallic cations, particularly Ca and Mg, has been established in all three experiments (IV, B, 3, (d), (i)). Whitehead (1966) reports that both Mg and Ca levels show an increase in grasses throughout spring and summer, when the effects of increasing maturity are avoided by frequent defoliation. Increasing soil temperature, besides its effects on nitrification, may have an indirect effect on the relative uptake of divalent cations, as the season progresses. Cunningham and Nielsen (1964) found that both increasing NO_3 availability and increasing soil temperature, with a marked positive interaction between the two, led to an increased content of Ca and Mg relative to Na and K in the herbage of Italian ryegrass. The herbage was, however, very mature at the time of harvest, after 8 weeks' regrowth.

3 SEASONAL DISORDERS IN FARM ANIMALS

It is extremely difficult to assess the contribution of seasonal variations in the soil N regime to the seasonal appearance of disorders in stock. Besides the associated changes in environment, and botanical composition and maturity of herbage, there may be associated physiological changes in the animals themselves; for instance, parturition and the onset of lactation. Other than their recognition, the latter factors are beyond the scope of this discussion. It is proposed to discuss briefly some possible implications of changes in the soil N

regime as they may affect stock health through changes in the chemical composition of grasses.

(a) "Grass Staggers"

This metabolic disorder occurs in New Zealand each spring, with varying severity. In dairy and beef cows, acute tetany is generally associated with lactation, most deaths occurring within a few weeks of calving. "Grass staggers" also occurs in pregnant cows and much less frequently in store cattle. No attempt is made to differentiate between hypomagnesaemia and hypocalceamia in this generalised discussion. The two may be frequently associated in "grass staggers" and are difficult to separate (Rook and Storry, 1962; Metson et al., 1966a). Successful therapy involves administration of Ca, Mg and glucose simultaneously, and it is not clear to which component(s) a cure can be attributed.

The chemical composition of pastures in relation to the incidence of "grass staggers" in beef cattle under New Zealand conditions, has been the subject of a recent review (Metson et al., 1966a). The following discussion is confined to a few comments about some possible contributions of the soil N regime during the period of maximum tetany incidence, to the occurrence of this metabolic disorder.

(i) Base levels in herbage: Reference has been made previously to a "safe" level of 0.2% Mg in herbage in relation to the onset of hypomagnesaemia (IV, C, 3, (d)). This is not an entirely correct concept; "grass staggers" has been observed on pastures containing more than this safe level, and there has been a failure

to observe this condition in animals grazing pasture containing less than the recommended safe level. There has been a general failure to correlate herbage Ca and Mg levels with the onset of "grass staggers", and besides animal factors, it is now believed that other chemical characteristics of the herbage can modify the "availability" of ingested Mg and Ca to the animal. There is, however, an overall picture which suggests that the lower the level of Mg, and possibly Ca, in herbage, the greater is the likelihood of tetany incidence (Rook and Storry, 1962; Metson et al., 1966a). Further, supplementation of Mg intake by various means gives a good measure of protection, which also suggests that total Mg uptake is important (loc.cit.).

As the occurrence of "grass tetany" coincides with that period when grasses are contributing the bulk of DM. production in a grass/clover sward (Metson et al., 1966a, 1966b), the chemical composition of grasses should have a major bearing on the nutritional value of the whole sward. In the present series of experiments, "predominantly NH_4" nutrition depressed the foliar content of both Mg and Ca in grasses (IV, B, 3, (d), (i)). Dutch workers have suggested that the ratio, me. K_{+}/me.(Mg + Ca)$_{+}$, may be important in determining the nutritional value of herbage, the incidence of tetany increasing as the proportion of divalent cations in herbage decreases (Kemp and 't Hart, 1957). In the present work, while this ratio has not been calculated, it has been found that the uptake of Ca and Mg has been more severely depressed by NH_4 than was that of K and Na (IV, C, 2, (d)). In the final experiment, where Na levels were very low, the uptake

of Ca and Mg was depressed by NH_4 to a greater extent than was that of K.

(ii) The level of total P in herbage: It has been suggested that the P/Mg and P/Ca levels in herbage may modify the "availability" of these divalent cations to the animal, higher relative levels of P being associated with lower "availabilities" (Metson et al., 1966a). In the two soil experiments of the current work, NH_4 nutrition resulted not only in a depression of herbage Mg and Ca, but also an increase in total P content. In experiment III, no significant increase in foliar P level was associated with this reduction of Mg and Ca during NH_4 nutrition, but this was probably an artefact of the experimental technique (IV, B, 4, (d), (i)). Clearly, grasses receiving NH_4 had higher P/Mg and P/Ca ratios than those receiving NO_3.

(iii) Organic acids: Recent work has implicated organic acids in the tetany syndrome. Burau and Stout (1965) found high levels of trans-aconitate in American range grasses during early spring, in areas which had a high incidence of tetany. Oral administration of comparatively low levels of trans-aconitate to heifers resulted in acute tetany and the death of some of the test animals (Anon., 1966). Burt and Thomas (1962) found that a dietary supplement of sodium citrate resulted in tetany symptoms in heifer calves. It has therefore been suggested that high levels of organic acid salts in early season forage may be associated with a reduction in the "availability" of dietary Mg, and possibly Ca, through the formation of chelates (Burau and Stout, 1965; Whitehead, 1966).

In the present study, grasses receiving NH_4 in the two soil experiments had a lower calculated organic anion content than those receiving NO_3 (IV, C, 2, (b)). The measured organic acid content of plants is characteristically reduced by NH_4 nutrition in relation to NO_3 (II, B, 3, (b), (iii)). In previous discussion associated with this project, evidence has been presented which suggests that organic acids may accumulate to comparatively high levels in either very mature ryegrass or ryegrass grown under conditions of N deficiency (II, B, 5, (d)). Neither of these conditions would appear to fit in with the characteristic pattern of "grass staggers" in New Zealand. A high incidence of this disorder is frequently associated with immature regrowth, a few weeks after grazing autumn-saved pasture. Further, available New Zealand data suggest that the N content of spring grass is embarassingly high when the maximum incidence of this disease occurs (Metson et al., 1966a).

No evidence has emerged from this study to link the N regime under New Zealand high fertility conditions, and its effects on organic anion accumulation, with the incidence of grass staggers. However, while the calculated total organic anion content of herbage has proved to be lower with NH_4 nutrition, particular organic acids may be important, and the spectrum of organic acids in the NH_4 herbage could be different from that receiving NO_3, although it is generally agreed that NH_4 nutrition reduces the content of malate, citrate and possibly oxalate, rather than other organic acids (II, B, 3, (b), (iii)). Further,

the relative levels of Mg (possibly Ca) and total organic acids may be important, and may be altered by NH_4 and NO_3 nutrition, an aspect which remains to be investigated.

It is possible that the situation under American and New Zealand conditions is quite dissimilar. American workers are at present investigating the possibility that accumulation of trans-aconitate in spring herbage may be the result of a low rate of organic matter mineralisation in rangeland soils during spring (Burau and Stout, 1966). With the stimulation of herbage growth under spring conditions, coupled with a deficiency of "anions" in the soil (N + P + S), there could result the accumulation of a large excess of metallic cations as their organic acid salts in herbage (loc.cit.), to maintain electrostatic balance in the tissues (see II, B, 4, (e)). This agrees with the finding that in mature ryegrass herbage grown under N deficient conditions (i.e. 1-1.5% total N in foliage), an excess of metallic cations over non-metallic ions is accumulated in the herbage, irrespective of the form of N available, and this excess of metallic cations must be equated by organic anions (II, B, 5, (d)).

