Mechanisms of Manganese Efficiency in Barley (*Hordeum vulgare*)

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DECLARATION

I HEREBY DECLARE that the work presented in this thesis has been carried out by myself and does not incorporate any material previously submitted for another degree in any university. To the best of my knowledge and belief, it does not contain any material previously written or published by another person, except where due reference is made in the text. I am willing to make the thesis available for photocopy and loan if it is accepted for the award of the degree.

J.L. Harbard

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DEDICATION

I wish to dedicate this work to my mother Marlene Joy Harbard in recognition of her love and support.

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ABSTRACT

This project sought to identify those mechanisms which confer Mn efficiency on some barley genotypes in the South Australian barley breeding program. Experiments were conducted under controlled environment conditions to test four areas which may contribute to Mn efficiency.

It was found that H^+ ions were extruded from roots of barley and wheat as a consequence of normal root growth and were not 'switched-on' as a consequence of Mn deficiency and these pH decreases were severely restricted in highly buffered calcareous soils of high pH. In addition, H^+ ion production was not responsible for the reduction of insoluble higher oxides of Mn as has been shown for Fe efficient dicots and some monocots. However an unidentified component of root cells was able to reduce Mn dioxide when leaked from damaged root cells of barley and wheat.

No difference between barley genotypes in seminal root morphology were identified in either soil or nutrient solution studies. However a more highly branched nodal root system was found in the more Mn efficienct genotypes which would enable greater exploration of the soil, an increase in the number of root tips which were shown to be areas of H^+ extrusion and an increased area of root exudate production.

A correlation between the severity of Mn deficiency symptoms on the plant and numbers of Mn oxidising populations in rhizosphere soil could not be established with certainty. At high soil Mn levels a decrease in numbers of colonies of Mn oxidising bacteria (not statistically significant) around the more Mn efficient cultivars was observed. This could prove to be important in increasing Mn availability to Mn efficient plants. Further studies would clarify whether this is a significant factor in determining Mn efficiency.

The critical level of Mn in whole tops was similar in Weeah and Galleon (16 and 18 μg/g respectively). However, the critical level of Mn in the young growing tissue (YEBs) as a function of YEB growth was higher in the Mn inefficient cultivar Galleon (12μg/g) than the Mn efficient cultivar Weeah (8μg/g).

Mn efficiency could not be wholly attributed to any of the mechanisms researched here. The results of this research suggests the presence of a plant produced compound released through the roots and capable of reducing unavailable Mn oxides. Further studies should therefore be directed at finding and identifying the component of root exudates in Mn efficient genotypes which can reduce insoluble Mn oxides in Mn deficient soils.

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1.1 INTRODUCTION

Gregory and Crowther (1928) observed differences among barley cultivars in plant yields and hypothesised that these differences were due to differential uptake and assimilation of soil constituents. Since this report only spasmodic attention has been given to the possibility of selecting plants according to variation in nutrient uptake. In the past it has been more economical to alter the soil environment to suit the plant than to breed for nutrient efficiency. Today however, there is greater acceptance of the fact that there are differential responses among cultivars to nutritional factors and that these may be successfully applied to solving specific problems of soil fertility or stress tolerance (Graham 1984).

Manganese deficiency is a widespread problem in southern Australia, particulary on calcareous sands where yields without Mn fertiliser range from 40 to 75% of those with Mn (Reuter *et al* 1973). Soluble Mn is quickly converted to unavailable Mn compounds at the high pH of these soils. Therefore there is little residual value of fertiliser applied at sowing. Thus, to correct Mn deficiency one or more foliar sprays must be applied during the growing season in addition to solid fertiliser at sowing. Foliar sprays often result in poor response due to such factors as low interception of spray and poor mobility and redistribution of Mn once it is within the plant (Henkens and Jongman 1965). In addition, if spraying is delayed until deficiency symptoms are noted it may already be too late to prevent some of the yield loss. Therefore, the alleviation of Mn deficiency requires a combination of both soil and foliar application of manganous sulphate at the right time. Because of the difficulties of correcting Mn deficiency by application of fertiliser and the additional labour, fuel and fertiliser costs necessary to perform the operations, sowing Mn 'efficient' cultivars in these problem soils may be the best approach to minimising yield losses (Graham 1984). There is also evidence which links nutrient deficiency to

susceptibility to disease (Graham and Rovira 1984, Wilhelm *et al* 1985). Thus, sowing a Mn efficient cultivar may reduce yield losses due to disease in addition to those due to Mn deficiency itself. Most importantly, the genetic potential for such a solution exists (Sparrow *et al* 1983).

It has recently become common practice in South Australia to assess current cultivars for increased resistance to Mn deficiency in field trials and to make recommendations based on these trials. A program specifically designed to select for a nutrient efficient genotype has been initiated. Such a program of selecting and breeding for nutrient efficient genotypes would be made easier if the mechanism(s) of Mn 'efficiency' could be identified. Nutrient efficiency of a genotype is defined as the ability to produce a high yield in a soil that is limiting in a particular element for a standard genotype (Graham 1984). A screening technique which could identify this mechanism(s) at the seedling stage would enable large numbers of progeny to be efficiently screened in the glasshouse before transferring selection to the field.

Mechanisms of nutrient efficiency may be based on physiological, morphological, and/or biochemical traits which can affect nutrient availability, absorption, translocation, or utilization. These mechanisms may also be described as general mechanisms affecting availability and uptake of several nutrients or as specific, affecting only one nutrient. Mn efficiency in barley cultivars may involve any of the mechanisms listed in Table 1.1:

Table 1.1 Plant mediated mechanisms of increasing Mn availability in alkaline soils

1) Root-induced acidification of the rhizosphere
2) Lowering the redox potential of the soil around the roots
3) Increased root absorbing surface
4) Increased root absorbing power
5) Reduced requirement for the element
6) Modification of the microbial balance in soil around roots
7) Production of Mn binding ligands
8) Mycorrhizal association
9) Increased translocation of Mn from root to shoot

Barley is considered to be more tolerant to Mn deficiency than either wheat or oats (Gallagher and Walsh 1943, Nyborg 1970, Graham 1983). The relative importance of each of these potential mechanisms will depend on the prevailing environmental conditions and the genotype involved. The aim of this research was to help identify those mechanisms which significantly contribute to the increased uptake, or tolerance to low available soil Mn in barley cultivars.

1.2 METABOLIC FUNCTIONS OF MANGANESE IN PLANTS

McHargue in 1922 showed by sand and water culture experiments that a number of species could not complete their life-cycle in the absence of Mn. By withholding Mn from the growth medium, Pirson (1937) found that depletion of Mn in higher plants or algae led to the loss of oxygen-evolving capacity and that this could be restored within 30 minutes after the readdition of Mn. The location of the site of function of Mn was clearly identified as photosystem II of photosynthesis by Cheniae and Martin (1970). It is now established that Mn is required for the water-splitting and oxygen evolving step in the light reaction of photosynthesis. Oxygen evolution is almost completely inhibited when fewer than four Mn atoms per 400 chlorophyll molecules are present (Amesz 1983).

Handley *et al* (1984) found Mn also acts as an electron source for superoxide radicals formed after photosystem I (see Figure 1.2.1). Superoxides are formed when a single electron is transmitted to oxygen and the inactivation of these superoxides is catalysed by superoxide dismutase (SOD). The CuZn-containing SODs are the most widespread in higher plants (Marschner 1986) although there is increasing evidence that Mn-containing SOD is also well distributed. Sevilla *et al* (1980) isolated SOD from pea leaf extracts with one atom of Mn per enzyme molecule. Later work by Palma *et al* (1986) found no Mn SOD activity in higher plant chloroplasts but there were Mn-containing proteins with SOD like activity. As yet only a few such Mn containing enzymes have been isolated from higher plants (Williams 1982).

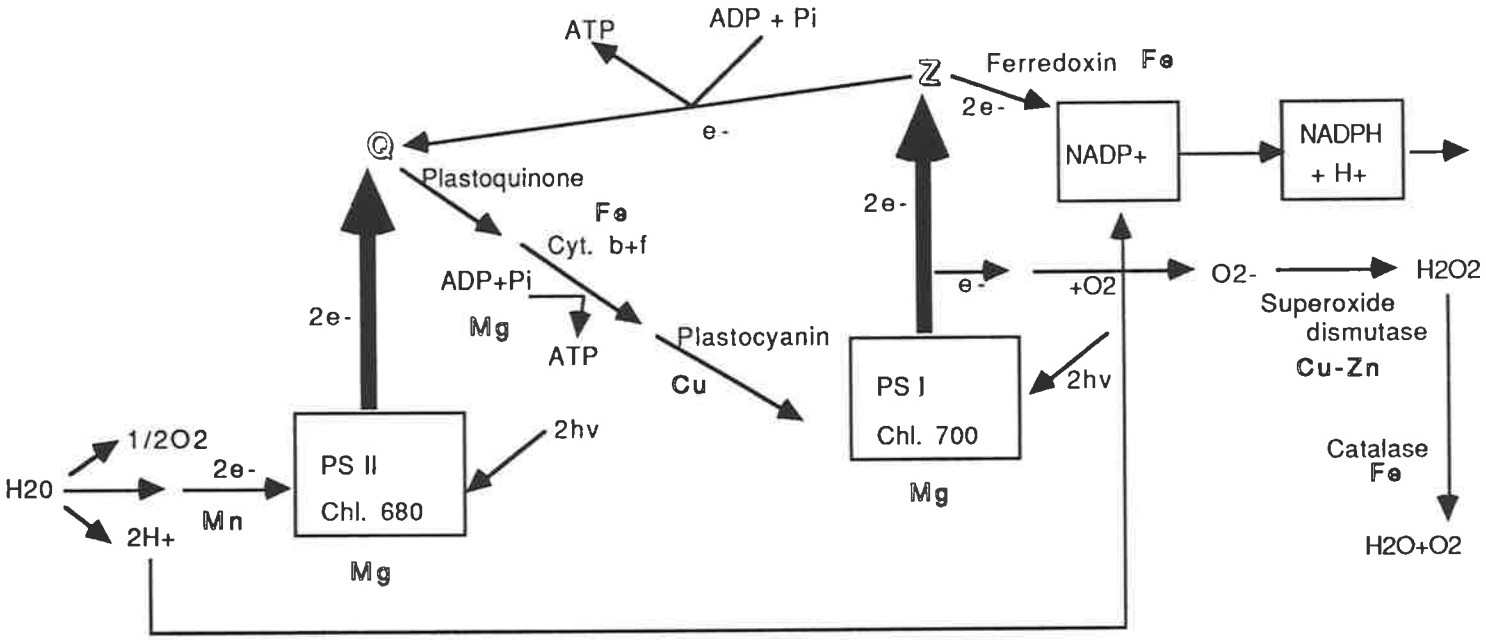

Figure 1.2.1 Photosynthetic electron transport chain with photosystems II and I (PSII and PSI) and photophosphorylation (ATP formation). Q,Quencher; Z,unknown compound; Cyt.,cytochrome. (After Marschner 1986)

Photosynthetic abnormalities in Mn deficient wheat plants have been detected by measurements of *in vivo* changes in chlorophyll a fluorescence (Kriedemann *et al* 1985, Norrish *et al* 1983). Characteristics of fluorescence transients obtained from dark adapted wheat leaves varied with the level of Mn supply.

In addition to photosynthetic dysfunction, disorganisation of the lamellar system of chloroplasts occurs as a result of Mn deficiency. When Mn is deficient, the structure of chloroplasts is markedly impaired even when other organelles show no visible alteration (Possingham *et al* 1964). In chloroplasts from slightly Mn deficient spinach plants the intergrana lamellae were detached from the grana and were scattered throughout the stroma, but the grana were normal. With increasing severity of deficiency, fewer intergrana lamellae were present, empty regions appeared in the stroma and the grana were abnormal in shape. In extreme deficiency, both grana and intergrana lamellae were absent, the chloroplast consisting only of a matrix with empty regions enclosed by a membrane (Mercer *et al* 1962). Khmara (1984) hypothesized that Mn^{2+} ions play a regulatory role in biosynthesis of structural protein components of photosysthetic membranes.

Apart from its role in photosysthesis Mn acts as an activator of many enzyme systems (Epstein 1972). Mn has been reported to be either required for or stimulatory to the activity of the indole-acetic acid (IAA) oxidase system from pea, bean, pineapple, lupin, wheat, Omphalia and cotton (Taylor *et al* 1968). In combination with other heavy metals Mn regulates phytohormonal metabolism through the activation of IAA oxidases (Amberger 1973).

Heenan and Campbell (1980) proposed an indirect role for Mn in nitrate reduction in soybean. The activity of nitrate reductase is dependent on malate or on photosynthetically produced substrate for generation of reductant in green leaves. As photosynthesis is decreased by Mn deficiency it is possible that the supply of reductant

decreases. Leidi and Gomez (1985) also proposed an indirect role of Mn but one independent of the photosynthesis effect. According to their model, Mn acts as a structural component or cofactor on the specific NADH-nitrate reductase inhibitor. Decarboxylases and dehydrogenases of the TCA cycle are also activated by Mn^{2+}. However, in most cases Mn^{2+} can be substituted by Mg^{2+} (Amberger 1973). Mg concentration is 50-100 times higher than that of Mn; therefore to be significant the activation of an enzyme by Mn would have to be more effective than that by Mg (Marschner 1986). Activation of RNA polymerase in chloroplasts for example requires 10 times higher concentrations of Mg than of Mn (Marschner 1986).

Mn has been shown to be involved in two or more steps of lignin biosynthesis (Gross 1980, Vance *et al* 1980). Graham (1983) proposed that a deficiency of Mn in wheat plants may result in a decrease in disease resistance by inhibiting the production of phenols and lignins. Brown *et al* (1984) confirmed that Mn deficieny decreased lignin in wheat roots.

Wilhelm *et al* (1985) and Graham (1983) have both shown tolerance to disease was reduced when plants were Mn deficient. Wilhelm *et al* (1985) found the tolerance of barley to cereal cyst nematode infection to be reduced by Mn deficiency and susceptibility to take-all in wheat to increase under Mn deficiency (Wilhelm 1991). Powdery mildews were more severe on Mn deficient barley, wheat, pumpkin seedlings, cucumber and common scab of potato and was decreased by application of Mn (Graham 1983).

Briefly, other functions of Mn include involvement in cell wall elongation and division and structural effects. Using both attached and excised roots Burstrom (1950) found cell elongation was reduced in Mn deficient plants. Mn has not been shown to be directly involved in protein synthesis although Mn deficiency does result in abnormalities in ribosome structures of *Aspergillus niger* (Ma *et al* 1985) and lamellar system of chloroplasts (Possingham *et al* 1964).

1.3 SYMPTOMS OF MANGANESE DEFICIENCY

The critical Mn concentration for deficiency in most plant species is in the range of 10 to 25 µg/g Mn in the dry matter of upper plant parts (Graham and Loneragan 1981, Mengel and Kirkby 1982, Ohki 1984, Graham *et al* 1985, Reuter and Robinson 1986, Hannam *et al* 1987). Reuter and Robinson (1986) have tabulated critical levels for barley from work done in Australia, USA and Canada and the critical levels range from 11 µg/g for field and soil grown plants to 20 µg/g in flowing solution culture systems. Many workers have commented on the variability of critical levels depending on plant age, plant part analysed, changes in environment and the method of plant culture (Bates 1971). Graham and co-workers compared the critical level of Mn in field grown wheat (Graham *et al* 1985) with that in solution culture grown wheat (Graham and Loneragan 1981). The critical level for young solution culture grown plants was 20 µg/g compared to 11 µg/g in field grown wheat. The method of determination of critical levels and the factors influencing the result will be discussed in more detail later as a mechanism of nutrient efficiency.

Below the critical level, dry matter production, net photosynthesis and chlorophyll content rapidly decline; however respiration and transpiration are less rapidly affected (Ohki 1985).

Mn deficiency symptoms vary with plant species but are characterised by interveinal chlorosis and reduced growth which are symptoms resulting from abnormalities in photosynthesis. Chlorosis is first visible in the younger leaves due to the immobility of Mn in the plant (Single 1958, Single and Bird 1958). There have been reports of small amounts of Mn (relative to the total Mn in the plant) being retranslocated (Williams and Moore 1953, Vose 1963). Observations have been made

in the field of symptoms appearing first on older leaves and extending gradually to the newer growth (Smith and Toms 1958).

In Mn deficient wheat and barley the leaves are pale green with interveinal necrotic spots developing under severe Mn stress. Mn deficient oats develop grey specks ("grey-speck" disease) and collapse, breaking over near the middle of the leaves (Samuel and Piper 1929). In wheat, barley and oats a deficient crop is characterised by pale leaves which become limp and soft to the touch (Smith and Toms 1958).

The reduction in plant vegetative growth is utimately reflected in harvestable yields. Cereal yields on the calcareous sands of South Australia without Mn fertiliser range from 40 to 75% of those with Mn (Reuter *et al* 1973). In severe cases the whole plant dies. The severity of these symptoms is also dependent on seasonal influences. Mn deficiency is worse in cold and wet seasons probably because of reduced root activity affecting Mn uptake (Batey 1971). Mn deficiency can cause decreased yields even in the absence of visual symptoms (Reuter *et al* 1973).

1.4 MANGANESE AVAILABILITY IN SOILS

Mn deficiency was the first recorded trace element deficiency in Australia (Samuel and Piper 1928). "Grey speck" disease of oats was cured by Mn application on the rendzina soils and on calcareous volcanic ash of the Mount Gambier district in South Australia (Samuel and Piper 1928). Other Australian soils affected to varying degrees by Mn deficiency are calcareous aeolian sands, terra rossas, solodized solonetz, lateritic podzolic soils and grey calcareous soils (Stephens and Donald 1958). On the world map Dudal (1976) has described eight classes of soils which have Mn deficiency as one of their characteristics. They are the quartz sands or Arenisols, Chernozems of the sub-humid steppes, Gleysols in which excess water was a major factor in their formation, Histosols which are related to the Gleysols with a high organic matter content, Kastanozems of the subarid steppes, the highly calcareous Rendzinas, Solonetz soils with subsurface clay horizons and Xerosols of the semi-arid areas.

In soils the concentration of total Mn shows an extreme range from values < 20 μg/g to high values > 6000 μg/g (Krauskopf 1972). As with other elements it is not the total amount of Mn present in soil that is important but that fraction of Mn which is "available" to plants (Leeper 1970). In its naturally occurring compounds Mn shows three valences (2^+,3^+,4^+). In reducing environments the stable Mn compounds are those of Mn^{2+} and in strongly oxidising environments the most stable compound is the dioxide, MnO_2. At intermediate redox potentials many compounds may form in which the metal shows two or possibly all three of its valences (Krauskopf 1972). As the divalent cation, Mn^{2+}, Mn is soluble, mobile, and available but as the tetravalent cation Mn^{4+}, practically insoluble, non-mobile and unavailable (Aubert and Pinta 1977). Soil concentrations of Mn "available" to plants are labelled as water-soluble (carbonates, bicarbonates, sulphates), exchangable (extractable by 1N ammonium acetate, pH 7),

dilute acid soluble (2.5% acetic acid, dilute sulphuric and hydrochloric acids) and easily reducible Mn ($MnO_2.nH_2O$ reducible by hydroquinone), (Aubert and Pinta 1977). The oxidation-reduction conditions in the soil such as pH, temperature, moisture and biological activities all affect the transitions and hence the availability of Mn through their effect on redox potential and the hydration-dehydration of Mn compounds (Cheng and Ouellette 1971, Christensen *et al* 1950).

Leeper (1970) represented the cycle of Mn through soils with the following two reactions:

$$(1)\quad Mn^{2+} + 2OH^- + (O) = MnO_2 + H_2O$$

$$(2)\quad MnO_2 + 4H^+ + 2e^- = Mn^{2+} + 2H_2O$$

In equation *(1)* atmospheric oxygen converts the divalent cation to the insoluble higher oxide, a reaction which requires some alkalinity. In equation *(2)* protons are required and the electrons may be supplied from the soil organic matter or from biological processes.

High organic matter content may also decrease Mn availability (Christensen *et al* 1950, Page 1968, Batey 1971, Cheng and Ouellette 1971, Cheng 1973, Pavanasasivam 1973). Page (1968) concluded that the formation of Mn complexes with organic matter at high pH is more important than biological oxidation of Mn; biological oxidation of Mn has long been considered to be one of the most important factors in decreased Mn availability. The work of Bromfield (1950, 1978), Gerretsen (1937), Leeper and Swaby (1940), Timonin (1946, 1950) and Maclachlan (1941) have shown that this oxidation of Mn to unavailable manganic oxides can be mediated by certain micro-organisms.

In highly calcareous soils low in organic matter, many workers believe that the chemical immobilisation of Mn may be more important than any biological effect (Reuter and Alston 1975). Ross and Bartlett (1981) theorized that Mn oxidation is

autocatalytic, involving specific adsorption of Mn^{2+} on existing Mn oxide surfaces. Jauregui and Reisenauer (1982) agreed with these findings but also found $CaCO_3$ depressed plant Mn uptake through adsorption and precipitation or formation of manganocalcite.

All of these factors and their interactions are influenced by soil pH (Sanders 1983, Cotter and Mishra 1968, Cheng 1973). Experiments by Cheng and Ouellete (1971) showed that pH, increased due to liming acid soils, resulted in a threefold decrease in the amount of exchangeable Mn in the soil and a two-fold decrease in the Mn content of the plant.

Conditions which lead to increases in Mn availability include lower pH, higher temperatures, decomposition of organic matter and water-logging. Palaniyandi and Smith (1979) found that leaf Mn contents in snap beans increased with ammoniacal N rather than nitrate-N due to the decrease in rhizopshere pH by absorption of the acidifying fertilizer. High temperatures accelerate reduction of Mn and account for much of the seasonal variation in Mn concentration and in turn, plant growth (Cotter and Mishra 1968). High temperatures enhance the activity of Mn reducing fungi and bacteria. Consequently, *Aspergillus niger* has been used as a microbial fertilizer in slightly alkaline soil to increase the amount of free divalent Mn available for plant uptake (Cheng 1973). Decomposing organic matter promotes reducing conditions through stimulation of reducing micro-organisms as well as through a direct chemical reductive effect (Cotter and Mishra 1968). Waterlogging enhances reduction due to the promotion of anaerobic conditions. Freezing and thawing cycles under flooded conditions increases exchangeable Mn (Cheng 1973). Sterilization of soil may result in toxic levels of Mn by the direct release of Mn complexed with the organic fraction of soil and the killing of Mn oxidising micro-organisms (Boyd 1971).

Physical characteristics also influence Mn availability. Goldberg *et al* (1983) found soil compaction increased Mn availability due to the increased root contact with

the soil. The crystalline structure of Mn oxide compounds also affects their availability. Jones and Leeper (1951) found the Mn oxides most available to plants were those with very small particles. The distribution of soil particles and porosity will influence drainage, aeration, pH and the chemical redox-potential and these overall physical effects interact with chemical and biological activity and govern the movement and availability of Mn. Christensen *et al* (1950) concluded that pH has the greatest effect on soil Mn availability, followed by organic matter decompostion and related microbial activity and moisture.

There are many estimates of soil Mn availability to plants (Baser *et al* 1971, Hoyt and Nyborg 1972, Duangpatra *et al* 1979, Khan 1979, Sheppard and Bates 1982, Schwab and Lindsay 1983); however, they remain unreliable. Bromfield and David (1978) concluded that many chemical procedures used tend to over-estimate easily reducible Mn and often changes in pool sizes may occur during sampling, storage and analysis as a result of changes in soil moisture and temperature (Shuman 1980). In addition, these tests often take no account of the individual requirements of various plant species and cultivars (Cox and Kamprath 1972).

1.5 MANGANESE ABSORPTION AND TRANSLOCATION WITHIN THE PLANT

The absorption of Mn has been shown to occur in two phases. These two phases are characterised by a rapid initial phase and a slower steady state phase (Stiles and Skelding 1940, Stiles and Dent 1946, Rees 1949). It is thought by some workers that the rapid phase is passive uptake, i.e. the diffusion of ions from the external solution into the root cell wall free space as far as the endodermis, and the second is active uptake i.e. requires metabolic energy (Pendias and Pendias 1984). Maas *et al* (1968) using excised barley roots found metabolic inhibitors (low temperature, dinitrophenol, cyanide) prevented Mn uptake during the second, steady state phase. Bowen (1981) using sugar cane leaf tissue found Mn absorption was inhibited by uncouplers of oxidative phosphorylation and was temperature dependent. He concluded that Mn uptake was under metabolic control. Page and Dainty (1964) however, using excised oat roots could find no evidence for active uptake of Mn and concluded the two phases function independently and both are entirely passive. Using sunflower Graham (1979) found no special metabolically-linked mechanism need be postulated to account for absorption of Mn other than that necessary to maintain the transmembrane potential. Evidence to date supports both passive and active uptake of Mn and more definitive studies are required before the mechanism of Mn absorption is resolved.

Modification of the rhizosphere by plant roots may solubilize unavailable oxides. Uren (1981, 1982) found both sterile and non-sterile roots of sunflower seedlings could reduce insoluble higher oxides of Mn directly (i.e. without assistance of rhizosphere organisms) via 'contact reduction'. Non-sterile roots of twelve other plant species could also reduce Mn oxides. These included corn, lucerne, lupins, navy bean, oats, wheat, buckwheat, choumollier, onions, soybeans, sugar beet and vetch.

He proposed that reducing agents were more likely to reduce Mn at the root surface rather than diffusing away from the root as the rapid oxidation of Mn^{2+} would render the reduced Mn unavailable again. Bromfield (1958a,b) found MnO_2 was made available to plants by substances released from the roots of oats and vetch. Other workers have isolated amino acids (Deb and Scheffer 1970, Barber and Gunn 1974), carboxcylic acids (Heintze and Mann 1947, Godo and Reisenauer 1980, Clarkson 1985), low molecular weight organic ligands (Linehan *et al* 1985) and chelating agents (Wallace 1963) from the rhizosphere of plant roots all of which aid in maintaining Mn in an available form. Deb and Scheffer (1970) found this solubility of MnO_2 varied with different amino acids. Linehan *et al* (1985) demonstrated the influence of the rhizosphere by measuring the mobilisation of Mn throughout the growing season which was very low prior to sowing and increased through the season to decline again at harvest. In addition to root-soil interactions, micro-organisms will also be involved. Barber and Lee (1974) found Mn uptake by barley plants grown in solution culture was stimulated in the presence of micro-organisms. They attributed this effect to an unknown water-soluble substance.

A number of cations have been shown to interfere with Mn uptake in short term experiments. Maas *et al* (1969) reported that Mg decreased the absorption of Mn by excised barley roots but that Ca did not. When Ca and Mg were both present, increasing either cation sharply reduced Mn absorption. Kannan (1969) on the other hand, showed that Ca alone markedly inhibited Mn uptake by isolated tobacco leaf cells. Kannan reported that Fe^{2+} also severely depressed Mn uptake an effect also reported by Vlamis and Williams (1962) using barley plants. Schener and Hoefner (1980) found in both maize and sunflower no interactions between Fe and Mn during uptake.

As with uptake, contradiction exists in the literature as to whether Mn is retranslocated within the plant. Williams and Moore (1952) found Mn in the stem of Algerian oats increased until flowering after which Mn decreased as it was translocated

to the developing grain. They found no mobilisation of Mn from the leaves. In contrast, Vose (1963) found what he considered to be physiologically significant redistribution of Mn in oats from older to younger leaves as a result of Mn deficiency stress. Henkens and Jongman (1965) when applying Mn externally to the leaves of wheat, oats, beet and peas found Mn was transported to younger plant parts; however, they found no such transportation in barley. Hannam *et al* (1985) found that Mn in lupins moved via the phloem, possibly mediated by transfer cells from pre-accumulated pools of Mn in roots, stems and petioles to developing sinks, including the seeds. This retranslocation of Mn prevented the development of split-seed in lupins (a symptom of Mn deficiency) if sufficient Mn had been accumulated from the soil prior to the onset of deficiency. In contrast, Single (1958) found no redistribution of Mn in wheat plants moved from high Mn culture treatments to a Mn deficient environment. However, external application of Mn resulted in small (in relation to the amount of Mn supplied and the total plant requirement) but measurable amounts of Mn being translocated to the growing points. He concluded however that Mn was not readily phloem mobile in wheat and that this lack of mobility could not be attributed to precipitation in the tissues (Single and Bird 1958). Nable and Loneragan (1984a,b) found no redistribution of Mn in phloem of subterranean clover during vegetative growth using ^{54}Mn nor did senescence promote Mn redistribution. They point out that as Mn is easily leached from leaves, earlier reports of Mn mobility in phloem from field data could have been a result of leaching by rain, mist or dew rather than internal redistribution.

The form of Mn, whether dissociated or bound, in the phloem and xylem is also unclear. Graham (1979) showed electron paramagnetic resonance spectra of xylem exudate of sunflower similar to that of Mn^{2+} probably bonded to water dipoles. Tiffin (1967) also found Mn^{2+} in tomato xylem exudate. White *et al* (1981) using a computer model determined that Mn, Zn, Ca and Mg were possibly bound by citric and malic acids in the xylem of soybean and tomato although Tiffin found no evidence of this in his experiments using tomato xylem exudates.

1.6 GENOTYPIC VARIATION

To facilitate discussions on genotypic variation in tolerance to Mn deficiency it is useful to define the term 'manganese efficiency'. Throughout this project the nutrient efficiency of a genotype is defined as the ability to produce a high yield in a soil that is limiting in a particular element for a standard genotype (Graham 1984). In this definition a soil type is defined as limiting in a particular element rather than deficient as in many cases the total amount of nutrient held in the soil is adequate but only a small part is available or easily converted by the plant into a useable form.

The most well known example of breeding for nutrient efficiency is that of iron efficiency. Improvement of tolerance to soils low in available iron has been achieved in a number of important crops including maize, sorghum, rice, citrus and soybean (Graham 1984). Clark (1983) has compiled a review citing examples of genotypic differences to ten elements in addition to iron. These include nitrogen, phosphorus, potassium, calcium, magnesium, manganese, boron, copper, zinc and molybdenum.

There is general agreement with respect to the ranking of cereal species to Mn efficiency. Rye is considered to be the most Mn efficient of the cereals followed by barley, wheat and the most susceptible, oats (Gallagher and Walsh 1943, Nyborg 1970, Graham *et al* 1983). In addition to variation between species genotypic differences in Mn efficiency have been observed in oats (Gallagher and Walsh 1943, Ryan 1958, Toms 1958, Munns *et al* 1963, Vose 1963, Deb and Scheffer 1970, Nyborg 1970, Brown and Jones 1974), wheat (Gallagher and Walsh 1943, Nyborg 1970, Graham 1984, Marcar 1986, Graham 1988), barley (Ryan 1958, Sparrow *et al* 1983, Graham 1984, Dick *et al* 1985, Graham 1988) lupins (Perry and Gartrell 1976)

and tolerance to toxicity in soybeans (Brown and Devine 1980, Ohki *et al* 1980, Heenan and Campbell 1980) and cowpea (Horst 1983).

Although there has been much work on identifying genotypic differences in tolerance to Mn deficiency the identification of the mechanism(s) involved and the corresponding genetic controls have yet to be determined. For example, Munns *et al* (1963) when investigating uptake and distribution of Mn in oat genotypes identified three pools of Mn in the roots. These were labelled as non-labile, replaceable and labile based on the ease with which each pool could be extracted. The varietal differences in Mn content of the shoot were attributed to variation in size and rate of turnover of the labile fraction. Vose (1963) however, also with oats found no varietal differences in the ability to retranslocate Mn in the above-ground plant parts. On investigating the effect of the amino acid fraction of root exudate on the absorption of Mn by eight oat varieties in both sterile and non-sterile media Deb and Scheffer (1970) found that under sterile conditions there was no difference in amino acid exudate and concluded that differences in Mn uptake could not be explained by exudation of different amino acids. Nyborg (1970) concluded that the sensitivity of oats to Mn deficiency was due to the poor ability of the plant to take up Mn and not to a higher requirement for the element.

As the mechanism of efficiency remains unidentified, screening for Mn efficiency remains based on factors such as yield, Mn concentration in aerial plant parts or a simple chlorosis score. However with no definite, identifiable mechanism of efficiency, the parameters being measured in the glasshouse or the field must correlate to final grain yield. Baker *et al* (1979) for example, tested P uptake from soils by corn hybrids selected for high and low P accumulation in the field and the glasshouse. They found that ranking depended on the parameter measured and the age of the plant (Table 1.6.1).

Table 1.6.1 Variation in ranking of corn hybrids with plant age and plant part.

Character Studied	Ranking
P in ear leaves (%)	2>3>4>1
P in seedlings in the field (%)	3>2=1>4
P in seedlings in the glasshouse at 3 weeks (%)	2>1>4>3
P in seedlings in the glasshouse at 16 days (%)	3>1>2>4
P in seedlings in the glasshouse at 29 days (%)	4>3>2>1
Weight of seedling plants at 3 weeks (g)	1>3>4>2
Weight of seedling plants at 16 days (g)	1>2>4>3
Weight of seedling plants at 29 days (g)	1>2>3>4
P uptake by seedling radicles (cts/min)	4>2=3=1
P uptake by root interception (%)	1>4>2>3
Grain Yields (kg/ha)	1>2>3>4

(After Baker *et al* 1979)

Other workers have also found poor agreement between rankings in the field compared to glasshouse or solution culture studies with both macro and trace elements such as P, K, Ca, and Mg in wheat and barley (Rasmusson *et al* 1971), P in corn (Fox 1978), and Fe in sorghum (Clark *et al* 1982 and Williams *et al* 1987).

Considerable evidence exists for single gene control of micronutrient efficiency characters (Graham 1984). McCarthy *et al* (1988) studied the inheritence of Mn efficiency in barley and found it to be controlled by a single major dominant gene.

1.7 MECHANISMS OF MANGANESE EFFICIENCY IN BARLEY

In the Introduction ten mechanisms were listed which could possibly explain the differences amoung plant cultivars in Mn efficiency. These can be divided into two groups consisting of those mechanisms which function outside the plant to affect the soil-root interface and those which function internally to affect translocation and utilisation (Table 1.7.1).

Table 1.7.1 Site of Action of Mechanisms of Mn Efficiency

Mechanism	Site of Action
Root induced acidification	rhizosphere
Lower redox potential	rhizosphere
Mn binding ligands	rhizosphere
Increased root absorbing surface	rhizosphere
Increased root absorbing power (Km)	rhizosphere
Modification of microbial balance	rhizosphere
Mycorrhizal associations	rhizosphere
Reduced requirement for the element	internal
Increased translocation from root to shoot	internal
Substitution by another element	internal

It is evident that most of the mechanisms operate in the rhizosphere to either increase the root area in contact with the soil or via exudates to solubilise unavailable Mn oxides. It is believed that in most instances, high nutrient efficiency is related primarily to root growth and activity and only in some instances to transport from the root to the shoot as Mn has low mobility within the plant (Läuchli 1976).

To date, only limited information is available on mechanisms of Mn efficiency but as mentioned in the previous section, much research has been devoted to elucidating the mechanisms of Fe efficiency in both monocots and dicots. As both Mn and Fe are multivalent cations, mechanisms of Fe efficiency may not be specific to Fe. Two seperate mechanisms operate in response to Fe deficiency. Firstly in dicots, and some

monocots, plants are able to solubilise Fe oxides by decreasing their rhizosphere pH through the release of H^+ (Brown 1978) thereby increasing their ability to reduce Fe^{3+} to the more soluble Fe^{2+}. Secondly, in grasses Römheld and Marschner (1986) have evidence for enhanced release of phytosiderphores and a highly specific uptake system for Fe^{3+} phytosiderophores.

1.7.1 The Effect of the Rhizosphere on Mineral Availability

The conditions in the rhizosphere (zone of soil immediately surrounding the root) differ in many respects from those in the bulk soil (soil some distance from the root). Although the chemical and physical properties of the bulk soil are important for root growth and nutrient availability the conditions of the rhizosphere and the influence of the root on it are vital in determining mineral nutrient uptake (Marschner 1986).

1.7.1.1 Changes in rhizosphere pH

Changes in rhizosphere pH are caused predominantly by differences in the cation/anion uptake ratio, in particular with nitrogen supplied as nitrate or ammonium. Ammonium supply is correlated with preferential cation uptake and thus higher net excretion rates of H^+ over HCO_3^- or OH^-; nitrate supply causes the reverse (Raven and Smith 1976, Marschner 1986). Weinberger and Yee (1984) using axenic cultures of wheat grown on nutrient agar found after three weeks that the rhizosphere pH of NO_3^- fed plants had increased to 8.5-9.5 while that of NH_4^+ fed plants had decreased to below 4.0 from an initial pH of 5.4. Plants fertilised with uncharged urea (H_2NCONH_2) showed no significant change. This study demonstrates that the root *per se* is capable of changing rhizosphere pH independent of interactions with rhizosphere micro-organisms.

