Resistance to Take-all Disease by Mn Efficient Wheat Cultivars.

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Declaration

I declare that this thesis is my account of my own work, none of which has been submitted previously in fulfilment of requirements for a degree at any University.

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

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Abbreviations and Terms used

Standard abbreviations for metric measurements are used throughout; for example, **kg** for kilogram, **mm** for millimetre, **D** for day. Other frequently used terms are:

purified water	Distilled and deionised water, having passed through a ultra-purifier Millepore system.
Ggt	*Gaeumannomyces graminis* var.*tritici*, fungal causative of the take-all disease.
+Ggt / -Ggt	Inoculum treatments with/without viable Ggt fungal hyphae.
+Mn / -Mn	Culture (usually soil) treatments with / without Mn added (as $MnSO_4$) as detailed in text. Where more than two Mn treatments are used, they are numbered from **Mn 0** (without added Mn) through **Mn 1** (lowest addition of Mn) to the highest Mn treatment, as detailed in the text.
Mn efficient / Mn inefficient genotype	A comparative term, indicating how successfully plant genotypes yield under Mn limiting conditions, compared to yield under Mn adequate conditions. The Mn efficiency of many cereal genotypes has been assessed in field variety trials (Graham 1990) and these findings have been used to select Mn efficient or inefficient genotypes for the work presented in this thesis.
ns	Non-significant interaction. All statistical analysis was carried out using the GENSTAT 5 package, Rothamsted, 1987.

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Thesis Summary

Manganese (Mn) deficiency has been shown to increase the susceptibility of wheat to infection by *Gaeumannomyces graminis* var *tritici* (Ggt). Screening of a variety of Australian wheat cultivars indicated that Mn efficient wheats (wheats less susceptible to Mn deficiency) were significantly less susceptible to infection by Ggt. These experiments were conducted under controlled conditions, in pots, using Mn-deficient soil.

A comparison of the levels of lignin and phenolics in the roots of a range of wheat genotypes did not show conclusively that Mn efficient genotypes constitutively produced more lignin or phenolics than inefficient genotypes. However, plants grown with adequate Mn had higher levels of lignin and phenolics in roots than plants grown under Mn limiting conditions.

A comparison of different sources of Mn (seed source Mn, soil source Mn and Mn efficient genotypes) on seedling growth and resistance to infection by Ggt did indicate that seed and soil sources of Mn were less effective in protecting the seedling from Ggt infection than the Mn efficiency of the wheat genotype itself. This experiment was conducted under similar conditions to the screening experiments.

Wheat plants from Ggt infested paddocks were assessed for Ggt infection, oxidising ability of Ggt isolated from the infected roots and the Mn content of the shoots. Plants with low root infection had higher levels of Mn in the shoot than plants severely infected. Of the 13 elements analysed, only Mn showed this effect. Plants with the greatest infection of roots were often those from which Ggt isolates of strong Mn oxidising ability were obtained. It seems possible that the oxidising power of virulent isolates of Ggt is sufficient to disrupt Mn availability to the plant roots.

Comparison of a range of Ggt isolates showed further evidence of the positive correlation of the pathogenicity and Mn oxidising ability of Ggt isolates. The rate of oxidation of Mn by a virulent isolate of Ggt was determined using a solution culture method.

These results suggest that competition exists between the wheat host and the pathogen, Ggt, in controlling the availability of Mn to the host. Since Mn is a requirement

for the expression of disease resistance mechanisms, conditions which favour the Mn status of the host are likely to lead to better control of the take-all disease in that crop.

Chapter 1

Literature Review

1.1 Introduction

The disease, take-all, causes yield loss in wheat crops world wide. Since the 1920's extensive screening of cultivars for resistance to take-all has been carried out, but with little success (Nilsson 1969, Penrose 1985, Scott and Hollins 1985). Recently, it has been claimed that there is no resistance to take-all in wheat (Scott and Hollins 1985).

In 1983, Graham proposed that low manganese (Mn) availability was the common factor linking many of the environmental conditions which increase the prevalence of take-all. Further work by Wilhelm (1992) has strongly supported this hypothesis and shown that the availability of Mn^{2+} to the wheat plant is important to the plant's resistance to infection by the take-all fungus. Plants grown under Mn limiting conditions, lacking adequate Mn supply to the roots, are more susceptible to infection by take-all disease.

Genotypic differences in Mn efficiency between wheat cultivars (or genotypes) have been observed; that is, under Mn limiting conditions, Mn efficient genotypes are less susceptible to Mn deficiency than other, inefficient genotypes, and may yield as well under Mn limiting conditions as under Mn adequate conditions (Graham 1984). It is my hypothesis that Mn efficient wheat cultivars have a greater resistance to the disease take-all than Mn inefficient cultivars.

Accordingly, the major topics discussed in this literature review are, firstly, Mn availability to wheat in highly alkaline soils and the need for Mn efficient genotypes in these soils.

Secondly the disease take-all, its causative organism, *Gaeumannomyces graminis* var *tritici* (Ggt) and methods used for its control.

Thirdly the extensive interaction of Mn and take-all, which leads to discussion of the hypothesis that:

Manganese efficient lines of wheat are better able to withstand infection by the fungus, Ggt, and so are less susceptible to yield loss due to take-all.

This literature review was finalised in August 1990. Work relevant to the hypothesis published after this date has been taken into consideration within the body of the thesis.

1.2 Manganese

1.2.1 Manganese in soil

Manganese exists in soils in at least three oxidation states (of a possible seven). Its most important forms in soils are:

Mn^{2+}	MnII	
MnOOH	MnIII	manganite
Mn_2O_3	MnIII	hausmannite
MnO_2	MnIV	pyrolusite

and in calcareous soil

$MnCO_3$	MnII	manganocalcite,

all of which are insoluble and crystalline, with the exception of Mn^{2+}, which exists as ions in the soil solution and exchange complex. Except at pH values below 5.0, the oxidation of Mn^{2+} to Mn(III) and Mn(IV) oxides is thermodynamically favoured in soils and natural waters (Norvell 1988), ie:

$$\mathrm{MnII} \xrightarrow[\text{pH 5.5}]{} \mathrm{MnIII} \xrightarrow[\text{pH 8.0.}]{} \mathrm{MnIV}$$

Furthermore, oxidation of Mn^{2+} at high pH is autocatalytic (Leeper 1970, Nealson et al. 1988) as the initial oxidation process results in adsorption of more Mn^{2+} (and other cations) onto the most reactive and freshly precipitated Mn oxide (Uren 1981, Nealson et al. 1988). The addition of Mn^{2+} as $MnSO_4$ to alkaline soil only briefly increases the level of soluble Mn in the soil solution; within days the Mn^{2+} is oxidised or trapped by ligands in humic matter (Marcar 1986).

Despite a high activation energy requirement for its reduction, Mn is highly active in the biotic interface, and many soil micro organisms are known to be oxidisers or reducers of Mn (Nealson et al. 1988).

1.2.2 Manganese in plants

Manganese is an essential trace element for the growth of higher plants (McHargue 1922, Samuel and Piper 1929) and one which has an important influence on many physiological and biochemical pathways (Clarkson 1988).

Manganese is vital for photosynthesis, being an integral requirement of a protein complex closely linked with photosystem II, and the splitting of water molecules. A superoxide dismutase containing Mn is thought to play roles in photorespiration and removal of H_2O_2 and O_2^- from the cell (Burnell 1988).

Manganese is necessary as an activator of many enzymes and also as a cofactor to many biosynthetic processes, especially within the production of lignin and phenolics. Mn is a cofactor for phenylalanine ammonia-lyase (PAL), an enzyme which has been proposed as having a regulatory role in the metabolism of phenolics, including lignin (cited by Kogel et al. 1985). A Mn activated peroxidase is also important in the lignification of plants (Kogel et al. 1985). Mn is involved in the synthesis, but also, and more precisely, in the oxidation of the auxin, indole acetic acid (IAA) (Campbell and Nable 1988).

At least two photosynthetic enzymes from C_4 plants are also known to have an absolute requirement for Mn (Burnell 1988).

1.2.3 Uptake of Mn by plants

1) Plant available Mn

It is generally recognised that uptake of manganese by plants is only of the ionic form, Mn^{2+}, which is present in the soil solution. Oxides are not utilised, except upon reduction to the Mn^{2+} form which is then made available to the plant. However, in high pH soils, Mn^{2+} is subject to oxidation and is less available to the plant from the soil solution. Other environmental conditions in the soil can also alter the availability of Mn^{2+} to the plant; soil temperature, water content and aeration of the soil, discussed in section 1.2.4 (1).

2) Mechanism of Mn uptake

Several mechanisms of Mn uptake have been put forward (Bowen 1981), the most recent and energetically supportable is by Nable and Loneragan (1984 a), as follows:

Mn^{2+} enters the cell by diffusion, under the electric potential difference set up across the cell membrane. Within the cytoplasm Mn is (energetically) removed to the vacuole (the 'labile' pool suggested by Munns et al. (1963) and is slowly released, to be transported to the shoot in the transpiration stream.

The uptake of Mn is thus controlled only by the availability of Mn^{2+} to plant roots and where in ample supply, Mn can accumulate within the plant to toxic levels.

There are many reports of mutual inhibition of nutrient uptake concerning Mn; with Fe (Zaharieva et al. 1988), Ca, Mg, Si, Co and Zn (Kabata-Pendias and Pendias 1984, Ruano et al. 1987, Foy et al. 1978). The existence of these interactions is widely accepted, but due to the inconsistent and complex nature of the evidence of these interactions, they are not considered further in this review.

Unlike Fe (Marschner et al. 1986) or Zn (Zhang et al. 1989), no specific chelators or ionophores have been found for Mn, to promote active uptake of the cation. There has been a suggestion that certain phenols may be specific chelators of Mn^{2+}, at least within the cell (Brown et al. 1984). Root exudates have been shown to increase the pool of available Mn by reducing MnO_2 (the most readily reduced form of oxidised Mn) (Uren 1981), but do not appear to be specifically linked with Mn uptake.

3) **Transport of Mn**

Manganese is not (or very poorly) retranslocated in the plant from senescing tissue. Nable and Loneragan (1984 a, b) discounted previous evidence of retranslocation of manganese in the plant (Boker 1960, Vose 1963) as loss from senescing leaves by leaching or as faulty interpretation. This infers that manganese is constantly demanded for new plant tissue, as it cannot be remobilised from old tissue.

It has been proposed that under Mn deficiency, stored vacuolar Mn is released into the transport system from root cells (Nable and Loneragan 1984 b, Vose 1963). The storage of Mn within roots has been documented (Vose 1963), but without evidence of how storage occurs or is maintained.

1.2.4 Soil Mn availability

1) Effects of soil conditions

At high pH and especially in calcareous soils, Mn^{2+} is immobilised, under chemical conditions which actively maintain Mn in higher oxidation states, and which also immobilise any Mn^{2+} added to the soil solution (Marcar 1986, section 1.2.1). Mn^{2+} added to these soils as fertiliser has no permanent effect on Mn availability, being rapidly oxidised and immobilised (Norvell 1988). These soils may have high levels of total Mn, but plants growing in them may be Mn deficient, the 'plant available' Mn levels being inadequate (Leeper 1970). Examples are seen in the alkaline and calcareous soils of Eyre and Yorke Peninsulas in S.A. where chronic Mn deficiency occurs in wheat and barley crops (Reuter 1972, Graham et al. 1983).

In other situations, the liming of acid soils may reduce the level of plant available Mn, and create Mn deficiency in plants (Norvell 1988). In contrast, acidification of the soil by the use of urea or ammoniacal fertilisers has been known to create Mn toxicity in the soil, where soil pH was previously moderate and Mn at levels sufficient for plants (Leeper 1970).

Manganese mobility, and thus the availability of Mn^{2+} to the plant, is also affected by organic matter in the soil. Mn^{2+} is readily complexed in the presence of organic compounds and may become more immobile as organic matter increases in the soil. A lack of organic matter in the soil under high pH conditions maintains the immobility of the manganese as an oxide.

2) Effects of climatic conditions

Seasonal conditions can alter the severity of the immobilisation of Mn^{2+} by effects upon plant metabolism which affect active uptake. High temperatures increase the reduction of Mn oxides in the soil (Ross and Bartlett 1981). Cold conditions tend to decrease plant uptake of Mn and may increase Mn deficiency in the plant (Batey 1971).

Oxygen levels in soil also affect Mn availability. Compaction of soil (light soils in particular), tends to increase the availability of Mn to the plant (Uren 1981, Leeper 1970), but whether by increasing the contact of roots to soil particles or by decreasing the soil aeration and oxygen levels, it is difficult to prove.

3) Effects of bacteria and fungi

Soil micro-organisms have three major effects upon plant nutrition within the rhizosphere (Sadasivan 1965): competition for available nutrients, mobilisation of unavailable complexes, and immobilisation of complexes.

The oxidation state of Mn in the soil, and thus its availability to the plant, is affected by the action of micro-organisms capable of oxidising or reducing Mn. Although these heterotrophic bacteria and fungi have been widely isolated and often characterised, neither the chemical mechanism of the reactions, nor the reason for the action has been fully explained (Nealson et al. 1988, Ghiorse 1988).

Possible reasons for the oxidation of Mn by micro-organisms are:

1) it is an energy releasing process; an exothermic reaction releasing 18.2 kcal per mole Mn (but it is unknown whether the energy is utilised),

2) that since Mn has mutagenic effects at high levels, oxidation is a self-protection mechanism by the organisms,

3) that other organisms are disadvantaged by the removal of Mn from the soil solution by oxidation, an antibiotic effect, analogous to the action of siderophores produced by some bacteria and fungi (Ghiorse 1988).

A more definite role for Mn oxidation has been identified in white rot fungi, as part of an extracellular lignin degrading pathway. Mn peroxidases are prominent in this fungal process (Kirk and Farrell 1987). The action of autocatalytic Mn oxidation increases energy input to oxidise (non-enzymic) bonds between lignin subunits and release them from the complex aggregate. Addition of H_2O_2 and Mn increases the rate of lignin degradation through "non-biological combustion" (Kirk and Farrell 1987).

Even less is known of the reduction of manganese by soil micro-organisms, due in part to the difficulty of controlling experiments (Nealson et al. 1988). It has been suggested that Mn oxides are utilised as terminal electron acceptors by micro-organisms (Stone 1987) and reduced in the process.

The uptake of manganese from soil by plants may be enhanced by the presence of Mn oxide-reducing bacteria (Barber and Lee 1974). It has been shown that phenolic exudates from plant roots also reduce Mn oxides within the rhizosphere (Bromfield 1958, Godo and Reisenauer 1980, Uren 1981, Jauregui and Reisenauer 1982), so the action of these bacteria is merely beneficial, not essential, to the plant.

1.2.5 Mn deficiency in the plant

In an early paper on Mn deficiency, Samuel and Piper (1929) showed that once the Mn present in the seed was exhausted, all species tested showed symptoms of Mn deficiency, and growth in some plants was almost immediately halted.

1) Symptoms in cereals

The precise symptoms of Mn deficiency are dependent upon the plant species, but include, in general, an interveinal chlorosis, seen in most cereals as pale striations or 'tram tracks' between the midrib and other veins along maturing leaves. Other typical symptoms are dark brown spots on leaves and premature senescence of older leaves. Growth is reduced in both root and shoot (Samuel and Piper 1928, 1929) as is tillering in barley (Reuter 1972) and wheat (Marcar and Graham 1987 a). Mn deficiency can also delay maturity in plants (Hannam et al 1987).

'Grey speck' disease in oats, characterised by necrotic patches, mottling of the leaves and poor growth, was diagnosed as Mn deficiency by Samuel and Piper (1928) and since then many other diseases have been either diagnosed as Mn deficiency, or linked with Mn deficiency, as application of Mn salts reduce the symptoms or prevalence of the disease (Graham 1983).

Mn deficient plants are weak and easily water stressed, and under very severe conditions of deficiency, death of the growing shoot occurs (Samuel and Piper 1929).

2) Effects on plant physiology

Due to the many biochemical requirements for Mn, deficiency has a profound effect on the plant. Mn deficiency reduces photosynthesis and the fixing of CO_2. By implication, respiration is also reduced. Photodestruction increases, due to lack of superoxide dismutase. As a less specific effect, the assimilation rate of nitrate (and other nutrients) is lowered. All of these factors contribute to the chlorosis and weak growth of chronically Mn deficient plants.

Mn deficiency also reduces production of phenolics, aromatics and lignins (Hirsch et al 1978, Brown et al. 1984) having a specific role in the metabolism of phenolic compunds (Hirsch et al 1978). These compounds are implicated as having roles in disease resistance (Friend 1981): Mn deficiency effectively weakens the plant against disease (Graham 1983).

Lastly, Mn deficiency (and toxicity) creates an imbalance in hormone levels (Campbell and Nable 1988) which disrupts growth throughout the plant.

3) Localised effects on plant growth

Overall, plant growth is reduced in Mn deficient conditions. However, reduction in root growth due to Mn deficiency increases the difficulty of Mn^{2+} uptake, which can only compound the problem of Mn availability to the roots, especially under high pH conditions.

Reuter (1972) suggested a major limitation of Mn uptake from Mn deficient soils is the ability of plant roots to encounter available pools of Mn within the soil. 'Available' Mn in this case refers to Mn^{2+} or MnO_2, as MnO_2 is the most readily reduced form of oxidised Mn (Uren 1981). The area of greatest Mn oxide reduction is in the rhizosphere of recently matured root cells, behind the root tip (Uren 1981). Page (1961) showed that more Mn was absorbed by the root tip than the remainder of the root. Thus limiting root growth of the root tip into new pools of Mn may limit Mn uptake by the already deficient plant.

Nable and Loneragan (1984 a) suggested that an uneven distribution of Mn in soils may lead to localised Mn deficient areas in the root system, as Mn is not retranslocated in the phloem. These areas of Mn deficiency in the root may be more susceptible to damage, leakage of nutrients and infection by soil pathogens.

4) Mn efficient plants

Plants showing tolerance to Mn deficient soils (ie Mn efficient plants) generally appear to have a larger root system (Marcar and Graham 1987 b) and more Mn in roots than in shoots (Vose 1963). With greater soil-to-root contact for excretion of root exudates, via more root hairs, and presumably a greater output of root exudates reducing Mn oxides (Reuter 1972), more Mn^{2+} is made available to the plant under Mn limiting conditions.

Marcar (1986) carried out a comparison of MnO_2 reduction by root exudates, from intact roots of wheat, barley and lupin plants, using the agar technique of Marschner et al. (1982). The zone of MnO_2 reduction around the roots was much larger for lupins than the less Mn efficient cereals.

Comparisons between the root growth of Mn deficient barley and wheat showed that the more Mn efficient species, barley, has a more extensive root system with more lateral branching (Marcar and Graham 1987 b). The larger root system was suggested as a possible mechanism of Mn efficiency in barley, since no difference between wheat and barley was observed in the reduction of Mn oxides by root exudates using the agar technique.

Genotypic differences in Mn efficiency, between and within species, are well known (Samuel and Piper 1929, Reuter 1972, Graham 1984, Harbard 1991). There are several possible mechanisms of Mn efficiency in wheat which will be discussed in a later section of this review, with reference to the advantages of breeding for Mn efficiency.

1.3 Take-all

1.3.1 *Gaeumannomyces graminis* var. *tritici*.

Gaeumannomyces graminis (Sacc) von Arx & Olivier var *tritici* Walker (Ggt) is a homothallic ascomycete, haploid throughout its vegetative phase, forming a transient, diploid zygote and ascospores. Asexual phialidic conidia are produced in soil but have not been found to germinate. Those conidia formed in culture are germinable and predominantly uninucleate (Asher 1981).

Although direct evidence is sparse, Ggt is thought to exist as a heterokaryotic mycelium (existence of nuclei with different genotypes in the same cell or in a common cytoplasm) which has advantages over the homokaryotic, since a genetically heterogeneous vegetative mycelia may exhibit greater plasticity and be able to respond more rapidly to selection (Asher 1981).

There is evidence of widespread intra-isolate variation; seen by examination of uninucleate conidial progeny for variation in virulence; and of hyphal sections formed by maceration of mycelium of a single isolate (Asher 1981). On growth of these hyphal sections, large differences in pathogenicity against seedlings were obvious.

The most probable origin of heterokaryosis is through the occurrence and accumulation of mutations. Anastomosis of these cells with new hyphal tips and/or movement of virus-like-particles (which exist in Ggt) spreads these mutations and combinations through new parts of the mycelium (Asher 1981). A high frequency of contact between strains in soil is unlikely, compared to airborne pathogens (Asher 1981).

1.3.2 Aetiology: infection process and disease

Ggt, the causative organism of the disease take-all, is found world-wide and can cause immense damage to cereal crops. Surviving in soils as a saprophyte, its action as a root parasite and pathogen is well known. The saprophytic growth of Ggt has recently been shown to be much more vigorous than was previously thought (Glenn and Parker 1988). Saprophytic growth of up to 6 cm in soil, from the inoculum point, resulting in infection of wheat roots was observed in a specially designed pot experiment. Leading hyphae of Ggt can grow trophically, towards a live host root from the inoculum source, to an extent of about 12 mm, while trophic growth by other soil fungi is limited to 4 or 5 mm (Gilligan 1980). Under conditions favouring pathogenicity (ie presence of live susceptible roots), the fungus is able to grow from root system to root system and spread up to 1.5m in diameter from an original inoculation point (Wehrle and Ogilvie 1956) within 8 months.

1) Infection of roots

Once leading hyphae have come into contact with the living root, darkly pigmented ectotrophic runner hyphae grow along the root surface or within the root cortex, at stages developing finer, hyaline hyphae which invade the root cortex, endodermis and the stele. Progressive invasion and colonisation of the phloem and xylem, through the stelar lesions, plug these vessels with hyphae. Translocation within the phloem and passage of water in the xylem is stopped below the plug, having a similar effect upon the root as amputating it at that point (Simmonds and Sallans 1933). New roots grown in response to the "amputation" may also be colonised, since the hyphae continue to grow up the root system toward the crown. Invasion and infection of the crown usually leads to plant death.

2) Disease expression

Depending on the number and extent of these stelar lesions, the infected plant suffers water and nutrient stress, resulting in reduced growth and yield. Severe infection of seedlings or young plants within an area of a paddock can stunt or kill the plants and 'take all' the yield in that patch. Less severe infection or infection of older plants allows normal growth until the plant nears maturity, when hot dry conditions cause water stress with which the impaired root system cannot cope. These plants are visible as 'whiteheads' in the crop, a symptom also known as 'hay-die'.

Moderate infection, whether due to a Ggt strain of low virulence, or conditions which favour plant growth above fungal growth, may be sub-clinical (Asher 1981) and never expressed as take-all or hay-die. However, the crop yield can still be considerably reduced from its potential, due to restricted root systems and diversion of resources into infection escape mechanisms, such as the growth of new roots, rather than shoot growth (Rovira and Venn 1985).

3) Effect of environmental conditions on disease

Severity of take-all disease is dependent on environmental conditions, being most severe where soil moisture is high and where soils are of neutral or alkaline pH. Thus, in irrigated wheat crops and in alkaline or limed soils in high rainfall areas, take-all has become devastating (Reis et al. 1982). Take-all is seldom severe in acidic soils and, where present at low pH, is regarded as an anomaly in the aetiology of the disease (Asher 1981).

Because of the interaction of disease severity with soil pH and other environmental conditions it is not always easy to differentiate between genetic variation of the pathogen and the variability in disease often caused by seasonal conditions.

Losses from take-all are generally severe when plants are deficient in any of the essential mineral elements (Reis et al. 1982, Wood and Robson 1984), but in particular when deficient in Mn (Graham 1983, Graham and Rovira 1984, Huber et al. 1987, Wilhelm et al. 1988). Addition of Mn salts to Mn deficient soils reduces take-all disease (Wilhelm et al. 1988). Use of soil fertilisers and other methods for the control of take-all disease are discussed in a later section.

1.3.3 Ggt inoculum

1) Inoculum survival

The saprophytic survival and growth of Ggt, in the debris of the previous host, maintains the fungus in the soil as viable inoculum. Further infection of host plants less susceptible than wheat (barley, rye, grasses) is less frequently expressed as take-all or hay-die disease, but ensures the survival of inoculum in the soil. Infection of volunteer cereals, especially wheat, is a more definite means of inoculum survival and spread (Brassett and Gilligan 1990).

Viability of Ggt inoculum decreases with time and certain environmental conditions. Warm, moist conditions decrease the viability of Ggt inoculum at the soil surface and within the soil (Garrett 1938, Kollmorgen 1985 a).

The horizontal distribution of Ggt within a paddock can vary dramatically (Cotterill and Sivasathamparam 1989 a, b) but in the soil profile, viable Ggt inoculum exists mostly in the top 10 - 15 cm of the soil (Kollmorgen et al. 1985 a). Deeper in the soil profile, pathogenic viability is lost with age, and the vegetative mycelium decomposes (Hornby 1981).

The survival of Ggt inoculum in the upper 15 cm of soil may be reduced by cultivation and crop rotation, but is also reduced by the action of soil bacteria antagonistic to Ggt. Antagonistic bacteria, particularly fluorescent pseudomonads, exist in take-all suppressive soils (Rovira and Wildermuth 1981) and are the subject of extensive study as biological controls for take-all (Weller et al. 1985).

Despite these various natural reductions in inoculum level, the survival rate of viable inoculum is usually sufficient to cause visible disease in subsequent wheat crops, often as whiteheads.

2) Pathogenic variation

Differences in pathogenicity have been observed in isolates of Ggt from roots of wheat and ryegrass plants (Dewan and Sivasithamparam 1990). Some isolates of Ggt from wheat plants were as pathogenic upon ryegrass as wheat, while other isolates, though pathogenic on wheat, were ineffective against ryegrass. Pathogenicity was measured as an infection index of plant roots. Isolates of Ggt from ryegrass were all more pathogenic on wheat than on ryegrass. Similar observations have been made recently by Hollins and Scott (1990) on isolates from rye and wheat roots.

Tests for pathogenicity of different isolates of Ggt (as opposed to ratings of yield loss due to the disease caused) tend to be in terms of the fraction of roots infected and the number and/or size of stelar lesions made on the roots. A suggestion by Garrett (cited by Walker 1981) was that the pathogenicity of an isolate be measured by the rate at which the ectotrophic runner hyphae reached the crown of the plant, since at this point, all roots can be infected and any further adventitious roots emerging will not escape infection. This may require continual assessment of the growth of hyphae along the roots, and does not take into account whether any actual invasion of the roots' vascular system occurs. Additionally, in Australia, infection of the crown is rarely seen in the field (Cotterill and Sivasithamparam 1989 a, Rovira and Wildermuth 1981), so perforce, the other methods are used.

3) Pathogenic attenuation

It is widely accepted that the continued subculturing of Ggt isolates can lead to a loss or attenuation of pathogenicity (Cunningham 1981). There is also evidence of attenuation of pathogenicity in the field (Hornby 1981). Virulence tests show that ageing inoculum in the field loses its capacity to infect and becomes less able to withstand higher temperatures. It seems likely that much of what is detected in host infection tests at these higher temperatures (eg 20 °C) may never function as inoculum in the field (Hornby 1981). Thus, greater survival of inoculum is not the only means by which severe disease expression occurs.

Although pathogenicity of inoculum may attenuate while the fungus is surviving saprophytically, it is assumed that upon contact of the Ggt hyphae with root exudates and/or the infection of roots, selection within the vegetative mycelium for pathogenic characteristics takes place. Hornby (1981), cites unpublished data of Thorpe (1970), where an increase in take-all activity did not seem to be solely a function of increased inoculum. Monocropping with susceptible crops has been shown to increase the level of viable inoculum in the soil (Kollmorgen et al. 1985 b) (prior to any development of take-all decline) but may also increase the level of pathogenicity of the inoculum population.

1.3.4 Take-all control

The control of take-all in the field is a difficult task and traditionally crop rotation practices have been used. Control of the disease by fungicides, biological control using fungi and bacteria, and control by crop nutrition methods are also practised.

1) Cultivation practices and crop rotation

Traditionally take-all disease has been controlled by methods of crop rotation, cultivation practices and the use of cleaning or break crops between susceptible cereal crops.

Cultivation to a fine tilth, or deep plowing and soil inversion have been shown to be effective measures in reducing the saprophytic survival of Ggt (Kollmorgen et al. 1985 a) but this practise is "inconsistent with the need to till the soil economically and with minimal risk of erosion" (Kollmorgen et al. 1985 a). While a firm seed bed has long been advised for reduction of take-all, the use of direct drilling techniques, while reducing soil erosion, has had varied effects on take-all; both increasing, maintaining and reducing take-all levels (Rovira and Venn 1985).

Fallowing treatments must be carefully monitored to be effective in reducing the level of active take-all inoculum. The carry over of inoculum in susceptible volunteer (self sown) cereals has been shown to increase take-all in subsequent susceptible crops (Brassett and Gilligan 1990). Removing grasses and volunteer cereals with the use of herbicides has been shown to reduce take-all in subsequent wheat crops (Geddens et al. 1990, Kollmorgen et al. 1985 a).

Rotation of wheat with legume, legume-pasture, and oats as break crops (crops not susceptible to Ggt) is, again, traditional but the effectiveness of these crops as cleaning crops (crops which reduce the level of Ggt inoculum in the soil) has recently been questioned.

Work by Kollmorgen et al. (1985 a) showed that the saprophytic survival of Ggt was increased by rotations of triticale and wheat and maintained by rotations of oats, barley and medic-grass pasture. Rotations including legumes, rapeseed and fallow reduced the saprophytic survival of Ggt. The singular benefit of N-fixation by legume crops in reducing take-all is called into doubt, as a similar yield increase in the subsequent wheat crop and a Ggt inoculum reduction was observed when using rape seed as a break crop (Kollmorgen et al. 1985 a).

Work by Maas and Kotze (1990) showed that while rotations of soybeans, sunflower, and fallow treatments increased take-all disease in wheat, break crops of maize and particularly tobacco not only reduced the disease, but increased the number and kinds of micro-organisms in the soil, which were presumably antagonistic to Ggt inoculum.

2) **Fungicides**

The fungicide triadimenol, applied in seed coating treatments, has been shown to suppress take-all in the field (Smiley et al. 1990). However, tillering, and thus grain yield, was reduced due to the phytotoxic action of the seed-applied fungicide.

The use of soil fungicides and fumigation techniques as sole measure of control against take-all is of doubtful long term benefit and the application of such chemicals to soil is not a preferred action of today's 'environmentally conscious' farmer. Although there are reports of take-all being controlled by fungicides such as triadimenol, benomyl and nuromyl (Bateman 1985, Rovira and Whitehead 1985, Bateman 1989, Smiley et al. 1990), they are of limited use and are regarded as effective only if used as part of an integrated program, including crop rotation, crop nutrition and the possible use of biological control (Bateman 1989).

3) **Biological control**

The thrust of biological control of take-all disease rests upon the phenomenon of take-all decline - the reduction in severity of disease expression after long term, continuous cropping with wheat, due to an increase in soil microflora populations, assumed antagonistic to Ggt. Take-all decline is not readily found in Australia. Long, hot, dry summers are assumed to reduce the populations of take-all suppressive soil micro-organisms and remove their beneficial effect (Cook and Rovira 1976). However bacteria and fungi isolated from take-all suppressive soils are frequently found to be antagonistic to Ggt and are proposed as biological control agents (Rovira and Wildermuth 1981, Weller et al. 1985). Of recent interest is *Trichoderma konigii*, an antibiotic-producing soil fungus which is extremely effective, in vitro, against six fungal soil pathogens, including Ggt (Simon 1989). The weakly pathogenic *Phialophora radicicola* - a fungus with similar action to Ggt - has been proposed as a biological control agent (Deacon 1976). Prior infection with *P. radicicola* has been shown to improve the resistance of roots to subsequent infection by Ggt (Cowan 1978).

Extensive work has been carried out on bacteria, mostly fluorescent pseudomonads, which have been isolated from take-all suppressive soils and found to reduce infection of wheat plant roots by Ggt and reduce take-all disease symptoms (Weller et al. 1985, Cook and Rovira 1976). The action of these bacteria against Ggt has generally been attributed to the production of antibiotics and siderophores, which are proposed to act against the pathogen.

The take-all suppressive bacteria *Pseudomonas fluorescens* strain 2-79 has been shown to inhibit the growth of Ggt *in vitro* and suppress the infection of wheat roots by Ggt in pot and field experiements. However, the effect of the antibiotic phenazine-1-carboxylic acid, purified from *P. fluorescens* strain 2-79, does not account fully for the suppressiveness of the pseudomonad on Ggt growth (Thomashaw and Weller 1990). The suggestion that siderophores produced by the bacteria further reduced the growth of Ggt (Thomashaw and Weller 1990) were rejected by Brisbane and Rovira (1988). Brisbane et al. (1989) observed no *in vitro* reduction of Ggt growth by a purified siderophore from *P. fluorescens* strain 2-79. In addition, contrary to previous results by Weller et al. (1985), no reduction of take-all by *P. fluorescens* strain 2-79 was observed *in vivo*, in any of three different soil types used, except in the presence of the fungicide biteranol. Plant growth was not improved by the presence of *P. fluorescens* strain 2-79 in the absence of Ggt inoculum.

4) Plant-beneficial micro-organisms

Beneficial micro organisms reduce take-all disease by increasing the growth of the whole plant, improving yield and aiding the escape of roots from infection by the Ggt fungus by increasing root system size (Ghisalberti et al. 1990). Plant growth promoting rhizobacteria and beneficial soil fungi create a general antagonism to root disease rather than a specific antagonism to Ggt (Cook and Rovira 1976). Disease may be suppressed, but whether a consequent reduction of Ggt inoculum occurs in the field is debatable (Simon and Sivasithamparam 1989), despite many of these microorganisms showing antagonism to Ggt *in vitro* (Dewan and Sivasithamparam 1988, Ghisalberti et al. 1990).

Many of these plant beneficial bacteria (and also take-all suppressive bacteria) are known to be reducers of Mn oxides (Huber and Wilhelm 1988) and may stimulate plant growth by improving the level of plant available Mn in the rhizosphere.

Take-all disease has been (indirectly) reduced by infection of wheat roots by a vesicular-arbuscular mycorrhiza (VAM) (Graham and Menge 1982). Under phosphate (P) deficient conditions, VAMs increase the availability of P to the roots. VAM infection also significantly increased uptake of Zn, under Zn deficient conditions, in citrus (Tinker and Gildon 1983) and of Mn, in Mn deficient conditions, in wheat and barley (Marcar 1986). The increased uptake of these mineral nutrients is probably indirect due to increased P uptake, but the overall effect is significant.

Some isolates of Ggt itself have been found to be plant beneficial: Maas et al. (1989) reported a non-pathogenic Ggt isolate which increased wheat plant growth compared to the uninoculated control plants. Dewan and Sivasithamparam (1990) isolated Ggt from rye roots which increased rye plant growth compared to an uninoculated control. The same isolate was pathogenic on wheat.

5) Crop nutrition

The prevalence of the disease take-all in low nutrient soils has been reported since 1940's (Garrett 1941, 1948) yet only recently has crop nutrition been seriously investigated as a control measure against the disease. Maintenance of plant growth under infection stress, by adequate crop nutrition, is regarded as the means by which most elemental fertiliser additions reduce take-all disease (Graham 1983), rather than a fungistatic effect upon the Ggt inoculum itself. In a field and pot study, Reis et al. (1982) showed that both macro and micronutrients were effective in reducing take-all in wheat. P and Zn gave the best field responses, but the pot trials also showed N, Mg, Cu and Mn to also be effective in reducing take-all. However, the levels of nutrients added to soil in Reis' experiments were extremely high (up to 4 x Hoagland's solution) and may have had debilitating, toxic effects upon the fungal inoculum as much as benefiting the plants.

Results of a more moderate study show the effect of Cu on take-all is small (Wood and Robson 1984), as a treatment considered luxuriant in Cu only reduced infection of roots by 10% from the nil Cu treatment, where plant growth was limited by Cu deficiency. Adequate plant nutrition in the higher Cu treatments would have strongly influenced the resistance of plant roots to infection.

Recent studies of the effect of P on take-all (Brennan 1988, 1989) generally indicate that plant growth is improved with sufficient P and the stronger plant has a greater ability to escape infection by Ggt. Increased uptake of other nutrients is a side effect of increased P nutrition (Section 1.3.4 (4)), and thus increase the chance of roots escaping infection.

The causality of take-all reduction by fertiliser addition can be difficult to unravel. Reduction of take-all by the application of Cl to soil was considered to be due to an improvement in the water relations of the plant, relative to the Ggt inoculum (Powelson et al. 1985). The low disease levels were seen as being due to adequate plant nutrition, rather than any effect of the fertiliser addition upon Ggt inoculum. Interestingly, Cl added to soil inhibits nitrification (Powelson et al. 1985) and the inhibition of nitrification has been shown to decrease take-all disease (Huber et al. 1968, Huber 1989).

5) i Nitrogen

Early reports on the effects of N fertiliser additions on take-all showed an increase in disease, but an accompanying increase in plant growth, which allowed roots to escape from infection. Yields were slightly better than those without N (Garrett 1941). Reis et al. (1982) showed that a similar percentage of roots per plant was found at a range of N levels, but that the number of roots per plant increased with N fertiliser addition.

These experiments were carried out using NO_3^- - N, but Huber et al. (1968) showed that take-all disease was increased by NO_3^- - N and reduced by fertilising with NH_4^+ - N. Further work by Smiley and Cook (1973) showed that the alkalinisation of the rhizosphere caused by uptake of N as NO_3^- appears to be sufficient to predispose roots to infection by Ggt. Uptake of NH_4^+, however, especially when nitrification is inhibited with nitropyrin (Huber and Dorich 1988), acidifies the rhizosphere, and infection by Ggt is less successful under the lower pH conditions.

Nitrification in soil has been associated with an increase in take-all (Huber and Wilhelm 1988). Application of nitrification inhibitors or NH_4^+ stabilisers, such as nitropyrin or N-serve not only reduce the incidence of take-all, but may increase plant growth significantly (Huber and Dorich 1988).

5) ii Manganese

The availability of Mn to plants is affected by different sources of N fertiliser. Uptake of NO_3^--N raises the rhizosphere pH, decreasing Mn^{2+} availability, while NH_4^+ uptake lowers rhizosphere pH and Mn becomes more available to plant roots. Nitrification, when not chemically controlled, is increased by the same environmental conditions which decrease the availability of Mn^{2+} in soil. The reduction of take-all by NH_4^+ fertiliser application and inhibitors of nitrification may, in reality, hide an interaction between Mn and take-all disease (Huber and Wilhelm 1988).

