ZINC AS A SUBSOIL NUTRIENT FOR CEREALS

A thesis submitted

by

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CONTENTS

ABSTRACT

In southern Australia, where subsoils are predominantly alkaline and pH increases with depth, the available zinc status of soils is low and cereals may suffer from zinc deficiency. This deficiency has traditionally been treated by application of zinc fertilizer to the cultivated layer from which the downward movement of zinc is unlikely. Evidence has accumulated over the past decade that a lack of zinc in the medium external to the root impairs the function of roots and that zinc may be required in subsoil as well as topsoil to correct the problem.

Field experiments were established at two sites at Minnipa in South Australia to measure the effects of deep placement to 0.4 m of zinc, nitrogen and phosphorus on wheat (*Tritium aestivum* L. cv. Machete) and barley (*Hordeum vulgare* L. cv. Stirling).

In view of the wide (0.45 m) spacing between the tynes of the deep ripper used for fertilizer placement, some clear effects were observed. Zinc concentrations in youngest emerged blades (YEBs) were generally highest where zinc was applied with nitrogen-phosphorus (NP) fertilizer. Grain yields were not highly correlated with zinc concentration in YEBs. Zinc concentration in grain was highest where a mixture in water of zinc sulphate, monoammonium phosphate and ammonium nitrate was applied to the subsoil. Subsoil placement of zinc and NP fertilizer significantly increased wheat grain yields and zinc concentrations in grain above placement in topsoil at one site in the second year. The apparent benefits of subsoil placement of zinc with NP fertilizers are worthy of further investigation in areas where alkaline subsoils occur.

In southern Australia, there exists a body of anecdotal evidence that wheat grown after field peas (*Pisum sativum* L.) is more productive than when grown after pasture legumes (principally *Medicago* spp.) The possibility that the different abilities of various species to mobilise zinc may be of benefit to following crops has only recently been considered. A deep pot experiment was conducted to compare the abilities of several antecedent species to cope with zinc deficient soil and to modify the available

zinc status of the soil to benefit wheat grown in the following year. Six species, *Lupinus pilosus* (cv. 20957), *Pisum sativum* L. (cv. Early Dun.), *Medicago truncatula* Gaertn. (cv. Parabinga), *Triticum durum* L. (cv. Durati), *Hordeum leporinum* Link and *Brassica juncea* Czern and Coss (cv. Pusa Bold) were grown in pots containing zinc-deficient Laffer sand fertilized with basal nutrients other than zinc. The same species, apart from *B. juncea,* were also grown in pots to which 0.25 mg Zn kg^{-1} soil was also added.

Of the five species, *L. pilosus* was the most zinc efficient and *H. leporinum* the least. *B. juncea* produced more dry matter in soil of low zinc status than other species and displayed no symptoms of zinc deficiency. The large seeded grain legumes produced significantly more dry matter to anthesis in zinc-deficient soil than *M. truncatula* or members of the *Poaceae*. *T. durum* (cv. Durati) was grown in the same pots and harvested three weeks after sowing when plants were almost completely necrotic. Durati shoots produced significantly more dry weight at harvest in soil of low zinc status following *P. sativum* than other species apart from *L. pilosus*. The data suggest that the reported better performance of cereals after *P. sativum* compared with *Medicago* based pastures is a real effect and may be due in part to an enhanced availability of zinc. In *T. aestivum* L. (cv. Excalibur) grown in the same soil for 20 weeks, zinc uptake following grain legumes in zinc deficient soil was significantly higher than after the *Poaceae* or *B. juncea*. Uptakes of several nutrients were significantly depressed in Excalibur grown after *H. leporinum* compared with other species. Durati appears to have a higher critical concentration for zinc than Excalibur.

The zinc efficiencies, root growth and production characterisitics of the wheat cultivars Gatcher (zinc-inefficient) and Excalibur (zinc-efficient) in infertile, alkaline subsoil typical of that which occurs on Eyre Peninsula were compared in a pot experiment. The principal hypothesis tested was that the zinc-efficiency of Excalibur, when compared with that of Gatcher, is due primarily to the ability of Excalibur to produce a greater surface area of roots. Zinc-efficient Excalibur wheat demonstrated a clear advantage in terms of grain yield compared with the inefficient cultivar Gatcher

when grown in a calcareous alkaline subsoil of low zinc status when other basal nutrients were added. Zinc uptake in Excalibur tops was the equivalent to that in Gatcher although Excalibur produced a total root length about half that of Gatcher. Excalibur also displayed a greater degree of internal efficiency for diverting zinc to grain formation.

Zinc efficiency offers a cost-effective approach to growing cereals on soils of low zinc status. However, more efficient grain production with respect to zinc supply does not necessarily imply higher zinc concentration in grain. The relationship between zinc placement in soil and grain concentration of zinc and other parameters in Excalibur were examined. Plants were grown in pots with three layers of sand each 20 cm deep. Basal nutrients were added to the whole soil but zinc was added at 0.5 mg Zn kg^{-1} soil in various combinations of layers. There were no differences in grain yield but the highest concentrations of zinc in grain occurred in pots containing added zinc in all three layers. Where only one layer was treated with zinc, concentrations of zinc in grain were highest where zinc was added to the bottom layer. In the zinc-efficient wheat cultivar Excalibur, high zinc concentrations in grain were dependent on a supply of adequate zinc throughout the root zone. Increasing the depth of placement to any degree above the standard 0.05 m used in the field in southern Australia is likely to have a beneficial effect on zinc concentration in grain.

The literature reveals a paucity of field studies of subsoil infertility, particularly with specific reference to zinc. The thesis describes investigations into field and pot studies of various aspects of subsoil infertility, including the possible roles of zinc efficiency in cereals and crop rotations in addressing this problem. The data indicate several promising avenues for further investigation.

STATEMENT

This thesis contains no material that has been accepted for the award of any other degree or diploma in any University, and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except when due reference is made in the text.

I consent to allow this thesis to be borrowed or copied, and any information contained herein cited elsewhere provided the author is duly acknowledged.

R. E. HOLLØWAY

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1.0 INTRODUCTION

There have been few field studies published in which subsoil infertility has been addressed, particularly with respect to zinc, although it is often acknowledged that where plants are grown on exposed subsoils (or "cut" soils), zinc deficiency is common (*e.g.* Takkar and Walker 1993). However, glasshouse and laboratory experiments have indicated that substantial benefits may accompany the correction of zinc deficiency in the subsoil. For instance, Nable and Webb (1993) demonstrated that withholding zinc from the subsoil in pots delayed head emergence, decreased water use and decreased grain yield in the zinc-inefficient wheat cultivar Gatcher by 20% compared with plants produced in pots to which zinc had been added in the subsoil. The zinc-efficient cultivar Excalibur was not affected in the same way, although zinc concentrations in grain were 50% less from pots with zinc-deficient subsoil than in grain from pots with fertilized subsoil.

In 1992, a national workshop was convened in Tanunda in South Australia to discuss "subsoil constraints to root growth and high soil water and nutrient use by plants". The workshop was a response to an increasing awareness that constraints to plant growth below the cultivated layer were of more significance than had been previously realised and that addressing these limitations could offer substantial increases in plant production. For example, Coventry *et al.* (1987) reported large grain yield increases in wheat over a five year period in north-eastern Victoria as a result of treating compacted acid soils with a combination of deep ripping and added lime.

Because the greater proportion of fertilizers are applied to the cultivated layer, seldom deeper than 0.05 m, nutrients become unavailable as the surface dries and it is possible that the placement of fertilizers in the subsoil will encourage root growth and enhance nutrient and water uptake from soil which remains wet as the surface dries. This has been clearly demonstrated with respect to copper by Graham (1991). Yield responses in crops and pastures have also been reported to deep placement of phosphorus (Jarvis and Bolland 1990, Simpson and Pinkerton 1989) and nitrogen

(Garwood and Williams 1967) and to nitrogen and phosphorus combined (Murphy *et al.* 1978, Slattery and Rainbow 1995).

Graham and Ascher (1993) have begun to address the problem of infertile, highly alkaline subsoils in South Australia by measuring responses to added organic matter or nutrients over a period of up to seven years. Subsoils were removed from pits in layers to a depth of 0.9 m, mixed with organic matter or nutrients and replaced. Control treatments consisted of soil removal and replacement with no additions of nutrients (physical disturbance) and a no-disturbance treatment. Substantial responses to the addition of organic matter or added nitrogen, phosphorus and trace elements in terms of vegetative and grain yields were recorded on a calcareous soil over a period of seven years. Large yield increases above the controls were recorded with the same treatments at most other sites (apart from more fertile red-brown earths). Interestingly, yield increases also occurred at sites in which the subsoils contained high concentrations of boron. It is estimated that the cropping area affected by high concentrations of boron exceeds 10 million hectares in the low rainfall (<350 mm per annum) cereal growing areas of southern Australia (Graham *et al.* 1992b). Upper Eyre Peninsula, in South Australia, constitutes about 20% of the State's wheat producing area and is characterised by coarse textured, calcareous soils, with low annual rainfall. In the majority of agricultural soils, alkalinity increases with depth and boron and salt concentrations are often sufficiently high to present a hazard to root growth (Holloway 1991, Holloway and Alston 1992). Such subsoils have been justifiably labelled "inhospitable" or "hostile" (Graham *et al.* 1992b).

1.1 THE PROBLEM DEFINED

In the calcareous soils of southern Australia, with highly alkaline subsoils, the available zinc status of the subsoil is likely to be low (Tiller 1983). Indeed, the low zinc status of these soils in general is becoming more evident as cropping frequency increases and high analysis fertilizers replace superphosphate, in which zinc has been a significant component (Riley *et al.* 1992), as the principal source of phosphorus. Where fertilizers are applied to correct zinc deficiency, the zinc is likely to be

retained at the surface (Jones *et al.* 1957), even in coarse textured sandy soils (Brennan and McGrath 1988).

There are few reports of where the effects of deep placement of zinc in the field have been investigated, particularly in alkaline soils. However, there are sound reasons for examining the effects of such a practice. While it has been shown that, given an adequate supply of zinc in part of the root zone, zinc can be transported in the phloem to maintain root growth rates in unsupplied zones (Loneragan *et al.* 1987, Webb and Loneragan 1990), it has also been demonstrated that adequate root function requires zinc in the external medium (Welch *et al.* 1982, Loneragan *et al.* 1987, Nable and Webb 1993). The integrity of root cell membranes is dependent on a supply of zinc in the external medium, as zinc is a component of superoxide dismutase which prevents the peroxidation of membrane proteins and lipids by superoxide radicals (Welch *et al.* 1982, Cakmak and Marschner 1988b). Where subsoils contain high concentrations of boron, low concentrations of zinc in the soil solution may allow boron to accumulate to toxic concentrations in wheat (Singh *et al.* 1990) and barley (Graham *et al.* 1987).

The identification of zinc efficiency in the South Australian wheat cultivar Excalibur (Graham *et al.* 1992a) has been an important development in an area where, for instance, a survey of farms in part of South Australia's low rainfall wheat producing area indicated that 96% of crops had foliar zinc concentrations of less than 20 mg Zn kg^{-1} (Hannam 1991). Zinc efficiency is likely to be of particular benefit where zinc fertilizer is concentrated at the surface and the topsoil is subject to drying. The physiological bases for zinc efficiency in Excalibur are not clear.

King *et al.* (1992) conducted a rotation experiment on a zinc-deficient deep red duplex soil in South Australia and reported that zinc uptake in cereals was generally greater when the cereals were grown after grain legumes than after mixed pastures, with grasses and *Medicago* species. There exists also a body of anecdotal evidence that cereal crops in southern Australia perform better after grain legumes (particularly field peas *Pisum sativum* L.) than after *Medicago* species.

It is axiomatic that where grain furnishes a high proportion of the human diet, adequate quantities of available zinc should be present in the grain. In his review of the requirements of humans for zinc in plants, Welch (1993) has indicated that marginal deficiency occurs in a sufficient number of human population groups and livestock as to be a cause for concern.

Although plant products contribute only a small proportion of the dietary zinc intake in some developed countries, it is likely that plant foods will constitute a higher proportion of zinc supply in future diets. While animal products contain substances which promote the availability of zinc in the human digestive system, there are several antinutritive factors, such as phytic acid and plant fibre which inhibit the absorption of zinc in the human gastro-intestinal tract. Welch (1993) has concluded that plant sources of zinc are poorly available compared with animal foods in the human diet because they contain lower quantities of those substances (possibly methionine and cysteine) which promote the availability of zinc. However, House and Welch (1989) have shown that it is possible to increase zinc concentration in grain without altering its availability to laboratory rats. In that case, increasing zinc concentration in grain by manipulation of fertilizers or plant genes has worthwhile benefits for human nutrition. Other benefits include enhanced growth and production of plants derived from seed with high concentrations of zinc grown in soils of low zinc status (Rengel and Graham 1995a,b).

In the experiment of Nable and Webb (1993) referred to above, the zinc efficiency of Excalibur in terms of grain yield and water usage was clearly demonstrated in comparison with the inefficient cultivar Gatcher when zinc was withheld from the subsoil. However, it is cause for concern that Excalibur had consistently lower zinc concentrations in grain than Gatcher, independent of the subsoil treatment and that when the subsoil was not fertilized with zinc, zinc concentrations in Excalibur grain were halved compared with grain from pots in which both soil layers were fertilized.

1.2 OBJECTIVES OF THESIS

Addressing the problem of infertile subsoils is likely to require more than one approach. Three avenues have been identified by Graham and Ascher (1993) and involve deep incorporation of green manure crops to a depth of about 0.3 m, injecting nutrients to a depth of 0.4 - 0.5 m as part of a deep tillage operation and breeding varieties which are tolerant to subsoil infertility. Graham and Rengel (1993) have postulated that roots of genotypes which are better able to mobilise zinc from the rhizosphere are more likely to be able to penetrate infertile, calcareous subsoils, since subsoil infertility with respect to micronutrients at least is usually one of availability rather than total quantity (Graham 1984).

Of the three avenues suggested by Graham and Ascher (1993) for approaching the problems of subsoil infertility, two have been investigated in the following thesis, with particular reference to zinc. Field experiments were established at Minnipa, on Eyre Peninsula in South Australia to investigate the effects of applying nutrients (principally zinc, nitrogen and phosphorus) to the subsoil to a depth of 0.4 m with a modified deep ripper.

The comparative performance of zinc-efficient and inefficient wheat cultivars has not been determined with respect to their adaptation to alkaline subsoils and their potentials for root growth in these soils. The question also remains: is zinc efficiency a function of measurable differences in root morphology? A laboratory pot experiment was designed to compare the growth of the zinc-inefficient wheat cultivar Gatcher with the efficient cultivar Excalibur in alkaline subsoil, either untreated or with added nutrients.

A deep pot experiment was designed to measure the zinc efficiencies (in terms of dry matter production) of a range of species grown in siliceous sand containing 0.3% added calcium carbonate and added nutrients with and without zinc. The effects of added zinc on root growth were compared. While it is apparent that some species are able to mobilise zinc from zinc-deficient soils, whether or not this is beneficial to

following crops is unknown. The hypothesis that zinc uptake in wheat plants grown in the same pots in the following year would not be affected by the antecedent species was tested.

Zinc efficiency in cereals offers a cost-effective approach to growing wheat on soils of low zinc status, but it is important that the relationship between zinc allocation to grain and the distribution of zinc in the root zone be better understood. To this end, a pot experiment was designed to measure the effects of zinc placement in the soil on the zinc concentrations and uptake in Excalibur, particularly with respect to concentrations in grain.

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

Understanding the role of zinc as an essential micronutrient is, to borrow a phrase from Tiller (1983), "a continuing process". The first steps were taken in the 19th century but only in the past 30 years has real progress been made in understanding the importance of zinc as a key component of a large number of enzyme systems, including those which are involved in gene expression. This review begins with a brief outline of the developing recognition of zinc as an essential micronutrient for plants. The role of zinc in stabilising root membranes is discussed, with the consequences of zinc deficiency for cell membrane integrity. Zinc uptake in plants is discussed in terms of zinc in the soil solution, with particular emphasis on the effects of pH on zinc availability, since the field work in this thesis was conducted in an area of highly alkaline soils. Uptake of zinc is also discussed with reference to zinc in the rhizosphere. Because zinc has a relatively low coefficient of diffusion, the rhizosphere assumes major significance for the zinc nutrition of plants and the changes which take place in the rhizosphere as a result of zinc and iron deficiency are of particular interest. Mechanisms of zinc uptake are not well understood but developments in this area are considered, based on the work of Kochian *et al.* (1991) and Welch *et al.* (1993) which addresses the putative roles of cation channels and phytosiderophores, induced by zinc deficiency, in mediating zinc uptake. While the majority of investigations into the role of vesicular arbuscular (VA) mycorrhizae in nutrient uptake in plants has focussed on phosphorus, it is apparent that VA mycorrhizae may play a significant role in the supply of zinc to host plants and some of the work in this area is discussed. The effects of crop rotations on zinc uptake are also considered, although the data published in this field are limited. The considerable literature on interactions between zinc and phosphorus are briefly considered, with interactions of zinc with other nutrients.

In recent years, the development of cereals with zinc efficiency traits, the heritable ability to tolerate soils of low zinc status, has been a promising development with considerable environmental and economic benefits. The agronomic implications of zinc efficiency in cereals and the state of progress in understanding the genetic and physiological bases for zinc efficiency are discussed.

Progress in dealing with zinc deficiency is also outlined - the diagnosis of deficiency through soil and plant tests and the correction of deficiency by fertilizer application.

Finally, because a portion of the work described in this thesis devolves on subsoil infertility with particular reference to zinc, a section of this review is devoted to the relationships between root growth and water and nutrient uptake in the subsoil. Only recently has attention been given to the subsoil as a source of nutrients, and some of the pioneering work in this field is discussed, concluding with reference to the possible role of zinc in the subsoil.

2.2 THE ROLE OF ZINC IN PLANT GROWTH

2.2.1 A brief history

Zinc is now understood to function in a considerable array of biochemical processes within plants, but fifty years were to pass between the Raulins' initial investigations in 1863 and 1869 which demonstrated the inability of certain cultured fungi to grow in media lacking zinc and Javillier's (1912) report that soil treatments of zinc sulphate increased the growth of some field crops. Mazé (1914, 1915, 1919) is credited with producing the first reliable evidence that zinc is needed for normal plant growth, although Sommer and Lipman's (1926) nutrient culture experiments marked the beginning of general acceptance that zinc is essential for plants.

During the next three decades, a slowly clarifying picture of the geographical extent of zinc deficiency emerged, most severely expressed in the United States and

Australia, but also in many other countries. Originally, zinc deficiency symptoms in plants were considered to be non nutritional because of the rapid and severe deterioration of the plant. Widespread recognition of zinc deficiency was retarded by the difficulty of removing zinc from nutrient solutions and plant containers (Thorne 1957). The earliest reports of responses to zinc in fruit trees come from Chandler *et al.* (1931) who in fact applied ferrous sulphate to a range of fruit trees in California exhibiting "little leaf" symptoms with some success. However, a batch of relatively pure ferrous sulphate failed to correct the condition and the probable role of zinc as an impurity in the ferrous sulphate was eventually deduced (Chandler *et al.* 1931).

In South Australia, it was realised by 1936 (Strickland 1937) that "mottle leaf" in citrus had been related in the United States to zinc deficiency and by the end of the year foliar applications of zinc sulphate had been able to correct the condition and corresponding recommendations were thereafter made to orchardists. Investigations into the need for zinc and copper in an extensive portion of aeolian calcareous and podsolized sands in the south-east of South Australia began in the same year, and spectacular responses to copper, zinc and superphosphate by pasture and cereal crops were reported from deficient areas by Riceman and Anderson (1943). Zinc deficiency in flax crops growing on red-brown earths with alkaline subsoils was also reported by Adams and Piper (1944). Zinc deficiency was discovered in plants growing on a range of virgin soils in South Australia - on acid sands, acid to neutral red-brown earths, calcareous sands, "mallee soils" (denoting the colloquial name for the predominant *Eucalypt* species), shallow grey and red calcareous soils, grey and black heavy textured soils and ground water rendzinas (Reuter *et al.* 1988). Virgin soils deficient in zinc and copper are also found in other states of Australia (Newman 1956), particularly Western Australia (Dunne and Elliott 1950, Toms 1958).

Thorne (1957) reviewed progress, suggesting a role for zinc in hormone and enzyme systems. By 1972, when Lindsay produced his exhaustive review, it was recognised that zinc played a vital role in oxidation within cells and a range of metabolic

processes, including the promotion of water absorption. Lindsay (1972) listed several dehydrogenases, proteinases and peptidases in which zinc is involved.

Price *et al.* (1972) also suggested the putative role of zinc in stabilising cytoplasmic ribosomes. Miller *et al.* (1985) reported the essential role of zinc in genetic transcription factor IIIA in *Xenopus laevis* oocytes and later confirmed the role of "zinc fingers" in gene expression and regulation. Many regulatory proteins which are bound to the recognition sequence of genes contain a domain specifically for binding to DNA. This structural motif for DNA recognition is the "zinc finger" - linearly structured repeated domains, each domain centred on a tetrahedral arrangement of zinc ligands to histidine and cysteine, the most common ligands in enzymes and proteins (Klug and Rhodes 1987, Miller *et al.* 1985). Klug and Rhodes (1987) have suggested that zinc is peculiarly suited to binding two parts of a protein in this way because, unlike a disulphide bridge, it could not be reduced within the cell (since it does not change its valency values) where redox reactions which would occur with copper or iron for example, would lead to the production of hydrolysing radicals able to attack RNA or DNA (Williams 1984).

As recently as 1972, carbonic anhydrase was, in the words of Lindsay (1972) "the only authenticated zinc metallo-enzyme in living organisms". A comprehensive review of the metabolic and physiological roles of zinc and its chemical forms in plants was recently produced by Brown *et al.* (1993) who noted that more than 70 metallo-enzymes containing zinc have now been identified although these represent only a minor fraction of total zinc in the plant. Zinc enzymes are known to occur in each of the six enzyme classes - oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (Barak and Helmke 1993).

2.2.2 Zinc and cell membrane integrity

While zinc appears to have a pervasive physiological role - in photosynthesis, reproduction, disease resistance (Graham 1983) and plant hormone and protein

metabolism - of particular interest is its role in cell membrane integrity. Impairment of the plasma membrane has been implicated in increased root exudation from zinc deficient plants, but other cell membranes, such as the tonoplast, are thought to be involved as well (Cakmak and Marschner 1988a). Both membranes comprise a continuous lipid bilayer which is relatively impermeable to the passage of ions so that macro and micronutrient transport into the cell depends on specific transport proteins incorporated within the lipid bilayer (Kochian 1991). The basis for understanding the role of zinc in membrane physiology was founded on extensive work with animal cell membranes. Most authors in this field refer to Chvapil (1973), Sunamoto *et al.* (1980) and Bettger and O'Dell (1981) as early sources for establishing the place of zinc in cell membrane physiology; membrane integrity was shown to require zinc in combination with sulphydryl groups of membrane proteins (Chvapil 1973), and phospholipids (Sunamoto *et al.* 1980, von Glos and Boursnell, 1981).

Welch *et al.* (1982) concluded from phosphorus accumulation studies with zinc-deficient plants and absorption studies on excised roots that zinc also played a fundamental role in the stability of plant cell membranes and that the effect of destabilising membranes was not easily reversed, as it is with calcium. Interestingly, these effects can occur very early in the growth cycle when the external root environment is deprived of zinc, despite adequate amounts for normal shoot growth (from seed reserves) in roots and shoots. An important conclusion from this work is that zinc is required (as is calcium, phosphorus, boron and manganese (Graham *et al.* 1992a)) in the external solution to maintain membrane integrity. In a range of plant genotypes grown without zinc, phosphorus accumulated to toxic levels in the oldest leaves but young wheat seedlings deprived of zinc absorbed less ^{32}P over eight days than those grown in a solution containing 2.5 μmol l^{-1} zinc - even though the plants not supplied with zinc were clearly displaying symptoms of phosphorus toxicity (Welch *et al.* 1982). This apparently contradictory result led to the astute hypothesis that the zinc deficient roots had in fact "leaked" (and exchanged) greater quantities of phosphorus during a thirty minute "desorption" period in unlabelled KH_2PO_4. This hypothesis was verified by an experiment in which deficient roots retained less

labelled phosphorus during the "desorption" period. It was also demonstrated that this effect was not specific to phosphorus alone - in a solution of sufficiently low phosphorus to avoid toxicity, zinc deficient roots retained 60% less Cl^- from labelled KCl than roots adequately supplied with zinc.

The fundamental role of zinc in maintaining cellular membrane integrity was thus deduced. It was proposed that zinc-deficient root cells, having impaired membrane integrity, could allow non selective mass flow of nutrient phosphorus into the roots, which could then accumulate particularly in actively transpiring older leaves. It was further hypothesised that zinc was involved in structural orientation of macro molecules in the membrane or with ion transport mechanisms.

Marschner *et al.* (1987) further reported on this phenomenon through studying root excretions of young cotton (*Gossypium hirsutum)* plants with severe, mild or no zinc deficiency. Roots of highly deficient plants excreted 3.3 times more amino acids and 2.6 times more carbohydrate than the zinc-sufficient control plants and the electrical conductivity of the root exudate solution was also tripled. In a more detailed study, Cakmak and Marschner (1988a) examined the effects of zinc deficiency on root exudation of cotton (*Gossypium hirsutum* L. cv. Deltapine 15/21), wheat (*Triticum aestivum* L cv. Cumhuriyet 75), tomato (*Lycopersicon esculentum* L cv. Super Marmande) and apple (*Malus domesticus* cv. M 26 rootstock) grown in controlled conditions. While zinc status did not affect potassium concentration within roots, K^+ leakage was significantly higher from roots of zinc-deficient plants. This leakage could be decreased by resupplying zinc for twelve hours.

It is of particular historical interest at this point that Chandler *et al.* (1931) noted that bark on the roots of fruit trees affected by "little leaf" (zinc deficiency was as yet unrecognised in these trees) contained "no more than 10% of the normal potassium content. It seems that this is due to an injury to the bark that permits leaking out of the potassium". Applying potassium to the trees did not help improve the condition.

Leakage of NO_3^- and amino acids was also observed in zinc deficient cotton plants, while tomato plants excreted significantly more amino acids (Cakmak and Marschner 1988a). Other low molecular weight compounds, excreted to the greatest degree by zinc-deficient apples, included sugars and phenols. The significant leakage of K^+ from zinc-deficient plants and the ability of added zinc to reduce this leakage provides strong evidence that a loss of membrane integrity is responsible for the increased exudation from zinc-deficient plants, rather than an excessive accumulation within the root cells. While calcium also plays a vital role in preserving the membrane integrity of root cells and is required in the external solution, calcium and zinc appear to operate at different sites (Cakmak and Marschner 1988a) in the root cell membrane.

There is now ample evidence for the importance of zinc in preventing the peroxidation of fatty acids in plant cell membranes (Cakmak and Marschner 1988a). Cakmak and Marschner (1988b) considered that the principal role of zinc in preserving membrane integrity lay with its ability to protect membrane proteins and lipids from the destructive effects of superoxide radicals ($O_2^{\cdot-}$) and their derivatives (*e.g.* $OH^{\cdot}$) produced by redox reactions within the cell. Again, work with animal cells has previously demonstrated the proclivity of these radicals for peroxidative damage of proteins and phospholipids (Demopoulos *et al.* 1980, Fridovich 1983, Autor *et al.* 1984). Zinc inhibits this damage by overriding the production of free oxygen radicals and scavenging those which occur through superoxide dismutase (SOD) one of which, a copper-zinc SOD, is the most abundant of the SODs in plant cells (Cakmak and Marschner 1988a).

Cells of zinc-deficient cotton roots have been shown to have a decreased level of SOD activity (Cakmak and Marschner 1987) and increased NADPH dependent $O_2^{\cdot-}$ production. These effects were later observed in bean and tomato roots as well (Cakmak and Marschner 1988b). NADPH oxidases catalyse peroxidative membrane damage and are themselves a source of $O_2^{\cdot-}$ (Cakmak and Marschner 1988c) in the microsomal membranes and the cytosol. Also associated with the protective role of SOD is catalase, an enzyme able to detoxify H_2O_2 (Autor *et al.* 1984). Cakmak and

Marschner (1988b) have clearly shown that both SOD and catalase levels fall rapidly in zinc-deficient root cells and that increased $O_2^{\cdot-}$ production coincides with the appearance of zinc deficiency symptoms in bean, cotton and tomato plants. As zinc deficiency symptoms become more severe, NADPH is oxidised at a greater rate and SOD and catalase activities decrease. Resupply of zinc to deficient plants significantly decreases $O_2^{\cdot-}$ generation. While there is some evidence for the direct effect of zinc on membrane integrity (Chvapil 1973), the indirect effects of zinc on root membranes through its role in SOD are more completely documented (Dhindsa *et al.* 1981, Cakmak and Marschner 1988a,b).

2.3 ZINC UPTAKE BY PLANTS

2.3.1 Zinc in the soil solution

Zinc is available to plants principally via the soil solution, although the greater part of soil zinc occurs in the solid phase associated with clay minerals, hydrous oxides and organic matter and a proportion of this is exchangeable (Armour *et al.* 1990, Barak and Helmke 1993). Lindsay (1979) represented the species contributing to total zinc in solution as:

$$[\text{Total soluble zinc}] = [Zn_{\text{inorganic}}] + [Zn_{\text{organic}}]$$

and: $$[Zn_{\text{inorganic}}] = [Zn^{2+}] + [ZnSO_4^0] + [ZnOH^+] + [ZnOH_2^0] + [ZnHPO_4^0]$$

Of the total amount of zinc contained in soil, the isotopically exchangeable zinc (commonly 4-6% in Australian acid soils (Tiller *et al.* 1972)) is that portion most likely to be available to plants (Barak and Helmke 1993). Zinc which may become available to plants occurs as exchangeable zinc on cation exchange sites or as organically complexed zinc in the colloidal soil fraction. Jeffery and Uren (1983) and Barber (1984) considered that most of the zinc in the soil solution occurs either as free metal ions or as labile complexes with organic matter. Zn(II) is a relatively effective Lewis acid in terms of its tendency to form covalent bonds with ligands (Barak and Helmke 1993). Zn^{2+} in solution is surrounded by six water molecules to

give $Zn(H_2O)_6^{2+}$, a multiprotic acid which can lose protons. The first step gives $ZnOH(H_2O)_5^{+}$ or $ZnOH^{+}$ (Barrow 1993). In their review of zinc chemistry as it affects plants, Barak and Helmke (1993) suggested that from the available evidence, the free hydrated cationic form of zinc is the only form which can be actively absorbed by plant roots (although this conclusion is based on a history of some controversy - see Kabata Pendias and Pendias 1984). Soluble complexed zinc (derived from reactions with organic acids, amino acids, or fulvic acids, Lindsay 1972) may also be transported to plant roots, (Hodgson *et al.* 1966) increasing the concentration of total soluble zinc and thereby indirectly enhancing zinc supply at the root surface (Hodgson *et al.* 1966, Treeby *et al.* 1989).

McGrath *et al.* (1988) measured a range of 15-65% of the zinc in displaced soil solutions as the free metal ion. More recently, Barak and Helmke (1993) suggested that "about one-half" of the dissolved zinc in the soil solution is in this form. McBride and Blasiak (1979) demonstrated that the proportion of zinc complexed in solution increased with increasing pH. Hodgson *et al.* (1965, 1966) showed that the amount of zinc complexed by organic matter increased as the amount of soluble organic matter present increased. Complexed zinc appears to be an important fraction of soluble zinc in calcareous soils (Hodgson *et al.* 1966). However, the possibility remains that at high pH, applied zinc may also be adsorbed by insoluble organic matter (Moraghan and Mascagni 1991). Humic acid ligands, for example, may form strong insoluble complexes with metal ions (Chairidchai and Ritchie 1990). It is worthy of note at this point that it is not easy to measure free metal ion concentrations separately from metal-ligand complexes and that the total concentration of zinc in solution can not be assumed to equate to free metal ion concentration - an implication often made in producing solubility graphs (McBride 1989). Nevertheless, it can be expected that if plants absorb only the free cationic form of zinc, then as absorption takes place, a shift in equilibrium will result in its replacement from other species of zinc present. The *rate* of zinc absorption will be a function of the concentration of the cation itself.

In the few attempts which have been made to measure them, values of concentrations of total zinc in surface soil solutions are usually extremely low (Linehan *et al.* 1989, Sinclair *et al.* 1990). For instance, while Barber (1984) cited the unpublished work of Warncke which indicated a range of 25 - 250 $\mu g\ l^{-1}$ from 60 Indiana soils, Hodgson *et al.* (1966) indicated average values from Colorado and New York soils ranging from less than 2 $\mu g\ l^{-1}$ to an average of 20 $\mu g\ l^{-1}$. Kabata-Pendias and Pendias (1984) reported from the literature a range varying from 4 to 270 $\mu g\ l^{-1}$ and in a Californian survey, Barak and Helmke (1993) reported that values of total dissolved zinc in water saturation extracts from 30 soil series had a median value of 40 $\mu g\ l^{-1}$. The values obtained will vary of course with soil and measurement technique. In Jeffery and Uren's (1983) study of the behaviour of zinc and copper in a sandy loam soil limed to give a range of pH values, the concentration of zinc in the soil solution decreased from 1 800 to 12 $\mu g\ l^{-1}$ between pH 4.4 and 7.5. Kiekens (1990) determined from the data of Lindsay (1972, 1979) that at pH 5, Zn^{2+} activity in solution is about 6.5×10^{3} $\mu g\ l^{-1}$ (10^{-4} M), decreasing to 6.5×10^{-3} $\mu g\ l^{-1}$ (10^{-10} M) at pH 8 - a 100 fold decrease for each unit rise in pH. The concentration of zinc in solution appears to be controlled by adsorption reactions with inorganic colloids and organic ligands so that with rising pH, zinc adsorption by soil and the formation of organic complexes is increased. The adsorption of zinc is more pH dependent at low concentrations (e.g. 6.5 $\mu g\ l^{-1}$) than at higher concentrations (*e.g.* 6.5×10^{6} $\mu g\ l^{-1}$) (Moraghan and Mascagni 1991). Soil pH controls the adsorption of zinc by changing either the availability of adsorption sites or the concentration of zinc species which are preferentially adsorbed (Barrow 1986a,b).

Lindsay (1979) has succinctly expressed the general solubility relationship for zinc in soils as:

$$\text{soil-Zn} + 2H^{+} \Leftrightarrow Zn^{2+} \quad (\log K^{0} = 5.8)$$

$$\text{and:} \quad \log Zn^{2+} = 5.8 - 2\ pH$$

$$\text{or:} \quad pZn \Leftrightarrow 2\ pH - 5.8$$

That is, Zn^{2+} activity varies directly with the square of proton activity, or decreases with increasing pH. Lindsay (1979) lists solubilities for a range of zinc minerals, all of which decrease 100 fold for each unit rise in pH. An alternative equation is that of Herms (1982 in Kiekens 1990).

$$pZn = 0.5\ pH + 1.02$$

The difference as Kiekens (1990) interprets it, is that dissolution-precipitation reactions are not the sole determinants of Zn solubility in soils, - complexation and adsorption-desorption reactions will also control zinc solubility.

For a range of Australian soils, Tiller (1983) suggested the relationship:

$$pZn \cong 0.75\ pH + 2.8$$

Below pH 7.7, Zn^{2+} appears to predominate as the major species in solution; between pH 7.7 and 9.1, $Zn(OH)^{+}$ appears to predominate and at higher pH, $Zn\ (OH)^{0}_{2}$. According to Tiller (1983), "*$Zn(OH)^{0}_{2}$ = Zn^{2+} at about pH 7.5....However, if the solubility of zinc as shown by Norvell and Lindsay (1969) has general applicability in the form they propose (Lindsay 1972), e.g. $[Zn^{2+}] = 10^{6}\ [H^{+}]$, then the actual concentration of $Zn(OH)^{0}_{2}$ in the soil solution should be about 10^{-9} M and relatively independent of pH within the range pH 6-8 covered by Norvell and Lindsay's data*". Complexes $ZnSO_4^0$ and $ZnHPO_4^0$ can also be significant in determining total zinc in solution, the latter mainly in neutral and alkaline soils, depending on pH and $H_2PO_4^-$ activity (Lindsay 1979).

The pK for

$$H_2PO_4^- \Leftrightarrow HPO_4^{2-} + H^+$$

is about 7, so that at pH 4, $H_2PO_4^-$ predominates while at pH 8, HPO_4^{2-} predominates, with the proportion of HPO_4^{2-} increasing by an order of magnitude for each unit increase in pH (Barrow 1987). When $H_2PO_4^-$ = 10^{-4} M, $ZnHPO_4^0$ has an activity equal to $ZnOH^+$. The activity of $ZnSO_4^0$ is equivalent to Zn^{2+} when SO_4^{2-} activity is

$10^{-2.33}$ M. Hence, fertilizers which contain high concentrations of sulphate such as $(NH_4)_2SO_4$ can increase zinc solubility and mobility (Lindsay 1979).

The large effects of pH on the availability of zinc contrast with the lower dependence of the availability of copper to changing pH (Loneragan 1975, Jeffery and Uren 1983). The major effect of pH on zinc availability is referred to throughout the literature as a prime determinant of zinc availability in soils, but the presence of biologically produced chelating ligands is also significant (Sinclair *et al.* 1990).

2.3.2 Zinc supply and the rhizosphere

Roots withdraw nutrients and water from a zone of soil immediately adjacent to the root surface and in this zone, plant exudates and lysates attract a host of microflora and microfauna which interact with the plant in highly complex ways. This zone of influence exerted by the root on the soil is known as the rhizosphere. With respect to terms used to describe root exudation in the rhizosphere, Gliński and Lipiec (1990) have taken some care to define the differences between exudates and secretions, but the terms appear to be used interchangeably in the literature in general.

Nutrients are delivered to the root surface by mass flow or diffusion. Mass flow is a process by which ions are carried to the root surface in water taken up by the plant to meet transpirational demands. (Barber's (1984) concept of mass flow did not infer that ions were taken into the plant by the water absorbed). When ions are required at a greater rate than can be supplied by mass flow, a concentration gradient between the root surface and soil generates the diffusion of ions to the root surface. Zinc concentrations in the soil solution are too low to allow the plant's requirements to be met by mass flow. Zinc supply to the roots is principally by diffusion, which Marschner (1993) suggested, is confined to a zone delineated by the limits of the root hair cylinder. For a rooting density of 2-4 cm cm^{-3}, about 10% of the soil could be expected to supply zinc to the roots by diffusion. Both Barber (1974) and Linehan *et al.* (1989) have concluded that mass flow is unlikely to provide more than a minor

part of the micronutrient requirement of plants and this has been discussed by Marschner (1993) who suggested that typically, about 2 mg of zinc might be provided by mass flow in total from a bulk soil solution of 6.5 μg Zn l^{-1} (10^{-7} M), with a normal plant demand of 10-30 mg zinc kg^{-1} dry weight. Calcareous soil solutions are more likely to have a zinc concentration of about 0.65 μg l^{-1} (10^{-8} M). Increasing pH lowers the diffusion coefficient for zinc (De_{Zn}), probably because a lower proportion of the zinc is present in solution (Clarke and Graham 1968). In calcareous soils De_{Zn} values may be 50 times lower than in acid soils (Marschner 1993). On the other hand, increasing organic matter in soil can lead to an increased value of De_{Zn} by desorption and complexation (Sharma and Deb 1988).

As roots grow in soil, they expand cylindrically and compress the soil around them, possibly enhancing diffusion characteristics of rhizosphere soil. A simplified model developed by Dexter (1987) allows for calculations of porosity changes around roots as they grow and the amounts of nutrients which lie within given distances of the root surfaces. In calculating nutrient inflow from soil solutions Sinclair *et al.* (1990) assumed a "zone of influence" extending 3.5 mm from the root as a minimum, based on Smith *et al.* (1986), which incidentally, use Dexter's (1987) initial estimates for the compression of soil by roots.

Supply to plants is determined by mobility and transport limitations so that in effect, the major potential source of zinc becomes the rhizosphere (Marschner 1993). Changes in the rhizosphere then, due to root exudation or ion exchange, have important implications for the zinc nutrition of plants, as they alter the mobility of zinc from that of the bulk soil. With the rhizosphere exerting such a large influence on zinc nutrition, root morphology patterns and root surface area may assume major importance in determining the availability of zinc in the soil.

The ability of roots to modify their environment by changing the pH of the rhizosphere confers a key nutritional advantage to some plants. The pH of the

rhizosphere changes according to imbalanced cation/anion uptake ratios which induce roots to excrete H^+ and HCO_3^- (or OH^-) to maintain neutrality of charge at the soil root interface.

Cation/anion uptake ratios and hence rhizosphere pH are most strongly influenced by the form of nitrogen supplied to the plants - whether NH_4^+ or NO_3^- (Dodge and Hiatt 1972, Raven and Smith 1976, Marschner *et al.* 1982, Marschner and Römheld 1983, Hausling *et al.* 1985). If nitrogen is absorbed as NO_3^-, the pH rises as HCO_3^- ions are excreted as a net effect. If NH_4^+ is absorbed, H^+ ions are excreted and the pH falls. Besides these differences, roots fed with NH^+_4 tend to have more and longer lateral branches than NO_3^--fed roots (Marschner *et al.* 1986). Marschner (1991) recorded a pH decrease in the rhizosphere of beans growing in a Luvisol from 6.8 to 5.4 with NH_4^+ nutrition and an increase to 7.3 with NO_3^- nutrition. Zinc concentrations increased from 34 mg Zn kg^{-1} dry matter in the shoots of the NO_3^--fed plants to 49 mg Zn kg^{-1} in those plants receiving NH^+_4. The degree of lowering of pH in the rhizosphere may be significantly decreased as the initial soil pH increases (Riley and Barber 1971).

Nyatsanga and Pierre (1973) measured a net excretion of 0.8 mmol H^+ g^{-1} of dry weight of roots into soil from lucerne (*Medicago sativa* L.) roots, and Marschner (1993) predicts that symbiotic nitrogen fixing legumes would take up more zinc than those relying on NO_3^- as their principal source of nitrogen. Nitrogen fixing legumes are also able to solubilize poorly soluble phosphates in the rhizosphere (Nye 1992).

The majority of monocotyledons, particularly the *Poaceae* (formerly *Gramineae*) reduce NO_3^- in the roots, (as opposed to those species in which the nitrate reductase system occurs in the shoots) and with NO_3^- the main source of nitrogen in most soils, HCO_3^- ions are excreted. Obviously the ability to change the pH will vary considerably with species and soil conditions. For instance, roots of phosphorus deficient rape *(Brassica napus)* are able to acidify the rhizosphere despite importing

NO_3^- as the principal source of nitrogen (Nye 1992), while rape roots supplied with phosphate do not have the same effect. In solution culture it was shown that rape roots stopped excreting HCO_3^- if NO_3^- was withdrawn. If phosphorus was withdrawn the uptake of NO_3^- was decreased much more severely than cation uptake, especially in the root apices, significantly decreasing HCO_3^- excretion (Moorby *et al.* 1985, 1988).

Other factors which allow roots to alter the rhizosphere pH are organic acid excretion, CO_2 released as a result of respiration and microbial acid production from excreted carbon (Nye 1992). Nye (1992) considers increased CO_2 from respiration and excretion of organic acids and bases from root surfaces to be of significantly less importance than the excretion of H^+ and HCO_3^- induced by imbalanced cation/anion uptake ratios. He reasons that the rise of CO_2 pressure near the root surface in aerobic soils is very low, (although CO_2 levels as high as 3% have been reported in soils (Cosby *et. al* 1985)) and that only small amounts of organic acids could be released because of the close control of cytoplasm pH at between 6 and 7, much higher than the pK of any potential organic acids, which would then be excreted as anions. Nye (1992) calculates that if a third of the non CO_2 carbon exuded by roots were to be microbially transformed to low molecular weight acids, 1 mmol charge g^{-1} plant dry weight could result, but mentions that nowhere near this amount has ever been measured "despite diligent research". On the other hand, Dinkelaker *et al.* (1989) have determined that 23% of the dry weight of *Lupinus albus* plants was excreted as citrate from proteoid roots as a specific response to phosphorus deficiency.

According to Riley and Barber (1971), pH in the rhizosphere may change by as much as two units from that of the bulk soil and the change of pH across the rhizosphere has been modelled on parameters including pH buffer capacity, soil water content, bulk soil pH and rate of acid release from the root surface (Riley and Barber 1971, Nye 1986). The ability of roots to modify the rhizosphere pH in a given soil differs widely between species and even between cultivars of the same species (Marschner *et al.* 1986).

2.3.3 Specific effects of proteoid roots

A particular response by some species to phosphorus deficiency is the formation of "proteoid" roots, (Gardner, *et al.* 1982a,b) so called because of their similarity to the root clusters of the *Proteaceae* (Lamont 1982). Proteoid roots comprise clusters of dense root branchlets about 5 - 10 mm long, supporting an intense profusion of root hairs. They are formed in white lupins (*Lupinus albus* L.) as a specific response to phosphorus deficiency (Gardner *et al.* 1982 b, Marschner *et al.* 1987) and may constitute 50% of total root dry weight (Dinkelaker *et al.* 1989). When white lupins were grown in a phosphorus-deficient calcareous (20% $CaCO_3$, pH (H_2O) 7.5) subsoil and fed with NO_3^- as the nitrogen source, pH in the proteoid rhizosphere of 13 week old plants fell to 4.8, and this was explained as a result of citric acid excretion, leading to dissociation of $CaCO_3$ in the rhizosphere and deposition of calcium citrate ($Ca_3(C_6H_5O_7)_2$). It was calculated that each plant exuded 1 g of citrate - 23% of the plant total dry weight (Dinkelaker *et al.* 1989). This is of interest in the light of studies of 30 years previously (*e.g.* Harmsen and Jager 1962) suggesting that vetch (*Vicia* spp) exuded 1.6 - 2.9% of the total carbon in the roots as exudates. Harmsen and Jager (1962) also measured a range of 2.6 to 22.5 mg carbon exuded by single wheat plants over two months. In recent years it has been commonly accepted that root exudates may represent 20-30% of the plant's photosynthetically fixed carbon (Uren and Reisenauer 1987, Marschner *et al.* 1990). Dinkelaker *et al.* (1989) measured a decrease in "available" (water extractable, calcium acetate/lactate extractable and sodium bicarbonate extractable) phosphorus concentration in the rhizosphere soil of proteoid roots, while "available" (DTPA extractable) iron, manganese and zinc increased. DTPA extractable zinc increased from 2.8 μmol Zn kg^{-1} in the dry bulk soil to 16.8 μmol Zn kg^{-1} in the proteoid rhizosphere - the sixfold increase recorded is probably a direct effect of lowered pH.

The ability of *L. albus* roots to decrease the pH in the proteoid rhizosphere from 7.5 to 4.8 is truly remarkable in a soil containing 20% $CaCO_3$. It would be of particular interest to test the rhizosphere acidifying capabilities of *L. albus* or *L. cosentinii* Walp. (which also forms proteoid roots) (Trinick 1977) in some southern Australian

soils which consist of 60-80% $CaCO_3$. Obviously the effects would be decreased (or delayed) in the presence of such high buffering capacity. In calcareous soils, soluble phosphorus depresses proteoid root formation and decreases uptake of zinc, iron and manganese (Marschner 1993). In the experiment of Dinkelaker *et al.* (1989), NO_3^- constituted the source of nitrogen, there were few root nodules and the small excess of cations absorbed above anions could produce much less than the amount of H^+ needed to lower the pH of the rhizosphere from 7.5 to 4.8. It was concluded that "citrate is excreted consistently with protons, presumably by a proton citrate co-transport".

Perhaps one of the most interesting aspects of the rhizosphere acidification phenomenon is the ability of *L. albus* to mobilise nutrients in excess of its own requirements as indicated by improved phosphorus, nitrogen and manganese uptake in wheat grown in mixed culture with *L. albus* (Gardner and Boundy 1983, Marschner *et al.* 1986).

2.3.4 Rhizosphere responses to iron and zinc deficiencies

Recent attention has focussed on the response of both monocotyledons and dicotyledons to iron deficiency and the consequent mobilisation of zinc (and other divalent micronutrient cations) as a result of modification of the rhizosphere, particularly in calcareous soils. Members of the family *Poaceae*, the grasses, have a unique response to iron deficiency - the release of non-protein amino acids - phytosiderophores. Siderophores are iron transport chelators produced microbially and the term phytosiderophore refers specifically to siderophores produced by grasses. Amino acid root exudates capable of chelating Fe(III) include avenic acid from oats (Fushiya *et al.* 1980, Marschner *et al.* 1989), 2'-deoxymugineic acid from wheat (Nomoto *et al.* 1981) and mugineic acid from rye and barley (Nomoto and Ofune 1982, Takagi *et al.* 1984). Rice does not appear to excrete mugineic acid and appears unable to take up iron from nutrient solutions where the pH is greater than 6, nor does the addition of synthetic chelators EDTA, HEDTA, EDDHA, DTPA or the

organic acids citric and malic acid have any stimulating effect on iron uptake. However, the addition of mugineic acid (1-15 μmol l^{-1}) was able to reverse the effects of iron deficiency in rice in a solution containing 20 μmol l^{-1} $^{-59}FeCl_3$ at pH 7 (Takagi *et al.* 1984). There is some difference of opinion about whether the phytosiderophore-complexed molecules transported into the cell are specific for iron (Marschner *et al.* 1989, Römheld and Marschner 1986), or may carry other divalent micronutrient cations (Crowley *et al.* 1991). For instance, Kochian (1993) proposes that zinc may be absorbed as Zn^{2+} across the plasma membranes via a putative cation channel or as a complex with an organic ligand. In the case of grasses, it was suggested that a zinc-phytosiderophore complex is transported into the cell by a transport protein. Phytosiderophores are certainly able to chelate zinc, copper and manganese, and in the case of zinc, appeared to be as effective as DTPA in mobilising zinc from calcareous soil (14.8% $CaCO_3$, pH 8.6) as demonstrated by Treeby *et al.* (1989) with exudates from iron-deficient barley roots. This property appears to be exclusive to the *Poaceae*. Because of the ability of phytosiderophores to complex more than iron alone, Crowley *et al.* (1987) have suggested the generic term phytochelate, or as Welch (1993) has recently proposed - phytometallophore. This term has been adopted in this review when referring to uptake of nutrients other than iron.

Marschner *et al.* (1989) have distinguished between non-specific reactions by plants to iron deficiency - principally root-induced changes of redox potential or pH, release of chelators or modifications of microbial activity in the rhizosphere - and specific mechanisms. The latter are categorised into "Strategy I" and "Strategy II" responses. Strategy I (or inducible) responses (stimulated reductase activity at the plasma membrane and enhanced net excretion of H^+ and phenols and other reducing compounds) are exhibited principally by dicotyledon and non *Poaceae* monocotyledon species, and Strategy II (or constitutive) responses (release of phytometallophores) by members of the *Poaceae*. Plants with Strategy II adaptations may be able to mobilize zinc, copper and manganese in calcareous soils, where such a mechanism may be crucial to an adequate supply of these nutrients. Enhanced zinc uptake by iron-

deficient dicotyledons is likely to be due to rhizosphere acidification (a Strategy I mechanism).

Because the majority of zinc taken up by plants is supplied from the rhizosphere, root-induced changes in the rhizosphere are likely to have a large effect on zinc uptake. Changes in the rhizosphere are induced by zinc deficiency. In dicotyledons, zinc deficiency leads to an increase in the cation/anion uptake ratio which in turn leads to acidification of the rhizosphere. Even when nitrogen is supplied as NO_3^-, the rhizosphere can be acidified by an increase in the ratio of cations to anions taken up (with a strong decrease in NO_3^- uptake in zinc deficient plants) (Cakmak and Marschner 1990) and, as has been discussed previously, a concomitant release of K^+, amino acids, sugars and phenols (Cakmak and Marschner 1988a, Zhang *et al.* 1991). The role of such exudates as chemotaxic stimuli to root pathogens and the enhanced susceptibility of zinc-deficient plants to root diseases has attracted increasing interest in recent years (*e.g.* Sparrow and Graham 1988, Brennan 1992a, Graham *et al.* 1992a, Thongbai *et al.* 1993a,b).

Zinc deficiency is also associated with a loss of membrane integrity and associated efflux of low molecular weight organic solutes. Iron concentrations have been increased in zinc-deficient Strategy I and Strategy II plants (Loneragan and Webb 1993). In navy beans *(Phaseolus vulgaris L.)* (Strategy I), zinc deficiency appears to increase the excretion of compounds which are able to reduce Fe(III) to Fe(II). Further, this response can also be related to the zinc efficiency status of the plants (Brown 1979).

In the *Poaceae*, some of the root exudates comprise phytometallophores which are able to mobilise zinc. For instance, it has been observed recently that the same exudates which mobilise Fe(III) also appear to be released in response to zinc deficiency and are able to enhance zinc uptake by wheat (Zhang *et al.* 1989, 1991). It is also likely that the profusion of other exudates (*e.g.* sugars, amino acids and

phenols) which are released in response to zinc deficiency (Marschner *et al.* 1990) - will enhance zinc mobility through increased microbial activity and ensuing formation of chelating agents (Marschner 1993).

Loneragan *et al.* (1987) used a split-root technique to show that the ability of part (50%) of the root system of wheat to acidify the external nutrient solution (containing NH_4^+) was depressed by the omission of zinc. The omission of zinc from the solution did not affect the root dry matter produced by that part, and while the concentration of zinc in the roots supplied by phloem transport appeared to be adequate, it was obviously insufficient to maintain normal root function.

2.3.5 Mechanisms of zinc uptake

The specific mechanisms of zinc uptake by plants are as yet speculative. Zinc appears to be absorbed by plant roots primarily as Zn^{2+} from the soil solution, and zinc uptake appears to be mediated by a protein with a strong affinity for zinc which transports the zinc across the plasma membrane (Kochian 1993). Kochian (1993) suggested that zinc transport across the plasma membrane is towards a large negative electrical potential, so that the process would be thermodynamically passive. He proposes that the negative electrical potential of the plasma membrane could provide the "driving force" for zinc uptake by means of a divalent cation channel in dicotyledons or monocotyledons which are not members of the *Poaceae*. In monocotyledons, it was proposed that non protein amino acids or phytometallophores, which are released into the rhizosphere as a result of iron or zinc deficiency, form a complex with Zn^{2+} which is transported to the outer face of the root-cell plasma membrane. The complex is then transported into the cell via a transport protein (Kochian 1993).

Kochian (1993) speculated that ion channels, known to facilitate high rates of transport into the root of macronutrients, may be involved in micronutrient uptake in dicotyledons and monocotyledons which are not members of the *Poaceae*. Because

ion channels do not appear to be so specific as to select a single ion, it is possible that a channel which transports divalent cationic macronutrients could also transport zinc (and other divalent cationic micronutrients) into the cell. The ionic radius of Zn^{2+} (which varies from 0.68 Å in four-fold co-ordination to 0.83 Å in six-fold co-ordination) occurs in the same range as Mg^{2+}, Cu^{2+}, and Fe^{2+}, but is significantly smaller than Ca^{2+}, which has an ionic radius of 1.08Å (CN=6) (Barak and Helmke 1993). The rate of entry would be limited of course by the low activity of free Zn^{2+} in the soil solution. This speculative model is based on research by Kochian *et al.* (1991) and Welch *et al.* (1993) indicating that iron deficiency in peas enhances absorption of zinc, manganese, copper and magnesium. Much of the speculation centres on the role of a ferric chelate reductase formed at the plasma membrane in response to iron deficiency. As a result of the reductase activity, Fe(III) is reduced to Fe(II), which is transported into the cell by a second mechanism involving a putative transport protein. The evidence that the reductase is involved in the regulation of ion channels responsible for uptake of divalent cations is compelling. The fact that zinc and magnesium concentrations were increased to a significant degree in the pea root tissue (Kochian 1991) negates any effect due to reduction, since they are not subject to redox reactions in the biological pH range. Secondly, the same group have measured a doubling of labelled $^{59}Fe^{2+}$ absorption by roots from solutions with Fe^{2+} activity controlled by chelate buffering - in other words, ion uptake was increased independent of reduction. In addition, Cu deficiency is now known to be able to induce Cu(II) reducing ferric reductase (Welch *et al.* 1993). Some species of navy bean (Jolley and Brown 1991) and peas (Kochian 1993) are also able to induce ferric reductase activity in the roots of zinc-deficient plants.

Kochian (1993) suggested that the stimulation of ferric reductase activity in the plasma membrane induced by iron, copper or zinc deficiency, in some way mediates zinc influx into the cell, possibly by reducing sulphydryl groups at the cytoplasmic face of the membrane. These sulphydryl groups appear to regulate the opening and closing of the channel. The relationship between the reductase and the reduction of the

sulphydryl groups which regulate access to the channel is unclear, but the model does provide a foundation for future research.

