SODIUM RELATIONS IN PASTURE AND FODDER PLANTS GROWN IN NEW ZEALAND

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G.S. Smith

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ABSTRACT

The results of an extensive geographical survey of sodium and potassium values in topdressed ryegrass-white clover pastures clearly indicate that in many areas of New Zealand, particularly inland where the influence of sea spray is minimal, herbage sodium levels are below those needed for grazing animals. Moreover, as a direct result of the increased use of potassic fertilisers, particularly in the North Island, most pastures now have potassium levels well in excess of plant needs. Because potassium suppresses plant uptake of sodium and increases the leaching loss of sodium from the soil, it was concluded that excessive use of potassic fertilisers would be expected to aggravate or even create an animal-nutrition problem involving sodium.

An additional animal-nutrition problem involving sodium is discussed in relation to the considerable differences measured in the extent to which the more important pasture and fodder species grown in New Zealand were able to absorb and translocate sodium from roots to shoots. In this respect plants can be classified into two distinct types according to the sites where sodium preferentially accumulates.

Natrophiles: plants in which sodium readily accumulates in the leaf tissues.

Natrophobes: plants in which sodium preferentially accumulates in the roots or lower stems with the result that only very small quantities of this element are present in the leaf tissue.

Because of this intrinsic physiological difference, natrophobes are unable to provide enough sodium for dietary needs of animals, even in a situation where there is enough sodium in the soil to produce satisfactory levels in natrophiles. In view of the depressing effects of potassium, the high risk of leaching under the humid weather

conditions which prevail in New Zealand, and the differences between plant species in their ability to translocate sodium, it seems that the most practical solution for correcting sodium deficiencies in grazing animals would be the direct supplementation with this element.

Apart from the fundamental differences in the sites of sodium accumulation in natrophiles and natrophobes there are a number of other important features which further characterise these two distinct types of plant. In both natrophiles and natrophobes there is a significant relationship between sodium and nitrogen. What is more, both nitrate nitrogen and nitrate reductase activity, as measured by an *in vivo* method, were found to be predominately located in tissues where sodium readily accumulates. In other words, nitrate reduction in natrophiles tends to occur in the aerial tissues whereas in natrophobes reduction occurs mainly in the roots and stems. Furthermore, it was concluded that the observed stimulation of nitrate reductase activity in tissues from both natrophiles and natrophobes, due to the addition of sodium chloride, was the result of an increase in nitrate nitrogen at the sites of reduction rather than any specific effect of sodium. However, the possibility that sodium has a stabilising effect on the nitrate reductase enzyme is discussed.

From an ecological point of view it seems reasonable to suggest that natrophobes have developed their characteristic distribution of sodium and nitrate reductase activity, along with lower transpiration rates, in order to tolerate more arid conditions than those which can be experienced by natrophilic plants.

INTRODUCTION

Outline of Research

Although many of the requirements for major and minor essential elements are similar in plants and animals significant differences exist, notably in the requirements of animals for sodium, cobalt, iodine, and selenium (Butler and Jones, 1973). Because plants can grow satisfactorily in the absence of these elements, a danger exists that in certain circumstances grazing animals will suffer from dietary deficiencies.

In pastoral agriculture, most of the loss of essential elements is a result of such animal activity as the inefficient return of elements in excreta, and their removal in saleable animal products (Karlovsky, 1975). Furthermore, as a result of increasing production, easily available reserves of nutrients accumulated in the soil will be depleted, and the rate of release from more permanent reserves will be too slow to maintain high productivity. Moreover, as the nitrogen status increases with the level of fertility of the soil, the plant develops a high shoot/root ratio (see Middleton, 1973) with the result that the main absorptive part of the root system is confined to a relatively shallow surface layer of soil thereby imposing further limitations on the availability of nutrients from natural sources.

It is therefore argued (Middleton and Smith, 1974) that for high producing pastoral agriculture fertiliser needs should be considered more and more in terms of complete fertiliser mixtures, and that the composition of such mixtures should satisfy both plant and animal needs (Middleton and Smith, 1976).

Because of the need to consider mineral requirements of both plants and animals, the following investigation into sodium nutrition of plants was considered from two points of view:-

(a) Agricultural

Investigations by Joyce and Brunswick (1975) into sodium deficiencies in grazing animals in the Taupo region, and reports of low sodium levels in mixed pasture plants from various parts of New Zealand, by Cullen *et al.* (1966), Metson *et al.* (1966) and McNaught and Dorofaeff (1968) made it evident that a comprehensive survey of sodium values in New Zealand pastures should be carried out.

In addition, the considerable differences between species in the extent to which they absorb and translocate sodium from root to shoot tissue, made it clear that the more important New Zealand pasture and fodder species needed to be classified according to their ability to translocate sodium. Pasture and fodder plants have therefore been grouped according to their ability to provide an adequate level of sodium in their leaf tissue for the needs of grazing animals.

(b) Physiological

Because sodium is not generally considered to be an essential element for higher plants, its physiological and biochemical role in the metabolism of the plant has been much neglected.

In order to elucidate some of the more basic physiological aspects of sodium nutrition, the effects of temperature, light, moisture stress, and potassium, on sodium content and associated chemical composition were studied in a number of plants.

An additional objective was a study of the role of sodium in nitrogen metabolism. Particular importance was attached to the induction and location of nitrate reductase activity in relation to the sites of sodium accumulation within different plant species.

REVIEW OF LITERATURE

(a) Sodium in Plants

(i) Function and requirements

For most higher plants sodium has not been found to have a specific essential function according to the criteria of Arnon and Stout (1939). However, sodium is essential for the blue-green alga *Anabaena cylindrica* with optimum growth obtained in media containing sodium greater than 218 µ eq/litre (Allen and Arnon, 1955). Small quantities of sodium are also essential for the halophyte *Atriplex vesicaria* (Brownell and Wood, 1957; Brownell, 1965), and recent evidence suggests that a number of other species having the C_4 dicarboxylic photosynthetic pathway also require sodium in minute amounts (Brownell and Crossland, 1972; 1974).

However, sodium has been observed to have a beneficial effect on dry weight production in a number of plants grown in field and pot experiments, notably members of the Chenopodiaceae family (see reviews of Harmer and Benne, 1945; Cope *et al.*, 1953; Lehr, 1953; and Hewitt, 1963).

Hewitt (1963) concluded that the response to sodium depends both on the plant species and composition of the nutrient supply, especially the level of potassium. He also pointed out that the increase in yield could be due to the effect of sodium modifying the soil, by increasing the uptake of other ions, or to the effect of the anion associated with the sodium upon the nutrition of the plant. Variation in the native sodium content of soils has also led to contradictory observations as to the effects of sodium on plant growth (Marschner, 1971).

Where sodium has had a pronounced beneficial effect on growth under conditions of insufficient supply of potassium, Marschner (1971) concluded that sodium may be partially replacing potassium in its non-specific role in maintenance of the cation/anion balance and its osmotic effects. Sodium can, for example, substitute for approximately two-thirds of the potassium requirements of Pangola grass (Digitaria descumbers Stent.) without causing any appreciable reduction in growth. This replacement by sodium could make potassium available for specific functions within the cell through retranslocation within the plant from older to younger leaves. However, this replacement was not effective when potassium content was very low (Gammon, 1953). A similar observation was reported for Rhodes grass (Chloris gayana) (Smith, 1974).

In water culture experiments (Ulrich and Ohki, 1965; El-Sheikh and Ulrich, 1970) and in field experimentation (Tinker, 1965) the yield of sugar beet (Beta vulgaris) was found to be substantially increased by the addition of sodium chloride to the growth medium, both under conditions where potassium was deficient and where potassium was adequately supplied. In this case sodium has an additional function, besides its ability to partially replace potassium, since it was found to increase the total sucrose content of the sugar beet plants even at high levels of potassium.

In enzymatic studies where the activity of enzymes have been compared in the presence of either potassium, or sodium, or in combination, sodium was able to substitute for potassium in some reactions but the rate of activity was very much reduced (Marschner, 1971). This failure was probably related to the alteration in steric configurations of the substrates by sodium (Evans and Sorger, 1966). However, Hansson and Kylin (1969) and Kylin and Gee (1970) have shown that the activity of ATPase in sugar beet roots and mangrove (Avicennia nitida) leaves is greatest when in an ionic environment of both potassium and sodium and not of potassium alone. Sodium has also been shown to be more effective than potassium in increasing esterification of phosphate in isolated mitochondria of rape (*Brassica rapa*) (Shah and Wedding, 1968). A requirement for high concentrations of sodium has been found for maximal activity in isolated enzymes from halophytic bacteria (Baxter and Gibbons, 1956; Brown, 1964; Marquez and Brodie, 1973). It was inferred that enzymes from these cells have adapted to high cytoplasmic ion concentrations (Greenway and Osmond, 1972). Similar enzymatic adaptions have also been proposed to account for salt tolerance in higher plants (Hayward and Wadleigh, 1949; Greenway, 1968). Marked responses in specific activity of certain enzymes have been noted in pea root tips under conditions of both sodium chloride and sodium sulphate salinity (Porath and Poljakoff-Mayber, 1964; Hason-Porath and Pojakoff-Mayber, 1969). However, Greenway and Osmond (1972) established that for four different enzymes, isolated from whole roots in both salt-sensitive and salt-tolerant plants, there was an equal sensitivity to sodium chloride. Furthermore, there was no alteration in the specific enzymatic activity when these plants were grown under saline conditions. A similar observation has been made by Weimberg (1968; 1970).

Sodium may also be implicated in nitrogen metabolism. Enhanced protein synthesis has been observed in a number of plants under conditions where sodium chloride has been increased (Kessler and Snir, 1969; Chatterton *et al.*, 1971; Langdale *et al.*, 1973; Helal *et al.*, 1975). Although there were no significant changes in total nitrogen in these plants when sodium was increased, there was, in association with the increased protein synthesis, a decrease in such non-protein nitrogen compounds as nitrate nitrogen (Langdale *et al.*, 1973; Helal *et al.*, 1975).

Observations that there was a connection between the absorption of sodium and increased succulence in plants led Jennings (1968) to propose a 'Unified theory' for the succulence of halophytes and sodium. Since the effect of sodium paralleled the effects of light and aridity in increasing succulence, it was concluded that the one unifying factor was ATP, as all these processes influence the level of ATP in plant tissue. The role which ATP might have on producing increased succulence was unclear, but it was concluded that the increased water content, through the homeostatic mechanism of sodium, would avoid, through dilution, the detrimental effect of high concentrations of toxic ions. Like potassium, sodium also influences the water economy of the plants by its effect on stomatal opening (Humble and Hsiao, 1969; Willmer and Mansfield, 1970). Differences observed in stomatal activity of excised leaves between plant species, with increasing sodium concentration in the incubation medium, could be related to membrane permeability to potassium and sodium ions (Marschner, 1971). Where a plant has a high membrane permeability to sodium such as sugar beet, sodium may partially replace potassium in this function of influencing the osmotic pressure in the chlorophyllous stomata cells.

(ii) Differences between genera, species, and varieties

Although sodium constitutes an appreciable fraction of the earth's crust (2.6 percent) and is the predominant soluble cation in saline and alkaline soils, there are however, considerable differences in the extent to which sodium is translocated to aerial tissues of plants. As early as 1934 van Itallie concluded "that more than any other plant constituent, sodium established differences between grasses". These intrinsic physiological differences are not confined to any one family or genera, but exist even at the varietal level (van Itallie, 1934; Collander, 1941; Garaudeaux, 1959; Kerguelen, 1960; Lehr, 1960; Butler et al., 1962; Copponet and Calvez, 1962; ap Griffith et al., 1965; Davies et al., 1966; Whitehead and Jones, 1969; Whitehead and

Van Itallie (1934)	Lehr (1960)	Garaudeaux (1959)	Copponet and Calvez (1962)	Kerguelen (1960)	Series I (1965)	ap Griffith et al Series 2 (1965)	Present series
Holcus lanatus	-	-	-	-	-	-	Holcus lanatus
	-	-	-	-	-	-	Cynosurus cristatus
Lolium perenne	Lolium perenne	-	-	-	Lolium perenne	Lolium perenne*	Lolium perenne
Anthoxanthum odoratum	-	-	-	-	-	-	Anthoxanthum odoratum
-	Dactylis glomerata	Dactylis glomerata*	Dactylis glomerata	Dactylis glomerata	Dactylis glomerata*	Dactylis glomerata	Dactylis glomerata
-	-	-	(Lolium perenne)	-	-	-	-
-	-	-	Festuca arundinacea	-	Festuca arundinacea	Festuca arundinacea	-
-	-	-	-	-	-	-	(Festuca rubra)
							Agrostis tenuis
Poa trivialis	Poa trivialis	-	-	-	-	-	Poa trivialis
-	-	-	Lolium multiflorum	Lolium multiflorum	Lolium multiflorum	-	-
-	Festuca pratensis	Festuca pratensis	Festuca pratensis	-	Festuca pratensis	Festuca pratensis	-
Poa pratensis	-	-	-	Poa pratensis	-	-	Poa pratensis
							(Lolium multiflorum)
Agrostis alba	-	-	-	-	-	-	-
Festuca rubra	-	-	-	Festuca rubra	-	-	-
Alopecurus pratensis	-	-	-	-	-	-	-
-	-	-	Festuca spadicea	-	-	-	-
-	-	-	Bromus inermis**	-	-	-	-
-	-	-	-	-	-	-	Arrhenathesum elatine
-	-	-	-	-	-	-	Phleum bertolonii
-	Phleum pratense	-	Phleum pratense	-	Phleum pratense**	-	Phleum pratense

Note: Species names in brackets indicate disagreement in order

* Some varieties were exceptionally low in Sodium eg, variety Latar of Dactylis glomerata & variety Norlea of Lolium perenne

** Pritchard et al. (1964) also find Bromus inermis & Phleum pratense to be low in Sodium

able 1: Grasses arranged in descending sodium content in leaves according to different workers (after ap Griffith and Walters, 1966)

Jones, 1972). Table 1 presents the sodium content in leaf tissue of some grasses in descending order according to different workers (after ap Griffith and Walters, 1966).

Plants can therefore be grouped into two distinct types according to the ability to translocate sodium into their leaf tissue:

Natrophilic plants (those with a high potential for sodium in their leaves). Natrophobic plants (those with a low potential for sodium in their leaves).

Although Marschner (1974) has previously employed both terms they were used in an undefined and very restricted sense.

Natrophilic plants are those which were grouped by Harmer and Benne (1945) into: (a) plants which benefitted from sodium when potassium was deficient; and (b) those that benefitted greatly from sodium when potassium was adequate for plant growth. Natrophobic plants on the other hand form the groups which showed little benefit from sodium when potassium was deficient and only slight to medium benefit when potassium was adequate. Ap Griffith and Walters (1966) also observed that natrophilic plants have a greater range of intervarietal variation in sodium content in leaf tissue than natrophobic plants. Likewise the sodium content in natrophilic plants was greatly influenced by environment, whereas natrophobic plants tended to be affected to a much lesser extent by such factors.

(iii) <u>Other factors influencing sodium in plants</u>

(a) <u>Soil sodium</u>

Sodium occurs in the soil as a constituent of silicate rocks, as an exchangeable cation, and as inorganic salts. Most silicates high in sodium are weathered and leached with relative ease, consequently, in humid climates sodium levels in soils are normally low (Russell, 1973). Exchangeable sodium in fertile soils, associated to a considerable extent with the organic fraction, is the major reserve available to plant roots in non-saline soils at any one time. In some arid and semi-arid soils, however, sodium may accumulate to such an extent that plant growth is inhibited, and it is not until these soils have been treated with gypsum and sufficient water to leach out the displaced sodium that important agricultural plants can grow (Tisdale and Nelson, 1970).

In many cultivated British soils, sodium accounts for up to 5 percent of the total exchangeable cations (Jacks, 1954). However, in New Zealand, typical exchangeable sodium levels in the top 8 cm of soil ranged in a survey of 54 different soil types from 0.0-2.5 me percent with a mean value of 0.4 me percent i.e. 2 percent of the total exchangeable cations (N.Z. Soil Bureau, 1968).

As a consequence of the rather low sodium status of New Zealand soils, low sodium levels in mixed pasture plants occur in various parts of New Zealand as recorded by Cullen _et al._, (1966), Metson _et al._, (1966), McNaught and Dorofaeff (1968).

(b) Fertiliser sodium

1. Natural

In addition to the sodium released from minerals by weathering, a certain amount is added to the soil in rainwater and this has considerably influenced the exchangeable sodium value in some soils, as found in Britain by Bolton (1971). Measurements in New Zealand by Blakemore (1953) and Gray (1888) have confirmed that the level of sodium in rainwater is critically dependent on the distance fine sea spray is carried inland by prevailing winds. The results, presented in Table 2, indicate that considerable quantities of sodium chloride (200 kg/ha/yr) may be deposited up to 6 kilometres inland, rapidly falling off thereafter as the distance from the coast increases.

Location	Distance from sea (km)	Quantities of sodium chloride (kg/ha/yr)
Rongotai	0.5	388
Baring Head	0.5	276
Pirinoa	6.0	206
Kelburn	6.0	185
Lincoln*	10.0	108
Levin	10.0	89
Palmerston North	32.0	84
Te Awa	48.0	61
Waingawa	61.0	67

* Grey (1888)

Table 2: Quantities of sodium deposited in rainwater in various parts of New Zealand (after Blakemore, 1953).

This influence is clearly shown in the composition of white clover in the Wairarapa. Sodium values in plants growing near the coast and exposed to salt bearing winds ranged from 0.4–0.5 percent whereas clover in relatively sheltered inland hill country valleys had between 0.1–0.2 percent sodium in their tissue (Wells, 1969).

2. Artificial

The commercial use of sodium as a fertiliser has largely been confined to members of the Chenopodiaceae family.

Durrant et al. (1974), from results of 216 field experiments with sugar beet over 12 years, point out some of the factors which influence the effect of added sodium on these plants. In the experiments they applied potassium and sodium fertilisers either separately or in combination, and found that the average response per kilogram of nutrient was more in favour of sodium. It was concluded that instances of near perfect substitution resulted from chance combinations of weather, crop husbandry, and with the level of potassium in the soil. This conclusion was based on the fact that potassium and sodium are only partially interchangeable and because the response to sodium was less influenced by season and time of sowing than that of potassium. Incidentally, it was recommended that both potassium and sodium should be added to sugar beet to avoid any possible yield loss.

Although sodium has not been extensively used as a fertiliser on pasture, it is present as an impurity in some low grade potassium fertiliser materials such as manure salts and kainite (Tisdale and Nelson, 1970). However, where it has been applied to pasture, it was for the following two reasons: first, as a means of increasing yield where potassium was deficient and this is the most commonly reported situation; second, to increase the plant sodium content where it has fallen below the minimum level required for grazing animals.

In New Zealand, investigations into sodium use on pastures have largely been confined to the first situation (Lynch, 1954; Bell, 1955); and by applying sodium chloride at a rate of 251 kg/ha/yr to a potassium deficient soil, McNaught and Karlovsky (1964) reported increases in pasture yields of up to 18 percent above that in control plots in the first two years, thereafter falling away in the third and fourth years as the level of potassium approached its specific requirement. These responses to sodium were however, much smaller than those where an equivalent rate of potassium chloride was applied.

Recent research has found that in widespread areas of Western Europe very low sodium levels exist in grass pastures and these levels are below those required for animal needs (Lehr, 1960). In a number of experiments on pastures in Holland, sodium fertilisers have been used to correct this deficiency (Lehr, 1960; Oostendorp, 1961; Bakker, 1963). It was found that the addition of sodium nitrate was more effective in maintaining suitable sodium contents in pastures than agricultural salt or kainite, where equivalent amounts of sodium and nitrogen (lime/ammonium nitrate) were applied. The sodium requirement was greater where the soil potassium status was high because of the competition effect of potassium ions during root absorption.

In a long term experiment where a total of 3450 kg/ha was applied to a pasture over 14 years, no systematic trend in sodium accumulation in the soil or in the plant was found. It was therefore concluded that large quantities of added sodium were rapidly leached from the root zone by winter rains (Lehr et al., 1963). A similar conclusion was also drawn by Tinker (1971) after an examination of the changes which occurred in the soil following a single heavy addition of sodium to sugar beet. By the end of the first year the level of sodium in the soil had fallen by half and by the third year the level was the same as that in control plots.

(c) Other fertiliser elements

The composition of fertilisers is one of the major factors influencing the sodium content in both natrophilic and natrophobic plants.

1. Nitrogen

Nitrogen has a specific effect on leaf sodium. In general there is a consistent increase in sodium content with increasing additions of nitrogen. However, the nitrogen effect is somewhat offset by the depressing effect of potassium (e.g. Stewart and Holmes 1953; Kemp, 1960; Lehr, 1960; Rahman et al., 1960; Hemingway, 1961; Lehr et al., 1963; Reith et al., 1964).

Table 3 shows clearly the effect of three levels of 'nitro-chalk' in the presence of low and high potassium on leaf sodium in mixed pasture herbage from Scotland (mean of three years).

Mean percent sodium in dry matter

	Nitrogen Application*					
	Nil		195 kg/ha		390 kg/ha	
Location	Low K	High K	Low K	High K	Low K	High K
Fingask	0.37	0.09	0.74	0.18	0.93	0.36
Kikhill	0.52	0.22	0.80	0.33	0.96	0.50
Balluskie	0.31	0.08	0.38	0.15	0.28	0.22
Crofthead	0.36	0.15	0.61	0.18	0.60	0.32

* N rates kg/ha/yr

Table 3: Effect of nitrogen, in the presence of potassium, on sodium contents in mixed herbage (after Reith *et al.*, 1964).

2. Superphosphate

The addition of superphosphate to well established permanent pasture does not appear to have any specific effect on the sodium content of leaf tissue (Stewart and Holmes, 1953; Hemingway, 1961; Reith *et al.*, 1964; Fleming, 1973). However, it may have an indirect effect on sodium. The results of Cullen *et al.* (1966) suggest that the significant increases in sodium content, where an application of lime-reverted superphosphate was made to a pasture in the initial stages of establishment, may be related more to the changes in botanical composition and nitrogen status. During *et al.* (1960) also observed that the addition of phosphorus and molybdenum to pasture consistently increased sodium in herbage; but both these elements were found to increase the nitrogen content of the pasture.

These results suggest that the increased sodium content, due to added superphosphate, was a nitrogen effect and not a specific fertiliser effect.

3. Potassium

Of the fertiliser elements, potassium has the most dominating influence on sodium levels in plants. It is well documented that the addition of potassic fertilisers to both natrophilic and natrophobic plants results in a depression of sodium (e.g. Stewart and Holmes, 1953; McNaught, 1959; During *et al.*, 1960; Hemingway, 1960; Hemingway, 1961; Reith *et al.*, 1964; Henkens, 1965; Harris *et al.*, 1966; Bolton, 1972).

The degree to which sodium in natrophobic plants is influenced by the application of potassic fertilisers is only slight in comparison to that in natrophilic plants (see Table 4).

	Mean percent sodium in dry matter Rate of potassium chloride (kg/ha)				
Type of plant	0	63	126	251	502
Natrophilic					
Cabbage	1.20	0.91	0.43	0.24	0.14
Sugar beet	1.80	1.62	1.35	1.51	1.05
Cocksfoot	0.91	0.70	0.16	0.11	0.04
Ryegrass	0.65	0.51	0.43	0.18	0.11
Natrophobic					
Potatoes	0.07	0.07	0.07	0.07	0.07
Paspalum	0.12	0.09	0.06	0.05	0.04

Table 4: Influence of potassic fertilisers on sodium concentration in leaf tissue of natrophilic and natrophobic plants (after McNaught, 1959).

4. Calcium and magnesium

Where calcium and magnesium have been reported to influence sodium in both natrophilic and natrophobic plants in field experiments (Cullen et al., 1966; Harris et al., 1966) the results suggest, as was found with superphosphate, that it was an indirect effect through their influence on nitrogen and potassium levels within the plant.

(d) Seasonal and temperature effects

Of the recorded investigations into the seasonal variations in sodium content of herbage, (e.g. Stewart and Holmes, 1953; Reith et al., 1964; McNaught and Dorofaeff, 1968), few have found any consistent trends. Where these trends have been observed (Fleming, 1973), sodium levels in the herbage tended to be high and were therefore, more associated with natrophilic type plants.

In general, sodium was found to be at a high level in the cooler months of the year. This observation is supported by solution culture work with perennial ryegrass. Mengel and Pfluger (1972) found that low atmospheric humidity and temperature promoted sodium uptake. A similar observation was made by Smith and Middleton (1974).

(e) Stage of maturity

Like most elements, sodium content declines with maturity of the plant (Thomas et al., 1952; Pritchard et al., 1964; Fleming and Murphy, 1968). On comparing the results of Pritchard et al., (1964) and Fleming and Murphy (1968), larger variations were found to occur in sodium content in the leaves of natrophiles as the plant ages, as expected, than that found in natrophobes.

(iv) Absorption and translocation

Although the mechanisms whereby natrophobic plants exert a selective effect are not well understood, the sites of restricted sodium movement have been attributed to the following tissues:

(a) Roots

It has been well documented that plants such as maize and bush beans readily accumulate sodium in root tissue so that only small quantities pass into the transpiration stream (e.g. Wybenga, 1957; Wallace et al., 1965; Shone et al., 1969; Marschner, 1974). To explain this, Gauch and Wadleigh (1945) considered that movement of sodium into the vascular tissue of the bean root might be restricted by the membranes of certain extrastelar tissues. Shone et al. (1969) also postulated that in maize the movement of sodium towards the basal part of the root was progressively slowed down by factors associated with either the walls of the xylem or the surrounding stelar parenchyma.

When the absorption of potassium and sodium was examined in root tissue over a wide concentration range, at least two distinct isotherms were noted (Epstein, 1966; Rains and Epstein, 1967). These two types have been taken to reflect transport systems or mechanisms common to both potassium and sodium. In contrast, Jacobson et al. (1961) postulated that there was a single common site for sodium and potassium absorption. However the former showed that potassium was preferentially taken up with respect to sodium by one of the two uptake mechanisms. The low concentration mechanism (referred to as system 1) was characterised by a high affinity for potassium with little effect from a large excess of sodium ions or from the anions in solution. By contrast, the low affinity mechanism (or mechanisms) for potassium absorption (system 2), which operated at a higher concentration than system 1, was considered to favour sodium markedly, and was also strongly affected by the nature of the anions in solution (Bange, 1959; Epstein, 1966; Rains and Epstein, 1967). Although controversy surrounds the location of these mechanisms, it is accepted that active ion absorption occurs at the plasmalemma and at the tonoplast of root cells (Epstein, 1966; Pitman and Saddler, 1967; Laties, 1969; Nissen, 1972). To account for the higher potassium/sodium ratio found in shoots, it has been considered (see Laties (1969) for a review) that only system 1, which favours potassium, is involved in the uptake of ions from the external solution. That is, it operates at the plasmalemma and therefore results in an enrichment of potassium in the cytoplasm. Further, it was assumed that it is the cytoplasmic salts which are delivered from the root cortex into the stele through a cytoplasmic continuum or symplasm (Crafts and Broyer, 1938; Lundegardh, 1950; Arisz, 1956). Moreover, system 2, which favours sodium, is situated at the tonoplast resulting in sodium being actively transported into the vacuole. The sodium concentration in the vacuole has been found to be three to four times that in the cytoplasm. The potassium concentration, on the other hand, was roughly the same in both compartments (Pitman and Saddler, 1967). Consequently, the enrichment of the cytoplasm, with potassium at the plasmalemma and by the active accumulation of sodium from the cytoplasm into the vacuole, results in a high potassium/sodium ratio in the shoots.

On the basis of electrochemical measurements, an alternative model has been proposed (Pitman and Saddler, 1967; Pitman _et al_., 1968). They attributed the high potassium/sodium ratio in the shoots to a sodium efflux pump operating at the plasmalemma, while an influx pump operates at the tonoplast. Increased selectivity for potassium at all concentrations, in contrast to Epstein's (1966) and Rains and Epstein's (1967) models, results from the transport of potassium inwardly and sodium outwardly from the cell at the plasmalemma, while at the tonoplast, both potassium and sodium are transported into the vacuole. A similar model was proposed by Jennings (1967).

Calcium ions have been shown since the early work by Viets (1944) to influence the absorption of potassium and sodium. Although calcium ions have been shown to play an indispensable role in selective ion transport mechanisms (Epstein, 1961; Foote and Hanson, 1964), no definite explanation has been offered except that calcium may influence the configuration of mechanism 1 such that, in its absence, the site which is normally highly selective for potassium will transport sodium as well as potassium (Rains and Epstein, 1967).

In rejecting the concept of a common absorption site, Bange (1975) has shown that sodium absorption was antagonised more by calcium than magnesium, whereas the inhibition of potassium uptake was associated more with magnesium than calcium. On this evidence, Bange (1975) concluded that there were two separate mechanisms in operation, one specific for potassium and the other for sodium. The interaction of these mechanisms was assumed to be due to a competition for some requirement in short supply such as ATP (Bange, 1962).

(b) Stems

Stems have also been considered as possible sites for sodium accumulation. It is the contention of both Bernstein *et al.* (1956) and Jacoby (1965) that sodium is actively withdrawn from the transpiration stream into cells adjacent to the vascular tissue. Introduction of metabolic inhibitors, such as low temperatures, anaerobic conditions, and such protein inhibitors as potassium cyanide, dinitrophenol, or chloramphenicol, have resulted in an increase in the upward movement of sodium into the leaf tissue (Pearson, 1962; Jacoby, 1965; Wallace *et al.*, 1965). These results suggested that sodium retention was dependent on a supply of metabolic energy (Jacoby, 1965). Further work by Rains (1969) established, as expected, that the highly selective mechanism associated with sodium accumulation in stems of natrophobic plants was not present in natrophilic plants. When the stem tissues of the natrophobic bean plant were separated into extra-cambial tissue (phloem, cortex, and endodermis) and xylary tissue, it was the latter which was responsible for the high rate of sodium absorption at this site.

It appears that this absorption mechanism can, under certain conditions, become saturated (Jacoby, 1964; 1965). Where natrophobes have been grown under conditions of high external concentrations of sodium, this ion has been observed to penetrate gradually into the leaf tissue (Bower and Wadleigh, 1948; Bernstein _et_ _al_., 1956; Huffaker and Wallace, 1959a, 1959b), such that sodium becomes more uniformly distributed throughout the plant (Jacoby, 1964).

In contrast to the theories of restricted sodium transport by preferential accumulation in root and stem tissues, a number of workers (e.g. Wallace and Hemaiden, 1963; Cooil _et_ _al_., 1965; Levi, 1970; Marschner, 1974) have invoked the idea of retranslocation of sodium away from the leaves. Wallace and Hemaiden (1963) have demonstrated that downward movement of sodium in the phloem is a characteristic associated only with natrophobic plants. Where labelled sodium has been applied to the leaves of such plants, it has resulted in the retranslocation of sodium away from the leaves by means of the phloem into the roots, where it accumulated. Subsequently it was released into the external solution (Levi, 1970; Marschner, 1974).

From such results it was concluded that downward movement of sodium in plants could partially account for the appearance that upward transport of this element was restricted (Cooil _et_ _al_., 1965). It is, therefore, apparent that natrophobic plants have developed very effective regulatory mechanisms to prevent the accumulation of sodium in the leaf tissue where, as shown by Marschner (1974), it may cause metabolic disturbances.

(b) Sodium in Animals

(i) Function and requirements

Sodium is an essential element for all animals and a deficiency causes serious physiological disturbances. In contrast to plants, sodium not potassium, is the dominant cation in maintaining cation/anion balance and osmotic effects in animals. Table 5 shows the distribution of sodium (total sodium in sheep and cattle is approx. 9.0 g/kg body weight) and potassium (total potassium in sheep and cattle is approx. 11.25 g/kg body weight) in various body tissues.

