GENOTYPIC VARIATION FOR MANGANESE EFFICIENCY IN CEREALS

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The research reported in this thesis is original and was conducted without collaboration. In specific instances reference has been made to the work of others.

Nico E. Marcar,

March, 1986.

I wish to dedicate this thesis

to my wife and son

and

to my parents

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Abstract

The experiments described in this thesis consider the extent of genetic variation for Mn efficiency (ie. tolerance to Mn deficiency) in cereals, with particular reference to wheat, and explore some possible plant factors associated with Mn efficiency. Emphasis has been given to the growth of seedling plants under growth cabinet conditions in pots containing Mn-deficient soil. A long term objective was to develop a screening technique for Mn efficient genotypes in a screening program.

Barley proved to be more Mn efficient than wheat, triticale or rye, when grown for 4-5 weeks in small (250 g cap.) pots at 15°C, having the highest dry matter production, Mn uptake and utilisation efficiency. The higher rate of Mn uptake for barley was much more strongly associated with root growth and root morphology (greater lateral root development) rather than with enhanced chemical or biological modification of the rhizosphere. From a study with wheat-barley addition lines it appeared that the Mn efficiency of barley could be transferred to wheat, but that the mechanism of this efficiency was not obvious nor simply inherited.

Differences in growth between wheat cultivars (in particular) without added Mn were confounded by differences in the Mn content of sown seeds. Nevertheless significant wheat cultivar variation in the rate of growth , Mn uptake and Mn utilisation became apparent when a little Mn (10 mg kg^{-1} soil) was added. Cultivar differences for a functional requirement of Mn in photosynthesis, as determined by room temperature chlorophyll *a* fluorescence, were small.

Further growth cabinet and field studies demonstrated that seed Mn content (natural ocurring as well as artificially increased by soaking in $MnSO_4$) played a vital role in determining early plant productivity as well as influencing grain yield. Manganese-inefficient genotypes could be distinquished by their greater response to seed soaking in the field.

Results of field experiments conducted mainly at Wangary on the same soil as used in the pot experiments showed that agronomic Mn efficiency (based on relative grain yield) varied considerably between cereal cultivars. Rye and Weeah barley were the most Mn-efficient and oats the least Mn-efficient cereal. Considerable differences were evident between cultivars of wheat, barley and triticale for actual and relative grain yields. The best wheat (Aroona) yielded about the same as the worst barley (Galleon). Yields of triticale were intermediate between wheat and rye. The least Mn-efficient cultivars were characterised by severe plant mortality mid-season as well as low grain yields per plant.

Table of contents

CHAPTER 2

TOLERANCE OF WHEAT, TRITICALE, RYE AND BARLEY SEEDLINGS TO MANGANESE DEFICIENCY

CHAPTER 3

FACTORS ASSOCIATED WITH MANGANESE AQUISITION BY WHEAT AND BARLEY SEEDLINGS

CHAPTER 4

GENOTYPIC VARIATION FOR MANGANESE EFFICIENCY IN WHEAT SEEDLINGS

CHAPTER 5

EFFECT OF SEED MANGANESE CONTENT ON SEEDLING GROWTH OF CEREALS, PARTICULARLY WHEAT, UNDER MANGANESE DEFICIENCY

CHAPTER 8

GENERAL CONCLUSIONS

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Manganese was the first trace element to be established as essential for higher plant growth (McHargue 1922). In Australia, Mn deficiency was first identified in 1927 by G. Samuel and C. S. Piper, working at the Waite Institute, South Australia. They correctly perceived that "road take-all" and "grey speck" disease of oats observed near Penola, South Australia, was caused by a decreased availability of Mn due to soil alkalinity. In 1929, they successfully produced grey speck disease in water culture, the first laboratory demonstration of a known micronutrient deficiency (Samuel and Piper 1929).

Manganese deficiency has subsequently been encountered in many crops grown on a wide range of Australian soils (Donald and Prescott 1975), but particularly on some soil groups of high lime status and pH, notably the coastal calcareous aeolian sands with up to 80% $CaCO_3$ (pH 8.5), the calcareous solonised brown soils (mallee soils) of uncertain origin, occurring in semi-arid southern areas, and various shallow soils over limestone (eg. rendzinas). Manganese deficiency is also prevalent on alkaline and/or high organic matter soils overseas (eg. Holland (Henkens and Jongman 1965), Sweden (Johannsen and Ekman 1956), United States (Cunningham 1972) and England (Batey 1971).

Amelioration of trace element-deficient soils of the so-called "90 mile desert" of South Australia in the 1950s increased the productivity of pasture legumes and hastened the development of cereal growing, particularly in more marginal farming country (Donald and Prescott 1975). Recently, there has been a renewed interest in application of Mn, Zn and Cu on cereal crops in South and Western Australia. This need has arisen primarily as a result of the increasing use of high analysis fertilisers.

Recent advances have been made in increasing the efficiency of soil and foliar applied Mn (Reuter et al 1973; Hannam 1984) and in the assessment of cultivars with increased tolerance to Mn deficiency (Graham et al 1983). Current research areas include (1) screening of genotypes for Mn efficiency (Graham 1984), (2) the relationship between Mn deficiency and the

susceptibility of plants to disease (Graham and Rovira 1984; Wilhelm et al 1985), (3) effects of Mn deficiency on plant metabolism and growth (Brown et al 1984; Nable et al 1984; Kriedemann et al 1985) and (4) the definition of critical concentrations of Mn for growth and grain yield (Graham et al 1985).

This review seeks to (1) discuss those soil conditions leading to the development of Mn deficiency and factors associated with plant tolerance to this stress, (2) describe the effects of Mn deficiency on plant growth and metabolism and (3) define characteristics of nutrient-efficient genotypes, with particular reference to Mn, and (4) comment on the efficacy of various screening procedures.

1.2 FORMS AND AVAILABILITY OF SOIL MANGANESE

1.2.1 Introduction

Total soil Mn content can vary between 20 and 6000 ppm (Krauskoff 1972). However, it is known that total soil Mn is poorly correlated with Mn availability to plants (Hoff and Mederski 1958; Page et al 1962; Leeper 1970; Aubert and Pinta 1977).

Weathering of the ferromagnesian and secondary soil minerals releases Mn mainly in a divalent (Mn^{2+}) form, which is then reoxidised and may become associated with other soil fractions, notably the clay minerals and organic matter (Taylor et al 1964). The reactions of these fractions in the soil determine the supply of Mn available for plants. The most important forms of Mn in soils are (Mengel and Kirkby 1982):

1. Mn^{2+} or divalent Mn
2. MnO_2 (Mn^{4+}) or pyrolusite (crystalline)
3. Mn_2O_3 (Mn^{3+}) or hausmannite (crystalline)
4. MnOOH or manganite (crystalline)

Soil Mn potentially available to plant roots has been termed "active Mn" (Mengel and Kirkby 1982) and refers to the contribution of divalent Mn and easily reducible Mn "pools". Divalent Mn occurs in the soil solution as water soluble Mn (in small quantities except in acid soils) and in association with the colloidal fraction (clay minerals and organic matter) as exchangeable Mn (Mulder and Gerretsen 1952). Types of organic matter differ in their ability to complex Mn^{2+} and therefore in their influence over Mn availability to plants (Mulder and Gerretson 1952). Easily reducible Mn is the fraction of potentially reactive Mn oxides that plant roots could have access to (Leeper 1970).

1.2.2 Measurement of soil Mn pools

Much effort has been devoted to the development and refinement of chemical procedures for estimating the size of soil Mn pools in order to determine the soil's potential for supplying Mn to plants. Chemical extraction of soil Mn in ionic, reduced or chelated forms have been used and these are summarised in Table 1.1. Critical levels for a particular Mn fraction can then be established (Lindsay and Norvell 1978).

Notwithstanding this effort, soil analysis remains an unreliable predictor of soil Mn availability, particularly on neutral to alkaline soils. This is partly due to the arbitrary nature of the chemical procedures used in estimating Mn pools (eg. overestimates of easily reducible Mn) and partly due to changes in pool sizes that occur during sampling, storage and analysis (Boken 1952; Bromfield 1978; Shumann 1980). Moreover, these tests take no account of the individual requirements of various plant species and cultivars (Cox and Kamprath 1972).

The emphasis in tests for soil Mn availability has been on the determination of divalent and chelate-extractable Mn, since it has been generally accepted that plants obtain soil Mn as Mn^{2+} (Geering et al 1969). Whilst this may be the case in neutral soils, it has been strongly argued that plants must obtain Mn^{2+} directly from Mn oxides by reduction processes when grown on alkaline soils (Jones and Leeper 1951a; Uren 1969; Bromfield 1978). Therefore, since Mn deficiency is most often associated with

Table 1.1: Chemical extractants used for measurement of soil Mn pools.

Mn pool	Extractant	Reference
A. Divalent		
1. water soluble	H_2O	Cox and Kamprath (1972)
2. exchangeable	ethanol	Uren (personal communication)
	1% CaNaHEDTA (pH=8)	Beckwith (1955)
	1 M CH_3COONH_4 (pH=7)	Skerman and Harmer (1942)
	$Mg(NO_3)_2$, $Ca(NO_3)_2$ CH_3COONa, $NH_4H_2PO_4$ $CaCl_2$	Sheppard and Bates (1982)
B. Chelate extractable		
	0.05 M EDTA	Viro (1955)
	0.005 M DTPA + 0.01 M $CaCl_2$ (pH=7.2)	Lindsay and Norvell (1978)
	2-ketogluconic acid	Berrow et al (1982)
C. Reducible		
1. easily reducible	0.2% hydroquinone + $Ca(NO_3)_2$	Jones and Leeper (1951b)
	CH_3COONH_4	Skerman and Harmer (1942)
	CaNaHEDTA (pH>7.5)	Beckwith (1955)
2. less easily reducible	dilute HCl, H_2SO_4 HNO_3, H_3PO_4 and HCl + H_2SO_4	Sheppard and Bates (1982)

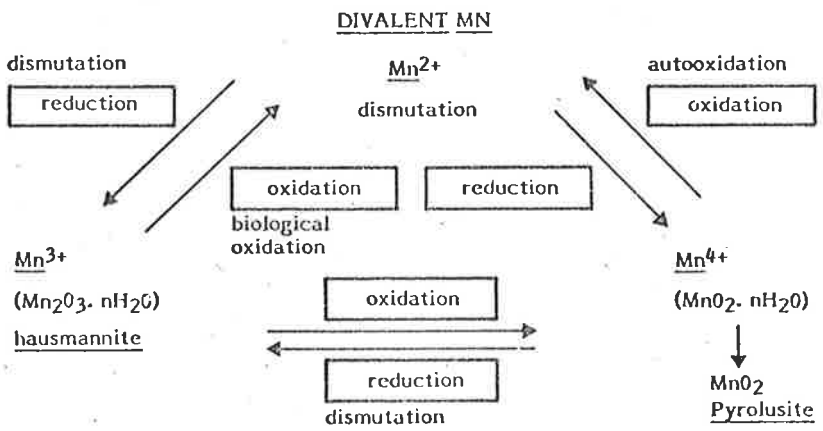

Figure 1.1 (adapted from Dion and Mann (1946) by Mengel and Kirkby 1982)

alkaline soils (Leeper 1970), more emphasis should be given in soil testing to the easily reducible fraction.

1.2.3 Manganese availability in calcareous soils

Most calcareous soils are in the range of pH 7.3 - 8.5 (Lindsay 1979) and contain variable amounts of free $CaCO_3$. In the presence of 1% CO_2 (eg around plant roots) pHs may be closer to 7.5 - 8.0 (Rowell 1981). Neither the soil nor the plant factors that determine the availability of Mn (and Fe) in calcareous soils are well understood (Jauregui and Reisenaeur 1981).

Calcium carbonate added to soil dramatically reduces the amount of exchangeable Mn even at neutral pH (Christensen et al 1950) and the amount of exchangeable Mn is highly correlated with the amount of $CaCO_3$ in calcareous soil profiles (Reuter et al 1973). A chemical association between Mn and $CaCO_3$ deposits was suggested some time ago by Mulder and Gerretson (1952) and Leeper (1952). It is now known that $CaCO_3$ influences Mn availability through its effects on pH, through surface adsorption of Mn^{2+} (Bromfield and David 1978; M^{c}Bride 1979) and through precipitation or formation of a manganocalcite (M^{c}Bride 1979). Manganocalcite ($MnCO_3$) may be nucleated at $CaCO_3$ surfaces at least initially, effectively coating calcite particles with $MnCO_3$ (M^{c}Bride 1979).

In addition, soils of recent marine origin may have Mn deposited within the calcite structure (Zajic 1969). Manganese is deposited in the calcareous shells of foraminiferous species feeding upon diatoms which contain Mn. Here the Ca is replaced by Mn in the calcite structure of the shells. Accretions of Mn oxides are common in molusc shells due to bacterially catalysed precipitation of Mn in seawater (Zajic 1969).

Bicarbonate ions per se may also reach toxic concentrations in the soil solution of calcareous soils (Lee and Woolhouse 1969; Hutchinson 1967) and limit root extension (Uren 1984), which will affect nutrient uptake. For example, uptake of Fe by beans was severely depressed by high concentrations of HCO_3^- (Rutland and Bucovac 1971) even in so called

Fe-efficient plant species such as sunflowers (Venkatraju and Marschner 1981).

1.2.4 Oxidation-reduction cycling of soil Mn

Soil Mn is subject to valency transformations, depending on the oxidation-reduction status of the soil system (Fig.1.1). The dynamics of oxidation-reduction cycling in soils are strongly affected by hydrogen (H^+) ion and electron (e^-) activities ie. pH and pe (Bartlett 1981). If oxidation and reduction are occurring simultaneously, the Mn^{2+} concentration in the equilibrium solution will be governed by the relative velocities of the oxidation and reduction reactions. Thus all soil factors influencing these processes have an effect on Mn availability to plants.

The redox equation governing the relative states of Mn in solution can be represented thus (Uren 1982):

$$MnO_x + 2xH^+ + 2(x-1)e \xleftrightarrow{\text{microorganisms}} Mn^{2+} + xH_2O$$

The value of x depends on the relative amounts of Mn^{4+} and Mn^{3+} present (Bromfield 1958). If the most stable oxide (MnO_2) is considered, the specific reaction is (Russell 1979; Rowell 1981):

$$MnO_2 + 4H^{2+} + 2e \longleftrightarrow Mn_{2+} + 2H_2O$$
$$pe = 1.23 - 0.030\log Mn^{2+} - 0.20pH$$

(a) Oxidation of Mn^{2+}

Both chemical and biological oxidation of Mn occur in soils (Dion and Mann 1946). The contribution of chemical reactions to immobilisation of Mn^{2+} increases as the pH increases (Dion and Mann 1946) and at very high pH chemical auto-oxidation predominates (Leeper 1970). Microbial oxidation is particularly important in neutral and slightly alkaline soils (Uren 1969; Geering et al 1969; Mengel and kirkby 1982).

The role of Mn-oxidising microbes in determining the availability of soil Mn to plants is still not fully understood (Bromfield 1978), but it is known that the ability of soil microbes to oxidise Mn^{2+} is greatly impeded below pH 5.5 (Leeper and Swaby 1949) and at alkaline pHs (Reuter and Alston 1975). Biological oxidation also requires an optimal O_2/CO_2 ratio in the soil for maximum efficiency (Uren 1969; Bromfield 1974).

Oxidation of Mn in soils has been shown to be microbially mediated, mainly by studies using soil sterilisation or microbial inhibition with antibiotics (Gerretsen (1937) and Leeper and Swaby (1940) used chloroform, Mann and Quastell (1946) used sodium azide and Timonin (1946) used calcium cyanide). However, some doubt has been recently cast on the validity of these studies by Ross and Bartlett (1981). These authors claimed that the apparent inhibition of microbial oxidation is in fact the reduction of Mn oxides by these chemicals.

Bacterial oxidation of soluble Mn salts to insoluble Mn oxides has been demonstrated in agar, liquid and solution cultures for Sphaerotilus discorphorus (Johnson and Stokes 1966), Arthrobacter and Pseudomonas (Mulder and Van Veen 1968; Bromfield 1974) and Pseudomonas III and Citrobacter freundii (Douka 1977). It is important to differentiate between indirect (eg pH changes or Mn adsorption onto bacterial cell surfaces) and direct (enzymatic action) effects of Mn-oxidising bacteria (Douka 1977). No direct evidence for microbial oxidation in soils is available. In fact, Ross and Bartlett (1981) found that none of the Mn-oxidising bacteria isolated from soils could oxidise Mn when reinoculated into the soil.

(b) Reduction of Mn oxides

The rate of reduction of Mn oxides is determined by the magnitude of pH and pe, the activity of soil microorganisms as well as the reactivity of the Mn oxides.

The concentration of Mn^{2+} in the soil solution increases 100-fold for each unit decrease in soil pH, due mainly to the provision of extra protons (Lindsay 1972) but also to enhanced oxidising power of the Mn oxides through a decreased redox potential (Russell 1979). Acidification may also slow or stop microbial oxidation.

The possible sources of electrons in a soil are the exudates (Bromfield 1958) and surfaces of roots (Brown et al 1961), microbial processes (Bromfield 1954) and soil organic matter (Mann and Quastel 1946; Leeper 1947). Of these sources organic matter appears to be the most important in the soil (Uren 1969), particularly at lower pH (Leeper 1947).

There is considerable evidence that microorganisms are able to bring about biological reduction of Mn oxides, particularly under anaerobic conditions, where MnO_2 may substitute for O_2 as an H^+ acceptor in bacterial respiration (Sherman and Harmer 1942). Indirectly, products of microbial metabolism, such as thiol and polyphenol groups, act as reducing agents, thus releasing Mn^{2+} (Mann and Quastell 1946; Webley and Duff 1965). These compounds would be acting in the same manner as the chemical reductants added to the soil by Fujimoto and Sherman (1948). Barber and Lee (1974) have shown that microorganisms introduced from the rhizosphere into nutrient solution can increase Mn uptake.

Wadsley and Walkley (1951) have shown that the reductive reactivity of Mn oxides was related to the oxide composition (higher oxidation states being more reactive), oxide particle size (small particle sizes expose a larger surface area for reduction), and the degree of crystallinity of the oxide (amorphous forms are more reactive). Jones and Leeper (1951a) demonstrated that Mn oxides with these specific properties (eg manganous manganite) were best in curing Mn deficiency. Other oxides (eg hausmannite) which did not possess these characteristics were inert soil amendments.

The most reactive oxides are likely to be the most freshly precipitated ones (Uren 1969), and in this respect they will be comparable to the microbial oxide prepared by Bromfield (1958), which had a fairly high

oxidation state and was amorphous and highly hydrated. Jones and Leeper (1951b) postulated that the loss of reactivity of the Mn oxide with length of time in contact with the soil was due to the reversion of the Mn oxide to a more ordered crystalline structure.

The form of Mn oxide most accessible to plant roots has been termed easily reducible Mn and is mainly MnO_2 rather than Mn_2O_3. This form of Mn oxide can be readily converted to Mn^{2+} by mild reducing agents (Leeper 1970).

1.3 MANGANESE UPTAKE AND TRANSLOCATION

1.3.1 Acquisition of Mn at the root-soil interface

Manganese availability in the rhizosphere is also affected by oxidation-reduction cycles, except that plant factors as well as microbial and soil factors must be invoked.

Soil Mn enters plant roots almost certainly as Mn^{2+} (Page et al 1962; Rivenbark 1961; Geering et al 1969). In soils which are mildly Mn-deficient it is possible that diffusion of exchangeable Mn to plant roots may provide an important pathway (Barber 1968; Halstead et al 1968). However in neutral to alkaline soils, which may be moderately to se verely Mn-deficient, the total reactive surface of the Mn oxides (Uren 1969) may be a better indicator of Mn availability. Thus reduction of the Mn oxides must occur at some stage before uptake.

The ability of growing roots to reduce Mn, by supplying electrons through root exudates, by altering the reducing capacity of the rhizosphere or by contact reduction, is widely recognised (Bromfield 1958, 1978; Passioura and Leeper 1963; Uren 1982). In order to understand how these three factors interact in determining the rate of arrival of Mn^{2+} at the root surface, it is important to review some literature relevant to the rhizosphere and root exudation.

(a) The rhizosphere and root exudates

The rhizosphere is defined as that zone of the soil in which the soil microorganisms are influenced by plant roots (Russell 1977). This zone overlaps with the bulk or non-rhizosphere soil and with the root surface. The transition zone between the root surface and soil rhizosphere has been termed the rhizoplane or rhizogel (Uren 1969), consisting of mucigel (Jenny and Grossenbacher 1963), which is the gelatinous material at the surface of roots grown in normal non-sterile soils (Rovira et al 1979).

Plant roots constantly release chemical substances into the rhizosphere. Substrates from roots have many origins. Rovira et al (1979) have classified these as:

1. Exudates (compounds of low molecular weight leaking from intact cells),
2. Secretions (compounds of low molecular weight as well as high molecular mucilages released actively from cells),
3. Mucilages (organic materials arising from the root cap, primary cell walls of root and root hair epidermal cells and bacterial degredation of epidermal cells),
4. Lysates (compounds released from autolysis of senescing cells).

Sloughed root cells should also be added to these (Bowen 1980).

In practise, it is usually impracticable to distinguish precisely between these different processes and the interest centres in their combined effect in providing substrates for the heterotrophic rhizosphere organisms (Russell 1977).

Rovira (1969) gives an extensive list of substances found in root exudates, including many carbohydrates, amino acids, organic acids, enzymes and miscellaneous compounds. The composition of exudates can also vary widely between species. Mucilage is dominantly carbohydrate and is probably a mixture of a glucan, in the form of cellulose microfibrils, and heterogeneous polysaccharides, including a pectic-like component (Oades

1978). The major source of exudates and mucilage is the zone immediately behind the root cap (Rovira 1969; Oades 1978). In addition diffusible exudates and mucilage may arise along various parts of the root, particularly between the root tip and the zone of elongation in the root hair region (Oades 1978).

Losses of substrates from soil grown plants (representing a drain on the carbon resources of the plant) can be very large indeed. Using ^{14}C-labelled CO_2, Barber and Martin (1976) showed 5-10% losses of the assimilated carbon by three-week old wheat plants into the soil. Between 3 and 9% of carbon in wheat roots was lost as water soluble substances and 17-25% was lost as water insoluble material (Martin 1977).

Mucilage may have a number of important roles in the uptake of nutrients and water by plants. Mucigel can vary in thickness but is commonly up to 10 um in diameter in wheat (Oades 1978). Mucilage is closely associated with the surface of the solid phase and this contact may facilitate exchange of ions between clay particles and plant roots (Jenny and Grossenbacher 1963) by diffusion and/or mass flow (Jenny 1966; Passioura 1966). Mucilage is a cation exchanger because the pectic substances contain uronic acids, and is also an important source of protons since it is probably exuded in the acid form (Oades 1978). Mucilage may also exert some selectivity on the uptake of ions e.g. K^+ over Na^+ (Oades 1978).

Organic acids such as citric, tartaric and malic are all found in exudates (Rovira 1969) and it is commonly believed that organic acids such as these are of vital importance in micronutrient availability, due to their chelating ability (Wallace 1963; Gardner et al 1983). The rhizosphere microbial production of powerful chelating agents in soil, for example, 2-ketogluconic acid, is also well established (Russell 1978). It has also been demonstrated that roots are capable of reducing dyes, such as methylene blue (Uren 1969).

Recently, the importance of anaerobic microsites in soil, associated with the rhizosphere, in determining plant nutrient availability has been suggested (Smith 1976). Decreases in O_2 and increases in CO_2 supply (due to microbial and root respiration) will lower the pe and pH in these microsites sufficiently to reduce Fe and Mn and also release P and other nutrients. These microsites are seen to be most important in undisturbed soils.

(b) Access to easily reducible Mn

Easily reducible Mn is the fraction of potentially reactive Mn plant roots could have access to by (Leeper 1970),

1. excreting reducing or chelating agents into the rhizosphere
2. direct contact exchange; here the root acts as an electron as well as a proton donor.

1. Non-contact mechanisms

Uren (1969) has argued convincingly why non-contact mechanisms become less efficient with increasing pH. Divalent Mn or chelated Mn can be readily oxidised (Bromfield 1978) and the closer the root surface is to the oxide, the greater is the chance the oxide will be reduced and the more likely the root is to absorb the Mn^{2+} produced. Bromfield (1978) showed that oxide particles adhered to roots in solution culture and reactive oxides added to soils have increased uptake without increases in exchangeable Mn (Jones and Leeper 1951a).

2. Contact mechanisms

Contact reduction as proposed by Leeper (1934, 1947), Passioura and Leeper (1963) and Uren (1969, 1982), refers to the reduction of Mn oxides at the root surface with the subsequent direct root absorption of the released Mn^{2+}. Roots can favour this reduction by (Uren 1981):

(a) releasing protons (H^+) and/or
(b) releasing electrons (reductants) and/or
(c) absorbing Mn^{2+} and (possibly)
(d) absorbing water (therefore decreasing the moisture content in the vicinity of the roots).

It has already been indicated that processes (a) and (b) occur in the rhizosphere and that they facilitate reduction of Mn oxides in soil.

Contact chelation of Mn^{2+} would appear to be less efficient since the complex between the chelating compounds from the root and Mn^{2+} needs to be stronger than EDTA to get much Mn from MnO_2 (Uren 1969). Lindsay (1974) indicated that the affinity of Mn^{2+} for synthetic chelates is quite low (eg. Fe displaces Mn from applied Mn EDTA). Contact exchange, as proposed by Jenny and Overstreet (1939), and later Jenny (1966), might aid in movement of Mn^{2+} through the mucigel after contact with the Mn oxide surface, but would have little effect in transfer of Mn^{2+} from Mn-colloid system to root cell because the plant cell wall could not get close enough to the soil colloid (Barley 1970).

Contact mechanisms may be very important in root hair Mn absorption (Uren 1981) because, as Barley (1970) points out, root hairs commonly increase the surface area of the epidermal walls by 2-10 fold. The region of greatest reducing activity appears to be associated with maturing or recently matured cells behind the root tip (Uren 1981), which is also the zone of maximal production of exudates and mucilage (see earlier section) and root hair production, as well as the area of maximal Mn absorption (Page 1961).

(c) Fe and Mn reduction at the root surface

Ferric oxides are more difficult to reduce than manganic oxides: pe's for Fe and Mn at pH 5 and pH 7 are respectively 170 and -180 and 640 and 410 mV (Russell 1973). Therefore any evidence which indicates that the reducing capacity of roots is important in Fe uptake can be taken as evidence for the Mn case as well.

A number of chemical reductants produced by roots of Fe-stressed plants grown in solution culture have been identified, primarily phenolic compounds (Brown and Ambler 1973), notably caffeic acid (Olsen et al 1982; Julian et al 1983). Reducing sugar exudates might also be involved in this reduction process (Stenlid 1950; Katznelson et al 1955; Rovira 1965).

Reductants reduce inorganic-Fe^{3+} (eg. $Fe(OH)_3$) and even chelated-Fe^{3+} (eg. FeHEDTA), if in sufficient quantity (Brown and Ambler 1973), in two ways (Uren 1982):

1. $FeIIIx + e^- \longrightarrow Fe^{2+} + x^{3-}$
2. $FeIIIx + 3H^+ + e^- \longrightarrow Fe^{2+} + H3x$

where x = an inorganic or organic "ligand".

Acid (H^+) production by roots (equation 2) enhances the reducing power of some reductants (Uren 1982) and has been shown to occur in several species (Kashirad and Marschner 1974; Kannan 1981). Irrespective of the effect of reductants, a decrease in pH will have dramatic effects on the Fe^{3+} concentration through $Fe(OH)_3$ solubilisation (Julian et al 1983), by the following mechanism (Uren 1982):

3. $FeIIIx + 3H^+ \longleftrightarrow Fe^{3+} + H3x$

Recently, Tagaki et al (1984) have isolated a phytosiderophore (mugineic acid), capable of chelating FeIII compounds, from barley roots. These Fe complexes are then absorbed without the need for reduction outside the plasma-membrane. Marschner et al (1985) described this as strategy II, in contrast with strategy I (discussed above) for dicotyledons and monocotyledons (except grasses).

No evidence appears to be available for specific reductants produced in response to Mn-stressed plants, although Uren (1981) has demonstrated direct chemical reduction of an insoluble hydrous oxide of Mn. Marschner et al (1982) have shown enhanced reduction of MnO_2 under Fe-deficiency, presumably via an Fe-stress induced reductant production.

In the examples cited above, conclusions have been drawn from plants or roots grown in solution culture or other artificial media at an acidic pH so that reductive processes would be favoured. The situation is not clear for rhizospheres in neutral or alkaline soils which may be highly buffered. For example, Bromfield's (1958) root exudates from oats and vetch were unable to dissolve MnO_2 at pH 7.0 and above; they would be even less likely to reduce Fe oxides at pH 7.5 to 8.0. Similarly Godo and Reisenhaeur (1980) found a significant role for root exudates in Mn uptake but only considered a bulk soil pH range of 4.5 to 6.5.

It has already been shown that roots can affect significant pH decreases across the rhizosphere. This reduction in rhizosphere pH probably results primarily from an imbalance between intake of cations and anions across the root surface, rather than, say, release of acidic compounds (eg. organic acids) in root exudates (Nye 1981). However, Nye (1981) has elegantly shown on theoretical grounds that with a high bulk soil pH (pH 8 or greater) very little change occurs in rhizosphere pH following release of H^+ at the root surface. This conclusion has been demonstrated experimentally using NH_4^+ vs NO_3^- N fertilisation with soybeans (Riley and Barber 1971) and wheat (Smiley and Cook 1973; Smiley 1974). Thus, if reduction processes are indeed important in soils of low Fe and Mn availability (eg. alkaline and calcareous soils), plants efficient for Fe and Mn uptake must secrete reductants which are effective at all pHs but particularly at pHs greater than pH 8 (Uren 1984).

(d) Oxidation of rhizosphere Mn

Rhizosphere organisms can sometimes reduce the availability of Mn to plants. Timonin (1946) established a correlation between the severity of Mn deficiency and the numbers of organisms capable of oxidising Mn and decomposing cellulose. However, he did not show convincingly that the absence of significant numbers of Mn oxidising bacteria from the resistant variety was primarily involved in its resistance since the soil disinfectant used (cyanogas) increased yields significantly in both resistant and susceptible varieties. Data from Timonin and Giles (1952)

suggests that cyanogas increased exchangeable Mn and this would most certainly influence the yields observed earlier. Bromfield (1978) found that oats could not absorb Mn from an MnO_2 source in solution culture, in the presence of Mn oxidising bacteria (Arthrobacter sp).

(e) Contribution of root members to Mn uptake

A dynamic picture of contact reduction is one of a reducing surface (associated with the region of root hair development), reducing because it secretes protons and electrons and actively takes up Fe^{2+} (and Mn^{2+}), being forced through the soil (Uren 1984). At any time, the total rate of Mn acquisition by plant roots will be determined by the size of the actively reducing and absorbing portion of the root system. Thus the rate of root proliferation and extension in soil is very important in the uptake of highly immobile nutrients such as Fe and Mn.

The zone of active root hair production (a few mms from the root tip) has been associated with Fe uptake by barley (Clarkson and Sanderson 1978) and Mn uptake by oats (Page 1961) as well as secretion of reductants (Branton and Jacobson 1962; Ambler et al 1971; Brown and Ambler 1974; Romheld and Marschner 1979) and acid (N.C. Uren, personal communication).

Root hairs can be regarded as an expansion of the root cylinder to a larger one whose surface is located near their tips (Nye 1966). Root hair density is commonly of the order of 40-100 per mm root length in cereals (Drew and Nye 1969; Bole 1973) with density tending to decrease from main axis to lateral roots of successively higher order (Dittmer 1937). Their importance in water (Newman 1974) and nutrient uptake (Nye 1966; Lewis and Quirk 1967; Barley and Rovira 1970) must be related to their persistance (Barley 1970) and the degree of root/soil contact made (Drew and Nye 1969; Russell 1977). Nye (1966) concluded that if the rate of diffusion and average concentration of nutrient at the root surface is small and the radius of the root hair cylinder large, then the importance of root hairs will be greater. Bole (1973) however, could not demonstrate that root

hairs controlled the supply of P where diffusion or contact were the major supply mechanisms.

Seminal roots and early formed nodal roots of wheat (Mackey 1973) and barley (Hackett 1968) are more efficient in nutrient uptake, particularly less mobile nutrients such as P (Hackett 1968) than later formed nodal roots, since the latter are thicker and have fewer laterals (Mackey 1973). However, significant nodal root growth can continue until maturity (Hurd 1974; Pianthus 1969) and these nodal roots may therefore be important in nutrient uptake later in ontogeny. Whilst a great deal of work has been conducted on water uptake by different members of the cereal root system (see reviews by Walter 1971; Ponsana 1975; Meyer 1977), very much less information is available for nutrient uptake, particularly in soil systems. No information is apparently available for uptake of Mn and Fe.

1.3.2 Manganese entry into roots

Manganese uptake by excised oat and barley roots is a two-step process, characteristic of the uptake of other cations (Page and Dainty 1964; Maas et al 1968), however, only Maas et al (1968) could demonstrate a metabolically mediated (ie. active) second uptake phase (after c. 30 mins absorption). Active uptake of Mn^{2+} by excised barley roots is characterised by multiphasic kinetics (Bowen 1981; Harrison et al 1983). A sharp transition occurs between active uptake and passive influx (to equilibrium) at 96 mM Mn (Bowen 1981). On the other hand Graham (1979) concluded that Mn absorption by sunflower roots was entirely a passive process.

The uptake of Mn has been shown to be modified by other cations of similar charge and ionic radius. Competition between similar cations has been demonstrated for Ca^{2+} (Van Diest and Schuffelen 1961; Schuman and Anderson 1976), Fe^{3+} (Rivenbark 1961; Heenan and Campbell 1983), Zn^{2+} (Rosell and Ulrich 1964), Cu^{2+} (Harrison et al 1983) and Mg^{2+} (Maas et al 1969). Manganese uptake from solution has also been shown to be strongly dependant on temperature (Bowen 1969) and pH (Maas et al 1968; Bowen 1969; Robson and Loneragan 1970).

Munns et al (1963b) proposed a model to account for different Mn fractions in oat roots in solution culture, and this remains the only such account to date. The proposed scheme involves three Mn fractions within the root; a replaceable fraction ("exchangeable" Mn identifiable with free space) and two non-replaceable fractions that were not exchangeable with the substrate. The latter two were termed "labile", since Mn from here could be transferred to the shoot and "non labile", concentrated in older regions of the root, largely bypassed in movement of Mn from root to shoot, except in times of Mn stress.

may have been washed off YMB

1.3.3 Translocation and redistribution of Mn

Currently absorbed Mn is preferentially translocated to the actively growing centres of the plant shoots (eg. young leaves or the primary shoot apices), largely bypassing the older leaves (Williams and Vlamis 1957; Vose 1963; Amberger 1973). Munns et al (1963b), Vose and Griffiths (1961) and Vose (1963), working with oats, have suggested that the root may act as a reservoir for Mn translocation to the shoot, which may be important during periods of Mn stress. When external supply is adequate the "labile" pool alone would be active, however, when the external supply was removed, translocation of Mn to the tops from the "non labile" pool was accelerated and became substantial (Munns et al 1963b). However, Single and Bird (1958) showed that no translocation of Mn occurred from wheat roots once a root Mn concentration of 10 ppm was reached, even though shoot concentrations were substantially lower.

The evidence which is available on the actual degree of Mn mobility and redistribution within the shoot system of plants is to some degree conflicting. Nable and Loneragan (1984), who showed that no Mn redistribution occurred from old subterranean clover leaves via the phloem, concluded that most studies reporting phloem mobility of Mn are unsatisfactory either because the experimentation was inadequate or the interpretation was faulty.

Single (1958) and Single and Bird (1958) showed in careful glasshouse experiments with wheat that little or no Mn redistribution occurred within the plant so that early formed (older) leaves retained most or all of their Mn and remained healthy (green), despite the development of Mn deficiency in late formed (younger) leaves. They suggested that Mn may accumulate at reaction centres remote from the phloem elements when present in only critical concentrations and therefore become very immobile. Vose (1963) demonstrated only very small amounts of Mn movement from expanded lower leaves of oats to younger leaves, although he suggested these might be physiologically significant, and that the deficiency stress per se in younger leaves is the most powerful factor in inducing export from older

leaves. Heenan and Campbell (1980) reported small losses of Mn from unifoliate leaves of soybeans growing into Mn deficiency.