Take-all disease has been significantly reduced, in pot and field experiments, by the application of Mn salts (Graham and Rovira 1984, Wilhelm et al. 1988, Huber 1989). Yield reduction (due to take-all) was significantly reduced upon the application of Mn fertiliser to Mn adequate soil in the field (Webb and Graham 1990). The application of MnO_2 fertiliser to Mn deficient soil did not reduce take-all in the field (Wilhelm et al. 1988). Immobilisation of rhizosphere Mn, by oxidation or chelation, has been shown to increase take-all (Huber 1987). Thus the effect Mn on take-all disease is likely to be related to the availability of Mn to the plant roots, in the form Mn^{2+}.

1.4 Take-all and Manganese

The relationship between Mn availability and prevalence of take-all disease is observable over a range of environmental conditions and agricultural practices. This relationship is best presented by the extensively researched table by Huber and Wilhelm (1988), reproduced below (Table 1.1), which contains many points mentioned earlier in this review.

Table 1.1 Similarity of conditions affecting take-all of wheat, nitrification and the availability of Mn. Taken from Huber and Wilhelm (1988) (Table 2)

Condition	Effect on		
	Take-all	Nitrification	Mn Availability
Acid pH soils	Decrease	Decrease	Increase
Ammonium N	Decrease	-	Increase
Nitrification inhibitors	Decrease	Decrease	Increase
Chloride fertilisation	Decrease	Decrease	Increase
Oats precrop	Decrease	-	Increase
Manganese fertilisation	Decrease	-	Increase
Tolerant cultivars	Decrease	-	Increase
Lupin precrop	Decrease	Decrease	-
Alkaline soils	Increase	Increase	Decrease
Liming	Increase	Increase	Decrease
Nitrate N	Increase	Increase	Decrease
Loose seedbed	Increase	Increase	Decrease
'Short' monocropping	Increase	-	Decrease
Dense seeding	Increase	-	Decrease
Manuring	Increase	Increase	Decrease
Soybean/alfalfa precrop	Increase	Increase	Decrease
Cool, wet soils	Increase	-	Decrease

Both the expression of take-all disease (Asher 1981) and the nature of Mn^{2+} availability in soil (Norvell 1988) are highly variable. However, in the face of such a large body of evidence, it is highly likely that there is a relationship between Mn nutrition and take-all which may be exploited in efforts to reduce the severity of take-all disease.

1.4.1 Possible mechanisms of interaction between Mn and take-all

Graham and Rovira (1984) made three proposals for the mechanism by which Mn reduced the infection of wheat roots by Ggt:

1) Mn may be directly toxic to the saprophytic survival of Ggt.

2) Lignin production is controlled by Mn activated enzyme systems. Ligneous material, part of the plant's wound response may be poorly developed in Mn deficient plants.

3) Acting through the physiology of the plant, Mn affects photosynthesis and thus the rate of exudation of organic compounds. These exudates affect microflora and the growth of Ggt.

These are each considered separately below.

1) Toxicity to Ggt

Mn has been found to be toxic (Timonin et al. 1972) and non toxic (Babich and Stotzky 1981) to various soil fungi. A specific case is the toxicity of Mn to *Streptomyces scabies*, the causal fungus of potato scab (Mortvedt et al. 1963). However, the hypothesis that Mn is directly toxic to Ggt has been discarded. There are several reasons:

1) While take-all symptoms on wheat roots were reduced in pot experiments by rates of Mn less than 10 mg kg^{-1}, rates of more than 150 mg kg^{-1} Mn were required to inhibit fungal growth on agar and in soil sandwiches (Wilhelm 1992). Thus the levels of Mn required to reduce fungal growth (on agar) are well above the Mn levels necessary for maximal yield in wheat (Wilhelm 1992).

2) Fungal growth was actually stimulated by low rates of soil-applied Mn, which had been shown to decrease take-all symptoms on wheat roots (Wilhelm 1992).

and 3) A reduction in Ggt infection of roots of wheat seedlings was seen when no Mn was added to the soil, but when high levels of Mn were present in the seed (Wilhelm et al. 1988). Reduction of infection by Ggt in the absence of a direct contact with the Mn treatment negates the hypothesis that Mn has a fungistatic action against the pathogen.

Ggt has been shown to oxidise Mn^{2+} to a brown precipitate identified as MnO_2 (Buchhorn 1988). It has been suggested that this may help protect the fungus from toxic levels of Mn, but oxidation of Mn in agar occurs at levels of Mn by which growth of the fungus is still stimulated (Wilhelm 1992). Oxidation of Mn by soil micro organisms has been previously discussed in this review (section 1.2.4 (3)).

2) Lignin production

The defence system of a plant is in three parts; the physical barriers of lignified tissue, the presence of phenolic and aromatic compounds which deter infective agents, and the response of the plant to wounding by the formation of lignitubers, suberised layers and callose (Bell 1981, Bostock and Stermer 1989). All of these defences have some reliance upon Mn in the plant (Friend 1981). Manganese is essential in the biosynthesis of lignin, phenolics and aromatic compounds, via the action of PAL, and Mn peroxidase.

Wheat plants deficient in Mn produce less total lignin; increasing Mn and NO_3^- levels in solution culture have an effect on both phenol and lignin levels in wheat seedlings (Brown et al. 1984). Mn deficiency in wheat plants decreased alkali labile phenols in shoots and decreased lignin in shoots and roots.

Lignitubers, produced as part of the plant wound response have a high Mn component (Skou 1981). Mn is thus required in the biosynthesis of the ligneous material, and in the actual, homogeneous structure. Under conditions of Mn deficiency, lignituber production in response to plant wounding is markedly reduced (Skou 1981).

However, even under conditions of Mn sufficiency, the effectiveness of lignitubers in preventing hyphal penetration of the endodermis of wheat roots, is doubtful (Skou 1981). Lignitubers may slow, but do not appear to prevent penetration of the stele by the fungus.

Excised root sections, from wheat plants grown under Mn deficient conditions, were infected by Ggt at a much faster rate than root sections from plants grown with sufficient Mn

(Wilhelm 1992). Lignification of the root pieces (whether exposed to Ggt or not) from Mn sufficient plants was much greater in the Mn deficient plant roots. This suggests that the difference in invasion of the roots was due to the defensive lignin structures formed prior to the roots' exposure to Ggt (Wilhelm 1992) - the constitutive lignin layers formed by the plant.

By this hypothesis, Mn sufficiency reduces Ggt infection through strong constitutive plant defence mechanisms; in phenolics production, and the physical barrier of lignin in the root, possibly enhanced by active lignituber production. The effective activity of the enzyme systems required for lignin production is reliant upon Mn (Gross 1980). Sufficient availability of Mn^{2+} to the root would ensure a consistent lignification of the roots, preventing easy penetration by root pathogens (Brown et al. 1984).

3) Root exudates

Plant root exudates include sugars, oligosaccharides, vitamins and amino acids; every soluble chemical species isolated from plants has been found in root exudates (Rovira 1956 a). Exudates may be specific species produced in the plant and secreted into the rhizosphere (such as mucigel, phytohormones, siderophores, reducing agents), or nutrients lost to the rhizosphere through leaky membranes or damaged tissue .

There are several possible mechanisms by which root exudates of a Mn sufficient plant may reduce Ggt infection, both directly acting against the fungus, or indirectly through the action of bacteria which may utilise the root exudates as metabolites.

Direct action against the Ggt fungus by wheat root secretions is unlikely, since this would constitute a resistance mechanism, as with the avenacins of oats. There is no evidence of such a mechanism in wheat. However, root secretions of rye have been suggested as having antibiotic properties, antagonistic to Ggt (T. Klein pers. comm.).

The micro-organisms that grow in the rhizosphere can affect the exudate patterns of plant roots by altering the integrity of the root and increasing leakage of exudates (rather than active secretion), or by modifying the environment of the roots and thus the root metabolism (Rovira 1956 c). Bacteria colonising the roots of a wheat plant may be antagonists of Ggt, and by some mechanism, reduce either the vegetative growth of the fungus, or its pathogenicity. These bacteria may also reduce Mn oxides and benefit the plant in this way. Mn oxide reducing agents secreted from plant roots also make Mn more available to the plant within the rhizosphere (Godo and Reisenauer 1980, Jauregui and Reisenauer 1982).

4) Oxidation of Mn by Ggt

A fourth proposal for the mechanism of Mn interaction in reducing take-all is suggested, which involves some aspects of the mechanisms described above.

As mentioned above (section 1.4.1 (1)) Ggt has been found to oxidise Mn (Buchhorn and Graham 1992, Wilhelm 1992) and further investigations have shown that there is a positive correlation between the pathogenicity of an isolate (measured as infection of seedling roots) with its ability to oxidise Mn^{2+} in agar (Buchhorn and Graham 1992). Wilhelm (1992) found nodules of Mn oxide at Ggt infection points on wheat roots, indicating the fungus may oxidise Mn in the rhizosphere and within the cortical free space.

The oxidation of Mn by Ggt may form part of the pathogenic action of the fungus. Pathogenic strains of Ggt may be capable of oxidising sufficient Mn^{2+} to create Mn deficiency in the rhizosphere, and alter the metabolic activity of the plant.

Mn is specifically required in the production of lignin, a major part in the plants' defence against pathogens (Gross 1980, section 1.4.1 (2)). The three mechanisms proposed by Graham and Rovira (1984) were examined by Wilhelm (1992), who suggested that the adequate lignification of wheat roots provided the best explanation of why plants sufficiently supplied with Mn were less susceptible to take-all disease. Disruption of Mn availability in the rhizosphere, whilst the plant is under stress from invasion by Ggt hyphae, may reduce the efficacy of active defence mechanisms such as lignituber production.

With the presence of sufficient Mn, the Mn oxidising capacity of the Ggt fungus may be nullified or "cancelled out", by the secretion of Mn oxide reducing agents (of plant or bacterial origin). Without the debilitating effect of rhizosphere Mn deficiency to weaken the plant, the fungal invasion of plant roots may be reduced.

The majority of these mechanisms depend upon the availability of Mn^{2+} to the plant roots. Plants capable of maintaining Mn as Mn^{2+} in the rhizosphere, or with a sufficient pool of Mn to overcome localised Mn deficiencies, may be more resistant to take-all.

Experimental evidence (Wilhelm et al. 1988) suggests that wheat genotypes less susceptible to Mn deficiency are more resistant to take-all disease.

1.5 Manganese efficiency

Graham (1984) has defined the nutrient efficiency of a genotype (for each element separately) as the ability to produce a high yield in a soil that is limiting in that element for a standard genotype. Additionally, an efficient genotype should also be expected to yield well in non-limiting conditions (Graham 1984).

Genotypic differences in nutrient efficiency are well documented, and a classic observation of variation in Mn efficiency was made by Samuel and Piper (1929) of chronic Mn deficiency symptoms in oats, while in the same paddock, rye plants were green and healthy.

The ranking of susceptibility to Mn deficiency (or of Mn efficiency) in cereal crops has been established as: oats, which is highly susceptible; wheat, triticale, barley and rye which is generally tolerant (Nyborg 1970, Reuter 1972, Marcar 1987).

Genotypic differences in Mn efficiency among cultivars of oats, wheat, triticale and barley have also been established (Gallaghar and Walsh 1943, Nyborg 1970, Reuter 1972, Graham et al. 1983, Marcar and Graham 1987 a, b).

Efficiency for Mn has been shown to be inherited as a major dominant gene (Graham et al. 1983, McCarthy et al. 1988, Longnecker et al. 1990). Heritable modifications of Mn efficiency may have effects through other physical or chemical mechanisms which affect the availability, uptake or use of Mn by the plant. The distinction between the actual efficiency mechanism and modifying effects upon Mn efficiency has not been made. However, a cross between efficient and inefficient barley genotypes, Weeah and Clipper respectively, yielded plants more efficient than the efficient parent, Weeah, due to a possible mixture of mechanistic efficiency and the vigorous root system of the inefficient genotype, Clipper (Graham et al. 1983).

Studies of triticale, the rye-wheat hybrid cereal, have shown that the Mn efficiency of triticale lies midway between its rye and wheat parents. Comparison of different triticale genotypes has indicated that Mn efficiency is inherited from rye on the 2R chromosome.

Triticales which lack the 2R chromosome have been shown to be susceptible to Mn deficiency. An example is the triticale cultivar, Coorong, which was shown to be less Mn efficient than wheat, in a field trial, under Mn limiting conditions (Marcar and Graham 1987 b, Graham et al. 1983).

Marcar noted that wheat-barley addition lines had an efficiency that lay between the parent cultivars, but no significant results to indicate on which chromosome of barley Mn efficiency lay.

Marcar also studied differences in Mn efficiency between genotypes of wheat, but was restricted by problems caused by different levels of seed source Mn (Marcar and Graham 1986). The nutritional status of the soil, often at a particular geographical site, plays a large part in the nutrient stored in the resulting seed, the source of nutrient for germination. Large differences in seed source nutrient may make significant differences upon the vigour of seedlings; effects which may still be observed in later plant growth, when nutrient from the seed is exhausted (Marcar and Graham 1986). High seed source nutrient may initially mask poor soil nutrition or nutrient inefficiency.

1.5.1 Why breed for Mn efficiency ?

Breeding for nutrient efficient crops is necessary in situations such as that of the highly alkaline, Mn deficient soils; where the nutrient is present, but is unavailable to the plant and not replaceable by fertiliser applications. The reduction of fertiliser requirements and of energy use are important economic considerations which favour the breeding of nutrient efficient cultivars, capable of high yield, under nutrient limiting conditions.

There is ample evidence of genetic variation for Mn efficiency in cereals (Nyborg 1970, Reuter 1972, Brown and Jones 1974, Graham 1983, Marcar and Graham 1987 a, b, Kaur et al. 1989). This provides a wide range of genotypes from which to select for nutrient efficiency. The possible mechanisms of Mn efficiency, which may differ in different species, is discussed below.

1.5.2 Mechanisms of efficiency

Means by which some plants could have a greater nutrient efficiency than others were listed by Graham (1984) as follows:

- better root geometry,
- chemical modification of the root : soil interface to increase the nutrient availability,
- faster rate of nutrient absorption at low concentrations,
- improved internal redistribution of the nutrient, and
- superior utilisation or lower functional requirement for the nutrient.

1) Root genetics and geometry

The genetic control of root growth is largely unknown; in tomato (Zobel 1975), 30% of the genome controls normal root (and shoot) growth, while 10% conditions root growth and development only.

The growth pattern of a plant's root system is important for the uptake of nutrient and water, which may be in growth limiting supply. Vose (1963) cites numerous examples where high rates of ion absorption were associated with large root systems, and Chvapin (1980) showed that species from nutrient poor habitats possess high root : shoot ratios.

Studies of root : shoot ratios in Australian wheats show that root density and root mass have been reduced dramatically in modern cultivars, compared to old landrace varieties (Evans and Dunstone 1970, Siddique et al. 1990). The general trend (pers. comm. A. J. Rathjen) in wheat breeding of reducing the root mass has produced plants less able to cope with nutrient deficient soils and root debilitating diseases, such as crown rot, rhizoctonia or take-all.

Qualitative observations by Marcar (1986) showed that while barley is more Mn efficient than wheat, neither the degree of rhizosphere acidification, nor the reduction of MnO_2 per unit root differed between species. Barley's greater Mn efficiency was attributed to its more extensive root system. This agrees with Uren's (1981) and Leeper's (1970) work on contact reduction of Mn (and Fe), where greater contact of roots with soil (by compaction of soil, or by soil contact with a more extensive root system) allows a greater uptake of nutrients.

However, although a general efficiency of nutrient uptake could be conferred by a large root system, efficiency for specific nutrients could not. The heritability of Mn efficiency appears to be simple (Graham et al. 1983), whereas root system genetics are complex (Zobel 1975). In addition, the Mn efficiency of modern wheats is equivalent to, or surpasses that of older cultivars, which presumeably have larger root masses. Thus the mechanism of Mn efficiency does not appear to reside in root system size.

2) Modification of soil by root exudates

Secretions of cereal roots include phenolics and acidic groups which reduce mineral complexes into ionic forms, making immobilised or oxidised nutrients available to the plant roots. Secretion of specific reducing agents or chelators (siderophores, ionophores and mugineic acid), often formed in response to nutrient deficiency by nutrient deficient plants, also increase nutrient availability to the plant's root system (Godo and Reisenauer 1980, Lehmann et al. 1987).

Proton efflux from root alters the pH of the rhizosphere, (Marschner et al. 1982, Bashan and Levanony 1989, Lehmann et al. 1987) and also reduces mineral complexes into plant-available forms. Rye has been shown to have a much greater efflux of reducing agents from roots than barley or wheat (Uren 1981). The great nutrient efficiency of rye may be attributed to its ability to alter nutrient availability in the rhizosphere.

3) Uptake and internal redistribution of Mn

As detailed earlier, Nable and Loneragan (1984 a, b) have proposed that the uptake of Mn by root cells is in two phases; a non-metabolic diffusion into the cytoplasm under a electrochemical gradient, followed by specific metabolic removal into the vacuole. It is possible that Mn efficiency could lie in the vacuolar removal of Mn from the cytoplasm and/or in the transport of Mn out of the cell to the growing shoot, for which mechanism there is only speculation in the literature.

4) Internal requirement and utilisation

The amount of Mn taken up into tissue by different genotypes varies, but actual utilisation may not. There is no evidence that Mn efficiency arises from a lower internal requirement for Mn in the shoot or whole plant. It was suggested by Nyborg (1970) that the greater sensitivity of oats to Mn deficiency was caused by poor ability for uptake, rather than a higher requirement for the element. A wide variety of plants (from cereals to sugar beet) with widely varying amounts of Mn in the whole plant, have been shown to have similar critical herbage levels of Mn (Marschner et al. 1986).

Although Mn efficient cereal varieties have much greater amounts of Mn in tissues, especially in the root, the actual critical value for Mn in the efficient plants is the same (or extremely similar) as for the inefficient plants (Graham et al. 1985). With this evidence of little useable variation in the utilisation of Mn in plants, this can be set aside as the means of efficiency for Mn nutrition.

Since the size and organisation of the root system is likely to be only part of the means of Mn efficiency, and the critical values, for Mn, of wheat genotypes (efficient and inefficient) are similar, one must look to other points where Mn efficiency may be improved. The role of root exudates in reduction of Mn oxides is one where breeding for improvement in Mn efficiency in wheat may be possible.

1.5.3 Breeding for Mn efficiency

In breeding for Mn efficiency in wheat, inheritance is assumed to be in the form of a single dominant gene. Additional heritable effects may modify the Mn efficiency of the genotype. The extent of efficiency in wheat is unknown, and probably unrealised. Use of Mn efficient genes from barley and rye to further improve the Mn efficiency of wheat may be possible, although a large yield reduction usually accompanies trans-species chromosomal additions and translocations.

1.5.4 Benefits of Mn efficient wheat

Australian wheats have been bred to yield well in harsh, water limiting conditions and have been adapted to poor conditions but sustained agriculture on the nutrient deficient soils of southern Australia can still only be carried out with the continued intensive use of fertilisers (Donald and Prescott 1975). The option is to continue to adapt crops to the conditions at hand; breeding for varieties capable of efficient, high yielding growth in nutrient limiting soils.

The simplest point in promoting breeding for nutrient efficient cereals is the reduction, particularly for micronutrients, in fertiliser treatments required to produce a profitable yield (Graham 1984). In the case of the chronically Mn deficient soils in South Australia, where foliar sprays of Mn are applied several times mid-season, this would be a significant reduction in energy costs to the farmer.

The annual addition of Mn fertiliser does not result in the accumulation of plant-available Mn and rates of Mn fertiliser application to the soil cannot be reduced unless the crop is Mn efficient and able to use available Mn in the native soil. More Mn is taken up by a Mn efficient plant throughout its growth under Mn limiting conditions than a Mn inefficient plant, often resulting in Mn efficient wheat varieties having higher concentrations of Mn in seed.

Higher Mn concentrations in grain has two benefits; higher Mn levels are passed on to human consumers of the grain, and high Mn levels are beneficial in grain used as seed under Mn limiting conditions (Marcar and Graham 1986).

A final benefit is an increase in resistance to disease, evident in nutritionally sufficient plants (Graham 1984). It has already been demonstrated that the disease take-all is less prevalent where wheat crops have sufficient Mn available (Wilhelm et al. 1988).

The similarity of ranking of cereals in resistance to take-all and in Mn efficiency is a further indication of the relationship discussed above. This is presented in table form (Table 1.2).

The more Mn efficient species are less susceptible to take-all with the notable exception of oats, which are regarded as resistant to Ggt infection, through the production of avenacins. It has been suggested that the lack of resisitance mechanisms to take-all disease in other cereals, such as wheat and barley, is due to the nature of take-all disease which does not create a large evolutionary pressure (Scott and Hollins 1985). The susceptibility of oats to Mn deficiency may have brought greater evolutionary pressure to bear on the genus, resulting in the development of resistance to the pathogen, in the development of avenacins.

Table 1.2 Relative rankings of take-all resistance and Mn efficiency by oats, rye, barley, triticale with and without the rye chromosome 2R (+2R, -2R) and wheat.

Resistance to Take-all	Mn Efficiency
oats	barley
rye	rye
barley = triticale	triticale (+2R)
wheat	wheat
	triticale (-2R)
	oats
Data from Scott (1981), Jensen and Jorgensen (1973)	Data from Marcar and Graham (1987 b)

This take-all resistance/Mn efficiency relationship may be expandable, to include cultivar differences in Mn efficiency as indicative of take-all resistance of that cultivar (or level of susceptibility to take-all) (Wilhelm et al. 1990). Under Mn limiting conditions, a Mn efficient wheat cultivar should be more resistant to take-all than an inefficient cultivar.

1.6 Resistance to Take-all of Mn efficient wheat cultivars

This section summarises the information reviewed and presents the hypotheses to be investigated in the body of this thesis.

It is established that Mn is a trace element essential for plant growth; a requirement of photosynthesis and of the production of lignin. Of the cereals, wheat is particularly susceptible to conditions of Mn deficiency, although more tolerant than oats.

Mn can only be taken up by the plant in the form Mn^{2+}. Mn cannot be retranslocated in the phloem of plants, creating a small, but constant demand for Mn^{2+} in the rhizosphere.

Mn efficient plants, with mechanism(s) that increase the Mn available to the plant, are less susceptible to Mn deficiency. Mn efficient wheats may be Mn sufficient under conditions which are growth limiting for Mn inefficient wheats.

Take-all of wheat is prevalent under conditions of Mn deficiency, ie low Mn^{2+} availability. Plants sufficiently supplied with Mn are less susceptible to take-all disease.

It is hypothesised that Mn efficient plants (genotypes) are less susceptible to take-all disease. In investigating this hypothesis the similarity of rankings of Mn efficiency of wheat cultivars and their levels of susceptibility to infection by the take-all fungus, Ggt, will be tested. Genotypes previously shown to be tolerant to take-all disease will be assessed for Mn efficiency.

Infection of wheat plant roots by the take-all fungus, Ggt, may be facilitated by poor lignin production of Mn deficient plants. Sufficient lignification of the roots, and healthy growth of the plant increase the chances of roots escaping infection by Ggt. Mn efficient genotypes may have more efficient production of lignin than less efficient genotypes.

A further hypothesis is that the Mn oxidising capacity of Ggt may alter Mn availability to the plant in the rhizosphere and render plant roots more susceptible to infection.

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Chapter 2

Effect of the Mn efficiency of wheat genotypes on their relative susceptibility to Ggt infection.

2.1 Introduction

The findings of Graham and Rovira (1984), on the effect of Mn in reducing the severity of the disease take-all, have been supported by pot and field experiments (Wilhelm et al. 1988, Huber and Wilhelm 1988). The form of Mn has been shown to be important in reducing take-all (Wilhelm et al. 1988): the less plant available forms of Mn, such as Mn oxides, have less effect, if any, in reducing take-all. Thus the availability of Mn^{2+} to wheat plant roots is an important factor in the susceptibility of wheat to take-all.

However, in soil conducive to take-all (high pH, high soil moisture, cool temperatures), Mn^{2+} is swiftly oxidised and made unavailable to the plant roots, causing Mn deficiency in the plant. In these soils Mn efficient wheat plants are at an advantage as they have a greater ability to grow in conditions of low Mn availability, perhaps by absorbing Mn more efficiently, or by utilising Mn more effectively (detailed in Chapter 1).

There is growing evidence that these efficient genotypes are less susceptible to infection by the take-all fungus, *Gaeumannomyces graminis* var *tritici* (Ggt) (Huber et al. 1991). Experiments by Wilhelm et al. (1990), using four wheat genotypes of varying Mn efficiency, showed that the most efficient (the breeding line C8MM) had the least root infection and the lowest total length of stelar lesions caused by the fungus. The least efficient, Bayonet, had the most disease, measured most significantly by the total length of stelar lesions (Fig. 2.1).

2.2 Method

Experiment 2.1

The initial experiment in this work was based upon Wilhelm's experiment (Wilhelm et al. 1990), but six wheat genotypes were used: the breeding line C8MM, and the cultivars Aroona, Schomburgk, Machete, Bayonet and Millewa. These wheats had been shown in field trials to differ significantly in Mn efficiency; C8MM and Aroona being the most tolerant in Mn deficient soil, Schomburgk and Machete being intermediate and Millewa and Bayonet highly susceptible to Mn deficiency.

The 'conetainer method', detailed below, is in common usage for similar experiments in our laboratory, frequently used as a means of ensuring infection of seedling roots by the take-all fungus. The method was initially developed by McDonald and Rovira (1988) and adapted by Wilhelm (1992).

A factorial experiment was carried out, of the six wheat genotypes named above, two soil treatments and two inoculum treatments. Each treatment was replicated five times, using a randomised complete block design for placement of the 120 pots.

2.2.1 Soil

Soil was collected from Wangary, on the Eyre Peninsula, South Australia; a site with chronically Mn deficient soil. Wangary soil is an aeolian, highly calcareous sand, (Vc H1, Northcote 1979) with a pH 8-9. Collected separately, topsoil (0 - 50 mm) and subsoil (50 - 100 mm), were sieved, air-dried and stored. The soil used in this experiment was from a collection made in 1989.

2.2.2 Soil treatment

Prior to all experiments using Wangary soil, equal weights of top- and subsoil were mixed, and portions taken for the various Mn treatments. The Mn treatments were added (as $MnSO_4$) in addition to 200 ml H_2O per kg of dry soil (20 % water content). The soil was then mixed thoroughly, and incubated (without light) at 10-15°C for two weeks.

Mn treatments were added at rates of nil (-Mn) and 18 mg Mn per kg soil (dry weight). The high rate of Mn addition to the soil and the incubation of the soil with the Mn treatments in place is required due to the swift oxidation of plant available Mn in such alkaline soil (Marcar 1986, Wilhelm 1992, Uren et al. 1988). A detailed study of this effect is presented by Webb et al. (1992). In this laboratory the procedure of incubation of Wangary soil with the Mn treatments in place is standard practice, so that the rate of change of available Mn has slowed to a more even level at planting.

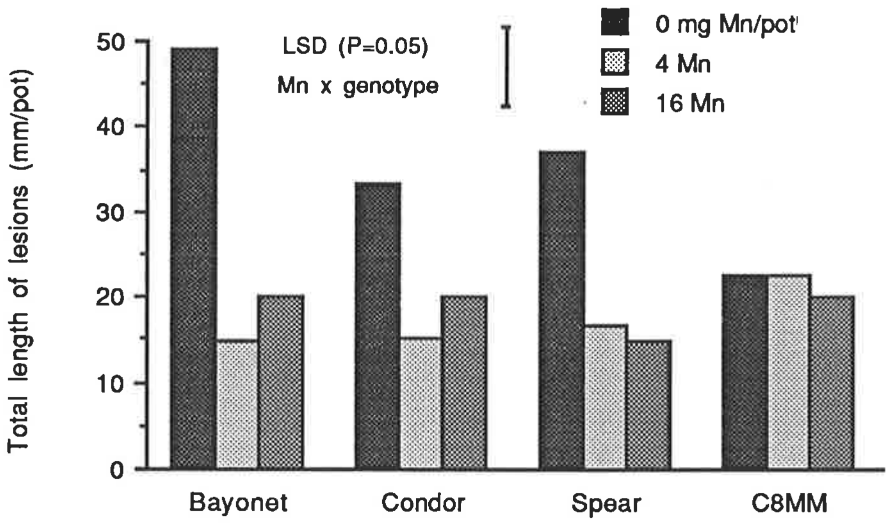

Fig. 2.1 Data from Wilhelm et al. (1990) (Figure 2A). Effect of soil applied Mn on total length of black stelar lesions, +Ggt treatment only. The conetainer method was used. Values are the average of five replicates.

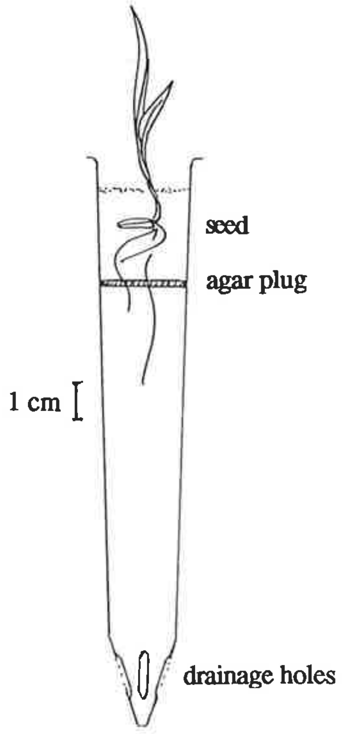

Fig. 2.2 Diagram of the conetainer method (from McDonald and Rovira 1988).

2.2.3 Basal nutrients

The rates at which basal nutrients were added to the soil were in mg / kg soil (dry weight): $Ca(NO_3)_2 4H_2O$ 918; KH_2PO_4 144; $MgSO_4.7H_2O$ 140; K_2SO_4 114; H_3BO_3 5.6; $ZnSO_4.7H_2O$ 26; $FeSO_4.7H_2O$ 17; NaCl 13, $CuSO_4.5H_2O$ 9; $CoSO_4.7H_2O$ 0.9 and $H_2MoO_4.H_2O$ 0.9.

2.2.4 Inoculum medium

The medium used to grow the fungal inoculum was a weak (1/25 of 'normal' strength) potato dextrose agar (1/25 PDA). PDA powder, 1.67 g and agar powder, 11 g were added to every 1.10 L of purified water and heated to boiling point to ensure the agars had dissolved. Agar was autoclaved at 121°C for 15 minutes, and then poured into sterile, 9 cm plastic petri dishes, such that each dish contained approximately 15 ml of agar.

2.2.5 Inoculum

The Ggt isolate used in all the pot experiments was the virulent isolate, Ggt 500, obtained from Dr A. D. Rovira, CSIRO, Division of Soils. The fungus was maintained on 1/25 PDA and original stock kept at 5°C. When required as inoculum, subcultures from the original plate were made, using a small, flame sterilised metal punch to cut sections from the edge of the hyphal growth. The sections were aseptically transferred to 1/25 PDA plates.

The inoculum used in experiments was grown in an incubator at 20°C on 1/25 PDA for 10 days, at which stage the mycelium covered the area of a 9 cm petri dish. Similar, sterile PDA plates were also prepared for the nil Ggt treatment.

2.2.6 Seed

Seed of each wheat genotype was obtained from a field trial at the Mn deficient site, Wangary. Analysis of this seed showed that the Mn concentration in the seed was very low; about 4 mg / kg. Seed was surface sterilised before germination by immersing in 30% ethanol for 30 seconds and rinsing in purified water, then immersing in 5% NaOCl for 1 minute and again rinsing in purified water. Seed was germinated by soaking in aerated purified water for 24 hours. Seeds with radicles of approximately equal length were used in the experiment.

2.2.7 Conetainer method

The pots used were the plastic 'conetainers' (Ray Leech Nurseries, Oregon, U.S.A.) which hold approximately 50 g (dry weight) of the Wangary soil. The conetainers were acid washed and air dried. The drainage holes were covered by a plug of cotton wool; in later experiments the drainage holes were covered with paper masking tape.

Soil was placed in each conetainer to a level 3 cm from the top of the pot (approximately 37 g dry weight soil). A plug of PDA, with or without inoculum, cut with a sterilised metal punch, was placed on top of the soil. The agar plug precisely fitted the internal diameter of the conetainer at that height, ensuring that the growing roots must pass through the agar / inoculum layer. A further layer of soil was placed on top of the agar plug (11 g dry weight) and then two germinated seeds. A final layer of soil (2 g dry weight) and 1 g of black plastic beads, as a mulch, was added (Fig 2.2). Finally the weight of each pot was checked in order that the water content of the soil might be maintained at 20% during the experiment, by watering each pot up to the original weight.

The pots, held in a rack, were placed in a growth chamber with a day / night cycle of 10 / 14 hours at 15 / 10°C. Pots were watered regularly (1-2 ml per day) with purified water and watered to original weight every five days. By the tenth day after sowing (D10) distinct symptoms of Mn deficiency had appeared in the -Mn treatments. These symptoms were more evident in the -Ggt than the +Ggt treatment. Moreover, by D17, even in the +Mn treatment, Mn deficiency symptoms were beginning to appear in the leaves of the inefficient genotypes.

The plants were harvested at D18. Shoots were weighed and dried, roots were washed, counted (number of roots per pot, NOR) and scored for infection. The number of stelar lesions per plant (NSL), total length of stelar lesions per plant (TLL) and location of the black stelar lesions was scored, as well as browning of the root cortex and the number of Ggt infected roots per plant (NIR). The roots were then weighed and dried. Shoots and roots were analysed by ICP for Mn concentration. Analysis of variance was carried out on infection and ICP data. Shoot and root fresh weight, shoot Mn concentration and content data are shown in Table 2.1, infection data are presented in Table 2.2.

Genotypes were ranked for Mn efficiency using the Mn content (mg / shoot) in the shoots of the - Ggt treatment plants. Efficiency was determined as the ratio of Mn content of -Mn to +Mn treatment. The percentage of roots with stelar lesions (PIR) was calculated from NIR / NOR.

2.3 Results

The Mn efficiency ranking of the six genotypes in this experiment was the same as found in field trials at Wangary in 1989 (Graham 1991).

Due to the severe Mn deficiency in the -Mn treatment, the plants were small and chlorotic, the inefficient genotypes especially so. There was a significant difference between Mn treatments for shoot and root weight, Mn content, and Mn concentration (Table 2.1), reflecting the effects of Mn deficiency in the -Mn treatment. However, Ggt treatment did not significantly affect plant measures and there was no significant interaction between genotype and Ggt treatment.

The infection levels in roots were low, possibly due to the short period of plant growth, permitting only a short time for infection to occur. However, infection measures were generally reduced in the +Mn treatment, most clearly shown by TLL and PIR. There was no difference in NSL between the +Mn and -Mn treatments, and only a small difference in NIR. The main effect of the +Mn treatment was to depress the infection measures. Individual genotypes did not necessarily follow this trend (Table 2.2, Fig. 2.3).

In the -Mn treatment the efficient genotypes clearly had less infection than the inefficient genotypes - Bayonet had three times the PIR of C8MM. However, within the +Mn treatment, the efficient genotypes, C8MM and Aroona, had infection levels equivalent to those of the inefficient Bayonet (Fig. 2.3). In the case of Schomburgk there were no stelar lesions or cortical browning due to Ggt in the +Mn treatment, but under Mn limiting conditions (-Mn) Schomburgk suffered infection. However, none of the interactions of genotype efficiency with Mn soil treatment for any of the disease measures were significant.

Table 2.1 Shoot and root fresh weights (g), shoot tissue Mn concentration (ShMn) (mg Mn / kg) and shoot tissue Mn content (Mn content) (μg) per plant, of six genotypes of wheat grown under two soil Mn treatments, -Mn (of no added Mn) and +Mn (18 mg Mn / kg), and without (-Ggt) or with (+Ggt) inoculum. Values are the mean of five replicates, standard errors are given.

Shoot Fresh Weight (g)	**-Mn**		**+Mn**			
	- Ggt	**+ Ggt**	**- Ggt**	**+ Ggt**	**mean**	**s.e. (±)**
C8MM	0.393	0.330	0.495	0.575	0.448	0.05
Aroona	0.348	0.315	0.416	0.484	0.390	0.04
Schomburgk	0.276	0.321	0.541	0.467	0.401	0.06
Machete	0.289	0.231	0.548	0.532	0.400	0.08
Millewa	0.227	0.224	0.505	0.378	0.209	0.07
Bayonet	0.184	0.214	0.503	0.432	0.333	0.08
mean	0.286	0.272	0.501	0.478	0.385	0.06
s.e. (±)	0.03	0.02	0.02	0.03	0.02	

ShMn (mg / kg)	**-Mn**		**+Mn**			
	- Ggt	**+ Ggt**	**- Ggt**	**+ Ggt**	**mean**	**s.e. (±)**
C8MM	4.02	4.55	31.35	31.51	18.03	8.96
Aroona	3.81	3.82	30.78	29.98	16.90	8.86
Schomburgk	3.16	3.19	29.44	30.87	17.03	8.99
Machete	2.85	2.50	31.65	31.03	16.76	9.62
Millewa	2.85	3.14	31.57	36.96	20.05	10.49
Bayonet	2.61	2.26	28.17	27.87	15.07	8.59
mean	3.22	3.24	30.49	31.37	21.70	9.23
s.e. (±)	0.23	0.35	0.57	1.24	0.60	

Mn content (μg)	**-Mn**		**+Mn**			
	- Ggt	**+ Ggt**	**- Ggt**	**+ Ggt**	**mean**	**s.e. (±)**
C8MM	0.107	0.172	2.06	2.01	1.41	0.62
Aroona	0.082	0.121	1.52	1.47	1.04	0.46
Schomburgk	0.074	0.089	1.89	1.95	1.31	0.61
Machete	0.061	0.068	1.88	1.96	1.30	0.62
Millewa	0.044	0.0647	1.91	2.24	1.41	0.68
Bayonet	0.044	0.050	1.93	1.90	1.29	0.62
mean	0.069	0.094	1.87	1.92	1.29	0.60
s.e. (±)	0.01	0.02	0.07	0.10	0.06	

Root Fresh Weight (g)	**-Mn**		**+Mn**			
	- Ggt	**+ Ggt**	**- Ggt**	**+ Ggt**	**mean**	**s.e. (±)**
C8MM	0.204	0.180	0.341	0.410	0.310	0.07
Aroona	0.177	0.151	0.365	0.342	0.286	0.07
Schomburgk	0.138	0.129	0.310	0.335	0.258	0.07
Machete	0.169	0.150	0.318	0.340	0.269	0.06
Millewa	0.131	0.134	0.316	0.264	0.238	0.05
Bayonet	0.119	0.106	0.275	0.255	0.212	0.05
mean	0.156	0.142	0.321	0.324	0.262	0.06
s.e.	0.01	0.01	0.01	0.02	0.01	

Table 2.2 The number of roots (NOR), the number of roots with stelar lesions (NIR), the number of stelar lesions (NSL) per plant, of six genotypes of wheat (one plant per pot), grown under two soil Mn treatments, -Mn (no added Mn) and +Mn (18 mg Mn / kg), without (-Ggt) or with (+Ggt) inoculum. The percentage of roots with stelar lesions (PIR) was calculated from NIR / NOR. Values are the mean of five replicates, standard errors are given. Analysis of disease measures did not include the -Ggt values.