Changes in rhizosphere pH due to nitrogen supply will affect uptake of other nutrients. Palaniyandi and Smith (1979) found leaf Mn contents were significantly higher in snap beans with ammoniacal-N than those fed with nitrate-N. In contrast, McGrath and Rorison (1982) found both *Bromus erectus* and *Holcus lanatus* grew better with nitrate-N than ammoniacal-N. In that study, ammoniacal-N inhibited Mn uptake and nitrate-N enhanced it. Vlamis and Williams (1962) found that NH_4, Ca, and Mg were effective in reducing Mn absorption and preventing toxic symptoms in barley under acid conditions, probably through ion competition during uptake, whilst Jackson and Williams (1968) (cited in McGrath and Rorison 1982) showed that nitrate stimulated uptake of Mn, Mg, and Sr by wheat. Marschner and Romheld (1983) point out that even within the root system of an individual plant, distinct pH gradients exceeding 2 pH units are sometimes observed along the root axis. Foy *et al* (1965) associated differential aluminium tolerance of two wheat cultivars with plant-induced pH changes around their roots. In a solution culture system the Al-sensitive wheat cultivar was found to lower the pH and the Al tolerant cultivar raised the pH. When considering genotypic differences in efficiency of Mn uptake it is possible that cultivars which can acidify the rhizosphere environment may have a greater capacity to reduce soil Mn oxides and release plant available Mn. Experiments testing this hypothesis are described in Chapter 2.

1.7.1.2 Root Exudates

Growing roots release considerable amounts of organic carbon into the rhizosphere. Three major components are involved: low molecular weight organic compounds (e.g amino acids), high molecular weight gelatinous material (mucilage) and sloughed-off cells and tissues. This organic carbon may affect the solubility in the rhizosphere and uptake of mineral elements either directly (e.g. by chelation) or indirectly (by stimulation of microbial acitivity) (Marschner *et al* 1986). Quantitatively, sugars and organic acids are generally the predominant compounds in root exudates.

However sugars have only minor effects on the mobilisation of mineral nutrients (Marschner 1986). Bromfield (1958ab) and Uren (1982) have shown solubilisation of MnO_2 by unidentified root exudates. Godo and Reisenauer (1980) attributed this mainly to organic acids, particularly in systems more acid than pH 5.5. The amount of Mn dissolved was considerably greater from the rhizosphere than from the bulk soil. They concluded that although pH is a major factor governing the availability of soil Mn, it is significantly influenced by rhizosphere exudates. Phenolics also contribute to the promotion of Mn reduction (Bromfield 1958b, Marschner *et al* 1986).

Invarson and Sowden (1969) investigated the free amino acid composition of the plant root rhizosphere under field conditions and found more amino acids in the rhizosphere than the bulk soil and that the composition varied with the plant species as well as with plant age and moisture conditions of the soil. Deb and Scheffer (1970) however found no effect of the amino acid fraction of root exudates on the absorption of Mn by eight oat cultivars.

In response to Fe deficiency, roots of grasses release non-proteinogenic amino acids such as avenic or mugineic acid (Takemoto *et al* 1978, Sugiuna *et al* 1984). These amino acids, called phytosiderophores, form stable chelates with Fe^{3+} and are highly efficient in mobilisation of both sparingly soluble inorganic Fe^{3+} compounds and Mn^{4+} compounds; however phytosiderophores are not stimulated by Mn deficiency (Zhang *et al* 1991). The rate of release of phytosiderophores is closely related to the iron nutritional status of the plants (Takagi *et al* 1984). Such a compound has not been found for Mn. Godo and Reisenauer (1980) suggest a chelating ligand is necessary to prevent adsorption and its subsequent reoxidation of divalent Mn released by reduction. They propose the hydroxy-carboxylates, citrate and malate may fill this role especially in acid soils. Jauregui and Reisenauer (1982) have shown that during the oxidation of one mole of malic acid to CO_2 at the surface of MnO_2, six moles of Mn^{2+} are released.

1.7.1.3 Micro-organisms

Timonin (1940) estimated the bacterial population in the rhizosphere of wheat, oats, lucerne and peas to be 7 to 71 times greater than in the bulk soil. Actinomyctes and fungi were 0.75 to 3.1 times more numerous. The population density of bacteria in the rhizosphere will vary with the amount of exudate, mucilage and sloughed-off cells. In soil-grown wheat plants under nonsterile conditions during a period of from three to eight weeks, between 20 and 40% of the carbon translocated from the shoots to the roots was lost as organic carbon into the rhizosphere (Martin 1977 cited in Marschner 1986). Most of this carbon is utilized fairly rapidly by rhizosphere micro-organisms. Rovira *et al* (1983) found the number of micro-organisms per unit surface area of roots increased basipetally and increased on older roots but usually covered less than 10% of the rhizoplane. Noninfecting micro-organisms affect the mineral nutrition of plants through their influence on *a)* use of microbial metabolites, *b)* the effect of growth regulators produced by micro-organisms, *c)* solubilisation of minerals, *d)* suppression of plant pathogens, *e)* production of enzymes and *f)* competition of micro-organisms with plants for essential nutrients (Gray and Williams 1971).

In the case of Mn availability, some micro-organisms tend to decrease a plant's ability to take up Mn from the soil by oxidising soluble Mn^{2+} to insoluble Mn^{4+} in the rhizosphere (Gerretsen 1937, Leeper and Swaby 1940, Tinker 1984). Barber and Lee (1974) however found the uptake of Mn by barley plants grown in solution culture to be stimulated in the presence of micro-organisms. Heintze and Mann (1947) found that polycarboxcylic and hydroxycarboxcylic acids as well as pyrophosphoric acids (all of which are normal metabolic products of bacteria), formed complexes with manganic Mn which are soluble over a wide pH range. They suggested that such complex formation would be important in maintaining Mn in an available form in soils. Timonin (1946) in a study on Mn deficient soils determined that a susceptible cultivar of oats harboured in its rhizosphere a denser population of Mn-oxidising bacteria than the

rhizosphere of a resistant cultivar when grown in the same soil and identical conditions. This possibility is examined in this thesis in Chapter 4.

The organic acid component of root exudates are the main solubilisers of mineral nutrients. The main carbon source for rhizosphere micro-organisms is sloughed-off cells and tissues and therefore it follows that at least some of the end products of microbial activity (e.g. organic acids) may have effects on mineral nutrient mobilisation that are similar to those of root exudates (Marschner 1986).

1.7.1.4 Mycorrhizae

Mycorrhizas are among the most widespread associations between micro-organisims and higher plants. Two major groups are involved: ectomycorrhizas and endomycorrhizas. Ectomycorrhizas predominate in tree species of the temperate zone. Endomycorrhizas include the vesicular arbuscular mycorrhiza (VAM) and have a broader range of plant species they can infect. VAM are found on the majority of the world's vegetation. A VAM has three important components; the root itself, the fungal structures within the cells of the root and a extra-matrical mycelium in the soil. As a rule the fungus is strongly or wholly dependent on the higher plant, whereas the plant may or may not benefit from the fungus.

It is well established that VAM can increase plant growth and that growth improvement is greatest in soils of low fertility (Gerdeman 1975). In the majority of cases enhanced P uptake is the primary cause of growth and yield increases. This may lead to a more rapid uptake of other mineral nutrients (Marschner 1986). Khan (1975) found improved growth of wheat seedlings which were infected with VAM in a field deficient in P and grain yield increased three-fold. This difference was eliminated by adding P fertiliser. Bolan *et al* (1987) using different forms of iron phosphates found the greatest benefit to subterranean clover when infected with VAM occured with the least soluble source of iron phosphate. Hall (1978) found highly significant differences in responsiveness to VAM in soil without added P in two cultivars of maize and one of

sweetcorn. He suggests breeders select for responsiveness to VAM at low soil P concentrations and high growth rates to reduce applications of P. This increase in uptake is primarily because of the greater surface area resulting from the growth of hyphae, which may reach distances of several centimetres from the root surface. This extension permits P uptake outside of the depletion zone in the rhizocylinder (Tinker 1984).

VAM infection can also increase the uptake of micronutrients. Manganese, Cu and Zn have low mobility in soil; therefore mycorrhizal infection could possibly improve trace element nutrition by a mechanism analogous to that for P (Tinker and Gildon 1983). Inoculation with VAM fungi alleviated Zn and Cu deficiencies in peach and citrus seedlings (Abbot and Robson 1984). Effects of VAM inoculation in growth of Zn deficient crops have also been shown for cotton and apples (Tinker and Gilden 1983). Killham and Firestone (1983) identified a possible adverse consequence of mycorrhizal fungal infection. Under conditions of acidic and heavy metal deposition VAM infection greatly enhanced metal uptake resulting in metal toxicity and reduced growth.

1.7.1.5 Root morphology

The root system of temperate cereal plants consists of seminal and nodal roots. The seminal roots are initiated first from primordia in the seed using the reserve food in the seed. Later, during tillering the nodal roots develop adventitiously from the basal nodes of the stem (Schuurman and De Boer 1970). The number and type of roots and presence or absence of root hairs clearly influence the nutrition of plants because of the greater soil volume that a large root system can explore and the variation in surface area caused by differences in the degree of branching. Under conditions of nutrient sufficiency growth rates are high in the early stages of plant development. When a luxury supply of nutrients is available root growth is reduced relative to shoot growth (May *et al* 1967). Schuurman and De Boer (1970) concluded seminal roots and the

early initiated nodal roots were the most important part of the root system. The seminal roots constituted the chief absorbing system until half-way into the growing season when the early formed nodal roots were more important on a weight basis than the seminal roots. Although they examined lateral root formation they did not discuss their contribution to absorption.

The development and morphology of the root system will vary under conditions of stress, be it either moisture stress (Hurd 1964, 1968) or nutrient stress. Hackett and Bartlett (1971) found 50-70% reduction in length of unbranched root axes of barley as a result of nutrient deficiency. They also found the form of the profile depended on the cultivar as well as nutrition. O'Brien (1979) concluded that the genetic variability in both extent and direction of the seminal root system in wheat was sufficient for root morphology to be altered by hybridization and selection. Raper and Barber (1970) found efficiency of K uptake per unit root surface was dependent on the soybean cultivar and that the cultivar with the smaller surface area had an absorption potential (Km) double that of larger root systems. Schenk and Barber (1979) concluded the P efficient corn genotypes could be developed on the basis of morphological and physiological root characteristics. Studies using barley showed laterals contributed significantly to P uptake (Scott Russell 1970, Drew and Saker 1978). Hackett (1968) however found K deficiency decreased the length of laterals and inhibited the development of secondary laterals.

Root hairs also contribute to mineral nutrient supply. Barley and Rovira (1970) found root hairs appreciably increased P uptake of pea from clay soil whereas in solution culture uptake of P was not affected by root hairs in wheat and barley. Root hairs enhanced K uptake by 77% in rye grass (Drew and Nye 1969). In contrast, Bole (1973) found root hairs did not increase P uptake in wheat.

Nutrient deficiency can also cause some redistribution of growth within the plant, with roots growing relatively more rapidly than the shoot (Clarkson and Hanson

1980). For example, both P and N deficiency lead to an increase in root length relative to shoot growth. Prolonging the duration of P starvation results in an increase in root dry weight, root length and the roots also become finer. This increases the soil area explored by the roots (Marschner 1986).

Responses to nutrient deficiency have dealt primarily with P and K nutrition. The effect of trace element deficiencies on root growth have not been investigated to the same extent. Cumbus (1985) studied the development of wheat roots under Zn deficiency in solution culture. As with P deficiency there was a change in the partitioning of the dry matter to reduce shoot demand and enhance ion uptake absorption area. The greater surface area of the roots was due to enhanced 1st order laterals, root number and length but there was no significant difference in seminal axes. However, in Mn deficient tomato plants, Abbot (1967) found the formation of lateral roots ceased completely and Burstrom (1950) found cell elongation in barley roots was inhibited by Mn deficiency. Experiments comparing root morphology of Mn efficient and Mn inefficient barley cultivars are described in Chapter 3.

1.7.2 Nutrient Efficiency due to Internal Utilisation and Translocation of Nutrients

1.7.2.1 Mobilisation

The extent of remobilisation of mineral nutrients is attracting increasing attention in connection with the selection of genotypes of high nutrient efficiency. Genotypes that grow well on soils of low nutrient availability may have a higher rate of uptake and translocation of a particular element as well as high rates of remobilisation from older to younger leaves or seeds (Marschner 1986). Furlani *et al* (1984) found P efficient genotypes could distribute P from older to younger developing tissues while inefficient genotypes did not possess this trait. The mobility of Mn in tissues was discussed in Section 1.5. Additionally, there may be benefits to plants in soils with low available Mn from seed loading of Mn or seed soaking with Mn solutions prior to sowing. Marcar and Graham (1986) found significant differences in growth of two wheat cultivars as a result of different seed Mn contents. Seed was obtained from different field sites (giving a Mn content range of 0.1 to 6.4 μg Mn $seed^{-1}$) as well as from soaking seed in $MnSO_4$ prior to sowing. Seed soaking greatly increased the seed Mn content. Only about 15-20% of this additional Mn was recovered in the seedlings after 26 days growth, but this was sufficient to improve seedling vigour. Increased grain yields from seed soaking were evident in the field. They concluded that the seed rather than the soil provided the major source of plant Mn during the first four weeks of growth. The Mn concentrations and contents for the two wheat cultivars used were measured from each of the sites where they were collected. The more Mn efficient cultivar 'Bodallin' could accumulate more Mn into the seeds than the Mn inefficient cultivar 'Bayonet' under identical growing conditions. Increased seed Mn gave 'Bodallin' an advantage over 'Bayonet' under conditions of low soil Mn. Marcar and Graham (1986) stressed that genotypic comparisons of Mn efficiency will be

confounded if seed Mn contents are not considered. Investigations into the heritability of seed Mn content are warranted. Under severe Mn deficiency in the field, the response of wheat plants to foliar Mn application could be improved by the use of seed treated with $MnSO_4$, especially in the absence of soil applied Mn.

1.7.2.2 Utilization

The efficiency with which a plant may utilize an element can be determined by measuring the critical nutrient concentration of that element in the plant. Above this level the plant is healthy and has normal growth but below this level the plant is deficient in the element and growth will be disrupted. Macy (1936) recognised this relationship and proposed a *critical percentage* of each nutrient in each kind of plant, above which there is a *luxury consumption* and below which there is *poverty adjustment* which is almost proportional to the deficiency until a *minimum percentage* is reached. Ulrich (1952) gave three definitions of the critical nutrient concentration: 'the nutrient concentration that is just deficient for maximum growth, that which is just adequate for maximum growth, or the concentration separating the zone of deficiency from the zone of adequacy'. Figure 1.7.2.1 represents the critical nutrient concentration as a narrow range of nutrient concentrations (the Transistion Zone) above which the plant is amply supplied with nutrients and below which the plant is deficient (Ulrich 1952). The relationship between nutrient concentration and yield of plant or product forms the basis of most schemes for using plant analysis to assess nutrient status. In practice the critical nutrient concentration (CNC) is defined arbitrarily as a single value at 90% maximum yield but with confidence limits.

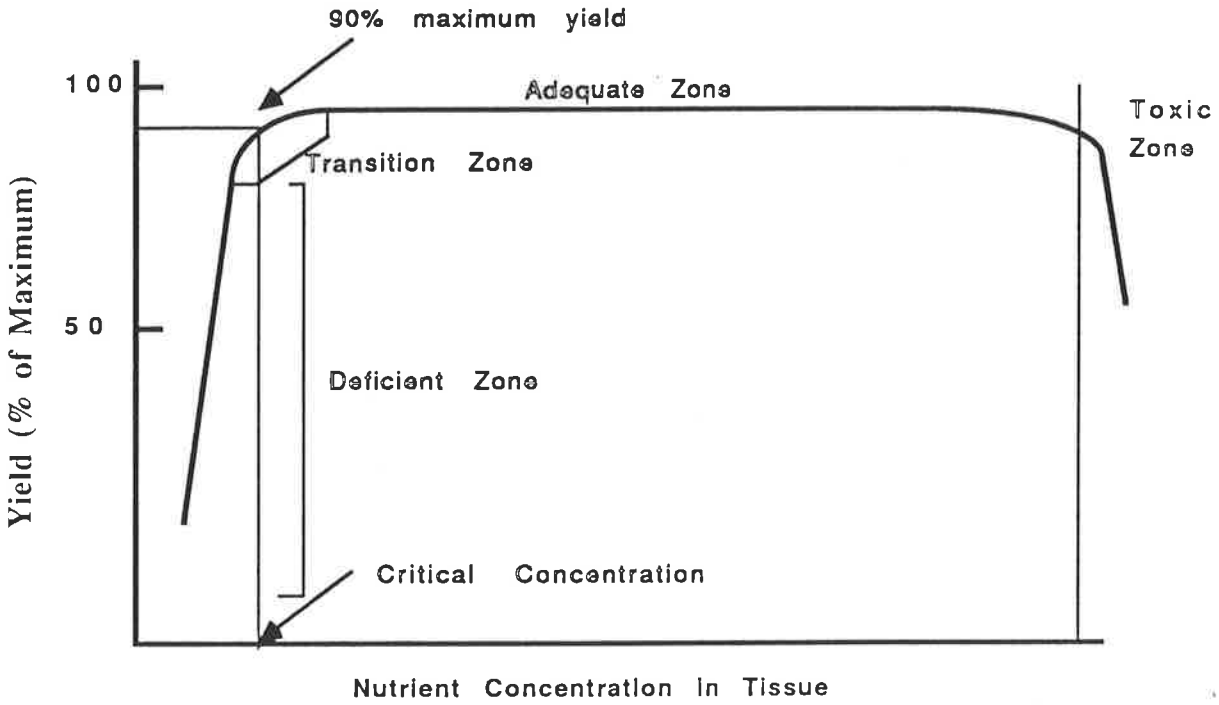

Figure 1.7.2.1 Derivation of critical nutrient concentration (Smith 1977)

Loneragan (1968) gave a formal definition of CNC which has been accepted by plant nutritionists since: 'The minimum concentration of nutrient present in plant organs at maximal growth'. He makes an important distinction between this and the functional nutrient requirement (FNC) which he defines as 'The minimal concentration of nutrient within the organ which can sustain its metabolic function at rates which do not limit growth'. These definitions distinguish between the concentration of nutrient *present* in tissue, organ or organism at maximal growth of the plant (CNC) and the concentration of nutrient *required* for function of that organ or tissue (FNC). These two values will not be equivalent for elements that are immobile in the plant. The CNC will be greater than the FNC.

Nable *et al* (1984) discussed the benefits of using FNC as a critical value for diagnosis of Mn deficiency in subteranean clover. They claimed FNC determined by correlation of nutrient concentrations in young leaves with their biochemical or physiological activities appeared to offer more accurate and consistent standards for diagnosis of plant nutrient status than critical values determined by correlation with plant dry weight. In the determination of the FNC for Mn they measured photosynthetic oxygen evolution. This would be a more sensitive measurement than dry matter yield because as old leaves remain green and retain relatively high Mn concentrations they may be expected to function normally while Mn deficiency is developing in young leaves. In such plants dry matter yield will not be depressed until sufficient leaves become Mn deficient to produce a measurable depression in growth. The sensitivity of biochemical assays is derived from the fact that they measure only the functional component of a nutrient pool whereas foliar analysis measures the total nutrient pool, including non-functional components (Bar-Akiva 1971). Similar assays based on the activity of enzymes have been developed for other elements (N, Zn, Fe, Cu) in other crops (citrus, wheat, maize, vegetable crops, subterranean clover) (Nable *et al* 1984) but as yet are not extensive enough to substitute for dry weight measurements.

Other factors which will influence CNC in plants and its evaluation include the type of culture conditions (solution, pots or field), age of the plant, or plant part analysed, environmental interactions, nutrient interactions (Bates 1971) and interpretation of results (hand drawn curves or computer fitting of non-linear regression models). The critical concentration of Mn in field grown and solution culture grown wheat has been determined and the value in solution culture for all tissues (20 µg/g) (Graham and Loneragan 1981), is considerably higher than that of field grown wheat (11 µg/g YEBS; 12 µg/g whole tops) (Graham *et al* 1985). Graham suggests the higher value may be an artifact of the solution culture system wherein nutrients are fully available until suddenly exhausted. Older parts of one leaf may be high in Mn and

younger tissues quite low as the solution becomes exhausted. The average concentration in such a leaf may then be higher than in the field where availability is controlled. Hannam and Riggs (1987) found no difference in critical level of Mn in barley (12μg/g) between growth chamber and field grown plants. Ohki and Ulrich (1977) found the CNC of Mn increased with plant age in cotton although it was unchanged for Zn. Moody and Edwards (1978) found CNC of P in whole tops of Townsville stylo decreased with plant age, but CNC of apical tissue and leaves did not decrease until after seed set while Robson *et al* (1984) found no change with plant age in CNC of Cu in wheat YEBs (youngest expanded blade).

CNC will also vary with plant part analysed particularly for the immobile elements. Karamanos *et al* (1984) found Mn CNC in oats varied from 9μg/g to 16 μg/g depending on the stage of growth but concluded that the CNC in 'boot' (Feekes 10) stage was the best indicator of yield. However application of fertilser was most effective when the first node of the stem was visible (Feekes 6). Martens *et al* (1977) collected flag leaves and seed of oats and found both to be satisfactory.

The approach taken by most workers is to sample tissue of similar physiological age. Young immature tissues are generally the most sensitive for immobile elements and older tissues the most sensitive for phloem-mobile elements (Smith 1986). The technique most commonly used to obtain tissue of the same age is sampling the most recently matured leaf blade or youngest expanded blade (YEB).

Environmental interactions become most relevant in the field when sampling might occur when growth is limited by factors other than the nutrient of interest such as, water stress, temperature, light, disease, insect attack, abnormal root development, irrigation management or interactions with other nutrient stresses such as nitrogen deficiency.

The most common method of deriving a CNC is by a hand-fitted curve. However computer techniques for fitting non-linear regression models permit more

objective derivation of CNC (Smith and Dolby 1977, Ware *et al* 1982, Graham *et al* 1985). Care must be taken when selecting the model to ensure all the parmeters in the model have a meaningful biological application.

Genotypic differences in CNC have not been demostrated for Mn. Graham *et al* (1985) and Marcar (1985) found the same CNC for Mn in two wheat genotypes differing in their ability to tolerate Mn deficiency. Snowball and Robson (1984) also found the same CNC for Cu in wheat, oats, and barley. Reuter *et al* (1983) found no difference in three subterranean clover cultivars in Cu CNC. In this thesis, (Chapter 5) genotypic differences in critical Mn concentrations are investigated. It is important to recognise that two genotypes having a similar CNC of an element may have very different external requirements for that element (Loneragan 1968). Reuter *et al* (1983) found three cultivars of subterranean clover had the same internal Cu requirement but one cultivar had a significantly higher requirement for external Cu. Snowball and Robson (1984) concluded that differences in growth responses among cereals to applied Cu appeared to be due to differences in Cu uptake rather than to differences in CNC.

1.8 THESIS PROLOGUE

The research in this thesis was undertaken in response to an Australian Research Grant Scheme proposal by Dr. R.D. Graham which was to identify the mechanisms which confer Mn 'efficiency' on some barley cultivars thus enabling them to tolerate soils low in available Mn. Due to the highly calcareous nature of Mn deficient soils, the treatment of Mn deficiency symptoms requires continued application of Mn containing fertilisers. The alternative to fertiliser application is the selection and breeding for Mn efficient cultivars.

To date, little is known about the factors which contribute to Mn efficiency. Ideally the identification of a mechanism followed by a quick and easy method of quantifying or testing for the presence of the mechanism(s) at the seedling stage would enable large numbers of cultivars or breeders lines to be screened for Mn efficiency.

This project sought to identify those mechanisms which confer Mn efficiency on some barley cultivars in the South Australian barley breeding program. Cultivars and breeders lines were classed as efficient or inefficient based on trials carrried out in soils of low available Mn by Dr. R.D. Graham and others on the Eyre Peninsula, South Australia. These cultivars were compared in pot experiments carried out in both soil and solution culture under controlled environment conditions to test four areas which may contribute to Mn efficiency.

The majority of research into mechanisms of trace element efficiencies has been devoted to that of Fe efficiency where root induced pH changes in the rhizosphere of dicots and some monocots play an important role in increasing unavailable Fe compounds. Although rhizosphere pH changes are not involved in Fe efficiency of cereals (Marschner 1986, Römheld and Marschner 1986) this has yet to be shown to be the case for Mn. The effect of root induced pH changes in the rhizosphere of Mn

efficient and inefficient barley cultivars in addition to wheat, triticale and rye cultivars were compared and their influence on the solubility of Mn dioxides assessed.

Secondly, a larger root system will increase contact with Mn compounds in the soil thus improving the plants ability to acquire Mn. The root system geometry of Mn efficient and inefficient cultivars were compared in both soil and solution culture.

Thirdly, a larger population of Mn oxidising micro-organisms was found in the rhizosphere of a Mn inefficient oat cultivar than a Mn efficient cultivar (Timonin 1946). The rhizosphere environment of an efficient and inefficient barley cultivar as well as wheat and rye were compared for presence of populations of Mn oxidising micro-organisms.

Finally, the critical Mn concentration was compared between a Mn efficient and inefficient barley cultivar. A lower internal concentration of Mn may be a mechanism whereby a Mn efficient cultivar would tolerate soils low in available Mn.

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CHAPTER 2

ROOT INDUCED CHANGES IN THE RHIZOSPHERE

2.0 ROOT INDUCED CHANGES IN THE RHIZOSPHERE

2.1 Introduction

Manganese deficiency can be severe on the calcareous sands (pH 8-9) of southern Australia (Reuter *et al* 1973, Graham *et al* 1983). The total Mn content of these soils should be sufficient to meet the demands of cereal crops (Graham 1984) but most of the Mn is present as insoluble Mn oxides. These oxides must be reduced before the Mn is available to plants. Reduction of Mn oxides is favoured by a decrease in pH (Leeper 1970).

Modification of the rhizosphere by plant roots can increase nutrient uptake through a number of non-specific mechanisms. The release of organic acids or preferential cation uptake will lower rhizosphere pH as will the root release of photosynthates which are substrates for rhizosphere micro-organisms (Marschner 1986). The major cause of changes in rhizosphere pH are due to differences in cation/anion uptake ratio, in particular with the form of nitrogen supply. Ammonium supply is correlated with preferential cation uptake (i.e., higher net excretion rates of H^+ over HCO_3^- or OH^-), nitrate supply causes the reverse (Marschner 1986, Raven and Smith 1976, Weinberger and Yee 1984).

The release of H^+ and the subsequent drop in pH of the rhizosphere may increase Mn availability to plants by reducing insoluble Mn oxides (Leeper 1970). The extent of acidification along cereal roots, was tested using the agar method of Marschner *et al* (1982). Cultivars of barley, wheat, triticale and rye (whose level of 'Mn-efficiency' had been assessed in field trials (Graham 1988, Marcar 1986) were grown in solution culture and transferred to nutrient agar 'plates' containing a pH indicator, bromocresol purple. The extent of reduction of Mn oxides by cereal roots was tested in nutrient agar infused with finely dispersed particles of MnO_2. Finally, the extent of pH decreases and reduction of Mn dioxide along barley roots of plants

which had been grown in a highly calcareous and hence, highly buffered soil was assessed on nutrient agar.

2.2 Material and Methods

Experiment 2(a) - pH Changes Along Roots Embedded in Agar

2.2.1 Seed

Eight barley (*Hordeum vulgare*), eight wheat (*Triticum aestivum*), two triticale (*Tritico-X:.secale*) and one rye (*Secale cereale*) genotypes were grown in nutrient solution in 2 litre pots, each pot containing 4 plants of the same genotype. All wheat and barley were grown without Mn while triticale and rye were grown both with and without Mn. The genotypes were chosen and rated as Mn 'efficient' or Mn 'inefficient' on the basis of their performance in field experiments conducted by Dr R.D. Graham on Mn deficient sites at Wangary (barley) and Tooligie (wheat) and by Dr N.E. Marcar at Murdinga (triticale and rye) on the Eyre Peninsula, South Australia.

Seeds were germinated on moistened filter papers in petri dishes at 20^{o}C for 48 hours and placed on grids in 0.5 mM $CaSO_4$ under lights to allow seedlings to deplete their seed reserves of Mn. Seeds were removed after one week and seedlings transferred to pots containing nutrient solutions.

Seeds from the same sources were analysed for Mn content (Table 2.1).

Table 2.1 Seed Mn contents of genotypes used in Experiment 2(a).

Genotype	Classification	Mn Content (µg/seed)
Barley		
Vic77015	Efficient¥	0.303
Stirling	Efficient	0.221
Weeah	Efficient	0.300
WA73S276	Efficient	0.349
WI2585	Inefficient	0.199
WI2616	Inefficient	0.207
WUM143	Inefficient	0.275
Galleon	Inefficient	0.211
Wheat		
Aroona	Efficient	0.259
RAC520	Efficient	0.631
RAC460B	Efficient	0.525
Raven	Efficient	0.328
Bayonet	Inefficient	0.283
Olympic	Inefficient	0.226
Condor	Inefficient	0.304
Millewa	Inefficient	0.250
Triticale		
Venus	Efficient	0.494
Coorong	Inefficient	0.043
Rye		
S.A. Rye	Efficient	0.163

¥ Classified by Graham (unpublished data)

2.2.2 Methods

Plants were grown at room temperature under mercury vapour lamps (photon flux densitiy of 300 µEinsteins m^{-2} $sec^{-1)}$ and a 12 hour day length. Plants were harvested after either 14 or 28 days. At each harvest, two intact plants of each genotype were placed in flat (20 cm by 20 cm and 2.5 cm deep) dishes. Three plants were put in each dish making sure roots from individual plants were not in contact with each other and that different genotypes were placed in separate dishes.

A basal nutrient solution, same concentration as in pots, was made up with agar, 0.75% w/v (Bacto-agar). The pH indicator, bromocresol purple was then added (0.006% w/v) and pH adjusted to 6.0 with NaOH. The agar preparation was poured over roots when it had cooled to approximately 28^0C or just prior to the agar setting. Agar around roots was examined for changes in pH after several hours at room

temperature. The indicator is yellow below pH 5.2, red at pH 6.0 and purple above pH 6.8. Shoots were then removed, weighed and oven-dried at 80^0C before digestion in nitric acid. Tissue was analysed by inductively-coupled plasma (ICP) emission spectrometry (ARL Model 3580) to determine Mn tissue concentrations.

2.2.3 Nutrient Solutions

The nutrient solution was adapted from Nable and Loneragan (1984) and contained the following: 2.5 mM $Ca(NO_3)_2$, 5.0 mM KNO_3, 1.0 mM $MgSO_4$, 0.5 mM $(NH_4)2HPO_4$, 0.015 mM H_3BO_3, 0.01 mM NaCl, $5x10^{-4}$ mM $CuSO_4$, $2x10^{-4}$ mM $CoSO_4$, $2.5x10^{-3}$ mM $ZnSO_4$, $1x10^{-4}$ mM H_2MoO_4, 0.03 mM Fe EDTA and $1x10^{-3}$ mM Mn as $MnSO_4$ where appropriate. Solutions were changed weekly and pH adjusted to 5.5 with NaOH. Double-deionised, distilled H_2O ($DDDH_2O$) was used for all solutions and macroelement stock solutions were stripped of micronutrient contaminants using an 8 hydroxyquinoline controlled pore glass bead column (Eskew *et al* 1984).

Two litre pots were lined with polyethylene bags and the plants supported with non-absorbent cotton wool in non-porous lids. Each pot was aerated from a source of compressed air to maintain circulation of nutrients around roots and to aerate the roots.

Experiment 2(b) - $Mn0_2$ Reduction By Roots Embedded in Agar

2.2.4 Seed

Seeds of two genotypes of barley and wheat and one each of triticale and rye were germinated as described in Experiment 2(a). The Mn efficient genotypes were WA73S276 (barley), Aroona (wheat), Venus (triticale) and South Australian Commercial (rye). The Mn inefficient genotypes were Galleon (barley) and Condor (wheat).

2.2.5 Methods

Plants were grown in solution culture as described in section *2.2.3* with and without added Mn and with five plants per pot. At harvest the plants were placed intact in nutrient agar which contained finely dispersed MnO_2. MnO_2 was prepared by dissolving $KMnO_4$ (final concentration 1mM) in the nutrient agar preparation and heating to 50^0C which was maintained for 2 hours. Finely dispersed MnO_2 was formed which turned the preparation brown. The pH of the preparation was adjusted with NaOH to 6.0. After pouring, plates were covered with aluminium foil (with the tops exposed) and sealed in plastic bags with a damp tissue to minimize water loss for up to 7 days. MnO_2 reduction was observed as disappearance of brown colour in the agar medium around the roots.

Experiment 2(c) -pH Changes and MnO_2 Reduction Along Roots Precultured in Mn Deficient Soil.

2.2.6 Seed

Six barley cultivars, three classed as efficient and three as inefficient were grown in Mn deficient soil. The Mn efficient genotypes were WA73S276, Weeah and VIC77015 and the inefficient genotypes, Galleon, WI2585 and WI2616. The seeds were germinated on moistened filter papers for 48 hours and then transferred to small pots, one plant per pot, filled with a Mn deficient soil.

2.2.7 Soil

Soil, which had previously been established to be low in available Mn (Graham *et al* 1985), was collected from Wangary in 1985. This soil (Uc 1.11, Northcote 1979) is a calcareous sand (80% $CaCO_3$) which has aeolian shell fragments of marine origin as the major component of its parent material. It was collected from two layers, the top (0-10cm) and sub (10-20cm) layers. The pH of these two layers (1:5 H_2O) was 8.9 and 9.1 respectively. After collection, the soil was air-dried and passed through a 2mm stainless steel screen to remove coarse organic matter and large

concretions. Fine organic material was blown from the soil surface by a stream of air. After sieving the soil was dried and sealed in bags. Storage of air dried soil will increase exchangable Mn levels (Boken 1952, Fujimoto and Sherman 1945, Shuman 1980). Therefore, prior to sowing the soil (equal mix of top and sub soil layers) was incubated in four plastic bags each containing 814g of soil at 21% moisture w/w for two weeks in the growth chamber at 15^0C constant temperature. Each bag corresponded to a seperate Mn and nitrogen treatment. $MnSO_4$ was added in the appropriate amounts to each bag prior to incubation.

2.2.8 Nutrients

The following nutrients were added in solution to each batch of soil after the two weeks incubation (mg salt/kg air dried soil) : 65.0 mg K_2SO_4, 37.0 mg $MgSO_4.7H_2O$, 3.0 mg H_3BO_3, 4.0 mg $CuSO_4.5H_2O$, 15.0 mg $ZnSO_4.7H_2O$, 0.5 mg $CoSO_4.7H_2O$, 7.5 mg NaCl, 0.5 mg $H_2MoO_4.H_2O$, 82.5 mg KH_2PO_4, 15.0 mg $FeSO_4.7H_2O$, 525.0 mg $Ca(NO_3)_2.4H_2O$ or 293.7 mg $(NH_4)_2SO_4$ and 552.2 mg $MnSO_4.4H_2O$ where appropriate.

All nutrients were thoroughly incorporated into the soil by hand mixing prior to potting. Each pot contained 44g of air dried soil which was watered during the experiment to 25% w/w at regular intervals.

2.2.9 Design

Three Mn efficient barley genotypes and three inefficient genotypes were grown in Mn deficient soil for 22 days in a growth chamber with 15^0C constant temperature and 12 hour photoperiod (c. 500 μEinsteins $m^{-2}sec^{-1}$). Plants were grown with Mn (+Mn, 136 mg Mn/kg air dried soil) or without Mn (-Mn) and with either NO_3-N or NH_4-N in a factorial design with three replicates. Both nitrogen treatments were added once a week for a total of 10mg N per plant to avoid depletion of NH_4 by nitrification. Pots were randomized every week to minimize shading and edge effects.

After 22 days, plants were harvested intact and the roots of one plant from each genotype were placed in either nutrient agar which contained a pH indicator (referred to as Plant 1) or with finely dispersed MnO_2 (Plant 2). The remaining plant was harvested and the fresh and dry weight of the roots recorded (Plant 3). All shoots were harvested from agar plates, weighed and dried before digestion in nitric acid for analysis by inductively-coulped plasma (ICP) emission spectrometry (ARL Model 3580).

2.3 Results

2.3.1 Experiment 2(a) - pH Changes Along Roots Embedded in Agar

Barley

The concentration of Mn in all genotypes was below the critical level (described in Chapter 5) in whole tops at both harvests with the exception of the Mn efficient cultivar Weeah (Figures 2.1 and 2.4). Top dry weights of barley genotypes (Figures 2.3 and 2.6) were always higher than wheat (Figures 2.9 and 2.12) and about the same as those for triticale and rye (Tables 2.3, 2.6 and 2,7).