(iv) Nitrogen and Carbohydrate fractions: The level of readily digestible carbohydrate in relation to the N content of herbage may be important in determining the "availability" of dietary Ca and Mg (Metson et al., 1966a). These authors suggest that the role of readily fermentable carbohydrates may be one of maintaining a sufficiently low pH in the "digestive apparatus" to allow Ca and Mg to persist in assimilable forms. A low level of total N in herbage has been related to a lower "safe" Mg level (Kemp,

1960; Rook and Storry, 1962). Teel (1962) found that forage with high levels of non-elaborated organic N reduced the voluntary DM. intake of heifers. He attributed this to the proneness of soluble organic N substances to more rapid deamination in the rumen than comparable amounts of true protein N.

Herbage produced with NH_4 nutrition has a characteristically higher organic N content and lower carbohydrate content than that receiving NO_3 (II, B, 3, (b), (i) and II, B, 3, (b), (ii)). Assimilation of NH_4 depletes carbohydrates with greater intensity than NO_3 assimilation, and results in herbage with higher levels of non-elaborated organic N constituents, as well as a lower soluble carbohydrate content, when compared with that receiving NO_3 (loc.cit.). Nowakowski et al., (1965) found that NH_4 nutrition of Italian ryegrass resulted in a lower total N content than NO_3, but the NH_4-fed grass had a much higher proportion of total N present in protein, soluble organic N and amide N forms, than the NO_3 grass. The latter had a much greater proportion of total N present as inorganic NO_3. At any level of soil N, ryegrass herbage receiving NH_4 contained less soluble carbohydrate than that receiving NO_3 (loc.cit.).

Carbohydrate and N fractions have not been investigated in the current work. However, luxury NH_4 supply in the culture solutions of experiment III resulted in an increase in total N content, from approximately 4.1% N with NO_3 to 4.5% N with NH_4, with no corresponding change in yield. This suggests that organic N compounds may have been formed at the expense of carbohydrates during NH_4 assimilation, as found by Nowakowski et al. (loc.cit.) with Italian ryegrass.

The "predominantly NH_4" regime during spring could contribute to the incidence of "grass tetany" through production of herbage characteristically high in soluble organic N compounds and low in readily fermentable carbohydrates. This may be particularly so under conditions where a considerable proportion of forage arises from grass growth in recent urine patches (Metson et al., 1966a), where the soil N level would be high (II, A, 2, (b)), and the effects of NH_4 on chemical composition intensified (Nowakowski et al., 1965). The effect of free ammonia production in the rumen, and the associated pH increase of rumen contents, on the "availability" of Mg and Ca to the animal is not clear. Reduced availability is apparently not associated with competition between NH_4 ions and metallic cations for absorption from the ingesta (pers.comm., C. W. Butler). While speculative, it is possible that the pH increase associated with digestion of foodstuffs with these characteristics may result in an increased passage of chelated or non-ionic Mg and Ca through the animals' digestive tract. If, for instance, the pH increase in the rumen were to cause an increase in the pH of the abomasum (normally pH ca. 2), even temporarily, non-ionic Ca and Mg compounds could pass from the alkaline environment of the rumen to the normally alkaline environment of the intestines, without being subjected to the normal acidic conditions of the abomasum. In this respect, the recent work of Deijs in Holland is of interest. He has found that blood serum Mg levels in cows may be depressed by feeds with a high lipid content, and suggests that the fatty acids released in the rumen during the breakdown of lipids may form insoluble

soaps with Mg and possibly Ca (pers.comm., A. J. Metson). Such soaps would normally be expected to dissociate at the low pH characteristic of the abomasum. If this were temporarily increased, the degree of dissociation of neutral soaps during their passage from the rumen to the intestines, may well be reduced. As the bulk of Mg is apparently absorbed from the ingesta during its passage through the intestines (Field, 1961) the increased passage of neutral soaps could lead to a reduced absorption and hence, a reduced "availability" of ingested Mg and Ca to the animal. Hawke (1963) found that the lipid content of short rotation ryegrass was higher in short, succulent regrowth (3.1% of DM.) than in more mature regrowth (5.1%). This study was conducted in New Zealand over several months, during two spring-early summer seasons. On the basis of evidence previously presented during this discussion, a high level of total N and soluble organic N, and a low level of readily available carbohydrates, would similarly be expected in leafy regrowth during the spring-early summer period. The possibility of a causal relationship with the incidence of "grass staggers" remains to be investigated.

To summarise, the "predominantly NH_4" soil N regime during spring may be related to the incidence of "grass staggers" through:

a. the antagonism of NH_4 with bases, Ca and Mg in particular, during uptake by grasses;

b. the synergism between NH_4 and P during uptake by grasses;

c. the production of herbage which is unbalanced in terms of organic N and readily fermentable carbohydrates.

(b) "Autumn Ill Thrift"

This condition is normally confined to the period of accelerated grass growth in autumn, coinciding with warm,moist conditions. Hoggets are particularly prone, leading to the condition known as "hogget ill thrift", with animals suffering from an apparent loss of appetite and associated weight losses (Butler, 1959; Butler and Johns, 1961).

The maximum concentration of free NO_3 in herbage coincides more or less with that period when NO_3 reaches greatest significance in the soil N regime (II, A, 2, (b)). A considerable amount of circumstantial evidence suggests that free NO_3 reaches maximum levels in grass on which animals are suffering from "autumn ill thrift" (Butler and Johns, 1961). It appears that NO_3 *per se* is no more than a contributor to the overall condition of the animal, which may be caused by the accumulation of metabolic intermediates during the reduction of NO_3 in the rumen (*loc.cit.*; Whitehead, 1966), or the accumulation of other non-protein N constituents in rapidly growing autumn grass (Butler, 1959). The most obvious characteristics of the chemical composition of such herbage are its high content of non-protein N, particularly free NO_3, coupled with a comparatively low level of soluble carbohydrates (Butler and Johns, 1961).

4 DISCUSSION AND CONCLUSIONS

The foregoing discussion of possible relationships between the soil N regime and the occurrence of "grass staggers" and "autumn ill thrift" under high fertility conditions in New Zealand,

has not added a great deal to present knowledge of the causes for these seasonal disorders in farm animals. However, recognition of the possible relationships between seasonal variations in the soil N regime and the occurrence of these disorders, would seem to justify further investigation into the relative effects of NO_3 and NH_4 on the chemical composition of pasture herbage.

The foregoing discussion has involved the tacit assumptions:

a. that the conditions of the current experiments were representative of field conditions; and

b. that the N regime in the field is "predominantly NH_4" during the occurrence of "grass staggers" and "predominantly NO_3" during the occurrence of "autumn ill thrift".