Tissue	Sodium	Potassium
Body fluids	39.0	2.0
Bone	44.0	0.4
Muscle	8.0	36.0
Nervous	8.0	31.0
Secretory	-	31.0
Fat	negligible	negligible

Table 5: Percentage distribution of total sodium and potassium in various tissues of sheep and cattle (Agricultural Research Council, 1965).

As a large proportion of sodium is found in body fluids, animals are unable to build up reserves of this element. Therefore a continuous adequate supply is essential, especially since over 80 percent of the total daily sodium intake in dairy cattle is excreted in faeces and urine, with only 6 percent being retained in the body (Hutton *et al.*, 1967). However, the ruminant can, under conditions of sodium deficiency, considerably reduce this loss by conservation of sodium by the kidneys and by re-absorption from fluids associated with faeces in the lower intestine against a concentration gradient (Bott _et al._, 1964; Brouwer, 1961).

Although the sodium requirements in the diet for different types of grazing animals are not exactly known it can be concluded that the requirement for lactating animals is greater than that for animals producing meat and wool, which, in turn is higher than that for store-fed animals on a maintenance ration. The following table (6) presents the estimated requirements for the different classes of grazing animals.

	Sheep*	Beef+ animals	Lactating @ dairy cows
Sodium (percentage of dry matter)	0.07	0.10	0.20

* Consuming 2 kg D.M./day
\+ " 8 kg D.M./day
@ " 12 kg D.M./day

Table 6: Minimum concentrations needed in pasture plants for satisfactory nutrition for different classes of grazing animals (National Research Council, 1957, 1963, 1966).

In New Zealand, deficiencies of sodium in grazing animals have been confirmed in the Taupo region. Joyce and Brunswick (1975) found significant live weight gains in both sheep and dairy animals fed on lucerne, with an average sodium concentration of 0.03 percent, when they were drenched with sodium chloride twice weekly.

Although hypomagnesaemic tetany is associated with low magnesium in the blood, a number of authors have suggested that low sodium intake may also contribute to the development of this disease (Kemp, 1958;

Lehr, 1960; Paterson and Crichton, 1960; Ross, 1960; Butler _et al._, 1963). Where cows have been deliberately deprived of sodium in their diet they have shown similar symptoms to hypomagnesaemia: "shivering, incoordination, weakness, cardiac arrhythmea, and death". However, when sodium was administered to these cows, they quickly recovered (Smith and Aines, 1959). A similar result was obtained with a herd of cows known to be suffering from hypomagnesaemia, in that where additional sodium was given to their diet, the symptoms of hypomagnesaemia disappeared (Paterson and Crichton, 1960). In a study of magnesium absorption from the gastro-intestinal tract of sheep, an increase in sodium concentration resulted in a net increase in magnesium absorption (Care _et al._, 1967). A similar result was also obtained with rats (Ross, 1960).

Relative to other animals, the volume of digestive secretions in ruminants is large. In cattle, for example, over 20 litres of saliva is produced daily (Bailey and Balch, 1961). Although 98 percent of the cations in ruminant saliva are made up of sodium and potassium, sodium is by far the major constituent (95 percent) and may represent more than five times the sodium content of the blood plasma (Bott _et al._, 1964; Bailey and Balch, 1961; Phillipson and Mangon, 1959).

Although much saliva sodium is recycled, any interference in sodium intake or increased stimulation in the rate of digestive secretions caused by a change of diet, could result in a considerable stress on the sodium balance and circulatory homeostasis (Bott _et al._, 1964). Although potassium, to a limited extent, can replace sodium as the predominant cation of the salivary secretion, this imbalance of sodium to potassium results in a substantial increase in aldosterone, an adrenal hormone which in excess causes hypertension

(Goding and Denton, 1959; Bott _et al_., 1964). Sodium stress is more likely to be found in young growing animals grazing sodium deficient pastures, but may also be aggravated in adult dairy cows through sodium lost in milk and saliva (Bott _et al_., 1964).

EXPERIMENTAL

Because of the extensive nature of this investigation, the experimental work is presented in four separate chapters. These chapters correspond to the four basic topics mentioned in the Introduction under the general headings of Agricultural and Physiological considerations. Each chapter contains an introduction, a description of the experimental procedure, results, and a discussion. An overall conclusion is given at the end of the experimental section.

CHAPTER 1

GEOGRAPHICAL SURVEY OF SODIUM AND POTASSIUM VALUES IN TOPDRESSED NEW ZEALAND PASTURES

INTRODUCTION

In 1962 Wells carried out a survey using sweet vernal (*Anthoxanthum ordoratum*) as an indicator plant to investigate the availability of sodium in soils from various parts of New Zealand; but all the 400 samples were taken from untopdressed sites. It was felt, however, that to permit an adequate geographical coverage of sodium values in topdressed ryegrass-white clover pastures throughout New Zealand, which are of greater agricultural importance, a further survey was required.

METHOD OF INVESTIGATION

(a) *Material*

Both sodium and corresponding potassium values were surveyed from the accumulated chemical leaf analysis data compiled by the Plant Nutrition Section at the Ruakura Agricultural Research Centre from 1952 to 1975. A quarter of a million plant samples were examined; they had either been taken from fertiliser field trials or from farmers paddocks where they had been sampled by Farm Advisory Officers. Where there was more than one sample from a geographical site, the mean values were taken. Additional samples were collected during this study from certain areas to permit an adequate coverage.

A study of potassium was included because of its dominating influence on sodium within the plant.

References to the procedures used for chemically analysing the plant samples are given in Chapter 2.

(b) Expression of Results

The results are presented according to geographical regions. These regions were chosen to represent, as far as possible, the major farming areas of New Zealand. The boundaries of the regions and distribution of the 2767 sample sites are given in Figs 1 and 2 for the North and South Islands respectively.

Although the number of sites in the South Island (381) was considerably less than that of the North Island (2386) it was considered that the major farming areas were adequately covered.

(i) Sodium

The distribution of sodium values in pastures was determined for each region by grouping the site results within a defined concentration range. The resulting number of sites within each group was expressed as a percentage of the total. In addition, the results were further summarised according to the estimated requirements for different classes of grazing animals (see Table 6 for estimations).

(ii) Potassium

Although the results for potassium were expressed in a similar fashion to sodium, the overall emphasis was on plant requirements as the animal needs for this element are considerably lower (Middleton

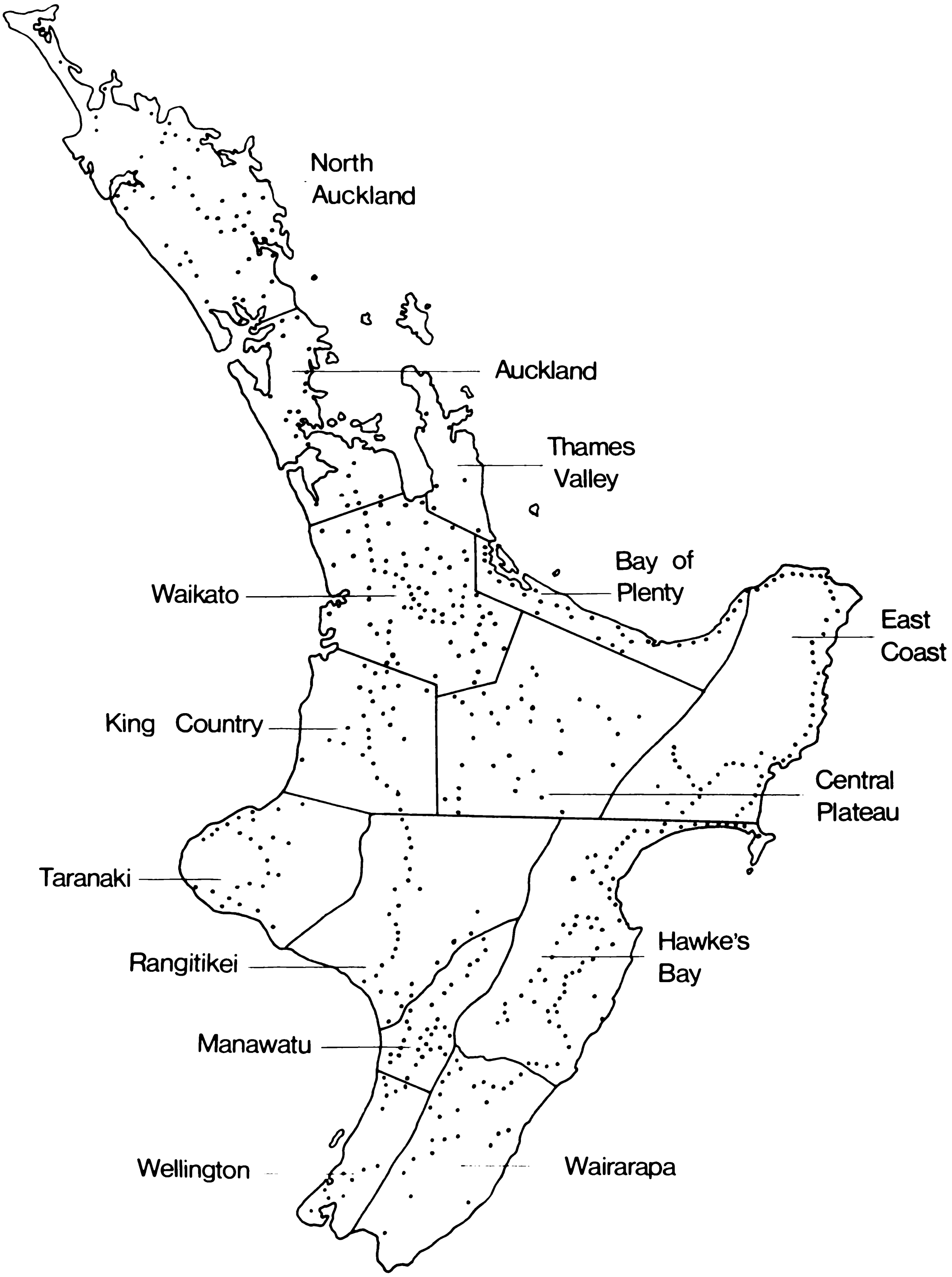

Fig. 1 – Distribution of sample sites along with the boundaries of the geographical regions within the North Island.

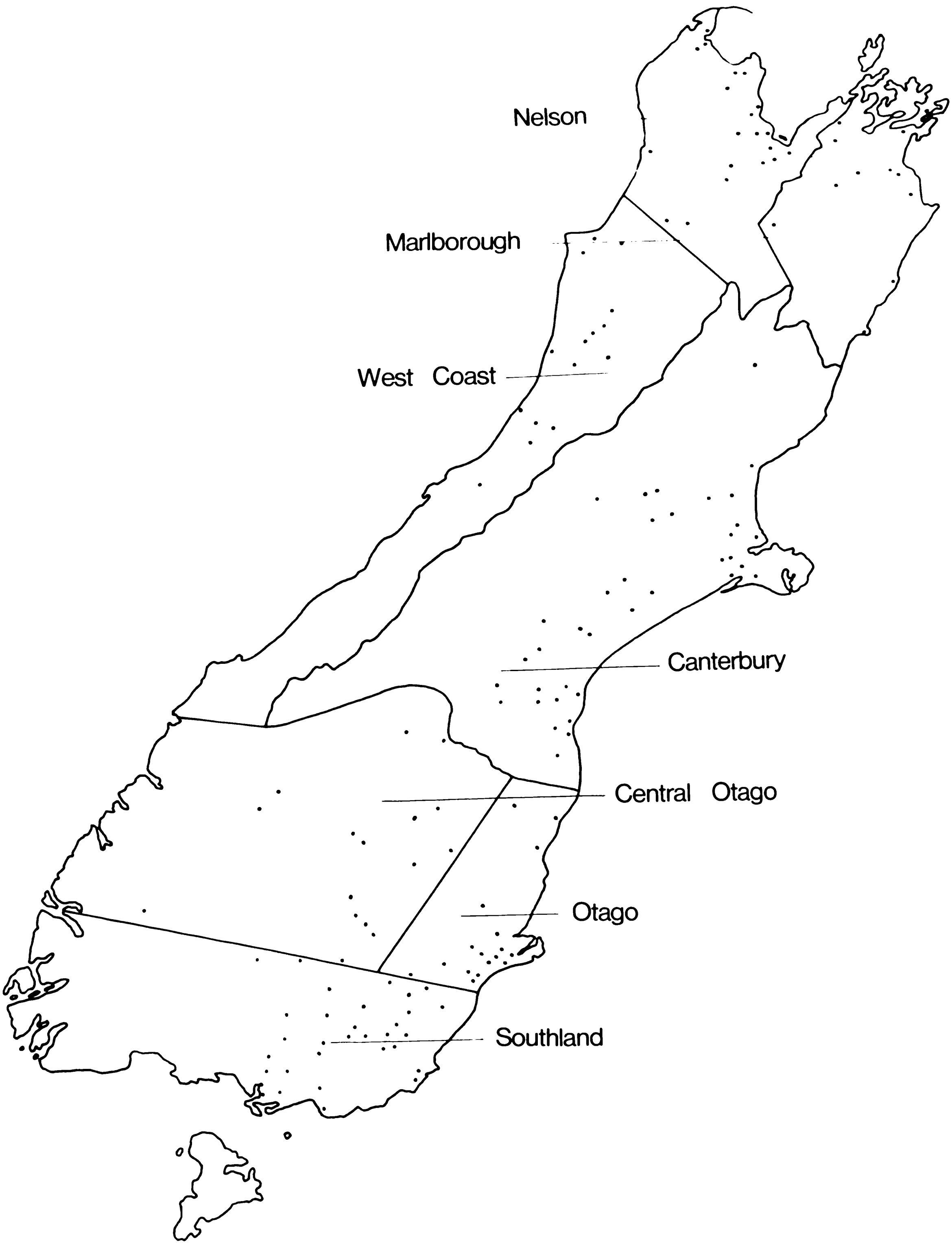

Fig. 2 - Distribution of sample sites along with the boundaries of the geographical regions within the South Island.

and Smith, 1976). Therefore, the results were summarised according to the levels suggested by McNaught (1970) as suitable for satisfactory plant growth. The ranges were as follows:-

Percent K	Plant Requirement
∠1.99	considered to be deficient
2-2.50	adequate for active growth
>2.50	excessive
>3.00	

RESULTS

Although the majority of individual plant samples came from field trials, the geographical locations these samples represented were considerably less than those from the Farm Advisory Samples. This is clearly shown in Table 7.

	Percentage of Total Number of Sites	
Sample Type	North Island	South Island
Field Trials	22	45
Farm Advisory	78	55
Total Number	2386	381

Table 7: Source of samples representing geographical locations.

To investigate the possibility that higher rates of potassic fertilisers were used in field trials than those normally associated with farming, which would produce a false picture of the overall status of potassium and indirectly sodium in pastures, mean values for both these elements were calculated from field trial and farm advisory samples (Table 8).

Sample Type	North Island Percent K	 Percent Na	South Island Percent K	 Percent Na
Field Trial	2.96±0.72	0.22±0.10	2.72±0.60	0.20±0.15
Farm Advisory	2.92±0.66	0.19±0.09	2.63±0.68	0.18±0.10

Table 8: Mean potassium and sodium concentrations calculated from herbage samples derived from different sources.

As there were no appreciable differences between field trials and farm advisory samples, the results from the two sources were combined to give an overall result for each of the elements.

Sodium

Detailed results for sodium are presented separately for North and South Islands in Figs 3 to 7 and summarised in Tables 9 and 10. These regional results are arranged within the figures and tables in descending order according to the number of sites (expressed as a percentage of the total) containing sodium values in pasture above 0.20 percent of the dry matter.

From these results it can be seen that a large number of sites in New Zealand are below the animal requirement for sodium, especially in relation to beef and dairy animals.

Although this survey indicates that Taranaki was the least deficient area in New Zealand, 35 percent of the sites were still below the acceptable dairy animal requirement, in fact in most of the areas investigated over 70 percent of the sites were below this level. Moreover, in two inland regions, Central Plateau and Central Otago, a serious sodium problem could exist for all classes of grazing animals as over 35 percent of the sites were below the minimal animal require-

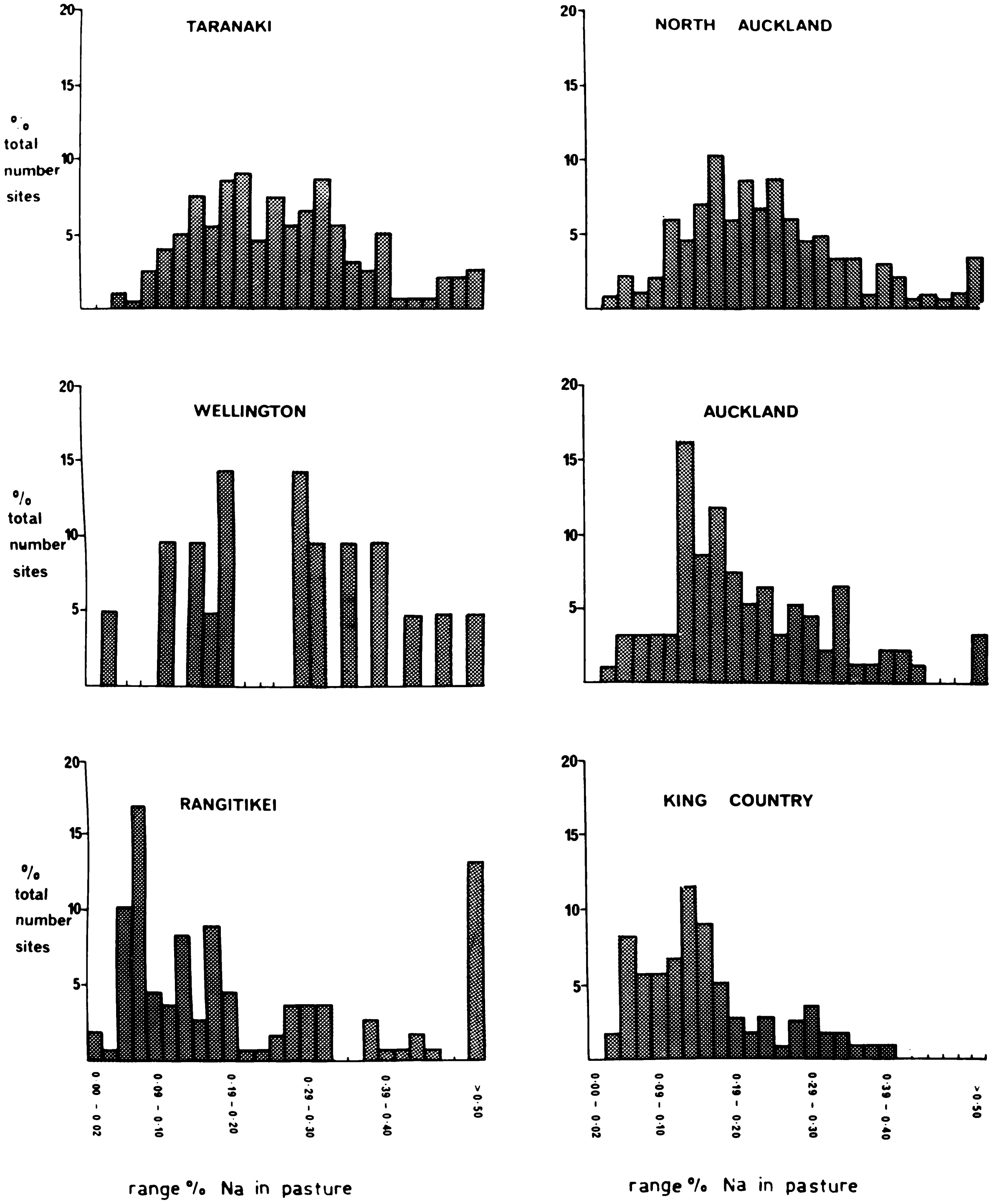

Fig. 3 - Distribution of sodium concentrations in herbage from topdressed North Island pastures.

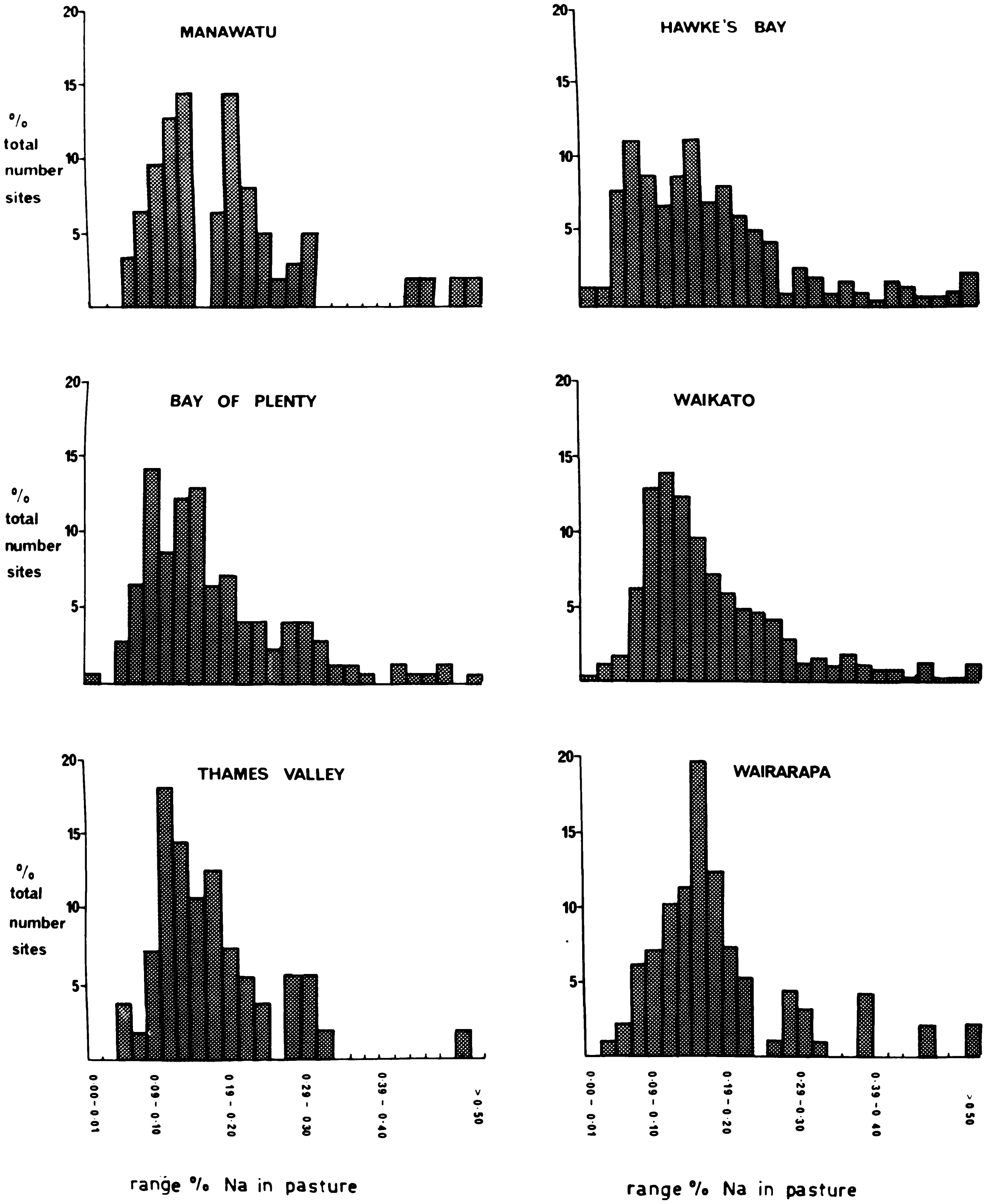

Fig. 4 - Distribution of sodium concentrations in herbage from topdressed North Island pastures.

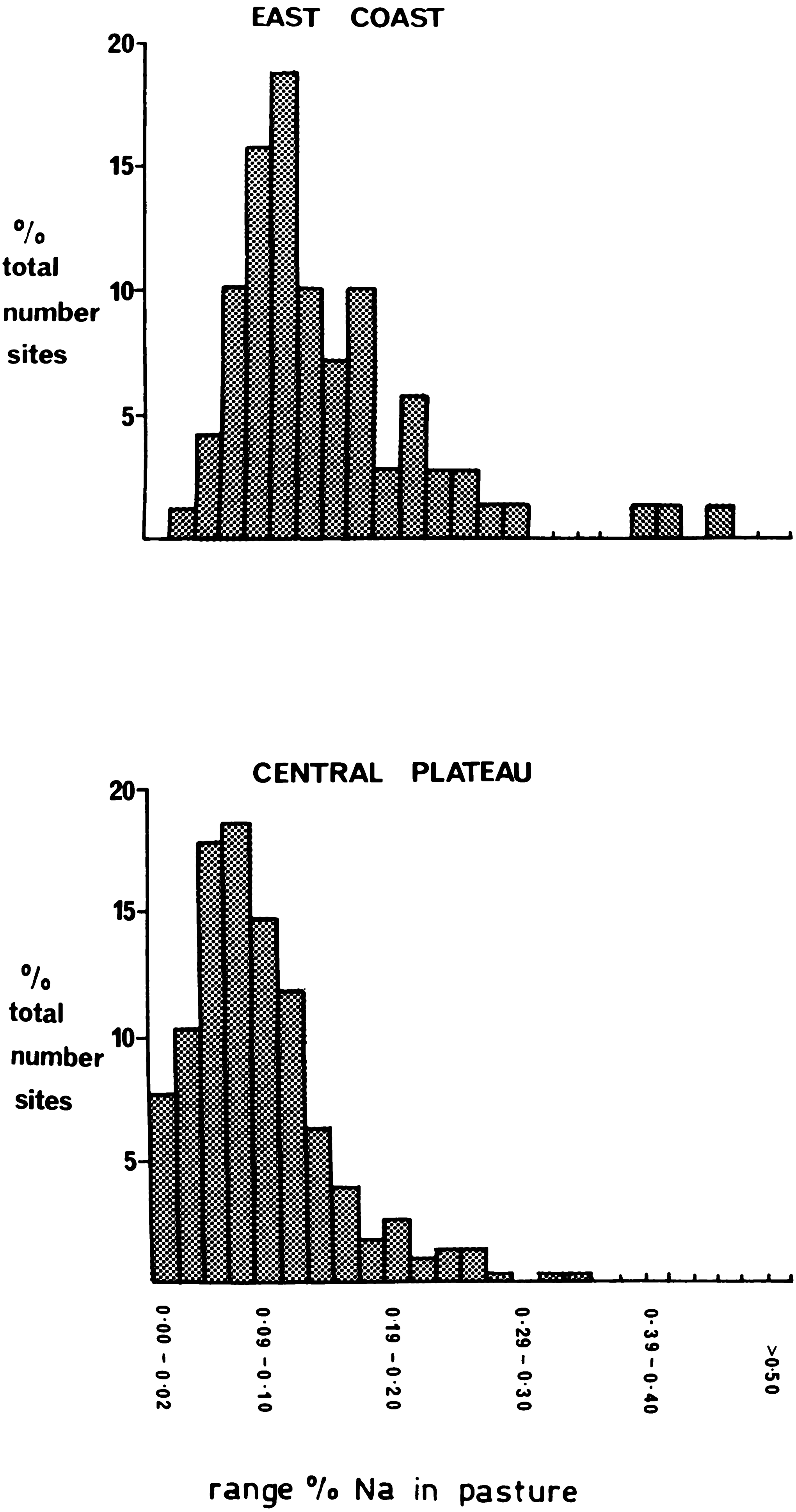

Fig. 5 – Distribution of sodium concentrations in herbage from topdressed North Island pastures.

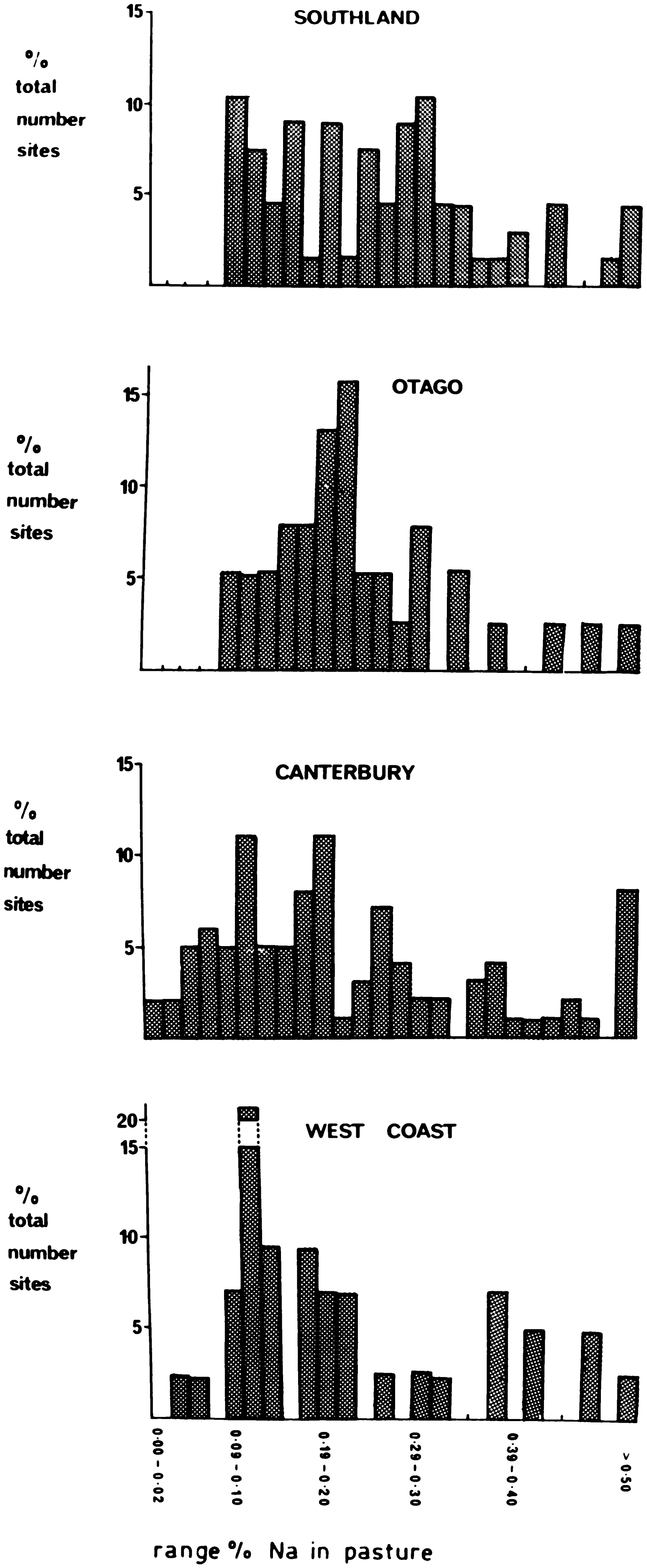

Fig. 6 – Distribution of sodium concentrations in herbage from topdressed South Island pastures.