Kochian (1993) has also proposed a model for uptake by members of the *Poaceae* in which phytometallophores are synthesised from nicotianamine in the cytoplasm as a response to either iron or zinc deficiency and are released into the rhizosphere. Either Zn^{2+} or Fe^{3+} ions are then complexed, mobilised and transported into the root cell via the same putative transport protein. The data of Marschner *et al.* (1989) and Zhang *et al.* (1989) indicate that iron uptake by deficient barley roots was two to three orders of magnitude greater when supplied as a complex with mugineic acid (ferric 3-hydroxy mugineic acid) than when supplied as Fe(III) EDTA or ferric hydroxide. Conversely, uptake rates of zinc as the zinc-mugineic complex were not different from uptake rates of zinc sulphate or Zn(II) EDTA. This would tend to suggest a high specificity for the iron-mugineic acid complex. However, Kochian's (1993) model is supported by the observations of Crowley *et al.* (1991) that both zinc and iron-mugineic acid complexes were absorbed at similar rates. More recently, Crowley *et al.* (1992) demonstrated that micro-organisms could strongly affect iron uptake rates from siderophores and phytosiderophores and could be "an unpredictable confounding factor in experiments examining mechanisms for utilization of microbial siderophores or siderophores under non sterile conditions". As Kochian (1993) has said, "this is an area of research that clearly awaits further investigation".

2.3.6 Effects of crop rotations on zinc uptake

Improvements in crop yields as a result of rotational effects - benefits conferred on one species by a previously grown species - have been sufficiently well tested to have stood the test of time. Macrae and Mehuys (1985) have referred to records of crop rotation in China as far back as the Han dynasty in 1000 BC. Bullock (1992) lists increased nitrogen supply and decreased weed, insect and disease problems as the principal sources of benefit, with nitrogen considered to be the main factor responsible. Bullock (1992) has pointed out that much of the improvement in yield

credited to nitrogen derived from grain legumes, may, in fact, be due to other factors because the net gain of nitrogen after the grain is removed from a legume crop is usually quite low and may even be negative (although not always - see for example Reeves *et al.* 1984). According to King (1990) microbial breakdown of organic matter constitutes a highly available source of plant nutrients as does the transformation of soil material by microbial byproducts such as organic acids released during respiration. Microbial enhanced chelation of zinc may also exert a considerable influence on the benefits conferred by antecedent species.

Chandi and Takkar (1982) considered the effect of a range of rotations on the fate of zinc fertilizer applied to the soil surface at a zinc-responsive site. There were differences between rotations in the maintenance of residual zinc and the rate of decrease of available zinc. Rotational effects were also recorded in rates of change of various labile zinc fractions. For instance, a continuous cereal rotation (wheat-maize) was responsible for the maximum decrease in exchangeable zinc while a cereal legume rotation (wheat-peanuts) led to the greatest decrease in the amount of zinc associated with organic matter. Most of the zinc added in fertilizer remained in the top 30 cm.

The amount of data relating to differences in micronutrient concentration and uptake due to rotations are limited. King *et al.* (1992) conducted a rotational experiment on a deep red duplex soil in the Upper South East of South Australia from 1976 to 1979. The rotations comprised wheat and barley (sown as split plot pairs) in alternate years to volunteer pasture (mainly grasses and broadleaved weeds), sown pasture (mainly grasses with introduced medic and subterranean clover), lupins (*L. angustifolius*), peas, tick beans (*Vicia faba*) or continuous wheat or barley. The rotations were phased so that each part of the rotation appeared in each season. Zinc uptake in wheat and barley was more dependent on rainfall than rotation. Zinc concentrations in whole wheat shoots were consistently lowest in wheat after lupins at tillering and anthesis, although the effect was small and not always significant. Similarly, zinc uptake was consistently lowest after volunteer and sown pasture. Zinc uptake in grain

from the peas-wheat, beans-wheat and continuous wheat rotations tended to be consistently higher than from pasture-wheat rotations. The influence of available zinc on the incidence of the root disease *Rhizoctonia solani* (which was significantly less in wheat sown after pulses (9.8%), than after grassy pastures (14.5%) or continuous wheat (14%) (King 1984)) was discussed. Oliver *et al.* (1993) reported higher concentrations of cadmium in wheat grain grown after lupins (*L. angustifolius*) than in wheat grown after wheat, barley, field peas, beans (*Vicia* spp.), volunteer pasture, sown pasture, or fallow. While the authors noted that the higher cadmium concentrations could not be explained solely by acidification, pH measurements were made on bulk soil samples: subtle changes in the rhizosphere would not be detected.

2.3.7 Mycorrhizal influence on nutrient uptake

The role of vesicular arbuscular (VA) mycorrhizae (the most common of the mycorrhizae in agricultural plants) in enhancing the effectiveness of nutrient uptake by crop plants is receiving increasing recognition, particularly because of their low host specificity level (Gianinazzi-Pearson 1984) and their ability to take up nutrients from infertile soils in biotrophic symbiosis with host plants (Tinker 1975). The role of VA mycorrhizae in modifying phosphorus uptake in plants has been a predominant subject of research in this field. Because zinc and phosphorus have low diffusion coefficients and are primarily supplied to the root by diffusion, it is likely that the research on effects of VA mycorrhizae on phosphorus uptake will have some relevance for uptake of zinc and possibly other immobile nutrients.

Marschner (1993) noted that there is widespread evidence that VA mycorrhizal infection enhances zinc uptake, particularly in soils with low zinc mobility. In mycorrhizal plants, phosphorus and zinc concentrations and total uptake both tend to increase. In conditions where VA mycorrhizal infection is depressed by high soil phosphorus, the consequential increase in phosphorus in the soil solution can offset the hyphal transfer but no such compensation occurs for zinc unless it too is provided.

Generally, zinc deficiency tends to be exacerbated where VA mycorrhizal infection is suppressed by high soil phosphorus or by some rotations (Thompson 1990). There is now some (albeit anecdotal) evidence that root degradation products of *Brassica juncea* for instance, may destroy mycorrhizae (A.J. Rathjen, pers. comm.) and some species which are poor hosts of mycorrhizae in previous year's rotations may induce lower infection levels in following crops and hence lead to decreased zinc uptake (Legget and Westermann 1986). VA mycorrhizal symbiosis may also account for absence of zinc deficiency symptoms in plants in soils with low zinc content (Thompson 1990). Obviously, VA mycorrhizae may play a crucial role in many circumstances in determining the zinc status of plants and as in the case of phosphorus efficiency (Baon 1994), zinc efficiency in cereals may well be a function, at least in part, of VA mycorrhizal infection.

Mycorrhizal infection also affects the partitioning of phosphorus between roots and shoots. A great deal of care needs to be exercised in interpreting data related to the utilisation of phosphorus by infected and non infected plants. As Baon *et al.* (1992) have noted, mycorrhizal infection may be positively correlated with efficiency of uptake in some species (*e.g.* barley) but not others (*e.g.* wheat or rye) whereas utilisation efficiency - the ability to produce a greater amount of dry matter per unit of phosphorus absorbed - may be negatively correlated with infection (*e.g.* barley and wheat in Baon's (1994) study). The addition of phosphorus fertilizer tends to decrease mycorrhizal colonisation of roots (Baon *et al.* 1992).

Inoculation of cereals with VA mycorrhizal fungi has led to positive dry matter and grain yield responses in phosphorus-deficient soils (Khan 1975, Jakobsen 1983) and the resulting symbiosis may enhance phosphorus uptake (Jakobsen 1983). Mycorrhizal plants are reported to take up phosphorus at a greater rate than uninfected plants (Sanders and Tinker 1971) and there is some evidence that mycorrhizal plants are able to extract phosphorus from sources of low availability such as rock phosphate (Pairunan *et al.* 1980). Besides being host dependent, a propensity for VA mycorrhizal infection may be an inherited trait (Mercy *et al.* 1990)

and there appear to be differences in infection among cultivars of wheat when inoculated with VA mycorrhizal fungi, the development of which appears to depend on the carbohydrate supplied by the host (Azcón and Ocampo 1981). Exudation of sugars from roots may be associated with infection of roots by mycorrhizae. Mycorrhizal infection can change the relative performances of cereals in terms of phosphorus efficiency in utilisation, uptake and agronomic efficiency (Baon 1994).

2.4 INTERACTIONS BETWEEN ZINC AND OTHER NUTRIENTS

2.4.1 Phosphorus

Zinc may interact with other nutrients in ways which affect its availability in the soil or its metabolism and transport within the plant. Loneragan and Webb (1993) have suggested that the most important interactions of zinc with other nutrients in crop production are those with NP fertilizers in soils where either zinc, phosphorus or nitrogen is limiting. Of all the interactions of zinc with other nutrients, zinc - phosphorus interactions have been studied in the greatest detail and have elicited the widest comment and engendered the strongest controversy. The pointed comments of Loneragan and Webb (1993) are worthy of reproduction in their entirety.

"There is a voluminous and confusing literature on P-Zn interactions. Much of the confusion has arisen from workers who failed to identify the factor operative in an interaction or who used conditions irrelevant to zinc deficiency. Others have compounded the confusion by accepting conclusions without critical evaluation of the experimental conditions and data or by looking for a single magic phenomenon, 'the P-Zn' interaction or the 'P-induced Zn deficiency' to explain the many phenomena now know to occur; some even look for an interaction under conditions where, since neither Zn or P are limiting or excessive, there is no reason to expect any".

"The value of careful, critical research in the understanding of nutrient interactions is well illustrated by recent research which has provided a new insight into the long puzzling phenomenon of 'P enhanced Zn requirements'. It contrasts with the huge volume of uncritical and unproductive research correlating P/Zn ratios with Zn deficiency and serves as a warning against the current trend of producing "desktop"

interactions from the uncritical development of relationships between every possible combination of nutrients resulting from the availability of multi-element analysers and high capacity computers".

Charitably, the authors do not produce an exhaustive list of examples. They distinguish two broad categories of phosphorus-zinc interactions - those in which increasing phosphorus applications decrease concentrations of zinc in the shoot and those in which zinc concentrations are not decreased. When phosphorus and zinc are both limiting or marginal, increased growth induced by added phosphorus can induce or exacerbate zinc deficiency - the growth dilution effect (Loneragan *et al.* 1979, Singh *et al.* 1988): adding both phosphorus and zinc corrects both limitations. At times, zinc concentrations are depressed below that which can be explained by dilution alone. Sharma *et al.* (1968) and Loneragan and Webb (1993) have suggested several mechanisms for this, based on phosphorus-induced depression of zinc absorption by roots, or on the translocation of zinc from roots to shoots.

There appears to be no concrete evidence for inhibition of zinc transport to the shoots by excess phosphorus. Loneragan and Webb (1993) refer to Chaudhry and Loneragan (1970) as evidence that excess phosphorus does not inhibit zinc transport to shoots although discussion in that paper refers to the effect of nitrogen (rather than phosphorus) fertilizer in increasing the proportion of zinc (and copper) in plant tops compared with roots, contradicting earlier suggestions that nitrogen fertilizers restricted zinc transport to shoots (Ozanne 1955).

Loneragan *et al.* (1979) related potentially toxic phosphorus concentrations to the intensity of symptoms with zinc deficiency in tissues which demonstrated "P enhanced zinc requirements". Loneragan *et al.* (1982) confirmed that zinc deficiency in Okra (*Abelmoschus esculentis* L Moench) strongly increased phosphorus transport to tops and accumulation in leaves, interfered with phosphorus metabolism and, with high phosphorus supplies, allowed phosphorus to accumulate to toxic concentrations in leaves, inducing or aggravating symptoms resembling zinc deficiency. In other words, symptoms previously attributed to zinc deficiency in leaves with adequate zinc

could be attributed to phosphorus toxicity. Similarly, leaf symptoms could also be attributed to phosphorus toxicity in subterranean clover (Loneragan *et al.* 1979), cotton (Cakmak and Marschner 1986) and wheat supplied with low zinc and high phosphorus concentrations (Webb and Loneragan 1988). Webb and Loneragan (1990) showed that the rate of phosphorus absorption by roots was depressed in a low zinc environment. This observation contrasts with some previous observations (Cakmak and Marschner 1986, Webb and Loneragan 1988), but is consistent with other experiments in which the role of zinc in membrane integrity was assessed and in which lower phosphorus absorption was attributed to phosphorus leakage (Welch *et al.* 1982). It is also possible for uptake of phosphorus by roots to decrease at the same time that translocation to shoots increases under conditions of phosphorus efflux from the cytoplasmic inorganic pool. This efflux may be induced in a low zinc environment (Loughman *et al.* 1982).

Thus, zinc deficiency appears to have at most only a temporary effect in enhancing phosphorus absorption and the controlling influence for the enhanced phosphorus concentrations in shoots induced by zinc deficiency seems to be a function of those mechanisms which determine phosphorus transport and distribution from roots to shoots (Marschner and Cakmak 1986, Webb and Loneragan 1990). Concentrations of water soluble zinc in roots and shoots can be lowered by high phosphorus supply and in cotton, visual symptoms of zinc deficiency and other detrimental effects of zinc deficiency (low levels of chlorophyll and compromised membrane integrity) have been closely correlated with concentration of water soluble but not total zinc in leaves. Hence, water soluble zinc appears to be a good indicator for zinc status (Cakmak and Marschner 1987). These authors suggested a physiological inactivation of zinc by high phosphorus concentrations, possibly the formation of sparingly soluble zinc phosphates. More recently Loneragan and Webb (1993) have concluded that zinc precipitation by high phosphorus concentrations within the plant (decreasing the proportion of water soluble zinc) is the principal cause of symptom development in the presence of undiminished (total) zinc concentrations.

2.4.2 Other nutrients

Nitrogen fertilizers can induce or exacerbate zinc deficiency in plants growing in soils with low zinc status by dilution of zinc in the plant through large increases in total growth (Chaudhry and Loneragan 1970). Nitrogen fertilizers may also affect zinc uptake as discussed previously, by changing the pH of the rhizosphere. Copper and zinc fertilizers did not alter vegetative or grain yields on a Western Australian infertile acid loamy sand in pot culture unless nitrogen was also added (Chaudhry and Loneragan 1970). There was a strong positive interaction between added nitrogen and zinc if copper was adequate, and a negative interaction in the absence of copper. The effects of nitrogen on zinc deficiency were aggravated by copper fertilizer. Copper has a higher stability constant than zinc and may displace zinc from co-ordination complexes with organic matter (Barber 1984). Copper and zinc appear to share competitive uptake mechanisms by roots, possibly utilising the same transport protein (Kochian 1993). However, as Loneragan and Webb (1993) point out, Zn^{2+} activity in *the soil* is likely to be much higher than Cu^{2+} activity at absorbing sites because a higher proportion of copper is complexed. They were unaware of any study in which growth or yield could be shown to have been affected by copper depressing zinc uptake. In the experiment of Chaudhry and Loneragan (1970), zinc was implicated in exacerbating the copper deficiency by depressing the uptake of copper and hence had a large effect on grain yield because of the key role of copper in grain production. Copper deficiency in wheat may also delay the senescence of, and export of zinc from, the oldest leaf (Hill *et al.* 1979).

Macronutrient cations such as NH_4^+, K^+, Ca^{2+} and Mg^{2+} have inhibited zinc uptake in solution culture studies (*e.g.* Chaudhry and Loneragan 1972) by both competitive and non competitive mechanisms but in soils, alkali and alkaline earth cations exert their major influence through their effect on pH. Absorption of zinc was not inhibited by anions (NO_3^- Cl^-, SO_4^{2-}, $H_2PO_4^-$) in a solution culture study by Chaudhry and Loneragan (1972).

The interactions between iron and zinc appear to be as intricate as those between phosphorus and zinc, and while lacking some of the controversial ambience, the specificity of the transport mechanism for the ferric phytometallophore complex into cells is subject to some debate. These interactions have been reviewed by Loneragan and Webb (1993). The specific reactions of dicotyledons and grasses to iron deficiency and the effects on zinc absorption have been discussed earlier in this review, as have the effects of zinc deficiency on iron absorption.

Excess manganese does not appear to inhibit zinc absorption by roots or shoots and wide variations in manganese concentrations and activities in chelate buffered solution between deficiency and near toxicity did not have pronounced effects on zinc concentrations in roots and shoots of barley (Loneragan and Webb 1993, Webb *et al.* 1993). The absorption of zinc may be affected by excess sodium in a similar but less effective mechanism than occurs with potassium - *i.e.* because the inhibitory effects of alkali and alkaline earth cations are mutually competitive, the inhibitory effect of potassium is greatest at low concentrations of calcium and decreases as calcium concentrations increase. Similarly, the inhibitory effects of sodium will decrease as the concentrations of calcium or other inhibitory cations increase (Chaudhry and Loneragan 1972).

In soils with high boron content, zinc deficiency appears to allow boron to accumulate to sufficient concentrations to reduce dry matter production in wheat - as demonstrated in pots by Singh *et al.* (1990), and in barley in a solution culture study where Graham *et al.* (1987) measured toxic concentrations of boron in the tissue of zinc deficient plants, although dry matter production in this instance was not affected. In the study by Singh *et al.* (1990), loamy sand was treated with 0, 2.5, 5.0, 7.5 or 10 mg kg^{-1} boron kg^{-1} soil and 0, 10 or 20 mg zinc kg^{-1} soil. Increasing zinc decreased manganese, magnesium and phosphorus uptake; copper, iron and potassium uptake were increased. Increasing boron decreased the uptake of copper, iron, manganese, calcium, magnesium, potassium and phosphorus, but this effect was reduced by adding zinc. Boron toxicity in wheat and barley is a common problem with many of

the cereal producing soils in the low rainfall areas of southern Australia (Cartwright *et al.* 1984, Hirsch and Manton 1989). Many of these soils are also characterised by low zinc availability (Reuter *et al.* 1988).

2.5 ZINC EFFICIENCY IN CEREALS

Where the ability of a crop plant to tolerate soils of low zinc status is heritable, this ability is termed zinc efficiency and is defined as "the ability of a cultivar or species to grow and yield well in soils too deficient in zinc for a standard cultivar". A tolerant cultivar exhibits little if any response to zinc fertilizer in deficient soil whereas a sensitive cultivar will exhibit a significant response (Graham 1984, Graham and Rengel 1993). Zinc efficiency is seen to be particularly advantageous where the effectiveness of zinc fertilizer is compromised, for instance where zinc fertilizer is concentrated in the topsoil over zinc-deficient subsoil and the topsoil is subject to drying. Nambiar's (1976) data has been referred to in this context (*e.g.* Simpson and Pinkerton 1989, Nable and Webb 1993) to show that zinc can be taken up from dry soil (having a matric potential less than -1.5 MPa) through the agency of excreted mucilage. However, it should be noted that the plants were able to absorb only 40% of that absorbed from wet soil. Graham and Rengel (1993) also refer to the environmental benefits of using zinc-efficient cultivars through the reduced need for fertilizer and machinery and the cost effectiveness of improved cultivars as means of technological advancement. They note that the development of zinc-efficient cultivars has been retarded by lack of progress in gaining a better understanding of the genetical, physiological and biochemical bases for the phenomenon. Rye (*Secale cereale* L.) is an example of general micronutrient efficiency in cereals and Graham and colleagues (*e.g.* Graham 1984, Graham *et al.* 1987, 1992a) have studied the effects of transferring copper and zinc efficiency genes into wheat.

While there are obvious differences between species in their ability to cope with low levels of zinc in soils (Tiwari and Dwivedi 1990), as Graham and Rengel (1993) have

pointed out, comparisons between species in terms of tolerance to zinc deficiency is fraught with danger because of the range of tolerances which occur within species.

Graham and Rengel (1993) have suggested that genetic factors responsible for the zinc efficiency trait are likely to be additive and that the development of increasingly efficient cultivars will depend on combining zinc efficiency mechanisms involving physiological processes and their underlying biochemical and molecular attributes. While the mechanisms of zinc efficiency are as yet poorly understood, the authors propose that mechanisms vary among crop species and while more than one mechanism may be involved in the expression of efficiency of a given genotype, increased efficiency is due either to the greater expression of a mechanism or additional mechanisms becoming operative. Genes responsible for zinc efficiency on sandy soil appear to differ from those which confer the trait on heavier textured soil and expressions of efficiency for other nutrients appear to be poorly linked with zinc efficiency (*e.g.* Brown and McDaniel 1978, Graham 1984, Graham *et al.* 1992a), indicating that mechanisms may vary for individual traits. Whatever the specific nature of the mechanism involved, most micronutrient efficiency traits in cereals appear to depend on a superior ability to take up the nutrient efficiently from the soil rather than to remobilize it, or an adaptation to survive on lower levels within the plant (Graham *et al.* 1992a). Of particular interest in South Australia has been the identification of a degree of zinc efficiency in the locally bred wheat cultivar Excalibur (Graham *et al.* 1992a). The experiment was conducted at Lameroo on a sand over clay soil in a low rainfall area and at Yeelana on a loam over structured medium clay. Both sites were in South Australia. DTPA extractable zinc at both sites was 0.2 mg Zn kg^{-1}. Zinc was applied as granules of zinc oxysulphate ($ZnSO_4.xZnO$ ~30% Zn) to the soil at 25 kg ha^{-1} at sowing and supplemented with a foliar spray of 200 g Zn ha^{-1} at tillering. The efficiency of Excalibur when compared with a range of cultivars was defined as performance (dry matter yield, grain yield or zinc uptake in grain) in zinc-deficient soil as a percentage of performance in zinc fertilized soil:

$$\text{Efficiency} = \frac{\text{Yield (-Zn)}}{\text{Yield (+Zn)}} \times 100\%$$

Unfortunately (in terms of human nutrition and the need for high concentrations of zinc in grain to be used for seed), Excalibur tends to follow the general trend across genotypes for lower zinc concentrations in grain to accompany higher yields. The range of zinc concentrations in grain from plants grown in this experiment on soil with low zinc content does suggest that cereal breeding offers an opportunity for improvement in this respect, although again there appears to be only a tenuous linkage of zinc content in grain with efficiency traits.

It is of interest that the mechanisms which confer zinc efficiency in soil of low zinc status do not appear to apply in solution culture. The relative efficiency of Excalibur in solution culture is considerably poorer than in soil. Excalibur's better performance in soil may be due to a relatively high proportion of roots of diameter less than 0.3 mm, given that root mass is similar (Graham and Rengel 1993). The generally poor linkage between efficiency traits for zinc and other micronutrients does indicate that zinc efficiency is not a primary function of gross root morphology (Graham *et al.* 1992a). It is not clear whether the efficiency of Excalibur in soil of low zinc status is due to differences in the root system, an enhanced symbiotic relationship with VA mycorrhizae, complex inter-relationships with phosphorus and/or iron metabolism, the release of phytometallophores in response to either iron or zinc deficiency, different physiological processes within the cell (principally compartmentalisation or utilisation differences), or different uptake or transport mechanisms. Even the amount of zinc in seeds has an important influence on performance in low zinc media, higher zinc conferring an initial advantage in terms of root and shoot mass and, if sufficiently high, may decrease (or even negate) the difference in performance between efficient and inefficient genotypes (Graham and Rengel 1993).

Over the past century, wheat breeding in South Australia has bestowed some measure of zinc efficiency on the currently grown bread wheats when compared with durum wheats which have a shorter history of development in soils of low zinc status. Durum wheats tend to be particularly sensitive to zinc deficiency in this region and the cultivar Durati has been shown to have a low efficiency on a range of soils from

sands to heavy clays. However, of special interest is the Durati derivative Kamilaroi which exhibits zinc efficiency on the black earths of New South Wales, but not on the sandy soils of South Australia where its efficiency appears to be less than that of Durati. Graham *et al.* (1992a) have explained this in terms of zinc deficiency being induced or aggravated by different causes in the two regions (*e.g.* high levels of native phosphorus in the black earths and low available zinc status in the sandy soils of South Australia).

2.6 RECOGNITION OF ZINC DEFICIENCY

2.6.1 Symptomatic diagnosis

The use of symptomatic diagnosis alone for the recognition of zinc deficiency has in recent years been largely superseded by plant tissue analysis, since it became recognised that sub clinical zinc deficiency can incur severe production losses in plants which do not exhibit zinc deficiency symptoms (Carroll and Loneragan 1968, Reuter *et al.* 1982).

Nevertheless, symptomatic diagnosis is still widely used for identification of severe deficiency because of the distinctive nature of zinc deficiency symptoms. In monocotyledons, symptoms of zinc deficiency are generally manifest in the centre of middle aged to older leaves as oily grey green patches which become necrotic and gradually extend to the leaf margins, so that leaves may collapse in the middle. In severe cases, young leaves are affected - and while the most severe symptoms are normally seen on older leaves, early symptoms can often be discovered first on young leaves (Brennan *et al.* 1993). Römheld and Marschner (1991) have suggested that symptoms on older leaves are, in fact, the symptoms of phosphorus toxicity. Occasionally, early symptoms may include longitudinal chlorotic bands parallel to the midrib - symptoms especially distinctive in maize (Römheld and Marschner 1991). In dicotyledons, stunted growth with small new leaves ("little leaf"), shortened petioles ("rosetting") and erect leaflets ("cupping") are common symptoms, with a change in colour to greyish green with interveinal chlorosis and necrotic spotting on upper leaf

surfaces (Snowball and Robson 1983). The aggravation of symptoms by high light intensity indicates the possible involvement of superoxide radicals in the development of symptoms (Marschner and Cakmak 1989). The rather severe effects of zinc deficiency on protein synthesis in higher plants are now considered to be due to a reduction in RNA and ribosome production (Kitagishi *et al.* 1987).

2.6.2 Soil indicators of zinc deficiency

Prediction of zinc deficiency from soil analyses is still largely empirical, and interpretation of soil data is something of an art. For some time, many of the parameters on which interpretive data are based have varied widely with little standardization of procedure although this situation is slowly changing. Tiller *et al.* (1975) suggested standard sampling procedures for surface soils and Khan and Nortcliff (1982) examined spatial variability of micronutrients in a given soil series and concluded that the definition of soil series from morphological and chemical properties did not imply homogeneity in terms of micronutrient distribution and that to achieve such homogeneity, considerably finer subdivision was necessary. A similar conclusion from published data was reached by Tiller (1983). It is self evident that any sampling strategy must, as a first step, include an assessment of the statistical distribution of the property to be measured to allow the sample size to be estimated for a given level of precision - this has been clearly shown by Beckett and Webster (1971) in their review of soil variability.

Further, there often exists a poor relationship between the total zinc content of a given soil and its ability to supply the plant with zinc (Tiller 1983). A range of extractants has been used to predict the availability of zinc in soil and in recent years, DTPA extraction has become a widely used indicator of plant-available zinc in soil (Lindsay and Norvell 1978, Soltanpour and Workman 1979). The Mehlich 3 extraction method (Mehlich 1984) has also attracted considerable interest because of its ability to allow a single extraction for bases (K, Ca, Mg), phosphorus, and micronutrients - a particular advantage for inductively coupled plasma spectrometry (Sims and Johnson 1991). As

Marschner (1993) has pointed out however, such tests provide at best an estimate of the probability of soil providing sufficient zinc to meet requirements for plant growth.

Average critical concentrations of DTPA extractable zinc range from 0.5 to 1.0 mg Zn kg^{-1} soil with a reported range of 0.1 to 1.0 mg Zn kg^{-1} soil (Cox 1987, Brennan and Gartrell 1990). However, the critical value is highly dependent on a range of soil parameters. For instance, Brennan and Gartrell (1990) conducted a series of five glasshouse experiments in which zinc was added to 54 Australian soils in which subterranean clover (*Trifolium subterraneum* L.) was grown and reported critical DTPA extractable zinc concentrations for sand of 0.13mg Zn kg^{-1} and for clay 0.55mg Zn kg^{-1}. Critical zinc levels were also related to pH (1:5 soil:water) and in soils with pH >7.5, the inclusion of free $CaCO_3$ percentage in the response prediction model improved its accuracy. Brennan *et al.* (1993) have more recently suggested that data on pH, organic carbon and clay can be used to enhance the correlation between soil test and plant growth and to increase the range of soils for which the test can provide a reliable indication of plant available zinc. However, Brennan *et al.* (1993) have noted that various extractant concentrations, pH values, added salts, soil:solution ratios, extraction periods, drying and grinding periods, intensity and speed of shaking have been reported in the literature with both EDTA and DTPA extraction.

Armour *et al.* (1990) adopted a procedure of combining estimates of the intensity factor (I) (the measurement of which in itself is not a simple task - see Black 1992) of zinc in the soil solution or extracted with 0.01 M $CaCl_2$ and the quantity or capacity factor (Q) (zinc extracted with 0.005 M EDTA). These combined estimates were able to account for 90% of variation of zinc concentration in leaf tissue through a multiple regression: 80% of the variation could be accounted for with HCl or DTPA extractable zinc (Q) and 0.01 M $CaCl_2$ extractable zinc (I). Brennan *et al.* (1993) have suggested that the adoption of standard tests such as these to measure I and Q would minimise the number of tests required and allow comparison of tests from different soils. Extremely sensitive measurement apparatus and rigid quality controls to avoid

contamination of samples are required to determine zinc concentrations in the soil solution. At present, the approach of Brennan and Gartrell (1990) and Brennan (1992b) of combining data on DTPA extractable zinc with those of other soil properties in a multivariate analysis seems to be the most useful for predicting plant growth from soil tests. It is of fundamental importance that laboratory procedures are standardised.

2.6.3 Tissue concentrations as indicators of zinc status

Recent expansion in the use of multi-element analysers has led to a simultaneous growth in the analysis of plant tissue as a means of diagnosis of zinc deficiency, although only a decade ago, plant samples analysed by commercial laboratories for the farming industry in the United States represented only 10% of the number of soil samples analysed (Jones 1991). Undoubtedly this situation has changed in recent years. A review of sampling methods and analytical procedures for plant tests may be found in Smith (1962), Smith (1986) and Jones (1991). As is the case with soil analysis, the need for standardised procedures is fundamental.

The critical deficiency concentration (CDC) of zinc in a given plant part is generally accepted as the concentration at which a 10% reduction in yield is established (Ulrich and Hills 1967). The range of concentrations between that responsible for maximum yield and a 10% reduction in yield is termed the transition zone (Ulrich and Hills 1973) or the critical nutrient range (CNR) (Dow and Roberts 1982). While these critical values are generally established in pot experiments in which different amounts of zinc are uniformly mixed through the root zone and with adequate supplies of other nutrients, there is an obvious need to test these values in the field for local application. The diagnostic critical concentration is defined as that concentration below which a yield decrease occurs (usually dry weight of the whole shoot) at the time of sampling. Prognostic values have also been used recently to predict zinc deficiency in plants before the appearance of symptoms or loss of production (the

prognostic critical concentration is that concentration below which yield of grain or dry matter is reduced at some future growth stage) (Riley *et al.* 1992).

Recent developments in sampling techniques have led to the wider use of young tissues (rather than whole shoots or grain) for the diagnosis of zinc deficiency. In some circumstances, where the quantity of leaf tissue available is limiting, testing whole shoots may be necessary. The youngest emerged blade (YEB) of cereals, or the youngest open leaf (YOL) for pasture legumes are generally selected for tissue analysis because zinc is poorly mobile in deficient plants and the earliest symptoms of deficiency generally appear on young leaves (when symptoms are fully developed, leaves are generally middle aged). Moreover, young leaves have more stable zinc concentrations at most stages of the plant's development (Reuter *et al.* 1982). The use of whole shoots to measure zinc status is considered to be generally unsatisfactory because the CDC normally decreases with age (Reuter *et al.* 1982). Zinc is not uniformly distributed throughout the plant (Ohki 1977) so that critical concentrations vary as plants change morphologically. A further obstacle in the path of interpreting whole-shoot analyses is the Piper-Steenbjerg effect (Piper 1942, Steenbjerg, 1951) in which very deficient plants may have higher zinc concentrations in whole shoots than plants which are only moderately deficient. Young leaves do not display the effect in cereals and using these leaves for tissue testing has been shown to be a preferable alternative in the case of copper deficiency (Loneragan *et al.* 1980). A further difficulty is presented by the steep nature of the deficient and transition zones of the general response curve for zinc and other micronutrients (*e.g.* Brennan *et al.* 1993, Jones 1991, Ohki 1984, Reuter and Robinson 1986) where a small increase in zinc concentration in the order of 1-2 mg kg^{-1} may determine whether a plant is deficient or sufficient with respect to zinc. On the other hand, this phenomenon may also be seen as advantageous, in that it is easier to categorise zinc status as deficient or sufficient.

Requirements for zinc may vary between cultivars within species (Shukla and Raj 1974, Clark 1978), suggesting that individual calibration may be required for accurate determination of the zinc efficiency status of a given cultivar. For instance, Graham

and Rengel (1993) have recorded typical symptoms of zinc deficiency in the durum wheat cv. Durati with the same concentrations of zinc in shoots as other wheat genotypes exhibiting no symptoms. In broader terms, Jones (1991) has suggested that the critical concentration of zinc in mature leaves in a wide range of crops is about 15 mg kg^{-1}.

For southern Australia, the critical concentration of zinc in the YEB of cereals at tillering appears to be 16-17 mg Zn kg^{-1} (R.J. Hannam and N.S. Wilhelm, pers. comm., Rengel and Graham 1995a). In terms of the prognostic critical concentration for zinc, Riley *et al.* (1992) proposed from their field data from the Eradu sandplain of Western Australia that concentrations in YEBs of about 16 mg zinc kg^{-1} at early tillering indicates sufficient zinc to avoid yield loss through deficiency, and that a concentration of 7.0 mg zinc kg^{-1} in the flag leaf post anthesis offers a similar assurance.

Although the use of biochemical methods to assess zinc status by measuring enzyme activities, specifically carbonic anhydrase in wheat (Dwivedi and Randhawa 1974), has offered promise as rapid, simple and accurate (Reuter and Robinson 1986, Brennan *et al.* 1993), relatively little progress has been made towards the wide adoption of these techniques.

2.7 ZINC FERTILIZATION

2.7.1 The need for zinc fertilization

Zinc deficiencies occur in cereal crops throughout the world, principally in alkaline soils (because of the effect of high pH on zinc activity), in leached acid sands and in submerged or flooded soils - hence the extensive literature on zinc deficiency in rice. As Graham *et al.* (1992a) have pointed out, zinc deficiency in cereals is more widespread than deficiencies of other micronutrients and occurs in cold and warm climates, in acid and alkaline soils and in the full gamut of soil textures. Sillanpää (1982), Welch *et al.* (1991) and more recently Takkar and Walker (1993) have reviewed the literature reporting the occurrence of zinc deficiency worldwide.

The need for zinc fertilizer in these areas and in general appears to be increasing because of more intense cropping (Mortvedt 1991), liming practices used to treat soil acidity (Takkar and Walker 1993) and, in Australia particularly, because of the greater use of high analysis or other fertilizers which do not contain zinc as a significant impurity - a feature of superphosphate manufactured from Christmas Island or Nauru rock phosphates (Williams 1974, Reuter *et al.* 1988). The increased use of sulphonyl urea herbicides which can exacerbate zinc deficiency (Robson and Snowball 1990) has also been implicated.

2.7.2 Zinc fertilizers

The most commonly used zinc fertilizer is zinc sulphate and in Australia, the most frequently used form is the heptahydrate - $ZnSO_4.7H_2O$. Zinc sulphate monohydrate, zinc oxide, zinc oxysulphate (zinc oxide partially acidulated with sulphuric acid), zinc carbonate, zinc chloride and zinc nitrate are other inorganic sources of zinc. Synthetic zinc chelates (such as ZnEDTA or zinc citrate) constitute efficient fertilizer sources of zinc and can be applied to the soil at lower rates but are costly in comparison with inorganic sources. Natural organic complexes (such as polyflavonoids, lignosulphates and phenols) formed by reacting zinc salts (usually zinc sulphate) with by-products of timber pulp manufacture and inorganic complexes (such as ammoniated zinc sulphate or zinc chloride solutions) are also commercially used sources of zinc. The latter are more commonly used in "starter" solutions (Mortvedt 1991, Mortvedt and Gilkes 1993).

Zinc fertilizers are most commonly applied to the soil to treat zinc deficiencies in field crops and because amounts less than 10 kg Zn ha^{-1} are often used, they (and other micronutrient fertilizers) are frequently mixed with NPK fertilizers either by incorporation during the initial synthesis of the fertilizer, by bulk blending of granular fertilizers, or by granular coating using a binding agent. Each of the processes may result in some decrease of the immediate availability of the zinc compared with separate application of the zinc source, *i.e.* its relative agronomic effectiveness (RE) may be decreased initially. On the other hand, the residual effectiveness of the zinc

fertilizer may be increased and insoluble zinc sources may become more soluble and more available.

2.7.3 Relative agronomic effectiveness

The RE of fertilizers (a comparison of their substitution value) is normally determined in a "horizontal" comparison procedure which is dependent on complete Mitscherlich response curves for the range of fertilizers under test all reaching a common yield maximum. The horizontal comparison indicates the amounts of fertilizers under comparison needed to produce the same yield (Barrow 1985). Mortvedt and Gilkes (1993) note that an alternative way of measuring RE is to compare zinc concentration or uptake of plants receiving a constant zinc application (in terms of zinc applied per unit area - a "vertical" comparison). Response curves describing zinc concentrations or uptake do not plateau when zinc sufficiency is attained as does yield. However, the RE values determined by this method are rate dependent, and horizontal comparisons of substitution values of fertilizers are the preferred method. Zinc fertilizers characteristically have a relatively long term residual effect and measurements of the residual RE (RRE) can be made by determining responses in subsequent crops. Ghosh (1990, cited by Mortvedt and Gilkes 1993) compared the RE and RRE of a range of zinc fertilizers in a glasshouse study conducted with a zinc-deficient acid sandy soil. In referring to this study, Mortvedt and Gilkes (1993) suggested that the results typify other investigations in this field. The most effective fertilizer was zinc sulphate solution mixed through the soil (RE = 1.00), with powdered ordinary superphosphate (OSP), monoammonium phosphate (MAP) and diammonium phosphate (DAP) (as zinc carriers with which zinc was incorporated before adding to the soil) having RE values of 0.93, 0.78 and 0.95 respectively. By way of contrast, zinc incorporated in granular fertilizers was significantly less effective, with RE values for OSP, MAP and DAP granules having values of 0.09, 0.11 and 0.03 respectively. RRE values for the granulated fertilizers were also very low, while that of zinc sulphate in the powdered OSP, MAP and DAP had fallen by about a third in the second year. In alkaline soils, the efficiency of DAP as a carrier for zinc is likely to be low and in these conditions, water soluble fertilizers are preferable. Further, the dissolution of zinc from fertilizer granules in alkaline soils is

strongly decreased in MAP and DAP which do not form acidic solutions during dissolution. Thorough mixing through the soil of finely divided zinc fertilizer appears to confer the maximum benefit in terms of efficient fertilization by optimising dissolution and root contact, and in alkaline soils water soluble zinc fertilizers are preferred (Mortvedt and Gilkes 1993).

2.7.4 Zinc fertilizer technology

Solid fertilizers

Bulk blending of granular fertilizers containing zinc and NP fertilizers can be successful, with little effect on the RE, providing problems with segregation during blending and handling, caused mainly by differences in size (but also differences in shape and density) are addressed (Silverberg *et al.* 1972 in Giordano and Mortvedt 1972, Mortvedt 1991). Alternatively the coating of granules with zinc fertilizer is efficient and effective in distributing zinc uniformly among granules, although care is needed with the choice of binding agent (ideally this should be a fertilizer solution (Mortvedt and Gilkes 1993)) and in ensuring a uniform thickness of the coating. The major disadvantage with the coating process is its cost (Mortvedt 1991). In the incorporation process, chemical changes may occur which alter the availability of zinc in the end product. For instance, the incorporation of soluble zinc with ammoniated orthophosphate fertilizers can lead to decreased zinc availability, possibly by the formation of insoluble zinc ammonium phosphate (Jackson *et al.* 1962, Mortvedt and Giordano 1969, Lehr 1972). On the other hand, insoluble sources of zinc (zinc oxide for example), are converted to soluble forms when added to acidic OSP (Lehr 1972, Gilkes 1977). The effectiveness of a given zinc fertilizer is related, in the first instance, to the proportion of water soluble zinc (Mortvedt 1968). Mortvedt and Gilkes (1993) point out that soil pH has a strong effect on dissolution of zinc from monoammonium phosphate (MAP) or diammonium phosphate (DAP) granules which do not have an acid reaction during dissolution. (MAP has a near neutral reaction and DAP has an alkaline reaction).

In contrast, the dissolution of zinc incorporated in OSP, which has an acid reaction, is not affected by soil pH. Further, about 90% of zinc in OSP granules is water soluble

(Gilkes and Sadleir 1981), whereas ammonium phosphate fertilizers into which zinc is incorporated contain a proportion of relatively insoluble zinc ammonium phosphate.

A singular advantage of using ammonium nitrogen fertilizers as a vehicle for zinc is their acidifying effect. Viets *et al.* (1957) measured enhanced zinc uptake from zinc sulphate by sorghum (*Sorghum bicolor* L. Moench) and ladino clover (*Trifolium repens* L.) when the zinc was applied with ammonium sulphate (greatest zinc uptake) compared with ammonium nitrate (intermediate) or sodium nitrate (lowest uptake). The pH in the bulk soil treated with ammonium sulphate was decreased from 7.2 to 5.0, that in the soil treated with ammonium nitrate fell to 6.0, while pH increased to 7.3 in soil treated with sodium nitrate.

Liquid fertilizers

Liquid fertilizers offer some advantages in ease of handling and uniformity of distribution and zinc fertilizers may be blended with NP fertilizers in liquid form. Clear liquids, either orthophosphate or polyphosphate solutions are commonly used as "starter" solutions and soluble zinc fertilizers can be readily used in this way (Mortvedt 1991, Mortvedt and Gilkes 1993). Agitated suspensions can also be used to carry zinc oxide (or zinc sulphate). Zinc sulphate or zinc oxide applied in orthophosphate or 16 - 40% polyphosphate suspensions appear to be equally as effective as the zinc fertilizer applied to the soil alone - an indication that unavailable products, resulting from chemical reactions, do not occur (Mortvedt 1979, Mortvedt 1991). However, the REs of the zinc sources is greater in 75% polyphosphate suspensions than in orthophosphate suspensions, suggesting that the availability of the zinc is enhanced by the polyphosphate suspension. The greater ability of polyphosphates to maintain zinc in solution is due to their ability to sequester metallic cations. However, polyphosphates lose their stability in soil and are hydrolysed to orthophosphates, particularly in calcareous soils (Mortvedt 1991). The solubility of zinc in fluid fertilizers is also strongly dependent on the pH of the fertilizer solution.

2.7.5 Methods and rates of zinc application

Application to soil

Zinc fertilizers may be broadcast and mixed with the soil by tillage or banded in the vicinity of the seed. In their review of fertilizer practices, Martens and Westermann (1991) suggested that applications of 11 - 17 kg ha^{-1} zinc are generally required on calcareous soils. The same authors reported that lower rates (about half) of banded fertilizer are commonly applied to achieve the same efficiency because of slower reversion due to lower zinc-soil contact. On the other hand, Brown and Krantz (1966) showed that the availability of zinc to plants could be reduced by banding. Takkar and Walker (1993) suggest that broadcasting and mixing of zinc sulphate or chelate with the soil, or banding below the seed are the most effective methods of correcting zinc deficiency in field crops as opposed to top dressing, side dressing, or side banding before sowing. Obviously, the method and rate chosen will depend on a range of factors including the sensitivity of the crop to zinc deficiency, the zinc status of the soil, the soil type, soil pH and texture, and the nature of the fertilizer used. For a comprehensive discussion of application rates and methods, see Takkar and Walker (1993). These authors suggest that local experimentation is necessary to determine the optimum combinations of fertilizers and methods for a given environment. Annual applications of zinc fertilizer can lead to increasing concentrations of zinc in plants because of their residual effect and fertilizer applications can be reduced or discontinued when zinc concentrations in plants increase to adequate levels, provided of course that monitoring of zinc concentrations in tissue (or soil) is continued (Mortvedt and Gilkes 1993). Brennan (1990) determined that the decline in availability of zinc with incubation was greater in alkaline soils or soils with high clay contents, high concentrations of organic carbon or free calcium carbonate. Applications of between 9 and 22 kg ha^{-1} of zinc appeared to be effective for about 10 years in correcting zinc deficiency on a range of non-flooded soils (Takkar and Walker 1993). Takkar and Walker's (1993) comment that tillage may reduce the residual effectiveness of zinc fertilizer because of its negative effects on VA mycorrhizal hyphae is of interest.

Foliar application

Both inorganic and organic zinc fertilizers are applied to foliage to correct zinc deficiency, normally at considerably lower rates than are applied to the soil, and at such low rates they have little residual value. For instance, as little as 240 g ha^{-1} of zinc is often applied as a foliar treatment for zinc deficiency to cereal crops in southern Australia (Reuter *et al.* 1988). However, in the case of moderate or severe deficiency, the ability of plants to take up sufficient zinc to correct the deficiency through leaf tissue may be inadequate and more than one application may be necessary (*e.g.* Khan and Soltanpour 1978, Martens and Westerman 1991). The use of adjuvants such as wetting agents and stickers can improve uptake (Mortvedt and Gilkes 1993). While foliar applications of zinc are relatively cheap and effective for correcting deficiencies identified by plant analyses and can provide a means of rapid response, it appears that application of zinc fertilizers to soil often results in higher grain yields (*e.g.* Brennan 1991) and offers the better long term solution to treatment of chronic deficiency, since relatively heavy applications can be effective for several years (Payne *et al.* 1988).

2.8 ROOT GEOMETRY, WATER AND NUTRIENT UPTAKE

2.8.1 General relationships

While it is undoubtedly true that the uptake of nutrients and water is a function of root geometry, it is also true that root geometry is, in part, a function of nutrient and water distribution through the soil, including the subsoil. The study of cereal root systems has tended to concentrate on root distribution and function in the cultivated horizon because of the concentration of both roots and nutrients in this zone. Just as rooting density declines rapidly with depth in most soils, the quantities of available nutrients in Australian soils follows a similarly steep decline. The decline in rooting density with depth can often be described by an exponential function (*e.g.* Gerwitz and Page 1974) although this is not invariably so. For instance, Russell (1977) used the data of Ellis *et al.* (1977) for spring barley (*Hordeum vulgare* L.) to derive a linear relationship in a dry season and an exponential relationship in a wet season. Greacen (1977) noted that the proportion of cereal roots in the topsoil in semi-arid environments in Australia may at times exceed that of a standard exponential function.

In the general case, water and nutrient uptake can be related to rooting density (expressed as length of roots per unit volume (L_v) or per unit mass of soil, or as length of root per unit of surface area L_a (Hurd 1974, Walter and Barley 1974, Meyer and Alston 1978). For instance, Ehlers *et al.* (1980) established that the rate of water uptake by roots was a function of rooting density and soil water potential, and a linear relationship between soil water loss and rooting density in cotton (*Gossypium hypogeum* L.) and maize (*Zea mays* L.) was demonstrated by Grimes *et al.* (1975). Similarly, Barber and Silberbush (1984) have explained a linear relationship between grain yield in soybean (*Glycine max* L.) and L_a in terms of nutrient supply. The Claasen-Barber (1976) mechanistic model which describes nutrient uptake by roots growing in soil and the Barber and Cushman (1981) modification of this model are based on parameters describing soil nutrient supply, root uptake kinetics and root morphology. The latter is described in terms of initial root length, rate of root growth (linear, exponential or logistic expressions) and average root radius. The assumption is made that roots are smooth cylinders and the models account for changes in root surface area and radial geometry with time. Root surface area is commonly accepted as the best indicator of the ability of a given root system to absorb nutrients (Black 1992). However, the estimated root surface area provides only an approximate index of the ability of roots to absorb nutrients and more appropriate indices will depend on detailed measurements of the surface area of active uptake for a given nutrient. The ability of the soil to provide nutrients and uptake kinetics of the root will determine the rate of nutrient uptake per unit of root surface (Barber and Silberbush 1984). Later models have included estimates of uptake by root hairs which are important for the uptake of nutrients such as phosphorus which have low coefficients of diffusion (Itoh and Barber 1983a,b, Barber and Silberbush 1984). However, Schenk and Barber (1980) obtained reasonably close correlations between predicted and observed uptakes of phosphorus in maize ($r = 0.91$) based on the Claasen-Barber model (1976) independent of root hair measurements, although it was considered that an underprediction in phosphorus uptake may have been due to uptake by root hairs. Russell and Clarkson (1976) determined that the abilities of root axes and laterals of barley to take up phosphate, calcium and potassium were more closely related to volume than length or surface area. This work was done in solution culture

however, where external nutrient concentration is similar over the entire root surface, whereas in soil, diffusion characteristics of various ions and soil properties have a controlling influence on rates of absorption by controlling concentrations at the root surface.

Hamblin and Tennant (1987) compared the water use of wheat, lupins (*Lupinus angustifolius* L.) and field peas (*Pisum sativum* L.) on four soil types in a dry season, and although the L_a values of cereal roots were consistently five to ten times larger than those of grain legumes, water loss during the growing season was similar for both wheat and lupins. Specific root water uptake (apparent water uptake per unit root length per day) was consistently higher for lupins and peas than for wheat. Maximum rooting depth was more closely correlated with water loss than was L_a for all species. For cereals grown on well drained, non compacted soil, rooting depth is generally a function of available water supply, provided shoots can produce adequate carbohydrate to sustain root growth (Russell 1973). In environments with low rainfall, root penetration is often a direct function of water penetration and the limits of root exploration are controlled by the regular wetting front (Russell 1973, Tennant 1976). The pioneer root ecologist Weaver (1926) noted that in the absence of subsoil water in the short grass plains of the USA, roots did not extend below 0.6 m. These conditions occurred in a climate where dry soil was watered by light showers and while the surface soil retained water, the subsoil was dry. When the same soil had a wet subsoil and the surface dried, roots penetrated deeper into the subsoil. The ability of roots to penetrate to depth and take up water assumes considerable importance in such circumstances. Indeed, where roots are prevented from reaching subsoil reserves, yields may be severely curtailed (Tennant 1976).

2.8.2 The subsoil as a source of nutrients

The subsoil may not only be a source of water in times of surface drought - a common occurrence in southern Australia's cereal zone - but may also be an important source of nutrients because useful quantities of nutrients are not absorbed from dry soil. There have been few studies in Australia, or anywhere else for that matter, to examine the relationships between root growth and function and the nutrient

status of subsoils. Where experiments have been conducted to examine the effects of subsoil constraints on root growth, some encouraging and spectacular results have been achieved. For instance, Graham *et al.* (1992b) established a series of imaginative experiments across South Australia on 15 soil types in which soil was removed in strata to a depth of 0.9 m, mixed with nutrients or organic matter and replaced. Because of the highly significant effect of soil strength on root growth (Dexter 1987), control treatments consisted of soil removal and replacement with no additions of nutrients (physical disturbance), besides a no-disturbance treatment. Large increases in dry matter production above the controls occurred at most sites, apart from fertile red-brown earths, when the subsoil was treated with organic matter or phosphorus + nitrogen + trace elements. The rates of inorganic fertilizers applied on a per hectare basis were: DAP 3 333 kg, Mo 1.3 kg, Ni 1.3 kg, Zn 33 kg, Cu 13 kg, Mn 333 kg. Striking yield increases (in one instance seven times the yield of the surrounding field) were recorded over a sustained period of seven years to the time of reporting. Similarly, Jarvis and Bolland (1990) demonstrated yield increases of 49% in Western Australia by banding superphosphate 5-8 cm below the seed rather than by drilling it with the seed. The yield increase above topdressing the same rate of superphosphate was 115%. Kuhlmann (1990) measured the uptake of potassium in spring wheat from the topsoil and subsoil (deeper than 0.30 m). Uptake from the subsoil ranged from 9-70% of total uptake and was a direct function of higher potassium concentrations and higher rooting densities in the subsoil and of rooting depth.

Garwood and Williams (1967) measured a greater uptake of nitrogen by perennial ryegrass (*Lolium perenne* L.) from fertilizer placed at 0.76 m than from a surface application with a dry topsoil and wet subsoil. Similarly, Simpson and Pinkerton (1989) demonstrated that monobasic calcium phosphate applied to the surface 40 mm of soil cultures could supply most of the phosphorus requirements of subterranean clover (as demonstrated by dry matter production) if sufficient water were added to this layer. As less water was added to the surface, dry matter production was proportionately higher in a treatment containing phosphorus in the subsoil between 40 and 70 mm. Graham (1991) showed that copper deficiency in wheat could be induced

in pots containing Laffer sand with adequate copper added to the topsoil (but none to the subsoil) if the topsoil was allowed to dry at various stages up to early stem elongation.

While root growth is closely dependent on water distribution in soils, the distribution of nutrients is also of considerable importance in determining root proliferation. For several decades now, research workers have noted a relationship between the depth and profusion of root growth and soil fertility. Weaver (1926) recorded that roots were more dense and more prolifically branched in soil layers rich in NO_3^- and tended to penetrate less into deeper soil. Fox *et al.* (1953) and Cooke (1954) recorded root proliferation around fertilizer bands and deduced that rooting density could reflect localised variations in soil fertility. In the case of nitrate or phosphate, plants are able to compensate to some degree for limited supplies to most of the root system if only part is supplied with favourable concentrations. In an elegant experiment, Drew and Saker (1975) supplied the entire root systems of barley plants with concentrations of 1 mmol NO_3^-, while others received this concentration in a 4 cm zone of a seminal axis only - the remainder of the root system received only 0.01 mmol NO_3^-. While the initial effect of this general decrease in NO_3^- was to lower shoot growth rates and nitrogen uptake, after 19 days both groups of plants displayed similar relative growth rates of shoots and concentrations of nitrogen. The compensatory growth was due to a proliferation of roots and a greater uptake of nitrogen per unit weight of roots in the treated segment. Similar data for nitrogen in maize were obtained by Edwards and Barber (1976) and for potassium in wheat by Lean *et al.* (1974).

The ability of root systems to compensate for unfavourable conditions in terms of water or nutrient concentrations in part of the root zone by increasing growth or uptake in more favoured parts has a considerable influence on root morphology. The compensatory growth phenomenon enables limited zones of soil to provide the plant's requirements of some nutrients - those most readily translocated through the phloem. While nitrogen and phosphate are readily retranslocated from shoots to roots and from older regions within roots to actively growing regions (Marschner 1986, Rufty *et al.* 1990, Smith *et al.* 1990), root growth may be inhibited if part of the external medium

is deprived of phosphate (Stryker *et al.* 1974). These differences may occur at the varietal level (Hackett 1968) and may provide some basis for the selection of cultivars which are more efficient in terms of uptake of a given nutrient. Hackett (1968) demonstrated such varietal differences but showed that in the general case for barley, potassium and phosphorus deficiency decreased total root weight and volume by 40 - 50% and total root length by 25 - 30%. However, while phosphorus deficiency did not affect the number of first order lateral roots, the number of second order lateral roots was increased by a factor of five. In the case of potassium deficiency, the number of first order lateral roots was decreased by 23% and no second order lateral roots were produced at all. Scott and Robson (1991) demonstrated that total root growth was decreased unless phosphorus, potassium and magnesium were supplied to both halves of vertically split root systems. However, decreased root growth does not necessarily imply a decrease in shoot growth. For instance, Nable and Loneragan (1984) demonstrated that shoot growth of subterranean clover plants was not affected when manganese was applied to only half of a vertically split root system, even though root growth in the deficient half was decreased. Manganese was not retranslocated from the supplied half of the root system to the deficient half.

2.8.3 A specific role for zinc in subsoil nutrition?

While the need for zinc in the environment external to the root has been demonstrated, the phloem mobility of zinc has been the subject of some controversy. As Longnecker and Robson (1993) have noted, the stunting of young leaves and decreased internode length in shoots of zinc-deficient plants has led to the incorrect conclusion that zinc is phloem-immobile, when in fact the retranslocation of zinc in the phloem appears to be a function of the zinc status of the plant. For instance, Loneragan *et al.* (1987) showed that zinc-adequate wheat plants could translocate sufficient zinc in the phloem to maintain root growth in part of the root system not supplied with zinc. However, deficiencies of zinc in the external environment can lead to impaired root function (Loneragan *et al.* 1987, Webb and Loneragan 1990). It is possible that the growth distortions reported in zinc-deficient plants may be due to the role of zinc in hormone synthesis.

While fertilizers are almost universally applied to topsoil, there is little opportunity for the added nutrients to benefit the subsoil, apart from soluble nutrients like nitrates which may move downwards (Burns 1980). Downward movement into the subsoil is unlikely to be the case with phosphorus or micronutrients. There is an increasing body of evidence that normal subsoil supplies of available nutrients are not able to meet plant requirements for optimum growth when they constitute the major source, as occurs when the topsoil is dry. In the case of zinc alone, there are many reports of zinc deficiency in plants growing on subsoils exposed by erosion or levelling ("cut" soils). Brennan and McGrath (1988) applied zinc fertilizer to the surface of an acid sand in Western Australia at a rate of 22.5 kg ha^{-1} of zinc and recovered 95% of the applied zinc from the top 5 cm of soil after 1, 400 mm of rain. The retention of zinc in the surface is likely to be even more evident in calcareous soils (Jones *et al.* 1957). This has important implications for roots growing in subsoils deficient in nutrients which are required in the external environment, principally calcium, boron and zinc. Low zinc concentrations in roots may lead to leakage of soluble organic substances into the soil, possibly attracting fungal pathogens, and reducing the uptake of phosphorus (Webb and Loneragan 1990).