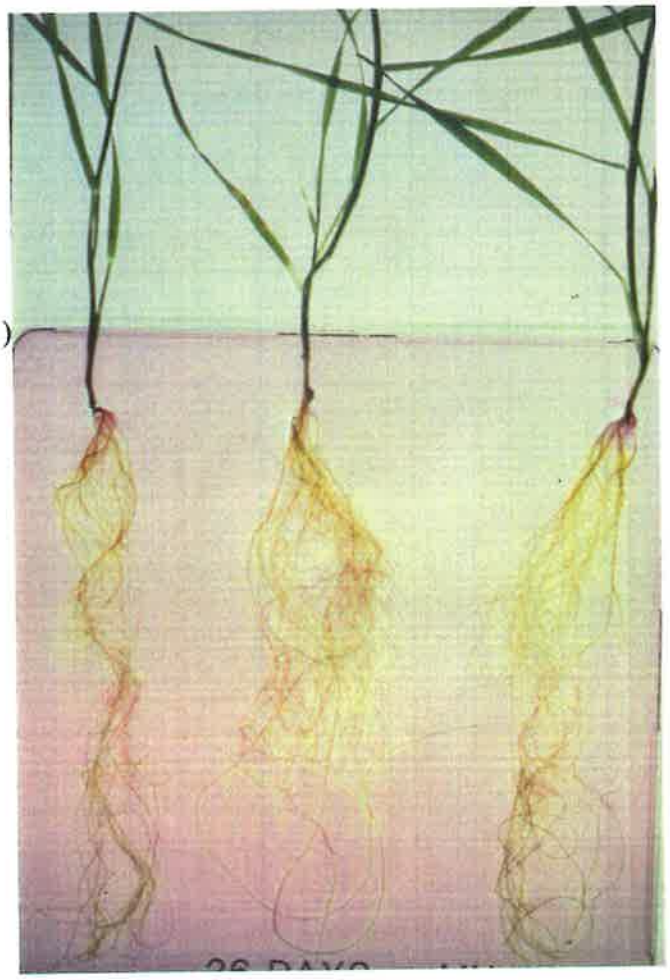
At fourteen days many plants were showing symptoms of Mn deficiency with pale, floppy leaves, floppy stems, stunted young leaves and old leaves becoming necrotic. These symptoms were most pronounced on the genotypes Galleon, WI2585 and WUM143 but Weeah remained a healthy green.

Decreases in pH were detectable in all genotypes at both harvests but there was no relationship between extent of H^+ extrusion and Mn efficiency. Plate (1) is a comparison of barley genotypes grown with and without Mn added to preculture nutrient solutions. The plants in Plate (1) were from a preliminary experiment, using the same culture techniques (but otherwise not discussed in this chapter) and produced identical effects in experiments presented here. Plants with adequate nutrition produced more H ions as a response to normal, healthy growth (Weisenseel *et al* 1979). The Mn deficient plants had a weaker response which could be representative of impaired metabolism. Plate (2) is of a typical plate showing Mn deficient barley genotypes at 14 days with a narrow zone of H^+ extrusion around the roots.

(A)

Plate 1: Effect of Mn nutrition and barley genotype on rhizosphere pH. Plants were grown in solution culture with (A) and without (B) added Mn and harvested after 26 days. Roots were then embedded in nutrient agar containing 0.006% bromocresol purple. L. to R.: Galleon, Clipper WA73S276.

(B)



(A)

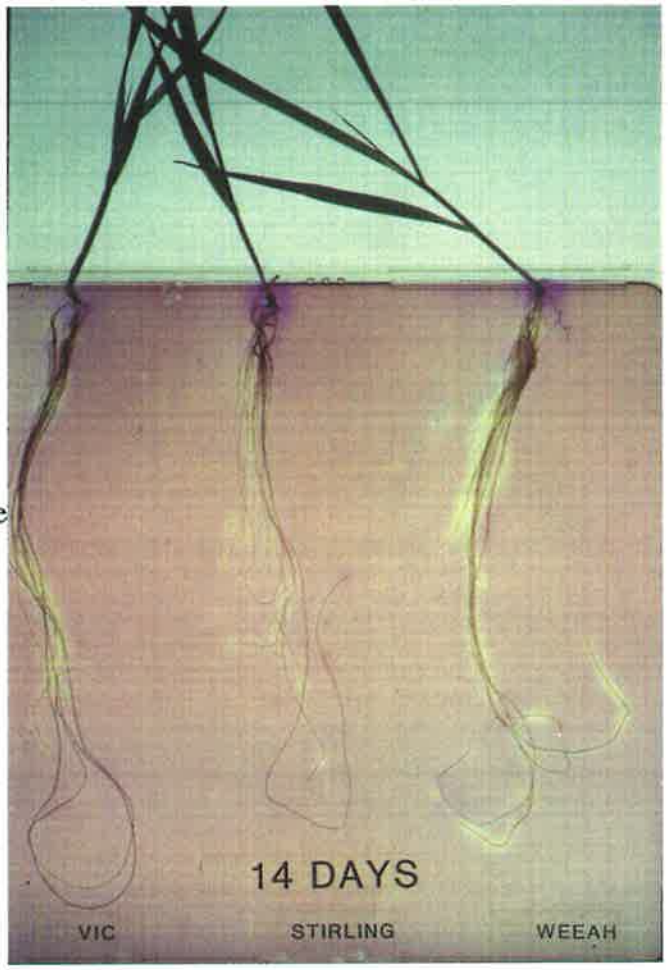


Plate 2: Effect of Mn deficiency on rhizosphere pH. Mn efficient (A) and Mn inefficient (B) barley genotypes were grown in solution culture with no added Mn. After14 days the roots were embedded in nutrient agar containing0.006% bromocresol purple.
Vic - Vic77015
WUM - WUM143

(B)

Wheat

Graham and Loneragan (1981) reported a critical level of Mn for wheat grown in solution culture of 20+/- 2 μg/g for all tissues. Figure 2.7 showed only three genotypes (Raven, Bayonet and Olympic) to be below the critical level. At 21 days all genotypes had 20 μg/g Mn and above in whole tops and these concentrations were much higher than in barley genotypes (Figure 2.10). Although wheat plants were smaller than barley plants the high Mn concentrations in tops of wheat could not be entirely explained by a concentration effect. Mn contents in tops of wheat genotypes were higher than for the barley genotypes at both harvests (Figure 2.8 and 2.11) .

Mn deficiency symptoms were however evident in the genotypes Raven, Bayonet and Olympic at the first harvest. After 21 days one Olympic plant had died but was placed in agar to determine whether associated micro-organisms would have any effect on the agar. It was found that there was no colour change in the absence of the living root.

H^+ extrusion was detectable in all genotypes at both harvests but there was no relationship between extent of H^+ extrusion and Mn efficiency. Plate (3) shows a typical response of the wheat genotypes. The extent of H^+ extrusion appeared to be related to the vigour of the plant. Condor is extremely sensitive to Mn deficiency and the poor vigour of the plant can be compared to the genotypes, Olympic and Millewa which are also sensitive to Mn deficiency.

Triticale

Coorong is a triticale genotype that is extremely sensitive to Mn deficiency. By the second harvest at 21 days all Coorong plants had died. The Mn concentration of 24.5 μg/g in whole tops (Table 2.4) at 14 days in the -Mn treatment would indicate the plants were Mn sufficient, however there was a 408% increase in dry weight of Coorong with the addition of Mn (Table 2.3).

Plate 3: Effect of Mn deficiency and wheat genotype on rhizosphere pH. Plants were grown in solution culture with no added Mn. After 21 days the roots were embedded in nutrient agar containing 0.006% bromocresol purple.

The obvious difference between the triticale response and that of wheat and barley at 14 days was that there were increases in pH around the roots as well as pH decreases (Plate 4). The regions around and immediately behind the root tips were acidic but the remainder of the root showed an increase in pH represented by the purple zone around the roots. At 21 days there was a much larger acidic zone around the roots but patches of increased pH remained.

Table 2.3 Dry weights of triticale genotypes at two harvests at 14 and 21 days (g/plant) †.

Genotype		14 Days	21 Days
Venus	- Mn	0.045	0.062
	+Mn	0.063	0.049
Coorong	- Mn	0.012	¥
	+Mn	0.049	0.060

¥ At the second harvest all plants of the Mn sensitive genotype, Coorong, had died.
† Average of two plants

Table 2.4 Mn concentrations of triticale genotypes at two harvests at 14 and 21 days (µg/g) †.

Genotype		14 Days	21 Days
Venus	- Mn	23.1	24.6
	+Mn	N.A.+	171.5
Coorong	- Mn	24.5	¥
	+Mn	141.9	179.4

¥ At the second harvest all plants of the Mn sensitive genotype, Coorong, had died.
† Average of two plants
+ Data not available

(A)

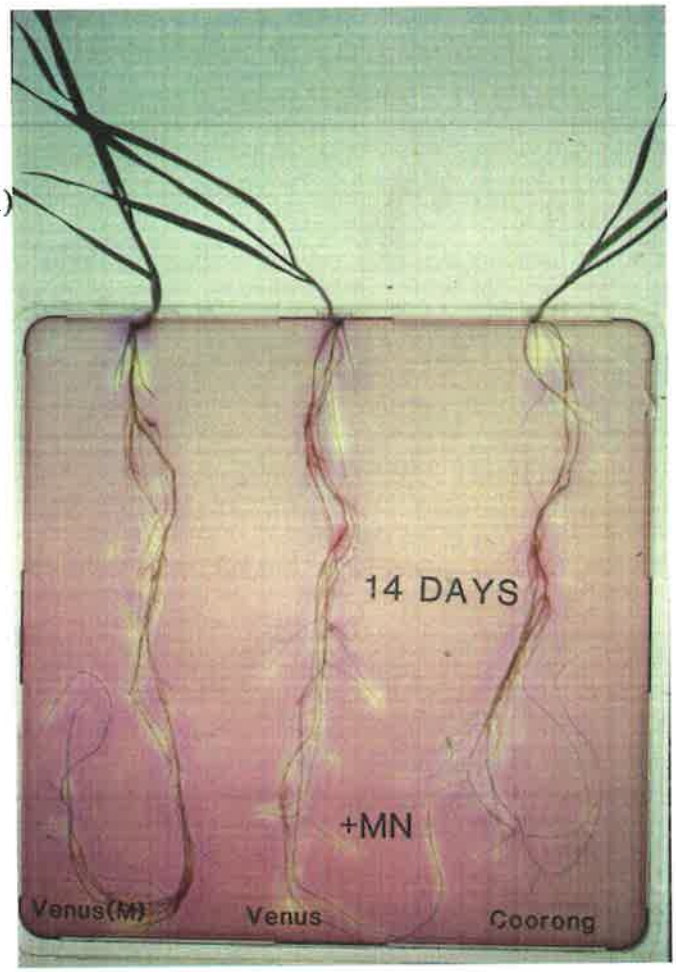


Plate 4: Effect of Mn nutrition and triticale genotype on rhizosphere pH. Plants were grown in solution culture with (A) and without (B) added Mn and harvested after 14 days. Roots were then embedded in nutrient agar containing 0.006% bromocresol purple.

(B)

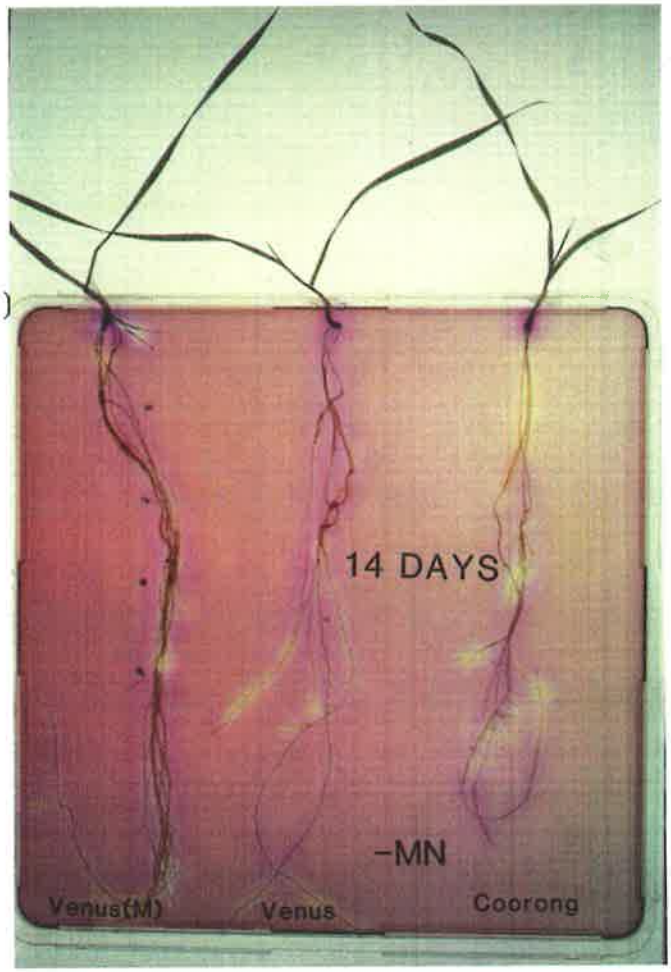


Table 2.5 Mn contents of triticale genotypes at two harvests at 14 and 21 days (µg) †.

Genotype		14 Days	21 Days
Venus	- Mn	1.04	1.53
	+Mn	N.A.+	8.40
Coorong	- Mn	0.29	.¥
	+Mn	6.95	10.76

¥ At the second harvest all plants of the Mn sensitive genotype, Coorong, had died.
† Average of two plants
+ Data not available

Rye

There was a yield increase with the addition of Mn at 21 days which would indicate that the Mn concentration of 14 µg/g in rye was not sufficient for maximum growth (Table 2.6 and 2.7).

The response of rye is different to that of wheat and barley with extensive alkaline zones around the roots at both 14 and 21 days. Unlike triticale, there was no acidification around the root tips. Rye is considered to be more nutrient 'efficient' than either wheat or barley (Graham *et al* 1987). The evidence from the agar plates suggests H^+ extrusion is not important in the solubilisation of unavailable nutrients by rye.

Table 2.6 Dry weights, Mn concentrations and contents of S.A. Rye at harvest at 14 days.¥

Cultivar	Dry Weight (g)	Mn Concentration (µg/g)	Mn Content (µg)
S.A. Rye - Mn	0.064	13.85	0.944
S.A. Rye +Mn	0.042	110.00	4.624

¥ Average of two plants

Table 2.7 Dry weights, Mn concentrations and contents of S.A. Rye at harvest at 21 days.*

Cultivar	Dry Weight (g)	Mn Concentration (μg/g)	Mn Content (μg)
S.A. Rye - Mn	0.107	13.99	1.504
S.A. Rye +Mn	0.233	83.72	19.15

* Average of two plants

2.3.2 Experiment 2(b) - MnO_2 Reduction By Roots Embedded in Agar

All plants in -Mn treatments were Mn deficient. Mn concentrations (Table 2.8) of plants in pH indicator agar were representative of the Mn status of plants after 21 days in solution culture. Mn concentrations of plants harvested after 21 days in solution culture followed by 6 days in agar containing MnO_2 were representative of the amount of Mn absorbed from the agar in addition to that gained in preculture. The Mn content of whole tops (Table 2.9) in the Mn inefficient barley genotype, Galleon was the lowest of all the genotypes in both Mn treatments. Galleon's growth was the most affected by Mn deficiency with dry weight of whole tops approximately one fifth of those of other genotypes (Table 2.10).

Table 2.8 Mn concentrations (μg/g) in whole tops of genotypes of barley, wheat, triticale and rye after preculture in nutrient solutions with or without Mn followed by several hours in nutrient agar containing a pH indicator (Plants 1 and 2) or 6 days in nutrient agar containing finely dispersed MnO_2 particles (Plants 3, 4 and 5).

Genotype	Mn Preculture	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
Barley						
Wa73S276	- Mn	5.9	7.1	38.8	110.2	48.3
	+Mn	37.6	32.3	67.4	82.2	80.7
Galleon	- Mn	9.5	10.1	75.6	141.2	64.2
	+Mn	43.3	52.1	84.6	121.5	77.9
Wheat						
Aroona	- Mn	9.3	10.7	53.2	23.2	33.8
	+Mn	55.2	57.6	78.4	97.2	95.4
Condor	- Mn	12.1	13.1	49.3	68.1	42.1
	+Mn	84.9	104.5	114.4	132.8	113.5
Triticale						
Venus	- Mn	10.5	9.2	42.1	27.9	32.6
	+Mn	67.2	66.8	100.5	112.8	89.3
Rye						
S.A. Rye	- Mn	12.4	14.6	88.9	71.1	56.3
	+Mn	67.2	66.8	129.5	109.8	141.7

Table 2.9 Mn contents (μg) in whole tops of genotypes of barley, wheat, triticale and rye after preculture in nutrient solutions with or without Mn followed by several hours in nutrient agar containing a pH indicator (Plants 1 and 2) or 6 days in nutrient agar containing finely dispersed MnO_2 particles (Plants 3, 4 and 5).

Genotype &	Mn Preculture	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
Barley						
Wa73S276	- Mn	0.5	0.8	6.1	13.0	6.3
	+Mn	14.7	10.4	25.5	35.2	34.9
Galleon	- Mn	0.2	0.2	3.2	4.9	2.4
	+Mn	4.9	7.0	6.3	9.1	8.4
Wheat						
Aroona	- Mn	1.7	1.7	7.4	4.5	5.8
	+Mn	12.8	15.9	20.8	19.9	23.8
Condor	- Mn	1.5	3.0	10.1	16.5	10.3
	+Mn	16.2	17.1	20.5	19.0	22.5
Triticale						
Venus	- Mn	3.3	2.8	15.5	10.3	12.1
	+Mn	19.9	20.5	34.9	43.7	35.6
Rye						
S.A. Rye	- Mn	1.2	4.2	15.7	12.0	5.7
	+Mn	7.5	12.6	26.4	15.4	25.2

Table 2.10 Whole top dry weights (g) of genotypes of barley, wheat, triticale and rye after preculture in nutrient solutions with or without Mn followed by several hours in nutrient agar containing a pH indicator (Plants 1 and 2) or 6 days in nutrient agar containing finely dispersed MnO_2 particles (Plants 3, 4 and 5).

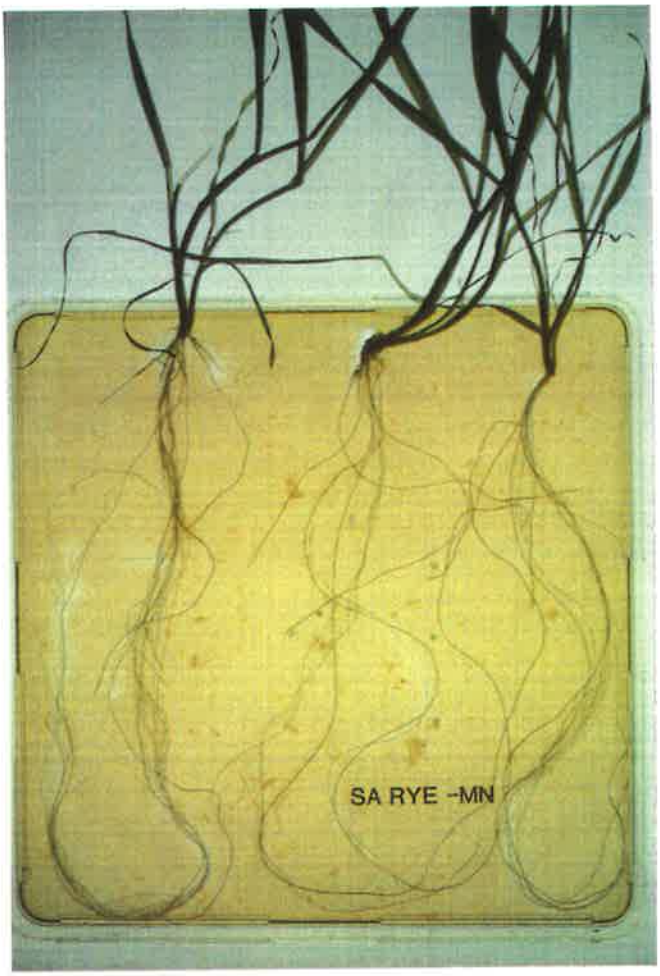
Genotype	Mn Preculture	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
Barley						
Wa73S276	- Mn	0.085	0.119	0.174	0.118	0.130
	+Mn	0.392	0.323	0.379	0.428	0.433
Galleon	- Mn	0.025	0.016	0.042	0.035	0.038
	+Mn	0.114	0.134	0.176	0.075	0.108
Wheat						
Aroona	- Mn	0.186	0.160	0.140	0.195	0.171
	+Mn	0.231	0.277	0.265	0.205	0.249
Condor	- Mn	0.124	0.228	0.205	0.243	0.244
	+Mn	0.191	0.164	0.179	0.143	0.198
Triticale						
Venus	- Mn	0.317	0.306	0.306	0.368	0.372
	+Mn	0.346	0.323	0.347	0.387	0.399
Rye						
S.A. Rye	- Mn	0.094	0.287	0.177	0.169	0.101
	+Mn	0.111	0.189	0.204	0.140	0.178

All genotypes absorbed Mn from Mn dioxide in agar (Table 2.9) but there was no evidence that plants precultured in -Mn solutions absorbed more Mn. Mn uptake was calculated by subtracting Mn contents of plants harvested from pH plates from contents of plants in Mn dioxide. Condor was the only genotype from -Mn precultures that had absorbed more Mn than the corresponding +Mn treatment.

The pH of solution cultures were measured daily (see Figure 2.13 and 2.14). The same trends were seen in both +Mn and -Mn precultures i.e., a steady decrease in pH. The barley genotype WA73S276 and the wheat genotype Aroona lowered the pH more in the +Mn pots in the last week of growth. These plants had the largest root systems which meant they had a greater influence on solution pH . In the first week of growth, roots had very little effect on the pH of the nutrient solution.

Those plants of each genotype which had been embedded in agar containing a pH indicator showed the same responses described in Experiment 2(a). Of the plants

Plate 5: Effect of Mn deficiency on rhizosphere reduction of manganic oxides by S.A. Rye. Plants were grown in solution culture with no added Mn. After 14 days the roots were embedded in agar containing finely dispersed MnO_2. Clearing of the agar represents reduction of MnO_2.



embedded in agar containing MnO_2, only the rye genotype had visible zones of reduction, represented by clearing of the brown colour in the agar, around the roots of every plant in both Mn treatments (Plate 5). Rye plants that had been precultured in -Mn solutions had more areas of reduction around roots than +Mn plants. The only other genotype that had zones of reduction around every plant was the triticale Venus, but only in plants that had been precultured in -Mn solutions and mainly confined to root tips. The wheat cultivar Aroona from the +Mn preculture had a clear zone of reduction around the nodal roots of one plant only. This was also seen around a single nodal root from Venus (+Mn preculture) which had been clearly broken allowing contents of the root cells to leak into the agar. The barley genotype WA73S276 from the +Mn preculture also had areas of reduction around one of the three plants. These isolated areas of reduction could be attributed to root damage which may have occurred at any time during transfer from the solution cultures to the agar plates. The wheat cultivar Condor and the barley cultivar Galleon had no areas of reduction in any plant from either Mn treatment.

2.3.3 Experiment 2(c) - pH Changes and MnO_2 Reduction Along Roots Precultured in Mn Deficient Soil.

After 21 days in Mn deficient soils, plants which had received NO_3 nitrogen were paler, with limper leaves compared to NH_4-N supplied plants. The root systems also differed between the two nitrogen treatments. Those plants which had received NH_4-N had shorter roots which appeared to have a higher branching rate, although this was not measured. This is the reverse of the effect seen in maize described by Marschner (1986). Manganese concentrations in tops of plants grown with nitrogen supplied as NH_4 were higher than those grown with NO_3 (Table 2.11). All genotypes from -Mn preculture were Mn deficient (Plant 3) after 21 days growth in Mn deficient soil and Mn concentration in all tops increased after 6 days on Mn dioxide agar (Plant 2).

Table 2.11 Mn concentrations (μg/g) of barley genotypes after preculture Mn deficient soil with two sources of nitrogen and with or without Mn followed by several hours in nutrient agar containing a pH indicator (Plant 1) or 6 days in nutrient agar containing finely dispersed MnO_2 particles (Plant 2). Plant 3 was not embedded in agar.

Genotype & Preculture			Plant 1	Plant 2	Plant 3
WA73S276	NO_3	- Mn	17.6	60.1	11.3
		+Mn	77.0	173.0	70.2
	NH_4	- Mn	19.2	79.9	16.2
		+Mn	99.9	147.6	75.3
Weeah	NO_3	- Mn	18.4	83.4	13.5
		+Mn	100.1	133.3	81.5
	NH_4	- Mn	21.6	98.4	19.6
		+Mn	105.8	162.9	91.7
Vic77015	NO_3	- Mn	13.4	50.8	11.8
		+Mn	72.0	123.7	64.1
	NH_4	- Mn	15.9	73.3	14.1
		+Mn	79.0	186.5	68.1
Galleon	NO_3	- Mn	14.7	45.4	11.5
		+Mn	92.3	131.4	77.7
	NH_4	- Mn	16.1	79.5	14.5
		+Mn	107.6	143.6	88.8
WI2616	NO_3	- Mn	12.2	104.8	13.2
		+Mn	79.2	120.1	73.8
	NH_4	- Mn	18.4	143.6	15.0
		+Mn	101.6	210.0	78.6
WI2585	NO_3	- Mn	8.5	65.0	9.1
		+Mn	73.3	105.4	63.7
	NH_4	- Mn	12.7	102.1	12.5
		+Mn	89.3	154.5	89.0

Table 2.12 Mn contents (μg) of barley genotypes after preculture in Mn deficient soil with two sources of nitrogen and with or without Mn followed by several hours in nutrient agar containing a pH indicator (Plant 1) or 6 days in nutrient agar containing finely dispersed MnO_2 particles (Plant 2). Plant 3 was not embedded in agar.

Genotype & Preculture			Plant 1	Plant 2	Plant 3
WA73S276	NO_3	- Mn	0.9	5.2	0.8
		+Mn	7.5	15.1	5.4
	NH_4	- Mn	1.6	7.2	1.3
		+Mn	9.2	13.1	5.9
Weeah	NO_3	- Mn	1.5	7.9	1.2
		+Mn	10.4	12.3	7.2
	NH_4	- Mn	2.0	8.4	1.4
		+Mn	8.9	14.7	6.0
Vic77015	NO_3	- Mn	1.2	4.6	0.8
		+Mn	6.3	12.6	4.7
	NH_4	- Mn	1.8	6.2	0.9
		+Mn	5.6	14.9	5.0
Galleon	NO_3	- Mn	1.4	3.7	0.8
		+Mn	10.2	13.1	5.6
	NH_4	- Mn	1.4	7.0	1.0
		+Mn	8.1	14.4	6.0
WI2616	NO_3	- Mn	1.0	8.0	0.8
		+Mn	10.0	13.2	6.3
	NH_4	- Mn	1.0	11.3	1.3
		+Mn	10.0	16.0	5.5
WI2585	NO_3	- Mn	0.4	3.5	0.5
		+Mn	7.5	12.1	5.8
	NH_4	- Mn	1.1	5.6	0.9
		+Mn	9.6	15.1	5.8

Mn contents of Mn deficient plants embedded in dioxide agar (Plant 2, Table 2.12) increased to the level reached by plants after 21 days in +Mn soil, regardless of the nitrogen treatment (Plant 3). The amount of Mn absorbed from the dioxide agar as a function of root dry weight was determined by subtracting the Mn content of tops harvested with no agar treatment (Plant 3) from the Mn content of plants after 6 days in dioxide agar (Plant 2) and dividing by the root dry weights (not shown) but no differences between genotypes or treatments were found.

Plate (6) of plants embedded in agar containing a pH indicator, clearly show the differences in root morphology between the two nitrogen treatments. Soil particles adhereing to the roots can also be seen. The high buffering capacity of the Mn

(A)

Plate 6: Effect of Mn deficiency and barley genotype on rhizosphere pH. Plants were grown in a Mn deficient calcareous soil supplied with nitrogen as either NH_4 -N (A) or NO_3 -N (B) prior to their roots being embedded in nutrient agar containing 0.006% bromocresol purple.

(B)

deficient calcareous soil restricted the decreases in pH along the roots that had been seen in plants grown in nutrient solutions (Experiment 2(a)). There were decreases in pH around root tips of plants grown with nitrogen supplied as NH_4 which was not present in plants supplied with NO_3. There were no differences in response between Mn treatments. An experiment conducted with soil grown barley plants (see Appendix) in which rhizosphere and bulk soil pH's were measured by shaking in 1:5 soil:$CaCl_2$ suspensions also showed no difference between rhizosphere and bulk soil pH.

Plants that had been embedded in agar containing MnO_2 had no areas along the roots which showed any clearing of the brown MnO_2, although the Mn concentration of the tops increased indicating that some Mn had been absorbed (Table 2.12).

2.4 Discussion

The experiments in this chapter tested the extent of H^+ extrusion along roots of different cereal genotypes and if patterns of H^+ extrusion along roots were related to the pattern of solubilization of Mn oxides. A decrease in pH was observed along roots of solution culture grown plants in both barley and wheat genotypes that had been embedded in agar containing a pH indicator. This method of detecting changes in pH enabled sites of pH decrease and increase to be located along the root. In more traditional methods of measuring rhizosphere pH, rhizosphere soil is collected from soil closely adhering to the root and measured with a pH electrode in 1:2 soil:0.01 M $CaCl_2$ suspensions or 1:2 soil:H_2O suspensions (Schofield and Taylor 1955, Smiley and Cook 1972). This method measures an average pH over the entire root system, thus ignoring differences in rhizosphere pH along an individual root. Marschner and Römheld (1983) observed pH differences of up to 3 units along roots of maize plants. Even in soils high in $CaCO_3$, if supplied with NH_4^+-N, microsites of low rhizosphere pH could be demonstrated (Marschner and Römheld 1983). This was also seen in Experiment 2(c) where barley plants grown in highly calcareous soil (pH 9.0) and supplied with NH_4^+-N decreased the pH of agar to below 5.2 at the root tips.

There was no evidence of increased H^+ extrusion along roots of Mn efficient genotypes grown without Mn; therefore Mn efficiency could not be attributed to an enhanced ability to extrude H^+ ions into the rhizosphere. Instead H^+ ions were pumped out in the course of normal growth as demonstrated by Weisenseel *et al* (1979) in young barley seedlings. The rye genotype used in these experiments however, had limited H^+ extrusion while the triticale genotypes were intermediate between rye and barley or wheat in their responses on agar. Rye is considered to be relatively nutrient efficient (Graham *et al* 1981, Harry and Graham 1981, Graham *et al* 1987) however; enhanced H^+ extrusion does not appear to be a mechanism of Mn efficiency in rye.

The chemical reduction of insoluble higher oxides of Mn to the manganous

ion (Mn^{2+}) is a necessary prerequisite for the uptake of Mn by plants. Uren (1981) described the chemical reaction involved with the following equation:

$$MnO_2 + 4H^+ + 2e^- \longrightarrow Mn^{2+} + 2H_2O.$$

The plant itself can be a source of both protons and reducing agents. The work of Uren (1981, 1982) and Marschner (Marschner *et al* 1982) has demonstrated the ability of a number of plants to reduce Mn oxides at the root surface. In this study, only the rye genotype consistently reduced Mn dioxide to an extent that was visible as clearing of the brown MnO_2 in the agar. All the genotypes however absorbed some Mn from the agar (Table 2.9). It is conceivable that Mn^{2+} ions were present in the agar and this would be available to plants (Jauregut and Reisenauer 1982, Jorgensen and Jensen 1984). The Mn content of rye however was not vastly greater than the other genotypes. Neither Uren nor Marschner measured the mineral contents of their plants so the extent of Mn uptake in shoots of plants following reduction of Mn oxides is uncertain.

As rye was the only genotype to visually reduce Mn dioxide but was the poorest H^+ ion extruder it suggests that reducing agents may have been extruded rather than H^+ ions. Uren (1981) observed that the pattern of reduction along the root did not follow that of acid production which suggested that the production of reducing agents was more important than acid production during the reducing process. The roots which had been damaged during transfer from nutrient solutions onto the agar plates had zones of Mn reduction around the damaged root section. Therefore, exudate (phenol) production as a function of wound response or some other components of cell contents were able to reduce Mn oxides.

Changes in rhizosphere pH caused by differences in cation/anion uptake ratio, in particular with nitrate and ammonium supply have been well documented (Marschner 1986, Raven and Smith 1976, Weinberger and Yee 1984). In the highly buffered, calcareous soil used in Experiment 2(c) the form of nitrogen supplied had limited influence on the reactions in the rhizosphere. Decreases in pH to below 5.2 in

agar by plants supplied with NH_4^+-N was restricted to root tips.

The conclusions drawn from these experiments are that H^+ ions are extruded from roots of barley and wheat as a consequence of normal root growth but any pH decreases are severely restricted in highly buffered calcareous soils of high pH. In addition, H^+ ion production was not responsible for the reduction of insoluble higher oxides of Mn as has been shown for Fe efficient dicots and some monocots (Marschner *et al* 1986). However an unidentified component of root cells was able to reduce Mn dioxide when leaked from damaged root cells of barley and wheat. This reducing agent may or may not be the same as that released by rye roots to reduce Mn oxides. Godo and Reisenauer (1980) found exuded compounds from wheat roots, such as hydroxy-carboxylates, increased soil Mn solubility through reducing MnO_2 and complexing the divalent Mn released.