That Mn is fairly mobile within plants is supported by studies with foliage applied Mn, however, as Nable and Loneragan (1984) point out, Mn painted onto (or supplied to) leaves may behave quite differently from Mn entering leaves via roots. Some studies have reported that radioactive Mn painted onto leaves moves to other leaves and to roots, from where it may be redistributed upwards) (for example, in beans (Bukovac and Wittwer 1957), soybeans (Romney and Toth 1954), oats (Boken 1960; Vose 1963) and wheat (Single 1958)). Henkens and Jongman (1965) concluded that although significant movement of Mn occurred from $MnSO_4$ sprayed leaves to roots, this movement and subsequent redistribution from roots is not considerable and insufficient to prevent Mn deficiency in the foliage formed after spraying.

Pate (1975) suggested that the mobility of Mn from leaves is intermediate between that of the freely mobile elements such as K and the highly immobile elements like Ca. In this he concurs with earlier findings of Bukovac and Wittwer (1957, 1961). Tiffin (1972) and Mengel and Kirkby (1982) concluded that there is insufficient evidence available to draw conclusions. The above findings certainly suggest that level of Mn supply may substantially influence mobility and redistribution of Mn. For example, Single and Bird (1958) suggested that when leaf Mn concentrations are high, Mn may be deposited as silicates, phosphates or molybdates. These depositions may control the concentration of Mn within the leaf, without participating in Mn utilisation or redistribution. Phloem (and xylem) transport of Mn is mainly as Mn^{2+} and not as an organic complex (Tiffin 1972; Mengel and Kirkby 1982; Graham 1979).

1.3.4 Distribution of Mn in the grain

Several studies have demonstrated a significant effect of seed nutrient status on plant performance in nutrient-deficient soil during seedling growth. For example, Massey and Loeffel (1969) showed that most of the Zn recovered in 33 day-old plants of 3 corn inbred lines, which differed in Zn

efficiency could be attributed to seed Zn supply; however, no general correlation between seed Zn content and plant Zn content could be established. Halim et al (1968) showed that the susceptibility of some corn inbred lines to Zn deficiency was clearly related to seed Zn supply but they did not make a big point of this. There have also been reports of growth effects from macronutrient seed reserves. Whiteaker et al (1976) found that seed P content was a source of variation in the P nutrition of homologous lines of beans. Up to 98% of the P in the seed (cotyledon) could be translocated to the seedling. To overcome this seeds of uniform size were used. Fox (1978) found that shoot yields of corn inbred lines grown for 3-4 weeks on low P soil were significantly correlated with seed weights and P content. Approx. 75% of the total P content of seedlings could have come from the seeds. Ozanne and Ascher (1965) found that the development made by seedlings of various species on K-deficient sand was an indication of the development permitted by the reserves of K in the seed.

Therefore aspects of Mn distribution in the cereal grain are considered. Apparently the two primary sources of Mn in the seed are the aleurone layer and the embryo radicle, at least in wheat. This has been established from early analyses of Mn in flour products and from later microscopic work. However, little quantitative data are available to indicate the sizes of Mn pools present in the grain.

Some early work of Bruere (1934) showed that approx. 70% of wheat grain Mn was contained in the bran (designated "envelope" by Bruere), containing the aleurone layer as well as the pericarp and testa, and 10% in the embryo. In addition, Bruere (1934) also found that the internal layers of the envelope contained c. three times as much Mn as the external layer. Presumably these internal layers include the aleurone layer.

Some indirect results of Hoffman et al (1943), showing approx. 90% loss of Mn in the whole grain during the milling process, concur with these results, since, during this process, germ, bran and residual adhering endosperm are separated from the endosperm.

More recent work by Duffus and Rosie (1976) on the Mn composition of barley grain, indicated a higher amount (approx. 25%) in the embryo and approx. 30% in the endosperm (containing the aleurone layer).

It is now known that the primary storage form of macro-nutrient and micro-nutrient mineral elements in seeds is phytin, a cationic salt of inositol hexaphosphoric acid (Lott and Spitzer 1980). This phytin is located in the electron dense globoid crystal portion of storage protein bodies found mainly in the aleurone layer. According to Stevens (1971) 90% of wheat seed phytin occurs in aleurone grains. However, chemical investigation of Mn containing globoid crystals has not been done, so it is open to question whether these elements occur as part of the phytin molecule, or whether they are present in some other form. The amount of Mn associated with phytin also varies with cereal species (Ashton and Williams 1958).

Some recent advanced microscopy experimentation has verified the localisation of Mn in the aluerone protein bodies (Tanaka et al (1974) using electron microscope X-ray analysis; Lott and Spitzer (1980) using energy dispersive X-ray analysis (EDX)). Lott and Spitzer (1980) also found Mn in globoid crystals located in the radicle of the embryo, but specifically located in the base and mid regions of the stele. No or little Mn was located in other parts of the embryo. Earlier, Buttrose (1978), using similar techniques, showed that Mn (and Fe) were definitely located in the globoid crystals of protein bodies of oat embryos, but he did not differentiate further. In a very recent paper, Mazzolini et al (1983), using proton induced X-ray emission (PIXE), have demonstrated that Mn is localised in both the primary and lateral roots of wheat seed embryo, particularly the outer cortex.

In order for mineral nutrients to be released, phytase must hydrolyse the insoluble salts of phytic acid. Phytase activity increases in aleurone and embryo tissue following imbibition. Translocation of the mineral ions from the aleurone tissue to the embryo appears to be by diffusion through the starchy endosperm followed by active uptake by the scutellum and redistribution to the developing axis (Laidman 1982). Eastwood and Laidman

(1971) have studied the mobilisation of macro-nutrients in the germination of wheat grain. During the first few days, at least, the root was predominant over the shoot in accumulating mineral elements. There was a loss of K and Mg to the filter paper. Presumably some losses (ie. leaching) would also occur for trace elements.

1.4 EFFECTS OF MANGANESE DEFICIENCY ON GROWTH AND YIELD

1.4.1 Functions of Mn in plant growth and development

(a) Role of Mn in photosynthesis

The most general symptom of Mn deficiency in higher plants is interveinal chlorosis followed by breakdown of tissues and the formation of necrotic areas. Such extreme breakdown of the photosynthetic apparatus might be expected to be preceded by a reduction in the rate of photosynthesis. Such a reduction has been observed, for example, with Mn-deficient algae (Pirson 1937; Emerson and Lewis 1939), oats (Gerretsen 1949; Vose 1963) and subterranean clover (Nable et al 1984).

The absolute requirement for Mn for a functional photosystem II and its involvement in the O_2 evolving step (photolysis) is now well documented (Cheniae and Martin 1968; Amesz 1983). Under conditions of Mn deficiency, the electron transport activity of photosystem II is much reduced, although never completely lost (Amesz 1983), while photosystem I activity is normal.

There are also alterations in the fluorescence induction kinetics of isolated chloroplast membranes (Anderson and Thorne 1968) and leaves (Simpson and Robinson 1984). Techniques for the measurement of photosynthetic fluorescence kinetics have only recently become available: leaf or chloroplast preparations are exposed to photosynthetically active light energy which leads to the commencement of the photosynthetic reactions. However some of the energy is not usefully consumed in photosynthesis and is emitted from the tissue as fluorescence in the red wavelengths. The use of room temperature fluorescence, particularly as an

indicator of PS2 activity, has been reviewed recently by Krause and Weiss (1983). The measurement of room temperature chlorophyll a fluorescence as an indicator of Mn-deficiency has been investigated with field-grown wheat as an alternative to Mn concentration (Graham et al 1985; Kriedemann et al 1985). This technique should provide a rapid, non-destructive assessment of the Mn-status of plant tissue.

Furthermore, the ratio of chlorophyll a/b is decreased, largely due to a loss of chlorophyll a (Anderson and Pyliotis 1969; Lerer and Bar-Akiva 1979). This loss may be due to the toxic effects of O_2 radicals (Powles 1983), since a reduced activity of superoxide dimutase is likely, or, due to a lesion in its biosynthesis (Simpson, personal communication). Simpson and Robinson (1984) have also shown a specific loss, under Mn deficiency, of three quarters of the particles from the endoplasmic fracture face of appressed thylakoid membranes, containing the photosystem II reaction centres and the chlorophyll a molecules within them. However, Mn may also be involved at other sites of the photosynthetic process, particularly in CO_2 fixation (Brown et al 1958; Pirson 1958).

(b) Role in metabolism

Manganese is considered to have an important role in the nitrogen metabolism of plants (Mengel and Kirkby 1982) specifically in NO_3^- assimilation. Manganese may have secondary effects on NO_3^- assimilation by influencing the supply of NADH for NO_3^- reduction or reducing equivalents for NO_2^{2-} reduction (Lerer and Bar-Akiva 1979). In a recent report, Watson and Hewitt (1980) demonstrated a 50% decrease in *in vivo* NO_3^- reductase activity of wheat leaves under Mn deficiency but no *in vitro* effects, therefore suggesting an indirect effect on the activity of this enzyme.

Manganese deficiency has been reported to induce marked changes in free amino acids of many plants (Hewitt 1951; Labanouskas 1970; Bowen 1983). In general it is the mono-amino acids which increase during Mn deficiency, since Mn^{2+} activates such enzymes as arginase (Kolloffel and Van Dijke 1975) which catalyses the hydrolysis of arginine to ornithine (Greenberg 1970).

Manganese has been implicated in a number of enzymes leading the the synthesis of phenolic compounds (Hirsch et al 1978). Many phenols with o-dihydroxy (catechol) groupings have an ability to chelate metals, for example, Mn^{2+} (Harborne 1980), and hence alter the level of free:bound Mn. This ratio may be involved in growth regulation since Mn^{2+} is known to affect the oxidation of IAA by specifically activating IAA oxidases (Mumford et al 1962; Tamazewski and Thimann 1966). For example, low levels of Mn^{2+} in root tissue may inhibit root growth via IAA stimulated ethylene production (Thimann 1977).

Manganese has been implicated in lignification and cell wall production by Gerretsen (1949) who attributed the effect to reduced carbon metabolism. Several of the major regulating enzymes in lignin biosynthesis have a requirement for Mn^{2+}, including phenyl alanine ammonia lyase, cinnamic acid hydroxylase (Engelsma 1972; Durst 1976) and peroxidase (Autor 1983). Mn is thought to be involved in the induction of rapid lignin synthesis as a defence during disease invasion (Krishna and Bharti 1983; Graham 1984).

more susceptible to disease.

1.4.2 Symptoms of Mn deficiency

One characteristic that appears to be common to many cereals is the observed "limpness" of plants. Gerretson (1949) suggested that this limpness wsa due to an effect of Mn deficiency on carbon assimilation, cellulose production and ultimately cell wall production. Specific symptoms vary with cereal species: for example, in wheat and rye leaves are chlorotic with interveinal white streaks (Wallace 1961; Batey 1971; Graham et al 1983); in barley, chlorosis in younger leaves leads to the development of grey-brown necrotic spots (Reuter et al 1973); and in oats, grey specks and lesions near the middle of the leaves lead to severe chlorosis (Wallace 1961; Brown and Jones 1974). In severe cases of Mn deficiency, leaf necrosis and plant death occurs (Graham, personal communication).

Degrees of symptom expression between genotypes can be characterised by a qualitative score, especially in the field (Batey 1971). Symptoms in the

field are frequently evident by early to mid tillering (Reuter et al 1973), although subclinical deficiency may occur.

There is apparent variation in the incidence and severity of Mn deficiency from season to season (Boken 1952; Batey 1971; Graham personal communication). Deficiency often appears worst in cold and wet seasons (Batey 1971). This may be due to reduced root activity affecting Mn uptake (Batey 1971) although it has been demonstrated that increased soil temperatures will increase the amount of exchangeable Mn (Boken 1952). The effect of poor drainage appears to be variable: perhaps the effect of poor drainage is to restrict root development which would tend to counteract any increase in exchangeable Mn in lower layers of soil (Batey 1971). It has also been observed that deficiency symtoms are often much worse when good growing conditions follow a cold spell (Batey 1971). This could be due to the inability of the root system to obtain sufficient Mn to meet the sudden increase in metabolic requirements (Batey 1971).

1.5 GENOTYPIC DIFFERENCES IN MANGANESE EFFICIENCY

1.5.1 Introduction

Whilst differential tolerance of plant species and cultivars to soils of low nutrient status has been established for many years, advances in the understanding of mechanisms associated with such tolerance have occurred only relatively recently. The term "nutrient efficiency" has been widely used in papers dealing with responses to nutrient deficiency but there is much latitude in its application.

In biological terms plant efficiency for the use of a nutrient is most simply described as dry matter production relative to the amount of that nutrient in the soil or other medium (Loneragan 1977). Thus a nutrient-efficient genotype is one having a high dry matter production at a low concentration of that nutrient in the medium, and conversely for a nutrient-inefficient one (Saric 1982). Sometimes (especially in the case of Fe deficiency), symptom expression rather than growth is emphasised (Clark 1977; Brown 1982).

Because of time and space limitations, vegetative growth under controlled conditions is the parameter most frequently considered, even though economic yield (ie. nutrient efficiency expressed in agronomical terms) should ultimately be the most important criterion (Loneragan 1977; Graham 1984). Therefore, studies which consider nutrient efficiency only during seedling and early growth stages may not necesssarily relate well to field performance.

An index of nutrient efficiency can be conveniently obtained by comparing growth under nutrient-deficient with nutrient-sufficient conditions: Graham (1984) proposed that nutrient-efficient genotypes would have relative yields approaching 100% or better, whilst inefficient ones would be in the range of 0-50%. It is thus possible to compare genotypes for degrees of nutrient efficiency, in a similar way as degrees of sensitivity and tolerance to nutrient deficiency, but this is seldom done.

Rather, the distinction between efficiency and inefficiency is usually sharp. It is also important to distinguish between efficiency for the use of a native nutrient (as in this thesis) and efficiency for the use of an applied nutrient.

1.5.2 Extent of Mn efficiency in cereals

Differences in tolerance of cultivated plant species to Mn deficiency have been reported for many years (Walsh and McDonnell 1956; Gerloff 1963). Cereals are generally classified as medium to highly sensitive, with oats being the most susceptible and rye the most tolerant (Gallagher and Walsh 1943; Puckridge 1958; Nyborg 1970). However, definitive comparative studies are lacking.

Differences between cultivars have also been widely reported. For example, such observations have been made for oats (Timonin 1946; Vose and Griffiths 1961; Deb and Scheffer 1970; Brown and Jones 1974; Berkenkamp et at 1978), wheat (Gallagher and Walsh 1943; Neenan 1960; Nyborg 1970; Graham et al 1983) and barley (Gallagher and Walsh 1943; Graham et al 1983). Significant varietal differences are also present in soybeans (Ohki et al 1980; Heenan and Campbell 1980) and lupins (Perry and Gartrell 1976; Walton 1978; Radjagukguk 1981).

1.5.3 Factors associated with Mn efficiency

At least three interrelated plant characteristics determine the degree of nutrient efficiency (Loneragan 1977; Clarkson and Hanson 1980):

1. adjustment of growth rate to make it compatible with nutrient supply
2. efficient nutrient uptake
3. efficient nutrient utilisation or internal economy.

In the following sections, these three characteristcs will be discussed in relation to Fe, P as well as Mn deficiency, since, only limited imformation is available for Mn-efficiency mechanisms. Like Mn, Fe also participates in oxidation-reduction cycling, whilst P also exists in a

number of plant available and unavailable soil pools. All three nutrients have decreased solubility at alkaline pHs. Most emphasis has been given to mechanism 2. since uptake is probably the primary limiting factor in nutrient efficiency at least for agriculturally important genotypes (Graham 1984).

1. Growth adjustment

The nutritional responses of wild plants adapted to less fertile habitats differ considerably from those of ill-adapted cultivated herbaceous plants eg. they do not have high relative growth rates and they do not respond well to nutrient supply (Chapin 1980). Plants with inherently low growth rates are particularly tolerant of low nutrient soils since they have a lower nutrient demand than those with high growth rates (Chapin 1980). This strategy would be less suitable for agriculturally useful plants bred for high growth rates. Figure 1.2 shows the two major types of strategies that are adaptive under conditions of high and low nutrient supply.

Genotypes may differ in efficiency for a certain nutrient partly due to differences in growth rates or response to another nutrient such as N (Loneragan 1977), depending on the rate of nutrient uptake.

2. Nutrient uptake

The ability to absorb nutrients has long been ackowledged as a major factor associated with differences among plants in their nutrient efficiency, and thus it is not surprising that most studies have concentrated on this. Emphasis has been given to absorption by the root rather than acquisition from the soil-root interface because solution culture studies have been most frequently used. But if access to soil nutrients is a limiting factor (as is mostly the case for Mn, Fe and P), morphological and biochemical aspects of roots will be critical and the efficiency of ion uptake mechanisms will be of secondary importance (Gerloff 1977; Graham 1984). Therefore soil cultures are preferable for genotype screening for Mn efficiency over solution cultures.

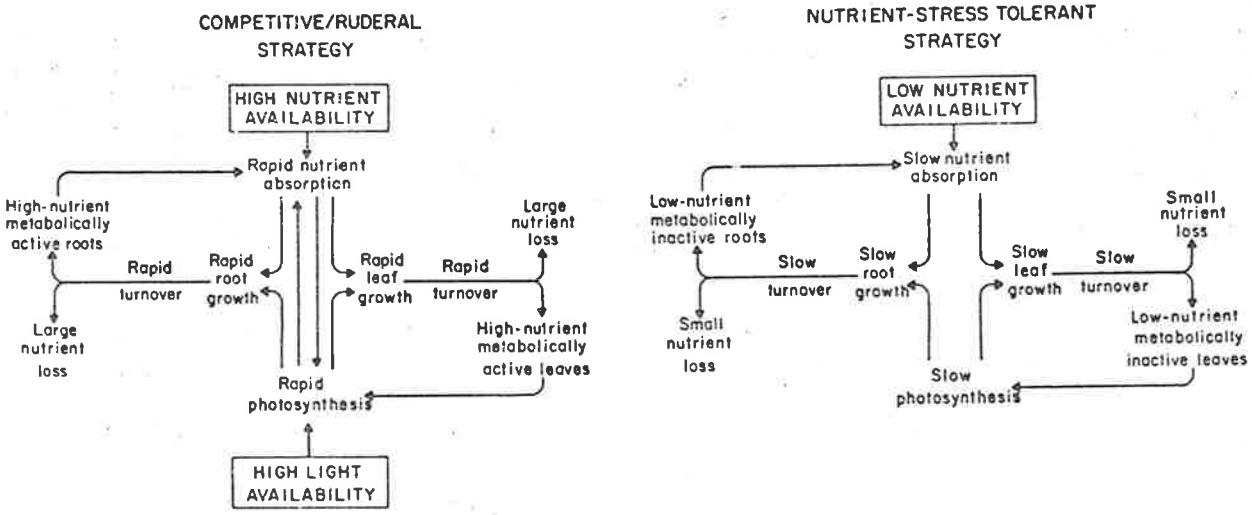

Figure 1.2 Interacting characteristics of plant strategies that are adaptive under conditions of high or low nutrient availability.

a. Nutrient absorption

Kinetic parameters of nutrient uptake have been obtained largely by short-term ion absorption with intact or excised roots over a wide range of ionic concentrations in solution cultures. Uptake efficiency can be expressed in terms of V_{max} or I_{max} (maximum uptake rate per weight or length of root) and K_m (affinity of roots for the nutrient). Nielsen (1979) also proposed that c_{min} (the minimum concentration of nutrient at the root surface for zero uptake), L (root length) and r (root radius) were important considerations where diffusion gradients existed (eg. for P).

On soils of low nutrient status the "affinity strategy" would be the most appropriate. However, several studies have shown that efficient genotypes of cultivated plants appear to employ the "velocity strategy" (eg. for P (Schenck and Barber 1979), S (Cacco et al 1976) and Mn (Landi and Fagioli 1983). This is because most of these nutrient efficient genotypes have relatively high growth rates: use of the "velocity strategy" would be predicted from the influence of growth rates on ion fluxes (Clarkson and Hanson 1980). In contrast, wild plants on infertile soils are characterised by both a low V_{max} and low K_m (Chapin 1980).

The only published account of genotypic diiferences for the rate of Mn absorption by whole plants is for two oat cultivars grown in solution culture (Munns et al 1963 a). However, these studies used unrealistically high solution Mn concentrations.

b. Nutrient acquisition

Perhaps the most complex processes are those which determine nutrient availability at the root surface (Nye 1977). Several plant factors can determine genotypic efficiency at this level, for example, radius, length, density and geometry of roots, root hair density, root exudates, adaptability of roots to soil microbes and mycorrhizal associations (Nielsen 1979; Lafever 1981).

Plant induced facultative reduction in rhizophere pH and release of reductants are primarily responsible for Fe-efficiency (Brown 1978), whilst acidification of the rhizosphere also has consequences for P solubilisation with P-efficient genotypes (Bekele et al 1983). Many studies dealing with exudates as a factor in genotypic differences in Fe-stress response have utilised mutants (eg. Brown et al 1971; Brown and Ambler 1973), but commercial cultivars have also been considered (eg. Brown and Jones 1977). Whilst the use of mutants facilitates the elucidation of genetic control (ie. single vs. multi-gene), the response of mutants is considerably different from adapted genotypes.

A role for reductants has also been ascribed to species differences in tolerance to Mn deficiency (eg. between vetch and oats (Bromfield 1958), between cereal species (Loneragan et al (cited by Brown et al 1978) and for lupins (Gardner et al 1982)). Only one study, with oat cultivars (Deb and Scheffer 1970), has related differences in Mn uptake between cultivars to exudate production.

Effective exploration and exploitation of the soil are ensured by the rapid elongation of main root axes and the subsequent proliferation of lateral branches. However, it is difficult experimentally to ascribe a primary role to growth or chemical modification of the rhizosphere enhancing growth through increased Mn uptake.

Species adapted to low nutrient soils frequently have high root:shoot ratios (Clarkson and Hanson 1980). Presumably, increased root growth will improve nutrient supply and therefore alleviate the stress, so switching reserves back to shoot growth. However, wild species adapted to infertile soils show much less phenotypic plasticity for shoot:root ratios (Chapin 1980).

Soil culture studies commonly consider root length or weight (eg. P-efficient genotypes are often associated with higher root lengths (Fawole et al 1982; Coltman et al 1985) or root radii (Schenck and Barber 1979)). Uptake per unit root length or weight has not been clearly linked with P efficiency (Coltman et al 1985), and certainly not with Mn efficiency.

Where nutrient solubilisation (eg Mn, Fe, P) or transformation (eg. Mn, Fe) are important factors limiting nutrient availability in soils, the extent of actively excreting root surfaces will be important. This has been shown for Banksia sp. (Jeffrey 1967) and Lupinus sp. (Gardiner et al 1982. Where diffusion to the root surface is important (eg. for P), density of roots and their geometry will also be important, especially where competition occurs between roots (Nye 1977).

Apart from a solution culture experiment dealing with Cu uptake by seedlings of several cereal species (Graham et al 1981), these morphological parameters have not been related to nutrient efficiency. Root development in solution cultures may be very different from that in soil due to phenotypic plasticity of the root system (Epstein 1972).

Even less attention has been given to the possible effects of root hair density or mycorrhizal associations in determining differences in nutrient efficiency. No clear picture emerges for a positive association between root hair density and P-efficiency: for example, although the higher P uptake of Brassica napus compared with Allium cepa was related to root hair abundance (Bhat and Nye 1974), no correlations were found between root hair density and P uptake by wheat genotypes (Bole 1973). Uren (1982) has suggested root hairs may be important in maximising root/soil contact and therefore plant access to reducible Mn but there is no experimental evidence. In contrast with P nutrition, no benefit would be expected for Mn nutrition from enhanced mycorrhizal associations with plant roots (Abbott and Robson 1984).

3. Nutrient utilisation

The efficiency of utilisation of a nutrient depends on both the plant's ability to move that nutrient to functional sites as well as the functional requirement of that nutrient in the plant's metabolism (Loneragan 1977). Although a genotype with efficient nutrient uptake characteristics may also have efficient usuage of that nutrient, this is not necessarily so.

(a) Translocation

There appear to be few studies which differentiate between the rates of absorption and xylem translocation in explaining observed differences in Mn contents between genotypes under Mn deficiency. Only one study (Munns et al 1963c), with two oat cultivars, has demonstrated differences in rates of transport from root to shoot. Differences also exist between genotypes in their capacity to retranslocate Mn in phloem. For example, Hocking and Pate (1978) indicated that seed of Lupinus albus acquired a greater proportion of Mn from vegetative parts than did seed from L. angustifolius.

(b) Functional requirement

There is no clear evidence that Mn efficiency arises from a lower functional requirement of Mn in the shoot. There appears to be a lack of evidence which makes genotypic comparisons of the relationship between Mn concentrations in cells and activity of Mn requiring enzymes in plants grown under the same conditions.

Because of the complexities associated with biochemical assays, critical nutrient concentrations (ie. nutrient concentrations in plant tissue corresponding to 90% of maximum plant growth (Ulrich and Hills 1967) are often resorted to since they have been considered related to (but not the same as) functional requirements of plants (Loneragan 1968). There are conflicting reports regarding the constancy or variation of critical nutrient concentrations with genotype, and there is certainly an inadequate knowledge at the cultivar level (Graham 1981).

Whilst dramatic examples exist for species variation in critical Mn concentration (eg. Labanauskas (1966) cites critical levels in leaves of Tung and Avena as >100 and 10-15 $\mu g\ g^{-1}$ Mn respectively), most examples are less dramatic.

The significance of these variations in critical Mn concentration with genotype may be viewed with suspicion, however, as factors such as choice of tissue, physiological age of tissue, plant age, nutrient interaction and culture medium (solution, sand, field) may impact on the derivation of critical levels (Bates 1971). Certainly comparisons should be made with plants grown under the same conditions, and, since Mn behaves as an immobile nutrient under conditions of limited supply, younger leaves should be sampled (Loneragan et al 1976).

1.5.4 Genetics of Mn efficiency

The only report dealing with the inheritance of Mn efficiency concerns F_2 studies with barley (Sparrow et al 1983). The results of this study suggested relatively simple genetic control but more information is needed. Much more is known about the physiology and genetics of Fe efficiency than for Mn efficiency. Early work of Weiss (1943) with soybean mutants revealed single major gene control, but subsequent studies have indicated contribution of minor genes also (Brown and Wann 1982; Fehr 1982). Much less is known about the inheritance of P deficiency: emphasis has been placed on quantitative inheritance owing to the importance of root extension parameters (Gabelman and Gerloff 1982).

There are many difficulties associated with determining the mode of inheritance of nutrient efficiency factors. The following examples are pertinant:

(1) it is important to decide what the assessment criteria will be eg. symptom expression, dry matter production, grain yield etc..
(2) it is important to decide which plant mechanisms (eg. chemical, biological and/or physical) are primarily responsible for determining efficiency,
(3) it is necessary to determine precisely which soil factors are responsible for inducing the deficiency stress, and,
(4) it is advantageous to assess the extent of genotype x environment interactions operating in the field and glasshouse since it is the phenotype that is being considered.

1.6 THESIS PROLOGUE

Interest in this research project was sparked by field observations of large differences in tolerance to Mn deficiency between wheat and, particularly, barley cultivars on chronically Mn-deficient calcareous soils of the lower Eyre peninsula, S.A. (Graham et al 1983). A number of other sites were also known to be marginally Mn deficient (R.D. Graham and D. Reuter, personal communications). It was felt that selection and breeding for Mn efficiency (see section 1.5.1) could provide a better alternative to the continued application of Mn containing fertilisers. It has already been pointed out that very little was known about factors contributing to Mn efficiency (see section 1.5.3) and virtually nothing was known about the inheritance of Mn efficiency (see section 1.5.4). The development of a rapid sreening technique for Mn efficiency that relied on seedling performance under controlled environment conditions rather than field evaluation was desirable, and therefore most pot experiments were conducted for approx. 4 weeks.

This project initially set out to investigate the extent of cultivar differences for and mechanisms of Mn efficiency in wheat, however, other cereals were included when it became obvious that the seedling studies uncovered very little variation for Mn uptake under Mn deficiency in wheat (Chapter 2). Genotypic differences in the ability to acquire Mn from the soil (rather than plant utilisation of acquired Mn) were considered to be primarily responsible for variation in Mn efficiency (see section 1.5.3) and therefore this project concentrates on identifying mechanisms associated with seedling Mn uptake (Chapters 2 and 3). Two cultivars of barley (Weeah and Galleon) and triticale (Venus and Coorong) were used because they had been shown to vary consistently in their Mn efficiency (R.D. Graham, personal communication). This thesis also considers the effect of seed Mn content on apparent Mn efficiency (Chapters 5 and 7), since the importance of this factor became obvious in the early experiments (Chapter 2). An attempt was also made to determine if there were any differences in the utilisation of Mn by wheat cultivars of differing Mn efficiency in photosynthesis by examining the kinetics of chlorophyll *a* fluorescence (Chapter 4). Field experiments have been included in this thesis (Chapter 6) because it was important to evaluate genotypic differences in agronomic Mn efficiency and to determine whether Mn uptake per se or other factors were associated with these differences.

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CHAPTER 2

TOLERANCE OF WHEAT, TRITICALE, RYE AND BARLEY SEEDLINGS TO MANGANESE DEFICIENCY

2.1 SUMMARY

An attempt was made to quantify the Mn efficiencies of wheat, barley, triticale and rye during the seedling stage. In particular, genotypic variation within wheat has been emphasised. Except for one small nutrient solution culture experiment, plants were grown in small pots containing a Mn-deficient calcareous sand over a 4-week period at 15 C.

Barley proved to be the most Mn-efficient cereal, having the highest dry matter production, Mn uptake and utilisation efficiency. The higher rate of Mn uptake for barley was stongly associated with greater lateral root development. Differences in growth between wheat cutivars without added Mn were confounded by differences in the Mn content of sown seeds. There appeared to be little genotypic variation for Mn uptake between these cultivars.

The results obtained from the screening of wheat-barley addition lines suggested that Mn-efficiency characters could be transferred from barley to wheat, since relative growth at MnO was significantly greater for some of the addition lines than for wheat. However, mechanisms for higher efficiencies were not clear.

2.2 INTRODUCTION

This Chapter describes several experiments which were aimed at quantifying differences between cereal species and cultivars for tolerance to Mn deficiency (ie. for Mn efficiency) at the seedling stage. Large cultivar differences in tolerance to Mn deficiency had already been demonstrated by R. D. Graham and associates at the Waite institute for barley, and, to a lesser extent for wheat, on a Mn-deficient soil near Wangary, S.A., in the period 1980-1982. The extent of genotypic differences in the field will be considered in Chapter 6.

Since the initial objective was to concentrate on Mn efficiency in wheat, more emphasis has been given to cultivars of wheat than of any other cereal. A long-term objective of this work was to develop a successful bioassay for Mn efficiency in wheat during seedling growth, in order to short cut the time and effort involved in field screening. This was considered feasible since good correlations had already been obtained between seedling growth in small pots and field performance under Mn deficiency for different barley cultivars (R.D. Graham, personal communication).

Three factors were considered to be primarily responsible for determining Mn efficiency during early growth: efficiency of Mn uptake, transport of absorbed Mn to the shoots and the utilisation of this transported Mn for growth. A detailed understanding of these factors as they affected genotypic differences in seedling performance would have important implications for the conduct of a breeding program.

Experiment 2.1 was concerned with quantifying growth and Mn uptake of wheat, triticale, rye and barley during seedling growth under deficient and adequate soil Mn supply, using soil collected from Wangary.

Experiment 2.2 was designed to calculate rates of Mn uptake at deficient and adequate soil Mn supply in order to gain more understanding of the species differences observed in Experiment 2.1.

Experiment 2.3 was undertaken in order to determine whether seedling Mn-efficiency characteristics of barley could be transferred into and be assessed in a wheat background by the use of wheat-barley disomic addition lines developed by Drs. A.K.M.R. Islam and K.W. Shepherd at the Waite Institute.

Experiment 2.4 compared the rates of Mn uptake for wheat, triticale and rye plants grown in nutrient solution, using a suitably low Mn concentration.

2.3 METHODS AND MATERIALS

2.3.1 General procedures for soil culture experiments

This section outlines methods and materials that were common to the soil culture experiments described in Chapters 2, 3 and 4. These are presented together for ease of reference; specific methods and materials for individual experiments are described separately.

The following cereal species were used in these experiments: wheat (*Triticum aestivum*), triticale (*Triticosecale x*), rye (*Secale cereale*) and barley (*Hordeum vulgare*).

(a) Seed germination and sowing

Seeds were surface sterilised for approx. 10 min with 1% NaOCl, rinsed several times with distilled, double deionised water (DDDI H_2O) and germinated for 2 days in glass petri-dishes at room temperature. Germinated seeds of uniform vigour, with coleoptiles just emerged, were then carefully planted into the soil.

(b) Soil description

A sandy calcareous soil from near Wangary, Eyre Peninsula, S.A., was used in these studies since an earlier study (Graham et al. 1983) had shown this to be suitabe for detecting differences in Mn efficiency between cultivars. This grey sand (Principal Profile Form Uc1.11 (King and Alston 1975)), was developed on shell fragments and has the following properties in the 0-10 cm (top) and 10-20 cm (sub) layers, respectively: pH (1:5 H_2O) of 8.9 and 9.1; 62 and 76% $CaCO_3$; 2.5 and 0.5% organic C; 0.35 and 0.25 ppm DTPA extractable Mn; 31 and 5 ppm available P ($NaHCO_3$ extractable); 100 and 50 ppm available K; 6.8 and 2.3 ppm nitrate N and field capacity moisture content (o.d. basis) of approx. 25%.

(c) Soil preparation and potting

It was not possible to use the same batch of Wangary soil for all experiments and this factor, coupled with duration of storage, resulted in small differences in soil Mn availability between experiments. In experiments 2.1-2.3 and 3.1 top and sub soil were mixed in equal proportions, whilst in experiment 3.2, 3.3 and 4.1 only top soil was used. All soils were sieved through a 2 mm stainless steel screen, after air drying, to remove concretions and coarse organic matter.

Basal nutrients were thoroughly incorporated into the soil by hand mixing specified amounts of stock solutions with 4-5 kg amounts of soil in plastic bags to give the following rates of nutrient addition: 450 mg $Ca(NO3)_2.4H_2O$, 52 mg K_2SO_4, 30 mg $MgSO_4.7H_2O$, 65 mg KH_2PO_4, 6 mg $FeSO_4.7H_2O$, 2 mg H_3BO_3, 3.2 mg $CuSO_4.5H_2O$, 20 mg $ZnSO_4.7H_2O$, 0.4 mg $CoSO_4.7H_2O$, 6 mg NaCl and 0.4 mg $H_2MoO_4.H_2O$ kg^{-1} a.d. soil. These rates were the same as those given by Graham and Rovira (1984) except that twice as much Zn was added, since these authors had observed somewhat low Zn concentrations in harvested plants.

Manganese was added as $MnSO_4$ in solution to the soil and again thoroughly mixed to give rates of either 0 (Mn0), 4 (Mn4), 10 (Mn10) or 40 (Mn40) mg Mn kg^{-1} a.d. soil. Graham and Rovira (1984) observed that maximum wheat seedling growth was attained at Mn40.

In experiments 2.1-2.3 and 5.1 clear plastic cups of 300 ml capacity (filled with 250 g a.d. soil) were used, whereas 500 ml cap. cylindrical plastic containers (filled with 450 g a.d. soil and covered with black plastic), were used in experiments 3.1, 5.2 and 5.3. All pots were thoroughly washed with detergent and rinsed with DDDI H_2O prior to potting. The soil was maintained at approx. 25% w/w moisture content (o.d. basis), except for experiment 5.2 where 30% w/w was used. Evaporative loss was minimised by covering the soil surface with inert black alkathene beads.