Neither of these assumptions is necessarily correct, although available information would suggest that the latter is correct. However, a high incidence of grass staggers and greater stock losses have been associated with a comparatively early spring flush of feed, combined with mild weather conditions, during a recent survey in Central Hawkes Bay (Metson et al., 1966a). Further, grass herbage containing high levels of free NO_3 has been observed in September in the Manawatu (Butler, 1959), accompanied by unusually dry, warm conditions. To what extent the soil N regime was changed by weather conditions in these cases is not known, although some nitrification and lack of leaching was obviously associated with the latter. Extrapolation of the data of Lyckiama (1963) for ryegrass would suggest that where NH_4 and NO_3 were simultaneously available to grasses, NH_4

could be expected to be assimilated while NO_3 accumulated in herbage. Possible interactions betwen the two N forms, when simultaneously available, could modify the chemical composition of herbage, and provides the basis for further investigation.

The elucidation of the precise physiological explanations for these seasonal disorders in farm animals is likely to be a lengthy programme, and will involve people from many disciplines. Most rapid progress may be made by the present, direct approach of making observations on herbage and animals during the actual course of these disorders. However, an incisive approach to the control of these disorders in the field will eventually have to be made. Whether control is effected by pasture and animal management, pasture plant breeding, manipulation of soil fertility, or any combination of these, basic knowledge will be required of those plant and soil factors which may be expected to modify the chemical composition of pasture herbage from season to season. Apart from moisture, the mineral N regime would appear to be the soil fertility factor which is most subject to seasonal variation under New Zealand high fertility conditions. The present study has been an attempt to examine some of the effects of NO_3 and NH_4 nitrogen on the mineral composition of pasture grasses, and to elucidate the plant physiological bases for the observed effects.

SUMMARY

Three glasshouse experiments were conducted to examine some of the effects of NO_3 and NH_4 nutrition on the mineral composition of two species of pasture grass. Ammonium nutrition, when compared with NO_3 nutrition, reduced the level of total bases in the herbage, especially Ca and Mg, and increased the levels of total S and total P. The two N forms were equally effective sources of mineral N, as determined by yield and total N content of the test plants.

With NO_3, plants assimilated a large excess of anions, and with NH_4, a large excess of cations. There were some compensatory changes in the uptake and accumulation of other ionic species from the medium when the ionic charge of mineral N was reversed, but these were small in relation to the difference in cation and anion assimilation by the plants resulting from the change in N form. It was concluded that ionic N caused only a small degree of interference with other ionic species during uptake and accumulation in the herbage of these grasses. Some possible plant physiological explanations for the observed effects were discussed.

It was found that in ryegrass the foliar content of several major elements varied widely among the plants, and was under genetic control. The data for sweet vernal were not investigated for genetic control over chemical composition.

Seasonal variation in the soil N regime and subsequent changes in the chemical composition of pasture grasses were considered as possible contributing factors to the seasonal appearance of some disorders in farm animals.

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APPENDIX 1 YIELD DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Calculated and Result
Replicates	9		
Treatments ‡	3	543.62	
N	1	1,092.02	1.36 N.S.
Sp	1	13.22	- N.S.
(N x Sp)	1	525.63	- N.S.
Error	27	803.66	
Total	39		

Coefficient of Variation (V) = 14.9%

MEAN YIELDS AND THEIR STANDARD ERRORS (g. D.M./Plant)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	1.88 ± 0.09	1.82	1.85 ± 0.06
NH_4-N	1.91	1.99	1.95
Species Means	1.89 ± 0.06	1.91	

‡ Treatments

N = Nitrogen Form
Sp = Species
(N x Sp) = (Nitrogen form x species) interaction

APPENDIX 2 YIELD DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	364,087.03	
N	1	93,122.50	4.83 *
Sp	1	838,102.50	43.52 **
(N x Sp)	1	161,036.10	8.36 **
Error	26	19,260.15	
Total	38		

V = 15.5%

MEAN YIELDS AND THEIR STANDARD ERRORS (g. D.M./Plant)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	10.57 ± 0.44	6.41 ± 0.46	8.49 ± 0.32
NH_4-N	10.27 ± 0.44	8.64 ± 0.44	9.45 ± 0.31
Species Means	10.42 ± 0.31	7.52 ± 0.32	

Detectable differences ‡ for nitrogen means within species or for species means within nitrogen forms:

$d_{.05}$ = 1.28

$d_{.01}$ = 1.73

‡ Detectable difference at the 5% probability level = $d_{.05}$

Detectable difference at the 1% probability level = $d_{.01}$

APPENDIX 3 YIELD DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	5	528,031.59	
N	2	549,235.22	20.85 **
Sp	1	1,513,046.40	57.43 **
(N x Sp)	2	4,318.05	- N.S.
Error	45	26,346.36	
Total	59		

V = 9.3%

MEAN YIELDS AND THEIR STANDARD ERRORS (g. D.M./Plant)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	18.05 ± 0.51	14.35	16.20 ± 0.36
NH_4 NO_3	20.62	17.99	19.30
NH_4-N	18.34	15.13	16.74
Species Means	19.00 ± 0.30	15.83	

Detectable differences for nitrogen means:

$d_{.05}$ = 1.03

$d_{.01}$ = 1.38

APPENDIX 4 POTASSIUM DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	13,754.42	
N	1	4,774.22	- N.S.
Sp	1	36,060.02	7.17 *
(N x Sp)	1	429.03	- N.S.
Error	27	5,029.13	
Total	39		

V = 8.9%

MEAN POTASSIUM CONTENTS AND THEIR STANDARD ERRORS (me.K%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	77.9 ± 2.2	84.6	81.3 ± 1.6
NH_4-N	76.4	81.7	79.1
Species Means	77.2 ± 1.6	83.2	

APPENDIX 5 POTASSIUM DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	46,549.40	
N	1	16,728.10	6.32 *
Sp	1	104,857.60	39.64 **
(N x Sp)	1	18,062.50	6.83 *
Error	26	2,645.42	
Total	38		

$$V = 8.2\%$$

MEAN POTASSIUM CONTENTS AND THEIR STANDARD ERRORS (me. K%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	53.3 ± 1.6	67.8 ± 1.7	60.6 ± 1.2
NH_4-N	61.6 ± 1.6	67.6 ± 1.6	64.6 ± 1.2
Species Means	57.5 ± 1.2	67.7 ± 1.2	

Detectable differences for nitrogen means within species or for species means within nitrogen forms:

$d_{.05} = 4.7$

$d_{.01} = 6.4$

APPENDIX 6 POTASSIUM DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and result
Replicates	9		
Treatments	5	80,071.22	
N	2	101,903.87	40.09 **
Sp	1	138,144.01	54.35 **
(N x Sp)	2	24,202.22	9.52 **
Error	45	2,541.91	
Total	59		

V = 4.3%

MEAN POTASSIUM CONTENTS AND THEIR STANDARD ERRORS (me. K%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	111.9 ± 1.6	127.2	119.6 ± 1.1
NH_4 NO_3	115.7	127.2	121.5
NH_4-N	107.0	108.9	108.0
Species Means	111.5 ± 0.9	121.1	

Detectable differences for nitrogen means:

$d_{.05}$ = 3.2

$d_{.01}$ = 4.3

Detectable differences for nitrogen means within species or for species means within nitrogen forms:

$d_{.05}$ = 4.5

$d_{.01}$ = 6.1

APPENDIX 7 SODIUM DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Calculated and Result
Replicates	9		
Treatments	3	127,700.95	
N	1	137,710.22	- N.S.
Sp	1	218,300.62	- N.S.
(N x Sp)	1	27,092.03	- N.S.
Error	27	337,333.52	
Total	39		

V = 26.8%

MEAN SODIUM CONTENTS AND THEIR STANDARD ERRORS (me. Na%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	23.3 ± 1.8	21.3	22.3 ± 1.3
NH_4-N	21.6	20.7	21.1
Species Means	22.5 ± 1.3	21.0	

APPENDIX 8 SODIUM DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	2,002,179.29	
N	1	5,888,260.22	14.59 **
Sp	1	104,550.62	- N.S.
(N x Sp)	1	13,727.03	- N.S.
Error	26	403,544.01	
Total	38		

$V = 30.4\%$

MEAN SODIUM CONTENTS AND THEIR STANDARD ERRORS (me. Na%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	25.4 ± 2.0	24.0 ± 2.1	24.8 ± 1.5
NH_4-N	17.4 ± 2.0	16.7 ± 2.0	17.1 ± 1.4
Species Means	21.4 ± 1.4	20.4 ± 1.5	

APPENDIX 9 SODIUM DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	5	33,768.24	
N	2	66,362.55	9.26 **
Sp	1	32,295.40	4.51 *
(N x Sp)	2	1,910.35	- N.S.
Error	45	7,163.00	
Total	59		

V = 32.8%

MEAN SODIUM CONTENTS AND THEIR STANDARD ERROR (me. Na%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	2.83 ± 0.29	3.48	3.15 ± 0.20
NH_4 NO_3	2.35	2.83	2.59
NH_4-N	1.87	2.13	2.00
Species Means	2.35 ± 0.15	2.88	

Detectable differences for nitrogen means:

$d_{.05}$ = 0.54

$d_{.01}$ = 0.72

APPENDIX 10 MAGNESIUM DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	288,719.03	
N	1	105,884.10	3.11 ($p<.10$)
Sp	1	760,104.90	22.30 **
(N x Sp)	1	168.10	- N.S.
Error	27	34,055.50	
Total	39		

V = 14.3%

MEAN MAGNESIUM CONTENTS AND THEIR STANDARD ERRORS (me. Mg%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	14.8 ± 0.6	12.0	13.4 ± 0.4
NH_4-N	13.7	11.0	12.4
Species Means	14.3 ± 0.4	11.5	

APPENDIX 11 MAGNESIUM DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and result
Replicates	9		
Treatments	3	254,465.76	
N	1	694,586.02	10.56 **
Sp	1	4.22	- N.S.
(N x Sp)	1	68,807.03	1.05 N.S.
Error	26	65,775.08	
Total	38		

V = 18.0%

MEAN MAGNESIUM CONTENTS AND THEIR STANDARD ERRORS (me. Mg%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	15.1 ± 0.8	16.0 ± 0.9	15.6 ± 0.6
NH_4-N	13.3 ± 0.8	12.5 ± 0.8	12.9 ± 0.6
Species Means	14.2 ± 0.6	14.2 ± 0.6	

APPENDIX 12 MAGNESIUM DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	5	50,734.00	
N	2	47,874.05	30.43 **
Sp	1	154,635.27	98.28 **
(N x Sp)	2	1,643.32	1.04 N.S.
Error	45	1,573.40	
Total	59		

V = 14.3%

MEAN MAGNESIUM CONTENTS AND THEIR STANDARD ERRORS (me. Mg%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	38.8 ± 1.3	27.6	33.2 ± 0.9
NH_4 NO_3	31.9	20.7	26.3
NH_4-N	27.8	19.7	23.8
Species Means	32.8 ± 0.7	22.7	

Detectable differences for nitrogen means:

$d_{.05}$ = 2.5

$d_{.01}$ = 3.4

APPENDIX 13 CALCIUM DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Calculated and Result
Replicates	9		
Treatments	3	26,632.02	
N	1	13,653.02	2.56 ($p < .15$)
Sp	1	65,529.02	12.29 **
(N x Sp)	1	714.03	- N.S.
Error	27	5,334.06	
Total	39		

V = 19.3%

MEAN CALCIUM CONTENTS AND THEIR STANDARD ERRORS (me. Ca%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	44.1 ± 2.3	35.2	39.6 ± 1.6
NH_4-N	39.6	32.3	35.9
Species Means	41.8 ± 1.6	33.7	

APPENDIX 14 CALCIUM DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	49,528.80	
N	1	85,377.60	13.75 **
Sp	1	56,550.40	9.11 **
(N x Sp)	1	6,658.40	1.07 N.S.
Error	26	6,210.48	
Total	38		

V = 26.8%

MEAN CALCIUM CONTENTS AND THEIR STANDARD ERRORS (me. Ca%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	36.5 ± 2.5	31.6 ± 2.6	34.0 ± 1.8
NH_4-N	29.8 ± 2.5	19.7 ± 2.5	24.8 ± 1.8
Species Means	33.2 ± 1.8	25.7 ± 1.8	

APPENDIX 15 CALCIUM DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Calculated and Result
Replicates	9		
Treatments	5	16,291.60	
N	2	33,239.85	63.07 **
Sp	1	14,539.27	27.59 **
(N x Sp)	2	219.52	- N.S.
Error	45	527.04	
Total	59		

V = 16.3%

MEAN CALCIUM CONTENTS AND THEIR STANDARD ERRORS (me. Ca%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	20.0 ± 0.7	16.5	18.3 ± 0.5
NH_4 NO_3	15.6	12.2	13.9
NH_4-N	11.3	9.0	10.1
Species Means	15.6 ± 0.4	12.5	

Detectable differences for nitrogen means:

$d_{.05}$ = 1.5 $d_{.01}$ = 2.0

APPENDIX 16 ΣC DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Calculated and Result
Replicates	9		
Treatments	3	36,374.03	
N	1	73,874.05	6.36 *
Sp	1	33,698.05	2.90 N.S.
(N x Sp)	1	1,550.00	- N.S.
Error	27	11,618.64	
Total	39		

V = 7.1%

MEAN ΣC CONTENTS AND THEIR STANDARD ERRORS (me.ΣC%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	160.1 ± 3.4	153.1	156.6 ± 2.4
NH_4-N	150.3	145.7	148.0
Species Means	155.1 ± 2.4	149.4	

APPENDIX 17 ΣC DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	100,356.96	
N	1	252,333.22	16.70 **
Sp	1	4,182.02	- N.S.
(N x Sp)	1	44,555.63	2.95 N.S.
Error	26	15,107.55	
Total	38		

V = 9.7%

MEAN Σ C CONTENTS AND THEIR STANDARD ERRORS (me. ΣC%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	130.4 ± 3.9	139.1 ± 4.1	134.7 ± 2.8
NH_4-N	121.2 ± 3.9	116.5 ± 3.9	118.9 ± 2.7
Species Means	125.8 ± 2.7	127.8 ± 2.8	