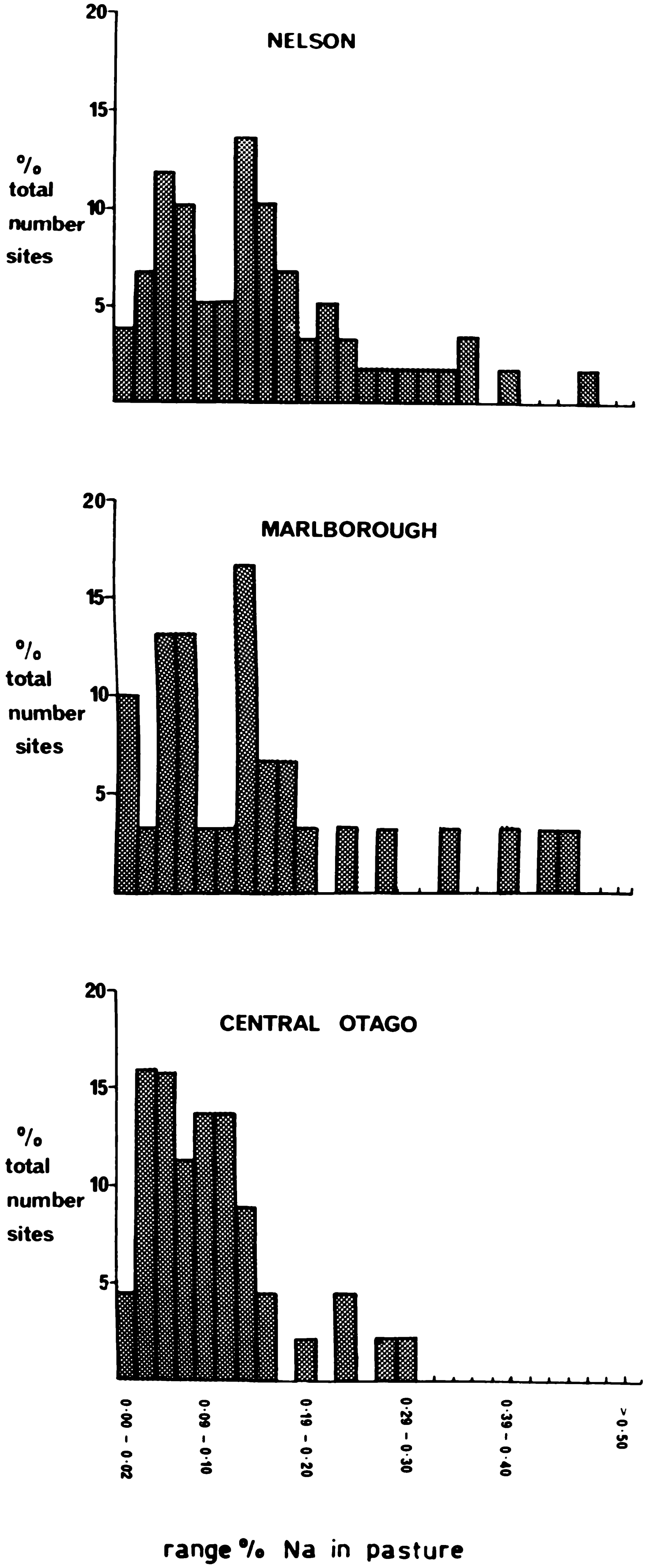

Fig. 7 - Distribution of sodium concentrations in herbage from topdressed South Island pastures.

Geographical region	Total number sites	Range sodium values*	Overall mean	The percentage of sites within a region which fall within the following specified sodium values – <0.07	<0.10	<0.20	>0–20
Taranaki	200	0.06–0.69	0.26	1.0	4.0	34.5	65.5
North Auckland	298	0.04–0.72	0.25	3.3	6.9	40.8	59.2
Wellington	21	0.03–0.57	0.28	4.8	4.8	42.9	57.1
Auckland	94	0.04–0.58	0.21	4.3	9.6	56.4	43.6
Rangitikei	106	0.00–1.14	0.23	13.2	34.0	64.1	35.9
King Country	122	0.04–0.45	0.18	9.8	21.2	67.2	32.8
Manawatu	63	0.05–0.55	0.19	3.2	19.0	69.8	30.2
Hawkes Bay	288	0.01–0.98	0.18	10.4	30.2	71.5	28.5
Bay of Plenty	170	0.01–0.48	0.18	3.5	24.1	71.8	28.2
Waikato	488	0.01–0.67	0.17	3.2	22.2	72.8	27.2
Thames Valley	55	0.06–0.49	0.16	3.6	12.7	76.3	23.7
Wairarapa	97	0.04–0.62	0.18	3.1	16.5	77.3	22.7
East Coast	69	0.04–0.45	0.15	5.7	31.7	80.8	19.2
Central Plateau	315	0.00–0.38	0.09	35.6	68.9	95.2	4.8
North Island	2386	0.00–1.14	0.20	9.2	25.7	67.3	32.7

* All sodium values expressed as percent of dry matter

Table 9: Summary of distribution of sodium values in topdressed North Island pastures.

Geographical region	Total number sites	Range sodium values*	Overall mean	The percentage of sites within which fall within the following specified sodium values -			
				<0.07	<0.10	<0.20	>0-20
Southland	67	0.10-0.65	0.25	0	10.4	41.9	58.1
Otago	38	0.10-0.63	0.24	0	5.3	44.9	55.1
Canterbury	100	0.02-1.02	0.23	9.0	20.0	60.0	40.0
West Coast	43	0.04-0.60	0.21	4.6	11.6	65.1	34.9
Nelson	59	0.01-0.47	0.15	22.1	37.4	76.5	23.5
Marlborough	30	0.00-0.46	0.15	26.6	43.2	79.7	20.3
Central Otago	44	0.02-0.30	0.10	36.3	61.3	90.7	9.3
South Island	381	0.00-1.02	0.19	12.6	25.2	63.5	36.5

* All sodium values expressed as percent of dry matter

Table 10: Summary of distribution of sodium values in topdressed South Island pastures.

ment. Although dairying is not the major farming concern in these two areas, over 90 percent of the sites were found to be below the sodium requirement for this type of stock. Apart from the Central Plateau and Central Otago, other areas, namely Nelson and Marlborough in the South and Rangitikei, East Coast, and Hawkes Bay in the North Island, show varying degrees of inadequacy of sodium for sheep and beef needs.

An overall summary of the distribution of sodium in pastures from the North and South Islands is given in Fig 8.

In general, the results also confirm expectations from the results presented in Table 2, that is coastal pastures would have higher sodium contents than inland areas as a consequence of the deposition of sodium chloride in sea spray. This is clearly shown in Table 11 in which the results are presented according to a transect taken from the sea coast to 43 km inland.

Location	Distance from sea (km)	Sodium content in pasture (percent dry matter)
Whakiki lagoon	0	0.29
Frasertown	11	0.15
Marumaru	18	0.11
Te Reinga	27	0.08
Ruakituri	33	0.07
Papuni	43	0.07

Table 11: Influence of sea spray on sodium content in pasture plants.

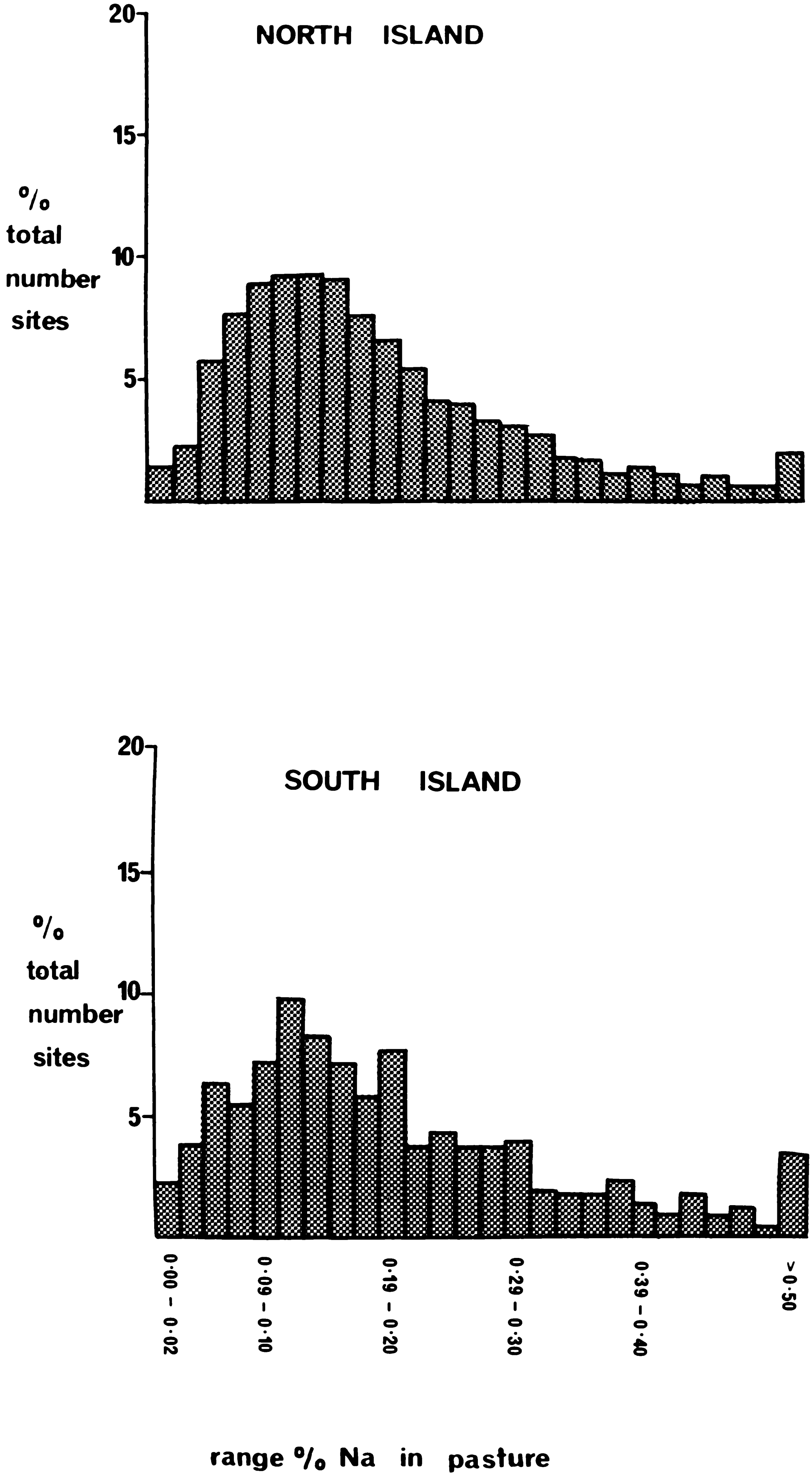

Fig. 8 – Summary of the distribution of sodium concentrations in herbage from topdressed New Zealand pastures.

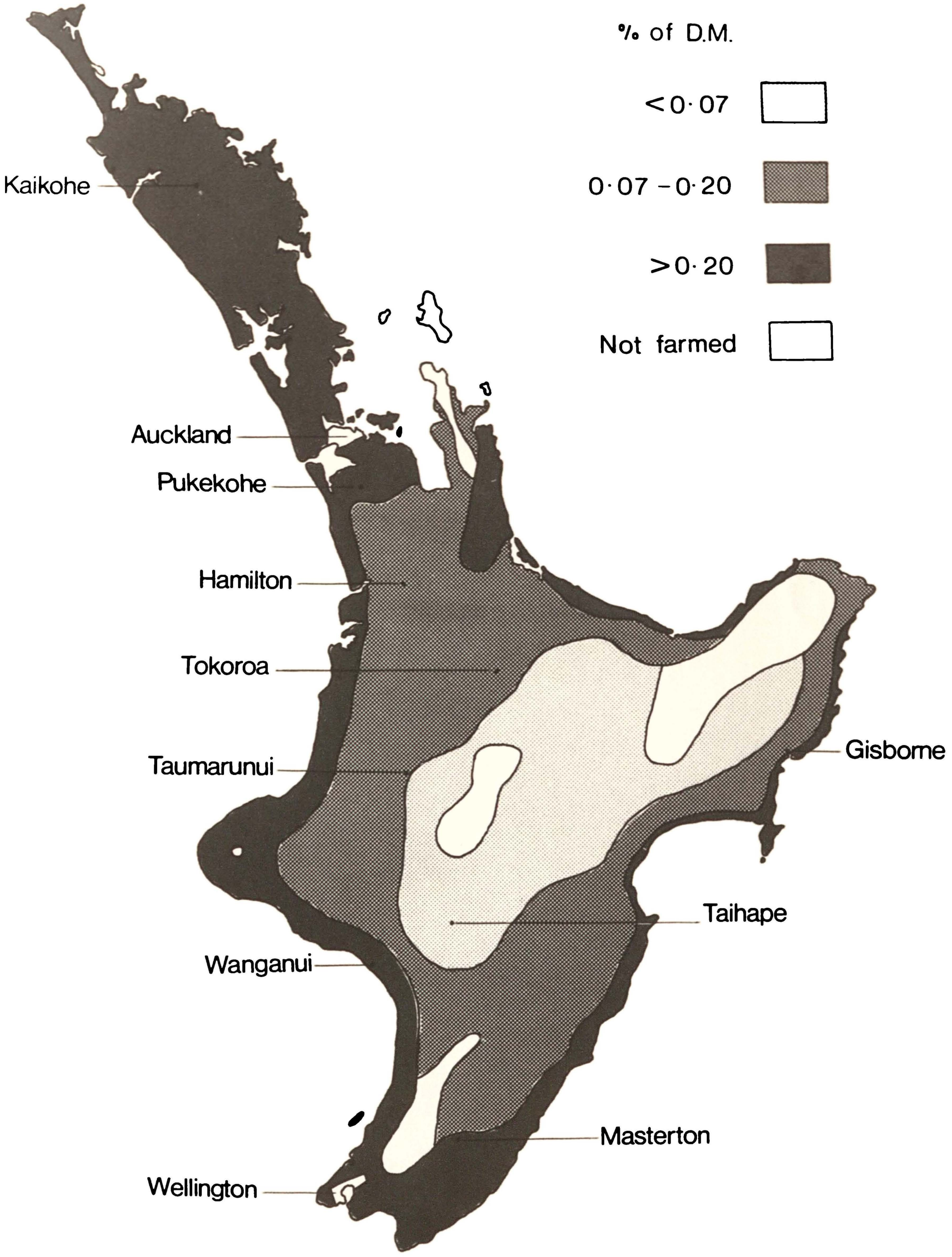

Fig. 9 – A generalised summary of sodium concentrations in herbage from topdressed North Island pastures.

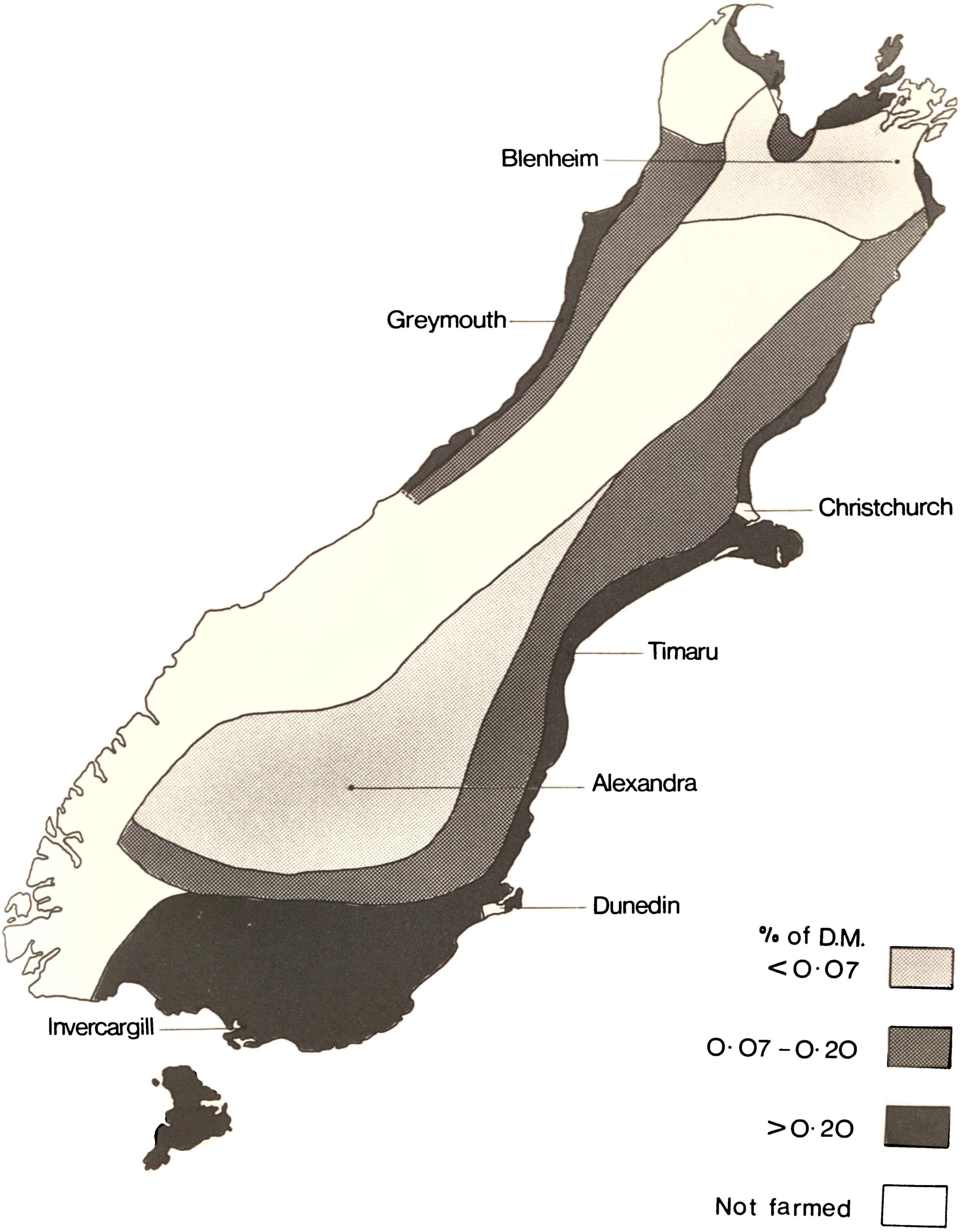

Fig. 10 - A generalised summary of sodium concentrations in herbage from topdressed South Island pastures.

Furthermore, this influence is greater on the West Coast than that of the East Coast of both Islands, due, presumably, to the direction of the prevailing winds.

A generalised summary of the above effects, along with the sodium status of inland areas of New Zealand is given in Figs 9 and 10.

Potassium

The results for potassium, Figs 11 to 15 and Tables 12 and 13, are summarised in a similar fashion to those for sodium, except that the arrangement of the regions was dependent on the number of sites in which the potassium values in the pasture were above 3.0 percent of the dry matter.

Only a small percentage of the sites throughout the country were shown in this survey to be deficient in potassium. Wellington and the West Coast had the highest number of sites with 29 percent and 23 percent respectively below 1.99 percent potassium. However, a more important feature of this survey was the number of sites in which the potassium levels were above those recommended for satisfactory plant growth. With the exception of Wellington and the West Coast, 50 percent of the sites within the remaining regions had potassium levels above 2.5 percent. Moreover, a number of areas, namely Taranaki, Bay of Plenty and the Central Plateau, over 60 percent of the sites had potassium levels greater than 3.0 percent.

The above results, summarised for both North and South Islands in Fig 16, are a reflection of the increased use of potassic fertilisers in New Zealand at least since 1950 when the sampling commenced (see Fig 17 for data on the increased use of potassium since 1950). As a direct consequence of this trend, many areas of both Islands now have potassium values in pastures which are well in excess of plant needs,

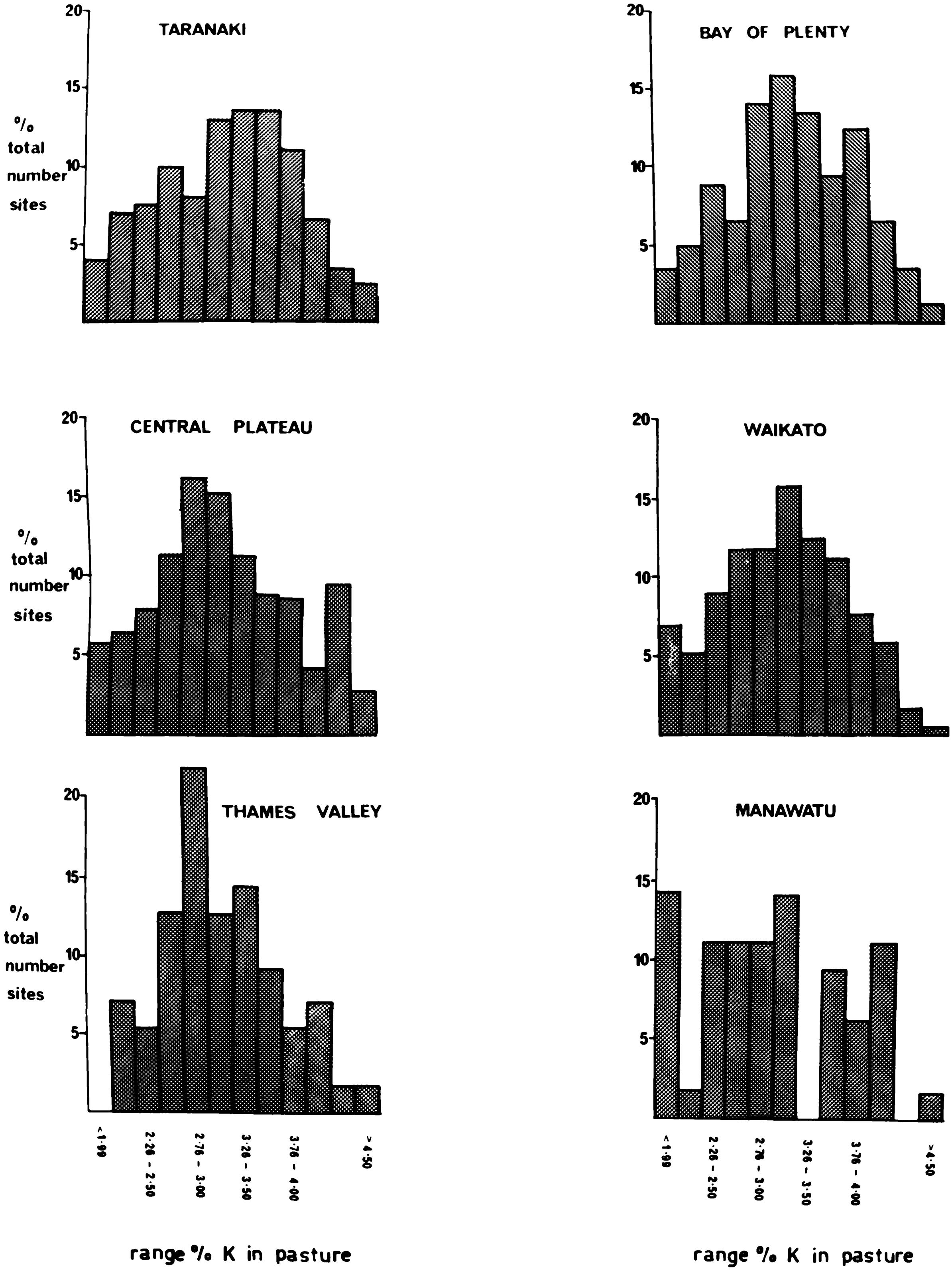

Fig. 11 - Distribution of potassium concentrations in herbage from topdressed North Island pastures.

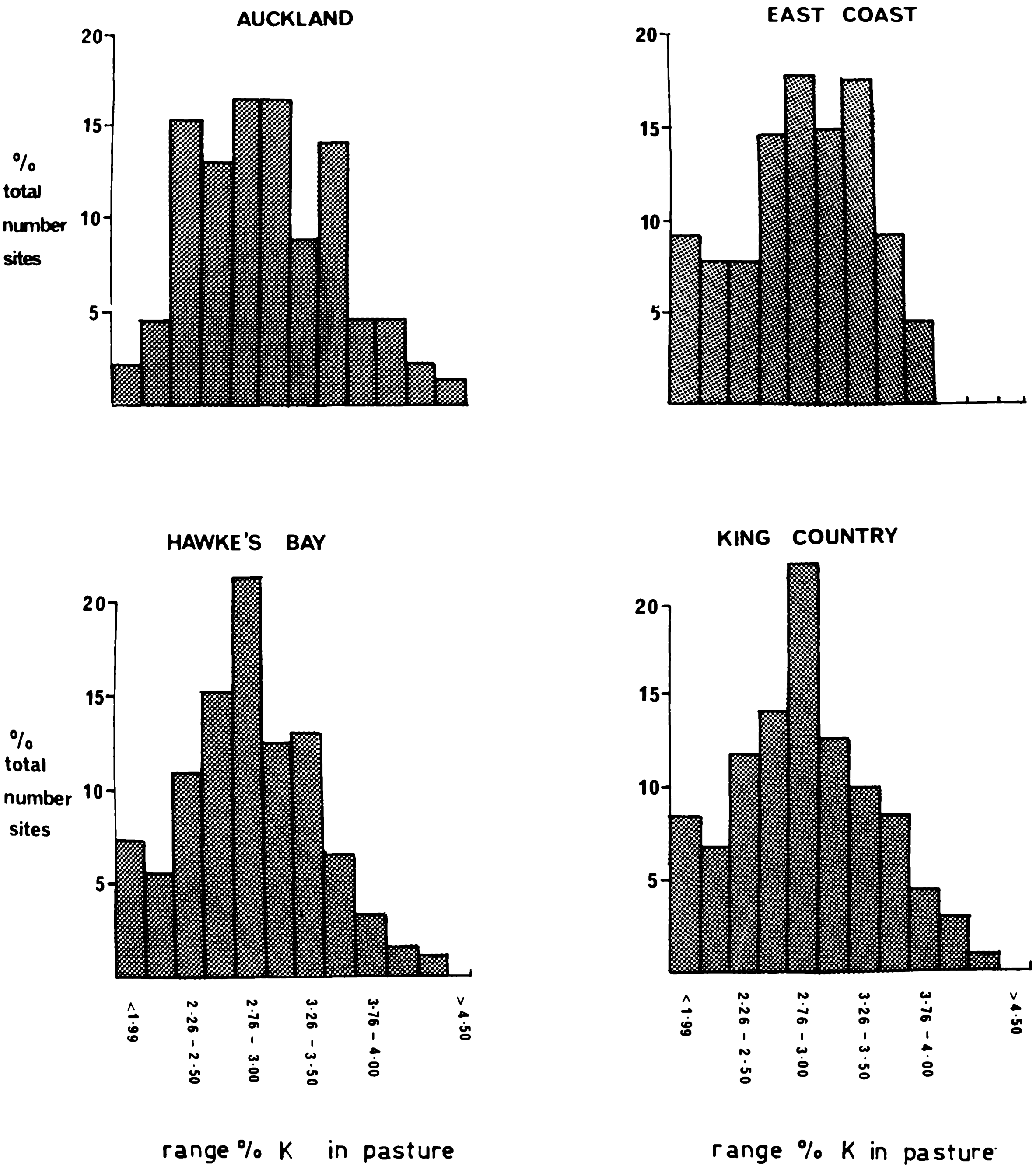

Fig. 12 - Distribution of potassium concentrations in herbage from topdressed North Island pastures.

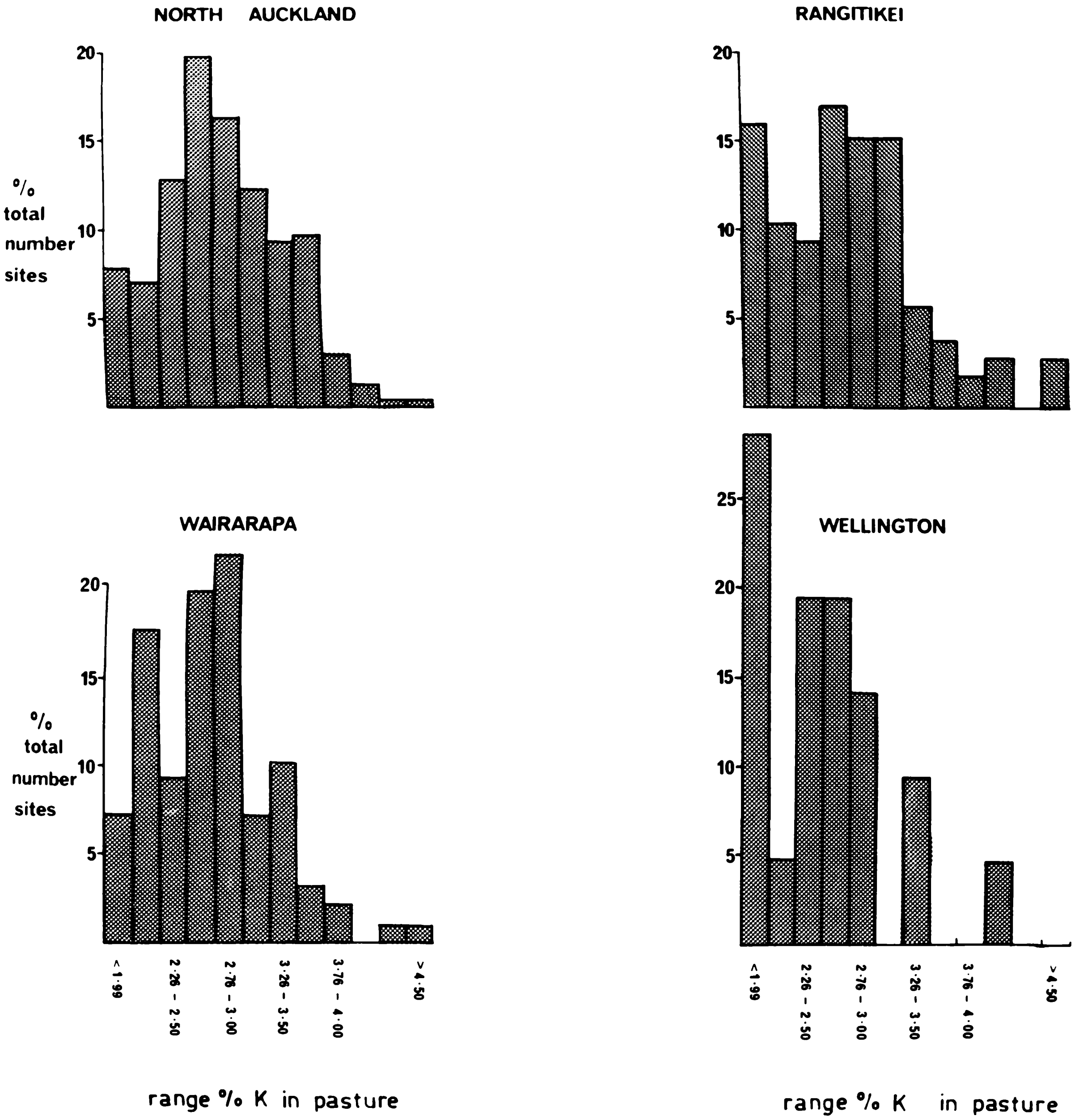

Fig. 13 - Distribution of potassium concentrations in herbage from topdressed North Island pastures.