(d) Harvesting procedures

At harvest, Mn-deficiency symptoms were noted, shoots were cut at soil level and fresh weight, tiller number (ie. stems other than the main culm) and leaf number (fully expanded blades ie. with ligules showing) recorded. In some cases the youngest expanded blade (YEB) was removed from the rest of the plant. Plant height was recorded as the height above the soil surface of the tip of the YEB when extended. Roots, including adhering seed coats, were thoroughly washed from the soil with deionised water, using a small stainless steel sieve, and fresh weights recorded. Dry weights of YEB, shoot and root tissue were obtained after oven drying at approx. 70°C for at least 24 h.

Subsamples of seeds used to grow plants were also taken for each cultivar for dry weight determination and subsequent Mn analyses after rinsing in DDDI H_2O for approx. 10 min.

(e) Analytical procedures

Analysis for Mn (as well as other micro- and macro-nutrients) was by flame emission atomic absorption spectrophotometry (AAS) following nitric-perchloric acid digestion. Details of the methods used (after Heanes (1980)) are shown below:

Sample dry weight g	Digestion flask cap. ml	Volume of nitric: perchloric acid ml	Final volume of digest ml
0.00 - 0.50	50	6:1	5
0.50 - 1.00	50	11:1	10
1.00 -	100	17.5:2.5	25

Shoot and root material was cut into small pieces with stainless steel scissors prior to digestion whereas seeds were digested whole (usually 10 seeds per sample). Care was taken to avoid contamination. Samples were digested in Erhlenmeyer flasks placed on frypans for heating.

All analyses were performed on a Phillips SP-9 AAS, with background correction used for Mn, Zn, Cu and Fe determination. One standard plant reference sample of known Mn concentration and 1 or 2 acid blank solutions were included per 30-40 samples.

(f) Statistical analyses

Most of the data was subject to analysis of variance or regression analysis using programs written in 'basic' for the Hewlard-Packard or Apple II micro-computer. In some cases data were log-transformed where non-normality of variances associated with the mean was suspected.

Significance of tabulated means is indicated in most cases by the use of the least significant difference (LSD) at the 5% level ($P<0.05$) and by the F-ratio (NS = not significant; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$). In some instances presentation of standard errors associated with the mean was deemed more appropriate.

2.3.2 Experiment 2.1

Eight wheat cultivars (Bodallin, Gatcher, Halberd, Bindawarra, Dundee, Oxley, Gluyas Early and Bayonet), two triticale cultivars (Venus and Coorong), two barley cultivars (Weeah and Galleon) and rye (S.A.) were used. They were chosen for comparison on the basis of results of an earlier experiment (Appendix 2.1). Sources of seed are indicated in Appendix 2.2.

The experiment was conducted in a growth room at a constant 15 °C and a photoperiod of 11 h at an average photon flux density of 240 µmole quanta m^{-2} s^{-1} at plant height. The experiment had a factorial, completely randomised design, comprising 13 genotypes, 2 levels of Mn (Mn0 and Mn40) and 3 replicates. Each pot contained three plants. Germinated seeds were sown approx. one week after soil potting. Plants were harvested 22 days after sowing.

Symptom expression was scored as follows: nil, mild (slight to distinct paling of YEBs), moderate (as above but with noticeably smaller plants, watery marks and/or necrotic spotting on YEBs) and severe (necrotic streaks and leaf collapse).

Net Mn uptake (µg $plant^{-1}$) was calculated by subtracting seed Mn content (µg $seed^{-1}$) from whole plant Mn content at harvest. Utilisation efficiency (UE) was calculated according to Siddiqi and Glass (1982) as:

$$UE = DW / [Mn] \quad (g^2\ \mu g^{-1})$$

where DW = whole plant dry weight and [Mn] = whole plant Mn concentration

2.3.3 Experiment 2.2

Four genotypes were used: wheat cv. Bayonet, barley cv. Galleon, triticale cv. Venus and rye cv. S.A. Except for rye, for which a low seed Mn source was not available, seeds of low Mn status were chosen (see Appendix 2.2).

This experiment was conducted in a growth cabinet at a constant 15°C and a photoperiod of 10 h at an average photon flux density of 400 μmole quanta m-2 s-1 at plant height. The experiment had a factorial, completely randomised design, comprising 4 genotypes, 3 levels of Mn (Mn0, Mn4, Mn40), 3 harvests (6, 16 and 26 days after sowing) and 3 replicates. Each pot contained two plants. Germinated seeds were sown 5 days after pots were filled with soil. Symptoms were scored as for experiment 2.1.

In addition to those measurements made in experiments 2.1, lengths of main axis and lateral roots were obtained by the line-intercept method modified by Tennant (1975), after spreading the roots in a large petri dish containing a little water and placing a grid of lines (1 cm diameter spacing) underneath. Average root diameter (mm) was calculated from the relationship:

$$r^2 = RFW / RL \times \pi$$

where r = average root radius, RFW = root fresh weight and RL = root length

Root specific gravity was assumed to equal 1.

Rates of Mn uptake were calculated according to Williams (1948):

$$(\ln W_2 - \ln W_1) / (W_2 - W_1) * (M_2 - M_1) / (t_2 - t_1) \text{ or,}$$

$$(\ln L_2 - \ln L_1) / (L_2 - L_1) * (M_2 - M_1) / (t_2 - t_1)$$

where W_1 and W_2 = root dry weight at harvest t_1 and t_2

L_1 and L_2 = total root length at harvest t_1 and t_2

M_1 and M_2 = whole plant Mn content at harvest t_1 and t_2

2.3.4 Experiment 2.3

Seed of barley (cv. Betzes), wheat (cv. Chinese Spring) and 6 addition lines were obtained from Dr. Islam (Waite Institute). One addition line (containing chromosome no. 5) was not available because of sterility (Islam and Shepherd 1981). An attempt was made to select seeds of similar seed weight (and therefore Mn content) but this was not possible because of inherent seed size differences between these lines (Appendix 2.9). Experimental procedures, cultural conditions and harvest measurements were the same as for experiment 2.1.

2.3.5 Experiment 2.4

Wheat cv. Bayonet, Bodallin and Gatcher, triticale cv. Venus and Coorong and rye cv. S.A. were used. Seed of each genotype was obtained from the same source as experiment 2.1. Twenty uniform 6 day old seedlings of each genotype, previously grown in 1/10 x Hoaglands solution containing 0.65 uM Mn, were transferred to an acid washed plastic container holding approx. 8 l of 1/2 x Hoaglands solution with 0.65 μM Mn added. Solution pH was kept in the range of pH 6.5 - 7.0. Iron was added at a rate of 0.004 μM $FeSO_4$ (Munns et al 1963), until just prior to the first harvest when chlorotic symptoms were noticed on young leaves. After this period Fe was added as FeEDTA. The experiment was conducted under the same conditions as described in experiment 2.2

Six plants were harvested at 18, 23 and 25 days from germination. Shoots were separated from roots and roots were washed for 5 min with DDDI H_2O, then 3-4 min in 0.5 mM $Ca(NO_3)_2$ at 5 C (to remove free space Mn) and then for 1 min in $DDDI_{2}O$ (to remove excess $Ca(NO_3)_2$ before they were blotted dry. Shoot and root samples were oven dried at 70° C for 24 h and assayed for Mn and Fe using AAS. Rates of Mn uptake were calculated as before (experiment 2.2).

2.4 RESULTS

2.4.1 Experiments 2.1 and 2.2

(a) Manganese-deficiency symptoms

Specific symptoms of Mn deficiency were generally evident only on leaves of the wheat cultivars, and did not occur when adequate Mn had been added (ie. at Mn40). In experiment 2.1, only mild symptoms were observed, on young leaves. Contrary to this, symptoms observed in experiment 2.2 varied from mild to severe and were first evident on the oldest (1st) leaf rather than on younger leaves. Grey specks and lesions, as observed by Brown and Jones (1974) for oats, were found in the middle of the leaf for some wheat cultivars.

(b) Shoot and root growth

Plants generally had 2-3 fully expanded leaves and 0-1 tillers. There were no effects of added Mn on tiller and leaf number. Shoot fresh and dry weights were highly correlated (r^2 = 0.95 and above) and thus fresh weights are not presented.

Significant variation was found between wheat cultivars in experiment 2.1 for shoot dry matter production at both deficient and adequate Mn supply (Table 2.1). However, the outstanding performance of Bodallin at Mn0 was apparently associated with its high seed Mn content. The responsiveness of cultivars to added Mn (ie. their relative yields) also varied considerably. The two barley cultivars and Venus triticale were the most productive genotypes at Mn0, and these and rye were least responsive to added Mn. The poorer performance of Coorong triticale compared with Venus triticale was also apparently related to seed Mn content.

Table 2.1: Shoot and root dry weight and the ratio of shoot:root dry weight after 24 days growth for genotypes studied in experiment 2.1. Data are means of 3 replicate pots. Relative yields (per cent of Mn40) are indicated in brackets.

Genotype	Shoot dry weight mg plant^{-1}		Root dry weight mg plant^{-1}		Shoot:Root ratio	
	Mn0	Mn40	Mn0	Mn40	Mn0	Mn40
Bodallin	54 (97)	55	37 (125)	30	1.45	1.87
Gatcher	41 (83)	47	24 (94)	26	1.69	1.91
Halberd	35 (79)	44	20 (94)	31	1.75	2.07
Bindawarra	41 (73)	56	22 (75)	20	1.83	1.91
Dundee	44 (71)	62	20 (69)	39	2.17	2.11
Oxley	34 (65)	52	17 (86)	21	1.90	2.54
Gluyas Early	44 (65)	67	22 (60)	36	2.04	1.86
Bayonet	34 (58)	59	16 (57)	27	2.17	2.17
Venus	75 (90)	84	46 (90)	52	1.62	1.61
Coorong	50 (91)	55	38 (105)	28	1.82	2.09
S.A.	37 (117)	32	21 (141)	15	1.79	2.16
Weeah	73 (93)	79	51 (103)	49	1.44	1.60
Galleon	71 (84)	84	49 (86)	57	1.44	1.48
LSD 5% G	6	***	5	***	0.24	***
Mn	2	***	2	***	0.10	**
G x Mn	9	***	7	***	0.32	*

Root fresh weights were also highly correlated with dry weights (eg. $r^2 = 0.85$ for experiment 2.2) and are not presented. Significant variation was also found between wheat cultivars for root dry matter production (Table 2.1). Responses to added Mn and ranking of genotypes for root growth, with and without added Mn, were similar to those for shoot growth.

Thus, differences between cultivars in shoot:root dry weight ratios between cultivars were small. However, genotypes with higher relative yields generally had lower shoot:root ratios. Only small increases in shoot:root ratios were found with Mn addition. Ratios for barley were consistently smaller than those of the other genotypes.

Root lengths, measured in experiment 2.2, are given in Figure 2.1. Large differences were found between cereals in main axis (eg. barley vs. wheat) and lateral (barley vs. the other cereals) root development. Main axis roots were predominantly seminal since there was little nodal root development. Barley had the greatest total root length at all levels of Mn supply and at each harvest. Bayonet had the smallest main axis root length at day 26. Addition of Mn had no effect on the length of main axis roots but resulted in significant increases in lateral root length for most genotypes by day 26. There was a diminished contribution of main axis roots to the total root length with increasing plant age and Mn supply.

Linear regression equations between root fresh weight, main axis and lateral root length are given in Appendix 2.3, whilst Appendix 2.4 shows the correlation coefficients. Reliable predictions of root length from the determination of root weight could only be made for wheat and rye.

Diameters of root members were not measured for experiment 2.2; however, calculations from the formula given in section 2.2 showed that average root diameters did not vary with genotype or Mn supply (Appendix 2.5).

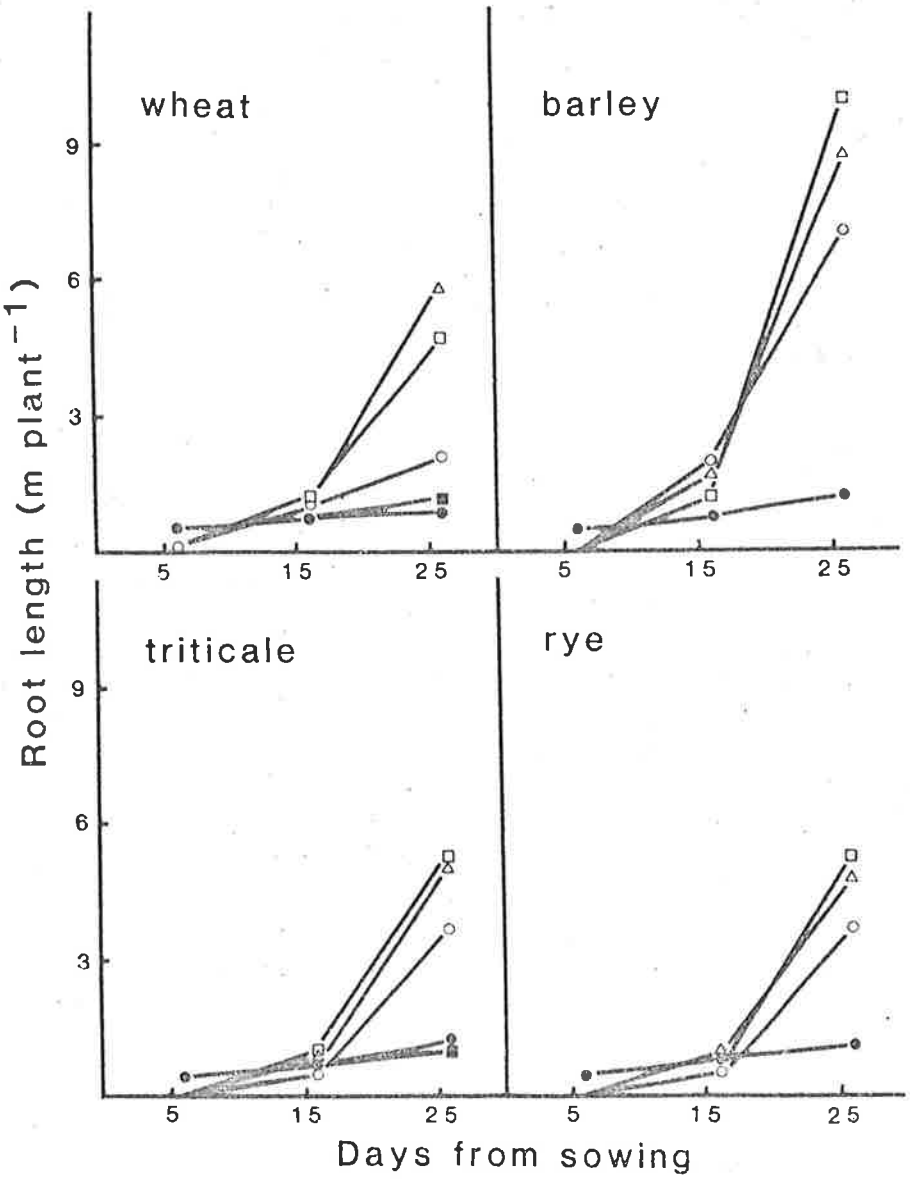

Figure 2.1: Increase in main axis (closed symbols) and lateral (open symbols) roots, between 6 and 26 days after sowing, for wheat, barley, triticale and rye plants grown at Mn0 (circles), Mn4 (squares) and Mn40 (triangles), in experiment 2.2.

(c) Manganese concentration

Shoot and root Mn concentrations for experiment 2.1 are given in Table 2.2. Data for YEB Mn concentrations are not included since they were very similar to and highly correlated (r^2 = 0.92) with shoot Mn concentrations. Of the wheats, only cv. Bodallin and Gatcher had significantly greater values at Mn0 and Mn40 than the rest. Overall, rye had a greater shoot Mn concentration at Mn0 and Mn40 than wheat, barley and triticale, but this was not the case for root Mn concentrations, which were similar for all genotypes. Zinc analyses on shoot material indicated that all plants were in the sufficiency range (>25 $\mu g\ g^{-1}$).

(d) Manganese content

Significant genotypic variation was found for shoot and root Mn contents at Mn0 and Mn40 in experiment 2.1 (Table 2.2). Barley, triticale cv. Venus and wheat cv. Bodallin had the highest plant Mn contents. There were significant differences between the wheat cultivars at both Mn0 and Mn40.

There were significant differences between cultivars in the ratio of shoot:root Mn content at Mn0 (for example, Bayonet had a low ratio and Gluyas had twice that ratio). Rye also had a high ratio but barley and triticale did not. At Mn40, only rye and wheat cv. Halberd had a significantly higher ratio than the others. The ratio increased for all genotypes, except Gluyas, with the addition of Mn ie when Mn supply was adequate relatively more Mn was transported to the shoot.

Shoot Mn content and shoot dry weight were highly correlated (r^2 = 0.91) at Mn0 and thus ranking of genotypes was similar using both parameters.

Table 2.2: Shoot and root Mn concentration and content, and the ratio of shoot:root Mn content for genotypes studied in experiment 2.1

Cultivar	Shoot Mn conc. $\mu g\ g^{-1}$		Root Mn conc. $\mu g\ g^{-1}$		Shoot Mn content $\mu g\ plant^{-1}$		Root Mn content $\mu g\ plant^{-1}$		Shoot:Root Mn content	
	Mn0	Mn40	Mn0	Mn40	Mn0	Mn40	Mn0	Mn40	Mn0	Mn40
Dundee	9.3	33.6	27.6	56.9	0.40	2.07	0.55	1.71	0.73	1.20
Halberd	9.9	37.7	23.2	42.0	0.34	1.60	0.41	0.85	0.83	1.89
Oxley	9.6	32.0	20.7	46.8	0.32	1.65	0.36	1.27	0.87	1.30
Bindawarra	10.0	34.1	24.4	60.6	0.41	1.91	0.55	1.81	0.74	1.05
Gluyas E.	10.6	36.6	23.9	51.7	0.46	2.42	0.41	1.87	1.11	1.10
Gatcher	12.7	36.3	32.3	53.5	0.50	1.78	0.75	1.50	0.67	1.18
Bodallin	18.3	47.2	36.1	68.1	1.00	2.54	1.30	2.03	0.75	1.25
Venus	15.0	54.5	26.9	57.9	1.12	4.49	1.27	3.04	0.88	1.47
Coorong	10.5	35.5	30.7	54.5	0.53	1.93	0.87	1.38	0.61	1.41
S.A.	17.9	69.1	31.0	61.9	0.66	2.18	0.60	0.90	1.04	2.43
Weeah	13.3	54.4	22.1	55.6	0.96	4.26	1.11	2.77	0.86	1.54
Galleon	11.9	49.4	22.5	60.0	0.83	4.26	1.13	3.38	0.73	1.24
LSD 5% G	3.1 ***		7.9 NS		0.26 ***		0.41 ***		0.14	**
Mn	1.1 ***		3.2 ***		0.10 ***		0.16 ***		0.06	***
G x Mn	4.5 ***		11.0 NS		0.36 ***		0.57 ***		0.20	***

(e) Manganese uptake

A high correlation (r^2 = 0.95) was found in experiment 2.1 between seed Mn content and whole plant Mn content, except for barley. In fact, only the two barley cultivars showed significant net Mn uptake at Mn0 (Table 2.3). Venus triticale had almost twice the Mn uptake of Coorong at Mn40, rye had only a relatively poor Mn uptake, whilst the two barley cultivars had the highest uptake.

The rate of Mn uptake between 16 and 26 days, calculated in experiment 2.2 is given in Table 2.4. Barley had twice the rate of actual Mn uptake than the other cereals at all levels of Mn supply. However, the rate of Mn uptake per unit root weight or length (relative uptake or efficiency of uptake) was similar for wheat and barley at low Mn supply, and greater for these two cereals than for triticale and rye. With Mn addition barley maintained a higher rate of actual Mn uptake per plant. All genotypes had equal efficiencies of Mn uptake at Mn4, whereas at Mn40 wheat was clearly least efficient.

Figure 2.2a shows the effect of genotype on net Mn uptake at Mn0 as a function of time. Little Mn uptake occurred until after 16 days. Manganese uptake for rye was negligible even at 26 days. This is also clearly evident in the data for shoot Mn concentration at Mn0 (Fig. 2.2b), which shows a decrease in Mn concentration between 16 and 26 days for rye, whilst there is an increase for the other genotypes over the the same period. Barley had the greatest uptake rate after 16 days: this was associated with a much higher lateral root production between 16 and 26 days growth (Fig. 2.1). However, the differences between wheat, triticale and rye were apparently not associated with differences in lateral root growth.

Table 2.3: Mn content of sown seeds and whole plants, net Mn uptake and utilisation efficiency for genotypes studied in experiment 2.1. Data are means of 3 replicates.

Genotype	Mn content			Net Mn uptake		Utilisation Efficiency	
	μg seed^{-1}	μg plant^{-1}		μg plant^{-1}		g^2 μg^{-1} x100	
		Mn0	Mn40	Mn0	Mn40	Mn0	Mn40
Bayonet	0.45	0.79	2.94	0.34	2.49	0.9	0.8
Halberd	0.73	0.75	2.47	0.02	1.74	1.2	0.5
Oxley	0.76	0.69	2.95	-0.07	2.19	1.2	0.5
Dundee	0.75	0.76	3.05	0.01	2.34	1.2	0.6
Bindawarra	0.78	0.98	3.74	0.20	2.96	1.2	0.6
Gluyas	0.84	0.88	4.33	0.04	3.49	1.5	0.7
Gatcher	1.55	1.31	3.52	-0.24	3.21	1.3	0.6
Bodallin	2.99	2.30	4.61	-0.69	1.62	1.1	0.5
Venus	2.13	2.41	7.60	0.28	5.47	1.8	0.7
Coorong	1.30	1.40	3.34	0.10	2.04	1.3	0.6
S.A.	0.95	1.31	3.11	0.36	2.16	0.8	0.2
Weeah	0.58	2.09	7.10	1.51	6.52	2.2	0.7
Galleon	0.62	1.98	7.71	1.36	7.09	2.2	0.8
LSD 5%	0.50	1.15 (GxMn)		-		0.3 (GxMn)	

Table 2.4: Rate of Mn uptake, between 16 and 26 days, for genotypes used in experiment 2.2. Data are means of 3 replicates

Genotype	Mn supply	Rate of Mn uptake		
	mg kg^{-1}	µg $plant^{-1}$ day^{-1}	µg g^{-1} day^{-1}	µg m^{-1} day^{-1}
wheat	0	0.05	1.94	0.017
	4	0.09	2.54	0.025
	40	0.22	5.82	0.060
barley	0	0.10	2.02	0.020
	4	0.17	2.67	0.029
	40	0.69	11.76	0.125
triticale	0	0.04	1.13	0.014
	4	0.08	2.33	0.022
	40	0.37	10.69	0.114
rye	0	0.02	0.61	0.006
	4	0.07	2.36	0.022
	40	0.43	15.12	0.131

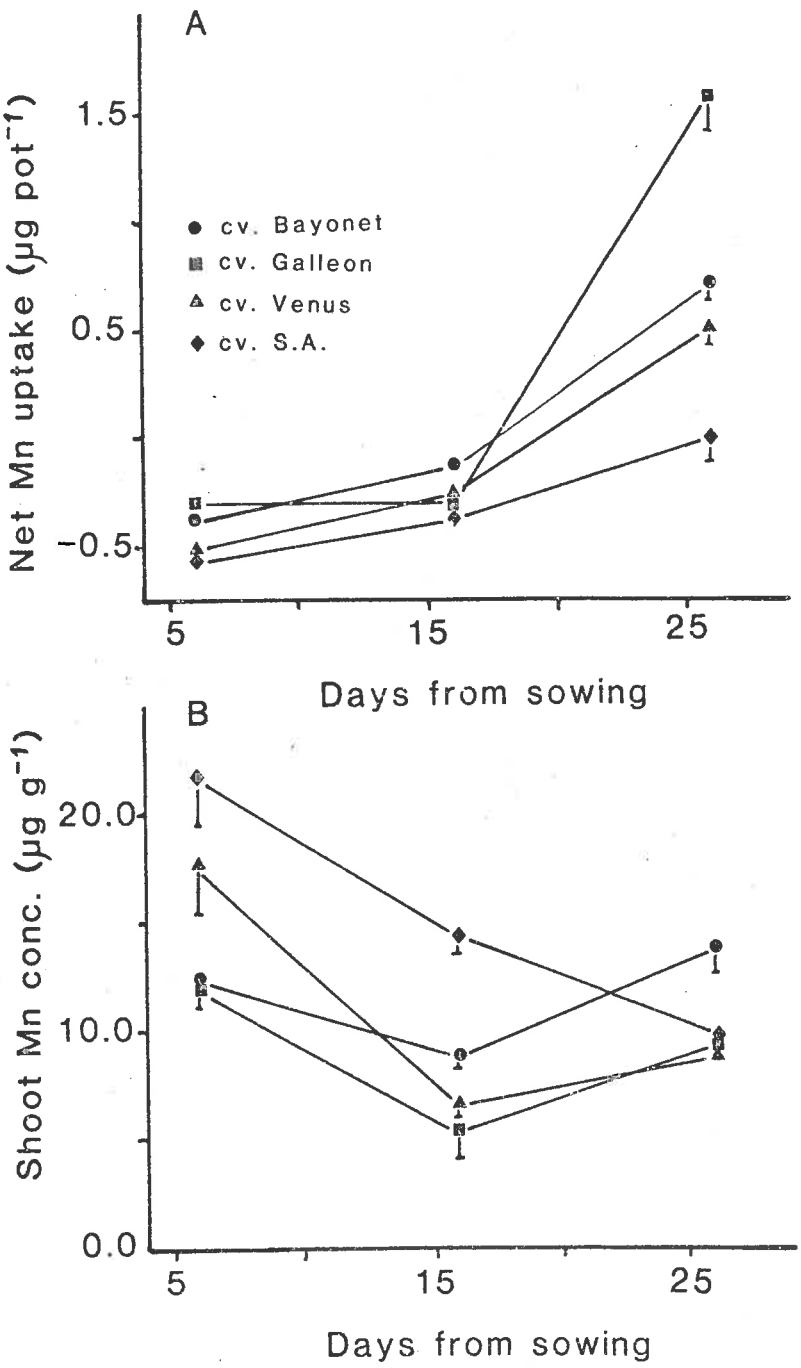

Figure 2.2: A. Net Mn uptake (ie. whole plant Mn content - initial seed Mn content) and B. Shoot Mn concentration for wheat, barley, triticale and rye at 6, 16 and 26 days after sowing (experiment 2.2). Vertical bars indicate standard errors.

(e) Utilisation efficiency

Table 2.3 shows that barley had the highest UE at Mn0, followed closely by Venus triticale. The other genotypes had considerably lower UEs. Of the wheats, Bayonet had the lowest value and Gluyas Early had the highest. All genotypes had similar UEs at Mn40, except for rye which had the lowest UE at both Mn0 and Mn40.

2.4.2 Experiment 2.3

Tables 2.5 and 2.6 show that Betzes had significantly more growth and Mn uptake than Chinese Spring at both deficient and adequate Mn supply, but a higher UE only at Mn0. Betzes also had a lower ratio of shoot:root dry weight and a significantly higher ratio of shoot:root Mn content at both Mn0 and Mn40. All the addition lines had similar actual dry weights, Mn uptake and the distribution of Mn between shoot and root at Mn0 and Mn40, and UE at Mn0, to Chinese Spring rather than to Betzes. However, relative dry weights (except lines 5 and 6) and the ratios of shoot:root dry weight at Mn0 (except line 1) were more like those of Betzes. The relative dry weight of line 2 was particularly high.

2.4.3 Experiment 2.4

The two triticale cultivars, Venus and Coorong, had the highest shoot and root dry weights, despite having the lowest Mn concentrations (Table 2.7). This resulted in much higher UEs for these cultivars. Shoot:root dry weight ratios were similar for all genotypes. Rye had considerably lower shoot Mn contents and higher root Mn contents than the other genotypes, resulting in a much lower ratio of shoot:root Mn content. Net Mn uptake and the rate of Mn uptake per plant were essentially similar for all genotypes, however, Coorong triticale was noticeably least efficient for Mn uptake, and had a lower net Mn uptake than Venus.

Table 2.5: Shoot and root dry weight and the ratio of shoot:ratio dry weight at deficient and adequate Mn supply for Betzes barley, Chinese Spring wheat and 6 addition lines (experiment 2.3). Relative yields (percent of Mn40) are given in brackets.

Line	Shoot dry weight g plant^{-1}			Root dry weight g plant^{-1}			Shoot:Root ratio	
	-Mn		+Mn	-Mn		+Mn	-Mn	+Mn
Betzes	234	(75)	310	123	(43)	289	2.00	1.08
C.S.	132	(50)	264	55	(30)	185	2.42	1.43
Add 1	162	(73)	221	65	(40)	162	2.63	1.37
2	149	(103)	145	69	(81)	85	1.97	1.78
3	106	(74)	143	54	(55)	98	1.99	1.47
4	141	(73)	192	82	(48)	170	1.72	1.25
5	137	(65)	210	81	(55)	146	1.74	1.44
6	127	(62)	206	67	(36)	188	1.90	1.47
LSD 5% lines	41 ***			51 ***			0.36 ***	
Mn	20 ***			25 ***			0.21 ***	
lines x Mn	58 ***			65 ***			0.49 ***	

Table 2.6: Shoot and root Mn contents, ratio of shoot:root Mn content, net Mn uptake and utilisation efficiency for Betzes barley, Chinese Spring wheat and 6 addition lines (experiment 2.3).

Line	Shoot Mn content $\mu g\ plant^{-1}$		Root Mn content $\mu g\ plant^{-1}$		Shoot:Root Mn content		Net Mn uptake $\mu g\ plant^{-1}$		U.E. $g^2\ \mu g^{-1} \times 100$	
	-Mn	+Mn	-Mn	+Mn	-Mn	+Mn	-Mn	+Mn	-Mn	+Mn
Betzes	1.01	5.83	1.17	4.99	0.94	1.17	1.58	9.22	5.85	3.28
C.S.	0.44	1.78	0.77	3.25	0.58	0.56	0.27	4.11	2.91	4.01
Add 1	0.58	1.50	1.03	4.37	0.65	0.35	-0.24	3.94	3.20	2.50
2	0.65	1.63	1.28	2.26	0.55	0.74	0.48	2.44	2.46	1.36
3	0.40	1.40	0.91	2.78	0.46	0.51	0.43	3.30	1.95	1.39
4	0.53	1.31	1.40	5.60	0.37	0.26	0.58	5.56	2.57	1.90
6	0.49	1.46	1.25	3.26	0.54	0.46	0.22	3.20	2.73	2.59
7	0.50	1.62	0.80	4.80	0.62	0.38	-0.66	4.46	2.90	2.42
LSD 5%										
lines	0.34 ***		NS		0.15 ***		-		-	
Mn	0.18 ***		0.88 ***		NS		-		-	
l x Mn	***		NS				-		-	

Table 2.7: Shoot and root dry weight, Mn concentration and Mn content, utilisation efficiency (UE) and Mn uptake after 28 days growth, and the rate of Mn uptake (between 18 and 28 days) for genotypes studied in experiment 2.4

Genotype	Dry weight		Mn conc.		Mn content		UE*	Net Mn Uptake	Rate of Mn Uptake#	
	Shoot	Root	Shoot	Root	Shoot	Root			I.	II.
	mg plant^{-1}		µg g^{-1}		µg plant^{-1}			µg plant^{-1}		
Bayonet	153	51	172	295	26.4	16.8	0.10	42.7	2.89	0.09
Bodallin	161	60	140	291	22.4	17.4	0.12	36.8	2.83	0.08
Gatcher	181	64	133	306	24.1	19.6	0.14	42.1	2.84	0.07
Venus	323	98	81	198	25.0	19.2	0.40	42.1	3.06	0.07
Coorong	241	84	91	190	22.0	15.9	0.28	37.9	2.53	0.05
S.A.	177	50	73	531	13.0	26.4	0.13	38.4	2.51	0.09
LSD 5%	36 ***	16 ***	14 ***	119 ***	5.4 ***	7.5 NS	-	-	-	-

* UE units are g^2 μg^{-1} x 100

\# I. units are µg plant^{-1} day^{-1}

II. units are µg g^{-1} day^{-1}

2.5 DISCUSSION

These experiments have demonstrated that young barley plants had considerably more capacity for Mn uptake from an acutely Mn-deficient soil than young wheat, triticale and rye plants (Table 2.3). The higher UE for barley also suggested a greater capacity for plant utilisation of the absorbed Mn. These two factors were associated with barley's high Mn-efficiency (ie. it s high relative yields). Relative yield assessment was the best method of ranking for Mn efficiency since it demonstrated the relative ability of genotypes to perform under deficient Mn supply. Although Venus triticale had similar actual yields to barley, and triticale, rye and Bodallin wheat had similar relative yields to barley (Table 2.1), these genotypes apparently relied much more on their seed Mn rather than Mn uptake to produce this growth.

Seed Mn contents were only determined at the conclusion of experiment 2.1. It then became clear that much of the variation in growth, Mn concentration and content between genotypes (particularly without added Mn) was apparently related to the use of different seed sources (and therefore seed Mn contents). For example, seeds of Bodallin had the potential to supply five times more Mn to developing seedlings than seeds of Bayonet (see Appendix 2.2). Similar conclusions were reached by comparing the Mn content of sown seeds with seedling shoot dry weight and Mn content for the 16 wheat cultivars studied in a preliminary experiment (Appendix 2.1).

The reserve of seed Mn proved to be very important in these studies because this soil was acutely Mn-deficient. Apart from the facts already highlighted above further evidence is provided in Appendix 2.8. These results clearly show tat the reason for not obtaining any significant differences between apparent Mn efficieny for Galleon and Weeah barley in experiment 2.1 was because of the seed source chosen (in contrast with results obtained by R.D. Graham and J. Ascher (personal communication) who used other sources). In most studies that have investigated nutrient

efficiency, nutrient contents of the seeds used would not have been as important because the soils used were not as deficient for the nutrient under consideration as that used here. Even for nutrient-inefficient genotypes, the nutrient content of sown seeds could be inferred to several times lower than that determined for the harvested plants (for example, in studies concerning Mn efficiency (Nyborg 1970; Brown and Jones 1974), Cu-efficiency (Nambiar 1976) and Zn-efficiency (Shukla and Raj 1976)). However, in other studies, seed nutrient content was shown to be a significant factor in apparent nutrient efficiency (for example, studies concerning Zn-efficiency in maize (Halim et al 1968; Massey and Loeffel 1969), P-efficiency in beans and maize (Whiteaker et al 1976; Fox 1978) and K-efficiency (Ozanne and Ascher 1965). A fuller discussion of the efects of seed Mn on cereal growth and yield is left until Chapters 5 and 6.

It might be expected that seedling root vigour would be greater for those seedlings derived from seeds of high seed Mn in the Mn0 treatment, and that this greater root proliferation might allow it to absorb more Mn and therefore produce more growth. However, the extreme Mn deficiency of this soil apparently precluded any benefit in Mn uptake. The amount of Mn absorbed by most genotypes at Mn0 in these experiments was clearly small even though calculated values for net Mn uptake could have underestimated real uptake because Mn potentially leached from the seed was not determined. This small amount of Mn uptake is consistent with the hypothesis that the predominant source of Mn available to these plants was from reducible rather than labile soil Mn. The extreme Mn deficiency of this soil was also highlighted by the fact that Mn uptake by Bayonet wheat was not increased by variation of soil moisture content and temperature (Appendix 2.8).

With Mn addition (ie. Mn40) much greater uptake was observed. Manganese added to this soil became readily unavailable (Appendix 2.7): presumably this Mn was oxidised, adsorbed onto MnO_2 surfaces or precipitated as $MnCO_3$. However, there would still have been considerable quantities of Mn^{2+} available in these experiments for uptake at least for the first half of the growth period. Presumably the Mn taken up by plants was mostly Mn^{2+} via diffusion to the root surface, but some Mn

solubilisation from freshly oxidised or precipitated Mn may have occurred.

Results from experiment 2.2 (Table 2.4 and Fig. 2.1) showed that under Mn deficiency the greater Mn uptake by barley compared with wheat, triticale and rye was associated with a greater amount of root (in particular lateral root length) rather than more efficient Mn uptake (per unit of weight or length of root). This experiment did not definitely prove that the greater root abundance of barley was responsible for its increased Mn uptake. However, indirect evidence that this may indeed be the case comes from the observation that barley also had a greater root length and higher Mn uptake at adequate Mn supply (Mn40). That is, barley has an inherently more vigorous seedling root system than the other cereals.