2.5 References

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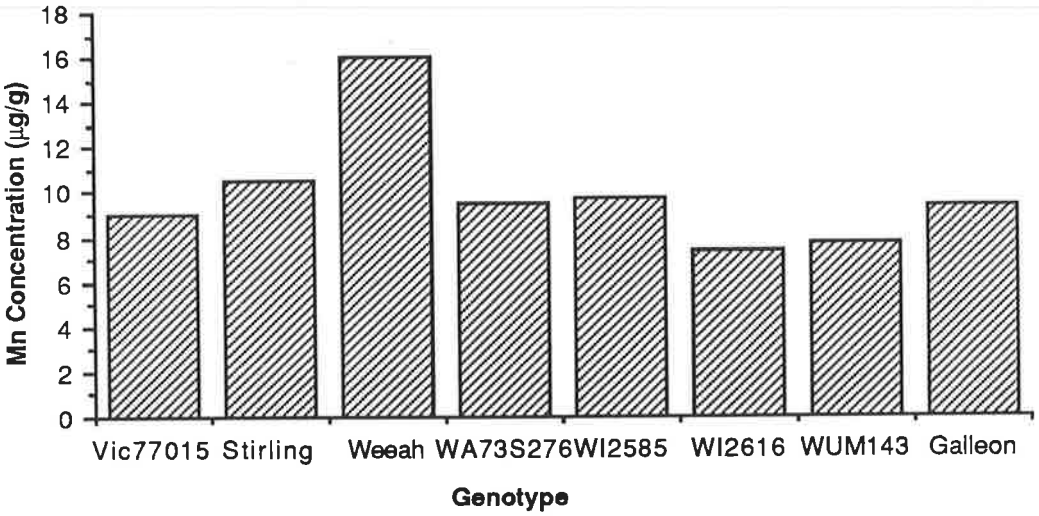
FIGURE 2.1 Mn concentrations (μg/g) in whole tops of eight barley genotypes after 14 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

FIGURE 2.2 Mn content (μg/plant) of whole tops of eight barley genotypes after 14 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

FIGURE 2.3 Dry weights (g) of whole tops of eight barley genotypes after 14 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

Fig. 2.1



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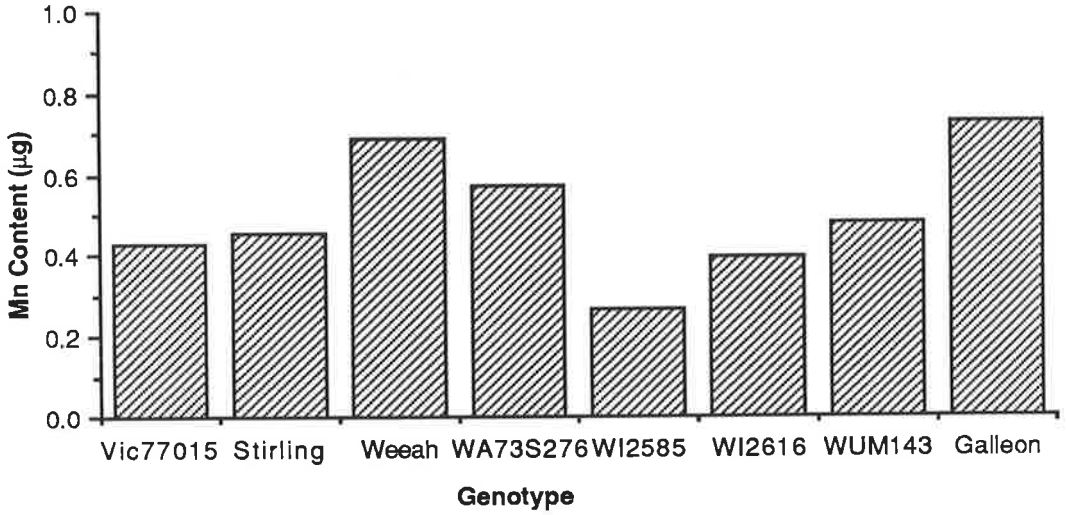

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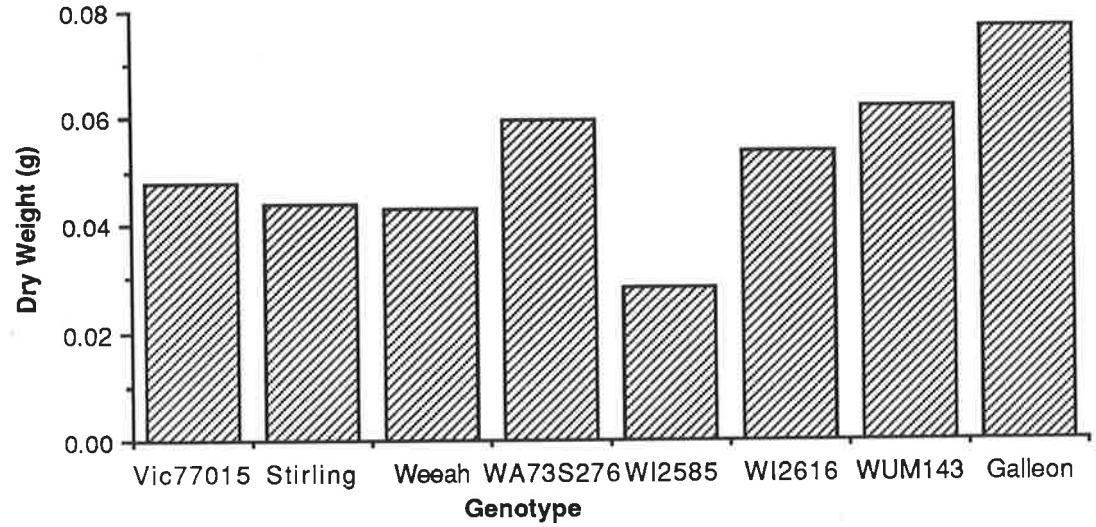
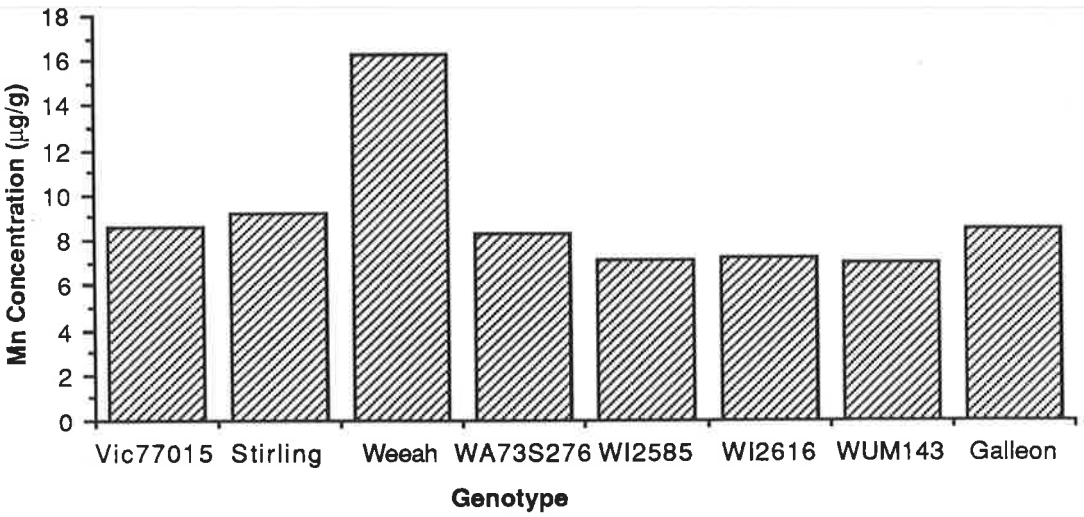


FIGURE 2.4 Mn concentrations (μg/g) in whole tops of eight barley genotypes after 21 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

FIGURE 2.5 Mn content (μg) in whole tops of eight barley genotypes after 21 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

FIGURE 2.6 Dry weights (g) of whole tops of eight barley genotypes after 21 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

Fig. 2.4



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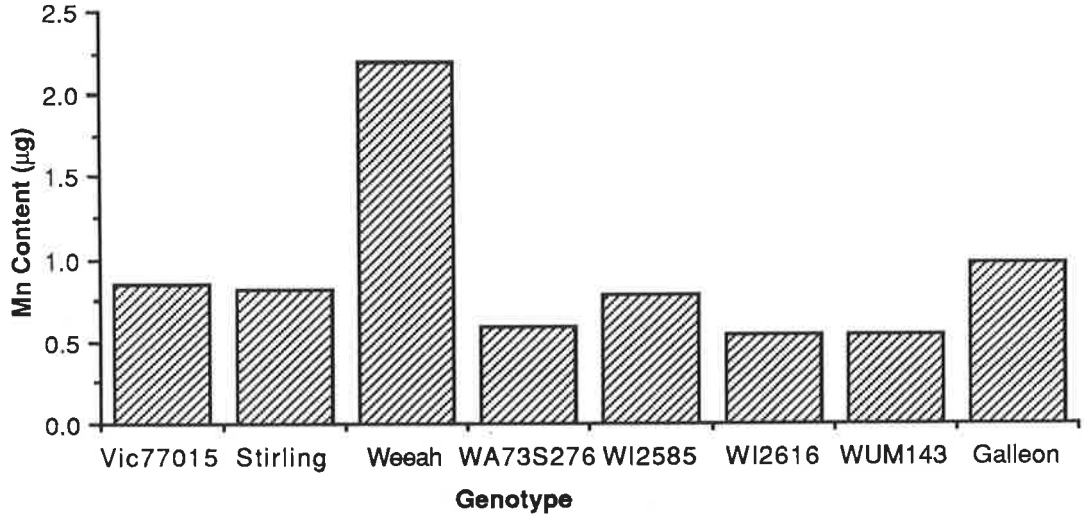

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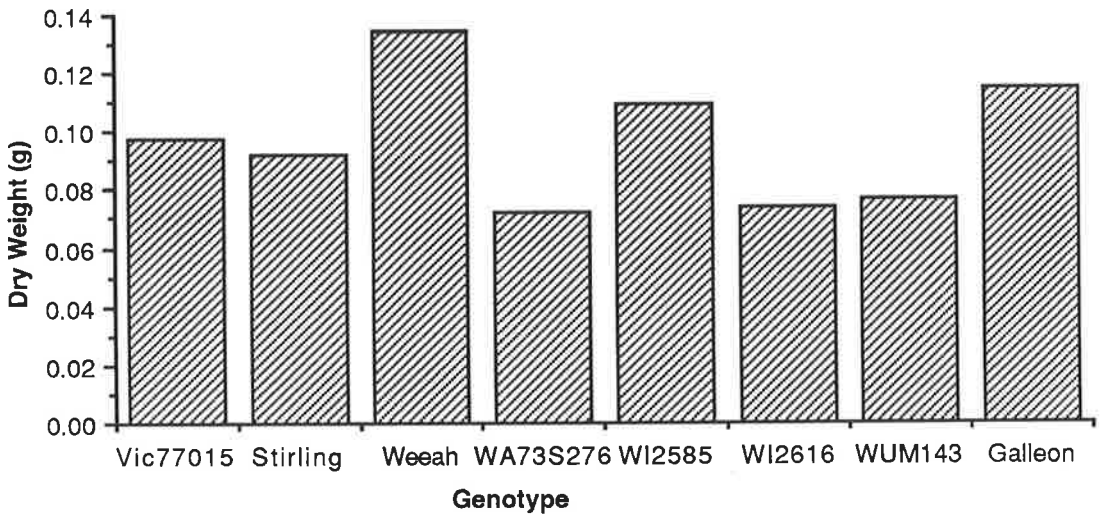
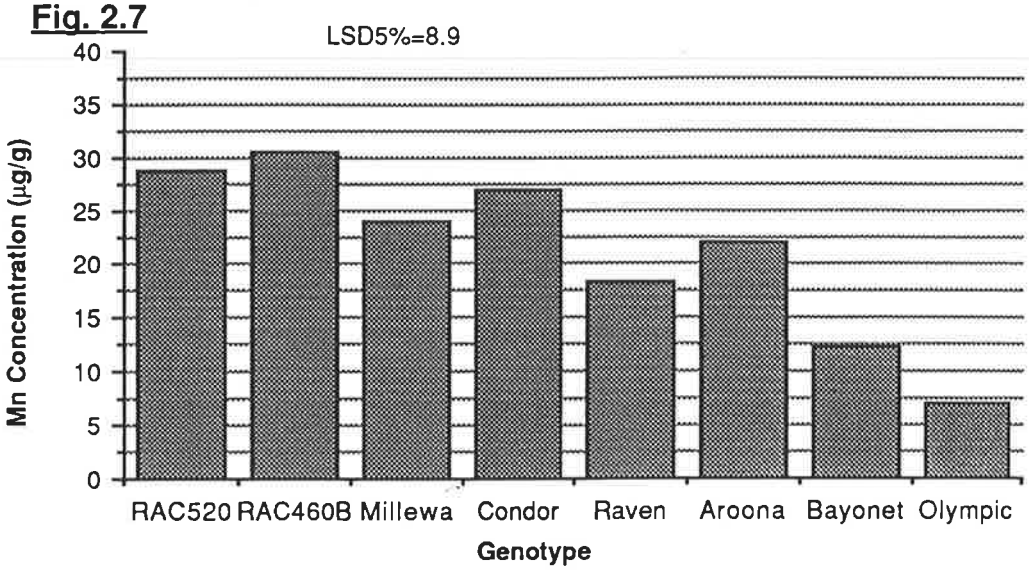


FIGURE 2.7 Mn concentrations (μg/g) in whole tops of eight wheat genotypes after 14 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

FIGURE 2.8 Mn content (μg/plant) of whole tops of eight wheat genotypes after 14 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

FIGURE 2.9 Dry weights (g/plant) of whole tops of eight wheat genotypes after 14 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

Fig. 2.7



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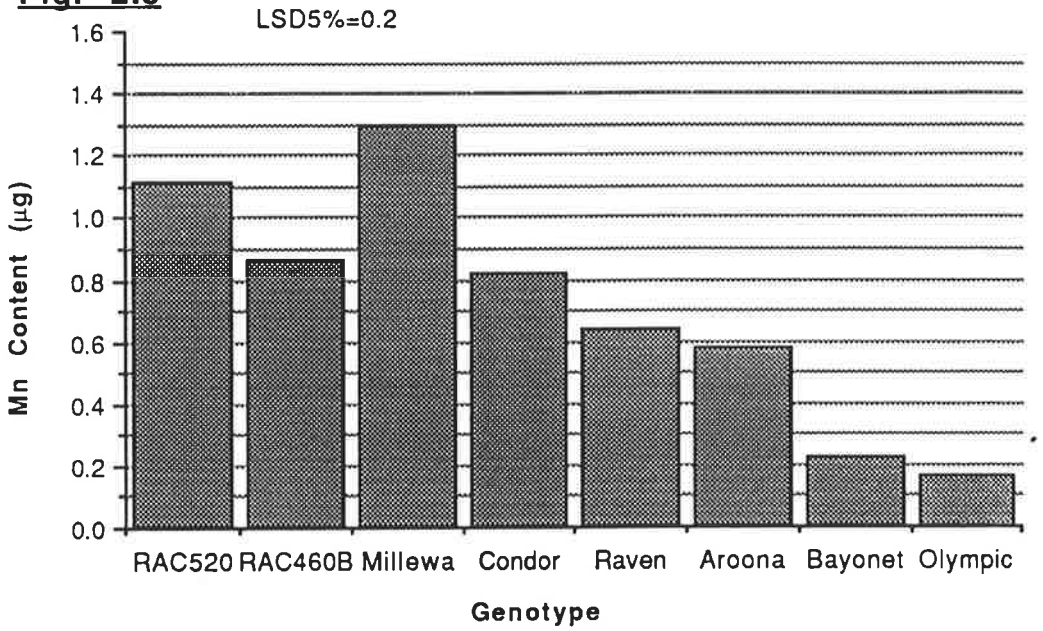

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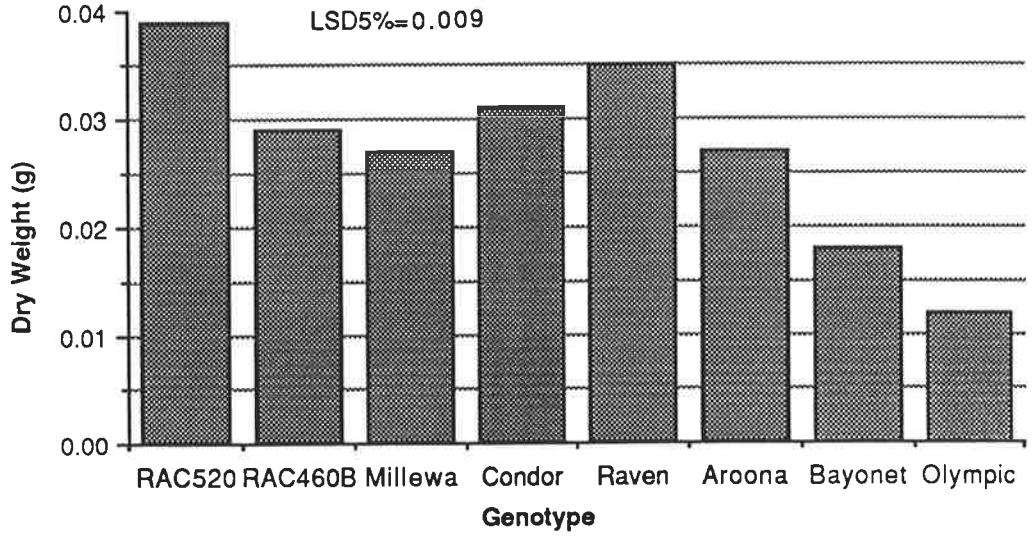


FIGURE 2.10 Mn concentrations (μg/g) in whole tops of eight wheat genotypes after 21 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

FIGURE 2.11 Mn content (μg/plant) of whole tops of eight wheat genotypes after 21 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

FIGURE 2.12 Dry weights (g/plant) of whole tops of eight wheat genotypes after 21 days preculture in nutrient solution without Mn followed by several hours in nutrient agar containing a pH indicator

Fig. 2.10

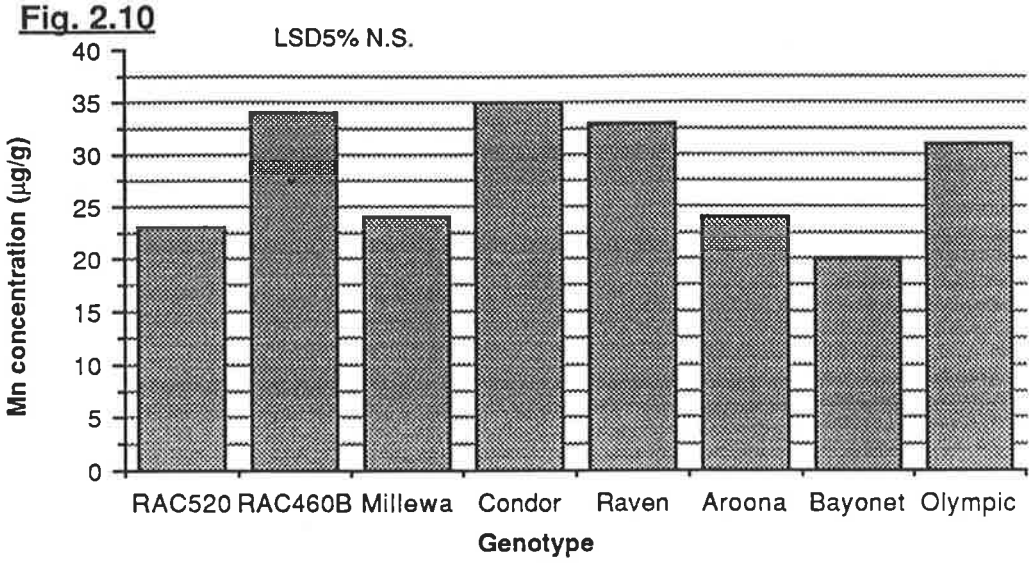


Fig. 2.11

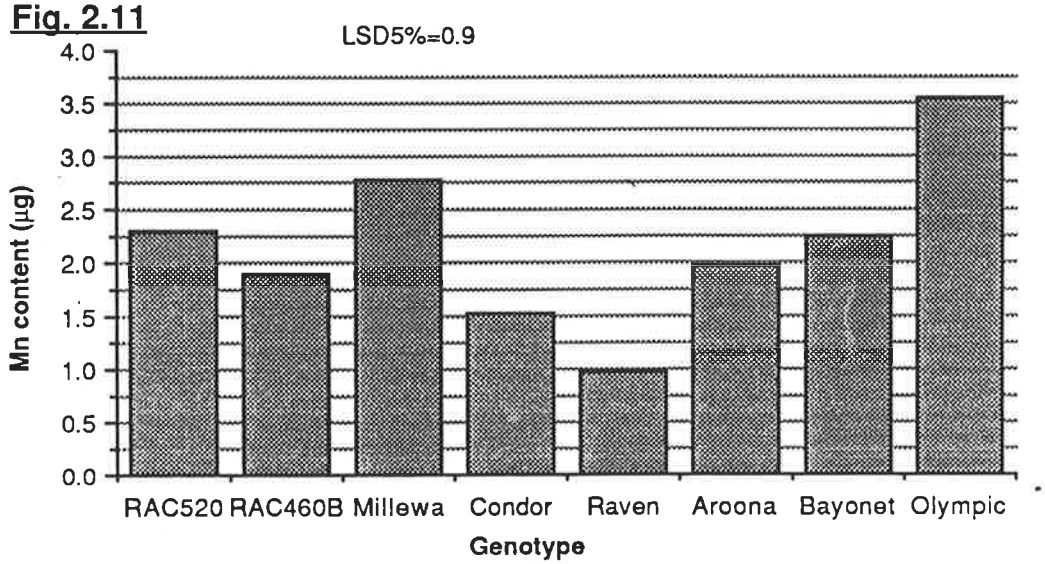


Fig. 2.12

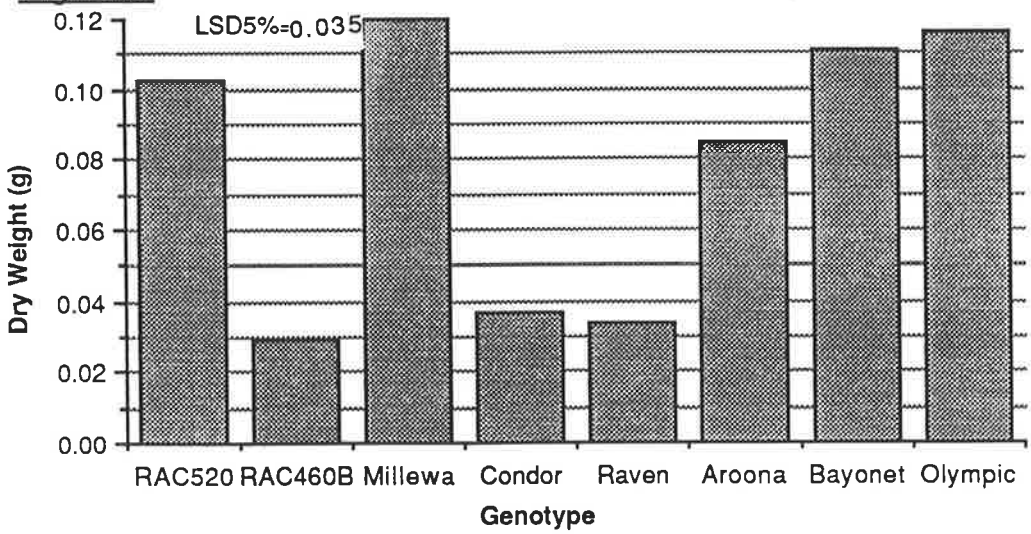


FIGURE 2.13 Changes in pH over 21 days of nutrient solutions with no added Mn supporting the growth of six genotypes. These were two barleys (WA73S276, Galleon), two wheats (Aroona, Condor) one triticale (Venus) and one rye (S.A. Commercial).

FIGURE 2.14 Changes in pH over 21 days of nutrient solutions with added Mn supporting the growth of six genotypes. These were two barleys (WA73S276, Galleon), two wheats (Aroona, Condor) one triticale (Venus) and one rye (S.A. Commercial).

Fig. 2.13

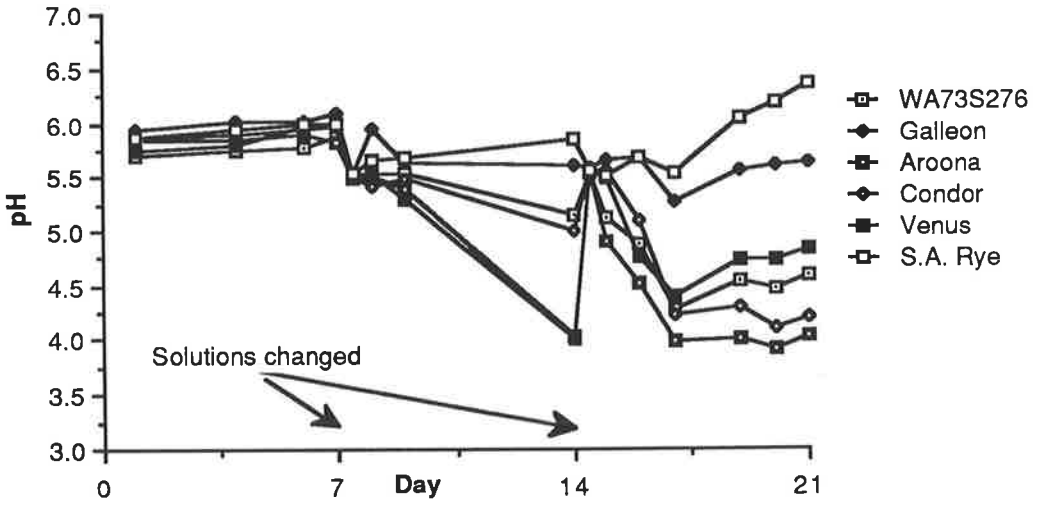
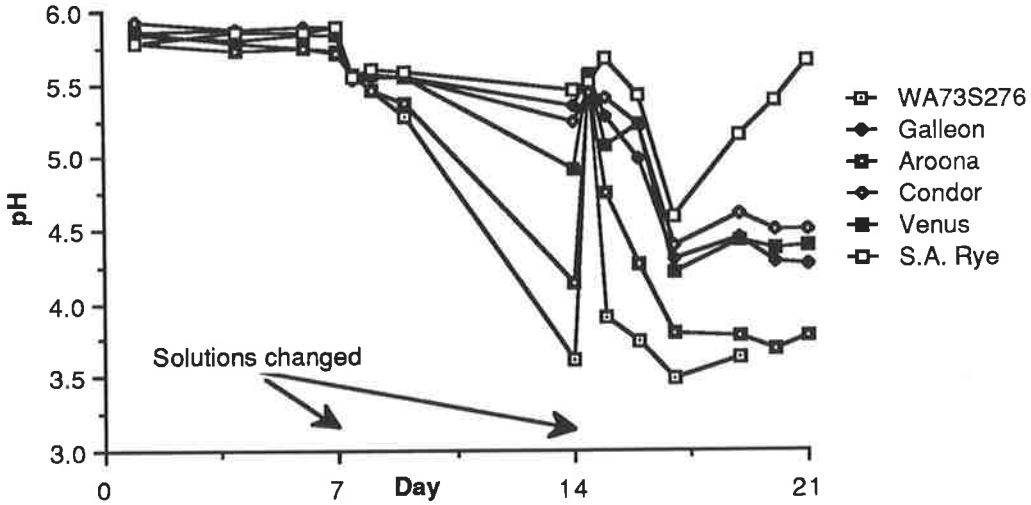


Fig. 2.14



2.6 APPENDIX 2.1

Rhizosphere and bulk soil pH's of soil in which three barley genotypes had been grown were measured. Nitrogen was supplied as either NO_3-N or NH_4-N to measure the effect of the two nitrogen sources on rhizosphere pH in a highly buffered, calcareous soil where Mn deficiency can be severe.

2.6.1 Design

Three barley genotypes were grown for 31 days in pots holding 180g air dried Mn deficient soil collected from the Eyre Peninsula, South Australia. The genotypes were Mn efficient WA73S276, Mn inefficient Galleon and Clipper which is intermediate. Plants were grown with Mn (10 mg Mn/pot) or without and with 10mg N/pot supplied as either $Ca(NO_3)_2$ or $(NH_4)_2SO_4$ with four plants/pot. Nitrogen was added once a week for a total of 40mg N to minimise depletion of NH_4 by nitrification. The experiment was a factorial design with 5 replicates and laid out in a randomized block in a growth chamber at 15^0C constant temperature and 10 hour photoperiod (c. 250 μEinsteins $m^{-2}sec^{-1}$). Seed and soil were from the same source as Experiments 2 (a,b and c) and nutrients were mixed through the soil as described in Section 2.2.8.

Following harvest at 31 days tops were weighed and dried before digestion in nitric acid for analysis by inductively-coupled plasma (ICP) emission spectrometry (ARL Model 3580).

Rhizosphere pH was measured in 1:5 soil:0.01M $CaCl_2$ suspensions with 1g rhizosphere soil (soil which remained on the roots after they had been vigorously shaken) and 5g bulk soil, which had been subsampled from a well mixed bulk. pH was measured after 15 minutes of intermittant hand shaking.

2.6.2 Results and Discussion

The more inefficient genotypes, Galleon and Clipper were Mn deficient whereas WA73S276 with a concentration of 13 μg/g was just sufficient in Mn (see Chapter 5). Mn concentrations were lower in plants that received NH_4-N than those with NO_3-N in both Mn treatments which is opposite to predictions of Raven and Smith (1976).

There was no effect on pH of the bulk soil but rhizosphere pH was decreased by NH_4-N and increased by NO_3-N. Godo and Reisenauer (1980) found the dissolution of MnO_2 increased with acidity and that the amount of Mn dissolved was considerably greater from rhizosphere than from bulk soil. Solubility changes in their studies were, however, drastically increased below pH 5.5 and were virtually undetectable above pH 6.5. Solubility of MnO_2 was attributed to root exudates such as citrate and malate. The pH of the rhizosphere in this experiment did not decrease below 7.65 and therefore would not be expected to influence the solubility of Mn oxides. This method of measuring pH records an average value over the entire root system and cannot take into account any microsites of lower or higher pH that may occur along the root and which could influence solubility of some compounds.

There was a significant genotype effect on rhizosphere pH with the more Mn efficient genotype WA73S276 increasing the pH. Again, such a limited change in pH may have little impact on soil reactions.

Mn Concentration of the Tops (µg/g/pot)

Mn / N	0 (mg) NO_3	NH_4	10 (mg) NO_3	NH_4
Galleon	6.74	5.45	50.41	45.29
Clipper	8.24	7.08	51.90	44.36
WA73S276	12.81	11.51	56.10	47.61

Genotype	***	LSD 0.5%=3.595
Mn	***	LSD 0.5%=2.936
N	***	LSD 0.5%=2.936
MnxN	*	LSD 0.5%=1.828

Bulk Soil pH

Mn / N	0 (mg) NO_3	NH_4	10 (mg) NO_3	NH_4
Galleon	7.784	7.752	7.722	7.716
Clipper	7.752	7.726	7.732	7.736
WA73S276	7.738	7.738	7.720	7.724

N.S.

Rhizosphere pH

Mn / N	0 (mg) NO_3	NH_4	10 (mg) NO_3	NH_4
Galleon	7.746	7.728	7.790	7.658
Clipper	7.802	7.732	7.792	7.650
WA73S276	7.836	7.738	7.822	7.716

Genotype	***	LSD 5%=0.022
Mn	*	LSD 5%=0.018
N	***	LSD 5%=0.018
MnxN	***	LSD 5%=0.026

Bulk Soil pH - rhizosphere pH

Mn / N	0 (mg) NO_3	NH_4	10 (mg) NO_3	NH_4
Galleon	-0.042	0.024	-0.060	0.058
Clipper	-0.050	-0.006	-0.060	0.086
WA73S276	-0.098	0.000	-0.102	0.008

N	***	LSD 5%=0.029

2.6.3 References

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CHAPTER 3

ROOT MORPHOLOGY OF SOME MN EFFICIENT AND MN INEFFICIENT BARLEY GENOTYPES

3.0 ROOT SYSTEM GEOMETRY

3.1 Introduction

The number and type of roots and presence or absence of root hairs will influence the nutrition of plants in a number of ways. A large root system can explore a greater volume of soil and differences in degrees of branching will change root surface area. Studies have shown that genetic variability in root morphology within a single species is large enough to warrant including root moropholgy in the selection process in breeding programs (O'Brien 1979).

Under conditions of nutrient stress the development and morphology of the root system may change. Mn efficient and Mn inefficient barley genotypes were grown in both soil and solution culture to test the hypothesis that under conditions of Mn stress the development and morphology of the barley root system will change.

Experiment 3(a) Root morphology of soil grown plants under conditions of both Mn sufficiency and deficiency.

3.2 Materials and Methods

3.2.1 Design

Six barley genotypes were grown in potted soil with three Mn levels and five replicates. The genotypes were the Mn efficient genotypes Vic77015, WA73S276 and Weeah and the Mn inefficient genotypes Galleon, WI2585 and WI2616. Mn levels ranged from no added Mn (Mn 0), to 68 mg Mn/kg air dried soil (Mn 68) and 273 mg Mn/kg air dried soil (Mn 273). These rates were higher than in previous experiments because the pots were smaller and the Mn was added before incubation. The pots were set out in a randomized block for 28 days in a growth chamber at 15^{o}C constant

temperature and a 12 hour photoperiod with a photon flux density of 250-350 μ Einsteins m^{-2} sec^{-1}. The pots were rerandomized every week to minimise shading and edge effects.

3.2.2 Seed

Seed which had been collected in 1985 from Wangary on the Eyre Peninsula, South Australia was used and two pre-germinated seeds (incubated at 20°C for 24 hrs) were sown per pot. The seed Mn contents were as follows;

Genotype	Mn content
Vic77015	0.30 μg
WA73S276	0.35 μg
Weeah	0.30 μg
Galleon	0.21 μg
WI 2585	0.20 μg
WI 2616	0.21 μg

3.2.3 Soil

Soil, which had previously been established to be low in available Mn (Graham *et al* 1985), was collected from Wangary in 1985. This soil (Uc 1.11, Northcote 1979) is a calcareous sand (80% $CaCO_3$) which has aeolian shell fragments of marine origin as the major part of its parent material. It was collected from two layers, the top (0-10cm) and subsoil (10-20cm) layers. The pH of these two layers (1:5 H_2O) was 8.9 and 9.1 respectively. After collection, the soil was air-dried and passed through a 2mm stainless steel screen to remove coarse organic matter and large concretions. Fine organic material was blown from the soil surface by a stream of air. After sieving the soil was dried and sealed in bags. Storage of air dried soil will increase exchangable Mn levels (Boken 1952, Fujimoto and Skerman 1945, Shuman 1980). Therefore, prior to sowing the soil was incubated in three 1.4 kg batches in plastic bags at 20% moisture w/w for two weeks in the growth chamber at 15°C constant temperature with an equal proportion of top and sub soil well mixed in each bag. Each bag corresponded to a Mn treatment. $MnSO_4$ was added in the appropriate

amounts to each bag prior to incubation.

3.2.4 Nutrients

The following nutrients were added in solution to each batch of soil after two weeks incubation (mg salt/kg air dried soil) : 65.0 mg K_2SO_4, 37.0 mg $MgSO_4.7H_2O$, 3.0 mg H_3BO_3, 4.0 mg $CuSO_4.5H_2O$, 15.0 mg $ZnSO_4.7H_2O$, 0.5 mg $CoSO_4.7H_2O$, 7.5 mg NaCl, 0.5 mg $H_2MoO_4.H_2O$, 82.5 mg KH_2PO_4, 15.0 mg $FeSO_4.7H_2O$, and 525.0 mg $Ca(NO_3)_2.4H_2O$.

All nutrients were thoroughly incorporated into the soil by hand mixing prior to potting. Each pot contained 44g of air dried soil which was watered during the experiment to 25% w/w at regular intervals.

3.2.5 Measurements

After 28 days the plants were harvested, the youngest expanded blades (YEBs) were sampled and fresh and dry weights of tops, roots and crowns were recorded. The roots were assessed for seminal and nodal number, seminal, lateral and nodal length and diameter, seminal, lateral and nodal branching rates and from the root length and diameters the surface areas and volumes of each root type were calculated. Branching frequency was estimated by placing a length of root along a 2cm grid and counting the branches. This was repeated 10 times at random for each root type. Diameters were measured using a calibrated eye-piece micrometer and low power microscope. Ten diameters for each root type were recorded. Root length was determined by spreading the roots in water in a large petri dish over a 2 cm or 1 cm grid of lines and using the line-intercept method (Tennant, 1975). The tops, YEBs and roots were digested in nitric acid and analysed by inductively-coupled plasma (ICP) emission spectrometry (ARL Model 3580).

3.2.6 Statistical Analysis.

The data were subject to analysis of variance using Genstat IV statistical package. Significant mean separation is indicated by the use of the least significant difference (LSD) at the 5% level where the F value is significant.

3.3 Results

Weeah had the highest Mn concentration in whole tops at Mn 0 with 16.2 µg/g and WI2585 the lowest with 7.3 µg/g (Table 3.1 & Fig 3.1). Mn concentrations in YEBs, which are sensitive to the level of nutrient supply, particularly for immobile nutrients such as Mn (Smith 1986), were lower than whole top concentrations but YEB Mn concentrations in the WI breeders lines only were below the critical level at Mn 0. At the higher Mn rates Mn concentrations were higher in YEBs than whole tops. The Mn inefficient breeders' lines WI2585 and WI2616 were both severely Mn deficient at Mn 0 and there was a large increase in biomass (total fresh and dry weights, Fig. 3.2 & 3.3) of both genotypes with the addition of Mn. Addition of Mn did not increase total biomass of the efficient lines, Weeah, WA73S276, and VIC77015. The biomass of Galleon, a Mn inefficient cultivar, with 13 µg/g Mn in its tissues at Mn 0 also increased with the addition of Mn, but only with the middle Mn rate. Weeah had the highest Mn content regardless of Mn treatments (not shown).

At the highest Mn treatment both shoot and root growth of the more Mn-inefficient genotypes were as good as the Mn-efficient genotypes. Differences in growth at Mn 0 are attributed to the sensitivity of the more Mn-inefficient genotypes to Mn deficiency.

Shoot biomass (Fig. 3.4) of the efficient genotypes was significantly greater

($P<0.05$) than the Mn-inefficient genotypes at Mn 0. Large increases in root biomass of the inefficient genotypes occured with the addition of Mn (Fig. 3.5). There was a small and non-significant increase in root biomass of the Mn efficient genotypes with the addition of Mn but no such increase occured in shoot biomass.

There were no significant differences in root:shoot ratios with Mn treatment in efficient genotypes (Fig. 3.6). Weeah maintained a high root:shoot ratio at all Mn levels. There were however significant increases ($P<0.005$) in root:shoot ratios with the addition of Mn in the inefficient genotypes. At high Mn treatments root:shoot ratios had increased to a level equal to that of the efficient genotypes so that under conditions of adequate nutrition there were sufficient roots supplying the shoots. Under Mn deficiency however the root system was much smaller relative to the shoots indicating the greater sensitivity of the roots of these genotypes to Mn stress.

Root morphology was assessed by measuring root lengths, diameters, surface areas, volumes and branching rates. Total root lengths of the WI lines at Mn 0 were significantly less ($P<0.005$) than for other genotypes (Fig 3.7). Both WI2616 and WI2585 had shorter 1st order laterals on seminal roots (Fig 3.8 & 3.9); while WI2616 also had shorter seminal roots, seminal root length of WI2585 was not significantly different from other genotypes. There were no significant differences in nodal root lengths between genotypes. Root lengths were significantly increased with the addition of Mn only in the WI lines.

Total root surface areas of the WI lines were significantly smaller than other genotypes at Mn 0 and increased with the addition of Mn to the greatest extent (Fig 3.10). There were no significant differences between genotypes in nodal surface areas. However there were large differences between genotypes in first order lateral surface areas but only small differences in seminal root surface areas (Fig 3.11 & 3.12). The trends reflected those of root length.

Root volumes showed similar trends to root lengths and surface areas, the WI

lines increasing in total volume with the addition of Mn at a greater rate than the more Mn-efficient genotypes, and Galleon was intermediate. The lateral volumes of the WI lines were more sensitive to Mn deficiency than the seminal roots (i.e. larger increases with the addition of Mn (Fig 3.13 & 14)). The relative sensitivity of lateral roots to Mn deficiency of Mn-inefficient barley genotypes was clearly demonstrated in the root length, surface area and volume data.

First order lateral branching rates (Fig 3.15) were measured on seminal and nodal roots (not shown) of each genotype at all Mn levels. Seminal branching significantly increased with the addition of Mn in the Mn-inefficient WI lines. Branching rates were also significantly less in the WI breeders lines at Mn 0 than other genotypes. No significant differences were found in second order lateral branching rates on either seminal or nodal roots (data not shown). In addition lateral roots initiating from nodal roots were poorly developed.

There were no significant differences in root diameters except in nodal roots (Fig 3.16) which were finer in the WI breeders lines at Mn 0. There were large increases in nodal diameter with the addition of Mn to the WI breeders lines. This was the only nodal root measurement which showed treatment differences. However, at harvest, nodal roots constituted only a small proportion of the total root system.