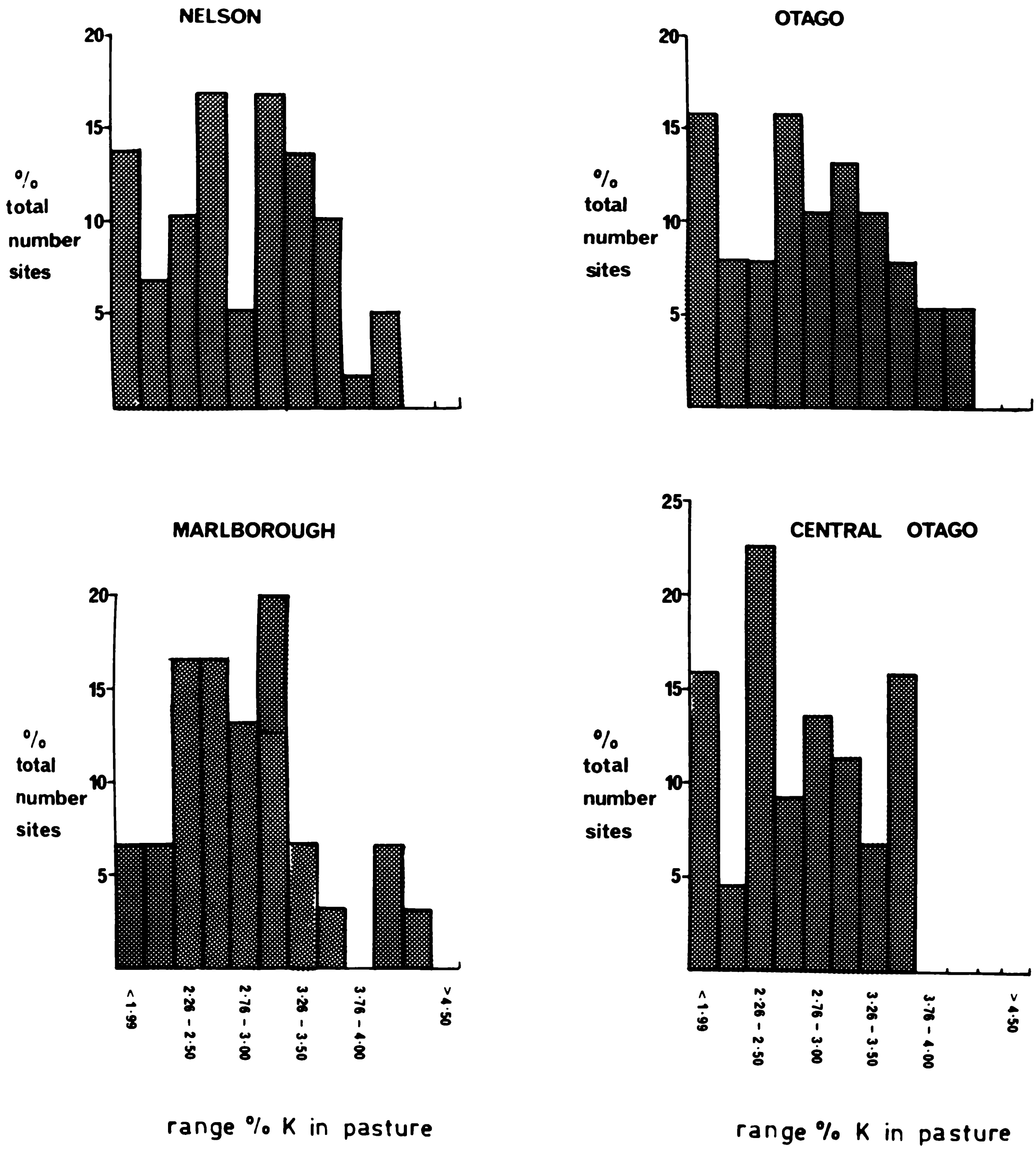

Fig. 14 - Distribution of potassium concentrations in herbage from topdressed South Island pastures.

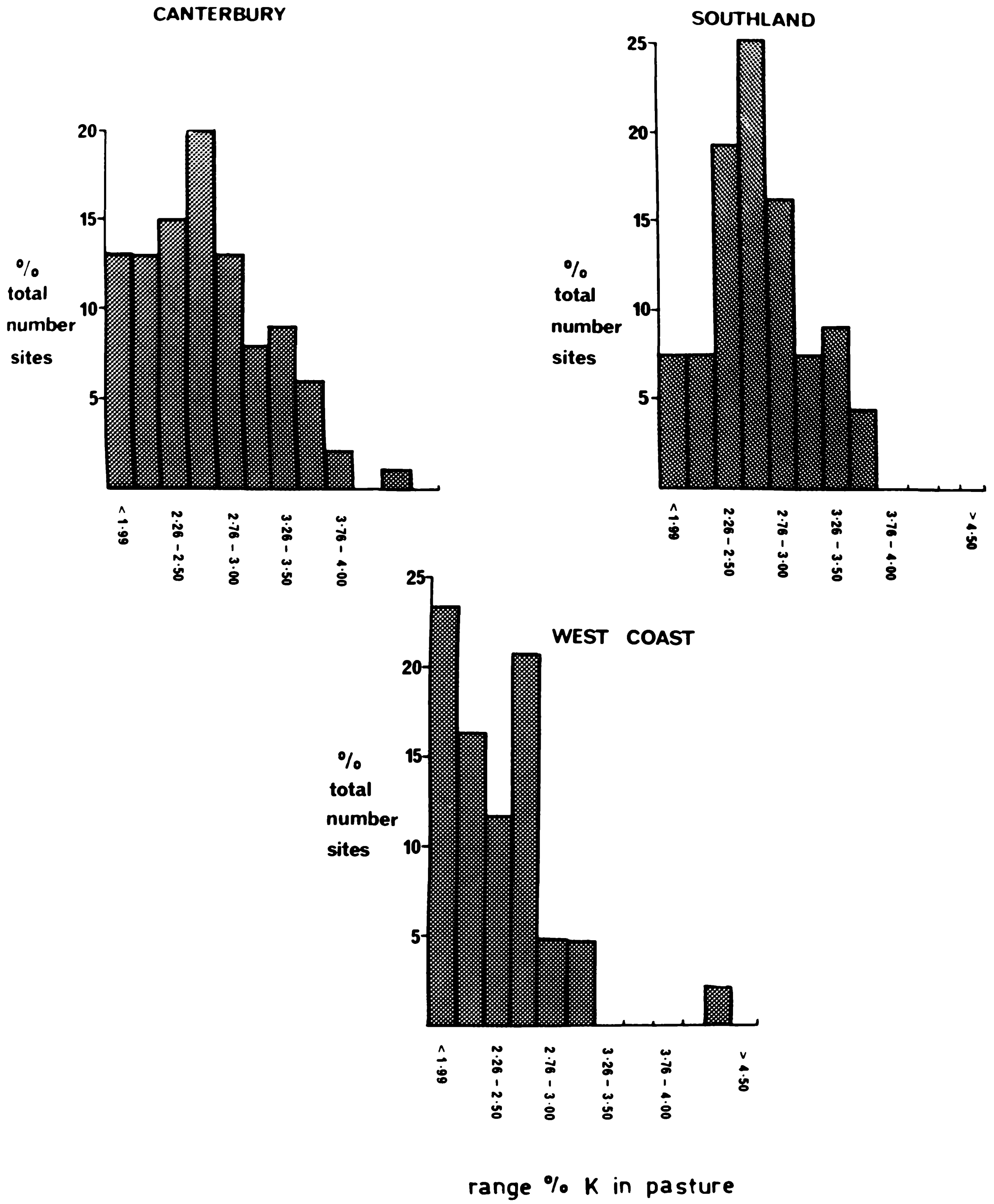

Fig. 15 - Distribution of potassium concentrations in herbage from topdressed South Island pastures.

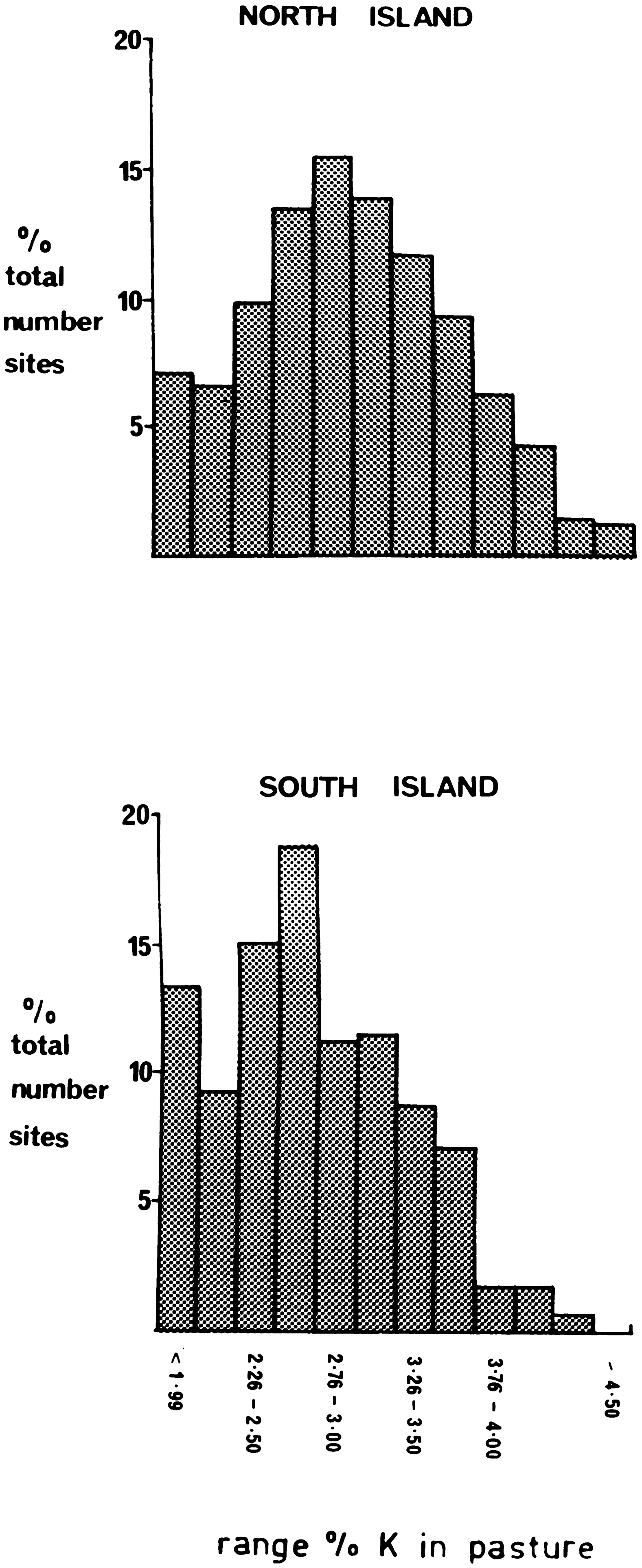

Fig. 16 – Summary of the distribution of potassium concentrations in herbage from topdressed New Zealand pastures.

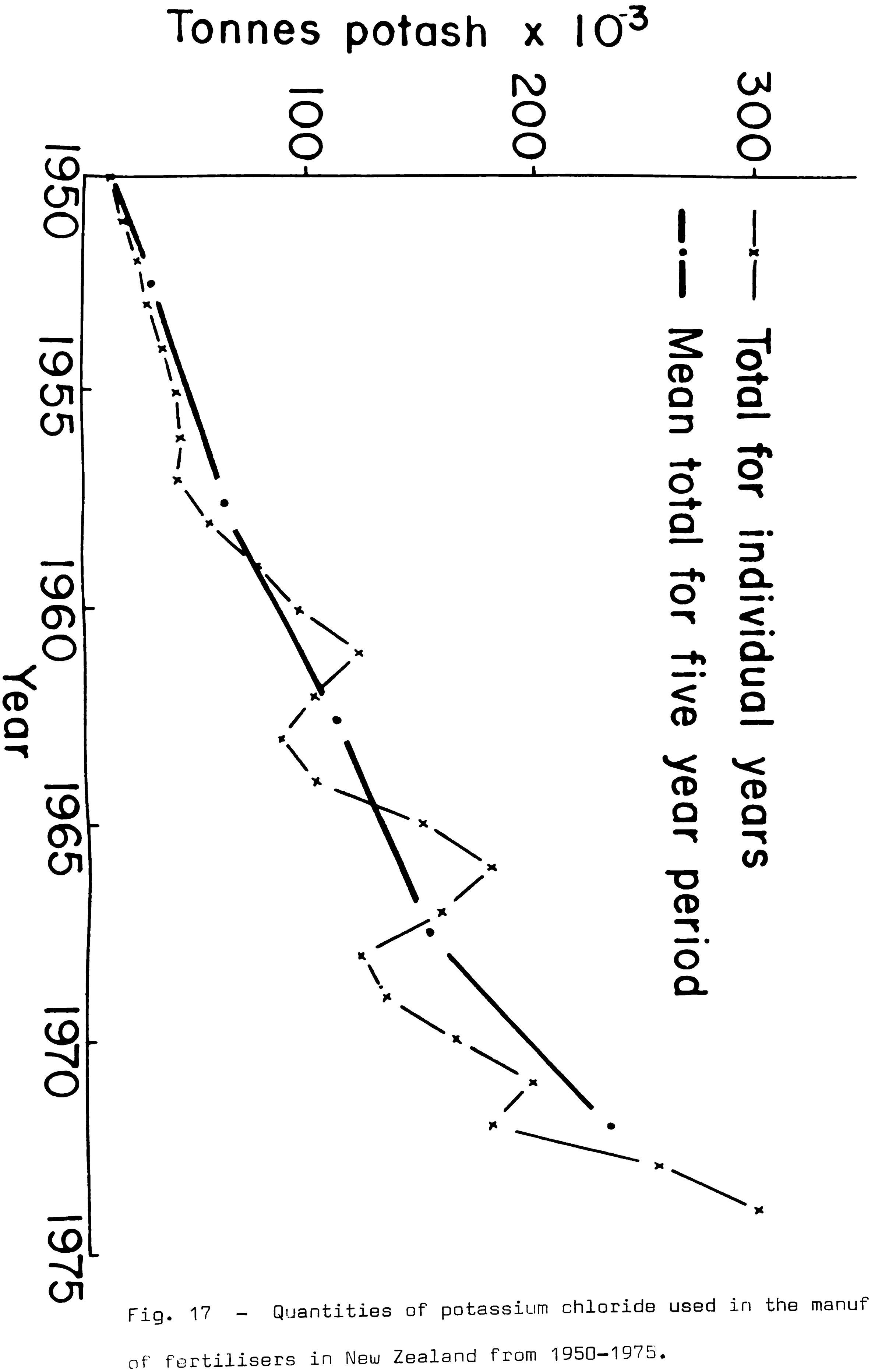

Fig. 17 - Quantities of potassium chloride used in the manufacture of fertilisers in New Zealand from 1950–1975.

Geographical region	Total number sites	Range potassium values*	Overall mean	The percentage of sites within a region which fall within the following specified potassium values – ≤1.99	2–2.50	>2.50	>3.00
Taranaki	200	1.37–5.21	3.23	4.0	14.5	81.5	63.5
Bay of Plenty	170	1.39–4.70	3.20	3.5	13.5	83.0	62.4
Central Plateau	315	1.31–5.21	3.04	5.7	14.2	80.1	60.9
Waikato	488	1.12–4.36	3.07	7.0	14.1	78.9	55.1
Thames Valley	55	2.00–4.81	3.10	0	12.8	87.2	52.7
Manawatu	63	1.18–4.84	2.98	14.3	12.7	73.0	50.7
Auckland	94	1.64–4.66	3.05	2.1	19.2	78.7	50.1
East Coast	69	1.01–3.86	2.86	8.7	14.4	76.9	44.9
Hawkes Bay	288	1.16–4.47	2.89	7.3	17.0	75.7	39.2
King Country	122	1.23–4.45	2.87	8.2	18.1	73.7	38.5
North Auckland	298	1.11–4.68	2.83	7.7	19.8	72.5	36.3
Rangitikei	106	1.03–5.13	2.65	16.0	19.8	64.2	32.1
Wairarapa	97	1.12–4.64	2.66	7.2	26.8	66.0	24.7
Wellington	21	1.52–4.19	2.53	28.6	23.8	47.6	14.3
North Island	2386	1.01–5.21	2.93	7.0	16.3	76.7	47.4

* All potassium values expressed as percent of dry matter

Table 12: Summary of distribution of potassium values in topdressed North Island pastures.

Geographical region	Total number sites	Range potassium values*	Overall mean	The percentage of sites within a region which fall within the following specified potassium values –			
				≤1.99	2–2.50	>2.50	>3.00
Nelson	59	1.15–4.18	2.83	13.6	17.0	69.4	47.5
Otago	38	1.21–4.22	2.78	15.8	15.8	68.4	42.2
Marlborough	30	1.57–4.30	2.85	6.7	23.4	70.0	40.0
Central Otago	44	0.98–3.70	2.49	15.9	27.2	56.9	34.1
Canterbury	100	1.23–4.43	2.67	13.0	28.0	59.0	26.0
Southland	67	1.34–3.83	2.70	7.5	26.9	65.6	24.0
West Coast	43	1.01–4.46	2.47	23.3	27.9	48.8	23.3
South Island	381	0.98–4.46	2.67	13.4	24.4	62.2	32.1

* All potassium values expressed as percent of dry matter

Table 13: Summary of distribution of potassium values in topdressed South Island pastures.

which suggests that in these areas excessive amounts of potassium are being applied. Furthermore, the results indicate that higher rates of potassic fertiliser are more widely used throughout the North Island than in the South Island.

DISCUSSION

The results from this survey clearly indicate that topdressed ryegrass-white clover pastures in many areas of New Zealand have sodium contents which are below animal requirements, especially in relation to beef and dairy animals. However, a clear cut picture of the sodium status of these pastures has been obscured for a number of reasons. First, many of the geological formations in New Zealand have yielded soils deficient in sodium for animal needs. Second, large amounts of sodium chloride are deposited from sea spray especially onto areas close to the Western Coasts of both Islands. Third, prior to 1950, low grade potassium fertilisers were commonly used on pastures (Bell, 1955). Moreover, these lower grade fertilisers contained considerable quantities of sodium as an impurity. However, since that date, there has been a considerable increase in the use of high grade potassic fertilisers on grassland in New Zealand. As a direct result of this increased use of potassium many pastures throughout New Zealand, as revealed by this survey, now have potassium levels well in excess of plant needs.

Apart from the competition between potassium and sodium ions in which the former depresses the uptake of the latter into the root system of plants, potassium also accelerates leaching of soil sodium from the root zone as indicated from the results of McNaught and Karlovsky (1964). Therefore, the excessive use of potassic fertilisers would aggrevate an animal-nutrition problem involving sodium or even create one where it may not have originally existed.

Although very little data is available on the incidence of dietary dependent disorders in grazing animals it is interesting to note the marked variation in the incidence of hypomagnesaemic tetany (a disease generally associated with low levels of magnesium in the blood) and associated chemical composition of pasture between coastal and inland areas (Table 14). Magnesium levels above 0.20 percent of dry matter in the herbage are not normally associated with the incidence of hypomagnesaemia (Kemp, 1960).

Location	Range in incidence of hypomagnesaemia in beef cows (percent)+	Pasture chemical composition* (percent dry matter)		
		Sodium	Magnesium	Potassium
Inland	2.2–3.5	0.04	0.25	2.95
Coastal	0.6–0.9	0.16	0.23	2.35

+ data from Cairney (1964)

* " " Saunders *et al.* (1964)

Table 14: Variation in the incidence of hypomagnesaemia and associated chemical composition of pasture between inland and coastal areas.

The data summarised in Table 14 was from a survey of cattle on 477 farms in the Hawke's Bay region (Cairney, 1964) and pasture samples were taken from a selection of these farms (Saunders *et al.*, 1964).

An important change in farm practice involving sodium should also be mentioned (see Middleton and Smith, 1976). This has entailed cessation in supplementation of hay with common salt as the hay stack has been replaced by the bale and barn, and concurrently, topdressing pastures with salt, which had been common practice in the past when meat-works were a cheap source of sodium chloride, has also been discontinued.

Finally, sources of sodium other than those supplied by plants should also be taken into account when considering grazing animals. In fact one of the first symptoms of sodium deficiency in grazing animals is an avid licking of wood, soil, and sweat of other animals (Underwood, 1966). In this regard, the ingestion of soil either directly or indirectly from soil contaminated herbage is considered to be an important source of mineral elements for grazing animals under certain circumstances (Healy, 1973). Furthermore, sodium in drinking water can also constitute a sizable fraction of the total intake (Smith and Anies, 1959). In this respect animals eating herbage low in sodium will show a marked preference for drinking water containing sodium. This is clearly seen from the results presented in Table 15 where water samples were collected from a farm on which cattle were found to preferentially drink from a spring while ignoring all other supplies. The sodium concentration in the spring water was over 50 times that found in the creek.

Concentration of elements in water*

Water Source	NH_4	NO_3	P	S	K	Mg	Ca	Na	Cl
Creek	0.1	0.1	0.04	5	1	4	26	11	13
Spring	2.2	0.1	0.16	0.5	5	3	5	600	94

* all figures are in ppm

Table 15: Chemical analysis of two water samples indicating the importance of drinking water as a source of sodium in an area low in herbage sodium.

CHAPTER 2

CLASSIFICATION OF THE MORE IMPORTANT NEW ZEALAND PASTURE AND FODDER SPECIES INTO NATROPHILIC AND NATROPHOBIC TYPE PLANTS

INTRODUCTION

Although there have been numerous reports on the differences which exist between plants in the extent to which they can absorb and translocate sodium, only a partial classification of some grass species into natrophilic and natrophobic types has been made (see Literature Review).

Therefore, the following investigation was undertaken to give a classification of some of the more important agricultural plants grown in New Zealand according to the sites where sodium preferentially accumulates within the plant.

EXPERIMENTAL TECHNIQUE

A glasshouse pot technique described by Middleton and Toxopeus (1973) which involves the growth of appropriate indicator plants in soil with the periodic addition of a complete balanced nutrient solution was used.

(a) Soil Preparation and Planting

The important agricultural plants listed in Table 16 were grown in a pumice derived soil, Atiamuri sand.

Before the soil was potted it was fumigated with 'Basamid Granular' (99 percent w/w dazomet) granules to destroy soil borne plant pests and diseases. The fumigant was added at the rate of one gram of 'Basamid' to one kilogram of soil. After the application of granules, which were thoroughly mixed into the sieved soil (a 6.5 mm wire mesh sieve was used), the soil was moistened and sealed within a black

	Common Name	Botanical Name
		Grasses
1	Ariki ryegrass	*Lolium* x *hybridum* Hausskn. cv. 'Grasslands Ariki'
2	Brown top	*Agrostis tenuis* Sibth.
3	Cocksfoot	*Dactylis glomerata* L. cv. 'Grasslands Apanui'
4	Kikuyu	*Pennisetum clandestinum* Rich.
5	Meadow grass	*Poa trivialis* L.
6	Nui ryegrass	*Lolium perenne* L. cv. 'Grasslands Nui'
7	*Paspalum*	*Paspalum dilatatum* Poir.
8	*Phalaris*	*Phalaris tuberosa* L.
9	Prairie grass	*Bromus unioloides* Syn.(*B. catharticus* Vahl.)
10	Ruanui ryegrass	*Lolium perenne* L. cv. 'Grasslands Ruanui'
11	Tall fescue	*Festuca arundinacea* Schred. cv. S170
12	Tama ryegrass	*Lolium multiflorum* Lam. cv. 'Grasslands Tama'
13	Timothy	*Phleum pratense* L. cv. 'Grasslands Kahu'
14	Yorkshire fog	*Holcus lanatus* L.
		Legumes
15	Alsike clover	*Trifolium hybridum* L.
16	*Desmodium*	*Desmodium uncinatum* Jacq. cv. Silver leaf
17	*Lotus*	*Lotus pedunculatus* cav. cv. 'Grasslands Maku'
18	Lucerne	*Medicago sativa* L. cv. 'Wairau'
19	Red clover	*Trifolium pratense* L. cv. 'Grasslands Turoa'
20	Soya bean	*Glycine max* L. cv. 'Amsoy'
21	Subterranean clover	*Trifolium subterraneum* L.
22	White clover	*Trifolium repens* L. cv. 'Grasslands Huia'
23	Lupin	*Lupinus angustifolius* L. cv. 'Unicrop'
		Fodder plants
24	Barley	*Hordeum vulgare* L. cv. 'Zephyr'
25	Chou moellier	*Brassica oleracea* L. cv. 'Giant chou moellier'
26	Kale	*Brassica oleracea* L. cv. 'Thousand-headed Kale'
27	Maize	*Zea mays* L.
28	Japanese millet	*Echinochloa frumentacea* Link.
29	Oats	*Avena sativa* L. cv. 'Algerian'
30	Rape	*Brassica napus* L. cv. 'Rangi'
31	Sudax	*Sorghum bicolor* (Lynn) x *Sorghum Sudanese* (Piper)

Table 16: Plants used to classify the more important New Zealand pasture and fodder species into natrophilic and natrophobic type plants.

plastic sheet for seven days. Subsequently the soil, which was continually kept moist, was exposed to the air for at least 14 days to allow toxic gases to escape. To make sure that no gases remained to interfere with seed germination, rape seeds were germinated in a small quantity of soil. Once the soil was shown to be free of the gases it was air dried ready for potting.

Each 15 centimetre plastic pot contained 500 grams of air dried soil loosely filled to three centimetres from the top. For plants with large seeds, maize, soyabean, sudax, and lupin, only 25 seeds were planted per pot, but all other test plants had 150 seeds sprinkled uniformly over the soil surface in each unit. The seeds were then covered with a one centimetre layer of soil which had been removed prior to planting.

After germination, all leguminous test plants received several applications of an aqueous suspension of their appropriate Rhizobium strain to ensure effective nodulation; the inoculum was cultured in yeast-mannitol agar (Van Schreven, 1964).

(b) Treatments and Design

The nutrient solution formulated by Middleton and Toxopeus (1973) was modified to give two contrasting levels of potassium for each plant species, with all other nutrients except chloride kept constant. The lower treatment of the two, was adjusted to give a potassium content in ryegrass of approximately 2.20 percent, considered to be adequate for satisfactory plant growth (McNaught, 1970). The corresponding sodium level was adjusted to give 0.30 percent of the dry matter.

Each potassium treatment was replicated six times. The pots were arranged in a randomised design on benches in a temperature controlled glasshouse (15 °C min - 25 °C max).

(c) Nutrient Solutions and Soil Moisture

The composition of the stock solutions of major elements (N, P, S, K, Na, Mg, and Ca), which varied according to treatment and plant species, is given in Table 17. A separate minor element (B, Co, Cu, Fe, Mn, Mo, and Zn) stock solution was prepared and the composition is given in Table 18.

Salt		Low K	High K
NH_4NO_3	a	26.7	26.7
	b	-	-
KH_2PO_4	a	3.0	3.0
	b	3.0	3.0
K_2HPO_4	a	3.3	3.3
	b	3.3	3.3
K_2SO_4	a	1.7	1.7
	b	1.7	1.7
KCl	a	-	3.0
	b	-	3.0
$MgSO_4 7H_2O$	a	2.7	2.7
	b	2.7	2.7
$CaCO_3$	a	0.5	0.5
	b	0.5	0.5
N HCl	a	10 ml	10 ml
	b	10 ml	10 ml
Na_2SO_4	a	1.6	1.6
	b	1.6	1.6

Table 17: Composition of stock solutions of major nutrients in grams or millilitres per litre, for (a) non-leguminous and (b) leguminous test plants grown in soil.

Salt	mg per litre
H_3BO_3	3.0
$COCl_2.6H_2O$	0.4
$CuCl_2.2H_2O$	1.0
$MnCl_2.H_2O$	10.0
$(NH_4)_6\ MO_7O_{24}.4H_2O$	0.4
$ZnCL_2$	1.5

Table 18: Composition of stock solution of minor nutrients in mg per litre, used with non-leguminous and leguminous test plants.

The dilute nutrient solutions for application to test plants were prepared as follows:–

200 ml of the appropriate major element solution plus 100 ml of the minor element supplement and made up to 4.5 litres with pure water containing 0.15 ppm of ferric citrate.

This solution was applied at the rate of 50 ml per pot, five times a week.

Soil moisture in each pot was maintained during the trial at 75 percent of field capacity by the addition of an appropriate nutrient solution, supplemented, according to transpiration rates of the growing plants, with pure water. This adjustment was important to ensure that leaching did not take place.

(d) Measurements

Aerial parts of the test plants were harvested by cutting at five centimetres above the soil surface when the leaf tissue had reached a height approximately 12 centimetres above the lip of the pot.

However, plants such as chou moellier, kale, lupin, rape, and soya bean, where the growing point was well above the normal cutting height, individual leaves were trimmed in such a way that enough young leaf tissue remained to give rapid regrowth.

The plants were cut four times, with each cut being taken after an interval of approximately 21 days. After the fourth cut the roots and stubble were also harvested. In this study the stubble was considered to be the five centimetre portion of tissue between the roots and harvested aerial parts. However, in certain cases, the stubble defined in this way was really the stem tissue. Soil was removed from the roots by washing in deionized water in such a manner that there were no appreciable losses of nutrients from the tissues within the root.

All harvested material was oven dried at 32.5 °C and weighed to give dry matter yields. Each replicate was then finely ground and chemically analysed for N, P, S, K, Na, Mg, and Ca. The methods used for the analysis of K, Na, Mg, and Ca in the nitric-perchloric acid digests have already been described (Dorofaeff and McNaught, 1962). Nitrogen was determined by the sodium phenate method; phosphorus, by a method of Jackson (1958) modified for use with an auto analyser; total sulphur, using the nitric-perchloric acid digestion procedure followed by a method adapted from Mottershead (1971).

Transpiration rates were determined for all plants except oats, barley, meadow grass, prairie grass, and soya bean. These five species were all planted at a slightly later date than that of the main group and were therefore at a different stage of growth. The rates were measured on five consecutive days prior to the first cut. All pots in the trial were adjusted to 75 percent of field capacity immediately before the commencement of the measurements. Transpiration rates were determined by measuring the weight loss within each pot from 75 percent field capacity three times a day. Water lost from the pots in this way was corrected for surface evaporation by subtracting the small mean loss of water from six pots containing bare soil over the same time period.

Exchangeable soil sodium and potassium cations were determined on a single soil sample composed of six replicates for each plant species and potassium treatment. The samples were taken at the end of the fourth cut before the roots were harvested. This method of determination involved leaching the soil with neutral normal ammonium acetate and measuring the displaced sodium and potassium by flame photometry.

(e) Expression of Results

All yields are expressed as grams dry weight per pot. On the other hand the concentration results from the chemical analyses are percentages of the dry matter.

Transpiration rates are expressed as millilitres of water per gram of dry matter per pot per day. The exchangeable soil cations as milligrams per pot.

Analysis of variance was carried out on all results.

RESULTS

The more detailed results of the effects of potassium on sodium levels and associated chemical composition in the above plants are discussed in chapter 3.

Although there were differences in the sodium concentration between the two potassium treatments, these differences were such that they had little influence on the overall ranking of the above plants in the following classification. Therefore, the results used for the classification of plants into natrophiles and natrophobes were calculated from the pooled means of the two potassium treatments for shoots, stubble, and roots. In the case of the shoots, the mean of the four cuts was used. These results are presented in Table 19. The plant species are arranged in the table in descending order according to the concentration of sodium in the leaf tissue.

From Table 19 it can be seen that most plants can be readily classified into two distinct types; on the one hand there is the group which has a high sodium concentration in leaf tissue and a relatively low concentration in roots, these are the natrophiles; whereas for the other group, the natrophobes, the reverse is generally found. Moreover, the results reveal a further important characteristic in some members of both natrophiles and natrophobes. In addition to the shoots or roots being major sites of sodium accumulation, there are a number of plants in which the stubble tissue is the principal site, with either the shoot or root, being the secondary site depending on whether the plants are natrophilic or natrophobic.

A classification of the above plants into natrophiles and natrophobes is given in Table 20, along with a further subdivision within each type according to the principal site of sodium accumulation. Pooled means of sodium concentrations of the plants in each subdivision, calculated from the results in Table 19, show the extent of the accumulation within the different tissues.

Although brown top, meadow grass, tall fescue, and rape, have been classified as natrophobes, according to the results from the initial cuts, they form a group of plants which, in some respects, could be regarded as intermediate between natrophiles and natrophobes (see Table 19). Another point is that with the exception of barley, subclover, tall fescue, meadow grass, and brown top, there were no significant differences in the sodium concentration in shoot tissue between cuts. However, in these five species, which

	Sodium (percent dry matter)		
Plant species	Shoots	Stubble	Roots
Phalaris	0.44	0.45	0.13
Oats	0.41	0.53	0.28
Chou moellier	0.41	0.23	0.14
Yorkshire fog	0.40	0.33	0.11
Kale	0.38	0.30	0.19
Subclover	0.36	0.43	0.25
Cocksfoot	0.35	0.40	0.18
White clover	0.35	0.34	0.20
Tama ryegrass	0.34	0.39	0.10
Nui ryegrass	0.34	0.37	0.14
Prairie grass	0.34	0.37	0.24
Ariki ryegrass	0.34	0.35	0.19
Barley	0.33	0.54	0.34
Ruanui ryegrass	0.33	0.38	0.15
Lotus	0.30	0.24	0.23
Tall fescue	0.24	0.23	0.19
Rape	0.23	0.48	0.25
Brown top	0.17	0.46	0.20
Meadow grass	0.17	0.29	0.24
Japanese millet	0.13	0.24	0.19
Red clover	0.07	0.25	0.44
Paspalum	0.06	0.15	0.28
Kikuyu	0.05	0.11	0.22
Timothy	0.04	0.37	0.24
Lucerne	0.04	0.26	0.42
Alsike clover	0.03	0.35	0.21
Lupin	0.03	0.14	0.74
Desmodium	0.01	0.29	0.49
Sudax	0.01	0.09	0.37
Maize	0.01	0.01	0.28
Soya bean	0.00	0.02	0.41
Standard error	±0.0067	±0.0068	±0.0059

Table 19: Plant species arranged in descending order according to the concentration of sodium in their leaf tissue.