APPENDIX 18 $\sum$C DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Calculated and Result
Replicates	9		
Treatments	5	199,010.19	
N	2	478.418.85	76.21 **
Sp	1	15,073.35	2.40 N.S.
(N x Sp)	2	11,569.95	1.84 N.S.
Error	45	6,277.64	
Total	59		

V = 9.7%

MEAN $\sum$C CONTENTS AND THEIR STANDARD ERRORS (me. $\sum$C%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	173.5 ± 2.5	174.8	174.2 ± 1.8
NH_4 NO_3	165.5	162.9	164.2
NH_4-N	147.9	139.7	143.8
Species Means	162.3 ± 1.4	159.1	

Detectable differences for nitrogen means:

$d_{.05}$ = 5.1

$d_{.01}$ = 6.8

APPENDIX 19 SULPHUR DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Calculated and Result
Replicates	9		
Treatments	3	560,740.69	
N	1	196,420.22	7.87 **
Sp	1	1,461,150.62	58.57 **
(N x Sp)	1	24,651.23	- N.S.
Error	27	24,945.49	
Total	39		

V = 7.6%

MEAN SULPHUR CONTENTS AND THEIR STANDARD ERRORS (me. S%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	21.77 ± 0.50	18.44	20.10 ± 0.35
NH_4-N	23.66	19.34	21.50
Species Means	22.71 ± 0.35	18.89	

APPENDIX 20 SULPHUR DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Calculated and Result
Replicates	9		
Treatments	3	48,863.30	
N	1	73,444.90	28.29 **
Sp	1	36,120.10	13.91 **
(N x Sp)	1	31,024.90	11.95 **
Error	26	2,596.25	
Total	38		

V = 21.2%

MEAN SULPHUR CONTENTS AND THEIR STANDARD ERRORS (me. S%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	20.0 ± 1.6	19.6 ± 1.7	19.8 ± 1.2
NH_4-N	34.2 ± 1.6	22.6 ± 1.6	28.4 ± 1.2
Species Means	27.1 ± 1.1	21.1 ± 1.2	

Detectable differences for nitrogen means within species or for species means within nitrogen forms:

$d_{.05}$ = 4.7

$d_{.01}$ = 6.3

APPENDIX 21 SULPHUR DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	5	154,474.31	
N	2	157,921.72	31.05 **
Sp	1	439,984.06	86.50 **
(N x Sp)	2	8,272.02	1.63 N.S.
Error	45	5,086.33	
Total	59		

V = 19.9%

MEAN SULPHUR CONTENTS AND THEIR STANDARD ERRORS (me. S%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	33.2 ± 2.3	20.8	27.0 ± 1.6
NH_4NO_3	45.6	25.8	35.7
NH_4-N	54.3	35.2	44.8
Species Means	44.4 ± 1.3	27.3	

Detectable differences for nitrogen means:

$d_{.05}$ = 4.5

$d_{.01}$ = 6.1

APPENDIX 22 PHOSPHORUS DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	5,234.16	
N	1	13,801.22	17.28 **
Sp	1	1,404.22	1.76 N.S.
(N x Sp)	1	497.03	- N.S.
Error	27	798.62	
Total	39		

V = 12.7%

MEAN PHOSPHORUS CONTENTS AND THEIR STANDARD ERRORS (me. P%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	20.7 ± 0.9	20.2	20.5 ± 0.6
NH_4-N	25.1	23.2	24.2
Species Means	22.9 ± 0.6	21.7	

APPENDIX 23 PHOSPHORUS DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	3,829.73	
N	1	8,584.70	24.05 **
Sp	1	1,488.20	4.17 (p = .06)
(N x Sp)	1	1,416.30	3.97 (p = .07)
Error	26	356.92	
Total	38		

V = 11.3%

MEAN PHOSPHORUS CONTENTS AND THEIR STANDARD ERRORS (me. P%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	14.05 ± 0.60	16.46 ± 0.63	15.26 ± 0.43
NH_4-N	18.17 ± 0.60	18.20 ± 0.60	18.19 ± 0.42
Species Means	16.11 ± 0.42	17.33 ± 0.43	

Detectable differences for nitrogen means within species or for species means within nitrogen forms:

$d_{.05}$ = 1.82

$d_{.01}$ = 2.46

(Calculated to allow for missing plot)

APPENDIX 24 PHOSPHORUS DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	5	2,946.18	
N	2	3,794.72	9.40 **
Sp	1	5,245.35	13.00 **
(N x Sp)	2	948.05	2.35 N.S.
Error	45	403.58	
Total	59		

V = 8.9%

MEAN PHOSPHORUS CONTENTS AND THEIR STANDARD ERRORS (me. P%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	21.56 ± 0.64	21.28	21.42 ± 0.45
NH_4-NO_3	25.48	22.82	24.15
NH_4-N	23.80	21.13	22.47
Species Means	23.61 ± 0.37	21.74	

Detectable differences for nitrogen means:

$d_{.05}$ = 1.28

$d_{.01}$ = 1.71

APPENDIX 25 CHLORIDE DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Calculated and Result
Replicates	9		
Treatments	3	1,805.43	
N	1	756.90	- N.S.
Sp	1	4,536.90	2.45 N.S.
(N x Sp)	1	122.50	- N.S.
Error	27	1,852.10	
Total	39		

V = 9.7%

MEAN CHLORIDE CONTENTS AND THEIR STANDARD ERRORS (me. Cl%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	44.9 ± 1.4	43.1	44.0 ± 1.0
NH_4-N	46.1	43.6	44.8
Species Means	45.5 ± 1.0	43.3	

APPENDIX 26 CHLORIDE DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	13,405.77	
N	1	13,987.60	3.66 N.S.
Sp	1	3,571.80	- N.S.
(N x Sp)	1	22,657.90	5.93 *
Error	26	3,818.93	
Total	38		

V = 27%

MEAN CHLORIDE CONTENTS AND THEIR STANDARD ERRORS (me. Cl%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	24.3 ± 2.0	17.7 ± 2.1	21.0 ± 1.4
NH_4-N	23.3 ± 2.0	26.2 ± 2.0	24.7 ± 1.4
Species Means	23.8 ± 1.4	22.9 ± 1.4	

Detectable differences for nitrogen means within species or for species means within nitrogen forms:

$d_{.05}$ = 6.0

$d_{.01}$ = 8.1

(calculated to allow for missing plot)

APPENDIX 27 ΣA DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	29,754.62	
N	1	35,700.62	15.00 **
Sp	1	51,051.02	21.45 **
(N x Sp)	1	2,512.23	1.06 N.S.
Error	27	2,380.50	
Total	39		

V = 5.6%

MEAN ΣA CONTENTS AND THEIR STANDARD ERRORS (me.ΣA%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	87.3 ± 1.5	81.8	84.5 ± 1.1
NH_4-N	94.9	86.2	90.5
Species Means	91.1 ± 1.1	84.0	

APPENDIX 28 ΣA DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	78,107.49	
N	1	201,498.02	50.11 **
Sp	1	31,753.22	7.90 **
(N x Sp)	1	1,071.23	- N.S.
Error	26	4,020.80	
Total	38		