A higher percentage and total amount of finer lateral roots in barley would result in more contact between root surfaces and soil constituents. This contact is probably the important limiting factor in Mn uptake from oxide and carbonate surfaces in calcareous soils (Mc Bride 1979; Uren 1981). Since mean root diameter was found to be no larger for barley than the other cereals, extension growth appeared to be the major mechanism whereby barley increased its total root surface area for Mn acquisition in these experiments. The small size of the pots used restricted root growth vertically and horizontally, and thus, the genetic potential for extension growth, particularly at adequate Mn supply, may not have been realised.

The only root morphological measurements made in this experiment were length and diameter. However, the degree of branching and lateral root number (and therefore contact with the soil solid phase) may also be a relevant factor in Mn efficiency. There is evidence that branching of the root system is positively correlated with length in barley (Hackett and Bartlett 1971). Total root length and surface area have been associated with the uptake of other less mobile nutrients, such as P, where diffusion to the root surface is a limiting factor (Schenk and Barber 1979 ; Coltman 1985). Although root hair parameters were not measured in these experiments, they may well have been involved in Mn uptake, as suggested by Uren (1981), since the rate of diffusion of Mn and the average

concentration at the root surface would be small (Nye 1966).

The importance of plant-induced rhizophere effects, such as H^+ ion extrusion and secretion of reducing and chelating agents, in determining genotype differences in Mn uptake cannot be discounted. These factors have been evaluated in further experiments that are discussed in Chapter 3. Both these and morphological characteristics may be very important in Mn acquisition from soils deficient in Mn, in contrast to the importance of root absorption characteristics for Mn uptake from solution culture (Munns et al 1963).

The UE under Mn deficiency was shown to be considerably higher for barley than for wheat and rye in experiments 2.2 and 2.3 (Tables 2.3 and 2.6), but Venus triticale also had a high ratio. The ratio as used here is according to Siddiqi and Glass (1981) ie. biomass/ nutrient concentration. This is different to the more accepted ratio of biomass/nutrient content (presumably absorbed) used by most workers eg. Loneragan and Ascher (1967). The former ratio takes into account growth dependent on tissue concentration and may reflect differences in a functional requirement for Mn. However, a smaller value for UE, reflecting a slower growth rate (eg. for rye), could also be regarded as an adaptive mechanism (Chapin 1980), since less Mn would be required for maintenance of growth.

Experiment 2.3 showed that Mn efficiency could be transferred from barley to wheat. Whilst all the wheat-barley addition lines had a similar yield potential to their wheat (Chinese Spring) parent, most of them had a similar Mn efficiency to their barley (Betzes) rather than to wheat parent since they had a better relative yield than wheat (Table 2.5). Confirmation of the high Mn efficiency of line 2 is warranted. These lines, however, had a similar Mn uptake and UE to their wheat rather than to their barley parent. Thus it appears that these factors are not mechanisms involved in determining Mn efficiency in these lines. The results obtained here do not clarify the basis of genetic control of Mn efficiency.

Some differences were found between the behaviour of genotypes in experiment 2.4 compared with experiments 2.1-2.3. For example, in experiment 2.4, Bayonet wheat had similar Mn uptake to Bodallin, and rye had a lower rate of Mn transport to shoots than wheat and triticale. The Mn concentrations and contents of plants in experiment 2.4 were much higher than those found in experiments 2.1-2.3. In retrospect, a lower solution Mn concentration than 0.65 µM should have been used because plant Mn contents were far too high for comparison with uptake efficiency in soils. Only one concentration was used owing to limitations of labour and space. However, most short-term Mn absorption studies have considered solution Mn concentrations greater than 2.0 uM (eg. Maas et al 1968; Ramani and Kannan 1982). Barber and Lee (1974) used a lower concentration (0.2 µM Mn), at which 3 week-old barley plants had shoot and root Mn concentrations of 11 and 45 ug g^{-1}, very similar to the Mn concentrations observed in experiments 2.1-2.3. Such differences highlight the importance of cultural factor(s) in determining growth, Mn uptake and transport by different genotypes.

2.6 CONCLUSIONS

Several conclusions can be made on the basis of the results of these experiments. Firstly, barley seedlings were shown to be more Mn efficient than those of wheat, triticale and rye. Secondly, barley's Mn efficiency was primarily related to greater Mn uptake (but also apparently to Mn utilisation). Thirdly, the amount of Mn uptake was correlated with the extent of seedling lateral root growth. Fourthly, the Mn content of sown seeds must be determined in order to properly calculate Mn uptake. Fifthly, the fact that wheat (as well as triticale and rye) seedlings had a net zero Mn uptake on this soil (in contrast to barley) means that any seedling screening technique to detect genotypic differences for Mn efficiency requires a soil less severely Mn deficient than is suitable for barley.

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Appendix 2.1: Preliminary screening of wheat cultivars for Mn efficiency during seedling growth

Sixteen wheat cultivars (as well as rye and barley) were grown in small pots containing Mn-deficient calcareous sand (see methods section) for 24 days in a glasshouse (average 22°C/15°C day/night temperature). The table below shows the original seed Mn content as well as the symptom score, shoot dry weight, Mn concentration and content of harvested plants. Data are means of three replicates. Relative yields (per cent of Mn40) are indicated in brackets.

Genotype	Seed Mn cont. $\mu g\ seed^{-1}$	Symptom * score	Shoot dry weight $mg\ plant^{-1}$		Shoot Mn conc. $\mu g\ g^{-1}$		Shoot Mn content $\mu g\ plant^{-1}$	
		Mn0	Mn0	Mn40	Mn0	Mn40	Mn0	Mn40
Purple Straw	0.96	0.5	32 (85)	37	9.7	56.2	0.26	1.84
Dundee	0.81	2.5	34 (83)	41	5.0	36.2	0.17	1.82
Gatcher	1.60	0	39 (81)	48	10.7	34.8	0.42	1.66
Bindawarra	0.78	0.5	33 (78)	43	5.6	40.5	0.19	1.70
Bencubbin	0.92	1.5	41 (74)	55	9.6	50.6	0.39	2.77
Gabo	1.05	2.0	37 (74)	51	7.8	41.6	0.29	2.09
Olympic	0.65	1.0	22 (72)	31	6.2	36.0	0.14	1.12
Songlen	0.50	3.0	29 (69)	41	7.6	35.9	0.22	1.50
Oxley	0.76	2.5	29 (65)	44	7.7	38.1	0.23	1.68
Halberd	0.73	2.5	24 (63)	38	7.4	44.8	0.18	1.68
Lance	0.58	2.5	32 (62)	51	5.0	27.5	0.16	1.36
Nabawa	0.88	1.5	35 (61)	57	9.0	39.4	0.26	1.78
Egret	0.67	1.5	22 (61)	35	7.9	48.3	0.17	1.71
Kite	0.82	1.0	31 (58)	53	9.8	38.2	0.29	2.03
Gluyas Early	0.84	2.5	30 (50)	60	9.3	49.9	0.28	2.65
Bayonet	0.45	2.5	27 (50)	54	7.7	36.0	0.21	1.87
S.A.	0.95	0	28 (106)	26	14.8	99.0	0.41	2.56
Weeah	0.58	0	50 (89)	57	10.4	91.9	0.58	5.19
Galleon	0.62	0	51 (80)	63	10.7	70.0	0.53	4.41
LSD 5% G			19 **		4.6 **		0.88 *	
Mn			6 **		1.6 **		0.30 ***	
G x Mn			NS		6.6 **		NS	

* 0 = nil, 0.5-1.0 = mild, 1.0-2.0 = moderate, 2.0-3.0 = severe

Appendix 2.2: Seed dry weight, Mn concentration and Mn content of cereal genotypes used in experiments 2.1-2.3. Data are means of 3 replicates of 10 seeds. Standard errors are given in brackets.

Cultivar	Exp. no.	Source	Dry weight mg seed^{-1}	Mn content µg seed^{-1}	Mn conc. µg g^{-1}
Dundee	1	(a)	38.0 (1.3)	0.81 (0.01)	21.3 (0.4)
Gatcher	1,3	(b)	37.6 (0.7)	1.60 (0.06)	40.2 (2.9)
Bindawarra	1	(a)	41.4 (1.9)	0.78 (0.01)	18.9 (0.2)
Oxley	1	(a)	37.4 (0.7)	0.76 (0.02)	20.2 (0.3)
Halberd	1	(a)	35.9 (1.0)	0.73 (0.03)	20.5 (1.3)
Gluyas E.	1	(a)	39.7 (2.4)	0.84 (0.08)	20.9 (0.9)
Bayonet	1,2,3	(a)	33.4 (2.4)	0.45 (0.05)	13.3 (0.4)
Bodallin	1,3	(b)	38.5 (2.5)	2.95 (0.44)	76.9 (4.6)
Venus	1,3	(c)	57.8 (1.4)	2.13 (0.04)	36.9 (1.2)
"	2	(d)			
Coorong	1,3	(c)	42.4 (1.3)	1.30 (0.03)	30.6 (1.0)
S.A. Rye	1,2,3	(e)	22.4 (0.9)	0.95 (0.01)	42.8 (2.3)
Weeah	1	(f)	45.1 (1.6)	0.58 (0.03)	12.8 (0.2)
Galleon	1,2	(f)	50.7 (1.1)	0.62 (0.03)	12.2 (0.5)

* (a) Rudall, S.A. (marginally Mn-deficient site)
(b) Roseworthy, S.A. (Mn-adequate site)
(c) Urrbrae, S.A. (Mn-adequate site)
(d) Yaninee, S.A. (marginally Mn-deficient site)
(e) Hodges Seed Co.
(f) Strathalbyn, S.A. (Mn-adequate site)

Appendix 2.3: Relationship between root fresh weight (Y) and main axis (X_1) and lateral (X_2) root length at 26 days (experiment 2.2)

Genotype	Equation
Wheat	$Y = 0.24 + 0.06X_1 + 0.06X_2$
Barley	$Y = -0.48 + 0.61X_1 + 0.04X_2$
Triticale	$Y = -0.14 + 0.34X_1 + 0.04X_2$
Rye	$Y = 0.43 + 0.01X_1 + 0.05X_2$

Appendix 2.4: Correlation coefficient (r^2) between root fresh weight (Y), main axis (X_1) and lateral root length (X_2) at 26 days (experiment 2.2)

Genotype	Y vs X_1	Y vs X_2	X_1 vs X_2
Wheat	0.56	0.54	0.86
Barley	0.09	0.18	0.47
Triticale	-0.36	0.71	0.26
Rye	0.73	0.66	0.90

Appendix 2.5: Mean root diameters for wheat, barley, triticale and rye after 26 days growth (experiment 2.2)

Genotpye	Diameter (mm)		
	Mn0	Mn4	Mn40
Wheat	0.17	0.17	0.15
Barley	0.15	0.15	0.17
Triticale	0.18	0.15	0.15
Rye	0.15	0.15	0.15

Appendix 2.6: Effect of soil moisture content and temperature on growth and Mn uptake of wheat cv. Bayonet.

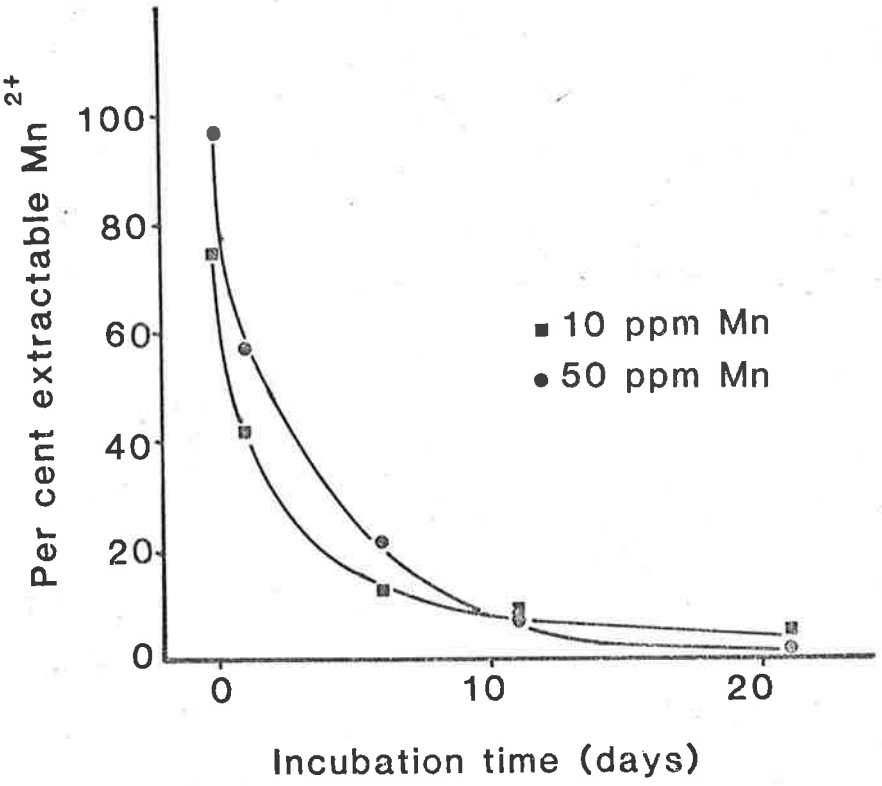
A small experiment was conducted to determine whether soil moisture content and temperature were critical factors influencing Mn uptake by wheat grown on the same soil used in experiments 2.1-2.3. Wheat (cv. Bayonet) plants were grown for 28 days in pots containing c. 450 g soil without Mn addition. The experiment was conducted at 15°C with 12 h light at an average photoflux density of 500 μmole quanta m^{-1} s^{-1}. It had a factorial, completely randomised design with 3 replicates, comprising the following treatments:

(a) 3 soil moisture contents viz. 15, 25 and 40 per cent (w/w), o.d. basis
(b) 2 soil temperatures viz. 15 and 25°C, the latter maintained by placing pots in a 25°C water bath.

Results are given in the table below. Only plants at 15 per cent moisture content had no Mn-deficiency symptoms. Higher soil moisture contents resulted in increased shoot growth but decreased shoot Mn concentrations because there was only a small (but significant) increase in Mn content. However, root dry matter production was reduced at higher soil moisture contents resulting in 3 to 4-fold increases in shoot:root ratios at 40 per cent moisture content. Higher soil temperatures reduced shoot growth and shoot Mn content but had no effect on shoot Mn concentration.

Treatment		Symptom	Shoot	Root	Shoot	Shoot
Temp. °C	Moisture g g^{-1}	score	dry wt. mg $plant^{-1}$	dry wt. mg $plant^{-1}$	Mn conc. μg g^{-1}	Mn cont. μg $plant^{-1}$
15	15	0	53	48	5.6	0.30
	25	3	88	33	4.4	0.38
	40	3.5	95	26	3.9	0.36
25	15	0	39	32	5.4	0.21
	2	3.5	60	38	4.1	0.25
	40	3.5	83	23	3.6	0.28
LSD 5%	Temp.	-	6	NS	NS	0.02
	Moisture	-	7	2	0.2	NS
	T x M	-	NS	3	NS	NS

initial seed Mn content = 0.72 ug $seed^{-1}$



Appendix 2.7: Rate of dissapearance of divalent Mn, added as MnSO to 10 g samples of the Mn-deficient calcareous soil used in experiments 2.1-2.3, at rates of 10 and 50 ppm Mn, incubated at 15°C.

Appendix 2.8: Effect of seed source on the growth and Mn uptake of two barley cultivars under Mn deficiency.

A small experiment was undertaken to determine the effect of seed source (seed from Wangary, Charlick and Urrbrae, S.A.), pot size (250 and 450 g soil cap.) and plant density (2 and 4 plants per pot) on the growth and Mn uptake of seedling (27 day old) plants of Weeah and Galleon barley. It was intended to resolve the discrepency between the similar Mn efficiencies for these cultivars found in this thesis (Chapter 2) and the higher efficiency found for Weeah by Graham et al (unpublished). Growth conditions and analyses were the same as described for Chapter 2 (experiment 2.1).

The higher plant density resulted in lower dry weights per plant only for the smaller pots. The dominant effect on biomass production was seed source and this is shown in the table below. Thus when seed from Charlick was used there was no difference between Galleon and Weeah at MnO (cf. table 2.1, Chapter 2), but there were when seed from Wangary and Waite were used (cf. J. Harbard, R.D. Graham, personal communications).

Seed source	Cultivar	Seed Mn content μg seed^{-1}	Shoot dw mg plant^{-1}	Shoot Mn content μg plant^{-1}
Wangary	Galleon	0.08	45	0.25
	Weeah	0.18	76	0.55
Charlick	Galleon	0.62	94	-
	Weeah	0.61	82	-
Waite	Galleon	0.69	84	0.65
	Weeah	0.99	107	1.12

Appendix 2.9: Seed dry weight, Mn concentration and Mn content of genotypes used in Experiment 2.3. Data are means of 3 replicates of 10 seeds each. Standard errors are given in brackets.

Addition Line	Dry weight mg seed^{-1}	Mn concentration μg g^{-1}	Mn content μg seed^{-1}
Betzes	36.0 (0.8)	16.6 (0.7)	0.60
C.S.	26.6 (0.6)	36.3 (0.3)	0.97
Add 1	32.8 (0.7)	59.4 (0.4)	1.95
" 2	23.1 (0.4)	63.5 (0.8)	1.47
" 3	24.0 (0.6)	37.3 (0.3)	0.90
" 4	25.5 (0.8)	52.6 (0.7)	1.34
" 6	29.4 (0.9)	52.8 (0.9)	1.55
" 7	33.2 (1.4)	60.4 (1.1)	2.01

CHAPTER 3

FACTORS ASSOCIATED WITH MANGANESE ACQUISITION BY WHEAT AND BARLEY SEEDLINGS

3.1 SUMMARY

This chapter describes several small experiments which attempted to identify some possible mechanisms associated with the greater acquisition of Mn by barley compared with wheat when seedling plants were grown in Mn-deficient calcareous soil (Chapter 2). Qualitative observations indicated that neither the degree of rhizosphere acidification nor reduction of MnO_2 per unit root were very different between wheat and barley, and that there was similar root infection with VA mycorrhiza. The higher Mn efficiency of barley in Mn-deficient soil may result from a greater total root surface area available for chemical modification.

3.2 INTRODUCTION

Several small experiments were undertaken in an attempt to identify mechanisms associated with the higher Mn uptake of barley compared with wheat under Mn deficiency as observed in experiments 2.1 and 2.2 (Chapter 2). Bayonet wheat and Galleon barley were compared because they were shown to have quite different Mn efficiencies in these experiments. The experiments reported here consider three possible factors associated with Mn acquisition at the soil-root interface:

(a) acidification of the rhizosphere: this would be expected to increase the amount of soluble Mn (Mn^{2+}) in the soil solution of the rhizosphere. For example, acidification of the rhizosphere has been demonstrated to be an important Fe-stress response for Fe-efficient plants in conjunction with reduction and chelation (Brown 1978).

(b) reduction of MnO_2: this would also be expected to render more Mn^{2+} available for absorption (Uren 1981).

(c) the degree of vesicular-arbuscular (VA) mycorrhizal infection: this might be expected to affect Mn uptake by altering the amount of contact made between roots and soil solids and/or soil solution.

3.3 MATERIALS AND METHODS

Experiment 3.1

Pregerminated seeds were sown into plexiglass boxes, with internal dimensions of 140 x 128 x 6 mm (after Marschner and Romheld 1983), containing Mn-deficient calcareous sand prepared as before (Chapter 2). Four boxes were assigned for each cereal. Plants were grown for 2 weeks at 15°C in a growth cabinet at c. 350 µmole quanta m^{-2} s^{-1}. At the end of the experiment, 2 boxes were infiltrated with agar containing phenol red (made up to pH 8.5) and colour changes observed, whereas plant roots from the 2 other boxes were harvested in order to separate strongly adhering (rhizosphere) soil from bulk soil. Soil pH was determined after shaking 0.5 g soil samples in 2.5 ml $CaCl_2$ for approx. 30 min.

Experiment 3.2

Roots of 8-12 day old solution-grown (1/10 x Hoaglands) plants were embedded into agar, containing 1/10 x Hoaglands and bromcresol purple pH indicator, in flat transparent plexiglass boxes (12 x 25 x 1.5 cm), after Marschner et al (1982). The N source used was NO_3^- since this was the form of N used in the soil studies (Chapter 2). The agar pH used was either pH 6 or 7. In one case, the agar was more strongly buffered by using 2 mM KH_2PO_4/ K_2HPO_4 buffer.

Experiment 3.3

Four to five day old solution-cultured wheat and barley plants were grown in a 'sandwich' of chromatography papers impregnated with MnO_2 after the method of Uren (1981). Preliminary studies showed that soaking papers in 5 x 10^{-2} M $KMnO_4$ solution for 1 h produced the optimal shade of brown for observing reduction. Plants were grown for 4-5 days, after placing the sandwich in a plastic bag and placing this bag inside a dark box at an angle of 85 , so that only the seedling shoot was exposed to the light.

Experiment 3.4

Roots of 8-12 day old solution-grown (Fe-deficient and Fe-adequate) and soil-grown plants were embedded into agar, containing 1/10 x Hoaglands and finely dispersed MnO_2, prepared according to Marschner and Romheld (1982). It was thought that Fe-deficient roots should have a greater ability to reduce MnO_2 than Fe-adequate roots. The medium was adjusted to pH 6. Three $KMnO_4$ concentrations (1, 0.5 and 0.1 mM) were used to prepare the MnO_2.

Photographs of roots from experiments 3.2 and 3.4 were made after placing the plexiglass boxes onto a lightbox.

Experiment 3.5

Plants were cultured and harvested (after 24 days growth) as described previously (see Chapter 2, part 1). Three VA mycorrhizal fungi, Glomus mosseae (GM), Gigaspora coralloidea (GC) and E3 were introduced into the soil prior to sowing using infected root material, but no attempt was made to standardise propagule numbers. Treatments were not replicated. Root systems were cleared in 10% KOH for 1.5 days at room temperature, washed in distilled water and 1N HCl and stained in 0.05% trypan blue in lactophenol for 10-15 min at 80°C (S. E. Smith, personal communication). Infection by VA mycorrhiza was simply scored as per cent total root length infected by hyphae, vesicles or arbuscles using the modified line method of Tennant (1975), with the aid of a binocular microscope at 125x magnification.

3.4 RESULTS

Whilst wheat plants showed mild to moderate Mn-deficiency symptoms, barley plants were usually quite healthy.

Experiment 3.1

There was no change in wheat or barley rhizosphere pH measured either directly by pH meter on mechanically separated bulk and rhizosphere soil fractions (Table 3.1) or in vivo as determined by colour changes in the

rhizosphere (photograph 3.1).

Experiment 3.2

Distinct colour changes were observed around roots embedded in agar both for wheat and barley, although the effect appeared to be slightly greater for barley. Most changes observed were due to acidification, ie. purple or red to yellow (photograph 3.2), although this was dependent on the initial pH of the agar as well as the degree of buffering. Most acidification occurred in the zone of elongation and in the area of lateral root production. Roots of lupins, included for comparison with wheat and barley showed a much greater rate of acidification (photograph 3.3).

Experiment 3.3

Narrow clear zones were apparent for roots of both wheat and barley. No consistent differences were found between several wheat cultivars.

Experiment 3.4

Distinct clear zones were evident around both wheat and barley roots after 3-4 days, particularly in the region of maximum lateral root production (photograph 3.4). Iron-deficient and Fe-adequate plants appeared to be able to reduce MnO_2 to the same degree. Roots of lupins, included for comparison, showed a much greater ability to reduce MnO_2 than wheat and barley (photograph 3.4).

Experiment 3.5

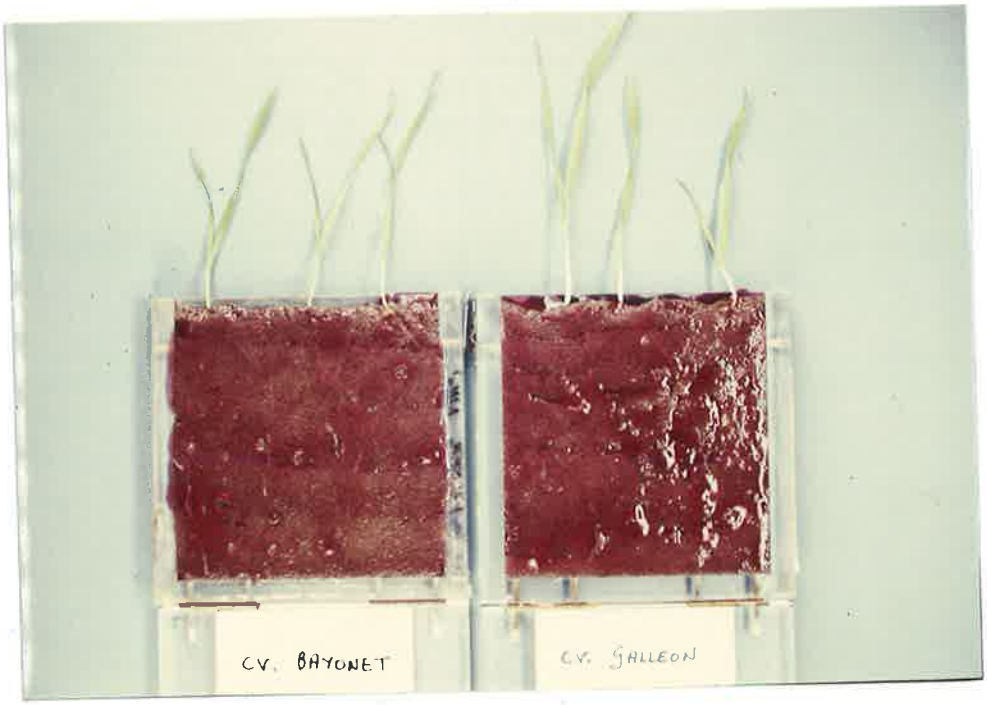
Percentage root length infection under control conditions was higher for wheat than for barley, in contrast to plant fresh weight (Table 3.2). Levels of infection were increased for barley but not for wheat with addition of VA mycorrhizal fungi into the soil. Plant fresh weights were similar for all treatments. Manganese uptake was not measured.

Table 3.1: Wheat (Bayonet) and barley (Galleon) rhizosphere pH compared with bulk soil pH. Data are means and standard errors of 5 replicates.

Genotype	Soil pH	
	Rhizosphere	Bulk
Bayonet	7.88 (0.03)	7.86 (0.03)
Galleon	7.99 (0.02)	7.99 (0.01)

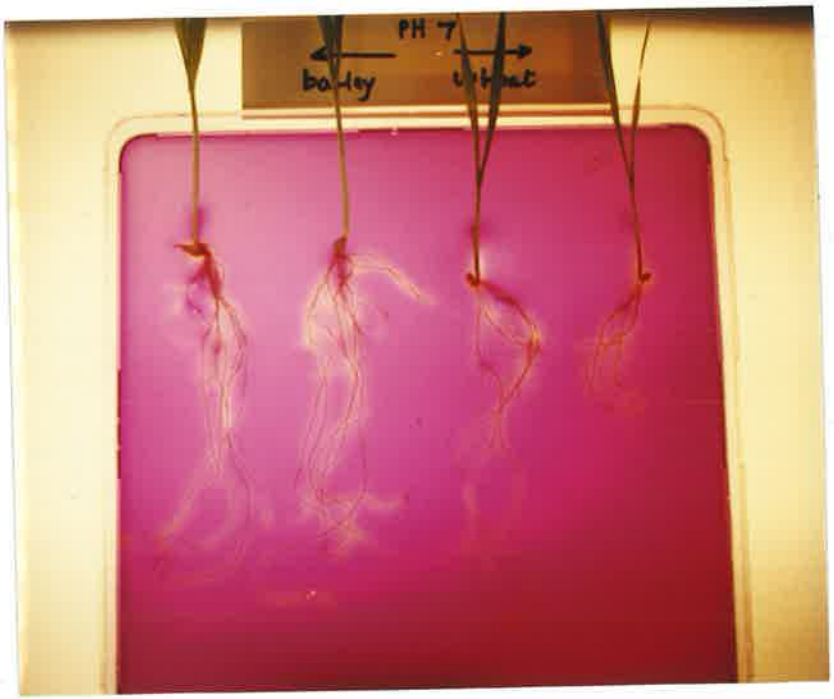
Table 3.2: Percentage VA mycorrhizal infection and growth of wheat (Bayonet) and barley (Galleon) after 24 days growth in a Mn-deficient calcareous soil from Wangary, S.A. Data for percent infection are means and standard errors of 3 measurements.

Fungal species	Percent infection		Total fresh weight g plant^{-1}	
	Bayonet	Galleon	Bayonet	Galleon
Control	15.3 (3.4)	5.7 (1.2)	2.25	3.66
GM	11.3 (3.3)	12.3 (3.4)	1.75	3.83
GC	5.7 (2.3)	11.0 (1.2)	2.37	3.96
E3	10.7 (1.5)	15.7 (3.0)	1.69	2.26



No visible difference in pH between rhizosphere and bulk soil (experiment 3.1)

Photograph 3.1



Both wheat and barley roots can acidify agar medium at pH 7 (experiment 3.2)

Photograph 3.2

Photograph 3.3

Lupin roots can acidify agar medium at pH 6 better than wheat and barley (experiment 3.3)

Lupin roots show much better ability to reduce MnO_2 than wheat or barley (experiment 3.4)

Photograph 3.4

3.5 DISCUSSION

From these studies it does not appear that seedling plants of barley and wheat can significantly lower their rhizosphere pH when grown in the calcareous soil used in experiments detailed in Chapter 2. It is still possible that more subtle changes occur at the rhizoplane which cannot be detected by the methods used here. These changes might occur, for example, in anaerobic micro-sites, as suggested by Smith (1976). However, it does not appear that pH reduction would be an important factor related to Mn uptake. Similar conclusions have been drawn for other well buffered alkaline soils (Riley and Barber 1971; Smiley 1974; Nye 1981). Uren (1984) pointed out that Fe- and Mn-efficient plants on calcareous soils must secrete reductants at all pH's but particularly at pH's greater than 8. Marschner and Romheld (1983) also found that addition of $CaCO_3$ to the soil resulted in a much reduced pH drop in the rhizosphere than without $CaCO_3$.

Results from the agar studies showed that roots of both wheat and barley appear to have a similar potential to acidify their rhizospheres and to reduce MnO_2. However these experiments had insufficient replication and lacked quantitative data. The superior ability of lupin roots to chemically modify their rhizosphere is in agreement with data of Gardner et al (1982). As the buffering capacity of the medium increased, pH changes were less dramatic. It is interesting that pH decreased for the first few hours, followed by a slower pH increase. This may have been due to a greater cation uptake compared with NO_3^- uptake because only a low strength nutrient solution was used. Such a suggestion was proposed by Marschner and Romheld (1983) to account for rhizosphere acidification of maize seedlings. The stress of Fe-deficiency did not enhance the ability of roots to reduce MnO_2.

If acidification and reduction are mechanisms associated with Mn efficiency of barley in soil it appears that this may be a consequence of total surface area available for chemical modification rather than more efficient modification per unit area root. However, until a method is available for quantifying the degree of acidification and reduction, definitive statements cannot be made as to the importance of these

mechanisms in determining genotypic differences in Mn uptake efficiency.

The greater ability of barley to absorb more Mn than wheat from this calcareous sand is apparently unrelated to a greater association with VA mycorrhiza. This is agreement with experimental evidence suggesting that VA mycorrhiza only modify nutrient acquisition by plants for nutrients that diffuse slowly towards the root, for example, P, Zn and Cu (Abbott and Robson 1984). Since Mn is extremely unavailable in this soil it s uptake would be unlikely to be influenced by mycorrhizal activity. While there are some reports of solubilising and chelating properties of mycorrhizal fungi, hard evidence is lacking (Coleman et al 1983).

Nevertheless, the level of infection was quite high considering the age of these plants (3.5 weeks) and the level of P supply (adequate for normal growth). For example, Jensen (1982) only found up to 10% infection for barley at 4.5 weeks. She also found that growth was not correlated with intensity of infection. Graminaceous plants generally have low levels of infection compared with clover and onions (S. E. Smith, personal communication). Wheat plants may not be significantly colonised in the field until after anthesis (Hetrick and Bloom 1983). Under conditions of P adequacy roots may be only sparsely infected and the degree of infection is unlikely to enhance the uptake of another limiting nutrient (Abbott and Robson 1984).

It has been suggested that high levels of available soil Mn can limit mycorrhizal growth (Menge et al 1982; P. McGee, personal communication), so that, conversely, low available Mn may encourage mycorrhizal activity, irrespective of P status. Certainly levels of infection in roots of wild oats (Avena fatua) , collected from the field (at Wangary), were much higher (>30%).

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CHAPTER 4

GENOTYPIC VARIATION FOR MANGANESE EFFICIENCY IN WHEAT SEEDLINGS

4.1 SUMMARY

Six wheat cultivars were grown in a growth cabinet at 15 °C for up to 30 days in pots containing a manganese (Mn) deficient calcareous soil with 0, 10 and 50 mg Mn kg^{-1} added. Measurements were made of shoot and root growth and Mn concentration, as well as Mn concentration, chlorophyll concentration and room temperature chlorophyll a fluorescence of the basal region of the youngest expanded blade. Significant differences were apparent between cultivars for shoot and root dry matter production, Mn uptake and Mn utilisation efficiency with added Mn. Manganese deficiency was accompanied by distinct changes in fluorescence induction kinetics (a large increase in the ratio Fo/Fv and a decrease in the ratio Fv/Fp), which reflected photosynthetic dysfunction, as well as a decrease in total chlorophyll content (Chl a+b). However, there were only small differences for these parameters between cultivars, suggesting only small cultivar differences for a functional requirement of Mn in photosynthesis. The critical Mn concentration for Fo/Fv, Fv/Fp and Chl a+b was in the range 13-15 $\mu g\ g^{-1}$, whilst that for shoot dry weight, although similar, appeared to differ with cultivar response to added Mn.

4.2 INTRODUCTION

The pot experiment described here was conducted in order to determine if wheat cultivar differences for Mn efficiency during early growth could be detected using more levels of soil Mn addition than were used in earlier studies (see Chapter 2). The soil used in these earlier studies had been too deficient for the screening of wheat cultivars. This experiment was commenced after a suitable soil-mix had been identified following the screening of a number of soils and soil mixes (Appendices 4.1, 4.2). Three wheats which had not been previously examined were included. It was intended to measure several factors related to Mn efficiency eg. Mn acquisition, transport of Mn to shoots and Mn utilisation.

Measurements of photosynthetic fluorescence kinetics were also made because very little information was available concerning cultivar

differences for utilisation of Mn in physiological processes (eg. photosynthesis) under Mn deficiency. Manganese deficiency is known to result in reduced oxygen evolution from photosystem 2 (Amesz 1983). Room temperature photosynthetic fluorescence is a useful indicator of photosystem 2 activity (Krause and Weiss 1984) and the value of the fluorescence ratio Fv/Fo is well correlated with Mn concentration (Graham et al 1985; Kriedemann et al 1985). The aim of these measurements was to ascertain whether cultivar differences existed for a critical Mn concentration for photosynthesis and growth. Some emphasis has also been given to highlighting the relationships between chlorophyll a fluorescence, total chlorophyll concentration, ratio of chlorophyll a/b and Mn concentration of the youngest expanded blades (YEBs).

4.3 MATERIALS AND METHODS

Six wheat cultivars (Rayonet, Bodallin, Millewa, Bindawarra, WT108 and Red Fife) were used. Red Fife, an historical Canadian cultivar, was included because of its supposedly high tolerance to Mn deficiency (Gallagher and Walsh 1943). The remaining wheats were selected on the basis of previous pot experiments and of field trials at Wangary and Karkoo, S.A. during 1983 (Graham personal communication). Seed was obtained from plants grown at Urbrae (1983) and Mn contents are indicated in Appendix 4.3.

The experiment was conducted at a constant 15°C and a 12 h photoperiod. Four sodium vapour lamps and 8 x 60 watt incandescent bulbs provided an average photoflux density of 500 μmole quanta m^{-2} s^{-1} at plant height. The experiment had a factorial, completely randomised design, comprising 6 genotypes, 3 levels of soil Mn (0 (Mn0), 10 (Mn10) and 50 (Mn50) mg Mn kg-1 soil), 2 harvests (22 and 30 days after sowing) and 4 replicates.