NOR	**-Mn**		**+Mn**			
	- Ggt	**+ Ggt**	**- Ggt**	**+ Ggt**	**mean**	**s.e. (±)**
C8MM	9.4	9.8	9.8	9.4	9.6	0.1
Aroona	8.4	9.6	9.4	10.8	9.9	0.4
Schomburgk	9.2	8.4	10.4	9.4	9.4	0.6
Machete	8.4	9.0	9.8	10.0	9.6	0.3
Millewa	7.2	7.2	8.8	9.8	8.6	0.8
Bayonet	8.4	7.6	8.6	9.6	8.6	0.6
mean	8.5	8.6	9.5	9.8	9.3	0.4
s.e. (±)	0.3	0.4	0.3	0.2	0.2	

NIR	**-Mn**		**+Mn**			
	- Ggt	**+ Ggt**	**- Ggt**	**+ Ggt**	**mean**	**s.e. (±)**
C8MM	0	0.6	0	1.2	0.9	0.3
Aroona	0	0.6	0	1.0	0.9	0.2
Schomburgk	0	0.8	0	0	0.6	0.4
Machete	0	1.2	0	0.6	1.0	0.3
Millewa	0	1.4	0	0.6	1.0	0.4
Bayonet	0	1.4	0	1.0	1.0	0.2
mean	-	1.0	-	0.7	0.9	0.1
s.e. (±)		0.2		0.2	0.1	

NSL	**-Mn**		**+Mn**			
	- Ggt	**+ Ggt**	**- Ggt**	**+ Ggt**	**mean**	**s.e. (±)**
C8MM	0	0.6	0	1.2	0.9	0.3
Aroona	0	0.6	0	1.0	0.8	0.2
Schomburgk	0	0.8	0	0	0.4	0.4
Machete	0	1.2	0	0.6	0.9	0.3
Millewa	0	1.4	0	0.6	1.0	0.4
Bayonet	0	1.4	0	1.0	1.2	0.2
mean	-	1.0	-	0.7	0.9	0.1
s.e. (±)		0.2		0.2	0.1	

PIR	**-Mn**		**+Mn**			
	- Ggt	**+ Ggt**	**- Ggt**	**+ Ggt**	**mean**	**s.e. (±)**
C8MM	0	6.2	0	12.4	9.3	3.1
Aroona	0	6.2	0	8.8	7.5	1.3
Schomburgk	0	8.8	0	0.0	4.4	4.4
Machete	0	13.6	0	6.0	9.8	3.8
Millewa	0	18.0	0	6.2	12.1	5.9
Bayonet	0	19.8	0	10.2	15.0	4.8
mean	-	12.1	-	7.3	9.7	2.4
s.e. (±)		2.4		1.8	1.5	

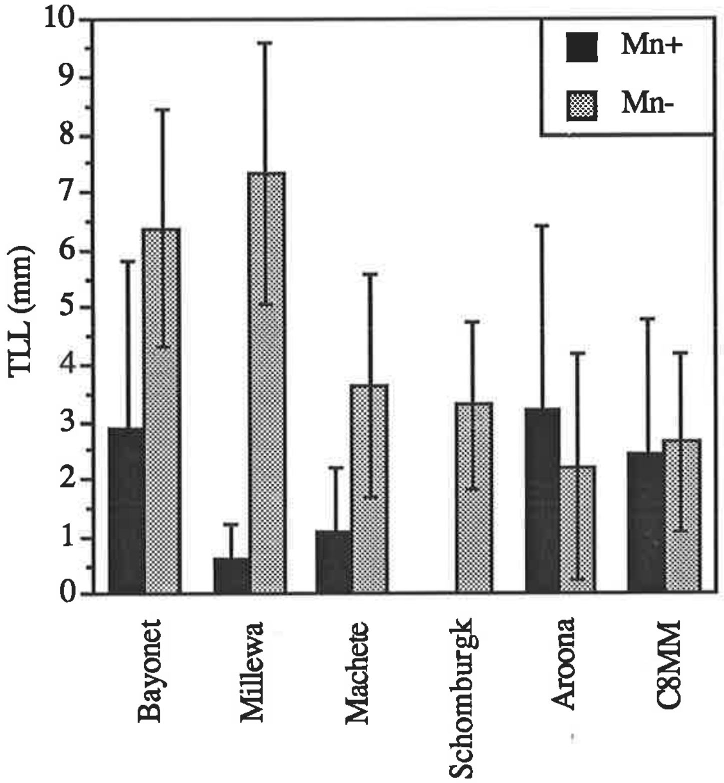

Fig. 2.3 Total length of stelar lesions (mm) per pot (TLL), caused by Ggt infection, inplants of six genotypes grown under + and -Mn soil treatments, applied to Mn deficient soil. Genotypes are arnked inorder of increasing Mn efficiency. Values are the mean of five replicates, standard error bars are shown.

Single replicates boosted the average disease levels in the -Mn treatment of Aroona and Bayonet (data not shown). Despite the nature of the inoculum method, whereby roots theoretically cannot avoid contact with the inoculum, large variations in infection still occurred between plants. The variability of either inoculum vigour or plant susceptibility to infection was not avoidable, even with such a contrived inoculum system as used here.

2.4 Discussion

The data obtained by Wilhelm et al. (1990) and the hypothesis that Mn efficient wheats are less susceptible to take-all disease in soils of low Mn status were supported by the results of this experiment. In the -Mn treatment, the most efficient genotypes, C8MM and Aroona, showed the least disease in the +Ggt treatment, and had the highest shoot fresh weights in the -Ggt treatment. The less efficient genotypes suffered more infection by the fungus and showed less tolerance of the low Mn conditions with subsequently lower shoot fresh weights.

In the +Mn soil treatment the overall level of infection was reduced. Most impressively, Schomburgk was completely free from infection; although some black runner hyphae were observed on the surface of the roots, no stelar lesions, or even penetration of runner hyphae into the cortex were found in any of the roots. The growth of runner hyphae on the root surfaces showed that the Ggt inoculum was viable in the pots of that treatment.

The Mn efficient genotypes were capable of maintaining near optimal vegetative growth (if not nutrient status) under Mn limiting conditions (-Mn), a factor which may have reduced their susceptibility to infection: C8MM and Aroona had similar, moderate levels of disease in both Mn treatments.

The genotypes used by Wilhelm et al. (1990) showed particularly clear differences in Mn efficiency and susceptibility to Ggt infection under Mn limiting conditions (Fig. 2.1). Since the mechanism of Mn efficiency is unknown, the determination of efficiency must rely on comparisons of +Mn and -Mn treatments within each experiment, and the exact ranking of genotypes for Mn efficiency may alter between experiments (Graham 1990). Although Mn efficiency has been shown to be heritable through a single, major gene (McCarthy et al. 1988), the screening of cultivars and advanced lines is likely to encounter high levels of variation within this particular trait, due to interference from genes which may add or detract from a genotype's Mn efficiency (Graham 1984). Similarly, the rankings of wheat genotypes for tolerance to take-all are rarely consistent from experiment to experiment (Penrose 1985, Riveros et al. 1987, Penrose 1991). However, the trends of this initial experiment encouraged a wider survey of wheat genotypes claimed to differ in Mn efficiency and in susceptibility to take-all.

2.5 Method

Experiment 2.2

The method described in Section 2.2 was followed, but with the following changes. Twenty-two genotypes were used in the experiment (listed in Table 2.3), with only four

Table 2.3 Sites from which seed was obtained and seed Mn concentration (mg / kg) of seed used in Experiment 2.2. (AWCC = Australian Winter Cereals Collection, Tamworth, N. S. W., Roseworthy = Roseworthy Agricultural College, S. A., Lameroo = Field trial at Lameroo, S. A., Yeelanna = Field trial at Yeelanna, S. A.. Lameroo and Yeelanna are Zn deficient sites but the seed was taken only from +Zn treatments and was not deficient in Zn.

Genotype	**Site**	**Mn seed**
Aroona	Lameroo	23.1
Bayonet	Lameroo	32.9
Bindawarra	Lameroo	33.0
Blade	Roseworthy	46.6
Bodallin	AWCC	29.1
C8MM	Lameroo	38.2
C8MM*MM*MMC	Lameroo	24.8
Cook	Lameroo	36.8
Halberd	Lameroo	23.3
Kite	Lameroo	25.2
Kulin	AWCC	32.6
Machete	Lameroo	29.6
Millewa	Lameroo	32.6
Molineux	Yeelanna	24.0
Red Fife	AWCC	42.4
Richelle Hative	AWCC	40.5
Schomburgk	Lameroo	23.1
Seite Cierros	AWCC	39.7
Songlen	Lameroo	26.7
Spear	Lameroo	38.6
Tatiara	Lameroo	24.9
Warigal	Lameroo	36.7
mean		32.0
s.e. (±)		1.5

replicates used. Seed was obtained from various sources: the Australian Winter Cereal Collection (Tamworth, New South Wales), Roseworthy Agricultural College (South Australia), and from field trials at Lameroo (South Australia). The seed used had much higher content of Mn than that used in the first experiment, ranging from 25 to 50 mg / kg, rather than 4 mg / kg. Additionally, Wangary soil from 1990 collection, rather than the 1989 collection was used. Finally, since seedling roots had grown into the cotton wool plugs in Experiment 2.1, the drainage holes of the conetainers were sealed with paper masking tape.

The plants were grown as before, under controlled conditions, and were harvested, by replicate, at 24, 26, 28 and 30 days after sowing. Shoots and roots were treated and scored for infection in the same way as before, and the roots were frozen after scoring.

2.6 Results

Symptoms of Mn deficiency were not apparent in shoots until 19 days after sowing and symptoms of severe Mn deficiency were not observed in the plants at any stage. Shoot dry weights were not significantly affected by Mn soil treatments or the presence of inoculum (+Ggt) (Table 2.4). The genotypes were ranked for Mn efficiency by the ratio of shoot Mn concentration of -Mn to +Mn treatment.

The disease scores gave unexpected results; showing that more infection of the roots occurred in the +Mn treatment than the -Mn treatment (Figs. 2.4 - 2.8). No significant interaction of genotype with Mn treatment was obtained for any of the disease measures, although both Mn treatment and genotype caused significant effects. The shoot Mn concentration was significantly affected by Mn soil treatment and to some extent by Ggt treatment (Table 2.5). Shoot Mn concentration (ShMn) was slightly lower in the +Ggt plants than the -Ggt plants, especially in the less efficient genotypes (Table 2.5). Generally the Mn concentration in shoots was not significantly affected by the presence of inoculum (+Ggt), indicating that plant metabolism, as affected by plant Mn status, had not yet been affected by Ggt infection of the roots.

Despite the greater infection of roots in the +Mn treatment plants, when genotypes were ranked for Mn efficiency (as in section 2.3) there was a recognisable trend of decreasing infection measures with increasing Mn efficiency (Figs. 2.4 - 2.8). At the ‘efficient’ end of the ranking, disease levels were reasonably low, with Bodallin, Tatiara, C8MM and Kite showing the lowest TLL and NSL over Mn treatments. In comparison, the

Table 2.4 Shoot dry weights (mg) of 22 genotypes of wheat, grown under two Mn treatments, -Mn (no added Mn) and +Mn (18 mg Mn / kg), without (-Ggt) and with (+Ggt) fungal inoculum. Genotypes are ranked in order of decreasing Mn efficiency. Values are the mean of four replicates, standard errors are shown.

Genotype	**-Mn**		**+Mn**		**mean**	**s.e.**
	- Ggt	**+ Ggt**	**- Ggt**	**+ Ggt**		**(±)**
Bodallin	98.3	98.0	87.5	87.5	93.9	6.2
Richelle Hative	102.8	101.5	112.5	109.8	106.6	9.4
C8MM	92.0	64.0	84.8	90.8	82.9	5.8
Seite Cierros	78.3	74.0	88.3	78.3	79.7	5.4
Blade	81.0	66.8	73.0	73.0	75.6	4.6
Kite	67.5	65.8	74.5	80.5	72.1	4.3
Aroona	70.0	67.8	85.8	85.5	77.3	4.6
Halberd	63.5	69.5	75.3	76.3	71.1	4.8
Bindawarra	71.8	58.0	66.3	72.5	67.1	2.9
Kulin	65.0	59.8	62.8	61.5	62.3	5.6
C8MM*MM*MMC	67.3	56.0	70.3	66.8	65.1	3.3
Machete	68.3	59.5	64.8	69.3	65.4	4.2
Tatiara	66.3	60.8	85.3	84.3	74.1	6.2
Warigal	61.0	63.3	97.3	76.8	74.6	6.6
Spear	74.3	54.8	75.8	82.3	71.8	5.3
Cook	52.5	54.8	60.8	63.0	57.8	4.1
Schomburgk	60.8	61.0	93.0	72.5	73.1	5.5
Red Fife	56.3	53.7	61.0	69.3	104.6	45.8
Bayonet	53.5	60.3	79.0	75.5	65.1	4.7
Millewa	48.0	48.3	77.8	65.8	59.9	4.7
Songlen	42.3	49.3	71.5	52.8	53.9	4.8
Molineux	49.3	40.8	85.3	61.5	59.2	6.9
mean	67.7	71.4	78.8	75.4	73.3	
s.e. (±)	2.2	8.5	2.9	2.7	3.0	

Table 2.5 Shoot Mn concentration (mg Mn / kg shoot dry weight) of 22 genotypes of wheat, grown under two Mn treatments, -Mn (no added Mn) and +Mn (18 mg Mn / kg), without (-Ggt) and with (+Ggt) fungal inoculum. Genotypes are ranked in order of decreasing Mn efficiency. Values are the mean of four replicates, standard errors are shown.

Genotype	-Mn		+Mn		mean	s.e.
	- Ggt	+ Ggt	- Ggt	+ Ggt		(±)
Bodallin	13.0	12.8	47.4	46.4	29.9	4.8
Richelle Hative	9.0	10.1	31.9	27.6	19.7	3.2
C8MM	11.5	12.8	58.3	53.2	33.6	6.1
Seite Cierros	10.8	11.2	51.3	49.1	30.6	5.3
Blade	10.8	11.5	54.8	36.8	28.5	5.3
Kite	10.4	11.7	44.8	42.4	27.3	4.6
Aroona	10.3	10.4	56.8	48.2	31.4	5.8
Halberd	9.8	11.2	51.3	47.9	30.1	5.4
Bindawarra	10.6	11.0	57.0	47.0	31.4	5.8
Kulin	12.3	11.1	51.5	43.5	29.6	5.0
C8MM*MM*MMC	10.7	11.9	56.7	52.4	32.9	6.0
Machete	9.8	11.4	45.3	40.1	26.6	4.5
Tatiara	10.0	11.9	58.5	57.0	34.3	6.7
Warigal	10.0	9.5	55.6	41.6	29.2	5.4
Spear	9.3	9.5	52.0	43.3	28.5	5.4
Cook	10.9	12.2	61.4	48.7	33.3	6.2
Schomburgk	9.4	10.1	54.1	45.0	29.6	5.7
Red Fife	9.3	10.6	50.4	40.7	27.8	5.0
Bayonet	8.4	8.7	35.4	28.6	20.3	3.3
Millewa	8.0	10.6	42.5	37.1	24.6	4.3
Songlen	8.9	10.9	60.5	51.4	32.9	6.6
Molineux	7.1	9.4	50.7	46.6	28.5	5.7
mean	10.0	10.9	51.3	44.3	29.1	
s.e. (±)	0.3	0.2	1.6	1.6	0.8	

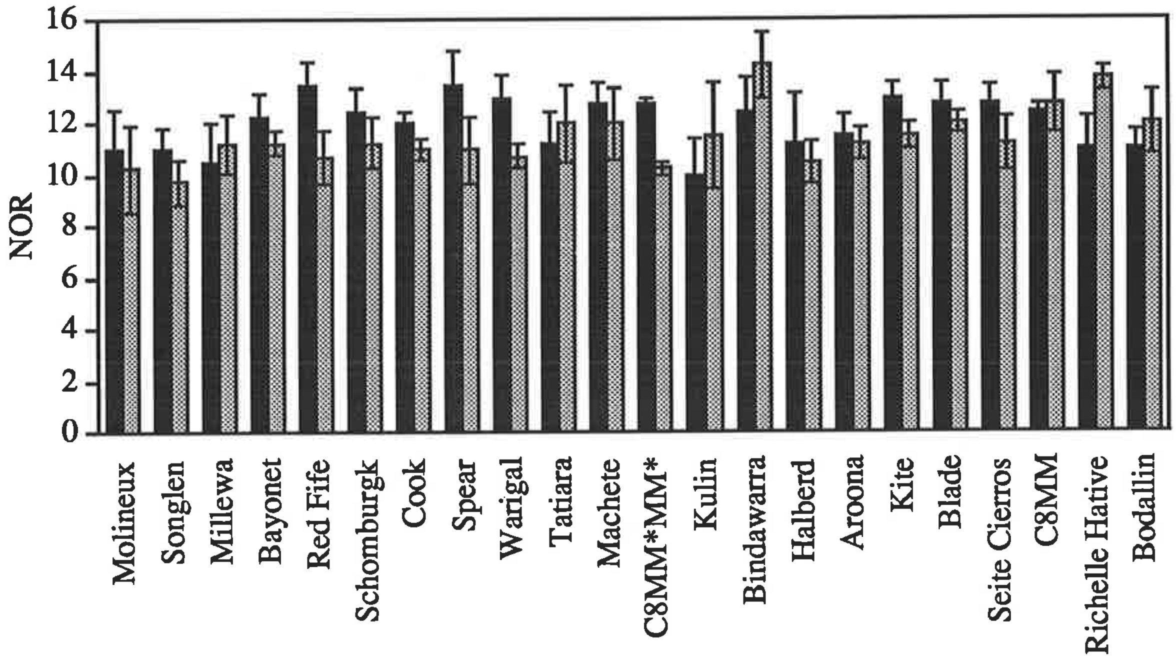

Fig. 2.4 Number of roots per plant (NOR) of 22 genotypes of wheat plants grown under +Mn ■ and -Mn ▨ treatments in Wangary soil with Ggt inoculum (+Ggt). Genotypes are ranked for increasing Mn efficiency. Values are the mean of four replicates, standard error bars are shown.

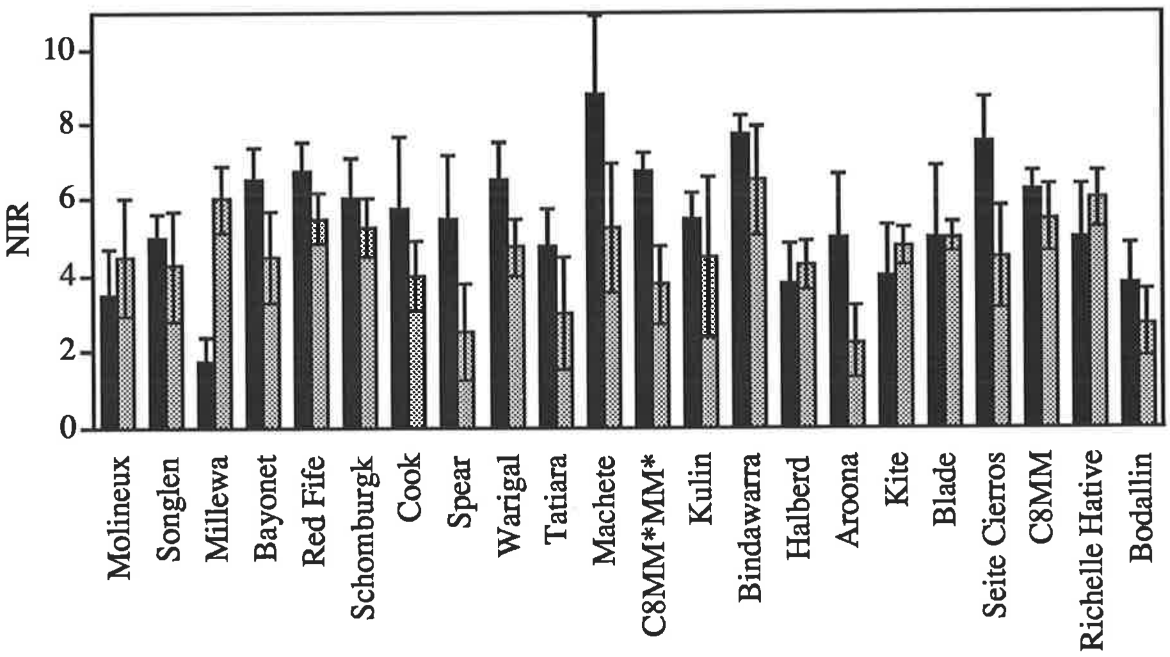

Fig. 2.5 Number of Ggt infected roots per plant (NIR) of 22 genotypes of wheat plants, grown under +Mn ■ and -Mn ▨ soil treatments with Ggt inoculum (+Ggt). Genotypes are ranked in order of increasing Mn efficiency. Values are the mean of four replicates, standard error bars are shown.

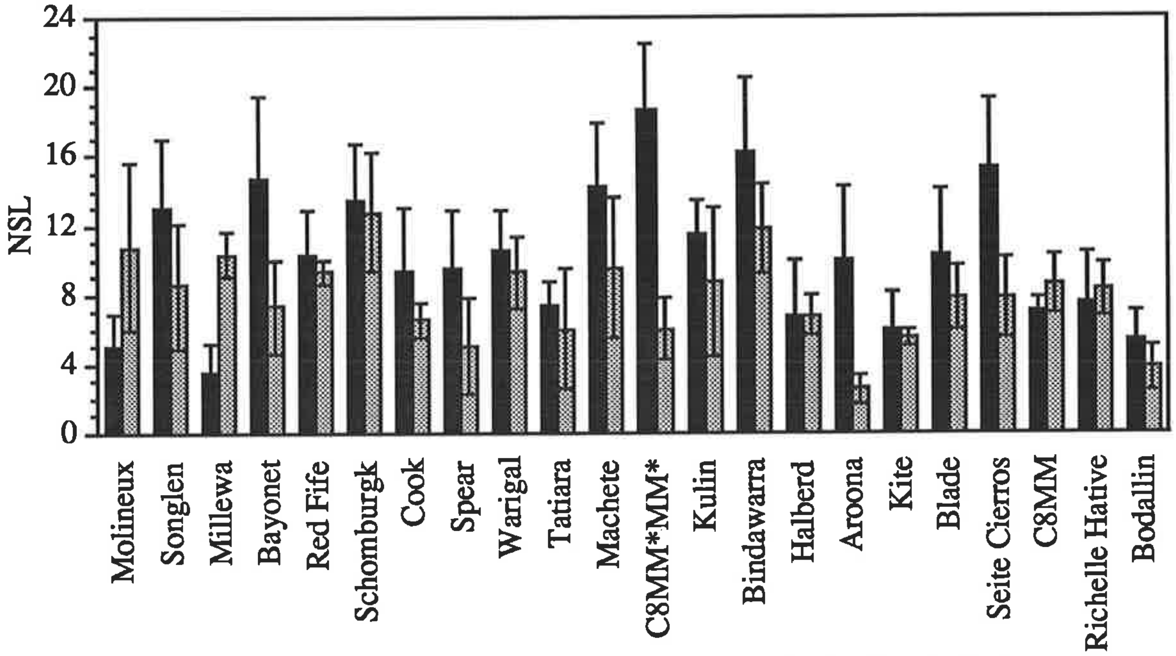

Fig. 2.6 Number of stelar lesions (NSL) in roots inoculated with Ggt (+Ggt) per plant of 22 genotypes of wheat plants, grown under +Mn ■ and -Mn ▨ soil treatments. Genotypes are ranked in order of increasing Mn efficiency. Values are the mean of four replicates and standard error bars are shown.

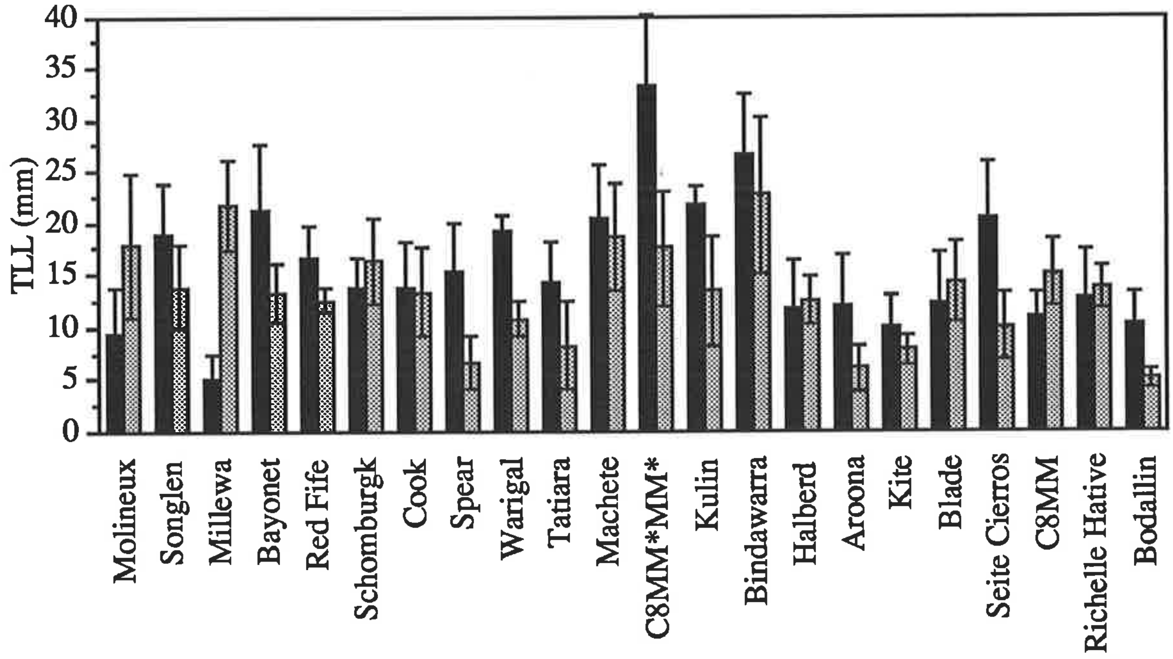

Fig. 2.7 Total length of stelar lesions (mm) per plant (TLL) in roots inoculated with Ggt (+Ggt) per plant of 22 wheat genotypes grown under +Mn ■ and -Mn ▨ soil treatments. Genotypes are ranked in order of increasing Mn efficiency. Values are the mean of four replicates and standard error bars are shown.

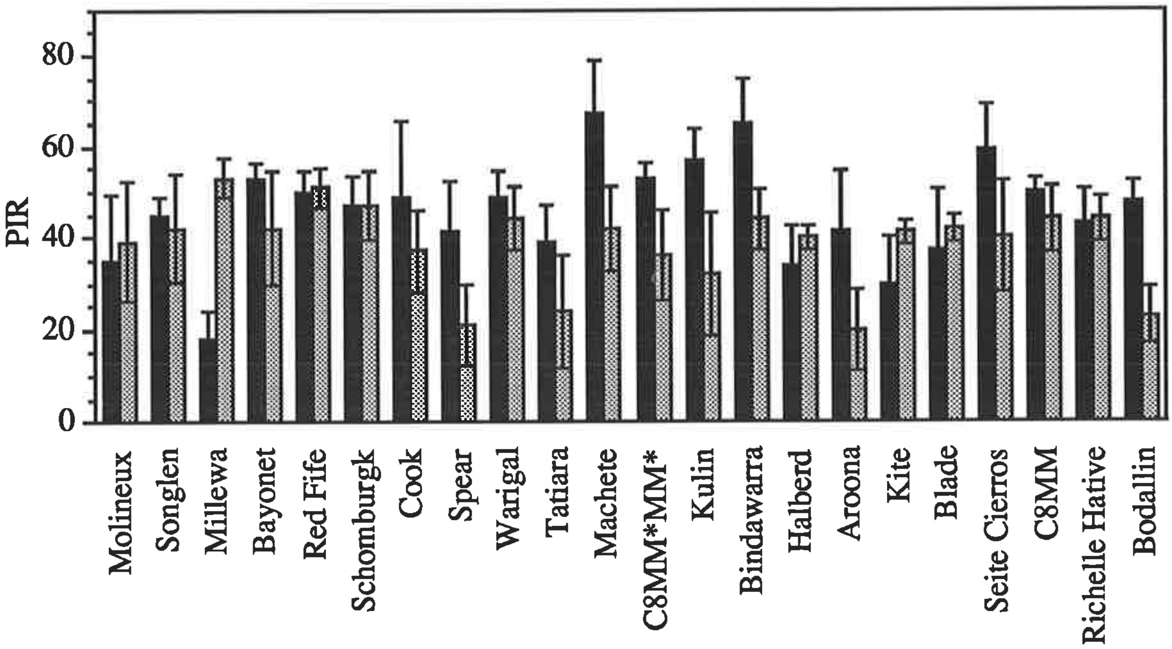

Fig. 2.8 Percent Ggt infected roots per plant (PIR) of 22 wheat genotypes, grown under +Mn ■ and -Mn ▨ soil treatments with Ggt inoculum (+Ggt). Genotypes are ranked in order of increasing Mn efficiency. Values are the mean of four replicates and standard error bars are shown.

breeding line C8MM*MM*MMC (related to C8MM and ranked as Mn efficient in this pot experiment) showed high disease levels, especially in the +Mn treatment (Figs. 2.4 - 2.8).

In the genotypes of intermediate Mn efficiency, generally higher levels of disease were seen; Bindawarra showed high TLL over both Mn treatments, and Schomburgk showed high NSL, also over both Mn treatments.

The Mn inefficient genotypes Molineux and Millewa were the only two out of the 22 genotypes to show decreased disease measures in the +Mn treatment. Both showed a large decrease in TLL and PIR in the +Mn treatments (Figs. 2.7, 2.8). Other inefficient genotypes, such as Bayonet and Songlen, showed high disease levels, but these were not decreased by the +Mn treatment.

2.7 Discussion

Many screening trials and experiments have been carried out in the attempt to find resistance to take-all (Scott and Hollins 1985, Penrose 1991), likewise extensive screening trials for Mn efficiency in wheat have been carried out (Marcar 1986, Marcar and Graham 1986, Graham 1991, Bansal et al. 1991). In order to further investigate the response of wheats differing in Mn efficiency to take-all, the larger number of wheat genotypes were selected on the basis of take-all resistance trials (Penrose 1985), Mn efficiency field trials (Graham 1991), and also to investigate the Mn efficiency and take-all tolerance of 'relatives' of some of these selected wheats.

For example, genotypes such as Kite and Richelle Hative (also published as AUS 1080) were chosen for tolerance to take-all (Penrose 1985), Tatiara and Red Fife for Mn efficiency (Marcar and Graham 1986) and Warigal was chosen for its close relation to the Mn efficient Aroona (a sibling) (pers. comm. A. J. Rathjen) Interestingly, in this experiment (not necessarily confirmed by performance in the field), the cultivar Kite was shown to be a Mn efficient genotype, and Tatiara showed reasonable tolerance to take-all. Red Fife performed disappointingly in both Mn efficiency and take-all tolerance and Warigal was both less Mn efficient and less tolerant to take-all infection than its sibling, Aroona.

Bodallin and Kulin (which has Bodallin in its pedigree) are both considered to be Mn efficient wheats (Graham pers. comm.). Bodallin had particularly low disease measures and was shown to be Mn efficient, while Kulin was both less Mn efficient and had higher levels of Ggt infection in roots.

Halberd and Songlen were respectively chosen for known tolerance to boron toxicity and intolerance of zinc deficiency, both trace elements which have been shown to have some bearing on response to take-all (Reis et al. 1984, Brennan 1992, R. O. Nable pers. comm.). Halberd had moderate disease measures in both +Mn and -Mn soil treatments, while Songlen, which performs poorly under nutrient deficient conditions (R D Graham pers. comm.), was highly susceptible to Ggt infection and disease.

Despite the inversion of effects from the +Mn and -Mn treatments (compared to Experiment 1), it can be seen that the more Mn efficient genotypes still had the lowest levels of disease in both the Mn soil treatments. The disease measures in the Mn efficient genotypes did not significantly differ over the soil Mn treatments, suggesting a tolerance to infection, even under Mn limiting conditions. The seedling tolerance of take-all disease by these efficient wheats may thus be a function of their Mn efficiency, rather than the plant available Mn in the soil.

The specificity of the effect of Mn fertiliser in reducing take-all has not only been shown where Mn is deficient in soil (Brennan 1992 a, Lucas and Sarniguet 1990), but also where the plants' Mn status (usually measured by shoot Mn concentration) is not deficient (Wilhelm 1992, Wilhelm et al. 1990, Webb and Graham 1990). The effects of other trace elements on take-all, such as Zn (Brennan 1992 b) and Cu (Wood and Robson 1984, Brennan 1991), have been shown to reduce disease severity upon the application of fertiliser to deficient soil, but once the deficiency is amended, even the application of luxurious rates of fertiliser do not further reduce take-all severity or improve grain yield in wheat (Brennan 1991). However, at a take-all infested site, where soil Mn was not deficient, the application of a luxury rate of Mn fertiliser was shown to increase grain yield of wheat (Webb and Graham 1990).

The results of Experiment 2.2, showing increased disease measures upon Mn addition to the soil, were puzzling. All previous experiments using Mn deficient soil in this laboratory had shown a reduction in disease upon the addition of Mn fertiliser (Graham and Rovira 1984, Wilhelm et al. 1988, Wilhelm et al. 1990, Rovira et al. 1985, Rengel et al. 1993) and other workers had shown similar effects (Huber and Dorich 1988, Lucas and Sarniguet 1990, Wilhelm and Huber 1988, Huber and McCay-Buis 1993).

The growth of all genotypes was increased by the addition of Mn to the soil (+Mn treatment). There was as much as three times the Mn in the shoots from the +Mn treatment as the -Mn treatment, with the average levels of Mn in the inefficient genotypes generally lower than those of the efficient genotypes. Vegetative growth responded to the Mn treatments but, with the exception of the inefficient genotypes Millewa and Molineux, disease reduction did not occur in the roots. Mn shoot concentrations were additionally affected by the high levels of seed source Mn, reducing the effect of the Mn deficient soil on the growth of the seedlings (Uren et al. 1988, Marcar and Graham 1986).

A possible explanation for the inversion of Mn effects on disease, from that expected in the experiment, is in two parts. It is suggested that the 1990 Wangary soil collection was a soil of extreme Mn deficiency, perhaps even to a point which may have caused a reduction in the saprophytic growth of the fungus (Wilhelm 1992), and thus a reduced virulence, and ultimately disease level. By this token, in the +Mn treatment, the availability of Mn to the fungus, as well as to the plant, was much higher and the vigour of the fungus may have been reflected in the higher disease levels. Secondly, the much higher levels of Mn in the seed used (in order to avoid early Mn deficiency in the seedlings) may have increased seedling vigour in *both* Mn treatments (Marcar and Graham 1986). The growth of the seedling plants would have been less reliant upon soil source Mn.

Thus, in the -Mn treatment, not only was plant growth less affected by Mn deficiency, being supported by seed source nutrient, but the fungal growth may have been less vigorous, as suggested above. The combination of these factors could lead to a lower level of disease in the -Mn plants compared to that seen in the +Mn plants. This is supported by the increase in disease measures seen in the least efficient genotypes under the +Mn soil treatment. Experiments testing this complex hypothesis are described in the next two chapters.

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Chapter 3

Effect of Mn availability in Wangary soil collections on growth of plants and Ggt.

3.1 Introduction

The results of Experiment 2.2 differed markedly from the results of other workers (Graham and Rovira 1984, Huber and Dorich 1988, Wilhelm et al. 1988, Wilhelm et al. 1990) and this chapter tests part of a hypothesis formed to explain these differences. In order to assess the hypothesis put forward at the end of the preceding chapter (section 2.7), several different components of the experimental system required testing.

Firstly, any differences between the soil collections in Mn content or Mn availability needed to be determined. Webb et al. (1993) have shown that long storage of collections of Wangary soil, used in these experiments, can lead to a decrease in the populations of Mn oxidising bacteria in the soil collection, and to a decrease in the ability of the soil to cause Mn deficiency in plants. The assumption that the 1990 collection of Wangary soil was more Mn deficient than the previously used 1989 collection was tested using three different methods.

Secondly, the effect of low levels of Mn in soil on the saprophytic growth of Ggt needed to be investigated, as the growth of the fungus may be retarded by extreme Mn deficiency in soil. Wilhelm (1992) showed that the growth of Ggt on potato dextrose agar was slightly benefited by the addition of 25 mg / kg Mn to the medium, but that in soil culture there was little or no stimulation of hyphal growth of Ggt by the lower levels of added Mn (25 and 50 mg / kg). However, under conditions where no Mn was added to the growth medium, saprophytic growth of Ggt hyphae was decreased (Wilhelm 1992).

Thirdly, the comparison of seed-source Mn and soil-source Mn on wheat plant resistance to infection by Ggt inoculum required investigation. High seed source nutrient levels have been proposed as a means of reducing seedling diseases (Marcar 1986, Wilhelm 1992, Sadasivan 1965) but the interaction of seed source and soil source nutrient against a pathogen has not been widely investigated. These Mn 'source' experiments are described in the next chapter while the soil and inoculum tests are sequentially described and discussed here.