V = 10.0%

MEAN ΣA CONTENTS AND THEIR STANDARD ERRORS (me. ΣA%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	58.4 ± 2.0	53.8 ± 2.1	56.1 ± 1.5
NH_4-N	73.6 ± 2.0	66.9 ± 2.0	70.3 ± 1.4
Species Means	66.0 ± 1.4	60.4 ± 1.5	

APPENDIX 29 Σ A DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	5	186,258.43	
N	2	179,850.32	35.92 **
Sp	1	541,880.06	108.23 **
(N x Sp)	2	14,855.72	2.97 N.S.
Error	45	5,006.52	
Total	59		

V = 12.1%

MEAN Σ A CONTENTS AND THEIR STANDARD ERRORS (me. Σ A%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	54.8 ± 2.2	42.1	48.4 ± 1.6
NH_4 NO_3	71.1	48.6	59.8
NH_4-N	78.1	56.4	67.2
Species Means	68.0 ± 1.3	49.0	

Detectable differences for nitrogen means:

$d_{.05}$ = 4.5

$d_{.01}$ = 6.0

EXPERIMENT 30 NITROGEN DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	13,701.67	
N	1	5,062.50	- N.S.
Sp	1	35,402.50	- N.S.
(N x Sp)	1	640.00	- N.S.
Error	27	56,349.14	
Total	39		

V = 9.5%

MEAN NITROGEN CONTENTS AND THEIR STANDARD ERRORS (me. N%)

Sp / N	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	245 ± 8	252	249 ± 5
NH_4-N	249	254	251
Species Means	247 ± 5	253	

APPENDIX 31 NITROGEN DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	15,446.49	
N	1	1,452.02	2.95 ($p < .10$)
Sp	1	41,409.22	83.99 **
(N x Sp)	1	3,478.23	7.05 *
Error	26	493.03	
Total	38		

V = 10.8%

MEAN NITROGEN CONTENTS AND THEIR STANDARD ERRORS (me. N%)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	170 ± 7	253 ± 7	211 ± 5
NH_4-N	176 ± 7	222 ± 7	199 ± 5
Species Means	173 ± 5	237 ± 5	

Detectsble differences for nitrogen means within species or for species means within nitrogen forms:

$d_{.05}$ = 20

$d_{.01}$ = 28

APPENDIX 32 NITROGEN DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	5	2,421.66	
N	2	3,631.52	11.29 **
Sp	1	4,318.01	13.42 **
(N x Sp)	2	263.62	- N.S.
Error	45	321.68	
Total	59		

V = 5.8%

MEAN NITROGEN CONTENTS AND THEIR STANDARD ERRORS (me. N%)

Sp / N	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	284.1 ± 5.7	309.4	296.8 ± 4.0
NH_4 NO_3	303.4	317.0	310.2
NH_4-N	317.7	329.7	323.7
Species Means	301.7 ± 3.3	318.7	

Detectable differences for nitrogen means:

$d_{.05}$ = 11.4

$d_{.01}$ = 15.3

APPENDIX 33 ΣA + N DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	246.57	
N	1	672.40	1.10 N.S.
Sp	1	14.40	- N.S.
(N x Sp)	1	52.90	- N.S.
Error	27	612.94	
Total	39		

V = 7.3%

MEAN ΣA + N CONTENTS AND THEIR STANDARD ERRORS (me. ΣA + N%)

Sp / N	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	333 ± 8	334	333
NH_4-N	343	340	342
Species Means	338 ± 6	337	

APPENDIX 34 ΣA + N DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	12,410.57	
N	1	122.50	- N.S.
Sp	1	32,947.60	66.51 **
(N x Sp)	1	4,161.60	8.40 **
Error	26	495.40	
Total	38		

V = 8.3%

MEAN ΣA + N CONTENTS AND THEIR STANDARD ERRORS (me. ΣA + N/l)

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	228 ± 7	306 ± 7	267 ± 5
NH_4-N	252 ± 7	289 ± 7	270 ± 5
Species Means	240 ± 5	297 ± 5	

Detectable differences for nitrogen means within species or for species means within nitrogen forms:

$d_{.05}$ = 21

$d_{.01}$ = 28

APPENDIX 35 ΣA + N DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	5	4,573.27	
N	2	10,612.27	29.54 **
Sp	1	56.10	- N.S.
(N x Sp)	2	792.85	2.21 N.S.
Error	45	359.21	
Total	59		

V = 5.1%

MEAN ΣA + N CONTENTS AND THEIR STANDARD ERRORS (me. ΣA + N%)

Sp / N	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	338.7 ± 6.0	351.3	345.0 ± 4.2
NH_4 NO_3	374.6	365.8	370.2
NH_4-N	395.8	386.2	391.0
Species Means	369.7 ± 3.5	367.8	

Detectable differences for nitrogen means:

$d_{.05}$ = 12.1

$d_{.01}$ = 16.1

APPENDIX 36 RATIO $\frac{\Sigma C}{\Sigma A}$ DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	183,895.42	
N	1	485,982.02	24.72 **
Sp	1	52,780.22	2.68 N.S.
(N x Sp)	1	12,924.03	- N.S.
Error	27	19,661.18	
Total	39		

V = 8.1%

MEAN VALUES FOR THE RATIO $\frac{\Sigma C}{\Sigma A}$ AND THEIR STANDARD ERRORS

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	1.841 ± 0.044	1.878	1.859 ± 0.031
NH_4-N	1.585	1.693	1.639
Species Means	1.713 ± 0.031	1.785	

APPENDIX 37 RATIO $\frac{\sum C}{\sum A}$ DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	2,318,728.22	
N	1	5,996,179.22	54.29 **
Sp	1	784,840.22	7.11 *
(N x Sp)	1	175,165.23	1.59 N.S.
Error	26	110,449.67	
Total	38		

V = 16.1%

MEAN VALUES FOR THE RATIO $\frac{\sum C}{\sum A}$ AND THEIR STANDARD ERRORS

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	2.241 ± 0.105	2.654 ± 0.111	2.448 ± 0.076
NH_4-N	1.599 ± 0.105	1.747 ± 0.105	1.673 ± 0.074
Species Means	1.920 ± 0.074	2.201 ± 0.076	

APPENDIX 38 RATIO $\frac{\Sigma C}{\Sigma A}$ DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	5	68,009.05	
N	2	111,413.62	91.87 **
Sp	1	111,718.35	92.12 **
(N x Sp)	2	2,749.85	2.27 N.S.
Error	45	1,212.69	
Total	59		

V = 11.9%

MEAN VALUES FOR THE RATIO $\frac{\Sigma C}{\Sigma A}$ AND THEIR STANDARD ERRORS

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	3.236 ± 0.110	4.193	3.715 ± 0.078
NH_4 NO_3	2.355	3.391	2.873
NH_4-N	1.928	2.524	2.226
Species Means	2.506 ± 0.064	3.369	

Detectable differences for nitrogen means:

$d_{.05}$ = 0.222

$d_{.01}$ = 0.296

APPENDIX 39 R VALUE DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated And Result	
Replicates	9			
Treatments	3	5,956.83		
N	1	13,690.00	6.27	*
Sp	1	3,572.10	1.64	N.S.
(N x Sp)	1	608.40	-	N.S.
Error	27	2,184.65		
Total	39			