3.4 Discussion

Marcar (1986) compared root length and diameters of two wheat cultivars at different Mn levels. He found there was an intrinsic difference in root morphology between the two cultivars with the more Mn-efficient cultivar having a larger root system due to proliferation of lateral rather than seminal roots regardless of the rate of supply of external Mn. In this experiment under conditions of Mn sufficiency there were no significant differences in root morphology between genotypes. At very low levels of external Mn supply (Mn 0) the root morphology of the more efficient genotypes remained unchanged. However, the WI lines proved to be extremely sensitive to low levels of soil Mn resulting in significantly smaller root systems particularly as a result of poor 1 st order lateral root development. The lowest Mn rate (Mn 0) did not induce Mn deficiency in all genotypes used in this experiment. The breeders lines WI2585 and WI2616 were suffering from severe Mn deficiency at Mn 0 whereas the efficient genotypes had sufficient Mn in their tissues to maintain normal growth, with Galleon intermediate. Therefore, whether or not the root morphology of the more Mn efficient genotypes changes under Mn deficiency could not be established. However, these genotypes were able to maintain Mn sufficiency in their tissues at a level of soil Mn that was insufficient to support normal growth for Galleon and the WI breeders lines. In the higher Mn treatments, the WI lines and Galleon had the same root dimensions as the more efficient genotypes with similar Mn concentrations and contents in shoots.

The level of available soil Mn would need to be an order of magnitude lower again so the more Mn efficient genotypes would be under the same Mn stress as the inefficient lines. If a change in root morphology was the mechansim of efficiency then

a change in root:shoot ratios in the direction of increasing root mass at the expense of shoot biomas would perhaps be expected as has been observed for example in wheat with the trace element Zn (Zhang *et al* 1989) and with the macro element P in buckwheat (Amann and Amberger 1989). In this experiment there was a small and non-significant increase in root biomass of the Mn efficient genotypes with the addition of Mn but no concurrent increase in shoot biomass which perhaps indicates a similar sensitivity of the roots to Mn deficiency as the more inefficient genotypes.

Marschner (1986) noted prolonged P starvation resulted in roots becoming finer. In this experiment the root diameters of seminal and their first order lateral roots did not change with Mn nutrition. However, nodal roots of the Mn-inefficient genotypes WI2585 and WI2616 at Mn 0 were significantly finer than other genotypes. Nodal roots however constituted only a small portion of the total root system at the time of harvest (28 days).

3.5 References

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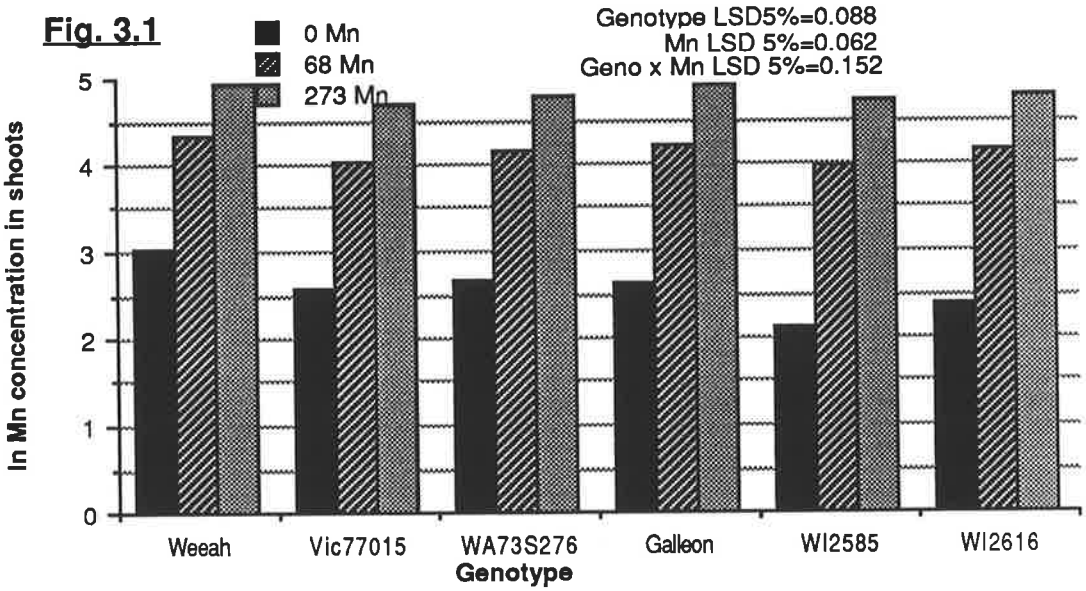
TABLE 3.1 Mn concentration in whole shoots of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

FIGURE 3.1 Ln transformation of the natural data from Table 3.1 to a obtain a normal distribution of variance when analysed. Ln Mn concentrations of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

TABLE 3.1 Mn concentrations in whole tops of six barley genotypes at 3 levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

Genotype	0 Mn	68 Mn	273 Mn
Weeah	16.2 (15.3)¥	74.8 (81.4)	135.1 (137.1)
Vic77015	12.1 (10.6)	57.2 (66.9)	109.8 (126.1)
WA73S276	13.3 (13.8)	63.4 (77.7)	120.2 (145.1)
Galleon	12.9 (11.6)	66.7 (83.0)	135.1 (168.4)
WI2585	7.3 (6.5)	53.0 (60.0)	112.5 (136.7)
WI2616	10.1 (8.4)	63.6 (64.8)	121.0 (118.1)

¥ Numbers in parentheses are YEB concentrations measured after bulking replicates to obtain sufficient material for analysis.



FIGURES 3.2 & 3.3 Total fresh and dry weights (shoot + root, g/pot) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

Fig. 3.2

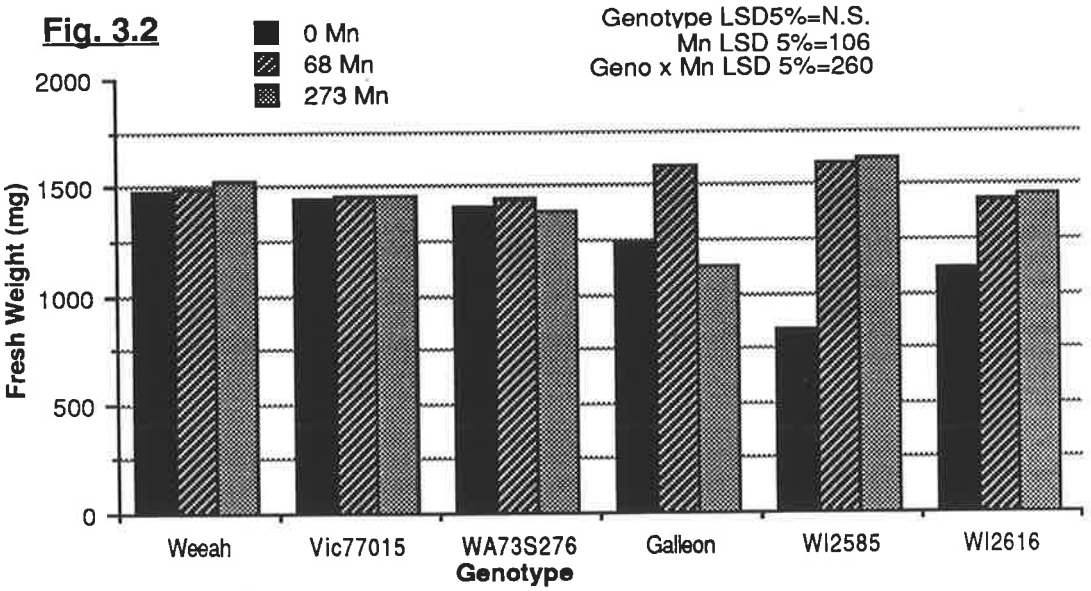

0 Mn
68 Mn
273 Mn
Genotype LSD5%=N.S.
Mn LSD 5%=106
Geno x Mn LSD 5%=260
Fresh Weight (mg)
2000
1500
1000
500
0
Weeah
Vic77015
WA73S276
Galleon
WI2585
WI2616
Genotype

Fig. 3.3

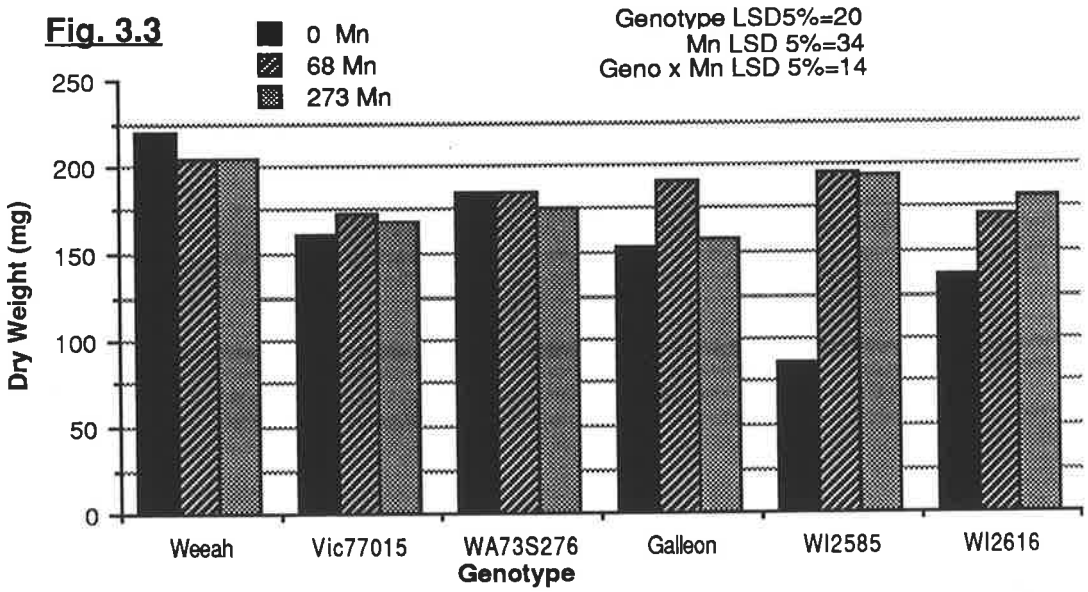

0 Mn
68 Mn
273 Mn
Genotype LSD5%=20
Mn LSD 5%=34
Geno x Mn LSD 5%=14
Dry Weight (mg)
250
200
150
100
50
0
Weeah
Vic77015
WA73S276
Galleon
WI2585
WI2616
Genotype

FIGURE 3.4 Dry weight of whole shoots (g/pot) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

FIGURE 3.5 Fresh weight of roots (g/pot) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

Fig. 3.4

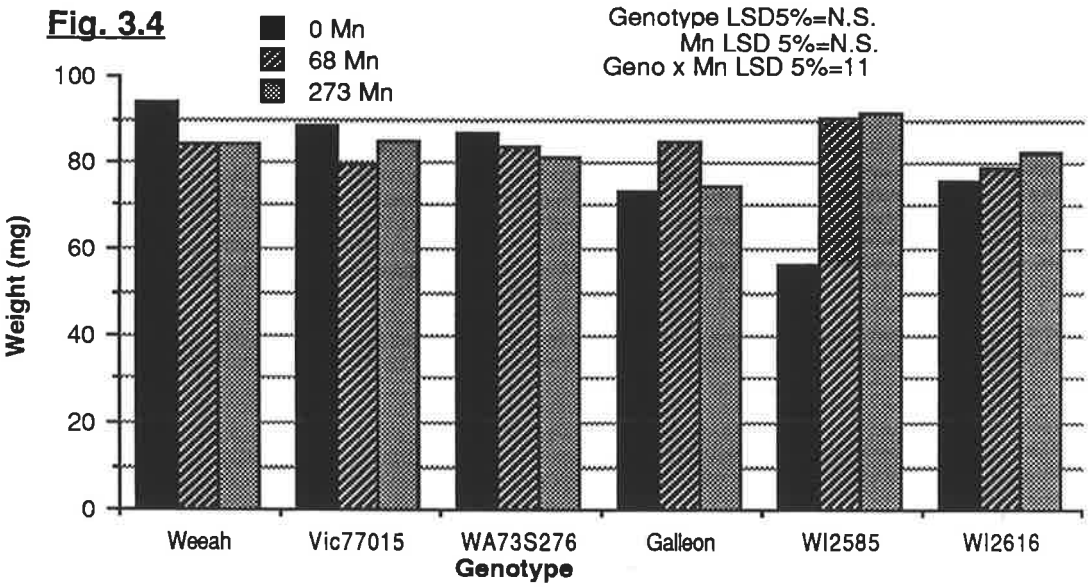

0 Mn
68 Mn
273 Mn
Genotype LSD5%=N.S.
Mn LSD 5%=N.S.
Geno x Mn LSD 5%=11
Weight (mg)
100
80
60
40
20
0
Weeah
Vic77015
WA73S276
Galleon
WI2585
WI2616
Genotype

Fig. 3.5

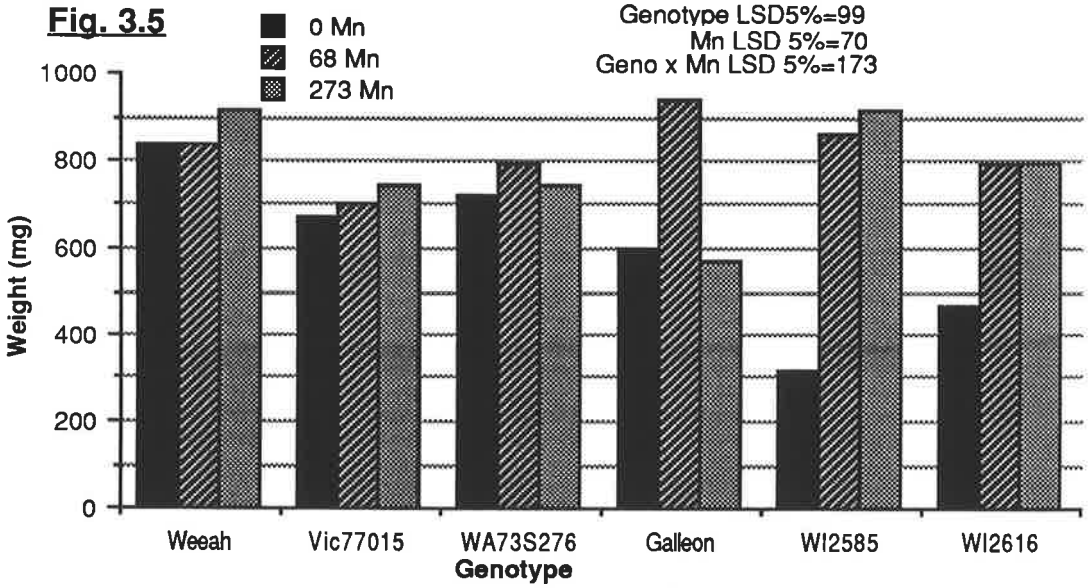

0 Mn
68 Mn
273 Mn
Genotype LSD5%=99
Mn LSD 5%=70
Geno x Mn LSD 5%=173
Weight (mg)
1000
800
600
400
200
0
Weeah
Vic77015
WA73S276
Galleon
WI2585
WI2616
Genotype

FIGURE 3.6 Fresh weight root:shoot ratios of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

Fig. 3.6

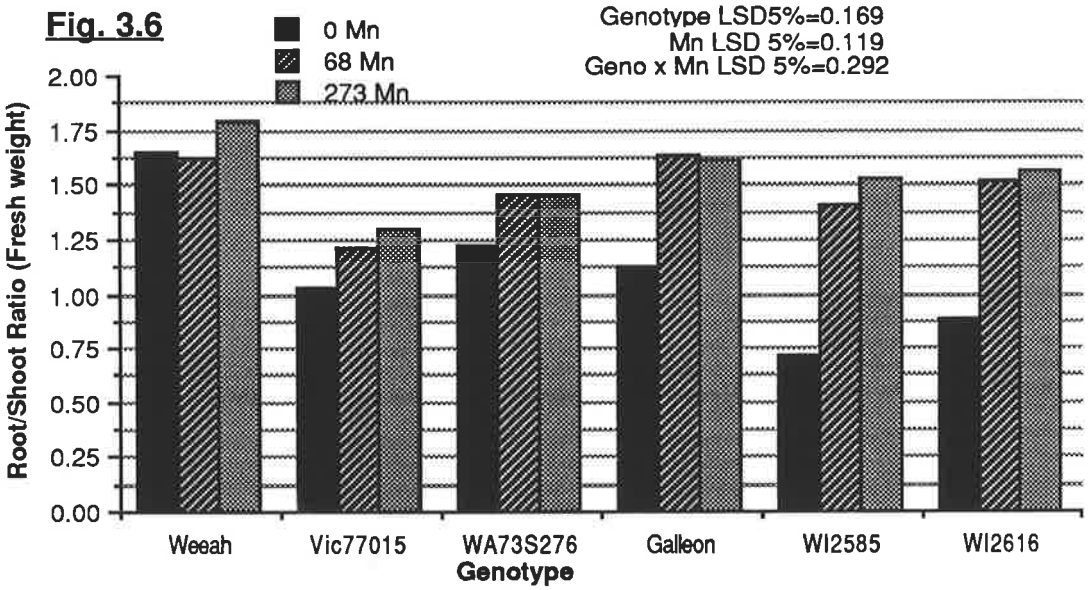

0 Mn
68 Mn
273 Mn
Genotype LSD5%=0.169
Mn LSD 5%=0.119
Geno x Mn LSD 5%=0.292
Root/Shoot Ratio (Fresh weight)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Weeah
Vic77015
WA73S276
Galleon
WI2585
WI2616
Genotype

FIGURE 3.7 Total root lengths (cm/pot) (seminal + nodal + lateral lengths) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

FIGURE 3.8 Seminal root lengths (cm/pot) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

FIGURE 3.9 Lateral root lengths (cm/pot) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

Fig. 3.7

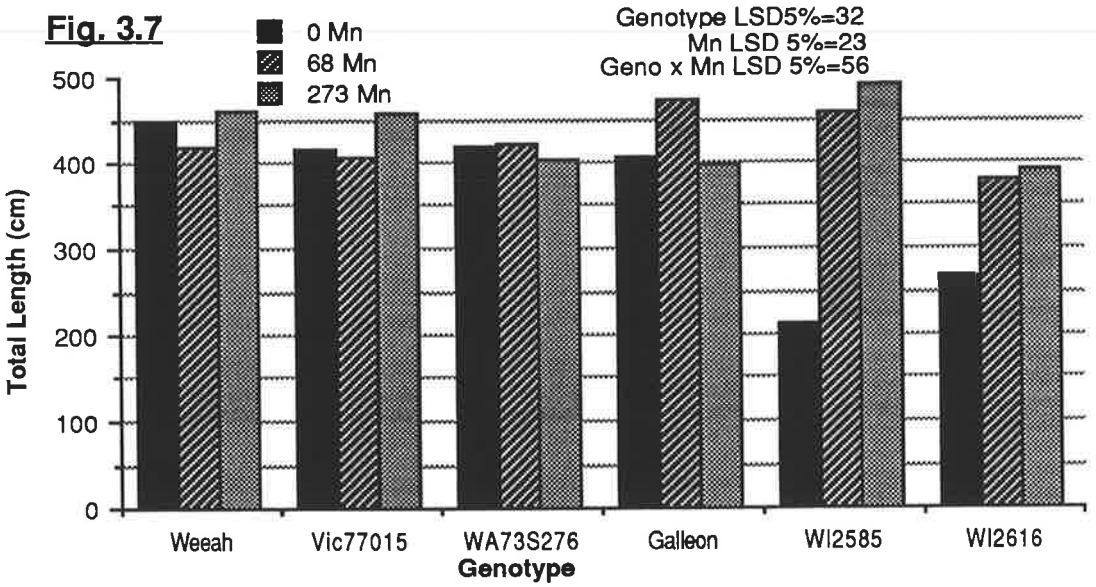


Fig. 3.8

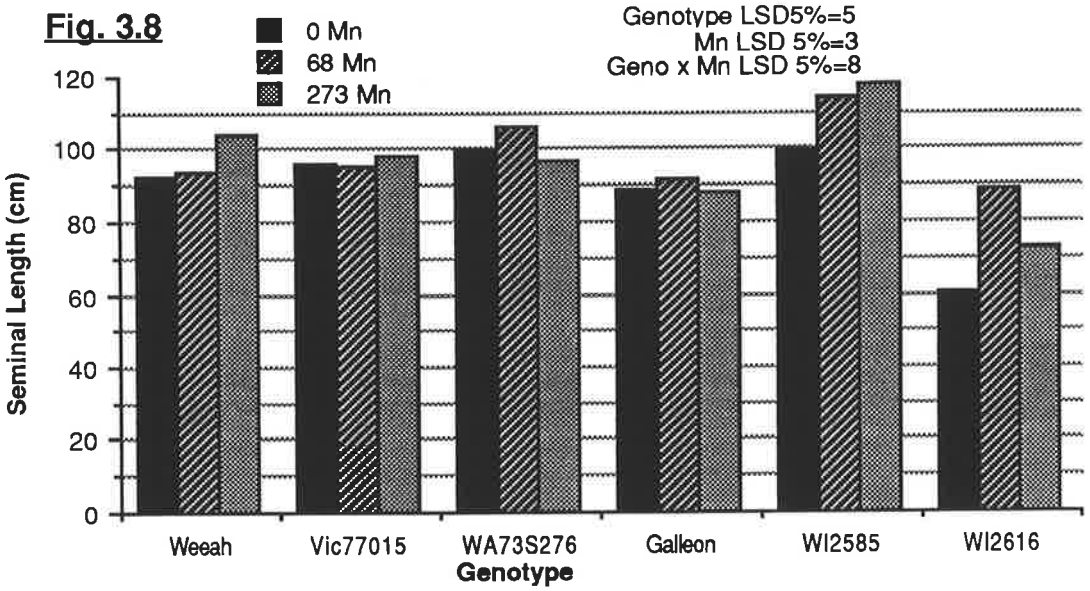


Fig. 3.9

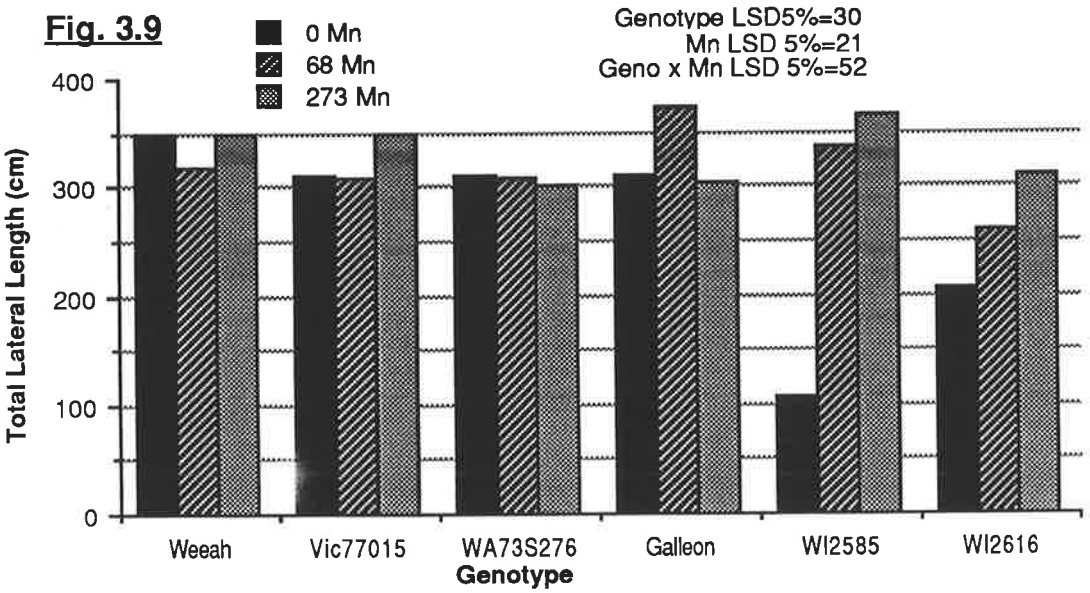


FIGURE 3.10 Total surface areas (cm^2/pot) (seminal + nodal + lateral surface areas) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

FIGURE 3.11 Seminal surface area (cm^2/pot) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

FIGURE 3.12 Lateral surface area (cm^2/pot) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

Fig. 3.10

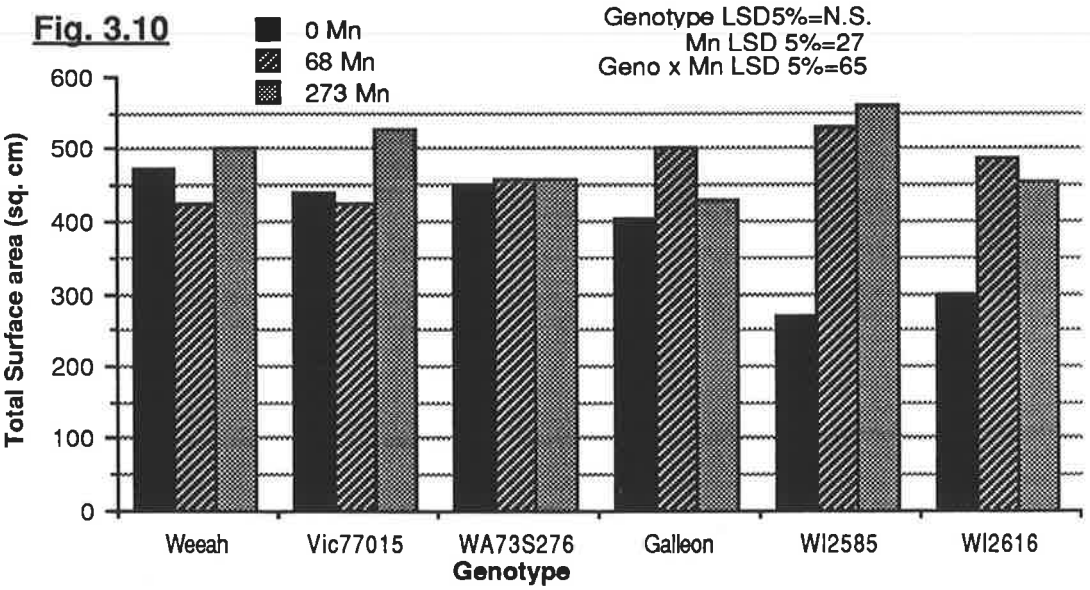


Fig. 3.11

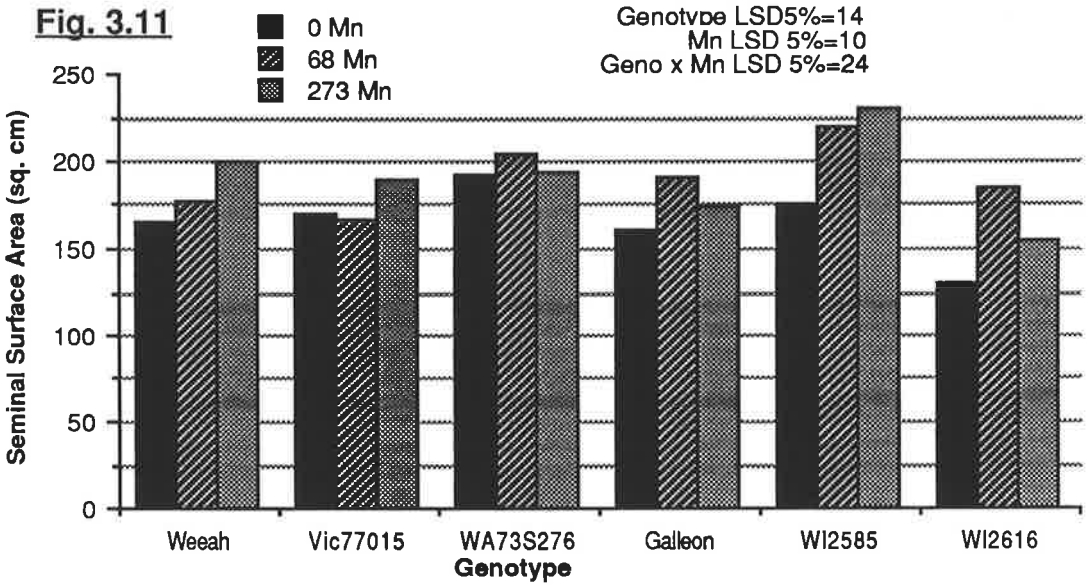


Fig. 3.12

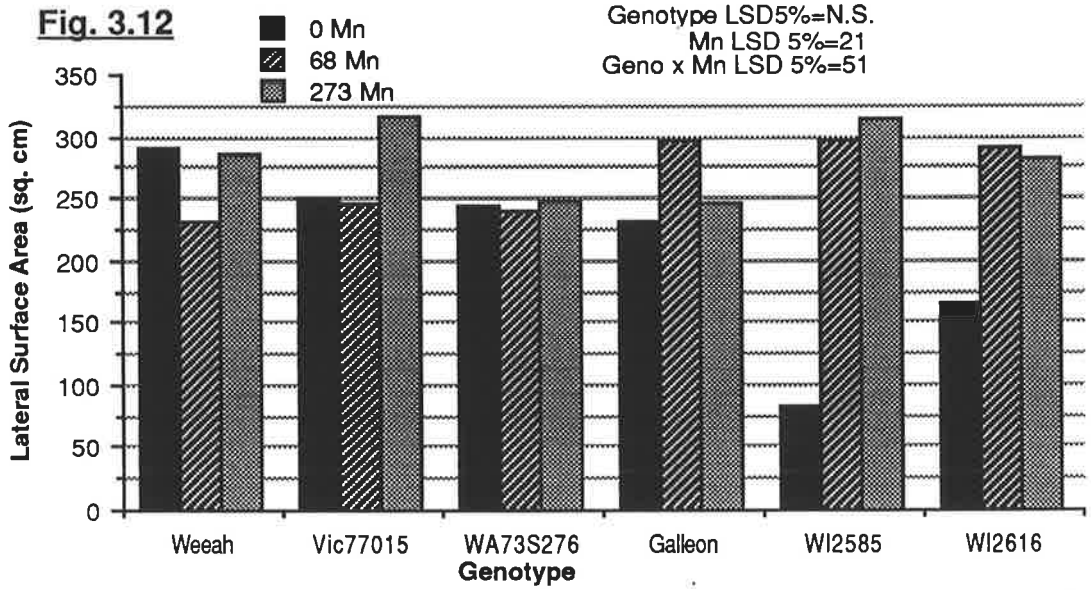


FIGURE 3.13 Total root volume (mm^3/pot) (seminal + nodal + lateral volume) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

FIGURE 3.14 Lateral root volume (mm^3/pot) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

Fig. 3.13

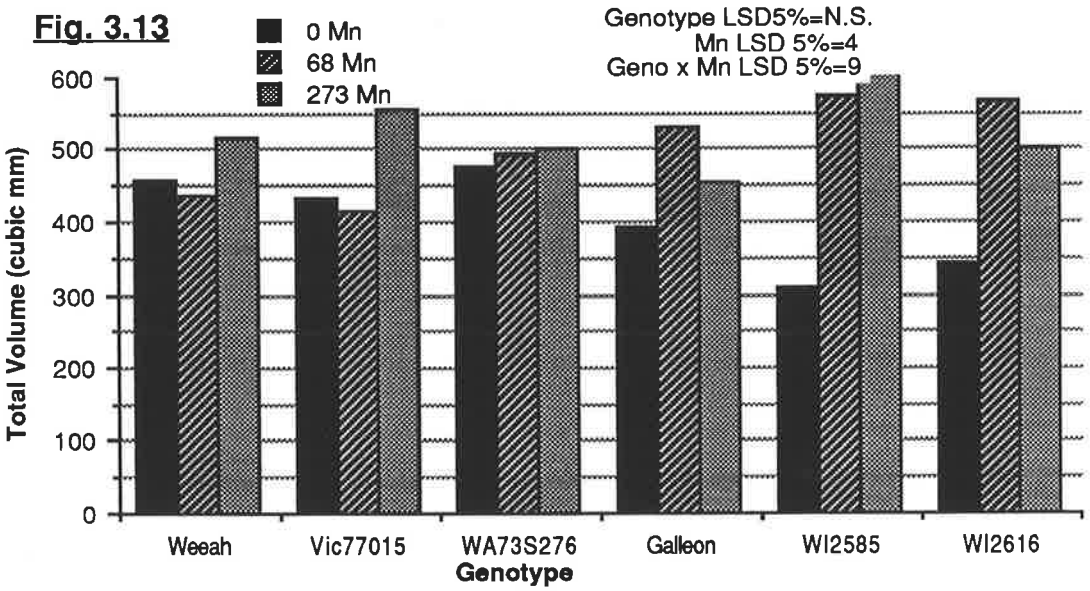


Fig. 3.14

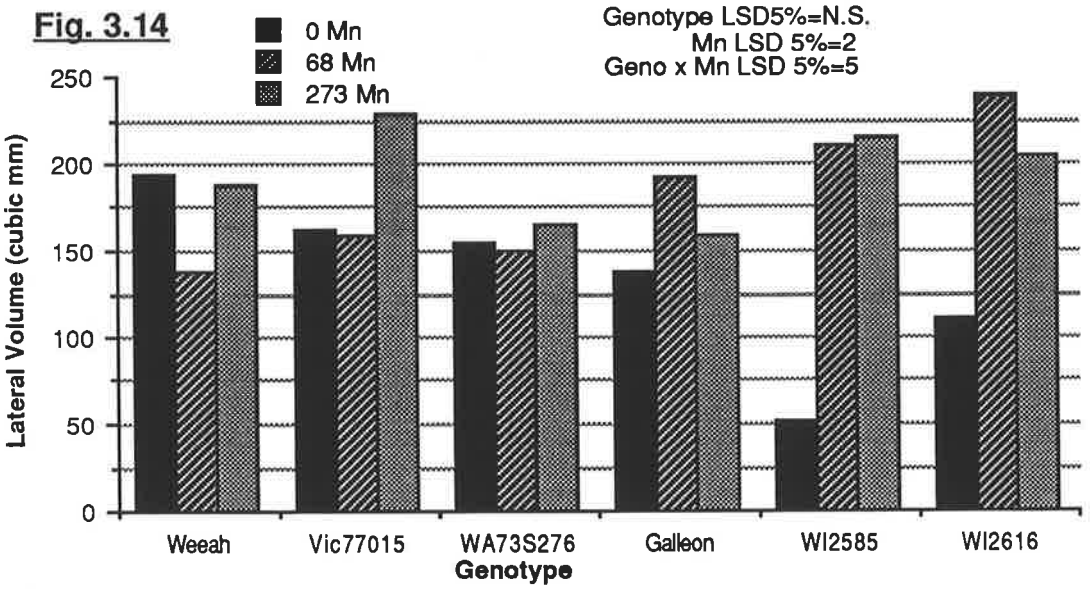


FIGURE 3.15 Seminal branching rate (number of branches/2 cm of root) of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

FIGURE 3.16 Diameter (mm) of nodal roots of *H. vulgare* cv. Weeah and Galleon and the breeders lines VIC77015, WA73S276, WI2585 and WI2616 after 28 days growth at three levels of soil Mn (0 Mn, 68 mg Mn/kg soil and 273 mg Mn/kg soil).