3.2 Soil Mn Tests

The availability of Mn in different collections of Wangary soil is the point which is addressed in the experiments described in this section. Manganese in the soil solution is usually in the available form Mn^{2+}, but the proportion of Mn in soil which is in the form Mn^{2+} is affected by both chemical and biological factors. Thermodynamically, the oxidation of Mn is possible at pH greater than 5.5, but in soils a mixture of effects (temperature, moisture, aeration) generally maintains sufficient levels of Mn available for plant growth until pH rises above pH 7 (Norvell 1988, Bartlett 1988). The autocatalytic nature of Mn oxidation at pH levels greater than 8.5 - 9 (Ross and Bartlett 1981) swiftly removes Mn from immediate availability to plants. However, reduction of Mn oxides to plant available form by root exudates has been shown (Godo and Reisenaur 1980, Jauregui and Reisenaur 1982, Marschner et al. 1986, Lehmann et al. 1987) and Mn oxides formed by microbial action have also been shown to be available to plants (Bromfield 1958). Microbial oxidation or chelation of Mn has been assumed to cause the greatest changes in soil Mn availability, rather than non-biological effects. A changing population of microorganisms in a soil may alter the availability of Mn quite dramatically (Ghiorse 1988, Gerretson 1937).

Work by Webb et al. (1993) has described the effect of storage of Wangary soil, over periods of several years, upon the ability of the soil to create severe Mn deficiency in plants. Changes in the microbial populations of oxidisers and reducers during storage of the soil collections was suggested as the reason for the increase in the availability of Mn to plants. In the experiments described below, a similar approach to Webb was used in assessing the plant available Mn in the 1989 and 1990 Wangary soil collections.

Assessing differences between collections of Wangary soil was carried out in three ways. The total Mn content was assayed, and plant available Mn was assessed by chemical assay and by plant response to the different soil collections. Three collections of Wangary soil were compared, the 1989 and 1990 collections used in experiments described in Chapter 2, and the 1988 Wangary collection was included to give further depth to the analysis. Two

Mn treatments were used for each collection, +Mn (18 mg Mn / kg soil dry weight) and -Mn (no Mn added), giving six soil treatments in all. The differences between the -Mn treatments of the collections were of most interest, but analysing the +Mn treatments might give an indication of any differences in oxidising power (biological or non-biological) between the collections.

The three collections of Wangary soil were tested for total Mn by X-ray fluorescence spectrometry, for available Mn by DTPA extraction (which estimates plant available Mn) and for plant available Mn in pot tests, using wheat plants.

3.3 Method

Wangary soil from the three collections (1988, 1989 and 1990) was prepared, divided for Mn treatments and incubated for two weeks with Mn treatments in place, as previously described (section 2.2.1, 2.2.2). Basal nutrients (other than Mn) were not applied to the soil used in the assays for total Mn or DTPA extractable Mn, but were applied after incubation, at usual rates (section 2.2.3), to the soil used in the pot experiment assessing plant available Mn.

3.3.1 X-ray fluorescence

Total Mn in the soil was determined by X-ray fluorescence spectrometry. The level of irradiation, the incident surface and homology of the particles, the effects of 'stronger' ions (such as Fe and Ca which have many excitation states) on the excitation of other particles and ions are accounted for by calculated matrix coefficients. Sensitivity of the technique is affected by the soil particle size.

In order to gain a smooth incident surface for the X-ray, soil samples are prepared by grinding, then pressing or dissolving the soil into a solid disc, with a smooth surface. Powder press disc samples were made using 10 g (dry weight) of each of the six soil treatments, dried in oven at 104°C overnight. The entire sample was ground for 15 seconds in a tungsten swingmill, then for a further 10 seconds with 2 ml of a 25% polyvinyl acetate solution (in ethanol and water). The discs were formed in a mould which consisted of several cylinders and a cap. The internal surface of the cap was covered in mylar film to give a smoother surface to the analytical face of the disc. The soil sample was pressed into form against the mylar film, and surrounded with sieved boric acid which formed the rest of the disc. The entire mould was placed under pressure (15 t / cm^2) compressing the boric acid and soil into a solid disc, approximately 8 mm thick, which could then be broken out of the mould. Two discs of each soil treatment were made and analysed by X-ray fluorescence spectrometry. Total soil Mn was expressed as % MnO in the soil.

3.3.2 DTPA extraction

A modification of the soil test developed by Lindsay and Norvell (1978) was used to assess DTPA extractable Mn in the three soil collections. The concentration of the DTPA extractant was adjusted to allow for moisture in the soil (from incubation) (Webb et al.1993). Extractant was thus: 0.149 g triethanolamine, 0.0197 g diethylene triamine pentaacetic acid (DTPA), 0.0147 g $CaCl_2$ in 900 ml H_2O, buffered at pH 7.3.

Three replicate samples of 12g (10 g dry weight) of each soil collection were taken from each soil treatment, shaken with 18 ml of extractant for two hours and filtered through a Whatmans No. 42 filter paper. The resulting solutions and appropriate standards containing the extractant were analysed by ICP spectrometry. The DTPA extraction assay is suited to estimation of plant available Mn, Fe, Cu and Zn in alkaline soils and the standard solutions included these trace elements. Using the ICP analysis results, standard curves were calculated and the levels of Mn, Cu, Fe and Zn in the soil were determined from them. Results were subjected to analysis of variance (Table 3.2).

3.3.3 Plant Responses

Seedling wheat plants were grown in small pots (50 g soil, dry weight) under the six soil treatments, with three replicates. Basal nutrients had been applied to the six soil treatments prior to potting. Two seeds per pot of surface sterilised and pre-germinated wheat (cv. Bayonet) were planted in each pot. Bayonet is a cultivar sensitive to Mn deficient conditions and the seed used had low Mn content (approximately 4 mg / kg). The plants were grown in a growth chamber (under conditions described in section 2.2.7) and the pots watered to original weight regularly with purified H_2O. Harvested after 29 days, fresh weights of shoots and roots were taken, and elemental tissue analysis of shoots and roots carried out by ICP spectrometry. Analysis of variance was carried out on shoot and root fresh weights and Mn concentrations (Tables 3.4, 3.5).

3.4 Results

3.4.1 X-ray fluorescence

X-ray fluorescence results showed no significant difference between the three soil collections for total soil Mn (Table 3.1). The -Mn treatments contained about 6 mg / kg MnO (4.6 mg Mn / kg soil), while the +Mn treatments gave results of about 22 mg / kg MnO (17.0 mg Mn / kg soil), showing the addition of Mn in the +Mn treatment (12 mg Mn / kg compared to the added 18 mg Mn / kg). The technique was used to the extent of its sensitivity, so little confidence is placed in the slight differences in total Mn seen between the 1988 collection and the more recent Wangary soil collections.

Table 3.1 Total Mn (mg Mn / kg soil dry weight), assessed by X-ray fluorescence spectrometry, in Wangary soil samples of three soil collections (1990, 1989, 1988) incubated with -Mn (no added Mn) and +Mn (18 mg Mn / kg soil) treatments. Values are the mean of two samples.

Year of collection	Soil treatment	MnO mg / kg	Mn mg / kg
1990	-Mn	5	3.9
	+Mn	21	16.2
1989	-Mn	5	3.9
	+Mn	21	16.2
1988	-Mn	8	6.2
	+Mn	23	17.8

3.4.2 DTPA extraction

DTPA extractable Mn was clearly much lower than the total Mn in the Wangary soil, assayed by X-ray fluorescence spectrometry. The analysis of the DTPA extracts showed

Table 3.2 DTPA extractable Mn, Fe, Cu and Zn in three collections of Wangary soil (1990, 1989, 1988), differing in storage age, under two Mn treatments, -Mn (no added Mn) and +Mn (18 mg Mn /kg soil dry weight).

Mn

mg / kg	1990	1989	1988	mean
-Mn	0.088	0.134	0.374	0.20
+Mn	19.16	23.39	24.05	22.20
mean	9.62	11.76	12.21	11.20
LSD	Mn tmt 0.976***, year ns , Mn tmt x year ns (-Mn results only: year 0.0127 ***)			

Fe

mg / kg	1990	1989	1988	mean
-Mn	1.44	1.76	3.20	2.13
+Mn	1.57	1.84	3.41	2.28
mean	1.50	1.80	3.31	2.20
LSD	Mn tmt 0.016 ***, year 0.02 ***, Mn x year 0.028*			

Cu

mg / kg	1990	1989	1988	mean
-Mn	0.152	0.150	0.151	0.151
+Mn	0.124	0.103	0.161	0.129
mean	0.138	0.127	0.156	0.140
LSD	Mn tmt ns, year ns, Mn tmt x year ns			

Zn

mg / kg	1990	1989	1988	mean
-Mn	0.440	0.123	0.667	0.410
+Mn	0.110	0.133	0.443	0.226
mean	0.275	0.128	0.550	0.318
LSD	Mn tmt ns, year ns, Mn tmt x year ns (+Mn results only: years 0.005***)			

Table 3.3 Results of assays of Wangary soil collections, with intention to look at storage effects on Mn extractability and availability to plants. Comparison of results of experiments carried out by Webb et al. (1993) (August -September 1989, when 1988 soil had been stored for approximately 18 months) and similar experiments carried out in this thesis (January 1992, when 1990 soil had been stored for approximately 22 months). Both sets of experiments were carried out under similar conditions. Wheat plants (Bayonet), using low Mn seed sources, were grown in collections of Wangary soil for 4 weeks. Values for shoot dry matter and shoot Mn concentrations are the means of 3 replicates and standard errors are given.

DTPA extractions: (mg/kg)

	In 1989 -Mn	**+Mn**	**In 1992 -Mn**	**+ Mn**
1988	0.09	no data	0.38	24.0
1989			0.13	23.4
1990			0.09	19.2

Shoot dry matter (mg)

	In 1989 -Mn		**+Mn**		**In 1992 -Mn**		**+Mn**	
Year	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
1988	65	25	375	35	23	4	115	18
1989	50	18	350	15	10	2	76	17
1990					13	3	78	12

Shoot Mn concentration (mg Mn / kg dry matter)

	In 1989 -Mn		**+Mn**		**In 1992 -Mn**		**+Mn**	
Year	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
1988	5.5	2.0	103	20	4.7	0.2	104	41
1989	5.0	0.5	30	5	3.1	0.3	126	32
1990					3.4	0.0	110	56

that the extractable Mn in the three soil collections did differ, the 1988 soil having more extractable Mn than either of the other collections, and the 1989 collection slightly more extractable Mn than the 1990 collection (Table 3.2). However, separate analysis of the Mn soil treatments showed that significant differences in Mn availability (as extractable Mn) did exist between the collections in the - Mn treatment, but not the + Mn treatment.

The extractability of iron (Fe), copper (Cu) and zinc (Cu) in the - Mn and +Mn treatments, determined in the same samples, is also shown (Table 3.2). The interaction of Mn soil treatment with collection year was significant for Fe extractability from the Wangary soil collections. Cu extractability appeared to be unaffected both by the storage of the soil and by the addition of Mn. An effect on Zn extractability was only found when the Mn soil treatments were analysed separately, showing that there were significant differences between the collections under the + Mn treatment.

Storage period affected the levels of DTPA extractable Mn in the collections of Wangary soil. These results are consistent with those of Webb et al. (1993), showing that the period of storage of Wangary soil increases the level of DTPA extractable Mn (Table 3.3). Shoot Mn concentrations of wheat plants grown in the Wangary soil collections are similarly affected by levels of plant available Mn increasing as the soil collections are stored. Similarities in shoot dry matter production are less clear (Table 3.3).

3.4.3 Plant Responses

Plant growth, affected by Mn availability, showed distinct differences between the Wangary soil collections. The fresh weight of roots showed a significant effect of the interaction of Mn treatment and year of collection, but shoot fresh weight did not show such a clear response to the treatments (Table 3.4). The plants grown in 1988 soil had the largest fresh weights, but shoot fresh weights were clearly greater in the 1990-soil plants than the 1989-soil plants. Dry weights were not significantly affected by the year of soil collection, although Mn treatments did have an expected significant effect (data shown in Table 3.3).

Mn concentration in the shoots and roots was significantly affected by Mn treatment (Table 3.5). The year of collection showed a significant effect on Mn concentrations in roots, but shoot Mn concentrations followed shoot fresh weights, with higher levels in the 1990 soil treatment than the 1989 soil.

Table 3.4 Fresh weight of shoots and roots (g) of wheat plants grown in three collections of Wangary soil (1990, 1989, 1988), under two Mn soil treatments, -Mn (no added Mn) and +Mn (18 mg Mn / kg soil dry weight) treatments. Values are the mean of three replicates. LSD values are given.

Shoot (g)

	1990	1989	1988	mean
-Mn	0.064	0.041	0.227	0.111
+Mn	0.588	0.524	0.862	0.658
mean	0.326	0.282	0.544	0.384
LSD	Mn tmt 0.048 ***, year 0.059 **, Mn tmt x year ns			

Root (g)

	1990	1989	1988	mean
-Mn	0.047	0.070	0.133	0.083
+Mn	0.607	0.667	0.910	0.728
mean	0.327	0.368	0.522	0.406
LSD	Mn tmt 0.022 ***, year 0.027 ***, Mn tmt x year 0.039**			

Table 3.5 Mn concentrations (mg / kg dry weight) in shoots and roots of wheat plants grown in three collections of Wangary soil (1990, 1989, 1988) under two Mn treatments, -Mn (no added Mn) and +Mn (18 mg Mn /kg soil dry weight). Data was adjusted (square root) to remove skew; adjusted data shown in italics. LSD values for adjusted data only.

Shoot Mn concentration

mg / kg	1990	1989	1988	mean
-Mn	3	3	5	4
	1.83	*1.75*	*2.18*	*1.92*
+Mn	110	126	147	128
	9.87	*11.02*	*12.11*	*11.00*
mean	57	64	76	66
	5.85	*6.38*	*7.14*	*6.46*
LSD	Mn tmt *1.02* ***, year ns, Mn tmt x year ns			

Root Mn concentration

mg / kg	1990	1989	1988	mean
-Mn	13	17	22	17.0
	3.57	*4.06*	*4.60*	*4.08*
+Mn	200	571	493	421.0
	14.09	*22.82*	*22.19*	*19.70*
mean	106	294	257	219.0
	8.83	*13.44*	*13.39*	*11.89*
LSD	Mn tmt *1.73* ***, year ns, Mn tmt x year ns			

3.5 Discussion

3.5.1 X ray Fluorescence

The three annual collections of Wangary soil were made at the same sites only a few metres from each other, from uncultivated soil and where no fertiliser had been added to the soil. Thus differences in total Mn between the soil collections here were unlikely, and the material from the collection site at Wangary has been shown to be a consistent material.

No significant differences were found between the soil collections (within -Mn or +Mn treatments) for total Mn (measured as %MnO).

Addition of Mn to the soil in the +Mn treatment was effectively measured by this method, but total Mn in the soil collections was not conclusively assessed.

3.5.2 DTPA Extraction

The effect of the period of storage of the soil collections on Mn extractability is clearly shown in these results. Extractable Mn increased with the age of the soil collections. Even in the +Mn treatment, there is a trend of increased Mn extractability with the age of the collection. The similar increase of Fe extractability with soil collection age is interesting since Mn and Fe are similar in their dependence on redox states for uptake by plants and are both actively transformed in soils.

Cu availability was not affected by the age of the collection or by the Mn treatment. Zn availability was only affected by age of the soil collection under the +Mn treatment. However, the effects of soil age and Mn treatment on Fe, Cu and Zn are not of great issue in the pot experiments carried out in these experiments, where native nutrient levels are masked by the addition of basal nutrient solutions.

Differences between microorganism populations in the collections of Wangary soil after storage was suggested (Webb et al. 1993) as being of extreme importance in the differences in plant available nutrient between soil collections. The microorganisms, both oxidisers and reducers, which appear to play a large part in the variability of the plant available pool of Mn in soils, would also affect the availability of Fe (Ghiorse 1988). The production of siderophores by rhizosphere bacteria (and of phytosiderophores by plants) enhances plant Fe uptake, and has been shown to additionally increase the uptake by plants of other trace elements, such as Zn, Mn and Cu (Zhang et al. 1989, Treeby et al. 1989).

The production of non-specific oxidising agents, chelating agents (such as siderophores) or reducing agents by plants and microorganisms represents great potential for altering nutrient availability in soil. However, the lack of change over time in Cu and Zn extractability, in the Wangary soil collections, may indicate that redox controlled reactions had a greater effect on the DTPA extractability of micronutrients than the production of microbial siderophores.

3.5.3 Plant Responses

Reisenauer (1988) and Webb et al. (1993) described discrepancies between the results from Mn DTPA extraction and plant responses to Mn in soils. The results from the DTPA extractions and the plant responses carried out on the three Wangary soil collections showed similar trends, but subtle differences (compared to the DTPA estimations) were apparent in the plant available Mn results.

The assay of plant uptake of Mn from the different soil collections showed that the more recent collections facilitated a greater Mn deficiency in plants. Extreme Mn deficiency was suffered by the plants grown in the -Mn treatment of the 1990 soil collection and root growth was significantly retarded, compared to the plants grown in the 1988 collection.

Root tissue provided a better measure of the effect of Mn availability under soil treatments than shoot tissue. Under these extremely Mn deficient conditions, Mn appears to be partitioned to the shoot, while under conditions of Mn adequacy, Mn is retained in the vacuole of root cells (Quiquampoix et al. 1993, Clarkson 1988, Munns et al. 1963, Vose 1963). The results showing 'pools' of Mn in root tissue may be showing this partitioning of excess Mn into the root tissue, or may simply be the effect of Mn being precipitated on the root surface, as Mn oxides. Plants grown in conditions of abundant available Mn often showed discoloured roots, due to the precipitation of Mn oxides onto the root surface (Wilhelm 1992).

3.5.4 Soil Mn Tests

These assessments of Mn in the three Wangary soil collections show that the collections differ in their ability to create extreme Mn deficiency in plants. The plant available Mn is clearly affected by storage time (and thus probably by microbial populations as discussed by Webb et al. 1993), not by the total Mn pool in the soil alone.

The differences in Mn extractability between the two soil collections used in the experiments in Chapter 2 is slight, but as has been shown, even slight differences in extractability can be associated with significant differences in plant growth, specifically root growth. ~~The rate of change in Mn availability in the Wangary soil collections appears to~~ The differences shown here between the 1989 and the 1990 soil collections are subtle, but sufficient to create significant differences in plant growth and in plant available Mn, thus supporting the first part of the hypothesis put forward (section 2.7), that the 1990 Wangary soil collection had greater facility to cause Mn deficiency than the 1989 collection.

3.6 Soil Sandwiches

It was suggested in the hypothesis put forward in Section 2.7 that fungal growth was decreased under conditions of extreme Mn deficiency. Fungal growth in soil (saprophytic growth) is dependent upon organic nutrient sources, and the availability of ions and sugars. Manganese has been suggested as being an essential trace element for fungi but quantification of the requirements of trace elements by fungi has not previously been a priority (Timonin et al. 1972, Cunningham 1981).

Sadasivan (1965) suggested that the requirement for trace elements in fungal pathogens was an important factor in the production of 'virulence enzymes'. Under Fe deficient conditions, production of pectin methyl esterase (PME) by *Fusarium vasinfectum* was reduced to the point where a normally pathogenic strain was considered hypovirulent. In contrast, under Mn deficient conditions, PME production was high, and decreased as Mn was increased in the growth medium (Subramanian 1956 (unseen) cited in Sadasivan 1965). Similarly, Jackson et al. (1989) showed that production of the toxin, fusarin C by *Fusarium moniliforme*, is decreased by the addition of Zn, Fe, Mn and Co to liquid culture medium, while dry matter production of the fungus is increased. Thus trace element deficiency in soil may affect the virulence of fungal pathogens separately from an effect on their saprophytic growth.

High soil pH increases the likelihood of take-all disease in susceptible crops. However, differing pH levels in soil (Glenn and Sivasithamparam 1991 and in agar (Webb and Fellows 1931) had no consistent effect on the saprophytic growth of Ggt isolates, and thus an increase in pH alone could not be the reason for an increase in take-all disease. Virulence of the isolates was not assessed. High soil pH also decreases the availability of several trace elements in the soil solution, including Mn. The availability of Mn in soil is certainly pH dependent, and may affect the saprophytic growth of Ggt in soil, if not the virulence of isolates.

Growth of Ggt inoculum on agar was shown to respond slightly to the addition of 50 mg / kg Mn to nutrient agar (Wilhelm 1992). Growth on agar has been strongly correlated with growth of hyphae on wheat roots (Holden 1976) and growth of Ggt in soil sandwiches has been used to indicate saprophytic growth strength (Simon et al. 1988, Gilligan 1985).

It must be remembered that saprophytic growth of the fungus does not necessarily indicate the virulence of the fungus.

3.7 Method

The experiment described here examines the effect of extremely low Mn levels on growth of Ggt inoculum, in soil sandwiches.

3.7.1 Soil

Wangary soil (1990 collection) was used in this experiment, prepared and incubated as usual (section 2.2.2), but with 10 Mn treatments. The soil used in these soil sandwiches was taken from the soil treatments prepared for the seedling pot experiment described in Chapter 4. The Mn treatments were 0, 4.5, 9, 13.5, 18, 24, 27, 36, 45, and 54 mg Mn /kg soil (dry weight). The rate of added Mn used in all previous experiments was 18 mg / kg. Soil treatments were numbered Mn 0 to Mn 9. Basal nutrients were added and mixed to the soil treatments after incubation as usual (section 2.2.3).

3.7.2 Soil Sandwiches

Approximately 60 g soil from each Mn treatment was packed loosely into the bottom half of a 9 cm, sterile plastic petri dish. A Whatmans No 1 filter paper was placed on top of the soil and Ggt (isolate 500) inoculum on a 4 mm diameter agar plug was positioned centrally on top of the paper. The lid of the petri dish was placed over the inoculum and filter paper and the whole lot turned upside-down. The rim of the filter paper, not covered by the soil and dish, was dampened with 1 ml of purified H_2O. The plates were placed in a growth cabinet for 10 days. The experiment was replicated five times, and proceeded simultaneously with the seedling pot experiment described in Chapter 4.

After 10 days the filter papers were removed from the petri dishes. Most of the soil clinging to the filter papers was removed, and hyphal growth on the filter papers was observed using a dissecting microscope (16 x magnification), without staining the hyphae. A hairline was used as a transect along which the hyphae were counted. Interception of the line by hyphae, usually black runner hyphae, were averaged over three fields of view. The radial growth of the hyphae outward from the edge of the agar plug was also measured.

3.7.3 Calculations

Newmann's method (Newmann 1966) was used to calculate the total length of hyphae. The equation for total length of roots, using a randomly placed hairline over a randomly placed and dispersed root system is:

$$R = \frac{N\,A}{2H}$$

where R= root length, N= total number of intersections of the hairline by roots (hyphae), A= area of dish in which the root system is observed and H= total length of hairlines (ie. all fields of view counted). A and H were constant, H was measured at 4.5 mm (a 4.5 mm F. O. V. at the stage surface), A = 5680 square mm (the area of the dish).

3.8 Results

It is clear from these results that the extreme Mn deficiency of the Wangary soil retarded the saprophytic growth of the Ggt hyphae; all other basal nutrients being added to the soil at usual rates (Table 3.6). Growth of hyphae from the edge of the agar plug on the filter paper was not as strong as expected. Few, unbranched hyphae emerged from the plug in the lowest Mn treatments and at the highest levels of Mn , although growth was strong near the inoculum plug, it did not extend further than a few millimetres from the inoculum source.

However, the differences between the soil treatments were large, the least growth in the Mn 0 treatment giving rise to only a few millimetres of hyphal growth, and addition of Mn to the soil (Mn 1) immediately increasing hyphal growth. Further addition of Mn to the soil gradually increased the length and density of hyphal growth, until reaching a plateau at the soil treatment Mn 6 (Figs. 3.1, 3.2). Growth of hyphae on the filters at the highest levels of Mn addition was restricted in diameter, but very dense.

Table 3.6 Effect of increasing levels of added Mn to soil on the growth Ggt 500 on filter paper in soil sandwiches, and the calculated total length of hyphae after 10 days. Mn was added to the soil as 1.0M MnSO4, at 10 rates. Growth was measured as the average radius of hyphae from the edge of the inoculum (mm), and the length of hyphae (R). Values are the average of five replicates.

Soil treatment	Growth observations	Growth (mm)	R (mm)
Mn 0	Sparse growth of black runner hyphae, not branched.	4.8	1.6
Mn 1		9.0	7.2
Mn 2		11.3	8.7
Mn 3	Some branching of black runner hyphae, but not forming a mat.	9.5	7.7
Mn 4		12.0	12.2
Mn 5	Thick growth from the agar plug for 3 mm, but then only thin, sparse, branched hyphae.	12.5	14.9
Mn 6		11.5	14.6
Mn 7	Thick mat of hyphae, but only within 5 mm of the edge of the agar plug	6.0	14.4
Mn 8		4.5	15.3
Mn 9		4.0	16.9

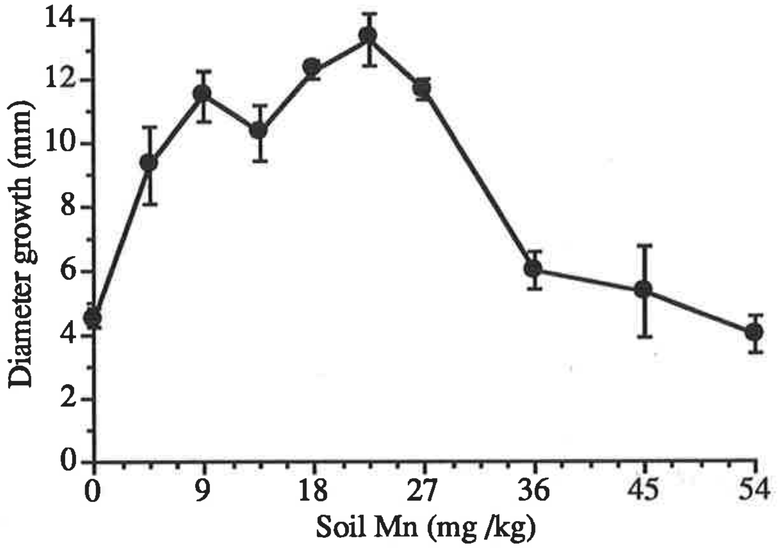

Fig. 3.1 Diameter of growth, ● (mm), after 10 days, of Ggt hyphae (Ggt 500) in Wangary soil sandwiches, under 10 soil Mn treatments (0 - 54 mg Mn / kg soil dry weight). Values are the mean of five replicates, standard error bars are shown.

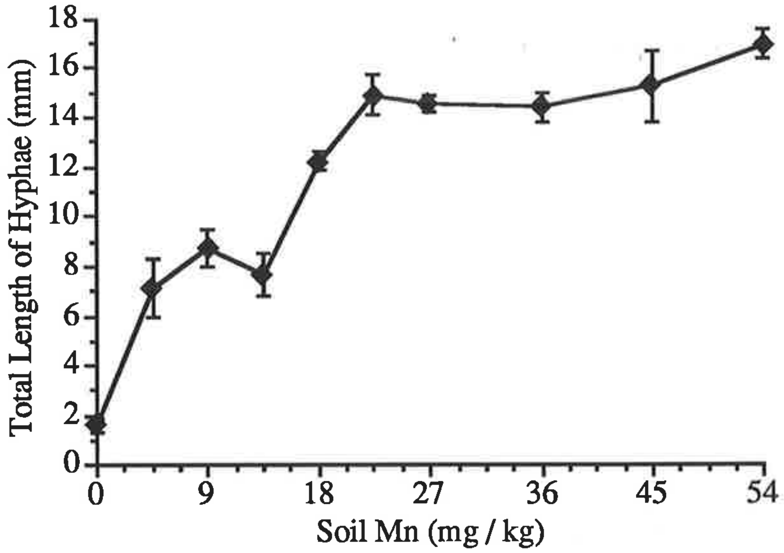

Fig. 3.2 Total length of hyphae, ◆ (mm), after 10 days, of Ggt hyphae (Ggt 500) in Wangary soil sandwiches, under 10 soil Mn treatments (0 - 54 mg Mn / kg soil dry weight). Values are the mean of five replicates, standard error bars are shown.

3.9 Discussion: Soil Sandwiches

Saprophytic growth of the hyphae was clearly depressed under Mn deficient conditions (soil treatment Mn 0). With the addition of 1/4 of the normal rate of Mn to the soil (Mn 1), growth of Ggt 500 (R) increased fourfold, while the diameter of growth nearly doubled. The growth was specifically affected by Mn availability, since all other basal nutrients were added to the soil mixture at levels suitable for plant nutrition.

Density of hyphal growth was not quantified. A very high density of hyphal growth within a small area on the filter paper was observed at the highest Mn treatments. The restricted area of this dense hyphal growth may have been due to the exhaustion of organic nutrients in the inoculum plug. This constricted, high density growth occurred in the Mn treatments Mn 7 to Mn 9, with 36 to 54 mg Mn / kg soil. It is unlikely that the restricted growth was a result of Mn toxicity, as Wilhelm (1992) has shown that hyphal growth is not depressed until Mn levels reach 800 mg / kg.

Saprophytic growth of the Ggt hyphae was thus shown to be retarded under conditions of extreme Mn deficiency in soil. The conditions of Experiment 2.2 (section 2.7) may thus have led to a poorer infective capacity of the inoculum and low levels of disease in the -Mn treatment. In the same experiment, addition of Mn to the soil would have increased the growth of the fungal inoculum, and a higher disease level was achieved in the +Mn treatment.

In the field, the saprophytic growth of Ggt inoculum in soil has been regarded as a means by which the likelihood of disease rises. Factors other than inoculum density in soil play a large part in determining the likelihood of take-all disease, the availability of Mn in the soil being one such factor (Lucas and Nignon 1987, Lucas and Sarniguet 1990). The effects of soil pH, nutrient availability and micro organism populations on both saprophytic growth of soil pathogens and on virulence of the isolates have been gathered under the term 'soil receptivity' (Alabouvette et al. 1982).

The receptivity of Wangary soil to take-all disease seems to be increased by the addition of low levels of Mn fertiliser. Addition of more Mn to the soil may decrease the susceptibility of plants to infection, while still promoting the saprophytic growth of the pathogen.

Sites such as Wangary and Marion Bay where Mn is chronically deficient should, by evidence of the experiments in Chapter 2 and other accounts, be permanently devastated by take-all but instead the root disease only occurs in mild, patchy way. The reason may well be that the extreme Mn deficiency of the soil inhibits saprophytic growth of the fungus and thus reduces its effectiveness as a pathogen.

3.10 References

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Chapter 4

Effects of seed source and soil source nutrient interactions upon seedling susceptibility to Ggt infection.

4.1 Introduction

In Chapter 2 the hypothesis was put forward that the extremely low levels of available Mn in the 1990 collection of Wangary soil may have had an effect on the growth of Ggt inoculum, and perhaps the capacity of the Ggt fungus to invade the seedling roots. (In this context 'capacity' differentiates between the innate virulence of the fungal isolate, and the capacity of that isolate to invade under poor conditions, a situation analogous to that of a skilled person who cannot perform without specific equipment, or a high yielding genotype which is nutrient inefficient.)

Having established in Chapter 3 that the growth of the Ggt fungus (and thus possibly its pathogenic effect) is reduced by the extremely low Mn levels in the 1990 collection of Wangary soil, this next experiment investigates the effect of the same ten soil Mn levels in Wangary soil on wheat plants and their susceptibility to infection by Ggt under these conditions.

Two other factors were integrated into this experiment - the effects of seed source Mn and Mn efficiency. These factors were included to give an indication of the relative power of Mn efficiency and seed source Mn to affect the susceptibility of wheat plants to Ggt infection in comparison with soil Mn treatments. The experiment was thus a study of the interactions of soil Mn nutrition, seed Mn nutrition and Mn efficiency of wheat genotypes, to provide information on the best way to reduce susceptibility of wheat seedlings to take-all disease.

The definition of Mn efficiency, and the effects of Mn efficiency in reducing the susceptibility of wheat genotypes to Ggt infection, have been previously discussed (section 1.5, Graham 1983, Wilhelm et al. 1990). A short review of the effects of seed source nutrition on plant growth and disease resistance follows.

4.1.1 Seed source nutrition

Seed source nutrient has been shown to have great importance in emergence and establishment of crop plants, leading to yield improvements where the seed source nutrition was optimal (Marcar and Graham 1986, 1987). High seed source Mn has been shown to have some effect upon tolerance of take-all by wheat (Wilhelm 1992). Plants grown from seed soaked in $MnSO_4$ prior to sowing in pot experiments had only slightly higher infection levels than plants grown with soil applied $MnSO_4$, compared to plants supplied with soil applied MnO_2 fertiliser, or with no applied Mn. Other experiments using seed soaked in micronutrients have shown that higher seed source nutrition creates better germination and resistance against *Fusarium* inoculum (Sadasivan 1965). Seed nutrient, whether natural, added by soaking, or applied as a coating, may have sufficient effect upon seedling establishment to affect the susceptibility of plants to disease.

Techniques of coating seed with fertiliser have been developed to add to or complement natural seed nutrient levels. Seed coating with fertiliser has been shown to be effective in increasing seedling vigour, which can lead to increases in yield through strong seedling establishment (Scott 1989). However, seed coating can also reduce germination and seedling establishment, and the close proximity of fertiliser (particularly more soluble sources of nutrients) to the emerging embryo can cause fertiliser injury of seedlings (Scott 1975, Klepper et al. 1983).

Plants grown at sites which differ in soil nutrition differ in the balance of mineral nutrients naturally translocated into their seed; plants grown at Mn deficient sites have low seed Mn concentrations (Marcar and Graham 1986, Longnecker et al. 1991, pers. comm. J. S. Ascher). The loading of seed nutrient may be additionally affected by genotypic factors. Under Mn limiting conditions a Mn efficient genotype is likely to produce seed with a higher Mn concentration, compared to seed of an inefficient genotype. However, the loading of Mn into cereal seed does not appear to be consistently affected by the Mn efficiency of the genotype (Graham 1988, Nable and Webb 1993) and it is not clear that Mn loading into seed is directly linked with Mn efficiency.

4.2 Method

4.2.1 Soil treatments

As detailed in Chapter 3 (section 3.8.1) Wangary soil (1990 collection) was mixed and incubated with ten different soil Mn treatments (Mn 0 - Mn 9), giving 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5 and 3.0 mg Mn per 50 g pot. Soil for each part of the experiment was incubated separately. Basal nutrients were added after incubation.

4.2.2 Inoculum

The conetainer procedure (section 2.2.1) was used again in this experiment. Inoculum used in the experiment, Ggt 500, was prepared as detailed in section 2.2.5. Agar medium preparation is detailed in section 2.2.4.

4.2.3 Seed

Seed of all three genotypes from the sites Wangary and Lameroo was used, with additional Spear seed from Tooligie, and Bayonet seed grown in a glasshouse (Glasshouse-source). Wangary is a Mn deficient site, while Lameroo is a Zn deficient site in the Mallee country of South Australia. Tooligie is a second, less Mn deficient site on the Eyre Peninsula (South Australia), and the glasshouse grown seed was grown in Wangary soil in large pots, with all basal nutrients except Mn added at sowing. The Lameroo-source seed was obtained from plants adequately supplied with Zn. The seed used in the experiment was analysed by ICP spectrometry (Appendix 2). The sites from which seed was obtained and the seed Mn concentrations are presented in Table 4.1.

All seed was surface sterilised and pregerminated prior to sowing in pots. Since seed differing in source often had slight differences in size and germination strength, care was taken in using only similarly sized, germinated seeds which had radicals of similar length (approximately 7 mm).

Table 4.1 Genotypes of wheat used in Experiment 4, the sites at which the seed was grown and the seed Mn concentrations (mg / kg).

Genotype	Site - Source	Mn (mg / kg)
C8MM	Lameroo	38.2
	Wangary	3.6
Spear	Lameroo	37.5
	Wangary	3.8
	Tooligie	8.6
Bayonet	Lameroo	32.9
	Wangary	3.2
	Glasshouse	25.2

4.2.5 Cultivation of seedlings

In order to score the plant roots for infection with as little delay as possible after harvesting, the experiment was not carried out in a single step but in a staggered fashion, divided by genotype into three parts. The three parts were sown at two week intervals and each harvested at 30 days. The experiment was completed within an eight week period, using the same growth chamber for each part. Preparation and growing conditions were identical for each part of the experiment.

Plants were grown in a growth chamber with a day / night cycle of 10 / 14 hours at 15 / 10°C and pots were watered to original weight with purified water every second day. Emergence of shoots in all three genotypes was quick (3 - 4 days), with the exception of Tooligie-source Spear plants. Although only germinated seed of similar growth status had been planted, one half of one replicate had to be resown (at D5) because of poor emergence. Germinated seed remaining from the initial sowing had been retained for this purpose, kept in the growth chamber, and was used to replace the damaged or stunted seed which had not emerged. Growth of seedlings of all genotypes and sources was satisfactory thereafter. Pots were watered to original weight with purified water every second day.

Seedlings were harvested after 30 days, shoot and root fresh weights taken, number of roots (NOR) counted and dry weight of shoots recorded, before analysis by ICP to determine the Mn concentration of shoots (ShMn). Roots of the +Ggt treatment plants were scored for number of infected roots (NIR), number of stelar lesions (NSL) and total length of lesions (TLL). The average length of lesions (AVL) was calculated by TLL / NSL. Analysis of variance was carried out on the plant data (which included + and -Ggt treatments) and disease measure results (+Ggt treatment only) for each genotype separately as well as for the whole experiment.

4.3 Results

4.3.1 Summary of results

This experiment was conducted to determine which of several different sources of Mn was the most effective in reducing infection of seedlings by Ggt. Under conditions of Mn deficiency, the additive effect of Mn efficiency and seed source Mn was shown to be as effective as soil fertiliser Mn in reducing Ggt infection of seedlings. Once adequate Mn was supplied to plant roots the response of disease measures to further soil Mn treatments and to differences in seed source was minimal. The Mn efficiency of plants was the greatest influence in reducing disease measures in plants adequately supplied with Mn. Overall, the interaction of Mn efficiency with soil Mn treatment had the greatest effect upon infection measures, reducing disease in Mn efficient plants with adequate soil Mn.

The vegetative plant data are presented first (Tables 4.2 - 4.19), showing clear differences between the genotypes, with C8MM showing the greatest growth and least impact of low Mn treatments, and Bayonet showing the most impact of the various Mn treatments. Seed source Mn affected shoot growth and shoot Mn (ShMn) levels more significantly than root growth measures, and had less effect on the infection of roots than soil Mn or genotype efficiency.

4.3.2 Plant growth results

While soil Mn treatment had an overriding effect upon ShMn, plant growth as a whole benefited from high seed source Mn, adequate soil Mn and by the absence of Ggt inoculum. The efficiency of each genotype affected the extent of the response to the Mn source treatments (Tables 4.2 - 4.19).