Natrophiles

Leaf			Stubble	
Grasses	**Legumes**	**Fodder**	**Legumes**	**Fodder**
Ariki ryegrass	Lotus	Chou moellier	Subclover	Barley
Cockfoot	White clover	Kale		Oats
Nui ryegrass				
Phalaris				
Prairie grass				
Ruanui ryegrass				
Tama ryegrass				
Yorkshire fog				

	Shoots	Stubble	Roots	Shoots	Stubble	Roots
percent Na (pooled mean)	0.36	0.35	0.17	0.37	0.50	0.29

Natrophobes

Stubble			Root		
Grasses	**Legumes**	**Fodder**	**Grasses**	**Legumes**	**Fodder**
Brown top	Alsike clover	Millet	Kikuyu	Desmodium	Maize
Meadow grass		Rape	Paspalum	Lucerne	Sudax
Tall fescue				Lupin	
Timothy				Red clover	
				Soya bean	

	Shoots	Stubble	Roots	Shoots	Stubble	Roots
percent Na (pooled mean)	0.14	0.35	0.22	0.03	0.15	0.41

Table 20: A classification of the more important New Zealand pasture and fodder species into natrophilic or natrophobic type plants according to the major sites of sodium accumulation.

includes this intermediate group, there was a significant ($p = 0.001$) trend towards an increase of sodium in shoots with each cut (Table 21).

Plant Species	Sodium (percent dry matter) Cut 1	Cut 2	Cut 3	Cut 4
<u>Natrophiles</u>				
Barley	0.23	0.32	0.33	0.40
Subclover	0.28	0.33	0.39	0.46
<u>Natrophobes</u>				
Tall fescue	0.12	0.22	0.25	0.37
Meadow grass	0.10	0.15	0.21	0.23
Brown top	0.08	0.16	0.17	0.25
Standard error	±0.0065	±0.0062	±0.008	±0.006

<u>Table 21</u>: Increase in sodium concentration in leaf tissue of certain natrophiles and natrophobes with cuts.

This result suggests that the main accumulation site within these plants is more readily saturated with sodium than in the other plants. Therefore, it is predictable that such natrophobic plants could eventually display natrophilic characteristics if they are supplied with a generous amount of sodium.

A notable feature of this classification is the number of tropical plants which are natrophobic. Furthermore, the mean results of the transpiration rates from the two potassium treatments, presented in Table 22, indicate that there is a highly significant difference in the rate of transpiration between natrophilic and natrophobic plants.

Plant Species	Transpiration rate (ml H_2O/gram dry weight/day/pot)	
	Temperate Natrophiles	
Ariki ryegrass	987	
Chou moellier	2181	
Cockefoot	1206	
Kale	2873	
Lotus	1067	
Nui ryegrass	1047	Standard error ± 69
Phalaris	1208	
Ruanui ryegrass	1209	
Subclover	1314	
Tama ryegrass	965	
White clover	1153	
Yorkshire fog	1445	
Mean	1388*	
	Temperate Natrophobes	
Alsike clover	660	
Brown top	831	
Lucerne	1182	
Lupin	625	Standard error ± 49
Rape	1653	
Red clover	938	
Tall fescue	649	
Timothy	1222	
Mean	970*	
	Tropical Natrophobes	
Desmodium	675	
Kikuyu	418	
Maize	371	Standard error ± 24
Millet	561	
Paspalum	777	
Sudax	401	
Mean	534*	

* All values significantly different from each other $p \leq 0.001$

Table 22: Transpiration rates of natrophilic and natrophobic plants.

In general, the natrophiles, which were comprised solely of temperate plants, had a considerably higher transpiration rate with a mean value of 1388 ml H_2O/gram dry weight/day/pot, than that of both temperate and tropical natrophobes. In fact, within the natrophobe group, the tropical plants had a transpiration rate far lower than their temperate counterparts, with mean rates of 534 and 970 ml/g/day/pot respectively.

This difference in transpiration rate between natrophiles and natrophobes is reflected not only in the sodium composition of these plants, but also in the concentration of other elements, notably nitrogen and potassium (Table 23).

Temperate Natrophiles

(Results expressed as percent dry matter)

Element		Shoots	Stubble	Roots
Nitrogen		4.67	2.99	2.21
Potassium		2.64	1.43	0.39
Sodium		0.36	0.38	0.19
			Temperate Natrophobes	
Nitrogen		4.99	2.75	2.30
Potassium		2.56	1.53	0.56
Sodium		0.11	0.29	0.32
			Tropical Natrophobes	
Nitrogen		3.67	2.39	1.97
Potassium		2.16	1.08	0.58
Sodium		0.04	0.16	0.32
Standard error	N	±0.11	±0.09	±0.08
	K	±0.02	±0.02	±0.01
	Na	±0.007	±0.007	±0.006

Table 23: Mean chemical composition of natrophilic and natrophibic plants.

Although no precise measurements were made in this study of the rate of sodium taken up by natrophiles and natrophobes, it is evident from the construction of a balance sheet for sodium from the results of the individual plant species, that there are considerable differences in the rate of uptake between these two types of plants. The balance sheet, Table 24, takes into account the initial amount of sodium in the soil, the total amount of sodium applied in the nutrient solution, the amount recovered in the plant, and finally a measure of the amount remaining in the soil after the completion of the experiment.

A close examination of Table 24 reveals that the total uptake of sodium by natrophiles is approximately twice that of natrophobes after a 90 day interval, in spite of the significant difference ($p = 0.001$) in total plant yield between natrophobes (pooled mean of 28.83 g/dry matter/pot) and that of natrophiles (pooled mean of 23.10 g/dry matter/pot). Moreover, this striking difference in uptake between natrophiles and natrophobes is further reflected in the amount of sodium remaining in the soil at the end of the experiment. The natrophiles, with their higher rate of sodium uptake, depleted the soil reserves of this element from an initial level of 30 milligrams/pot down to a mean level for this group of 17 milligrams/pot. On the other hand for the natrophobes, in accord with their lower rate of uptake, an accumulation of sodium occurred in the soil. In fact, the mean soil level for the natrophobes of 40 milligrams of sodium/pot was 10 milligrams higher than the original soil level, and over twice that found for the natrophiles.

Table 24: Sodium balance sheet for individual plant species.

Plant Species	Sodium (Results expressed as milligrams/pot) Total 4 cuts Shoots	Stubble	Roots	Total Plant	Total Applied	Soil Contribution by difference	Soil Sodium* after 4 cuts
Temperate Natrophiles							
Ariki ryegrass	67.1	10.6	10.0	87.7	66.7	21.0	10.4
Barley	31.4	7.0	9.0	47.4	66.7	-	21.9
Chou moellier	59.0	11.1	6.1	76.2	66.7	9.5	19.6
Cocksfoot	69.2	12.8	7.2	89.2	66.7	22.5	10.4
Kale	59.2	13.1	6.2	78.5	66.7	11.8	21.9
Lotus	45.0	4.6	3.5	53.1	66.7	-	25.3
Nui ryegrass	70.8	11.1	6.1	88.0	66.7	21.3	10.4
Oats	39.2	5.1	7.9	52.2	66.7	-	15.0
Phalaris	65.6	12.9	5.9	84.4	66.7	17.7	17.3
Prairie grass	37.0	6.2	10.2	53.4	66.7	-	17.3
Ruanui ryegrass	60.5	16.5	7.2	84.2	66.7	17.5	12.7
Subclover	51.0	6.4	4.0	61.4	66.7	-	23.0
Tama ryegrass	70.3	7.5	4.1	81.9	66.7	15.2	13.8
White clover	56.5	4.3	2.8	63.6	66.7	-	27.6
Yorkshire fog	74.8	7.5	4.1	86.4	66.7	19.7	9.2
Mean	57.1	9.1	6.3	72.5	66.7	10.4	17.1
Temperate Natrophobes							
Alsike clover	4.8	4.4	2.1	11.3	66.7	-	54.1
Brown top	32.4	18.1	7.6	58.1	66.7	-	35.7
Lucerne	3.4	2.0	3.8	9.2	66.7	-	52.9
Lupin	2.9	8.2	20.7	31.6	66.7	-	27.6
Meadow grass	19.0	4.0	5.1	28.1	66.7	-	28.8
Rape	41.6	15.9	12.5	70.0	66.7	3.3	18.4
Red clover	6.0	1.8	5.5	13.3	66.7	-	41.4
Tall fescue	46.2	9.6	9.0	64.8	66.7	-	25.3
Timothy	4.8	5.9	5.7	16.4	66.7	-	29.9
Soya bean	0.4	2.0	29.8	32.2	66.7	-	36.8
Mean	16.2	7.2	10.2	33.6	66.7	-	34.1
Tropical Natrophobes							
Desmodium	1.1	5.1	11.4	17.6	66.7	-	34.5
Kikuyu	16.2	7.6	11.3	35.1	66.7	-	73.6
Maize	1.9	0.5	33.4	35.8	66.7	-	27.6
Millet	38.6	14.2	8.1	60.9	66.7	-	42.6
Paspalum	17.3	11.2	24.0	52.5	66.7	-	40.3
Sudax	2.6	5.1	28.3	36.0	66.7	-	64.4
Mean	13.0	7.3	19.4	39.7	66.7	-	47.2

* Initial soil sodium = 29.9 milligrams/pot

DISCUSSION

A most significant feature of this classification of plants into natrophiles and natrophobes is in relation to animal nutrition. Because of their intrinsic physiological difference, natrophobes are unable to provide feed containing enough sodium for dietary needs of animals, even in a situation where there is enough sodium in the soil to produce a satisfactory level in natrophiles. As a consequence, direct supplementation of the animals with sodium will, in general, be necessary where natrophobes form a substantial part of the diet. On the other hand, supplementation of natrophiles will only be required when the plants are grown on sodium deficient soils.

Moreover, with the trend towards the increased use of such high yielding plants as maize, sudax, and millet, as fodder crops, their natrophobic characteristics should be taken into account when assessing their potential as animal feeds.

Apart from the fundamental differences in the sites of sodium accumulation in natrophiles and natrophobes, there are two closely related physiological aspects which further characterise these two distinct types of plant.

It is well known that plant species differ in their transpiration rates (e.g. Briggs and Shontz, 1914), moreoever, plants possessing the C_4-photosynthetic pathway have lower rates than those with the C_3-pathway (Downes, 1969; Forde *et al.* 1974). However, it is of interest that the natrophiles, which in this study were solely made up of C_3 temperate plants, not only had higher transpiration rates than their natrophobic counterparts, but also higher ion uptake rates, particularly sodium. Furthermore, within the natrophobes, the temperate C_3 group had a higher mean transpiration rate, as expected, than the C_4 plants.

Therefore, the observed difference in the rate of sodium uptake by natrophiles, as opposed to natrophobes, could be a result of the differing transpiration rates.

CHAPTER 3

ENVIRONMENTAL FACTORS AFFECTING THE SODIUM CONTENT IN PLANTS

INTRODUCTION

Because sodium is not generally considered to be an essential element for higher plants, many of the basic physiological aspects of sodium nutrition are unknown. Therefore, to elucidate some of these aspects, three separate experiments were undertaken in which the effects of the following environmental factors on sodium nutrition were examined:

Experiment 1) Climatic factors
2) High substrate potassium
3) High substrate sodium

The results and discussion from each experiment are presented separately.

EXPERIMENT 1: CLIMATIC FACTORS

Due to the lack of suitable experimental facilities few controlled investigations have been made into the climatic factors which may influence mineral composition of plants. Therefore, to examine the effects of light, temperature, and moisture stress on sodium content in natrophiles and natrophobes, the following study was carried out in the D.S.I.R. Climate Laboratory facilities at Palmerston North.

EXPERIMENTAL TECHNIQUE

In general, the experimental procedures were the same as those described in Chapter 2.

(a) Soil As for Chapter 2.

(b) Test Plants

Three species* were used:–

* Botanical names are given in Table 16.

Natrophiles	Natrophobes
"Grasslands Ariki" ryegrass	"Wairau" lucerne
"Grasslands Huia" white clover	

Initially the plants were grown in a temperature controlled glasshouse (15° min – 25° max) until they were four centimetres above the pot rim. At this stage the plants were transferred to the Climate Rooms.

During this establishment period, the clover and lucerne plants received several applications of an aqueous suspension of their appropriate <u>Rhizobium</u> strain to ensure effective nodulation.

(c) <u>Design and Treatments</u>

The design of the experiment was as follows:

Room 1	High Light High Temperature	High moisture stress
		Low moisture stress
Room 2	High Light Low Temperature	High moisture stress
		Low moisture stress
Room 3	Low Light High Temperature	High moisture stress
		Low moisture stress
Room 4	Low Light Low Temperature	High moisture stress
		Low moisture

The values selected for temperature and light in the high treatments were considered to be representative of mid-summer conditions found in the more temperate parts of New Zealand. The contrasting low temperature and light treatments were chosen to simulate typical mid-winter conditions.

The plots were arranged in six randomised blocks per plant species on benches within each Climate Room.

(d) Detailed Climate Room Conditions

The lighting system consisted of a combination of 1000 Watt Sylvania Metalarc high pressure discharge lamps, and 100 Watt Philips tungsten iodide incandescent lamps. The high light rooms had four each of the above two types of lamps; while the low light rooms had only two of each.

Temperature in the high temperature rooms was maintained at 25 °C (±0.5 °C) during the day with a change to 16 °C (±0.5 °C) at night; low temperature rooms had a day temperature of 12 °C (±0.5 °C) and a night temperature of 5 °C (±0.5 °C).

The relative humidity was kept constant (±5 percent) in all four rooms during the day at 85 percent changing to 75 percent at night.

The photoperiod for the high light rooms was 16 hours and for the low light rooms 12 hours. There was an abrupt light-dark change. On the other hand, the day to night humidity changes occurred over two hours; the photoperiod beginning or ending halfway through the change-over.

Carbon dioxide concentration was ambient at 300-400 ppm and the air flow down through the plants was 0.3-0.5 m/sec^{-1}, measured at the top of the canopy.

A summary of the climatic conditions in each room is given in Table 25.

Room	Treatment	Temperature ($\pm$0.5 °C)		Relative Humidity ($\pm$5%)		Light* Intensity (Wm^{-2})
		Day	Night	Day	Night	
1	High light High temp.	25	16	85	75	174
2	High light Low temp.	12	5	85	75	183
3	Low light High temp.	25	16	85	75	87
4	Low light Low temp.	12	5	85	75	87

* mean light intensity - measured at the start and finish of the experiment

Table 25: Summary of climatic conditions.

(e) Nutrient Solutions and Soil Moisture Stress

For a description of the nutrient solutions, see details of the "low" potassium complete nutrient solutions for leguminous and non-leguminous plants given in Chapter 2.

The dilute solution was applied at a rate of 50 ml per pot three times a week.

Two soil moisture stress treatments were imposed; the soil in the low stress pots was maintained by addition of an appropriate nutrient solution, supplemented, where required, with pure water to give 75 per-cent of field capacity; whereas in the high moisture stress situation, the addition of moisture to the soil was adjusted to give only 50 per-cent of field capacity.

(f) Measurements

The methods of harvesting and chemically analysing the plants were the same as those described in Chapter 2. However, the time of cutting was determined by the height of plants grown under the low moisture stress (75 percent of field capacity) treatment. Four cuts were taken.

Analysis of variance was carried out on all results.

RESULTS

The results are presented according to plant species.

(a) Perennial ryegrass

Treatment means for sodium contents in shoots, stubble, and roots are given in Table 26.

A combination of high light, low temperature, and a high moisture stress, resulted in a marked increase in sodium concentration in all tissues of the plants compared with other treatment combinations. On the other hand, low light, temperature, and moisture stress resulted in sodium concentrations within the plant which were much lower than that found for the other treatment combinations.

Treatments			Sodium (percent dry matter)		
Light	Temp.	Moisture stress	Shoots	Stubble	Roots
H	H	H	0.37	0.47	0.19
H	H	L	0.29	0.42	0.17
H	L	H	0.58	0.81	0.32
H	L	L	0.44	0.72	0.22
L	H	H	0.37	0.61	0.23
L	H	L	0.27	0.52	0.21
L	L	H	0.20	0.31	0.17
L	L	L	0.15	0.28	0.12
		SE =	±0.04	±0.08	±0.03

H = High
L = Low

Table 26: Mean sodium concentration in perennial ryegrass as affected by combinations of light, temperature, and moisture stress.

An examination of the results of the main effects from the three treatments, presented in Table 27, indicates that light has the most dominating influence on sodium within ryegrass plants. An increase in light intensity from typical midwinter to that of midsummer conditions, resulted in a significant increase in sodium throughout the plant. Total plant dry matter yields (shoots + stubble + roots) were also significantly increased (P = 0.001) from 3.18 grams per pot to 8.20 grams per pot with increasing light.

High moisture stress also significantly increased sodium throughout the plant. However, in contrast to light, this effect can be accounted for by a marked reduction in yield. Total yields for the high moisture stress conditions were significantly lower (P = 0.001), 5.09 grams dry matter per pot, than for the low moisture stress plants which yielded 6.29 trams per pot.

Temperature on the other hand had no significant effect on sodium concentration.

		Sodium (percent dry matter)		
Treatment		Shoots	Stubble	Roots
Light	High	0.42***	0.61***	0.23**
	Low	0.25	0.43	0.18
Moisture stress	High	0.38***	0.55***	0.23**
	Low	0.29	0.49	0.18
Temperature	High	0.33 N.S.	0.51 N.S.	0.23 N.S.
	Low	0.34	0.53	0.21

*** Sign. diff. P = 0.001
** " " P = 0.01
N.S. Not sign. diff.

Table 27: Effects of light, moisture stress, and temperature on sodium concentration in perennial ryegrass.

(b) White clover

Although the sodium content in white clover responded to the various combinations of light, temperature, and moisture stress, in a similar manner to that found for ryegrass, the degree of change was somewhat less in this species (Table 28).

Treatments			Sodium (percent dry matter)		
Light	Temp.	Moisture stress	Shoots	Stubble	Roots
H	H	H	0.28	0.24	0.30
H	H	L	0.24	0.27	0.29
H	L	H	0.36	0.39	0.45
H	L	L	0.35	0.35	0.41
L	H	H	0.30	0.27	0.25
L	H	L	0.30	0.21	0.29
L	L	H	0.22	0.21	0.27
L	L	L	0.21	0.17	0.27
H = High L = Low		SE	±0.02	±0.02	±0.02

Table 28: Mean sodium concentration in white clover as affected by combinations of light, temperature, and moisture stress.

The results of the main effects of the three treatments are presented in Table 29.

As observed in ryegrass, light intensity significantly ($P = 0.001$) increased sodium in all tissues of the plant. However, increased moisture stress only slightly ($P = 0.05$) increased sodium in both shoots and stubble with no change in root sodium.

In spite of the highly significant ($P = 0.001$) decrease in sodium in the roots with increasing temperature, it did not significantly affect sodium in other parts of the plant.

Treatment		Sodium (percent dry matter) Shoots	Stubble	Roots
Light	High	0.31***	0.31***	0.36***
	Low	0.26	0.22	0.27
Moisture stress	High	0.29*	0.28*	0.32 N.S.
	Low	0.28	0.25	0.32
Temperature	High	0.28 N.S.	0.25 N.S.	0.28***
	Low	0.29	0.28	0.35

*** Sign. diff.	P = 0.001
* " "	P = 0.05
N.S.	Not sign. diff.

Table 29: Effects of light, moisture stress, and temperature on sodium concentration in white clover.

Dry matter yields were adversely affected (P = 0.001) by both high temperature and moisture stress, whereas, increased light intensity greatly (P = 0.001) increased yields.

(c) Lucerne

As expected, the major changes in sodium concentration in lucerne, in response to light, temperature, and moisture stress, occurred in the roots. Moreover, in contrast to the natrophilic ryegrass and white clover, there was no particular combination of factors which increased or decreased sodium consistently throughout the plant (see Table 30 for treatment means).

Treatments			Sodium (percent dry wt)		
Light	Temp.	Moisture stress	Shoots	Stubble	Roots
H	H	H	0.09	0.12	0.49
H	H	L	0.08	0.12	0.30
H	L	H	0.09	0.26	0.76
H	L	L	0.07	0.24	0.63
L	H	H	0.12	0.20	0.25
L	H	L	0.10	0.22	0.20
L	L	H	0.10	0.16	0.28
L	L	L	0.06	0.17	0.27
H = High	L = Low		±0.02	±0.03	±0.07

Table 30: Mean sodium concentrations in lucerne as affected by combinations of light, temperature, and moisture stress.

Contrary to the results for natrophiles, increased light intensity influenced the sodium content only in the roots, where there was a marked ($P = 0.001$) increase (Table 31).

Treatment		Sodium (percent dry matter) Shoots	Stubble	Roots
Light	High	0.08 N.S.	0.19 N.S.	0.55***
	Low	0.10	0.19	0.25
Moisture stress	High	0.10**	0.19 N.S.	0.45***
	Low	0.08	0.19	0.35
Temperature	High	0.10*	0.17**	0.31***
	Low	0.08	0.21	0.49

*** Sign. diff. $P = 0.001$
** " " $P = 0.01$
* " " $P = 0.05$
N.S. Not sign. diff.

Table 31: Effects of light, moisture stress, and temperature, on sodium concentration in lucerne.

As a result of a decrease in dry matter yield, sodium accumulated in shoots and roots of plants growing under a high moisture stress condition. In addition, high temperature enhanced the translocation of sodium from the roots, and to a lesser extent the stubble, into the shoot tissue of lucerne.

An additional feature, common to all three test plants, was the effect of light on shoot nitrogen, phosphorus, and potassium. An examination of the results presented in Table 32 reveals that an increase in the light intensity resulted in significant increases in all three elements.

	Mean percentage of shoot dry matter					
	Nitrogen		Phosphorus		Potassium	
Plant Species	LL	HL	LL	HL	LL	HL
Ryegrass	3.90	4.00*	0.26	0.43***	2.59	2.93***
White clover	3.60	4.22***	0.25	0.30***	2.18	2.40***
Lucerne	3.81	4.53***	0.27	0.35***	2.36	2.88***

LL = Low light HL = High light

*** Sign. diff. P = 0.001

* " " P = 0.05

Table 32: Effect of light on the concentrations of nitrogen, phosphorus, and potassium in shoot tissue.

DISCUSSION

There is little doubt that of the three factors investigated, light intensity had the greatest influence on sodium concentrations in both natrophiles and natrophobes, especially in areas of high sodium accumulation. Sodium concentration also increased in plants growing under conditions of high moisture stress, however, this effect can be accounted for by the marked decrease in plant yields, as measured for example in this experiment.

Sodium on the other hand was less sensitive to temperature changes, except in lucerne where there was a slight enhancement of the translocation of this element from roots to shoots.

It is interesting to note that there have been a number of observations to the effect that changes in sodium concentration can influence succulence in plants (see Jennings, 1968). Moreover, increased light intensity and aridity can also cause an increase in plant succulence (Watson, 1942). As a possible explanation to the above phenomena, Jennings (1968) proposed that succulence is controlled by changes in sodium concentration within the plant through the action of light and aridity on ATP synthesis. How these changes in sodium concentration directly influenced plant succulence were not revealed.

The level of nitrogen in plants has long been known to cause changes in tissue succulence (see Russel, 1973; Bailey, 1973). Moreover, the results from this experiment reveal that changes in the concentration of both nitrogen and phosphorus, brought about by light intensity, were identical to those for sodium. In addition, both phosphorus and sodium (see Chapter 4 for the role of sodium in nitrogen metabolism) are implicated in nitrogen metabolism. Therefore, these results suggest that changes in plant succulence, resulting from alterations in sodium concentration, are most likely caused by fundamental changes in nitrogen metabolism within the plant.

EXPERIMENT 2: EFFECT OF HIGH SUBSTRATE POTASSIUM

It is well documented (see Literature Review) that an increase in potassium depresses sodium in leaf tissue of both natrophiles and natrophobes. However, the extent to which potassium depresses sodium in other sites within the plant, especially in natrophobes, is unknown. Therefore, the influence of potassium on the main sites of sodium accumulation along with the effect on other major nutrient elements was investigated in a wide range of plant species.

EXPERIMENTAL TECHNIQUE

See Chapter 2 for details.

RESULTS

The results from the two potassium treatments are presented according to the classification of natrophiles and natrophobes given in Table 10.

Sodium

Increased potassium significantly ($P = 0.001$) depressed sodium in shoot tissue of both natrophiles and natrophobes. However, an examination of the results presented in Table 33 indicates that the most pronounced effect of potassium on sodium tended to be centred on the main sites of sodium accumulation. It is also interesting to note that with the exception of the natrophobes in which the root system is the main site of sodium accumulation, sodium increased significantly ($p = 0.001$) in the roots as a result of additional potassium.

Plant Type	Main site of sodium accumulation	Tissue	Sodium (percent dry matter)	
			Low K	High K *
Natrophiles	Leaf	Shoots	0.38	0.34
		Stubble	0.35	0.33
		Roots	0.16	0.18
	Stubble	Shoots	0.38	0.35
		Stubble	0.52	0.48
		Roots	0.28	0.30
Natrophobes	Stubble	Shoots	0.15	0.11
		Stubble	0.38	0.34
		Roots	0.21	0.24
	Roots	Shoots	0.06	0.05
		Stubble	0.16	0.16 N.S.
		Roots	0.40	0.36

* All high potassium values, except those marked N.S., are significantly different from the low treatment. (Shoots $P = 0.001$; Stubble $P = 0.01$; Roots $P = 0.001$)

Table 33: Effect of potassium on sodium concentration in natrophiles and natrophobes.

Nitrogen

dditional potassium, significantly ($P=0.001$) decreased the nitrogen content in leaf tissue of all plants (Table 34). Moreover, the nitrogen concentrations in leaves of natrophobes (pooled mean of 4.44 percent of dry matter) were much lower than their natrophilic counterparts (pooled mean of 4.72 percent dry matter).

Plant Type	Main site of sodium accumulation	Tissue	Nitrogen (percent dry matter)	
			Low K	High K
Natrophiles	Leaf	Shoots	4.73	4.55
		Stubble	3.02	2.97
		Roots	2.20	2.10
	Stubble	Shoots	4.82	4.79
		Stubble	2.99	3.01
		Roots	2.49	2.40
Natrophobes	Stubble	Shoots	4.37	4.31
		Stubble	2.42	2.62
		Roots	2.24	2.25
	Roots	Shoots	4.57	4.50
		Stubble	2.82	2.94
		Roots	2.08	1.95

* All high potassium values are significantly different from the low treatment (Shoots $P = 0.001$; Stubble $P = 0.001$; Roots $P = 0.05$.)

Table 34: Effect of potassium on nitrogen concentration in natrophiles and natrophobes.

Although there were no consistent trends in the nitrogen content of stubble and roots, in general, additional potassium tended to increase the nitrogen content of the stubble while decreasing it in the roots.

Another important point is the close relationship between sodium and nitrogen levels in the tissues of both natrophiles and natrophobes, as revealed by an examination of Tables 33 and 34. In fact, the results from a correlation matrix, calculated from the pooled means of the two potassium treatments for the individual tissues and presented in Table 35, clearly indicate a highly significant positive correlation ($P = 0.001$) between the level of sodium in shoots, stubble, and roots, and the corresponding nitrogen content in these tissues.

	Tissue	Sodium Shoots	Stubble	Roots
Nitrogen	Shoots	0.230	-	-
	Stubble	-	0.346	-
	Roots	-	-	0.291

Critical value of r for P = 0.001 = 0.173

Table 35: A correlation matrix between the level of sodium and corresponding nitrogen content in various plant tissues.

In other words, any increase or decrease in the sodium level within the plant is accompanied by a corresponding change in the relative level of nitrogen and vice versa.

Potassium

As expected, the potassium levels increased throughout the plant as a result of additional amounts of this element in the nutrient solution. However, there was a relatively larger change in the potassium concentration in the leaf tissue than in the other tissues. In addition, the potassium concentrations in the natrophobes, like nitrogen, were much lower than those recorded for natrophiles (Table 36).

Plant Type	Main site of sodium accumulation	Tissue	Potassium (percent dry matter) Low K	Potassium (percent dry matter) High K*
Natrophiles	Leaf	Shoots	2.30	2.88
		Stubble	1.11	1.52
		Roots	0.32	0.41
	Stubble	Shoots	2.55	3.12
		Stubble	1.64	2.19
		Roots	0.40	0.60
Natrophobes	Stubble	Shoots	2.25	2.86
		Stubble	1.09	1.66
		Roots	0.34	0.47
	Roots	Shoots	2.07	2.50
		Stubble	1.09	1.53
		Roots	0.57	0.78

* All high potassium values are significantly ($P = 0.001$) different from the low treatment.

Table 36: Distribution of potassium in natrophiles and natrophobes.

The effect of extra potassium on total plant yields over the 90 day growth period was minimal, in fact, in most species there was only a slight increase in dry matter production as indicated by the following pooled means; low potassium 25.0 grams dry matter per pot (S.E. ±0.45), high potassium 26.8 grams dry matter per pot (S.E. ±0.48). The pooled means were calculated from the results of all the individual species.

Transpiration Rates

Additional potassium significantly reduced the transpiration rate in both natrophiles and natrophobes (Table 37).

Plant Type	Transpiration Rate* (ml H_2O/gram dry weight/day/pot) Low K	 High K
Natrophiles	1448	1334
Natrophobes	731	701

* All values are significantly different from each other ($P = 0.001$).

Table 37: Effect of potassium on transpiration rates in natrophiles and natrophobes.

A discussion concerning the pronounced differences in transpiration rates between natrophiles and natrophobes is given in Chapter 2.

DISCUSSION

The most notable feature recorded in this experiment was the significant relationship between sodium and nitrogen in both natrophiles and natrophobes. Furthermore, an increase in the level of potassium above that normally associated with satisfactory growth resulted in a reduction, not only of the concentration of sodium in the aerial parts of plants but also in the nitrogen content.

In his book "Soil Conditions and Plant Growth", Russell (1973), when discussing the functions of potassium with respect to nitrogen, makes the following statement: "Thus potassium acts as a corrective to the harmful effects of nitrogen, ...". It may be deduced from the above statement, in light of the results presented in this experiment, that this "corrective effect" of potassium is brought about by a reduction in the overall sodium and nitrogen levels within the plant.

Furthermore, in relation to the assumption that succulence in plants is related to the levels of nitrogen and sodium (see Chapter 3: Experiment 1), Goodall et al. (1957) reported that potassium deficient lettuce plants were more succulent than plants with adequate amounts of potassium. In addition, tropical grasses, which have much lower sodium and nitrogen contents than their temperate counterparts (see Chapter 2) contain less water within their tissues (Bailey, 1973).

A further feature of this experiment was the observed reduction in the transpiration rate due to an increase in applied potassium. It is relevant to note that the addition of sodium chloride to cotton plants (Gossypium hirsutum) resulted in an increase in the rate of transpiration (Boyer 1965). Moreover, in some plants such as Commelina communis, sodium is reported to be more effective in keeping the stomata of the leaves open than corresponding amounts of potassium (Willmer and Mansfield, 1970). It is also known that with an increase in the rate of transpiration there is a decrease on the part of the root to exert a selective effect on the ratio of potassium to sodium reaching the shoot (Pitman, 1965, 1966; Jennings, 1967). In other words, as the rate of transpiration increases the ratio of potassium to sodium reaching the shoot changes in favour of sodium. This would explain the decrease in sodium content in aerial tissue and the corresponding accumulation of this element in the root systems observed in most of the plants receiving additional potassium.