Fig. 3.15

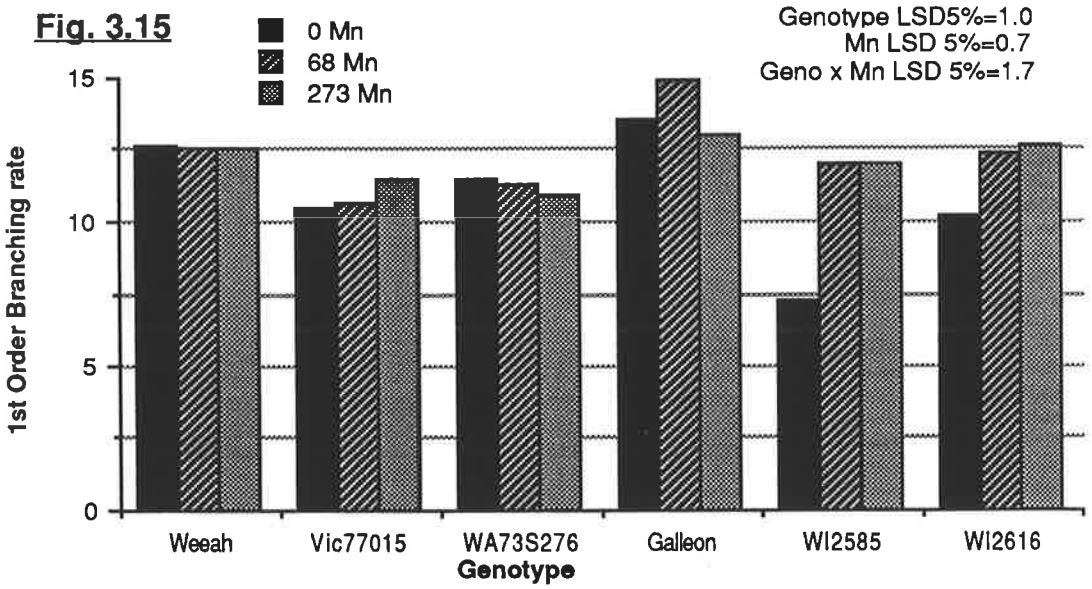
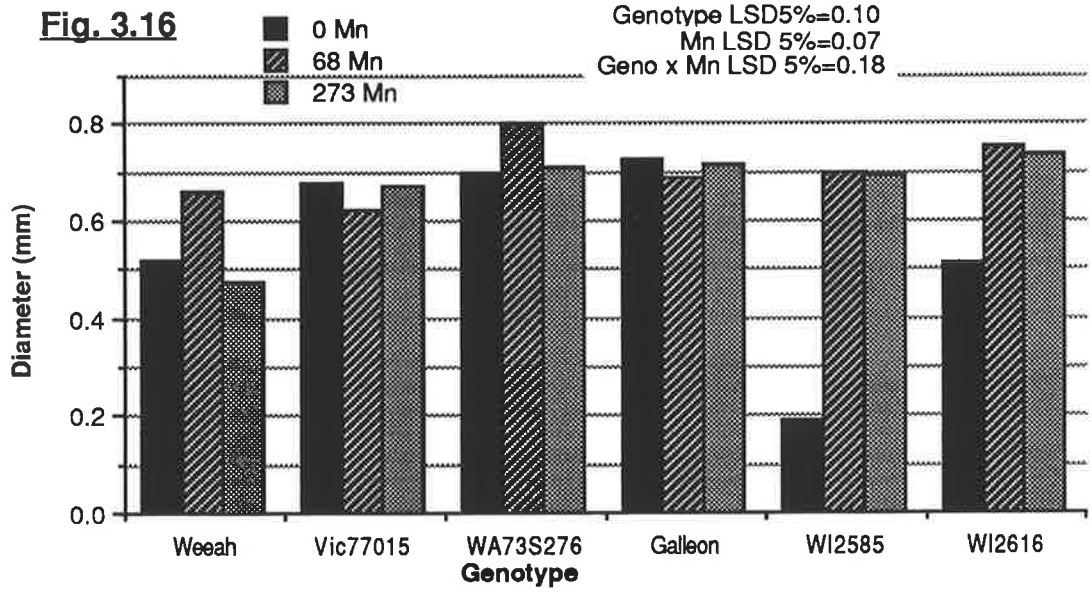


Fig. 3.16



Experiment 3(b)

3.6 Introduction

The previous experiment investigated root morphology of 4 week old barley seedlings grown in Mn deficient soil. Plants at this stage had only one to two tillers and consequently little nodal root development; therefore it was difficult to assess the contribution nodal roots may make to Mn efficiency. Prolonging the duration of the experiment was not desirable for a number of reasons. Pot size would be a limiting factor in addition to plants running into nutrient deficiencies after 4 weeks growth. Most importantly the larger the root system the more difficult it would be to seperate roots from soil and each other and to reliably assess all parameters. Schuurman and De Boer (1970) found that in the later part of the growth period of cereals the early formed nodal roots were more important on a weight basis than the seminal roots and so may constitute the chief absorbing system. By growing plants in solution culture it was possible to produce large root systems in 4 weeks which did not present the same problems of assessment.

3.7 Materials and Methods

3.7.1 Design

Barley plants were grown in solution culture and harvested 4 weeks after germination when the plants were tillering (up to four tillers) and the nodal roots had developed.

Three barley (*H. vulgare)* genotypes were grown in two litre pots of solution with three replicates. The genotypes were the Mn-efficient genotype WA73S276, the Mn-inefficient genotype Galleon and Clipper which is classed as intermediate for Mn efficiency based on their performance in field experiments (Graham *et al* 1983). Mn treatments were no added Mn (-Mn) and 1 μM Mn (+Mn). Plants were grown under

mercury vapour lamps with a 12 hour photoperiod (photon flux density of 300 μ Einsteins m^{-2} sec^{-1}).

3.7.2 Seed

Seed which had been collected in 1983 from Wangary on the Eyre Peninsula, South Australia was used. Seeds were germinated at 20°C for 48 hours on moistened filter papers in petri dishes and placed on grids in 0.5 mM $CaSO_4.2H_2O$ under lights to enable the seedlings to utilize the seed reserves of Mn. Seed Mn contents were WA73S276 0.35 μg Mn /seed, Clipper 0.28 μg and Galleon 0.21 μg. After one week these were transferred to nutrient treatment solutions in 2L pots with four plants per pot.

3.7.3 Nutrient Solutions

The nutrient solution was adapted from Nable and Loneragan (1984) and contained the following: 2.5 mM $Ca(NO_3)$, 5.0 mM KNO_3, 1.0 mM $MgSO_4$, 0.5 mM $(NH_4)2HPO_4$, 0.015 mM H_3BO_3, 0.01 mM NaCl, $5x10^{-4}$ mM $CuSO_4$, $2x10^{-4}$ mM $CoSO_4$, $2.5x10^{-3}$ mM $ZnSO_4$, $1x10^{-4}$ mM $H2MoO4$, 0.03 mM Fe EDTA and $1x10^{-3}$ mM Mn as $MnSO_4$ where appropriate. Solutions were changed weekly and pH adjusted to 5.5. Double-deionised, distilled H_2O ($DDDH_2O$) was used for all solutions and macroelement stock solutions were stripped of micronutrient contaminants using an 8 hydroxyquinoline controlled pore glass bead column (Eskew *et al* 1984).

Plastic lined pots were covered by paraffin-wax coated masonite lids and the plants supported in holes with non-absorbent cotton wool. Each pot was aerated from a source of compressed air to maintain circulation of nutrients around roots and to aerate the roots.

3.7.4 Measurements

Harvest was at 28 days when shoots were cut off and the roots carefully separated. Tillers were counted and fresh weights taken of tops and roots. Roots were assessed for the same parameters as in Expt. 3(a). Plant material was dried and digested in nitric acid and analysed by inductively-coupled plasma (ICP) emission spectrometry (ARL Model 3580).

3.7.5 Statistical Analysis

The data were subject to analysis of variance using Genstat IV statistical package. Significant mean separation is indicated by the use of the least significant difference (LSD) at the 5% level where appropriate.

3.8 Results

At harvest both Galleon and Clipper were exhibiting symptoms of Mn deficiency. Necrotic lesions were present on the oldest leaves with the remaining leaves being pale green.

Mn concentrations of shoots were below the critical level (described in Chapter 5) for all genotypes in the -Mn treatment (Fig. 3.18). Accordingly there was a significant growth response with the addition of Mn. Averaged over all genotypes, the addition of Mn resulted in a 152% increase in shoot growth and 158% increase in root growth. Mn contents of whole shoots at Mn 0 were 3-4 times greater than seed Mn contents indicating a little Mn was absorbed from nutrient solutions. At the high Mn treatment Mn content of the Mn inefficient genotype Galleon was half that of Clipper and WA73S276. This indicated that when Mn was available WA73S276 and Clipper were able to absorb Mn faster. There were no significant differences in fresh weights between genotypes of either shoots or roots at Mn 0 (Fig. 3.19 & 3.20). Of the plants supplied with Mn, Galleon was the smallest while Clipper had the largest shoots and the same root weight as WA73S276. Root:shoot ratios (not shown) were the same for all genotypes at both Mn treatments; therefore there were no changes in partitioning of plant biomass.

Root morphology was assessed in the same way as the previous experiment. All parameters increased with the addition of Mn and the number of both seminal and nodal roots also increased. There were no significant differences between genotypes in root lengths either seminal or first order laterals (branching from the seminals) or in surface areas and volumes (Figures 3.22-3.25) at Mn 0. Total root length, surface area and volume were greatest in the genotype Clipper regardless of the Mn treatment.

The surface area and volume of nodal roots were larger at Mn 0 in the Mn efficient genotype WA73S276 and smallest in Galleon (Figs. 3.26 & 3.27). At high Mn Clipper had the largest surface area and volume of nodal roots. Nodal roots had a higher branching rate than seminal roots and at Mn 0 WA73S276 had eight times the number of branches as Galleon and twice the number of Clipper (Fig. 3.21). First order lateral roots branching from nodal roots had a completely different distribution between genotypes. The Mn efficient genotype WA73S276 had greater surface areas and volumes of first order laterals at Mn 0 than the +Mn treatments of Clipper and Galleon (Figures 3.28 and 3.29). At the high Mn treatment WA73S276 had four times the root volume of Clipper and six times that of Galleon and approximately three times the surface area of Clipper and eight times that of Galleon.

3.9 Discussion

Compared to soil grown plants from Experiment 3(a) the plants in this experiment were approximately seven times larger with up to four tillers per plant compared to only one or two tillers in the soil grown plants and were at a later growth stage with nodal roots constituting a larger proportion of the total root system. All genotypes were Mn deficient at the Mn 0 treatment and there were no differences between genotypes in either root or shoot fresh weights at Mn 0. Fresh weights of roots and shoots were reduced by Mn deficiency by the same proportions i.e. roots were not more sensitive to deficiency than tops, which was the case with soil grown plants.

There were no significant differences between genotypes in root fresh weights at Mn 0; however there were significant differences in root geometry between genotypes in both Mn treatments. First order lateral roots, branching from nodal roots of the Mn efficient genotype WA73S276, were more highly developed than those of Clipper or Galleon at both Mn levels (Figures 3.28 and 3.29). The branching rate (Figure 3.21) was much higher for WA73S276 at Mn 0 and although the branching rate was the same as Clipper at +Mn the total lateral root length on the nodals of Clipper were much shorter. This difference in root geometry may affect mineral aquisition in a number or ways. Firstly, an increase in the number of root tips as a result of higher branching rates in the Mn efficient genotype WA73S276 would lead to increased microsites of lower pH. Secondly, an increase in the number of root tips results in a higher production of sloughed off cells which would in turn encourage greater numbers of possibly Mn reducing rhizosphere micro-organisms (see Chapter 4 for discussion on interactions with rhizosphere micro-organisms). Thirdly, Marschner *et al* (1987) found that apical root zones of seminal and lateral roots in barley were the sites for phytosiderophore release and uptake in 15 day old seedlings. The corresponding

proliferation of lateral roots in the more Mn efficient genotypes seen here may also increase the number of sites available for exudate release and absorption.

In Experiment 3(a) with plants grown in potted soil, the Mn inefficient genotype Galleon grew as well as the efficient genotypes under conditions of Mn sufficiency. In this experiment however Galleon did not reach the same growth plateau as Clipper and WA73S276 in shoots and Clipper in the roots. In addition, it had half the Mn content of the other genotypes when Mn was freely available in solution. Therefore, under the same conditions of freely available Mn, Galleon did not take up as much Mn as the other genotypes. This may be a result of a decrease in the number of absorbing sites due to the difference in number of lateral roots. In addition, the lack of a rhizosphere in the solution culture system prevents the accumulation of plant exudates and associated rhizosphere micro-organisms both of which may disrupt nutrient uptake in Galleon.

Several conclusions can be drawn from these two experiments. No changes in root:shoot ratios as described by Cumbus (1985) with Zn deficient wheat plants and Marschner (1986) with P deficient beans, in which shoot growth was reduced relative to root growth, occurred under conditions of Mn deficiency in any of the barley genotypes tested here. However, differences in root morphology between genotypes were found at both high and low Mn treatments in solution culture grown plants but this did not result in a larger root system (i.e. root fresh weight). Rather, the geometry of the root system was changed because of a higher branching rate of nodal roots which were initiated at tillering. A highly branched, early initiated nodal root system may enhance seedling vigour in a number of ways including increasing the number of root tips, more lateral roots which may be the site of absorption and more sloughed off cells at root tips.

There were no differences in root morphology in soil grown plants at high Mn and at Mn 0 differences in root morphology were due to the extreme sensitivity of the

WI breeders lines and Galleon to Mn deficiency which severely restricted their root growth. The more efficient genotypes were not Mn deficient at Mn 0 and had the same root system as at high Mn. The contribution made by nodal roots in soil culture could not be assessed, neither should the contribution to efficiency made by root hair length and density be ruled out.

The solution culture grown plants had differences in root morphology between genotypes in nodal root development. As the soil grown plants had limited nodal root development at harvest after 28 days growth, the Mn efficiency of the more Mn efficient genotypes could not be attributed to their root geometry. The efficient genotypes were Mn sufficient at a level of soil Mn that was insufficient to support normal growth for Galleon and the WI breeders lines. Thus, they were able to accumulate Mn from soil using a mechanism unrelated to total root length or changes in morphology. Possibilities include the presence of different populations of micro-organisms (this is discussed in Chapter 4), a lower critical nutrient requirement (discussed in Chapter 5) or the release of exudates which may be either specific as in the release of phytosiderophores as a response to Fe deficiency or general such as low molecular weight organic solutes which can mobilise the more insoluble elements. Zhang *et al* (1991) has shown that Zn deficiency induced phytosiderophore release in barley was able to mobilise Mn in a calcareous soil. Under Mn deficiency however Zhang (1989) was unable to find phytosiderophore release in either wheat or barley.

3.10 References

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FIGURE 3.18 Mn concentrations (μg/g) of whole shoots in 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

FIGURE 3.19 Fresh weights (g/plant) of whole shoots of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

FIGURE 3.20 Fresh weights (g/plant) of roots of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

Fig. 3.18

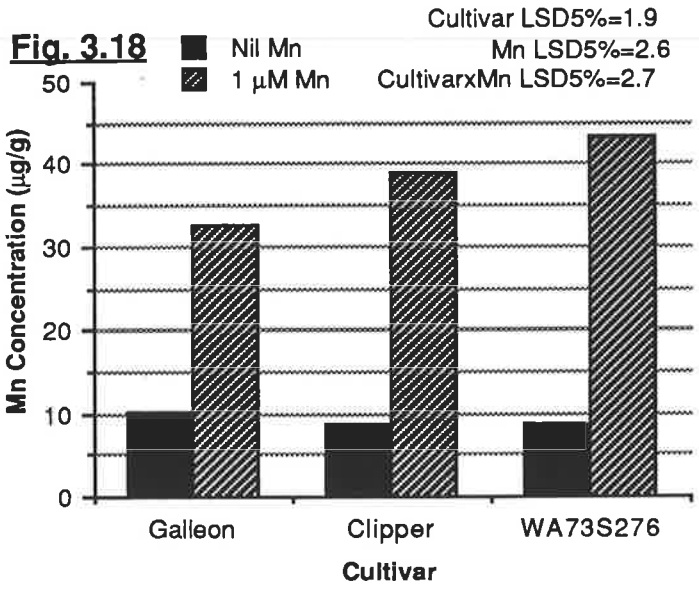


Fig. 3.19

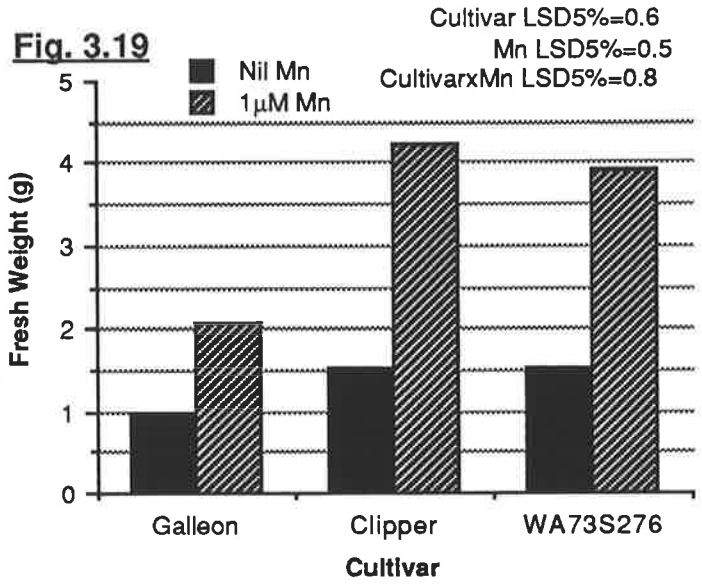


Fig. 3.20

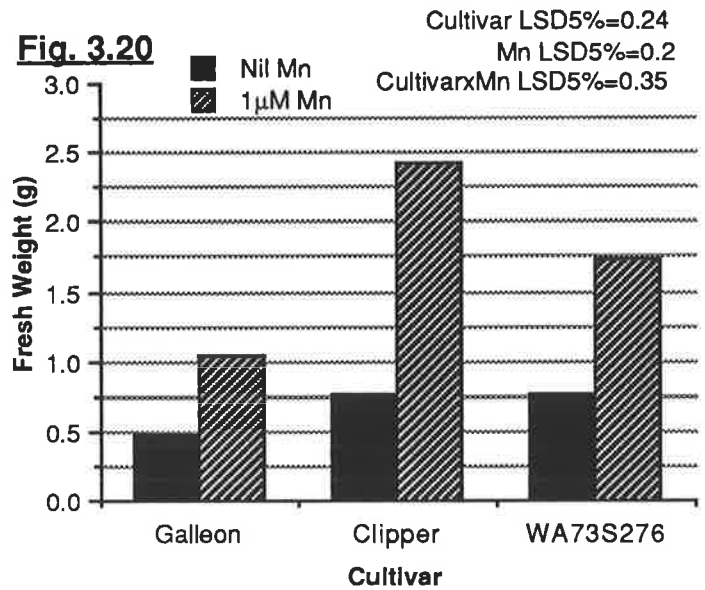


FIGURE 3.21 Nodal branching rate (number of 1st order lateral branches/2 cm of root) of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

FIGURE 3.22 Surface area of seminal roots(mm^2/plant) of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

FIGURE 3.23 Seminal root volume (mm^3/plant) of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

Fig. 3.21

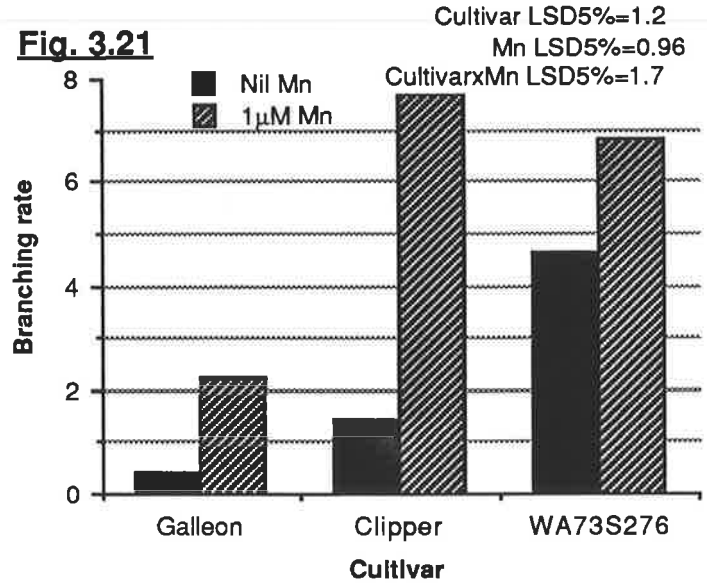


Fig. 3.22

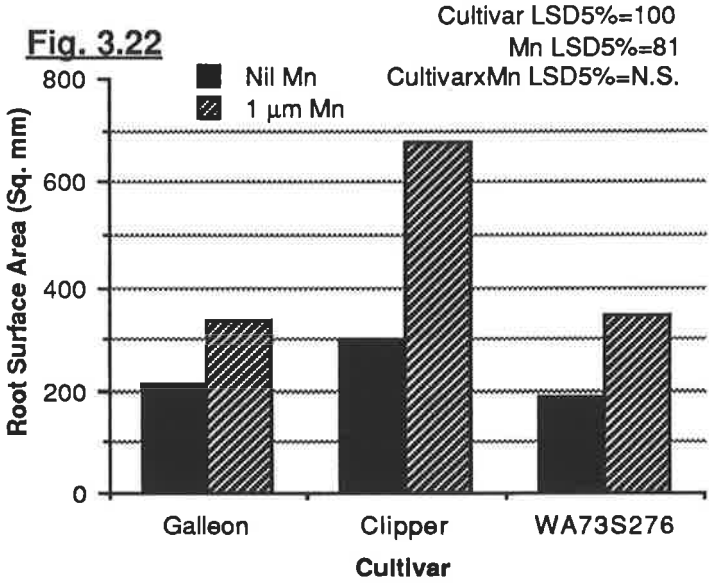


Fig. 3.23

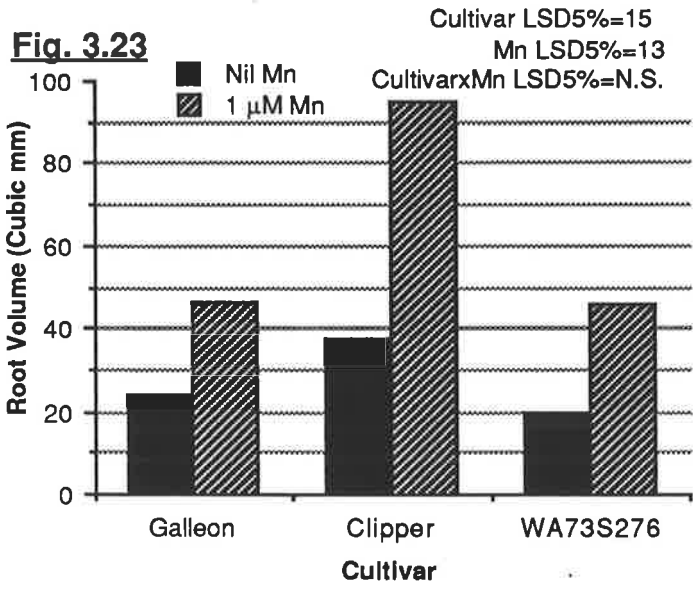


FIGURE 3.24 Surface areas (mm^2/plant) of lateral roots branching from the seminal root system of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

FIGURE 3.25 Root volumes (mm^3/plant) of lateral roots branching from the seminal root system of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

Fig. 3.24

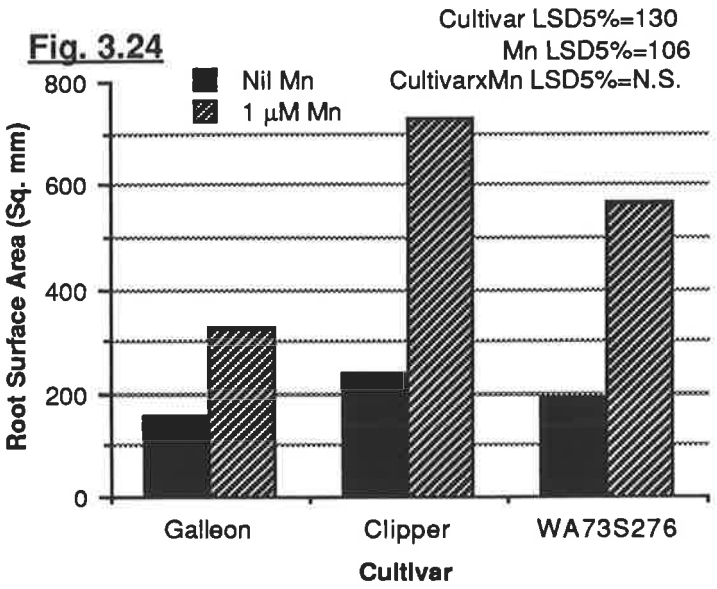


Fig. 3.25

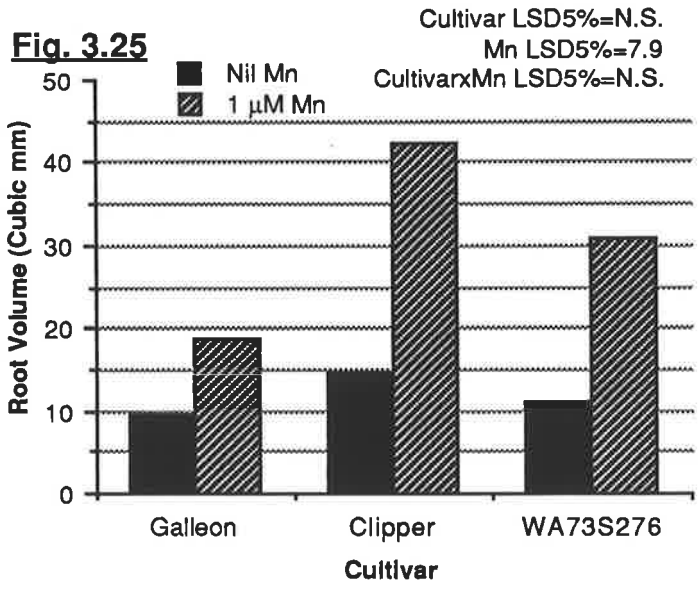


FIGURE 3.26 Surface areas (mm^2/plant) of nodal root axes of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

FIGURE 3.27 Volume (mm^3/plant) of nodal root.axes of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

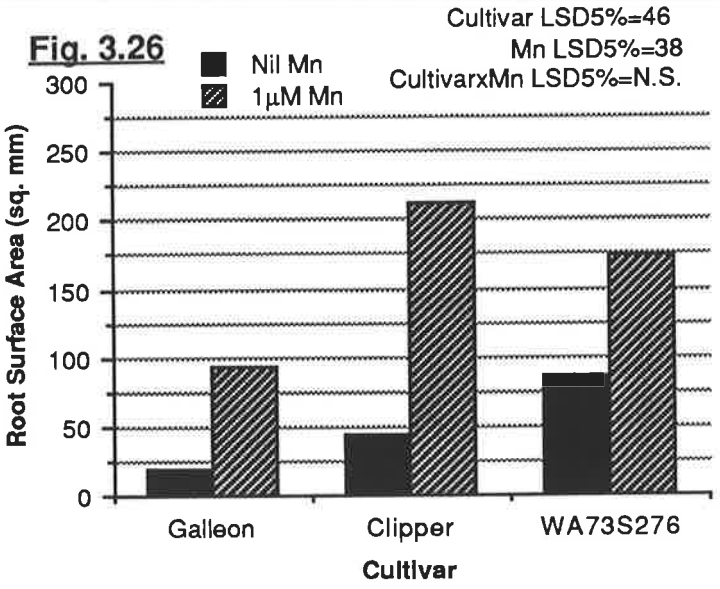


Fig. 3.26
Nil Mn
1μM Mn
Cultivar LSD5%=46
Mn LSD5%=38
CultivarxMn LSD5%=N.S.
Root Surface Area (sq. mm)
300
250
200
150
100
50
0
Galleon
Clipper
WA73S276
Cultivar

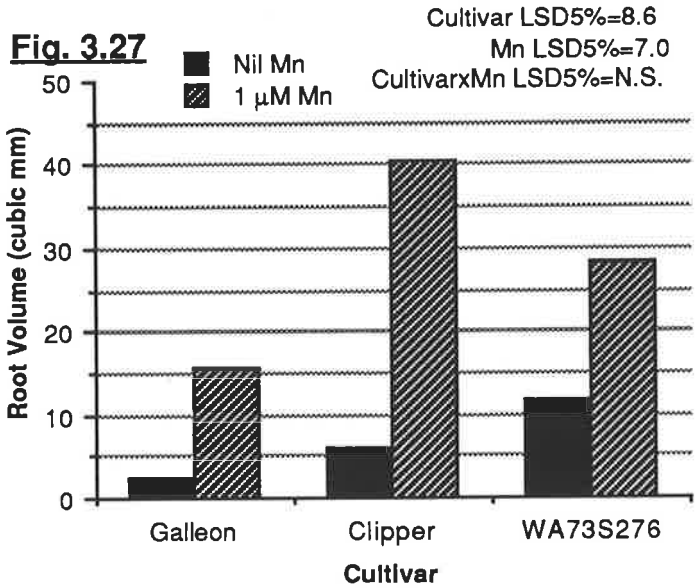


Fig. 3.27
Nil Mn
1 μM Mn
Cultivar LSD5%=8.6
Mn LSD5%=7.0
CultivarxMn LSD5%=N.S.
Root Volume (cubic mm)
50
40
30
20
10
0
Galleon
Clipper
WA73S276
Cultivar

FIGURE 3.28 Surface area (mm^2/plant) of lateral roots attached to the nodal root axes of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

FIGURE 3.29 Volume (mm^3/plant) of lateral roots attached to the nodal root axes of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

FIGURE 3.30 Mn content (μg) of whole shoots of 28 day old barley (*H. vulgare*) plants grown in nutrient solutions with two Mn treatments.

Fig. 3.28

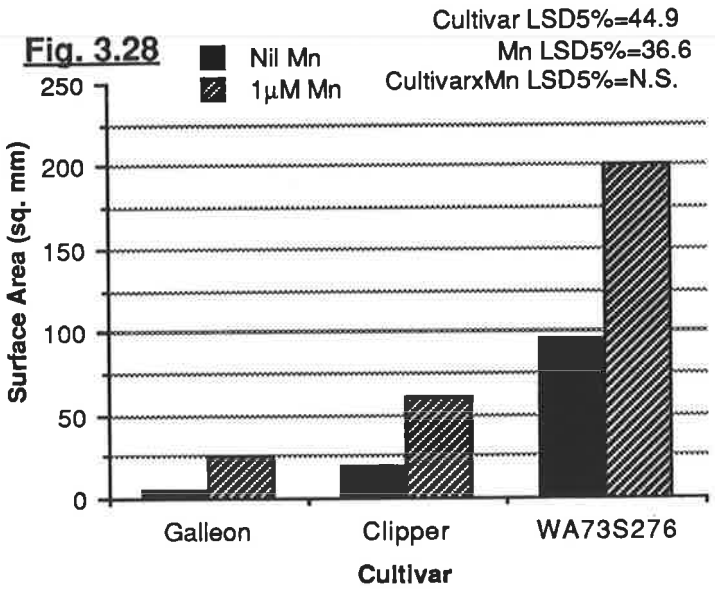


Fig. 3.29

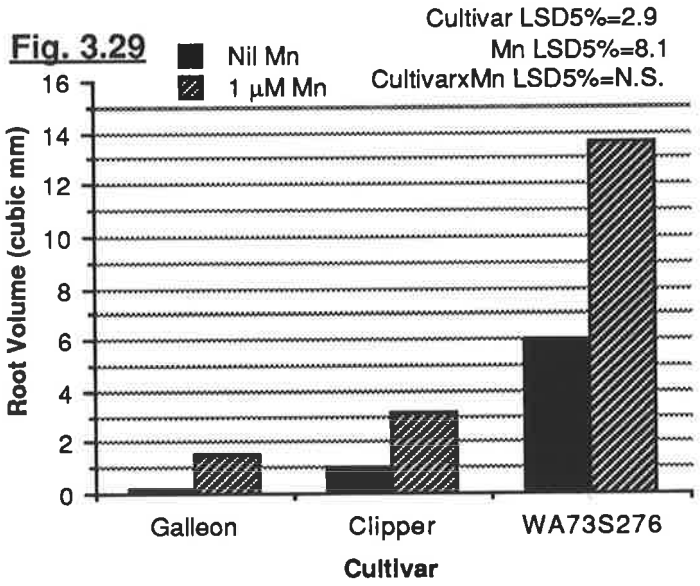
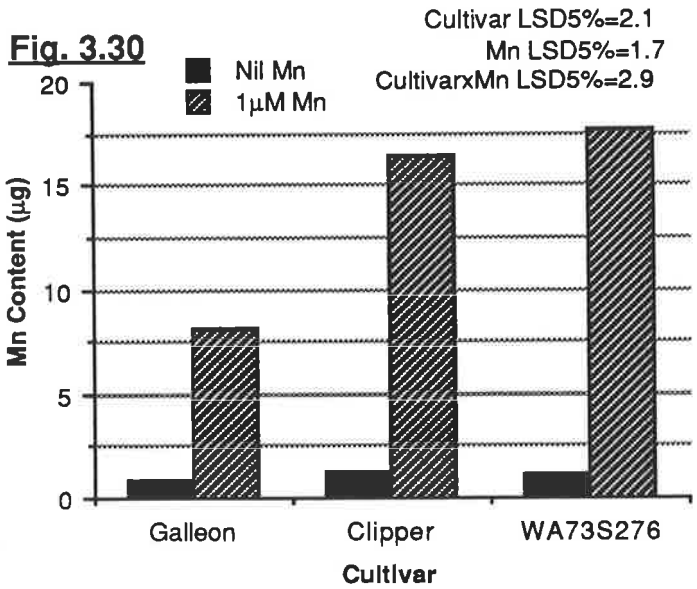


Fig. 3.30



CHAPTER 4

BIOLOGICAL OXIDATION OF MN IN RHIZOSPHERE SOIL

4.0 BIOLOGICAL OXIDATION OF MN IN RHIZOSPHERE SOILS

4.1 Introduction

The biological oxidation of manganous ions into unavailable manganic oxides by soil micro-organisms can lead to Mn deficiency in cereals (Bromfield and Skerman 1950, Gerretsen 1937, Leeper and Swaby 1940, Maclachlan 1941, Timonin 1950, Bromfield 1978). Timonin (1946) found that the rhizosphere of a Mn inefficient oat cultivar harboured a denser population of Mn-oxidising organisms than a Mn efficient cultivar and that there was a significant correlation between the severity of Mn deficiency symptoms and numbers of Mn oxidising bacteria.

The type and number of micro-organisms colonising plant roots will depend in part on the nature of root exudates. Exudation of different compounds by roots of various cultivars could result in different populations of micro-organisms. If the rhizosphere environment of a Mn-efficient barley cultivar favoured Mn reducing micro-organisms over Mn oxidising micro-organisms this may then result in greater availability of Mn to the host plant.

In the experiment described in this chapter, the method of Leeper and Swaby (1940), modified from Gerretsen (1937), was used to prepare plates of manganese oxidising micro-organisms from rhizosphere soil. This was done to determine whether there was a difference in numbers of rhizosphere populations of Mn oxidising micro-organisms firstly between species (wheat, barley and rye) and between two barley genotypes.

4.2 Materials and Methods

4.2.1 Design

A factorial experiment set out in a randomized block was undertaken with one cultivar of wheat *Triticum aestivum* cv. Condor, two of barley *Hordeum vulgare* cv.Weeah and Galleon and *Secale cereale* cv. South Australian Commercial. Condor wheat is considered to be Mn inefficient (R.D. Graham, pers. comm.), Weeah barley is Mn efficient (Relative Yield 97%) (Graham *et al* 1983) and Galleon is Mn inefficient (Relative Yield 8%) while rye is considered to be relatively nutrient efficient (R.D. Graham, pers. comm).

Plants were grown in 500g of Mn deficient soil in pots at 2 Mn levels (nil and 40 mg Mn/ kg soil as $MnSO_4$) with five replicates for 29 days in a growth chamber with 15^0C constant temperature and 12 hour photoperiod (c. 500 μEinsteins $m^{-2}sec^{-1}$). The pots were rerandomized every week to minimise shading and edge effects.

4.2.2 Seed

The seed Mn contents were as follows:

Cultivar	**Seed Mn content**
Weeah	0.21 μg/seed
Galleon	0.29 μg/seed
Condor	0.30 μg/seed
S.A. rye	0.16 μg/seed

Not all the seed was available from the same source and came from the following areas, Weeah and Galleon from Wangary on Eyre Peninsula, South Australia collected in 1985, Condor from Tooligie on Eyre Peninsula also collected in 1985 and S.A. Rye from Murdinga on Eyre Peninsula collected in 1982. Three seeds were sown per pot and thinned to two, 1 week after emergence.

4.2.3 Soil

Soil, which had previously been established to be low in available Mn (Graham *et al* 1985), was collected from Wangary in 1986. This soil (Uc 1.11, Northcote 1979) is a calcareous sand (80% $CaCO_3$) which has aeolian shell fragments of marine origin as the major part of its parent material. It was collected in two layers, the top (0-10cm) and sub (10-20cm) layers. The pH of these two layers (1:5 H_2O) was 8.9 and 9.1 respectively. After collection, the soil was air-dried and passed through a 2mm stainless steel screen to remove coarse organic matter and large concretions. Fine organic material, which could be a possible source of contaminants, was blown from the soil surface by a stream of air . Prior to sowing the soil was incubated in two, 8 kg batches in plastic bags at 21% moisture w/w for two weeks in the growth chamber at 15^0C constant temperature with an equal proportion of top and sub soil well mixed in each bag. Each bag corresponded to a Mn treatment. $MnSO_4$ was added in the appropriate amounts to each bag prior to incubation.

4.2.4 Nutrients

The following nutrients were added in solution to each bag of soil (mg salt/kg air dried soil) : 65.0 mg K_2SO_4, 37.0 mg $MgSO_47H_2O$, 3.0 mg H_3BO_3, 4.0 mg $CuSO_45H_2O$, 15.0 mg $ZnSO_47H_2O$, 0.5 mg $CoSO_47H_2O$, 7.5 mg NaCl, 0.5 mg $H_2MoO_4H_2O$, 82.5 mg KH_2PO_4, 15.0 mg $FeSO_47H_2O$, and 525.0 mg $Ca(NO_3)_24H_2O$.

All nutrients were thoroughly incorporated into the soil by hand mixing prior to potting. Each pot contained 400g of air dried soil which was watered during the experiment to 25% w/w twice a week.

4.2.5 Measurements

The plants were scored for chlorosis once a week (Table 4.1). Plants were harvested after 29 days and fresh and dry weights of tops and roots recorded. The tops were digested in nitric acid and analysed by inductively-coupled plasma (ICP) emission spectrometry (ARL Model 3580).