The subsoils of southern Australia's cereal growing areas contain high concentrations of calcium and boron, are infertile, highly alkaline, often sodic and/or saline and of heavy texture. The availability of micronutrients to plants is low and they have been labelled "inhospitable" or "hostile". Many of the subsoils in this region contain sufficiently high concentrations of boron (some subsoils contain in excess of 100 mg boron kg^{-1} soil) and sodium to pose a hazard to root growth (Holloway 1991). The estimated cropping area affected by subsoils with high concentrations of boron is greater than 10 million hectares in the low rainfall areas of southern Australia (Graham *et al.* 1992b). In many parts of Upper Eyre Peninsula in South Australia and in the Victorian mallee (Griffiths and Walsgott 1985) the penetration of wheat roots into subsoils below 0.5 m is often limited, as extensive examination of pit faces has shown. The depth of wet soil (wetting front) often extends below this depth and as a result, not all subsoil water is used by the crop. There is evidence (*e.g.* Walter and Barley 1974) that water within the rooting zone is not fully utilised. The role of

salinity and boron in relation to this problem has been examined for Upper Eyre Peninsula (Holloway 1991, Holloway and Alston 1992) but the role of nutrient deficiencies or deep placement of nutrients has not been studied in detail, apart from the initial pioneering investigations of Graham *et al.* (1992b) referred to above.

The role of zinc deficiency in exacerbating boron toxicity has been referred to earlier, as has the data of Graham *et al.* (1987) demonstrating the more rapid accumulation of toxic quantities of boron in the tissue of zinc-deficient barley than in plants adequately supplied with zinc. Similarly, Singh *et al.* (1990) have demonstrated decreasing boron concentrations in wheat with increasing amounts of zinc added to a zinc-deficient loamy sand in pots. Of particular interest here is the study by Nable and Webb (1993) who compared the growth of two wheat cultivars Gatcher (zinc-inefficient) and Excalibur (zinc-efficient) in pots containing zinc added to the top soil (0.10 m deep) and either added to or withheld from the subsoil (0.25 m deep). The soil in the experiment was Laffer sand to which 0.3% $CaCO_3$ was added. Withholding zinc from the subsoil had no effect on head emergence or grain yield of Excalibur, but with Gatcher, the period to 50% head emergence was delayed by 10 days, grain yield was depressed by 20% and water use was depressed by 12% during the final 60 days before maturity. The water use of Excalibur was not affected by withholding zinc from the subsoil. At maturity, zinc treatments did not affect vegetative yields, root growth (in topsoil or subsoil), number of tillers, numbers of heads with grain or numbers of grains per head. The differences in grain yield in the two Gatcher treatments were ascribed to smaller grains in the Gatcher grown with zinc deficient subsoil. The reduction in water uptake by Gatcher grown in pots containing deficient subsoil was explained in terms of loss of membrane integrity. Withholding zinc from the subsoil halved the total zinc content of shoots in both cultivars. Such data invite further investigation into the role of zinc deficiency in discrete portions of the root zone.

2.9 SUMMARY

Zinc deficiency in crop plants has been recognised for many years although the complex of functions in which zinc is involved in higher plants is only partially

understood. Zinc deficiency in general imposes its effects most strongly on the metabolism of carbohydrates, proteins, and auxins, and on reproduction where it is integral to the production and stability of RNA and DNA. Of particular import in this review is the vital role of zinc in maintaining cellular membrane integrity; zinc is involved in preventing the peroxidation of membrane proteins and lipids by superoxide radicals through its role in superoxide dismutase. Zinc deficient root cells release significantly more amino acids, carbohydrates, phosphorus, potassium, nitrate, chloride and low molecular weight compounds including sugars and phenols than zinc sufficient cells. The maintenance of membrane integrity requires zinc in the external solution.

The immediate source of zinc for plants is the soil solution in which most of the zinc present (generally in micromolar concentrations) occurs as the free hydrated metal ion or complexed with organic matter. Organic complexes with zinc assume greater importance for plants as the soil solution pH rises. Zinc in solution is also a function of adsorption-desorption reactions and these are controlled by pH, so that the availability of zinc to plants decreases strongly with increasing pH, particularly at low zinc concentrations. Because zinc is supplied to roots principally by diffusion, the rhizosphere, in which conditions differ significantly from the bulk soil, assumes major significance in controlling zinc nutrition. The ability of plants to modify the rhizosphere pH is a function of cation/anion uptake ratios which are most strongly influenced by the form of nitrogen supplied. The availability of zinc can be enhanced by NH_4^+ nutrition which leads to a decrease in the pH of the rhizosphere. Specific and even varietal differences occur in the ability of plants to modify the pH of the rhizosphere according to nutritional stimuli. Legumes which fix nitrogen are also able to lower the pH of the rhizosphere. Proteoid roots, an adaptation of some species (*e.g.* white lupins) to infertile soils, are formed as a response to phosphorus deficiency. They appear to be particularly efficient at lowering the pH of the rhizosphere and increasing the availability of phosphorus, iron, manganese and zinc through the excretion of citric acid, even with NO_3^- nutrition. Zinc may be mobilised by general non-specific responses to iron or zinc deficiency (root - induced changes in pH or redox potential or microbial activity in the rhizosphere) or by specific iron

deficiency mechanisms. Specific uptake mechanisms in dicotyledons and monocotyledons which are not members of the *Poaceae* include enhanced production of ferric reductase in the plasma membrane and net excretion of H^+ and reducing compounds (Strategy I). The ferric reductase may also be involved in the regulation of divalent cationic channels which may mediate zinc uptake in plants. In the *Poaceae*, specific responses involve the release of iron transport chelators, the phytosiderophores, or preferably, phytometallophores (Strategy II). The phytometallophores from Strategy II plants appear to be more efficient in terms of mobilizing zinc in calcareous soils than low molecular weight organic solutes. Mycorrhizal symbiosis enhances zinc uptake, particularly in soils low in available zinc.

The study of zinc nutrition can not be undertaken without considering interactions with other nutrients. The investigation of phosphorus-zinc interactions has a controversial history. The major interactions occur when both phosphorus and zinc supplies are marginal and the addition of phosphorus induces or enhances zinc deficiency by dilution. When increasing phosphorus depresses zinc concentrations to a greater degree than can be explained by dilution, it is possible that phosphorus-induced mechanisms may depress zinc absorption or inhibit zinc transport from roots to shoots. A further puzzling family of interactions occurs when increasing phosphorus induces symptoms of zinc deficiency or reduces growth with no effect on zinc concentration, while the addition of zinc corrects the disorder. Water soluble zinc in the tissue, (zinc which is physiologically active), can be lowered by high phosphorus supply, perhaps by precipitation, and the symptoms may be due to phosphorus toxicity. Zinc deficient plants appear to be more susceptible to boron toxicity and this is of particular importance in much of the low rainfall cereal growing area of southern Australia where soils have a low available zinc status and high boron concentrations in the subsoil. The role of zinc in maintaining the structural and functional integrity of root cell membranes is of crucial importance in protecting plants from the ingress of toxic amounts of boron into root cells.

Zinc-efficient wheat cultivars offer significant environmental benefits through their cost effectiveness and reduced fertilizer requirements, since efficiency mechanisms for micronutrient uptake depend on a superior ability to extract the nutrient from the soil. The actual mechanisms which confer zinc efficiency are not clear, but those which apply in soil do not apply in solution culture.

Zinc deficiency has distinct symptoms which are easily recognised but subclinical symptoms may incur severe production losses. Consequently, tissue analysis has become the primary means of detecting zinc deficiency. Critical zinc concentrations (the concentration just less than that needed for maximum growth) need to be determined for individual species. In wheat, the critical zinc concentration in the youngest emerged blade (YEB) at tillering is about 17 mg kg^{-1}. The critical concentration falls with the age of the plant. An early tillering YEB concentration of 16 mg Zn kg^{-1} appears to indicate adequate zinc for future growth.

The need for zinc fertilization is increasing because of more intense cropping, liming practices to treat soil acidity and the increased use of relatively pure fertilizers low in zinc impurities. Zinc is often applied in conjunction with NP fertilizers by bulk blending, coating of granules, or by incorporation during the formulation process. The effectiveness of a given zinc fertilizer is related in the first instance to the amount of water soluble zinc. Thorough mixing through the soil of finely divided zinc appears to be the most efficient means of zinc fertilization. Zinc incorporated in granular fertilizer, particularly DAP, offers a less efficient alternative in alkaline soils. Applications of between 9 and 22 kg ha^{-1} zinc may be effective in correcting zinc deficiency for about 10 years on a range of soil types. Foliar applications are effective at much lower rates than soil applications but more than one application may be necessary and even then are only effective for the crop being treated.

Zinc fertilizer is almost universally applied to the topsoil and is unlikely to be leached to any great extent into the subsoil. Southern Australian subsoils are commonly low in available plant nutrients and the soil pH usually increases with depth in the root zone. Zinc availability is generally extremely low. Further, zinc is supplied to the roots

principally by diffusion and uptake is a function of the actively absorbing surface area. Since rooting densities decline rapidly with depth, zinc uptake from the subsoil can be expected to be low. This is likely to confer a serious disadvantage in conditions of topsoil drying, a frequent occurrence in the cereal growing areas of southern Australia where rainfall is low. The deleterious effects of high concentrations of boron in the subsoil may be exacerbated by low zinc concentrations in the soil solution. The literature contains a paucity of information on the role of zinc in subsoil nutrition, particularly in field soils and on the role of root geometry and zinc placement in zinc uptake. Little information is available on the ability of various species to mobilise zinc from deficient soil for use by following crops. These fields of investigation form the basis of this thesis.

3.0 The effects of zinc, nitrogen and phosphorus fertilizer placement on the growth of wheat and barley in field experiments.

3.1 INTRODUCTION

Zinc deficiency in cereals is becoming increasingly common in southern Australia, a result of increased cropping frequency and an almost universal change in fertilizer from ordinary superphosphate (high in zinc impurity) to high analysis NP fertilizer (e.g. Riley *et al.* 1992). Throughout the greater part of the low rainfall agricultural areas of southern Australia, the problem is exacerbated by root zone alkalinity which tends to increase with soil depth. Zinc deficiency in these areas is corrected either by foliar application or by applying zinc fertilizer to the cultivated layer - 0.05 m deep. The movement of zinc below this layer by leaching is improbable (Brennan and McGrath, 1988). There is increasing laboratory evidence that root function is impaired in the absence of external zinc (Nable and Webb, 1993). Field studies in this area are limited, although where attempts have been made to enhance subsoil fertility, results have been impressive (Graham *et al.* 1992b). The correction of subsoil infertility by mechanical means may be expensive although if benefits extend over several years then the costs per year can be reduced proportionately.

A practical means for treating soil compaction and ameliorating high subsoil acidity by injecting lime slurry into the subsoil was developed in North-Eastern Victoria by Brooke *et al.* (1986). A machine similar in principle to that described by Brooke *et al.* (1986) was constructed for field experiments with nitrogen, phosphorus and zinc fertilizer on Upper Eyre Peninsula, a low rainfall cereal growing area with subsoils characterised by high pH and frequently, salinity and high extractable boron concentrations below 0.50 m. In these dry areas, surface drought is common during the growing season and there is some evidence that placement of phosphorus fertilizer below the normal depth of cultivation enhances phosphorus uptake and plant growth if the surface dries out (Simpson and Pinkerton, 1989). The initial experiment described here was conducted in 1993 and was designed to examine the effects of placement of

zinc in the subsoil (Ap, A and Btk horizons, to a depth of 0.40 m) on the growth of wheat in the field. A second site with modified treatments, was established nearby in 1994. The 1993 site was re-sown to barley in 1994. Both sites were re-sown to wheat in 1995.

3.2 MATERIALS AND METHODS

Sites were selected in adjacent fields N6 (1993, 1994 and 1995) and S4 (1994 and 1995) at Minnipa Research Centre on Upper Eyre Peninsula (lat. 32° 51'S, long. 135° 09'E), approximately 400 km NW of Adelaide, the capital of South Australia. The soil on which the experiments were conducted was a solonised brown soil (Stace *et al.* 1968), or a Calcixerollic Xerochrept, fine silty, mixed, thermic (Soil Taxonomy - Soil Survey Staff 1975). Site details are given in the Appendix, (Table A3.1).

3.2.1 Experiment A. field N6 - 1993

The experiment was laid out in a randomised block design with four replicates. There were eight treatments. Treatments are described by abbreviations in which "C" represents controls, "R" represents deep ripped, "Z", "N" and "P" represent added zinc, nitrogen and phosphorus respectively, "T" represents treatments in which nutrients were added to the topsoil only and "S" represents subsoil treatments.

Treatments were as follows:

* **[C]** non ripped control plots.

* **[RC]** ripped (to 0.40 m) control plots.

* **[ZT]** zinc applied as solid fertilizer to the topsoil (0 - 0.05 m) (10 kg Zn ha^{-1} as $ZnSO_4.7H_2O$)

* **[ZS]** zinc applied in water to a depth of 0.40 m (10 kg Zn ha^{-1} as $ZnSO_4.7H_2O$).

* **[NPT]** nitrogen and phosphorus applied to the topsoil as granulated solid fertilizer (41 kg N ha^{-1} (67% as NH_4NO_3, 33% as diammonium phosphate (DAP)) and 15 kg P ha^{-1} as DAP).

* **[NPS]** nitrogen and phosphorus applied in water to a depth of 0.40 m (41 kg N ha^{-1} (83% as NH_4NO_3, 17% as monoammonium phosphate (MAP) and 15 kg P ha^{-1} as MAP).

* **[NPZT]** nitrogen, phosphorus and zinc applied to the topsoil as granulated fertilizer (10 kg Zn ha^{-1} as $ZnSO_4.7H_2O$, 41 kg N ha^{-1} (67% as NH_4NO_3, 33% as DAP) and 15 kg P ha^{-1} as DAP).

* **[NPZS]** nitrogen, phosphorus and zinc applied in water as a single application to a depth of 0.40 m (10 kg Zn ha^{-1} as $ZnSO_4.7H_2O$, 41 kg N ha^{-1} (83% as NH_4NO_3, 17% as MAP, and 15 kg P ha^{-1} as MAP).

Plots were 30 m long, with 5 m spacings between plot centres. Each plot consisted of two adjacent strips sown with a 10 row combine drill, with 0.18 m row spacings. All plots (both topsoil and subsoil treatments apart from the C treatment) were ripped in the same direction on May 27 1993 at a speed of 0.9 m s^{-1} with a three tyned Howard "Paraplow" with tyne spacings of 0.45 m. Plots consisted of three ripped strips, each 1.35 m wide - a total ripped width of 4.05 m. Spray nozzles were attached to the back of each tyne to allow nutrient solutions to be pumped into the subsoil below 0.05 m at a pressure of 210 kPa. Surface treated plots (ZT, NPT, NPZT and RC) were also ripped, and received water only. The water application rate of 16 700 l ha^{-1} was common to all plots. After ripping, fertilizer was applied to the surface treated plots using a small plot drill and all plots received the same number of passes. In the subsoil treatments (ZS, NPS, NPZS), 82% of total nitrogen, 60% of total phosphorus and 70% of total zinc (totals include basal dressings) were applied in water to the subsoil below 0.05 m.

A light roller was used to break up surface clods. Plots were then sown to Machete wheat on June 16 at a sowing rate of 59 kg ha^{-1} with a basal ("starter") dressing of 9 kg N ha^{-1} and 10 kg P ha^{-1} as DAP applied with the seed. On June 18, gravimetric water content was determined at 0.1 m intervals to a depth of 1 m from 50 mm core samples taken from each plot. Concomitantly, soil bulk densities were determined for 0.1 m intervals from thin walled stainless steel core tubes inserted at random horizontally into pit walls dug in adjacent ripped and non ripped control plots. Six replicates were taken from each depth.

Penetrometer resistances to a depth of 0.45 m were also measured at 30 mm intervals using a Bush recording penetrometer (Anderson *et al.* 1980) on June 18. The penetrometer cone had a diameter of 12.6 mm and a total enclosed angle of 30°. Five replicates were measured at random sites in each plot.

At Feekes 4 (Large 1954), 20 youngest emerged wheat leaf blades (YEBs) were collected from each plot for nutrient analysis. The samples were oven dried for 24 hours at 80°C and ground in a stainless steel mill. The samples were then digested in nitric acid and analysed for nutrient concentration (boron, copper, magnesium, manganese, phosphorus, potassium, sodium, sulphur and zinc) by inductively coupled plasma spectrometry (ICPS) (Zarcinas and Cartwright 1983, Rengel and Graham 1995a).

At Feekes 7, wheat dry matter production was determined from two x 1 m rows from each plot (0.36 m^2). The cut material was dried for 24 hours at 80°C and dry weight determined. On October 6 at Feekes 10.5.4, dry matter production was determined again, with three replicates of four x 1 m rows from each plot (0.72 m^2).

On November 8, near crop maturity, penetrometer resistances were measured. Nine replicate measurements were made at random in each of the four C plots and an adjacent ripped plot.

On November 23 at crop maturity, plots were harvested with a small plot harvester and wheat grain yield determined. Grain samples were retained for ICPS analysis and for protein determination (at 11% water content) using near infra red spectrophotometry (NIR). An area of each plot was left unreaped and four replicates of 0.5 m x 2 row quadrats (0.54 m^2 $plot^{-1}$) were cut from each plot to determine total dry matter production, heads m^{-2}, hundred grain weight and harvest index. Harvest index was calculated as the ratio of grain to total weight of tops. Immediately after harvest, three 50 mm soil cores were taken from each plot to a depth of 1 m. The cores were divided into 0.2 m intervals and the three samples were bulked for each depth interval and weighed. The bulked samples were first air dried as described by Hignett (1976) and then oven dried to determine gravimetric water content. Bulked samples from 0.2 - 0.4, 0.4 - 0.6, 0.6 - 0.8 and 0.8 - 1.0 m depth intervals for each plot were retained for the determination of rooting density (L_v), based on the method of Hignett (1976). Roots washed from soil were floated onto A4 blotting paper and photocopied. Root lengths and diameter were determined from scanned images using SCI-SCAN root measurement software.

Water use efficiency (kg ha^{-1} mm^{-1}) was calculated in terms of grain production from the difference in total water loss to the depth of maximum rooting in each plot between sowing and maturity plus the rainfall in that period. Maximum rooting depth was determined according to the criterion of Forrest *et al.* (1985). It was assumed that losses through deep drainage were negligible.

3.2.2 Experiment B. field N6 - 1994

On June 9 1994, the N6 experimental area used in 1993 was resown to Stirling barley at 60 kg ha^{-1} with a basal fertilizer dressing of 6 kg N ha^{-1} and 13 kg P ha^{-1} as MAP applied with the seed. Soil water measurements in 0.2 m intervals were taken to 1.0 m on July 1, a week after plants began to emerge. A single 50 mm core sample was taken from each plot. Penetrometer resistances to 0.45 m were also measured from five replicates in each of the C and RC plots to assess changes in soil strength in the year after ripping. Barley tissue samples consisting of 25 YEBs

collected from each plot at Feekes 5 were dried, ground, digested in nitric acid and analysed for nutrient content by ICPS.

Dry matter production of barley shoots was estimated at Feekes 8 from three replicates of 0.5 m x 2 rows cut from each plot (0.54 m^2). The cut material was dried for 24 hours at 80°C and weighed. Total dry matter production was estimated in the same way at crop maturity on November 29. Heads were counted to determine heads m^{-2}; hundred grain weights and harvest indices were also measured. Soil water contents in 0.2 m intervals to 1.0 m were also determined from bulked samples from three 50 mm cores taken from each plot.

Plots were harvested using a small plot harvester on December 5. Grain samples were retained for determination of protein (NIR) and nutrient content (ICPS).

3.2.3 Experiment C. field N6 - 1995

In 1995, the experimental plots in N6 were re-sown to Machete wheat on May 29 at a sowing rate of 65 kg ha^{-1} with a basal fertilizer dressing of 50 kg ha^{-1} DAP.

At Feekes 5, 70 YEBs were taken from each plot and dried for 24 hours at 80°C before digestion in nitric acid and ICPS analysis.

3.2.4 Experiment D. field S4 - 1994

Because of the positive responses to nitrogen, phosphorus and zinc applied in the N6 experiment at relatively high rates for this low rainfall environment, a site was established in an adjacent field (S4) in 1994 to investigate higher applications of nitrogen and phosphorus alone and with zinc.

The experiment was laid out in a randomised block design with six replicates. The C, RC, NPT, NPS, NPZT and NPZS treatments applied in Experiment A at the N6 site were repeated. The same abbreviations used to represent the treatments at the N6 site apply, with the inclusion of "H" to represent higher rates of nitrogen and phosphorus.

The following additional treatments were included:

* **[NPHT]** nitrogen and phosphorus applied to the topsoil with the drill at a higher rate (93 kg N ha^{-1} (61% as NH_4NO_3, 39% as DAP) and 40 kg P ha^{-1} as DAP).

* **[NPHS]** nitrogen and phosphorus applied in water at a higher rate to a depth of 0.40 m with the paraplow (91 kg N ha^{-1} (80% as NH_4NO_3, 20% as MAP) and 40 kg P ha^{-1} as MAP).

* **[NPZSS]** the NPZS treatment was repeated except that 10 kg Zn ha^{-1} was applied as $ZnSO_4.7H_2O$ in water in a separate ripping pass as distinct from the NPZS treatment in which MAP, NH_4NO_3 and $ZnSO_4.7H_2O$ were applied together. The final "S" in this treatment indicates separate application of NP fertilizer and zinc.

* **[NPHZT]** the NPHT treatment was repeated except that 10 kg Zn ha^{-1} as $ZnSO_4.7H_2O$ was applied at the same time through a separate compartment of the drill.

* **[NPHZS]** the NPHS treatment was repeated except that 10 kg Zn ha^{-1} was also applied as $ZnSO_4.7H_2O$ in solution in a separate ripping pass.

The experiment was established on the same soil type described for the 1993 experiment. Full details are given in the Appendix (Table A3.1). Ripping and fertilizer treatments were applied on April 11 in very dry soil (matric potential (Ψ_m) = - 1.5 MPa). Water was applied in every case at a rate of 16 700 l ha^{-1} per pass. Plot centres were at 3.0 m and each plot consisted of two ripped strips 2.6 m wide with a single (1.8 m) sown strip (10 rows) in the centre. All plots were ripped (other than the non ripped control) and they received the same application of water. All plots other than the non ripped control received the same number of ripping passes, the same rate of water application and the same number of passes with the combine

drill on the same day. Topsoil fertilizer treatments were applied with a drill after ripping.

In the NPHS and NPHZS treatments, 91% of total nitrogen and 80% of total phosphorus were applied to the subsoil. To all subsoil treatments in which zinc was applied, 70% of the zinc was placed in the subsoil.

A light roller was used immediately after ripping to break down surface clods. The plots were then cultivated and rolled again just before sowing on June 10. The plots were sown with 60 kg ha^{-1} Machete wheat at a depth of 0.05 m with a basal fertilizer dressing of 9 kg N ha^{-1} and 10 kg P ha^{-1} as DAP. The soil was moist to 0.20 m but below this depth was close to the -1.5 MPa matric potential .

On June 27, as the wheat plants were emerging, a 50 mm soil core was taken from each plot to measure soil water content in 0.2 m intervals to a depth of 1.0 m. Penetrometer resistances were also measured. Eight replicate readings were taken on adjacent C and RC plots in four of the six replicates chosen at random. On July 17, plants in the early tillering stage were already suffering the visible effects of water stress. Plants grew rapidly to the late tillering stage (Feekes 3) on July 27 and had reached Feekes 5 by August 11, when 25 YEBs were taken from each plot. The samples were dried for 24 hours at 80°C, ground and digested in nitric acid before ICPS analysis for nutrient concentrations as for the 1993 experiment.

Two pits 2 m deep were dug at the end of two adjacent C and RC plots for site description and measurement of bulk density. Six stainless steel cores of 75 mm diameter and 100 mm depth were inserted into the pit face in the centre of each 0.1 m depth interval for the measurement of bulk density.

Three replicates of 2 rows x 0.5 m (0.54 m^2) areas were cut from each plot on August 30 at Feekes 7. Plants were wilting at the time from severe water stress. On October 14, plants had reached Feekes 11.2 and were ripe by November 1. On November 8, two cuts (0.36 m^2) were taken from each plot to determine dry matter of tops, harvest

index, heads m^{-2} and hundred grain weight. Grain yield was determined with a small plot harvester on November 17. Grain samples were retained for measurement of protein (NIR) and ICPS analysis as for the 1993 field N6 experiment.

At crop maturity, three 50 mm soil cores were taken at random to a depth of 1.0 m. The three cores were cut at 0.2 m intervals and bulked for each depth interval. After the soil was thoroughly mixed, a small subsample was collected to determine gravimetric water content. The bulk sample was then dried as described by Hignett (1976). Root measurements were determined from each sample as described for the N6 site. Root measurements were taken in the 0.2 - 0.4 and 0.4 - 0.6 m depth intervals only. Water use efficiency was calculated as previously described.

3.2.5 Experiment E. field S4 - 1995

In 1995, the experimental plots in S4 were resown to Machete wheat on May 29 at a sowing rate of 65 kg ha^{-1} with a basal fertilizer dressing of 50 kg ha^{-1} DAP. At Feekes 5, 70 YEBs were taken from each plot and dried for 24 hours at 80°C before digestion in nitric acid and ICPS analysis. At maturity, the plots were reaped with a small plot harvester and grain yields determined. Grain samples were retained for determination of protein concentrations and ICPS analysis as described above.

3.2.6 Statistical Analysis

Results were analysed by analysis of variance using the Statistix software package. The basic assumptions of the analysis of variance were tested with each analysis. The assumption of additivity of replicate and treatment effects was tested using Tukey's 1 degree of freedom test. Homogeneity (constant error variance) was assessed by plotting residuals against fitted values. The assumption of normality of the data distribution was tested using the Wilk Shapiro statistic and the rankit plot. Where significant *F* values were obtained for analysis of variance, probability terms are shown as *, ** or *** for $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. Treatments were generally compared by the least significant difference (LSD), but only where significant *F* values were obtained for the analysis of variance. Raw means are

presented in all tables and graphs, but in the case of transformed data, the indication of significance of differences between means are based on analyses of the transformed data.

3.3 RESULTS

3.3.1 Experiment A. field N6 - 1993

3.3.1.1 Rainfall

Rainfall data for Minnipa for 1993, 1994 and 1995 are shown in Fig. 3.1 The total rainfall at Minnipa in 1994, the driest year ever recorded, was 160.4 mm. The mean annual rainfall is 325 mm.

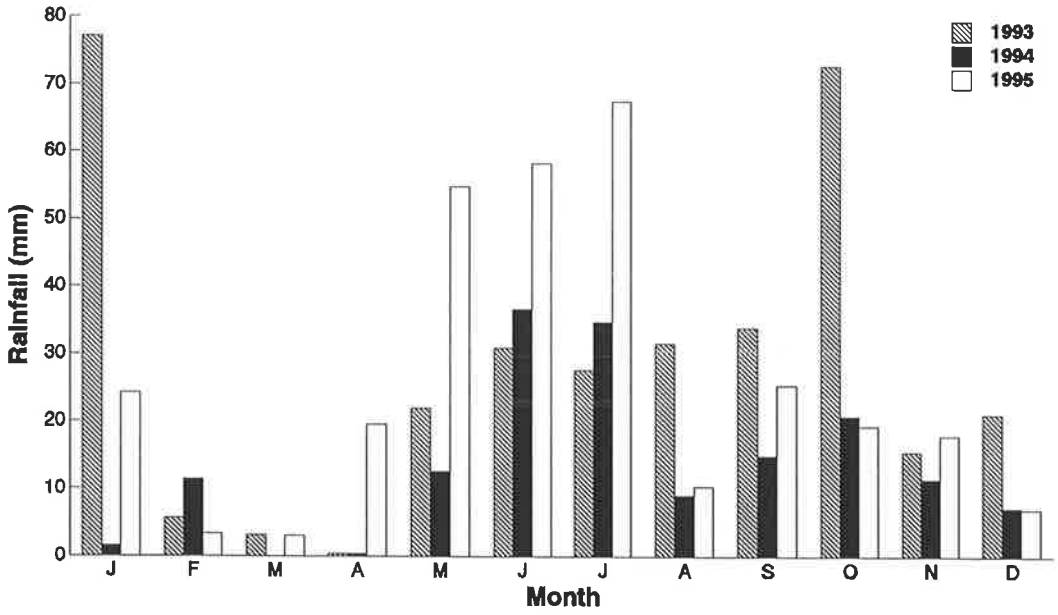

Fig. 3.1 Monthly rainfall at Minnipa, South Australia, in 1993, 1994 and 1995.

3.3.1.2 The effects of deep ripping on soil strength

The effects of deep ripping to 0.40 m on soil strength shortly after sowing are shown in Fig. 3.2. The graph compares mean penetrometer resistance at each depth for the C plots (n_1) and the remaining ripped plots (n_2). LSD was calculated for each depth as:

$$LSD = t\sqrt{EMS}\ (^1/n_1 + {}^1/n_2)$$

where t = t for error mean square (EMS) df.

The ripped plots were significantly weaker in terms of soil strength than the C plots between 0.09 and 0.36 m. (There were no significant differences between treatments in terms of soil water content to 0.50 m at the time of measurement, Table 3.1). By crop maturity, soil strength in the non ripped plots exceeded the capacity of the penetrometer (~7 MPa) below 0.12 m (Fig. 3.3). Excessive soil strength as a cause of restricted root growth has been well documented. It is apparent from the work of Dexter (1987) that penetrometer resistances of the order of 4-5 MPa measured at a depth between 0.15 and 0.27 m (Fig. 3.2) at sowing in the C plots would seriously restrict the penetration of roots into the subsoil in this environment (Ehlers *et al.* 1983) without the presence of low resistance pathways or biopores (Jakobsen and Dexter 1988).

Plate 3.1 Equipment used in the field experiments to inject nutrients into the subsoil to a depth of 0.40 m.

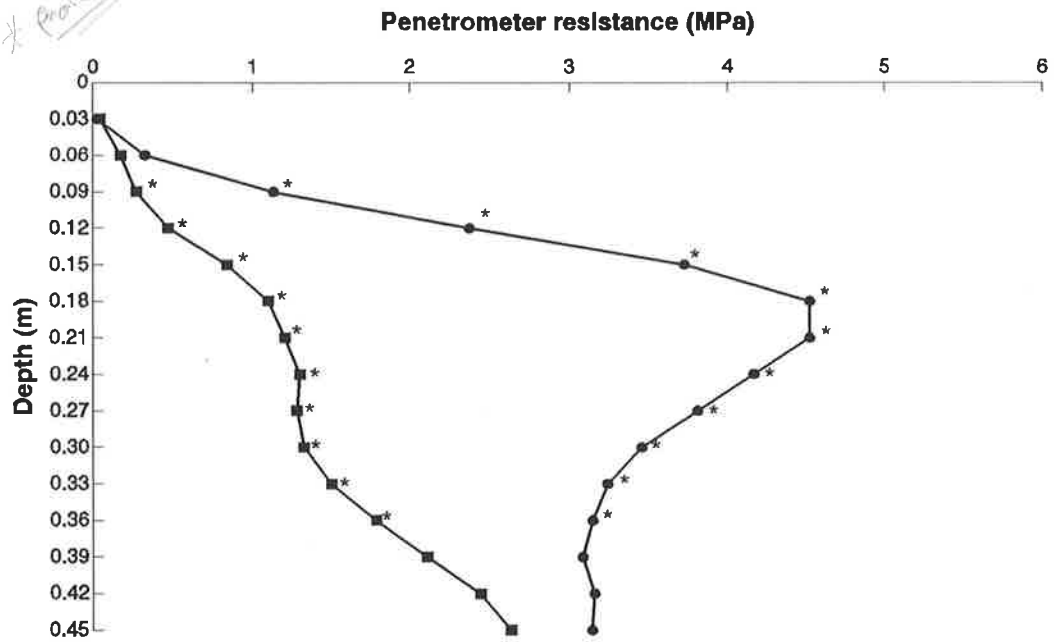

Fig. 3.2 Penetrometer resistance (MPa) as a function of depth for ripped (■) and non ripped (●) soil on June 18 in field N6, Minnipa, 1993. Values at the same depth marked with an (*) are significantly different ($P \leq 0.05$).

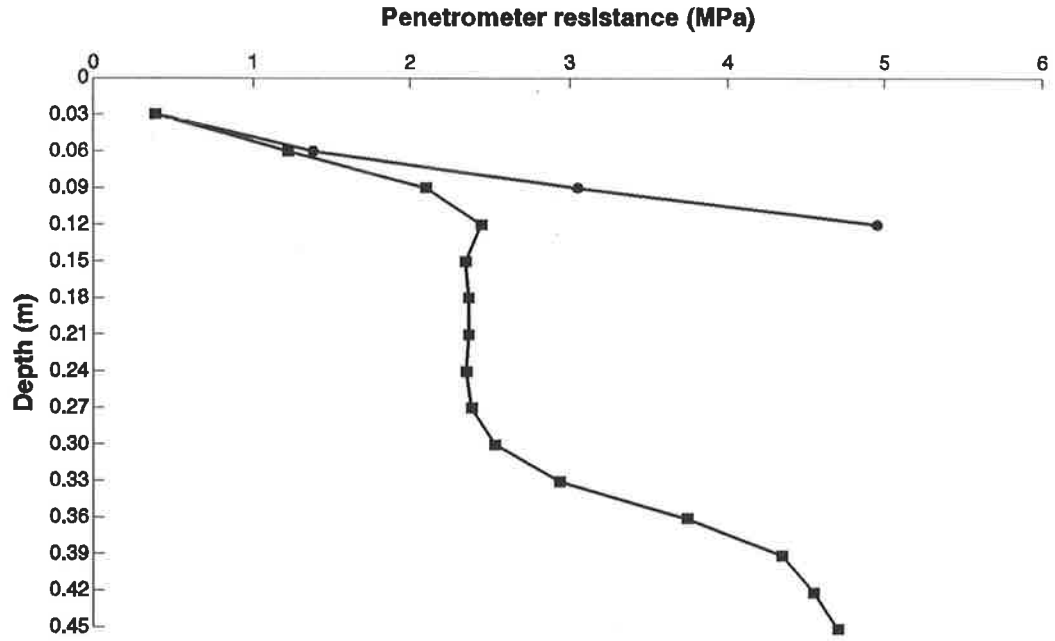

Fig. 3.3 Penetrometer resistance (MPa) as a function of depth for ripped (■) and non ripped soil (●) at crop maturity in field N6, Minnipa, 1993.

Table 3.1 Gravimetric soil water content as a function of depth in ripped and non ripped plots on June 18, field N6, Minnipa, 1993.

Depth	Ripped	Non ripped	*F*
(m)	(kg kg^{-1})	(kg kg^{-1})	
0.0 - 0.1	0.132	0.137	ns
0.1 - 0.2	0.121	0.125	ns
0.2 - 0.3	0.104	0.124	ns
0.3 - 0.4	0.114	0.118	ns
0.4 - 0.5	0.116	0.123	ns

Table 3.2 Zinc and phosphorus concentrations in YEBs of Machete wheat shoots at Feekes 4, in field N6, Minnipa 1993, as a function of fertilizer placement and ripping treatments. Values in the same column with the same letter are not significantly different ($P \leq 0.05$)

Treatment	Zn[A]	P
	(mg kg^{-1})	(g kg^{-1})
NPZT	20.9 b	2.67 d
NPZS	35.9 a	3.01 b
NPT	12.7 d	2.85 c
NPS	11.4 de	3.24 a
ZT	17.8 c	2.67 d
ZS	19.5 bc	2.60 d
C	10.5 e	2.74 cd
RC	11.5 de	2.60 d
F	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

3.3.1.3 Nutrient concentrations in tissue

The results of ICPS analyses of YEB tissues for zinc and phosphorus concentrations at Feekes 4 are shown in Table 3.2. Zinc concentrations in YEBs from the C and RC treatments and from the NPT and NPS treatments indicate at best marginal zinc concentrations. That is, while there were no obvious symptoms of zinc deficiency, a reduction in growth can be expected (Reuter and Robinson 1986). With all treatments where zinc was added, zinc concentrations in tissues were significantly enhanced. Plants in the NPZS plots had significantly higher zinc concentrations than all other treatments, more than three times those of the control plots. Zinc concentrations in YEBs were enhanced by the addition of NP and zinc fertilizer to the topsoil (NPZT) compared with the addition of zinc alone (ZT). Phosphorus concentrations in tissues were relatively low and in all but the NPS and NPZS plants, concentrations of phosphorus were marginal (Reuter and Robinson 1986).

3.3.1.4 Production of dry matter

Dry weights of shoots determined at Feekes 7 and Feekes 10.5.4 are shown in Table 3.3. While ripping had no statistically significant effect compared with non ripping on dry matter production at Feekes 10.5.4, all treatments in which nitrogen and phosphorus were added, irrespective of whether they were added to the topsoil or subsoil, produced significantly more dry matter than the non ripped control. Low zinc concentrations in tissue (as determined from YEBs collected at Feekes 4) did not appear to have affected dry matter production. At Feekes 10.5.4, the NPS treatment had produced 28% more dry matter than the C treatment and it was the only treatment to have produced significantly more than the RC. The addition of zinc alone had no significant effects on dry weight of shoots, compared with the RC and C plots.

Table 3.3 Dry weight of shoots of Machete wheat at Feekes 7 and Feekes 10.5.4 as a function of fertilizer placement and ripping treatment in field N6, Minnipa 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Dry weight of shoots Feekes 7	Dry weight of shoots Feekes 10.5.4
	(kg ha^{-1})	(kg ha^{-1})
NPZT	1 311	3 751 abc
NPZS	1 489	3 800 ab
NPT	1 350	3 905 ab
NPS	1 417	4 140 a
ZT	1 211	3 494 bcd
ZS	1 188	3 257 cd
C	1 211	3 225 d
RC	976	3 417 bcd
F	ns	*

3.3.1.5 Grain yield and other production parameters

Grain yield and other production parameters are shown in Table 3.4.

Table 3.4 Grain yield, head density, harvest index, grain protein and water use efficiency as a function of fertilizer placement and ripping treatments in field N6, Minnipa 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Grain yield	Head density	Harvest index	Grain protein	Water use efficiency
	(kg ha^{-1})	(heads m^{-2})	(%)	(%)	(kg grain ha^{-1} mm^{-1})
NPZT	1 702 abc	215	54.9 ab	13.7	7.8 abc
NPZS	1 830 a	263	55.7 a	13.9	9.2 a
NPT	1 750 a	231	53.1 c	13.6	7.9 abc
NPS	1 731 ab	263	49.1 d	13.9	8.2 ab
ZT	1 664 abc	196	53.8 bc	13.2	7.7 abc
ZS	1 376 c	216	54.4 abc	13.6	6.6 bc
C	1 392 c	265	52.8 c	13.6	6.6 bc
RC	1 411 bc	232	53.4 bc	13.5	6.4 c
F	*	ns	*	ns	*

The NPZS treatment produced the highest grain yield, 31% higher than the non ripped control and 30% higher than the ripped control. The supply of zinc alone had no such effect. Three treatments - NPZS, NPS and NPT produced yields significantly higher than the non ripped control. Only one treatment with added zinc, NPZS, gave yields significantly higher than the ripped control. The highest harvest index occurred in the NPZS treatment and the lowest in the NPS treatment. It is worthy of note that the NPS treatment had a significantly lower harvest index than other treatments suggesting (by contrast with the NPZS treatment) that both zinc and NP fertilizer are required in the subsoil for efficient grain formation. A common difficulty with the application of nitrogen fertilizer in excess of 10-15 kg ha^{-1} in this environment is that

crops with lush growth tend to "burn off" in times of soil water stress and high temperatures at the end of the season. The absence of zinc in the subsoil may contribute to this problem because of the role of zinc in stabilising root membranes and optimising water use efficiency.

Grain protein percentages did not differ significantly between treatments (although analysis of variance gave a close to significant value of F for treatment effects on grain protein, $P \leq 0.06$). Highest values occurred in the NPS and NPZS treatments.

Water use efficiency was calculated from water loss in the root zone between sowing and harvest, plus the rainfall in that period (see Appendix, Table A3.5). The most water use efficient plants were with treatment NPZS which produced 39% more grain per mm of water lost from the root zone than plants from the C treatment. This increase in efficiency cannot be ascribed to the presence of zinc alone because water use efficiency of the ZS treatment was not different from the C treatment. The NPZS treatment alone was more water use efficient than both controls. This suggests again that both NP fertilizer and zinc are required in the subsoil for optimum root performance.

3.3.1.6 Root growth

There were no significant differences in rooting density (L_v) between treatments at any depth interval (Appendix, Fig. A3.1). The maximum rooting depth for all treatments was between 0.6 and 0.8 m. Root data were generally highly variable with coefficients of variation for data from the 0.2 - 0.4, 0.4 - 0.6 and 0.6 - 0.8 m depth intervals of 37%, 43% and 73% respectively. The NPZS treatment had the highest mean L_v at the bottom of the ripped zone and a relatively low value in the underlying 0.4 - 0.6 m interval. Soil water measurements, which also offer a useful guide to rooting density for given depth intervals (*e.g.* Ehlers *et al.*, 1980, Grimes *et al.* 1975, Hammel *et al.* 1985) indicate for the 0.4 - 0.6 m interval that the NPZS treatment contained significantly more soil water than all other treatments ($P \leq 0.05$), suggesting less root growth or impaired root function at this depth (Appendix, Table A3.7). There were no other significant differences in soil water content for any depth

interval. It is interesting that the variance of the L_v data was lower for the NPZS treatment than for other treatments.

Treatments had no significant effects on mean root diameters in the three depth intervals, nor on the proportion of fine roots (<0.3 mm diameter) in the total length of roots measured (Appendix, Table A3.6).

3.3.1.7 Nutrient concentrations in grain

Concentrations of zinc, phosphorus and boron in grain are shown in Table 3.5. As with tissue concentrations of zinc, grain zinc concentrations from the NPZS treatment were about three times those of the control plots and double the grain concentration of zinc in the ZT plots in which the same amount of zinc was applied to the topsoil. Applying zinc alone to the subsoil (ZS) did not increase mean grain zinc concentration significantly above that where zinc was applied to the topsoil (ZT). Where NP fertilizer was applied to the subsoil (NPS) zinc concentration in the grain was 10.1 mg kg^{-1}, significantly lower than in grain from the non ripped control. The reduction in grain zinc with this treatment is likely to be due to dilution (see Table 3.6).

Plate 3.2 The Howard "Paraplow" used in the field experiments to inject nutrients into the subsoil to a depth of 0.40 m.

Table 3.5 Concentrations of zinc, phosphorus and boron in Machete wheat grain, in field N6, Minnipa 1993, as a function of fertilizer placement and ripping treatments. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Zn[A]	P	B
	(mg kg^{-1})	(g kg^{-1})	(mg kg^{-1})
NPZT	23.5 b	2.92 b	2.1c
NPZS	35.3 a	2.81 b	2.3 c
NPT	11.2 de	2.86 b	3.5 a
NPS	10.1 e	3.32 a	3.3 ab
ZT	17.2 c	2.99 b	2.4 c
ZS	20.3 bc	2.88 b	2.3 c
C	12.0 de	3.09 ab	2.5 bc
RC	12.4 d	2.97 b	2.5 bc
F	***	*	*

[A] Analysis of variance performed on $\log_e$ - transformed data.

Concentrations of phosphorus, (Tables 3.2, 3.5), copper, manganese and magnesium (see Appendix, Tables A3.2, A3.3, A3.9 and A3.10) were significantly higher in the NPS tissues and grain than with the NPZS treatment, suggesting either competition with zinc for uptake sites, or zinc moderated control of uptake through its role in membrane function. This may be particularly important in the case of boron concentrations in grain (Table 3.5). Cartwright *et al.* (1984) considered that boron concentrations in grain of > 3 mg kg^{-1} were indicative of boron toxicity in barley. It is of particular interest that the NPT and NPS treatments exhibited high concentrations of boron in grain (3.5 and 3.3 mg kg^{-1} respectively). The addition of zinc to these treatments (NPZT and NPZS) lowered grain boron significantly.

3.3.1.8 Nutrient uptake in grain

Table 3.6 Effect of fertilizer placement and ripping treatment on uptake of zinc, phosphorus and boron in grain of Machete wheat in field N6, Minnipa 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.5$).

Treatment	Zn uptake [A]	P uptake [A]	B Uptake
	(g ha^{-1})	(kg ha^{-1})	(g ha^{-1})
NPZT	38.2 b	4.91	3.6 b
NPZS	65.4 a	5.17	4.1 b
NPT	19.5 d	4.96	6.2 a
NPS	17.3 d	5.73	5.5 a
ZT	28.2 c	4.98	3.9 b
ZS	28.3 c	3.98	3.0 b
C	17.2 d	4.41	3.4 b
RC	17.5 d	4.10	3.3 b
F	***	ns	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Zinc uptake in grain (Table 3.6) was significantly enhanced with the NPZS treatment, and was 71% higher than with the next highest treatment, NPZT, which in turn produced a 35% greater grain zinc uptake than the ZT and ZS treatments. There was no difference in zinc uptake due to placement of zinc alone in the subsoil or topsoil (ZS or ZT), but zinc uptake in grain from both ZT and ZS treatments was significantly higher than in grain from the NPT, NPS or control treatments. Phosphorus uptake tended to be higher in the NPS and NPZS treatments and lower in the ZS treatment, which was not supplied with additional phosphorus. The NPT and NPS grain uptake of boron was significantly higher than other treatments and it may be inferred that the addition of zinc (NPZT, NPZS) reduced boron uptake to a significant degree, presumably through its effect on the stability of root cell membranes.

3.3.2 Experiment B. field N6 - 1994

3.3.2.1 The effects of deep ripping in the previous season on soil strength

Although Minnipa is located in a low rainfall area (mean annual rainfall 325mm) and drought occurs frequently, 1994 was the driest year on record, with a total annual rainfall of only 160 mm. Rain fell infrequently and was restricted to light showers. Even at the beginning of the season, as crops were emerging, soil water potential below 0.2 m was close to -1.5 MPa (Appendix, Table A3.11). As it eventuated, June and July were the wettest months and little useful rain fell after the beginning of August. There were no significant differences in soil water content between treatments for any depth interval at crop emergence.

Penetrometer resistance for the RC and C treatments to a depth of 0.45 m is shown in Fig. 3.4. Although the data suggest that the ripped soil was weaker between 0.15 - 0.30 m, there were no significant differences ($P \leq 0.05$) in penetrometer resistance at any depth interval. While the data were highly variable, they do suggest that the physical effect of ripping in this environment is unlikely to remain for more than one, or at most two, seasons.

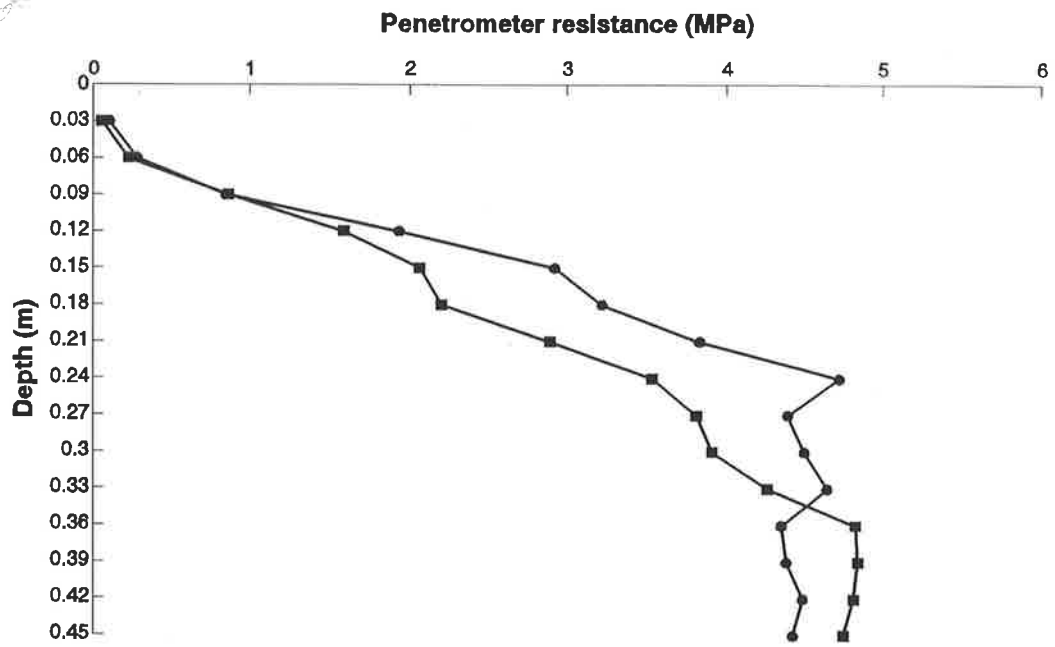

Fig. 3.4 Penetrometer resistance (MPa) in June 1994 as a function of ripped (■) and non ripped (●) treatments applied in 1993 in field N6, Minnipa.

3.3.2.2 Nutrient concentrations in tissue

Concentrations of zinc and phosphorus measured at Feekes 5 are shown in Table 3.7. Zinc concentrations in barley tissue differed widely between treatments. The application of zinc to the surface soil in 1993 (NPZT, ZT) resulted in significantly higher zinc concentrations in tissues in 1994 than where it was applied to the whole soil profile to 0.4 m. It is postulated that the higher zinc concentrations from surface applied zinc are due to the controlling influence of soil water - the topsoil was the principal source of water during the growing season and with the topsoil treatments, the applied zinc was concentrated in this zone. All treatments which received zinc produced plants with significantly higher zinc concentrations than NPT, NPS or control treatments, which did not differ significantly from each other. In all treatments, phosphorus concentrations appear to have been in the critical range for barley plants at Feekes 5 (Reuter and Robinson 1986). This may in part be a reflection of the rapid transition through the various growth stages while under water stress. Tissue sodium concentrations in Stirling barley (Appendix, Table A3.12) were an order of magnitude greater than in Machete wheat in 1993, with a mean concentration of 2,404 mg kg^{-1}. However, these concentrations are commonly recorded in barley in South Australia (A.J. Rathjen, pers. comm), and do not necessarily reflect excessive uptake from the subsoil.

Table 3.7 Concentrations of zinc and phosphorus in YEBs of Stirling barley at Feekes 5, in field N6, Minnipa 1994, as a function of fertilizer placement and ripping treatments applied in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 Treatment	Zn[A]	P
	(mg kg^{-1})	(g kg^{-1})
NPZT	28.4 a	2.70
NPZS	24.2 b	2.52
NPT	15.3 d	2.76
NPS	14.4 d	2.84
ZT	28.6 a	2.50
ZS	22.0 c	2.50
C	14.1 d	2.68
RC	13.7 d	2.62
F	***	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

3.3.2.3 Grain yield and other production parameters

Grain yield and other production parameters are shown in Table 3.8. Grain yields were very low and results must be considered in the context of severe water stress. Water use efficiency in terms of grain production (Table 3.9) was significantly higher in the non ripped control than all other treatments, and grain yield was higher than all treatments other than the ripped control (Table 3.8). The increased yield in the non ripped control may be explained in terms of the number of grain heads m^{-2} which it was able to support, 32% more than the ripped control and 94% more than the NPZT treatment and in a greater harvest index. There were no significant differences in grain size as expressed by hundred grain weight (Appendix, Table A3.8). Grain in general was small and shrivelled. Harvest indices also indicate that both treatments in which zinc was placed in the topsoil (NPZT and ZT) produced significantly less grain

as a proportion of total dry matter than the control treatments. There were no significant differences between treatments in terms of total dry weight of above ground plant parts (tops) at crop maturity (Appendix, Table A3.14). While the NPZS and NPS treatments produced only 59% and 56% of the grain reaped from the non ripped control plots respectively, the total dry weight of tops as a proportion of the non ripped controls were 99% and 98% respectively. Similarly, water use efficiencies for total dry weight of tops were not significantly different between treatments.

Table 3.8 Grain yield, head density and harvest index of Stirling barley in field N6, Minnipa 1994, as a function of fertilizer placement and ripping treatments applied in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 Treatment	Grain yield [A]	Head density	Harvest index [B]
	(kg ha^{-1})	(heads m^{-2})	(%)
NPZT	169 d	133	13.1 d
NPZS	205 bcd	211	19.7 abcd
NPT	217 bcd	188	19.6 abcd
NPS	196 cd	142	17.5 bcd
ZT	242 bc	166	16.5 cd
ZS	228 bcd	205	25.4 ab
C	348 a	258	26.1 a
RC	272 ab	196	20.7 abc
F	**	ns ($P \leq 0.1$)	*

A Analysis of variance performed on $\log_e$ - transformed data.

B Analysis of variance performed on square root transformed data.

Table 3.9 Grain protein, grain water use efficiency and dry matter water use efficiency of Stirling barley in field N6, Minnipa 1994, as a function of fertilizer placement and ripping treatments applied in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 Treatment	Grain protein	Water use efficiency Grain [B]	Water use efficiency [B] Dry Matter
	(%)	(kg grain ha^{-1} mm^{-1})	(kg dry matter ha^{-1} mm^{-1})
NPZT	17.0	1.4 c	15.4
NPZS	17.4	1.7 b	19.6
NPT	16.7	1.8 bc	18.2
NPS	16.7	1.7 bc	16.8
ZT	16.8	1.9 bc	18.4
ZS	17.1	1.9 bc	15.6
C	16.2	3.0 a	20.8
RC	16.3	2.22 b	18.1
F	ns	**	ns ($P \leq 0.1$)

[A] Analysis of variance performed on $\log_e$ - transformed data.

[B] Analysis of variance performed on square root-transformed data.

3.3.2.4 Nutrient concentrations in grain

Zinc concentrations in barley grain in 1994 (Table 3.10) indicate the significant effects of zinc fertilization in 1993. Interestingly, despite the harshness of the drought and the lack of penetrating rain, the NPZS treatments produced higher concentrations of zinc in grain than the ZT treatment. The NPT treatment produced a significantly higher grain zinc concentration than the NPS, possibly due to enhanced root growth in the topsoil in which the added fertilizer, native zinc and the growing season rainfall were concentrated, or perhaps due to phosphorus - zinc interactions.

There were no differences between treatments in concentrations of boron in grain (Table 3.10) with no grain (surprisingly) reaching the concentration of 3.0 mg kg^{-1} which indicates potential boron toxicity (Cartwright *et al.* 1984). This suggests that the soil wetting front in 1994 did not penetrate beyond 0.4 m into soil with high boron. Unpublished computer simulation data (B. Jakobsen pers. comm.) suggests that the wetting front is likely to have been between 0.4 - 0.6 m in 1994. Soil water potential in the 0.4 - 0.6 m depth interval was close to -1.5 MPa at the beginning of the season and at crop maturity. Grain from the C treatment was generally low in nutrient concentrations, suggesting restricted root growth and nutrient uptake earlier in the season coincident with reduced water uptake and top growth which, in an extremely dry season, proved advantageous as the plants matured.

Table 3.10 Concentrations of zinc, phosphorus and boron in Stirling barley grain in field N6, Minnipa 1994, as a function of fertilizer placement and ripping treatments applied in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 treatment	Zn[A]	P	B
	(mg kg^{-1})	(g kg^{-1})	(mg kg^{-1})
NPZT	37.0 ab	4.04 a	2.3
NPZS	37.4 a	3.38 c	2.3
NPT	19.3 c	3.57 bc	2.4
NPS	16.4 d	3.79 ab	2.4
ZT	30.7 b	3.72 abc	2.7
ZS	33.8 ab	3.45 bc	2.5
C	15.5 d	3.40 c	2.3
RC	18.0 cd	3.65 bc	2.2
F	***	*	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

3.3.2.5 Nutrient uptake in grain

Uptake of zinc in grain from added zinc treatments was significantly higher than in grain from the NPT and NPS treatments (Table 3.11). There were no effects of placement between NPZT and NPZS and ZT and ZS treatments. Phosphorus uptake in grain was significantly greater with the C treatment than all treatments other than the RC.

Table 3.11 Uptake of zinc, phosphorus and boron by Stirling barley grain, in field N6, Minnipa 1994, as a function of fertilizer placement and ripping treatments applied in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Zn uptake [A]	P uptake	B uptake [A]
	(g ha^{-1})	(kg ha^{-1})	(g ha^{-1})
NPZT	6.2 abc	0.67 c	0.37 c
NPZS	7.5 ab	0.75 c	0.48 bc
NPT	4.1 de	0.77 bc	0.51 bc
NPS	3.1 e	0.74 c	0.52 bc
ZT	7.4 ab	0.90 bc	0.67 ab
ZS	7.6 a	0.78 bc	0.61 bc
C	5.3 bcd	1.17 a	0.80 a
RC	4.9 cd	1.00 ab	0.61 bc
F	***	**	*

[A] Analysis of variance performed on $\log_e$ - transformed data.

3.3.3 Experiment C. field N6 - 1995

3.3.3.1 Nutrient concentrations in tissue

The results of ICPS analysis of YEBs for field N6 (third consecutive year of cropping) are shown in Table 3.12.