V = 10.3%

MEAN R VALUES AND THEIR STANDARD ERRORS

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	0.485 ± 0.015	0.459	0.472 ± 0.010
NH_4-N	0.441	0.430	0.435
Species Means	0.463 ± 0.010	0.444	

APPENDIX 40 R VALUE DATA: EXPERIMENT II

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	3	52,250.41	
N	1	52,056.22	20.95 **
Sp	1	99,700.22	40.12 **
(N x Sp)	1	4,995.23	2.01 N.S.
Error	26	2,485·02	
Total	38		

V = 10.4%

MEAN R VALUES AND THEIR STANDARD ERRORS

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	0.577 ± 0.016	0.454 ± 0.017	0.515 ± 0.011
NH_4-N	0.482 ± 0.016	0.405 ± 0.016	0.443 ± 0.011
Species Means	0.529 ± 0.011	0.429 ± 0.011	

APPENDIX 41 R VALUE DATA: EXPERIMENT III

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Treatments	5	38,272.79	
N	2	94,590.82	106.67 **
Sp	1	1,041.66	1.17 N.S.
(N x Sp)	2	570.32	- N.S.
Error	45	886.79	
Total	59		

V = 6.8%

MEAN R VALUES AND THEIR STANDARD ERRORS

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	0.514 ± 0.009	0.498	0.506 ± 0.007
NH_4 NO_3	0.444	0.447	0.445
NH_4-N	0.376	0.363	0.369
Species Means	0.444 ± 0.005	0.436	

Detectable differences for nitrogen means:

$d_{.05}$ = 0.019

$d_{.01}$ = 0.025

CATION UPTAKE

APPENDIX 42 RATIO ANION UPTAKE DATA: EXPERIMENT I

ANALYSIS OF VARIANCE

(a) Data from NO_3-N Treatment

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Species	1	3,537.80	1.24 N.S.
Error	9	2,853.91	
Total	19		

V = 11.3%

(b) Data from NH_4-N Treatment

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Species	1	913,781.25	11.29 **
Error	9	80,899.31	
Total	19		

V = 6.4%

CATION UPTAKE

MEAN VALUES FOR THE RATIO ANION UPTAKE, AND THEIR STANDARD ERRORS

Sp / N	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	0.485 ± 0.017	0.459 ± 0.017	0.472 ± 0.012
NH_4-N	4.212 ± 0.090	4.640 ± 0.090	4.426 ± 0.064

CATION UPTAKE

APPENDIX 43 RATIO ANION UPTAKE DATA: EXPERIMENT II

ANALYSES OF VARIANCE

(a) Data from NO_3-N Treatment

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Species	1	69,738.05	12.12 **
Error	8	5,756.18	
Total	18		

V = 14.7%

(b) Data from NH_4-N Treatment

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Species	1	6,544,824.05	28.69 **
Error	9	228,121.38	
Total	19		

V = 10.6%

MEAN VALUES FOR THE RATIO $\frac{\text{CATION UPTAKE}}{\text{ANION UPTAKE}}$, AND THEIR STANDARD ERRORS

Sp / N	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	0.577 ± 0.024	0.458 ± 0.025	0.518 ± 0.017
NH_4-N	3.944 ± 0.151	5.088 ± 0.151	4.516 ± 0.114

APPENDIX 44 CATION UPTAKE
RATIO ANION UPTAKE DATA: EXPERIMENT III

ANALYSES OF VARIANCE

(a) Data from NO_3-N Treatment

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Species	1	1,248.20	- N.S.
Error	9	1,642.87	
Total	19		

V = 8.0%

(b) Data from NH_4-N Treatment

Source of Variation	Degrees of Freedom	Mean Square	F calculated and Result
Replicates	9		
Species	1	29,248,129.80	27.40 **
Error	9	1,067,434.13	
Total	19		

V = 14.2%

CATION UPTAKE
MEAN VALUES FOR THE RATIO ANION UPTAKE, AND THEIR STANDARD ERRORS

N \ Sp	Ryegrass	Sweet Vernal	Nitrogen Means
NO_3-N	0.514 ± 0.013	0.498 ± 0.013	0.506 ± 0.009
NH_4-N	6.073 ± 0.327	8.492 ± 0.327	7.283 ± 0.237

APPENDIX 45 SUMMARY OF NITROGEN RESULTS AVERAGED OVER BOTH SPECIES

	YIELD	METALS (me. %)					NON-METALS (me. %)						RATIOS		
	(g.D.M. /plant	K	Na	Mg	Ca	me. sum of metals ($\sum$C)	S	P	Cl	me. sum of anions ($\sum$A)	N	me. sum of non-metals ($\sum$A + N)	$\frac{\sum C}{\sum A}$	R Value	Cation Uptake / Anion Uptake
EXPERIMENT I															
NO_3-N	1.85	81.3	22.3	13.4	39.6	156.6	20.1	20.5	44.0	84.5	249	333	1.859	0.472	0.472
NH_4-N	1.95	79.1	21.1	12.4	35.9	148.0	21.5	24.2	44.8	90.5	251	342	1.639	0.435	4.426
Result	N.S.	N.S.	N.S.	p<.10	p<.15	*	**	**	N.S.	**	N.S.	N.S.	**	*	**
EXPERIMENT II															
NO_3-N	8.49	60.6	24.8	15.6	34.0	134.7	19.8	15.26	21.0	56.1	211	267	2.448	0.515	0.518
NH_4-N	9.45	64.6	17.1	12.9	24.8	118.9	28.4	18.19	24.7	70.3	199	270	1.673	0.443	4.516
Result	*	*	**	**	**	**	**	**	N.S.	**	N.S.	N.S.	**	**	**
EXPERIMENT III															
NO_3-N	16.20	119.6	3.15	33.2	18.3	174.2	27.0	21.42	-	48.4	296.8	345.0	3.715	0.506	0.506
NH_4 NO_3	19.30	121.5	2.59	26.3	13.9	164.2	35.7	24.15	-	59.8	310.2	370.2	2.873	0.445	-
NH_4-N	16.74	108.0	2.00	23.8	10.1	143.8	44.8	22.47	-	67.2	323.7	391.0	2.226	0.369	7.283
Result	**	**	**	**	**	**	**	**	-	**	**	**	**	**	**