The different Mn source treatments did not create large differences in the growth of C8MM plants. Only slight differences were seen between the soil Mn treatments, with slight stunting in the highest Mn treatment (Mn 9) and slight Mn deficiency symptoms in the lowest Mn treatments, especially in the +Ggt treatments. Plants of the less efficient Spear and Bayonet genotypes showed more Mn deficiency symptoms (especially in the lower soil Mn and seed Mn treatments) and greater responses in plant growth measures to the higher soil Mn treatments than C8MM plants.

1) Genotypes

Overall, the strongest effects on plant growth resulted from the Mn efficiency of genotypes and from soil Mn treatments. The Mn efficiency of the genotypes affected all measures of plant growth, as well as ShMn; Bayonet having the weakest vegetative growth in the lower soil treatments, C8MM the strongest. A combination of high genotype Mn efficiency and the absence of inoculum increased root fresh weight (Tables 4.11 - 4.13, 4.17 - 4.19).

As soil Mn treatments rose, plant growth improved for each genotype. Increasing Mn soil treatments caused plant growth (Tables 4.2 - 4.4) and ShMn concentrations to rise to a plateau at Mn 5 or Mn 6, sometimes falling slightly at Mn 9 (Table 4.8 - 4.10). With the further interaction of seed source, Lameroo-source plants of all genotypes had higher ShMn than Wangary source plants, and the higher soil Mn treatments further increased Mn concentrations in the shoots of these plants.

The comparison of genotypes show similarities in plant response to soil and inoculum treatments across genotypes, but also show clearly that the differences in plant growth caused by seed source treatments are not as great as differences in plant growth due to Mn efficiency. The efficient C8MM generally outyielded the inefficient Bayonet, even when Bayonet was grown under advantageous conditions (soil treatment Mn 9, Glasshouse-source; shoot fresh weight 0.508 ±0.032 g), and C8MM under the most minimal (soil treatment Mn 0, Wangary-source; shoot fresh weight 0.634 ± 0.115 g. -Ggt treatments, the averages of 3 replicates).

2) Seed source

The interaction of seed source and soil Mn treatment was a significant factor affecting seedling vegetative growth in all three genotypes (Tables 4.2 - 4.4, 4.5 - 4.7, 4.11, 4.13). Higher seed source nutrient in combination with the absence of inoculum tended to increase shoot growth in the less Mn efficient Spear and Bayonet. Root fresh weight was not consistently increased by high Mn seed source, while NOR was increased by high seed source Mn in Spear and Bayonet (Tables 4.15, 4.16) but not in C8MM (Table 4.14).

In general, plants with higher seed Mn grew better than those with low seed Mn, but there were two clear exceptions. In these cases some factor of the seed sources other than seed Mn concentration had a dominant effect on plant growth. Tooligie-source Spear plants had higher levels of seed Mn than Wangary-source Spear, but were generally out-performed in all measures of plant growth by Wangary-source Spear plants (Tables 4.1, 4.3, 4.6, 4.9, 4.15). Only in soil treatment Mn 0 did Tooligie-source plants yield consistently better than Wangary-source plants.

Similarly, Lameroo-source Bayonet plants had a higher level of seed source Mn than Glasshouse-source Bayonet plants (Table 4.1), but were consistently out-performed by Glasshouse-source plants. All measures of plant growth were significantly higher in the Glasshouse-source plants than those of Wangary and Lameroo-source plants (Tables 4.4, 4.7, 4.13, 4.16, 4.19), although in soil Mn treatments above Mn 3, the ShMn of Glasshouse-source plants was often below that of both Wangary and Lameroo-source plants (Table 4.10).

3) Soil Mn treatments and shoot Mn concentration

Shoot Mn concentrations were significantly responsive to genotype efficiency, seed source, soil treatment and inoculum presence (Tables 4.8 - 4.10). Shoot Mn concentration

(ShMn) was greatly affected by soil Mn treatment, clearly increasing as soil Mn levels increased. Similarly, the more Mn efficient genotypes, C8MM and Spear, had higher ShMn levels than the inefficient Bayonet. The presence of Ggt inoculum significantly depressed ShMn across the soil Mn treatments, with seed source Mn appearing to have little direct effect on ShMn.

The concentration of Mn in shoots was much higher in the C8MM plants at low soil Mn levels than in Spear or Bayonet shoots in the same soil treatments (Tables 4.8 - 4.10). Mn deficiency symptoms were observed in Spear and Bayonet plants from soil treatments Mn 0 and Mn 1, with ShMn below 20 mg / kg.

In Spear and Bayonet plants, ShMn rose sharply as soil Mn treatments increased from Mn 0 to Mn 3. C8MM plants, with ShMn above 20 mg / kg in Mn 0, did not show such a sharp rise in shoot Mn over the same soil treatments. In soil treatments Mn 5 or 6, ShMn in all genotypes reached a level of about 110 mg Mn / kg (shoot dry weight), usually rising in Mn 9 to about 140 mg Mn / kg.

Shoot fresh weight, root fresh weight and NOR were affected to a lesser extent by increasing soil Mn treatments, beyond a large initial increase from Mn 0 to Mn 1 (Tables 4.2 - 4.4, 4.11 - 4.13, 4.15, 4.16). Root length, however, increased dramatically with the first few soil Mn treatments (Mn 1 - Mn 3), levelling out, usually at Mn 3, and then dropping slightly from the plateau in the Mn 9 treatment (Tables 4.18, 4.19).

4) Ggt inoculum and root growth

The presence of Ggt inoculum consistently decreased root length, across genotypes and seed sources (Tables 4.17 - 4.19), with Bayonet plants the most clearly affected. NOR was decreased by +Ggt in Spear plants (Table 4.15) but in Bayonet plants slightly increased (Table 4.16), rising further with increasing soil Mn treatments. Root fresh weight was significantly increased by an interaction of the +Ggt treatment and high Mn seed source in Bayonet (Table 4.13). C8MM root growth was largely unaffected by inoculum treatments.

Shoot fresh weight and ShMn were significantly decreased in +Ggt treatment plants of all genotypes (Tables 4.2 - 4.4, 4.8 - 4.10). Shoot dry weight was also reduced by the +Ggt treatment (Tables 4.5 - 4.7).

Table 4.2 Shoot fresh weights (g) of wheat seedlings, genotype C8MM, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Two sources of seed (Source) were used: low Mn Wangary-source (Wan), and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

C8MM Shoot fresh weight (g) Soil Mn treatment

	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	0.634	0.707	0.592	0624	0.651	0.678	0.608	0.654	0.513	0.554	0.622
	Lam	0.846	0.554	0.449	0.706	0.83	0.687	0.755	0.755	0.694	0.579	0.686
+ Ggt	Wan	0.386	0.609	0.475	0.351	0.312	0.540	0.390	0.393	0.328	0.312	0.409
	Lam	0.606	0.560	0.468	0.496	0.607	0.627	0.4647	0.600	0.519	0.308	0.526
mean		0.618	0.607	0.496	0.544	0.601	0.633	0.555	0.600	0.514	0.438	0.561

LSD Source 0.022 ***, Inoculum 0.022 ***, Mn tmt 0.05 **, Source x Mn tmt 0.07 **

Table 4.3 Shoot fresh weights (g) of wheat seedlings, genotype Spear, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), low Mn Tooligie-source (Too),and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

Spear	Shoot fresh weight (g)				Soil Mn treatment						
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Too	0.182	0.519	0.411	0.497	0.555	0.363	0.434	0.460	0.384	0.482	0.429
	Wan	0.166	0.592	0.664	0.572	0.581	0.553	0.633	0.508	0.503	0.575	0.535
	Lam	0.384	0.450	0.580	0.595	0.559	0.606	0.443	0.444	0.429	0.591	0.508
+Ggt	Too	0.147	0.210	0.220	0.370	0.191	0.139	0.318	0.319	0.248	0.164	0.233
	Wan	0.076	0.256	0.391	0.398	0.239	0.268	0.448	0.425	0.272	0.306	0.385
	Lam	0.175	0.258	0.453	0.414	0.221	0.429	0.458	0.420	0.323	0.333	0.348
mean		0.186	0.381	0.453	0.474	0.391	0.393	0.456	0.430	0.360	0.408	0.393

LSD Source 0.019 ***, Inoculum 0.015 ***, Mn tmt 0.034 ***, Source x Mn tmt 0.06 *,

Inoculum x Mn tmt 0.05 **

Table 4.4 Shoot fresh weights (g) of wheat seedlings, genotype Bayonet, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), high Mn Lameroo-source (Lam), and medium Mn Glasshouse-source (Gla). Values are the average of three replicates; means and LSD values are given.

Bayonet	Shoot fresh weight (g)				Soil Mn treatment						
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	0.019	0.323	0.287	0.413	0.374	0.454	0.440	0.347	0.407	0.376	0.344
	Lam	0.164	0.420	0.481	0.326	0.353	0.425	0.400	0.358	0.447	0.363	0.374
	Gla	0.368	0.379	0.425	0.470	0.412	0.478	0.460	0.455	0.419	0.508	0.436
+ Ggt	Wan	0.012	0.120	0.194	0.252	0.153	0.206	0.244	0.152	0.219	0.279	0.183
	Lam	0.101	0.183	0.168	0.202	0.115	0.297	0.195	0.215	0.219	0.202	0.190
	Gla	0.182	0.254	0.197	0.250	0.268	0.308	0.411	0.232	0.334	0.330	0.277
mean		0.141	0.280	0.292	0.319	0.277	0.361	0.358	0.293	0.341	0.343	0.301

LSD Source 0.014 ***, Inoculum 0.017 ***, Mn tmt 0.026 ***, Source x Mn tmt 0.045 *

Table 4.5 Shoot dry weights of wheat seedlings, genotype C8MM, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Two sources of seed (Source) were used: low Mn Wangary-source (Wan), and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

C8MM	Shoot dry weight (g)			Soil Mn treatment							
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	0.1180	0.1177	0.0973	0.0963	0.1033	0.1143	0.1030	0.1193	0.0927	0.1050	0.1067
	Lam	0.1303	0.0770	0.1057	0.1297	0.1160	0.1237	0.1180	0.1300	0.1047	0.1283	0.1163
+ Ggt	Wan	0.0783	0.1187	0.0920	0.0723	0.683	0.1127	0.0780	0.0847	0.0680	0.0670	0.0840
	Lam	0.1193	0.0713	0.1077	0.0923	0.1067	0.1180	0.0880	0.1153	0.1023	0.0617	0.0983
mean		0.1115	0.0962	0.1007	0.0977	0.0986	0.1172	0.0967	0.1123	0.0919	0.0905	0.1013

LSD Source 0.0044 **, Inoculum 0.0044 ***, Mn tmt ns, Source x Mn tmt 0.0140 *

Table 4.6 Shoot dry weights of wheat seedlings, genotype Spear, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), low Mn Tooligie-source (Too),and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

Spear	Shoot dry weight				Soil Mn treatments						
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Too	0.0210	0.0677	0.0347	0.0363	0.0773	0.0493	0.0857	0.0667	0.0570	0.0713	0.0567
	Wan	0.0207	0.0733	0.0923	0.0760	0.0803	0.0753	0.0903	0.0713	0.0733	0.0897	0.1013
	Lam	0.0477	0.0603	0.0813	0.0793	0.0740	0.0843	0.0630	0.0623	0.0677	0.0900	0.0710
+Ggt	Too	0.0223	0.0290	0.1947	0.0507	0.228	0.0219	0.0407	0.0473	0.0407	0.0290	0.0499
	Wan	0.0150	0.0407	0.0623	0.0567	0.0470	0.0373	0.0637	0.0573	0.0403	0.0493	0.0740
	Lam	0.0333	0.0413	0.0633	0.0540	0.0310	0.0640	0.0623	0.0580	0.0540	0.0477	0.0509
mean		0.0267	0.0521	0.0881	0.0588	0.0554	0.0554	0.0676	0.0605	0.0555	0.628	0.0740

LSD Source 0.23 ***, Inoculum 0.19 ***, Mn tmt 0.43 ***, Source x Inoculum 0.33 **

Table 4.7 Shoot dry weights of wheat seedlings, genotype Bayonet, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), and high Mn Glasshouse source and Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

Bayonet	Shoot dry weight (g)	Soil Mn treatments									
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	0.0090	0.0320	0.0410	0.0493	0.0417	0.0523	0.0523	0.0413	0.0560	0.0500	0.0425
	Lam	0.0193	0.0540	0.0513	0.0527	0.0463	0.0683	0.0543	0.0487	0.0550	0.0553	0.0505
	Gla	0.0503	0.0527	0.0543	0.0603	0.0500	0.0667	0.0713	0.0613	0.0570	0.0783	0.0602
+ Ggt	Wan	0.0240	0.0380	0.0417	0.0400	0.0383	0.0470	0.0493	0.0377	0.0433	0.0633	0.0423
	Lam	0.0147	0.0283	0.0297	0.0350	0.0213	0.0460	0.0363	0.0357	0.0343	0.0300	0.0314
	Gla	0.0300	0.0533	0.0467	0.0460	0.0610	0.0530	0.0590	0.0487	0.0633	0.0550	0.0516
mean		0.0246	0.0431	0.0441	0.0472	0.0431	0.0556	0.0538	0.0456	0.0515	0.0558	0.0460

LSD Source 0.031 ***, Inoculum 0.0026 ***, Mn tmt 0.0057 ***, Source x Inoculum 0.0044 *

Table 4.8 Whole shoot Mn concentrations (ShMn) of wheat seedlings, genotype C8MM, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Two sources of seed (Source) were used: low Mn Wangary-source (Wan), and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

C8MM			ShMn (mg / kg)			Soil Mn treatment					
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	40.1	41.3	55.0	63.0	87.0	160.8	101.1	116.1	176.7	140.3	94.8
	Lam	42.5	64.4	49.6	64.3	79.8	142.1	134.4	140.3	180.6	135.1	103.3
+ Ggt	Wan	27.4	38.6	39.2	41.8	61.7	134.0	81.8	91.2	128.1	103.1	74.7
	Lam	28.2	42.7	41.0	63.4	77.1	129.9	83.1	112.2	136.4	173.0	88.7
mean		34.5	46.7	46.2	58.2	76.4	141.7	100.1	115.0	155.4	137.9	91.2

LSD Source 4.67 *, Inoculum 4.67 ***, Mn tmt 10.45 ***

Table 4.9 Whole shoot Mn concentrations (ShMn) of wheat seedlings, genotype Spear, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), low Mn Tooligie-source (Too),and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

Spear		ShMn (mg / kg)				Soil Mn treatment						
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Too	4.3	15.3	24.1	57.7	74.7	91.0	118.0	174.0	160.7	203.0	92.3
	Wan	15.0	26.3	40.0	63.7	65.0	94.3	113.7	158.3	165.0	207.3	94.9
	Lam	8.0	30.3	48.0	85.0	77.0	101.7	107.7	178.7	201.7	197.7	103.6
+Ggt	Too	5.4	13.7	21.0	38.3	51.3	72.6	87.3	149.0	96.3	191.7	72.7
	Wan	6.5	23.0	22.3	44.7	55.0	76.7	114.7	140.0	145.3	155.3	78.3
	Lam	8.5	33.3	58.0	58.7	76.0	93.3	116.7	147.3	142.3	196.3	93.0
mean		7.9	23.7	35.6	58.0	66.5	88.3	109.7	157.9	151.9	191.9	89.1

LSD Source 4.1 ***, Inoculum 3.3 ***, Mn tmt 7.4 ***

Table 4.10 Whole shoot Mn concentrations (ShMn) of wheat seedlings, genotype Bayonet, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), high Mn Lameroo-source (Lam),and high Mn Glasshouse-source (Gla). Values are the average of three replicates; means and LSD values are given.

Bayonet		ShMn (mg / kg)				Soil Mn treatment						
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	3.2	17.1	32.1	50.7	71.9	84.7	109.4	124.0	184.6	170.1	84.8
	Lam	10.0	23.7	54.1	56.3	76.1	91.8	117.0	146.4	186.4	199.7	96.1
	Gla	8.7	21.2	37.2	47.9	68.2	82.7	95.4	107.6	167.9	173.3	81.0
+ Ggt	Wan	6.0	15.1	32.9	48.3	86.3	95.0	90.8	120.4	107.3	186.2	78.8
	Lam	9.6	19.9	32.2	37.3	51.5	67.7	77.1	99.8	161.1	157.5	71.4
	Gla	10.1	20.2	25.8	42.5	55.6	86.6	98.8	102.1	155.5	181.8	77.9
mean		7.9	19.6	35.7	47.2	68.3	84.8	98.1	116.7	160.5	178.1	81.7

LSD Source ns, Inoculum 3.8 **, Mn tmt 8.4 ***, Source x Inoculum 6.5 *

Table 4.11 Root fresh weights (g) of wheat seedlings, genotype C8MM, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Two sources of seed (Source) were used: low Mn Wangary-source (Wan), and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

C8MM	Root fresh weight (g)				Soil Mn treatments							
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	1.076	1.147	1.057	0.930	0.891	1.141	0.853	1.004	0.756	0.905	0.976
	Lam	1.187	0.636	1.019	1.074	1.201	1.353	1.144	1.211	1.127	0.999	1.095
+ Ggt	Wan	0.957	1.192	1.185	0.922	0.745	1.078	0.834	0.860	0.871	0.763	0.940
	Lam	1.138	1.154	1.045	1.026	1.177	1.364	0.965	1.190	1.032	0.655	1.074
mean		1.086	1.030	1.066	0.988	1.035	1.231	0.949	1.066	0.946	0.638	1.021

LSD Source 0.043**, Inoculum ns, Mn tmt 0.096*, Source x Mn tmt 0.1355 *

Table 4.12 Root fresh weights (g) of wheat seedlings, genotype Spear, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), low Mn Tooligie-source (Too),and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

Spear	Root fresh weight (g)				Soil Mn treatments						
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Too	0.214	1.158	0.728	0.935	1.010	0.779	0.754	1.09	0.904	0.785	0.836
	Wan	0.155	0.888	1.121	0.943	0.879	0.841	1.061	0.760	0.795	0.925	0.836
	Lam	0.364	0.740	1.081	0.980	1.021	1.030	0.787	0.772	0.420	0.816	0.800
+Ggt	Too	0.192	0.447	0.437	0.727	0.398	0.333	0.615	0.716	0.470	0.408	0.474
	Wan	0.151	0.519	0.621	0.712	0.463	0.486	0.748	0.787	0.474	0.531	0.549
	Lam	0.361	0.562	0.642	0.635	0.375	0.733	0.856	0.665	0.625	0.653	0.611
mean		0.240	0.719	0.770	0.822	0.691	0.694	0.803	0.798	0.615	0.686	0.684

LSD Source ns, Inoculum 0.030 ***, Mn tmt 0.067 ***, Inoculum x Mn tmt 0.095 **

Table 4.13 Root fresh weights (g) of wheat seedlings, genotype Bayonet, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), high Mn Lameroo-source (Lam),and high Mn Glasshouse-source (Gla). Values are the average of three replicates; means and LSD values are given.

Bayonet	Root fresh weight (g)				Soil Mn treatments							
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	0.027	0.352	0.398	0.544	0.577	0.677	0.610	0.531	0.829	0.796	0.534
	Lam	0.186	0.570	0.743	0.537	0.557	0.723	0.789	0.750	1.040	0.743	0.664
	Gla	0.352	0.637	0.564	0.849	0.690	0.849	0.869	0.935	0.909	0.968	0.762
+ Ggt	Wan	0.192	0.395	0.745	0.576	0.620	0.846	0.964	0.859	0.980	1.730	0.790
	Lam	0.405	0.650	0.783	0.870	0.545	1.048	0.915	0.942	1.112	1.040	0.830
	Gla	0.703	1.167	0.975	1.121	1.353	1.401	2.173	0.995	1.638	1.610	1.314
mean		0.311	0.629	0.701	0.749	0.724	0.924	1.053	0.835	1.084	1.147	0.816

LSD Source 0.06 ***, Inoculum 0.05 ***, Mn tmt 0.11 ***, Source x Inoculum 0.089 **

Table 4.14 Number of roots per wheat seedling (NOR), genotype C8MM, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Two sources of seed (Source) were used: low Mn Wangary-source (Wan), and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

C8MM	NOR	Soil Mn treatments									
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	7.7	8.3	8.0	7.3	7.7	9.0	7.7	8.0	8.3	7.3	7.9
	Lam	9.3	8.3	8.7	8.0	7.7	9.0	7.0	6.7	8.3	7.3	8.0
+ Ggt	Wan	9.0	9.0	8.3	7.7	8.7	9.0	8.0	8.7	8.7	7.7	8.5
	Lam	7.7	8.3	7.3	8.3	7.3	7.3	8.0	9.7	8.3	7.3	8.0
mean		8.4	8.5	8.1	7.8	7.8	8.6	7.7	8.3	8.4	7.4	8.1

LSD Source ns, Inoculum ns, Mn tmt ns

Table 4.15 Number of roots per wheat seedling (NOR), genotype Spear, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), low Mn Tooligie-source (Too),and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

Spear	NOR	Soil Mn treatments									
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Too	4.3	6.7	7.3	7.0	7.7	8.0	6.3	7.7	7.3	7.3	7.0
	Wan	4.7	6.3	7.7	8.0	7.7	7.7	7.7	7.7	7.7	8.0	7.3
	Lam	6.0	7.0	8.7	7.3	8.0	7.3	7.0	7.3	6.7	7.3	7.3
+Ggt	Too	5.7	6.0	7.3	6.3	8.0	6.9	6.3	7.0	6.3	8.0	6.8
	Wan	5.3	10.3	8.3	9.0	9.7	8.7	8.3	8.0	8.0	8.0	8.4
	Lam	7.3	10.3	9.3	7.7	8.0	10.3	9.0	8.7	8.7	8.0	8.7
mean		5.6	7.8	8.1	7.6	8.2	8.2	7.4	7.7	7.4	7.8	7.6

LSD Source 0.23 ***, Inoculum 0.19 ***, Mn tmt 0.43 ***, Source x Inoculum 0.33 **

Table 4.16 Number of roots per wheat seedling (NOR), genotype Bayonet, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), and high Mn Glasshouse source and Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

Bayonet	NOR	Soil Mn treatments									
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	4.0	5.3	5.3	6.7	7.0	6.7	5.7	5.3	7.3	6.3	6.0
	Lam	5.0	6.3	7.0	6.0	6.3	7.0	5.3	6.7	6.7	6.3	6.3
	Gla	7.0	5.3	6.3	6.7	7.0	7.7	6.0	7.7	6.7	7.3	6.8
+ Ggt	Wan	4.7	6.7	7.3	7.0	5.3	7.7	8.7	5.7	7.7	7.7	6.8
	Lam	5.7	6.3	6.3	8.0	6.7	8.3	6.7	7.0	7.7	7.3	7.0
	Gla	6.0	7.0	6.7	7.7	8.0	9.0	9.0	7.0	8.3	9.3	7.8
mean		5.4	6.2	6.5	7.0	6.7	7.7	6.9	6.6	7.4	7.4	6.8

LSD Source 0.23***, Inoculum 0.18***, Mn tmt 0.41***, Inoculum x Mn tmt ns (Fprob = 0.077)

Table 4.17 Root length of wheat seedlings, genotype C8MM, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Two sources of seed (Source) were used: low Mn Wangary-source (Wan), and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

C8MM	Root length (mm)	Soil Mn treatments									
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	169.3	191.7	165.3	162.0	176.7	172.7	171.3	163.0	177.0	164.3	171.3
	Lam	182.3	176.0	168.3	194.0	193.3	183.3	173.3	169.0	178.0	171.7	178.9
+ Ggt	Wan	160.3	175.3	176.7	161.3	166.3	164.7	165.0	170.3	168.7	167.7	167.6
	Lam	165.7	170.0	177.0	173.3	176.0	175.0	162.0	174.7	171.7	170.0	171.5
mean		169.4	178.3	171.8	172.7	178.0	173.9	167.9	169.3	173.8	168.4	172.4

LSD Source 2.46*, Inoculum 2.46*, Mn tmt ns

Table 4.18 Root length of wheat seedlings (mm), genotype Spear, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), low Mn Tooligie-source (Too),and high Mn Lameroo-source (Lam). Values are the average of three replicates; means and LSD values are given.

Spear	Root length (mm)			Soil Mn treatments							
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Too	146.0	179.7	174.7	182.7	180.7	168.7	183.0	178.0	174.7	184.0	175.2
	Wan	140.7	187.3	174.0	173.3	179.0	182.0	186.0	193.0	177.7	178.0	177.1
	Lam	174.0	170.3	173.7	176.0	179.7	180.3	178.7	186.7	175.7	178.0	177.3
+Ggt	Too	121.7	146.7	175.7	178.7	171.7	155.3	202.0	177.7	177.0	173.0	167.9
	Wan	97.7	173.7	209.3	188.7	179.0	190.7	184.7	196.0	179.3	169.7	176.9
	Lam	151.0	172.3	182.7	182.0	163.0	173.0	173.7	170.3	163.7	187.0	171.9
mean		138.5	171.7	181.7	180.2	175.5	175.0	184.7	183.6	174.7	178.3	174.4

LSD Source ns, Inoculum ns, Mn tmt 6.03 ***, Inoculum x Mn tmt 8.53 *

Table 4.19 Root length of wheat seedlings (mm), genotype Bayonet, grown without (- Ggt) and with (+ Ggt) Ggt inoculum, under 10 soil Mn treatments. Three sources of seed (Source) were used: low Mn Wangary-source (Wan), high Mn Lameroo-source (Lam),and high Mn Glasshouse-source (Gla). Values are the average of three replicates; means and LSD values are given.

Bayonet	Root length (mm)			Soil Mn treatments							
	Source	0	1	2	3	4	5	6	7	8	9	mean
- Ggt	Wan	96.7	176.3	179.0	182.7	191.0	190.3	185.7	174.0	171.0	185.0	173.2
	Lam	170.7	178.7	199.0	178.0	174.0	177.0	181.0	175.3	178.7	184.0	179.6
	Gla	181.0	170.3	168.0	187.3	175.3	179.3	174.0	178.3	174.0	173.7	176.6
+ Ggt	Wan	61.3	118.3	153.3	123.7	133.7	169.0	149.0	138.3	168.7	143.3	135.9
	Lam	105.3	121.3	144.7	156.7	116.7	149.7	141.0	170.7	178.0	121.7	140.6
	Gla	133.0	159.0	151.3	158.3	175.3	162.7	187.0	151.7	179.0	166.3	162.4
mean		124.7	154.0	165.9	164.4	161.7	171.3	169.6	164.7	174.9	162.3	161.4

LSD Source 3.82 ***, Inoculum 3.12 ***, Mn tmt 6.97 ***, Source x Inoculum 5.40 **,

Source x Mn tmt 12.06 ***, Inoculum x Mn tmt 9.85 *

4.3.2 Infection and disease results

In general, higher soil Mn and seed source nutrient benefited plant growth and had the effect of decreasing Ggt infection of the roots, but the effect of high Mn efficiency had an even greater impact in decreasing Ggt infection. Infection was consistently reduced as Mn efficiency of the wheat genotypes increased (Figs. 4.1 - 4.5). Significant differences in disease susceptibility were seen between the three genotypes, best seen in the measure AVL (average length of lesions) (Fig. 4.4). Only low levels of disease were found in the Mn efficient C8MM plants, while the most extensive infection of roots was seen in Bayonet plants.

1) Soil Mn treatments

A consistent pattern in the disease measures was seen in Spear and Bayonet plants, affected by the soil Mn treatment, although the disease measures in Bayonet plants were higher than in Spear plants. TLL (total length of lesions), NIR (number of infected roots), NSL (number of stelar lesions) and PIR (percent infected roots) measures were significantly affected by the Mn soil treatment: lowest in soil treatment Mn 0, rising sharply in the next treatments to be reduced slightly in Mn 3 or 4, and distinctly reduced in Mn 6 or 7 (Figs. 4.1 - 4.3, 4.5). Disease levels tended to fluctuate in the highest soil Mn treatments (Mn 8 and 9) (Figs. 4.1 - 4.3) where yields indicated some effects of possible Mn toxicity, although symptoms were not observed.

In contrast, in C8MM plants the disease measures TLL, NIR and NSL followed a pattern of high infection in soil treatment Mn 0, which was immediately reduced in Mn 2 and 3, but slowly increasing with further additions of Mn to the soil (Figs. 4.1-4.2, 4.4). It is suggested that strongly growing roots of the Mn efficient C8MM plants had greater contact with the inescapable fungal inoculum plug, which may have permitted even weakened fungal inoculum (section 4.3.2 (3)) to invade roots, albeit at low level, compared to the other genotypes used. Further addition of Mn to the soil did not decrease the disease levels in C8MM plants, similar to the effect on Spear and Bayonet.

Generally, the addition of Mn to the Wangary soil resulted in infection levels increasing dramatically, peaking over the soil treatments Mn 1 - 3 and then decreasing (Mn 3

- 6). Infection levels then either remained the same until rising in Mn 9, or rose immediately to previous severities. Although root infection was generally reduced by the soil treatments Mn 3 - 7, the highest soil Mn treatments (Mn 8 - 9) did not reduce NIR or NSL in any of the three genotypes.

Both seed source and soil Mn treatments had significant effect upon NOR in the +Ggt treatment of Spear plants, with more roots in Lameroo-source plants, and more roots in the higher soil Mn treatments than the lower Mn treatments. Soil Mn treatment had a significant effect upon NOR in the +Ggt treatment of Bayonet plants, with slight differences also seen between the seed source treatments. NOR in C8MM plants was not affected by the inoculum treatment.

2) Seed source

C8MM NIR was reduced by high Mn seed source, with more diseased roots in Wangary-source plants than in Lameroo-source plants. A large difference between seed source treatments was also seen with TLL, NSL and AVL, with Wangary-source plants showing between two to three times the total length , twice the number and larger lesions than the Lameroo-source plants. Spear AVL was remarkably consistent across both seed source and soil Mn treatments, with the exception of some higher levels in Wangary-source plants (Fig. 4.4).

However, Bayonet TLL, and AVL (Figs. 4.1, 4.4) (greatly influenced by TLL), showed an impressive decrease in lesion length in the higher soil Mn treatments, and large differences between the seed source treatments. Wangary-source plants showed a gradual decrease in lesion length as soil Mn treatment increased. Lameroo-source plants showed very strong levels of infection in soil treatments Mn 1, 2 and 3, which dropped in the higher Mn treatments to levels below those of the Wangary-source plants. Glasshouse-source plants had consistently small lesions (similar in size to those of C8MM plants) across the soil treatments.

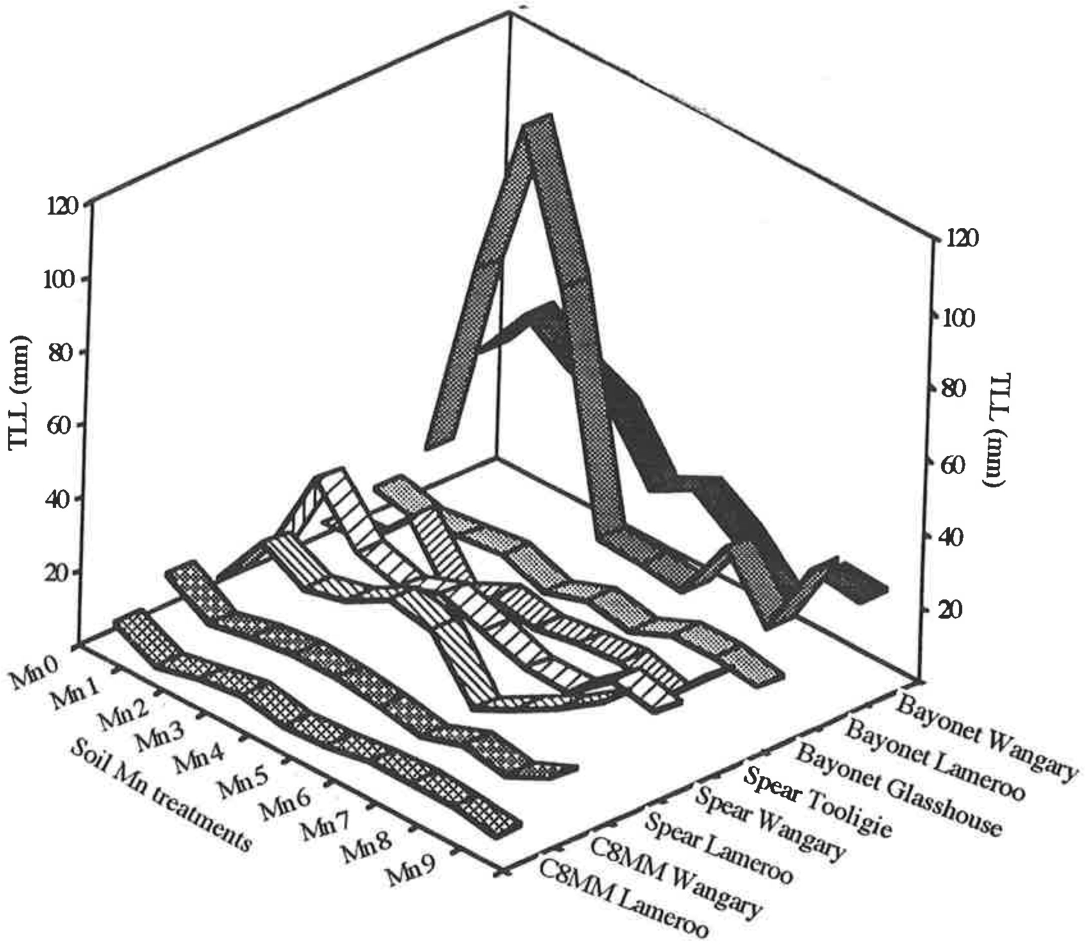
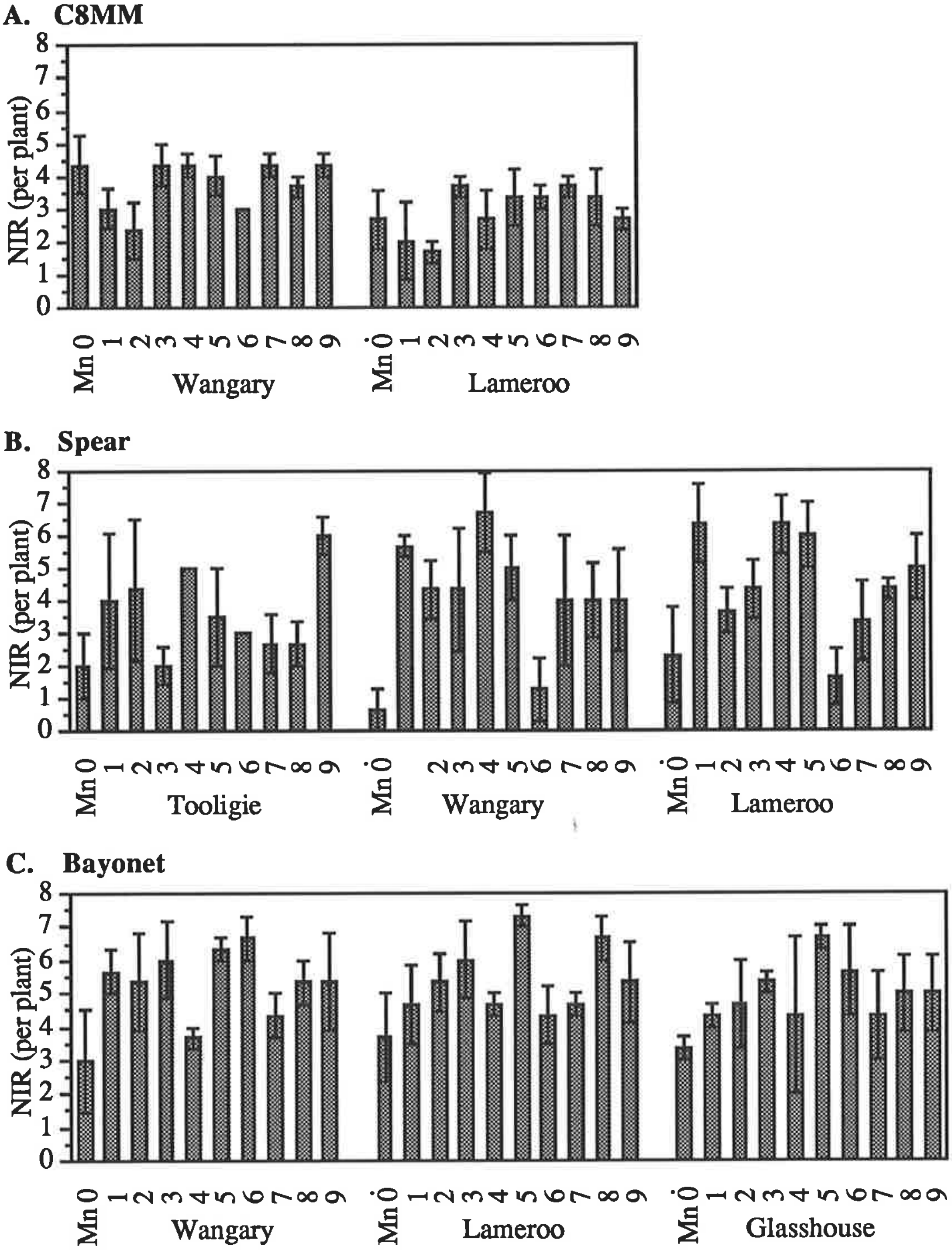


Fig. 4.1 Total length of stelar lesions (mm) per plant (TLL) on roots of plants of three wheat genotypes (C8MM, Spear and Bayonet), grown under ten Mn soil treatments (Mn 0 - Mn 9), from four seed sources, differing in Mn concentration (Wangary - low Mn, Tooligie - low Mn, Lameroo - high Mn, Glasshouse - high Mn). Values are the mean of three replicates.



A. C8MM

B. Spear

C. Bayonet

Fig. 4.2 Number of Ggt infected roots (NIR) of plants of three wheat genotypes (C8MM, Spear and Bayonet), grown under ten Mn soil treatments (Mn 0 - Mn 9), from four seed sources, differing in Mn concentration (Wangary - low Mn, Tooligie - low Mn, Lameroo - high Mn, Glasshouse - high Mn). Values are the mean of three replicates, standard error bars are shown.