Each pot contained 2 plants. Pots contained a 1:1 mixture of calcareous and siliceous sand (the latter soil has been described by Hannam 1984) and were filled 10 days prior to sowing. This mix was used in an attempt to decrease the severity of soil Mn deficiency. The following rates of basal nutrient application were used: 155 mg NH_4NO_3, 78 mg K_2SO_4,

50 mg $MgSO_4.2H_2O$, 100 mg KH_2PO_4, 25 mg $CaSO_4.2H_2O$, 9 mg $FeSO_4.2H_2O$, 0.9 mg $Na_2MoO_4.2H_2O$, 3 mg H_3BO_3, 0.7 mg $CoSO_4.7H_2O$, 4.8 mg $CuSO_4.5H_2O$ and 15 mg $ZnSO_4.7H_2O$ kg^{-1} a.d. soil.

Root length measurements were made (as for experiment 2.2, Chapter 2)) only for Bayonet and Bodallin. Root member diameters were determined with a Leitz binocular microscope at 16x magnification using a micrometer eyepiece. Relative growth rate was calculated as:

$((\ln W_2 - \ln W_1) / (t_2 - t_2)) \times W_2 - W_1$

Rates of Mn uptake were calculated again as for experiment 2.2 (Chapter 2).

A number of measurements were made on the basal region of the YEB of one plant from each pot (ie. 4 leaves per treatment):

(a) the induction kinetics for chlorophyll a fuorescence were measured on the adaxial surface of attached YEBs, in the dark, at 22°C, after at least 30 min dark adaptation, using the same equipment and operating conditions of Simpson and Robinson (1984). Light from a 100-W halogen lamp, filtered by a blue cut-off filter, had an intensity of approx. 600 µmole quanta m^{-2} at the sample. The fluorescence was measured by a photodiode. The change in fluorescence yield with time was displayed by a two pen recorder, and the fast kinetics (0-200 ms) were captured by a microprocessor and stored for later analysis on a Hewlard-Packard HP85 microcomputer.

(b) total chlorophyll and the ratio of chlorophyll a/b were determined on leaf segments (50-150 mg fresh weight) taken from the leaf portion used for fluorescence measurement by absorbance measurements at 663, 652 and 645 nm, after tissue extraction in cold 80 % acetone (Arnon 1949), followed by centrifugation at approx. 1000 rpm for 3 min.

(c) the Mn concentration of similar amounts of leaf tissue as (b) was determined by atomic absorption spectrophotometry (Perkin Elmer model 400), using the grahite furnace (HGA 2100) technique, after digestion of oven dried material with conc. HNO_3 (D. Paskett, personal communication). The following control settings were used for the furnace:

Stage 1 Drying 30 sec. at 110°C

Stage 2 Charring 15 sec. at 1000°C
Stage 3 Atomisation 5 sec. at 2600°C

Curves were either fitted by least squares regression or by eye using treatment means as a guide. Critical Mn concentrations for the parameters measured were estimated as those associated with 90% of the maximum value (Ulrich and Hills 1967).

4.4 RESULTS

4.4.1 Growth

Symptoms of Mn deficiency were evident for most cultivars at Mn0 and Mn10: they ranged from chlorosis per se to chlorosis with interveinal white streaks (as observed by Wallace (1961) and Batey (1971) for wheat and rye).

Significant differences were found for shoot and root growth between cultivars at all levels of Mn supply at H2 (Table 4.1). Data for H1 are given in Appendix 4.4. The poorest growth was generally made by Bayonet, whereas Red Fife had the lowest dry matter production at Mn0 but highest at Mn10 and Mn50. There were no cultivar differences in the ratio of shoot:root dry weight.

Shoot and root relative growth rates are shown in Figure 4.1. Bayonet had the lowest rates at all levels of Mn supply whereas Red Fife had the highest rates of shoot growth at Mn10 and Mn50. There was no root growth at Mn0 between 22 and 30 days. Thus, at Mn0, shoot growth was apparently at the expense of root growth. Subtantiallly more growth was made by Mn-adequate plants than Mn-deficient ones between 22 and 30 days, and therefore relative yields at Mn0 were much smaller for H2 than for H1.

Root lengths and diameters were only measured for Bayonet and Bodallin (Tables 4.2 and 4.3). Bodallin had a higher root length than Bayonet at all levels of Mn supply. This was associated predominantly with a greater lateral root length development. There were no significant differences in

Table 4.1: Shoot and root dry weights for 6 wheat cultivars after 30 days growth. Data are means of 4 replicate pots. Relative yields (per cent of Mn50) are in brackets.

Cultivar	Shoot dry weight mg plant^{-1}			Root dry weight mg plant^{-1}			Shoot:Root ratio		
	Mn0	Mn10	Mn50	Mn0	Mn10	Mn50	Mn0	Mn10	Mn50
Bayonet	119 (32)	253 (68)	371	33 (18)	92 (51)	182	3.64	2.84	2.11
Bodallin	170 (36)	340 (72)	474	47 (17)	164 (60)	272	3.57	2.19	1.78
Red Fife	97 (15)	461 (72)	637	24 (5)	337 (75)	446	4.02	1.44	1.45
Millewa	156 (39)	320 (80)	398	44 (14)	167 (52)	323	3.60	2.07	1.41
WT108	135 (32)	347 (83)	421	33 (12)	248 (88)	282	4.26	1.46	1.64
Bindawarra	151 (36)	326 (78)	418	42 (17)	204 (82)	247	3.74	1.93	1.73
LSD 5% cv		34	***		43	***		NS	
Mn		28	***		34	***		0.34	***
cv x Mn		68	***		85	***		NS	

Table 4.2: Main axis, lateral and total root lengths for Bayonet and Bodallin after 22 (H1) and 30 (H2) days growth

Cultivar	Mn Supply	Main axis m plant^{-1}		Lateral m plant^{-1}		Total m plant^{-1}	
		H1	H2	H1	H2	H1	H2
Bayonet	Mn0	1.13	1.38	2.88	5.57	4.01	6.95
	Mn10	1.39	1.55	13.63	16.37	15.17	17.35
	Mn50	1.51	2.54	11.08	29.98	12.59	32.52
Bodallin	Mn0	1.51	2.61	4.14	10.80	5.66	13.40
	Mn10	2.01	3.25	15.11	25.71	16.91	28.95
	Mn50	2.70	3.17	16.85	32.58	19.55	35.86
LSD 5%	cv	0.30 ***	0.66 ***	NS	5.95 *	NS	6.48 *
	Mn	0.36 ***	NS	4.44 ***	7.21 ***	4.60 ***	7.91 ***
	cv x Mn	0.72 ***	NS	NS	NS	NS	NS

Table 4.3: Diameters of main axis (M.A.) and lateral (Lat) roots for Bayonet and Bodallin under Mn-deficiency (Mn0) or with added Mn (average of Mn10 and Mn50), after 22 (H1) and 30 (H2) days growth. Standard errors are given in brackets.

Root Member	Cultivar	Diameter (mm)				
		-Mn		+Mn		Average
		H1	H2	H1	H2	
M.A.	Bayonet	0.55(0.02) (32)*	0.54(0.03) (40)	0.62(0.02) (13)	0.70(0.03) (36)	0.58(0.03) (121)
	Bodallin	0.59(0.01) (18)	0.56(0.02) (42)	0.67(0.01) (13)	0.73(0.03) (30)	0.62(0.02) (103)
Lat.	Bayonet	0.25(0.02) (34)	0.20(0.00) (47)	0.24(0.02) (14)	0.20(0.01) (42)	0.22(0.01) (137)
	Bodallin	0.22(0.01) (23)	0.19(0.01) (56)	0.23(0.02) (15)	0.20(0.01) (25)	0.21(0.01) (119)

* number of observations

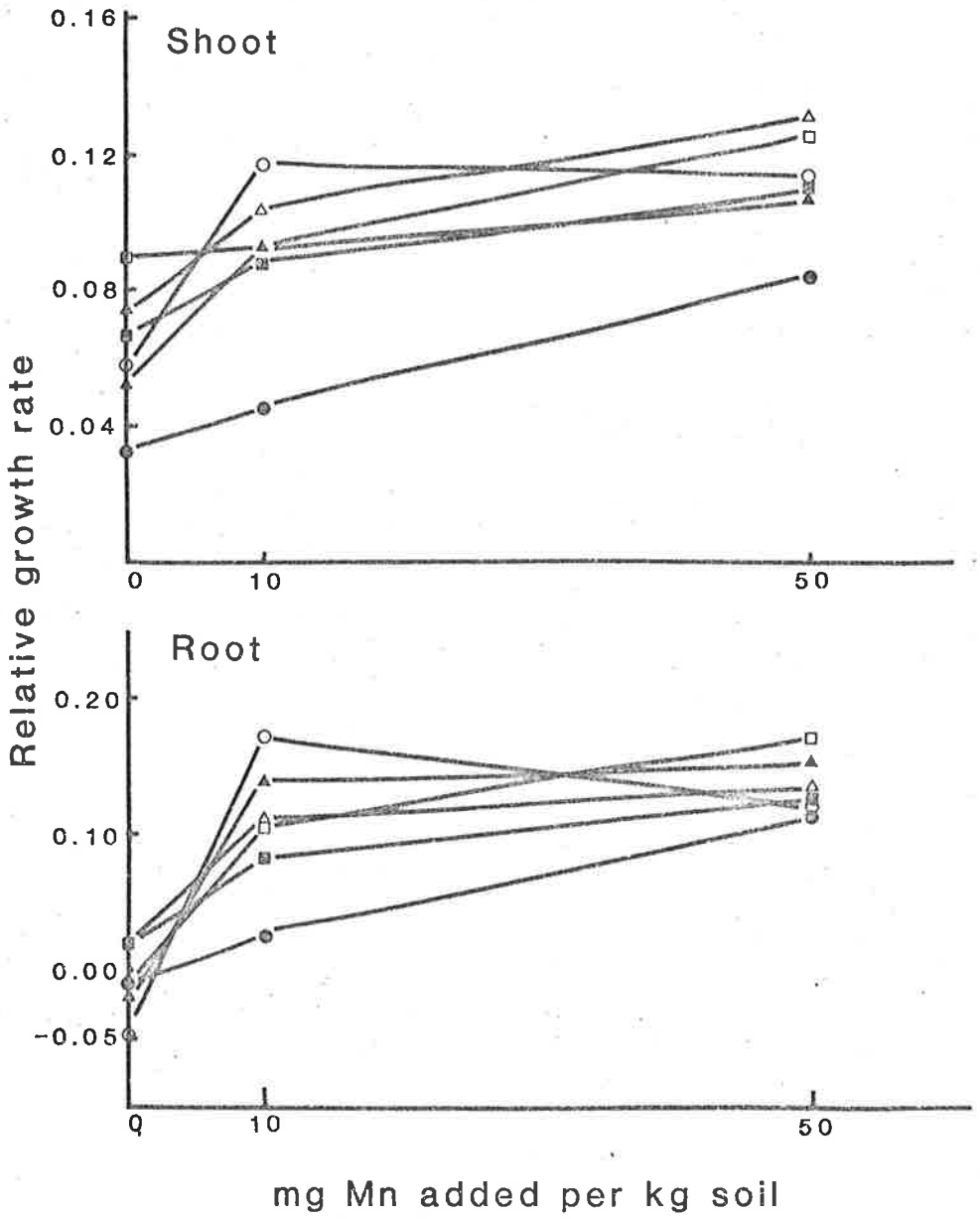

Figure 4.1. Relative shoot and root growth rates for 6 wheat cultivars in response to added Mn between 22 and 30 days after sowing. Symbols refer to Bayonet (●), Bodallin (■), Red Fife (▲), Millewa (△), Bindawarra (□) and WT108 (○).

diameters of main axis and lateral roots between these cultivars. Diameters of lateral roots were somewhat larger at H1 than at H2, probably fewer finer secondary lateral roots were present at H1.

4.4.2 Manganese uptake, translocation and utilisation efficiency

Table 4.4 shows the shoot and root Mn concentrations at H2. A significant reduction in shoot but not root Mn concentration was observed between H1 and H2, irrespective of cultivar. This reflected a dilution of Mn in shoot tissue. Significant cultivar differences were only evident for root Mn concentrations at Mn0; however, shoot differences did occur with added Mn (eg. Bayonet had lowest and Red Fife and WT108 had highest values). Figure 4.2 shows the relationship between relative shoot dry weight and shoot Mn concentration at H2 for three of the cultivars studied. The critical Mn concentration appeared to vary from approx. 12-17 $\mu g\ g^{-1}$.

Significant differences between cultivars in shoot and root Mn contents were found only at Mn10 and Mn50 (Table 4.5). Ratios of shoot:root Mn content were significant between cultivars at all levels of Mn supply.

Significant differences between cultivars were found for the absolute and relative rate of Mn uptake at Mn10 and Mn50 (Fig. 4.3). The performances of Red Fife and Bayonet are particularly well contrasted.

Utilisation efficiencies (UE) for these cultivars are indicated in Figure 4.4. Bodallin had the highest value at Mn0, but Red Fife had the highest value at Mn10 and Mn50. There was a clear decline in the UE after Mn10 for all cultivars.

4.4.3 Chlorophyll fluorescence

Chlorophyll a fluorescence induction kinetics

The room temperature fluorescence induction kinetics of control, Mn-deficient and severely Mn-deficient leaves are shown in Figure 4.5. The

Table 4.4: Shoot and root Mn concentration for 6 wheat cultivars after 30 days growth. Data are means of 4 replicate pots.

Cultivar	Shoot Mn conc. $\mu g\ g^{-1}$			Root Mn conc. $\mu g\ g^{-1}$		
	Mn0	Mn10	Mn50	Mn0	Mn10	Mn50
Bayonet	5.1	8.0	27.1	16.8	14.9	48.4
Bodallin	5.5	9.5	31.4	14.1	19.2	59.9
Red Fife	4.8	13.8	33.1	16.7	23.7	62.3
Millewa	4.8	10.9	29.4	20.1	19.1	51.4
WT108	5.9	13.0	55.1	24.6	22.0	76.3
Bindawarra	5.4	10.0	30.8	19.2	20.3	53.6
LSD 5% cv		2.1 ***			4.1 ***	
Mn		1.8 ***			3.6 ***	
cv x Mn		4.4 ***			8.6 ***	

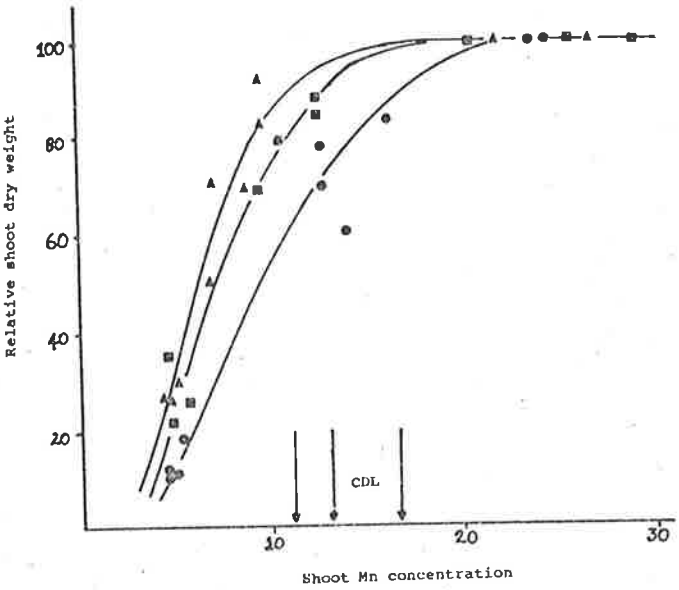

Figure 4.2: Relative shoot dry weight vs. shoot Mn concentration for the estimation of the critical deficiency level (CDL). Symbols represent Bayonet (▲), Red Fife (●) and Bodallin (■).

Table 4.5: Shoot and root Mn content and the ratio of shoot:root Mn content for 6 wheat cultivars after 30 days growth.
Data in brackets are log-transformed values.

Cultivar	Shoot Mn content µg plant^{-1}			Root Mn content µg plant^{-1}			Shoot:Root ratio		
	Mn0	Mn10	Mn50	Mn0	Mn10	Mn50	Mn0	Mn10	Mn50
Bayonet	0.45 (-0.57)	2.05 (0.68)	9.41 (2.28)	0.53 (-0.67)	4.01 (0.28)	13.51 (2.13)	1.11	1.55	1.20
Bodallin	0.83 (-0.13)	3.16 (1.16)	14.13 (2.70)	0.64 (-0.46)	3.19 (1.10)	16.40 (2.78)	1.50	1.10	0.94
Red Fife	0.47 (-0.79)	6.16 (1.82)	20.88 (3.02)	0.44 (-0.85)	7.58 (2.02)	27.53 (3.31)	1.09	0.82	0.77
Millewa	0.75 (-0.31)	3.58 (1.23)	11.18 (2.40)	0.83 (-0.18)	3.26 (1.09)	15.76 (2.70)	0.88	1.20	0.78
WT108	0.79 (-0.24)	4.33 (1.44)	22.50 (3.13)	1.12 (-0.27)	5.20 (1.64)	20.60 (2.99)	1.08	0.82	1.18
Bindawarra	0.81 (-0.22)	3.18 (1.16)	12.81 (2.55)	0.78 (-0.25)	3.88 (1.28)	12.95 (2.56)	1.03	0.96	1.18
LSD 5% cv		0.19	***		0.25	***		0.23	***
Mn		0.13	***		0.18	***		NS	
cv x Mn		0.33	***		0.43	***		0.43	***

LSD values for shoot and root Mn content are for the log-transformed data

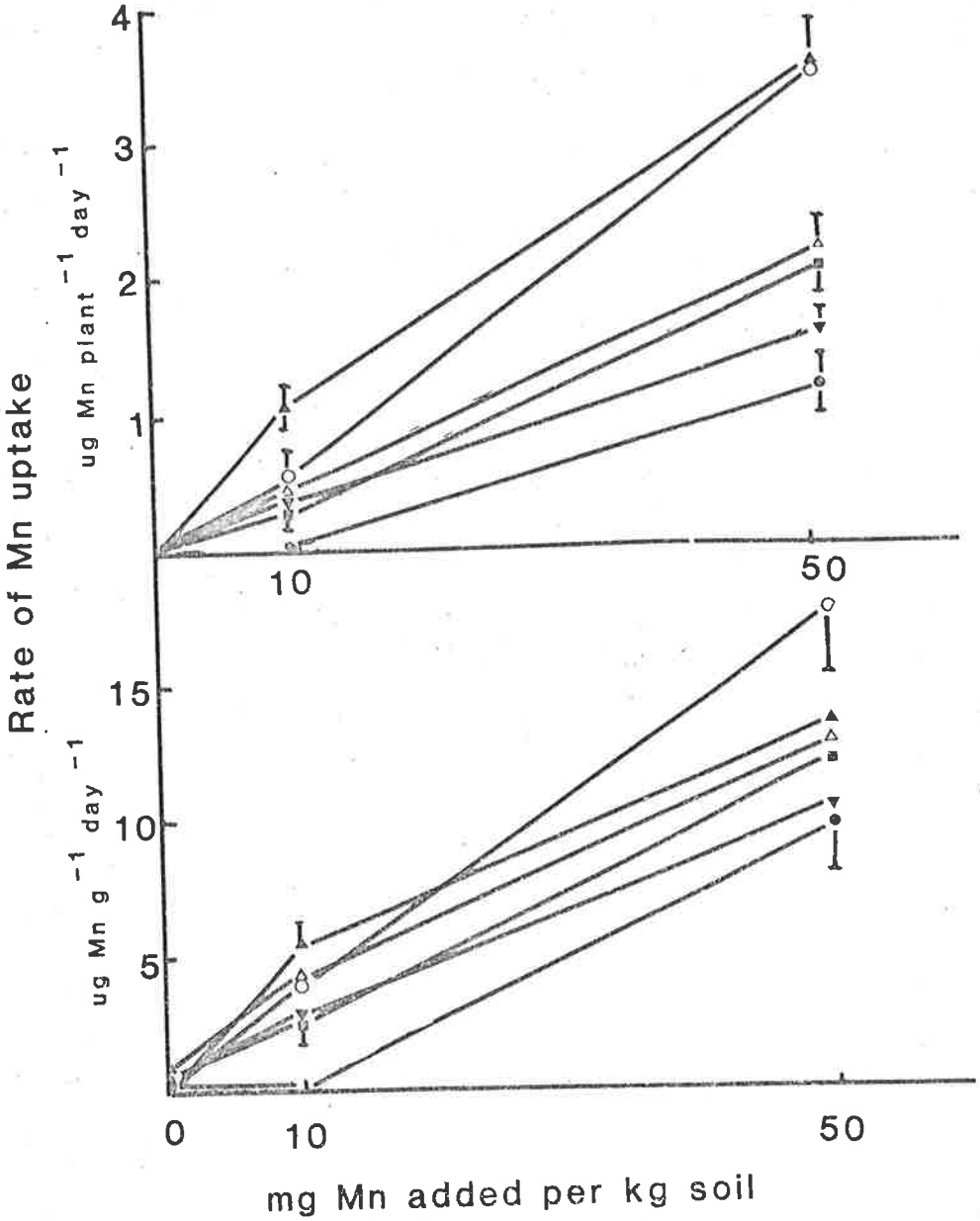

Figure 4.3. Rate of Mn uptake for 6 wheat cultivars in response to added Mn between 22 and 30 days after sowing. Standard errors are indicated by bars. Symbols refer to Bayonet (●), Bodallin (■), Red Fife (▲), Millewa (△), Bindawarra (□) and WT108 (○).

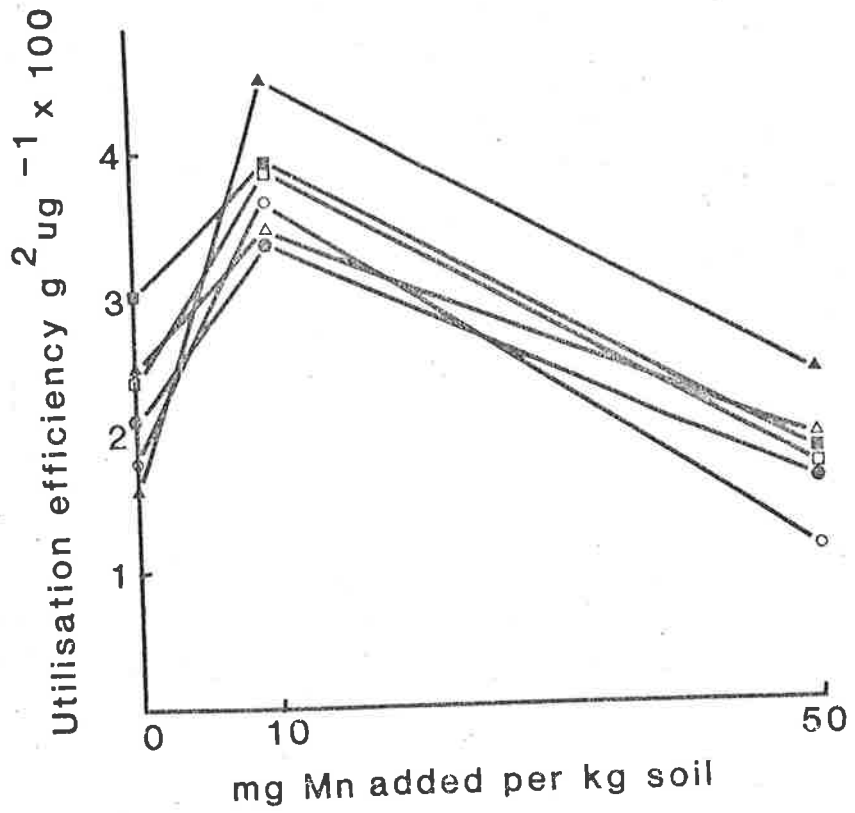

Figure 4.4. Manganese utilisation efficiency for 6 wheat cultivars in response to added Mn between 22 and 30 days after sowing. Symbols refer to Bayonet (●), Bodallin (■), Red Fife (▲), Millewa (△), Bindawarra (□) and WT108 (○).

data are for Bayonet but these are representative of all the cultivars studied. The fluorescence properties averaged over the 6 wheat cultivars are shown in Table 4.6. The small standard errors indicate only small cultivar differences in these properties: values for individual cultivars are given in Appendix 4.5.

There were 3 consistent differences between Mn-deficient and Mn-adequate (healthy) leaves. Manganese deficiency resulted in (1) increase in the initial level of fluorescence (Fo), (2) a lower level of maximum yield fluorescence (Fp) and therefore (3) substantially less variable fluorescence (Fv= Fp-Fo). As a result, Mn-deficient leaves had a lower ratio Fv/Fp and a higher ratio Fo/Fv than Mn-adequate leaves. It is also clear from the examination of the fast kinetics that there was a slower rise from Fo to Fp for Mn-deficient leaves.

Chlorophyll a fluorescence, chlorophyll concentration and Mn concentration

Only data for the ratio Fv/Fp is presented here, whilst data for the ratio Fo/Fv is given in Appendix 4.6. The critical Chl a+b for Fv/Fp was approx. 1.2 mg g^{-1} fresh wt. (Fig. 4.6a). Furthermore, no further decrease in Fv/Fp occurred at Chl a+b of less than 0.75 mg g^{-1} fresh wt. There was no clear relationship between the ratio of chlorophyll a/b (Chl a/b) and Fv/Fp (data not shown). This agrees with the observation that Mn deficiency was associated with only a small reduction in Chl a/b (Table 4.6). It is possible that cultivar differences are again involved, since only one cultivar (Bayonet) demonstrated any decrease in Chl a/b (from 3.0 (at Mn50) to 2.5 (at Mn0)). There was also a strong relationship between leaf Mn concentration, Fv/Fp (Fig. 4.6b) and Chl a+b (data not shown). The critical Mn concentration for Fv/Fp was approx. 15 µg Mn g^{-1} dry wt. The closeness of fit of the data points to the fitted curves suggested little cultivar variation in the estimated critical values.

It is noteworthy that visible symptoms of Mn deficiency were only evident at Mn concentrations of less than approx. 12 µg g^{-1} whereas the fluorescence measurements indicated dysfunction at approx. 15 µg g^{-1}.

Table 4.6: Effect of soil Mn supply on room temperature chlorophyll *a* fluorescence yield (arbitrary units), chlorophyll concentration, chlorophyll a/b and Mn concentration, averaged over 6 wheat cultivars after 30 days growth.
Data are means and standard errors of 24 observations, unless otherwise given in brackets.

Parameter	Mn supply (mg kg^{-1} soil)		
	0 (Mn0)	10 (Mn10)	50 (Mn50)
Fo	1248 ± 58	842 ± 27	453 ± 11
Fv	843 ± 37	1436 ± 86	2317 ± 73
Fp	2094 ± 86	2279 ± 100	2770 ± 82
Fo/Fv	1.49 ± 0.11	0.62 ± 0.03	0.20 ± 0.01
Fv/Fp	40.8 ± 0.9	62.2 ± 1.3	83.6 ± 0.3
Chl a+b	0.71 ± 0.03 (15)	1.03 ± 0.03 (16)	1.25 ± 0.05 (15)
Chl a/b	2.50 ± 0.04 (15)	2.48 ± 0.06 (16)	2.69 ± 0.07 (15)
Mn concentration	3.6 ± 0.2 (18)	11.4 ± 0.9 (19)	19.7 ± 1.5 (15)

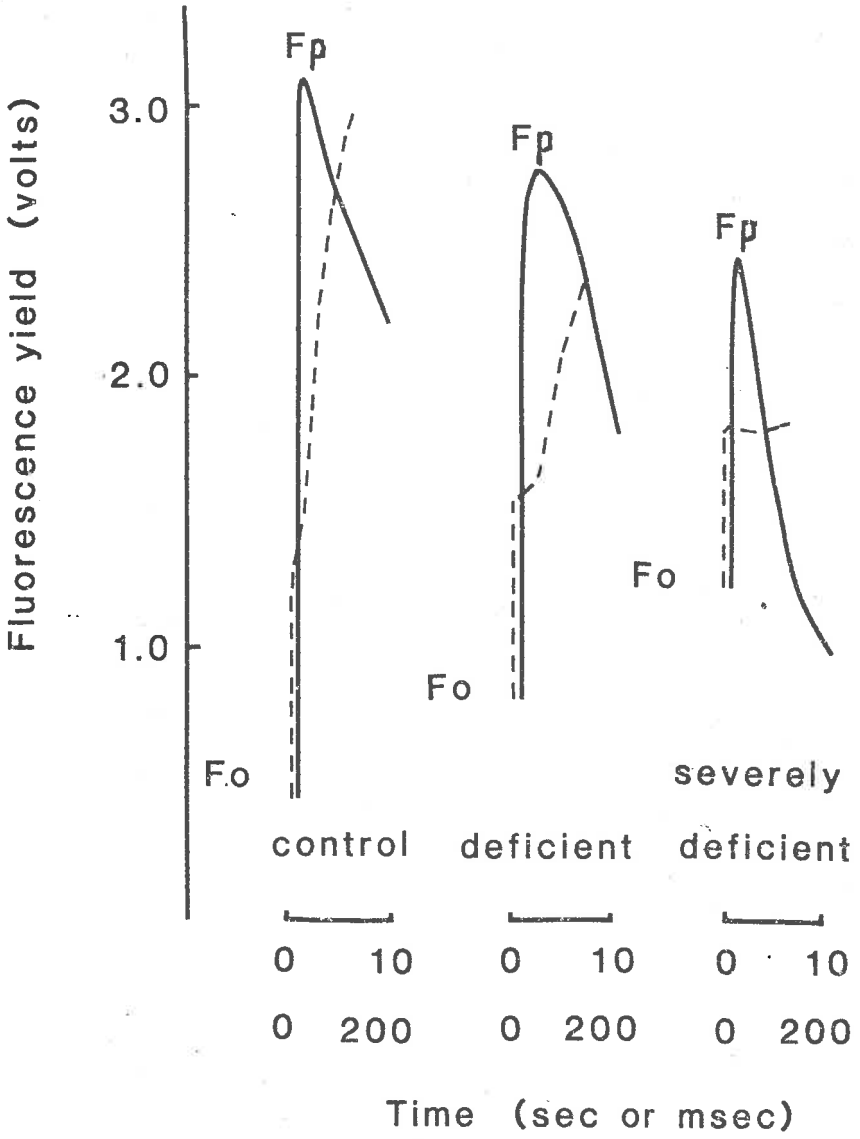

Figure 4.5. Fluorescence induction kinetics of the basal region of dark-adapted attached YEBs of wheat cv. Bayonet, showing constant (Fo), maximum (Fp) and variable (Fv = Fp-Fo) fluorescence yield, for control (Mn50), Mn-deficient (Mn10) and severely Mn-deficient (Mn0) plants. Slow kinetics (0-10 sec) are indicated by unbroken (-) lines and fast kinetics (0-200 msec) by broken (---) lines.

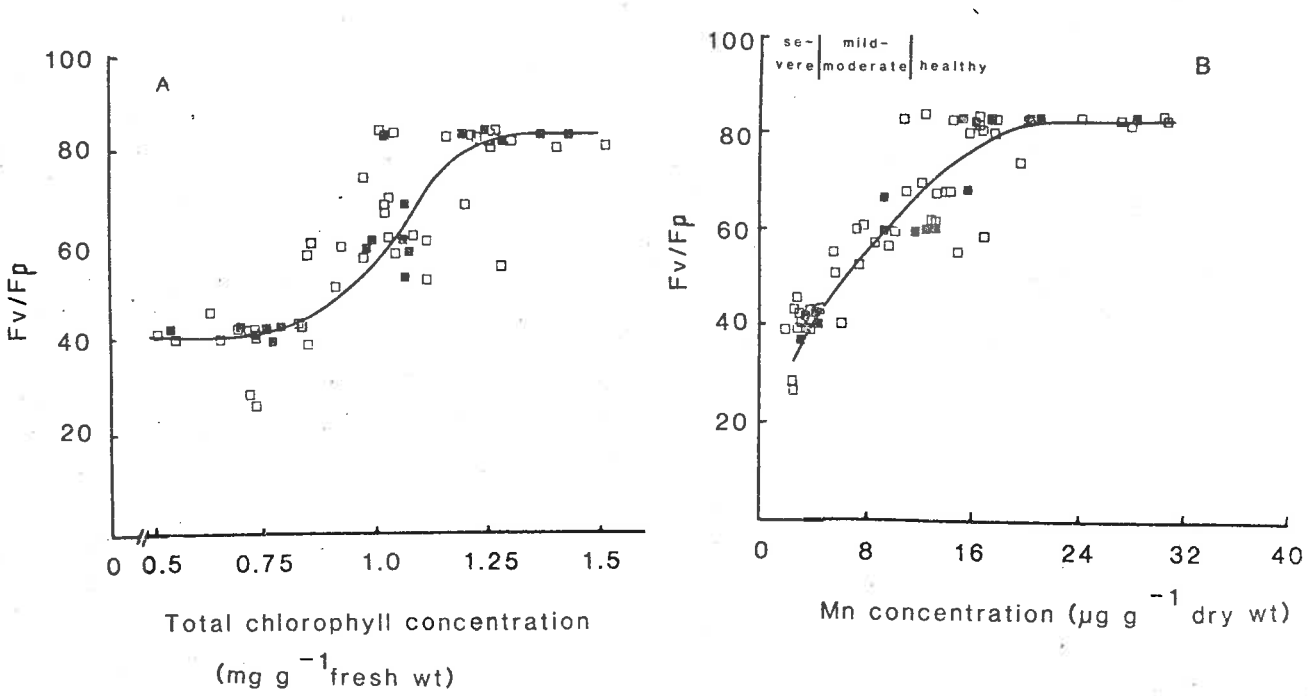

Fig. 4.6. The relationship between the fluorescence ratio Fv/Fp and (A) total chlorophyll concentration (Chl a+b) and (B) Mn concentration of the basal region of Mn-deficient and Mn-sufficient YEBs. Data points are for 6 wheat cultivars. Means indicated by closed symbols

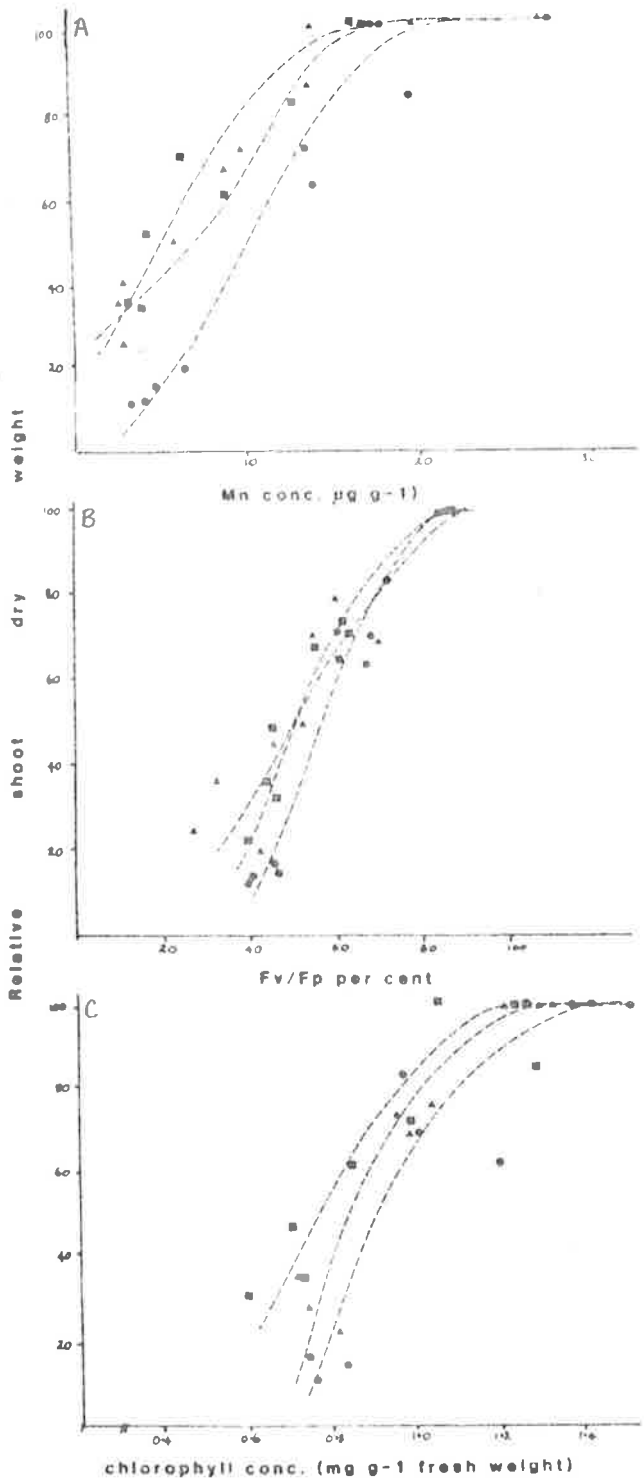

Figure 4.7. Relationship between relative shoot dry weight and A. Mn concentration B. Fv/Fp and C. Chl a+b concentration for the estimation of critical values. Symbols represent Bayonet (▲), Red Fife (●) and Bodallin (■).