However, in the natrophobes in which the main site of sodium accumulation is in the root, additional potassium not only decreased sodium in the aerial tissues but also caused a reduction of sodium in the roots. This overall decrease could be due to the operation of a potassium stimulated sodium efflux pump in the root tissue as suggested by Pitman and Saddler (1967) and confirmed by Jeschke (1972).

EXPERIMENT 3: EFFECT OF HIGH SUBSTRATE SODIUM

Although sodium is not normally translocated into the leaf tissue of natrophobes in any quantity, it appears that under conditions of high external concentrations of sodium the main sites of accumulation become saturated such that sodium slowly accumulates in the leaf tissue (see Literature Review).

In order to investigate this phenomenon further, a detailed examination was made into the effects of high concentrations of sodium in the root zone on the yield and chemical composition of three plant species.

EXPERIMENTAL TECHNIQUE

The basic glasshouse pot technique employed in this study is described in Chapter 2.

(a) Growth Medium and Test Plants

Plants were grown in 15 centimetre plastic pots containing one kilogram of acid (hydrochloric) washed river sand. The particle size of the sand was between 0.2–0.7 millimetres.

The three plant species investigated are listed below according to the main sites of sodium accumulation.

Main site of sodium accumulation	Plant species*
Leaf	"Grasslands Ariki" ryegrass
Stubble	Timothy
Root	"Wairau" lucerne

* Botanical names are given in Table 16.

(b) Design and Treatments

Five levels of sodium were imposed on the three test plants. The pots were arranged in six randomised blocks per plant species on benches within a temperature controlled glasshouse (15 °C min – 25 °C max).

(c) Nutrient Solutions and Moisture Stress

The composition of the dilute complete major element solution added to the test plants is given in Table 38.

	Milliequivalents/litre							
Sodium level	N	P	S	K	Mg	Ca	Na	Cl
1	18.3	1.8	1.4	2.6	1.1	2.4	0.9	0.9
2	"	"	"	"	"	"	3.6	3.6
3	"	"	"	"	"	"	6.2	6.2
4	"	"	"	"	"	"	9.0	9.0
5	"	"	"	"	"	"	11.6	11.6

Table 38: Composition of the dilute major element solution used in the sodium sand experiment.

Details of the preparation of the dilute complete nutrient solution from separate concentrated stock solutions for major and minor elements are given in Chapter 2.

Nutrients were supplied three times a week, and the moisture level in each pot maintained at 75 percent of field capacity.

(d) Measurements

The cutting interval and methods for harvesting and chemically analysing the plants are described in detail in Chapter 2.

RESULTS

Details of the effects of high sodium concentration in the root zone on the accumulation of this ion in various tissues from the test plants are presented in Table 39.

As expected, sodium readily accumulated in the aerial parts of the natrophilic perennial ryegrass at all levels. However, no substantial accumulation of sodium occurred in the roots of this plant at any level. Although sodium increased in the shoots of both natrophobes at the higher levels of sodium, it was not until after the second cut that any appreciable quantities of sodium were translocated into the leaves. This slow initial movement into the shoots at these high levels suggests that there is a progressive saturation of the main accumulation sites. Once these sites reached a certain level, sodium readily moved into the leaves. This is clearly recorded in the third and fourth cuts. However, the degree of saturation at the end of the experiment was such that it was still possible to distinguish the main sites of accumulation even at the highest level of sodium chloride. It is also important to point out that in order to produce a comparable sodium concentration in the shoots of lucerne and timothy (refer to the mean of four cuts) to that found in the natrophilic ryegrass for the lowest sodium treatment, it was necessary to apply a nutrient solution containing approximately ten times the concentration of sodium.

Sodium (percent dry matter)

Perennial Ryegrass

		Shoots				Shoot		
Treatment		Cut 1	2	3	4	Mean	Stubble	Roots
Sodium chloride	0.9*	0.30	0.15	0.20	0.23	0.22	0.25	0.07
	3.6	0.44	0.33	0.43	0.45	0.41	0.41	0.07
	6.2	0.63	0.54	0.79	0.67	0.66	0.54	0.08
	9.0	0.76	0.80	1.08	1.13	0.94	0.62	0.08
	11.6	0.98	1.20	1.74	1.82	1.44	0.64	0.07
Standard error				±0.03			±0.03	±0.01

Timothy

Sodium chloride	0.9	0.04	0.04	0.07	0.03	0.05	0.10	0.12
	3.6	0.04	0.05	0.07	0.09	0.06	0.28	0.17
	6.2	0.05	0.09	0.28	0.27	0.17	0.56	0.24
	9.0	0.07	0.18	0.46	0.48	0.30	0.80	0.27
	11.6	0.09	0.21	0.54	0.91	0.44	0.84	0.26
Standard error				±0.01			±0.03	±0.03

Lucerne

Sodium chloride	0.9	0.01	0.03	0.00	0.01	0.01	0.06	0.09
	3.6	0.04	0.06	0.05	0.09	0.06	0.29	0.34
	6.2	0.07	0.12	0.12	0.20	0.13	0.61	0.63
	9.0	0.15	0.23	0.30	0.50	0.30	0.80	1.11
	11.6	0.18	0.27	0.49	0.90	0.46	0.97	1.37
Standard error				±0.01			±0.02	±0.02

* Milliequivalents per litre in dilute nutrient solution added to plants.

Table 39: Effects of increasing sodium chloride in the root zone on the accumulation of sodium in various tissues of three different test plants.

Perennial Ryegrass

Treatment		(grams dry matter/pot) Shoots[+]	 Stubble	 Roots
Sodium chloride	0.9[*]	9.65	2.22	4.28
	3.6	9.74	2.26	5.52
	6.2	9.91	2.24	4.20
	9.0	9.57	2.05	4.92
	11.6	8.42	1.58	3.43
Standard error		±0.61	±0.23	±0.76

Timothy

Sodium chloride	0.9	10.32	1.64	4.14
	3.6	10.11	1.43	4.44
	6.2	9.67	1.26	3.59
	9.0	8.43	0.81	2.62
	11.6	4.96	0.58	1.20
Standard error		±0.45	±0.15	±0.54

Lucerne

Sodium chloride	0.9	8.85	2.27	4.59
	3.6	8.92	2.53	4.73
	6.2	9.02	2.35	4.66
	9.0	8.24	2.23	4.05
	11.6	5.92	1.41	2.27
Standard error		±0.77	±0.19	±0.32

[+] Total for four cuts

[*] Milliequivalents per litre in dilute nutrient solution added to plants.

Table 40: Effects of increasing sodium chloride in the root zone on dry matter yields of perennial ryegrass, timothy, and lucerne.

Perennial Ryegrass

Treatment		Shoots		Stubble		Roots	
		percent N	percent K	percent N	percent K	percent N	percent K
Sodium chloride	0.9*	4.11	2.06	2.16	1.71	1.20	0.20
	3.6	4.12	1.64	1.97	0.68	0.91	0.16
	6.2	4.16	1.28	2.27	0.54	1.06	0.18
	9.0	4.21	0.87	2.43	0.40	1.11	0.13
	11.6	4.33	0.49	2.79	0.30	1.10	0.08
Standard error		±0.14	±0.05	±0.15	±0.11	±0.18	±0.02

Timothy

Treatment		Shoots		Stubble		Roots	
		percent N	percent K	percent N	percent K	percent N	percent K
Sodium chloride	0.9	4.14	2.18	2.00	1.26	0.73	0.20
	3.6	4.16	1.80	1.84	1.01	1.01	0.15
	6.2	4.42	1.40	2.01	0.68	1.06	0.14
	9.0	4.73	1.07	2.74	0.51	1.32	0.14
	11.6	5.08	0.77	3.15	0.45	1.37	0.11
Standard error		±0.14	±0.07	±0.16	±0.08	±0.14	±0.03

Lucerne

Treatment		Shoots		Stubble		Roots	
		percent N	percent K	percent N	percent K	percent N	percent K
Sodium chloride	0.9	3.54	2.56	1.57	2.28	2.35	1.17
	3.6	3.67	2.28	1.90	1.63	2.07	0.77
	6.2	3.87	1.94	1.96	1.37	2.44	0.52
	9.0	4.13	1.53	1.95	0.76	2.43	0.31
	11.6	4.44	1.13	2.63	0.36	2.13	0.20
Standard error		±0.11	±0.08	±0.16	±0.08	±0.14	±0.06

* Milliequivalents per litre in dilute nutrient solution added to plants.

Table 41: Influence of sodium chloride on the concentration of nitrogen and potassium in perennial ryegrass, timothy and lucerne.

Dry matter yields for both ryegrass and lucerne were only adversely affected by the highest sodium treatment. On the other hand, for timothy, which appears to be less tolerant of high levels of sodium chloride, yields decreased progressively at all cuts as the concentration of this salt increased in the nutrient solution (Table 40).

The pronounced effects of sodium on nitrogen and potassium composition of the test plants are clearly shown in Table 41. The shoot values presented in this table are the means of four cuts. Without exception, sodium chloride increased the nitrogen concentration in the shoots at all levels. Potassium, on the other hand, was severely depressed in all tissues as a result of increased sodium. Although not shown, there were no significant effects on the other nutrient elements.

DISCUSSION

The results confirm earlier observations of Huffaker and Wallace (1959a; 1959b) and Jacoby (1964; 1965) that in order to increase substantially the translocation of sodium into leaf tissue of natrophobes, very high concentrations of this element are required in the root zone.

Because of the location of the main accumulation sites and the lower rate of uptake recorded for natrophobes (see Chapter 2), considerable differences still existed between natrophiles and natrophobes in the level of sodium in the shoots even at high external concentrations.

Although sodium severely depressed the uptake of potassium such that the concentrations in the shoots were well below the level considered by McNaught (1970) to be required for satisfactory growth in all but the lowest sodium treatment, dry matter yields for both ryegrass and lucerne were not adversely affected until the concentrations in the nutrient solutions were very high. Therefore, it must be concluded that sodium was maintaining plant growth by substituting for potassium in a number of metabolic processes. However, once the level of potassium fell below an absolute minimum, which varied according to plant species, plant yields were affected. This beneficial effect of sodium on plants growing under conditions of marginal potassium deficiency has been recorded in numerous experiments (see Literature Review).

Timothy, on the other hand, belongs to the group of plants in which the addition of sodium under conditions of potassium deficiency has no beneficial effect.

One possible cause of the detrimental effect of sodium on yields of salt sensitive plants, apart from that due to the depression of potassium uptake, is that observed by Marschner and Mix (1973). They found that if leaves of the natrophobic bean plant (Phaseolus vulgaris) were exposed to high concentrations of sodium for any length of time, pronounced swellings occurred in the fine structure of the chloroplasts. Moreover, there was a strong efflux of potassium from these leaves. However, it is unlikely that a similar effect could have contributed to any extent to the decline in the yields of the two natrophobes in this experiment. In both plants the concentration of sodium in the leaves did not increase to any high level until after the second cut, by which time the yields were noticeably depressed. In fact for timothy, yields had declined even at the lower sodium treatments.

Considering the significant relationship between sodium and nitrogen, it is more likely that the decline in plant yields at high concentrations of sodium is related to some aspect of nitrogen metabolism.

The most significant biochemical change which takes place in plants growing under conditions of high sodium in relation to nitrogen metabolism, is the accumulation of intermediaries of protein synthesis, in particular amino acids (Strongonov, 1962; Pluenneke and Joham, 1972). A similar accumulation of amino compounds takes place under conditions of water stress (Barnett and Naylor, 1966); a condition, incidentally, which also significantly increases the sodium concentration within the plant (see Chapter 3: Experiment 1).

As both potassium and magnesium ions are essential for the incorporation of amino acids into proteins (Lubin 1963; Lubin and Ennis, 1964; Evans and Sorger, 1966; Boulter, 1970; Wilson, 1971) it is therefore not unexpected to find these non protein compounds, in particular the diamine putrescine, accumulating in the shoot tissue of plants suffering from such deficiencies (Richards and Coleman, 1952; Murty, *et al.*, 1971; Basso and Smith, 1974). Moreover, where sodium has been substituted for potassium in enzyme studies, the rate of amino acid incorporation into protein is markedly reduced (Lubin and Ennis, 1964; Ben-Zioni *et al.*, 1967; Kahane and Poljakoff-Mayber, 1968).

Considering the specific requirements of potassium, it is not surprising that small additions of this element to high sodium plants enhances the incorporation of labelled soluble amino compounds into protein, thereby reducing the free amino pool (Helal *et al.*, 1975).

Because the concentration of magnesium in plants is either not affected by sodium, as recorded in this experiment, or can in fact be enhanced (Whitehead and Jones, 1972), this ion is not considered to play any significant part in the accumulation of amino compounds under conditions of high external sodium.

Therefore, the detrimental effect of sodium on plant growth at high concentrations is not a direct effect, but rather the result of an excessive accumulation of intermediaries of protein synthesis caused by an induced potassium deficiency.

CHAPTER 4

THE ROLE OF SODIUM IN NITROGEN METABOLISM, ESPECIALLY IN RELATION TO NITRATE REDUCTION

INTRODUCTION

In discussing the functions and requirements of sodium in plants (see Review of Literature) a number of references suggest that sodium may be implicated in certain aspects of nitrogen metabolism (Kessler and Snir, 1969; Chatterton *et al.*, 1971; Pluenneke and Joham, 1972; Langdale *et al.*, 1973; Helal *et al.*, 1975). In the experiments concerned where various fractions of total nitrogen had been measured, reduced forms of nitrogen were found to be promoted by increases in the concentration of applied sodium. Moreover, Sankhla and Huber (1975) have observed that sodium chloride markedly stimulated the *in vivo* activity of nitrate reductase in leaves of *Phaseolus aconitifolius* seedlings. A similar observation was made by Marquez and Brodie (1973) using an extreme halophilic bacterium *Halobacterium salinorium*. They found that no nitrate reductase activity occurred in the absence of sodium chloride.

In general, plants can be broadly grouped into those in which the root is the major site where incoming nitrate is reduced and those where the reduction process is mainly confined to leaf tissue (Wallace and Pate, 1967). However, between these two extremes there is a wide spectrum of plants which show varying degrees in their ability to reduce nitrate within their roots and shoots (Bollard, 1957; 1960; Pate, 1973). In fact it is significant that of the major cations present in the plant, sodium is the only element which preferentially accumulates in different sites throughout the plant according to plant species.

Therefore, to investigate the possibility of a link between sodium and nitrate reduction within the plant, the following three experiments were undertaken:-

Experiment 1) Distribution of nitrate reductase activity in a selection of natrophilic and natrophobic plants.

Experiment 2) Influence of sodium chloride on nitrate reductase activity in perennial ryegrass and timothy.

Experiment 3) The effects of sodium and potassium on the induction of nitrate reductase activity in a selection of natrophiles and natrophobes.

(1) EXPERIMENTAL TECHNIQUE

Although the basic experimental procedures are described in Chapter 2, the additional techniques employed in the three experiments are as follows:-

(i) EXPERIMENT 1

(a) Growth Medium and Test Plants

The growth medium was the same as that described in Chapter 3: Experiment 3.

The ten test plants investigated are listed in Table 42 according to the main sites of sodium accumulation.

Natrophiles*	Natrophobes*	
Leaves	Stubble	Roots
'Ariki' ryegrass	Alsike clover	Lucerne
Chou moellier	Timothy	Maize
Cocksfoot	Rape	Sudax
'Huia' white clover		

* Botanical names for the above plants are given in Table 16.

Table 42: Plants used to investigate the distribution of nitrate reductase activity in natrophiles and natrophobes.

(b) Design

Three pots of each test plant were arranged in randomised blocks on benches within a temperature controlled glasshouse (15 °C min - 25 °C max).

(c) Nutrient Solution and Moisture Level

The composition of the dilute major element solution used for all plants is given in Table 43.

	N	P	S	K	Na	Mg	Ca	Cl
Milliequivalents/litre	18.3	1.8	1.4	2.6	0.9	1.1	2.4	0.9

Table 43: Composition of dilute major element nutrient solution applied to the test plants in all sand experiments.

Details of the preparation of the dilute nutrient solution from separate concentrated stock solutions for major and minor elements, rate of nutrient addition, and moisture level maintained in each pot, are given in Chapter 2.

(d) Harvesting

The plants were harvested 21 days after seed germination and dissected into shoot, stubble, and root tissue according to the description given in Chapter 2. A further explanation of the standardisation of assay samples is given in the results.

(e) Determination of Nitrate Reductase Activity

A modification of the intact tissue assay method of Jaworski (1971) was used. Because of the diurnal activity of this enzyme, as indicated by Thibodeau and Jaworski (1975), all tissues were sampled at 11 am.

Approximately two grams of finely chopped fresh plant tissue was prepared from each replicate. The finely chopped material was thoroughly mixed and two 500 milligram wet weight sub-samples were taken. Each sub-sample was placed in a 25 millilitre screw cap vial containing five millilitres of the following potassium incubation medium:-

Potassium Incubation Medium

0.1 M potassium phosphate buffer pH 7.5

0.1 M potassium nitrate

5% n-propanol

2 drops chloramphenicol (0.5 μg/ml) as
an antiseptic

The vials were sealed and kept in the dark at 28 °C for two hours. Nitrite released into the medium after the two hour incubation period was determined by treating two millilitre aliquots with five millilitres of the following solution:-

0.001% N-1-napthylethenediamine d-hydrochloride

0.06% Sulfanilamide in 0.2 N HCl

The reaction mixture was diluted if necessary and its optical density measured at 540 nm after 20 minutes.

Blank incubations minus tissue were run identically.

(f) Expression of Results

Nitrate reductase activity is expressed in μmoles NO_2 per gram fresh weight per hour.

(ii) EXPERIMENT 2

The plant material assayed for enzymatic activity in this experiment was derived from the two test plants ryegrass and timothy used in Chapter 3: Experiment 3. Except for a description of the time of harvest and the determination of nitrate, all experimental procedures including a description of the assay samples are given elsewhere.

(a) Harvesting

Fresh tissue was dissected from the test plants at the time of the fourth cut and immediately assayed for enzymatic activity.

(b) Determination of Nitrate Nitrogen

Nitrate content was determined in shoots (tissue from the fourth cut), stubble, and roots, according to a modification of the automated method described by Kamphake et al. (1967).

(iii) EXPERIMENT 3

Except for the sodium assay medium, all experimental details and plants used to assay for enzymatic activity are described in Experiment 1 of this Chapter.

(a) Sodium Incubation Medium

0.1 M sodium phosphate buffer pH 7.5

0.1 M sodium nitrate

5% n-propanol

2 drops chloramphenicol (0.5 μg/ml) as an antiseptic.

(2) RESULTS

In order to standardise on the selection of samples for assay, a preliminary experiment was undertaken. In this experiment nitrate reductase activity and corresponding molybdenum concentrations were measured in various segments of leaf, stubble, and root tissue. The segments were selected from young maize plants harvested 21 days after seed germination. Details of the dissected segments are as follows:-

Basic tissue	Segment	Comments
Leaf	tip	0-5 centimetre segment from leaf tip
	base	remaining leaf tissue
	whole	complete tissue
Stubble	upper	0-5 centimetre segment immediately below leaf
	lower	remaining stubble tissue
	whole	complete tissue
Roots	apical zone	0-5 centimetre root tip segment
	mature zone	remaining root tissue
	whole	complete tissue

The results from this investigation, displayed in Table 44, demonstrate the considerable differences in nitrate reductase activity according to the basic type of tissue selected and also according to the position from within these tissues that the assay samples were taken.

In addition, the concentration of molybdenum is to a large extent a measure of the degree of activity of this enzyme in the various tissue segments.

Tissue	Segment*	Nitrate reductase activity (μmoles NO_2/g fresh wt/hr)	Molybdenum ppm +
	tip	2.18±0.16	0.21
Leaf	base	1.96±0.18	0.25
	whole	1.98±0.13	0.31
	upper	1.09±0.18	0.17
Stubble	lower	0.84±0.11	0.20
	whole	0.88±0.02	0.12
	apical	2.34±0.10	0.44
Root	mature	0.84±0.11	0.32
	whole	0.79±0.08	0.39

* see text for explanation + stand error ±0.05

Table 44: Distribution of nitrate reductase activity and molybdenum concentration in various tissue segments from young maize plants.

Because the variation in nitrate reductase activity was relatively small within the various segments of tissue taken from leaves and stubble, no preferential selection of assay samples was considered necessary for these two tissues. Therefore, for the following investigations, enzyme activity was measured using the leaf and stubble tissue dissected according to the description given in Chapter 2. On the other hand, because the measured level of nitrate reductase activity in the root was critically dependent on the position from which the assay sample was taken, it was decided to standardise on the segment of tissue with the highest enzyme activity for all subsequent experiments. In addition to being the area of highest nitrate reductase activity the apical region of the root is also one of the major zones of ion uptake (Loneragen, 1973) as well as being an area of high metabolic activity (Machlis, 1944). Therefore, it was concluded that any metabolic changes occurring in the plant which could affect nitrate reductase activity would tend to be reflected more readily in the root tip than in the remaining root tissue.

Results from the individual experiments outlined in the introduction to this chapter are presented separately as follows:-

(i) EXPERIMENT 1

Distribution of nitrate reductase activity in a selection of natrophilic and natrophobic plants.

Results

Detailed results of the distribution of nitrate reductase activity in a selection of natrophilic and natrophobic plants are given in Table 39. The results are arranged in the table according to the main sites of sodium accumulation.

Although there were considerable differences in the degree of enzymatic activity between plant species, the results in Table 45 indicate that within the individual plants, nitrate reductase activity was most pronounced in the sites where there is a preferential accumulation of sodium. As a definite example, a more detailed examination of data for maize roots (Table 44) showed that the concentration of sodium and nitrate reductase activity were both highest in the root apex (Table 46).

Plant Species	Nitrate Reductase Activity (μmoles NO_2/g fresh wt/hr) Shoot	 Stubble	 Root
Leaf* Natrophiles			
Chou moellier	15.69±0.40	10.03±0.77	10.84±0.75
White clover	5.43±0.14	4.51±0.54	1.14±0.41
Cocksfoot	3.53±0.15	1.73±0.30	1.51±0.11
Perennial ryegrass	2.91±0.17	1.48±0.16	0.87±0.02
Stubble* Natrophobes			
Rape	8.86±0.54	10.08±0.11	9.75±0.63
Alsike clover	5.16±0.81	4.78±0.62	0.81±0.16
Timothy	2.08±0.11	2.64±0.30	1.68±0.41
Root* Natrophobes			
Maize	1.98±0.13	0.88±0.11	2.34±0.10
Lucerne	1.25±0.11	7.21±0.13	8.56±0.22
Sudax	1.12±0.05	0.60±0.05	1.42±0.10

* Refers to the main site of sodium accumulation within the plant (see Chapter 2).

Table 45: Distribution of nitrate reductase activity in a selection of natrophiles and natrophobes.

Root Segment	Nitrate reductase activity* (μmoles NO_2/g fresh wt/hr)	Sodium (percent dry matter)
Apical zone	2.34±0.10	0.31±0.02
Mature zone	0.84±0.11	0.18±0.01
Whole root	0.79±0.08	0.22±0.02

* Taken from Table 44.

Table 46: Variation in nitrate reductase activity and corresponding sodium concentrations in maize root segments.

Of the individual plant species studied, the two brassicas (chou moellier and rape), which are both temperate plants, were found to have by far the highest enzymatic activity; by contrast the lowest enzymatic activities were measured in the two tropical plants, maize and sudax.

(ii) EXPERIMENT 2

Influence of sodium chloride on nitrate reductase activity in perennial ryegrass and timothy.

Results

The pronounced effects of increasing concentrations of sodium chloride on nitrate reductase activity in various tissues of perennial ryegrass and timothy are displayed in Table 47.

Sodium chloride markedly increased nitrate reductase activity in both test plants, particularly in the tissues where sodium accumulated. In natrophilic ryegrass these changes were largely confined to the shoot tissue with only small differences occurring in the stubble and roots. Although changes in nitrate reductase activity were very marked in the stubble of timothy, it was also prominent in the shoot tissue at the higher concentrations of sodium.

Nitrate Reductase Activity
(μmoles NO_2/g fresh wt/hr)

Perennial Ryegrass

Treatment		Shoot	Stubble	Root
Sodium chloride	0.9*	2.10±0.05	1.60±0.07	0.76±0.06
	3.6	2.48±0.07	2.14±0.17	0.56±0.02
	6.2	2.93±0.21	1.98±0.03	0.64±0.06
	9.0	3.48±0.29	2.09±0.20	0.84±0.06
	11.6	4.36±0.08	1.86±0.05	0.80±0.06

Timothy

Sodium chloride	0.9*	1.29±0.15	1.68±0.09	1.13±0.16
	3.6	2.30±0.08	2.64±0.54	1.85±0.25
	6.2	2.49±0.05	2.81±0.18	1.32±0.23
	9.0	2.71±0.60	3.17±0.20	1.07±0.14
	11.6	6.26±0.23	6.07±0.26	1.03±0.02

* Milliequivalents per litre in dilute nutrient solution applied to plants

Table 47: Influence of sodium chloride on nitrate reductase activity in various tissues of perennial ryegrass and timothy.

Nitrate Nitrogen

Perennial ryegrass

		Leaf		Stubble		Roots	
Treatment		NO_3 (ppm)	percent of total leaf N	NO_3 (ppm)	percent of total stubble N	NO_3 (ppm)	percent of total root N
Sodium chloride	0.9*	500	1.24	407	1.89	113	0.94
	3.6	475	1.13	382	1.94	125	1.37
	6.2	600	1.42	500	2.20	88	0.83
	9.0	875	2.02	763	3.14	83	0.75
	11.6	1000	2.18	691	2.48	93	0.85
Standard error		±89	-	±60	-	±10	-

Timothy

Treatment							
Sodium chloride	0.9*	875	2.10	813	4.07	75	1.03
	3.6	890	2.10	763	4.15	63	0.62
	6.2	975	2.23	1000	4.98	138	1.30
	9.0	1025	2.15	1435	5.24	425	3.22
	11.6	1425	2.60	2683	8.52	517	3.77
Standard error		±116	-	±120	-	±24	-

* Milliequivalents per litre in dilute nutrient solution applied to plants.

Table 48: Influence of sodium chloride on nitrate concentrations in various tissues of perennial ryegrass and timothy.

Nitrate nitrogen was also influenced by sodium chloride. Although the actual concentration of nitrate increased within most tissues of both plants with increasing sodium, these changes were very small when compared to the overall increases in total nitrogen (Table 48). Another important point concerning nitrate nitrogen is in relation to the accumulation of this compound in various sites within the test plants. In ryegrass the highest nitrate concentrations were measured in the leaf tissue, whereas in timothy nitrate tended to accumulate in the stubble (see Table 48). Moreover, in conjunction with the abrupt increase in the concentration of sodium in the aerial parts of both plants at the higher concentrations of applied sodium chloride there were also sharp increases in both the concentration of nitrate and nitrate reductase activity within these sites. (A summary of the above effects are given in Table 49a for perennial ryegrass and for timothy in Table 49b.)

Perennial ryegrass

Shoots

Treatment		Nitrate$^+$ reductase activity	Nitrate (ppm)	Sodium (percent dry matter)
Sodium chloride	0.9*	2.10	500	0.23
	3.6	2.48	475	0.45
	6.2	2.93	600	0.67
	9.0	3.48	875	1.13
	11.6	4.36	1000	1.82

Stubble

Sodium chloride	0.9*	1.60	407	0.25
	3.6	2.14	382	0.41
	6.2	1.98	500	0.54
	9.0	2.09	763	0.62
	11.6	1.86	691	0.64

Roots

Sodium chloride	0.9*	0.76	113	0.07
	3.6	0.56	125	0.07
	6.2	0.64	88	0.08
	9.0	0.84	83	0.08
	11.6	0.80	93	0.07

+ μ moles NO_2/gram fresh weight/hour

* Milliequivalents per litre in dilute nutrient solution applied to plants

Table 49a: Summary of the influence of sodium chloride on nitrate reductase activity, nitrate content, and sodium concentration in perennial ryegrass.

Timothy

Shoots

Treatment		Nitrate[+] reductase activity	Nitrate (ppm)	Sodium (percent dry matter)
Sodium chloride	0.9*	1.29	875	0.03
	3.6	2.30	890	0.09
	6.2	2.49	975	0.27
	9.0	2.71	1025	0.48
	11.6	6.26	1425	0.91

Stubble

Treatment		Nitrate[+] reductase activity	Nitrate (ppm)	Sodium (percent dry matter)
Sodium chloride	0.9*	1.68	813	0.10
	3.6	2.64	763	0.28
	6.2	2.81	1000	0.56
	9.0	3.17	1435	0.80
	11.6	6.07	2683	0.84

Roots

Treatment		Nitrate[+] reductase activity	Nitrate (ppm)	Sodium (percent dry matter)
Sodium chloride	0.9*	1.13	75	0.12
	3.6	1.85	63	0.17
	6.2	1.32	138	0.24
	9.0	1.07	425	0.27
	11.6	1.03	517	0.26

[+] μ moles NO_2/gram fresh weight/hour

* Milliequivalents per litre in dilute nutrient solution applied to plants

Table 49b: Summary of the influence of sodium chloride on nitrate reductase activity, nitrate content, and sodium concentration in timothy.

(iii) EXPERIMENT 3

The effects of sodium and potassium on the induction of nitrate reductase activity in a selection of natrophiles and natrophobes.

Results

The results, which are pooled means of the individual plant species presented in Table 50, indicate that the potassium based incubation medium induced slightly higher nitrate reductase activity in all assay samples. However, the variation in enzymatic activity (as indicated by the standard error) was in general lower for the sodium based medium than that associated with the potassium medium.

Nitrate Reductase Activity
(µmoles NO_2/g fresh wt/hr)

	Shoots		Stubble		Roots	
	Medium		Medium		Medium	
Plant type	K	Na	K	Na	K	Na
Leaf natrophile	6.89	5.80	4.44	3.70	3.59	2.77
Standard error	±0.22	±0.22	±0.44	±0.29	±0.32	±0.18
Stubble natrophobe	5.37	4.48	5.83	5.44	4.08	3.54
Standard error	±0.49	±0.32	±0.34	±0.23	±0.40	±0.29
Root natrophobe	1.45	1.33	2.90	2.63	4.11	3.52
Standard error	±0.10	±0.08	±0.22	±0.10	±0.14	±0.17

Table 50: Influence of potassium and sodium incubation media on the induction of nitrate reductase activity in a selection of natrophiles and natrophobes.

A possible cause of the slight difference between the two incubation media may have been related to the somewhat higher conductivity of the potassium medium as opposed to that measured for sodium. The conductivities of the two solutions were 25.9 x 10^{-3} mhos and 20.5 x 10^{-3} mhos respectively. However, when the conductivity of the sodium medium was adjusted with either sodium chloride or sodium sulphate to the level measured in the potassium medium, the level of nitrate reductase activity (induced in 21 day old perennial ryegrass leaves) was still below that measured in the potassium medium. There was however, a slight increase in enzymatic activity with the additional sodium salts (Table 51).

Incubation medium	conductivity (at 25 °C)	Nitrate reductase activity (μmoles NO_2/g fresh wt/hr)
potassium	25.9 x 10^{-3} mhos	3.26±0.17
sodium	20.5 x 10^{-3} mhos	2.31±0.09
sodium + sodium chloride	25.7 x 10^{-3} mhos	2.61±0.07
sodium + sodium sulphate	25.9 x 10^{-3} mhos	2.67±0.12

<u>Table 51</u>: The effect of increasing the conductivity of the sodium incubation medium on nitrate reductase activity in young Ariki ryegrass leaves.