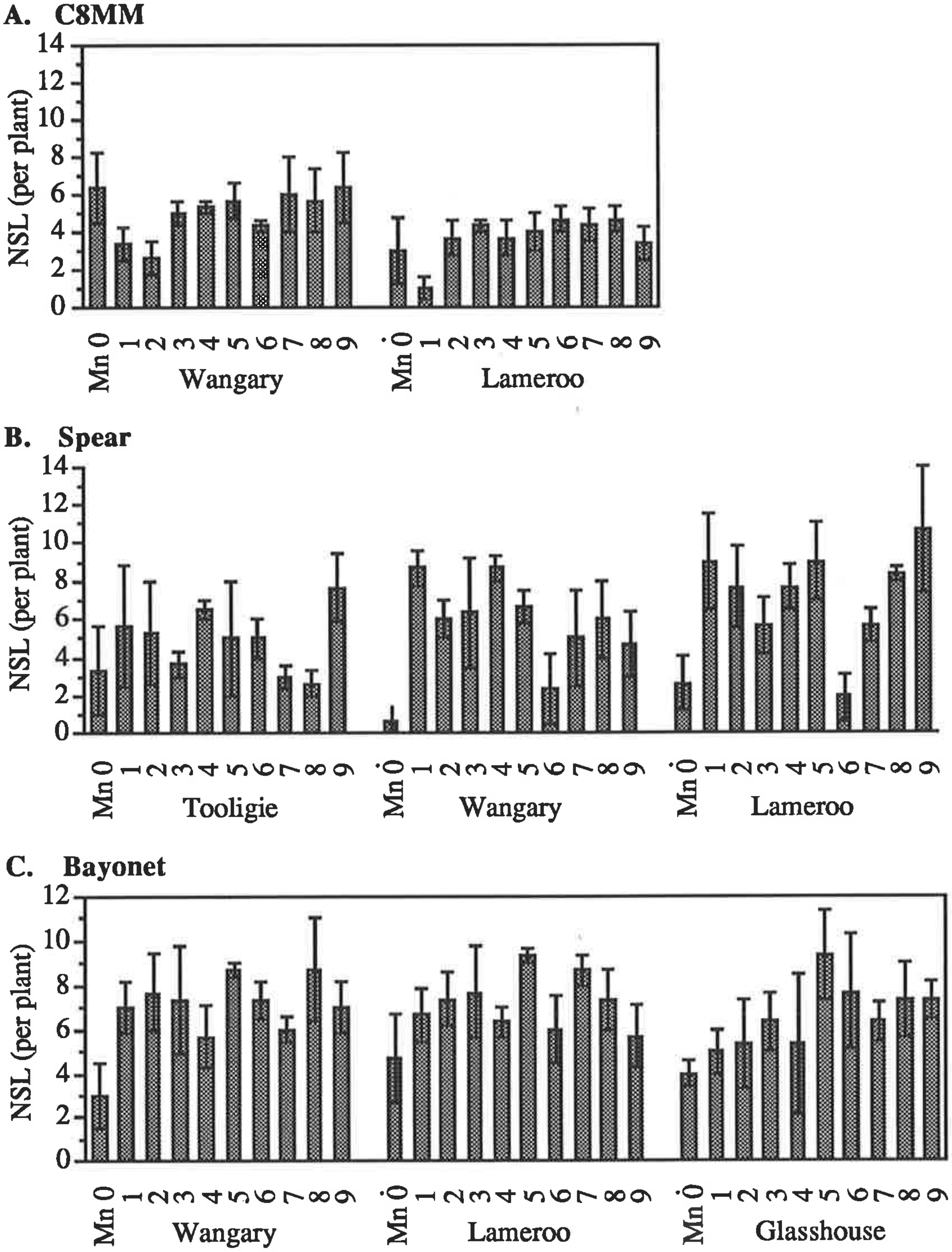


Fig. 4.3 Number of stelar lesions (NSL) on roots of plants of three wheat genotypes (C8MM, Spear and Bayonet), grown under ten Mn soil treatments (Mn 0 - Mn 9), from four seed sources, differing in Mn concentration (Wangary - low Mn, Tooligie - low Mn, Lameroo - high Mn, Glasshouse - high Mn). Values are the mean of three replicates, standard error bars are shown.

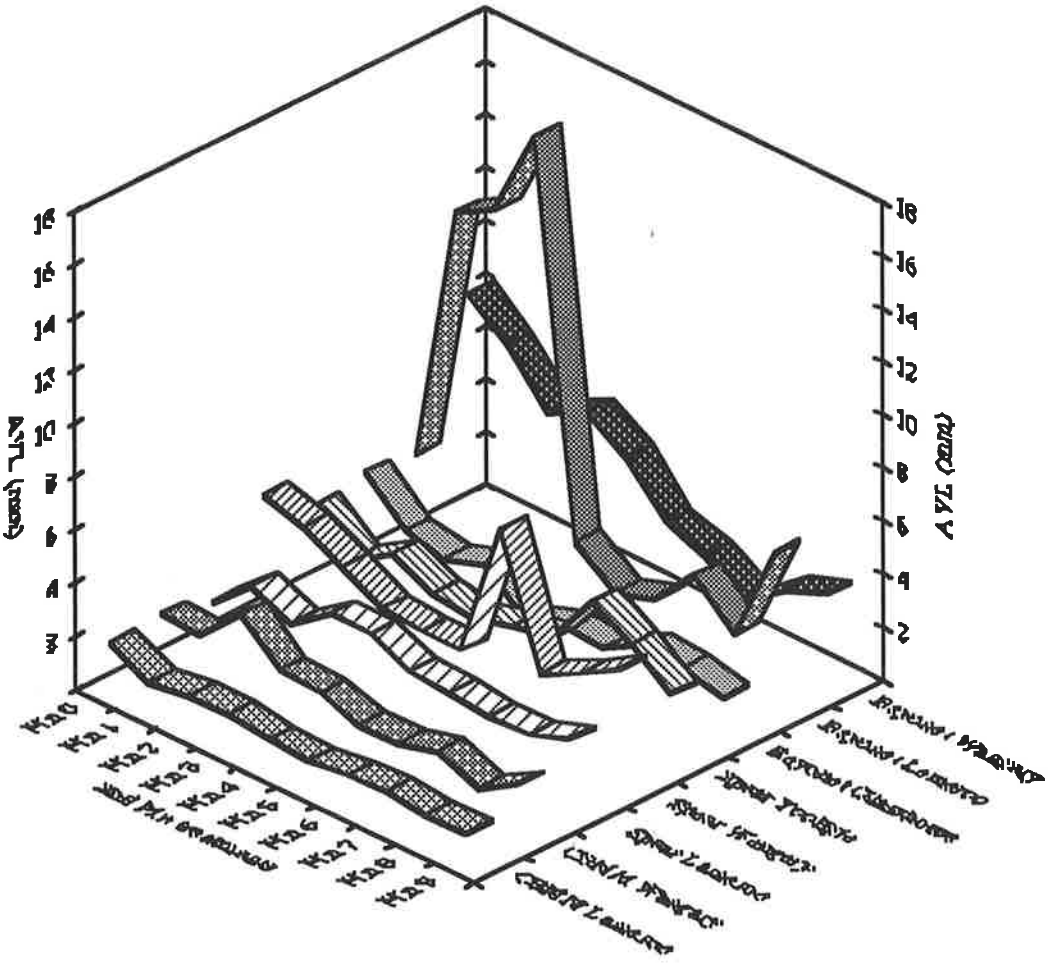
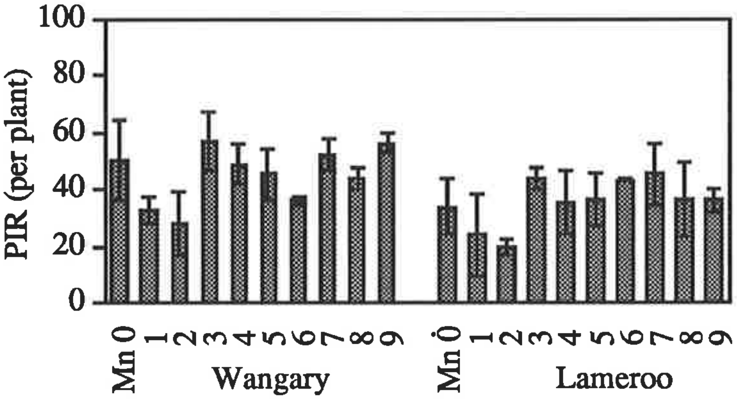
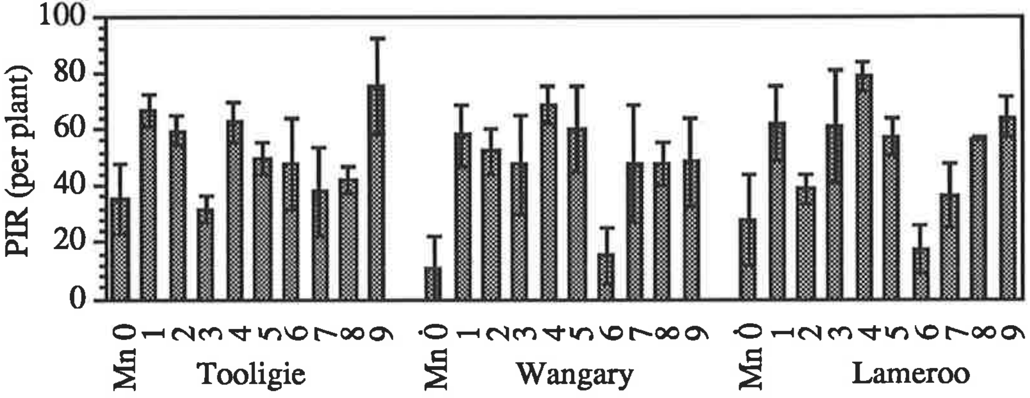


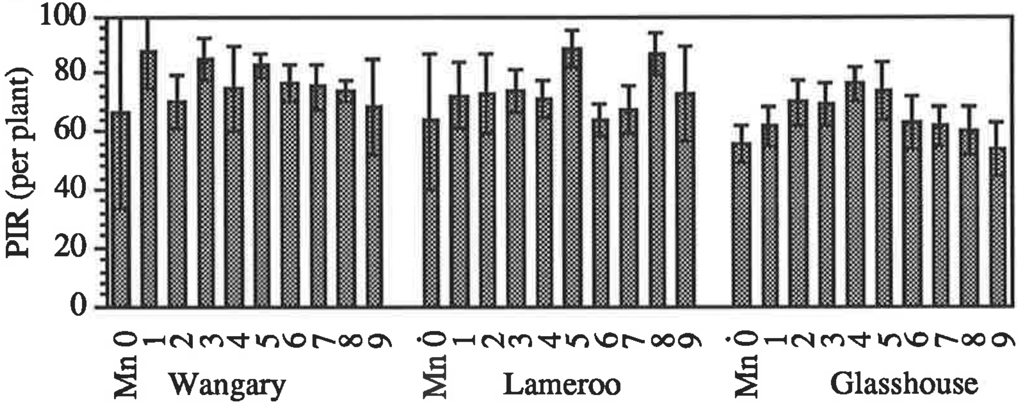
Fig. 4.4 Average length of stelar lesions (mm) per plant (AVL) of plants of three wheat genotypes (C8MM, Spear and Bayonet), grown under ten Mn soil treatments (Mn 0 - Mn9), from four seed sources, differing in seed concentration (Wangary - low Mn, Tooligie - low Mn, Lameroo - high Mn, Glasshouse - high Mn). Values are the mean of three replicates.



A. C8MM



B. Spear



C. Bayonet

Fig. 4.5 Percent infected roots (PIR) of plants of three wheat genotypes (C8MM, Spear and Bayonet), grown under ten Mn soil treatments (Mn 0 - Mn 9), from four seed sources, differing in Mn concentration (Wangary - low Mn, Tooligie - low Mn, Lameroo - high Mn, Glasshouse - high Mn). Values are the mean of three replicates, standard error bars are shown.

3) Inoculum strength and disease

Further addition of Mn in the soil treatments decreased disease levels in all three genotypes, and in Mn 4 (the +Mn treatment used in Experiments 2.1 and 2.2) disease measurements were often as low as those found in the Mn 0 treatment, where the growth of the inoculum was weakest.

The high level of Mn added to the soil in Mn 9 (with no similar increase in other nutrients) may have caused nutrient imbalance in the plants, distressing plant systems. However, examination of the elemental analysis of plants from treatments Mn 8 and 9, showed no deficiencies in shoot uptake of other nutrients (Appendix 3). It is more likely that intense growth of the Ggt inoculum in the highest soil Mn treatments caused the increase of infection of roots. The growth of Ggt inoculum was shown to be most intense at the highest levels of added Mn (Mn 8 - 9) in Chapter 3 (section 3.9).

The very low infection levels in soil treatment Mn 0 (no Mn added), which sometimes continued in Mn 1 (0.25 mg Mn per 50 g soil), were indicative of the poor growth of Ggt fungus under conditions of extreme Mn deficiency (section 3.9). These low infection levels were seen particularly in Spear and Bayonet plants. Growth of the fungus is not necessarily a measure of disease, but disease reduction under extreme Mn deficiency in the Wangary soil was confirmed by the low infection levels in the Mn 0 soil treatments, with an immediate increase in disease in Mn 1 and 2 soil treatments. Growth of Ggt inoculum has previously been shown to be increased upon the addition of Mn to agar growth media (Wilhelm 1992).

4.4 Discussion

Disease measures in this experiment showed a consistent pattern; increasing as the plants were attacked by an initially weak, then (as the fungal inoculum received enough Mn for adequate growth) stronger fungal inoculum. This is consistent with the hypothesis of section 2.7 that the extreme Mn deficiency of Wangary soil (1990 collection), which reduced growth of the Ggt inoculum, also reduced infection of plant roots by the fungus. In soil Mn treatments where the Mn in the soil was sufficient for stronger growth (and pathogenic action) by the Ggt inoculum, but not sufficient for the plant to tolerate invasion of roots, there was a high level of disease.

Plants grown with sufficient Mn to tolerate, or resist invasion of roots had reduced disease levels. The requirement for Mn in the production of lignin (Brown 1984, Burnell 1988) is suggested as the reason for increased resistance to the root disease in plants sufficiently supplied with Mn (Wilhelm 1992). This is further discussed in Chapter 5.

As superoptimal Mn was added in soil treatments, disease was not consistently reduced. A further fluctuation of fungal strengths and plant resistances may explain some of the erratic results, especially in the highest soil Mn treatments. Hyphal growth of Ggt inoculum at high levels of Mn (soil treatments Mn 8 - 9) was denser (section 3.9) and may have led to greater infection of roots in these soil treatments. The use of superoptimal soil Mn fertiliser has been effective in improving grain yield of wheat in the presence of take-all inoculum, but the severity of root infection of the field plants was not investigated (Webb and Graham 1991).

Within genotypes, seed Mn showed strong influences on infection measures. This supports the hypothesis (section 2.7) that high seed source nutrition engenders plants with lower Ggt infection levels.

However, across genotypes the differences between these seed sources were less important in affecting susceptibility to root infection by Ggt. Overall, seed Mn affected shoot growth and ShMn more significantly than root growth measures, and thus had less effect on the infection of roots than soil. Mn content of seed can dominate over genotypic differences in contributing to seedling vigour and yield (Marcar 1986, Uren et al. 1988), but it appears that in root growth, the effect of seed source nutrition is overborne by soil Mn availability and genotype efficiency.

Over and above the effects of soil and seed source Mn, the Mn efficiency of plant genotypes has been shown to reduce the susceptibility of plants to infection by inescapable Ggt inoculum. C8MM, although not showing the initial low levels of infection seen in the less efficient genotypes in soil treatment Mn 0 and Mn 1, seemed to respond more consistently to the soil Mn treatments than Spear and Bayonet, especially in Lameroo-source plants. Furthermore, the low infection levels seen in Spear and Bayonet plants in the Mn 0 soil treatment were higher than infection levels in C8MM plants from the same soil treatment, showing that the Mn efficient genotype was less susceptible to infection.

The Mn efficiency ranking of genotypes tested in Experiment 2.2 was thus actually made up of components of seed source nutrient and genetically determined efficiency. This aids in explaining why genotypes which showed only moderate Mn efficiency had high tolerance to take-all infection under Mn limited conditions, and why genotypes with higher Mn efficiency rankings showed only moderate tolerance to the Ggt infection. Seed source nutrient differences did not entirely mask the differences in Mn efficiency between the genotypes used in Experiment 2.2 (section 2.5) and the conclusion can still be drawn that the Mn efficient genotypes suffered less infection from (inescapable) Ggt inoculum than Mn inefficient genotypes.

Field trials and pot trial alike have rarely shown consistent rankings of take-all tolerance within a species (Nilsson 1969, Scott 1981, Riveros et al. 1987). In the field the variable nature of Ggt inoculum, seed and soil nutrition and ultimately the growing season combine to obfuscate results and affect the inoculum virulence or saprophytic strength, the growth of the host genotypes, and the availability of nutrients such as Mn. Only large differences in susceptibility to the disease could be consistently observed through such a range of factors. In addition, the genotypes used here are adapted cultivars and advanced breeding lines, which differ in many traits other than Mn efficiency. Differences in susceptibility to take-all disease between the 22 wheat genotypes used in Experiment 2.2 were barely evident, with the complicating factor of seed nutrient affecting susceptibility, as well as genotype.

The extreme system used in the pot experiments described above perhaps exaggerates the Mn effects. The use of Wangary soil is a great advantage in producing extreme Mn deficient conditions. Some other workers (Hornby and Bateman 1991, Brennan 1992), using soils of a less extreme nature, have not shown effective decrease of take-all by the application of Mn fertiliser. In an investigation of the physicochemical characteristics of soils susceptible to take-all, Lucas and Sarniguet (1990) showed that the level of available Mn in soil actually rose upon the addition of Ggt inoculum. However, effective decrease of take-all disease by Mn fertiliser application, or has been shown in a variety of soils which are less Mn deficient than Wangary soil (Wilhelm et al. 1988, Huber 1989, Webb and Graham 1990, Lucas and Sarniguet 1990, Huber and McCay Buis 1993).

The extreme Mn deficiency of the Wangary soil used in these pot experiments has its obvious experimental drawbacks, in particular, retarding fungal inoculum growth when no Mn is added to the soil. Where sufficient Mn for fungal growth is available, the inescapable nature of the inoculum may create higher infection levels than might occur under more natural, or field conditions, but is useful in providing a definite means of infection.

4.5 Conclusion

Although high seed source Mn can depress disease measures in seedling plant roots, the factor of Mn efficiency overrides seed source nutrient, providing an even greater reduction in susceptibility to root infection.

The overall conclusion of the experiments described in Chapters 2, 3 and 4 confirms the initial premise that plants better supplied with Mn, whether by genetic efficiency, seed source nutrient, or soil available Mn, are less susceptible to infection by the Ggt inoculum (Wilhelm et al. 1988, 1990).

Within genotypes, seed source was shown to have strong effects on plant growth and resistance to Ggt infection, but between genotypes, Mn efficiency had better ability to improve resistance to infection than seed source. The wide range of soil Mn treatments used in the experiment described in this chapter provided background levels to compare genotype efficiency for Mn with seed Mn nutrient as a means of reducing seedling root infection, and also provided a static impression of changing Mn availability in the soil pool, as Mn availability affects the resistance of seedlings to Ggt.

Mn availability was additionally shown to affect the ability of Ggt fungus to infect plant roots. The saprophytic growth of the fungus and its pathogenicity were decreased under conditions of extreme Mn deficiency, and were also affected by the highest Mn soil treatments in the range used here.

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Chapter 5

The effect of Mn nutrition and genotypic differences on lignin production in wheat seedling roots.

5.1 Introduction

The effect of Mn nutrition on the lignification of roots has been suggested as the most supportable mechanism hypothesised for the increased resistance to take-all by Mn adequate plants (Graham and Rovira 1984, Wilhelm 1992). The production of the innate defence mechanisms of all plants, lignin and phenolic compounds, is affected by the Mn status of the plant (Gross 1980, Brown et al. 1984, Burnell 1988). Mn deficient plants, with inadequate lignification of the stele, are provided with less physical protection against penetration by the Ggt fungus than Mn adequate plants.

This chapter investigates the effects of Mn nutrition and differences in Mn efficiency of wheats on lignin production in roots, as a mechanism of resistance to infection by Ggt. The synthesis of lignin and its role in plant defence against pathogens was briefly considered in Chapter 1 (section 1.2.2) but a deeper and updated review of the literature is presented here.

5.1.1 Composition and synthesis of lignin

The structural role of lignin in the plant is that of a strengthening and frame making layer. Lignin is an inert and complex hydrophobic macromolecule with an irregular structure. Synthesis of the lignin macromolecule is a process of non-enzymic polymerisation; the linking and cross-linking of three aromatic alcohol precursors by a large number of bonds (Wardrop 1971). The alcohols are *p*-coumaryl (which form the *p*-hydroxyphenyl subunits in the polymer), coniferyl (the guaicyl subunits) and sinapyl alcohol (the syringyl subunits). While gymnosperm lignin contains a high proportion of guaicyl subunits, dicot angiosperm lignin contains approximately equal amounts of guaicyl and syringyl subunits, but only small amounts of p-hydroxyphenyl (Kirk and Farrell 1987). Monocot angiosperm (grass) lignins contain roughly equal amounts of all three aromatic alcohol subunits and are further chemically distinct from wood lignins by possessing *p*-hydroxcinnamyl residues (Morrison 1972, Brett and Waldron 1990).

These lignin precursors, the coumaryl, coniferyl and sinapyl alcohols, are synthesised by enzymes of the shikimate pathway, which is also the synthetic pathway for other phenolic compounds, plant hormones such as indole acetic acid (IAA), and a variety of aromatic amine derived compounds (Whitmore 1978, Burnell 1988).

As previously mentioned (Chapter 1) some of the enzymes within the shikimate pathway require Mn^{2+} as a co-factor or as a stimulator of the enzymic process. Other trace elements, such as Cu and Zn, are similarly required in the formation of the lignin precursors (Robson et al. 1981, Graham 1983). In some cases Mg^{2+} can be replaced as a cofactor or stimulator by Mn^{2+} (Gross 1980).

The enzymes involved in the production of lignin precursors which are affected by Mn are phenylalanine ammonia lyase (PAL) (Burnell 1988) and 4CL (4-coumarate:CoA ligase) (Gross 1980). PAL is the rate setting enzyme in the process of precursor formation (Jones 1984), which catalyses the deamination of L-phenylalanine to cinnamic acid (Gross 1980). 4CL activates the cinnamic acids prior to polymerisation, requiring the presence of a divalent cation (Mn^{2+}) as co-factor and ATP as a co-substrate (Gross 1980). The enzyme cinnamoyl alcohol dehydrogenase (CAD) contains Zn, and specifically requires NADPH to finalise the activation of the precursors; now cinnamyl alcohols. CAD can be inhibited by sulphinamoyls, which bind to the Zn in the enzyme and prevent enzymic action (Moerschbacher et al. 1990 b).

The synthesis of the lignin macromolecule is not enzymically controlled, but relies upon enzymic provision of precursors and substrates. The lignin precursor alcohols are oxidised by a peroxidase / H_2O_2 system, and link at the oxidation sites (Gross et al. 1977, Gross 1980, Askerlund et al. 1987). Synthesis of H_2O_2 requires a wall-bound malate dehydrogenase, a NADPH oxidase, and is stimulated by the presence of Mn^{2+} and monophenol precursors (Gross 1980). Polymerisation of the lignin precursors can continue to occur as long as activated precursors, H_2O_2 and space in the cell wall are available (Brett and Waldron 1990). Interestingly, a Mn peroxidase is also of importance in the degradation of lignin by the white rot fungus, *Phanerochate chrysosporium* (Glenn et al. 1986).

5.1.2 Lignin formation as a responsive defence mechanism

Although the lignin layer forms a passive barrier against pathogens, the active lignification of cell walls upon wounding (Doster and Bostock 1988, Bowles 1990) or in response to pathogenic infection or stimulus is well documented (Kogel et al. 1985, Bostock and Stermer 1989, Niemann et al. 1990, Cahill and McComb 1992). One of the earlier reports is from Hijwegen (1963), who suggests that the lignification response in resistant cucumber plants is a form of active resistance against pathogens. He noted that not only is the presence of the peroxidase enzyme necessary for lignification, but also the presence of substrate, and that resistance might be conferred by a higher peroxidase activity.

With the development of more sophisticated techniques for determining resistance genes and enzyme purification, the general message is the same. Plants with resistance genes to specific pathogens (usually aerial pathogens) show strong, swift and sensitive lignification responses to infection, or simply to "antigenic" aspects of the pathogen: chemical elicitors of the defensive lignification response (Kogel et al. 1985, Southerton and Deverall 1990). Fungal factors such as toxins, non-pathogenic fungal factors such as chitin (Sherwood and Vance 1976), and also chemicals such as mercuric and cadmium chlorides (Barber and Ride 1988) have been shown to elicit a lignification response. This response includes the systemic enhancement of hydroxyprolines (Hammerschmidt et al. 1984), the increased production and activity of enzymes such as PAL, CAD and peroxidase (Moerschbacher et al. 1990 a, Madan et al. 1991) and stimulation of the process by further provision of substrates such as the lignin precursors (variously described as monophenols or cinnamyl alcohols), H_2O_2 and cofactors such as Mn.

The effect of Mn on lignification response is twofold: lignin precursor synthesis (Engelsema 1972, Hirsch et al. 1978, Halliwell 1978, Robson et al. 1981, Graham 1983) and the activity of key enzymes such as PAL (Jones 1984) and Mn peroxidase (Graham 1983, Southerton and Deverall 1990) which are both affected by the availability of endogenous Mn.

Vance and Sherwood (1976) showed that the lignification response to fungal pathogens could be inhibited by the presence of cyclohexamide, which inhibits protein formation. Specific inhibition of the PAL and CAD enzymes stopped the lignification process in cucumber and permitted infection by a pathogen to occur (Moerschbacher et al. 1990 b). The active lignification response is thus dependent upon protein (enzyme) formation and function. Inhibition of enzymes such as PAL and peroxidase by Mn deficiency in the plant may similarly reduce lignin synthesis.

However, the response of plant roots to the pathogenic take-all fungus, Ggt, is not a specific response. Lignification occurs in response to invasion of the cortical tissue, in a way which is similar to wound response, the formation of lignin structures (lignitubers) around the invading hyphae after the penetration of the cell wall by pectolytic and other degradative enzymes (Weste 1972). Lignituber formation begins approximately eight hours after hyphal invasion and does not cease until after phloem tissue has been fully disrupted (Weste 1972). The lignitubers continue to form around the invading hyphae, restricting hyphal growth. However further growth of invasive hyphae is not prevented, since the fungal cell wall degrading enzymes dissolve the lignituber material in the same way as the cell wall material (Skou 1981). Lignituber formation is thus not completely effective as a method of resistance, since hyphal growth and further invasion of the root is only slowed, not prevented. Lignin in lignitubers differ in composition from normal lignin; the presence of phenolic hydroxyl groups is shown by a positive reaction to safranin, but a negative reaction to basic fuschin indicates that aldehyde groups are absent from the polymer, and variable reactions to HCl-phloroglucinol stains shows that cinnamaldehydes are not always present in the lignituber complex (Skou 1981). Differences in the availability of precursors may cause different compositions of lignin, and enzyme activity in the production of precursors can affect the proportions of the three different precursors made. CAD treats all three precursors in the same manner, so the effects are not CAD determined (Gross 1980, Jones 1984).

5.1.3 Effect of Mn upon lignin formation

Brown (1984) investigated the combined effect of Mn and N nutrition in wheat seedlings on the synthesis of lignin, aromatic amino acids, chlorophyll and phenolic compounds. The critical values of Mn concentration in the wheat seedlings for synthesis of these compounds were determined (using graphs constructed from the results). Brown et al. (1984) found that low Mn did reduce the lignin in roots and shoots of the wheat seedlings. However, according to the critical (Mn) values, the process of lignin synthesis was one of the least sensitive to Mn deficiency, requiring only 9 µg / g Mn (shoot dry matter) for normal lignin synthesis in the roots, and an even lower requirement for synthesis of lignin in the shoot (Brown et al. 1984). Synthesis of phenols was more sensitive to Mn deficiency, with the various critical values dependent on N levels, but between 12 and 19 µg / g Mn (dry matter).

However, work by Webb and Dell (1990) on the effect of Mn deficiency on lignin synthesis in wheat roots has provided a different aspect. While symptoms of Mn deficiency had not developed in plants not supplied with Mn, observation of stained transverse root sections showed gaps in the layer of lignified tissue. These gaps in consecutive sections indicated a hole in the lignin layer of the stele; a weak place where either subunits for lignin production were not supplied, or the polymerisation of the subunits did not take place and tissue was not lignified. No such gaps were found in the lignin layer of Mn adequate plants. Observed in this way, the effect of Mn deficient conditions on lignin production appears to be more sensitive than was indicated by the critical values found by Brown et al. (1984). This provides a different, and probably more sensitive viewpoint of the effect of Mn nutrition on lignin synthesis, since Brown's critical values were calculated using a chemical assay for total lignin, and did not determine the spatial distribution of lignin.

The effect of Mn upon lignin formation and the resistance of roots to infection by Ggt was investigated by Wilhelm (1992). Wheat plants were grown with and without Mn in solution culture; the excised roots of the plants were then exposed to Ggt inoculum on agar plates. Infection was assessed by microscopic examination of the hyphal invasion of the roots. Excised roots from plants grown with Mn had a slower rate and lower extent of infection by Ggt than plants grown under Mn deficient conditions. Lignin production was assessed by staining root sections with phloroglucinol-HCl and was not measured or chemically assayed. However, the preformed, constitutive lignin barriers in the excised roots were shown to be strongly affected by the Mn status of the plants, lignified material being less evident in Mn deficient plant root tissue.

The availability of Mn to plants roots affects the constitutive production of lignin in roots. It is likely that the requirement for Mn in the induced production of lignitubers in an infection response is also be affected by Mn availability. There is thus a strong link between Mn availability to plant roots and the ability of plants to resist or tolerate infection of roots.

5.2 Experimental work

5.2.1 Mn efficiency and lignin production

Under conditions of low Mn availability, Mn efficient plants are capable of normal growth and would be regarded as Mn adequate, while Mn inefficient plants under the same conditions grow poorly and may show symptoms of Mn deficiency. Nutrient deficiency, particularly Mn deficiency, may reduce the lignin defence barrier against invasive pathogens. Wounding, or penetration of the plants cells by a pathogen induces the response of lignification at that site. The demand on the lignin and phenolic pathway under such a response may create a demand for Mn as a co-factor. Under Mn limiting conditions, can these responses be effectively elicited ? Are Mn efficient plants more effective at lignin production under these conditions than inefficient plants ? Do Mn efficient plants constitutively produce more lignin than Mn inefficient plants ? Experiments were designed to answer some of these questions.

5.2.2 Preliminary experiments

Initially plants of several wheat genotypes differing in Mn efficiency were grown in Wangary soil, with and without Mn. Roots, once washed free of soil, were stained with a lignin specific stain, phloroglucinol-HCl, and compared for lignification patterns under low power magnification. Under Mn adequate conditions, the phloroglucinol-HCl stain showed strong lignification of stele tissue in efficient and inefficient genotypes. However, under Mn limiting conditions the Mn inefficient wheat, Bayonet, showed patches in the root stele where a lack of staining indicated that lignification had been poor. The Mn efficient wheat, C8MM, showed no such patches and lignification of the stele was consistent along the length of the roots. Results were limited to these simple observations.

5.2.3 Mn source and genotypic variation

Experiments initially used the same assays as Brown (1984), determining total lignin and phenolics content of root samples, to investigate any differences in constitutive lignin or phenolics production between wheat genotypes differing in Mn efficiency. The technique of Morrison (1972), adapted by Brown (Brown 1984), was chosen for these experiments due to its quantitative nature. An assay for phenolics (Jennings 1981) was used in Experiment 5.2.

5.3 Method

Experiment 5.1

5.3.1 Soil

Two soils were used, Wangary soil and Laffer sand.

The Wangary soil (1990 collection) was incubated at 20 % water content for two weeks prior to the experiment, in treatments of nil (-Mn) and 18 mg / kg Mn (+Mn) (see section 2.2.2). Basal nutrients were added after incubation (section 2.2.3).

Laffer sand is a siliceous white sand, an infertile soil collected from Tintinara, South Australia, sieved and air dried before storage. The Laffer sand was used as a 'sand culture' treatment and, to further reduce levels of contaminants in the soil, the sand was washed in 10% HNO_3 and rinsed in purified water, then again air dried. The acid washed sand was considered to be sufficiently sterile and was not incubated.

Basal nutrients were added to Laffer sand at rates (in mg / kg dry weight) $CaCO_3$ 3000; $Ca(NO_3)_2.4H_2O$ 918; KH_2PO_4 71.8; $MgSO_4.7H_2O$ 140; H_3BO_3 1.4; $ZnSO_4.7H_2O$ 26; NaCl 13; $CuSO_4.5H_2O$ 9; $CoSO_4.7H_2O$ 0.9 and $H_2MoO_4.H_2O$ 0.9. Upon the addition of the $CaCO_3$, Laffer sand had a pH of approximately 6.5.

Mn (as $MnSO_4.H_2O$ in solution) was added to give Mn treatments of nil, 0.01, 0.02 and 0.04 mg / kg (designated Mn 0, Mn 1, Mn 2, Mn 4).

5.3.2 Seed

Two wheat genotypes, the Mn efficient Aroona and Mn inefficient Bayonet, were used in this experiment. Seed Mn concentrations of both genotypes were approximately 30 mg / kg. Seed was surface sterilised and germinated prior to sowing, one seed per pot, in 50 g conetainers.

5.3.3 Procedure

Plants were grown in a growth chamber with a day / night cycle of 10 / 14 hours at 15 / 10°C and watered to original weight with purified water every second day. At D28, shoots were harvested, weighed, and analysed by ICP spectrometry. Roots were washed, weighed and dried. Roots from four replicates were assayed for lignin as detailed below (section 5.3.5). Roots from the fifth replicate were analysed by ICP spectrometry.

5.3.4 Method

Experiment 5.2

A wider range of genotypes was assayed for lignin production and for production of phenolics, which would include lignin precursor phenolics. Roots of 22 genotypes of wheat seedlings from Experiment 2.2 (section 2.5) were assayed for content of phenolics and lignin, using the combination of assays used by Brown (1984).

Two replicates of roots were used, from the -Ggt treatment only. Assays of diseased roots, from Ggt treatment plants, would show lignification responses to infection (Skou 1981). The infection levels within the +Ggt treatment of Expt 2.2 varied greatly, and it is likely that the lignification responses to infection levels also varied. Thus only the constitutive lignin and phenolics levels of the uninoculated plants are presented here, affected by genotype and the soil Mn treatment, not infection by Ggt.

5.3.5 Lignin assay

The assay used was adapted from that of Morrison (1972). After extraction of phenolics from the roots (Morrison 1972, Brown 1984), the root tissue samples (10 - 20 mg) were digested with 25% acetyl bromide in glacial acetic acid, at 70°C for 30 minutes. The cooled root tissue was transferred to flasks containing NaOH and glacial acetic acid. Hydroxylammonium chloride solution was added and the sample was made up to 100 ml with acetic acid, shaken and allowed to stand for at least 1 hour, allowing protein sediment to settle. A reagent blank was run with every set of samples.

Absorbance values (optical density) of the samples correlate directly to the total lignin content (Morrison 1972). Optical density of the sample solutions was read at 280 nm. The absorption values (Abs_{280}, in L/ cm g^{-1}) were calculated from the equation:

$$Abs = ODs\text{-}ODb / C$$

where ODs = optical density of the sample (cm^{-1}), ODb = the optical density of the reagent blank and C = dry weight of sample matter in the final solution (g / L).

5.3.6 Phenolics assay

The assay for total and alkali-labile phenolics was adapted from Jennings (1981) by Brown et al.(1984), and is detailed in Rengel et al. (1993). Root material was ground to a paste with a methanol-chloroform-water extraction mixture, homogenised and centrifuged. Separation of phases in the samples was achieved by adding chloroform, with further centrifugation. Part of the aqueous phase was used to determine total and alkali-labile phenolics, using the Folin Ciocalateau reagent (Jennings 1981), measuring absorbance at 760 nm.

In Experiment 5.2 the lignin assay described above was carried out on the residue from the phenolics assay.

5.4 Results

5.4.1 Experiment 5.1

In Wangary soil, root lignin content of Bayonet plants decreased under Mn deficient conditions, while levels of lignin in Aroona plant roots were similar in both Mn treatments. In Laffer sand, Aroona plants did not show a consistent response of root lignin to soil Mn treatments (Fig. 5.1). Root lignin content of Bayonet plants grown in Laffers sand increased as Mn soil treatments decreased. Lignin content of plant roots was not affected significantly by the shoot or root concentrations of other elements assayed by ICP spectrometry (Appendix 4).

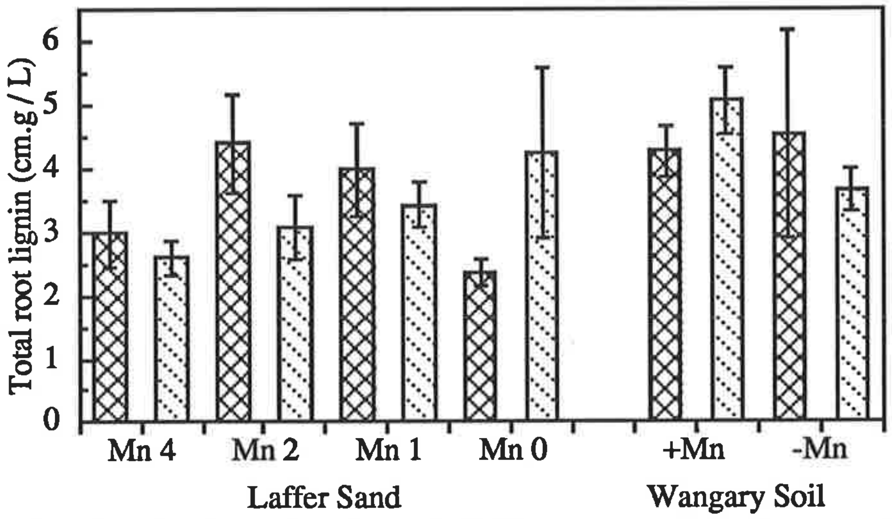

Fig. 5.1 Total root lignin content (Abs $_{280}$, cm.g / L) of two genotypes of wheat (Aroona ▩ and Bayonet ░) grown in Laffer sand under four Mn soil treatments and Wangary soil under two Mn soil treatments. Values are the mean of four replicates, standard error bars are shown.