Table 4.1: Scoring technique used to measure extent of Mn deficiency symptoms

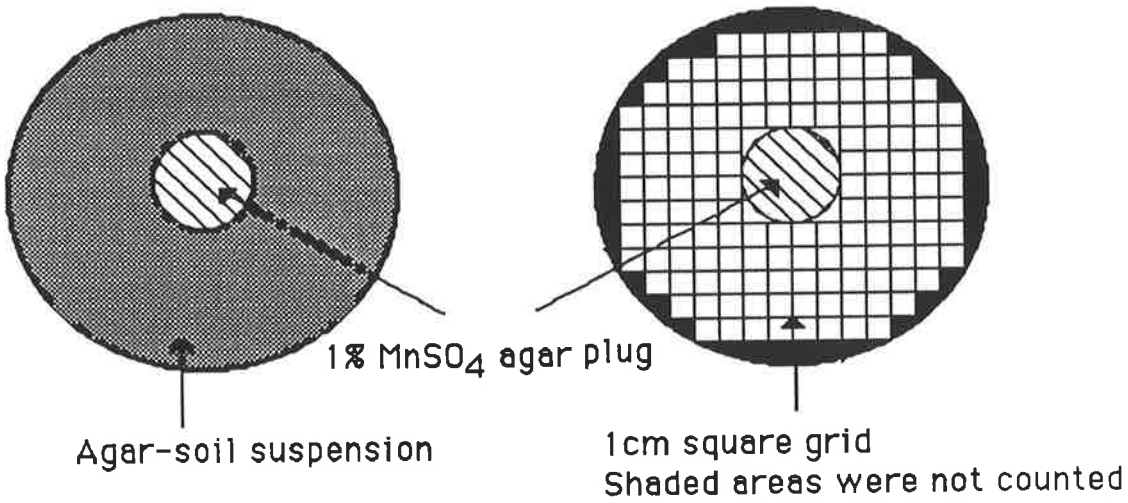
Scores	
1	Healthy
2	Pale green
3	Interveinal chlorosis
4	Severe chlorosis/necrosis
5	Stunted

4.2.6 Assessment of Mn oxide deposits

Five grams of rhizosphere soil were collected from each pot by shaking off soil tightly adhered to roots. This soil was added to 20ml of 2% Bacto-Agar (cooled to approximately 27^0C) and poured aseptically into petri dishes. When solid, a hole 2.5cm in diameter was cut from the centre of each plate and filled with agar containing 1% $MnSO_4$. The petri dishes were then incubated at 25^oC for 14 days. Manganous ions diffused from the centre well of $MnSO_4$ agar and through the soil agar. Mn^{2+} was oxidised at sites of Mn-oxidising microbial colonies. The number and extent of Mn oxide deposits are a measure of the number of Mn-oxidisers in the rhizosphere .

After 14 days the bottom surfaces of the petri dishes were divided into 1 cm^2 grids and the number of discrete deposits per whole grid in the soil counted under a dissecting microscope (Figure 4.1).

Figure 4.1 The technique used to count the number of Mn oxide deposits formed after 14 days incubation at 25 ^{0}C. A grid was laid over each petri dish and the number of discrete deposits were counted in each whole grid under a dissecting microscope



4.2.7 Statistical Analysis

The data was subject to analysis of variance using Genstat IV statistical package. Significant mean separation is indicated by the use of the least significant difference (LSD) at the 5% level where appropriate.

4.3 Results

At high Mn all cultivars appeared healthy (Fig 4.2). However at nil Mn the Mn efficient barley cultivar Weeah and S.A. rye were significantly greener than the Mn inefficient barley cultivar Galleon or the Mn inefficient wheat cultivar Condor. Both barleys outyielded Condor and S.A. Rye at high Mn (Fig. 4.3-4.6) but at nil Mn Weeah had the greatest biomass. Roots were more sensitive to Mn deficiency than tops but the roots of both barley cultivars increased with added Mn at a greater rate than those of Condor or S.A. rye. The number of manganic oxide deposits per petri dish was not significantly different between Mn treatments or between cultivars (Fig. 4.7).

The Mn concentrations of whole shoots were all below the critical level at nil Mn and there was no significant difference in Mn concentrations between cultivars (Fig. 4.8). At the high Mn treatment however, the Mn concentration of shoots of Weeah was significantly greater than that of S.A. rye which in turn was greater than Condor which was greater than Galleon. Both Weeah and rye were in the Mn sufficiency range in their tissues at the high Mn treatment. However, for the more Mn inefficient cultivars Condor and Galleon the tissue Mn levels were marginal (see Chapter 5 for discussion of critical nutrition concentrations). As no chlorosis was evident at the high Mn treatment (Fig. 4.2) the Mn inefficient cultivars Condor and Galleon were deemed to be sub-clinically deficient. It is probable that a higher level of soil Mn would have shown an increase in biomass of both Galleon and Condor as they reached sufficiency in their tissues. The Mn contents of both of the barleys were significantly higher than Condor or rye at the high Mn treatment (Fig. 4.9).

4.4 Discussion

No statistically significant differences in pattern or number of Mn oxide deposits were observed for the Wangary soil between Mn treatments, cultivars or species. However, the trend of increased Mn oxide deposits at high Mn for the more Mn inefficient cultivars Condor wheat and Galleon barley may be important (Fig. 4.7); this contrasted with decreasing trends for the two efficient genotypes. It is not possible however to place any significance to these trends in view of the high variability among replicates in these measurements. The amount of Mn absorbed by all cultivars at nil Mn was extremely small due to the severe deficiency of this soil. Under these conditions root exudation from these severely stressed plants may have been inhibited. At the high Mn treatment the Mn concentrations of the Mn inefficient cultivars Galleon and Condor were still in the critical level range. This level of added Mn (40 mg Mn/kg soil) separated the cultivars into Mn sufficiency and deficiency. Mn was more readily absorbed and plant growth was not severely depressed at this rate of Mn thus results from this treatment may be more meaningful for comparison of rhizosphere activity. At this level the number of Mn oxide deposits associated with the rhizosphere of the Mn efficient plants was lower than for the Mn inefficient plants. The higher levels of Mn-oxidising rhizosphere micro-flora of Mn inefficient plants may have oxidised sufficient available Mn to restrict uptake by plants. Conversely, the rhizosphere soil from Mn efficient cultivars produced lower populations of Mn-oxidisers in the rhizosphere thereby possibly leaving more soil Mn available for plant uptake.

Although this technique measured the relative amount of oxidation taking place between plates it did not indicate the type of organisms involved. The conditions of incubation may have favoured some but not all rhizosphere micro-organisms. Other workers such as Leeper and Swaby (1940) using the same method on different soil types collected from South Australia, Victoria and Western Australia did not quantify

the amount of oxidation taking place but observed differences in behaviour between soil types with each soil giving a characteristic, reproducible pattern. Some soils developed a uniform brown zone. Others developed a ring of brown spots. Others slowly formed large spots up to 5mm diameter, scattered over the entire plate. None of these patterns was observed in this experiment. The trends observed in this work tend to support the hypothesis that Mn efficient cultivars had less microbial Mn oxidation in their rhizosphere than Mn inefficient cultivars. This could be tested in future experiments using more Mn treatments. It is also possible that experiments which measured the activities of Mn-reducing organisms may show differences between genotypes.

In conclusion, a correlation between the severity of Mn deficiency symptoms and numbers of Mn oxidising populations could not be established with certainty. At high Mn a decrease in Mn oxidation (not statistically significant) by the more Mn efficient cultivars was observed. This could prove to be important in increasing Mn availability to Mn efficient plants. Further studies would clarify whether this is a significant factor in determining Mn efficiency.

4.5 References

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FIGURE 4.2 Symptoms of Mn deficiency in *H. vulgare* cv. Weeah and Galleon, *T. aestivum* cv. Condor and *S. cereale* cv. S.A. Commercialwere measured by a chlorosis score after 29 days growth. Scores were assessed according to the table below.

Scores	
1	Healthy
2	Pale green
3	Interveinal chlorosis
4	Severe chlorosis/necrosis
5	Stunted

FIGURE 4.3 Shoot fresh weight (g/pot) after 29 days growth of *H. vulgare* cv. Weeah and Galleon, *T. aestivum* cv. Condor and *S.cereale* cv. S.A.Commercial at two levels of soil Mn.

Fig. 4.2

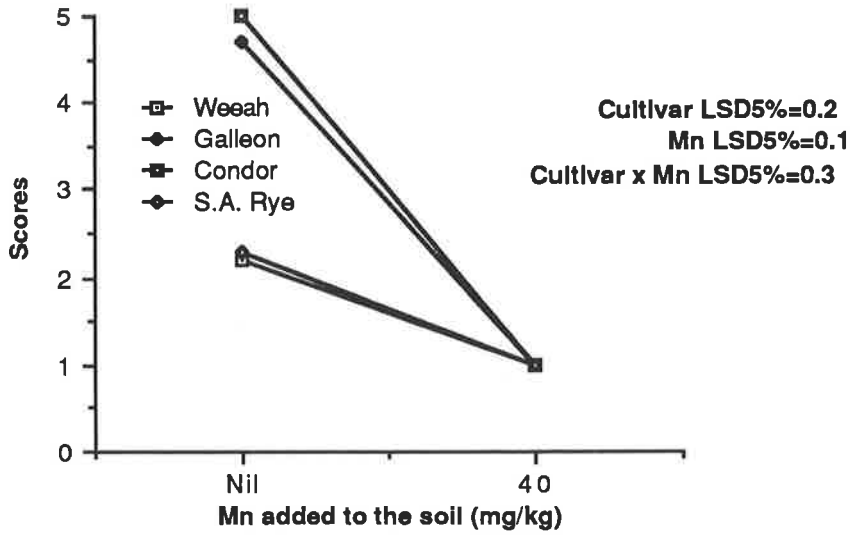
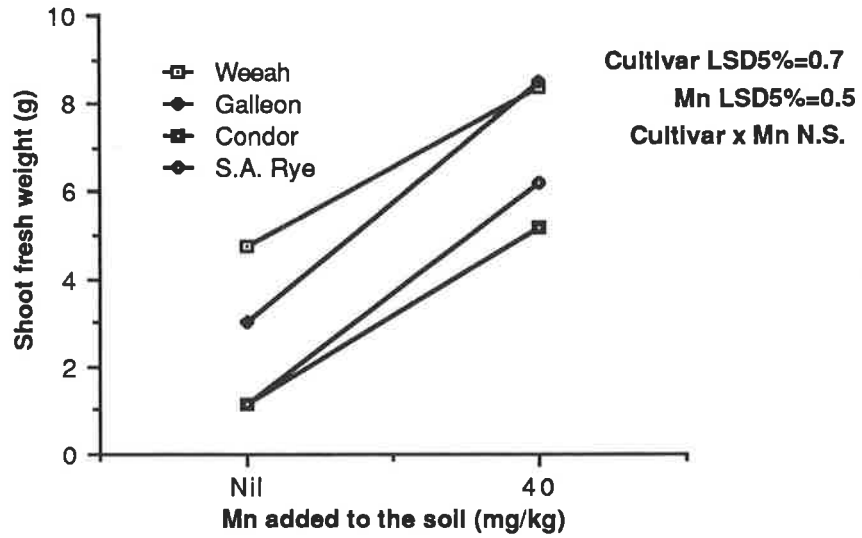

Scores
5
4
3
2
1
0
Weeah
Galleon
Condor
S.A. Rye
Cultivar LSD5%=0.2
Mn LSD5%=0.1
Cultivar x Mn LSD5%=0.3
Nil
40
Mn added to the soil (mg/kg)

Fig. 4.3


Shoot fresh weight (g)
10
8
6
4
2
0
Weeah
Galleon
Condor
S.A. Rye
Cultivar LSD5%=0.7
Mn LSD5%=0.5
Cultivar x Mn N.S.
Nil
40
Mn added to the soil (mg/kg)

FIGURES 4.4-4.6 Fresh and dry weights of roots and dry weight of shoots (on a per pot basis) after 29 days growth of *H. vulgare* cv. Weeah and Galleon, *T. aestivum* cv. Condor and *S. cereale* cv. S.A.Commercial at two levels of soil Mn.

Fig. 4.4

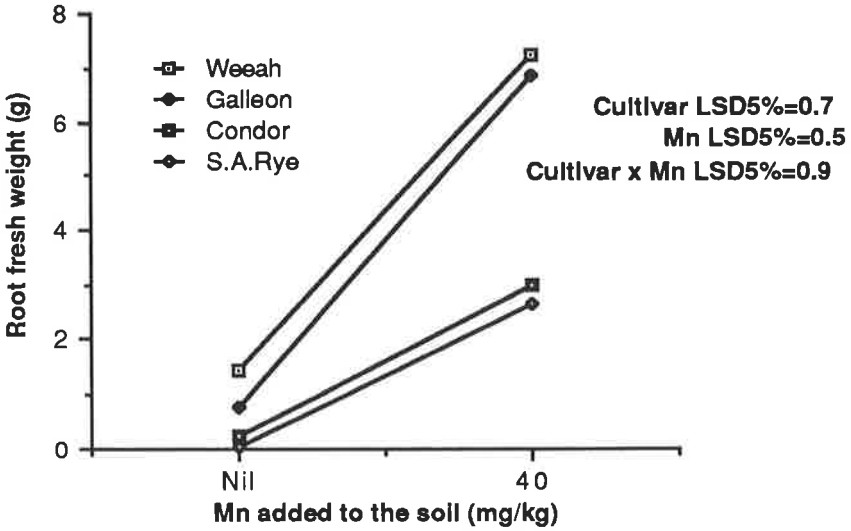


Fig. 4.5

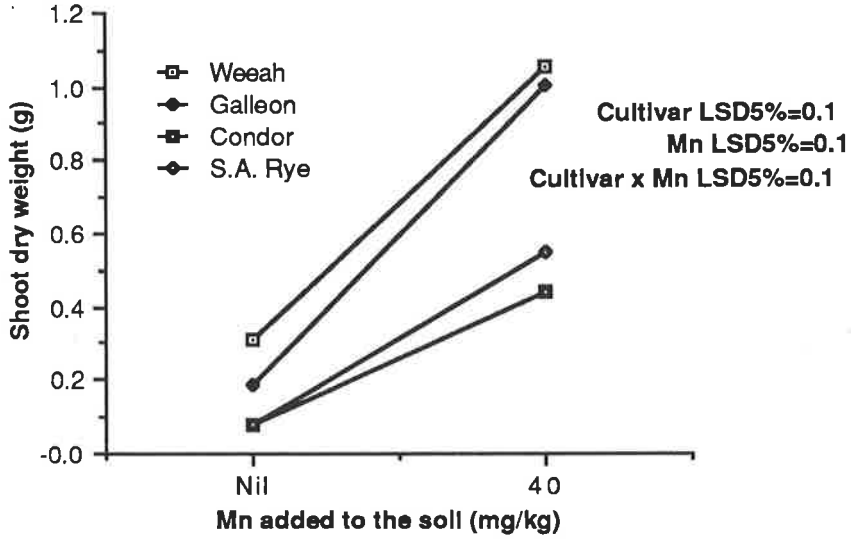


Fig. 4.6

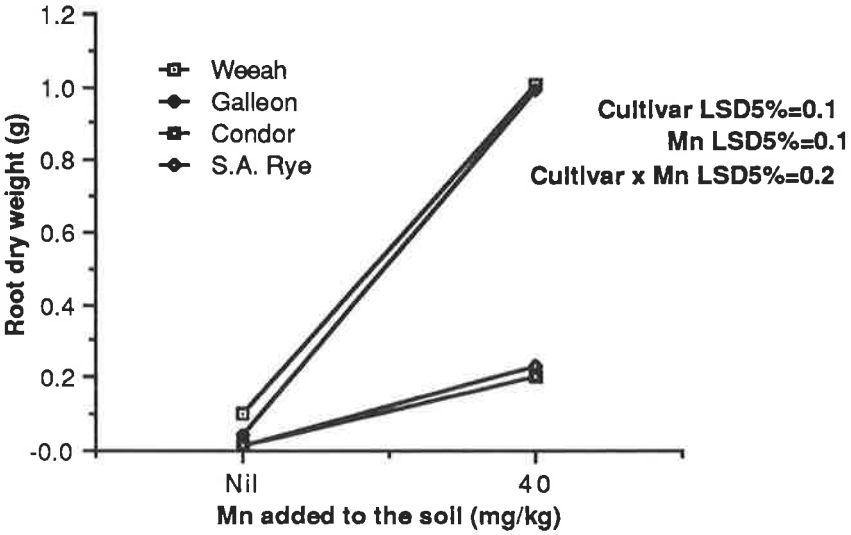


FIGURE 4.7 The number of Mn oxide deposits (see text for method) per petri dish developed on rhizosphere soil collected from *H. vulgare* cv. Weeah and Galleon, *T. aestivum* cv. Condor and *S. cereale* cv. S.A. Commercial at two Mn levels after 29 days growth.

Fig. 4.7

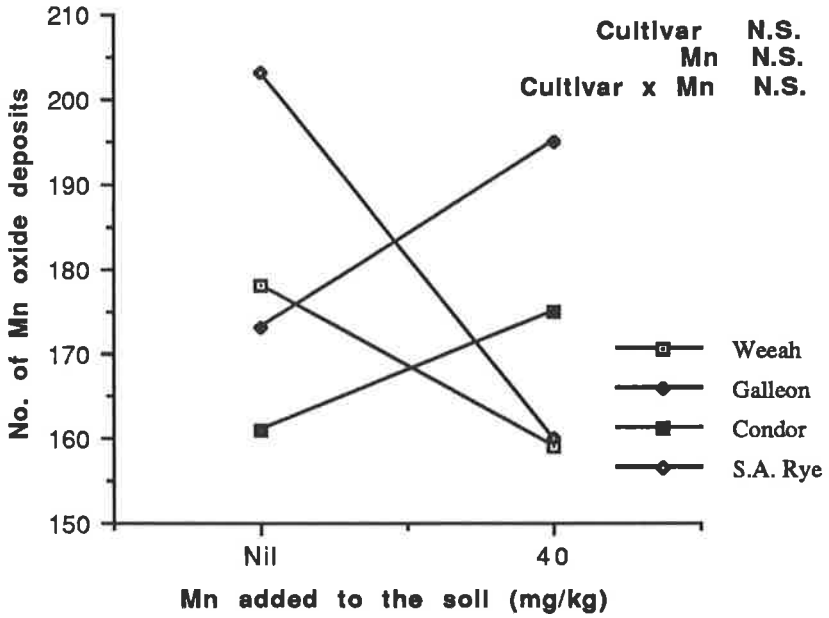

Cultivar N.S.
Mn N.S.
Cultivar x Mn N.S.
No. of Mn oxide deposits
210
200
190
180
170
160
150
Nil
40
Mn added to the soil (mg/kg)
Weeah
Galleon
Condor
S.A. Rye

FIGURE 4.8 & 4.9 Mn concentration and contents in whole shoots of *H. vulgare* cv. Weeah and Galleon, *T. aestivum* cv. Condor and *S. cereale* cv. S.A. Commercial after 29 days growth at two levels of soil Mn.

Fig. 4.8

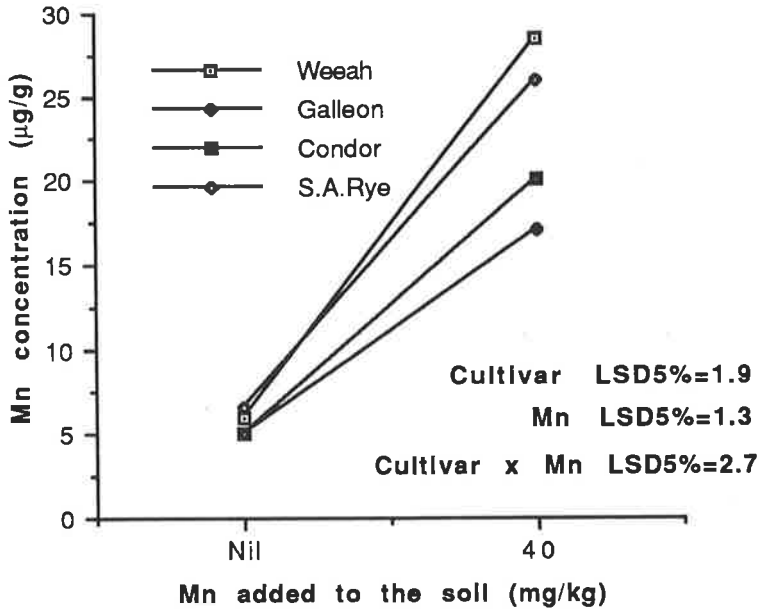
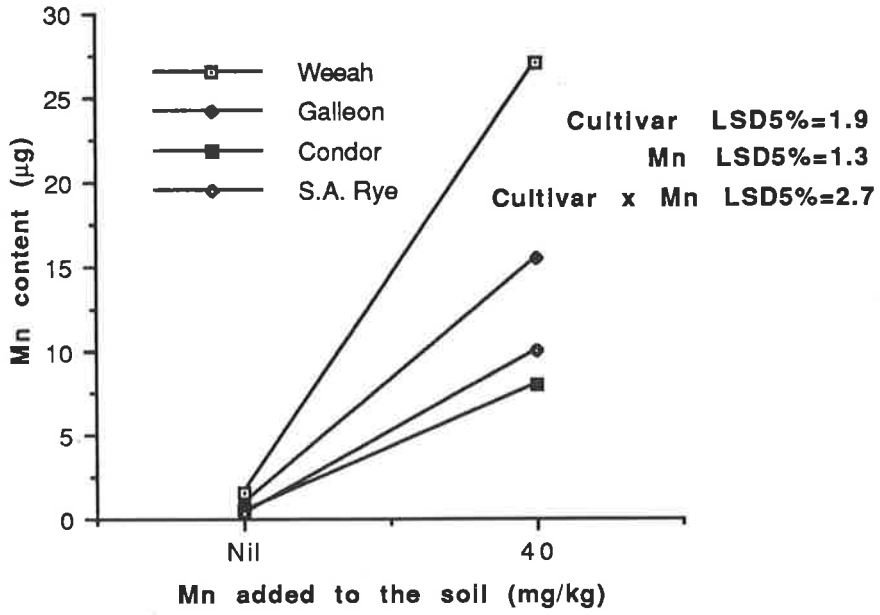

30
25
20
15
10
5
0
Mn concentration (µg/g)
Weeah
Galleon
Condor
S.A.Rye
Cultivar LSD5%=1.9
Mn LSD5%=1.3
Cultivar x Mn LSD5%=2.7
Nil
40
Mn added to the soil (mg/kg)

Fig. 4.9


30
25
20
15
10
5
0
Mn content (µg)
Weeah
Galleon
Condor
S.A. Rye
Cultivar LSD5%=1.9
Mn LSD5%=1.3
Cultivar x Mn LSD5%=2.7
Nil
40
Mn added to the soil (mg/kg)

CHAPTER 5

CRITICAL MN CONCENTRATION IN BARLEY

5.0 CRITICAL MN CONCENTRATION IN BARLEY

5.1 Introduction

The mechanisms of efficiency discussed in the previous chapters have all functioned outside the plant to increase nutrient availability. Some of these can be classed as general mechanisms if they affect availability of nutrients other than Mn. A lower internal requirement for Mn however, is a nutrient specific mechanism and one which functions inside the plant. A lower internal requirement for an element will be reflected in a lower 'critical level' for that element. The 'critical level' is used to designate the concentration of the element in plant tissues which is just adequate for plant growth. Above this concentration a plant is healthy and has normal growth but below it the plant is deficient in the element and growth is depressed. The critical level has been defined as the concentration of the element present in tissue at 90% maximal plant growth (Reuter and Robinson 1986) and is derived from critical concentration curves which are a relationship between nutrient concentration and plant yield.

To date, there is contradictory evidence as to whether genotypes within a species have different critical nutrient concentrations. Marcar (1986) used three wheat cultivars differing in Mn efficiency and found critical nutrient concentrations varied between 12 and 17 µg/g depending on the genotype. Conversely, Graham *et al* (1985) found the critical nutrient concentration for Mn in field grown wheat was the same for both a Mn efficient and a less Mn efficient cultivar.

Critical nutrient concentrations between cultivars of barley differing in Mn efficiency under a controlled environment have not been examined. This experiment was undertaken to determine whether differences existed between a Mn efficient and a Mn inefficient cultivar of barley in their critical Mn concentrations.

5.2 Materials and Methods

5.2.1 Design

A factorial experiment set out in a randomized block was undertaken with Mn 'efficient' *Hordeum vulgare* cv. Weeah (Relative Yield 97%) and Mn 'inefficient' *Hordeum vulgare* cv Galleon (Relative Yield 8%) (Graham *et al* 1983) with eight Mn treatments and five replicates. Mn treatments were nil, 1, 2, 5, 10, 20, 40 and 80 mg Mn/kg soil as $MnSO_4$. Pots were rerandomized every week to minimise shading and edge effects. The experiment was carried out under controlled environment conditions in a growth chamber with 15^0C constant temperature and 12 hour photoperiod (c. 500 μEinsteins $m^{-2}sec^{-1}$).

5.2.2 Seed

Seed which had been collected in 1985 from Wangary on the Eyre Peninsula, South Australia, was used and three pre-germinated seeds (20°C for 24 hrs) were sown per pot and thinned to two 5 days after emergence. The seed Mn content was 0.29μg/seed for Weeah and 0.21μg/seed for Galleon.

5.2.3 Soil

Soil, which had previously been established to be low in available Mn (Graham *et al* 1985), was collected from Wangary in 1986. This soil (Uc 1.11, Northcote 1979) is a calcareous sand (80% $CaCO_3$) which has aeolian shell fragments of marine origin as the major part of its parent material. It was collected from two layers, the top (0-10cm) and sub (10-20cm) layers. The pH of these two layers (1:5 H_2O) was 8.9 and 9.1 respectively. After collection, the soil was air-dried and passed through a 2mm stainless steel screen to remove coarse organic matter and large concrétions. Fine organic material was blown from the soil surface by a stream of air. After collection the soil was dried and sealed in bags. Storage of air dried soil will

increase exchangable Mn levels (Boken 1952, Fujimoto and Sherman 1945, Shuman 1980). Therefore, prior to sowing the soil was incubated in eight, 4 kg batches in plastic bags at 21% moisture w/w for two weeks in the growth chamber at 15^0C constant temperature with an equal proportion of top and sub soil well mixed in each bag. This was done to decrease the level of plant available Mn probably through encouraging Mn-oxidising micro-organisms. Each bag corresponded to a Mn treatment. $MnSO_4$ was added in the appropriate amounts to each bag prior to incubation.

5.2.4 Nutrients

The following nutrients were added in solution to each 4kg batch of soil after two weeks incubation (mg salt/kg air dried soil) : 65.0 mg K_2SO_4, 37.0 mg $MgSO_4.7H_2O$, 3.0 mg H_3BO_3, 4.0 mg $CuSO_4.5H_2O$, 15.0 mg $ZnSO_4.7H_2O$, 0.5 mg $CoSO_4.7H_2O$, 7.5 mg NaCl, 0.5 mg $H_2MoO_4.H_2O$, 82.5 mg KH_2PO_4, 15.0 mg $FeSO_4.7H_2O$, and 525.0 mg $Ca(NO_3)_2.4H_2O$.

All nutrients were thoroughly incorporated into the soil by hand mixing prior to potting. Each pot contained 385g of air dried soil which was watered during the experiment to 25% w/w.

5.2.5 Measurements

The plants were scored for chlorosis once a week (Table 5.1). Plants were harvested after 28 days and fresh and dry weights of tops, roots and crowns and tiller number were recorded. YEBs (youngest expanded blade) were separated from the tops for separate analysis. All plant parts were digested in nitric acid and analysed by inductively-coupled plasma (ICP) emission spectrometry (ARL Model 3580).

Table 5.1: Scoring technique used to measure extent of Mn deficiency symptoms

Scores	
1	Healthy
2	Pale green
3	Interveinal chlorosis
4	Severe chlorosis/necrosis
5	Stunted

5.2.6 Statistical Analyses

The data were subject to analysis of variance suitable for a randomized block design using Genstat IV. Significant mean separation is indicated by the use of the least significant difference (LSD) at the 5% level where F values indicated significant treatment effects.

The critical nutrient concentration was determined with two computer generated models using SAS (Statistical Analysis System, SAS Institute, Inc. Box 8000, Cary, NC). The models were those that had been used by Ware *et al* (1982) and Graham *et al* (1985). For convenience the model used by Graham *et al* (1985) has been labelled Model 1 and that used by Ware *et al* (1982) Model 2 and they took the following form;

Model 1.....$y_{cl}=a + b.c^{x}$

where y_{cl} is 90% maximum yield
a is the asymptotic maximum yield
b = ymax - yo
c is constant of proportionality
x is tissue Mn concentration

Model 2.....$y_{cl}=b\ (1 - ce^{-ax})$

where y_{cl} is 90% maximum yield
b is the asymptotic maximum yield as x approaches infinity
$c = (b - y_O)/b$
a is the constant of proportionality
x is tissue Mn concentration

Computer generated models were chosen to determine critical Mn levels as they provided quick and objective comparsions between cultivars. Two models were used to assess whether they produced the same critical levels.

5.3 Results

The Mn inefficient cultivar Galleon was pale yellow after the first week of growth, at all but the two highest levels of Mn. Symptoms were first evident on the oldest leaves and chlorosis progressed with time (Figs. 5.1- 5.4). Plants at harvest in the nil Mn treatment were severely stunted with some dead tillers whereas in the Mn efficient cultivar Weeah, Mn deficiency symptoms were confined to expressions of interveinal chlorosis on the youngest leaves. Mn deficiency symptoms did not appear on Weeah at 20mg Mn/kg soil and above; however, 40mg Mn/kg soil were required to prevent the appearence of symptoms in Galleon.

Weeah had more fresh weight biomass at low Mn levels (e.g. at nil Mn, Weeah had 47% more biomass than Galleon Fig. 5.5). At the higher Mn levels there were no significant differences in shoot biomass between the two cultivars (Fig. 5.5 & 5.6). The root fresh and dry weights showed similiar trends with the roots being more sensitive to Mn deficiency (Figs. 5.7 & 5.8). Weeah was more responsive to added Mn than Galleon with the root biomass of Weeah increasing with increasing Mn at a higher rate than Galleon. These trends were the same for total fresh and dry weights (not shown). In all cases Weeah reached its growth plateau at a lower level of added Mn than Galleon.

Under conditions of Mn sufficiency, Galleon had more tillers than Weeah (Fig. 5.9) however the total biomass of Galleon was significantly less than that of Weeah. Tillering of both cultivars increased with the addition of Mn but Weeah again reached its plateau at a lower level of added Mn than Galleon.

The Mn efficient cultivar Weeah had a higher Mn content and a higher Mn

concentration than Galleon at all Mn treatments above 10mg Mn/kg soil in both YEBs and whole tops (Fig. 5.10 - 5.13). To reach a level of Mn sufficiency Galleon required approximately twice the level of added Mn than did Weeah. There were no differences between cultivars in Mn uptake (per unit of root dry weight) at low levels of soil Mn (Fig. 5.20). At high soil Mn, Weeah had a higher Mn uptake per unit of root dry weight than Galleon.

Critical nutrient concentrations calculated using the models are in Tables 5.1 and 5.2. Figures 5.14-5.19 are critical concentration curves in whole tops and YEBs. Critical concentrations fall in the same range for both Weeah and Galleon. The values are more variable between the models for Galleon probably because of lack of real definition of the growth plateau in Galleon.

Table 5.1: Critical Mn concentrations (μg/g) of whole tops and YEBs as a function of whole top dry weight using two computer generated models.

	Weeah		Galleon	
	Tops	**YEBs**	**Tops**	**YEBs**
Model 1*	16.1 (0.97)	14.5 (0.97)	17.0 (0.96)	13.8 (0.96)
Model 2†	16.1 (0.97)	14.6 (0.97)	20.0 (0.96)	17.3 (0.96)

(r^2 values in parentheses)

* $y_{cl} = a + bc^x$

† $y_{cl} = b(1-ce^{-ax})$

A plot of YEB dry weights as a function of YEB concentrations (Figs.5.18 & 5.19) revealed that the critical Mn concentrations for YEB growth (Table 5.2) were lower than those for whole shoots which is consistent with results in cereals from other workers (Ohki 1984). Both models calculated a higher critical Mn concentration in Galleon compared to Weeah.

Table 5.2: Critical Mn concentrations (µg/g) of YEBs as a function of YEB dry weight using two computer generated models.

	Weeah	Galleon
Model 1*	10.0 (0.98)	12.1 (0.96)
Model 2†	6.0 (0.98)	12.7 (0.96)

(r^2 values in parentheses)

* $y_{cl} = a + bc^x$

† $y_{cl} = b(1-ce^{-ax})$

The root:shoot ratio of Weeah (Fig. 5.21) was higher than that for Galleon at Mn 5 when both genotypes were Mn deficient. Weeah had the ability to partition more plant biomass in roots at the expense of shoots and this trend was present at all Mn levels above Mn 5.

Seed Mn contents for the two cultivars were essentially the same i.e. 0.21 µg/seed for Weeah and 0.29 µg/seed for Galleon. Therefore differences in seedling growth could not be attributed to differences in seed Mn contents.

5.4 Discussion

This experiment was conducted in order to determine whether a Mn-efficient and a Mn inefficient barley cultivar had different critical Mn concentrations. Reuter *et al* (1983) used three procedures for fitting relationships between shoot yields and Cu concentrations in the youngest open leaves of three subterranean clover cultivars. They used nonrectanglular hyperbola regressions (Smith and Dolby 1977), the Mitscherlich plant growth model (Model 2 in this chapter) and hand fitted curves. Two harvests were made at 39 and 70 days and the critical Cu concentrations for the three procedures varied from 2.5 to 6.0 µg/g at 39 days and 1.8 to 3.4 µg/g at 70 days. They concluded

that their studies confirmed a Cu concentration of 3 μg/g as a critical nutrient concentration despite the fact that there was a 140% variation in values at 39 days and 89% at 70 days. The value of 3 μg/g was obtained by averaging the critical Cu concentrations over all treatments. This paper also demonstrates the variation in calculation of critical values depending on the method used to fit the relationships.

Two widely accepted models were chosen from the literature to construct curves from which critical Mn concentrations could be calculated (Table 5.1). There was no difference between the models in critical levels for Weeah but the critical levels predicted for Galleon were overall higher than those for Weeah and the value predicted for Model 2 was higher than that for Model 1. Okhi proposed the use of the Mitscherlich plant growth model (Model 2 in this chapter) to determine critical nutrient deficiency levels and found that this model produced critical levels significantly greater than previously published values using hand-fitted curves (Ware *et al* 1982). This variation in values between models may be caused by the limitation of the Galleon data where strong definition of the growth plateau had not been reached. Ideally, another treatment of high soil Mn would have strengthened this plateau. Figures 5.18 and 5.19 of YEB dry weight as a function of YEB Mn concentration in both models gave a higher critical Mn concentration for Galleon than Weeah. According to Nable *et al* (1984) the Mn concentration measured in these young tissues may correspond more closely to the functional Mn requirement than analysis of whole tops which would include non-functional Mn accumulated in old tissues. No difference in critical Mn concentrations in whole tops between barley genotypes could be found from this experiment, yet in young growing tissue the Mn efficient cultivar Weeah had a lower critical concentration (8μg/g) than the inefficient cultivar Galleon (12μg/g). Hannam *et al* 1987) determined the critical level in Galleon barley both in glasshouse experiments and in the field and found a critical level based on dry matter production of 12μg/g in YEBs which corresponds with this experiment. They compared this with the functional Mn requirement by measuring chlorophyll 'a' fluorescence as a measure of disruption

in photosynthesis. Using this parameter YEBs were shown to be suffering from Mn deficiency at a slightly higher level of Mn in the tissues of 14μg/g. This proved to be a more sensitive criterion than a decrease in yield for measuring a critical Mn concentration. However, they noted that this did not apply to all species. In wheat for example the critical Mn concentration in YEBs were similar for both dry matter and chlorophyll 'a' fluorescence (Graham *et al* 1985).

Growth data and chlorosis scores showed that under conditions of Mn deficiency Weeah remained greener and was more vigorous than Galleon. Although Weeah had the highest Mn concentration and content this was not due to more efficient Mn uptake (uptake per unit weight of root) at the lowest levels of soil Mn (Fig. 5.20). When Mn was readily available however, as in the highest Mn treatment, Weeah was able to take up Mn more efficiently.

Root:shoot ratios were found to differ between the genotypes under conditions of Mn deficiency with Weeah partioning more plant biomass into the roots at the expense of the shoots. This had not been observed in earlier experiments due to the lack of severe Mn deficiency in the soil in those experiments. Having this ability may contribute to Weeah's Mn efficiency. This genotype can reach a level of Mn sufficiency in its tissues at a lower level of soil available Mn than Galleon, although there was no difference in rate of Mn uptake per gram of root at the time of harvest at low levels of soil Mn. Thus, a larger root system would enable Weeah to maintain Mn sufficiency in its shoots.

In conclusion, the critical level of Mn in whole tops was similar in Weeah and Galleon (16 and 18 μg/g respectively). However, the critical level of Mn in the young growing tissue (YEBs) as a function of YEB growth was higher in the Mn inefficient cultivar Galleon (12μg/g) than the Mn efficient cultivar Weeah (8μg/g). In addition the critical levels for YEBs was lower than whole tops for both genotypes. The Mn present in whole tops would include both functional and stored Mn whereas that in the

would be more closely related to the functional level of Mn.

A distinction can be made between critical internal Mn concentration and critical external Mn concentration. The two genotypes may have similar internal Mn requirements in whole tops, but the cultivars differed markedly in their external requirements with Galleon needing twice the level of soil Mn to reach Mn sufficiency than that of the Mn efficient genotype Weeah.