Table 3.12 Concentrations of zinc and phosphorus in YEBs of Machete wheat at Feekes 5 as a function of fertilizer placement and ripping treatments applied in 1993, in field N6, Minnipa 1995. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 Treatment	Zn	P
	(mg kg^{-1})	(g kg^{-1})
NPZT	33.2 a	3.37 b
NPZS	34.2 a	3.41 b
NPT	18.9 c	3.39 b
NPS	17.2 c	3.63 a
ZT	27.9 b	3.28 b
ZS	27.8 b	3.26 b
C	17.6 c	3.42 b
RC	17.7 c	3.39 b
F	***	*

Zinc concentrations in YEBs varied significantly ($P \leq 0.001$) between treatments in N6 in the third consecutive crop after the initial treatment application in 1993. The order of treatment differences reflected those of 1993, although zinc concentrations in the RC and C treatments were considerably higher in 1995, with a mean of 17.65 mg kg^{-1} compared with 11.0 mg kg^{-1} in 1993. As in 1993, while there were no differences in zinc concentrations in tissue between the ZT and ZS treatments, the addition of zinc, nitrogen and phosphorus in 1993 (NPZT, NPZS) significantly increased zinc concentrations above those with the ZS and ZT treatments. There were no

differences between treatments for phosphorus concentration in YEBs, apart from the significantly higher concentrations in the NPS treatment. Again, this treatment produced plants with significantly higher tissue phosphorus than other treatments in 1993. This effect is not likely to be a function of placement in soil of higher soil water content which is often suggested as a reason for enhanced phosphorus uptake in deep placement studies (Jarvis and Bolland 1990). From crop emergence on June 12 to the time of sampling, soil water in the topsoil was continually replenished by frequent light rains but is unlikely to have penetrated into the subsoil in any useful quantity. It is of interest that such a response to phosphorus was obtained in the third year after application.

3.3.4 Experiment D. field S4 - 1994

3.3.4.1 The effects of deep ripping on soil strength

The lack of soil water in 1994 undoubtedly had a major influence on the performance of all treatments. There were no differences in soil water content at any depth interval between treatments at the time of crop sowing (Table 3.13). Penetrometer resistances as a function of depth for C and RC treatments are shown in Fig. 3.5. Ripping had a significant effect on soil strength between 0.12 and 0.33 m.

Plate 3.3. Method of delivery of nutrients to the subsoil.

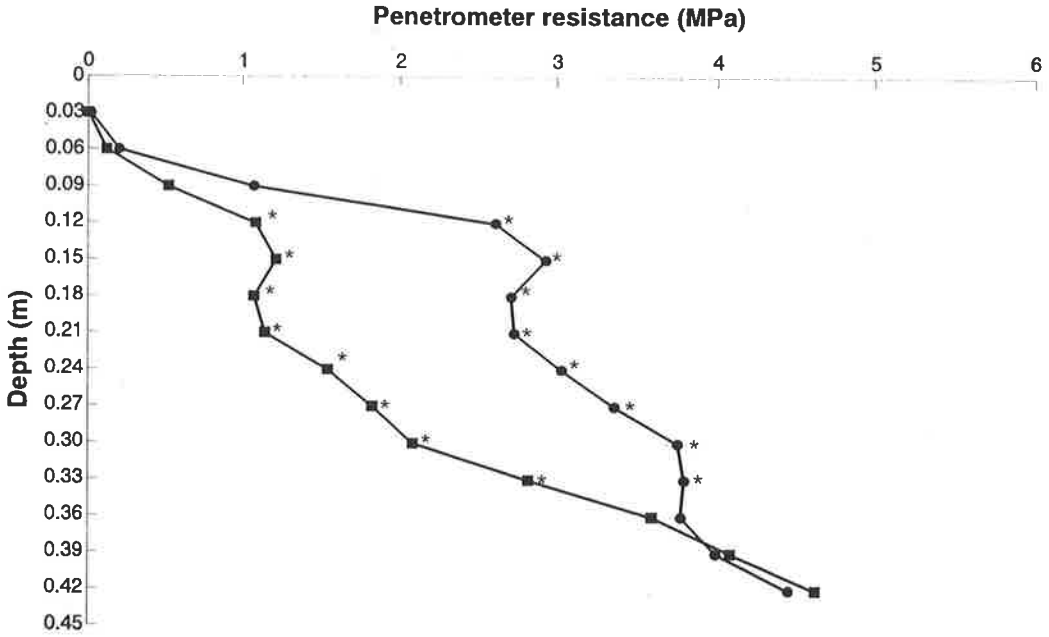

Fig. 3.5 Penetrometer resistance (MPa) as a function of depth in ripped (■) and non ripped (●) control plots at sowing in field S4, 1994. At a given depth, values marked with an (*) are significantly different (P≤0.05).

3.3.4.2 Soil water content at sowing

Table 3.13 Gravimetric soil water content as a function of depth in ripped and non ripped treatments in field S4, Minnipa at sowing 1994.

Depth	Ripped	Non ripped	*F*
(m)	(kg kg^{-1})	(kg kg^{-1})	
0.0 - 0.2	0.136	0.155	ns
0.2 - 0.4	0.105	0.110	ns
0.4 - 0.6	0.137	0.142	ns

3.3.4.3 Nutrient concentrations in tissue

Concentrations of zinc and phosphorus in YEB tissue at Feekes 5 are shown in Table 3.14.

Table 3.14 Concentrations of zinc and phosphorus in YEBs of Machete wheat at Feekes 5 as a function of fertilizer placement and ripping treatments in field S4, Minnipa 1994. Values in the same column with the same letter are not significantly different (P≤0.05).

Treatments	Zn[A]	P[A]
	(mg kg^{-1})	(g kg^{-1})
NPHZT	26.1 bc	4.99 abc
NPHZS	27.6 b	4.79 bcd
NPZT	36.4 a	4.62 cd
NPZSS	29.6 b	4.74 bcd
NPZS	36.8 a	4.61 d
NPT	17.8 de	4.54 d
NPS	16.2 e	4.62 d
NPHT	18.8 d	5.31 a
NPHS	16.6 de	5.04 ab
C	23.5 c	4.86 bcd
RC	18.6 de	4.60 d
F	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

The highest concentrations of zinc in YEBs were measured in plants from the NPZS and NPZT treatments, concentrations about twice as high as in plants from the ripped control. Zinc concentrations in those treatments not receiving zinc fertilizer (other than the non-ripped control) were marginal (Reuter and Robinson 1986). It is worthy of note that applying zinc to the subsoil did not result in increased tissue zinc

concentrations in plants from the NPHZS or NPZSS treatments above those in the corresponding topsoil treatments (NPHZT, NPZT) - indeed the NPZT tissues contained significantly higher zinc concentrations than the NPZSS. However, the application of zinc sulphate with ammonium nitrate and MAP mixed in water (NPZS) resulted in zinc concentrations in tissue which were significantly higher than in plants from the other subsoil treatments. While the method of delivery was the same, in the NPZS treatment, the zinc and NP fertilizer were applied together in solution whereas with the NPHZS and NPZSS treatments, the zinc was applied in a separate pass. (In the second pass in the NPZS treatment, only water was applied).

Phosphorus concentrations in tissue were adequate in plants from all treatments, although concentrations tended to be higher in plants with the high phosphorus (NPHT, NPHS, and NPHZT) treatments. Placement of phosphorus in the subsoil led to no significant increases in phosphorus concentrations in tissue compared with the corresponding topsoil treatments. Simple correlations performed on nutrient concentrations of tissues indicate that zinc concentrations were negatively correlated with manganese (r = -0.38 **), calcium (r = -0.29 *) and magnesium (r = -0.64***) (Appendix, Tables A3.18, A3.19). This corresponds with similar relationships in Machete wheat at the 1993 site - manganese (r = -0.42*), calcium (r = -0.29 ns) and magnesium (r = -0.41*). Loneragan and Webb (1993) have suggested that while calcium and magnesium inhibit zinc absorption from solutions, in soils their inhibitory activity is likely to be less important than the effects of their salts on soil pH.

3.3.4.4 Production of dry matter

Dry matter production of shoots at Feekes 7 is shown in Table 3.15. The NPHZS treatment produced more dry matter than other treatments at that stage, 83% higher than the ripped control. On August 30 1994, when the measurements were taken, plants were beginning to show signs of severe water stress. There were no increases in terms of dry matter production from soil disturbance alone, as indicated by the lack of significant difference between the RC and C treatments. At maturity, the NPHZS treatment produced the highest quantity of dry matter per hectare although significantly higher only than the controls and the NPZS and NPHS treatments. The NPHS plots were identical to the NPHZS in terms of fertilizer additions apart from the inclusion of

zinc but they produced significantly less dry matter. At crop maturity, both controls produced significantly less total dry weight of tops than most other treatments for which data could be compared.

Table 3.15 Dry weight of shoots at Feekes 7 and dry weight of above ground plant parts (tops) at crop maturity as a function of fertilizer placement and ripping treatments in field S4, Minnipa 1994. Values with the same letter are not significantly different (P≤0.05).

Treatments	**Dry weight of shoots at Feekes 7**	**Total dry weight of tops at maturity** [A]
	(kg ha^{-1})	(kg ha^{-1})
NPHZT	677 b	2 563 [Z]
NPHZS	835 a	2 503 a
NPZT	679 b	2 264 a
NPZSS	658 b	2 495 a
NPZS	558 bc	2 272 [Z]
NPT	552 bc	2 297 a
NPS	457 c	2 272 [Z]
NPHT	658 b	2 208 ab
NPHS	511 c	1 926 bc
C	478 c	1 692 c
RC	457 c	1 873 c
F	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

[Z] Analysis of variance performed without these treatments due to non additivity which could not be corrected by data transformation. The variance in the data for these treatments was higher than for other treatments.

3.3.4.5 Grain yield and other production parameters

Grain yield, head density and hundred grain weight are shown in Table 3.16. other production parameters are shown in Table 3.17.

Table 3.16 Grain yield, head density and hundred grain weight of Machete wheat as a function of fertilizer placement and ripping treatments in field S4, Minnipa 1994. Values in the same column with the same letter are not significantly different (P≤0.05).

Treatment	Grain yield	Head density	Hundred grain weight
	(kg ha^{-1})	(heads m^{-2})	(g)
NPHZT	938 ab	177 ab	3.82 a
NPHZS	982 a	176 ab	3.63 cd
NPZT	857 abcd	162 abc	3.84 a
NPZSS	908 abc	181 a	3.70 abcd
NPZS	965 a	160 bcd	3.65 bcd
NPT	849 abcd	159 bcd	3.80 ab
NPS	794 bcd	167 ab	3.70 abcd
NPHT	884 abc	157 bcd	3.69 abcd
NPHS	763 cd	145 cd	3.59 d
C	730 cd	140 d	3.60 d
RC	841 abcd	141 d	3.76 abcd
F	*	***	*

Table 3.17 Harvest index, grain protein and grain water use efficiency of Machete wheat as a function of fertilizer placement and ripping treatments in field S4, Minnipa 1994. Values of the same column with the same letter are not significantly different (P≤0.05).

Treatment	Harvest index	Grain protein	Water use efficiency Grain
	(%)	(%)	(kg grain ha^{-1} mm^{-1})
NPHZT	50.1 abcde	13.9 cd	6.05 ab
NPHZS	51.2 abc	14.3 ab	6.3 a
NPZT	50.8 abc	13.5 e	5.3 bcde
NPZSS	52.3 a	13.6 e	5.9 abc
NPZS	52.2 a	13.6 e	6.2 ab
NPT	51.1 abc	13.4 e	5.4 abcde
NPS	49.7 bcde	13.7 de	5.2 cde
NPHT	49.0 cde	14.1 bc	5.7 abcd
NPHS	48.3 de	14.4 a	5.0 de
C	47.9 e	13.0 f	4.7 e
RC	50.6 abcd	13.1 f	5.7 abcde
F	*	***	*

The highest grain yield was reaped from the NPHZS treatment - 35% higher than the non ripped control and 17% higher than the ripped control. Statistically however, none of the treatments to which extra fertilizer (above the basal dressing) was added yielded significantly more than the ripped control.

Compared with the non ripped control, the only measured parameter significantly increased by ripping alone was harvest index. The addition of high rates of nitrogen and phosphorus to the subsoil (NPHS) did not increase the number of fertile tillers

(heads m^{-2}) compared with the ripped control; adding zinc to this treatment (NPHZS) increased the number of fertile tillers by 21% above the NPHS and by 25% above the ripped control. As occurred in 1993, harvest index was low with subsoil treatments to which nitrogen and phosphorus were applied alone, suggesting that zinc is also required for efficient grain formation. Plants in the NPHZS treatment produced significantly more protein in grain than from the NPHZT treatment although the NPHS treatment also produced grain with significantly higher protein than from the NPHT treatment. However, 1994 was the driest year on record and rainfall was restricted to light showers. It is probable that any benefits of adding nutrients to the subsoil would be lower in a year when plants had to rely heavily on soil water and nutrient supplies in the topsoil. High protein concentrations in grain from the NPHZS and NPHS treatments may, in part, be due to water stress at grain filling, which is also reflected in slightly smaller grain size with these treatments. Water use efficiency was significantly higher with the NPHZS treatment than the NPHS. The ripped control had a lower number of fertile tillers than most of the treatments to which zinc was applied and significantly lower grain protein than all treatments other than the non ripped control.

The addition of zinc with higher rates of nitrogen and phosphorus than normally applied in this region (5 - 10kg N ha^{-1}, 10kg P ha^{-1}), had positive effects on several yield components. General contrast comparisons between groups of treatments containing zinc (NPZT, NPZSS, NPZS, NPHZT, NPHZS) (Zn+) and those not containing zinc (NPT, NPHT, NPS, NPHS) (Zn-), regardless of whether applied to the subsoil or topsoil, were performed on the yield component data using the t statistic for *a priori* comparisons. The results are shown in Table 3.18.

Table 3.18 General (*a priori*) contrasts between means of treatment groups (+Zn) and (-Zn) for grain yield, head density, hundred grain weight (HGW), harvest index (HI), grain protein, and grain water use efficiency (GWUE) of Machete wheat in field S4, Minnipa 1994.

Treatment group	Grain yield	Head density	HGW	HI	Grain protein	GWUE
	(kg ha^{-1})	(heads m^{-2})	(g)	(%)	(%)	(kg ha^{-1} mm^{-1})
Zn-	823	157	3.70	49.5	13.63	5.3
Zn+	930	170	3.73	51.7	13.81	6.0
P (t statistic)	**	**	ns	***	ns	**

Soil to which zinc was added produced significantly more grain (13% increase) with more fertile tillers and higher water use efficiency. Similar statistical analyses performed on treatment groups comparing those with zinc placed in the topsoil and subsoil showed no significant differences for any yield component other than hundred grain weight, in which case grain in treatments with surface applied zinc was 4.6% heavier. Similarly, when all treatments to which extra fertilizer (above the basal level) was applied to the topsoil were compared with subsoil treatments, hundred grain weight was reduced by 3.7% in the subsoil treatments. Apart from hundred grain weight, grain protein was the only yield component to be significantly affected by fertilizer placement, and was the only parameter to be increased significantly ($P \leq 0.001$) by subsoil placement. The mean grain protein concentration for all topsoil (T) treatments was 13.7% and for all subsoil (S) treatments 13.9%. Grain from the NPHZS treatment produced grain with a 10% higher concentration of grain protein than the ripped control. However, this increase in grain protein concentration may also be a function of water stress at grain filling.

3.3.4.6 Root growth

There were no effects of treatments on rooting density at either of the depth intervals measured (Table 3.19; Appendix, Table A3.20). However, mean rooting density for all treatments for the 0.2 - 0.4 m interval was almost four times less than the overall mean rooting density for the same soil type at the 1993 (N6) site, and seven times less for the 0.4 - 0.6 m depth interval, indicating the large negative effects of water stress on root growth.

Table 3.19 Rooting density and gravimetric soil water content in Machete wheat plots at a depth interval of 0.2 - 0.4 m as a function of fertilizer placement and ripping treatments at crop maturity in field S4, Minnipa 1994.

Treatment	**Rooting Density 0.2 - 0.4 m**	**Soil water content 0.2 - 0.4 m**
	(L_v cm cm^{-3})	(kg kg^{-1})
NPHZT	0.74	0.099 ab
NPHZS	0.77	0.094 b
NPZT	0.98	0.090 b
NPZSS	0.65	0.093 b
NPZS	0.68	0.095 b
NPT	0.84	0.097 b
NPS	0.73	0.098 b
NPHT	0.70	0.097 b
NPHS	0.70	0.099 ab
C	0.78	0.094 b
RC	0.60	0.108 a
F	ns	*

Soil water measurements taken at crop maturity may also be used as an indication of root activity and the correlation coefficient for gravimetric water content and rooting density in the 0.2 - 0.4 m depth interval was - 0.53 (***). Rooting density was lowest at the bottom of the ripped zone with the ripped control treatment, and soil water content was correspondingly high (Table 3.19).

Analysis of variance gave significant values of F for treatment effects on mean root diameters and proportions of fine roots (<0.3 mm diameter) in the 0.2-0.4 m depth interval (Appendix, Table A3.21). However, absolute differences between treatments were small.

There is no indication from the raw data that ripping and subsoil application of nutrients induced greater variability in rooting density - indeed the rooting data from the NPZS treatment tended to be more uniform in terms of rooting density values from 0.2 - 0.4 m depth interval than other treatments including the controls. The highest variance for L_v data from this interval occurred in the RC treatment.

3.3.4.7 Nutrient concentrations in grain

Nutrient concentrations in grain are shown in Table 3.20. The highest concentration of zinc in grain occurred with the NPZS treatment in which zinc sulphate was mixed with ammonium nitrate and MAP in solution and applied as a single application. This treatment could not be included in the analysis of variance because of the effect of non additivity - the standard error of the mean was 3.6 with upper and lower 95% confidence intervals about the mean 17.2 and 35.6 respectively. (For all other treatments in which zinc was included, standard error of the mean was 0.69 and upper and lower confidence intervals 19.2 and 16.3 respectively). Nevertheless, the data suggest that this treatment was singularly effective in increasing grain zinc concentrations. The same treatment applied in 1993 also produced high tissue and grain zinc concentrations.

Table 3.20 Concentrations of zinc, phosphorus and boron in Machete wheat grain as a function of fertilizer placement and ripping treatments in field S4, Minnipa 1994. Values in the same column with the same letter are not significantly different (P≤0.05).

Treatment	Zn[A]	P[A]	B
	(mg kg^{-1})	(g kg^{-1})	(mg kg^{-1})
NPHZT	15.2 b	2.4 de	9.1
NPHZS	17.9 a	2.5 bc	9.4
NPZT	18.7 a	2.2 ef	9.1
NPZSS	19.3 a	2.4 cd	8.7
NPZS	26.4 [Z]	2.4 cd	9.3
NPT	10.1 c	2.3 de	10.4
NPS	8.8 c	2.5 b	10.6
NPHT	9.3 c	2.5 bcd	9.3
NPHS	8.9 c	2.8 a	9.6
C	9.3 c	2.1 f	9.3
RC	9.9 c	2.4 de	9.7
F	***	***	ns

[A] Analysis performed on $\log_e$ - transformed data.

[Z] Data were analysed without treatment NPZS due to non additivity which could not be corrected by data transformation. The mean and variance for this treatment were much greater than for other treatments.

Subsoil treatments in all cases produced significantly higher grain phosphorus than their corresponding topsoil treatments and the ripped control. Ripping had a significant effect compared with non ripping on phosphorus (Table 3.20) and magnesium (Appendix, Table A3.24) concentrations in grain which are closely associated as they were in 1993 (1993 r = 0.91 ***; 1994 r = 0.82 ***).

Grain protein and phosphorus concentrations were highly correlated in this experiment (r = 0.66 ***). Ripping alone led to a higher grain phosphorus concentration than non ripping but there were no differences in grain protein between these treatments. Undoubtedly there were complex interactions between ripping, nitrogen, phosphorus, zinc and water use in determining grain protein. Treatments to which zinc was added tended to have lower grain manganese and iron than the added NP treatments (Appendix, Table A3.23). In 1993 (N6) grain manganese in wheat was negatively correlated with grain zinc (r = - 0.43*) and again in 1994 (S4) (r = - 0.49 ***).

There were no treatment effects on concentrations of boron in wheat grain - probably the best indicator of boron toxicity. It is highly likely that excess boron affected yield to some extent in all treatments and it is of interest that boron concentrations in grain were four times higher than those in barley at the N6 site in the same season. Zinc added to the subsoil had no effect on grain boron although the concentration of zinc added was much less than that found to be ameliorative by Singh *et al.* (1990).

3.3.4.8 Nutrient uptake in grain

Uptake of zinc, phosphorus and boron in grain is shown in Table 3.21. Zinc uptake was greatest in the NPZS treatment - significantly higher (47%) than the next highest treatment (NPHZS). Otherwise there were no differences between treatments to which zinc was added, all of which showed a higher uptake of zinc than grain from nil zinc treatments. Phosphorus uptake was significantly increased by ripping compared with non ripping.

Table 3.21 Uptake of zinc, phosphorus and boron in Machete wheat grain as a function of ripping and fertilizer placement in field S4, Minnipa 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Zn[A]	P	B
	(g ha^{-1})	(kg ha^{-1})	(g ha^{-1})
NPHZT	14.4 b	2.23 ab	8.5
NPHZS	17.5 b	2.45 a	9.2
NPZT	15.9 b	1.90 ab	7.8
NPZSS	17.3 b	2.19 b	7.9
NPZS	25.8 a	2.30 ab	9.0
NPT	8.7 c	2.00 b	8.7
NPS	7.0 c	2.02 ab	8.4
NPHT	8.3 c	2.18 ab	8.3
NPHS	6.8 c	2.16 ab	7.2
C	6.7 c	1.54 c	6.8
RC	8.3 c	1.99 b	8.2
F	***	**	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

3.3.5 Experiment E. Field S4 - 1995

3.3.5.1 Nutrient concentrations in tissue

Nutrient concentrations in YEBs from the S4 experiment site in 1995 are shown in Table 3.22. The highest zinc concentration occurred in the NPZS treatment, 68% higher than the RC and C. This treatment in the third year at the N6 site also produced the highest tissue zinc, although not significantly greater than in the NPZT treatment. In contrast to 1994, which was extremely dry, placing zinc with nitrogen

and phosphorus in the subsoil at the S4 site in the previous year significantly increased zinc concentration in tissue above topsoil placement in 1995. Significantly higher concentrations of boron in the 1995 YEBs in the subsoil zinc treatments (NPHZS, NPZSS) than the NPS and NPHS treatments are difficult to explain, although they may represent more extensive root growth. Root growth was not measured in 1995.

Table 3.22 Concentrations of zinc, phosphorus and boron in YEBs of Machete wheat at Feekes 5 in field S4, Minnipa 1995, as a function of fertilizer placement and ripping treatments applied in 1995. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Zn	P	B[A]
	(mg kg^{-1})	(g kg^{-1})	(mg kg^{-1})
NPHZT	19.3 c	3.36 cde	11.6 e
NPHZS	24.9 b	3.60 ab	17.3 a
NPZT	19.7 c	3.42 cde	13.9 bcd
NPZSS	24.4 b	3.33 e	14.7 ab
NPZS	27.0 a	3.35 de	14.2 bc
NPT	15.5 d	3.70 cde	12.8 bcde
NPS	15.5 d	3.47 bcde	11.9 cde
NPHT	16.3 d	3.51 abc	11.6 e
NPHS	15.3 d	3.62 a	11.9 de
C	16.1 d	3.49 abcd	13.0 bcde
RC	16.1 d	3.33 e	13.7 bcd
F	***	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

3.3.5.2 Grain yield and protein

Results of grain yield and protein determinations are shown in Table 3.23.

Table 3.23 Grain yield and protein concentration of Machete wheat in field S4, Minnipa 1995, as a function of deep ripping and fertilizer placement treatments applied in 1994. Values with the same letter are not significantly different ($P \leq 0.05$).

1994 Treatment	Grain yield[A]	Grain protein
	(kg ha^{-1})	(%)
NPHZT	1 481 b	14.45 cde
NPHZS	1 441 bc	14.80 abc
NPZT	1 455 b	14.33 e
NPZSS	1 614 a	14.32 e
NPZS	1 593 a	14.42 de
NPT	1 344 cde	14.45 cde
NPS	1 288 e	15.03 ab
NPHT	1 335 cd	14.78 bcd
NPHS	1 129 f	15.17 a
C	1 391 bcd	13.42 f
RC	1 406 bcd	14.17 e
F	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data

Analysis of variance gave significant values of *F* for the effects of treatments applied in the previous year on grain yield and protein concentrations in 1995. Grain yields with two subsoil treatments, NPZS and NPZSS, were significantly higher than all other treatments. Grain yields from the NPZSS treatment exceeded those from the ripped control by 15%. Ripping alone had no effect on grain yield. By contrast, yields from NPHS plots were significantly lower than those from all other treatments, including the controls. It is of interest that protein concentrations were highest with this treatment. Protein concentrations were significantly lower in grain from non ripped control plots than from other treatments, and in this instance, ripping in the previous season significantly enhanced grain protein compared with not ripping.

3.3.5.3 Nutrient concentrations in grain

Concentrations of zinc, phosphorus and boron in grain from field S4 in 1995 are shown in Table 3.24. The data indicate a clear advantage for subsoil placement of zinc (with NP fertilizer) in terms of increasing zinc concentration in grain. Grain yield was highly correlated with zinc concentration in grain (r = 0.40 **). The NPZS treatment was superior to all others in this regard, with zinc concentrations in grain double those of the ripped control. It is of interest that ripping in the previous year resulted in a significant increase in zinc concentration in grain compared with not ripping. Grain phosphorus concentrations were significantly higher in grain from the two subsoil treatments given NP fertilizer only, without added zinc - NPHS and NPS. There was a highly significant negative correlation between grain phosphorus and yield (r= -0.47 **). There were no significant effects of ripping in the previous year on grain phosphorus concentrations. Grain boron concentrations were also significantly negatively correlated with yield (r = - 0.27 *). Zinc placement in the subsoil appeared to have no consistent effect on boron concentrations in grain.

Table 3.24 Concentrations of zinc, phosphorus and boron in grain of Machete wheat in field S4, Minnipa 1995, as a function of deep ripping and fertilizer placement treatments applied in April 1994. Values with the same letter are not significantly different ($P \leq 0.05$).

1994 Treatment	Zn	P	B
	(mg kg^{-1})	(g kg^{-1})	(mg kg^{-1})
NPHZT	15.4 cd	3.11 bc	10.0 cd
NPHZS	22.4 b	3.24 b	11.2 abc
NPZT	16.8 c	3.10 bc	11.7 ab
NPZSS	22.3 b	3.10 bc	9.6 d
NPZS	26.0 a	3.12 bc	10.2 bcd
NPT	12.3 ef	3.16 b	10.9 bcd
NPS	13.5 de	3.54 a	10.8 bcd
NPHT	13.4 de	3.24 b	10.6 bcd
NPHS	12.7 ef	3.69 a	11.5 abc
C	10.9 f	2.98 c	11.7 ab
RC	13.2 e	3.14 bc	12.6 a
F	***	***	*

3.3.5.4 Nutrient uptake in grain

Table 3.25 Uptake of zinc, phosphorus and boron in Machete wheat grain in field S4, Minnipa 1995, as a function of deep ripping and fertilizer placement treatments applied in 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1994 Treatment	Zn[A]	P	B
	(g ha^{-1})	(kg ha^{-1})	(g ha^{-1})
NPHZT	22.9 c	4.61	14.7 bcd
NPHZS	31.9 b	4.65	16.2 abc
NPZT	24.2 c	4.48	17.0 ab
NPZSS	36.3 b	5.01	15.3 abcd
NPZS	41.7 a	4.99	16.2 abc
NPT	16.6 de	4.25	14.6 bcd
NPS	17.3 de	4.30	13.9 cd
NPHT	17.7 d	4.16	14.2 cd
NPHS	14.3 f	4.48	12.9 d
C	18.5 ef	4.15	16.2 abc
RC	15.3 d	4.41	17.6 a
F	***	[Z]	*

[A] Analysis of variance performed on $\log_e$-transformed data.

[Z] Data not analysed by analysis of variance due to non additivity which could not be corrected by data transformation. Individual pairwise comparisons were made by the two sample t-test (Appendix, Table A3.31).

Uptakes of zinc, phosphorus and boron in grain are shown in Table 3.25. Zinc uptake was significantly higher in grain from subsoil treatments to which zinc was added than from other treatments, including those in which zinc was added to the topsoil. Zinc uptake in grain from the latter was also significantly higher than from treatments not receiving added zinc. Phosphorus uptake in grain was not changed significantly by the treatments compared with the ripped control (Appendix, Table A3.31). Grain boron uptakes varied significantly between treatments but there were no apparent patterns of fertilizer placement or ripping effects.

3.4 GENERAL DISCUSSION

The addition of zinc fertilizer to cereal plots in 1993 (N6) and 1994 (S4) increased zinc concentrations in tissues (YEBs) to a significant degree in the year of application and in following seasons above those with treatments to which zinc was not applied. At the N6 site, significant increases in tissue zinc were measured in the third consecutive crop, and at the S4 site, in the second consecutive crop. The application of zinc with NP fertilizer consistently produced the highest concentrations of zinc in YEBs and grain in 1993 and 1995. In the very dry 1994 season, in which rainfall was largely restricted to light showers with little subsoil water available, topsoil treatments were equally as effective (S4) or more effective (N6 - barley) than subsoil treatments in increasing concentrations of zinc in plants.

Zinc concentrations in grain were substantially enhanced by zinc fertilization but subsoil fertilization with nitrogen, phosphorus and zinc produced significant increases in grain zinc concentration above other treatments in the majority of cases. This has implications for human nutrition in areas where cereals constitute a major portion of the diet. As Welch (1993) has pointed out, increasing the concentration and quantity of zinc and its bioavailability in plants used for food is of increasing importance. The importance of high zinc concentrations in seed has also recently been demonstrated by Rengel and Graham (1995a,b), who showed that high zinc concentrations in both efficient and inefficient cultivars produced more and bigger grain than seed with low

zinc concentrations within each cultivar, when grown in soil of low zinc status. Responses to zinc, nitrogen and phosphorus placement in the subsoil (in terms of zinc concentrations) were more obvious in grain than in YEBs presumably because of a higher dependance on subsoil water at grain filling. These effects were more pronounced in the better seasons. Phosphorus concentrations in YEB tissues were at times (but not consistently) enhanced by subsoil placement of NP fertilizer.

For all treatments in the 1993, 1994 and 1995 experiments with Machete wheat, there was a close association between YEB zinc and grain zinc (1993 r = 0.94 ***; 1994 r = 0.86 ***; 1995 r = 0.71***). At the higher levels of NP fertilizer applied with zinc at the S4 site in 1994 (NPHZT and NPHZS), grain from the subsoil treatment in 1994 and 1995 contained significantly more zinc than the topsoil treatment. Zinc uptake in wheat grain was consistently highest in NPZS treatments where nitrogen, phosphorus and zinc were mixed as a single application, but, as reported by King *et al.* (1992) zinc uptake was also affected by seasonal rainfall.

The application of NP fertilizer and zinc sulphate together in water to a depth of 0.4 m substantially and consistently enhanced zinc concentrations in wheat tissue and grain and zinc uptake in grain compared with ripped and non ripped controls. Nable and Webb (1993) reported that zinc concentrations in grain of both the zinc-efficient cultivar Excalibur and zinc-inefficient cultivar Gatcher (Graham *et al.* 1992a) were doubled by adding zinc to the subsoil in a pot experiment. The effects on tissue and grain in the N6 and S4 experiments extended beyond the year of application to the second year at least. This treatment (NPZS) also enhanced zinc concentrations in tissue and grain in the better seasons 1993 and 1995, compared with applying the same rates of nutrient to the topsoil, applying zinc alone to the subsoil, or with zinc sulphate applied at the same time as NP fertilizer in separate applications. There is no simple explanation for this result. It is probable that relatively insoluble zinc ammonium phosphates (*e.g.* $ZnNH_4PO_4$) would form as a result of mixing MAP, ammonium nitrate and zinc sulphate in water (Lehr 1972). Brown and Krantz (1966) noted that zinc ammonium phosphate powder was "very satisfactory" as a fertilizer if

it was mixed with the whole soil volume in pots in which maize was grown, but was much less effective if granulated, indicating that the availability of zinc was greater when the powder was thoroughly mixed with the soil. It is possible that a suspension of zinc ammonium phosphate applied as it was in this experiment forms an effective "slow release" fertilizer in this soil. There is however, some (unpublished) evidence that such a combination produces an unexplained increase in zinc availability in hydroponic solutions (K Millhouse, pers. comm.).

Murphy *et al.* (1978) suggested that when ammonia and 11-37-0 (liquid ammonium polyphosphate) were injected to a depth of 0.15 m, consistent responses to the simultaneous placement of nitrogen and phosphorus independent of soil available phosphorus levels were due to "a possible change in phosphorus chemistry produced by high concentrations of ammonium nitrogen in the phosphorus retention zone". In the current experiments, a change in zinc chemistry with the addition of ammonium and phosphate may have altered the immediate and long term agronomic effectiveness of the zinc. Mortvedt and Gilkes (1993) have suggested that relative agronomic effectiveness of zinc fertilizer applied in the first year is more dependent on zinc-soil reactions for the second crop than the first crop.

Ammonium nitrate, technical grade MAP and DAP were applied to the plots in different proportions because of the initial imperative to apply the same total amounts of nitrogen and phosphorus to the "T" (topsoil) and "S" (subsoil) plots. Because of difficulties with calibration and application, (drill application rates are not continuously variable but change in discrete increments), the proportions of nitrogen supplied as ammonium and nitrate varied with treatments. In general however, "T" plots received 57% of their total applied nitrogen as ammonium and 43% as nitrate. "S" plots received lower proportions of ammonium nitrogen - about 47%.

At soil depths below 0.05 m, "S" plots treated with NP fertilizer received 36% of total nitrogen as ammonium. Evidence exists from solution culture studies (which are

independent of soil factors which affect the availability of ammonium or nitrate nitrogen during the growing season) that a ratio of between 25 - 75% ammonium to nitrate nitrogen enhance dry matter production and root growth in wheat compared with the provision of either ammonium or nitrate alone (Gashaw and Mugwira 1981, Villa *et al.* 1992). Some studies have suggested that pH changes in the bulk soil due to the form of nitrogen applied may significantly alter nutrient availability (Thomson *et al.* 1993). There was no evidence of changes in bulk soil pH due to fertilizer treatments in the S4 experiment in 1994. Core samples to 0.40 m from the C and NPZS treatments taken for rooting density measurements were subsampled and pH (1:5 soil:water) measured. There were no changes in soil pH due to treatment effects. No attempt was made to measure changes in the pH of rhizosphere soil. The most prominent influence on cation/anion uptake ratios and hence on rhizosphere pH, is the form of nitrogen taken up by roots (Marschner and Romheld 1983), and in alkaline soils, mobilisation of phosphorus and zinc can be enhanced by supplying nitrogen in the ammonium form (Marschner *et al.* 1989, Marschner 1991). In the current experiment "T" plots received a higher proportion of the total added nitrogen as ammonium than the "S" plots.

Dry matter production mid season was generally highest with NPZS treatments. Similarly, grain yield was generally highest with NPZS treatments but this apparent advantage was not always significant and at the N6 site in the 1994 drought, the C treatment produced significantly more barley than other treatments. The data suggest that soil water deficiency had the overriding control of responses to treatments in this experiment. The plants in the non ripped control may have grown less rapidly earlier in the season, due to restricted root growth and nutrient supply and this may have led to more available supplies of soil water during the latter part of the season and grain filling. This phenomenon has been recorded by Meyer and Alston (1978) who were able to increase yield in wheat with a limited water supply by reducing the number of seminal roots at the beginning of the season, conserving more water for grain filling. Depth of fertilizer placement had no significant and consistent effect on grain yield. Grain protein in wheat was generally enhanced by subsoil placement of NP fertilizer with or without zinc, significantly so at the S4 site in 1994, but this did not apply to

4.0 The effects of antecedent species on the zinc nutrition of wheat.

4.1 INTRODUCTION

The benefits of growing crops in rotations have been recognised for centuries, particularly where nitrogen fixing legumes form part of the sequence. Enhanced yields and quality of produce (increased protein, for example) due to nitrogen fixation and reduction in the incidence of pests and diseases are well documented responses to appropriate crop rotations (Bullock 1992).

It is possible that one advantage of rotating crops is the effect that the different species have on the availability of nutrients other than nitrogen. Besides the specific effects of microbial activity in modifying the availability of plant nutrients (King 1990), attention has been focused recently on the ability of some plants to influence the availability of nutrients by modifying the rhizosphere. For instance, Cakmak and Marschner (1990) have demonstrated how cotton (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.) and buckwheat (*Fagopyrum esculentum* Möench.) in zinc deficient media could alter the cation-anion uptake ratio by decreasing nitrate uptake, resulting in a lowering of external pH. Zinc deficient monocotyledons did not, by comparison, decrease the pH of the nutrient solution.

As Cakmak and Marschner (1990) have pointed out, zinc is poorly available to plants in calcareous soils, but acidification of the rhizosphere induced by ammonium, rather than nitrate, nutrition or excretion of citric acid as a response to phosphorus deficiency may increase zinc availability. The authors considered that the low molecular weight organic solutes released by zinc deficient dicotyledons (one response to zinc deficiency) were not as effective as the phytometallophores released by zinc (or iron) deficient grasses in mobilizing zinc in calcareous soil.

While some species are able to mobilize zinc from zinc deficient soils, the question remains: is this mobilization likely to be beneficial to the following crop? Only

recently has the possibility been raised that the ability of crops to mobilize nutrients for their own use may enhance the uptake of these nutrients by following crops. It has been demonstrated that *Lupinus albus,* for example, is able to mobilize nutrients such as phosphorus and manganese to the benefit of wheat (*Triticum aestivum* L.) intercropped with it (Gardner and Boundy 1983). However, there is little evidence of increased micronutrient uptake as a result of rotational sequences. Leggett and Westermann (1986) measured enhanced zinc uptake in beans (*Phaseolus vulgaris* L.) grown after maize (*Zea mays* L.) compared with beans grown after sugar beet (*Beta vulgaris* L.). Zinc uptake in beans grown after maize exceeded the uptake in plants fertilized with 11.2 kg ha^{-1} zinc and grown on fallow or after sugar beet. There were no significant differences in DTPA-extractable zinc in soil after fallow, sugar beet or maize.

The authors considered that the enhancement in zinc uptake after maize was due to mycorrhizal effects - mycorrhizae did not proliferate in sugar beet or fallow. Enhanced zinc uptake in beans grown after maize was not observed in greenhouse or growth chamber experiments, the authors presuming that mycorrhizae were absent.

King *et al.* (1992) demonstrated strong effects of seasonal rainfall on zinc uptake in wheat grain in an experiment conducted on a zinc-deficient soil and recorded higher zinc uptake in wheat from legume-wheat or continuous wheat rotations than from grassy pasture-wheat rotations. Zinc concentrations in wheat were consistently lower after lupins (*L. angustifolius*) than after pasture (*Medicago* spp. + grasses), field peas (*Pisum sativum* L.), tick beans (*Vicia faba*), wheat or barley (*Hordeum vulgare L.*). These effects were small and not always statistically significant. Oliver *et al.* (1993) reported higher grain cadmium concentrations in wheat grown after lupins than in wheat grown after barley, beans, volunteer pasture, sown pasture or fallow.

The southern Australian farming system is based largely on crop rotations with legume based pastures (principally *Medicago* spp.), with common rotations being wheat-barley-pasture, or wheat-barley-pasture-pasture. Recently, however, an

increasing number of farmers have included field peas in their cropping sequences and there exists a body of anecdotal evidence that wheat grown after medic pasture no longer produces the yields which were once expected and that wheat grown after field peas consistently produces superior crops. While there may be many reasons for this, the incidence of zinc deficiency in these areas has increased concurrently. It is possible that part of the reason for the enhanced performance of wheat following pea crops is due to enhanced mobilization of zinc by peas. While zinc fertilizer can be economically applied to the topsoil it is difficult to apply to the subsoil, particularly below 0.4 m where available zinc concentrations in southern Australia are invariably low. The possibility exists that some species may be able to mobilize sufficient zinc from soils low in available zinc to meet their own requirements and enhance zinc uptake in following crops. The aims of the deep pot experiment described here were to compare the abilities of several antecedent species to cope with zinc-deficient soil and to modify the available zinc status of the soil to the benefit of wheat grown in the following year.

4.2 MATERIALS AND METHODS

4.2.1 Experimental design and statistical analysis

The experiment, begun in 1993, consisted of a completely randomised design with eight replicates of each treatment - with 11 treatments, there were 88 pots in all. Plants were sown in PVC pots 0.15 m diameter and 1.0 m deep, lined with sealed polythene bags.

The experiment was conducted in Laffer sand - a zinc deficient (DTPA extractable zinc 0.05 mg kg^{-1}) siliceous sandy soil from virgin forest at Tintinara in the south-east of South Australia. To half the pots were added complete nutrients, including 0.25 mg Zn kg^{-1} dry soil as $ZnSO_4.7H_2O$ (Zn+ pots) and to the other half, complete nutrients with the exception of zinc (Zn- pots). Seeds of rough seeded lupin (*Lupinus pilosus* cv. 20957 (Atwell 1991) - an accession from Haifa in Israel), field pea (*Pisum sativum*) cv. Early Dun, annual medic (*Medicago truncatula* Gaertn.) cv. Parabinga, wheat (*Triticum durum*) cv. Durati, barley grass (*Hordeum leporinum* Link) and Indian mustard (*Brassica juncea* (L.) Czern and Coss.) cv. Pusa Bold were sown in

1993; *B. juncea* seeds were sown in eight surplus Zn- pots only, and these were then randomised within the total 88 pots. Four of the eight replicates were dismantled at harvest for measurements of root growth. The remaining four replicates were stored over summer and sown to wheat in 1994.

The data were subjected to analysis of variance using Statistix software. Assumptions of constant error variance (homogeneity), normality of data distribution and non-additivity of replicate and treatment effects were tested for each analysis as described in Chapter 3. *A priori* linear contrasts were used to compare the performance of legumes with grasses as antecedent genera in terms of affecting zinc uptake in wheat. Student's t-test was used to identify significant differences.

4.2.2 A. 1993

4.2.2.1 Preparation of materials

When the soil was collected, the surface 0.05 m was removed to decrease the amount of organic matter present. The soil was air dried in a sealed glasshouse and sieved through a stainless steel 1 mm sieve. The dry soil was mixed with $CaCO_3$ at 3000 mg kg^{-1} soil. This procedure raised the pH (1:5 soil: water) to 8.2. Basal nutrients were added in solution separately as follows (mg kg^{-1} dry soil) and thoroughly mixed: $Ca(NO_3)_2.\ 4H_2O$ (918); KH_2PO_4,(72); K_2SO_4 (114); $MgSO_4.7H_2O$ (140); H_3BO_3 (1.4); NaCl (3.2); $MnSO_4.H_2O$ (2.0); $CuSO_4.5H_2O$ (2.2); $CoSO_4.5H_2O$ (0.22); $H_2MoO_4.H_2O$ (0.22); $FeSO_4.7H_2O$ (0.7). The total soil was then halved and to one half was added $ZnSO_4.7H_2O$ in solution at a rate equivalent to 0.25 mg Zn kg^{-1} dry soil (Zn+ soil). No zinc was added to the remaining half (Zn- soil). Both halves of the bulk soil were mixed with triple deionised glass distilled water (TD water) to field capacity (0.12 kg kg^{-1}; ψ_m = -10 kPa). Wet soil, equivalent to 19 kg dry soil, was added to the PVC pots. Seeds were surface sterilized in a solution of 1.5% sodium hypochlorite, thoroughly rinsed in TD water and sown on September 21, 1993.

4.2.2.2 Experimental Procedures

After emergence, plants were thinned to give two *L. pilosus, P. sativum, T. durum* and *B. juncea* plants pot^{-1}, five *H. leporinum* and eighteen *M. truncatula* plants pot^{-1}.

The plants were grown under controlled conditions in a laboratory where the temperature was maintained between 10°C and 20°C, with a light flux density of 500 μmol quanta m^{-2} s^{-1} and 11 hour daylength. Low intensity incandescent lighting was also provided for 13 hours daily. After the plants emerged, 150 g of polythene beads were added to each pot as mulch. The beads were washed in detergent, triple rinsed in TD water, soaked in 10% nitric acid for 24 hours and triple rinsed again in TD water before addition to the pots. All glassware used in the experiment was treated in the same way before use.

Pots were weighed (and re-randomized) weekly and watered to field capacity when the weight of the pot indicated that the soil water content had decreased to 50% of field capacity. The amount of water added to each pot was recorded. On any day, pots were given a maximum of 500 ml of water.

When all plants of a given species had reached mid anthesis in the zinc treated (Zn+) soil, all plants of that species were harvested, (including plants in the Zn- soil) by cutting the plants 1 cm above the soil surface.

Shoots were dried for 24 hours at 80°C, weighed, ground and digested in nitric acid for ICPS analysis for concentrations of boron, copper, iron, manganese, magnesium, sodium, potassium, phosphorus, sulphur and zinc (Zarcinas and Cartwright 1983, Rengel and Graham 1995a). Subsamples were also analysed for nitrogen content using the Kjeldahl procedure. Harvesting of tops began with *P. sativum*, *M. truncatula* and *T. durum* on December 7 and concluded with *L. pilosus* and *H. leporinum* on January 9. *B. juncea* plants were harvested on December 22. At harvest, half of the pots in which each species were grown were weighed and dismantled. The column of soil was cut into depth intervals of 0 - 0.1, 0.1 - 0.2, 0.3 - 0.5, 0.5 - 0.7 and 0.7 - 0.9 m. Each sample was dried as described by Hignett (1976) for measurements of root weight. The dried samples were later soaked and washed over a fine mesh sieve to separate roots from soil. Care was taken to avoid contamination of roots with soil particles. The roots were then re-dried and weighed. Nutrient concentrations in roots were not determined because of the large amount of

washing required to separate roots from soil and the difficulty in avoiding contamination. It was assumed that roots would have reached their maximum depth at anthesis (Troughton 1962, Hurd and Spratt 1975).

The remaining pots were watered to field capacity after harvest and allowed to incubate until May 6 in a sealed storage room in which the temperature varied between 10°C and 30°C with a median temperature of 25°C. On May 6, calcium nitrate ($Ca(NO_3)_2.4H_2O$) equivalent to 26.3 mg N kg^{-1} dry soil (500 mg N pot^{-1}) was added with sufficient TD water to bring the soil in each pot to field capacity.

4.2.3 B. 1994

4.2.3.1 Experimental procedures

Seeds of wheat (*Triticum durum* L.) cv. Durati (containing about 330 ng Zn $seed^{-1}$) were surface sterilized in 1.5% sodium hypochlorite solution for 20 minutes and rinsed in TD water. Seeds were allowed to germinate on moistened filter paper (Whatman no. 42, ashless) and four seeds from which normal radicles had emerged were sown in each pot. When the plants had established, the number of plants in each pot was reduced to two.

By May 24, at the 1½ leaf stage, severe zinc deficiency symptoms had appeared in plants in the Zn- group and by May 30 it was apparent that most plants were on the verge of death. Subsequently (22 days after sowing), all plants were removed from both Zn+ and Zn- pots and, after cutting off the seed and roots, were dried, weighed and digested in nitric acid for ICPS analysis.

On June 6, all pots were re-sown with four germinated seeds of wheat (*Triticum aestivum* L.) cv. Excalibur, which contained 900 ng Zn $seed^{-1}$. The rationale for this was that Excalibur has a high degree of zinc efficiency (Graham *et al.* 1992a) and, presumably, behaves in a similar fashion to Aroona, a zinc efficient cultivar. Aroona, in comparison with Durati, releases more phytometallophores (predominantly 2'-deoxymugineic acid (DMA)) in response to zinc deficiency (Cakmak *et al.* 1994). The soil was re-watered to field capacity before sowing. After emergence, the plants

were thinned to two in each pot. Surplus plants were removed with their seeds. As in 1993, the pots were weighed and re-randomized weekly and watered to field capacity when the pot weight fell to that at which the soil water content was 50% of field capacity. Each pot received a maximum of 500 ml TD water on any day.

On July 8, all pots were treated with an equivalent of 0.17 mg Mn kg^{-1} soil applied during watering as $MnSO_4.H_2O$ in solution and were treated again on July 17 and September 19. In addition, the Zn+ pots received the same application weekly between August 24 and September 25 - a total of 1.36 mg Mn kg^{-1} dry soil in the Zn+ pots and 0.51 Mn kg^{-1} dry soil in the Zn-pots.

Plants in the Zn- pots were severely affected by zinc deficiency and by October 24, when these pots were reaped (140 days after sowing) some plants had died. The plants were cut 1 cm above the soil surface and oven dried for 24 hours at 80°C and weighed. They were then digested in nitric acid for ICPS analysis.

Plants in the Zn+ pots were harvested as they matured. Plants were cut 1 cm above the crown, oven dried for 24 hours at 80°C and weighed. Heads were separated and hand threshed. Dried straw and grain samples were ground and digested in nitric acid for ICPS analysis. Particular emphasis in this chapter has been placed on the effects of treatments on concentrations and uptake of zinc and phosphorus in wheat. Data are also presented for potassium and boron, because of the relationship between zinc deficiency in plants and potassium leakage from roots (Cakmak and Marschner 1988a) and because of the reported relationships between zinc deficiency and boron accumulation in cereals (Graham *et al.* 1987, Singh *et al.* 1990). Sodium was considered because of the high uptake of this nutrient by some species in 1993. Little is known about the interactions of zinc and manganese in plants and manganese was considered also for this reason.

It was not possible to measure rooting densities in the second year because sufficient undecomposed root material remained from 1993 to make separating the old and new root material impossible.

Uptakes of nutrients were calculated as the product of nutrient concentration and dry weight. In the following, "shoots" refers to above ground plant parts until grain formation, after which the term "tops" is used to describe all above-ground plant parts, including grain. Total nutrient uptake of tops was calculated by adding straw uptake to grain uptake. Harvest index was calculated as the ratio of grain weight to total weight of tops (straw + chaff + grain).

4.3 RESULTS

4.3.1 A. 1993

4.3.1.1 Production of dry matter

In 1993, *T. durum, M. truncatula* and *H. leporinum* growing in Zn- pots began to display symptoms of zinc deficiency soon after emergence (see Plate 4.3). Symptoms in *P. sativum* and *L. pilosus* appeared more slowly and *B. juncea* plants at no time showed signs of nutrient stress (see Plate 4.1). The most severe early symptoms were demonstrated in *T. durum* and *H. leporinum* plants. By October 31, some *T. durum* plants in the Zn- pots were almost completely necrotic and by November 15, plants in two Zn- pots had died. When Zn- pots were harvested (i.e. when plants of the same species in Zn+ pots had reached mid anthesis) species other than *B. juncea*, *L. pilosus* and *P. sativum* had made little growth (Fig. 4.1). *H. leporinum* produced more dry matter than other species in the Zn+ pots and *M. truncatula* less than other species. In zinc- deficient (Zn-) soil, the performance of *B. juncea* was remarkable and it produced 3.5 times more dry matter than *L. pilosus*. There were no symptoms of zinc deficiency in *B. juncea* plants at any time, although zinc concentrations in tissue at anthesis were relatively low (8.1 mg Zn kg^{-1}). It is possible that *B. juncea* has a low internal requirement for zinc but this has not been demonstrated. The ability of *B. juncea* to mobilize zinc from soil so low in "available" zinc is worthy of further study. *B. juncea* also appears to be efficient in extracting boron from soils of low boron status (J. Stangoulis, pers. comm.).

The most zinc-efficient of the five species for which zinc efficiency could be calculated (*B. juncea* was not grown in Zn+ pots) was *L. pilosus*.

Zinc efficiency is defined as :

$$\frac{\text{dry weight of tops in Zn- soil}}{\text{dry weight of tops in Zn+ soil}} \times 100\%$$

Relative zinc efficiencies in terms of dry matter production of shoots were *L. pilosus* (34%), *P. sativum* (19%), *T. durum* (10%), *M. truncatula* (9%) and *H. leporinum* (5%).

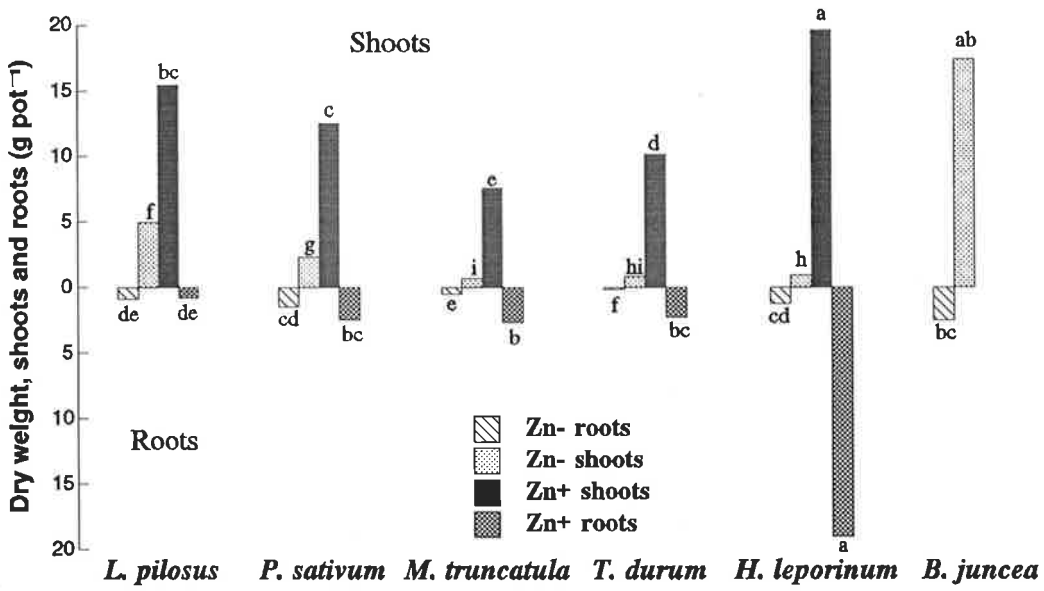

Fig. 4.1 Dry weight of shoots and roots (at anthesis of zinc-treated plants) in Laffer sand, 1993. Bars representing roots or shoots labelled with the same letter are not significantly different ($P \leq 0.05$).

4.3.1.2 Root weights

For *L. pilosus* or *P. sativum*, total dry weights of roots pot^{-1} (Fig. 4.1) were not significantly lower in Zn- soil than in Zn+ soil. *M. truncatula* grown in Zn- pots produced only 19% of total root mass in Zn+ pots, and *T. durum* and *H. leporinum* only about 6%. In Zn+ soil, *H. leporinum* produced a large quantity of root dry matter - an order of magnitude greater than the mean of the other species. Rooting densities, in terms of root weight per unit weight of soil of the different species in Zn- soil are shown in Fig. 4.2 and in Zn+ soil in Fig. 4.3. Rooting densities were most severely affected by low soil zinc levels in *T. durum, P. sativum* and *M. truncatula* - no roots were found in the lowest depth interval of *T. durum* and

P. sativum pots. The growth of *L. pilosus* roots was unaffected by the zinc status of the soil and rooting density profiles were similar in Zn- and Zn+ pots. It is interesting that the effect of zinc-deficient soil on rooting density was not uniform with depth between species - this effect would not be apparent in solution culture studies. For instance, rooting densities of *P. sativum* were significantly less in Zn- soil than in Zn+ soil below 0.4 m, *M. truncatula* rooting densities were significantly less below 0.2 m and rooting densities of *T. durum* and *H. leporinum* were significantly less for all depth intervals in Zn- soil. These differential responses are likely to be further confounded in the field by changes in available zinc status with depth.

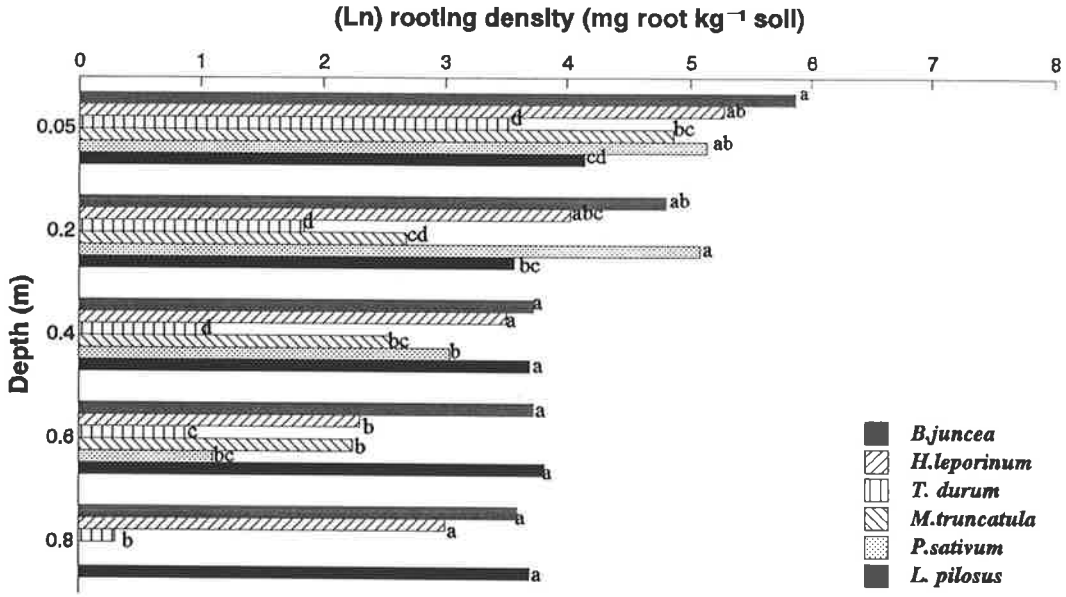

Fig. 4.2 (Ln) rooting density (mg root kg^{-1} soil) in 1993 as a function of depth in zinc-deficient Laffer sand, 1993. At a given depth interval, bars marked by the same letter are not significantly different ($P \leq 0.05$).