4.4.4 Determination of incipient yield depression.

Figures 4.7 a-c show the relationships between relative shoot dry weight and Fv/Fp, Chl a+b and Mn concentration of the basal segment of the YEB for three of the cultivars (Bayonet, Bodallin and Red Fife). Since there was a considerable spread of the data, particularly for Mn-adequate (healthy) tissue because of differences in cultivar response to added Mn, relative yields were plotted. Estimates of the critical values for these parameters appeared to vary with cultivar* e.g. the critical Mn concentration ranged from approx. 12-17 $\mu g\ g^{-1}$ dry wt.

4.5 DISCUSSION

This experiment confirmed the findings of Chapter 2 that wheat roots were unable to extract any meaningful amounts of Mn from this calcareous soil, even when this soil had been mixed (1:1) with a less severely Mn-deficient siliceous sand. This was not expected since a pilot study had indicated that Bayonet was able to extract signficantly more Mn from this mix than the calcareous soil alone (Appendix 4.2).

The addition of only a small amount of Mn (10 mg kg^{-1}) was sufficient to differentiate between the wheat cultivars on the basis of both growth and Mn uptake. There appeared to be no differences between wheat cultivars in the transport of Mn from root to shoot but there were differences in the efficiency of Mn utilisation. It did not appear that cultivar differences in photosynthetic efficiency (as judged by chlorophyll a fluorescence) under Mn deficiency were involved in differences in Mn utilisation. This suggests that Mn deficiency was limiting the effectiveness of other physiological processes differently for different cultivars. The fact that cultivar differences in fluorescence properties under Mn deficiency were small highlights the potential for fluorescence measurements, which are non-destructive, in detecting subclinical Mn deficiency.

Fo fluorescence is regarded as that fluorescence arriving prior to any quenching of excitation energy by functional PS2 reaction centres. The increase in Fo is likely to be a result of a reduction in the number of

* (for all parameters)

functional reaction centres under Mn deficiency as shown by Simpson and Robinson (1984). This reults in a lowering of the efficiency of quenching of excitation energy and therefore a rise in the Fo fluorescence (Krause and Weiss 1984). The changes observed here for Fo/Fv and Fv/Fp were in agreement with those of Kriedemann et al (1985) for wheat and Simpson and Robinson (1984) for spinach.

The Chl a+b values found here are in agreement with values for solution-culture grown wheat (Ohki 1985) and field-grown oats (Vose 1962), amongst others, although they are lower than those found by Kriedemann (1985). Discrepencies could arise from cultivar differences (eg. in this experiment, Millewa and WT108 had a Chl a+b content of 1.45 and 1.03 mg Chl g^{-1} respectively under control conditions) and/or differences in experimental conditions. The absence of an effect on Chl a/b here was different to reports with wheat (Brown 1983; Kriedemann et al 1985) and other species (Weilland et al 1975; Simpson and Robinson 1984) which showed a significant lowering of Chl a/b accompanying Mn deficiency. The reason for this discrepency was not apparent to the author.

Curve fitting would have been facilitated if more levels of Mn addition had been used since the true source of variation between data points was masked to some extent by variation amongst cultivars. Nevertheless estimates of critical Mn concentrations are discussed below. The critical Mn concentration for Fo/Fv of approx. 15 $\mu g\ g^{-1}$ is a little higher than the value of 10-11 $\mu g\ g^{-1}$ found for youngest expanded leaves of older field-grown plants (Graham et al 1985; Kriedemann et al 1985). These differences may be a reflection of cultural conditions or cultivars used. The range of critical Mn concentrations for shoot growth estimated from shoot and YEB Mn concentrations plotted against relative shoot yields are also a little higher than that obtained by Graham et al (1985) for whole YEBs of field-grown plants, but they are consistent with 15 $\mu g\ g^{-1}$ dry wt found for fluorescence parameters and Chl a+b. A close similarity between critical values for growth and photosynthetic parameters was also found for soybeans (Ohki 1981) but not for wheat (Ohki 1985) or lupins (Hannam 1984). In the latter case a lower critical level was obtained for chlorophyll content than for growth ie. growth was more sensitive to Mn deficiency than

synthesis of chlorophyll and growth is being limited (presumably) by other processes which require Mn. It is also clear that significant yield depression occurred in this experiment for values of all the parameters where no Mn deficiency symptoms were evident ie. these parameters were able to detect sub-clinical Mn deficiency.

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Appendix 4.1: Growth and Mn uptake of Bayonet wheat on several soils.

In a further attempt to select a more suitable soil for screening wheat cultivars for Mn efficiency, a number of soils, selected on the basis of DTPA extractable Mn concentrations, were screened.

Soil samples were collected from several sites in S.A.; near Coonalpyn (A2), Yeelana (0-10 cm., 2 paddocks, 1 & 2), Roseworthy (0-10 and 10-20 cm, paddock E12), Pinaroo (0-10 cm) and Wangary (0-10 cm). The following table indicates the field texture, DTPA extractable Mn concentration and the moisture content that each soil was kept at during the experiment:

Soil	Texture	Mn content (ppm)	Moisture content (per cent)
C A2	siliceous sand	0.07	14
Y top 1	sandy loam	2.84	20
Y top 2	sandy loam	N.D.	20
R top	clay loam	1.37	20
R sub	clay loam	0.75	20
P top	mallee sand	1.92	15
W top	calcareous sand	0.26	25

Basal nutrient additions for soils from Yeelana and Roseworthy were the same as those for Wangary (see Chapter 2). For the soils from Pinaroo and Coonalpyn, twice the rates of $KH_2(PO_4)_2$, K_2SO_4, $MgSO_4.7H_2O$ and micronutrients were added as for the soil from Wangary, and, NH_4NO_3 (150 mg kg^{-1}) and $CaSO_4.2H_2O$ (50 mg kg^{-1}) were added instead of $Ca(NO_3)_2$. No Mn was added to these soils.

Results of this experiment are given in the following table. Plant growth on the calcareous sand from Wangary was much poorer than any of the soils used. For example, shoot growth was approx. 10% that of the soil from Pinaroo and root growth approx. 15% that of the soil from Coonalpyn. This poor growth was associated with lack of tillering and reduced leaf

production as well as smaller leaf areas (not shown). Shoot Mn contents for plants grown on the soil from Wangary were dramatically less than those for the other soils. Shoot Mn concentrations for plants grown on Wangary soil were well below the CDL values for wheat (see Chapter 5), in contrast to those grown on the other soils.

Treatment *	Tiller number plant^{-1}	Shoot dry wt. mg plant^{-1}	Root dry wt. mg plant^{-1}	Shoot Mn conc. $\mu g\ g^{-1}$	Shoot Mn content μg plant^{-1}
W top	0	73	41	4.8	0.35
Y top 1	2	544	310	32.7	17.82
Y top 2	2	448	273	74.7	33.45
R top	2	395	187	35.1	13.93
R sub	2	315	183	37.7	11.90
P top	2-3	669	234	55.9	37.19
C A2	2-3	393	340	19.7	7.71
LSD 5% ***	N.D.	62 ***	75 ***	5.7 ***	3.47

* W = Wangary, Y = Yeelana, R = Roseworthy, P = Pinnaroo, C = Coonalpyn

Appendix 4.2: Shoot and root dry weights and shoot Mn concentration and content of wheat cv. Bayonet after 35 days growth in several mixes of a calcareous sand (from Wangary, S.A.) and a siliceous sand (from Coonalpyn, S.A.).

Treatment W : C *	Shoot dry wt mg plant^{-1}	Root dry wt mg plant^{-1}	Shoot Mn conc µg g^{-1}	Shoot Mn cont µg plant^{-1}
1 : 0	130	58	3.8	0.49
2 : 1	150	51	4.2	0.63
1 : 1	182	47	4.1	0.76
1 : 2	220	74	4.6	1.00
1 : 4	309	82	5.3	1.42
0 : 1	691	448	14.1	9.71
LSD 5%	46 ***	39 ***	0.5 ***	0.38 ***

* W = Wangary, C = Coonalpyn

Appendix 4.3: Seed dry weight, Mn concentration and Mn content of the wheat cultivars used in experiment 4.1. Data are means of 3 replicates of 10 seeds each. Standard errors are given in brackets.

Cultivar	Dry weight mg seed^{-1}	Mn content µg seed^{-1}	Mn conc. µg g^{-1}
Bayonet	42.1 (1.2)	2.05 (0.07)	48.5 (3.2)
Bodallin	43.2 (1.8)	2.93 (0.08)	67.9 (1.7)
Red Fife	30.6 (1.4)	1.23 (0.08)	40.2 (1.4)
Bindawarra	43.3 (1.0)	2.18 (0.15)	50.3 (4.6)
Millewa	43.5 (2.6)	1.69 (0.08)	38.8 (1.2)
WT108	40.5 (1.6)	1.88 (0.08)	46.4 (1.0)

Appendix 4.4: Shoot and root dry weights and the ratio shoot:root dry weight for 6 wheat cultivars after 22 days growth (H1) (experiment 4.1). Data are means of 4 replicate pots. Relative yields (percent of Mn50) are given in brackets.

Cultivar	Shoot dry weight mg plant^{-1}			Root dry weight mg plant^{-1}			Shoot:Root ratio mg plant^{-1}		
	Mn0	Mn10	Mn50	Mn0	Mn10	Mn50	Mn0	Mn10	Mn50
Bayonet	89.4 (51)	175 (99)	176	32 (47)	74 (100)	73	2.67	2.39	2.40
Bodallin	98 (50)	197 (100)	196	44 (43)	85 (83)	103	2.50	2.02	2.01
Red Fife	65 (24)	219 (74)	276	31 (20)	108 (70)	155	2.22	2.06	1.98
Millewa	86 (61)	143 (100)	143	36 (48)	72 (95)	76	2.39	2.04	1.90
WT108	85 (49)	137 (78)	174	48 (42)	62 (54)	115	1.85	2.28	1.67
Bindawarra	77 (49)	154 (97)	158	36 (43)	76 (93)	81	2.28	2.08	1.95
LSD 5% cv	20 ***			19 ***			0.21 *		
Mn	15 ***			15 ***			NS		
cv x Mn	36 ***			NS			NS		

Appendix 4.5: Total chlorophyll content and the ratio chlorophyll a/b of basal region of the youngest expanded blade (YEB) of 6 wheat cultivars after 30 days growth (experiment 5.1).

Cultivar	Chlorophyll content mg g^{-1} fresh wt. leaf			Chlorophyll a/b		
	Mn0	Mn10	Mn50	Mn0	Mn10	Mn50
Bayonet	0.78	0.98	1.23	2.5	2.8	3.0
Bodallin	0.68	1.07	1.18	2.4	2.4	2.5
Red Fife	0.78	1.07	1.42	2.4	2.4	2.6
Bindawarra	0.75	0.99	1.27	2.6	2.3	2.6
Millewa	0.72	1.09	1.45	2.6	2.7	2.8
WT108	0.53	1.09	1.03	2.4	2.5	2.6

Appendix 4.6: The chlorophyll fluorescence ratio Fv/Fp of the basal region of YEBs of 6 wheat cultivars after 30 days growth (expt 5.1).

Cultivar	Fv/Fp per cent		
	Mn0	Mn10	Mn50
Bayonet	36.3	59.1	84.5
Bodallin	42.7	58.5	83.9
Red Fife	42.1	68.2	82.6
Bindawarra	41.6	60.0	81.7
Millewa	40.0	60.3	84.2
WT108	41.5	67.0	84.5

CHAPTER 5

EFFECT OF SEED MANGANESE CONTENT ON SEEDLING GROWTH OF CEREALS, PARTICULARLY WHEAT, UNDER MANGANESE DEFICIENCY

5.1 SUMMARY

The importance of seed Mn content for early growth of wheat, triticale and barley on a severely Mn-deficient calcareous soil was investigated in two pot experiments. Seed was obtained from various sources to provide a range of Mn contents. This natural range of seed Mn content was extended by soaking seeds in $MnSO_4$ prior to sowing. Response curves showed that at least 5 μg Mn $seed^{-1}$ (approx. 130 $\mu g\ g^{-1}$ in the seed) was needed for maximum growth of 35 day old wheat plants. Seed soaking greatly increased the seed Mn content; however, only about 15-20% of this additional Mn was recovered in the harvested plants. Manganese analysis of different aged leaves also permitted the establishment of Mn critical deficiency levels.

It is concluded that, in cases of acute soil Mn deficiency, seed Mn content plays a vital role in determining seedling growth. Under such circumstances seed Mn contents must be taken into account so that real Mn uptake may be calculated. These studies have reinforced earlier observations that genotype comparisons for Mn efficiency must be made with the same or similar seed sources.

5.2 INTRODUCTION

One conclusion from Chapters 2 and 4 was that seed of similar Mn content should be used when comparing cereal genotypes for early growth on Mn-deficient soil in order to avoid erroneous inferences regarding differences in Mn efficiency between genotypes. However, there are no reports dealing with effects of natural variation in seed Mn content on plant growth under Mn deficiency. In cases of severe soil Mn deficiency seed reserves of Mn would appear to play an important role in early plant vigour.

On the other hand, a number of examples have been reported in the literature demonstrating increased growth and delayed onset of Mn deficiency symptoms in cereals as a consequence of soaking seed in high concentrations of Mn salts (Roberts 1948; Drennan et al 1961; Khalid and

Malik 1982). The effectiveness of seed Mn, either naturally or artificially supplied, as a source of Mn for plants under Mn-deficiency stress, would be expected to depend on such factors as (1) the initial seed Mn content, (2) the amount translocated to the embryo and retained by it, (3) the rate and extent of Mn translocation to roots and shoots , and (4) the functional Mn requirement of shoot tissue.

The two experiments described here were designed to demonstrate the importance of seed Mn more clearly. Experiment 5.1 tested the hypothesis that Bayonet and Bodallin wheat had similar Mn-efficiencies when the same seed sources were used. Experiment 5.2 investigated the growth response of wheat, barley and triticale to increasing seed Mn content. The wide range of wheat seed Mn contents used in these studies enabled some attention to be given to the Mn critical deficiency level (CDL) in shoots, leaves and roots.

This chapter is an excursion into the effects of seed Mn supply on plant growth under Mn deficiency. This excursion is relevant to the thesis theme because of the interaction that exists between seed source and genotype ranking for Mn efficiency. It was also of interest to compare the separate effects of seed source and genotype on plant growth under Mn deficiency.

5.3 MATERIALS AND METHODS

Details of the soil used and plant culture have been given in Chapter 2. Only a summary with variations from these methods is provided here.

In order to obtain a wide natural range of seed Mn contents, seeds were obtained from a number of experimental sites in S.A. known to vary in soil Mn availability (Table 5.1), since it was not possible to obtain a sufficiently large range of seed Mn contents from plants grown at one site. Artificially high seed Mn contents were produced by allowing 2 g seed samples to soak for 5 h in 5 ml of 125 mole m^{-3} $MnSO_4$ (expt. 5.1) or 250 mole m^{-3} $MnSO_4$ (expt. 5.2) and, after thorough rinsing, to dry at approx. 35 ^{o}C prior to sowing. Preliminary studies indicated that these

concentrations and this duration of seed soaking did not result in significant reduction in germination capacity (Appendix 5.1).

At harvest, symptoms of Mn deficiency were noted, tiller and leaf numbers recorded and shoot and root dry weights were determined as outlined in Chapter 2. Seed and plant analysis for Mn and calculation of Mn uptake was also the same as outlined in Chapter 2. Curves were fitted by eye using all the data points (except for Fig. 5.1 which used only means). The critical Mn deficiency level (CDL) was determined as that Mn concentration corresponding to 90% of maximum dry matter production (Ulrich and Hills 1967). Computer fitted hyperbolic curves produced almost identical CDL values.

5.3.1 Experiment 5.1

This experiment was conducted in a growth cabinet at 15°C and with a 12 h photoperiod. Two wheat cultivars, Bodallin and Bayonet, were used: for both cultivars, two seed sources (from Palmer and Urrbrae) were combined factorially with two rates of soil Mn application, 0 (Mn0) and 40 (Mn40) mg kg^{-1} a. d. basis, in addition to the seed soaking treatment at Mn0. Four 300 cm^3 capacity pots, containing three plants each, were allocated per treatment. Plants were harvested 26 days after sowing. The first (oldest), second (usually the youngest expanded blade or YEB) and third + younger blades (blades 1, 2 and 3+ respectively) were separated for Mn determination.

5.3.2 Experiment 5.2

This experiment was conducted in a temperature controlled glasshouse (14°C mean max. and 10°C mean min.). The experiment used Bodallin and Bayonet wheat, Galleon barley and Venus triticale. Five seed sources were used for wheat and three for barley and triticale, in addition to Mn-soaked seeds (Table 5.1). No Mn was added to the soil. Three pots, containing three plants each and holding twice the weight of soil (450 g air dry) as for experiment 5.1, were allocated per treatment. Plants were harvested 36 days after sowing.

5.4 RESULTS AND DISCUSSION

5.4.1 Seed Mn content

Bodallin appeared to accumulate more Mn into seeds than Bayonet under identical growing conditions (Table 5.1). Less Mn accumulated in barley seeds than those of wheat or triticale. In agreement with this, seed Mn concentrations of only approx. 50 $\mu g\ g^{-1}$ were recorded by the author for barley grown on acid (Mn-toxic) soils near Wagga, NSW, compared with approx. 150 $\mu g\ g^{-1}$ for wheat. If differences in seed accumulation of Mn could be shown to be heritable, differences in seed Mn content of sown seed between genotypes may well contribute to variation in Mn efficiency.

Appendix 5.2 shows that seed Mn contents and concentrations associated with the various Mn-soaking treatments used in preliminary studies were greatly increased above natural levels. These values correspond to the germination percentages given in Appendix 5.1.

5.4.2 Symptoms

The degree of expression of Mn-deficiency symptoms, the rate of symptom development and the ranking of leaves in order of susceptibility, for wheat in experiment 5.2, was primarily related to the seed source but also to the cultivar used (Table 5.2). Symptoms ranged from severe (chlorotic streaking, necrosis and leaf collapse) through to moderate (flecking and necrosis) or mild (general chlorosis)*. The effect of seed source was also evident for barley but not for triticale, which showed at worst only slight symptoms (Appendix 5.3).

Only those wheat plants derived from seeds with relatively high Mn contents showed symptoms first in the youngest formed leaves ie. older leaves were healthy. This latter observation is most frequently made (eg. for oats (Vose 1963) and wheat (Single and Bird 1958)). Since this soil supplied very little Mn to these plants (see Table 5.4) and assuming minimal remobilisation of Mn from old to young leaves (Nable and Loneragan

* (photograph 1)

1984) developing leaves would be primarily dependent on direct or indirect (via roots) supply from the seed. Thus, when the supply of Mn from the seed was small (eg. seed from Wangary), the first leaf became severely deficient. This was already evident by day 18, at which time the second leaf was moderately deficient. By day 36, all leaves showed severe symptoms and plants were close to death. When seed Mn supply was high (eg. seed from potting soil), there were no symptoms on day 18, and only the third leaf had any symptoms by day 36.

There appeared to be no difference between Bayonet and Bodallin in the leaf Mn concentration associated with specific Mn deficiency symptoms. This is illustrated in Table 5.3 and Appendix 5.4. The critical leaf Mn concentration associated with the appearance of symptoms was approx. 5.0 $\mu g\ g^{-1}$ for both cultivars.

5.4.3 Growth

The growth and development of wheat, triticale and barley was strongly affected by the seed Mn content (Table 5.2, Appendix 5.5 and Fig. 5.1)*. Plants derived from seeds of higher Mn content had greater shoot dry weights, leaf and tiller numbers (Appendix 5.3). Critical seed Mn levels, i.e. the seed Mn content associated with 10% reduction from the maximum dry matter production, for 35 day old wheat plants, were approx. 5.3 μg (shoot) and 4.2 μg (root) $seed^{-1}$ for experiment 2 (Fig. 5.1). There were no apparent differences between cultivars. Similar values were apparent for triticale, but there was insufficient data for barley (Appendix 5.5). The critical values calculated here would be expected to increase with a longer period of growth because the Mn supply to the recent leaves from the seed would become limiting. Best plant growth was obtained from seeds soaked in $MnSO_4$. The optimal duration of soaking would be dependent on the amount of Mn absorbed by the seed and retained by it during seedling growth, the rate of translocation of Mn to developing leaf tissue as well as the age of plants at harvest.

* (and photograph 2)

Table 5.1: Seed Mn concentration and content for wheat, barley and triticale from different seed sources (experiment 5.1, 5.2). Data are means and standard errors of 3 replicate analyses.

Genotype	Seed source		Mn concentration $\mu g\ g^{-1}$	Mn content $\mu g\ seed^{-1}$
Bayonet	1	Wangary	4.1 (1.1)	0.15 (0.03)
	2	Murdinga	8.7 (1.7)	0.31 (0.05)
	3	Palmer	27.6 (0.7)	1.13 (0.04)
	4	Urrbrae	48.5 (3.2)	2.05 (0.08)
	5	potting soil	134.8 (8.9)	5.18 (0.31)
		Mn-soaked (1)	505.2 (9.8)	20.10 (0.42)
	6	" (2)	846.0(12.1)	29.20 (0.47)
Bodallin	1	Wangary	5.1 (0.7)	0.24 (0.03)
	2	Murdinga	12.5 (0.9)	0.49 (0.05)
	3	Palmer	33.7 (1.6)	1.35 (0.09)
	4	Urrbrae	67.9 (1.7)	2.94 (0.08)
	5	potting soil	153.1 (2.6)	6.38 (0.10)
		Mn-soaked (1)	542.3 (6.5)	22.15 (0.51)
Venus	1	Murdinga	13.7 (0.6)	0.47 (0.03)
	2	Urrbrae	50.6 (1.5)	2.37 (0.07)
	3	potting soil	129.5 (3.2)	5.39 (0.21)
	4	Mn-soaked (2)	765.3 (9.3)	35.81 (0.42)
Galleon	1	Warooka	2.4 (0.6)	0.08 (0.02)
	2	Strathalbyn	12.2 (0.9)	0.62 (0.07)
	3	Urrbrae	26.7 (1.0)	1.20 (0.18)
	4	Mn-soaked (2)	1151.1(15.6)	53.22 (0.62)

(1) = experiment 5.1, (2) = experiment 5.2

Table 5.2: Effect of seed source on the extent of Mn-deficiency symptoms in wheat (Bayonet and Bodallin) after 18 and 36 days growth (experiment 5.2).

Seed source	Day 18		Day 36	
	Bayonet	Bodallin	Bayonet	Bodallin
Wangary	L1 severe L2 moderate	L1 moderate L2 slight	all severe	all severe
Murdinga	all moderate	nil	L1 severe L2,3 moderate	L1 nil L2,3 moderate
Palmer	L1,2 slight	nil	L1,2 moderate L3 severe	L1,2 nil L3 moderate
Urrbrae	nil	nil	all moderate	nil
potting soil	nil	nil	L1 nil L2,3 slight	nil
Palmer (Mn-soaked)	nil	nil	L1,2 nil L3 slight	nil

L1, L2 and L3 = leaf 1, leaf 2 and leaf 3.

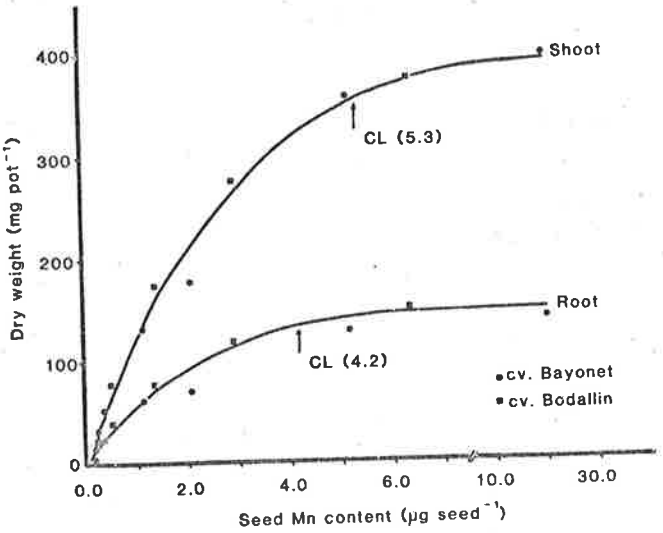


Figure 5.1: Effect of seed Mn content on shoot and root dry weights of wheat 36 days after sowing on a Mn-deficient soil (experiment 5.2), showing the critical level (CL) for Mn in seeds.

Manganese addition to the soil eliminated symptoms of Mn deficiency and growth was improved by up to 50% (Table 5.3). Soil Mn addition resulted in more shoot and root growth for Bayonet and a greater plant Mn content compared with the addition of Mn to the seed.

5.4.4 Manganese uptake

Calculations of net Mn uptake indicated that less Mn was found in harvested plants than was initially present in seeds for wheat and triticale, but not for barley (Table 5.3, and comparison of Table 5.1 with Appendix 5.5). This confirms the findings of Chapter 2.

Presumably both native and artificially supplied Mn was leached from the germinating seed and young seedling roots. Because of the highly oxidising nature of this alkaline soil much of this leached Mn would have become unavailable for re-absorption (see section 5.2). Only a large drop in rhizosphere pH would enable sufficient Mn^{2+} to remain close to the root surface; however, even the use of NH_4-N did not alter bulk or rhizosphere pH in this soil (J. Harbard, personal communication).

Leaching of native Mn from the seed is quite likely for native Mn since during the first few hours of germination the root is predominant over the shoot in accumulating mineral elements (Eastwood and Laidman 1971). Manganese could also be lost from the seed coat since preliminary data indicated that approx. 60% of the Mn contained in 4 day old germinated wheat seeds was in the seed coat.

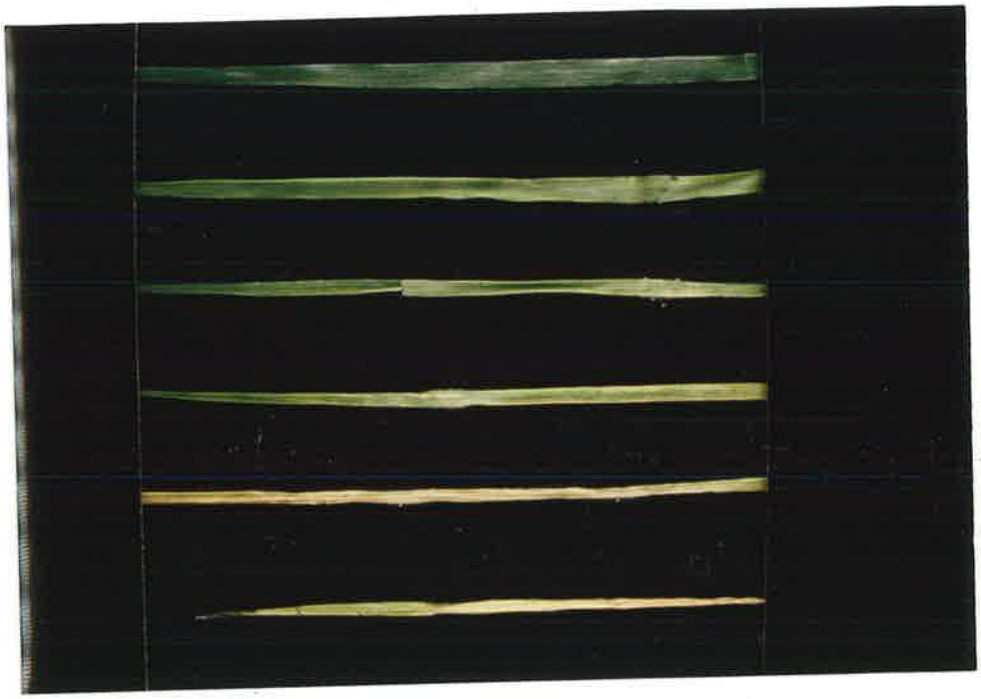
Only approx. 15-20% of the Mn applied to the seeds through seed soaking was recovered in the harvested plants. This is consistent with data from a preliminary experiment (Appendix 5.6) showing approx. 75% of this, presumably mobile Mn, being found in the endosperm region, and therefore more likely to be leached.

It was apparent that the seed rather than the soil had provided the major source of Mn for these plants since there was no net Mn uptake at MnO. It is possible that seeds harvested from different sites could vary

Table 5.3: Effect of seed and soil Mn supply on growth and Mn content of 26 day old wheat plants (seed from Palmer, S.A.) (experiment 5.1).

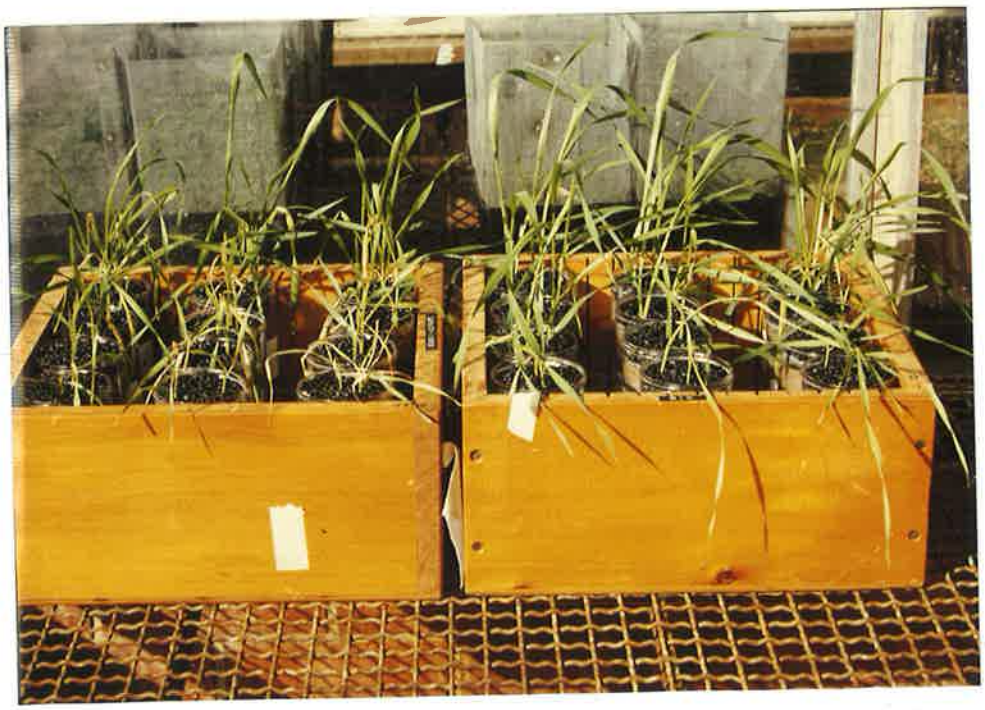
Treatment		Symptom expression	Mn conc of YEB $\mu g\ g^{-1}$	Shoot dry wt $mg\ pot^{-1}$	Whole plant Mn content $\mu g\ pot^{-1}$	Net Mn uptake $\mu g\ pot^{-1}$
Bayonet	1	severe	2.1	167	2.88	- 0.51
	2	nil	24.5	242	17.31	13.89
	3	nil	6.8	185	11.06	- 8.94
Bodallin	1	moderate	4.6	162	2.97	- 1.09
	2	nil	40.8	202	20.24	16.19
	3	nil	9.3	200	9.56	-12.54
LSD 5%			1.8	37	0.90	-

1 = no added Mn
2 = Mn added to soil (incorporation of MnSO4)
3 = Mn added to seed (soaked in MnSO4)



photograph 1

Top to bottom: increasing Mn deficiency in wheat leaves



photograph 2

Bayonet Bodallin

Effect of increasing seed Mn content (foreground to background) on growth of wheat.

in other seed characters, for example seed vigour, due to differences in protein content (Torres and Paulsen 1982) or seed size (Evans and Bhatt 1977). However, since uniformly germinated seedlings were used in these experiments, it appears that seed Mn is the major source of variation in growth. It might be expected that the more vigorous root systems found with plants derived from high Mn seed (Fig. 5.1) would lead to greater Mn uptake by these plants, but this was not found.

Seed soaking greatly elevated plant Mn contents, particularly of the youngest leaves, thus enabling increased growth of new tissue. The infuence of seed soaking (and soil Mn addition) would have been even more evident by a later harvest, since plants derived from these treatments would have a more rapid growth rate than those from seeds of low seed Mn.

5.4.5 Manganese critical deficiency levels

Manganese CDLs were only calculated for wheat and those from experiment 5.2 are presented in Figures 5.2 a and b. The CDL for whole shoots was 11.0 $\mu g\ g^{-1}$, much lower than reported elsewhere for wheat (Fales and Ohki 1982; Ohki 1984) but similar to that of Graham et al (1985). The CDL for roots was somewhat higher at 19.0 $\mu g\ g^{-1}$. Similar CDL values were found for experiment 5.1, even though plants were younger. The similarity of CDLs for Bodallin and Bayonet wheat suggests that there were no differences in a functional Mn requirement between these cultivars.

The CDL for leaves 1, 2 and 3+ was 25, 11 and 8 $\mu g\ g^{-1}$ respectively (Figs. 5.3 a, b, c). The CDL for leaves 1 and 2 are much lower than the values of 82 and 39 $\mu g\ g^{-1}$ reported by Okhi (1984) for solution culture grown wheat. The CDL for leaf 2 is comparable with the value of 11 $\mu g\ g^{-1}$ obtained by Graham et al (1985) for YEB's of older, field grown wheat plants, but lower than the value of 18 $\mu g\ g^{-1}$ found by Graham and Loneragan (1981) for wheat seedlings grown in solution culture. The CDL curves for blades 2 and 3 were associated with a much sharper transition zone between deficiency and sufficiency than for blade 1, in agreement with Okhi (1984).

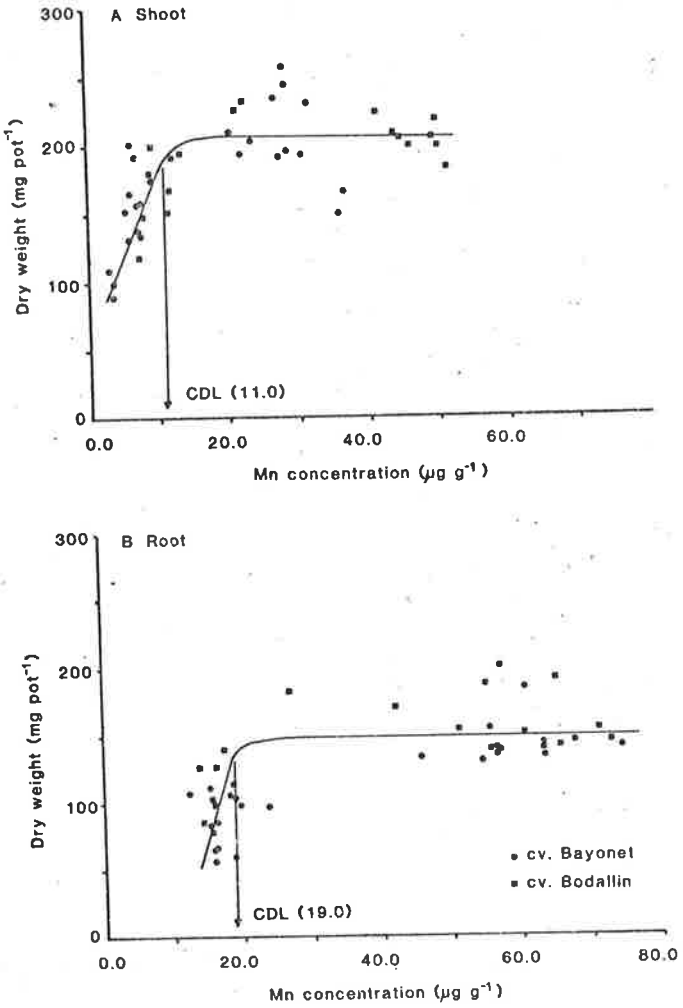


Figure 5.2: Manganese concentration in A. shoot and B. root relative to dry weights of 35 day old wheat plants for the estimation of the Mn critical deficiency level (CDL) in experiment 5.2.