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Fig. 5.1

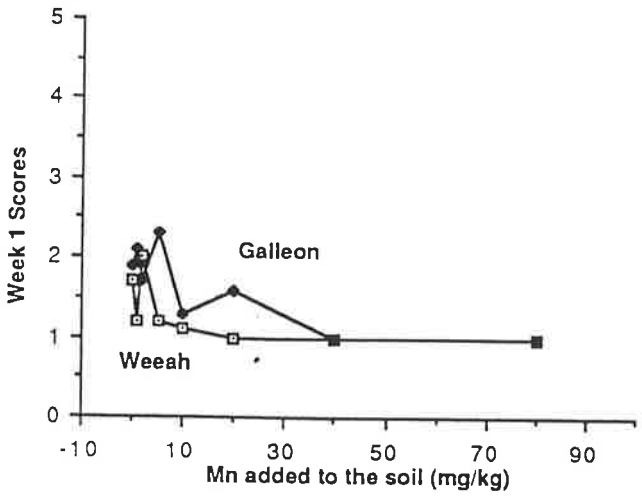
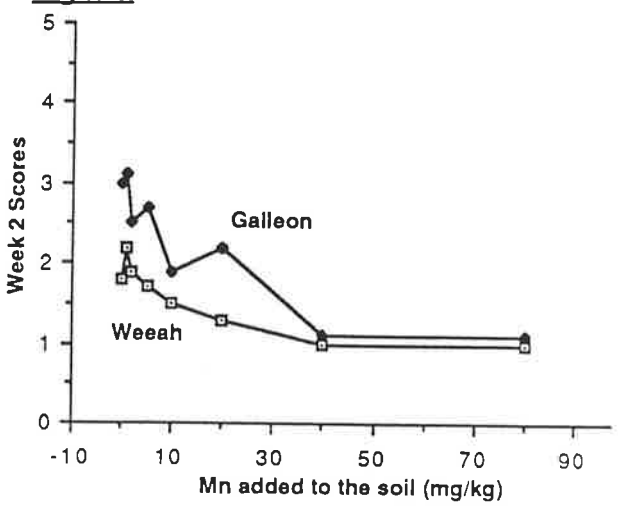


Fig. 5.2



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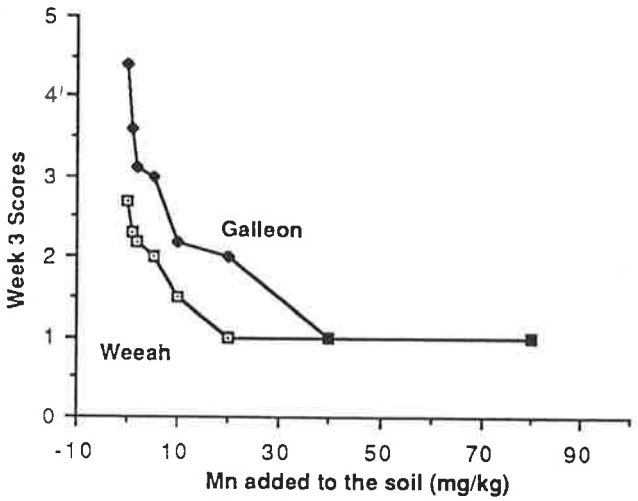
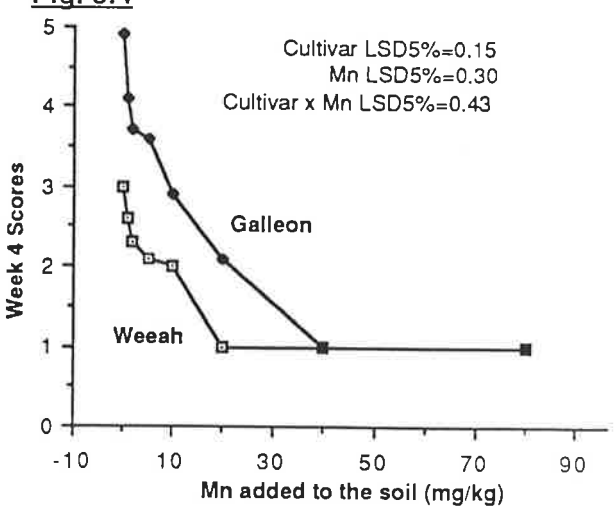


Fig. 5.4



FIGURES 5.1-5.4 Symptoms of Mn deficiency as a function of Mn additions to the soil in *H. vulgare* cv. Weeah and Galleon were measured by a chlorosis score once a week until harvest at 28 days. Scores were assessed according to the table below.

Scores	
1	Healthy
2	Pale green
3	Interveinal chlorosis
4	Severe chlorosis/necrosis
5	Stunted

Fig. 5.5

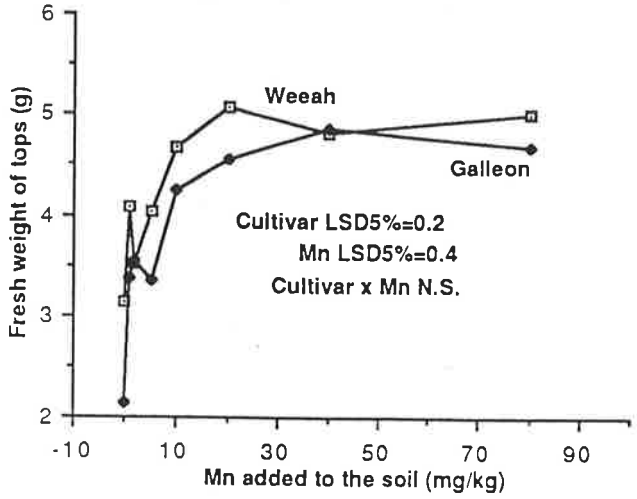


Fig. 5.6

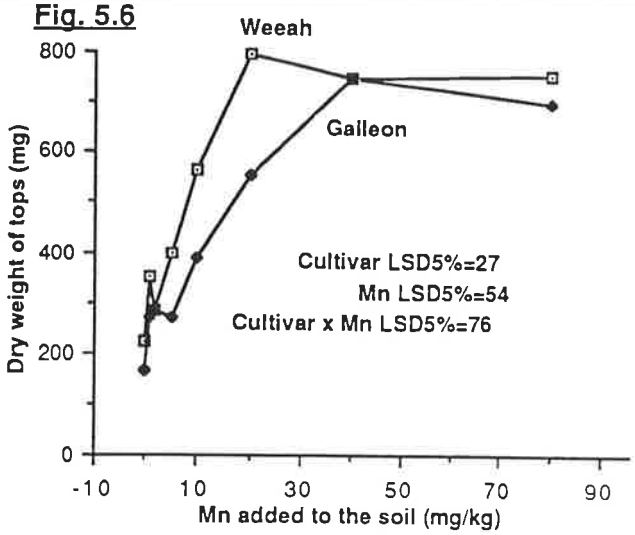


Fig. 5.7

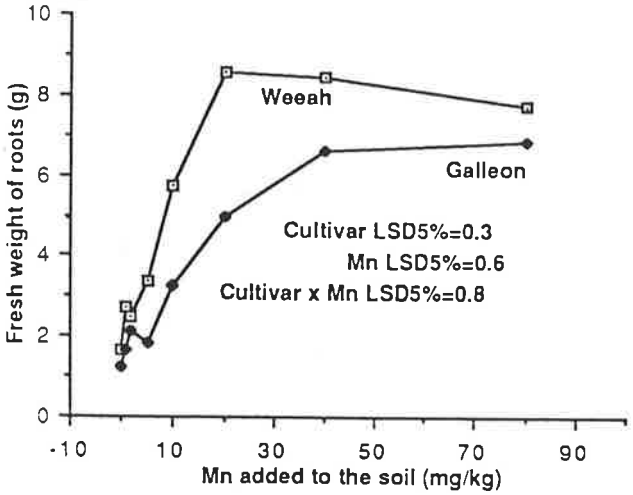
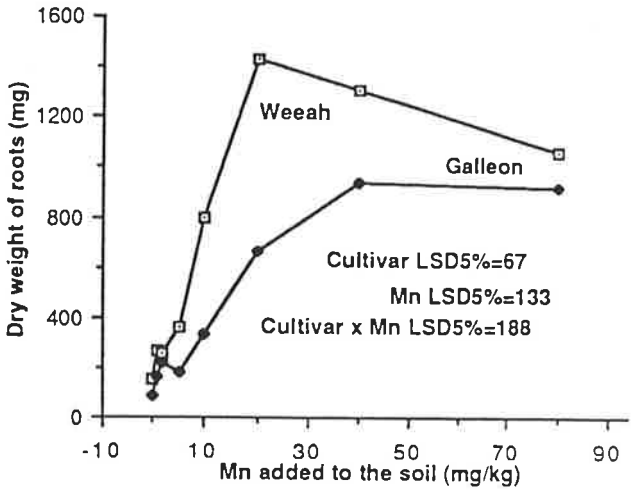


Fig. 5.8



FIGURES 5.5-5.8 Fresh and dry weights of tops and roots (on a per pot basis) at harvest at 28 days of two barley cultivars *H. vulgare* cv. Weeah and Galleon at eight levels of Mn additions to the soil.

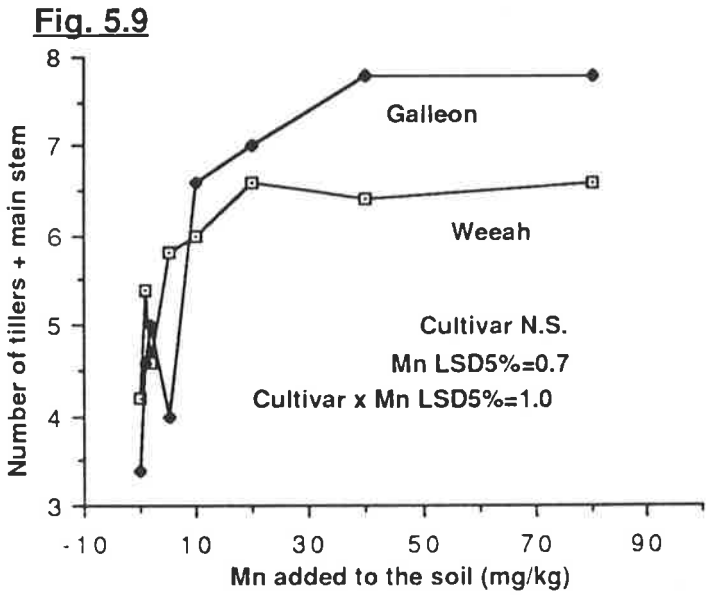


FIGURE 5.9 The number of tillers + the main stem (on a per pot basis) of two barley cultivars *H. vulgare* cv. Weeah and Galleon at eight levels of Mn additions to the soil at harvest at 28 days.

Fig. 5.10

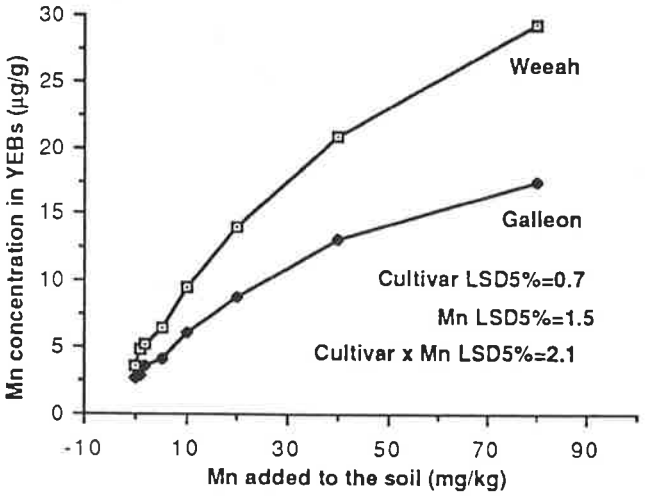


Fig. 5.11

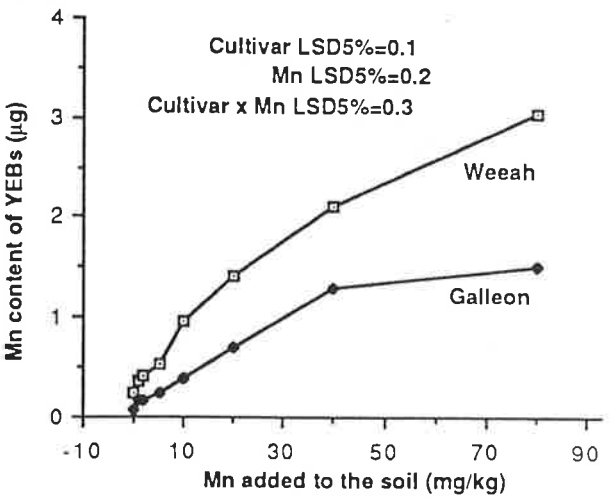


Fig. 5.12

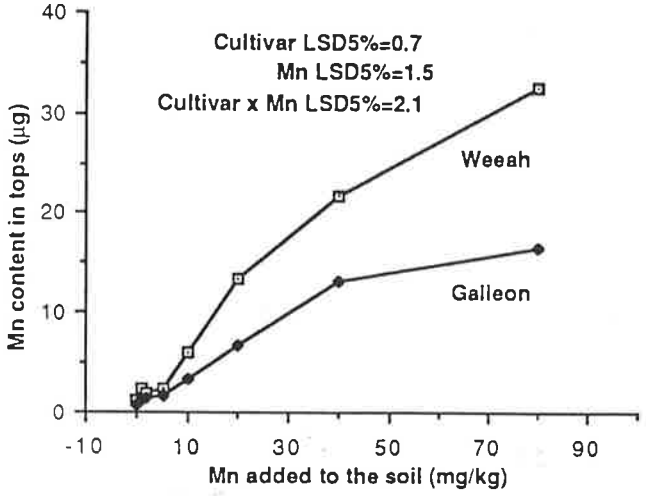


Fig. 5.13

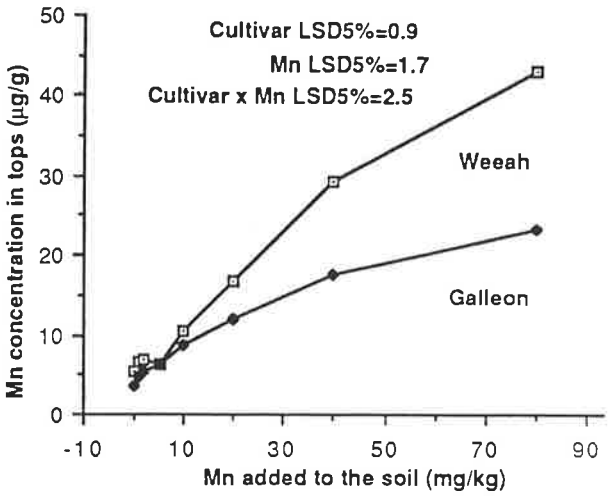


FIGURE 5.10-5.13 Mn concentrations and contents in whole tops and YEBs of two barley cultivars *H. vulgare* cv. Weeah and Galleon at eight levels of Mn additions to the soil at harvest at 28 days.

FIGURES 5.14-5.17 Critical nutrient curves constructed with computer generated models using SAS (Statistical Analysis System, SAS Institute, Inc. Box 8000, Cary, NC) for two barley cultivars *H. vulgare* cv. Weeah and Galleon for whole tops and YEBs. The equation of the line for each figure is listed below for both

Model 1 $y_{cl}=a + b.c^{x}$ and

Model 2 $y_{cl}=b\ (1 - ce^{-ax})$.

FIG. 5.14 Model 1 $Y_{cl}=0.782786751-1.087359319 \text{ X } 0.835342186^{x}$

Model 2 $Y_{cl}=0.782712786(1-1.387750285e^{-0.179729372x})$

FIG. 5.15 Model 1 $Y_{cl}=0.8137825046-0.8782612946 \text{ X } 0.8718438673^{x}$

Model 2 $Y_{cl}=0.813751431(1-1.079460246e^{-0.137196904x})$

FIG. 5.16 Model 1 $Y_{cl}=0.776482106-1.438269022 \text{ X } 0.834912137^{x}$

Model 2 $Y_{cl}=0.776513609(1-1.852375754e^{-0.180424140x})$

FIG. 5.17 Model 1 $Y_{cl}=0.775560808-1.018055132 \text{ X } 0.879024642^{x}$

Model 2 $Y_{cl}=0.775521843(1-1.312782617e^{-0.128966066x})$

Fig. 5.14

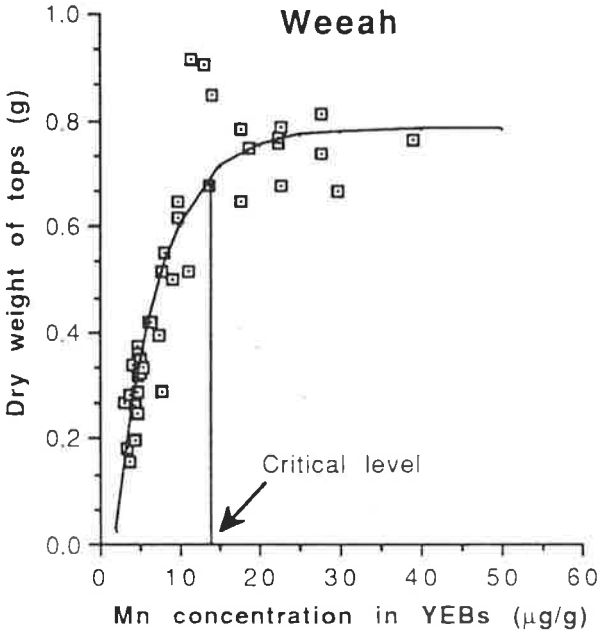


Fig. 5.15

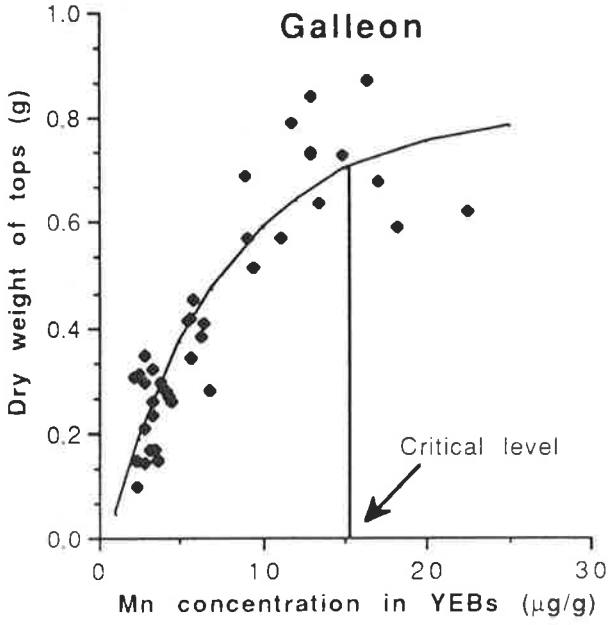


Fig. 5.16

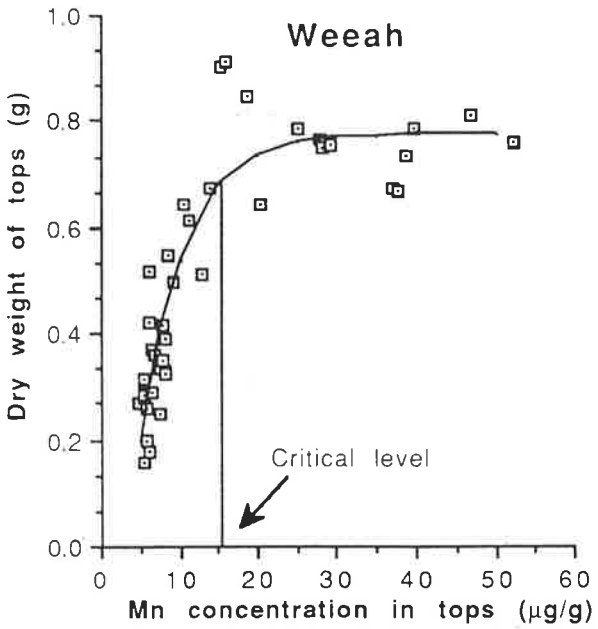
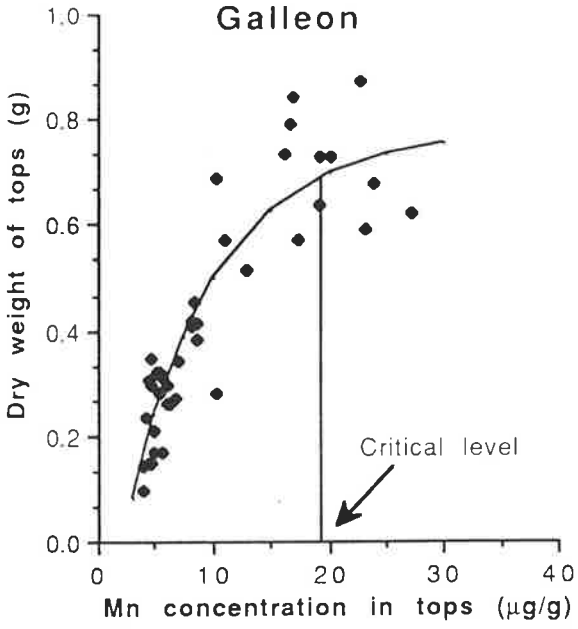


Fig. 5.17



FIGURES 5.18-5.19 Critical nutrient curves constructed with computer generated models using SAS (Statistical Analysis System, SAS Institute, Inc. Box 8000, Cary, NC) for two barley cultivars *H. vulgare* cv. Weeah and Galleon for YEBs. The equation of the line for each figure is listed below for both Model 1 $y_{cl}=a + b.c^{x}$ and

Model 2 $y_{cl}=b\,(1 - ce^{-ax})$.

FIG. 5.18

Model 1 $Y_{cl}=0.1037101310-0.0897325469 \text{ X } 0.7875422370^{x}$

Model 2 $Y_{cl}=0.097010059(1-2.731220694e^{-0.554450180)}$

FIG. 5.19

Model 1 $Y_{cl}=0.0978511866-0.0915533478 \text{ X } 0.8390860350^{x}$

Model 2 $Y_{cl}=0.0978425802(1-0.9357092825e^{-0.1755050121x)}$

Fig. 5.18

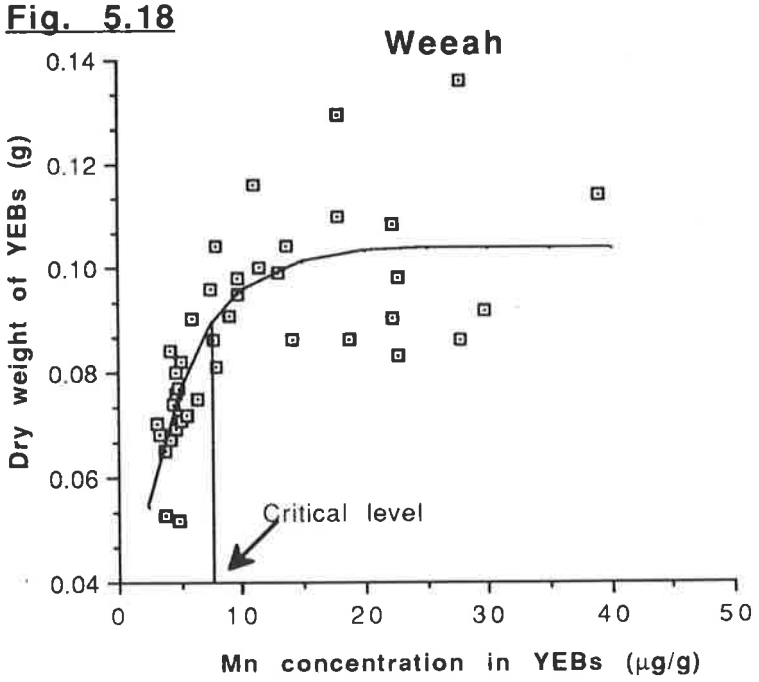


Fig. 5.19

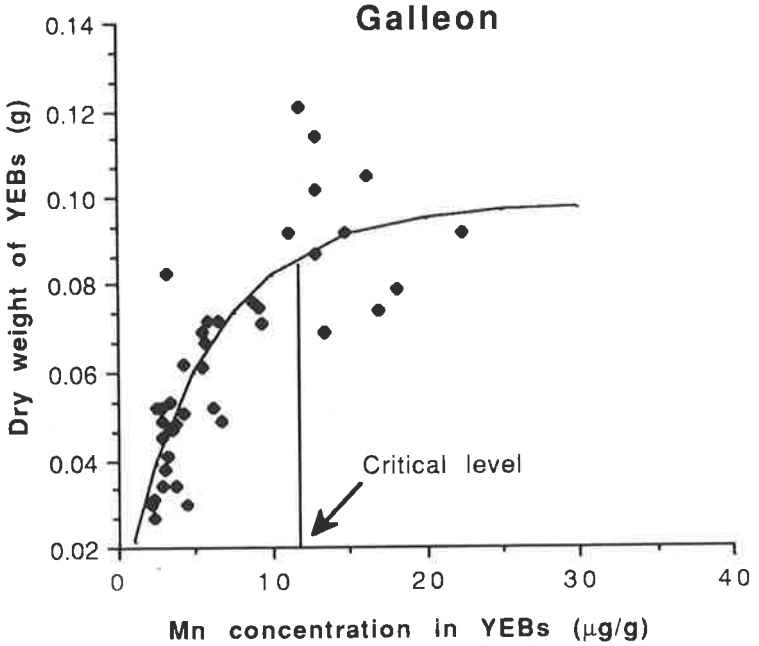


Fig. 5.20

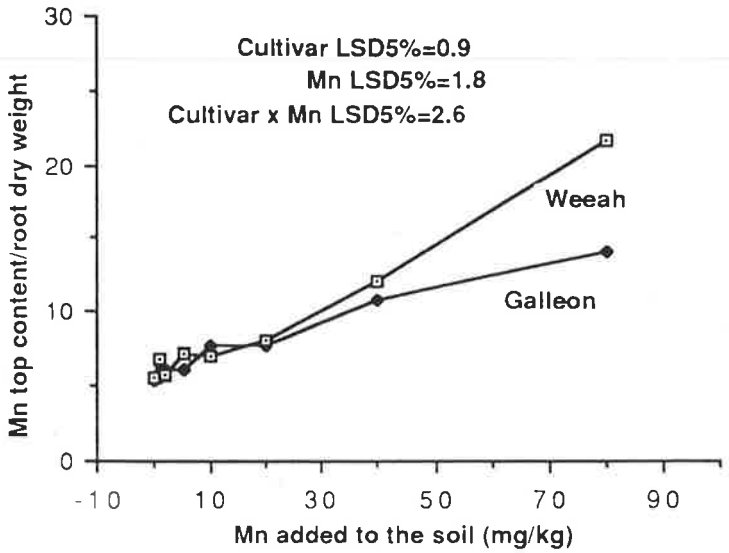


FIGURE 5.20 Mn uptake per unit of final root weight of two barley cultivars *H. vulgare* cv. Weeah and Galleon as a function of Mn additions to the soil after 28 days growth.

Fig. 5.21

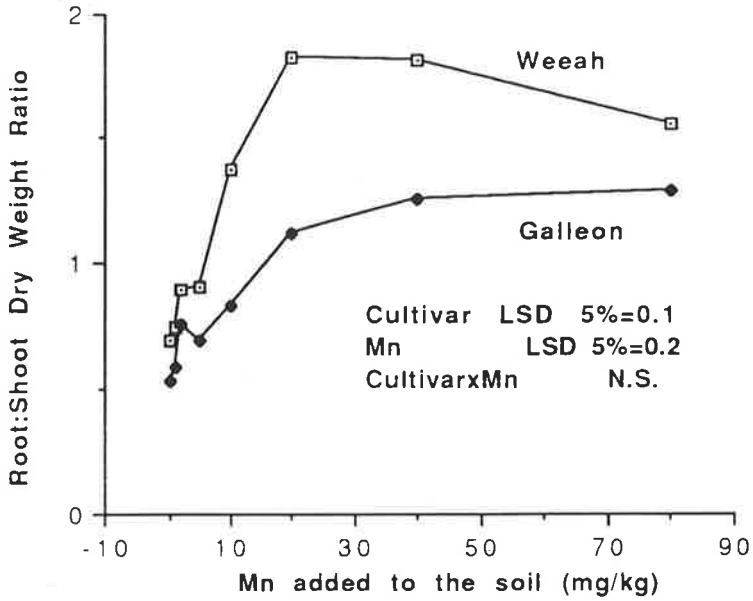


FIGURE 5.21 Root:shoot dry weight ratios of two barley cultivars *H. vulgare* cv. Weeah and Galleon as a function of Mn additions to the soil after 28 days growth.

CHAPTER 6

GENERAL DISCUSSION

6.1 Discussion

The aims of the experiments in this thesis were to identify the mechanism(s) which confer Mn efficiency on some genotypes of barley. Four areas were investigated and those were; (1) a possible lowering of the pH in the rhizosphere soil of efficient plants, (2) whether differences in root morphology existed between efficient and inefficient genotypes, (3) if a lower population of Mn oxidising micro-organisms were associated with Mn efficient genotypes and (4) whether efficient genotypes had a lower critical level. The identification of a mechanism of efficiency followed by a method of assessing its efficacy at the seedling stage could then be incorporated into the screening stage of a breeding program. Screening for Mn efficient barley seedlings at present relies on such factors as Mn concentration in aerial parts, chlorosis scores and in later screenings, final yields. All of these are indirectly related to mechanisms of efficiency. Uren *et al* (1988) found that a chlorsis score was successful in mimicking field rankings of barley genotypes, more than Mn uptake but again this has no direct relationship with a possible mechanism of efficiency.

The agar method of assessing H^+ extrusion was a valuable tool in determining the ability of a plant to acidify it's rhizosphere under different growing conditions. It had limitiations however as it could not be used for quantitative comparsions although this was achieved in some degree by Marschner *et al* (1986) using colour coding. The location of sites of acidification around the roots were identified at root tips and around lateral roots both in solution culture and in the highly buffered Mn deficient calcareous soil. However, the response at the root/agar interface may not resemble what is actually happening at the root/soil interface. The quantity of Mn sequestered in roots could not be determined due to the constraints of the experimental system which prevented whole plant analysis. Roots were not analysed after embedding in agar. This Mn may be important if it was shown to be transported to shoots in times of Mn stress. Observation of reduction of Mn oxides by roots embedded in agar impregnated

with brown Mn oxide may have been affected by variations in Mn oxide density and agar thickness between plates. Also the presence of free Mn^{2+} in the agar was established as all plants absorbed Mn from the agar but a corresponding clearing around the roots was not observed. Therefore the agar method may not have been sensitive enough to distingish between genotypes in their ability to reduce Mn oxides.

Experiments using areated solution culture as a nutrient medium have a major limitation as they have no functional rhizosphere. There were higher Mn concentrations in soil grown plants where the rhizosphere is present, than the solution culture grown plants. There was a finite amount of Mn in solution which may suddenly become exhausted leaving the plants in a state of deficiency. When the solutions were renewed the roots would again be in a state of sufficiency. The flux of deficiency/sufficiency may inhibit or impair root functions. Experiments in potted soil would provide a steady supply of Mn to the plant. The presence of micro-organisms in the rhizosphere contributes to root activity which again are probably quite different in nutrient solution. Therefore nutrient solution studies, especially under sterile conditions, can not be fully representative of the field situation.

There have been suggestions to breed for root morphological traits (Marschner 1988) in breeding for nutrient efficiency. Study of the root systems in soil grown plants were carried out at the seedling stage when the seminal roots were the major nutrient gatherers. Although seminal roots decrease in importance as nutrient gatherers as the nodal root system develops, a strong seedling seminal root system will promote early seedling vigour which may then be carried through to seed set. No difference between genotypes in seminal root morphology were identified in either soil or nutrient solution studies. Experiments using solution culture enabled study of the roots at a later developmental stage and the contribution made by nodal roots could be established. It was found that the more Mn efficient genotypes had a more highly branched nodal root system than the inefficient genotypes. This trait would have a number of advantages including greater exploration of the soil, an increase in the

number of root tips which have been shown to be areas of H^+ extrusion and an increased area of root exudate production. However root form in an aerated solution culture system would be expected to differ from the field situation. Ideally, nodal root development in soil grown plants should be assessed. A change in root:shoot ratios as a response to nutrient deficiency has been observed by a number of workers (Cumbus 1985) and this was seen in Chapter 5 where root growth was increased at the expense of the shoot. Under severe Mn deficiency however, root dry weight was depressed to a greater extent than shoot dry weight in all genotypes assessed. This root sensitivity to Mn deficiency was noted in both soil and solution cultures and has been observed by other workers in Mn deficient barley (M. Webb pers. comm).

Seed Mn content has been found by Marcar (1986) and Uren *et al* (1988) to enhance seedling vigour. Better accumulation of Mn in the seed leading to greater seedling vigour and viability may be a manifestation of Mn efficiency or a second contributing factor to efficiency. In these investigations the contribution made by high seed loading was eliminated by using seed with as similar Mn content as possible. This sometimes entailed use of seed from different sources in the same experiment. Prior to sowing, the seeds were allowed to grow for one week in a solution of $CaSO_4$ to consume these seed reserves.

Microbial activity in the rhizosphere is greater than in the bulk soil due to the better supply of organic carbon provided by plant roots. Whether this will increase or decrease Mn availability to the plant depends on the relative populations of Mn oxidising and Mn reducing microorganisms. Within the pH range of 6-8, Mn-oxidising microorganisms are far more effective than any non-biological system of oxidising Mn^{2+} (Uren 1981). It is likely that some benefit is derived from associated microorganisms as at least some of the end products of microbial activity such as organic acids may have effects on mineral nutrient mobilisation that are similiar to root exudates. Thus, although no significant differences were found between genotypes in numbers of Mn oxidising colonies in soil collected from the rhizosphere such as

Timonin (1946) found with oats, a trend was evident of more Mn oxidising microorganisms present in the rhizospheres of the more Mn inefficient genotypes. Microorganisms may yet prove to play an important role in Mn nutrition.

No difference was found in critical level between a Mn efficient and Mn inefficient genotype when using whole tops. In the youngest plant tissue the critical level for the efficient genotype was 8µg/g compared to 12µg/g in the inefficient genotype and 17µg/g in whole tops. The higher level in whole tops is a reflection on the total Mn content in tissues that is, both functional and stored Mn. The fact that Weeah has a lower concentration of Mn in YEBs may indicate that those functions which require Mn may be more sensitive to the element and function at a lower level or that another element may substitute. This experiment estimated the total amount of Mn in a particular plant part and did not discriminate between functional Mn and total Mn as described by Nable *et al* (1984) where functional Mn was measured through the participation of Mn in the water splitting process of photosynthesis. Hannam *et al* (1987) measured the functional nutrient requirement of Mn in Galleon by measuring enhanced chlorophyll 'a' fluorescence as a measure of disruption in photosynthesis and found this to be a more sensitive parameter than dry matter yield.

Mn efficiency could not be wholly attributed to any of the mechanisms researched here. The additive effect of a combination of any of the processes studied here in the one genotype may ensure an adequate level of tissue Mn under conditions of low available soil Mn. McCarthy *et al* (1988), however, have shown that Mn efficiency in barley seems to be controlled by a single, major, dominant gene. It is unlikely that all of these processes could be under single gene control. Studies on absorption (Mn uptake per gram dry weight of root) between an efficient and inefficient genotype in solution culture have shown a higher absorbance for the more efficient genotypes at all Mn levels (Webb pers. comm.) but this has not been a consistent response and there was no difference in absorbance in soil culture in this study at low soil Mn levels.

The results of this research suggests the presence of a plant produced compound released through the roots and capable of reducing unavailable Mn oxides. Such compounds have been found for example in Zn deficient barley and wheat where Zn deficiency increased root exudation of amino acids, sugars and phenolics which increased mobilisation of zinc from Zn chelite, a synthetic resin (Zhang *et al* 1991). With Zn deficiency the integrity of cell membranes is impaired so an increase in root exudate can be attributed in part to membrane breakdown. These exudates were highly effective in mobilizing Zn, Fe, Cu and Mn in calcareous soil. Zhang *et al* (1989) have identified the dominant component in wheat root exudates as the phytosiderophore 2'--deoxymugineic acid released under both Zn and Fe deficiency and capable of mobilising both Zn and Fe at comparable rates. Therefore phytosiderophore release was not a response mechanism specific for Fe deficiency but one that also occured under Zn deficiency in both wheat and barley. Experiments with barley showed under both Zn and Fe deficiency another phytosiderophore, epi-3-hydroxymugineic acid, was released which was capable of mobilizing Fe, Zn Cu and Mn from calcareous soil (Treeby *et al* 1989). However, no such release of phytosiderophores have been found in either wheat or barley under Mn deficiency (Zhang *et al* 1989). In this study, Mn inefficient genotypes required twice the amount of added Mn to the soil than efficient genotypes to reach Mn sufficiency in their tissues. Therefore these efficient genotypes were able to mobilise and absorb insoluble Mn oxides from the soil and absorb more Mn from solution culture, when Mn was freely available, than inefficent genotypes indicating the existence of both a mechanism to reduce Mn oxides and an enhanced capacity to take up Mn^{2+}. Further studies should therefore be directed at finding and identifying the component of root exudates in Mn efficient genotypes which can reduce insoluble Mn oxides in Mn deficient soils.

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