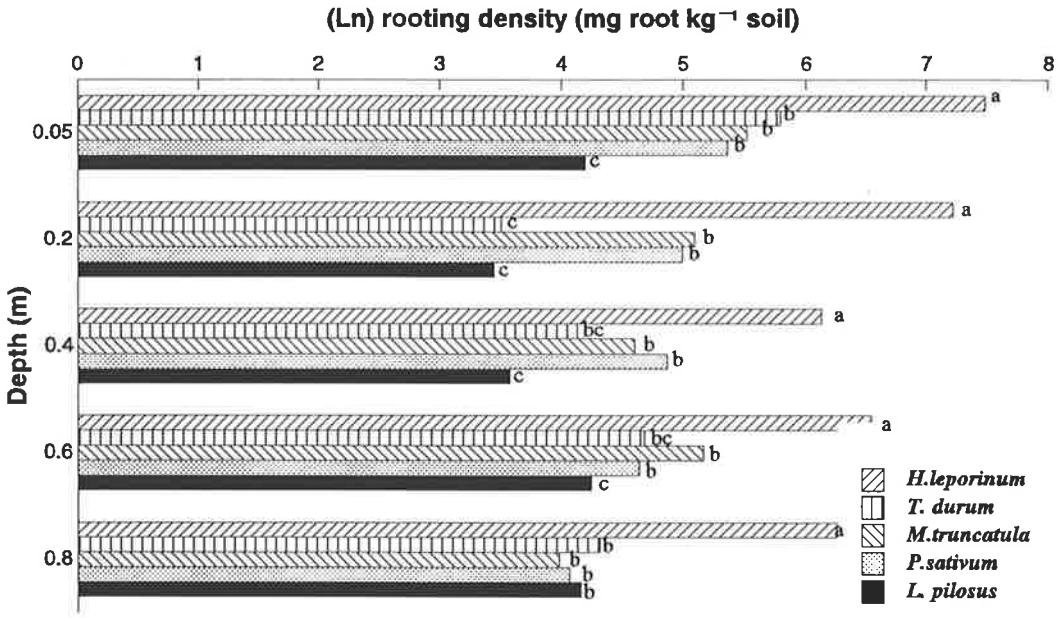

Fig. 4.3 (Ln) rooting density (mg root kg^{-1} soil) in 1993 as a function of depth in Laffer sand to which has been added 0.25 mg Zn kg^{-1} soil, 1993. At a given depth interval, bars marked with the same letter are not significantly different ($P \leq 0.05$).

4.3.1.3 Nutrient concentrations in whole shoots

Of the five species for which comparisons can be made, tissue from Zn+ pots contained significantly higher concentrations of zinc than tissue from Zn- soil (Table 4.1a,b). *L. pilosus* tissue from Zn- soil contained 72% of the zinc concentration of tissue from Zn+ pots. Zinc concentrations in tissue from Zn- pots as a percentage of those from corresponding Zn+ pots reflected the order of zinc efficiencies in terms of dry weight ratios - *P. sativum* (62%), *M. truncatula* (45%), *T. durum* (40%) and *H. leporinum* (31%).

The effects of withholding zinc on concentrations of other nutrients varied between species and there were differences in the ratios of nutrients absorbed between plants of the same species from Zn- and Zn+ soil. These differences may reflect different roles for zinc in the uptake mechanisms of the different species (Loneragan and Webb 1993).

For instance, sodium concentrations in *L. pilosus* (Zn-) tissue were significantly higher than in other species. *L. pilosus* tissue in Zn- soil contained significantly less boron than Zn+ tissue, while boron concentrations in *P. sativum* shoots did not differ between Zn- and Zn+ pots. *P. sativum* shoots from Zn- soil contained significantly lower concentrations of phosphorus than in tissue from Zn+ soil. Phosphorus concentrations in *T. durum*, *M. truncatula*, and *H. leporinum* shoots were, as would be expected, significantly higher in Zn- tissue than in Zn+ tissue.

Boron concentrations were significantly higher for *M. truncatula* shoots from Zn- pots than other treatments and 69% higher than for *M. truncatula* shoots from Zn+ pots. Manganese concentrations in shoots of *T. durum* grown in Zn+ pots were relatively low, although plants did not exhibit any signs of deficiency (Plate 4.2).

Plate 4.1 *B. juncea* grown in Laffer sand with added basal nutrients other than zinc. The plants exhibited no symptoms of zinc deficiency.

Plate 4.2 ***T. durum*** **grown in Laffer sand with added basal nutrients. Zinc was omitted from the pot at left and added at 0.25 mg Zn kg^{-1} soil on the right.**

Table 4.1a Concentrations of zinc, manganese, copper and boron in whole shoots (harvested in 1993 at anthesis in zinc treated plants) of different species as a function of zinc treatment. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 species	Zn Treatment	Zn [A]	Mn	Cu [A]	B
		(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
L. pilosus	Zn+	9.0 cd	14 fg	5.5 g	41 d
L. pilosus	Zn-	6.4 f	23 e	5.6 g	30 e
P. sativum	Zn+	10.2 c	10 g	4.8 h	24 fg
P. sativum	Zn-	6.3 f	21 ef	6.0 g	29 ef
M. truncatula	Zn+	9.6 c	14 fg	7.1 f	63 c
M. truncatula	Zn-	4.3 g	31 d	7.9 ef	107 a
T. durum	Zn+	17.9 a	8 g	8.9 de	21 g
T. durum	Zn-	7.1 ef	68 b	19.6 b	38 d
H. leporinum	Zn+	15.3 b	12 g	9.1 d	27 ef
H. leporinum	Zn-	4.8 g	190 a	35.7 a	40 d
B. juncea	Zn-	8.1 de	42.1 c	12.6 c	75 b
F		***	***	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table 4.1b Concentrations of phosphorus, nitrogen, potassium and sodium in whole shoots (harvested in 1993 at anthesis of zinc treated plants) of different species as a function of zinc treatment. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 species	Zn Treatment	P	N	K	Na [A]
		(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
L. pilosus	Zn+	1.63 gh	37.0 d	25.0 e	1.90 b
L. pilosus	Zn-	1.32 hi	49.2 b	28.2 de	2.51 a
P. sativum	Zn+	1.84 g	31.8 fg	24.2 e	0.38 ef
P. sativum	Zn-	0.90 i	43.4 c	36.4 c	1.15 e
M. truncatula	Zn+	2.83 f	32.3 ef	32.0 d	0.46 e
M. truncatula	Zn-	5.20 c	54.9 a	30.6 d	0.51 e
T. durum	Zn+	4.62 d	27.4 g	37.9 c	0.75 d
T. durum	Zn-	7.32 b	-	49.5 ab	1.76 b
H. leporinum	Zn+	4.13 e	36.6 de	48.4 b	0.32 f
H. leporinum	Zn-	12.29 a	35.1 def	51.8 ab	0.11 g
B. juncea	Zn-	7.01 b	44.5 c	53.1 a	0.43 ef
F		***	***	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

4.3.1.4 Nutrient uptake in whole shoots

Nutrient uptakes in shoots for all species for both Zn- and Zn+ pots are given in (Tables 4.2a, 4.2b, 4.3a, 4.3b). Zinc uptake in shoots was corrected for the contribution made by seeds but does not include zinc content in the roots, which was not measured. The result demonstrates the importance of seed zinc, particularly in the case of the larger seeds grown in zinc-deficient soil. Rengel and Graham (1995a,b) have recently shown the important contribution made by seed zinc to early wheat growth in zinc-deficient soil. In *L. pilosus* and *P. sativum*, seeds may contain considerable quantities of zinc. For instance, the mean weight of *L. pilosus* seeds was 0.386 g with a zinc concentration of 46 mg Zn kg^{-1}. The two seeds sown in each pot contained a total of 35.5 μg zinc. With a mean dry weight of shoots of 5.16 g pot^{-1} and a mean zinc concentration of 6.47 mg kg^{-1}, total zinc uptake in shoots was 33.02 μg pot^{-1} - less than originally contained in the seeds. *P. sativum* seeds contributed 12.2 μg pot^{-1}, *M. truncatula* 3.1 μg pot^{-1} (18 seeds), *T. durum* 0.66 μg pot^{-1}, *H. leporinum* 0.75 μg pot^{-1} and *B. juncea* 0.23 μg pot^{-1}. In cases of extreme zinc deficiency, as occurred in the Zn- pots, the contribution of seed zinc is obviously of critical importance to growth and survival. In the *L. pilosus* and *M. truncatula* Zn- pots, there was no net removal of zinc in the shoots because of the large contribution of the seed, while in the *B. juncea* pots, 134 μg zinc was removed in the shoots.

Table 4.2a Uptakes of zinc, manganese, copper and boron in shoots from Zn- pots 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Species	Zn [S A]	Mn [A]	Cu [A]	B [A]
	(μg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})
L. pilosus	-2.10 d	114 b	23 c	124 b
P. sativum	2.30 bc	47 c	15 d	69 c
M. truncatula	-0.08 c	21 d	6 e	75 c
T. durum	5.50 b	58 c	16 d	30 e
H. leporinum	4.50 b	207 a	36 b	40 d
B. juncea	134.0 a	738 [Z]	196 a	1 180 a
F	***	***	***	***

[S] Net uptake in shoots corrected for seed zinc

[A] Analysis of variance performed on $\log_e$ - transformed data

[Z] Analysis of variance performed without this treatment due to non-additivity which could not be corrected by data transformation. The mean and variance for this treatment were much greater than for other treatments.

Table 4.2b Uptakes of phosphorus, nitrogen, potassium and sodium in shoots from Zn- pots 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Species	P	N	K	Na
	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})
L. pilosus	7 c	245 b	140 b	12.40 [Z]
P. sativum	2 e	101 c	84 c	2.65 b
M. truncatula	4 d	38 d	21 f	0.37 d
T. durum	8 b	-	43 e	1.48 c
H. leporinum	11 b	38 d	57 d	0.12 e
B. juncea	121 a	762 a	911 a	7.23 a
F	***	***	***	***

[Z] Analysis of variance performed without this treatment due to non-additivity which could not be corrected by data transformation. The mean and variance for this treatment were much greater than for other treatments.

Table 4.3a Uptakes of zinc, manganese, copper and boron in shoots from Zn+ pots, 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Species	Zn [A S]	Mn [A]	Cu [A]	B [A]
	(μg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})
L. pilosus	103 cd	225 a	85 b	626 a
P. sativum	116 c	128 b	60 c	300 c
M. truncatula	70 d	104 cd	53 c	475 b
T. durum	183 b	81 d	90 b	210 d
H. leporinum	299 a	233 a	178 a	529 ab
F	***	***	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

[S] Net uptake in shoots corrected for seed zinc.

Table 4.3b Uptakes of phosphorus, nitrogen, potassium and sodium in shoots from Zn+ pots, 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Species	P [A]	N [A]	K [A]	Na
	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})
L. pilosus	25.3 c	575 b	389 b	29.5 a
P. sativum	22.9 c	398 c	301 c	4.8 c
M. truncatula	21.4 c	245 d	242 d	3.0 d
T. durum	47.1 b	276 d	375 bc	7.7 b
H. leporinum	80.2 a	716 a	945 a	6.0 bc
F	***	***	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Zinc uptake from Zn+ pots differed significantly between species (Table 4.3a). *H. leporinum* withdrew most zinc from the Zn+ soil - about 6% of the total zinc added and also extracted large amounts of phosphorus, nitrogen and potassium (Table 4.3b). *L. pilosus* withdrew relatively large amounts of sodium from the Zn+ and Zn- soil. In general, legumes took up less zinc from Zn+ soil than the two representatives of the *Poaceae* (formerly *Gramineae*).

4.3.1.5 Water use efficiency

Water use efficiencies (kg m^{-3}) for shoots were calculated for each species for both Zn- and Zn+ pots and are shown in Table 4.4. Water use efficiency was highest in *P. sativum* (Zn+). Water use efficiencies for *P. sativum*, *M. truncatula* and *H. leporinum* were significantly lower in Zn- pots than in corresponding Zn+ pots. The lowest water use efficiency occurred in *H. leporinum* with no added zinc.

Table 4.4 Water use efficiency of shoots (WUE shoots) as a function of species and zinc treatment, 1993. Values with the same letter are not significantly different ($P \leq 0.05$).

	Zn treatment	WUE shoots
		(kg shoots m^{-3})
L. pilosus	Zn+	4.08 b
L. pilosus	Zn-	3.66 bc
P. sativum	Zn+	5.43 a
P. sativum	Zn-	4.09 b
M. truncatula	Zn+	3.55 bc
M. truncatula	Zn-	2.30 d
T. durum	Zn+	3.42 bc
T. durum	Zn-	3.73 b
H. leporinum	Zn+	2.83 cd
H. leporinum	Zn-	0.96 e
B. juncea	Zn-	3.67 bc
F		***

4.3.2 B. 1994

4.3.2.1 Production of dry matter - Zn- soil

Dry weights of shoots - Durati in Zn- soil

Total dry weights of Durati shoots produced from Zn- soil are shown in Fig. 4.4. Dry weights of shoots were significantly higher following *P. sativum* than other species apart from *L. pilosus*. Durati grown after *M. truncatula* produced significantly less dry matter than when grown after the two grain legumes.

Dry weight of shoots - Excalibur in Zn- soil

Dry weights of Excalibur shoots grown in Zn- soil are shown in Fig. 4.4. After the Durati plants were removed on May 30, Excalibur plants fared better than Durati in the Zn- soil. However, symptoms of zinc deficiency were soon apparent with all treatments. Indeed, symptoms were so severe that by October 24, 140 days after sowing (140 DAS), some plants had died. In only a single case were plants able to produce heads - this occurred in a pot where *P. sativum* was grown in 1993 (Plate 4.4, Plate 4.5). Mean zinc concentration in this plant (whole shoot) was 3.85 mg kg^{-1}, compared with an overall treatment mean of 3.4 mg kg^{-1}. Excalibur plants produced significantly more dry weight after the two grain legumes in Zn- soil than when grown after *T. durum* or *B. juncea*.

Dry weights of shoots - Durati + Excalibur in Zn- soil

As a group, wheat plants grown in rotation with legumes in zinc-deficient soil produced significantly more total dry matter of shoots (Durati + Excalibur) than did plants following representatives of the *Poaceae*, - *T. durum* and *H. leporinum* ($P \leq 0.05$) or *B. juncea* ($P \leq 0.01$) (Appendix, Table A4.4). While relative differences between treatments in Zn- soil were high (mean dry weight of Durati+Excalibur following *L. pilosus* was 72% greater than when following *T. durum*), absolute values were low, considerably lower than those from Zn+ pots. Mean total dry weight of Durati+Excalibur shoots from Zn- soil was 1.15 g compared with 16.0 g (straw + chaff + grain) from Zn+ soil.

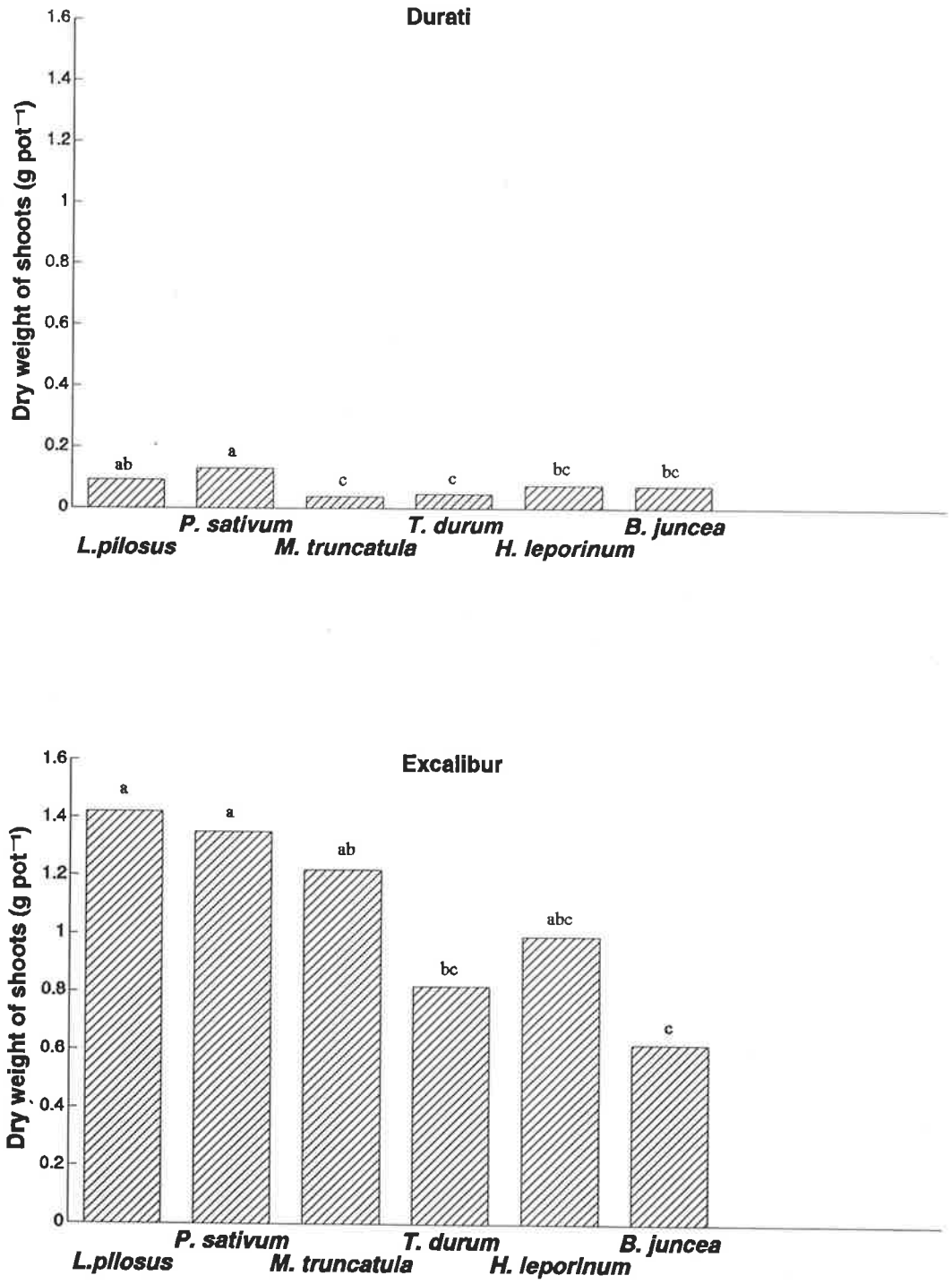

Fig. 4.4 Total dry weights of shoots of Durati wheat 22 days after sowing (above) and Excalibur wheat 140 days after sowing (below) in zinc deficient Laffer sand (Zn-) as a function of species grown in 1993. Bars labelled with the same letter are not significantly different ($P \leq 0.05$).

4.3.2.2 Production of dry matter in Zn+ soil

Dry weight of tops (straw + grain) and grain yield - Excalibur in Zn+ soil

Total dry weights of Excalibur tops (straw + grain) and grain yields in Zn+ pots are shown in Fig. 4.5. All plants in Zn+ soil matured and produced grain. Dry weights of tops are presented for Excalibur only because the contribution of Durati to the final weight was minimal and significant differences between treatments for the totals (Durati+Excalibur) reflected those for Excalibur alone. Excalibur following *H. leporinum* produced the least quantity of tops and grain, significantly less grain than all other treatments.

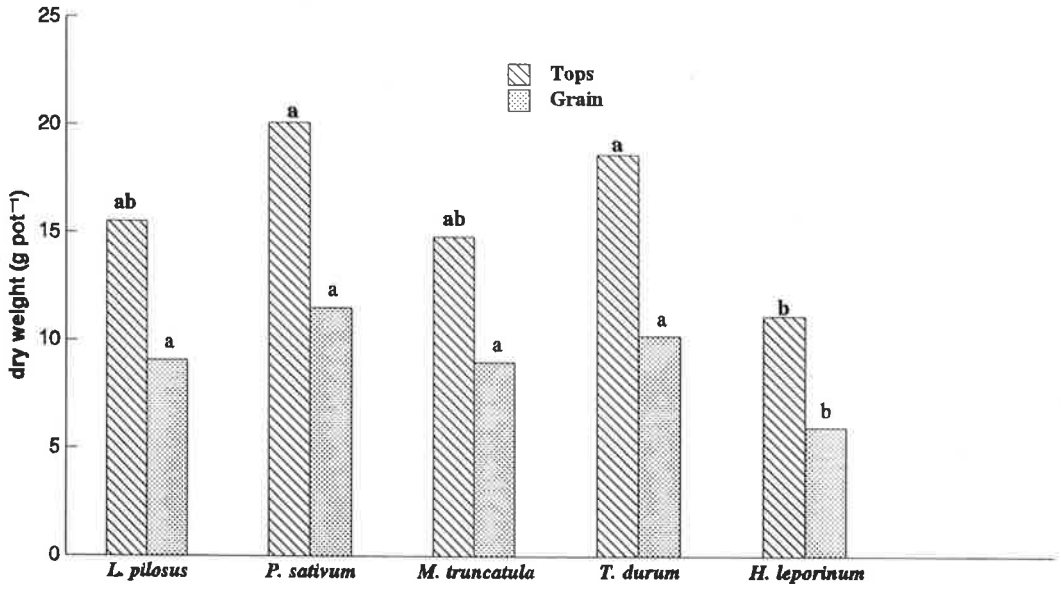

Fig. 4.5 Total dry weight of tops and grain of Excalibur wheat grown in Zn+ pots, 1994, as a function of species grown in 1993. Bars representing the same parameter labelled with the same letter are not significantly different ($P \leq 0.05$).

4.3.2.3 Nutrient concentrations and uptake

Nutrient concentrations - Durati

Nutrient concentrations in Durati wheat shoots 22 DAS are shown in Tables 4.5a, 4.5b. There were no significant differences in zinc concentrations in wheat shoots as a function of prior species in either Zn- soil (Table 4.5a) or Zn+ soil (Table 4.5b). Zinc concentrations in whole shoots from Zn- soil were significantly lower than those for the corresponding treatments in Zn+ soil.

The data from the Zn- pots were highly variable in the case of Durati grown after *L. pilosus* and *T. durum*, as is evident in the standard errors of the means. There were visible differences between plants in the Zn+ and Zn- pots. At 22 DAS, many plants in the Zn- soil had become necrotic and were collapsing, while those in Zn+ soil were vigorous and displayed no signs of zinc deficiency.

Durati grown after *M. truncatula* in zinc-deficient soil contained considerably higher manganese concentrations than other treatments. Manganese concentrations in Zn+ pots appeared to be low, but even in the pots containing *H. leporinum* in 1993, which extracted the greatest quantity of manganese, only about 2% of the amount originally added was removed. Concentrations of potassium were higher in Zn+ plants following each species ($P \leq 0.05$) than in corresponding Zn- plants. Commonly, zinc-deficient plants contain higher concentrations of most nutrients other than potassium (the "concentration effect"). The lower concentrations of potassium in the Zn- pots may be indicative of root membrane leakage.

Table 4.5 Concentrations of zinc, phosphorus, potassium, boron, manganese and sodium in Durati wheat tissue (whole shoots) 22 days after sowing in Zn- soil and Zn+ soil 1994, as a function of species grown in 1993. Values in the same column with the same letter are not significantly different (P≤0.05). Values in brackets are standard errors of the means.

Zn- Soil

1993 spp.	**Zn**	**P**	**K**	**B**	**Mn**	**Na**
	(mg kg $^{-1}$)	(g kg $^{-1}$)	(g kg $^{-1}$)	(mg kg $^{-1}$)	(mg kg $^{-1}$)	(g kg $^{-1}$)
L. pilosus	16.8 (4.6)	5.50	45.9	17.5 ab	14.6 b	0.51 bc
P. sativum	10.0 (2.2)	4.57	39.5	15.6 bc	17.8 b	0.95 ab
M. truncatula	13.5 (1.5)	3.90	29.3	13.5 bc	31.6 a	1.35 a
T. durum	17.7 (4.9)	5.35	39.3	20.3 a	18.4 b	0.97 ab
H. leporinum	9.0 (0.6)	5.68	40.8	17.8 ab	19.2 b	0.48 c
B. juncea	9.4 (0.8)	4.27	40.9	9.3 d	14.8 b	0.41 c
F	ns	ns	ns	***	***	***

Zn+ soil

1993 spp.	**Zn**	**P**	**K**	**B**	**Mn**	**Na** [A]
	(mg kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(g kg^{-1})
L. pilosus	25.3 (1.8)	4.71 b	60.7	9.6 c	9.0	0.401 ab
P. sativum	25.0 (3.0)	5.93 a	60.4	14.1 a	8.8	0.204 c
M. truncatula	22.2 (2.6)	4.81 b	49.1	12.3 ab	9.9	0.279 bc
T. durum	28.6 (3.0)	5.91 a	54.4	12.6 a	10.3	0.174 c
H. leporinum	25.1 (2.1)	4.00 b	56.4	9.8 bc	10.2	0.476 a
F	ns	**	ns	***	ns	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Nutrient uptake in whole shoots - Durati

Uptake of nutrients by Durati wheat plants 22 DAS is shown in Table 4.6. Zinc uptake in Durati wheat shoots in Zn- pots differed significantly between treatments (Table 4.6). Durati following *L. pilosus* and *P. sativum* exhibited the greatest uptake, significantly more than in Durati grown after *M. truncatula*. This is of particular import in the case of *P. sativum* and *M. truncatula*, which did not take up significantly different quantities of zinc in 1993. That is, the difference here cannot be explained in terms of different net uptakes in 1993. When comparing zinc uptakes from Zn- and Zn+ pots, all Zn+ treatments significantly exceeded their Zn- counterparts ($P \leq 0.05$). There were no significant differences in uptake of zinc or other nutrients among treatments in Zn+ pots, nor were there significant differences between treatments in dry weights of shoots (Appendix, Table A4.3).

Table 4.6 Uptake of zinc (corrected for seed zinc), phosphorus, potassium, boron, manganese and sodium in Durati wheat tissue (whole shoots) 22 days after sowing in Zn- soil and Zn+ soil, as a function of species grown in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Zn- soil

1993 spp.	**Zn**	**P** [A]	**K**	**B**	**Mn**	**Na**
	(μg pot^{-1})	(μg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})
L. pilosus	0.97 a	511 ab	4.16 a	1.56 ab	1.28 b	441 bc
P.sativum	0.92 ab	603 a	5.18 a	2.03 a	2.29 a	116 a
M. truncatula	0.16 c	144 c	1.45 c	0.51 d	1.02 b	68 b
T. durum	0.43 abc	263 bc	1.85 bc	0.96 bc	0.88 b	44 bc
H. leporinum	0.35 bc	443 ab	3.15 ab	1.35 abc	1.45 b	36 c
B. juncea	0.33 c	304 ab	3.03 ab	0.67 cd	1.04 b	31 c
F	*	**	**	**	*	***

[A] Analysis of variance performed on $\log_e$ transformed data.

Zn+ soil

1993 spp.	Zn	P	K	B	Mn	Na
	(μg pot^{-1})	(μg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})
L. pilosus	2.61	553	7.23	1.15	0.99	49.0
P. sativum	3.01	805	8.54	1.99	1.99	30.8
M. truncatula	1.57	403	4.23	0.99	0.99	23.5
T. durum	2.65	636	5.96	1.34	1.34	18.8
H. leporinum	2.57	460	6.65	1.15	1.15	57.0
F	ns	ns	ns	ns	ns	ns

Nutrient concentrations in whole shoots - Excalibur in Zn- soil

Concentrations of nutrients in whole shoots of Excalibur in Zn- soil at 140 DAS are shown in Table 4.7. Nutrient concentrations in Excalibur shoots do not indicate deficient concentrations of nutrients other than zinc, which were consistently low. Cakmak *et al.* (1994) reported that visual zinc deficiency symptoms appeared "first and more severely" in Durati compared with the zinc-efficient cultivar Aroona although tissue concentrations were the same in both cultivars. The authors also suggested that in comparison to Aroona, Durati may have a higher critical deficiency concentration for zinc and may utilize zinc internally less effectively than Aroona.

The current experiment supports these hypotheses. Although the Excalibur plants - the efficiency mechanisms of which are assumed to operate in a similar manner to those of Aroona - were sampled at a later growth stage than Durati (140 DAS cf 22 DAS), both genotypes had reached a similar stage of necrosis and collapse when harvested. However, Durati had a mean zinc concentration for all treatments of 12.7 mg kg^{-1} and Excalibur 3.4 mg kg^{-1}.

Table 4.7 Concentrations of zinc, phosphorus, potassium, boron, manganese and sodium in whole shoots of Excalibur wheat 140 days after sowing in Zn- soil, 1994 as a function of species grown in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 spp.	Zn	P	K	B	Mn	Na
	(mg kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
L. pilosus	3.69	13.5 b	49.0	35.8	49.8	109
P. sativum	3.59	12.3 b	46.7	34.6	55.8	179
M. truncatula	3.19	13.8 b	51.0	36.1	47.4	94
T. durum	3.43	16.5 a	48.5	40.5	56.0	161
H. leporinum	3.13	12.0 b	48.2	36.3	61.5	75
B. juncea	3.63	9.9 c	44.3	31.3	50.5	129
F	ns	***	ns	ns	ns	ns

There were no differences in nutrient concentrations between treatments in Zn- soil other than for phosphorus. Plants grown after *T. durum* showed significantly higher phosphorus concentrations than other treatments and plants grown after *B. juncea* significantly lower phosphorus concentrations. *B. juncea* removed 38% of the phosphorus added to the soil in 1993. Nevertheless, all treatments displayed the commonly observed high concentration of phosphorus in zinc-deficient tissue.

Nutrient concentrations in straw and grain - Excalibur in Zn+ soil

Nutrient concentrations in mature straw of Excalibur grown in Zn+ pots are shown in Table 4.8. Concentrations for mature straw appeared to be adequate for the nutrients shown. Phosphorus concentrations were significantly lower in Excalibur straw following *H. leporinum* than after other species. *H. leporinum* extracted a large

amount of phosphorus in 1993 (26% of the total added) and this was not replaced in 1994. However, phosphorus concentration in grain with this treatment (Table 4.9) appeared to be within the adequate range (Reuter and Robinson 1986).

Table 4.8 Concentrations of zinc, phosphorus, potassium, boron, manganese and sodium in mature Excalibur wheat straw in Zn+ soil 1994, as a function of species grown in 1993. Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

1993 spp.	Zn	P [A]	K	B	Mn	Na
	(mg kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
L. pilosus	9.93	2.27 a	49.1	34.3 b	8.68	113
P. sativum	7.38	2.10 a	49.8	41.3 ab	8.90	87
M. truncatula	7.23	1.60 a	51.6	34.8 b	6.93	64
T. durum	8.05	2.22 a	48.3	54.4 a	11.47	133
H. leporinum	6.50	0.82 b	49.5	30.2 b	10.65	52
F	ns	**	ns	**	ns	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

Zinc concentrations in grain (Table 4.9) did not differ significantly between treatments and indicate adequate concentrations. Phosphorus and potassium concentrations in grain were significantly lower in the *H.leporinum* rotation.

Table 4.9 Concentrations of zinc, phosphorus, boron, manganese and sodium in Excalibur grain from Zn+ soil, 1994 as a function of species grown in 1993. Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

1993 spp.	Zn	P	K	B	Mn	Na
	(mg kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
L. pilosus	18.2	4.75 a	6.00 a	2.0	7.1	19.5
P. sativum	18.1	4.64 a	6.20 a	2.0	7.0	21.8
M. truncatula	18.2	4.61 a	6.02 a	2.1	5.9	29.8
T. durum	17.6	4.50 a	6.26 a	2.2	6.2	23.0
H. leporinum	19.6	3.83 b	5.36 b	2.3	7.9	19.0
F	ns	**	**	ns	ns	ns

Nutrient uptake in whole shoots - Excalibur in Zn- soil

Nutrient uptakes by whole shoots of Excalibur from Zn- soil are shown in Table 4.10. The uptake of zinc in plants grown after legumes was significantly greater ($P \leq 0.001$) than when grown after the *Poaceae* or *B. juncea*. There were no differences in uptake among the legume rotations. Plants grown after *B. juncea* took up less phosphorus, potassium, boron and manganese than when grown after legumes.

Plate 4.3 ***M. truncatula*** **grown in Laffer sand with added basal nutrients. Zinc was omitted from the pot at left and added at 0.25 mg Zn kg^{-1} soil on the right.**

Table 4.10 Uptake of zinc (corrected for seed zinc), phosphorus, potassium, boron, manganese and sodium in whole shoots of Excalibur wheat harvested from Zn- soil 140 days after sowing as a function of species grown in 1993. Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

1993 spp.	Zn	P	K	B	Mn	Na
	($\mu g\ pot^{-1}$)	($mg\ pot^{-1}$)	($mg\ pot^{-1}$)	($\mu g\ pot^{-1}$)	($\mu g\ pot^{-1}$)	($\mu g\ pot^{-1}$)
L. pilosus	4.34 a	18.6 a	70.7 a	40.7 a	55.1 ab	101 b
P. sativum	3.90 a	16.5 a	61.5 ab	44.3 a	72.6 a	218 a
M. truncatula	3.03 ab	16.8 a	62.4 ab	43.2 a	58.6 ab	115 b
T. durum	1.89 bc	13.4 ab	39.7 bc	32.8 a	45.6 bc	115 b
H. leporinum	1.24 c	11.0 bc	46.6 bc	35.2 a	51.7 abc	72 b
B. juncea	1.32 c	5.7 c	27.5 c	18.9 b	30.3 c	66 b
F	***	**	**	*	*	*

Zinc uptake in whole shoots - Durati+Excalibur in Zn- soil

A significantly greater uptake of zinc in whole shoots occurred from zinc-deficient soil in Durati+Excalibur following *L. pilosus* and *P. sativum* than occurred in plants following all other treatments ($P \leq 0.001$) (Appendix, Table A4.5). Zinc uptake in wheat shoots was also greater after *M. truncatula* than after *H. leporinum* or *B. juncea*. In this respect, legumes as a group induced a significantly higher uptake of zinc in following wheat plants than the *Poaceae* ($P \leq 0.001$). The results for the Zn+ soil are shown in Fig 4.5.

Nutrient uptake in straw and grain - Excalibur in Zn+ soil

Uptake of zinc and phosphorus in tops (straw + grain) (Table 4.11) was significantly less in plants grown after *H. leporinum* than all other species.

Table 4.11 Uptake of zinc (corrected for seed zinc), phosphorus, potassium, boron, manganese and sodium in tops (straw + grain) of Excalibur wheat grown in Zn+ soil 1994, as a function of species grown in 1993. Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

1993 spp.	Zn [A]	P	K	B [A]	Mn	Na
	(μg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})	(mg pot^{-1})
L. pilosus	228 a	56.3 ab	372 a	240 b	121	92 ab
P. sativum	249 a	60.2 ab	381 a	255 b	125	38 bc
M. truncatula	207 ab	49.4 b	346 ab	229 bc	93	68 b
T. durum	242 a	65.6 a	470 a	467 a	157	38 a
H. leporium	141 b	28.5 c	214 b	126 c	97	24 c
F	*	***	*	**	ns	**

[A] Analysis of variance performed on $\log_e$ - transformed data.

Zinc uptake by rotations

Zn - soil

Zinc uptakes in shoots for the various rotations were calculated by adding zinc uptake (corrected for seed zinc) in shoots from the species grown in 1993 to total uptake by both Durati and Excalibur in 1994. The results are shown in Fig. 4.6.

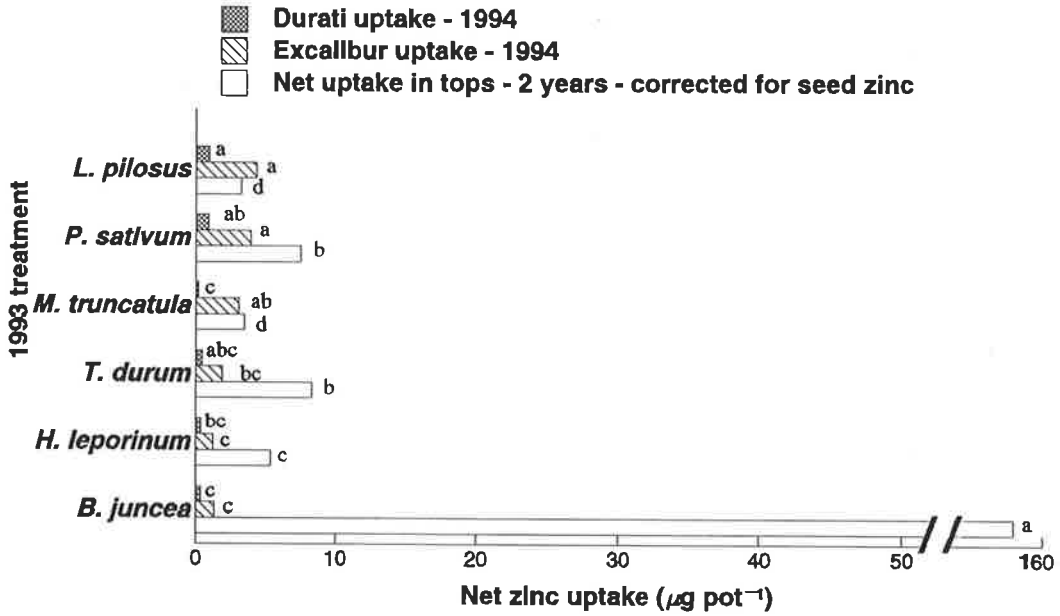

Fig. 4.6 Net zinc uptake in shoots (μg pot^{-1}) from zinc deficient (Zn-) Laffer sand by Durati and Excalibur wheat in 1994 and total uptake over the two seasons corrected for 1993 and 1994 seed zinc content. Within each set of data represented, bars labelled with the same letter are not significantly different ($P \leq 0.05$).

Within the legumes, net uptake of zinc over the two seasons was significantly higher with *P. sativum* as the antecedent species than with *L. pilosus* or *M. truncatula*. While second year uptake was high in the *L. pilosus* rotation, when the contribution of the zinc content in the massive *L. pilosus* seed is included, net uptake over the two seasons was significantly less than in the *P. sativum* and *T. durum* rotations. It is apparent that *B. juncea* has a highly effective mechanism for mobilising zinc, compared with the other species grown.

Zn+ soil

Total zinc uptakes for the rotations corrected for seed zinc are shown in Fig 4.7. There were no significant differences in uptake over the two years, although in the second year, Excalibur grown in the *H. leporinum* rotation displayed a low uptake of zinc compared with other treatments.

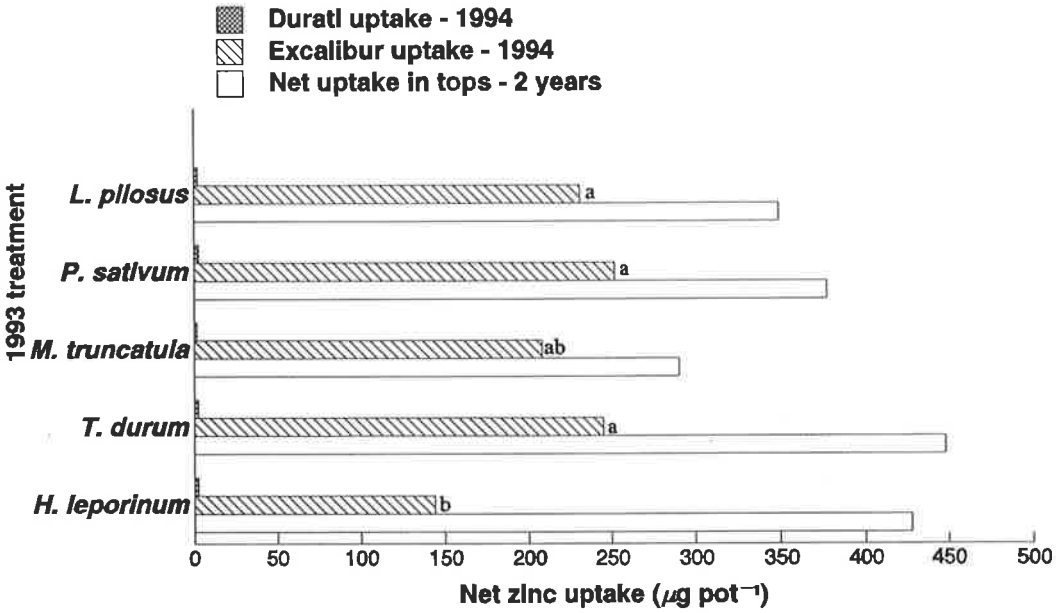

Fig. 4.7 Net zinc uptake in shoots (μg pot^{-1}) by Durati and Excalibur wheat in 1994 from Zn+ soil and total uptake over the two seasons corrected for 1993 and 1994 seed zinc content. Bars representing uptake by Excalibur in 1994 labelled with the same letter are not significantly different ($P \leq 0.05$).

4.3.2.4 Water use efficiency

Water use efficiency was calculated for grain (kg grain m^{-3}) (Zn+ pots), shoots (kg shoots m^{-3}) (Zn- pots), or tops (tops = straw + grain) (kg tops m^{-3}) (Zn+ pots). Analysis of variance produced significant values of F ($P \leq 0.001$) for the effects of antecedent species on water use efficiency (kg shoots m^{-3}) in Zn- soil (Table 4.12), but not for tops or grain in Zn+ soil (Appendix, Table A4.15). Wheat grown after *P. sativum* and *M. truncatula* was significantly more efficient in water use in Zn- soil than when following other species. The least water use efficient plants followed *B. juncea*. The results from the Zn- soil may be partially explained in terms of the significant relationship between water use efficiency and zinc uptake in the shoots (r = 0.62**).

Table 4.12 Water use efficiency (WUE shoots) of shoots of wheat (Durati + Excalibur) in 1994 as a function of species grown in 1993 in Zn- soil. Values with the same letter are not significantly different ($P \leq 0.05$).

1993 spp.	**WUE shoots**
	(kg shoots m^{-3})
L. pilosus	1.15 bc
P. sativum	1.67 a
M. truncatula	1.65 a
T. durum	1.23 b
H. leporinum	1.01 bc
B. juncea	0.83 c
F	***

4.4 GENERAL DISCUSSION

Zinc efficiencies (expressed as percentages of dry weights) of the five species grown in zinc-deficient and zinc-adequate Laffer sand varied widely among the species. *L. pilosus* was the most zinc-efficient (34%) and *H. leporinum* the least (4.9%). *M. truncatula* was the least efficient of the legumes (9%). At harvest, relative

concentrations of zinc in whole shoots grown in Zn- soil as a proportion of concentrations in shoots of the same species grown in Zn+ soil reflected the order of zinc efficiencies with *L. pilosus* having the highest relative concentration and *H. leporinum* the least. While *B. juncea* was not grown in Zn+ soil, it produced 3.5 times more dry matter than *L. pilosus* in Zn- soil and was clearly better adapted to the zinc-deficient soil than other species. *B. juncea* appears to have a remarkable capacity to mobilise zinc. The role of allelopathy in influencing the performance of species grown in rotation was not investigated in the current experiment, but may explain some of the differences in growth and nutrient uptake measured, particularly in terms of generally poor performance of wheat grown after *H. leporinum* in Zn+ soil. For instance, Halsall *et al.* (1995) demonstrated significant decreases in the germination, radicle elongation, nodulation and root elongation of *M. truncatula* cv. Jemalong when exposed to sterile cold water extracts of *H. leporinum* shoots. The production of lateral roots and root hairs was severely restricted. While residues of shoots were removed in the current experiment, it is possible that allelopathic chemicals are also released as a result of root decomposition. On the other hand, the degree of decomposition of roots was not determined and it is possible that the tie-up of nutrients in undecomposed roots may also be a significant contributor to the poor growth of subsequent wheat plants.

The high concentration of boron in *M. truncatula* grown in Zn- soil is of particular interest because the cultivar Parabinga is widely grown in an area of southern Australia where soil boron concentrations are high. Because zinc deficiency is an increasingly common phenomenon in this region and because medic production appears to be declining simultaneously, the role of zinc in ameliorating the negative impact of excessive boron uptake in *Medicago* spp. appears to be a worthwhile area for research. It is interesting that for all species other than *M. truncatula* grown in 1993, potassium concentrations were higher (although not always significantly so) in shoots from Zn- pots than from Zn+ pots, while in *M. truncatula*, potassium concentrations were slightly lower. While the differences in potassium concentrations were not always significant, it may be hypothesized that taken together, the large increase in boron concentrations and possible leakage of potassium indicate that the

Plate 4.4 ***P. sativum*** **grown in Laffer sand with added basal nutrients. Zinc was omitted from the pot at left and added at 0.25 mg Zn kg^{-1} soil on the right.**

adverse effects of zinc deficiency on root membranes may be particularly severe in *M. truncatula*. This species may be particularly vulnerable to zinc deficiency in terms of impairment of the integrity of plasma membranes in root cells. The deleterious effects of zinc deficiency were more severe in *M. truncatula* than the two grain legumes. Selection of medic for zinc efficiency traits may result in worthwhile production increases in an area where zinc deficiency is an increasing problem.

The zinc content of plants grown on Zn- soil was strongly influenced by original seed zinc content. When sown, the two *L. pilosus* seeds contained a total of 35.5 μg zinc and at harvest 33.4 μg zinc was removed in the plant tops. Similarly, slightly less zinc was removed in *M. truncatula* tops than was added originally in the seed. The content of zinc in the seed of *P. sativum, H. Leporimum, T. durum* and *B. juncea* as a proportion of the zinc content of shoots at harvest was 84%, 14%, 11% and 0.2% respectively. Apart from *B. juncea*, the onset and severity of zinc deficiency symptoms were delayed as the contribution of seed zinc to the total uptake in shoots at harvest increased. This may be a significant adaptational advantage, although more research is needed to determine the final outcome of allowing the various species to grow through to maturity. It may be argued that those plants with higher seed zinc reserves would be disadvantaged in terms of their ability to reproduce (because of the consequential greater need for zinc for seed production). In the current experiment, where zinc deficiency was severe, it is doubtful that any of the species tested (other than *B. juncea*) would have been able to produce viable seed, but in terms of the ability to grow in zinc-deficient soil alone, the large seeded legumes certainly performed better than *M. truncatula* or members of the *Poaceae*.

In discussing the relative merits of the abilities of the monocotyledons and dicotyledons to mobilize zinc in calcareous soils of low zinc availability, Cakmak and Marschner (1990) suggested that the ability of dicotyledons to acidify the rhizosphere in zinc-deficient soil may be of ecological relevance. They considered however that low molecular weight organic solutes released by zinc-deficient dicotyledons were not highly efficient in mobilizing zinc from calcareous soils. By contrast, they were able to demonstrate that phytometallophores released by zinc- (or iron-) deficient members

of the *Poaceae* were highly efficient in mobilizing zinc. In this respect, *T. durum* and *H. leporinum* were able to extract significantly more zinc from zinc-deficient soil in 1993 up to anthesis than were *L. pilosus* or *M. truncatula* (Table 4.2a). However, in terms of ecological relevance, it should be borne in mind that several *T. durum* and *H. leporinum* plants had died before harvest in 1993. Further, the performance of the two grain legumes in terms of dry matter production and total zinc uptake in shoots (not corrected for seed zinc) in zinc-deficient (Zn-) soil in 1993 was considerably greater than the other treatments, apart from the *B. juncea* rotation. Any advantage due to better mobilisation of zinc by the *Poaceae* was more than compensated for to the time of harvest by the large contributions of zinc in the seed of the large seeded grain legumes. It may also be conjectured that because the plants were harvested before maturity, the presence of relatively large quantities of seed zinc may have retarded the onset of zinc deficiency and hence delayed the activation of specific responses to zinc deficiency in the grain legumes. That is, the relative uptake of zinc by the grain legumes may have increased over time. In soil to which zinc was added, *H. leporinum* and *T. durum* extracted significantly more zinc than the grain legumes in 1993 (Table 4.3a).

The zinc status of the soil had different effects on root growth of the various species. These differences occurred in soil in which zinc was uniformly distributed throughout the profile. In southern Australian soils, adequate concentrations of plant-available zinc normally occur only in the top 0.05 m of the profile. Below this depth, zinc concentrations are almost invariably low and in this situation, root growth would be a complex function of the degree of variability of the concentrations of zinc (and other nutrients) with depth and the response of the species to this variablity.

In 1994, zinc concentrations in Durati plants harvested 22 DAS did not differ between treatments in Zn- pots or in Zn+ pots, but plants grown in Zn+ soil contained significantly higher zinc concentrations than corresponding treatments in Zn- soil. This indicates that plants had outgrown, at least in part, their dependence on seed reserves. Potassium concentrations were also significantly higher in Zn+ plants than

in corresponding Zn- plants, an indication of impaired root cell membrane integrity in the Zn- plants (Cakmak and Marschner 1988a).

Zinc uptake by Durati in Zn- soil differed significantly between treatments. It may be argued that these differences are due to different uptake patterns in 1993. For instance, zinc uptake in Durati was significantly higher following *L. pilosus* than when following *M. truncatula, H. leporinum*, or *B. juncea*. The net zinc uptake by *L. pilosus* shoots in 1993 was less by 7.6 μg pot^{-1} than the zinc uptake in *H. leporinum* shoots. The (significant) difference in zinc uptake by Durati in 1994 between these two treatments was only 0.62 μg pot^{-1}. The difference in this case may be due to the fact that less (net) zinc was removed by *L. pilosus* in 1993. The same argument may apply when comparing the *L. pilosus* rotation with the *M. truncatula* and *B. juncea* rotations. However, the significant difference between zinc uptake in Durati following *P. sativum* and *M. truncatula* cannot be explained in this way because net zinc uptake in *P. sativum* was not significantly different from that in *M. truncatula* in 1993. It is unlikely that *M. truncatula* had diverted a disproportionate amount of zinc into the roots compared with *P. sativum* since the latter produced about three times the total root mass in Zn- soil. Furthermore, the differences in uptake of less than 1 μg pot^{-1} represent only a small fraction of the total soil zinc. It can be estimated that each Zn- pot contained about 950 μg of DTPA extractable zinc at the beginning of the experiment. There is evidence, then, that Durati grown after *P. sativum* had access to slightly but significantly more available zinc than when grown after *M. truncatula*.

Zinc concentrations in Excalibur 140 DAS in Zn- soil were much lower than in Durati 22 DAS when both cultivars had reached a similar stage of necrosis and tissue collapse. The fact that Excalibur plants grew longer is not only a reflection of the relative zinc efficiencies of the two cultivars, but the original seed concentration of zinc in Excalibur was about three times higher than in Durati and this difference would have had a significant influence (Rengel and Graham 1995a,b). However, the fact that mean tissue concentrations were considerably lower in Excalibur when symptoms were similar suggests that Durati may have a higher critical deficiency

concentration for zinc and may be less effective in its internal utilization of zinc, as suggested by Cakmak *et al.* (1994).

Over the two seasons, total net uptake of zinc in shoots was much greater in the *B. juncea* rotation than others, although zinc uptake by Durati and Excalibur in 1994 constituted only a small proportion of the total. Total net zinc uptake in shoots over the two seasons was significantly less in the *M. truncatula* rotation than in the *P. sativum*, *T. durum* or *H. leporinum* rotations. In this respect, there were no differences between the legumes and the *Poaceae*.

Despite the low uptake of zinc in Durati and Excalibur wheat grown after *B. juncea*, the fact that the plants were able to grow for a total of 162 days suggests that some of the zinc mobilized by *B. juncea* may have remained in an available form in the soil. No measurements were made of root zinc content and this aspect - the rate of root breakdown and the proportion of available zinc remaining - needs further investigation. After the 1994 harvest, it was apparent that a considerable amount of root material from 1993 was still present in an undecomposed form, despite the period of incubation. In the field, however, warmer temperatures, higher microbial populations and tillage may all hasten the rate of decomposition although soil water content is generally low over summer in the southern Australian environment. It is possible that the quantity of plant-available zinc remaining after *B. juncea* may increase significantly given sufficient time for adequate decomposition of the plant residues. On the other hand, it is possible that in a soil with more clay than that used in the current experiment, small amounts of zinc released from root decomposition would be rapidly adsorbed. The availability of this zinc to following plants would be a complex function of decomposition rates, microbial action and complexation and sorption reactions in the soil. There is some evidence that most of the zinc contained in subcellular fractions of roots is 'soluble' (Polar 1976, cited by Longnecker and Robson, 1993) and that a large proportion of the remainder occurs in cell walls (Longnecker and Robson, 1993). If it is assumed that roots of *B. juncea* in the current experiment contained about the same concentration of zinc as in the shoots (Banuelos *et al.* 1993), the total root mass would have contained about 20 μg of zinc at harvest.

The *H. leporinum* rotation in Zn+ soil produced significantly less tops and grain of wheat with a lower zinc uptake than most other rotations. It is possible, given the large amount of root material produced by *H. leporinum* in Zn+ soil in 1993, that allelopathic or mycorrhizal effects or root breakdown reactions may have had some adverse effect on the growth of wheat plants in the following year. On the other hand, the lack of decomposition of *H. leporinum* roots may also have contributed to the poor growth of the wheat plants. There is a need for further caution in interpreting the data because of the large amounts of phosphorus removed by *H. leporinum* in 1993 (Fig. 4.8). However, while the possibility exists that phosphorus transport to roots was curtailed by removal in the previous season, phosphorus uptake by Excalibur from the Zn- soil was also relatively low after *H. leporinum*. However, tissue concentrations of phosphorus were very high. Also, total uptake of phosphorus over the two seasons in the *H. leporinum* rotation in Zn+ soil was slightly less than occurred in the *T. durum* rotation. *H. leporinum* also withdrew large amounts of potassium from Zn+ pots in 1993 although the concentrations of potassium in Excalibur straw and grain do not appear to indicate deficiency.

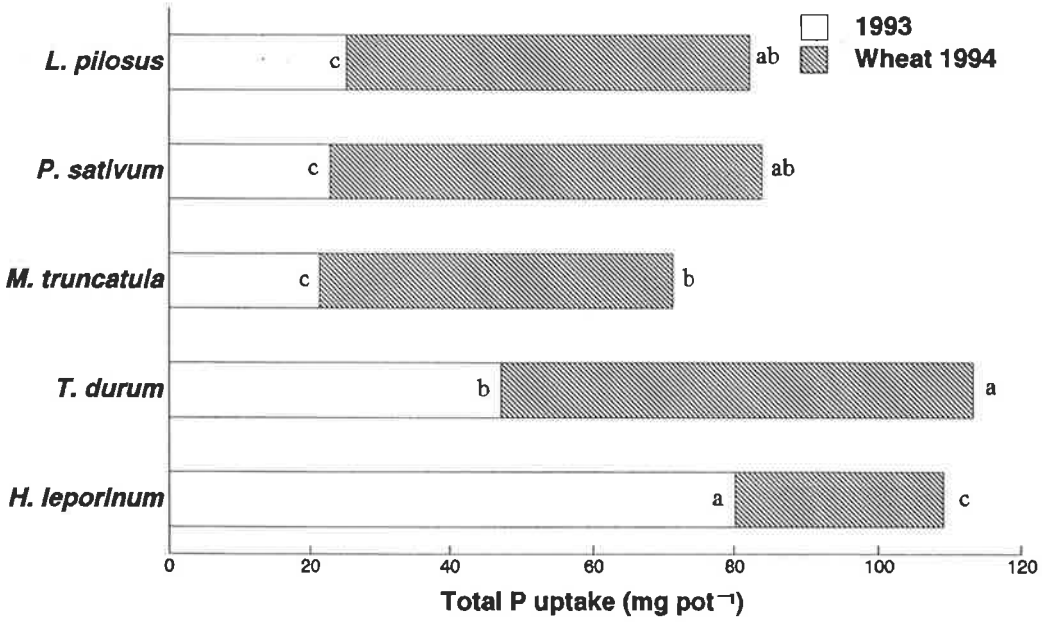

Fig. 4.8 Total phosphorus uptake (μg pot^{-1}) by different species in 1993 and by wheat in 1994 from Zn+ pots. For a given year, values labelled with the same letter are not significantly different ($P \leq 0.05$).