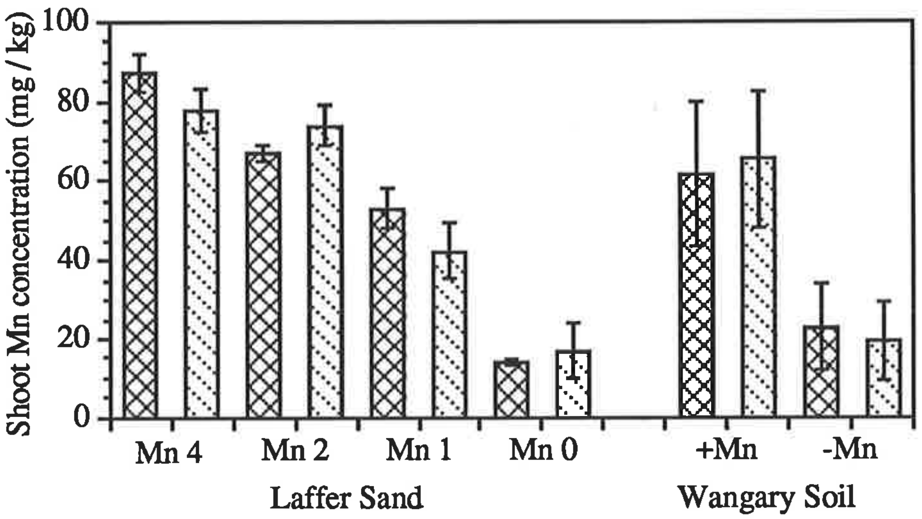

Fig. 5.2 Shoot Mn concentration (mg Mn / kg dry weight) of two genotypes of wheat (Aroona ▩ and Bayonet ░) grown in Laffer sand under four Mn soil treatments and Wangary soil under two Mn soil treatments. Values are the mean of five replicates, standard error bars are shown.

In both soils, shoot Mn concentrations showed clear responses to the soil Mn treatments (Fig. 5.2), rising with increasing soil Mn. There were no differences between the two genotypes in shoot Mn concentrations. Root Mn concentrations showed similar, responses to two Mn soil treatments in Laffer sand (Fig. 5.3).

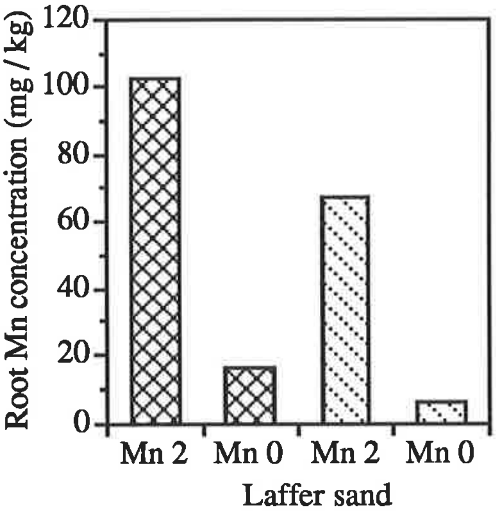

Fig. 5.3 Root Mn concentration (mg Mn / kg dry weight) of two genotypes of wheat, (Aroona ▩ and Bayonet ░), grown in Laffers sand under two Mn treatments. Values are from one replicate only.

Despite low shoot Mn concentrations in the Mn 0 soil treatment, the plants grown in Laffer sand did not show Mn deficiency symptoms. In Wangary soil, the Mn inefficient Bayonet showed Mn deficiency symptoms in the -Mn treatment plants; leaves were chlorotic and shoot growth poor. Aroona plants grown in the Wangary soil did not show deficiency symptoms.

The two soils used, although both highly infertile sands, differed greatly. It is recognised in our laboratory that, even with the addition of $CaCO_3$ to the basal nutrients, and temperature control during plant growth, that it is difficult to obtain Mn deficiency symptoms in plants grown in Laffer sand. Webb and Dell (1990) showed that microscopic differences in lignin distribution in roots occurred before Mn deficiency symptoms were visible in plants grown without Mn in solution culture.

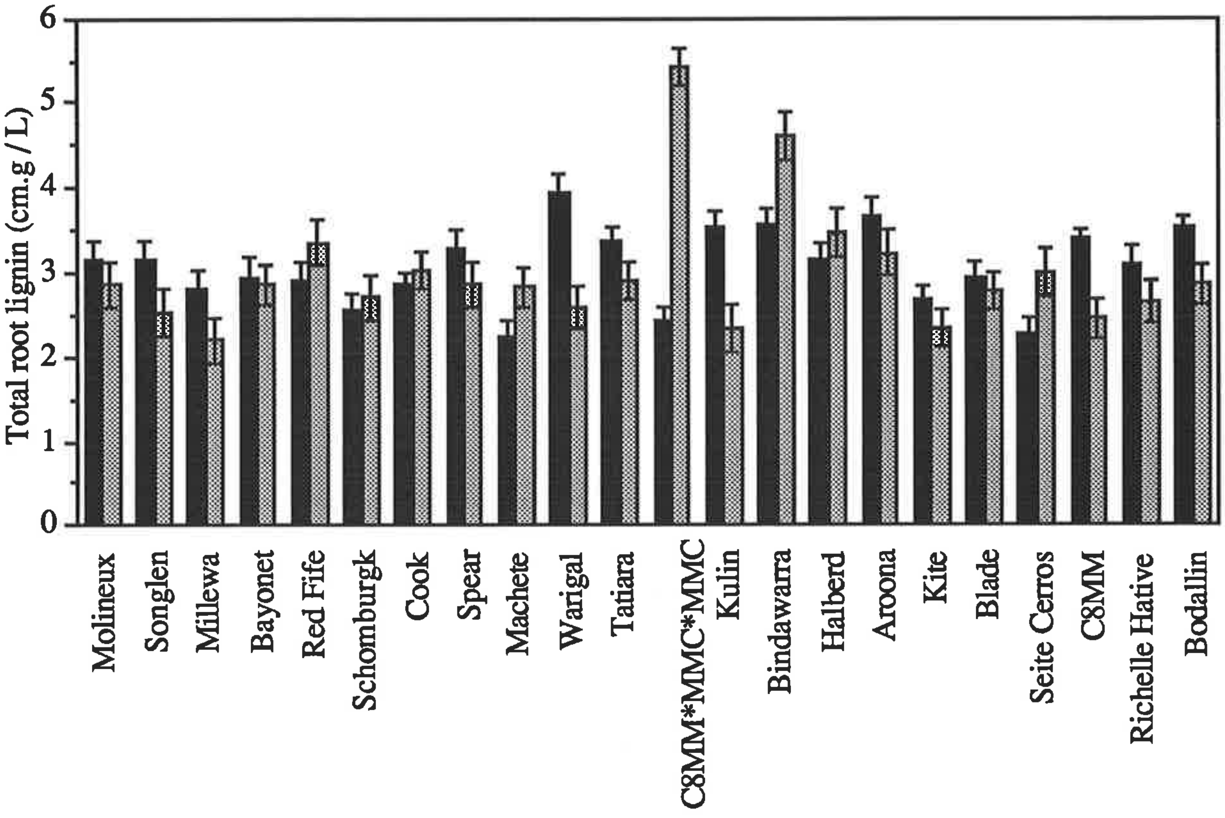

Fig.5.4 Lignin content of roots of 22 wheat genotypes grown under two soil Mn treatments, -Mn ■ (no added Mn), or +Mn ▨ (18 mg / kg soil). Genotypes are ranked in order of increasing Mn efficiency (calculated as a ratio of shoot Mn concentration -Mn:+Mn). Values are the mean of two replicates, standard error bars are shown.

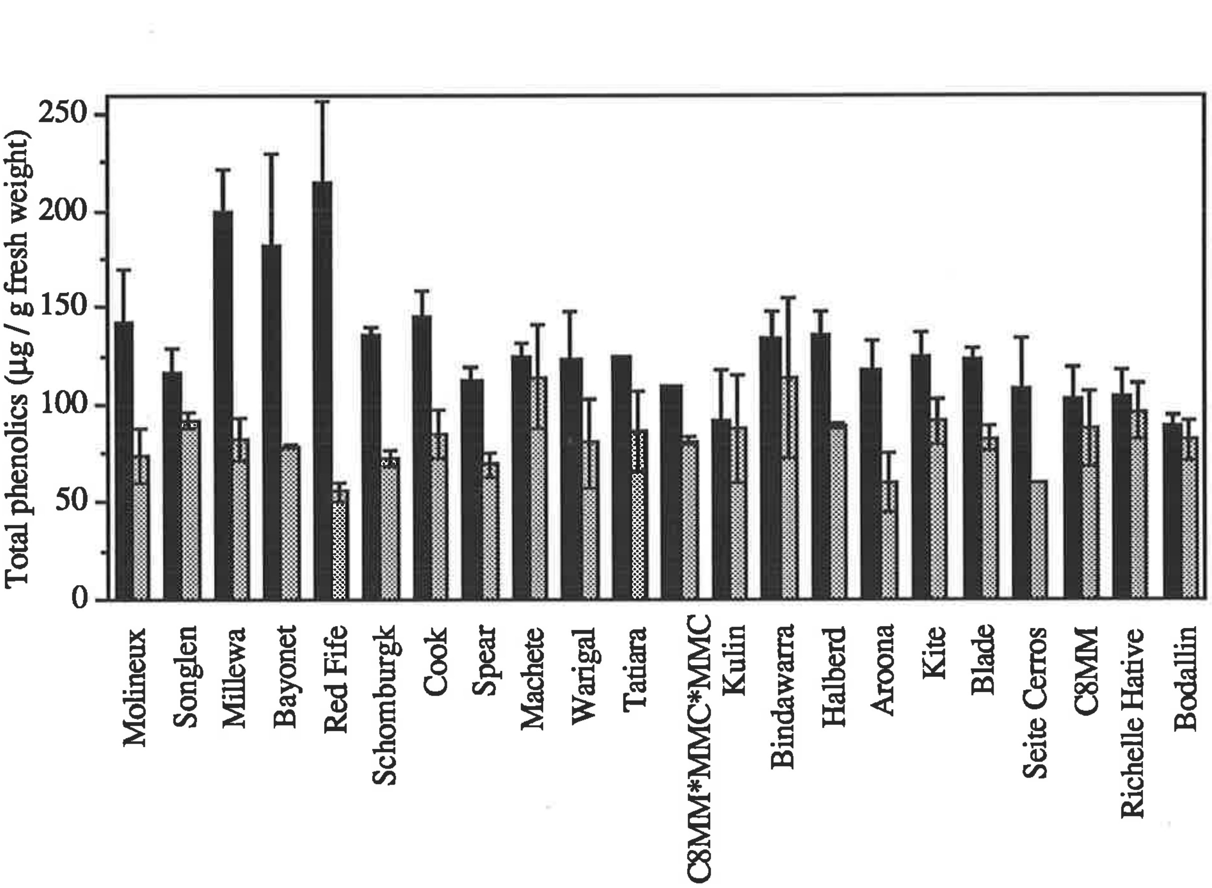

Fig. 5.5 Total phenolic content (µg / g fresh weight) of roots from 22 wheat genotypes, grown under two soil Mn treatments, -Mn ■ (no added Mn) and +Mn ▨ (18 mg / kg soil). Genotypes are ranked in order of decreasing Mn efficiency (calculated as a ratio of shoot Mn concentration, -Mn:+Mn). Values are the mean of two replicates, standard error bars are shown.

5.4.2 Experiment 5.2

The results of the lignin assay did not show any significant interactions of lignin production with genotype or Mn treatment. However trends are apparent, as less lignin was generally found in the Mn deficient (-Mn) plants, and across the genotypes slightly less lignin was found in the inefficient genotypes (Fig. 5.4).

The interaction of soil Mn treatment and wheat genotype affected total phenolic production with the more efficient genotypes producing (or accumulating) a lower level of total phenolics under Mn deficiency than the less efficient genotypes (Figure 5.5). Plants with adequate Mn (+Mn soil treatment) showed less variation between the genotypes, generally producing less phenolic compounds. In contrast to Brown et al. (1984) only minute amounts of alkali-labile phenolic compounds (monophenols) were found by the assay and the alkali stable phenolic compounds remaining followed the same trends as the total phenolics. The phenolics assayed here are monophenols, whereas the alkali-labile phenolics assayed by Jenning's test are dihydroxy phenols (typified by catechol), which include the cinnamyl alcohols - the lignin precursors.

5.5 Localised quantification of lignin content

If the reduction in lignification, due to Mn deficiency, is significant in permitting easier infection of the stele by fungi, but the effect of reduced lignification is localised to certain parts of the root only, the effect is not easily detectable with the total chemical assay. Thus a different approach is required.

In the light of the recent work by Webb and Dell (1990) it would appear that the assay used here (Brown et al. 1984), although quantitative, is too insensitive to resolve the apparently small but important differences in lignin production between genotypes, until Mn deficiency stress has made the differences overt.

Events in lignin production have been shown to occur on a small scale; such localised effects which are not effectively measured by gross tissue analysis (Webb and Dell 1990, Graham and Graham 1991), and the painstaking technique of microscope observation of root sections does not easily provide quantitative data. Another possible approach is also semi-quantitative, using a gel densitometer to measure lignin stained with phloroglucinol-HCl (Barber and Ride 1988).

An attempt to use Magnetic Resonance Imaging, a technique that can be used to give quantitative data to measure differences in lignin production, is described below.

5.5.1 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is an adaptation of nuclear magnetic resonance (NMR) spectrometry, using the resonance of free carbohydrate, organic groups, water molecules and protons to provide signal data, transformed to produce a chemical image of the tissue structure. The technique is non-invasive and has the added bonus of being able to create a "slide library" (gaining a sequence of transectional images by moving the focus of the signal). ^{1}H MRI provides a map of the distribution of free H_2O in the tissue structure and is a quantifiable technique. Some success in imaging root transections had been achieved using chive roots (Pope 1991), and images of roots at different points along the root system showed the vascular system of the root quite clearly. The chive plants had been maintained in the tubes for some days during the collection of signal data. If this could be done as effectively with wheat plants, perhaps the progressive development of fungal infections on the roots could be followed.

Lignin is a hydrophobic molecule and so appears black in the image (a negative image). Under conditions which reduced lignin production, an increase in the black area of the image would be expected. Resolution of 10 μm is considered possible in an image of biological tissue, providing microscopic, as well as quantitative detail.

5.6 Method

The trial of MRI micro-imaging of wheat roots was carried out at the NMR micro-imaging facility at the University of New South Wales, under the direction of Professor J. M. Pope. The facility is based around a 4.7 Tesla superconducting magnet, with 15cm diameter, room temperature bore, and a Bruker MSL200 pulsed NMR spectrometer.

5.6.1 Plant culture

Wheat plants (genotype Bayonet) were grown in solution culture, under +Mn and -Mn treatments. Seed was of low Mn source (3.2 mg Mn / kg), and was surface sterilised and germinated prior to sowing in the solution culture system. Three plants per pot were grown for two weeks in strong daylight (a day / night cycle of approximately 14 / 10 hours).

The pots used had a volume of 350 ml. Solution culture nutrients were as follows: $Ca(NO_3)_2.4H_2O$ 500; KH_2PO_4 13.6; $MgSO_4.7H_2O$ 123; K_2SO_4 173; H_3BO_3 0.13; $ZnSO_4.7H_2O$ 0.40; NaCl 2.9; $CuSO_4.5H_2O$ 0.25; $CoSO_4.7H_2O$ 0.023 and $H_2MoO_4.H_2O$ 0.023 mg / L. Fe was added as FeH EDTA, with a final concentration of 20 µM. Mn was added, as $MnSO_4.H_2O$, at 0.09 mg / L to the +Mn treatment only .

5.6.2 MRI procedure and results

Entire root systems of intact single plants from the +Mn treatment were placed in the chamber inside 5 mm glass NMR tubes. The resonance 'beam' hits the bottom 1.5 cm of the tube, so the sample must be placed deeply in the tube. A dry tube is preferably used to ensure a minimum of interference from external free water with the signal from the roots. Additionally, air bubbles may form in water around the sample, which can obscure the signal and thus distort the image.

However, a wheat root system eased into a dry tubes provided a poor signal, with very poor resolution.

A root system in a NMR tube completely filled with water (ensuring that no air bubbles were present) provided the best signal and images were obtained from overnight sampling of the signal (Fig. 5.6). The image showed a transect of the tangle of roots in the bottom of the tube, the white circles are the root cortex, the black is the lignified tissue, with the central white non-lignified xylem elements.

Quantification of the elements of the image (Fig. 5.6) by NMR assessment of the signal showed a large H_2O peak at 8 Hz, and a small unidentified peak at 50 Hz, of 4.8 ppm. The significance of this peak is unknown.

This most successful sample still did not achieve a useful level of signal definition and the resolution of the lignin layer was not sufficient to be used as a measurement.

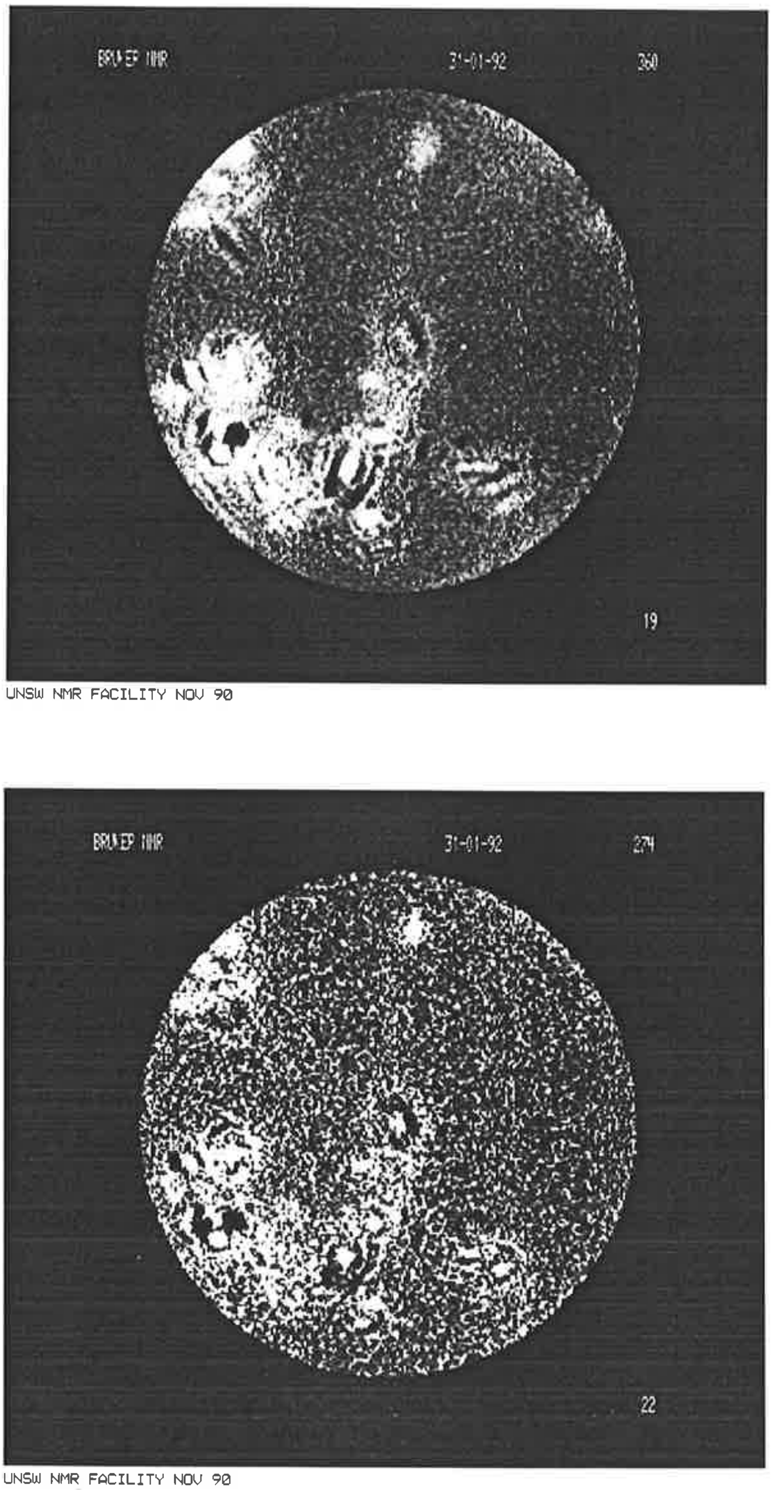

Fig. 5.6 Transverse section images of wheat roots, obtained through Magnetic Resonance Imaging techniques. Images were obtained after overnight sampling of signal from wheat roots, grown in solution culture.

Resolution of the lignin layer was not sufficient to be able to calculate amounts of lignin (as negative signal) in the 'slices' of the image. Pectins, oils and lipids are the molecules which create the strongest and most useful resonance for NMR and MRI, having many free proton and carbohydrate groups. Cereals have very low levels of these molecules in roots and low, indistinct signal from wheat roots was to be expected. However, this technique may still be useful for determining lignin levels (amongst other, less recalcitrant compounds) in plants with a higher level of lipid or oils in the root tissue, which provide a stronger signal and thus a clearer image. Whole root systems have been studied for water movement using NMR techniques (Ratkovinc and Vucinic 1992) and MRI techniques (Bottomley et al. 1986).

5.7 Discussion

Overall, total lignin production was not reduced significantly in roots grown under Mn limiting conditions. Lignin content in plant roots has previously been shown to be decreased by Mn deficient conditions (Brown et al. 1984, Webb and Dell 1990) but also to be decreased when Mn fertiliser was added to a Mn deficient sand (Rengel et al. 1993).

However, the slight trends found in the lignin results from Experiment 5.2 do support the hypothesis (section 5.2.1), slight differences between genotypes in constitutive lignin levels showing the Mn efficient genotypes to be better able to produce lignin under Mn deficiency stress. It is doubtful whether the very slight differences in lignin production seen here could useful as a specific breeding trait, if indeed separable from Mn efficiency. Genotypic traits such as high peroxidase activity have been linked with field resistance and are suggested as useful selection criteria in breeding programs (Reuveni et al. 1991).

The Mn efficient genotypes did not constitutively produce more lignin than the inefficient genotypes under Mn adequate conditions. However, Rengel et al. (1993) showed that in plants infected with Ggt inoculum, Mn efficient wheat genotypes had smaller infection lesions than inefficient genotypes, regardless of Mn soil treatment. The lignification response to Ggt infection appeared to be more effective in the Mn efficient wheats, while constitutive lignin levels did not differ significantly between the genotypes (Rengel et al. 1993).

Fewer phenolics were accumulated in root tissue under Mn adequate conditions than under Mn deficient conditions. Accumulation of these phenolic compounds under Mn deficient conditions could be explained by the requirement for Mn in the final production of the lignin macromolecule. Additionally, under Mn limiting conditions, total phenolics levels accumulated in plant roots rose as Mn efficiency of the genotypes decreased.

Assuming that all the phenolics are lignin precursors, phenolics would be accumulated under subclinical Mn deficiency, reducing lignin production in the -Mn treatment, and utilised when adequate Mn was available to the plant in the +Mn soil treatment. Although it is probable that the higher levels of phenolics accumulated in the -Mn treatment and in Mn inefficient genotypes does indicate an accumulation of lignin precursors, not all of these phenols, even those specific alcohols which are the subunits of lignin, are dedicated to the production of the macromolecule (Southerton and Deverall 1990).

In the most Mn efficient genotypes (C8MM, Bodallin), the phenolics levels in plants from both the Mn soil treatments were similar, perhaps indicating that the pathways utilising phenolic compounds were still adequately supplied with Mn, even in the -Mn treatment. It is likely that Mn efficient genotypes have sufficient levels of Mn^{2+} (under Mn limiting conditions) to stimulate the peroxidase / H_2O_2 system which polymerises the lignin precursors (Engelsema 1972). Under conditions of Mn deficiency, especially in inefficient genotypes, the peroxidase controlled precursor polymerisation process may be stalled and the lignin layer imperfectly formed.

Holes in the constitutive lignin layer around the stele, as observed by Webb and Dell (1990) in Mn deficient plants, would admit invasive hyphae more readily. Wilhelm (1992) showed that excised roots from Mn deficient plants were more rapidly and more extensively infected by Ggt. The sheath habit of Ggt runner hyphae on the root surface, and in the root cortex, may be 'searching' for a weakened area in the lignified tissue before invasion of the xylem can occur.

The invasion of the phloem tissue is as important in the development of the disease, if not more important than invasion of the xylem (Clarkson et al. 1975). Formation of constitutive lignin only occurs in the xylem tissue and thus will only protect xylem vascular tissue but not phloem or cortical cells from invasion by Ggt fungus. The formation of lignitubers in the cortex and phloem is ineffective in halting invasion of root tissue by hyphae (Weste 1972, Skou 1981). In addition, the formation of lignitubers in cells invaded by Ggt hyphae does not appear to continue past cessation of phloem flow (Clarkson et al. 1975). Further upward invasion of the phloem and cortical tissue by fungal hyphae is ensured, although the rate of cell invasion of may be reduced. The lignified material (lignitubers) formed in response to cell invasion does not prevent further Ggt hyphal penetration, and active resistance to infection in the plant only occurs where root function is still adequate (Holden 1976, Clarkson 1988).

Under disease stress the availability of Mn may be restricted, and the lignification response to infection may suffer in consequence. The lignification response as well as constitutive lignin formation may be affected by Mn efficiency and Mn availability. The more Mn efficient wheats have shown better responses in lignification upon infection than inefficient wheats (Rengel et al. 1993).

Heightened levels of resistance to Ggt may be elicited in plant roots by a resistance response to an avirulent fungus (Wong 1981). Prior infection of wheat roots by the fungus *Phialophora radicicola* has been shown to reduce infection of roots by Ggt, a suggested means of biological control of take-all disease (Deacon 1976). Cowan (1978) showed that levels of cinnamic acids were increased in roots infected with *P. radicicola*, and that the phenolic compound, caffeic acid, had an impressive effect in reducing the growth of the Ggt fungus on agar. Cinnamic and caffeic acids are produced as part of the shikimate pathway and are important in the formation of lignin precursor phenolics. Accumulation of lignin precursors may reduce growth of Ggt fungus. B and Si are also accumulated in defensive tissue, but their role in wound response or disease resistance, if any, is not known (Kunoh 1990, Billa and Monties 1991).

5.8 Conclusion

Lignin production was hypothesised by Wilhelm (1992) as the probable means of take-all resistance with reference to Mn nutrition. The resistance of plant roots to invasion by take-all is likely to be more reliant upon the consistent distribution of the lignin layer, rather than the total amount of lignin in the root system. Availability of Mn to plants is important in innate resistance to infection (Wilhelm 1992) and to the lignification response to infection by Ggt (Rengel et al. 1993).

Lignin production in roots was not consistently affected by Mn availability in the soil. However, some trends showing reduction in lignin production under Mn limiting conditions were observed, supporting evidence of other workers (Brown et al. 1984, Webb and Dell 1990, Wilhelm 1992). Genotypic differences in Mn efficiency were not clearly reflected by differences in lignin production between genotypes. However, constitutive levels of lignin production and phenolics accumulation in Mn efficient genotypes were not affected under Mn limiting conditions, whereas Mn inefficient genotypes did show an accumulation of phenolic products and some reduction in lignin levels, where Mn availability was a limiting factor.

Since localised differences in lignin levels are important in an investigation into the area of disease susceptibility (Hammerschmidt and Kuc 1982), an assay or technique sensitive enough to determine quantitative differences in lignin levels at specific points along the root system is required. Suitable methods are needed to investigate the distribution of lignin in roots rather than assaying the total lignin content. MRI techniques were not effective for the clear resolution of root lignin in cereals. The effect of Mn nutrition on the lignification response to infection in plant roots may be better studied through investigating the effect of Mn deficiency on the enzymic production of lignin precursors and enzymic activity.

5.9 References

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Chapter 6

Manganese oxidising ability of isolates of the take-all fungus correlates to virulence

6.1 Introduction

Micronutrients have a role in host-pathogen interactions and show a wide range of effects on plant-disease interactions (Graham 1983, Graham and Webb 1991). Addition of a particular micronutrient to deficient soil may affect either the status of the plant, the pathogen or both, thus affecting the host-pathogen relationship. Manganese affects a wide range of diseases in many plant hosts (Huber and Wilhelm 1988). Graham and Rovira (1984) have shown that Mn added to a Mn-deficient calcareous soil significantly decreased take-all in wheat. Wilhelm et al. (1988) found that strongly virulent isolates of *Gaeumannomyces graminis* var *tritici* (Ggt) oxidized Mn^{2+}, forming brown Mn oxide precipitate on agar to which Mn^{2+} had been added. Moreover, they noted that a non virulent isolate of Ggt was a poor oxidizer of Mn in agar. Expanding this concept, Buchhorn and Graham (1992) established a significant correlation between virulence (expressed as the total length of stelar lesions on infected roots) and the Mn oxidation capacity of 14 isolates of Ggt.

Variation in virulence between isolates of Ggt occurs in the field, and it has been suggested that isolate virulence may be inversely related to the susceptibility of the host plant from which the fungus was isolated (Dewan and Sivasithamparam 1990). However, the virulence (or invasiveness) of an isolate has not been found to be related to growth rate of the fungus on agar (Asher 1981), to production of the enzyme polygalacturonase (Holden and Ashby 1978) or to the production or absence of melanin in the runner hyphae (Asher 1981).

Many soil bacteria and fungi have been identified as transformers of Mn in soil and oxidation of Mn by soil microorganisms is a common ability (Bromfield 1958 a, Timonin et al. 1972, Ghiorse 1988). Thus the oxidation of Mn by a soilborne fungus such as Ggt is not unusual, but combined with the known effects of Mn fertiliser in reducing the take-all disease (Wilhelm et al 1988), more attention to a possible linkage between the virulence of isolates of Ggt and their ability to oxidize Mn^{2+} was warranted.

6.2 Materials and methods

Two experiments comparing the virulence and the Mn oxidising ability of Ggt isolates were carried out; the first in 1988 (Buchhorn and Graham 1992) using 14 isolates of Ggt, the second in 1989 using 26 isolates. Both experiments involved a virulence test carried out on seedlings in pots of soil, plus ranking the Mn oxidising ability of isolates grown on agar. A means of quantifying the oxidation of Mn by Ggt was investigated, using solution culture medium.

6.2.1 Inoculum

The solid agar medium used throughout was a weak potato dextrose agar medium ($^1/_{25}$PDA) (1.6 g l^{-1} potato dextrose agar and 10 g l^{-1} 'Difco' bacto-agar, poured after autoclaving into 9 cm, sterile plastic Petri dishes). Ggt isolates grown and subcultured on $^1/_{25}$PDA, were stored at 5°C. Fourteen isolates were used in 1988. These were subcultured six times on $^1/_{25}$PDA before use in the 1989 experiment, in which 11 more isolates were included. The sites from which all the isolates were collected are presented in Table 6.1. As we were concerned that the virulence of the isolates used in 1988 may have been attenuated due to the subculturing, two forms of the Ggt 8 isolate were used in the 1989 experiment. One had undergone the subculturing described above (8S), while the other was grown from rye seed that had been inoculated with Ggt 8 at the same time as the 1988 experiment (8M). The inoculated rye seed had been stored for the interim under sterile conditions, at 2°C.

6.2.2 Soil

The soils used, a Dy5 Mallee sand (Northcote 1979) and a washed river sand, were autoclaved in 2 kg aliquots, at 121°C and 1.5 kPa for 30 min.

Table 6.1 *Gaeumannomyces graminis* var *tritici* (Ggt) isolates used in virulence and Mn oxidation experiments, ranked for Mn oxidation capacity, showing the Mn oxidation rankings (0 = no oxidation, 10 = greatest oxidation) for both experiments (1988 and 1989) and the sites from which the isolates were collected. Isolates were obtained courtesy of H.J. McDonald, A.D. Rovira and P. Harvey.

Ggt isolate	Ranking		Collection Site [a]
	1988	1989	
82	1.3	0	Turretfield, S. A.
143 WUF		0.1	W.A.
KS1	2.0	0.3	Kapunda, S.A.
43	7.3	0.6	Avon, S.A.
AbV.V.15		0.6	Palmer, S.A.
162	5.3	0.9	Avon, S.A.
68	4.0	3.4	Mallala, S.A.
TW1		3.4	Terrawinda, N.S.W.
8 (8S)	8.7	3.6	Avon, S.A.
168	6.0	4	Avon, S.A.
94	2.7	4	Kapunda, S.A.
233	4.7	4.9	Kapunda, S.A.
211	8.0	5.4	Avon, S.A.
T.2		5.4	Temora, N.S.W.
91	3.3	6.2	S.A.
VVP		6.3	Palmer, S.A.
104	6.7	6.8	Turretfield, S.A.
153	10	7	Freeling, S.A.
500	9.3	7.1	W.A.
Tem		7.4	Temora, N.S.W.
TW II		7.6	Terrawinda, N.S.W.
8M		7.8	Avon, S. A.
GW		7.9	Gainman, N.S.W.
G		8	Gainman, N.S.W.
TCool		8.9	Coolamon, N.S.W.
T->C		9.1	Coolamon, N.S.W.

[a] Collection sites are named by locality, if known, and state; S. A. South Australia, W. A. Western Australia, N. S. W. New South Wales

6.2.3 Seed

Wheat was used in both experiments (1988 cv Spear, 1989 cv Bayonet). After wetting with 30% ethanol for 1 minute, seed was surface sterilised with 5% NaClO for 2 minutes, then rinsed in deionized water.

6.2.4 Virulence test

The virulence test described here followed the technique developed by McDonald and Rovira (1988). The inoculum for the virulence tests was grown on $^1/_{25}$PDA for 10 days at 20°C. Uninoculated $^1/_{25}$PDA plates were used for the nil Ggt control. Conetainers were used, with drainage holes blocked with cotton wool. Each conetainer was filled as follows: river sand to 18 cm, an agar plug (3 cm diameter, 3 mm thick, inoculated or uninoculated) exactly filling the internal diameter of the pot, Mallee sand to 20 cm, one wheat seed, Mallee sand to 21 cm. Deionised water was added to maintain the water content at 15% w/w throughout the experiments. The experiments were maintained in a growth chamber with 10 h day length, 15/10°C day/night temperature, a photon flux density of 300 μmol m^{-2} s^{-1}. Each treatment, including the control, was replicated ten times.

In the 1989 experiment, germination and emergence was poor, and although pots were resown with pre-germinated seed, there were several missing plants. In the 1988 experiment all the plants grew.

Plants were harvested after D 21 , shoot fresh weights taken and roots carefully washed free of sand. Roots were scored for number of infected roots (NIR), number of stelar lesions (NSL) and total length of lesions (TLL) per plant. All data was analysed using Genstat 5 (Lane et al. 1987).

6.2.5 Mn Oxidation Ranking

The oxidising ability of the isolates was ranked by growing the isolates on $^1/_{25}$PDA amended with 50 mg l^{-1} Mn^{2+}. Autoclaved stock of $MnSO_4$ was added and mixed with sterile $^1/_{25}$PDA immediately prior to pouring the plates. Isolates were grown on this agar for 14 d (at 20°C) and compared with colonies of the same age grown on $^1/_{25}$PDA containing no added Mn. Isolates were ranked for Mn oxidation ability (the intensity of brown Mn oxide formed in the agar) and scored on a scale of 0 - 10 (0 = no oxidation, 10 = greatest oxidation). That the brown precipitates formed by the fungus under this type of system are Mn oxides was confirmed by Wilhelm (1992) using the benzidine reaction to Mn oxides (blue colour of stain on contact with Mn oxides) (Leeper 1970), and by Arnott et al. (1991) using energy dispersive X-ray micro-analysis.

6.2.6 Rate of Mn oxidation by Ggt 500 in solution culture

Preliminary experiments suggested that Mn^{2+} oxidized in undisturbed solution media by Ggt would be physically removed from the solution; either sinking as residue or precipitating on the surface of hyphae. Samples taken from the solution phase would effectively show a gradual depletion of Mn^{2+} as the ion was oxidized by the fungus.

A defined, sucrose-based solution medium was used, containing (in $g.L^{-1}$ H_2O) $NaNO_3$ 2; KH_2PO_4 0.3; $MgSO_4.7H_2O$ 0.5; KCl 0.5; $FeSO_4$ 0.01 and sucrose 30. The solution medium was adjusted to pH 7.0 with 0.1M NaOH, dispensed into conical flasks (providing a large surface area for the fungus to grow) which were plugged with cotton wool and capped with aluminium foil. The flasks were autoclaved for 15 minutes at 121°C and 1.5 kPa. Separately autoclaved stock of 10 mM $MnSO_4$ was prepared.

Small plugs of agar covered in actively growing Ggt 500 inoculum were aseptically transferred into six flasks containing 20 ml of solution medium. Flasks were recapped and kept undisturbed at 20°C. After 10 days a mat of diffuse grey or hyaline hyphae had grown and was floating in the solution around the agar plug.

Under sterile conditions (laminar flow cabinet), hyphal masses from four flasks were rinsed in deionized H_2O and transferred into four 1 L conical flasks containing 500 ml of autoclaved solution medium to which 0, 1.0 (two flasks) and 5.0 ml of sterile $MnSO_4$ stock had been added (giving levels of 0, 0.02 mM and 0.1mM Mn). Similar flasks were prepared as controls for non-biological oxidation of Mn in the solution medium, without hyphal mass and with autoclaved hyphal mass. Flasks were kept in this laminar flow cabinet for the duration of the experiment. Samples (5 ml) were aseptically taken, prior to the addition of fungal hyphae, at the time of addition, and then at 1, 2, 3, 4, 6, 8, 11, 14, 18, 22, 30, 34, 45 and 57 hours, using an autopippete and a sterile tip for each separate sample. The samples were immediately transferred to sterile 5 ml plastic tubes, capped and analysed by ICP spectrometry. The pH of the solution media remained stable at pH 7.0 for the duration of the experiment.

After final sampling, the flasks were autoclaved for 15 min and allowed to cool before the contents of each was filtered through a pre-weighed filter paper (Whatman No.41). Each Ggt sample (hyphal mass and filter paper) was rinsed with 250 ml of purified H_2O, dried at 80°C, weighed and analysed by ICP spectrometry. Control papers soaked in solution medium (without Mn), were similarly treated and analysed. Thus, filter paper contaminants could be subtracted from the Ggt samples, and the dry weight of the hyphal mass and the amount of Mn in the hyphal mass calculated.

6.3 Results

6.3.1 Correlation of Mn oxidation ability with virulence of Ggt isolates

No symptoms of disease or nutrient deficiency were seen in the shoots of the seedlings during the experiment, and no effect of inoculum was seen on plant growth or development (Fig. 6.1). Shoot fresh weight was not significantly correlated with Mn oxidation ability in either experiment, showing that the development of seedlings were not significantly affected by disease at the time of harvest.