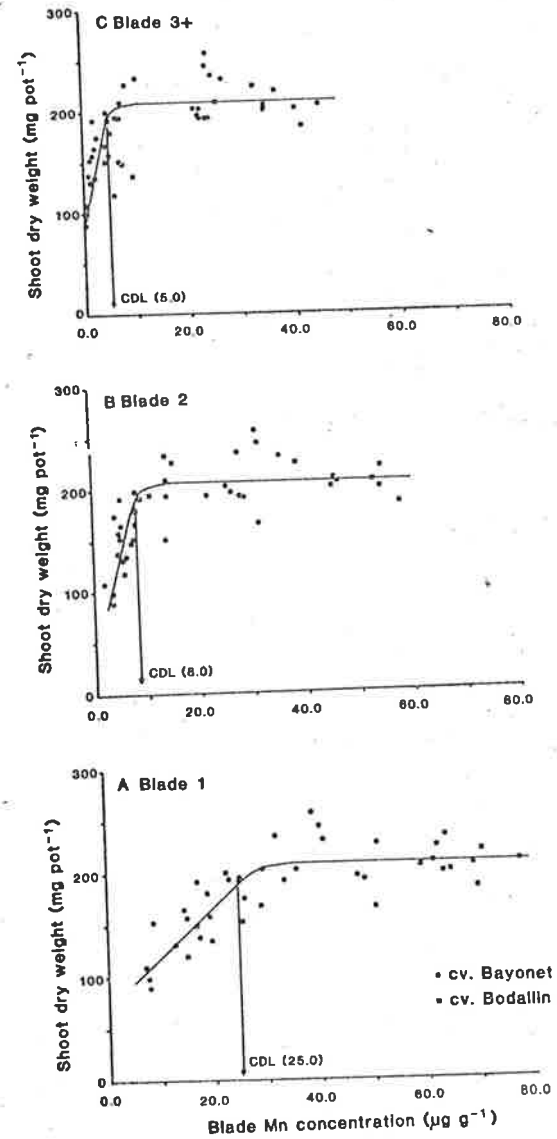


Figure 5.3: Manganese concentration in A. leaf blade 1 (B1), B. leaf blade 2 (B2) and C. leaf blade 3 (B3+) relative to shoot dry weights of 26 day old wheat plants for the estimation of the CDL in experiment 5.1.

Critical curves will differ in shape depending on the nutrient and aerial environment, plant age, tissue sampled etc. (Bates 1971). Certainly, a definite effect of the culture system under which the plants are grown into deficiency is evident for solution vs. soil culture. This issue has been very ably discussed by Nable et al (1984) for subterranean clover. They distinguished plants grown under a constant Mn supply in the culture system from those transferred to nil Mn from an adequate supply. In the latter situation CDLs were much smaller. Since the soil used in these experiments supplied essentially no Mn to the plants, it is not surprising that smaller CDL values were obtained than those published elsewhere. In this case, variation in seed Mn content, rather than substrate Mn, loaded the plants with Mn to differing degrees.

5.5 CONCLUSIONS

These studies have conclusively shown that seedling growth of several cereals on severely Mn-deficient soil can be significantly improved when seed of high Mn content is used. Comparison of genotypes for Mn efficiency, when seed sources are dissimilar, will lead to erroneous conclusions. It is also clear that under conditions of severe Mn deficiency, real Mn uptake can only be accurately determined if the initial seed Mn content is known.

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Appendix 5.1: Effect of soaking seed of wheat, triticale and barley in several concentrations of $MnSO_4H_2O$ for 3, 6 and 12 hours on germination percentage (based on plumule emergence). Figures in brackets indicate percentage of slower germinating seeds at the time of assessment.

Genotype	$MnSO_4.H_2O$ concentration M	Time 3 hours	Time 6 hours	Time 12 hours
wheat	0	89 (7)	68 (10)	71 (9)
	0.10	97 (3)	97 (3)	80 (13)
	0.25	87 (13)	83 (10)	87 (7)
	0.50	93 (3)	93 (3)	30 (57)
triticale	0	100 (0)	100 (0)	94 (6)
	0.10	90 (10)	100 (0)	90 (10)
	0.25	87 (10)	97 (0)	93 (7)
	0.50	100 (0)	93 (7)	97 (3)
barley	0	89 (4)	73 (18)	88 (3)
	0.10	80 (17)	80 (7)	27 (47)
	0.25	70 (20)	80 (13)	44 (38)
	0.50	87 (3)	80 (13)	33 (33)

initial germination percentages were:
Bayonet (82 (15)), Venus (95 (5)) and Galleon (77 (8))

Appendix 5.2: Effect of soaking seed of wheat, triticale and barley in several concentrations of $MnSO_4H_2O$ for 3, 6 and 12 hours on seed Mn concentration and content.

Genotype	$MnSO_4.H_2O$ concentration M	Mn concentration $\mu g\ g^{-1}$			Mn content $\mu g\ seed^{-1}$		
		3	6	12	3	6	12
wheat	0.10	562	510	584	18.9	18.4	19.2
	0.25	865	846	985	30.5	29.2	33.8
	0.50	1065	1233	1100	37.5	39.1	36.0
triticale	0.10	520	649	692	24.3	30.3	32.8
	0.25	711	781	1148	35.4	35.8	54.2
	0.50	936	895	1367	46.2	43.3	63.1
barley	0.10	655	718	769	29.3	33.6	34.8
	0.25	998	1201	1238	47.8	56.5	54.9
	0.50	1349	1496	1790	62.2	68.5	82.0

initial seed Mn concentrations (and contents) were:
wheat (20 (0.7)), triticale (51 (2.4)) and barley (27 (1.2))

Appendix 5.3: Leaf number, tiller number and symptom expression for wheat, barley and triticale after 18 and 36 days growth (experiment 5.2).

Genotype	Seed source	Leaf number pot^{-1}		Tiller number pot^{-1}		Symptom expression score	
		18 days	36 days	18 days	36 days	18 days	36 days
Bayonet	1	2.0	2.7	0.0	0.0	3.0	3.0
	2	2.0	4.0	0.0	0.0	3.0	3.0
	3	2.3	4.7	0.0	0.0	0.5	3.0
	4	2.3	5.7	0.0	0.0	0.0	2.5
	5	3.3	6.0	1.0	3.3	0.0	2.0
	6	4.0	6.0	0.0	2.3	0.0	1.0
Bodallin	1	2.0	3.0	0.0	0.0	2.0	3.0
	2	2.0	4.0	0.0	0.0	0.0	2.5
	3	2.0	5.0	0.0	0.0	0.0	2.0
	4	2.0	6.0	0.0	0.3	0.0	0.0
	5	2.0	6.0	0.0	2.0	0.0	0.0
Galleon	1	1.7	3.7	0.0	0.0	2.5	3.0
	2	4.0	6.0	0.0	0.3	0.0	2.0
	3	4.0	6.0	0.0	2.0	0.0	1.0
	4	4.0	6.0	0.0	4.0	0.0	0.0
Venus	1	2.0	4.0	0.0	0.0	0.0	0.5
	2	2.0	5.3	0.0	0.0	0.0	0.0
	3	2.0	6.0	0.0	1.3	0.0	0.0
	4	2.0	6.0	0.0	0.0	0.0	0.0

Appendix 5.4: Scores for Mn-deficiency symptoms and Mn concentrations for individual wheat leaves at 24 days (experiment 5.1)

Genotype	Seed source	Leaf number	Symptom score	Mn concentration $\mu g\ g^{-1}$	
Bayonet	1	1	0	8.8	33.9
		2	0/1	3.7	24.8
		3+	3/4	0.7	21.9
	2	1	0	13.8	37.9
		2	0/1	5.4	31.5
		3+	1/2	1.9	24.7
	3	1	0	20.6	48.4
		2	0	5.0	31.5
		3+	0/1	1.9	24.5
Bodallin	2	1	0	18.2	68.0
		2	0	7.4	55.1
		3+	1	4.5	40.8
	3	1	0	26.1	61.2
		2	0	9.0	43.6
		3+	0/1	5.0	33.3

1 = Wangary, S.A. ; 2 = Rudall, S.A. ; 3 = Palmer, S.A.

Appendix 5.5: Shoot and root dry weights, Mn contents and Mn concentrations for wheat (cv. Bayonet and Bodallin), triticale (cv. Venus) and barley (cv. Galleon) from several seed sources (see Table 5.1) after 35 days growth (experiment 5.2).

Genotype	Seed source	Dry weight g plant^{-1}		Mn conc. µg g^{-1}		Mn content µg plant^{-1}	
		Shoot	Root	Shoot	Root	Shoot	Root
Bayonet	1	0.009	0.003	5.1	13.1	0.04	0.05
	2	0.028	0.012	4.3	10.2	0.12	0.13
	3	0.066	0.032	5.2	15.5	0.35	0.50
	4	0.089	0.033	5.5	18.1	0.47	0.60
	5	0.180	0.064	10.3	18.7	1.85	1.23
	6	0.200	0.071	14.2	64.7	2.88	4.48
Bodallin	1	0.018	0.012				
	2	0.041	0.021	3.6	11.4	0.15	0.26
	3	0.088	0.040	5.2	19.9	0.46	0.79
	4	0.139	0.060	7.2	18.5	1.00	1.11
	5	0.188	0.076	11.0	22.6	2.06	1.87
Venus	1	0.052	0.021	4.7	23.9	0.25	0.54
	2	0.138	0.059	5.8	21.4	0.80	1.21
	3	0.217	0.104	10.6	21.6	2.28	2.23
	4	0.219	0.103	21.7	79.8	4.74	8.18
Galleon	1	0.021	0.011	5.1	12.0	0.11	0.14
	2	0.087	0.036	3.6	16.7	0.31	0.59
	3	0.124	0.045	5.3	17.0	0.65	0.77
	4	0.228	0.099	15.2	63.8	3.46	6.42

Appendix 5.6: Mobility of Mn absorbed by Bayonet wheat seeds soaked in manganous sulphate.

An attempt was made to determine the rate of efflux of Mn absorbed by wheat seed soaked in $MnSO_4$, and to determine the amount of Mn associated with the embryo and endosperm.

Seeds which had been soaked in 250 mole m^{-3} $MnSO_4.H_2O$ for 9 h were placed in DDDI H_2O for different times then rinsed, dried at 70°C and assayed for Mn. Some seeds were dissected by hand into embryo and endosperm regions.

The figure below shows that about half the absorbed Mn was leached out in the first 30 min. Subsequently, only a small amount of Mn was lost. However, it is very likely that Mn influx might have occurred with time since no attempt was made to immobilise the leached Mn, for example, by adding a chelating compound such as EDTA to the water.

Approximately 25% of the absorbed Mn was found in the embryo, and therefore less liable to being leached out of the seed, but its Mn concentration was about 4 times that of the endosperm (see table below).

Section	Dry wt mg	Mn conc. $\mu g\ g^{-1}$	Mn cont. μg
Embryo	2.6	4321	11.15
Endosperm	40.9	1056	43.18
LSD 5%	3.2	949	3.23

CHAPTER 6

COMPARATIVE TOLERANCE OF CEREALS TO MANGANESE DEFICIENCY IN THE FIELD

6.1 SUMMARY

This Chapter describes two field experiments which compared the growth, Mn uptake and agronomic Mn efficiency of several cereal genotypes on the same Mn-deficient soil used to screen several genotypes in pots (Chapter 2). Agronomic Mn efficiency was defined as grain yield without added Mn compared to its yield with Mn added (ie. relative yield). On this basis rye and Weeah barley were most Mn-efficient whereas oats was least efficent. Considerable differences were evident between cultivars of wheat, barley and triticale for actual and relative yields. The best wheat (Aroona) yielded about the same as the worst barley (Galleon). Yields for triticale were intermediate between wheat and rye. The least Mn-efficient genotypes (eg. Olympic wheat and Avon oats) were characterised by severe plant mortality mid-season as well as low grain yields per plant. High grain yields per plant were associated with high grain number and grain weight.

6.2 INTRODUCTION

The occurrence of Mn deficiency in cereal crops has been ackowledged as a serious problem on certain soil types in southern Australia for a over half a century (Donald and Prescott 1975). The most extensive areas of Mn deficiency in cereal growing districts occur on the calcareous sands of the lower Yorke and Eyre Peninsulas of South Australia (Scott 1932; Higgs and Burton 1955; Puckridge 1958; Carter and Heard 1962; Reuter et al 1973; King and Alston 1975).

It has also been known for some time that yield losses due to Mn deficiency could be largely overcome by the application of superphosphate containing $MnSO_4$, drilled in with the seed (Higgs and Burton 1955; Carter and Heard 1962; Reuter et al 1973). Annual applications of soil Mn are necessary as well as several foliar sprays in order to maximise grain yields (Reuter et al 1973).

In the period prior to 1980 most studies concerned with correcting Mn deficiency used only one cultivar of barley or oats. Subsequently (1980-1983), R. D. Graham and associates (Waite Institute) evaluated the tolerance of a large number of barley and wheat cultivars on this Mn-deficient calcareous sand at a site near Wangary, Eyre Peninsula. Ranking of cultivars was based on plant colour, vigour and grain yield. The use of cultivars tolerant to Mn deficiency should lessen the need for a dependence on Mn fertilisers and sprays.

The field experiments described in this section compare the growth, Mn uptake and grain yielding ability of several cereal cultivars on this Mn-deficient soil, in order to (1) confirm the results of earlier years (2) elucidate factors affecting agronomic Mn efficiency in the field and (3) compare field performance with seedling performance in pots (Chapter 2). Agronomic Mn efficiency is defined here after Graham (1984), as grain or vegetative yield of a genotype without Mn compared to its yield with Mn. Foliar rather than soil Mn was applied in order to obtain the yield potential of each genotype, since this was easier in the experimental system used.

6.3 MATERIALS AND METHODS

6.3.1 Experiment 6.1

Twelve genotypes were chosen to represent a range of tolerances to Mn deficiency, on the basis of observations made during the 1983 growing season at Wangary (Graham, personal communication). There were five wheat cultivars (Aroona, Bayonet, Bodallin, Olympic and Egret), three triticale cultivars (Venus, Coorong and Currency), two barley cultivars (Galleon and Weeah), rye (S.A.) and oats (Avon). Seed sources are indicated in Appendix 6.1.

The experiment was located on a deep grey calcareous sand (Uc1.11 (King and Alston 1975)) and situated on the property of Mr. D. Ottens, c. 10 km E of Wangary, S.A.. Plant breeder's plots (4 rows (0.75 m) x 4.2 m) were sown with a precision drill on 14/6/84. The experiment was designed as a

split-plot randomised complete block, with 5 replicates. All plots were given a basal application of approx. 200 kg ha^{-1} superphosphate, containing Cu, Zn, Co and Mo, and 80 kg ha^{-1} ammonium nitrate at sowing.

Three harvests (H1-H3) were made: H1 on 14/8/84 (60 days), H2 on 20/9/84 (98 days) and H3 on 6/12/84 (178 days) for barley and on 23/12/84 (195 days) for the other cereals. At each harvest, plots were assessed on the basis of vigour and colour (degree of greenness) by giving a score of 0-5, and scored for phenological development (according to Zaddocks et al 1974). Shoots of entire plants from 1.0 m of row were sampled at the root surface. Subsequently, plant, tiller and leaf numbers of harvested plants were measured. Plants harvested at H1 and H2 were washed with DDDI H_2O prior to oven drying at approx. 70 °C, whereas those harvested at H3 were dried at approx. 35 °C without washing. Manganese analyses were only performed at H1 and H2 for those cultivars harvested from plots not srayed with Mn. Harvest index was calculated at maturity as follows:

$$HI = GW / PW$$

where HI = harvest index, GW = grain weight and PW = total plant weight

Foliar Mn was applied at H1 to a randomly allocated sub plot of each pair of plots, so that a total of 60 plots were sprayed, at the rate of approx. 3 ml 'mangasol' (ie. 0.4 g Mn as $MnSO_4$) per plot. The weather was fine. The second foliar application intended at the time of H2, was not made owing to inclement weather.

6.3.2 Experiment 6.2

This experiment was conducted in order to determine the responses of wheat (Aroona and Bayonet) and barley (Galleon) to Mn fertiliser addition. Seed of wheat was obtained from Rudall and that of barley from Urrbrae, S.A., in order to obtain similar Mn contents. It was known from an experiment at Wangary during 1983 that the two wheat cultivars were known to vary in grain yield with and without foliar Mn, but their ability to utilise fertiliser Mn was unknown. Such knowledge was not only of intrinsic interest but might also be relevant to fertiliser recommendations.

The experiment was located on the property of Mr. K. Murdoch, approx. 20 km SW of Warooka, S.A., on a soil type similar to that at Wangary. A soil description of this site has been given by Reuter et al (1973).

Three levels of feriliser Mn (0, 3 and 9 kg Mn ha^{-1}), were applied as a superphosphate/manganous sulphate compound mix ('Warooka' mix). All plots had a basal dressing of approx. 200 kg ha^{-1} superphosphate containing Cu. The experiment had a factorial design with 5 blocks. Plot sizes and harvesting procedures were the same as for the experiments at Wangary. Plots were harvested at 55, 85 and 170 days after sowing. At maturity, plots were mechanically harvested with a 'Wintersteiger' Nursery Master plot harvester. At this time the wheat plots were approx. 1-2 weeks from harvest ripeness.

6.4 RESULTS

6.4.1 Experiment 6.1

(a) Plant density

There were significant differences in plant density between genotypes at each harvest (Tables (6.1, 6.2 and 6.4). The differences at H1 reflected differences in percentage emergence, resulting from variation in the number of seeds sown per m of row. Plant density was noticeably decreased at maturity owing to plant mortality for some genotypes (eg. Olympic and Avon). Plots with significant plant mortality were much more susceptible to weed growth. Except for Bayonet and Bodallin, foliar Mn application maintained a constant plant density over the growing season.

(b) Symptoms of Mn deficiency

The main observation was a gradient of leaf colour from very pale green to dark green, which enabled a qualitative score to be given (Appendix 6.2). Rye and triticale always had the darkest green leaves. This type of scoring was not as rigorous as the system outlined by Batey

(1971), where specific symptoms and percentage leaf death are considered as well as the degree of chlorosis; however it was sufficient to differentiate between genotypes.

Specific symptoms of Mn deficiency were noticeable at both 60 and 98 days for oats, barley and wheat. Oats had the most severe symptoms. Leaves had grey-brown spots and necrotic zones leading to doubling over of leaves near their base. This gave the oat plots, in particular, a mottled appearance. Bayonet also had severe symptoms (leaf necrosis). Symptoms on Galleon consisted mainly of distinct leaf chlorosis but also whitish streaks and leaf bending.

There was a significant negative correlation between the score for colour and shoot Mn concentration (eg. for H2 of experiment 6.1, $r = 0.70$). Thus a greener leaf did not necessarily reflect a higher Mn concentration.

(c) Phenological development

At H1 plants were in the early tillering stage (Feekes Stage 2 or FS 2). At H2 there were large differences between the genotypes in phenological development; oats were at tillering (FS 3-4), most wheats were at late tillering to late stem extension (FS 5-8), barley was at stem extension to early boot (FS 8-10) whereas triticale and rye were at early booting to early ear emergence (FS 10-10.2). There was no apparent significant effect of Mn addition on phenological development.

(d) Tiller number

Tiller numbers at H1 and H2 are given in Tables 6.1 and 6.2. The trends for both harvests were similar. Barley and rye had significantly more tillers than the other cereals, and, by H2, barley, rye and the other cereals had approx. 2, 1 and 0 tillers per plant respectively. The number of tillers for barley was reduced by about 50% at H3. Application of foliar Mn increased tiller numbers for all genotypes but genotype rankings were not altered.

(e) Shoot dry weight

Significant differences occurred between genotypes for shoot dry matter production over the growing season (Figs. 6.1 a, b). Rye was the most productive and oats the least productive cereal. Cultivar differences were particularly apparent for wheat (Aroona and Olympic) and barley (Weeah and Galleon) at H2 and subsequently. There was no increase in plant biomass for Olympic and Avon between H2 and H3: in fact there was a significant decrease in productivity per unit area between H2 and H3 directly attributable to a large reduction in the number of plants per m^2 (see (a)). Details for all the genotypes are given in Tables 6.1 and 6.2.

Significant differences between genotypes in response to foliar Mn were clear by H2: Galleon, Avon and Currency were the most responsive (approx. 40% increase on a per plant basis) with the other genotypes showing much less response (approx. 15% increase or less). By H3 only Weeah barley, S.A. rye and and Bayonet wheat had maintained a low response (approx. 15% or less) whereas the other genotypes, particularly Avon oats, were much more responsive. Relative yields for the Mn-inefficient genotypes were smaller when expressed on a per unit area basis because of an increased plant survival with foliar Mn application (Table 6.4).

(f) Shoot Mn content and concentration

Genotype ranking for shoot Mn content was similar at both H1 and H2 with rye = barley > triticale > wheat > oats.

At H1, cultivar differences were evident for barley and triticale but not for wheat (Table 6.1). By multiplying shoot Mn content by a factor of 2, in order to estimate total plant Mn content (results from 1983 had shown that shoot and root Mn contents were similar at this stage of growth), it is clear that, except for barley and rye, this content was comparable with the Mn content of the sown seed. Shoot Mn concentrations were low for all genotypes.

Table 6.1: Number of plants per m^2, tiller number, shoot dry weight, Mn content and utilisation efficiency (UE) for several cereal genotypes, 60 days after sowing (experiment 6.1).

Cultivar	Plant density plants m^{-1}	Tiller number plant^{-1}	Shoot dry wt g plant^{-1}	Shoot Mn conc $\mu g\ g^{-1}$	Shoot Mn content ug plant^{-1}	U.E. $g^2\ \mu g^{-1}$
Aroona	112	0.2	0.090	10.2	0.95	0.88
Bodallin	146	0.0	0.080	10.1	0.80	0.79
Egret	129	0.1	0.084	8.9	0.73	0.94
Bayonet	126	0.2	0.082	9.2	0.80	0.89
Olympic	154	0.0	0.071	11.5	0.83	0.62
Venus	111	0.0	0.111	10.3	1.13	1.08
Currency	147	0.0	0.130	10.2	1.34	1.27
Coorong	111	0.0	0.091	9.1	0.83	1.00
S.A.	122	0.4	0.101	12.2	1.22	0.83
Weeah	160	0.6	0.112	13.0	1.45	0.86
Galleon	147	0.6	0.099	10.5	1.05	0.94
Avon	138	0.1	0.051	9.4	0.48	0.54
LSD 5%	37	0.2	0.023	2.4	0.35	-
	**	***	***	*	***	-

Table 6.2 Number of plants per m^2, tiller numbers, shoot dry weight Mn contents and U.E.s for several cereal cultivars after 98 days (experiment 6.1). Relative yields (percent of + foliar Mn) are given in brackets

Genotype	Plant density plants m^{-2}	Tiller number	Shoot dry weight g $plant^{-1}$	Shoot Mn. conc. $\mu g\ g^{-1}$	Shoot Mn. cont. μg $plant^{-1}$	U.E. #
Aroona	141	0.2	0.50 (83)	8.1	3.93	6.2
Bodallin	167	0.0	0.33 (92)	10.2	3.24	3.2
Egret	126	0.3	0.33 (72)	11.2	3.52	3.0
Bayonet	122	0.3	0.44 (94)	13.3	4.11	3.3
Olympic	175	0.1	0.30 (100)	11.7	3.49	2.6
Venus	123	0.1	0.77 (84)	8.6	6.70	9.0
Currency	182	0.0	0.53 (59)	6.6	3.51	8.0
Coorong	172	0.0	0.46 (75)	9.9	4.73	4.6
S.A.	110	1.2	1.20 (86)	7.1	8.09	16.9
Weeah	157	1.5	0.92 (100)	6.9	6.40	13.3
Galleon	157	1.8	0.46 (61)	16.7	8.21	27.5
Avon	159	0.1	0.17 (61)	14.2	2.38	1.2
LSD 5% G	31	NS	0.24 ***	3.2 ***	2.11 **	-
Mn	NS	0.1 ***	0.08 *	N.A.	N.A.	N.A.
G x Mn	NS	0.4 ***	NS	N.A.	N.A.	N.A.

N.A. = not applicable

= $g^2\ \mu g^{-1}$ x 100

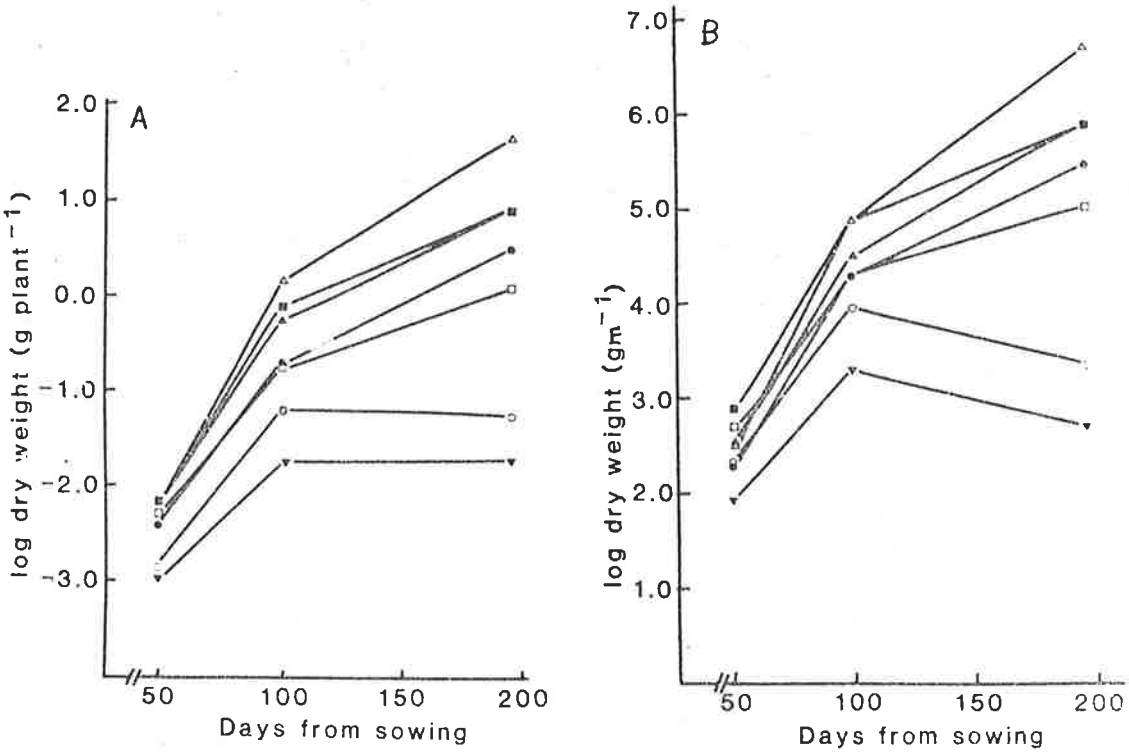

Figure 6.1: Shoot dry matter production (log scale), expressed A. per plant or B. per m^2, for some of the genotypes studied in experiment 5.1. The genotypes corresponding to the symbols used are: Aroona (●), Olympic (○), Weeah (■), Galleon (□), Venus (▲), S.A. (△) and Avon (▼).

Trial site at Wangary in mid-October 1984. Plants at mid-late tillering. Tall dark green plants (rye and triticale) and smaller, paler green plants (barley and wheat).

Trial site at Wangary in mid-November, 1984. Plants at stem extension-ear emergence. Taller, more productive plants in 2nd row (rye and triticale) and smaller, much poorer plants in foreground (wheat).

By H2, shoot Mn contents had increased 5-6 fold (Table 6.2). Cultivar differences were still only evident for barley and triticale. For example, Galleon had both a higher shoot Mn content and concentration than Weeah. This occurred even though it had much lower shoot growth. Therefore the calculated UE for Weeah was much higher than for Galleon. Aroona and S.A. had high UEs for similar reasons.

(g) Grain yield

Grain yields were expressed on both a per plant and a per unit area basis (Table 6.3). Rye and Weeah barley had the highest actual and relative yields wheres oats had the lowest. Considerable differences in actual and relative yields were evident for cultivars of wheat, triticale and barley. The best wheat (Aroona) yielded about the same as the worst barley (Galleon). Yields for triticale were intermediate between wheat and rye. Differences between wheat and barley cultivars were much more pronounced for grain yield per m^2 because of significant plant mortality for less tolerant cultivars (Table 6.4).

High grain yield, expressed on a per plant basis, was also associated with both the number and weight of grains produced per plant (Table 6.4). This is clearly illustrated for Weeah and Galleon barley. Weeah also had a significantly higher harvest index than Galleon (Table 6.3). As well as this, the percentage of grain bearing (ie. fertile) tillers was found to be much higher for Weeah (97 and 96 with and without foliar Mn) than for Galleon (61 and 37 with and without foliar Mn). This parameter was only assessed for barley. The high yield of rye was associated with a large number of relatively low weight seeds produced on the main culm, but not with a high harvest index.

Table 6.3: Grain yield and harvest index of some cereal genotypes at Wangary in 1984, with (+) and without (-) foliar Mn (experiment 6.1). Relative yields (per cent of foliar Mn) are given in brackets.

Cultivar	Grain Yield g plant^{-1}		Grain Yield g m^{-2}		Harvest Index	
	-	+	-	+	-	+
Aroona	0.54 (65)	0.95	69.3 (55)	144.5	0.33	0.37
Bodallin	0.06 (19)	0.32	5.1 (10)	53.2	0.07	0.22
Egret	0.20 (40)	0.50	13.6 (20)	66.6	0.27	0.25
Bayonet	0.30 (81)	0.37	12.2 (48)	25.3	0.14	0.14
Olympic	0.03 (50)	0.06	1.7 (30)	5.6	0.03	0.01
Venus	0.84 (74)	1.13	107.5 (56)	191.5	0.31	0.32
Currency	0.58 (53)	1.09	127.4 (55)	232.4	0.31	0.35
Coorong	0.55 (59)	0.94	99.1 (57)	174.4	0.34	0.36
S.A Comm	1.38 (88)	1.56	223.6(101)	221.2	0.26	0.24
Weeah	1.18 (96)	1.23	162.3(105)	154.7	0.45	0.46
Galleon	0.39 (59)	0.66	50.7 (55)	91.7	0.25	0.30
Avon	0.02 (14)	0.14	0.5 (2)	23.0	0.07	0.12
LSD 5% cv	0.43 ***		69.5 ***		0.10 ***	
F	0.08 ***		12.5 ***		0.03 ***	
cv x F	0.26 *		75.3 *		NS	

Table 6.4: Number of plants per m^2, number of grains per plant and weight per grain at maturity for several cereal genotypes at Wangary in 1984, with (+) and without (-) foliar Mn (experiment 6.1).

Cultivar	Plant number (plants m^{-2})		Weight per grain (mg)		Number of grains per plant	
	-	+	-	+	-	+
Aroona	127.9	152.4	28.2	31.5	19.6	29.6
Bodallin	85.3	166.3	- *	30.0	-	9.2
Egret	68.2	133.2	21.8	24.3	14.8	18.4
Bayonet	40.5	68.2	-	-	-	-
Olympic	56.5	93.8	-	-	-	-
Venus	127.9	169.5	34.7	35.6	23.9	30.8
Currency	219.6	213.2	32.5	36.9	17.5	25.5
Coorong	180.1	185.5	26.9	32.3	20.0	28.8
S.A.	162.0	141.8	22.9	22.6	64.8	73.2
Weeah	137.5	125.8	39.0	43.4	30.5	28.3
Galleon	130.1	138.6	30.6	32.6	11.1	17.9
Avon	25.6	164.2	-	14.3	-	11.7
LSD 5% cv	43.0 ***		-		-	
F	12.4 ***		-		-	
cv x F	43.3 ***		-		-	

Table 6.5: Shoot dry weight, Mn concentration and Mn content after 55 days and grain yield at maturity for wheat (Aroona and Bayonet) and barley (Galleon) at Warooka in 1984 (experiment 6.2).

Genotype	Mn rate	Shoot dry weight	Shoot Mn conc.	Shoot Mn content	Grain yield
	kg ha^{-1}	g m^{-2}	μg g^{-1}	μg $plant^{-1}$	g m^{-2}
Bayonet	0	5.2	18.0	0.66	162.5
	3	5.3	21.9	0.93	173.3
	9	7.9	24.5	1.31	209.8
Aroona	0	7.5	18.4	0.93	183.5
	3	6.0	22.0	0.92	163.7
	9	8.9	28.6	1.68	208.4
Galleon	0	13.2	16.5	1.23	302.4
	3	15.4	16.6	1.38	309.8
	9	18.2	23.2	2.27	364.3

There was considerable variation in growth and grain yield between replicate blocks. Plots situated on the lowest part of the slope produced the highest dry matter and grain yield. Even with the use of paired plots, this factor increased replicate errors if randomly assigned plots for a genotype favoured any particular slope.

6.4.2 Experiment 6.2

Aroona and Bayonet wheat had similar grain yields, with and without added Mn, although Aroona had a higher shoot dry matter production at early tillering (Table 6.5). Galleon yielded almost twice as either wheat throughout the season. However, relative yields without added Mn were similar to those of wheat.

This site was considerably less Mn-deficient than that at Wangary: for example, grain yield for Bayonet at Warooka approx. 10 times that at Wangary. Although plant density was not assessed at maturity, it is unlikely that there was significant plant mortality.

6.5 DISCUSSION

Relative yields, which reflected responses to foliar Mn, varied significantly between genotypes (Table 6.4), even though only one spray was used. Only Weeah barley and S.A. rye would be classified as agronomically Mn-efficient, according to the definition of Mn efficiency in terms of relative grain yield given by Graham et al (1983). However, since Mn analyses were not determined for plants of Mn sprayed plots, it is not possible to say whether the all genotypes sprayed with foliar Mn were Mn sufficient nearer maturity and therefore whether yields with Mn spray represent the true yield potential for each genotype.

The ranking of wheat genotypes for dry matter production, utilisation efficiency and grain yield with and without Mn is in agreement with the results obtained by R. D. Graham and associates in 1982 (data not shown) and 1983 at Wangary (Appendix 6.3-6.5). Grain yield data from these years

was only available on a per unit area basis, however, it is likely that plant mortality occurred at least to the same degree since there was less rainfall in the spring of 1983 than 1984 (Appendix 6.6).

All wheat cultivars were much more responsive to foliar Mn in 1983 than in experiment 6.1, most likely because two sprays were used in 1983. Reuter et al (1973a) demonstrated the benefit of 2 sprays on the yield of barley, and in particular the extra benefit of the 2nd spray at stem extension, since this was the period of maximum LAI (Puckridge and Donald 1967). However, the degree of benefit accruing from more than one spray is season and site dependent (Graham, pesonal communication). There may also be a significant genotype x spraying time interaction owing to differences in maturity (and therefore time to attainment of maximum LAI) between cultivars, but this was not investigated.

It appears that differences in the degree of Mn efficiency between wheat cultivars may be smaller under conditions of more marginal soil Mn deficiency. This contention is supported by comparison of data from the two experiments described here as well as data obtained from a cultivar trial situated on a slightly Mn-deficient mallee sand near Karoonda, S.A. in 1984 (Appendix 6.7). For example, Aroona and Bayonet had similar shoot growth, dry weight and grain yield and only Millewa (which showed Mn-deficiency symptoms in mid-season) had consistently lower yields. Differences between wheat, triticale and rye were much less pronounced than at Wangary.