(3) DISCUSSION

It is clearly evident from the above results that sodium is in some way associated with the reduction of nitrate within the plant. Moreover, it is significant, in light of the well documented observation that plants have different abilities to reduce nitrate within their shoots and roots (eg. Wilson, 1943; Bollard, 1957; 1960; Wright and Davison, 1964; Miflin, 1967; Wallace and Pate, 1967; Beevers and Hageman, 1969; Weissman, 1972; Pate, 1973), that both high nitrate nitrogen and nitrate reductase activity should occur in tissues where sodium accumulates. In other words, it is a feature of natrophilic plants that the reduction of nitrate occurs mainly within their aerial tissues with only small amounts of nitrate being reduced within their roots; whereas in natrophobic plants reduction occurs mainly within the root and stem tissues. But once the accumulation sites within the roots or stems of natrophobes have been saturated, both sodium and nitrate nitrogen greatly increase in the aerial tissues along with a marked stimulation of nitrate reductase activity. In view of the fact that nitrate reductase is induced by nitrate (see review by Beevers and Hageman, 1969) it is not surprising to find that a high nitrate content within plants coincides with a corresponding high nitrate reductase activity.

Although the addition of sodium chloride to both natrophiles and natrophobes resulted in the stimulation of nitrate reductase activity and increased the level of nitrate, the level of nitrate nitrogen always remained a small, relatively constant fraction of the total nitrogen content within these plants.

In order to maintain chemical neutrality within the plant during reduction of nitrate to an amminical form, it has been suggested (eg. Burstrom, 1945; Dijkshoorn, 1958) that there is a simultaneous production of organic anions at the time of reduction. Such a contention is supported by observations that plants have higher carboxylate contents when growing in media in which the concentration of nitrate ions has been increased (Dijkshoorn, 1962; Kirkby and Mengel, 1967; Dijkshoorn _et al._, 1968; van Tuil, 1970). As a consequence of such a hypothesis, it would be expected that the site at which nitrate is reduced would not only influence the translocation of cations but also the accumulation of organic acids. In other words, nitrate must be translocated to the site of reduction with an accompanying inorganic cation and on reduction of nitrate the charge on the accompanying ion would pass directly to an organic anion. Therefore, the leaves of natrophiles would be expected to have higher concentrations of both cations and organic acids than corresponding natrophobic plants if nitrate was predominantly reduced in the roots of the latter. In fact, data by Pierce and Appleman (1943), Kirkby (1969) and Coic (1971) presented in Table 52, and also the notable differences in cation composition between natrophiles and natrophobes recorded in the preceding chapters of this study, would support the observed differences in location of nitrate reductase activity between these two different types of plant.

The above observations would suggest that in contributing, with potassium, calcium, and magnesium, to the maintenance of cation-anion balance within the plant, sodium tends to be associated with nitrate.

Although it has been reported (Tottingham _et al._, 1934) that nitrate is taken up more readily from nutrient solutions prepared with potassium nitrate than those prepared with calcium or sodium nitrate, thereby lending support to the theory that potassium is the preferential carrier of nitrate within the plant (Dijkshoorn, 1969; Lips _et al._, 1971), field evidence and experimental observations from

Leaf tissue

Plant	Total organic acids	potassium	sodium	nitrogen source
Natrophiles				
Beet^{+}	428	152	-	NO_3
Spinach^{+}	380	187	-	"
Tomato^{+}	270	82	-	"
Tomato*	242	58	19	"
Tomato$^{\bullet}$	26	11	-	"
Natrophobes				
Alfalfa^{+}	186	93	-	NO_3
Wheat^{+}	122	173	-	"
Blue grass^{+}	109	142	-	"
Rye*	78	86	1	"
Maize$^{\bullet}$	12	13	-	"

$^{+}$ Data from Pierce and Appleman (1943)
* " " Kirkby (1969)
$^{\bullet}$ " " Coic (1971)
All values are given in milliequivalents per 100 g of dry matter.

Table 52: Variation in total organic acids, potassium, and sodium in leaves of natrophiles and natrophobes.

this investigation do not support such a finding. For example, in the field Lowry *et al.* (1936) found that adding potassium decreased the nitrate content of xylem sap exuded by corn plants. A similar effect has been recorded in blue panic grass where potassium had no effect on nitrate content (Wright *et al.*, 1960). In addition, the results of Sorensen (1960) clearly indicate that in petioles of sugar beet, where nitrate preferentially accumulates, there is an inverse relationship between nitrate content and potassium. Furthermore, as a consequence of adding nitrogen as 'nitro-chalk' to pastures, Reith et al. (1964) observed that the concentration of sodium in the leaves increased with increasing additions of nitrogen even in the presence of additional potassium (see Table 3). On the other hand, additional nitrogen reduced the corresponding concentrations of potassium within these tissues. This inverse relationship between potassium and nitrogen is confirmed in the results presented by Jones (1961) and Freeman (1967). They found that nitrate accumulated in tissues of plants growing under potassium deficient conditions. Such conditions are also known to increase the uptake of sodium significantly.

Studies on the effects of light intensity on chemical composition of plants have also yielded some interesting results. In addition to promoting nitrate reductase activity (Candela et al., 1957; Hageman and Flesher, 1960), light also increases the uptake of sodium (MacRobbie and Dainty, 1958) along with nitrate (Nagai and Tazawa, 1962; Beevers et al., 1965), but contrary to the preferential nitrate carrier theory, light causes substantial losses of potassium, even as high as eightfold (MacRobbie and Dainty, 1958).

Considering the close association between nitrate reduction and organic acid formation along with the apparent ability of sodium to accompany the nitrate ion within the plant, it is not surprising therefore to find sodium equally effective as potassium in promoting malate formation in red beet (Splittstoesser and Beevers, 1964). In fact, malate production persisted longer with sodium phosphate than it did with the corresponding potassium salt. The effectiveness of malate as an organic anion to counterbalance inorganic cations is well documented (Ulrich, 1941; Jacobson and Ordin, 1954; Van

Steveninck, 1966; Jacoby and Laties, 1971), especially during the reduction of nitrate (Ben-Zioni et al., 1970). Moreover, malic acid is located predominantly in the vacuoles of beet cells and not in the cytoplasm (Osmond and Laties, 1969). It is therefore significant that the concentration of sodium in the vacuoles of barley roots is reported to be three to four times that in the cytoplasm, while the concentration of potassium on the other hand was roughly the same in both compartments (Pitman and Saddler, 1967). In addition, a recent investigation by Poole (1971) indicates that the uptake of sodium in red beet cells inhibits the transfer of potassium into the vacuole, however sodium had no effect on the concentration of potassium in the cytoplasm which remained at a constant level. This is particularly important in view of the fact that the oxalate isolated from Setaria sphacelata was sodium oxalate and not potassium (Jones and Ford, 1972). It has also been observed that the total organic acid content increases in certain potassium deficient plants (Vickery and Palmer 1957; Freeman, 1967; Dijkshoorn et al., 1968), particularly in the presence of sodium (van Tuil, 1970). Such a condition would not be expected to occur if there were a direct relationship between nitrate content and potassium.

Therefore, contrary to the unfounded suggestion by Lips et al. (1971) that the presence of sodium in the plant would prevent the preferential uptake of nitrate by the roots at moderate concentrations, the results of this investigation would suggest that sodium is highly effective in accompanying the nitrate ion within the plant. Moreover, there is no reason to believe that sodium would immobilise malate, or any other major organic acid for that matter, in the sense that Lips et al. (1971) imply, as all sodium salts of the important organic acids found in the plant are only slightly less soluble than the corresponding potassium salts. What is more, sodium is highly mobile within the phloem of a number of plants (Cooil _et al._, 1965; Levi, 1970; Clarkson, 1974; Marschner, 1974).

In view of the above findings it is proposed that one of sodium's main functions in the plant is to accompany the nitrate ion to the major site of reduction, thereby forming a pool of reducible nitrate. On reduction of nitrate, the resulting sodium ion, along with the other cations accompanying nitrate, would immediately combine with an organic anion. Some of these organic salts, particularly sodium, would subsequently accumulate in the vacuoles of the cells and thereby contribute to the osmotic pressure. Such a proposal would account for the irregular distribution of sodium in plants, in fact corresponding to the uneven disposition of the nitrate reductase enzyme. Moreover, it appears that the stimulation of nitrate reductase activity by sodium chloride in plants, as recorded in this investigation and by Sankhla and Huber (1975) is most likely the result of the increased translocation of nitrate into the tissues where nitrate reduction preferentially occurs.

In addition to the general role of maintaining chemical neutrality within the plant it is possible that sodium may have a specific effect on the nitrate reductase enzyme as suggested by Marquez and Brodie (1973) for halophilic bacteria.

It is well documented that many enzymatic systems in halophilic bacteria require high concentrations of sodium chloride for maximal activity (Baxter and Gibbons, 1954; 1956; 1957; Larsen _et al._, 1962; Brown, 1964; Larsen _et al._, 1967; Lanyi, 1969; Lieberman and Lanyi, 1971). Several hypotheses have been suggested concerning the stability of halophilic enzymes in salt solutions. Baxter (1959) considered that this stability could be ascribed to electrostatic interactions, this suggestion was later extended to implicate the shielding of negative charges found on proteins (Brown, 1965; Holmes and Halvorson, 1965; Kushner and Onishi, 1966). The latter hypothesis was supported by the findings that halophilic proteins contain an excess of acidic amino acids, namely aspartic and glutamic acids, which could be shielded by sodium and other cations (Brown, 1963; Bayley, 1966). In addition to electrostatic shielding, Lanyi and Stevenson (1970) suggested that the high salt requirement of halophilic enzymes is due to the presence of hydrophobic bonds. These bonds are considered to give the enzyme stability and are promoted by sodium chloride. In the absence of salt it is suggested that the enzymes are almost completely unfolded and inactive. However, as the concentration of sodium chloride is increased, the native conformation is reached through the interaction of hydrophobic groups as their solubility in water is decreased.

Therefore, it appears that sodium chloride, and to a lesser extent other sodium salts (Lanyi and Stevenson, 1970) have the ability of promoting and stabilising certain enzyme systems in bacteria, this effect has also been observed in certain higher plants. Prisco and Vieira (1976) reported that sodium chloride delayed the breakdown and turnover of proteins in germinating serido been _Vigna sinensis_ seeds. This stabilising effect of sodium salts may account for the generally lower variation in nitrate reductase activity recorded in this study when sodium rather than potassium was in the incubation medium. However, having due regard for the non specific nature of the substrate required for inducing the nitrate reductase enzyme (Beevers and Hageman, 1969; Knyph, 1974) and the slight advantage of a potassium based incubation medium over a similar sodium medium in short term _in vivo_ experiments (two hours), it would appear that sodium does not have a significant overall effect on this enzyme by comparison with potassium.

CONCLUSIONS

Because of the fragmentary nature of the data concerning both the practical and physiological significance of sodium in plant nutrition an attempt is made in this section to draw the results of this investigation, together with existing data, into a generalised conclusion presented under the following three general headings:- agricultural, physiological, and ecological.

(a) AGRICULTURAL

It is now evident from this investigation that a large number of topdressed ryegrass-white clover pastures in New Zealand have sodium levels which are below those required for grazing animals. Low sodium in the diet of animals can result in a number of serious metabolic disorders.

There are a number of factors which have contributed to the generally low sodium status of New Zealand pastures. In addition to the geological formations which have yielded soils naturally deficient in sodium, there have been a series of changes in farming practices over the past thirty years. Since 1950 there has been a considerable increase in the use of high grade potassic fertilisers on pastures throughout the country. As a direct consequence of this trend pastures in most areas now have potassium levels which are well in excess of plant needs. Moreover, potassium not only depresses the uptake of sodium into the plant, but also accelerates the leaching losses of soil sodium from the root zone, especially under the humid climatic conditions which prevail in New Zealand. Therefore, the excessive use of potassic fertilisers would be expected to aggrevate an animal-nutrition problem involving sodium or even create one where it may not have originally existed. What is more, the abandoning of the practices of applying sodium as a fertiliser and in many cases the direct supplementation of animal feeds with salt has coincided with the increased use of potassium. However, in comparison to inland areas, the problems associated with low herbage sodium in coastal regions are offset by the large quantities of sodium chloride deposited from sea spray, as a consequence of which higher sodium levels are maintained. In addition to herbage sodium other sources of this element, such as drinking water and in certain circumstances ingested soil, can form important fractions of the total daily intake by animals.

An additional animal-nutrition problem involving sodium arises from the considerable differences between plant species in the extent to which they accumulate sodium in root and shoot tissue. In this respect plants can be classified as follows:-

Natrophiles: plants in which sodium readily accumulates in leaf tissues.

Natrophobes: plants in which sodium preferentially accumulates in the roots or lower stems with the result that only very small quantities of this element are present in the leaf tissue.

As a consequence of their inherent physiological differences, natrophobes are unable to provide food rations containing enough sodium for dietary needs of grazing animals, even in a situation where there is enough sodium in the soil to produce a satisfactory level in natrophiles; an animal nutrition problem is therefore likely to occur where natrophobic plants form a substantial part of the animals diet. From a practical point of view the use of sodium fertilisers to increase the sodium content in plant tissues to an acceptable level for animal nutrition would only be effective if natrophilic plants were involved. Furthermore, the success of sodium fertilisers would depend to a large extent on the potassium status of the soil in which the plants were grown and also on the sodium salt involved. In this context it appears that sodium nitrate is more effective in increasing the sodium content in leaves of plants than other sodium salts (Lehr, 1960; Wallace _et al._, 1965). This result is not surprising in view of the close association between sodium and nitrate nitrogen within the plant. Although it is possible to increase the sodium content in the leaves of natrophobes under certain conditions, the high concentration of sodium required in the root zone to raise the level of this element to one which is acceptable for animal nutrition could result in depressed plant growth and would certainly be uneconomic to achieve. Moreover, the application of foliar sprays containing sodium to the leaves of natrophobes would be equally ineffective as sodium is rapidly translocated away from the leaves of natrophobic plants via the phloem into the roots (Wallace and Hemaidan, 1963; Cooil _et al._, 1965; Levi, 1970; Marschner, 1974). Therefore, the most practical solution for correcting sodium deficiencies in grazing animals, particularly in view of the depressing effects of potassium and the high risk of leaching, would appear to be direct supplementation of the animal either by means of salt licks or by the addition of sodium to the drinking water.

In the absence of any field trial data it is predictable from the close relationship which exists between sodium, nitrate nitrogen, and the activity of the molybdenum containing nitrate reductase enzyme within the plant, that both the levels of sodium and molybdenum in the aerial parts of natrophilic plants should follow closely the seasonal changes in nitrate nitrogen. In Britain for example, high levels of nitrate are reported to occur in pastures during spring and autumn

(Jones et al., 1961). Seasonal changes in leaf sodium in natrophobes on the other hand would be very small considering the location of this element within these plants. Sodium would also be expected to increase in the leaves of natrophiles as the level of nitrate nitrogen in the ecosystem increases with pasture development. In fact, experimental observations by Cullen et al. (1966) would lend support to this point.

(b) PHYSIOLOGICAL

One of the most notable features of this study was the significant relationship measured between sodium and nitrogen in the tissues of both natrophiles and natrophobes. Moreover, the greater part of nitrate reductase activity in plants, as measured by an intact tissue method, was centred in areas where both sodium and nitrate nitrogen preferentially accumulate. In other words, in natrophiles nitrate tends to be reduced predominantly in the aerial tissues, whereas in natrophobes reduction of nitrate is generally confined to the stems and roots. Moreover, nitrate reductase activity in roots occurs mainly in the apical zone. It is also evident from this study that the application of sodium chloride to both natrophilic and natrophobic plants, in the presence of adequate amounts of nitrogen, not only resulted in a marked stimulation of nitrate reductase activity but also increased the level of nitrate nitrogen and the overall total nitrogen content. However, a most important point was that the level of nitrate nitrogen always remained at a small, relatively constant fraction of the total nitrogen content within the plants.

In addition to stimulating nitrogen metabolism, high external concentrations of sodium can result, as measured in this investigation, in the preferential accumulation sites for sodium in natrophobes becoming saturated such that both sodium and nitrate nitrogen move freely into the leaves. In their experiments Wallace and Pate (1965; 1967) have clearly shown that on saturating the reductase capacity in the roots of certain plants with high levels of nitrate, free nitrate nitrogen moves up into the shoots. As expected the capacity to saturate the nitrate reductase enzyme with nitrate differs greatly for different plant species (Beevers and Hageman, 1969). It is also interesting to note in light of the above observations, that the application of metabolic inhibitors to the roots or stems of natrophobes greatly enhances the movement of sodium into the leaves (Pearson, 1962; Jacoby, 1965; Wallace _et al._, 1965). Unfortunately no measurements were made of the nitrate levels in the plants from these experiments.

In view of the fact that the synthesis of the nitrate reductase enzyme is induced by nitrate (see review by Beevers and Hageman, 1969) and the close association between sodium and nitrate within the plant, it would appear that the stimulation of nitrate reductase activity by sodium chloride is the result of an increase in nitrate nitrogen at the site of reduction. Therefore the circumstantial evidence strongly suggests that one of the main roles of sodium in plants is to accompany the nitrate ion. Moreover, it is envisaged that in order to maintain chemical neutrality when nitrate is reduced, sodium combines with the resulting carboxylic anion. Such a proposition would in fact account for the irregular accumulation of sodium in plants, particularly in view of the uneven distribution of both nitrate and the nitrate reductase enzyme. It would also explain why, of the three environmental factors, light, moisture stress, and temperature, light has the most dominating influence on sodium. In this respect light not only stimulates nitrate reductase activity

(Candela _et al_., 1957; Hageman and Flesher, 1960) but also the uptake of nitrate into the plant (Nagai and Tazawa, 1962; Beevers _et al_., 1965). It is also interesting to note that plants which have high molybdenum requirements (During, 1972) and high nitrate contents in their tissues (Wright and Davison, 1964) are species which, in general, have been found to benefit from sodium fertilisers, as for example members of the Chenopodiaceae and Cruciferae families. It may also be conjectured that one of the many causes of the irregular and often conflicting reports about the stimulating effects sodium has on dry matter yields could be closely related to the level of nitrate present.

Apart from the general role of maintaining chemical neutrality within the plant, sodium does not appear to have a specific effect on the nitrate reductase enzyme when compared with an identical potassium based incubation medium in short term (two hours) _in vivo_ experiments, as carried out for example in this study. However, there have been a number of speculations to the effect that sodium may stabilise and prevent the breakdown of proteins (Lanyi and Stevenson, 1970; Prisco and Viera, 1976). Moreover, in relation to the nitrate reductase enzyme, Huffaker and Peterson (1974) state that this enzyme is the only specific plant protein for which definite evidence of protein 'turnover' has been obtained so far (see Zielke and Filner, 1971). Various estimates have been reported for 'turnover' rates of this enzyme and these rates are found to vary considerably according to plant species and pretreatment (Huffaker and Peterson, 1974). Therefore, in order to examine more fully the possibility that sodium could have a stabilising effect on nitrate reductase, more detailed and longer term experiments than those employed in this study will be required.

(c) ECOLOGICAL

In light of the fundamental characteristics which distinguish natrophilic and natrophobic plants as revealed in this investigation, it is interesting to speculate on the possible ecological reasons why plants should have evolved such contrasting sites for an identical physiological activity.

It is apparent from the study of plants possessing the C_3 and C_4 photosynthetic pathways that water conservation within the plant has played a major part in the evolution of the C_4 system (Brown, 1958; Black *et al.*, 1969; Downes, 1969; Black, 1971; Hatch and Boardman, 1974; Laetsch, 1974). Thus transpiration rates in C_4 plants are much lower than those measured in C_3 plants (Downes, 1969; Forde *et al.*, 1974). It is therefore most significant that all C_4 plants examined were natrophobes; also the rates of transpiration and ion uptake, particularly of sodium, in all natrophobes, that is both C_3 and C_4 members, were much lower than those measured in natrophilic plants grown under the same conditions. As expected, however, C_3 natrophobes had higher transpiration rates than the C_4 natrophobes. Therefore, in view of the similarities between C_3 and C_4 natrophobes in a number of basic physiological features namely, the preferential sites of sodium and nitrate accumulation, the distribution of nitrate reductase activity, the rate of ion uptake, and the rate of transpiration, it would seem most likely that natrophobes have developed these modifications in order to conserve water. Furthermore, these adaptions would enable natrophobes to tolerate more arid conditions than those which could be endured by natrophilic plants.

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BIBLIOGRAPHY

Agricultural Research Council, London. 1965. *The nutrient requirements of farm livestock*. No.2. Ruminant technical reviews and summaries. A.R.C. pp 67–85.

Allen, M.B.; D.I. Arnon. 1955. Studies on nitrogen-fixing blue-green algae. II. The sodium requirement of *Anabaena cylindrica*. *Physiologia Pl.* 8: 653–660.

Arisz, W.H. 1956. Significance of the symplasm theory for transport in the root. *Protoplasma* 46: 5–62.

Arnon, D.I.; P.R. Stout. 1939. The essentiality of certain elements in minute quantity for plants with special reference to copper. *Pl. Physiol.* 14: 371–375.

Bailey, C.B.; C.C. Balch. 1961. Saliva secretion and its relation to feeding in cattle. 2. The composition and rate of secretion of mixed saliva in the cow during rest. *Br. J. Nutr.* 15: 383–402.

Bailey, R.W. 1973. *Water in Herbage* in Chemistry and Biochemistry of Herbage Vol 2 pp 13–24. ed. G.W. Butler and R.W. Bailey. Academic Press, London.

Bakker, Y. 1963. Recommendation for the fertilisation of grassland. *Landbouwroorl* 26: 633–636.

Bange, G.G.J. 1959. Interactions in the potassium and sodium absorption by intact maize seedlings. *Pl. Soil* 11: 17–29.

Bange, G.G.J. 1962. The carrier theory of ion transport: a reconsideration. *Acta. bot. neerl.* 11: 139–146.

Bange, G.G.J. 1975. Divalent cations and K/Na selectivity in excised barley roots. *Physiologia Pl.* 34: 110–116.

Bange, G.G.J.; J. Tromp; S. Henks. 1965. Interactions in the absorption of potassium, sodium, and ammonium ions in excised barley roots. *Acta bot. neerl.* 14: 116–130.

Barnett, N.M.; A.W. Naylor. 1966. Amino acid and protein metabolism in Bermuda Grass during water stress. Pl. Physiol. 41: 1222–1230.

Basso, L.C.; T.A. Smith. 1974. Effect of mineral deficiency on amine formation in higher plants. Phytochemistry. 13: 875–883.

Baxter, R.M. 1959. An interpretation of the effects of salts on the lactic dehydrogenase of *Halobacterum salinarium*. Can. J. Microbiol. 5: 47–57.

Baxter, R.M.; N.E. Gibbons. 1954. The glycol dehydrogenases of *Pseudomonas salinaria*, *Vibro costuoles* and *Escherichia coli* in relation to bacterial halophilism. Can. J. Biochem. Physiol. 32: 206–217.

Baxter, R.M.; N.E. Gibbons. 1956. Effects of sodium and potassium chloride on certain enzymes of *Micrococcus halodenitrificans* and *Pseudomonas salinaria*. Can. J. Microbiol. 2: 599–606

Baxter, R.M.; N.E. Gibbons. 1957. The cysteine desulphydrase of *Pseudomonas salinaria*. Can. J. Microbiol. 3: 461–465.

Bayley, S.T. 1966. Composition of ribosomes of an extremely halophilic bacterium. J. molec. Biol. 15: 420–427.

Beevers, L.; R.H. Hageman. 1969. Nitrate reduction in higher plants. A. Rev. Pl. Physiol. 20: 495–522.

Beevers, L.; L.E. Schrader; D. Flesher; R.H. Hageman. 1965. The role of light and nitrate in the induction of nitrate reductase in radish cotyledons and maize seedlings. Pl. Physiol. 40: 691–698.

Bell, J.E. 1955. Fertilisers. N.Z. Jl. Agric. 90: 81.

Ben-Zioni, A.; C. Itai; Y. Vaadia. 1967. Water and salt stresses, kinetin and protein synthesis in tobacco leaves. Pl. Physiol. 42: 361–365.

Ben-Zioni, A.; Y. Vaadia; H.S. Lips. 1970. Correlations between nitrate reduction, protein synthesis and nitrate accumulation. *Physiologia Pl*. 23: 1039-1047.

Bernstein, L.; J.W. Brown; H.E. Hayward. 1956. The influence of rootstock on growth and salt accumulation in stone-fruit trees and almonds. *Proc. Am. Soc. Hort. Sci*. 68: 86-95.

Black, C.C. 1971. *Advances in Ecological Research*, Vol 7 pp 87-114. New York. Academic Press.

Black, C.C.; T.M. Chen; R.H. Brown. 1969. Biochemical basis for plant competition. *Weed Sci*. 17: 338-343.

Blakemore, L.C. 1953. The chloride content of rain-water collected in the Wellington Province. *N.Z. Jl. Sc. Technol*. 35 B: 193-197.

Bollard, E.G. 1957. Translocation of organic nitrogen in the xylem. *Aust. J. biol. Sci*. 10: 279-301.

Bollard, E.G. 1960. Transport in the xylem. *A. Rev. Pl. Physiol*. 11: 141-166.

Bolton, J. 1971. Quantity - intensity, relationships for labile sodium in field soils. *J. Soil. Sci*. 22: 417-429.

Bolton, J. 1972. Effects of potassium, magnesium, and sodium fertilisers and lime on the yield and composition of crops in a ten-year experiment at Rothamsted. *Rothamsted Experimental Station Report* for 1972, part 2, pp 102-110.

Bott, E.; D.A. Denton; J.R. Goding; J.R. Sabine. 1964. Sodium deficiency and corticosteroid secretion in cattle. *Nature, Lond*. 202: 461-463.

Boulter, D. 1970. Protein synthesis in plants. *A. Rev. Pl. Physiol*. 21: 91-114.

Bower, C.A.; C.H. Wadleigh. 1948. Growth and cationic accumulation by four species of plants as influenced by various levels of exchangeable sodium. *Soil. Sci. Soc. Am. Proc.* 13: 218–226.

Boyer, J.S. 1965. Effects of osmotic water stress on metabolic rates of cotton plants with open stomata. *Pl. Physiol.* 40: 229–234.

Briggs, L.J.; H.L. Shantz. 1914. Relative water requirements of plants. *J. agric. Res.* 3: 1–63.

Brouwer, E. 1961. *In. Digestive Physiology and Nutrition of the Ruminant.* pp 154 ed. D. Lewis, London. Butterworths.

Brown, A.D. 1963. The peripheral structures of gram-negative bacteria. IV. The cation-sensitive dissolution of the cell membrane of the halophilic bacterium, *Halobacterium halobium.* *Biochim. biophys. Acta.* 75: 524–435.

Brown, A.D. 1964. Aspects of bacterial response to the ionic environment. *Bact. Rev.* 28: 296–329.

Brown, A.D. 1965. Hydrogen ion titrations of intact and dissolved lipoprotein membranes. *J. molec. Biol.* 12: 491–508.

Brownell, P.F. 1965. Sodium as an essential micronutrient element for a higher plant (*Atriplex vesicaria*). *Pl. Physiol.* 40: 406–468.

Brownell, P.F.; C.J. Crossland. 1972. The requirement for sodium as a micronutrient by species having the C_4 dicarboxylic photosynthetic pathway. *Pl. Physiol.* 49: 794–797.

Brownell, P.F.; C.J. Crossland. 1974. Growth responses to sodium by *Brophyllum tubiflorum* under conditions inducing crassulacean acid metabolism. *Pl. Physiol.* 54: 416–417.

Brownell, P.F.; J.G. Wood. 1957. Sodium as an essential micronutrient element for *Atriplex vesicaria*, Herward. *Nature. Lond.* 179: 635–636

Burström, H. 1945. Studies on the buffer systems of cells. Ark. Bot. 32A: 1–18.

Butler, E.J.; Veterinary Officers of the East and West of Scotland Agricultural Colleges. 1963. The mineral element content of spring pasture in relation to the occurrence of grass tetany and hypomagnesaemia in dairy cows. J. agric. Sci. Camb. 66: 329–340.

Butler, G.W.; P.C. Barclay; A.C. Glenday. 1962. Genetic and environmental differences in the mineral composition of ryegrass herbage. Pl. Soil. 16: 214–228.

Butler, G.W.; D.I.H. Jones. 1973. Mineral biochemistry of herbage. In Chemistry and Biochemistry of Herbage Vol 2 ed. G.W. Butler and R.W. Bailey pp 127–162. Academic Press, London.

Cairney, I.M. 1964. Grass staggers in beef cattle. The disease. N.Z. Jl. Agric. 109: 47–49.

Candela, M.I.; E.G. Fisher; E.J. Hewitt. 1957. Molybdenum as a plant nutrient. X. Some factors affecting the activity of nitrate reductase in cauliflower plants grown with different nitrogen sources and molybdenum levels in sand culture. Pl. Physiol. 32: 280–288.

Care, A.D.; L.E. Vowles; S.O. Mann; D.B. Ross. 1967. Factors affecting magnesium absorption in relation to the aetiology of acute hypomagnesaemia. J. agric. Sci. Camb. 68: 195–204.

Chatterton, N.J.; J.R. Goodin; C. Duncan. 1971. Nitrogen metabolism in Atriplex polycarpa as affected by substrate nitrogen and NaCl salinity. Agron. J. 63: 271–274.

Clarkson, D.T. 1974. Ion transport and cell structure pub. McGraw-Hill U.K. p 253.

Coïc, Y. 1971. Influence du métabolisme de NO_3^- dans les racines sur l'etat nutritionnel de la plante. In Recent Advances in Plant Nutrition. Vol 1 pp 217–227. ed. R.M. Samish. pub Gordon and Breach. N.Y.

Collander, R. 1941. Selective absorption of cations by higher plants. Pl. Physiol. 16: 691–720.

Cooil, B.J.; R.K. de la Fuente, R.S. de la Pena. 1965. Absorption and transport of sodium and potassium in squash. Pl. Physiol. 40: 625–632.

Cope, J.T.; R. Bradford; M. Peech. 1953. Effect of sodium fertilisation on yield and cation content of some field crops. Soil Sci. 76: 65–74.

Copponet, M.; J. Calvez. 1962. Variations de la composition minérale de dix varietes de graminées fourrogieres au cours d'une annee d'explantation au rythme pâture. Annls agron. 13: 203–219.

Crafts, A.S.; T.C. Broyer . 1938. Migration of salts and water into xylem of the roots of higher plants. Am. J. Bot. 25: 529–535.

Cullen, N.A.; K.J. McNaught; N.S. Mountier. 1966. The establishment of pasture on yellow-brown loams near Te Anau. IV. The effect of lime and phosphate on establishment of clover and grass oversown on uncultivated ground. N.Z. Jl. agric. Res. 9: 375–387.

Davies, W.E.; ap G. Griffith; A. Ellington. 1966. The assessment of herbage legume varieties. II In vitro digestibility, water soluble carbohydrate, crude protein and mineral content of primary growth of clover and lucerne. J. agric. Sci. Camb. 66: 351–357.

Dijkshoorn, W. 1958. Nitrate accumulation, nitrate balance and cation-anion ratios during regrowth of perennial ryegrass. Neth. J. agric. Sci. 6: 211-222.

Dijkshoorn, W. 1962. Metabolic regulation of the alkaline effect of nitrate utilization in plants. Nature, Lond. 194: 165-167.

Dijkshoorn, W. 1969. The relation of growth to the chief ionic constituents of the plant. In Ecological aspects of the mineral nutrition of plants. Oxford, Blackwall. pp 201-213.

Dijkshoorn, W.; D.J. Lathwell; C.T. Wit. 1968. Temporal changes in carboxylase content of ryegrass with stepwise changes in nutrition. Pl. Soil 29: 369-390.

Dorofaeff, F.D.; K.J. McNaught. 1961. Magnesium deficiency in white clover (Trifolium repens L.) on a pumice soil. N.Z. Jl. agric. Res. 5: 310-317.