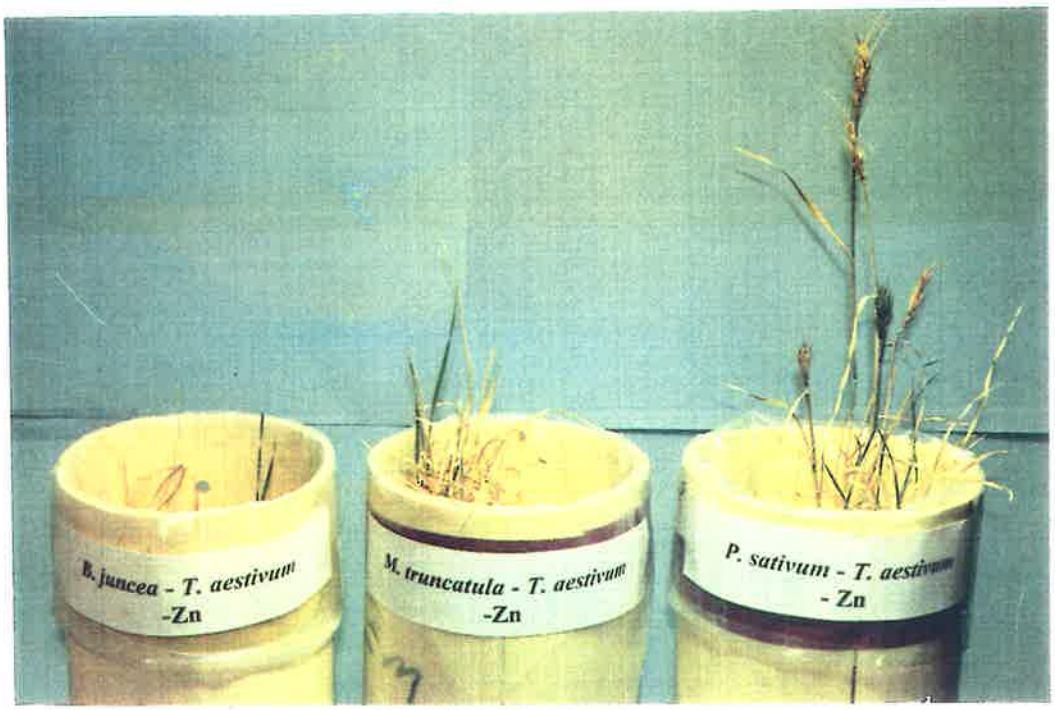

Plate 4.5 **Excalibur wheat grown in Laffer sand with added basal nutrients other than zinc, following, from left to right, *B. juncea, M. truncatula* and *P. sativum*.**

Peas (Kochian 1993) and some species of navy bean (Jolley and Brown 1991) are able to induce ferric reductase activity in the roots of zinc-deficient plants and this may enhance zinc influx and leave zinc in an available form for some time after harvest. While there were no statistically significant benefits conferred by any single rotation in Zn+ soil on yield or zinc uptake parameters it is interesting to note that wheat grown after *P. sativum* exhibited relatively high dry matter and grain production, high grain zinc uptake, high total zinc uptake and high water use efficiency for grain and shoot production. Taken together, these data suggest a trend for enhancement of zinc availability in the *P. sativum* rotation.

While the evidence is strongest from Zn- soil that wheat grown after *P. sativum* had access to more available zinc than when grown after *M. truncatula*, the fact that wheat grown after *P. sativum* performed consistently (but not significantly ($P \leq 0.05$)) better in most of the parameters measured in both Zn+ and Zn- soil suggests that the reported better performance of cereals after *P. sativum* than *M. truncatula* is a real effect and may be due in part to enhanced availability of zinc. Further research in this area is warranted because of the economic significance to South Australian farmers.

5.0 The effects of added zinc and other nutrients on the growth of zinc efficient and inefficient wheat cultivars in an alkaline soil.

5.1 INTRODUCTION

Subsoil constraints to root growth have only recently been studied in any detail because of the difficulties involved in obtaining unequivocal data, as Graham *et al.* (1992b) have observed. In the lower rainfall (<350 mm per annum) cereal growing areas of southern Australia, the majority of soils on which cereals are grown are naturally infertile and this problem has traditionally been addressed by fertilizing the top 0.05 m of soil.

Subsoils in this area generally become more alkaline with depth and the texture becomes finer, so that the immobilisation of nutrients such as zinc tends to increase with depth. Subsoil infertility may be exacerbated by removal of nutrients in plant tops (Graham *et al.* 1992b). The problem is further compounded in areas such as Eyre Peninsula by high concentrations of boron and salt at depths below 0.40 m (Holloway 1991).

While subsoil amelioration may be practicable to a depth of 0.40 m, treating the soil below this depth mechanically is likely to be too difficult and too expensive to consider, particularly in environments with low rainfall. As Graham and Rengel (1993) have noted, the most tenable solution to this problem lies with the breeding and selection of efficient cultivars. The authors note that "wheat grows poorly in potted subsoil even when fertilized with nitrogen and phosphorus..... we believe the better approach to this problem is to breed cereals with root systems which will penetrate subsoils of low phosphorus and micronutrient availability".

Graham *et al.* (1992a) have identified a wide range of genetic diversity for efficiency in uptake of zinc (and other micronutrients) in wheat. The South Australian cultivar Excalibur was outstanding in this respect in field experiments. By contrast, the cultivar Gatcher was shown to be consistently zinc inefficient.

There is little information concerning root growth parameters (in soil) which may affect zinc efficiency (zinc efficiency in soil and nutrient culture do not appear to be related - Graham and Rengel 1993). Dong *et al.* (1995) indicate that in zinc- deficient soil, Excalibur is able to produce more fine roots (diameter <0.2 mm) than Gatcher and the surface to volume ratio increases as root diameter falls. This would allow the exploration of a greater volume of soil if root mass is similar. It is possible that this difference could account to some degree for the difference in zinc efficiency between the cultivars.

There also exists the unexplored possibility that a zinc efficient cultivar such as Excalibur would be better able to extend roots into infertile, alkaline, subsoils which often contain high concentrations of boron. To compare the zinc efficiency, root growth and production characteristics of the two cultivars Excalibur and Gatcher in subsoils of this type, a pot experiment was designed in which the cultivars were grown in subsoil typical of that occurring on Eyre Peninsula. The subsoil was either untreated or was mixed with complete nutrient solutions with and without zinc.

5.2 MATERIALS AND METHODS

The experiment consisted of a completely randomised design with factorial combinations of added zinc (*Zn+*, *Zn-*), added nutrients (*nu+*, *nu-*) and cultivar, (Excalibur "*E*", or Gatcher "*G*"). The treatments are labelled: *E nu+ Zn+*, *E nu+ Zn-*, *E nu- Zn-*, *E nu- Zn+*, *G nu+ Zn+*, *G nu+ Zn-*, *G nu- Zn-*, *G nu- Zn+*. There were four replicates of each treatment.

5.2.1 Soil preparation

Soil for the experiment was collected from a newly excavated pit in virgin woodland at Minnipa, on Eyre Peninsula, South Australia. As defined by Stace *et al.* (1968), the soil is a solonised brown soil, or according to Soil Taxonomy (Soil Survey Staff 1975), a Calcixerollic Xerochrept (fine silty, mixed, thermic). The soil was taken from the 0.5 - 0.8 m depth interval (Bk1 horizon), part of a IIIA carbonate layer - a layer consisting of a compact yellowish mixture of finely divided carbonate and sand

containing less than 30% calcrete fragments (Wetherby and Oades 1975). The soil was sieved through a 2 mm stainless steel sieve and air dried in a dust free environment.

Table 5.1 Some properties of the soil used in the experiment.

Field texture		light clay
pH	(1:5 soil:water)	9.5
Electrical conductivity	(EC_{e25} dS m^{-1})	0.91
Gravimetric water content at -10 kPa	(kg kg^{-1})	0.279
Gravimetric water content at -100 kPa	(kg kg^{-1})	0.158
Gravimetric water content at -1.5 MPa	(kg kg^{-1})	0.106
Hot 0.01 M $CaCl_2$ extractable B	(mg kg^{-1})	13.2
DTPA extractable Zn	(mg kg^{-1})	0.10
DTPA extractable Mn	(mg kg^{-1})	0.43
DTPA extractable Cu	(mg kg^{-1})	0.07
Exchangeable sodium percentage	(%)	13.0
$CaCO_3$ content	(%)	28.0

Soil boron concentrations were determined by hot 0.01 M $CaCl_2$ extraction (Bingham 1982) and ICPS analysis (Zarcinas and Cartwright 1983). The zinc, copper and manganese status of the soil were determined by DTPA extraction. Electrical conductivities (EC_{e25}) were determined from saturated paste extracts. Soil pH was measured in a 1:5 soil:water suspension. Water retention characteristics of the soil were also determined. For water potentials of -100 kPa and -1.5 MPa, samples were dried from saturation on ceramic pressure plate extractors. To determine gravimetric water content at -10 kPa, samples were drained from saturation on "porosity 4" sintered glass funnels.

The soil used in this experiment was non saline (EC_{e25} ≤4.0 dSm^{-1}) but was relatively high in extractable boron. Concentrations of extractable boron greater than 15.0 mg kg^{-1} are considered to be indicative of a potential boron hazard in the subsoil (B. Cartwright pers. comm.). DTPA extractable zinc concentrations were low.

Basal nutrients were added in solution and thoroughly mixed with half of the bulk soil as follows (mg kg^{-1} dry soil): $Ca(NO_3)_2.4H_2O$ (918); KH_2PO_4 (216); K_2SO_4 (114); $MgSO_4.7H_2O$ (140); $MnSO_4.H_2O$ (2.8); $CuSO_4.5H_2O$ (2.2); $FeSO_4.7H_2O$ (0.7); $Co(NO_3)_2.6H_2O$ (0.247); $Na_2 MoO_4.\ 2H_2O$ (0.303). To half of this soil and to half of the untreated soil was also added 7.5 mg Zn kg^{-1} dry soil as $ZnSO_4.7H_2O$ in solution. The remainder of the soil was mixed with water only.

The soil was mixed with triple deionized glass distilled water (TD water) to the optimum consistency for packing (13.4% gravimetric water content, (determined beforehand by empirical tests). Soil was packed to a dry bulk density of 1290 kg m^{-3} - about optimum for root growth for a soil of this texture (Jones 1983). Mixed soil was added to cylindrical PVC pots 15 cm diameter and 60 cm deep lined with sealed polythene bags. Water was then added to the pots to bring the weight to the equivalent of 20.5% gravimetric water content. This then became the maximum water content to which pots were rewatered, since empirical tests had shown that adding more water than this caused the soil to "slump" and lose its aggregated structure. Each pot contained the equivalent of 12.5 kg of dry soil.

5.2.2 Experimental procedure

The experiment was conducted in a laboratory under controlled conditions, with the temperature maintained between 10 and 20°C. Plants were grown under artificial lighting with 11 hours of high intensity lighting each day from metal halide lamps providing a light flux density of 500 μmol quanta m^2 s^{-1}. Incandescent globes provided 14 hours of low intensity lighting each day, overlapping the 11 hours of high intensity lighting.

Pots were packed and watered on September 19, 1993. On September 20, seeds of the zinc efficient cultivar Excalibur and the inefficient cultivar Gatcher were surface sterilised with a solution of 1.5% sodium hypochlorite and after rinsing them in TD water, six seeds were sown in each pot. Seeds of cultivar Gatcher had a mean zinc content of 340 ng Zn $seed^{-1}$ and Excalibur seeds 320 ng Zn $seed^{-1}$.

After emergence, the number of plants in each pot was reduced to three and 150 g of black polythene beads were added to the pot as mulch. (The beads had previously been washed in detergent, rinsed in TD water, soaked for 24 hours in 10% nitric acid solution and triple rinsed in TD water). Pots were weighed twice weekly and were watered to the maximum weight when the weight of the pot fell to the weight equivalent to -100 kPa matric potential.

Pots were watered with a maximum of 500 ml water on any day. At Feekes 8, youngest emerged blades (YEBs) were taken from 'representative' pots from a single replication of Gatcher and Excalibur with *nu*+ *Zn*+ and *nu*+ *Zn*- treatments. The YEBs were dried for 24 hours at 60°C and digested in nitric acid before ICPS analysis for minerals, principally zinc and phosphorus (Zarcinas and Cartwright 1983, Rengel and Graham 1995a).

Plants were allowed to grow to maturity and were harvested as they ripened. Plants were cut 1 cm above the crown and dried for 24 hours at 60°C. The heads were separated from the stems and the number of sterile and fertile heads determined. Heads were threshed and grains weighed. Dried straw samples were ground, and with grain samples, were digested in acid and analysed by ICPS for concentrations of zinc, boron, manganese, phosphorus and sodium. Phosphorus was considered in this study because of the likelihood of interactions with zinc (Loneragan and Webb 1993). Boron and sodium were considered because they often occur at high concentrations in these soils (Holloway and Alston 1992) and manganese was considered because of the negative relationship between manganese and zinc concentrations in grain observed in the field experiments reported in Chapter 3 and because the interactions between zinc and manganese are not well understood.

Uptakes of nutrients by shoots and grain were calculated as the product of nutrient concentration and dry weight. Total nutrient uptake of tops was calculated as the sum of uptakes in straw and grain. Harvest index was calculated as the ratio of grain weight to total weight of tops (straw + chaff + grain).

At harvest, the polythene bags containing the soil columns were removed from each pot and the soil profile cut into three sections: 0 - 0.15 m, 0.15 - 0.35 m and 0.35 - 0.55 m. Soil was dried as described by Hignett (1976). Dried soil samples were shaken through a 1 cm stainless steel sieve and long root pieces were cut to about 1 cm length, then remixed with the soil. The bulk sample was then split several times to give a subsample of about 180 g.

Roots were washed from this subsample using a high speed mixer and a fine mesh sieve (250 μm). The roots were floated onto A4 sized blotting paper, photocopied and lengths and diameters measured using SCI-SCAN root measurement software. From these data, rooting densities (L_v cm cm^{-3}) and total root surface areas (RSA cm^2) were calculated.

5.2.3 Statistical Analysis

Analysis of data was conducted by analysis of variance for a three way factorial design using the Statistix software package.

Assumptions of constant error variance (homogeneity), normality of data distribution and additivity of treatment and replicate effects were tested for each analysis. All data presented in tables and graphs are raw means but when data transformation was necessary, LSDs are presented from the analysis of transformed data.

5.3 RESULTS

5.3.1 Early growth

The first symptoms of zinc deficiency had appeared by October 6 at the 1.5 leaf stage in Gatcher plants supplied with nutrients other than zinc (*G nu+ Zn-*). By October 23, symptoms had also appeared on Excalibur plants with the corresponding treatment and a week later, plants of both cultivars were displaying severe symptoms of zinc deficiency with this treatment (*nu+ Zn-*). Gatcher plants grown in the untreated subsoil (*G nu- Zn-*) were stunted and withered, with a purplish tinge. Excalibur plants with the corresponding treatment (*E nu- Zn-*) were slightly larger but were nevertheless considerably stunted when compared with plants grown on *nu+ Zn+* treated soil. Plants grown in soil to which zinc alone was added (*G nu- Zn+* and *E nu- Zn+*) were slightly taller and more vigorous than their *G nu- Zn-* and *E nu- Zn-* counterparts, but were also stunted.

Results of ICPS analysis of YEBs (flag leaf -1) from *G nu+ Zn+*, *G nu+ Zn-*, *E nu+ Zn+* and *E nu+ Zn-* soil at Feekes 8 are shown in Table 5.2.

Table 5.2 Concentrations of zinc and phosphorus in 'representative' YEBs (flag leaf-1) of Gatcher and Excalibur wheat at Feekes 8 grown in Minnipa subsoil 1993, as a function of nutrient treatments, 1993.

Treatment	**Zn**	**P**
	(mg kg^{-1})	(g kg^{-1})
E nu+ Zn+	123	3.81
E nu+ Zn-	6	7.48
G nu+ Zn+	98	4.33
G nu+ Zn-	8	8.11

The measurements shown in Table 5.2 were not replicated but they indicate large contrasts between zinc and phosphorus concentrations in the leaf tissue of both cultivars, depending on whether or not zinc was added.

5.3.2 Head emergence and growth to maturity

By December 1993, heads had begun to emerge from plants in the *nu*+ *Zn*+ treated soil. Both Gatcher and Excalibur plants grown in *nu*+ *Zn*- pots displayed delayed head emergence compared with plants grown in *nu*+ *Zn*+ soil (Fig. 5.1). In the case of Gatcher, however, some plants in the *nu*+ *Zn*- pots did not produce heads at all (Plate 5.1).

Plate 5.1 **Gatcher wheat grown in Minnipa subsoil with added basal nutrients. Zinc was omitted from the pot at right and added at 7.5 mg Zn kg^{-1} soil on the left.**

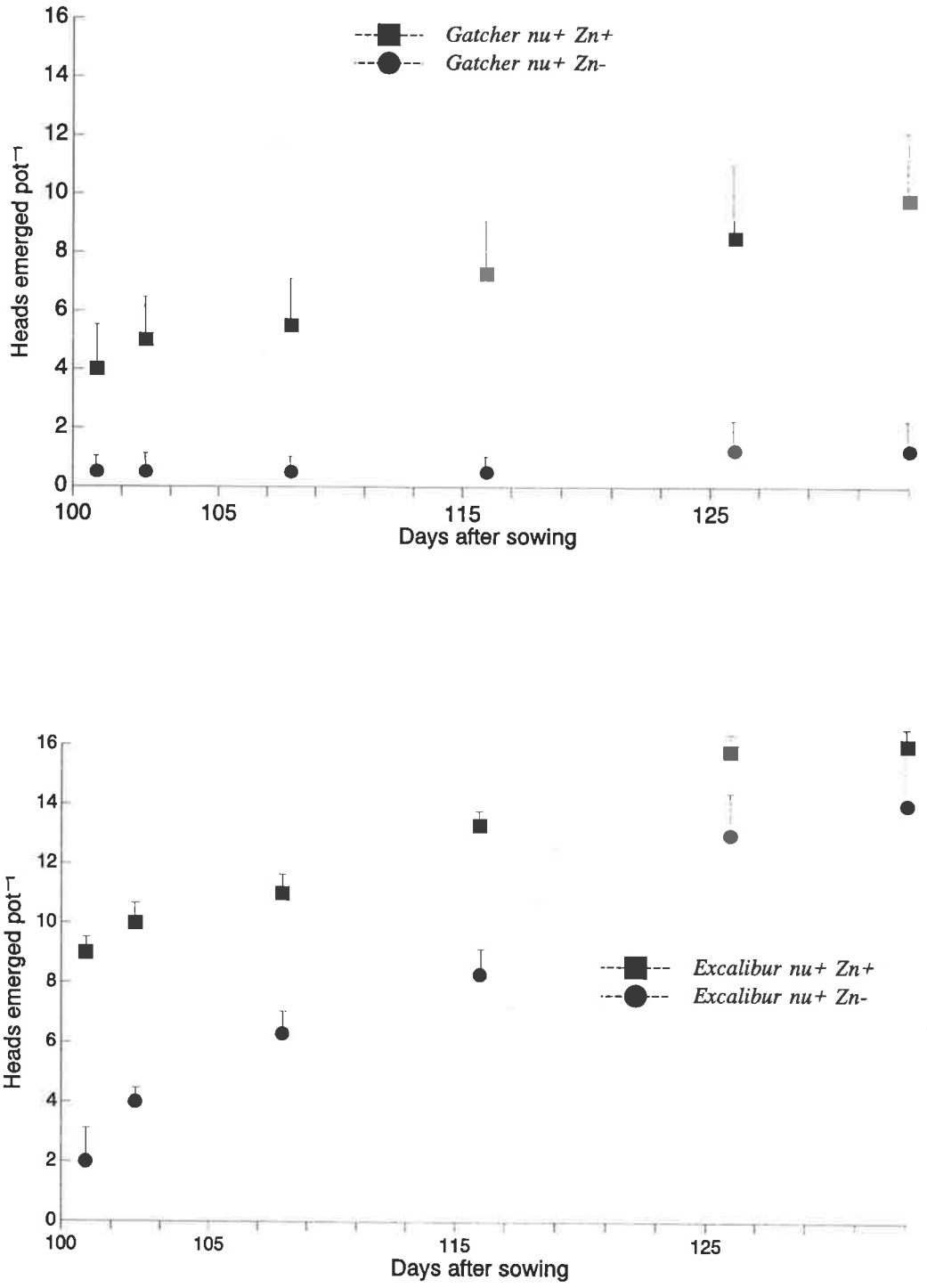

Fig 5.1 Head emergence of Gatcher (above) and Excalibur (below) wheat between 100 and 133 days after sowing in *nu*+ pots as a function of zinc treatment (Zn+ and Zn-). Error bars represent standard errors of the means.

Analysis of variance produced significant values of *F* for main effects *Zn, nu* and *cv* for the number of days taken to reach maturity. Gatcher plants took longer (221 days) to reach maturity than Excalibur (183 days) (P≤0.001) although there were significant interactions between *cv* and *nu* (P≤0.001). The time to reach maturity in Gatcher was not affected by nutrient treatment, but maturity was hastened with Excalibur in *nu*- treatments compared with *nu*+ treatments. The data are shown in Table 5.3.

Table 5.3 Length of growing season (days from sowing to maturity) for Gatcher and Excalibur wheat grown in Minnipa subsoil 1993, as a function of nutrient treatment. Values with the same letter are not significantly different (P≤0.05).

Cultivar Effect *(cv)*	**Basal Nutrient Effect** *(nu)*	**Zinc Effect** *(Zn)*		*(cv* x *nu)* **Means**
		Zn+	**Zn-**	
		(days)		
Excalibur	+	192	212	202 b
	-	161	167	164 c
Gatcher	+	225	224	225 a
	-	211	224	218 a
	Zn means	197 y	207 x	

The behaviour of Excalibur in this instance is of particular interest. Maturity with Excalibur in the *E nu+ Zn-* treatment was delayed by 20 days in comparison with plants with the *E nu+ Zn+* treatment. Rengel and Graham (1995b) reported delays in maturity in the order of three to four weeks in Gatcher and Excalibur plants grown in zinc-deficient Laffer sand, compared with plants provided with adequate zinc. The role of zinc in modifying the ability of plants to mature is an interesting field for further study, particularly in low rainfall environments where growing seasons tend to finish abruptly.

5.3.3 Production of dry matter

Table 5.4 Dry weight of grain + chaff + straw (tops) of Gatcher and Excalibur wheat grown in Minnipa subsoil 1993, as a function of nutrient treatment. Values in the same analysis group with the same letter are not significantly different (P≤0.05).

Main Effect	Dry weight of tops
Cultivar	(g pot^{-1})
Excalibur	11.1 b
Gatcher	16.4 a
Basal nutrients	
nu+	26.4 c
nu-	1.2 d
Zinc	
Zn+	15.7 e
Zn-	11.8 f

Mean dry weights of tops (grain + straw + chaff) for the main effects *cv, nu* and *Zn* are shown in Table 5.4. Overall, Gatcher produced 48% more dry weight of tops than Excalibur (P≤0.001). Dry matter production was significantly higher with Zn+ treatments than Zn- treatments but clearly the greatest difference occurred between *nu+* and *nu-* treatments. The greatest amount of dry matter was produced in *G nu+ Zn+*. In comparison, dry weight of tops was decreased by 37% in *G nu+ Zn-*. Excalibur was not affected to the same degree, however. (There were no significant interactions for any of these factors: Appendix, Table A5.2).

5.3.4 Grain yield and related parameters

Grain yield and related parameters are shown in Table 5.5. There were significant three way interactions (*cv* x *nu* x *Zn*) for each of the parameters shown.

Table 5.5 Grain yield, fertile heads and hundred grain weight (HGW) of Gatcher and Excalibur wheat grown in Minnipa subsoil 1993, as a function of nutrient treatment. Values in the same analysis group with the same letter are not significantly different ($P \leq 0.05$).

Basal Nutrient Effect *(nu)*	**Zinc Effect** *(Zn)*	**Cultivar Effect** *(cv)*	
		Excalibur	**Gatcher**
		Grain Yield [B] ($g\ pot^{-1}$)	
nu-	*Zn-*	0.34 d	0.45 cd
	Zn+	0.56 cd	0.50 cd
nu+	*Zn-*	6.75 b	1.09 c
	Zn+	9.13 a	9.21 a
		Fertile heads [B] ($heads\ pot^{-1}$)	
nu-	*Zn-*	3.3 b	4.3 b
	Zn+	3.3 b	4.0 b
nu+	*Zn-*	29.5 a	5.0 b
	Zn+	22.0 a	23.3 a
		HGW [A] (g)	
nu-	*Zn-*	4.16 b	4.61 ab
	Zn+	3.15 c	5.39 a
nu+	*Zn-*	4.36 b	4.74 ab
	Zn+	4.34 b	4.35 b

[A] Analysis of variance performed on $\log_e$ - transformed data.

[B] Analysis of variance performed on square root - transformed data.

5.3.4.1 Grain yield

Grain yield was highest in Excalibur and Gatcher plants grown with added nutrients and zinc (*nu+ Zn+*) - there was no significant difference between the performance of the two cultivars in the *nu+ Zn+* treatment. However, there was a significant *cv* x *Zn* interaction ($P \leq 0.001$) and the absence of zinc when other nutrients were added (*nu+ Zn-*) had a much greater effect on Gatcher than on Excalibur.

Zinc efficiency, defined as

$$\frac{\text{(grain yield } nu+\ Zn-)}{\text{(grain yield } nu+\ Zn+)} \times 100\%$$

was 74% for Excalibur compared with only 12% for Gatcher. Excalibur produced six times more grain in the *nu+ Zn-* soil than Gatcher. Plants grown with *nu-* treatments produced only 4 - 6% of the grain produced in *nu+ Zn+* soil. All two-way and three-way interactions were significant for the effects of the three factors (*cv, nu* and *Zn*) on grain yield (Appendix, Table A5.3).

5.3.4.2 Fertile Heads

The number of fertile heads in Gatcher decreased greatly from 23.3 heads pot^{-1} in *G nu+ Zn+* to only 5 heads pot^{-1} in *G nu+ Zn-*, contrasting with the *E nu+ Zn-* and *E nu+ Zn+* treatments with which the numbers of fertile heads did not differ significantly.

The number of fertile heads in plants grown in *nu-* soil was significantly lower than with all *nu+* soil treatments, other than *G nu+ Zn-*. The addition of zinc alone to the soil (*nu- Zn+*) had no effect on the number of fertile heads pot^{-1}. The number of fertile heads counted included those with only a few grains in each head. The proportion of sterile florets in each head was not determined but the mean number of grains $head^{-1}$ (Table 5.6) was calculated from the total number of fertile heads, hundred grain weight and grain yield pot^{-1}. When nutrients other than zinc were added, *G nu+ Zn-* plants produced significantly fewer grains $head^{-1}$ than *E nu+ Zn-*.

Hundred grain weight

Hundred grain weight was significantly higher with *G nu- Zn+* grain than with *E nu- Zn+* grain. However, there were no significant differences between hundred grain weights with any of the *nu+* treatments.

5.3.4.3 Infertile heads

Excalibur plants grown in *nu+ Zn-* soil produced a relatively large number of totally infertile heads (Table 5.8), many of which emerged after the main body of heads had reached anthesis. There were also a number of heads in which a high proportion of florets were sterile. In this case, heads were considered to be fertile.

5.3.4.4 Water use efficiency - grain and tops

Analysis of variance produced significant values of F for *cv* as a main effect and a significant two way interaction for *nu x Zn.* The data are shown in Table 5.6 (ii) and (iii). Across all *nu* and *Zn* treatments, Excalibur was almost three times more water use efficient for grain production than Gatcher. Across both cultivars, water use efficiency for grain was significantly higher when basal nutrients and zinc were supplied than when either was absent. However, in the case of production of tops, the presence of basal nutrients other than zinc increased water use efficiency significantly above those with the *nu- Zn-* and *nu- Zn+* treatments.

Grains head^{-1}

Excalibur produced significantly more grains head^{-1} than Excalibur with both the *nu+ Zn-* and *nu- Zn+* treatments. In the untreated subsoil *(nu- Zn-)* and in the *nu+ Zn+* soil, both cultivars produced similar numbers of grains head^{-1}.

Table 5.6 Mean grains head^{-1}(i), grain water use efficiency (WUE grain)(ii) and water use efficiency of tops (WUE tops)(iii) of Gatcher and Excalibur wheat grown in Minnipa subsoil 1993, as a function of nutrient treatment. Values in the same analysis group with the same letter are not significantly different (P≤0.05).

(i) Grains head^{-1}

Basal Nutrient Effect *(nu)*	Zinc Effect *(Zn)*	Cultivar Effect *(cv)*	
		Excalibur	**Gatcher**
		(grains head^{-1}) [A]	
nu-	*Zn-*	2.43 c	2.33c
	Zn+	5.69 b	1.25 d
nu+	*Zn-*	5.33 b	2.58 cd
	Zn+	9.63 a	9.82 a

[A] Analysis of variance performed on $\log_e$ - transformed data.

(ii) WUE grain

Basal Nutrient Effect *nu*	Zinc Effect *Zn*	Cultivar Effect *(cv)*		*(nu x Zn)* Means
		Excalibur	**Gatcher**	
		(kg grain m^{-3} water)		
nu-	*Zn-*	0.46	0.20	0.33 b
	Zn+	0.58	0.07	0.33 b
nu+	*Zn-*	0.40	0.19	0.30 b
	Zn+	1.26	0.50	0.88 a
	cv means	0.67 x	0.24 y	

(iii) WUE tops

Basal Nutrient Effect *(nu)*	Zinc Effect *(Zn)*	Cultivar Effect *(cv)*		*(nu x Zn)* Means
		Excalibur	**Gatcher**	
		(kg tops m^{-3} water)		
nu-	*Zn-*	0.98	0.47	0.73 c
	Zn+	0.88	0.57	0.73 c
nu+	*Zn-*	1.80	1.37	1.59 b
	Zn+	3.00	2.04	2.52 a
	cv means	1.7 x	1.1 y	

Table 5.7 Harvest index of Gatcher and Excalibur wheat grown in Minnipa subsoil, as a function of nutrient treatment. Values in the same analysis group with the same letter are not significantly different ($P \leq 0.05$).

Basal Nutrient Effect *(nu)*	**Zinc Effect** *(Zn)*	**Cultivar Effect** *(cv)*		***(nu x Zn)* Means**
		Excalibur	**Gatcher**	
		% [B]		
nu-	*Zn-*	45.9	39.2	42.6 a
	Zn+	45.4	33.5	39.5 a
nu+	*Zn-*	32.3	4.1	18.2 b
	Zn+	42.0	24.3	33.2 a
	cv means	41.4x	25.3 y	

Cultivar Effect *(cv)*	**Basal Nutrient Effect** *(nu)*	**Zinc Effect** *(Zn)*		***(nu x cv)* Means**
		Zn+	**Zn-**	
		% [B]		
Excalibur	*nu-*	45.4	45.9	45.7 a
	nu+	42.0	32.3	37.2 a
Gatcher	*nu-*	33.5	39.2	36.4 a
	nu+	24.3	4.1	14.2 b
	Zn means	36.3 x	30.4 y	

[B] Analysis of variance performed on square root - transformed data.

5.3.4.5 Harvest Index

Harvest index indicates the ability of plants to channel photosynthates and nutrients efficiently into grain production (Table 5.7, Appendix Table A5.2). Analysis of variance produced significant values of F for each of the three main effects *cv*($P \leq 0.001$), *nu*($P \leq 0.001$) and *Zn*($P \leq 0.05$). Across all *nu* and *Zn* treatments, Excalibur was significantly more efficient with respect to harvest index than was

and *nu* x *cv* interactions. Across all *nu* and *Zn* treatments, Excalibur was significantly more efficient with respect to harvest index than was Gatcher. For both cultivars, harvest index was significantly lower ($P \leq 0.05$) with the *nu+ Zn-* treatment than for other *nu x Zn* treatments. It is also of interest that harvest index with the *E nu+ Zn-* treatments was eight times greater than the equivalent Gatcher treatment, *G nu+ Zn-*.

Table 5.8 Infertile heads produced by Gatcher and Excalibur wheat grown in Minnipa subsoil 1993, as a function of nutrient treatments. Values in brackets are standard errors of the means.

Treatment	Infertile heads
	(heads pot^{-1})
E nu+ Zn+	2.75 (1.25)
E nu+ Zn-	7.75 (2.10)
E nu- Zn+	0
E nu- Zn-	0
G nu+ Zn+	2.25 (1.60)
G nu+ Zn-	2.25 (0.85)
G nu- Zn+	1.25 (0.75)
G nu- Zn-	1.25 (0.94)
Mean	2.19 (0.55)

5.3.5 Nutrient concentrations in grain and straw

Nutrient concentrations in grain are shown in Table 5.9. Analysis of variance produced significant values of *F* for two way interactions for zinc, phosphorus, manganese and boron concentrations in grain. These data are shown in Table 5.9 and in the Appendix, Table A5.4. Across cultivars, zinc concentrations in grain were highest predictability with *Zn+* treatments. Grain produced from *nu- Zn-* soil had significantly higher zinc concentrations than grain from nu+ Zn- soil. Phosphorus

concentrations were significantly higher in *nu+ Zn-* grain than *nu+ Zn+* grain for both cultivars.

Across all *nu* and *Zn* treatments, Gatcher grain had a significantly ($P \leq 0.001$) higher concentration of manganese (57.6 mg Mn kg^{-1}) than Excalibur (36.3 mg Mn kg^{-1}) and for both cultivars, the lowest concentration of manganese in grain occurred in the *nu+ Zn+* treatment.

Grain boron was relatively high in all treatments, exceeding the concentration established in barley by Cartwright *et al.* (1984) as indicating a boron hazard (3.0 mg B kg^{-1}). Differences in grain boron uptake (Table 5.10) in Gatcher between plants with the *G nu+ Zn+* and *G nu+ Zn-* treatments were large and may partly explain the differences in concentration. There were no significant interactions with Zn for grain boron concentration and mean grain boron across all treatments was 4.5 mg B kg^{-1} for *Zn+* and 5.5 mg B kg^{-1} for *Zn-* treatments - an increase of 22% ($P \leq 0.05$).

Table 5.9 Concentrations of zinc, manganese, phosphorus and boron in Gatcher and Excalibur wheat grain grown in Minnipa subsoil, as a function of nutrient treatment. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Basal Nutrient Effect *(nu)*	Zinc Effect *(Zn)*	Cultivar Effect *(cv)*		*(nu x Zn)* Means
		Excalibur	Gatcher	
		Zn concentration in grain [A] (mg kg^{-1})		
nu-	*Zn-*	41	39	40 b
	Zn+	126	106	116 a
nu+	*Zn-*	11	14	13 c
	Zn+	96	81	89 a
	cv means (ns)	69	60	
		Mn concentration in grain [A] (mg kg^{-1})		
nu-	*Zn-*	40.2	64.5	52.4 a
	Zn+	37.6	62.4	50.0 a
nu+	*Zn-*	37.8	59.8	48.8 a
	Zn+	29.5	44.6	37.1 b
	cv means	36.3 y	57.6 x	
		P concentration in grain (g kg^{-1})		
nu-	*Zn-*	5.1	4.8	5.0 ab
	Zn+	4.2	4.7	4.5 bc
nu+	*Zn-*	5.2	5.5	5.4 a
	Zn+	4.2	4.3	4.3 c
	cv means (ns)	4.7	4.8	

Cultivar Effect *(cv)*	Basal Nutrient Effect *(nu)*	Zinc Effect *(Zn)*		*(nu x Zn)* Means
		Zn+	Zn-	
		B concentration in grain [A] (mg kg^{-1})		
Excalibur	*nu-*	3.5	4.5	4.0 c
	nu+	4.3	4.8	4.6 ab
Gatcher	*nu-*	4.1	4.7	4.4 bc
	nu+	6.2	8.2	7.2 a
	Zn means	4.5 y	5.5 x	

[A] Analysis of variance performed on $\log_e$ - transformed data.

Nutrient concentrations in straw were measured and the data indicate that the lowest concentrations of zinc occurred with the *E nu+ Zn-* and *G nu+ Zn-* treatments with zinc concentrations of 6 and 7 mg Zn kg^{-1} respectively. There were no significant differences between the main effect *cv* across all treatments in terms of zinc concentrations in straw (Appendix, Table A5.5, A5.6).

5.3.6 Nutrient uptake in grain and straw

Analysis of variance of nutrient uptake in grain gave significant values of *F* for two and three way interactions between *cv*, *nu* and *Zn* for all of the nutrients listed in Table 5.10, (see also Appendix, Table A5.7). Zinc uptake was significantly higher with *E nu+ Zn+* and *G nu+ Zn+* grain than with other treatments. Uptake of zinc in *E nu+ Zn-* grain was significantly higher than in *E nu- Zn-* grain, but there was no difference in zinc uptake between *G nu+ Zn-* and *G nu- Zn-*. Zinc, phosphorus, boron and manganese uptake in *E nu+ Zn-* grain were significantly greater than with *G nu+Zn-*.

Table 5.10 Uptakes of zinc, phosphorus, boron and manganese in Gatcher and Excalibur wheat grain grown in Minnipa subsoil, as a function of nutrient treatment. Values in the same analysis group with the same letter are not significantly different ($P \leq 0.05$).

Basal Nutrient Effect *(nu)*	**Zinc Effect** *(Zn)*	**Cultivar Effect** *(cv)*	
		Excalibur	**Gatcher**
		Zn uptake (μg pot^{-1}) [A]	
nu-	*Zn-*	13 c	18 c
	Zn+	64 b	47 b
nu+	*Zn-*	75 b	13 c
	Zn+	880 a	752 a
		P uptake (mg pot^{-1}) [B]	
nu-	*Zn-*	1.7 c	2.2 c
	Zn+	2.4 bc	2.3 c
nu+	*Zn-*	35.2 a	5.7 b
	Zn+	37.8 a	39.5 a
		B uptake (μg pot^{-1}) [A]	
nu-	*Zn-*	1.5 d	2.0 d
	Zn+	1.8 d	1.9 d
nu+	*Zn-*	31.9 b	7.6 c
	Zn+	38.6 ab	57.7 a
		Mn uptake (μg pot^{-1}) [A]	
nu-	*Zn-*	13 d	29 c
	Zn+	21 cd	30 bc
nu+	*Zn-*	254 a	59 b
	Zn+	271 a	415 a

[A] Analysis of variance performed on $\log_e$ - transformed data.

[B] Analysis of variance performed on square root - transformed data.

The mean uptake of zinc in Gatcher straw (1.21 mg pot^{-1}) was significantly ($P \leq 0.001$) greater than in Excalibur (0.72 mg pot^{-1}). There were no significant interactions with *cv*, *nu* or *Zn* as components in terms of zinc uptake in straw (Appendix, Table A5.9).

Total nutrient uptake in tops

Nutrient uptake in grain and straw were added to give total uptake in tops and the data are shown in Table 5.11(i) for zinc and phosphorus and Table 5.11(ii) for manganese.

Total zinc uptake in tops was greatest in *G nu+ Zn+*, significantly higher than with the corresponding Excalibur treatment *E nu+ Zn+*. While zinc uptake in grain (Table 5.10) was significantly greater in *E nu+ Zn-* than *G nu+ Zn-*, there were no significant differences in zinc uptake of tops between plants with these treatments, an indication that the zinc efficiency in Excalibur may be a function of its ability to divert zinc into grain production when the supply of zinc is limited. Across all nutrient treatments, total zinc uptake in Gatcher was significantly greater than in Excalibur (Appendix, Table A5.10). There were significant three-way interactions among *cv, nu* and *Zn* for phosphorus uptake in tops. Uptake of phosphorus in *nu-* treatments was very low, but was significantly higher with *nu+ Zn-* treatments than with *nu+ Zn+* treatments. In the latter, phosphorus uptake was significantly higher in Gatcher than in Excalibur. There were significant main effects for *cv* and *nu* ($P \leq 0.001$) and significant two way interactions for *nu x Zn* ($P \leq 0.01$) with respect to total manganese uptake and the data are shown in Table 5.11(ii). Across all nutrient treatments, uptake of manganese in Gatcher was twice that in Excalibur. There were no significant interactions for total boron uptake (Appendix, Table A5.10) but the main effect for *cv* was significant ($P \leq 0.05$), Gatcher taking up 78% more boron than Excalibur.

Table 5.11 Uptake of zinc, phosphorus (i) and manganese (ii) in Gatcher and Excalibur wheat tops (grain + straw) grown in Minnipa subsoil, as a function of nutrient treatment. Values in the same analysis group with the same letter are not significantly different (P≤0.05).

(i)

Basal Nutrient Effect *(nu)*	Zinc Effect *(Zn)*	Cultivar Effect *(cv)*	
		Excalibur	**Gatcher**
		Zn uptake (μg pot^{-1}) [A]	
nu-	*Zn-*	20 f	30 e
	Zn+	250 c	210 cd
nu+	*Zn-*	160 d	180 d
	Zn+	3480 b	5230 a
		P uptake (mg pot^{-1}) [A]	
nu-	*Zn-*	2 e	3 d
	Zn+	3 de	4 d
nu+	*Zn-*	124 ab	155 a
	Zn+	55 c	108 b

(ii)

Basal Nutrient Effect *(nu)*	Zinc Effect *(Zn)*	Cultivar Effect *(cv)*		*(nu x Zn)* Means
		Excalibur	**Gatcher**	
		Mn uptake (μg pot^{-1}) [A]		
nu-	*Zn-*	80	150	120 c
	Zn+	150	230	190 b
nu+	*Zn-*	2870	5000	3940 a
	Zn+	2540	5700	4120 a
	cv means	1410 y	2770 x	

[A] Analysis of variance performed on $\log_e$ - transformed data.

5.3.7 Physiological zinc efficiency

As an index of how absorbed nitrogen is utilised for grain production, Isfan (1990) and Isfan *et al.* (1991) measured the physiological efficiency (PE) of absorbed nitrogen by above ground plant parts (grain plus straw) at maturity - based on the original work of Novoa and Loomis (1981). Rengel and Graham (1995b) adapted the index for zinc in cereals as the ratio of grain produced per unit of zinc absorbed and translocated to above ground parts of the plant. The PE of the various treatments is shown in Table 5.12. Rengel and Graham (1995b) reported that for Gatcher and

Excalibur plants grown in Laffer sand (with high and low concentrations of zinc in the seed) PE was highest in plants fertilized with moderate amounts of zinc (0.05 and 0.2 mg Zn kg^{-1}), but the PE was reduced significantly in deficient soil, or soil provided with toxic concentrations (3.2 mg Zn kg^{-1}).

Table 5.12 Physiological zinc efficiency of Gatcher and Excalibur wheat grown in Minnipa subsoil, as a function of nutrient treatment. Values with the same letter are not significantly different ($P \leq 0.05$).

Basal Nutrient Effect *(nu)*	**Zinc Effect** *(Zn)*	**Cultivar Effect** *(cv)*	
		Excalibur	**Gatcher**
		(kg grain produced g^{-1} zinc translocated to tops) [A]	
nu+	*Zn+*	2.7 cd	1.7 d
	Zn-	42.2 a	5.8 c
nu-	*Zn+*	2.2 d	2.3 d
	Zn-	17.5 b	15.8 b

[A] Analysis of variance performed on $\log_e$ - transformed data.

In the current experiment there were significant three way *cv x nu x Zn* interactions ($P \leq 0.05$) and PE was lowest in plants fertilised with zinc (*Zn+*). There were no significant differences between the cultivars with the *Zn+* treatments. PE was significantly higher with the *nu- Zn-* treatments than the *nu- Zn+*, and there were no differences between cultivars. However, the PE for *E nu+ Zn-* was exceptionally high compared with *G nu+ Zn-*, an indication of the superior ability of Excalibur to channel zinc into grain production in this instance.

5.3.8 Relative transport to grain

The relative transport of zinc to grain ("zinc harvest index") (% of zinc in grain of the total content in tops) is another indicator of the ability of plants to load seed with zinc. The data are shown in Table 5.13 and Appendix, Table A5.11). There were significant *F* values for main effects for *cv* ($P \leq 0.001$), *Zn* ($P \leq 0.001$) and *nu* ($P \leq 0.001$) and significant two way interactions for *cv, nu* and *Zn*.

Table 5.14 Mean root diameter of Gatcher and Excalibur wheat at maturity in Minnipa subsoil, as a function of depth and nutrient treatment. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Root Diameter 0.0 - 0.15 m	0.15 - 0.35 m	0.35 - 0.55 m
	(mm)	(mm)	(mm)
E nu+ Zn+	0.453 cd	0.422	0.426 bc
E nu+ Zn-	0.500 bc	0.418	0.369 c
E nu- Zn+	0.348 ef	0.396	0.367 c
E nu- Zn-	0.325 f	0.361	0.368 c
G nu+ Zn+	0.564 ab	0.536	0.517 a
G nu+ Zn-	0.604 a	0.471	0.506 ab
G nu- Zn+	0.438 cde	0.428	0.403 c
G nu- Zn-	0.372 def	0.400	0.416 c
F	***	ns	**
Excalibur	0.406	0.404	0.382
Gatcher	0.492	0.458	0.460
F	**	ns	**
nu+	0.503	0.467	0.454
nu-	0.371	0.396	0.388
F	***	*	**
Zn+	0.451	0.450	0.428
Zn-	0.450	0.412	0.415
F	ns	ns	ns
F (Zn x cv)	ns	ns	ns
F (nu x Zn)	ns	ns	ns
F (nu x cv)	ns	ns	ns
F (nu x cv x Zn)	ns	ns	ns

The ability of Excalibur to divert zinc to grain with the *E nu+ Zn-* treatments was almost seven times greater than that of Gatcher (Table 5.13(ii)), given the same soil environment (*G nu+ Zn-*). Across all treatments, Excalibur was clearly more efficient than Gatcher in terms of transporting zinc to grain (Table 5.13(ii)).

5.3.9 Root growth

5.3.9.1 Rooting densities and total root length

Rooting densities for the three depth intervals and total root length pot^{-1} are shown in Table 5.14 and in the Appendix, Table A5.12.

The greatest effect on rooting density over all depths was due to the addition of nutrients other than zinc - presumably the principal nutrients involved in promoting root growth were nitrogen and phosphorus as the soil is relatively more deficient in these. The cultivar effect was also highly significant at the lower two depth intervals, Gatcher producing twice as many roots as Excalibur (Table 5.14). The presence or absence of added zinc had no effect on rooting density at any of the depth intervals. The results in terms of differences between the two cultivars were unexpected. It had been hypothesised before the experiment that the greater zinc efficiency of Excalibur may have been due to an ability to generate a more extensive root system, thus enhancing its ability to absorb zinc.

Total root lengths (m pot^{-1}) were also calculated (Appendix, Table A5.13a). In the *nu+* treatments, Gatcher plants produced about twice as much length of root in each pot as Excalibur. Across all nutrient treatments, Gatcher also produced 88% greater root length than Excalibur. The addition of zinc had no effect on total root length.

Table 5.14 Rooting densities of Gatcher and Excalibur wheat in Minnipa subsoil 1993, as a function of depth and nutrient treatment.

Treatment	0.0 - 0.15 m [A]	0.15 - 0.35 m [A]	0.35 - 0.55 m [B]
	(cm cm^{-3})	(cm cm^{-3})	(cm cm^{-3})
Excalibur	3.21	2.43	2.13
Gatcher	4.86	4.98	4.45
F (main effect)	ns	***	***
nu+	7.17	6.20	5.45
nu-	0.89	1.21	1.13
F	***	***	***
Zn+	4.00	3.41	3.25
Zn-	4.07	4.00	3.33
F	ns	ns	ns
F (*Zn* x *cv*)	ns	ns	ns
F (*nu* x *Zn*)	ns	ns	ns
F (*nu* x *cv*)	ns	ns	ns
F (*nu* x *cv* x *Zn*)	ns	ns	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

[B] Analysis of variance performed on square root - transformed data.

5.3.9.2 Root parameters

Mean root diameters (mm) were also measured for each of the three depth intervals (Table 5.15). There were no significant interactions among *cv*, *Zn* or *nu*. Excalibur produced significantly ($P \leq 0.01$) finer roots than Gatcher in the surface layer (0.41 and 0.49 mm mean diameters respectively) and in the base layer (0.38 and 0.46 mm mean diameters respectively).

Table 5.15 Mean root diameter of Gatcher and Excalibur wheat at maturity in Minnipa subsoil, as a function of depth and nutrient treatment.

Treatment	Root Diameter		
	0.0 - 0.15 m	**0.15 - 0.35 m**	**0.35 - 0.55 m**
	(mm)	(mm)	(mm)
Excalibur	0.406	0.404	0.382
Gatcher	0.492	0.458	0.460
F	**	ns	**
nu+	0.503	0.467	0.454
nu-	0.371	0.396	0.388
F	***	*	**
Zn+	0.451	0.450	0.428
Zn-	0.450	0.412	0.415
F	ns	ns	ns
F (Zn x cv)	ns	ns	ns
F (nu x Zn)	ns	ns	ns
F (nu x cv)	ns	ns	ns
F (nu x cv x Zn)	ns	ns	ns

The proportions of fine roots (<0.3 mm diameter) in each depth interval are shown in Table 5.16. There were no significant interactions between *cv, nu* or *Zn* for any depth interval, but the main effects for *cv* and *nu* were significant.

In the upper soil layer, Excalibur had a significantly ($P \leq 0.01$) higher proportion of fine roots (51.6%) than Gatcher (42.4%) and also in the base soil layer ($P \leq 0.05$) with 49.6% for Excalibur and 41.4% for Gatcher (Table 5.16).

Table 5.16 Treatment means of proportions of fine roots (<0.3 mm diameter) in Gatcher and Excalibur wheat at maturity in Minnipa subsoil, as a function of depth and nutrient treatment.

Treatment	**% Fine Roots**		
	0.0 - 0.15 m	**0.15 - 0.35 m**	**0.35 - 0.55 m**
	(%)	(%)	(%)
Excalibur	51.6	47.3	49.6
Gatcher	42.4	40.5	41.4
F	**	ns	*
nu+	40.4	41.0	42.0
nu-	53.6	46.8	49.0
F	***	ns	*
Zn+	44.9	41.4	43.4
Zn-	49.1	46.5	47.3
F	ns	ns	ns
F (Zn x cv)	ns	ns	ns
F (nu x Zn)	ns	ns	ns
F (nu x cv)	ns	ns	ns
F (nu x cv x Zn)	ns	ns	ns

Total root surface areas (cm^2) were also calculated for each depth interval (Table 5.17, Appendix Table A5.15). For the 0.35 - 0.55 m depth interval, there was a significant ($P \leq 0.01$) *nu x cv* interaction and the data are shown in Table 5.17(ii).

Across nutrient treatments and at all depths, Gatcher in general produced a greater root surface area than Excalibur. Root surface areas were considerably greater with *nu+* soil than with *nu-* soil (Table 5.17(i)). Calculations of root surface area were based on the assumption that the roots were smooth cylinders of uniform radius, an assumption made by Barber and Cushman (1981) in their model of nutrient uptake by roots growing in soil. No measurements were made of the effects of root hairs on total root surface area and so it is likely that the calculations considerably underestimate the total surface area. Because zinc, like phosphorus, has a low effective diffusion coefficient, the role of root hairs is likely to be important in zinc absorption. Itoh and Barber (1983a) estimated a ratio of root hair surface:root surface for wheat of 0.7. However, Barber and Silberbush (1984) reported that while data on root hairs was generally important for accurate predictions of phosphorus uptake in cereals, phosphorus uptake could be predicted with reasonable accuracy based on estimates of root surface area from length and diameter dimensions.

Table 5.17 Treatment means of total surface area of roots of Gatcher and Excalibur wheat at maturity in Minnipa subsoil, as a function of depth and nutrient treatment.

(i)

Treatment	Surface Area of Roots		
	0.0 - 0.15 m [A]	0.15 - 0.35 m [A]	0.35 - 0.55 m [B]
	(cm^2)	(cm^2)	(cm^2)
Excalibur	1270	1160	950
Gatcher	2350	2830	4300
F (main effect)	*	***	***
nu+	3330	3450	2940
nu-	287	539	505
F	***	***	***
Zn+	1820	1940	1710
Zn-	1800	2040	1740
F	ns	ns	ns
F(*Zn* x *cv*)	ns	ns	ns
F(*nu* x *Zn*)	ns	ns	ns
F(*nu* x *cv*)	ns	ns	** (see below)
F(*nu* x *cv* x *Zn*)	ns	ns	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

[B] Analysis of variance performed on square root - transformed data.

(ii)

Cultivar Effects *(cv)*	Basal Nutrient Effect *(nu)*	Zinc Effect *(Zn)*		*(cv x nu)* Means
		Zn+	Zn-	
		Surface Area of Roots (0.35 - 0.55 m)		
Excalibur	+	1580	1420	1500 b
	-	360	434	397 c
Gatcher	+	4260	4510	4385 a
	-	640	580	610 c
	Zn means (ns)	1710	1740	

5.4 GENERAL DISCUSSION

The poor performance of both Gatcher and Excalibur in the untreated Minnipa subsoil is an indication of its low fertility status. The addition of zinc alone increased dry weights of tops by 33% ($P \leq 0.01$) but the addition of other nutrients (*nu+*) increased dry weights of tops by a factor of 22, compared with the untreated soil.

Excalibur is clearly superior to Gatcher in several yield components when the performance of the two cultivars is compared in *nu+ Zn+* and *nu+ Zn-* soil. The effect on grain yield of omitting zinc from the added nutrients was much more dramatic in Gatcher, which had a zinc efficiency of 12%, compared with 74% for Excalibur. Similarly, Excalibur was less affected than Gatcher by the omission of zinc in terms of fertile heads, water use efficiency of grain production, grains $head^{-1}$ and harvest index.

It had been hypothesised that the zinc efficiency of Excalibur in comparison with Gatcher was a function of an ability to produce a root system with a higher proportion of fine roots and hence a higher surface area to volume ratio, given a root system of approximately the same mass (Dong *et al.* 1995). Excalibur did produce a higher proportion of fine roots - an indication of more efficient use of assimilates for root growth (Horst and Wiesler 1986). However, the data indicate that differences in root diameter between Excalibur and Gatcher are unlikely to be responsible for the superior zinc efficiency of Excalibur. Indeed, the total uptake of zinc in tops (straw + grain) was similar in both cultivars in *nu+ Zn-* soil, while Excalibur produced about half the total length of roots and estimated root surface area in this soil. This suggests a more efficient uptake mechanism in Excalibur. Presumably, if zinc is required in the external medium, a root system of half the length would be proportionately less subject to the leakage of water and nutrients suffered by a more extensive root system in an environment in which available zinc is limited.

Excalibur also appears better able than Gatcher to divert zinc to grain production in soil with low available zinc status. It is suggested that a physiological difference between the two cultivars in this respect is also responsible for the superior zinc efficiency of Excalibur. While total zinc uptake in tops was the same in both cultivars in the *nu+ Zn-* soil, uptake in grain was almost six times greater in Excalibur than in

Gatcher. Uptake of zinc in straw on the other hand, was 1.7 times higher in Gatcher than in Excalibur. The difference between the cultivars in this respect is most evident in the two parameters, PE and relative transport to grain. Excalibur grown in *nu+ Zn-* soil displayed a PE of 42.2, seven times greater than the PE of 5.8 for Gatcher grown in the same soil. (When grown in the *nu+ Zn+* soil, the PE of both cultivars was similar). Of the total zinc content of tops in *nu+ Zn-* soil, 46.9% was contained in Excalibur grain, and only 7.2% in Gatcher. Clearly, the ability of Excalibur to channel zinc into grain production in an environment where zinc availability is low is superior to that of Gatcher. The number of fertile heads did not differ whether Excalibur was grown in *nu+ Zn+* or *nu+ Zn-* soil, but the number of fertile heads in Gatcher was reduced by 79% in *nu+ Zn-* soil compared with *nu+ Zn+* soil. *E nu+ Zn-* plants produced 55% of the number of grains in each *E nu+ Zn+* head , but *G nu+ Zn-* plants produced only 26% of the grains in *G nu+ Zn+* heads. The higher proportion of fertile florets in Excalibur indicates a more efficient use of zinc within the plant. It is also of interest here that Excalibur plants demonstrated a significantly higher harvest index than Gatcher plants across all nutrient treatments (Table 5.7).

While concentrations of zinc in grain of Excalibur and Gatcher were not different, concentrations of manganese were 59% higher in Gatcher. Pearson and Rengel (1995a) have recently suggested that zinc and manganese are transported to grain in fundamentally different ways. While zinc may be transported and remobilised from several pools within the plant, manganese appears to be transported from roots to the stem via the xylem for temporary storage and later translocated to the grain via the xylem. Pearson and Rengel (1995b) have also suggested that the immobility of manganese pools within above ground plant organs indicates that roots may provide an important storage for manganese until grain formation. The higher manganese concentration in Gatcher grain may be a function of its more extensive root system.

In the untreated subsoil (*nu- Zn-*) both cultivars grew poorly and produced little grain and there were no significant differences between the cultivars in terms of grain produced. Total zinc and phosphorus uptake in tops from the untreated soil were significantly greater in Gatcher than Excalibur (Table 5.11), although grain uptake was not significantly different. Excalibur then displays a significant adaptational advantage in the untreated soil - more efficient use of limited nutrients for grain production. The

data also indicate that in this soil, root growth can be promoted considerably by the addition of nutrients. Further studies are needed to define which combinations of nutrients are needed, and in what quantities.

It is unlikely that fertilizing the soil below 0.40 m in low rainfall environments such as Upper Eyre Peninsula will be practicable, but cultivars which are able to utilise the water and nutrients available offer the most tenable solution to the problem of "inhospitable subsoil".

6.0 The effects of localised zinc supply on the growth and grain uptake of zinc in a zinc efficient wheat cultivar.

6.1 INTRODUCTION

The effects of uneven or localised zinc supply in the root zone of wheat appear to be subtle. For instance, Loneragan *et al.* (1987) utilised a "vertical" split root technique (*i.e.* half of the roots were grown in separate solutions with different zinc concentrations) to test the capacity for translocation of zinc in the phloem to maintain the function and growth of wheat roots in a low zinc environment. Omitting zinc from half of the root system did not affect the dry matter production of that half, and zinc concentration in the roots appeared to be adequate. However, while the pH of the nutrient solution was progressively lowered throughout the duration of the experiment, at its conclusion, the pH of the nutrient solution without zinc was 0.6 units higher than the solution containing zinc. While it appears that zinc translocation between an adequately supplied portion of the root system and a portion in a zinc-deficient medium is sufficient to maintain root dry matter production, other root functions may suffer. However, because an aim of the experiment was to measure changes in pH of the nutrient solution, the pH was allowed to drift to levels which may have been detrimental to root growth.