It is apparent from data presented for biomass and grain yield that the Mn efficiency of rye has been transferred to triticale. Even Coorong, which was classed as agronomically Mn-inefficient by Graham et al (1983), showed no plant mortality. Whilst the yields for rye in these experiments accord well with those of Graham et al (1983), those for Coorong are considerably lower. Overall it is clear that triticale and rye are much better adapted to these soils than are wheat and oats, with barley of intermediate adaptation. The most Mn-efficient wheat at Wangary had relative grain yields without Mn of only approx. 55% compared with approx. 95% for barley.

Seeds from the same source were used, as far as possible, since results from a 1983 field experiment established that the degree of Mn efficiency may be have been at least partly related to the seed source (and therefore the Mn content) used. Seed Mn concentrations and contents for barley were 2-3 times lower than for the other cereals, even though seed was obtained from Urrbrae for all cultivars tested, except for Olympic wheat and S.A. rye (Appendix 6.1). The lower seed Mn concentrations for barley may have been associated with a smaller translocation of Mn to the seed, since (a) similar differences have been found for other seed sources (see Chapter 2) and (b) seed weights for barley were similar to those of wheat. The number of seeds sown per m of row varied between genotypes: however, the range of plant densities observed at H1 (approx. 100-150 plants m^2) would not have been expected to result in significant differences in inter-plant competition (Walter 1971).

It was found that Mn-inefficient genotypes exhibited significant plant mortality post-late tillering. Mid-season plant death on this chronically Mn-deficient soil type has been reported previously (e.g. for oats (Puckridge 1958) and barley (Graham et al 1983)), and has also been observed on other Mn-deficient soils (Hoyt and Myovella 1974). This cessation of growth may have been due to a Mn deficiency per se or to some other factor(s), such as water stress resulting from a much reduced root system. The root systems of even the Mn-efficient genotypes under Mn sufficiency were concentrated in the first 10 cm of darker grey sand so that top soil drying could significantly affect Mn uptake.

The variability in yield and symptom development between replicate blocks added an undesirably large error component to the measurements. The increased growth on the lower slopes was probably due to a more favourable supply of soil Mn resulting from a higher soil moisture content or even temporary waterlogging, which would enhance reduction of MnO_2 (Piper 1943; Uren 1969). Unfortunately it was not possible to select a more uniform site with the degree of Mn deficiency required.

Dry matter production and Mn status of plants at H1 (60 days) did not correlate well with yield at maturity except for oats which performed poorly throughout the season (Cf. Tables 6.1 and 6.3). This is probably because most of the Mn in seedling plants was seed derived and therefore did not reflect differences in Mn uptake. Cultivar differences in shoot dry matter production mid-season (H1) were more closely associated with final grain yield This suggests that factors, such as maturity type, moisture stress and/or disease resistance, operating later in the season, may have had more influence in determining yield differences between genotypes than those earlier in the season, unless these are more difficult to detect.

As a matter of interest shoot Ca concentrations were determined for all genotypes at H1, since it was thought that Ca toxicity might result from excessive uptake from this soil. However all genotypes had concentrations of approx. 1%, except for barley and rye which had approx. 1.3%. This result suggested that Ca toxicity was not associated with Mn inefficiency.

In the experiments described here and in other trials, Aroona was consistently rated as Mn-efficient; Oxley, Bodallin and Egret as moderately Mn-efficient; and Bayonet, Olympic and Songlen as Mn-inefficient. Unlike the case for Mn-efficient barley cultivars which could be related genetically to traditional English landraces (Graham et al 1983), there did not appear to be a common parental type for the Mn-efficient and Mn-inefficient wheats, although there appears to be some evidence that some derivatives from Raven (including Aroona) have greater Mn efficiency (A.J. Rathgen, personal communication). The difficulty in obtaining a consistent relationship is probably due to the hexaploid background of wheat which may mask a simply heritable trait. There was also no apparent consistent relationship between maturity type and Mn efficiency, although Aroona was early maturing and Mn efficient. The use of relative yield as an index of Mn efficiency should have minimised the possible effect of maturity differences. Neverthelss it was thought possible that since late maturing genotypes often have a more abundant root system (Pianthus 1969, O'Brien 1979), they might have more opportunity for Mn uptake, and therefore be

more Mn-efficient, but this did not appear to be the case.

Rye, triticale and barley were clearly more Mn-efficient than wheat and oats but there were significant cultivar differences. On the basis of these experiments, factors associated with higher Mn efficiency (relative grain yield per unit area) appear to be greater biomass production and a higher UE at and after mid-season, low plant mortality later in the season and more higher weight grains per plant. Mn efficiency during early growth in the field does not correlate well with Mn efficiency at maturity. Generally, genotypic differences for Mn uptake assessed during seedling growth in pots (Chapter 2) agreed well with those observed for early growth in the field. The poorer performance of rye seedlings in the pot studies (Chapter 2) compared with their growth in the field may have been due to the use of a less vigorous seed source in the former case.

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Appendix 6.1: Seed dry weight, Mn concentration and content for genotypes used in experiment 6.1. Data are means and standard errors of 3 replicates of 10 seeds each.

Genotype	Source	Dry weight mg seed^{-1}	Mn concentration μg g^{-1}	Mn content μg seed^{-1}
Aroona	(a)	44.5 (3.1)	55.9 (0.5)	2.49 (5.15)
Bayonet	(a)	43.7 (5.0)	58.4 (0.1)	2.55 (0.03)
Bodallin	(a)	41.3 (4.0)	70.3 (3.4)	2.91 (0.11)
Olympic	(b)	33.4 (1.2)	106.6 (9.3)	3.57 (0.44)
Egret	(a)	42.5 (6.6)	46.8 (0.9)	1.98 (0.27)
Weeah	(a)	49.3 (0.6)	20.2 (0.7)	1.00 (0.05)
Galleon	(a)	43.9 (0.8)	15.7 (0.2)	0.69 (0.00)
Venus	(a)	54.7 (3.2)	46.3 (1.3)	2.54 (0.22)
Currency	(a)	59.6 (3.0)	52.9 (0.4)	3.16 (0.13)
Coorong	(a)	51.1 (2.3)	50.3 (1.8)	2.56 (0.02)
S.A.	(c)	28.5 (0.5)	30.7 (0.3)	0.88 (0.01)
Avon	(a)	31.6 (1.1)	49.9 (4.7)	1.59 (0.20)

(a) Urrbrae, S.A. (Mn-adequate site)
(b) Wagga, N.S.W. (Mn-toxic site)
(c) Strathalbyn, S.A. (Mn-adequate site)

Appendix 6.2: Colour and vigour score for genotypes in experiment 6.1 at Wangary 1984, for harvests 1 and 2 (ie. H1 and H2).

Genotype	Foliar * Mn	Colour + score		Vigour + score	
		H1	H2	H1	H2
Aroona	-	3.0	2.7	3.0	2.9
	+	3.0	3.0	3.0	3.2
Bodallin	-	3.0	2.5	3.5	2.5
	+	3.0	2.8	3.5	3.0
Egret	-	3.0	2.2	2.5	2.3
	+	3.0	2.7	2.5	2.8
Bayonet	-	2.5#	2.0	2.5	1.8
	+	2.5#	2.4	2.5	2.3
Olympic	-	3.0	2.0	3.0	1.8
	+	3.0	2.5	3.0	2.4
Venus	-	3.0	3.3	3.5	3.3
	+	3.0	3.6	3.5	3.9
Coorong	-	3.0	2.9	3.5	3.0
	+	3.0	3.5	3.5	3.6
Currency	-	4.0	3.6	4.0	3.4
	+	4.0	4.1	4.0	4.0
Galleon	-	3.0	2.1	3.5	2.4
	+	3.0	2.6	3.5	2.8
Weeah	-	4.0	2.9	4.0	3.1
	+	4.0	3.3	4.0	3.2
S.A.	-	3.5	4.0	3.5	3.9
	+	3.5	4.1	3.5	4.2
Avon	-	3.0	1.7	2.5	1.0
	+	3.0	2.9	2.5	2.1

* + and - represent plots designated for + and - foliar Mn application at harvest 1

+ visual and colour assessment based on score value from 0.0 to 5.0 (5.0 = greenest and most vigorous)

some Mn deficiency symptoms visible in addition to chlorosis

Appendix 6.5: Grain yield and harvest index for several wheat cultivars grown at Wangary in 1983, with (+) and without (-) foliar Mn (calculated from unpublished data of R. D. Graham). Relative yields (per cent of foliar Mn treatment) are given in brackets.

Genotype	Grain yield $g\ m^{-2}$			Harvest index	
	-		+	-	+
Aroona	65.1	(39)	168.5	0.47	0.48
Purple Straw	41.1	(24)	169.6	0.31	0.36
Oxley	61.7	(30)	208.4	0.47	0.47
Egret	42.8	(22)	191.9	0.43	0.48
Bodallin	41.1	(21)	192.4	0.43	0.48
Halberd	19.4	(17)	111.9	0.29	0.43
Bayonet	14.9	(12)	122.2	0.25	0.42
Bindawarra	16.0	(10)	161.6	0.34	0.48
Olympic	6.9	(06)	109.6	0.35	0.42

Appendix 6.6: Mean monthly rainfall data for Wanilla (near Wangary site) and Warooka (near Warooka site) for 1983 and 1984.

Year	Site	Rainfall (mm)											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1983	Wanilla	5	33	46	151	40	46	183	65	64	17	13	19
	Warooka	4	6	86	77	37	27	81	64	32	43	20	12
1984	Wanilla	5	4	41	18	42	35	136	152	147	27	7	23
	Warooka	11	1	13	18	31	38	68	104	46	19	26	12

Appendix 6.3: Shoot dry weights for several wheat cultivars grown at Wangary in 1983, with (+) and without (-) foliar Mn (calculated from unpublished data of R. D. Graham). Relative yields (per cent of foliar Mn treatment) are given in brackets.

Cultivar	Shoot dry weight ($g\ m^{-2}$)						
	(55 days)	(95 days)			(195 days)		
	-	-		+	-		+
Aroona	8.9	49.6	(66)	75.7	148.2	(42)	352.2
Purple straw	9.3	38.5	(78)	49.7	133.2	(28)	474.9
Oxley	8.3	42.5	(44)	96.1	131.8	(30)	442.9
Egret	8.5	28.8	(43)	67.5	98.5	(24)	403.0
Bodallin	10.3	28.7	(54)	67.5	95.9	(24)	401.7
Halberd	7.4	26.8	(74)	36.2	67.1	(26)	257.6
Bayonet	6.6	26.0	(38)	67.7	60.2	(21)	288.1
Bindawarra	7.6	22.9	(41)	56.0	47.3	(14)	338.6
Olympic	4.6	17.3	(47)	37.2	19.3	(07)	262.6
LSD 5% cv	-	21.7			96.5		
Mn	-	3.1			23.3		

Appendix 6.4: Shoot Mn concentration and utilisation efficiency (UE) for several wheat cultivars at Wangary in 1983, for plots without foliar Mn (calculated from unpublished data of R. D. Graham).

Cultivar	55 days		95 days	
	Mn conc. $\mu g\ g^{-1}$	U.E. $g^2\ \mu g^{-1}$	Mn conc. $\mu g\ g^{-1}$	U.E. $g^2\ \mu g^{-1}$
Aroona	11.6	0.77	4.2	11.81
Purple Straw	14.3	0.65	7.5	5.13
Oxley	9.6	0.86	4.6	9.25
Egret	11.4	0.74	6.5	4.43
Bodallin	10.6	0.97	5.7	8.41
Halberd	10.0	0.74	7.6	3.53
Bayonet	11.2	0.59	6.3	4.13
Bindawarra	10.1	0.76	8.9	2.57
Olympic	11.2	0.41	7.5	2.30

Appendix 6.7: Shoot dry weight, Mn concentration and content at 7 weeks, shoot Mn concentration at 12 weeks and grain yields for some wheat and triticale cultivars and rye grown on a slightly Mn-deficient soil near Karoonda, S.A. in 1984.

Genotype	8 weeks			12 weeks	19 weeks
	Dry wt	Mn conc.	Mn cont.	Mn conc.	Grain yield
	mg plant^{-1}	μg g^{-1}	μg plant^{-1}	μg g^{-1}	g m^{2}
Aroona	0.24	33.7	7.93	11.2	120
Bayonet	0.21	33.6	7.08	8.2	120
Millewa	0.18	25.8	4.70	6.0	91
Venus	0.30	40.8	12.22	10.4	124
Currency	0.31	40.0	12.60		114
Coorong	0.35	22.5	8.15	7.5	110
S.A.	0.28	29.7	8.45	7.9	136

CHAPTER 7

EFFECT OF SEED MANGANESE CONTENT ON AGRONOMIC MANGANESE EFFICIENCY IN CEREALS

7.1 SUMMARY

Three field experiments have established the importance of seed source, particularly seed soaking, on dry matter production and grain yield of wheat and barley cultivars. Rye and triticale showed very little response to seed Mn treatment. Even less Mn was recovered in field-grown plants derived from Mn-soaked seeds than in the pot studies. Foliar Mn was much more effective when applied to plants derived from seed of higher Mn content. Manganese-inefficient genotypes could be distinquished by their greater response to seed soaking.

7.2 INTRODUCTION

One conclusion from Chapter 6 was that seed of similar Mn content should be used when comparing cereal genotypes for growth and grain yield on Mn-deficient soil in order to avoid erroneous inferences regarding the degree of agronomic Mn efficiency. This chapter describes three field experiments designed to assess in more detail the effect of seed Mn content (natural and artificially raised) on the performance of several cereals under Mn deficiency. Experiment 7.1 was designed to evaluate the effect of seed Mn content on the growth and grain yield of wheat, barley and triticale. Experiment 7.2 set out to determine whether genotype ranking for growth and grain yield under Mn deficiency would be altered with a large increase in seed Mn content by seed soaking in $MnSO_4$ prior to sowing. Experiment 7.3 was designed to compare the effect of seed Mn with soil Mn application for wheat and barley.

7.3 MATERIALS AND METHODS

7.3.1 Experiment 7.1

The experiment was conducted during 1984 at Wangary (see experiment 6.1, Chapter 6). The effect of three seed sources (seed from Wangary and Urrbrae and seed soaked in $MnSO_4$) were evaluated in a randomised complete block design, with and without foliar Mn applied to split plots. Seed

soaking was accomplished by placing 500 g lots of seed in 1 l of 0.25 M $MnSO_4.H_2O$ for 6 h. Plot sizes, basal fertiliser and foliar Mn application and harvesting procedures were the same as for Chapter 3.

7.3.2 Experiment 7.2

This experiment was also conducted at Wangary during 1984. The following genotypes were used: wheat (Aroona, Bayonet and Egret), triticale (Venus and Coorong), barley (Weeah and Galleon) and rye (S.A.). Application of Mn to the seed was as per experiment 7.1.

7.3.3 Experiment 7.3

The experiment was conducted at Warooka (see experiment 6.2, Chapter 6) during 1984. The experiment utilised a 2^4 factorial randomised complete block design, with 2 genotypes, 2 seed Mn contents (+ and - seed soaking in 0.25 M $MnSO_4$), 2 Mn fertiliser treatments (+ and - 9 kg Mn ha^{-1} as the 'Warooka' mix) and 2 foliar Mn treatments (+ and - a spray at 7 weeks after sowing). Superphosphate (210 kg ha^{-1}) was applied at sowing and foliar Cu and Zn were applied to all plots at 7 weeks.

7.4 RESULTS AND DISCUSSION

7.4.1 Experiments 7.1 and 7.2

The three 1984 field experiments described here have highlighted the importance of seed Mn (natural and Mn-soaked) on plant growth throughout the growing season. Tables 7.1 and 7.2 show results for experiment 7.1 from harvests at 8 weeks (early tillering) and maturity respectively. Table 7.3 shows data for grain yield from experiment 7.2. Data for colour and vigour scores for experiment 7.1 is given in Appendix 7.1. There was a significant improvement in leaf colour with seed soaking in both experiments, particularly at early tillering (H1) for Galleon and Bayonet. Least growth was made by plants derived from seed harvested at Wangary. Significant plant mortality was observed for Bayonet (ex Wangary) at 8

weeks and severe mortality for Bayonet (ex Wangary and Waite) and Galleon (ex Wangary) at maturity. No significant plant mortality was observed for Venus. There were no significant effects of seed Mn-soaking on grain yield at Wangary in 1983 probably because the natural Mn content of the seed used was already relatively high (seeds used for the 1983 experiment came from Urrbrae, S.A.).

It is noteworthy that the shoot Mn content at 8 weeks for plants derived from Mn-soaked seeds was much lower here than after 5 weeks in the controlled environment study (Table 7.1). This is probably due in part to lower soil temperatures in the field (less than $10^{\circ}C$) reducing the rate of germination and emergence, and consequently, increasing the duration of Mn leaching from seed and seedling roots. This hypothesis is favoured by some data from a preliminary experiment, showing only approx. 15 % retention of Mn from soaked seeds in one week old soil emerged seedlings, at $10^{\circ}C$ and lower (Appendix 7.2).

At maturity, highest grain yields were obtained for plants derived from Mn-soaked seed which had foliar Mn applied (Table 7.3). This effect was most evident for yield of Bayonet and Galleon per m^2 owing to the absence of plant mortality. None of the treatments resulted in plant mortality for Venus. It is not clear at this stage whether plant motality was due to a lack of Mn per se or to some interacting factor, such as reduced water uptake resulting from reduced root activity. Observation of root systems did not indicate significant pathogen activity.

Grain yields and harvest indices were extremely poor for experiment 7.1. No Mn fertiliser was applied to ascertain the yield potential that existed. Results from an adjacent experiment indicated that soil applied Mn gave much higher grain yields than observed for any treatment used here (R. D. Graham unpublished). However, even the one Mn foliar treatment used here was clearly most effective on plants derived from seeds of higher Mn content, probably because these plants had a higher dry weight (and therefore leaf area) at 8 weeks.

Table 7.1: Plant density, tiller number and shoot dry weight, Mn concentration and content for wheat, triticale and barley from 3 seed sources after 56 days growth (experiment 7.1).

Cultivar	Seed source	Plant number m^{-2}	Tiller number $plant^{-1}$	Shoot dry wt $mg\ plant^{-1}$	Shoot Mn conc $\mu g\ g^{-1}$	Shoot Mn cont $\mu g\ plant^{-1}$
Bayonet	Wangary	106.4	0.0	30.6	13.0	0.41
	Urrbrae	122.2	0.1	89.2	11.0	0.97
	Wangary (Mn-soaked)	162.0	0.2	68.4	13.3	0.92
Venus	Wangary	122.4	0.0	69.8	11.5	0.80
	Urrbrae	95.7	0.0	104.0	10.1	1.07
	Wangary (Mn-soaked)	139.3	0.0	89.6	16.4	1.54
Galleon	Wangary	127.4	0.6	81.8	9.8	0.80
	Urrbrae	137.1	0.4	86.0	10.7	0.90
	Wangary (Mn-soaked)	106.1	1.0	100.8	13.6	1.43
LSD 5%	ss, gen *	NS	0.2	15.3	1.8	0.18
	ss x gen	42.5	0.3	26.6	3.2	0.31

* ss = seed source, gen = genotype

Table 7.2: Plant density, grain yield and harvest index for wheat, triticale and barley from 3 seed sources at maturity grown , with and without foliar Mn (experiment 7.1).

Cultivar	Seed source	Foliar Mn	Plant number m^{-2}	Grain dry wt g $plant^{-1}$	Grain dry wt g m^{-2}	Harvest index per cent
Bayonet	Wangary	-	6.4	0.02	0.1	-
		+	20.3	0.00	0.1	0.8
	Urrbrae	-	58.6	0.09	3.2	5.0
		+	132.2	0.04	5.4	5.8
	Wangary (Mn-soaked)	-	98.1	0.01	0.7	1.4
		+	182.2	0.06	14.0	6.4
Venus	Wangary	-	145.0	0.36	66.8	27.0
		+	139.0	0.43	65.7	28.0
	Urrbrae	-	149.2	0.44	69.0	25.8
		+	154.6	0.93	141.6	32.8
	Wangary (Mn-soaked)	-	188.6	0.43	81.8	29.4
		+	170.6	0.94	155.0	32.8
Galleon	Wangary	-	65.0	0.29	14.2	30.8
		+	105.5	0.52	56.8	38.2
	Urrbrae	-	151.3	0.19	69.0	21.2
		+	165.0	0.61	102.6	39.0
	Wangary (Mn-soaked)	-	118.3	0.47	54.9	34.4
		+	131.1	0.75	97.6	39.6
LSD 5%	ss, gen *		21.2	0.13	21.5	3.4
	foliar		12.1	0.06	8.6	3.6
	ss x gen x foliar		34.8	0.17	27.3	NS

* ss = seed source, gen = genotype

Table 7.3: Number of plants per m^2, grain yield and harvest index at maturity for several cereal genotypes with (+) and without (-) seed soaking in $MnSO_4$ (experiment 7.2).

Cultivar	Plant number plants m^{-2}		Grain dry weight g $plant^{-1}$		Grain yield g m^{-2}		Harvest index	
	-	+	-	+	-	+	-	+
Aroona	134.3	172.7	0.22	0.16	29.5	27.6	0.22	0.20
Bayonet	19.2	116.2	0.00	0.02	0.0	23.2	0.00	0.03
Egret	71.4	133.2	0.06	0.06	4.3	8.0	0.07	0.08
Venus	199.3	152.4	0.49	0.53	97.7	80.8	0.29	0.29
Coorong	170.6	167.4	0.22	0.41	37.5	68.6	0.26	0.30
S.A.	175.9	178.0	1.20	1.32	211.0	235.0	0.27	0.27
Weeah	159.9	166.3	1.00	0.86	159.9	143.0	0.44	0.46
Galleon	53.3	134.3	0.17	0.23	9.1	30.9	0.15	0.25
LSD 5% cv	39.3 ***		0.17 ***				0.08 ***	
seed	19.5 ***		NS				0.02 ***	
cv x seed	58.2 *		NS				NS	
blocks	NS		*				NS	

Table 7.4: Number of grains per plant and weight per grain for barley and triticale grown from 3 seed sources with (+) and without (-) foliar Mn (experiment 7.1).

Genotype	Seed source	Number of grains plant^{-1}		Mean weight per grain mg	
		-	+	-	+
Galleon	Wangary	8.2	14.7	35.3	35.2
	Waite	7.8	16.7	31.6	32.7
	Wangary (Mn-soaked)	14.9	20.5	32.9	36.0
Venus	Wangary	9.6	13.7	29.9	33.0
	Waite	13.3	25.6	30.2	36.1
	Wangary (Mn-soaked)	13.7	25.8	30.9	36.0

Table 7.5: Effect of seed Mn-soaking, Mn fertiliser (Fert Mn) and foliar Mn (Fl Mn) on grain yield per m^2 of wheat and barley (experiment 7.3).

Genotype	- Mn soaking				+ Mn soaking			
	- Fert Mn		+ Fert Mn		- Fert Mn		+ Fert Mn	
	- Fl Mn	+ Fl Mn	- Fl Mn	+ Fl Mn	- Fl Mn	+ Fl Mn	- Fl Mn	+ Fl Mn
Bayonet	144	159	277	279	195	201	279	333
Galleon	110	171	281	299	134	146	265	344

Ranking of genotypes for Mn efficiency was not altered in experiment 7.2, except for the positions of Aroona and Galleon. This was primarily due to a large decrease in plant mortality for Galleon with seed soaking. The Mn inefficiencies of Galleon, Coorong, Bayonet and Egret are highlighted by the fact that only these cultivars responded to seed soaking (Table 7.3).

Seed Mn content had a large effect on the number of grains produced per plant but no effect on mean grain weight, whereas foliar Mn application increased both grain number and grain weight (Table 7.4). Data are only given for Galleon and Venus because of insufficient grain production by Bayonet.

7.4.2 Experiment 7.3

The effect of seed Mn-soaking and foliar Mn on grain yield was small but significant (approx. 10-15 %), whereas large responses (approx. 50 %) were found for Mn fertiliser addition (Table 7.5). However, foliar and soil Mn application to plants derived from Mn-soaked seeds resulted in approx. 15-20 % more grain than their application to plants derived from unsoaked seeds. The relatively poor response to seed Mn-soaking resulted from the relatively high Mn content of seed (ex Waite) used here coupled with the more marginally Mn-deficient nature of this soil.

7.5 CONCLUSIONS

Manganese-inefficient cultivars could be distinguished by their greater response to seed soaking. Responses to foliar Mn application in the field should be improved by the use of seed soaked in $MnSO_4$. Application of Mn to the seed could lessen the dependence on soil Mn fertiliser application. Certainly farmers would be advised to import seed of barley and wheat from Mn-sufficient or even Mn-toxic sites if they are considering growing these crops on acutely Mn-deficient sites without Mn fertiliser.

Appendix 7.1: Colour and vigour scores and establishment count at H1 from experiment 7.1 .

Genotype	Seed source	Foliar# Mn	Colour+ score	Vigour+ score	Establishment (m-1	s.e.)
wheat cv. Bayonet	Wan	-	1.0###	1.0	24.6	0.5
		+	1.0###	1.0	24.4	2.0
	Wai	-	2.5	3.0	23.6	1.4
		+	2.5	3.0	24.4	1.6
	S	-	3.0	3.5	28.0	1.5
		+	3.0	3.5	33.8	4.1
triticale cv. Venus	Wan	-	3.0	2.5	24.2	2.2
		+	3.0	2.5	24.2	2.0
	Wai	-	4.0	4.0	20.8	0.4
		+	4.0	4.0	21.4	1.8
	S	-	4.0	4.0	26.2	1.5
		+	4.0	4.0	26.6	2.6
barley cv. Galleon	Wan	-	2.5#	2.5	24.0	1.1
		+	2.5#	2.5	21.8	1.8
	Wai	-	3.0	3.5	28.0	1.5
		+	3.0	3.5	26.8	1.5
	S	-	3.5	3.5	20.2	1.4
		+	3.5	3.5	23.0	0.9

* + and - foliar Mn designates plots to receive a + and - Mn spray application at the time of harvest 1
+ visual assessment score based on scale 0.0 to 5.0 (5.0 = most green and vigorous)
# some Mn deficiency symptoms in addition to general chlorosis
severe " " " "

Appendix 7.2: Effect of soil temperature and Mn content of Bayonet wheat seeds soaked in manganous sulphate.

An experiment was conducted to test the hypothesis that the effectiveness of soaking seeds in manganous sulphate in order to promote growth on Mn-deficient soil would be reduced in the field because of low soil temperatures. Low soil temperatures would increase the degree of Mn leaching from germinating seeds as a result of slow germination and emergence.

Ten seeds, previously soaked in 250 mole m^{-3} $MnSO_4.H_2O$ for 9 h, were germinated in 9 cm diam. glass petri dishes containing 80 g of Mn-deficient sand made up to 25 % w/w moisture content. Six temperature treatments were used, viz 6, 9, 11, 15, 19 and 25 ^{o}C, by placing dishes into temperature controlled germination cabinets. Three dishes were allocated per treatment. The experiment was terminated for each treatment when at least some of the germinated seeds had emerged.

The rate of germination and emergence was significantly increased at lower soil temperatures, whilst the per cent germination was not affected (see table below). Seeds germinated at 11 and 15 ^{o}C had the highest Mn concentration and content, whilst those germinated at higher and lower temperatures had lower values. Even so, considerable quantities of Mn (approx. 75 %) were lost at optimal temperatures.

It is possible that at lower temperatures there is greater opportunity for Mn leaching due to slower germination as well as effects on membranes, whilst at higher temperatures membrane effects predominate.

Temperature °C	Germination percentage	Time to germination days	Mn conc µg g^{-1}	Mn content µg $seed^{-1}$
25	73	3	291	10.10
19	77	4	274	10.88
15	80	5	414	16.05
11	73	6	437	17.25
9	80	9	277	10.98
6	70	13	278	11.52
initial	-	-	1445	60.40
LSD 5%	NS	-	136	5.24

CHAPTER 8

GENERAL CONCLUSIONS

8.1 EXPERIMENTAL OVERVIEW

The experiments described in this thesis have attempted to (1) quantify the degree of genotypic variation for Mn efficiency within wheat as well as between wheat, barley, triticale and rye, both during seedling growth and at grain maturity and (2) evaluate possible mechanisms associated with Mn efficiency between cereal genotypes. A long term objective of this research was to develop a rapid screening procedure for Mn efficiency, with particular emphasis on wheat.

The majority of controlled environment experiments considered the growth and Mn uptake of seedling (4-5 week old) plants cultured in small pots containing Mn-deficient soil. Soil cultures, rather than nutrient solution or sand cultures, were used because the former encompassed the possibility of chemical modification of the rhizosphere, as well as root exploration, in determining root acquisition of Mn. Agar and filter paper media were used to evaluate the possible importance of rhizosphere mechanisms (acidification and reduction) in differential Mn uptake between barley and rye. Less effort was devoted to this aspect because of the inability to select cultivars of wheat with significant differences in Mn uptake ability under soil Mn deficiency. Seed Mn content was shown to have a great impact on apparent Mn efficiency.

Field experimentation was considered necessary in order both to rank genotypes on the basis of grain yield production under Mn deficiency (ie. for agronomic Mn efficiency) and also to determine if plant factors later in the growing season were more associated with Mn efficiency than those during seedling growth.

8.2 GENERAL CONCLUSIONS

8.2.1 Genotypic variation for Mn efficiency

In the pot studies, only barley roots were able to acquire significant amounts of Mn from the acutely Mn deficient soil used in the pot experiments, and there were no wheat cultivar differences. Therefore any seedling screening technique for cultivar variation in Mn efficiency within wheat, triticale and rye should consider a less severely Mn deficient soil than is suitable for barley. Larger plant/soil ratios than used here should be used in order to avoid a pot bound root system, part of which would not be participating in Mn uptake. The results obtained from the screening of wheat-barley addition lines suggested that Mn-efficiency characters could be transferred from barley to wheat, since relative growth at MnO was significantly greater for some of the addition lines than for wheat. However, mechanisms for the higher Mn efficiencies were not clear.

In the field, rye and triticale, as well as barley, were shown to be more agronomically Mn-efficient than wheat, but there were significant cultivar differences. However, the former cereals (except for barley) were not more efficient for Mn uptake during field seedling establishment. Thus it is apparent that results from pot tests are not consistently applicable to the field, and that therefore a simple screening procedure based on such tests will be difficult to develop for cereals other than perhaps barley. Such a discrepency may reflect adaptation of certain cultivars to local climatic conditions enabling them to produce higher grain yields, a factor superimposed upon tolerance to Mn deficiency per se.

8.2.2 Mechanisms associated with Mn efficiency

One factor strongly associated with apparent genotypic variation for seedling Mn efficiency was the Mn content of sown seeds. The impact of seed Mn would of course be expected to be more pronounced with an acute deficiency of Mn in the substrate, which was the case with the calcareous soil from Wangary used in these experiments. Irrespective of its effect on genotype ranking for Mn efficiency, knowledge of the seed Mn content is

necessary in order to determine actual Mn uptake. Heritability of seed Mn content could become an important issue if seed Mn content differences between genotypes were sufficiently large to affect Mn efficiency ranking. The genotype with the higher seed Mn content could have a distinct advantage if the extra Mn available from the seed resulted in better root exploration of the soil with consequent improved uptake.

The field experiments reported in Chapter 6 also clearly showed that genotype ranking for agronomic Mn efficiency was influenced by seed Mn content. The experiments of Chapter 7 also showed that Mn application to the seed for sowing could lessen farmer's dependence on soil Mn fertiliser application. Certainly farmers would be advised to import seed of barley and wheat from Mn-sufficient or even Mn-toxic sites if they are considering growing these crops on acutely Mn-deficient sites without Mn fertiliser. One interesting observation was that agronomically Mn-efficient genotypes could be distinquished by their greater response to seed soaking in $MnSO_4$.

The extent of the seedling lateral root system was strongly associated with the greater Mn acquisition of barley compared with other cereals (Chapter 2) and Bodallin vs Bayonet wheat (Chapter 4). It would seem prudent to evaluate genotypic variation for root physical dimensions under Mn deficiency and sufficiency in greater detail (eg. first, second etc. order laterals from seminal and nodal roots and root hairs) in order to determine if causal relationships exist between these paramaters and Mn uptake from Mn-deficient soil.

The experiments outlined in Chapter 3 did not suggest that chemical modification (acidification and reduction) of the rhizosphere was significantly related to the higher Mn seedling Mn efficiency of wheat compared with wheat. Such modification has been shown to be important in the Fe efficiency of dicots. However, it is suggested that quantitative data for chemical modification are now required before definitive statements can be made as its importance in genotype variation for Mn efficiency. Rhizosphere chemistry might be expected to play a more important role in non-calcareous neutral-alkaline soils (Uren 1969).

Differences between wheat cultivars for the activity of photosystem II, as measured by room temperature chlorophyll a fluorescence kinetics, under Mn deficiency, were small (Chapter 4), suggesting that differences in growth reduction between wheat cultivars might have resulted from differences in the activity of other physiological processes (eg. nitrate assimilation) or internal distribution/redistribution of Mn (at the tissue and cellular level). Certainly differences in Mn utilisation efficiencies were shown to exist for these cultivars.

From the field experiments reported in Chapter 6, it is concluded that cultivars with high agronomic Mn efficiency have low plant mortality mid-season as well as high grain yields per plant. Differences in Mn uptake and Mn utilisation after mid-tillering also appear to be associated with Mn efficiency for some genotypes (eg Aroona wheat, S.A. rye in Chapter 6).

8.3 FUTURE RESEARCH

The major screening effort of this thesis centred around wheat. Considerably more wheat cultivars (including, for example, cultivars of Triticum aestivum from overseas and several T. durum cultivars) could profitably be screened for tolerance to Mn deficiency at the seedling stage. A milder level of Mn-deficiency stress than that used in the experiments reported in this thesis should be used so that Mn uptake (rather than seed Mn) will be the determinant of Mn efficiency.

Solution culture experiments should be conducted if it is intended to determine genotypic differences in Mn uptake from known external Mn concentrations; uptake kinetic parameters (eg. Km and Vmax) could also be determined (see for example Landi and Fagioli 1983). However, since the importance of root growth and root morphology as well as rhizosphere effects cannot be adequately evaluated in agitated solution cultures, it would seem necessary to devote considerable effort to the selection of a suitable soil with reproducible and low Mn availability.

If the aim of the screening is to consider Mn efficiency per se it would be more expedient to screen a wide range of both adapted and non-adapted genotypes at the seedling stage. The inclusion of related grass species (eg. Elytigia) and perhaps wheat-Eltrigia crosses could provide potentially more tolerant material (cf. their use by Storey et al (1985) in salt tolerance studies). The limited range studied did not suggest any significant variability in the ability to absorb Mn from very deficient subtrates. Thus in this sense a successful screening procedure was not forthcoming. However, if a non-adapted wheat cultivar was identified with superior Mn uptake ability this character could be incorporated into an adapted cultivar and both pot and field tests conducted to determine its effect on Mn efficiency of the latter cultivar. Future screening should incorporate field trials for the reasons outlined in Chapter 6 and section 8.2.

The results of this thesis infer that pot tests for screening wheat cultivars will only relate to field performance if similar seed sources are used and a less severe Mn deficient soil used than is suitable for barley. It would still appear necessary to analyse plants for Mn content to calculate the amount of Mn uptake and the degree of Mn utilisation, although only shoots or perhaps youngest expanded blades would be sufficient for ranking purposes. The use of relative dry weight as the index of Mn efficiency still requires at least one (or better two) soil applied (or foliar applied) Mn treatments to provide a control with maximum yield. Ideally the most inefficient genotype should yield approximately the same as the inefficient one under normal conditions.

Although most emphasis should be given to Mn uptake it would seem to also study the extent of genotypic differences in the utilisation of Mn in more detail. Efficiency of utilisation has been stressed by Saric (1982).

In order to make more rapid progress in breeding for Mn efficiency for a given cereal, the heritability (and the genetic mechanism) for Mn uptake should be determined. One way that this can be achieved would be to screen the F1 and F2 progeny of a cross between an efficient and inefficient cultivar, bearing in mind the points mentioned above. Such a study was

attempted by the author using Bayonet and Red Fife wheat but the results are not included because of difficulties in dicriminating between seed and soil obtained Mn for individual plants. Removal of a large part of the seed endosperm (to lower the Mn content) was unsatisfactory because this resulted in marked dwarfing of plants.

8.4 REFERENCES

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