APPENDIX 46 SUMMARY OF RYEGRASS RESULTS

	YIELD	METALS (me. %)					NON-METALS (me. %)						RATIOS		
	(g. D.M. /plant)	K	Na	Mg	Ca	me. sum of metals ($\sum$C)	S	P	Cl	me. sum of anions ($\sum$A)	N	me. sum of non-metals ($\sum$A + N)	$\frac{\sum C}{\sum A}$	R Value	Cation Uptake / Anion Uptake
EXPERIMENT I															
NO_3-N	1.88	77.9	23.3	14.8	44.1	160.1	21.8	20.7	44.9	87.3	245	333	1.841	0.485	0.485
NH_4-N	1.91	76.4	21.6	13.7	39.6	150.3	23.7	25.1	46.1	94.9	249	343	1.585	0.441	4.212
Result	N.S.	N.S.	N.S.	p<.10	p<.15	*	**	**	N.S.	**	N.S.	N.S.	**	*	**
EXPERIMENT II															
NO_3-N	10.57	53.3	25.4	15.1	36.5	130.4	20.0	14.05	24.3	58.4	170	228	2.241	0.577	0.577
NH_4-N	10.27	61.6	17.4	13.3	29.8	121.2	34.2	18.17	23.3	73.6	176	252	1.599	0.482	3.944
Result	N.S.	**	**	**	**	**	**	**	N.S.	**	N.S.	*	**	**	**
EXPERIMENT III															
NO_3-N	18.05	111.9	2.83	38.8	20.0	173.5	33.2	21.56	-	54.8	284.1	338.7	3.236	0.514	0.514
NH_4 NO_3	20.62	115.7	2.35	31.9	15.6	165.5	45.6	25.48	-	71.1	303.4	374.6	2.355	0.444	-
NH_4-N	18.34	107.0	1.87	27.8	11.3	147.9	54.3	23.80	-	78.1	317.7	395.8	1.928	0.376	6.073
Result	**	**	**	**	**	**	**	**	-	**	**	**	**	**	**

APPENDIX 47

SUMMARY OF SWEET VERNAL RESULTS

	YIELD	METALS (me. %)					NON-METALS (me. %)						RATIOS		
	(g. D.M. /plant)	K	Na	Mg	Ca	me.sum of metals (∑C)	S	P	Cl	me. sum of anions (∑A)	N	me.sum of non-metals (∑A + N)	∑C / ∑A	R Value	Cation Uptake / Anion Uptake
EXPERIMENT I															
NO_3-N	1.82	84.6	21.3	12.0	35.2	153.1	18.4	20.2	43.1	81.8	252	334	1.878	0.459	0.459
NH_4-N	1.99	81.7	20.7	11.0	32.3	145.7	19.3	23.2	43.6	86.2	254	340	1.693	0.430	4.640
Result	N.S.	N.S.	N.S.	p<.10	p<.15	*	**	**	N.S.	**	N.S.	N.S.	**	**	**
EXPERIMENT II															
NO_3-N	6.41	67.8	24.0	16.0	31.6	139.1	19.6	16.46	17.7	53.8	253	306	2.654	0.454	0.458
NH_4-N	8.64	67.6	16.7	12.5	19.7	116.5	22.6	18.20	26.2	66.9	222	289	1.747	0.405	5.088
Result	**	N.S.	**	**	**	**	N.S.	*	**	**	**	N.S.	**	**	**
EXPERIMENT III															
NO_3-N	14.35	127.2	3.48	27.6	16.5	174.8	20.8	21.28	-	42.1	309.4	351.3	4.193	0.498	0.498
NH_4-NO_3	17.99	127.2	2.83	20.7	12.2	162.9	25.8	22.82	-	48.6	317.0	365.8	3.391	0.447	-
NH_4-N	15.13	108.9	2.13	19.7	9.0	139.7	35.2	21.13	-	56.4	329.7	386.2	2.524	0.363	8.492
Result	**	**	**	**	**	**	**	**	-	**	**	**	**	**	**

APPENDIX 48 SUMMARY OF SPECIES RESULTS AVERAGED OVER NITROGEN FORMS

	YIELD	METALS (me. %)					NON-METALS (me. %)						RATIOS		
	(g. D.M. /plant)	K	Na	Mg	Ca	me.sum of metals (ΣC)	S	P	Cl	me.sum of anions (ΣA + N)	N	me.sum of non-metals (ΣA + N)	ΣC / ΣA	R Value	Cation Uptake / Anion Uptake
EXPERIMENT I															
RYEGRASS	1.89	77.2	22.5	14.3	41.8	155.1	22.7	22.9	45.3	91.1	247	338	1.713	0.463	-
SWEET VERNAL	1.91	83.2	21.0	11.5	33.7	149.4	18.9	21.7	43.3	84.0	253	337	1.785	0.444	-
RESULT	N.S.	*	N,S.	**	**	N.S.	**	N.S.	N.S.	**	N.S.	N.S.	N.S.	N.S.	-
EXPERIMENT II															
RYEGRASS	10.42	57.5	21.4	14.2	33.2	125.8	27.1	16.11	23.8	66.0	173	240	1.920	0.529	-
SWEET VERNAL	7.52	67.7	20.4	14.2	25.7	127.8	21.1	17.33	22.9	60.4	237	297	2.201	0.429	-
RESULT	**	**	N.S.	N.S.	**	N.S.	**	p=.06	N.S.	**	**	**	*	**	
EXPERIMENT III															
RYEGRASS	19.00	111.5	2.35	32.8	15.6	162.3	44.4	23.61	-	68.0	301.7	369.7	2.506	0.444	-
SWEET VERNAL	15.83	121.1	2.88	22.7	12.5	159.1	27.3	21.74	-	49.0	318.7	367.8	3.369	0.436	-
RESULT	**	**	*	**	**	N.S.	**	**	-	**	**	N.S.	**	N.S.	-

APPENDIX 49 SUMMARY OF THE ANALYSES OF VARIANCE FOR GENETIC DIFFERENCES AMONG RYEGRASS PLANTS

Character		F calculated	Result	Standard Error	V(%)
Yield	NO_3-N	2.46	N.S.	± 0.6	10.1
	NH_4-N	1.70	N.S.	± 0.9	15.4
K	NO_3-N	3.13	*	± 3.2	6.8
	NH_4-N	1.46	N.S.	± 2.8	5.9
Na	NO_3-N	4.60	**	± 2.8	28.4
	NH_4-N	4.44	**	± 2.6	33.0
Mg	NO_3-N	3.33	*	± 1.8	13.4
	NH_4-N	3.34	*	± 1.8	16.7
Ca	NO_3-N	5.85	**	± 3.7	10.9
	NH_4-N	7.57	**	± 2.9	18.7
ΣC	NO_3-N	5.15	**	± 5.2	5.8
	NH_4-N	6.42	**	± 4.9	6.0
S	NO_3-N	-	N.S.	± 3.1	21.5
	NH_4-N	1.10	N.S.	± 4.9	31.5
P	NO_3-N	5.41	**	± 0.8	7.3
	NH_4-N	4.57	**	± 1.3	10.4
Cl	NO_3-N	1.70	N.S.	± 2.4	11.8
	NH_4-N	-	N.S.	± 2.3	11.3
ΣA	NO_3-N	1.47	N.S.	± 3.5	9.1
	NH_4-N	1.34	N.S.	± 4.6	9.6
N	NO_3-N	6.91	**	± 7.7	5.7
	NH_4-N	3.90	**	± 9.0	6.4

Contd...

APPENDIX 49 CONTD...

		F calculated	Result	Standard Error	V(%)
$\Sigma A + H$	NO_3-N	4.95	**	± 9.0	5.2
	NH_4-N	2.80	*	±11.0	5.7
R-value	NO_3-N	8.49	**	± 0.023	7.4
	NH_4-N	12.59	**	± 0.015	6.1
$\frac{Mg + Ca}{Na + K}$	NO_3-N	-	-	-	-
	NH_4-N	7.15	**	± 0.04	14.4
Organic anions	NO_3-N	-	-	-	-
	NH_4-N	6.29	**	± 4	10.7

(See tables 3 and 4 for the units of measurement)