Webb and Loneragan (1990) conducted a similar experiment but maintained the nutrient solution pH between 5 and 7 and arrived at similar conclusions - that when the supply of zinc to the shoots was adequate, omitting zinc from half the root system did not affect dry matter production (of roots or shoots) but that zinc translocation may not be adequate to support all plant functions requiring zinc through phloem transport. It has also been demonstrated that low zinc concentrations depress the absorption and retention of phosphorus (and chlorine) in wheat (Welch *et al.* 1982, Webb and Loneragan 1990).

Webb and Loneragan (1990) postulated that if zinc translocation is unable to support root functions, then regions of deficiency may occur in deeper soil layers which are

not fertilized with zinc and which, in many areas of southern Australia at least, are inherently of low zinc status.

Robson and Snowball (1990) also conducted a vertical split-root experiment with paired compartments with and without zinc. In this instance, root weights were lower in compartments to which zinc was not added compared with associated compartments supplied with zinc. However, it was considered that zinc applications were sub-optimal: the aim of the experiment was to assess the effects of herbicide on zinc uptake and utilisation. It was considered that zinc was less readily translocated in phloem when supplied at sub-optimal levels. Zinc deficiency was also associated with thickening of roots (decreased length g^{-1} root).

Nable and Webb (1993) compared the performance of zinc-efficient (Excalibur) and zinc-inefficient (Gatcher) wheat cultivars in a laterally split root system in which topsoil (0.1 m deep) was supplied with zinc but the subsoil (0.25 m deep) was either supplied with zinc or zinc was withheld. Withholding zinc from the subsoil had no effect on vegetative yield or root growth in either zone, number of tillers, number of fertile heads, number of grains per head, or on plant appearance before booting in either cultivar. However, Gatcher plants grown in pots containing subsoil without added zinc demonstrated delayed head emergence and lower grain yield and water usage in the 60 day period preceding maturity, compared with plants grown in pots with zinc fertilized subsoil. Excalibur displayed none of these effects with zinc-deficient-subsoil.

In both cultivars, zinc concentrations in the flag leaf were higher in pots containing subsoil zinc. While only 20% of the root weight occurred in the subsoil, about 50% of the zinc appeared to be taken up by these roots. Excalibur had consistently higher zinc concentrations in flag leaves and lower concentrations in grain than Gatcher, independent of the subsoil treatment.

While Excalibur was obviously more zinc-efficient than Gatcher in terms of its ability to produce grain in pots with zinc-deficient subsoil, the low concentration of zinc in grain of Excalibur (10 mg kg^{-1}) is of concern. High concentrations of zinc in seed are

important for nutritional reasons (Welch 1993) and for vigour and yield of crops grown from the seed (Rengel and Graham 1995a,b).

Because zinc efficiency in cereals offers a cost effective way of dealing with zinc-deficient soils over a wide area (Graham and Rengel 1993), it is important that the relationship between the placement of zinc fertilizer and transport to grain be better understood. An experiment was designed to test the hypothesis that the growth and zinc concentration and uptake in grain of Excalibur wheat are independent of the effects of zinc placement in the root zone.

6.2 MATERIALS AND METHODS

6.2.1 Experimental design and statistical analysis

The experiment consisted of a completely randomised design with three replicates of each treatment. Excalibur wheat plants were grown in cylindrical PVC pots lined with polythene bags (0.15 m diameter, 0.70 m deep). The soil profile was divided into three layers, each 0.20 m deep.

Soil was added to each layer to give various combinations of zinc placement. The treatments were labelled as follows Zn +++ (*i.e.* zinc added to all three layers top to bottom, reading from left to right), Zn ++−, Zn +−−, Zn +−+, Zn −++, Zn−−+, Zn −+−. As described in the experiment of Nable and Webb (1993), plants were also grown in single pots to which no zinc was added.

Analysis of data was conducted by analysis of variance using the Statistix software package. Assumptions of constant error variance (homogeneity), normality of data distribution and additivity of treatment and replicate effects were tested for each analysis as described previously. All data presented in tables and graphs are raw means but when data transformation was necessary, significant differences are indicated from the analysis of transformed data. In the case of grain zinc concentrations, differences in variance between treatment groups could not be satisfactorily resolved by data transformation, Bartlett's test for data sets with unequal variances remaining significant. In this instance, two-sample t-tests were used to compare each treatment.

6.2.2 Experimental procedure

The experiment was conducted in Laffer sand, the characteristics of which are described in Chapter 4. The soil was air dried and sieved through a stainless steel sieve. The dry soil was mixed with $CaCO_3$ at 3 000 mg kg^{-1} soil. Basal nutrients were added separately in solution and thoroughly mixed as follows (mg kg^{-1} dry soil): $Ca(NO_3)_2.4H_2O$, (918); KH_2PO_4, (72); K_2SO_4, (114); $MgSO_4.7H_2O$, (140); H_3BO_3, (1.4); NaCl, (3.2); $MnSO_4.H_2O$, (4.6); $CuSO_4.5H_2O$, (2.2); $CoSO_4.5H_2O$, (0.22); $H_2MoO_4.H_2O$, (0.22); $FeSO_4.7H_2O$, (0.7).

The total soil was then divided into two parts and to one part was added $ZnSO_4.7H_2O$ in solution at a rate equivalent to 0.5 mg Zn kg^{-1} soil. Both lots of soil were mixed with triple deionised glass distilled (TD) water to field capacity (0.12 kg kg^{-1}).

As the pots were filled, the edges were carefully tamped to prevent water draining down the side of the pot during watering. Polythene gloves were used in the filling process and a fresh pair of gloves was used with each layer, to avoid contamination. To prevent contamination of the sides of the pot with zinc-treated soil, polythene sleeves were inserted into the pot to the appropriate depth and soil added. The sleeve was then twisted to reduce its diameter and carefully removed. Samples taken after the soil had been allowed to equilibrate for 60 h were allowed to air dry and the zinc status of the Zn- and Zn+ soil was assessed by DTPA extraction.

Preparation of seed, water, utensils and laboratory conditions were as described in Chapter 4. Seeds were sown on April 20, 1995. On April 27, plants were thinned to two in each pot. On May 4, 100 g of acid-washed and TD water-rinsed polythene beads were added to each pot as mulch.

To each layer was added an equivalent of 4.3 kg dry soil, a total of 12.9 kg pot^{-1}. On May 16, at early tillering (Feekes 2) two youngest emerged blades (YEBs) were taken from each pot for nutrient analysis. The YEBs were oven dried at 80°C for 24 hours and digested in nitric acid before ICPS analysis for concentrations of zinc, phosphorus, potassium, manganese and boron (Zarcinas and Cartwright 1983, Rengel and Graham 1995a). Concentrations of potassium and boron have been included in this study because of the effects of zinc deficiency on the leakage of potassium from

roots (Cakmak and Marschner 1988a) and the relationship between low zinc supply and the accumulation of boron in cereals (Graham *et al.* 1987, Singh *et al.* 1990). Manganese has also been considered because the relationships between zinc and manganese concentrations in grain are not well understood.

Pots were weighed weekly and the amount of water required to bring the pots to their field capacity weight was added over the next week. No more than 200 ml of water was added at each watering to reduce the possibility of leaching or free drainage down the sides of the pot.

The emergence of heads was recorded during each week and at mid anthesis, two flag leaves were taken from each pot for nutrient analysis. The flag leaves were prepared for analysis in the same way as the YEBs taken earlier in the season.

Pots were watered until it was estimated that grain in two-thirds of the heads which had emerged was dead ripe. Watering was then stopped. At maturity, plants were cut 1 cm above the soil surface and were dried for 24 hours at 80°C, then weighed. Tillers and fertile heads were counted and grain threshed from the heads by hand. Grain samples were weighed to determine grain yield and digested in nitric acid for ICPS analysis as described above. Subsamples of grain were counted and weighed to determine hundred grain weights. Straw samples were ground and subsampled for ICPS analysis.

Uptakes of nutrients in shoots and grain were calculated as the product of nutrient concentration and dry weight. Harvest index was calculated as the ratio of grain weight to total weight of tops (straw + chaff +grain).

Immediately after harvest, pots were laid on a table and the polythene lining bags extracted from the PVC cylinders. The bags were then cut open with a scalpel and the soil column rolled into a semi-circular 1mm mesh sieve. The soil was carefully washed away with a fine jet of low pressure water so that the root system remained intact. The washed root system was then laid out on a grid and two-cm sections of the

entire root system were cut from the centre of each layer. The roots were stored in a solution of 70% ethanol.

Roots were then floated onto A4 sized blotting paper from which the water was drained so that the roots remained on the surface of the paper. The paper was covered with an acetate sheet, inverted and photocopied. Root lengths and diameters were then calculated using the SCI-SCAN root measurement software. Rooting densities were calculated from this data. Total root lengths for each pot were calculated based on the assumption that the rooting density in the centre of each layer represented the mean rooting density for that layer.

6.3 RESULTS AND DISCUSSION

DTPA extractable zinc concentrations in the mixed soil from which zinc was omitted were 0.19 mg Zn kg^{-1} soil and in the soil to which zinc was added, 0.53 mg Zn kg^{-1}. On May 2, signs of zinc deficiency had begun to appear on plants at the 2.5 leaf stage in the $--+$ and $-+-$ treatments and on May 10, symptoms had also appeared in plants with the $-++$ treatment.

After May 2, plants where zinc was added to the topsoil were visibly larger and more vigorous than plants grown or where zinc was omitted from the topsoil (Plates 6.1 and 6.2). The first heads began to emerge by June 23.

6.3.1 Head emergence, fertile heads, grain yields and other yield parameters

The times to 50% head emergence were measured for each treatment and are shown in Table 6.1. The placement of zinc induced significant differences in the rate of head emergence. Generally, head emergence was delayed in the $--+$ and $-+-$ soil, although differences were not consistently significant. In Nable and Webb's (1993) experiment, the time to 50% head emergence was delayed by about 10 days in Gatcher when zinc was omitted from the subsoil compared with plants grown in subsoil to which zinc had been added (*i.e.* $+-$ compared with $++$). There were no differences in head emergence times in Excalibur. Similarly, in the current

Plate 6.1

Plate 6.2

Wheat, 26 days after sowing in zinc deficient Laffer sand in which zinc was withheld from the top layer (0.20 m deep)(Plate 6.1) and plants of the same age grown in Laffer sand to which 0.5 mg Zn kg^{-1} was added to the top layer (Plate 6.2).

experiment, there were no significant differences in time to 50% head emergence between the +++ and +−− treatments. The time to 50% head emergence in the −−+ soil was delayed by about 11 days compared with the ++− soil.

The results suggest that despite the zinc efficiency of Excalibur, head emergence may be delayed if the plants do not have access to zinc early in the growth cycle. When grouped means across treatments for days to 50% head emergence are compared, emergence was delayed by five days in plants grown in soil from which zinc was omitted in the top layer, compared with those with added zinc ($P \leq 0.01$). Zinc placement in the centre or base layers had no effect.

Analysis of variance gave significant values of F for the number of tillers and fertile heads pot^{-1} (Table 6.1). The mean number of tillers in plants grown in soil with added zinc in the base layer (21.1) was significantly ($P \leq 0.05$) higher than in plants with treatments from which zinc had been omitted from the base layer (19.4).

Table 6.1 Days to 50% head emergence (D50HE), numbers of tillers and numbers of fertile heads in Excalibur wheat as a function of zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	D50HE	Tillers	Fertile heads
+++	87.0 abc	19.3 bc	18.0 bc
++−	81.7 c	18.0 c	17.7 c
+−+	85.3 bc	22.7 ab	21.7 ab
−++	86.7 abc	24.0 a	23.0 a
+−−	82.0 c	20.0 bc	20.0 abc
−+−	89.3 ab	20.3 bc	19.7 abc
−−+	92.3 a	18.3 c	18.0 bc
F	*	*	*

Zinc placement had no effect on grain yield, nor were there significant differences between treatments in terms of total dry weight of tops, harvest index, hundred grain weight or grains head^{-1} (Appendix, Table A6.5). It should be noted that the lack of large differences in yields is in one sense an advantage for interpreting results with respect to differences in zinc concentrations in tissue or grain because of the relationship between concentration and uptake and the confounding effects which may be introduced with gross differences in yields.

6.3.2 Root measurements

Rooting densities measured from the centre of each of the three layers and mean root diameters at crop maturity are shown in Table 6.2. Rooting densities and mean root diameters were not significantly different between treatments in the upper two layers, but significant differences did occur in the base layer. Mean rooting densities were significantly higher in the base layer where the layer was not supplied with zinc (10.4 cm cm^{-3}) compared with treatments supplied with zinc (8.1 cm cm^{-3}) ($P \leq 0.01$). Roots from each pot were also weighed but the data are not presented because of the

unreliability induced by the difficulties in removing all of the sand grains which were tightly bound in the denser root mats, even small amounts of contamination having a large effect on weight. Similar difficulties were reported by Ellington (1986).

Table 6.2 Rooting density (L_v) and mean root diameter (RD) of Excalibur wheat at crop maturity as a function of depth and zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	L_v	RD	L_v	RD	L_v	RD
	0.08 - 0.10 m		**0.28 - 0.30 m**		**0.48 - 0.50 m**	
	(cm cm^{-3})	(mm)	(cm cm^{-3})	(mm)	(cm cm^{-3})	(mm)
+++	4.55	0.185	5.05	0.221	9.77 ab	0.236 a
++−	5.70	0.184	7.12	0.218	9.41 abc	0.202 bc
+−+	5.75	0.174	4.83	0.209	6.96 c	0.202 bc
−++	6.29	0.185	5.63	0.226	8.56 c	0.222 ab
+−−	4.94	0.186	6.52	0.217	11.68 a	0.187 c
−+−	6.08	0.196	5.84	0.205	10.02 ab	0.210 abc
−−+	5.48	0.178	3.83	0.226	7.00 c	0.218 ab
F	ns	ns	ns	ns	*	*

As with rooting densities, mean root diameters did not differ significantly in the upper two layers but did differ in the base layer. Roots from the base layer with +−− soil were significantly finer than in the −−+, −++ and +++ soil. Rooting density was also highest in the base layer of the +−− soil. Mean root diameters were significantly ($P \leq 0.05$) finer where zinc was not added to the base layer (0.20 mm) compared with those where zinc was added (0.22 mm). The placement of zinc in other layers had no effect on root diameter in the base layer.

Plate 6.3 indicates the differences in root growth between Excalibur plants grown in soil to which no zinc was added, compared with plants grown in soil to which zinc was added to the centre only ($-+-$). Zinc translocation in both directions (*i.e.* above and below the site of uptake) appears to be sufficient to maintain root growth, if not root function, since rooting density and diameter with the $-+-$ treatment was not significantly different from that with the $+++$ treatment in any layer.

Total root length was estimated for each pot based on the assumption that mean rooting densities in the centre of the layer were average values for the entire layer. The data suggest that total root length was least with the $--+$ treatment and greatest with the $+--$ treatment (Appendix, Table A6.12).

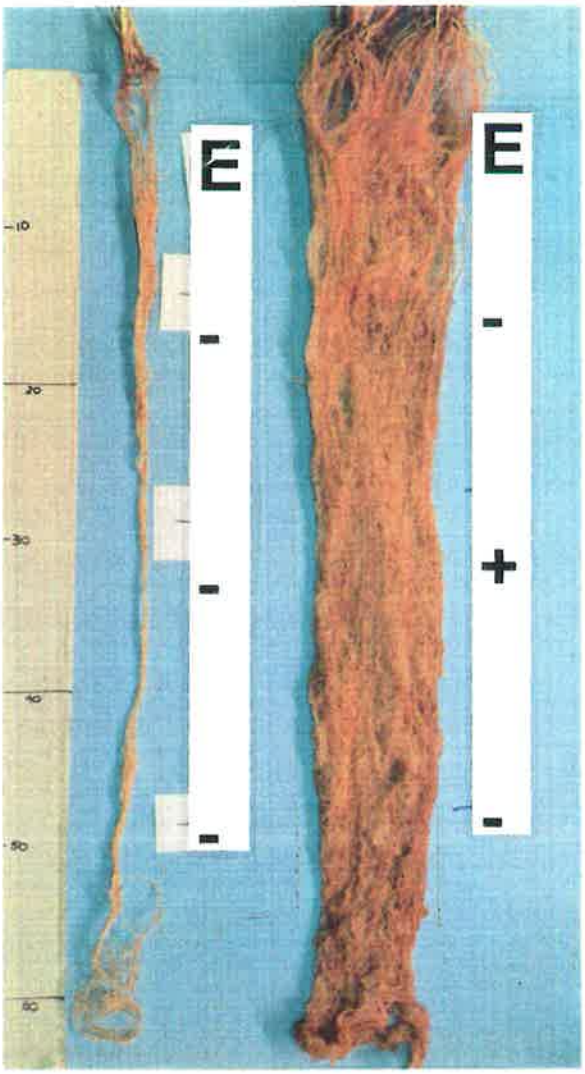

Plate 6.3 **Roots of Excalibur wheat grown in Laffer sand with added basal nutrients other than zinc (left) and in the same soil to which 0.5 mg Zn kg^{-1} soil was added to the central layer (0.2-0.4 m) only.**

6.3.3 Nutrient Concentrations in YEBs

Analysis of variance showed significant values of *F* for the effect of zinc placement on the concentration of several nutrients in YEBs collected from each pot at early tillering Feekes 2 (Table 6.3).

Table 6.3 Concentrations of zinc, phosphorus, potassium, manganese and boron in YEBs of Excalibur wheat as a function of zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Zn	P	K	Mn	B
	(mg kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
+++	44.5 a	7.8	78.8	19 b	35.1 b
++−	42.7 a	7.9	71.5	20 b	45.9 ab
+−+	34.0 b	7.0	74.2	24 b	37.8 b
−++	23.9 c	6.2	61.8	57 [z]	54.7 a
+−−	27.3 c	6.8	71.7	23 b	36.0 b
−+−	25.7 c	6.3	64.9	32 a	35.0 b
−−+	17.0 d	11.0 [z]	61.6	104 [z]	54.4 a
F	***	ns	ns	**	*

[z] Analysis of variance performed without this treatment due to non additivity which could not be corrected by data transformation. The mean and variance for this treatment were higher than for other treatments.

Zinc concentrations were significantly higher in pots with zinc added to the top two layers (+++ and ++−). Zinc concentrations reflect the probable relations between the total quantity of added zinc and root distribution. Because rooting density declines with depth (at least in the early stages of growth in pots), often in an exponential manner (*e.g.* Russell 1977), it is understandable that YEBs in the ++− treatment contained significantly higher zinc concentrations than the +−+ treatment, which in

turn had significantly higher concentrations than the $-++$ treatment. The significant difference in zinc concentrations between the $+-+$ (34.0 mg Zn kg^{-1}) and $+--$ treatments (27.3 mg Zn kg^{-1}) suggests that even at Feekes 2, plants had been absorbing some zinc from below 0.40 m.

YEBs from plants with the $--+$ treatment had the lowest (probably deficient) concentrations of zinc. However, concentrations were higher than would be expected had the plants been grown in an environment where zinc was withheld from the entire profile, again suggesting some uptake from below 0.4 m.

Phosphorus concentrations in YEBs did not differ between treatments. YEB concentrations of phosphorus in plants with the $--+$ treatment (which was not included in the analysis of variance) were not significantly higher than with other treatments when compared by the two-sample t-test. Plants with the $--+$ treatment also demonstrated the highest concentrations of manganese in YEBs. This treatment was not included in the analysis of variance but two sample t-tests indicate that the manganese concentration was significantly higher ($P \leq 0.05$) than in all but the $-+-$ and $-++$ soil.

Manganese concentration was inversely related to zinc concentration as indicated by concentrations in YEBs, and the correlation coefficient for zinc and manganese in YEBs was -0.7 ***. It is of interest that manganese concentrations were much higher in YEBs from plants grown in pots from which zinc was withheld from the top layer. For instance, manganese concentrations in YEBs were 23.6 mg kg^{-1} in $+-+$ pots, 56.7 mg kg^{-1} in $-++$ pots and 22.9 mg kg^{-1} in $+--$ pots. Although zinc and manganese concentrations in YEBs were inversely related, Kochian (1993) has indicated that the main competitive interaction for zinc (Zn^{2+}) is copper (Cu^{2+}), and this has been confirmed by Rengel and Graham (1995a) in zinc-deficient plants. Kochian (1993) also referred to studies by Schmid *et al.* (1965) which demonstrated that in contrast to copper, manganese did not affect zinc uptake. Similar findings were reported by Loneragan and Webb (1993).

6.3.4 Nutrient concentrations in flag leaves

Concentrations of nutrients in flag leaves are shown in Table 6.4.

Table 6.4 Concentrations of zinc, phosphorus, potassium, manganese and boron in flag leaves of Excalibur wheat at Feekes 10.5.2 as a function of zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Zn	P	K	Mn	B
	(mg kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
+++	25.4 a	3.68	22.5 bc	13.3 c	61.2
++−	21.6 b	3.90	24.4 ab	17.3 b	47.6
+−+	22.6 b	3.39	21.1 bc	16.8 b	59.4
−++	21.6 b	3.36	21.4 bc	16.2 b	60.3
+−−	15.0 d	3.31	16.9 d	21.8 a	56.3
−+−	17.5 cd	3.53	19.5 cd	21.3 a	44.3
−−+	18.8 c	3.79	27.4 a	18.4 b	59.5
F	***	ns	**	*	ns

Analysis of variance gave significant values of *F* for treatment effects on concentrations of zinc, potassium and manganese in flag leaves. Zinc concentrations in flag leaves in pots containing two or three layers with added zinc tended to reflect the total amount of zinc available more than placement effects. It is of interest that the arrangement of layers which most closely resembles the field situation for zinc placement in southern Australia (*i.e.* +−−) had the lowest zinc concentration in the flag leaves. With all treatments except −−+, zinc concentrations in YEBs were much greater than concentrations in flag leaves which were harvested later. This may indicate a temporal effect as more roots entered the bottom soil layer. The correlation coefficient for the relationship between flag leaf zinc concentration and grain zinc concentration was 0.87 ***, while the relationship for YEB zinc and grain zinc

concentrations was 0.44 *, suggesting that flag leaves and grain may draw on similar pools of zinc.

The differences in potassium concentrations in flag leaves between treatments are not easily explained in terms of zinc placement effects. Concentrations of potassium in flag leaves of plants with $+--$ and $-+-$ treatments tended to be lower than with other treatments, which may be explained as leakage in areas of the root zone deficient in zinc. It has been shown that transport of zinc in the phloem from a region of adequate zinc to a deficient region is sufficient to maintain dry matter production but not root function (Loneragan *et al.* 1987, Webb & Loneragan 1990). However, this may not apply to xylem transport from roots in a lower zone having adequate zinc, and may explain the significantly higher potassium concentrations in flag leaves from the $--+$ soil compared with plants from the $-+-$ and $+--$ soil. However, this does not explain the significant difference between the $--+$ treatment and $+++$ treatments.

Manganese concentrations in flag leaves differed significantly between treatments. The order of concentrations between treatments was different from that with YEBs and tended to reflect the change in zinc status of the plants. Indeed, the correlation coefficient for zinc and manganese concentrations in flag leaves was -0.88 ***. The inverse relationship may reflect the different translocation patterns of the two nutrients as the plant begins to form reproductive tissue.

6.3.5 Concentrations of nutrients in straw

Concentrations of nutrients in straw are shown in Table 6.5.

Table 6.5 Concentrations of zinc, phosphorus, potassium, manganese, and boron in Excalibur wheat straw as a function of zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Zn	P	K	Mn	B
	(mg kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
+++	6.80	0.79	26.3	7.83 cd	34.5 bc
++−	4.72	0.80	26.2	10.10 ab	42.8 a
+−+	4.55	0.66	22.8	6.83 cd	28.3 cd
−++	7.48	0.65	22.0	6.43 d	21.9 d
+−−	6.27	0.68	24.1	10.93 a	36.7 ab
−+−	3.57	0.61	21.4	7.60 cd	24.7 d
−−+	4.68	0.62	21.4	8.52 bc	33.5 bc
F	ns	ns	ns	***	***

Analysis of variance produced no significant values of *F* for treatment differences in concentrations of zinc, phosphorus or potassium in straw. However, there were significant treatment effects for manganese and boron concentrations. Manganese concentrations were not significantly (negatively) correlated with zinc concentrations in straw as they were in flag leaves. The +−− soil contained the highest concentration of manganese in both flag leaves and straw. It is also of interest that the order of differences in concentrations in straw between the various treatments of the phloem immobile nutrients calcium and boron were similar, with highest concentrations in ++− soil and lowest in −++ soil (Table 6.5; Appendix, Table A6.9).

6.3.6 Zinc concentrations in grain

Although analysis of variance gave highly significant values of *F* for the effects of zinc placement on concentrations of zinc in grain, the assumption of data homogeneity could not be satisfied by data transformation. Consequently, pairwise t-tests were conducted to determine significant differences. The results are displayed in Table 6.6.

Table 6.6 Zinc concentrations in Excalibur wheat grain as a function of zinc placement. Significant differences in grain zinc concentrations between treatments were determined by pairwise t-tests and are indicated below.

Treatment	+++	++−	+−+	−++	+−−	−+−	−−+
Zinc concentration (mg kg^{-1})	23.8	16.3	19.0	20.1	9.7	10.3	15.1
+++	-						
++−	*	-					
+−+	ns	ns	-				
−++	ns	ns	ns	-			
+−−	*	*	P<0.1	*	-		
−+−	*	*	P<0.1	*	P<0.1	-	
−−+	*	ns	ns	P<0.1	***	***	-

The data show a general trend for zinc concentration in grain to reflect the total amount of zinc available in the root zone. Zinc concentrations were highest in plants grown in +++ pots and were lowest in plants grown in pots in which zinc was added to a single layer. The data also demonstrate significant effects of placement on zinc concentrations in grain. It is perhaps surprising that while grain zinc concentrations were significantly different between the ++− and +++ soil, there were no such differences between the +++ and +−+ or −++ soil.

Where zinc was added to a single layer, concentrations of Zn in grain differed to a significant degree ($P \leq 0.05$) or close to it, grain zinc increasing as the depth of placement increased. It is also of interest that the lowest concentration of zinc in grain occurred in the $+--$ treatment, which most closely simulates the field situation for zinc concentration gradients. Over all treatments, withholding zinc from the base of the pot had a highly significant effect on grain zinc ($P \leq 0.001$). Mean grain zinc for pots with zinc withheld from base layers was 12.8 mg Zn kg^{-1} and for pots containing added zinc in the base layer, 19.5 mg Zn kg^{-1}. Concentrations of other nutrients in grain are shown in the Appendix, Tables A6.6 and A6.7.

6.3.7 Nutrient uptake - straw and grain

Zinc uptake in straw (Appendix, Table A6.10) did not differ significantly between treatments, and there were no significant differences for nutrients other than manganese (Appendix, Table A6.11) where significant values of *F* were obtained from the analysis of variance ($P \leq 0.01$).

Nutrient uptake in grain is shown in Table 6.7. Analysis of variance produced significant values of *F* for treatment effects on grain zinc uptake but there were no significant differences for other nutrients. Grain zinc uptake reflected grain zinc concentrations and was highest where zinc was added to all three layers ($+++$) and lowest in the $+--$ soil. However, in the case of treatments where zinc was added to two layers, zinc uptake was significantly higher in the $-++$ than the $++-$ soil. Mean zinc uptakes were significantly higher with treatments in which zinc was added to the base layer (546 μg pot^{-1}) than in plants grown in soil with zinc-deficient base layers (347 μg pot^{-1}) ($P \leq 0.001$).

Table 6.7 Uptake of zinc, phosphorus, potassium, manganese and boron in Excalibur wheat grain as a function of zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Zn[A]	P	K	Mn	B
	(μg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})
+++	619 a	96	115	313	69.6
++−	451 bc	97	115	319	72.0
+−+	555 ab	100	125	261	74.7
−++	578 a	99	118	282	76.8
+−−	277 d	100	129	325	84.6
−+−	315 d	102	134	304	86.0
−−+	434 c	93	119	282	77.0
F	***	ns	ns	ns	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

6.3.8 Physiological zinc efficiency and relative transport to grain

Physiological efficiency (PE) is an index of how absorbed zinc which has been translocated to plant tops is utilised for grain production (Rengel and Graham, 1995b). The percentage of total zinc in tops contained in the grain is also indicated by the relative transport of zinc to grain (RTP). Both parameters are shown in Table 6.8.

Table 6.8 Physiological zinc efficiency and relative transport of zinc to grain in Excalibur wheat as a function of zinc placement. Values in the same column with the same letter are not significantly different.

Treatment	Physiological Zinc Efficiency	Relative Transport to grain
	(kg grain produced g^{-1} Zn translocated to tops)	(% of zinc in grain of total content in above ground parts)
+++	28.2 d	66
++−	47.0 bc	76
+−+	27.1 d	64
−++	34.7 cd	70
+−−	62.3 ab	60
−+−	69.2 a	71
−−+	52.1 abc	78
F	**	ns

Zinc placement had a highly significant effect on PE ($P \leq 0.01$), with the highest values occurring with treatments in which only one layer contained added zinc. Mean PEs were compared (by pairwise t-tests) for groups of treatments with or without zinc in each layer. The PE for placement of zinc in the top or centre layers were not significantly different but mean PEs for soil without zinc in the base layer (59.5) was significantly higher ($P \leq 0.01$) than for soil to which zinc was added to the base layer (36.3).

6.3.9 Water use

Water use was calculated for each treatment and the results are shown in Fig. 1 for the treatments +++, +−− and −−+.

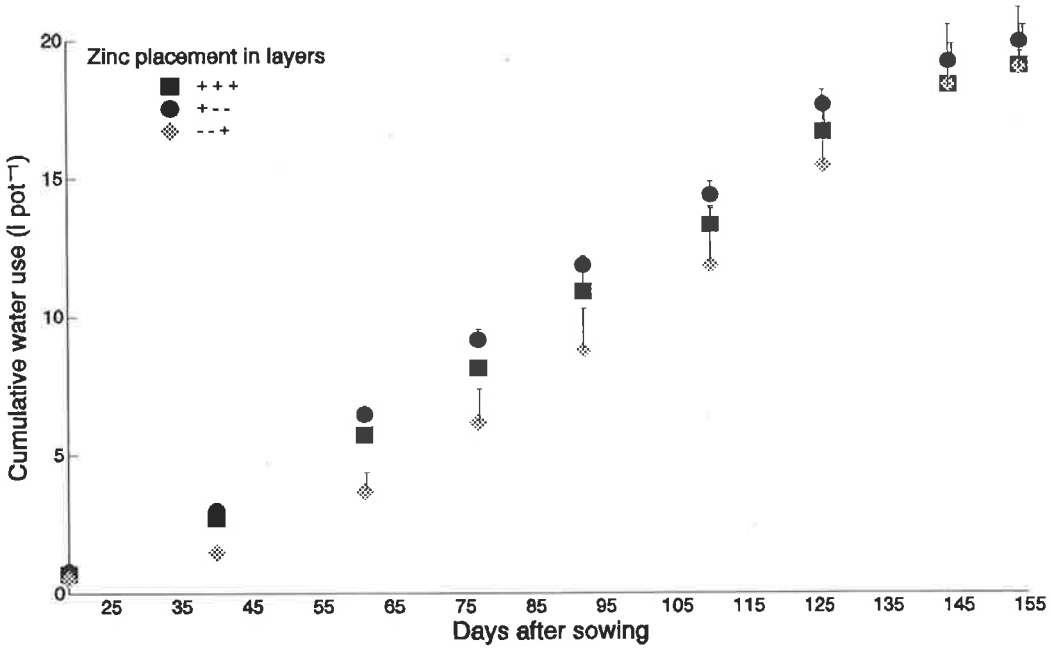

Fig 6.1 Cumulative water use (l pot^{-1}) in Excalibur wheat grown in three different combinations of zinc placement in 0.2 m layers in pots 0.6 m deep. Bars represent standard errors of the means and are not shown where they do not exceed the size of the symbol.

During the first 84 days after sowing (84 DAS), cumulative water use differed significantly between treatments. As a group, plants in pots lacking added zinc in the top layer used significantly ($P \leq 0.05$) less water (8.70 l pot^{-1}) than plants in pots where zinc was added to the top layer (10.1 l pot^{-1}). To that point, plants in the −−+ soil displayed the lowest cumulative water use (7.32 l pot^{-1}) and the +−− soil the highest (10.58 l pot^{-1}). Analysis of variance of total water use showed that total water use was not different between the treatments. However, significant values of *F* were obtained for the comparison of water use between treatments from 117 DAS to 154 DAS (Table 6.9).

Table 6.9 Water use of Excalibur wheat plants over a 37 day period approaching maturity. Values with the same letter are not significantly different.

Treatment	Water use (117 DAS - 154 DAS)
	(l pot^{-1})
+++	6.28 bcd
++−	5.43 d
+−+	6.08 cd
−++	7.12 abc
+−−	5.97 cd
−+−	7.30 ab
−−+	7.97 a
F	*

As the plants approached maturity, water use was higher in plants grown in pots lacking zinc in the top layer. The mean water use over the period for this group was 7.46 l pot^{-1} compared with 5.96 l pot^{-1} for plants grown with topsoil zinc ($P \leq 0.001$). The greatest water use over the period occurred in the −−+ soil, 34% more than in the +−− soil.

Plants grown in soil lacking added zinc in the surface 0.20 m grew more slowly and used less water in the first twelve weeks, but compensated for this by using more water during the final stages of the growing season so that total cumulative water use was similar for all treatments.

6.4 GENERAL DISCUSSION

When Excalibur wheat was grown in deep pots containing Laffer sand with various combinations of zinc placement, absence of added zinc from the top layer induced symptoms of zinc deficiency and visibly retarded growth for several weeks. Water use was significantly less in these plants up to 12 weeks after sowing and significantly

greater over the last five weeks of the growing season, so that total water use was the same for all treatments. There were no significant differences in total dry matter of tops or grain produced. However, in southern Australia where plants are likely to suffer water and temperature stress as they approach maturity, the early retardation induced by lack of zinc in the topsoil may be irrecoverable. Absence of zinc from the topsoil was also reflected in lower zinc and higher manganese concentrations in YEBs. Zinc concentrations in YEBs were significantly higher in plants grown in soil to which zinc had been added to the top two layers. It was also apparent that, as early as 26 days after sowing, plants had begun to absorb zinc from below 0.4 m.

Concentrations of zinc in flag leaves increased with the total quantity of zinc added to the soil, with the lowest concentrations in plants grown in pots with a single Zn+ layer. While zinc placement had no effect on concentrations in flag leaves from plants in pots with two Zn+ layers, plants grown in $--+$ pots had significantly higher concentrations than plants from $+--$ pots. Zinc and manganese in flag leaves were negatively correlated to a highly significant degree and changes in zinc concentrations between YEBs and flag leaves were the antithesis of changes in manganese concentrations with the same treatments.

While Nable and Webb (1993) measured no differences in time to 50% head emergence in Excalibur grown in $+-$ or $++$ treatments, data from the current experiment indicate that head emergence may be delayed in Excalibur as the result of a lack of zinc early in the growth period. Time to 50% head emergence was 82 days with the $+--$ treatment, 89 days with the $-+-$ treatment, and 92 days with the $--+$ treatment. Head emergence was delayed by 5 days in treatments lacking zinc in the topsoil (mean 89 days) compared with treatments with added zinc (84 days) ($P \leq 0.01$). While plants in controlled conditions may have had access to adequate water as they approached maturity, such compensation may not be possible in the field situation in southern Australia where water stress and increasing temperatures accompany ripening,

Rooting densities and root diameters differed only in the base layer where roots tended to be longer and finer with the + − − than with the other treatments. Mean rooting densities were higher and root diameters finer in the base layer when zinc was omitted. Robson and Snowball (1989) associated zinc deficiency with thickened roots in a split root glasshouse experiment, but this may apply only where the entire root zone is deficient.

The data suggest that patterns of retranslocation and seed loading of zinc and manganese differ. Developing grains are provided with nutrients from stems, roots and leaves. Because xylem transport into grains does not occur, (it is generally considered that tissues with low transpiration rates are supplied by the phloem although Longnecker and Robson (1993) suggest the possibility of symplastic transport from the xylem sap) nutrient loading into seeds appears to be largely dependent on phloem retranslocation (Rengel and Graham 1995b). However, work by Pearson and Rengel (1995a) has provided evidence that manganese is transported to the spikelet principally through the xylem. A high proportion of the manganese transported to the spikelet accumulates in the palea, lemma and glumes which have their own vascular system and high transpiration rates (Pearson and Rengel 1994, Pearson *et al.* 1995), but only a relatively small amount of this manganese is retranslocated to the grain. The precise pathway of manganese loading into the grain is as yet unknown. Pearson *et al.* 1995) have suggested that zinc, on the other hand, may enter grain via phloem pathways used by macronutrients. Nutrient concentrations in chaff were not measured in the current experiment.

Zinc concentrations in flag leaves were significantly different between treatments but there were no differences between treatments in terms of concentrations of zinc in straw. Concentrations of zinc in flag leaves and grain were highly correlated ($r = 0.87$ ***) and the relationship may provide a useful prognostic tool for predicting zinc in grain. Concentrations of manganese in flag leaves were poorly correlated with those in grain. Pearson and Rengel (1995b) have shown that while zinc taken up by roots may be distributed to leaves during grain development, little if any manganese is transported to leaves during this phase. Manganese is immobile from wheat leaves

but can be stored in the stem for remobilisation in the xylem (Pearson and Rengel 1994). Overall, the mean zinc concentration in straw was 27% of the mean flag leaf concentration. In contrast, mean manganese concentrations in straw were 47% of those in flag leaves, and manganese concentrations in straw differed significantly between treatments. While zinc concentrations in grain differed widely between treatments, manganese concentrations were relatively uniform. These data indicate that the mobilisation of zinc from shoots into grain may be higher than for manganese. Zinc retranslocation varies with the supply of zinc to the plant and zinc concentrations decrease more rapidly from leaves in plants deficient in zinc than from adequate plants (Pearson and Rengel 1995a). While plants with some treatments in the current experiment exhibited symptoms of zinc deficiency in the early stages of growth, all treatments appeared to have supplied adequate zinc in terms of there being sufficient to produce dry matter and grain.

Nable and Webb (1993) reported that concentrations of zinc in grain were halved in both Excalibur and Gatcher when the plants were grown in soil with little zinc in the subsoil compared with plants grown in pots where zinc was added to the entire soil. In the current experiment, zinc concentrations in grain from the $+--$ soil were 47% of those from the $+++$ soil.

Zinc concentration in grain was highest where the entire soil was supplied with zinc, but where one or two layers were lacking zinc, zinc concentrations increased with deeper placement. Indeed, mean zinc concentrations in grain were most strongly influenced by the zinc status of the base layer. Mean zinc concentrations in grain were 52% higher where zinc was added to the base layer compared with grain grown in soil where the base layer was zinc-deficient. Similar results applied to zinc uptake in the grain which was significantly greater in soil containing added zinc in the base layer. Zinc placement in the top or centre layers had no overall effect on grain concentration or uptake of zinc in grain. The same results apply if the $+++$ treatment is excluded from the data analyses.

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Ion absorption and transport vary along the length of roots and are subject to variation in structural and physiological conditions (Lüttge 1983). For instance, calcium absorption in the roots of young maize seedlings was shown by Marschner and Richter (1973) to be highest in the region of the root tip, possibly because of preferential adsorption of polyvalent ions into the mucigel (Luttge 1983). Ferguson and Clarkson (1976) showed that suberisation of hypodermal cells in roots greatly reduced phosphorus uptake in maize and Clarkson *et al.* (1978) demonstrated that suberised layers in the outer cortex of roots of *Carex arenia* also strongly reduced the uptake of phosphorus and calcium.

It may be argued that if zinc uptake is similarly affected, the higher proportion of young roots, with which are associated the most active absorption sites, are deeper in the soil, (see for example Taylor and Klepper 1973), then it is from this area where zinc would be most easily absorbed as grain filling and maturity approach. However, rooting densities may be closely associated with the number of root apices (Lungley 1973) and in the −−+ soil, rooting density was significantly lower than in the +−− soil in the base layer. It is possible that the differences in zinc concentrations in grain are due to redistribution of zinc within the plant as a function of a temporal variation in supply. While it appears that stems and leaves constitute an important source for the loading of seed with zinc, a constant or increasing supply of zinc may be necessary to maintain zinc concentrations and hence cell membrane integrity in maturing plant parts. This is unlikely to occur in the field environment of much of southern Australia where adequate concentrations of available zinc are confined to the top 0.05 m of the soil and pH increases and zinc availability decreases with depth.

The supply of zinc later in the season may be maintained by foliar application but results have been inconclusive in dicotyledons at least (Longnecker and Robson 1993) and the problem of maintaining root function remains. Certainly, more investigation is needed in this area.

It may be concluded that, given the value of zinc efficiency in Excalibur in terms of grain production, optimum zinc concentrations in grain depend on a supply of adequate zinc throughout the root zone. It can be expected that for the majority of other wheat cultivars which do not display a high degree of zinc efficiency, the need for an adequate supply of zinc throughout the root zone will be of greater importance. Increasing the depth of placement in the field to any degree above the standard 0.05m is likely to have a beneficial effect on zinc in grain.

7.0 GENERAL DISCUSSION

This thesis has addressed several aspects of subsoil infertility with particular emphasis on zinc, an area in which the literature is not well served. In the field experiments described, benefits resulting from subsoil placement of nitrogen, phosphorus and zinc fertilizers in the field included significant increases in grain yield of wheat. Wheat yields with the NPZS treatment alone consistently and significantly outyielded the non ripped control in each of the years 1993, 1994 and 1995. Similarly, significantly higher zinc concentrations were measured in the grain over the same period with the NPZS treatment compared with the non ripped control, while zinc uptake by grain was significantly greater with the NPZS treatment than with all other treatments. In the wheat experiments described, grain water use efficiency was measured in 1993 and 1994 and at both sites only the NPZS wheat was produced with significantly greater water use efficiency compared with the non ripped control in both seasons. The results are of particular interest, given the confounding effects of drought in 1994 and the variance associated with soil disturbance induced by the deep ripping tynes.

In the context of the Australian research which has been published, the term "deep placement" often refers to the practise of placing fertilizer a few centimetres below the seed (*e.g.* Slattery and Rainbow 1995). The machine used in the field experiments described in this thesis was designed to apply liquid fertilizers to a depth of 0.4 m and to allow mixing with the soil as a result of soil flow around the ripping tynes. However, with ripper tyne spacings of 0.45 m, nutrients would not be well mixed through the soil. The development of techniques for more complete mixing to a given depth may provide even greater responses to subsoil fertilization.

The greatest difference in zinc concentrations in YEBs from the field experiments occurred between plants in plots provided with added zinc and those grown where zinc was not added. Zinc concentrations were, however, significantly increased in wheat YEBs where zinc was applied with added NP fertilizer compared with plants receiving zinc alone. This was the case whether the NP fertilizer was applied

separately to the topsoil or when applied in liquid to the subsoil and the difference remained into the third season.

In terms of zinc concentrations and uptake in grain, the data support the contention of Welch and House (1984) that the zinc content of food crops can be most efficiently increased by applying zinc in an available form, possibly in excess of those rates required for maximum yields. Moreover, the current data also suggest that (in the alkaline soils under study at least) for a given application rate of zinc fertilizer, zinc concentration in grain can be efficiently increased by application with NP fertilizer to the subsoil, and that the effect may extend well beyond the year of application. For instance, at the S4 site in 1995, the second year after application of the fertilizer, zinc concentrations in and uptake by grain were significantly higher with subsoil treatments to which zinc was applied than with corresponding topsoil treatments. This occurred despite the fact that zinc was delivered to the subsoil via tynes spaced 0.45 m apart and to the topsoil via tynes 0.18 m apart.

The most effective of the subsoil treatments however was the application of a single mixture of zinc sulphate, MAP and ammonium nitrate (NPZS). Ghosh (1990) suggested that the reaction of zinc with MAP will normally yield insoluble zinc phosphates. However, there was no evidence of negative effects of insoluble reaction products in these experiments.

Lehr (1972) suggested that in general, mixing zinc with NP solutions will produce poorly soluble zinc ammonium phosphates. Reaction products which do form as a result of dissolving zinc with MAP and ammonium nitrate will be the result of complex ionic equilibria and solubility product relationships. Mortvedt (1991) indicated that reaction products are likely to be $ZnNH_4PO_4$ and $Zn(NH_4)_2P_2O_7.H_2O$ at pH greater than 6, the predominant end product depending on the proportion of phosphorus present as pyrophosphate in the solution. However, there is evidence that insoluble forms of zinc can provide effective fertilizers when mixed thoroughly with the soil. For instance, Brown and Krantz (1966) reported that zinc ammonium phosphate was "very satisfactory" as a fertilizer if mixed thoroughly with the soil.

Although hopeite ($Zn_3(PO_4)_2.4H_2O$) is relatively insoluble, it was as effective as zincite (ZnO), smithsonite ($ZnCO_3$) and zinc sulphate ($ZnSO_4.7H_2O$) as a source of zinc for sorghum when mixed with soil (Boawn *et al.* 1957). Suspensions of relatively insoluble sources of zinc, when mixed thoroughly with the soil, may be as effective as more soluble sources. In the current experiment, the mixture of zinc sulphate, MAP and ammonium nitrate applied to the subsoil was more effective in increasing zinc concentrations in grain than when the zinc sulphate was applied separately. It is possible that the formation of a relatively insoluble source of zinc may have produced a form of "slow release" fertilizer which provides a sustained release of zinc, even in a highly alkaline soil environment, into the soil solution over a period of years. It remains to be determined however, whether the mixture used would be as effective if applied in solution to the topsoil. The data indicate that for the S4 site in 1995, separate subsoil placement of zinc and NP fertilizer increased grain yields and zinc concentrations in grain significantly above topsoil placement. It follows then that the subsoil placement of the NP-Zn mixture (NPZS) would provide the most effective immediate and long term use of these fertilizers. Because of the general absence of published work in this area, the data establish a basis for further investigation.

The thesis also considers options available to address the problem of subsoil infertility other than fertilization, which may not be economically feasible to depths below 0.4 m at least. The concept of zinc efficiency in cereals assumes considerable importance for growth in soils with subsoils of low zinc status. The possibility of efficient species extending roots more effectively into the subsoil and mobilising zinc for their own use and for other crops which follow is intriguing. Of the species grown in the experiment described in Chapter 4, *L. pilosus* was the most zinc-efficient and *H. leporinum* the least. The zinc-efficiency of *B. juncea* was not determined but its ability to produce a greater amount of dry weight of shoots in soil of low zinc status was significantly greater than all other species grown. Grewal *et al.* (1996) have reported the results of testing a range of genotypes of canola (*B. napus*) and *B. juncea* for zinc efficiency. The *B. juncea* genotype Pusa Bold, which was used in the experiment described in

Chapter 4, was the most zinc-efficient in terms of dry matter production of roots and shoots at six weeks of age.

The data also indicate that the widely grown *M. truncatula* cultivar Parabinga demonstrated a low zinc efficiency in comparison with the other legumes grown. Parabinga also displayed extremely high concentrations of boron in zinc-deficient tissue. Parabinga has been described as moderately sensitive to boron toxicity - the tolerance of a cultivar is a function of its ability to control the uptake of excessive boron (Paull 1990). There appear to be no published data relating zinc efficiency to boron tolerance in *Medicago* species although there is a need for investigation in this area because of the key role of medic pastures in the farming systems of southern Australia. It is possible that zinc inefficiency in Parabinga may decrease its ability to control the uptake of excessive boron.

Large seeded grain legumes with a high content of zinc in the seed displayed an inherent advantage over the other species (apart from *B. juncea*) in terms of their abilities to produce dry matter early in the growing season in zinc-deficient soil. The data indicate that Durati wheat harvested three weeks after sowing produced significantly more dry weight of shoots when following *P. sativum* than other species apart from *L. pilosus*. It is postulated that enhanced growth of Durati following *P. sativum* may be due to a slight increase in available zinc. The mechanism responsible for this increase is unknown. Such an increase did not occur when Excalibur was grown following Durati. There was, however, a significant increase in zinc uptake in Excalibur wheat grown after legumes as a group than after the *Poaceae* or *B. juncea*. King *et al.* (1992) reported from field studies that cereals grown after grain legumes absorbed more zinc (and other nutrients) than when grown after grassy pasture. Given the strong effect of seed zinc concentration on growth of wheat in soil of low zinc status (Rengel and Graham 1995a,b) a small increment in available zinc in the soil at the beginning of the season is likely to have a significant effect on early growth. With a generally low zinc status in southern Australian subsoils, a small increase in available zinc in soil following *P. sativum* crops compared with *M. truncatula* pastures may be sufficient to benefit following wheat crops. The data also indicate a

suppression of zinc uptake in Excalibur wheat grown after *B. juncea* and *H. leporinum* in Zn- soil. In soil to which zinc was added, uptakes of several nutrients were also depressed following *H. leporinum*. It is open to speculation whether these differences are due to allelochemicals produced from the breakdown of roots. In the case of *H. leporinum*, cold water extracts from shoots have been shown to have had detrimental effects on the growth of *M. truncatula*. Similarly, it has been reported that breakdown products in stems and roots of *B. juncea* are toxic to fungi and soil nematodes (Kirkegaard *et al.* 1993). It is also possible that the suppressed nutrient uptake in wheat grown after *H. leporinum* is due in some degree to the lack of breakdown of roots, since at harvest there was clearly a large amount of root material remaining from the first year.

Durati appears to have a higher critical concentration for zinc than Excalibur and in hindsight proved to be a poor choice for the second year of the experiment because of its extreme sensitivity to zinc deficiency. Both Durati and Excalibur exhibited a similar degree of leaf necrosis and collapse at harvest, with Durati having a mean zinc concentration of 12.7 mg Zn kg ha^{-1} and Excalibur 3.4 mg Zn kg^{-1} Cakmak *et al.* (1994) reported the earlier appearance and greater severity of symptoms of zinc deficiency in Durati than in the zinc-efficient cultivar Aroona at the same zinc concentrations in shoots: it was concluded that the lower zinc-efficiency of Durati was due to a lower efficiency of zinc utilization. Caution is required in extrapolating results of a pot experiment conducted in sand to field conditions as the data are not directly applicable. Nevertheless, the results of the pot studies have provided several avenues for further investigation. Evidence has been provided that the reported improvement in the growth of wheat following field peas compared with medic pastures may be due in part to an increase in zinc uptake. More detailed field investigations are warranted. There exists also a strong case for selection of pasture species for zinc efficiency traits. Further investigation into the mechanisms of zinc uptake of the *B. juncea* cultivar Pusa Bold is also worthy of consideration.

The relative abilities of zinc-efficient (Excalibur) and zinc-inefficient (Gatcher) wheat cultivars to produce grain and roots in an alkaline subsoil with low zinc status were

assessed. When nutrients other than zinc were supplied, the zinc efficiency of Excalibur was clearly evident in terms of grain production and other yield parameters. Excalibur appeared to have a more efficient uptake mechanism for zinc than Gatcher in soil of low zinc status, since both cultivars took up the same quantity of zinc in tops (straw + grain) while Excalibur had a root system of about half the total length of Gatcher, possibly due to the greater release of phytometallophores in response to zinc deficiency (Cakmak *et al.* 1994). However, Excalibur also exhibited a greater internal efficiency for allocating zinc to grain formation. The data also indicate differences between the two cultivars with respect to manganese loading in grain. Possibly the higher manganese concentrations in Gatcher grain are a product of its more extensive root system, since roots act as a storage organ for manganese until grain formation (Pearson and Rengel 1995b). The superior zinc efficiency of Excalibur was not expressed in terms of grain yield in untreated subsoil although water use efficiency was superior in terms of grain yield and total weight of dry matter produced. The major effect on the production of shoots, grain and roots, however, was due to the addition of nutrients other than zinc. In both cultivars, grain yield was increased more than 20 times by the addition of complete nutrients, including zinc, in comparison with the untreated soil. However, the effects of fertilizing different portions of the root zone and the effects of combinations of nutrients and soil types remain the object of future investigations. The data produced in the present study indicate that such investigations will be worthwhile.

Both pot and field experiments have demonstrated the effects of zinc placement on zinc concentration in grain. In the pot experiment described in Chapter 6, plants grown in soil with all three layers containing added zinc produced the highest concentrations of zinc in grain of the zinc-efficient cultivar Excalibur, significantly higher than where just one layer was treated. However, where zinc was added to a single layer, highest concentrations in the grain occurred where zinc was placed in the bottom layer. It is not clear whether this is due to differences in root geometry or to redistribution of zinc within the plant due to temporal variation in supply. Further research is needed to identify the mechanisms involved.

7.1 Results in terms of meeting original objectives

The original objectives of the thesis were outlined in the Introduction (Chapter 1). Because of the limited reports in the literature with respect to subsoil fertilization, the objectives of the field work described in Chapter 3 were to compare the effects of placement of zinc, nitrogen and phosphorus fertilizers into the subsoil and the topsoil using the most practicable means currently available. Despite the confounding effects of drought and soil disturbance induced by the ripping tynes, the evidence available from the field experiments indicates substantial benefits in terms of grain yield, water use efficiency and zinc uptake by grain as a result of treating the subsoil with NP and zinc fertilizers. There exists a strong case for more detailed investigation of application methods, depth of application and forms and combinations of various fertilizers.

The rotation experiment described in Chapter 4 was established to measure the zinc efficiencies in terms of dry weight of shoots of various species in Laffer sand and to measure their relative root growth abilities. Large differences in zinc efficiency and root growth were recorded. It was shown that zinc content in the seed was an important contributor to the ability of species to survive in conditions of extreme zinc deficiency. *B. juncea* cv. Pusa Bold was shown to have a high ability to mobilise zinc from zinc-deficient sand, but there was no evidence that this ability was of benefit to wheat plants grown in the following season. Indeed, the primary objective of the experiment was to identify whether differences in the abilities of various species to mobilise zinc would be of benefit to wheat plants grown in rotation. The data indicate that wheat grown after *P. sativum* in zinc-deficient sand had access to more available zinc than did plants grown after *M. truncatula*. There was also some evidence that the uptake of several nutrients grown after *H. leporinum* is inhibited by an unidentified mechanism. Because crop and pasture rotations form an important part of agriculture in southern Australia, the results do have implications for farming systems.

In addressing the question of whether or not root morphology plays a major part in determining zinc efficiency in wheat, it was shown that in fact, the efficient cultivar Excalibur had a root system which was considerably less extensive than that of the

inefficient cultivar Gatcher. The data support the contention of Graham and Rengel (1993) that genetic factors responsible for zinc efficiency are likely to be additive. In comparison with Gatcher, Excalibur displayed a more efficient uptake mechanism for zinc, and also a higher degree of internal efficiency for allocating zinc to grain production.

The objective of the final experiment was to examine the relationship between zinc placement and zinc uptake and its allocation to grain in Excalibur. High zinc concentrations in grain are important from the point of view of human nutrition and for seedling vigour in plants. Although Excalibur displays a high degree of zinc efficiency, the data from Chapter 6 indicate that zinc is required throughout the root zone to maximise zinc concentrations in grain. In terms of extrapolation to the field, the problem of enhancing zinc concentration in southern Australia, where topsoil drying is a common occurrence as crop maturity approaches can best be addressed by subsoil placement of zinc.

Taken together, the data indicate that the placement of zinc, nitrogen and phosphorus (and other nutrients where they are likely to be deficient), to a depth defined by cost and practicality but certainly below 0.05 m, promise to enhance cereal production in areas where subsoils are infertile. While zinc efficiency is of obvious importance, the extension of the concept of nutrient efficiency to other micro and macronutrients which also limit growth because of their unavailability in the subsoil and combining them in single genotypes is a challenge for the future. In a world where cereal grains are an increasingly important component of the human diet, the effective enhancement of the micronutrient concentration in grain is worthy of more detailed study. Indeed, understanding the role of subsoils in plant nutrition is likely to be a key area of research for the next decade or so.

APPENDIX

Table: A3.1 Minnipa Research Centre: typical soil profile representing the experimental sites in fields N6 and S4.

Soil classification: Calcixerollic Xerochrept: fine silty, mixed, thermic (Soil Taxonomy - Soil Survey Staff 1975).