Differences in transpiration rates between tropical and temperate grasses under controlled conditions

Downes, R.W. 1969. Planta. 88: 261-273.

During, C. 1972. Fertilisers and soils in New Zealand farming 2nd edition A.R. Shearer Govt Printer, Wellington.

During, C.; P.B. Lynch; K.J. McNaught; R.S. Scott. 1960. A study of the interaction of P, K, S, Mg, Mo, and B fertilisers, and ground limestone, applied to grass-clover pasture. N.Z. Jl. agric. Res. 3: 950-993.

Durrant, M.J.; A.P. Draycott; D.A. Boyd. 1974. The response of sugar beet to potassium and sodium fertilisers. J. agric. Sci. Camb. 83: 427-434.

El-Sheikh, A.M.; Ulrich, A. 1970. Interactions of rubidium, sodium, and potassium on the nutrition of sugar beet plants. Pl. Physiol. 46: 645-649.

Epstein, E. 1961. The essential role of calcium in selective cation transport by plant cells. Pl. Physiol. 36: 437–444.

Epstein, E. 1966. Dual pattern of ion absorption by plant cells and by plants. Nature, Lond. 212: 1324–1327.

Evans, H.J.; G.J. Sorger. 1966. Role of mineral elements with emphasis on the univalent cations. A. Rev. Pl. Physiol. 17: 47–76.

Fleming, G.A. 1973. Mineral composition of herbage. In Chemistry and Biochemistry of Herbage. ed G.W. Butler and R.W. Bailey. Vol 1. pp 529–566. Academic Press. London.

Fleming, G.A.; W.E. Murphy. 1968. The uptake of some major and trace elements by grasses as affected by season and stage of maturity. J. Br. Grassld. Soc. 23: 174–185.

Foote, B.D.; J.B. Hanson. 1964. Ion uptake by soyabean root tissue depleted of calcium by ethylenediaminetetraacetic acid. Pl. Physiol. 39: 450–460.

Forde, B.J.; M.F. Beardsell; H.C.M. Bassett. 1974. Comparative transpiration rates of three grass species. In Mechanisms of Regulation of Plant Growth. eds. R.L. Bieleski, A.R. Ferguson, M.M. Cresswell. Bulletin 12, The Royal Society of New Zealand pp 417–421.

Freeman, G.G. 1967. Studies on potassium nutrition of plants. II. Some effects of potassium deficiency on the organic acids of leaves. J. Sci. Fd. Agric. 18: 569–576.

Gammon, N. 1953. Potassium requirements of Pangola and pasture grasses. Soil Sci. 76: 81–90.

Garaudeaux, J. 1959. Etude de la composition minérale de quelques souches de graminées. Proceedings of the World Congress of Agricultural Research. Rome. pp 593–597.

Gauch, H.G.; C.H. Wadleigh. 1945. Effect of high concentrations of sodium, calcium, chloride, and sulphate on ionic absorption by bean plants. Soil Sci. 59: 139–153.

Goding, J.R.; D.A. Denton. 1959. The response to Na$^+$ depletion in adrenalectomized sheep with parotid fistulae. Aust. J. Expt. Biol. Med. Sci. 37: 211–238.

Goodall, D.W.; W.G. Slater; A.E. Grantlipp. 1957. Nutrient interactions and deficiency diagnosis in the lettuce. II. Effects of nutrition on water content. Aust. J. biol. Sci. 10: 57–65.

Grey, G. 1888. On the dissolved matter contained in rain-water collected at Lincoln, N.Z. Aust. N.Z. Ass. Advanc. Sci. 138–152.

Greenway, H. 1968. Growth stimulation by high chloride concentrations in halophytes. Israel J. Bot. 17: 169–177.

Greenway, H.; C.B. Osmond. 1972. Salt responses of enzymes from species differing in salt tolerance. Pl. Physiol. 49: 256–259.

ap Griffith, G.; D.I.H. Jones; R.J.K. Walters. 1965. Specific and varietal differences in sodium and potassium in grasses. J. Sci. Fd. Agric. 16: 94–98.

ap Griffith, G.; R.J.K. Walters. 1966. The sodium and potassium content of some grass genera, species and varieties. J. agric. Sci. Camb. 67: 81–89.

Hageman, R.H.; D. Flesher. 1960. Nitrate reductase activity in corn seedlings as affected by light and nitrate content of nutrient media. Pl. Physiol. 35: 700–708.

Hansson, G.; A. Kylin. 1969. ATP-ase activities in homogenates from sugar beet roots, relation to Mg^{++} and (Na$^+$ + K$^+$) stimulation. Pflanzenphysiol. 60: 270–275.

Harmer, P.M.; E.J. Benne. 1945. Sodium as a crop nutrient. Soil Sci. 60: 137–148.

Harris, P.B.; K.J. McNaught; P.B. Lynch. 1966. The main effects and interactions of nutrients on the growth of irrigated lucerne. N.Z. Jl. agric. Res. 9: 653–690.

Hason-Porath, E.; A. Poljakoff-Mayber. 1969. The effect of salinity on the malic dehydrogenase of pea roots. Pl. Physiol. 44: 1031–1034.

Hatch, M.D.; N.K. Boardman. 1973. Biochemistry of photosynthesis. In Chemistry and Biochemistry of Herbage Vol 2 eds. G.W. Butler and R.W. Bailey pp 22–55 Academic Press, London.

Hayward, H.E.; C.H. Wadleigh. 1949. Plant growth on saline and alkali soils. Advan. Agron. 1: 1–38.

Healy, W.B. 1973. Nutritional aspects of soil ingestion by grazing animals. In Chemistry and Biochemistry of Herbage Vol 1 eds G.W. Butler and R.W. Bailey pp 567–588. Academic Press London.

Helal. M.; K. Koch; K. Mengel. 1975. Effect of salinity and potassium on the uptake of nitrogen and on nitrogen metabolism in young barley plants. Physiologia Pl. 35: 310–313.

Hemingway, R.G. 1960a. Effects of salt and other fertilisers on yield and mineral composition of forage crops. II Turnips. J. Sci. Fd. Agric. 11: 349–355.

Hemingway, R.G. 1960b. Effects of salt and other fertilisers on yield and mineral composition of forage crops. III Kale. J. Sci. Fd. Agric. 11: 355–362.

Hemingway, R.G. 1961. Magnesium, potassium, sodium, and calcium contents of herbage as influenced by fertiliser treatments over a three year period. J. Br. Grassld. Soc. 16: 106–116.

Henkens, C.H. 1965. Factors influencing the sodium content of meadow grass. Neth. J. agric. Sci. 13: 21–47.

Hewitt, E.J. 1963. The essential nutrient elements: Requirements and interactions in plants. In Plant Physiology Vol III. ed. E.C. Steward pp 137–360. Academic Press, New York.

Holmes, P.K.; H.O. Halvorson. 1965. Properties of a purified halophilic malic dehydrogenase J. Bact. 90: 316–326.

Huffaker, R.C.; L.W. Peterson. 1974. Protein turnover in plants and possible means of its regulation. A. Rev. Pl. Physiol. 25: 363–392.

Huffaker, R.C.; A. Wallace. 1959a. Sodium absorption by different plant species at different potassium levels. Soil Sci. 87: 130–134.

Huffaker, R.C.; A. Wallace. 1959b. Effect of potassium and sodium levels on sodium distribution in some plant species. Soil Sci. 88: 80–82.

Humble, G.D.; T.C. Hsiao. 1969. Specific requirements of potassium for light activated opening of stomata in epidermal strips. Pl. Physiol. 44: 230–234.

Hutton, J.B.; K.E. Jury; E.B. Davies. 1967. Studies of the nutritive value of New Zealand dairy pasture. V. The intake and utilization of potassium, sodium, calcium, phosphorus, and nitrogen in pasture herbage by lactating dairy cattle. N.Z. Jl. agric. Res. 10: 367–388.

van Itallie, T.B. 1934. Chemical composition of some grass varieties at different stages of growth. Versl. Landbouw. Onderz. 40: 639–693.

Jacks, C.V. 1954. Soil Thomas Nelson and Sons Ltd. London pp 40–41.

Jackson, M.L. 1958. Soil Chemical Analysis. Constable pp 148–151.

Jacobson, L.; R.J. Hannapel; D.P. Moore; M. Schaedle. 1961. Influence of Ca on selectivity of ion absorption process. Pl. Physiol. 36: 58–61.

Jacobson, L.; L. Ordin. 1954. Organic acid metabolism and ion absorption in roots. Pl. Physiol. 20: 70–75.

Jacoby, B. 1964. Function of bean roots and stems in sodium retention. Pl. Physiol. 39: 445–449.

Jacoby, B. 1965. Sodium retention in excised bean stems. Physiologia Pl. 18: 730–739.

Jacoby, B.; G.G. Laties. 1971. Bicarbonate fixation and malate compartmentation in relation to salt-induced stoichiometric synthesis of organic acid. Pl. Physiol. 47: 525–531.

Jaworski, E.G. 1971. Nitrate reductase assay in intact plant tissues. Biochem. Biophys. Res. Commun. 43: 1274–1279.

Jennings, D.H. 1967. Electrical potential measurements, ion pumps and root exudation – a comment and a model exploring cation selectivity by the root. New Phytol. 66: 357–369.

Jennings, D.H. 1968. Halophytes, succulence, and sodium in plants – a unified theory. New Phytol. 67: 899–911.

Jeschke, W.D. 1972. K$^+$ – stimulated Na$^+$ efflux and selective transport in barley roots. In Ion Transport in Plants (Proceedings of Liverpool International Workshop on Ion Transport) ed. W.P. Anderson. pp 285–296.

Jones, D.I.H.; G. ap Griffith; R.J.K. Walters. 1961. The effect of nitrogen fertiliser on the water – soluble carbohydrate content of perennial ryegrass and cocksfoot. J. Br. Grassld. Soc. 16: 272–275.

Jones, L.H. 1961. Some effects of potassium deficiency on the metabolism of the tomato plant. Can. J. Bot. 39: 593–606.

Jones, R.J.; C.W. Ford. 1972. Some factors affecting the oxalate content of the tropical grass Setaria sphacelata. Aust. J. exp. Agric. Anim. Husb. 12: 400–406.

Joyce, J.P.; L.C.F. Brunswick. 1975. Sodium supplementation of sheep and cattle fed lucerne. N.Z. Jl. expt. Agric. 3: 299–304.

Kahane, I.; A. Poljakoff-Mayber. 1968. Effect of substrate salinity on the ability for protein synthesis in pea roots. Pl. Physiol. 43: 1115–1119.

Kamphake, L.J.; S.A. Hannah; J.M. Cohen. 1967. In Water Research Vol pp 206–216. Pergqman Press, Gt Britain.

Karlovsky, J. 1975. How much phosphate do we really need? N.Z. agric. Sci. 9: 149–153.

Kemp, A. 1958. Influence of fertiliser treatment of grassland on the incidence of hypomagnesaemia and hypomagnesaemic tetany (grass tetany) in milking cows. Neth. J. agric. Sci. 6: 281–297.

Kemp, A. 1960. Hypomagnesaemia in milking cows. The response of serum magnesium to alterations in herbage composition resulting from potash and nitrogen dressings on pasture. Neth. J. agric. Sci. 8: 281–304.

Kerguelen, M. 1960. Annls. Amel. Pl. 10: 177–236.

Kessler, B.; I. Snir. 1969. Salt effects on nucleic acids and protein metabolism in citrus seedlings. Proc. 1st Int. Citrus symp. 1: 381–386.

Kirkby, E.A. 1969. Ion uptake and ionic balance in plants in relation to the form of nitrogen nutrition. pp 215–235. In Ecological Aspects of the Mineral Nutrition of Plants. ed. I.H. Rorison. pub. Blackwell Scientific Pub. Oxford.

Kirkby, E.A.; K. Mengel. 1967. Ionic balance in different tissues of the tomato plant in relation to nitrate, urea or ammonium nutrition. Pl. Physiol. 42: 6–14.

Knypl, J.S. 1974. Induction of nitrate reductase activity by benzylaminopurine, succinic acid-2, 2-methyl-hydrazide, Chloramphenicol, and Chlorogenic acid in dark grown cucumber cotyledons. *In* *Mechanisms of Regulation of Plant Growth.* ed. R.L. Bieleski, A.R. Ferguson, M.M. Cresswell. Bulletin 12 Royal Soc N.Z. pp 71-76.

Kushner, D.J.; H.J. Onishi. 1966. Contribution of protein and lipid components to the salt response of envelopes on an extremely halophilic bacterium. *J. Bact.* 91: 653-660.

Kylin, A.; R. Gee. 1970. Adenosine triphosphatase activities in leaves of the mangrove *Avicennia nitida* Jacq. *Pl. Physiol.* 45: 169-172.

Laetsch, W.M. 1974. The C_4 syndrome: A structural analysis. *A. Rev. Pl. Physiol.* 25: 27-52.

Langdale, G.W.; J.R. Thomas; T.G. Littleton. 1973. Nitrogen metabolism of Stargrass as affected by nitrogen and soil salinity. *Agron. J.* 65: 468-470.

Lanyi, J.K. 1969. Studies of the electron transfer chains of extremely halophilic bacteria. III Mechanisms of the effect of sale on menadione reductase. *J. biol. Chem.* 244: 4168-4173.

Lanyi, J.K.; J. Stevenson. 1970. Studies of the electron transport chain of extremely halophilic bacteria IV Role of hydrophobic forces in the structure of menadione reductase. *J. biol. Chem.* 245: 4074-4080.

Larsen, H.; A.H. Rose; J.F. Wilkinson. 1967. *Advances in microbial physiology.* Vol 1, p 97. Academic Press. New York.

Larsen, H.; I.C. Gunsalus; R.Y. Stanier. 1962. *The Bacteria* Vol 4. Academic Press. New York.

Latiea, G.G. 1969. Dual mechanisms of salt uptake in relation to compartmentation and long-distance transport. A. Rev. Pl. Physiol. 20: 89-116.

Lehr, J.J. 1953. Sodium as an essential plant nutrient. J. Sci. Fd. Agric. 4: 460-467.

Lehr, J.J. 1960. The sodium content of meadow grass in relation to species and fertilization. Proc. 8th Int. Grassland Cong. pp 101-103.

Lehr, J.J.; J. Grashuis; E.E. Van Koetsveid. 1963. Effect of fertilization on mineral-element balance in grassland. Neth. J. agric. Sci. 11: 23-37.

Levi, E. 1970. Penetration retention and transport of foliar applied single salts of Na, K. Rb, and Cs. Physiologica Pl. 23: 811-819.

Lieberman, M.M.; J.K. Lanyi. 1971. Studies of the electron transport chain of extremely halophilic bacteria. V. Mode of action of salts on cytochrome oxodase. Biochem. biophys. Acta. 245: 21-33.

Lips, S.H.; A. Ben-Zioni; Y. Vaadia. 1971. K^+ recirculation in plants and its importance for adequate nitrate nutrition. In Recent Advances in Plant Nutrition. Vol 1 pp 207-214. ed. R.M. Samish. pub. Gordon and Breach. Lond.

Loneragan, J.F. 1973. Mineral absorption and its relation to the mineral composition of herbage. In Chemistry and Biochemistry of Herbage Vol 2. pp 103-125. ed. G.W. Butler and R.W. Bailey, Academic Press. Lond.

Lowry, M.W.; W.C. Huggins; L.A. Forrest. 1936 Georgian Agri. Expt. Sta. Bull. 193.

Lubin, M. 1963. Cell potassium and the regulation of protein synthesis In The Cellular Functions of Membrane Transport. pp 193–211. ed. J.E. Hoffman. Prentice-Hill Inc., New Jersey.

Lubin, M.; H.L. Ennis. 1964. On the role of intracellular potassium in protein synthesis. Biochem. biophys. Acta. 80: 614–631.

Lundegårdh, H. 1950. The translocation of salts and water through wheat roots. Physiologia Pl. 3: 103–151.

Lynch, P.B. 1954. Pasture responses to trace elements. N.Z. Jl. Agric. 89: 17–26.

Machlis, L. 1944. The respiration gradient in barley roots. Am. J. Bot. 31: 281–282.

McNaught, K.J. 1959. Effect of potassium fertilizer on sodium, magnesium, and calcium in plant tissue. N.Z. Jl. Agric. 99: 442.

McNaught, K.J. 1970. Diagnosis of mineral deficiencies in grass-legume pastures by plant analysis. Proc. XI Int. Grassland Congress. pp 334–338.

McNaught, K.J.; F.D. Dorofaeff. 1968. Effect of magnesium fertilisers and season on levels of inorganic nutrients in a pasture on Hamilton clay loam. II. Nitrogen, phosphorus, sulphur, potassium, sodium, and trace elements. N.Z. Jl. agric. Res. 11: 551–559.

McNaught, K.J.; J. Karlovsky. 1964. Sodium chloride responses in pasture on a potassium deficient soil. N.Z. Jl. agric. Res. 7: 386–404.

MacRobbie, E.A.C.; J. Dainty. 1958. Sodium and potassium distribution and transport in the seaweed Rhodymenia palmata (L) Grev. Physiologia Pl. 11: 782–811.

Marquez, E.D.; A.F. Brodie. 1973. The effect of cations on the heat stability of a halphilic nitrate reductase. Biochem. biophys. Acta. 321: 84-89.

Marschner, H. 1971. Why can sodium replace potassium in plants. 8th Colloq. Int. Potash Inst. Uppsala. pp 50-63. (Int. Potash Inst. Berne).

Marschner, H. 1974. Mechanisms of regulation of mineral nutrition in higher plants. In Mechanisms of Regulation of Plant Growth ed. R.L. Bieleski, A.R. Ferguson, M.M. Cresswell. Bulletin 12. The Royal Society of New Zealand. pp 99-109.

Marschner, H.; G.Mix. 1974. Einfluss von Natrium chlorid und mycostatin auf den Mineral-stoffgehalt im blattgewebe und die Feinstruktur der chloroplasten. Z. Pflanzenernähn. Bodenkd. (In press).

Mengel, K.; R. Pfluger. 1972. Sodium and magnesium contents of different varieties of perennial ryegrass as affected by temperature and atmospheric humidity. Zeitschr. Pf fur Acker-und Pflanzenbau. 4: 272-279.

Metson, A.J.; W.M.H. Saunders; T.W. Collie, V.W. Graham. 1966. Chemical composition of pastures in relation to grass tetany in beef breeding cows. N.Z. Jl. agric. Res. 9: 410-436.

Middleton, K.R. 1973. Nutrient balance and leaf analysis. Proc. 14th Technical Conference of the New Zealand Fertiliser Manufacturers Research Association pp 129-135.

Middleton, K.R.; G.S. Smith. 1974. Estimating the fertiliser rate for maintaining permanent pasture. Proc. 10th Int. Soil Congr. Moscow 4: 151-159.

Middleton, K.R.; G.S. Smith. 1976. Plant and animal nutrition and animal health. N.Z. Jl. Agric. 133: 71-72.

Middleton, K.R.; M.R.J. Toxopeus, 1973. Diagnosis and measurement of multiple deficiencies by a subtractive technique. Pl. Soil. 38: 219-226.

Miflin, B.J. 1967. Distribution of nitrate and nitrite reductase in barley. Nature, Lond. 214: 1133-1134.

Mottershead, B.F. 1971. Estimation of sulphur in biological materials using the technicon autoanalyser. Lab. Practice 20: 483-484.

Murty, K.S.; T.A. Smith; C. Bould. 1971. The relation between putrescine content and potassium status of black currant leaves. Ann. Bot. 35: 687-695.

Nagai, R.; M. Tazawa. 1962. Changes in the resting potential and ion absorption induced by light in a single cell. Plant Cell Physiol. 3: 323-329.

National Academy of Sciences - National Research Council 1966. Publ. 1349. Nutrient requirements of domestic animals. No III Nutrient requirements of dairy cattle. Third revised edition.

National Academy of Sciences - National Research Council 1963. Publ. 1137 Nutrient requirements of domestic animals. No IV Nutrient requirements of beef cattle. Fourth revised edition.

National Academy of Sciences - National Research Council 1957. Publ. 504 Nutrient requirements of domestic animals. No V Nutrient requirements of sheep.

Nissen, P. 1972. Multiphasic ion uptake in roots. In Ion Transport in Plants (Proc. Liverpool Ind. Workshop on Ion Transport). Ed. W.P. Anderson.

New Zealand Soil Bureau Soils Part 3 1968. Soil Bureau Bulletin 26 (3). Compiler N. Wells ed J. Luke. N.Z.D.S.I.R.

Oostendorp, D. 1961. The sodium supply of cattle. Landbouwvoorl 18: 8-9, 609-614.

Osmond, C.B.; G.C. Laties. 1969. Compartmentation of malate in relation to ion absorption in beet. *Pl. Physiol.* 44: 7–14

Pate, J.S. 1973. Uptake, assimilation and transport of nitrogen compounds by plants. *Soil.Biol. Biochem.* 5: 109–119.

Paterson, R.; C. Crichton. 1960. Grass staggers in large scale dairying on grass. *J. Br. Grassld. Soc.* 15: 100–105.

Phillipson, A.T.; J.L. Menon 1959. Bloat in cattle XVI. Bovine Saliva: The chemical composition of the parotid, submaxillary, and residual secretions. *N.Z. Jl. agric. Res.* 2: 990–1001.

Pierce, E.C.; C.O. Appleman. 1943. Role of soluble organic acids in the cation-anion balance in plants. *Pl. Physiol.* 18: 224–238.

Pitman, M.G. 1965. Transpiration and selective uptake of potassium by barley seedlings (*Hordeum vulgare* c.v. Bolivia). *Aust. J. biol. Sci.* 18: 987–998.

Pitman, M.G. 1966. Uptake of potassium and sodium by seedlings of *Sinapis alba*. *Aust. J. biol. Sci.* 19: 257–269.

Pitman, M.G.; A.C. Courtice; B. Lee. 1968. Comparison of potassium and sodium uptake by barley roots at high and low salt status. *Aust. J. biol Sci* 21: 871–881.

Pitman, M.G.; H.W. Saddler. 1967. Active sodium and potassium transport in cells of barley roots. *Proc. Nat. Acad. Sc. Wash.* 57: 44–52.

Pluenneke, R.H.; H.E. Joham. 1972. The influence of low substrate sodium levels upon the free amino acid content of cotton leaves. *Pl. Physiol.* 49: 502–505.

Poole, R.J. 1971. Effect of sodium on potassium fluxes at the cell membrane and vacuole membrane of red beet. *Pl. Physiol.* 47: 731–734.

Porath, E.; A. Poljakoff-Mayber. 1964. Effect of salinity on metabolic pathways in pea root tips. Israel J. Bot. 13: 115-121.

Prisco, J.T.; G.H.F. Vieira. 1976. Effects of NaCl salinity on nitrogenous compounds and proteases during germination of *Vigna sinensis* seeds. Physiol. Plant. 36: 317-320.

Pritchard, G.I.; W.J. Pigden; L.P. Folkins. 1964. Distribution of potassium, calcium, magnesium, and sodium in grasses at progressive stages of maturity. Can. J. Pl. Sc. 44: 318-324.

Rahman, H.; P. McDonald; K. Simpson. 1960. Effect of nitrogen and potassium fertilizers on the mineral status of perennial ryegrass (*Lolium perenne*). I. Mineral content. J. Sc. Fd. agric. 11: 422-432.

Rains, D.W. 1969. Cation absorption by slices of stem tissue of bean and cotton. Experientia 25: 215-216.

Rains, D.W.; E. Epstein. 1967. Sodium absorption by barley roots: Its mediation by mechanism 2 of alkali cation transport. Pl. Physiol. 42: 319-323.

Reith, J.W.S.; R.H.E. Inkson, W. Holmes; D.S. MacLusky; D. Reid; R.G. Heddle; G.J.F. Copeman. 1964. The effects of fertiliser on herbage production. II. The effect of nitrogen, phosphorus, and potassium on botanical and chemical composition. J. agric. Sc., Camb. 63: 209-219.

Richards, F.J.; R.G. Coleman. 1952. Occurrence of putrescine in potassium-deficient barley. Nature, Lond. 170: 460.

Ross, D.B. 1960. Influence of sodium on the transport of magnesium across the intestinal wall of the rat *in vitro*. Nature, Lond. 189: 840-841.

Russell, E.W. 1973. Soil Conditions and Plant Growth 10th Edition. London. Longman Group Ltd.

Sankhla, N.; W. Huber. 1975. Effect of S-H and abscisic acid on *in vivo* activity of nitrate reductase in seedlings of *Phaseolus aconitifolius*. *Z. Pflanzenphysiol Bot.* 76: 467–470.

Saunders, W.M.H.; A.J. Metson, I.J. Pohlen. 1964. Grass staggers in beef cattle. Soil studies. *NZ Jl. Agric.* 109: 53–56.

Schreven, D.A. Van. 1967. The effect of some Actinomycetes on Rhizobia and *Agrobacterium radiobacter*. *Pl. Soil.* 21: 283–302.

Shah, B.H.; R.T. Wedding. 1968. Sodium ion influence on phosphorylations associated with oxidation of succinate by turnip root mitochondria. *Science.* 160: 304–306.

Shone, M.G.T.; D.T. Clarkson; J. Sanderson. 1969. The absorption and translocation of sodium by maize seedlings. *Planta* 86: 301–314.

Smith, F.W. 1974. The effect of sodium on potassium nutrition and ionic relations in Rhodes grass. *Aust. J. agric. Res.* 25: 407–414.

Smith, G.S.; K.R. Middleton. 1974. Effects of temperature, water-stress, and liming, on the concentration of certain essential elements found in pasture plants during rapid vegetative growth. *In* *Plant Nutrition and Ion Uptake.* A Workshop Seminar. Grasslands and Applied Biochemistry Division D.S.I.R.

Smith, S.E.; P.D. Aines. 1959. Salt requirements of dairy cows. *Cornell University Agriculture Experimental Station* New York. Bulletin No 938. p 26.

Sorensen, C. 1960. The influence of nutrition on the nitrogenous constituents of plants. II. Field experiments with heavy dressings of nitrogen to fodder sugar beet. *Acta Agri. Scand.* 10: 17–44.

Splittstoesser, W.E.; H. Beevers. 1964. Acids in storage tissue. Effects of salts and aging. Pl. Physiol. 39: 163-169.

Stewart, A.B.; W. Holmes. 1953. Manuring of grassland. I. Some effects of heavy dressings of nitrogen on the mineral composition of grassland herbage. J. Sc. Fd. Agric. 4: 401-408.

Strogonov, B.P. 1962. Physiological basis of salt tolerance of plants as affected by various types of salinity. Trans. from Russian and ed. by A. Poljakoff-Mayber and A.A. Hayer 1964. Jerusalem: Israel Program for Scientific Translocations p 279.

Thibodeau, P.S.; E.G. Jaworski. 1975. Patterns of nitrogen utilization in the soybean. Planta 127: 133-147.

Thomas, B.; A. Thompson; V.A. Oyenuga; R.H. Armstrong. 1952. The ash constituents of some herbage plants at different stages of maturity. Emp. J. expt. Agric. 20: 10-22.

Tinker, P.B.H. 1971. Changes in soil sodium following its addition in fertilizers. M.A.A.F. Technical Bull. 20: 318-25.

Tinker, P.B.H. 1965. The effects of nitrogen, potassium, and sodium fertilizers on sugar beet. J. agric. Sci. Camb. 65: 207-212.

Tisdale, S.L.; W.L. Nelson. 1970. Soil Fertility and Fertilizers 2nd Edition London. Collier-MacMillan International.

Tottingham, W.E.; H.L. Stephens; E.J. Lease. 1934. Influence of shorter light rays upon absorption of nitrate by the young wheat plant. Pl. Physiol. 9: 127-142.

Tuil, H.D.W. Van 1970. The organic-salt content of plants in relation to growth and nitrogen nutrition. pp 45-68. In Nitrogen nutrition of plants. Ed. E.A. Kirkby University of Leeds.

Underwood, C.B.E. 1966. Sodium and chlorine pp 80-91. In The Mineral Nutrition of Livestock. Pub. by arrangement with the Food and Agriculture Organisation of the United Nations by Comm. Agric. Bur. Aberdeen.

Ulrich, A. 1941. Metabolism of non-volatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. Am. J. Bot. 28: 526-537.

Ulrich, A.; K. Ohki. 1956. Chlorine, bromine, and sodium as nutrients for sugar beet plants. Pl. Physiol. 31: 171-181.

Van Steveninck, R.F.M. 1966. Some metabolic implications of the tris effect in beetroot tissue. Aust J. biol. Sci. 19: 271-281.

Vickery, H.B.; J.K. Palmer. 1957. The metabolism of the organic acids of tobacco leaves. XII. Effect of culture of excised leaves in solutions of malonate at pH 4 to pH 7. J. biol. Chem. 225: 629-640.

Viets, F.G. Jr. 1944. Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. Pl. Physiol. 19: 466-480.

Wallace, A.; N. Hemaidon. 1963. Sodium transport from roots to shoots in bush bean and radish. Pl. Physiol. 38: viii

Wallace, A.; N. Hemaidon; S.M. Sufi. 1965. Sodium translocation in bush beans. Soil Sci. 100: 331-334.

Wallace, W.; J.S. Pate. 1965. Nitrate reductase in field pea (Pisum arvense L.) Ann. Bot. 29: 655-671.

Wallace, W.; J.S. Pate. 1967. Nitrate assimilation in higher plants with special reference to the Cocklebur (Xanthium pennsylvanicum Wallr). Ann. Bot. 31: 213-228.

Watson, R.W. 1942. The mechanism of elongation in palisade cells. New Phytol. 41: 206.

Weimberg, R. 1968. An electrophoretic analysis of isozymes of malate dehydrogenase in several different plants. Pl. Physiol. 43: 622-628.

Weimberg, R. 1970. Enzyme levels in pea seedlings grown on highly salinized media. Pl. Physiol. 46: 466-470.

Weissman, G.S. 1972. Influence of ammonium and nitrate nutrition on enzymatic activity on soybean and sunflower. Pl. Physiol. 49: 138-141.

Wells, N. 1962. 'Available' sodium (Sodium in Sweet Vernal Grass) in unfertilised soils. Single Factor Map 59 and 60. Dept. Sci. and Ind. Res., Wellington.

Wells, N. 1969. A reconnaissance of the element content of Wairarapa pastures and white clover plants. Proc. N.Z. Grassld. Ass. 31: 71-79.

Whitehead, D.C.; E.C. Jones. 1969. Nutrient elements in the herbage of white clover, red clover, lucerne, and sainfoin. J. Sci. Fd. Agric. 20: 584-591.

Whitehead, D.C.; L.H.P. Jones. 1972. The effect of replacing potassium by sodium on cation uptake and transport to the shoots in four legumes and Italian ryegrass. Ann. appl. Biol. 71: 81-89.

Willmer, C.M.; T.A. Mansfield. 1970. Active cation transport and stomatal opening a possible physiological role of sodium ions. Pflanzenphysiol 61: 398-400

Wilson, J.K. 1943. Nitrate in plants: Its relation to fertilizer injury, changes during silage making, and indirect toxicity to animals. J. Am. Soc. Agron. 35: 279-290.

Wilson, R.W. 1971. The role of univalent cations in enzyme systems. In Recent Advances in Plant Nutrition. Vol 1 pp 167–187. ed. R.M. Samish, New York. Gordon and Breech.

Wright, M.J.; K.L. Davison. 1964. Nitrate accumulation in crops and nitrate poisoning in animals. Ad. Agron. 16: 197–247.

Wright, N.; R.J. Trautman; L.J. Streetman. 1960. Nitrate accumulation in Blue Panicgrass. Agron. J. 52: 671–672.

Wybenga, J.M. 1957. A contribution to the knowledge of the importance of sodium in plant life. Investigation with radioactive sodium. D. Agric Sc. Thesis (Kontoordruk de Goede Wageningen).

Zielke, H.R.; P. Filner. 1971. Synthesis and turnover of nitrate reductase induced by nitrate in cultured tobacco cells. J. biol. Chem. 246: 1772–1779.