Horizons	**Texture**	**pH**	**pH**	**$CaCO_3$**
(m)	(field)	(1:5 soil:water)	(0.01M $CaCl_2$) (1:5)	(%)
0.00 - 0.07 Ap	loam, fine sandy	8.3	7.7	5
0.07 - 0.18 A	loam	8.6	7.9	9
0.18 - 0.43 Btk	clay loam	8.8	8.0	9
0.43 - 0.75 Bk1	clay loam	9.4	8.5	28
0.75 - 1.50 Bk2	light clay	9.4	8.6	41

Horizons Depth	**Na**	**Exchangeable K**	**Cations Ca**	**Mg**	**Cation exchange capacity**
(m)			(mmol charge kg^{-1})		
0.00 - 0.07	2.0	25.0	410	27.0	176
0.07 - 0.18	3.3	24.0	680	35.0	193
0.18 - 0.43	23.0	6.4	690	71.0	178
0.43 - 0.75	36.0	10.0	620	130.0	163
0.75 - 1.50	49.0	6.7	620	150.0	163

Horizons	EC_{e25}	Exchangeable sodium percentage	Calcium chloride extractable boron	DTPA extractable zinc	Water saturation percentage
(m)	dS m^{-1}	(%)	mg kg^{-1}	mg kg^{-1}	(%)
0.00-0.07	1.4	1	3.2	0.27	28
0.07-0.18	1.1	2	3.0	0.27	30
0.18-0.43	3.4	13	3.7	0.38	30
0.43-0.75	11.6	22	12.5	0.49	51
0.75-1.50	9.7	30	22.0	0.22	71

Table A3.2 Concentrations of copper, manganese, iron, boron and sodium in YEBs of Machete wheat shoots at Feekes 4 as a function of fertilizer placement and ripping treatments in field N6, Minnipa 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Cu	Mn	Fe	B	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
NPZT	8.1 e	80 cde	81 b	10.1	155 ab
NPZS	8.8 b	87 bcd	80 b	12.4	178 a
NPT	8.4 bc	92 bc	85 b	12.0	133 bc
NPS	9.3 a	114 a	86 ab	15.3	136 bc
ZT	8.3 bc	78 de	80 b	11.1	143 bc
ZS	8.1 c	72 e	85 b	10.6	129 e
C	8.1 c	89 bcd	82 b	11.8	129 c
RC	8.6 b	97 b	92 a	12.3	121 c
F	***	***	*	ns	**

Table A3.3 Concentrations of magnesium, calcium, sulphur and potassium in YEBs of Machete wheat shoots at Feekes 4 as a function of fertilizer placement and ripping treatments in field N6, Minnipa 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Mg[A]	Ca	S	K
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
NPZT	1.46 cd	3.39	3.97 bc	44.3 a
NPZS	1.59 bc	3.43	3.97 bc	44.3 a
NPT	1.67 ab	3.42	3.97 bc	44.8 a
NPS	1.85 a	2.47	4.20 ab	45.5 a
ZT	1.45 cd	3.15	3.91 cd	43.7 a
ZS	1.43 d	3.23	3.91 cd	44.3 a
C	1.67 a	3.63	3.68 d	40.4 b
RC	1.80 a	3.19	4.24 a	44.5 a
F	***	ns	**	*

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A3.4 Dry weight of above ground plant parts (tops) of Machete wheat at crop maturity as a function of fertilizer placement and ripping treatments in field N6, Minnipa 1993. Values with the same letter are not significantly different ($P \leq 0.05$).

Treatment	**Dry weight of tops at crop maturity 1993**
	(kg ha^{-1})
NPZT	4 231 bc
NPZS	5 044 a
NPT	4 418 ab
NPS	4 091 bc
ZT	3 718 bc
ZS	3 508 c
C	3 950 bc
RC	4 195 bc
F	*

Table A3.5 Water loss to the depth of maximum rooting between sowing and crop maturity as a function of fertilizer placement and ripping treatments in field N6, Minnipa 1993. Values labelled with the same letter are not significantly different ($P \leq 0.05$).

Treatment	**Water loss**
	(mm)
NPZT	216 ab
NPZS	198 c
NPT	220 a
NPS	212 ab
ZT	216 ab
ZS	209 bc
C	211 ab
RC	216 ab
F	**

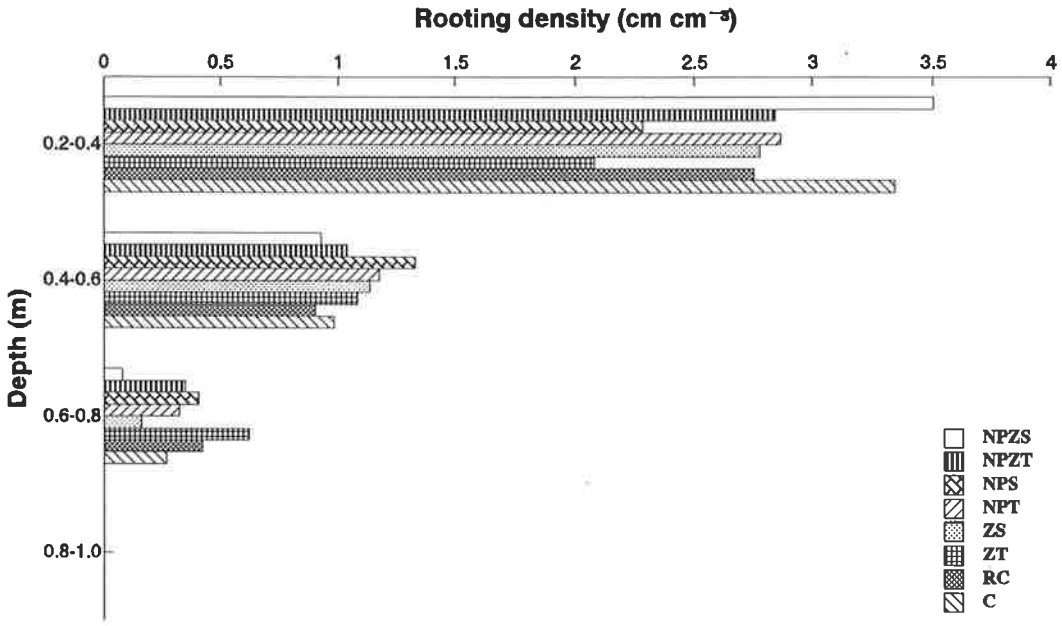

Fig A3.1 Rooting density (cm cm^{-3}) at crop maturity as a function of depth, fertilizer placement and ripping treatments in field N6, Minnipa, 1993.

Table A3.6 Mean root diameter (RD) and proportion of fine roots (% fine) of total roots <0.3 mm diameter as a function of depth, fertilizer placement and ripping treatments in field N6, Minnipa 1993.

Treatment	0.2 - 0.4 m		0.4 - 0.6 m		0.6 - 0.8 m	
	RD	%fine	RD	%fine	RD	%fine
	(mm)	(%)	(mm)	(%)	(mm)	(%)
NPZT	0.39	77	0.30	87	0.30	86
NPZS	0.41	75	0.31	85	0.31	88
NPT	0.40	75	0.30	86	0.30	83
NPS	0.37	75	0.36	80	0.36	87
ZT	0.36	81	0.34	83	0.34	90
ZS	0.36	80	0.33	84	0.33	86
C	0.35	81	0.35	82	0.35	88
RC	0.36	80	0.32	84	0.32	86
F	ns	ns	ns	ns	ns	ns

Table A3.7 Gravimetric water content as a function of depth, fertilizer placement and ripping treatments at crop maturity in field N6, Minnipa 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	0.0 - 0.2 m	0.2 - 0.4 m	0.4 - 0.6m[A]	0.6 - 0.8 m	0.8 - 1.0 m
	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})
NPZT	0.082	0.107	0.112 b	0.127	0.127
NPZS	0.091	0.106	0.131 a	0.134	0.127
NPT	0.091	0.096	0.105 b	0.117	0.131
NPS	0.082	0.095	0.107 b	0.123	0.130
ZT	0.092	0.101	0.107 b	0.122	0.124
ZS	0.093	0.090	0.108 b	0.128	0.131
C	0.093	0.099	0.103 b	0.123	0.132
RC	0.093	0.097	0.095 b	0.122	0.131
F	ns	ns	*	ns	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A3.8 Hundred grain weight of Machete wheat grain (1993) and Stirling barley grain (1994) as a function of fertilizer placement and ripping treatments in field N6, Minnipa.

Treatment	N6 1993 Hundred grain weight	N6 1994 Hundred grain weight
	(g)	(g)
NPZT	4.32	2.44
NPZS	4.16	2.61
NPT	4.37	2.49
NPS	4.33	2.38
ZT	4.30	2.48
ZS	4.37	2.52
C	4.35	2.56
RC	4.39	2.49
F	ns	ns

Table A3.9 Concentrations of copper, manganese, iron and sodium in Machete wheat grain as a function of fertilizer placement and ripping treatments in field N6, Minnipa 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Cu	Mn	Fe	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
NPZT	5.4 c	52.3 b	11.6	48.3
NPZS	5.1 c	52.3 b	18.5	40.8
NPT	6.1 b	51.8 ab	11.5	40.3
NPS	7.0 a	67.8 a	10.6	40.8
ZT	5.9 b	55.5 b	10.5	41.0
ZS	5.5 bc	52.5 b	14.7	40.5
C	6.1 b	55.3 b	13.9	42.5
RC	6.0 b	54.3 b	11.6	39.3
F	**	**	ns	ns

Table A3.10 Concentrations of magnesium, calcium, sulphur and potassium in Machete wheat grain as a function of fertilizer placement and ripping treatments in field N6, Minnipa 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Mg	Ca	S	K
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
NPZT	1.32 c	0.45 a	1.69	3.97
NPZS	1.30 c	0.43 ab	1.70	3.91
NPT	1.34 bc	0.36 d	1.64	3.88
NPS	1.44 a	0.38 cd	1.73	4.14
ZT	1.34 bc	0.39 cd	1.66	4.00
ZS	1.32 c	0.41 abc	1.69	3.88
C	1.40 ab	0.40 bc	1.68	4.03
RC	1.36 bc	0.39 cd	1.67	4.03
F	*	**	ns	ns

Table A3.11 Gravimetric soil water content at sowing in field N6, Minnipa 1994, as a function of depth and fertilizer placement and ripping treatments applied in 1993.

Treatment	Gravimetric water content				
	0.0-0.2 m	**0.2-0.4 m**	**0.4-0.6 m**	**0.6-0.8 m**	**0.8-1.0 m**
	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})
NPZT	0.140	0.092	0.111	0.123	0.122
NPZS	0.143	0.081	0.107	0.126	0.125
NPT	0.135	0.089	0.112	0.127	0.123
NPS	0.135	0.088	0.105	0.116	0.122
ZT	0.139	0.084	0.089	0.150	0.149
ZS	0.136	0.088	0.108	0.130	0.125
C	0.135	0.085	0.106	0.126	0.124
RC	0.135	0.085	0.106	0.124	0.126
F	ns	ns	ns	ns	ns

Table A3.12 Concentrations of copper, manganese, boron, iron and sodium in YEBs of Stirling barley at Feekes 5 in field N6, Minnipa 1994, as a function of fertilizer placement and ripping treatments applied in 1993.

Treatment	Cu	Mn	B	Fe	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(g kg^{-1})
NPZT	9.1	50.5	8.0	63.0	2.53
NPZS	8.8	50.8	7.7	61.0	2.42
NPT	8.9	49.5	7.6	66.8	2.20
NPS	8.7	48.8	7.8	65.5	2.32
ZT	8.7	49.8	8.4	61.5	2.47
ZS	8.6	47.5	8.0	60.5	2.56
C	8.2	46.8	8.2	64.5	2.56
RC	9.0	50.8	8.0	65.5	2.18
F	ns	ns	ns	ns	ns

Table A3.13 Concentrations of magnesium, calcium, sulphur and potassium in YEBs of Stirling barley at Feekes 5 in field N6, Minnipa 1994, as a function of fertilizer placement and ripping treatments applied in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Mg	Ca	S	K
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
NPZT	1.91 bc	7.00	3.51	39.8
NPZS	1.94 abc	6.75	3.39	38.2
NPT	2.05 a	6.99	3.57	41.2
NPS	1.98 ab	6.93	3.55	40.9
ZT	1.87 bc	6.52	3.26	38.4
ZS	1.81 c	6.58	3.30	38.0
C	2.05 a	6.54	3.30	39.6
RC	2.00 ab	6.94	3.57	40.0
F	*	ns	ns	ns

Table A3.14 Dry weight of shoots at Feekes 8 and of total above ground plant parts (tops) at crop maturity of Stirling barley in field N6, Minnipa 1994, as a function of fertilizer placement and ripping treatments applied in 1993.

Treatment	Dry weight of shoots at Feekes 8, 1994	Dry weight of tops at crop maturity 1994
	(kg ha^{-1})	(kg ha^{-1})
NPZT	1 368	1 883
NPZS	1 403	2 395
NPT	1 288	2 226
NPS	1 257	2 361
ZT	1 392	2 329
ZS	1 285	1 856
C	1 260	2 418
RC	1 358	2 225
F	ns	ns

Table A3.15 Gravimetric water content at crop maturity in field N6, Minnipa 1994, as a function of depth and fertilizer placement and ripping treatments applied in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

	Gravimetric water content				
Treatment	**0.0-0.2 m**	**0.2-0.4 m**	**0.4-0.6 m**	**0.6-0.8 m**	**0.8-1.0 m**
	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})
NPZT	0.069	0.094	0.112 ab	0.121	0.144
NPZS	0.066	0.093	0.111 ab	0.119	0.129
NPT	0.068	0.092	0.114 ab	0.121	0.134
NPS	0.067	0.088	0.109 abc	0.121	0.128
ZT	0.066	0.087	0.109 c	0.115	0.129
ZS	0.069	0.094	0.114 ab	0.128	0.131
C	0.069	0.095	0.117 a	0.123	0.143
RC	0.068	0.083	0.106 bc	0.119	0.138
F	ns	ns	*	ns	ns

Table A3.16 Concentrations of copper, manganese, iron and sodium in Stirling barley grain in field N6, Minnipa 1994, as a function of fertilizer placement and ripping treatments applied in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Cu	Mn	Fe	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
NPZT	7.4 a	20.6	41.5	250
NPZS	6.8 b	19.7	23.8	280
NPT	6.2 c	21.1	34.3	270
NPS	6.1 c	20.3	23.5	270
ZT	7.2 ab	20.9	29.5	280
ZS	6.9 ab	21.8	36.0	300
C	5.8 d	18.4	34.0	270
RC	6.3 c	19.5	31.0	270
F	***	ns	ns	ns

Table A3.17 Concentrations of magnesium, calcium, sulphur and potassium in Stirling barley grain in field N6, Minnipa 1994, as a function of fertilizer placement and ripping treatments applied in 1993.

Treatment	Mg	Ca	S	K
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
NPZT	1.09	0.55	2.14	6.55
NPZS	1.06	0.50	2.01	5.57
NPT	1.09	0.53	2.03	5.93
NPS	1.07	0.52	1.96	6.19
ZT	1.10	0.52	2.09	6.16
ZS	1.07	0.54	2.07	5.88
C	1.12	0.50	1.98	5.68
RC	1.06	0.51	2.01	6.10
F	ns	ns	ns	ns

Table A3.18 Concentrations of copper, manganese, iron, boron and sodium in YEBs of Machete wheat at Feekes 5 as a function of fertilizer placement and ripping treatments in field S4, Minnipa 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Cu	Mn[A]	Fe	B	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
NPHZT	7.1	105 [z]	124 bc	10.7	346
NPHZS	6.9	95 de	120 c	11.4	320
NPZT	6.9	101 bcde	124 bc	10.9	334
NPZSS	6.6	95 e	118 c	9.7	332
NPZS	6.9	97 de	118 c	10.8	331
NPT	6.8	107 ab	138 a	10.3	392
NPS	6.9	109 a	143 a	10.5	401
NPHT	6.8	111 [z]	132 ab	10.2	357
NPHS	6.8	104 abc	122 bc	10.3	340
C	6.9	103 abcd	138 a	9.4	339
RC	6.8	96 de	124 bc	10.0	364
F	ns	**	***	ns	*

[A] Analysis of variance performed on $\log_e$ - transformed data.

[z] Analysis of variance performed without these treatments due to non additivity which could not be corrected by data transformation.

Table A3.19 Concentrations of magnesium, calcium, sulphur and potassium in YEBs of Machete wheat at Feekes 5 as a function of fertilizer placement and ripping treatments in field S4, Minnipa 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Mg	Ca	S[A]	K
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
NPHZT	1.84 de	4.44 ab	4.72 abc	45.1 e
NPHZS	1.76 e	3.84 d	4.78 ab	45.8 de
NPZT	1.83 de	4.35 bc	4.79 ab	47.0 bcd
NPZSS	1.76 e	3.92 d	4.75 ab	46.5 cde
NPZS	1.79 de	3.91 d	4.67 bcd	47.0 bcd
NPT	2.00 b	4.59 ab	4.56 d	47.8 bc
NPS	2.16 a	4.54 ab	4.66 bcd	48.2 b
NPHT	2.08 b	4.79 a	4.87 a	47.8 bc
NPHS	1.99 bc	3.97 cd	4.60 cd	47.2 bcd
C	1.96 bc	4.81 a	4.57 d	47.1 bcd
RC	1.88 cd	3.91 d	4.53 d	50.6 a
F	***	***	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A3.20 Rooting density in Machete wheat plots at depth interval of 0.4 - 0.6 m as a function of fertilizer placement and ripping treatments at crop maturity in field S4, Minnipa 1994.

Treatment	**Rooting density 0.4-0.6 m**
	(cm cm^{-3})
NPHZT	0.20
NPHZS	0.17
NPZT	0.17
NPZSS	0.18
NPZS	0.16
NPT	0.17
NPS	0.23
NPHT	0.14
NPHS	0.18
C	0.18
RC	0.09
F	ns

Table: A3.21 Mean root diameter (RD) and proportion of fine roots (% fine) of total roots <0.3 mm diameter as a function of depth, fertilizer placement and ripping treatments in field S4, Minnipa 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	0.2-0.4 m		0.4-0.6 m	
	RD[A]	% fine	RD	% fine
	(mm)	(%)	(mm)	(%)
NPHZT	0.36 abc	81 abc	0.45	68
NPHZS	0.34 bcd	84 ab	0.45	72
NPZS	0.35 abcd	82 abc	0.44	72
NPZSS	0.33 cd	85 a	0.42	74
NPT	0.33 cd	85 a	0.42	75
NPS	0.35 abcd	82 abc	0.45	73
NPHT	0.36 abc	81 abc	0.45	73
NPHS	0.37 ab	80 bc	0.50	64
C	0.31 d	85 a	0.44	71
RC	0.39 a	78 c	0.46	69
F	0.36 abc	81 abc	0.40	78
	*	*	ns	ns

[A] Analysis of variance performed on $\log_e$ - transformed data

Table A3.22 Gravimetric soil water content at crop maturity as a function of depth, fertilizer placement and ripping treatments in field S4, Minnipa, 1994.

Treatment	Gravimetric water content			
	0.0-0.2 m	**0.4-0.6 m**	**0.6-0.8 m**	**0.8-1.0 m**
	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})	(kg kg^{-1})
NPHZT	0.066	0.128	0.147	0.142
NPHZS	0.067	0.128	0.142	0.134
NPZT	0.063	0.128	0.138	0.141
NPZS	0.061	0.127	0.143	0.144
NPZSS	0.064	0.132	0.142	0.141
NPT	0.062	0.124	0.142	0.139
NPS	0.066	0.129	0.138	0.136
NPHT	0.062	0.129	0.143	0.145
NPHS	0.067	0.132	0.143	0.137
C	0.069	0.133	0.143	0.146
RC	0.069	0.140	0.145	0.133
F	ns	ns	ns	ns

Table A3.23 Concentrations of copper, manganese, iron and sodium in Machete wheat grain as a function of fertilizer placement and ripping treatments in field S4, Minnipa 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Cu[A]	Mn	Fe	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
NPHZT	3.0 cde	51.1 bcd	37.2 bcd	103
NPHZS	2.8 de	47.2 d	36.9 cd	103
NPZT	2.8 de	49.0 cd	37.0 bcd	103
NPZSS	2.7 e	48.0 d	36.6 cd	94
NPZS	3.1 bcde	49.5 cd	35.2 d	116
NPT	3.4 ab	55.0 ab	40.1 ab	107
NPS	3.1 abcd	54.8 ab	38.8 abc	109
NPHT	3.1 bcde	55.0 ab	41.1 a	75
NPHS	3.7 a	58.3 a	38.7 abc	99
C	3.3 abc	49.5 cd	36.9 cd	103
RC	3.4 ab	52.6 bc	38.7 abc	111
F	**	***	*	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A3.24 Concentrations of magnesium, calcium, sulphur and potassium in Machete wheat grain as a function of fertilizer placement and deep ripping treatments in field S4, Minnipa 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Mg	Ca	S	K
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
NPHZT	1.29 cde	0.36 abc	1.80 a	3.80 cde
NPHZS	1.31 cd	0.36 abc	1.77 a	3.96 a
NPZT	1.27 de	0.35 abcd	1.73 ab	3.67 e
NPZSS	1.31 cd	0.37 ab	1.75 ab	3.81 bcd
NPZS	1.31 bcd	0.38 a	1.75 ab	3.89 abc
NPT	1.32 bc	0.32 d	1.68 bc	3.70 de
NPS	1.36 b	0.32 d	1.68 bc	3.78 cde
NPHT	1.33 bc	0.33 cd	1.75 ab	3.76 cde
NPHS	1.42 a	0.34 bcd	1.73 ab	3.94 ab
C	1.26 e	0.35 abc	1.63 c	3.52 cde
RC	1.31 cd	0.33 cd	1.65 c	3.76 cde
F	***	**	***	***

Table A3.25 Concentrations of copper, manganese, iron, boron and sodium in YEBs of Machete wheat at Feekes 5 in field N6, Minnipa 1995, as a function of fertilizer placement and ripping treatments applied in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Cu	Mn	Fe	B	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(g kg^{-1})
NPZT	10.5	64.3 cd	98	7.8	3.56
NPZS	10.5	66.8 bc	113	9.6	3.77
NPT	10.8	66.8 bc	100	9.2	4.06
NPS	10.1	73.0 a	107	8.6	3.10
ZT	10.4	65.6 bcd	104	8.2	3.58
ZS	10.1	66.0 bc	106	8.1	3.43
C	9.9	60.8 d	109	8.1	3.49
RC	10.8	69.3 ab	109	8.0	3.56
F	ns	**	ns	ns	ns

Table A3.26 Concentrations of magnesium, calcium, sulphur and potassium in YEBs of Machete wheat at Feekes 5 in field N6, Minnipa 1995, as a function of fertilizer placement and ripping treatments applied in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Mg	Ca	S	K
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
NPZT	2.02 cd	4.60	3.94 b	41.6
NPZS	2.10 d	4.90	4.09 a	39.0
NPT	2.22 ab	5.17	3.93 b	41.6
NPS	2.08 bcd	4.28	3.99 ab	42.0
ZT	2.03 cd	4.77	3.93 bc	39.4
ZS	1.94 d	4.62	3.96 b	38.8
C	2.15 abc	4.53	3.82 c	40.1
RC	2.25 a	4.91	4.00 ab	41.4
F	**	ns	**	ns

Table A3.27 Concentrations of copper, manganese, iron, boron and sodium in YEBs of Machete wheat at Feekes 5 in field S4, Minnipa 1995, as a function of fertilizer placement and ripping treatments applied in 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1994 Treatment	**Cu**	**Mn[A]**	**Fe**	**B[A]**	**Na[A]**
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(g kg^{-1})
NPHZT	5.8 bcde	81.7 cd	163	11.6 e	0.90 e
NPHZS	6.4 a	87.2 ab	164	17.3 a	1.08 abc
NPZT	5.7 de	79.8 cd	167	13.9 bcd	0.97 bcde
NPZSS	6.1 abcd	80.7 cd	155	14.7 ab	1.08 ab
NPZS	6.1 abc	79.2 d	164	14.2 bc	1.03 abcd
NPT	5.8 bcde	82.0 cd	181	12.8 bcde	1.02 abcd
NPS	6.2 ab	86.3 ab	156	11.9 cde	0.95 de
NPHT	5.5 ef	84.2 bc	158	11.6 e	0.88 e
NPHS	5.8 cde	90.3 a	188	11.9 de	0.96 cde
C	5.2 f	73.8 e	182	13.0 bcde	1.10 a
RC	5.9 bcd	84.0 bc	198	13.7 bcd	1.10 ab
F	***	***	ns	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A3.28 Concentrations of magnesium, calcium, sulphur and potassium in YEBs of Machete wheat at Feekes 5 in field S4, Minnipa 1995, as a function of fertilizer placement and ripping treatments applied in 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1994 Treatment	Mg	Ca	S	K
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
NPHZT	1.89	3.18	3.95 ab	38.0 bcd
NPHZS	2.14	3.30	4.02 a	37.2 cd
NPZT	1.91	3.04	4.00 a	38.7 ab
NPZSS	1.94	3.26	3.96 ab	37.0 d
NPZS	1.92	3.18	3.91 abc	37.2 cd
NPT	2.18	3.14	3.89 bc	38.7 ab
NPS	2.03	3.22	3.95 ab	38.5 abc
NPHT	1.94	3.17	3.89 bc	39.4 a
NPHS	2.05	3.43	4.01 a	38.6 ab
C	1.96	3.21	3.84 c	36.7 d
RC	2.04	3.46	3.89 bc	37.6 bcd
F	ns	ns	*	**

Table A3.29 Concentrations of copper, iron and manganese in Machete wheat grain in field S4, Minnipa 1995, as a function of fertilizer placement and ripping treatments applied in 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Cu	Fe[A]	Mn[A]
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
NPHZT	3.68 bcd	18.5 b	52.4 bc
NPHZS	3.13 e	16.5 b	46.2 ef
NPZT	3.63 bcd	18.8 ab	51.1 cd
NPZSS	3.23 de	15.5 b	46.2 f
NPZS	3.55 cde	17.3 b	49.2 de
NPT	4.23 a	19.6 ab	55.6 ab
NPS	4.12 ab	24.0 a	55.3 ab
NPHT	3.97 abc	19.5 ab	53.4 bc
NPHS	4.22 a	23.8 a	58.3 a
C	3.72 bcd	17.9 b	50.9 cd
RC	3.98 abc	20.0 ab	54.2 bc
F	***	*	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A3.30 Concentrations of potassium, sodium, magnesium, calcium and sulphur in Machete wheat grain in field S4, Minnipa 1995, as a function of fertilizer placement and ripping treatments applied in 1994. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	K	Na[A]	Mg	Ca	S
	(g kg^{-1})	(mg kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
NPHZT	4.03 cde	62.3 ab	1.36 bc	0.30 ab	1.88 ab
NPHZS	4.18 bc	70.3 a	1.34 cd	0.27 a	1.85 bc
NPZT	4.04 cde	66.0 a	1.35 bcd	0.26 ab	1.85 bc
NPZSS	4.00 de	57.2 abc	1.31 d	0.26 ab	1.85 bc
NPZS	4.06 cde	73.3 a	1.35 bcd	0.27 a	1.89 ab
NPT	3.99 de	47.3 c	1.40 b	0.23 d	1.88 ab
NPS	4.23 b	47.2 c	1.47 a	0.25 bc	1.94 a
NPHT	4.13 bcd	61.2 abc	1.38 bc	0.25 bc	1.89 ab
NPHS	4.41 a	48.5 bc	1.49 a	0.26 ab	1.95 a
C	3.94 e	56.3 abc	1.37 bc	0.24 cd	1.78 c
RC	4.02 cde	50.0 bc	1.40 b	0.25 bc	1.88 ab
F	***	**	***	**	*

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A3.31 Comparison of phosphorus uptake differences in Machete wheat grain in field S4, Minnipa 1995, as affected by deep ripping and fertilizer placement treatments applied in April 1994. Values have been compared by the two sample t test.

	C	RC	NPZS	NPT	NPS	NPHT	NPHS	NPZT	NPZSS	NPHZT
C										
RC	ns									
NPZS	*	ns								
NPT	ns	ns	ns							
NPS	ns	ns	ns	ns						
NPHT	ns	ns	ns	ns	ns					
NPHS	ns	ns	*	ns	ns	ns				
NPZT	ns	ns	ns	ns	ns	ns	ns			
NPZSS	*	ns	ns	ns	ns	ns	*	ns		
NPHZT	ns	ns	ns	ns	ns	ns	ns	ns	ns	
NPHZS	ns	ns	ns	ns	ns	ns	*	ns	ns	ns

Table A4.1 Concentrations of magnesium, sulphur and iron in whole shoots (harvested in 1993 at anthesis of zinc treated plants) as a function of species and zinc treatment. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Species	Zn Treatment	Mg [A]	S [A]	Fe
		(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})
L. pilosus	Zn+	5.85 c	3.50 ef	58
L. pilosus	Zn-	8.41 a	5.00 d	52
P. sativum	Zn+	4.56 d	3.50 ef	43
P. sativum	Zn-	6.51 b	3.83 e	45
M. truncatula	Zn+	6.51 b	3.33 f	84
M. truncatula	Zn-	7.97 a	10.10 b	177
T. durum	Zn+	1.99 g	3.24 f	56
T. durum	Zn-	3.89 e	7.27 c	223
H. leporinum	Zn+	2.29 f	3.61 ef	66
H. leporinum	Zn-	3.57 e	3.60 ef	488
B. juncea	Zn-	5.95 c	12.10 a	81.5
F		***	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A4.2 Uptakes of magnesium, sulphur, copper and iron in whole shoots (harvested in 1993 at anthesis of zinc treated plants) from Zn- pots (Table A4.2a) and Zn+ pots (Table A4.2b), 1993. Values in the same mn with the same letter are not significantly different ($P \leq 0.05$).

Table A4.2a

Species	Mg	S [A]	Cu [A]	Fe [A]
	(mg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(mg pot^{-1})
L. pilosus	42 b	25 a	27 e	0.26 c
P. sativum	15 c	9 b	14 d	0.11 d
M. truncatula	6 d	7 bc	5 e	0.12 d
T. durum	3 e	6 bc	17 d	0.19 c
H. leporinum	4 e	4 d	39 b	0.54 b
B. juncea	104 a	213 [Z]	218 a	1.42 a
F	***	***	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

[Z] Analysis of variance performed without this treatment due to non additivity which could not be corrected by data transformation. The mean and variance for this treatment were much higher than for other treatments.

Table A4.2b

Species	Mg [A]	S [A]	Cu [A]	Fe
	(mg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(mg pot^{-1})
L. pilosus	90.7 a	53.8 b	85 b	0.90 b
P. sativum	56.9 b	43.7 b	60 c	0.54 c
M. truncatula	48.9 bc	25.0 c	53 c	0.67 bc
T. durum	19.8 d	32.5 c	90 b	0.57 c
H. leproinum	45.0 c	70.5 a	178 a	1.29 a
F	***	***	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A4.3 Dry weight of Durati wheat shoots 22 DAS in Zn+ soil as a function of species grown in 1993.

Species	Dry weight of shoots
	(g pot^{-1})
L. pilosus	1.18
P. sativum	1.38
M. truncatula	0.85
H. leporinum	1.15
F	ns

Table A4.4 Total dry weights of shoots of Excalibur plus Durati wheat in Zn- soil as a function of species grown in 1993. Values with the same letter are not significantly different ($P \leq 0.05$).

1993 species	Total dry weight
	(g pot^{-1})
L. pilosus	1.49 a
P. sativum	1.48 a
M. truncatula	1.26 ab
T. durum	0.87 bc
H. leporinum	1.06 abc
B. juncea	0.69 c
F	*

Table A4.5 Uptake of zinc (corrected for seed zinc) in shoots of Durati (22 DAS) plus Excalibur (140 DAS) wheat grown in Zn- soil, as a function of species grown in 1993. Values with the same letter are not significantly different ($P \leq 0.05$).

1993 species	**Zinc uptake**
	(μg pot^{-1})
L. pilosus	5.39 a
P. sativum	4.82 a
M. truncatula	3.19 b
T. durum	2.31 bc
H. leporinum	1.50 c
B. juncea	1.65 c
F	***

Table A4.6 Concentrations of calcium, magnesium, sulphur and iron in Durati wheat shoots 22 DAS in Zn- soil (Table A4.6a) and Zn+ soil (Table A4.6b) 1994, as a function of species grown in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Table A4.6a

1993 species	Ca	Mg	S	Fe
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})
L. pilosus	16.8 b	2.25	5.53	182
P. sativum	21.5 ab	2.84	5.81	149
M. truncatula	25.3 a	2.73	4.30	112
T. durum	25.0 a	3.15	5.97	182
B. juncea	15.9 b	2.17	5.10	183
F	*	ns	ns	ns

Table A4.6b

1993 species	Ca	Mg	S	Fe
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})
L. pilosus	19.1	1.94	4.80	99
P. sativum	19.2	2.10	5.48	107
M. truncatula	21.5	1.87	4.79	113
T. durum	21.7	1.91	4.01	138
H. leporinum	21.1	1.89	4.08	121
F	ns	ns	ns	ns

Table A4.7 Concentrations of calcium, magnesium, sulphur and iron in Excalibur wheat shoots 140 DAS in Zn- soil 1994, as a function of species grown in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 species	Ca	Mg	S	Fe
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})
L. pilosus	15.2	4.92	4.19	230
P. sativum	16.5	5.52	4.17	231
M. truncatula	16.3	5.36	4.28	246
T. durum	17.4	5.30	3.61	215
H. leporinum	15.4	5.09	3.59	211
B. juncea	18.0	6.00	6.30	239
F	ns	ns	ns	ns

Table A4.8 Uptakes of calcium, magnesium, sulphur and iron in Excalibur wheat shoots 140 DAS in Zn- soil 1994, as a function of species grown in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 species	Ca	Mg	S	Fe
	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})
L. pilosus	19.5	7.06 a	5.31	343
P. sativum	21.6	7.56 a	5.50	320
M. truncatula	20.0	6.48 ab	5.20	302
T. durum	13.6	4.29 bc	2.72	177
H. leporinum	14.5	3.42 c	3.58	220
B. juncea	10.8	3.65 c	3.83	149
F	ns	**	ns	ns

Table A4.9 Uptake of zinc by Durati (22 DAS) plus Excalibur (140 DAS) wheat shoots in Zn- soil as a function of species grown in 1993. Values with the same letter are not significantly different ($P \leq 0.05$).

1993 species	Zn uptake in wheat
	(μg pot^{-1})
L. pilosus	5.39 a
P. sativum	4.82 a
M. truncatula	3.19 b
T. durum	2.31 bc
H. leporinum	1.50 c
B. juncea	1.65 c
F	***

Table A4.10 Concentrations of calcium, magnesium, sulphur, copper and iron in mature Excalibur wheat straw in Zn+ soil 1994 as a function of species grown in 1993. Values with the same letter are not significantly different ($P \leq 0.05$).

1993 species	Ca	Mg	S	Cu	Fe [A]
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
L. pilosus	12.3	3.92	5.54	6.30	46.3 a
P. sativum	12.7	3.50	5.36	5.43	45.9 a
M. truncatula	11.7	3.15	5.20	5.23	38.4 a
T. durum	14.6	4.11	6.06	6.13	45.1 a
H. leporinum	12.4	3.62	5.58	4.53	26.1 b
F	ns	ns	ns	ns	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A4.11 Concentrations of calcium, magnesium, sulphur, copper and iron in Excalibur wheat grain in Zn+ soil 1994, as a function of species grown in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 species	Ca	Mg	S	Cu	Fe [A]
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
L. pilosus	561	1.79	1.87	7.70	44.0 a
P. sativum	589	1.75	1.85	7.10	48.0 a
M. truncatula	612	1.77	1.94	6.95	46.0 a
T. durum	591	1.83	2.05	7.45	46.0 a
H. leporinum	608	1.69	1.93	6.60	39.0 b
F	ns	ns	ns	ns	**

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A4.12 Uptake of zinc, phosphorus, potassium, boron, manganese and sodium in mature Excalibur wheat straw grown in Zn+ soil 1994, as a function of species grown in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 species	Zn [A]	P [A]	K	B	Mn	Na [A]
	(μg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})
L. pilosus	63.8 a	14.5 a	317 a	222 b	55.7 b	6.4 ab
P. sativum	47.1 a	13.8 a	314 a	232 b	45.7 b	5.2 c
M. truncatula	40.5 a	8.3 b	292 ab	210 b	39.0 b	5.7 bc
T. durum	64.1 a	17.0 a	405 a	444 a	92.1 a	7.0 a
H. leporinum	17.8 b	3.8 c	180 b	110 b	48.3 b	4.8 c
F	**	***	*	**	**	**

[A] Analysis of variance performed on $\log_e$ transformed data.

Table A4.13 Uptake of zinc, phosphorus, potassium, boron, manganese and sodium in grain of Excalibur wheat grown in Zn+ soil 1994, as a function of species grown in 1993. Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

1993 species	Zn [A]	P [A]	K	B	Mn	Na
	(μg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})
L. pilosus	165 ab	41.8 a	54.4 a	18.5	65.4	179
P. sativum	215 a	51.9 a	72.5 a	23.8	85.2	265
M. truncatula	167 ab	41.1 a	53.7 a	18.5	53.6	286
T. durum	179 a	48.6 a	64.5 a	22.2	64.7	240
H. leporinum	122 b	23.3 b	32.4 b	14.9	48.7	116
F	*	***	**	ns	ns	ns

[A] Analysis of variance performed on $\log_e$ transformed data.

Table A4.14 Uptake of calcium, magnesium, sulphur, copper and iron in tops (grain + straw) of Excalibur wheat in Zn+ soil 1994, as a function of species grown in 1993. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

1993 species	Ca	Mg	S	Cu [A]	Fe [A]
	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})
L. pilosus	84 ab	41.6 ab	52.7 ab	111 ab	698 b
P. sativum	76 ab	32.8 bc	49.9 ab	109 ab	779 ab
M. truncatula	78 b	34.2 bc	48.3 b	91 b	488 c
T. durum	130 a	52.8 a	71.6 a	124 a	839 a
H. leporinum	45 b	23.7 c	33.3 b	58 c	327 d
F	*	**	*	***	***

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A4.15 Water use efficiency (WUE tops) of tops (straw + grain) and grain (WUE grain) of Excalibur wheat in Zn+ soil in 1994, as a function of species grown in 1993.

1993 spp.	WUE tops	WUE grain
	(kg tops m^{-3})	(kg grain m^{-3})
L. pilosus	1.74	1.02
P. sativum	2.09	1.21
M. truncatula	1.86	1.14
T. durum	1.91	1.05
H. leporinum	1.83	1.10
F	ns	ns

Table A5.1 Concentrations of potassium, boron, manganese and sodium in "representative" YEBs (flag leaf-1) of Gatcher and Excalibur wheat at Feekes 8, grown in Minnipa subsoil as a function of nutrient treatments, 1993.

Treatment	K	B	Mn	Na
	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
E nu+ Zn+	44.2	280	131	134
E nu+ Zn-	46.4	279	178	97
G nu+ Zn+	43.5	302	116	89
G nu+ Zn-	43.8	340	152	97

Table A5.2 Treatment means of dry weight of tops, harvest index and water use efficiency of tops (WUE tops) of Gatcher and Excalibur wheat grown in Minnipa subsoil, as a function of nutrient treatment.

Treatment	**Dry weight of tops**	**Harvest index** [B]	**WUE tops**
	(g pot^{-1})	(%)	(kg tops m^{-1})
Excalibur	11.1	41.4	1.7
Gatcher	16.4	25.3	1.1
F	***	***	***
nu+	26.4	25.8	2.05
nu-	1.2	41.0	0.72
F	***	***	***
Zn+	15.7	36.3	1.63
Zn-	11.8	30.4	1.15
F	**	*	***
F (nu x Zn)	ns	**	***
F (nu x cv)	ns	*	ns
F (Zn x cv)	ns	ns	ns
F (nu x cv x Zn)	ns	ns	ns

[B] Analysis of variance performed on square root - transformed data.

Table A5.3 Treatment means of grain yield, fertile heads, hundred grain weight (HGW) and grain water use efficiency (WUE grain) of Gatcher and Excalibur wheat grown in Minnipa subsoil, as a function of nutrient treatment.

Treatment	**Grain yield** [B]	**HGW** [A]	**Fertile heads** [B]	**WUE grain**
	(g pot^{-1})	(g)	(heads pot^{-1})	(kg grain m^{-3})
Excalibur	4.19	4.00	14.5	0.67
Gatcher	2.81	4.78	10.5	0.24
F	***	**	***	***
nu+	6.55	4.45	20.0	0.60
nu-	0.46	4.33	3.7	0.31
F	***	ns	***	***
Zn+	4.85	4.47	13.2	0.58
Zn-	2.16	4.31	10.5	0.33
F	***	ns	*	***
F (Zn x cv)	***	ns	***	ns
F (nu x Zn)	***	ns	*	***
F (nu x cv)	***	*	***	**
F (nu x Zn x cv)	***	*	***	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

[B] Analysis of variance performed on square root - transformed data.

Table A5.4 Treatment means of concentrations of zinc, phosphorus, boron, manganese and sodium in Gatcher and Excalibur wheat grain grown in Minnipa subsoil, as a function of nutrient treatment.

Treatment	Zn [A]	P	B [A]	Mn [A]	Na
	(mg kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
Excalibur	68.5	4.73	4.3	36.3	27.7
Gatcher	60.1	4.83	5.8	57.6	17.3
F	ns	ns	**	***	**
nu+	50.5	4.78	5.85	42.7	24.2
nu-	78.0	4.78	4.19	51.2	20.8
F	***	ns	***	***	ns
Zn+	102	4.40	4.51	43.5	26.1
Zn-	26	5.16	5.53	50.4	18.9
F	***	***	*	***	*
F (Zn x cv)	ns	ns	ns	ns	ns
F (nu x Zn)	***	*	ns	*	*
F (nu x cv)	ns	ns	*	ns	*
F (nu x cv x Zn)	ns	ns	ns	ns	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A5.5 Concentrations of zinc, phosphorus, boron, manganese and sodium in Gatcher and Excalibur wheat straw grown in Minnipa subsoil, as a function of nutrient treatment. Values in the same column with the same letter are not significantly different (P≤0.05).

Treatment	Zn	P	B	Mn	Na
	(mg kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
E nu+ Zn+	206	1.35	479	180	2.78
E nu+ Zn-	6	6.35	453	187	1.45
E nu- Zn+	284	1.18	245	199	0.75
E nu- Zn-	15	1.83	390	184	0.91
G nu+ Zn+	157	2.36	409	183	0.53
G nu+ Zn-	7	6.24	431	205	0.50
G nu- Zn+	180	1.83	210	211	0.68
G nu- Zn-	19	2.04	314	179	0.89

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A5.6 Concentrations of zinc, phosphorus, boron, manganese and sodium in Gatcher and Excalibur wheat straw grown in Minnipa subsoil, as a function of nutrient treatment.

Treatment	Zn [A]	P	B	Mn	Na
	(mg kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
Excalibur	128	2.68	392	187	1.47
Gatcher	91	3.12	341	194	0.65
F	ns	*	*	ns	***
nu+	94	4.07	443	188	1.32
nu-	124	1.72	289	193	0.81
F	***	***	***	ns	***
Zn+	207	4.11	335	193	1.19
Zn-	12	1.68	397	189	0.94
F	***	***	*	ns	*
F (Zn x cv)	*	*	ns	ns	**
F (nu x Zn)	**	***	*	*	**
F (nu x cv)	ns	ns	ns	ns	***
F (nu x cv x Zn)	ns	ns	ns	ns	*

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A5.7 Treatment means of uptakes of zinc, phosphorus, boron, manganese and sodium in Gatcher and Excalibur wheat grain grown in Minnipa subsoil, as a function of nutrient treatment.

Treatment	Zn [A]	P [B]	B [A]	Mn [A]	Na [A]
	(μg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})
Excalibur	258	19.3	18.5	140	149
Gatcher	208	12.4	17.3	133	47
F	***	***	ns	ns	***
nu+	430	29.6	33.9	250	187
nu-	36	2.1	1.8	23	10
F	***	***	***	***	***
Zn+	436	20.5	25.0	184	155
Zn-	30	11.2	10.7	89	41
F	***	***	***	***	***
F (Zn x cv)	*	***	**	*	ns
F (nu x Zn)	***	***	***	*	***
F (nu x cv)	**	***	*	**	**
F (nu x cv x Zn)	**	***	***	**	*

[A] Analysis of variance performed on $\log_e$ - transformed data.

[B] Analysis of variance performed on square root - transformed data.

Table A5.8 Uptakes of zinc, phosphorus, boron, manganese and sodium in Gatcher and Excalibur wheat straw grown in Minnipa subsoil, as a function of nutrient treatment. Values in the same column with the same letter are not significantly different (P≤0.05).

Treatment	Zn	P	B	Mn	Na
	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})
E nu+ Zn+	2.60	16.6	6.1	2.21	35.1
E nu+ Zn-	0.09	89.2	6.4	2.62	20.3
E nu- Zn+	0.18	00.7	0.2	0.13	0.5
E nu- Zn-	0.06	00.7	0.2	0.70	0.4
G nu+ Zn+	4.48	68.9	12.0	5.28	14.5
G nu+ Zn-	0.17	149.1	10.4	4.94	10.8
G nu- Zn+	0.17	1.8	0.2	0.20	0.8
G nu- Zn-	0.12	1.29	0.3	0.12	0.7

Table A5.9 Treatment means of uptakes of zinc, phosphorus, boron, manganese and sodium in Gatcher and Excalibur wheat straw grown in Minnipa subsoil, as a function of nutrient treatment.

Treatment	Zn [A]	P [A]	B [B]	Mn [A]	Na [B]
	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})
Excalibur	0.72	26.8	3.19	1.27	14.1
Gatcher	1.21	55.3	5.70	2.64	6.7
F	***	***	***	***	***
nu+	1.83	81.0	8.71	3.78	20.2
nu-	0.92	1.1	0.19	0.13	0.6
F	***	***	***	***	***
Zn+	1.86	22.0	4.62	1.92	12.7
Zn-	0.07	60.1	4.27	1.94	8.1
F	***	***	ns	**	*
F (Zn x cv)	ns	*	ns	ns	ns
F (nu x Zn)	ns	***	ns	**	*
F (nu x cv)	ns	ns	***	ns	***
F (nu x cv x Zn)	ns	ns	ns	ns	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

[B] Analysis of variance performed on square root - transformed data.

Table A5.10 Treatment means of total uptake of zinc, phosphorus, boron, manganese and sodium in Gatcher and Excalibur wheat tops (grain + straw) grown in Minnipa subsoil, as a function of nutrient treatment.

Treatment	**Zn** [A]	**P** [A]	**B** [A]	**Mn** [A]	**Na** [A]
	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})
Excalibur	0.98	46	3.21	1.41	14.2
Gatcher	0.14	68	5.72	2.77	6.7
F	**	***	*	***	ns
nu+	2.26	111	8.74	4.03	20.4
nu-	0.13	3	0.19	0.15	0.6
F	***	***	***	***	***
Zn+	2.29	43	4.65	2.16	12.8
Zn-	0.98	71	4.29	2.03	8.1
F	***	*	ns	**	ns
F (*Zn* x *cv*)	ns	ns	ns	ns	ns
F (*nu* x *Zn*)	***	***	ns	**	ns
F (*nu* x *cv*)	ns	ns	ns	ns	ns
F(*nu* x *cv* x *Zn*)	**	*	ns	ns	*

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A5.11 Treatment means of relative transport to grain in Gatcher and Excalibur wheat tops (straw + grain) grown in Minnipa subsoil, as a function of nutrient treatment.

Treatment	**Relative transport to grain** [B]
	(% zinc in grain of total content in tops)
Excalibur	42.0
Gatcher	25.4
F	***
nu+	23.1
nu-	44.3
F	***
Zn+	22.2
Zn-	45.1
F	***
F (Zn x cv)	*
F (nu x Zn)	***
F (nu x cv)	**
F (nu x cv x Zn)	ns

[B] Analysis of variance performed on square root - transformed data.

Table A5.12 Treatment means of rooting densities of Gatcher and Excalibur wheat at maturity in Minnipa subsoil, as a function of depth and nutrient treatment.

Treatment	Rooting Density 0.0 - 0.15 m [A]	0.15 - 0.35 m [A]	0.35 - 0.55 m [B]	Total Root Length [B]
	(cm cm^{-3})	(cm cm^{-3})	(cm cm^{-3})	(m pot^{-1})
E nu+ Zn+	4.82	4.08	3.31	389
E nu+ Zn-	6.58	4.10	3.31	436
E nu- Zn+	0.70	0.87	0.90	81
E nu- Zn-	0.73	0.67	0.98	78
G nu+ Zn+	9.41	7.43	7.37	772
G nu+ Zn-	7.88	9.20	7.80	810
G nu- Zn+	1.03	1.25	1.42	122
G nu- Zn-	1.10	2.03	1.22	144

Table A5.13 Treatment means of total root length in Gatcher and Excalibur wheat grown in Minnipa subsoil 1993, as a function of depth and nutrient treatment. Values with the same letter are not significantly different (P≤0.05).

Treatment	Total root length
	(m pot^{-1})
Excalibur	246
Gatcher	462
F	***
nu+	602
nu-	106
F	***
Zn+	341
Zn-	367
F	ns
F (Zn x cv)	ns
F (nu x Zn)	ns
F (nu x cv)	*
F (nu x cv x Zn)	ns

Table A5.13a

Cultivar Effect *(cv)*	Basal Nutrient Effect *(nu)*	Zinc Effect *(Zn)*		*(nu x cv)* Means
		Zn+	Zn-	
		Total root length (m pot^{-1})		
Excalibur	+	389	436	413 b
	-	81	78	80 c
Gatcher	+	772	810	791 a
	-	122	144	133 c
	Zn means (ns)	341	367	

Table A5.14 Proportions of fine roots (<0.3 mm diameter) in Gatcher and Excalibur wheat at maturity in Minnipa subsoil, as a function of depth and nutrient treatment. Values in the same column with the same letter are not significantly different (P≤0.05).

Treatment	% Fine roots		
	0.0 - 0.15 m	**0.15 - 0.35 m**	**0.35 - 0.55 m**
	(%)	(%)	(%)
E nu+ Zn+	44.5	42.2	42.8
E nu+ Zn-	42.3	45.7	50.1
E nu- Zn+	57.4	47.3	51.7
E nu- Zn-	62.4	54.2	53.8
G nu+ Zn+	35.4	34.6	34.2
G nu+ Zn-	39.4	41.7	40.7
G nu- Zn+	42.2	41.4	46.0
G nu- Zn-	52.4	44.5	44.7

Table A5.15 Total surface area of roots of Gatcher and Excalibur wheat at maturity in Minnipa subsoil as a function of depth and nutrient treatment. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Surface Area of Roots		
	0.0 - 0.15 m	**0.15 - 0.35 m**	**0.35 - 0.55 m**
	(cm^2)	(cm^2)	(cm^2)
E nu+ Zn+	1950	2020	1580
E nu+ Zn-	2720	1940	1420
E nu- Zn+	210	380	360
E nu- Zn-	205	284	434
G nu+ Zn+	4730	4770	4260
G nu+ Zn-	3930	5060	4510
G nu- Zn+	400	600	640
G nu- Zn-	335	889	580

Table A6.1 Concentrations of copper, iron and sodium in YEBs of Excalibur wheat at Feekes 2, as a function of zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Cu [A]	Fe [A]	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
+++	17.3 bc	103 c	129
++−	18.4 b	113 bc	102
+−+	16.6 bc	104 bc	116
−++	17.5 bc	133 b	101
+−−	16.3 bc	102 c	107
−+−	15.4 c	106 bc	116
−−+	27.8 a	238 a	118
F	***	***	ns

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A6.2 Concentrations of calcium, magnesium and sulphur in YEBs of Excalibur wheat at Feekes 2, as a function of zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Ca	Mg	S
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
+++	7.4	2.86 b	5.77
++−	9.2	3.19 b	6.37
+−+	7.7	2.79 b	6.21
−++	10.4	3.80 a	6.22
+−−	7.0	2.88 b	6.10
−+−	8.1	2.92 b	5.93
−−+	9.2	3.78 a	6.84
F	ns	***	ns

Table A6.3 Concentrations of copper, iron and sodium in flag leaves of Excalibur wheat at Feekes 10.5.2, as a function of zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Cu	Fe [A]	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
+++	10.7	85 c	32.0 a
++−	10.5	98 ab	22.7 bc
+−+	10.2	89 bc	22.0 bc
−++	10.5	89 bc	21.0 bc
+−−	10.8	109 a	19.3 c
−+−	10.6	93 bc	27.3 ab
−−+	10.3	88 bc	20.7 bc
F	ns	**	*

[A] Analysis of variance performed on $\log_e$ - transformed data.

Table A6.4 Concentrations of calcium, magnesium and sulphur in flag leaves of Excalibur wheat at Feekes 10.5.2, as a function of zinc placement. Values in the same column with the same letter are not significantly different (≤ 0.05).

Treatment	Ca	Mg	S
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
+++	13.9	4.07	5.34
++−	13.5	4.23	5.75
+−+	14.0	5.02	6.06
−++	14.2	4.86	5.29
+−−	13.4	4.68	5.89
−+−	13.0	5.31	5.93
−−+	15.0	4.36	5.39
F	ns	ns	ns

Table A6.5 Grain yield, total dry weight of tops, harvest index, hundred grain weight (HGW) and grains per head (GPH) of Excalibur wheat as affected by zinc placement.

Treatment	Grain yield	Dry weight of tops	Harvest index	HGW	GPH
	(g pot^{-1})	(g pot^{-1})	(%)	(g)	
+++	26.4	56.7	46.4	4.37	34.0
++−	27.3	57.6	47.3	4.45	34.8
+−+	28.7	60.3	47.5	4.29	31.3
−++	29.0	63.3	45.8	4.24	29.9
+−−	28.6	62.0	46.2	4.11	35.0
−+−	30.6	66.4	46.0	4.44	35.2
−−+	28.8	54.6	48.8	4.51	35.6
F	ns	ns	ns	ns	ns

Table A6.6 Concentrations of calcium, magnesium, phosphorus, potassium and sulphur in Excalibur wheat grain, as a function of zinc placement.

Treatment	Ca	Mg	P	K	S
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
+++	0.54	1.43	3.68	4.36	1.70
++−	0.53	1.44	3.56	4.21	1.67
+−+	0.50	1.44	3.48	4.35	1.72
−++	0.50	1.44	3.44	4.08	1.73
+−−	0.46	1.48	3.50	4.54	1.69
−+−	0.48	1.40	3.36	4.39	1.67
−−+	0.50	1.36	3.22	4.13	1.70
F	ns	ns	ns	ns	ns

Table A6.7 Concentrations of boron, copper, iron, manganese and sodium in Excalibur wheat grain, as a function of zinc placement.

Treatment	B	Cu	Fe	Mn	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
+++	2.67	6.13	34.3	12.1	13.3
++−	2.63	6.17	34.9	12.0	13.7
+−+	2.60	6.37	34.7	9.2	13.3
−++	2.67	6.30	35.2	9.6	13.7
+−−	2.97	6.40	36.5	11.4	14.7
−+−	2.80	6.30	34.2	9.9	14.0
−−+	2.67	6.27	37.8	9.9	14.0
F	ns	ns	ns	ns	ns

Table A6.8 Concentrations of copper, iron and sodium in straw of Excalibur wheat, as a function of zinc placement.

Treatment	Cu	Fe	Na
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
+++	3.33	24.2	38.0
++−	3.65	26.6	26.0
+−+	4.23	23.1	49.0
−++	5.02	36.2	53.3
+−−	4.47	37.1	24.7
−+−	3.83	25.7	40.0
−−+	3.32	43.1	42.3
F	ns	ns	ns

Table A6.9 Concentrations of calcium, magnesium and sulphur in straw of Excalibur wheat, as a function of zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Ca	Mg	S
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
+++	9.6 ab	3.35	3.52
++−	10.3 a	4.00	3.84
+−+	8.2 bcd	3.23	3.35
−++	6.7 d	2.70	3.26
+−−	9.1 abc	4.04	3.94
−+−	7.6 cd	3.32	3.29
−−+	9.1 abc	3.51	3.49
F	*	ns	ns

Table A6.10 Uptake of zinc, boron, copper, iron, manganese and sodium in Excalibur wheat straw, as a function of zinc placement. Values in the same column with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Zn	B	Cu	Fe	Mn	Na
	(μg pot^{-1})	(mg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})	(μg pot^{-1})	(mg pot^{-1})
+++	355	1.04	101	735	237 c	1.15
++−	143	1.31	111	806	304 ab	0.80
+−+	148	0.90	134	731	216 c	1.54
−++	265	0.76	178	1 286	223 c	1.88
+−−	213	1.22	149	1 239	365 a	0.82
−+−	129	0.89	137	919	273 bc	1.45
−−+	154	0.89	86	1 209	269 bc	1.19
F	ns	ns	ns	ns	**	ns

Table A6.11 Uptake of calcium, magnesium, phosphorus, potassium and sulphur in Excalibur wheat straw, as a function of zinc placement.

Treatment	Ca	Mg	P	K	S
	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})	(mg pot^{-1})
+++	291	101	24.1	1 795	106
++−	316	123	24.0	789	117
+−+	259	102	20.9	722	106
−++	230	93	22.5	752	113
+−−	305	135	22.7	804	132
−+−	271	119	22.0	765	119
−−+	238	107	18.6	646	108
F	ns	ns	ns	ns	ns

Table A6.12 Estimated total root length of Excalibur wheat at maturity as a function of zinc placement. Values with the same letter are not significantly different ($P \leq 0.05$).

Treatment	Total root length
	(m pot^{-1})
+++	692 ab
++−	809 a
+−+	622 b
−++	728 ab
+−−	830 a
−+−	782 a
−−+	579 b
F	*

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