However, the results from both experiments (Figs. 6.2, 6.3) show that the disease measures NSL and TLL were positively correlated with variation in virulence of the isolates. Thus the infection scores (NSL and TLL) could be used as a comparative measurement of the virulence of an isolate, since the infection had not yet affected plant shoot growth, which would in turn affect the overall resistance of the plants to the invasive pathogen. The number of infected of roots (NIR) (data not shown), the frequency of lesion formation (NSL) and the extent of lesion formation (TLL) all show an aspect of virulence of the fungus, and in this text these disease scores will be referred to generally as a measure of the virulence of an isolate.

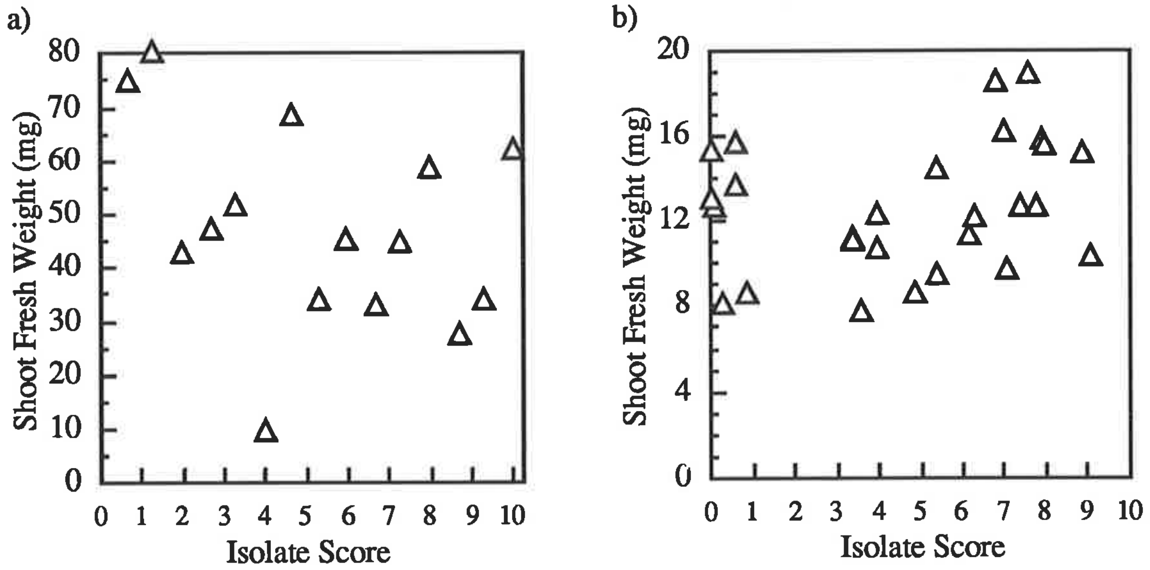

Fig. 6.1 Absence of asociation of shoot fresh weight of wheat plants with Mn oxidation capacity scores (0 =no oxidation, 10 = greatest oxidation) of isolates used in the virulence tests: (a) 1988 experiment, (b) 1989 experiment.

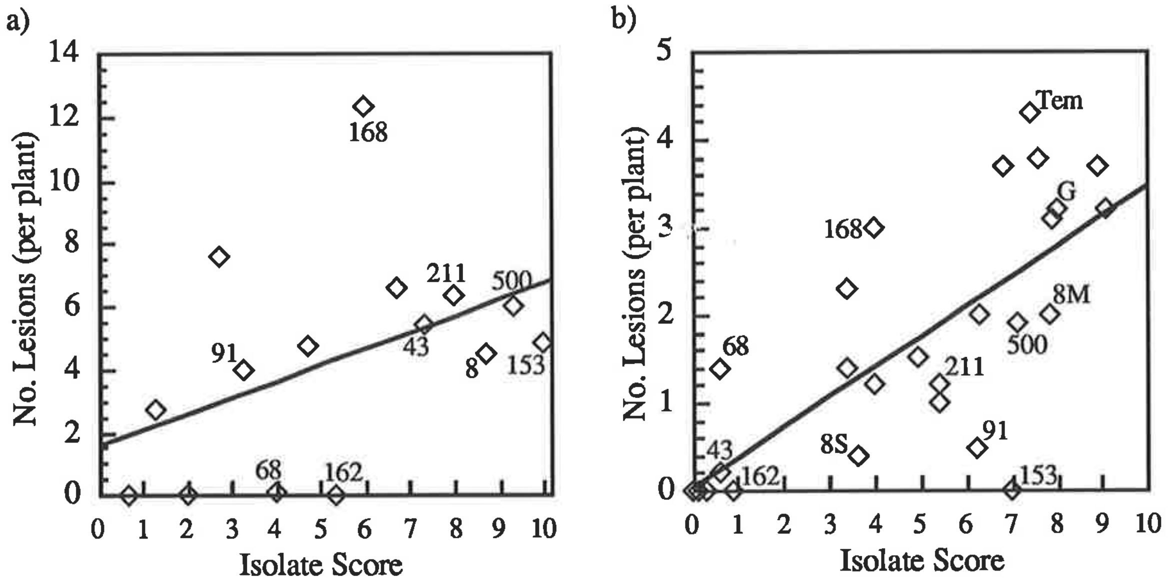

Fig. 6.2 Correlation of number of lesions (NSL) per plant with Mn oxidation capacity scores ((0 =no oxidation, 10 = greatest oxidation) of isolates used in the virulence tests: (a) 1988 experiment, with 14 isolates and nil Ggt control (C), (b) 1989 experiment with 26 isolates and nil Ggt control. Positive, significant correlations were obtained in both experiments; 1988 $R^2 = 0.16$; 1989 $R^2 = 0.54$. Isolates mentioned in the text (such as 8, 8S and 8M) and from Table 1 (Tem , G) are labelled, showing the effect of subculturing upon Mn oxidation capacity and virulence.

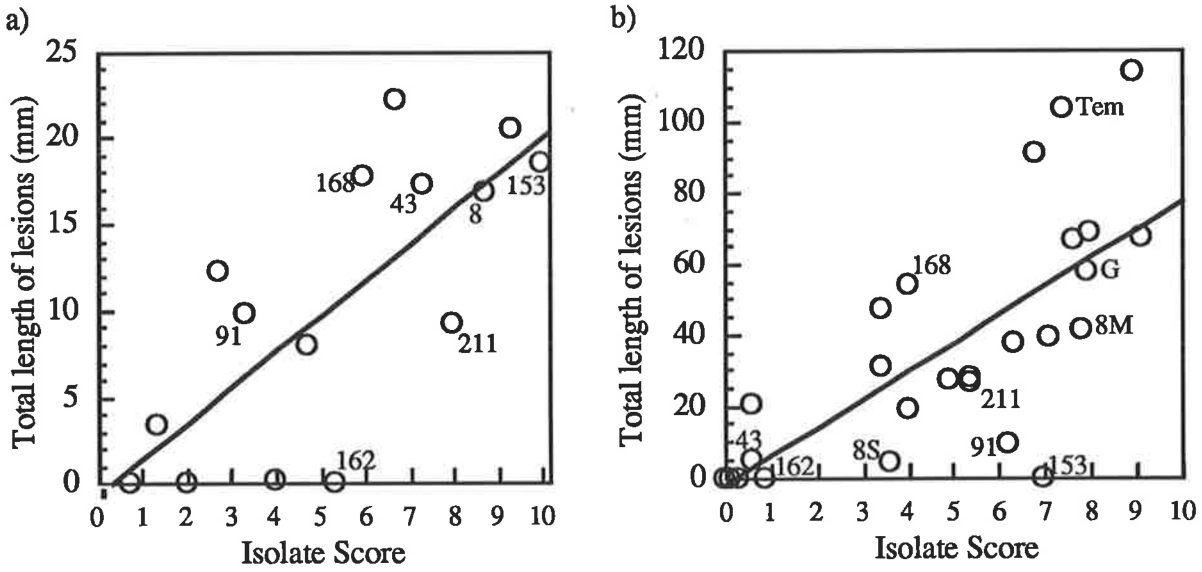

Fig. 6.3 Correlation of total length of stelar lesions (TLL) (mm plant^{-1}) with Mn oxidation capacity scores (0 =no oxidation, 10 = greatest oxidation) of isolates used in the virulence tests: (a) 1988 experiment, with 14 isolates and nil Ggt control. (b) 1989 experiment with 26 isolates and nil Ggt control. Positive, significant correlations were obtained in both experiments; in 1988 $R^2 = 0.59$; in 1989 $R^2 = 0.53$. Isolates mentioned in the text (such as 8, 8S and 8M) and from Table 1 (Tem, G) are labelled, showing the effect of subculturing upon Mn oxidation capacity and virulence.

In both experiments, disease scores were positively correlated with the Mn oxidising ability of the isolates. Over 50% of the variation in TLL (Fig. 6.2), NSL (Fig. 6.3) and NIR (data not shown) was explained by the ranking of the Ggt isolates' Mn oxidation ability.

Differences were seen in Mn oxidation ability rankings and in virulence of isolates between the 1988 and 1989 experiments. The general trend shown was that of decreasing Mn oxidising ability, accompanied by decrease or stability of virulence. The isolates Ggt 153 and 8S show this most clearly.

The Mn oxidation ranking and virulence of Ggt 8 differed distinctly between the experiments, shown clearly by comparison in the 1989 experiment between Ggt 8S (subcultured form of Ggt 8) and Ggt 8M (maintained form of Ggt 8). In the 1988 experiment Ggt 8 had high Mn oxidising ability and virulence. In the 1989 experiment Ggt 8M had Mn oxidising ability and virulence similar to that of Ggt 8 (1988 results) but through subculturing not only the virulence of Ggt 8S had attenuated but Mn oxidising ability was also markedly decreased (Figs. 6.2, 6.3).

However, while Ggt 43 showed a reduced ability to oxidize Mn^{2+}, its virulence remained much the same. A similar, but less marked effect was seen in Ggt 162 and 211. In comparison, Ggt 91 appeared to have an increased oxidising ability, moving up the oxidation ranking while its virulence remained the same, and Ggt 68, an increased virulence with a stable Mn oxidising capacity (Table 6.1, Figs. 6.2, 6.3).

The addition of Mn to the $^1/_{25}$PDA medium did not significantly affect the growth rate of the Ggt isolates, compared to growth rates of the same isolates on $^1/_{25}$PDA without Mn. The diameter of hyphal growth was measured during the growth of the isolates and although there were differences in growth rate and growth pattern between isolates, there were no differences due to Mn in the agar medium (data not shown).

However, there were distinct differences in shoot fresh weight between the experiments; the Spear wheat (1988 experiment) growing significantly larger than the Bayonet (1989). There were also greater TLL values in the Bayonet experiment, than for the Spear wheat but the positive correlation of the virulence of Ggt isolates with their Mn oxidation ability was maintained.

6.3.2 Mn oxidation by Ggt 500 in solution culture

The concentration of Mn in the samples from the solution media containing Ggt and Mn decreased over time, particularly in the 0.02mM treatment, where the total Mn in the sample was halved by the end of the period (Fig. 6.4). The second treatment of Mn (0.1mM Mn) was not reasonably affected by the presence of viable Ggt until the final sampling period, when total Mn in solution dropped dramatically from 83 µmol to 8 µmol Mn (data not shown).

The controls, 0 Mn and Nil Ggt, showed no changes in Mn concentration over the sampling period, as would be expected (Fig. 6.4). No other element measured altered significantly or consistently in concentration during the sampling time (data not shown).

The rate at which Mn was lost from the 0.02 mM Mn solutions in this experiment was 4 nmol g^{-1} (dry weight hyphae) h^{-1} , over the 30 hour period after Mn levels began to fall.

The initial presence in the solution medium treatments of the added Mn as free Mn^{2+} was confirmed by NMR spectroscopy in preliminary experiments. Unfortunately the levels of Mn used in this experiment were too low to carry out the whole experiment using NMR spectroscopy (G. P. Jones pers. comm.), precluding confirmation of free Mn^{2+} levels in the solution medium during the experiment.

Since brown Mn oxide precipitate was progressively deposited on the hyphal mass, it is unlikely that non-biological reaction in the solution caused the reduction of total Mn in the samples. No other precipitate was observed in any of the flasks during the experiment. Although the oxidation of Mn by the Ggt isolate was only one means by which the total Mn may have been depleted in solution, extreme care was taken to minimise contamination (biological or chemical) and the brown precipitate observed on the surface of the hyphal mass gives credence to our interpretation of the results.

Elemental analysis of the hyphal mass and residue showed that only Mn and Zn concentrations varied with respect to the treatments (Table 2). Mn accumulation in the residue and hyphae was increased by the Mn treatments but Zn accumulation in the hyphae did not appear to be related to the Mn treatments. Hyphal mass dry weights are also presented in Table 6.2.

Table 6.2 Dry weight of hyphal mass (mg), and Mn and Zn concentrations of the hyphal mass and residue (mg kg^{-1} dry weight) after 57 hours in defined solution media. Values given in this table were calculated by subtracting mean dryweights and elemental contents of the control filter papers from that of the sample. The nil-hyphae treatment had no inoculum in the solution medium. Values for the Ggt 0.02 mM Mn treatment are the average of two replicates.

Hyphae	Mn level (mM)	Dry weight (mg)	Mn (mg / kg)	Zn
Ggt	0	44	0.27	1.74
Ggt	0.02	68	112	1.04
Ggt	0.10	21	444	1.16
Nil	0.02	2	0.31	0.77

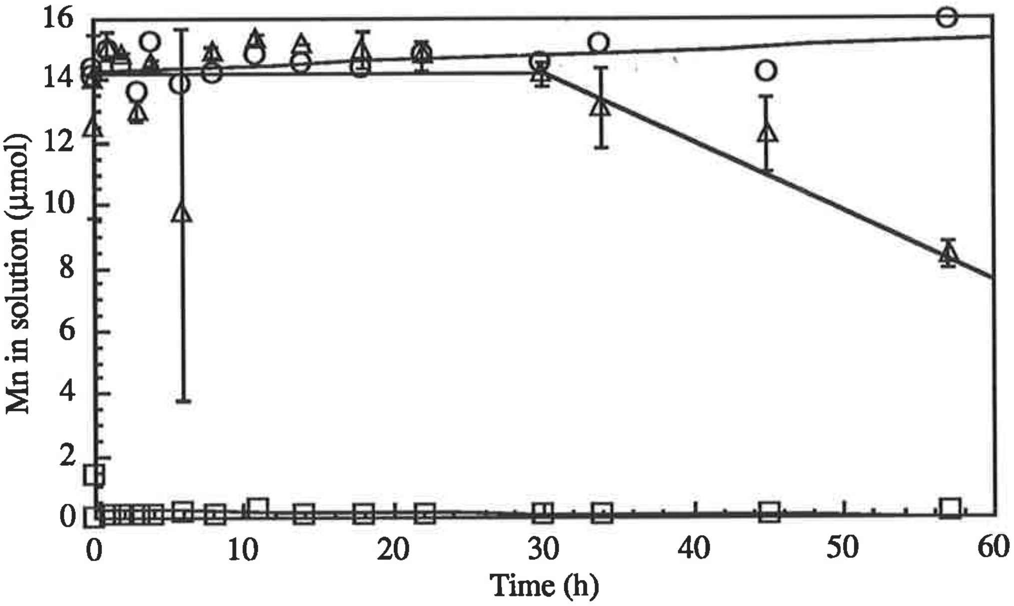

Fig. 6.4 Changes in total Mn concentration (µg l^{-1}) in the defined solution medium over time, caused by the presence of a viable Ggt 500 hyphal mass, under three treatments : nil Mn control with active Ggt (□); nil Ggt control with 0.02mM Mn in the solution medium (○); and active Ggt with 0.02mM Mn in the solution medium (△). A rate of Mn oxidation by Ggt, over the period 30 h - 60 h, was calculated as 4 nmol g^{-1}(hyphae dry weight) h^{-1}.

6.4 Discussion

6.4.1 Correlation of Mn oxidation ability with virulence of Ggt isolates

A mode of pathogenic action by Ggt is suggested by the significant correlation of the virulence of a Ggt isolate with the ability of that isolate to oxidize Mn. It is possible that the oxidation of Mn in the rhizosphere by virulent isolates of Ggt may deplete plant available Mn and even cause a localized Mn deficiency at the root surface, or in the root cortex.

Since no symptoms of disease or nutrient deficiency were seen in the shoots of the seedlings during growth, and no growth differences were seen between the inoculated plants and the uninoculated control, the differences seen between disease measures for the isolates indicate real differences between the isolates and not indirect effects. Differences were seen between the experiments, in the shoot fresh weights of plants and the levels of disease scores. Bayonet is more susceptible to Ggt infection than Spear, by virtue of its poor Mn efficiency (Wilhelm et al. 1990), presumably resulting in the higher disease scores in the 1989 experiment. However, the difference in plant size and cultivar susceptibility to Ggt did not alter the nature of the positive correlation of disease scores with the Mn oxidising ability of the isolates.

We believe that the current method is measuring virulence of the Ggt isolates against a constant resistance of the host, and because the plant roots must grow through the agar plug. The method used for the virulence test does not permit roots to escape from contact with the inoculum. Only the innate resistance of the plant roots, or the low virulence of the inoculum prevents invasion of the stele. Total lesion length (TLL) gives an indication of plant response to infection, once it has occurred; low TLL measurements may indicate either strong resistance response by the plant, or, in the system used here, low virulence of the Ggt isolate. In these experiments, where the plants cannot escape contact with the fungus, the disease scores are a measurement of virulence of the inoculum.

A positive correlation between virulence and Mn oxidising capacity of Ggt isolates was also found by Rosemann et al. (1991). In their experiments it was shown that temperature plays an important role in both the virulence and Mn oxidising capacity of Ggt isolates and the optimum temperature for Mn oxidation varied between isolates. Moreover the greatest virulence of each isolate was found at the temperature optimum for Mn oxidation.

6.4.2 Attenuation

It is well documented that the virulence of isolates of Ggt attenuates upon subculturing, causing difficulties in the long term storage of isolates (Asher, 1981). The difference between the Mn oxidation rankings in the experiments shows that the rate of attenuation is not entirely consistent across isolates as some appear to be more stable than others (in particular isolates 68 and 168 increased in virulence), but in general, the more recently obtained isolates were the more virulent and were also the strongest oxidizers.

Observations between the experiments suggest that the Mn oxidising capacity of the isolate varies with virulence over time. Comparing Ggt 8 with Ggt 8S, the virulence of Ggt 8 was attenuated by subculturing. There is an obvious difference in both virulence and Mn oxidation ability when Ggt 8S is compared to Ggt 8M which shows more similarity to the rankings and scores obtained by Ggt 8 in the 1988 experiment. This reasonably consistent linkage of the attenuation of virulence with oxidation ability further supports our hypothesis that Mn oxidation is a component of an isolates' virulence. Oxidation of Mn by virulent isolates of the fungus may occur at rates which would deplete plant available Mn, and perhaps cause Mn deficiency in the rhizosphere.

6.4.3 Mn oxidation by Ggt 500 in solution culture

With an estimated rate of Mn oxidation by a virulent Ggt isolate of 4 nmol g^{-1} (dry weight hyphae) h^{-1} it is possible that the loss of plant available Mn in the rhizosphere, due to oxidation by the Ggt fungus, could become significant. This creates an intriguing problem of Mn levels, rhizosphere chemistry and the availability of Mn in the rhizosphere. Oxidation of Mn by soil microorganisms can decrease Mn availability to the plant (Gerretson 1937; Bromfield 1958 a), but the Mn oxides formed are not necessarily permanently unavailable to plant roots (Bromfield 1958 b).

6.5 Implications

The oxidation of Mn by Ggt may be an active pathogenic action; the fungus 'taking advantage' of conditions in which the oxidation of Mn is electrochemically and kinetically favourable (pH > 5.5), and perhaps by making the acquisition of Mn by roots less effective, even causing a localized deficiency of plant available Mn. The habit of Ggt in sheathing roots with runner hyphae prior to infection may create an environment where a small hyphal mass can oxidize enough Mn in the root zone to disturb the Mn supply to the besieged root. Wilhelm (1992) showed that root segments from plants deficient in Mn were more readily invaded by Ggt fungi (in vitro) than root segments sufficiently supplied with Mn. A deficiency in Mn in the zone of infection may reduce the efficacy of the plants' defensive response to infection (lignituber formation), since Mn acts as a catalyst in the condensation of lignin precursors (Burnell, 1988).

Take-all is prevalent under conditions which decrease Mn availability to plants (Huber and Wilhelm 1988); the addition of Mn fertiliser to soils reduces take-all (Wilhelm et al. 1988) and wheat genotypes less susceptible to Mn deficiency are also less susceptible to infection by Ggt (Wilhelm et al. 1990). The positive correlation of the virulence of Ggt isolates and their Mn oxidising capacity is a further indication that the importance of Mn nutrition in the control take-all is not simply a problem of deficient soils, but an aspect of the epidemiology of take-all disease.

6.6 References

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Chapter 7

Take-all in the field: Mn oxidation ability of Ggt isolates and plant responses

7.1 Introduction

The highly variable expression of take-all disease in the field has frustrated cereal breeders and researchers for many years, with field experiments disrupted by the occurrence of take-all disease and by the normally patchy distribution and unpredictability of disease expression (Hornby and Bateman 1991, Read 1993). The seasonal variation of soil moisture and temperature, the susceptibility of the crop plant to nutrient deficiency and the state of the soil microflora, all affect the growth of the fungal pathogen Ggt, the growth of its cereal hosts and the expression of take-all disease (Cook and Rovira 1976, Trolldenier 1981, Cotterill and Sivasithamparam 1989 a, Murray et al. 1991). Although agronomic factors such as crop rotations, fertiliser treatments, sowing dates and weed control can be managed to reduce the likelihood of severe disease in susceptible crops, yield loss due to take-all disease may still occur (Huber 1981, Kollmorgen et al. 1985, Rovira and Venn 1985, Simon and Sivasithamparam 1988, Huber and McCay-Buis 1993).

In the field, presence of take-all disease is characterised by the formation of irregular patches of diseased plants, visible as plants with stunted growth or whiteheads. The distribution of Ggt inoculum in soil has been shown to vary widely across fields and between sites (Hornby 1981, Cotterill and Sivasithamparam 1989 b). Infection of root systems by the Ggt fungus is not restricted to plants within these patches, although roots of plants outside of take-all patches may be less severely infected. Distinct take-all patches appear to persist from year to year, although size of the patch may alter over time (Hornby 1981, Hornby and Bateman 1991). The "bare-patch" caused by Rhizoctonia root rot of cereal crops also shows this pattern of distinct patches of diseased plants, although plants outside the bare-patches may also be infected (Thongbai et al. 1993).

Natural take-all patches in cereal crops, and areas where Ggt inoculum has been applied in field trials, have shown up in subsequent crops as patches where plants are Mn deficient (D. M. Huber pers. comm., Huber and McCay-Buis 1993). Soybeans grown after take-all infected wheat in the field have shown symptoms of Mn deficiency. The only recorded instances of take-all infection of a non graminaceous crop have been found in soybeans (Roy et al. 1982) by fungus identified as *Gaeumannomyces graminis* var. *graminis*, not Ggt. Soybeans are a crop susceptible to Mn deficiency. The reports of take-all infection of soybeans were from the Midwest, U.S.A., where they are often grown in rotation with wheat, which is highly susceptible to take-all (Huber 1981).

Adequate Mn nutrition of wheat has been shown to reduce take-all in the field (Wilhelm et al. 1988, Huber 1989). The importance of the availability of Mn to plant roots in affecting the susceptibility of plants to take-all disease has been established in many aspects of the plant-fungus interaction (Wilhelm 1992, this thesis). Immobilisation of Mn by seed coating treatments has been shown to increase the susceptibility of seedlings to take-all (Huber 1987, Rosemann et al. 1988). The effects of the ability of Ggt isolates to oxidise Mn is positively correlated with the virulence of the isolates (Rosemann et al. 1991, Buchhorn and Graham 1992, this thesis). It is hypothesised that Mn oxidation by Ggt in the rhizosphere may be sufficient to disrupt Mn availability to plants and create Mn deficiency stress within the plant. The requirements for this oxidation to be successful would probably be very similar to those climatic and agronomic factors which are known to increase the likelihood of take-all (Huber and Wilhelm 1988), such as high soil pH, low soil moisture and loose seedbed soil.

7.2 Ggt isolates from field plant samples

Whole plant samples were taken late in the 1990 season (at head filling) from farmers' paddocks and from field trials, where white-heads or patchiness typical of take-all was clearly visible in cereal crops. Isolates of the take-all fungus were assessed for Mn oxidation capacity.

The field samples discussed in this chapter were taken to investigate further the findings of Buchhorn and Graham (1992) and determine whether the Ggt isolates from plant roots inside take-all patches were more likely to oxidise Mn than Ggt isolates from plant roots outside the take-all patches.

7.3 Method

7.3.1 Sites

Whole plant samples were taken from various take-all prone sites in South Australia during October, 1990 (Fig. 7.1). Plants were taken from take-all patches (TAp) and from adjacent, 'ordinary' areas (Ord) of the crop, which showed no disease symptoms. The Ord samples were made from plants growing within 3 m of the edge of the take-all patch from which the TAp samples had been taken.

The details of sites are as follows:

Palmer. Oct 4. Farmer's paddock, 5 km east of Palmer township; soil - alkaline, red-brown earth. Wheat (cultivars Spear and Machete) had been sown by the farmer in the same paddock and both cultivars were affected by take-all. Spear and Machete are both considered to be only moderately Mn efficient wheats (R. D. Graham pers. comm.).

Karoonda. Oct 28. Farmer's property; soil - alkaline red Mallee sand.

Lameroo. Oct 30. Farmer's property (Mr Wayne Hayward); soil - alkaline sand over clay. Whiteheads found consistently in wheel tracks from farm machinery.

Beer. Oct 22. Farmer's paddock, 20 km NE of Streaky Bay township; soil - fine calcareous soil. One corner of the paddock had been devastated by take-all disease and was almost completely bare.

Forrest. Oct 22. Farmer's paddock within the Hundred of Forrest, 10 km N of Streaky Bay township; soil - red-brown earth. Take-all patches found throughout the crop.

Johnson. Oct 22. Farmer's paddock, 15 km NE of Streaky Bay township; soil - fine calcareous soil.

Mudamuckla. Oct 23. Field trial of cereal varieties; soil - fine calcareous sand. Whiteheads were found consistently through the entire cereal varieties trial. Samples were taken of Schomburgk and Kulin wheat plants. Kulin is considered Mn efficient, Schomburgk moderately efficient (R. D. Graham pers. comm.).

Piednippie. Oct 24. Farmer's paddock on the Streaky Bay - Piednippie road, 22 km E of Streaky Bay township; soil - red-brown earth. Whiteheads found in irregular patches in the crop.

Sceale. Oct 24. Farmer's paddock on the edge of the Sceale Bay township; soil - white sand. Boron toxicity symptoms (brown speckling of leaf blade) seen in plant leaves (Cartwright et al. 1984).

Sceale E. Oct 24. Farmer's paddock, 5 km east of Sceale Bay township; soil - white sand. Boron toxicity symptoms in the leaves, as in samples from the Sceale site.

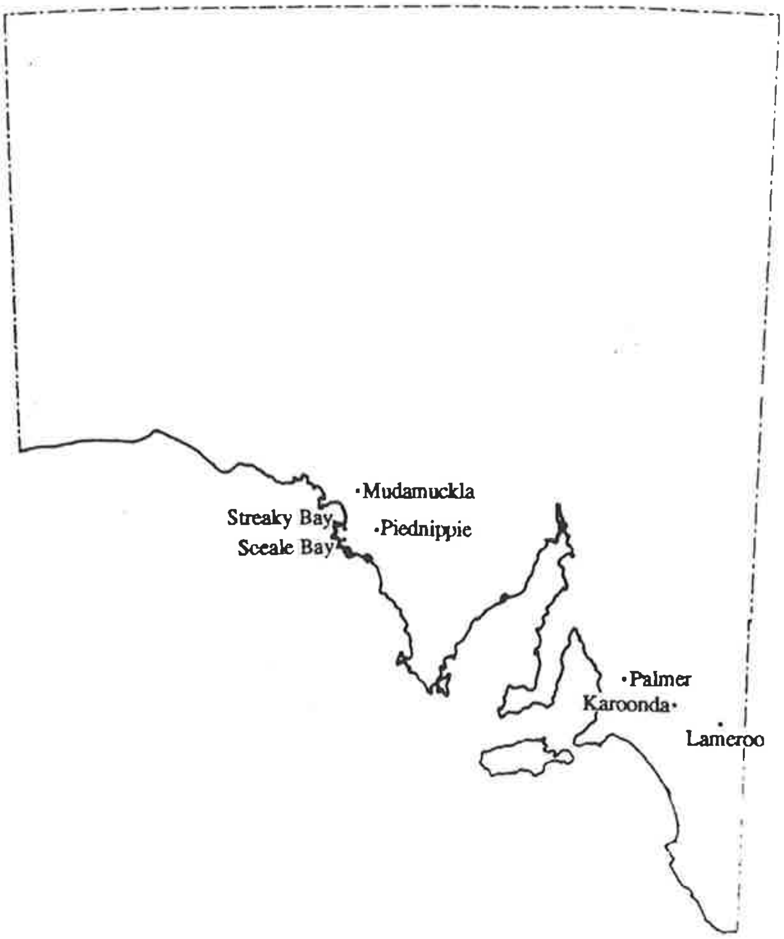

Fig. 7.1 Map of South Australia, showing towns adjacent to sites where whole plants were sampled from cereal crops infested with take-all disease. Site details are described in the text.

7.3.2 Procedure

Upon sampling, plants were placed in plastic bags with great care that shoot material should not come into contact with soil from the roots. In the laboratory, tops were separated from the roots, dried and weighed. The dry tops consisted of stems, upper leaves, flags and maturing heads. Lower leaves were removed, often being contaminated with soil. Samples differed in variety and maturity and some samples were made up of several plants. No effort was made to standardise the number of plants per sample or the maturity of the plants in the sample. The whole samples were ground, in a hammer mill, and elemental analysis carried out on 1.0 g sub-samples by ICP spectrometry.

Roots were washed free of soil and assessed for take-all disease (percent infected roots) and presence or absence of *Heterodera avenae* or cereal cyst nematode (CCN), evidenced by cysts and 'knotted' roots. Seminal and nodal roots which had clearly visible black stelar lesions were scored as infected with take-all (even if Ggt was not isolated from the roots) and the percentage of all roots infected (PIR) calculated as an average of all the plants in the sample. Root systems were often damaged and broken during removal from the soil, so PIR was only an indicative scoring of infection.

Roots were then surface sterilised briefly in 5% sodium hypochlorite and cut roughly into 5 cm lengths which were placed on weak (4%)potato dextrose agar (PDA) plates. PDA plates were incubated at 20°C for 48 hours, and fungal growth from the roots assessed. Growth was often so vigorous that fungal colonies had merged. Isolated fungi were subcultured onto PDA plates and identified.

Ggt isolates were subcultured onto PDA and Mn PDA (4% PDA media containing approximately 30 mg / kg Mn, added as 50 ml of 10 mM $MnSO_4$ in 1 L of agar), and grown for five days to assess growth vigour (measured as radial growth of the hyphae) and Mn oxidising capacity (scored on a scale of 1 to 5; 1 - no oxidation, 5 - formation of dark brown (Mn) oxide throughout the fungal hyphal growth). Four isolates used in the work presented in Chapter 6 (Ggt 500, 8, 43 and 162) were simultaneously grown on similar plates and also scored for Mn oxidation capacity. Ggt 500 was scored as 5, while Ggt 162 was scored as 1.

Samples from the Palmer site were replicated three times, and provided more information about plant genotype response to take-all disease. It was decided to analyse those samples separately from the other sites, and more strictly. Treating cultivar as the main plot effect and patch type (TAp or Ord) as the split plot, analysis of variance of sample dry weights and the results of the elemental analysis of plant tops was carried out.

Samples from the remaining sites were treated as replicates (*ie* a TAp and an Ord sample from each site made up a replicate) and not differentiated by cultivar or species. The Karoonda samples were treated as if from two sites, and the single Johnson (TAp only) sample was removed from the data set.

7.4 Results

A negative correlation between the Mn concentration of plant tops and the severity of take-all disease (as PIR) in the corresponding root systems was found (Fig. 7.2). Mn oxidising capacity of Ggt fungi isolated from the root systems appeared to be associated with both disease severity and with Mn concentrations in the plant tops (Table 7.2).

7.4.1 Roots and Fungal Isolates

None of the plants sampled escaped root disease (Table 7.1). Ggt was isolated from most of the roots taken from take-all patches, but the surface sterilisation may have reduced the viability of some hyphae. Evidence of cereal cyst nematode (CCN) was frequently seen on roots. Several common root pathogens beside Ggt were isolated from the roots; *Rhizoctonia solani, Bipolaris* sp. and *Fusarium graminiarum.* Also isolated were *Alternaria* sp. and *Trichoderma* sp. Nematodes were seen in the fungal isolates, as well as a nematode-catching fungus (not identified), both in colonies from the Palmer samples.

Growth of Ggt isolates on agar was generally not affected by the presence of Mn in agar. Growth of some isolates, all strong Mn oxidisers, was slightly promoted on the Mn-PDA (data not shown). Ggt was not isolated from all the infected roots and was more successfully isolated from TAp plants than ORd plants (Tables 7.1, 7.2).

Table 7.1 Root disease in cereal plants sampled from take-all infested paddocks at various sites in South Australia. Where known, the cereal cultivars are named. Disease status of the whole plant samples is shown as TAp (from a take-all or whitehead patch) or Ord (from an adjacent, symptom-free, 'ordinary' area in the crop). Fungi isolated from the roots are shown as: Gg- *Gaeumannomyces graminis*, Fg- *Fusarium* sp, Rs-*Rhizoctonia solani*, Bp-*Bipolaris* sp, Alt -*Alternaria* sp., Tr -*Trichoderma* sp., CCN - presence of CCN cysts on roots.

		Root Disease	
Site	Cultivar	Ord	TAp
Palmer	Spear	Gg, Alt, Fg	Gg, Tr, Rs, CCN
		Gg, Rs,Tr	Gg, Tr, Fg
		Gg, CCN	Gg, Fg, CCN
Palmer	Machete	Gg, Alt, CCN	Fg, Gg, CCN
		Gg, CCN	Fg, Gg, CCN
		Fg	Fg
Karoonda	Schooner	Bp, Gg, Tr, CCN	Gg, Fg, Tr, CCN
		Gg, Fg, Rs	Gg, Fg, Alt
Lameroo	Spear	Tr	Gg, Tr
Mudamuckla	Schomburgk	Fg, Gg	no sample
	Kulin	Gg	
Beer	Machete	Gg, Fg, CCN	
			Gg, CCN
Forrest	Machete	Gg, Fg, CCN	Gg, Fg, Bp, CCN
Johnson	Machete	no sample	Gg, Fg, CCN
Piednippie	Spear	Fg, Gg, CCN	Fg, Gg, Bp, CCN
Sceale	Barley	Gg, Tr, CCN	Fg, Gg, Bp
Sceale E	Schooner	Fg, Gg, CCN	Fg, Gg, Tr, CCN

Table 7.2 Take-all disease of of cereal plants, sampled from take-all infested paddocks at various sites in South Australia., measured as PIR (percent roots with black stelar lesions). Mn oxidising ability of Ggt isolates from the plant roots, and Mn concentration of plant tops (mg Mn / kg shoot dry weight). Disease status of the whole plant samples is shown as TAp (from a take-all or whitehead patch) or Ord (from an adjacent, symptom-free, 'ordinary' area in the crop). Mn oxidising ability of Ggt isolates from the plant sample roots was scored 1 to 5 [a]. A dash (-) indicates no Ggt isolate.

	Ord			TAp		
Site	PIR	Mn ox	Mn (mg / kg)	PIR	Mn ox	Mn (mg / kg)
Palmer Spear	10	1	31.7	75	5	34.8
	20	-	41.4	75	5	33.4
	60	4	43.4	80	4	24.9
Palmer Machete	10	4	46.9	75	1	16.2
	10	-	51.5	90	5	16.5
	10	-	47.0	75	-	21.7
Karoonda	10	4	5.0	50	1	5.8
	10	3	11.3	40	1	16.5
Lameroo	50	-	37.4	75	1	9.0
Mudamuckla Sch	60	1	11.0	no plant sample		
Kulin	10	1	48.1			
Beer	80	1	11.3	90	3	57.0
Forrest	10	3	10.9	40	1	46.5
Johnson	no plant sample			100	3	57.0
Piednippie	30	1	26.4	50	3	24.6
Sceale	15	1	34.7	70	1	7.2
Sceale E	10	1	15.9	60	5	10.8
mean	25.3	2.1	29.6	69.7	2.8	25.5
s.e.	5.9	0.4	4.1	4.7	0.5	4.4

[a] 1 - no oxidation, 5 - formation of dark brown (Mn) oxide throughout the fungal growth (1- white or grey hyphae, 2- brown oxide in the central, oldest hyphae, 3 - pale brown oxide uniform, 4- concentric rings or patches of dark brown oxide and 5 - dark brown oxide uniform).

Isolates of Ggt from the TAp samples often had higher Mn oxidation scores than Ggt isolates from Ord samples (Table 7.2). This was not consistent across the samples or sites, and some of the strongest Mn oxidisers were isolated from Ord sample plants. However, plants infected by strong Mn oxidising isolates of Ggt, from either TAp or Ord sample plants, tended to have lower Mn concentrations in shoot tissue, while plants infected by Ggt isolates that were weak (low scoring) Mn oxidisers had higher Mn concentrations (Tables 7.2, 7.3).

7.4.2 Palmer Site samples

Plants from the TAp samples had higher PIR than from the Ord samples but differences between the cultivars in PIR were small.

There was a strong interaction of patch and cultivar affecting Mn concentration in the plant tops, Spear wheat having a significantly lower level of disease (measured as PIR) than Machete wheat. Additionally, patch had a significant effect on Mn concentration with the Ord samples having higher Mn concentrations in the tops than in the TAp samples. Spear wheat tended to have slightly higher concentrations of nutrients in plant tops than Machete wheat, in both TAp and Ord samples. In particular, Spear had higher concentrations of Mn in plant tops than Machete.

Boron (B) and Copper (Cu) concentrations in the plant tops were significantly reduced in TAp plant samples, with B concentration showing a trend of interaction between the patch and cultivar effects. Sulphur (S) concentrations in plant tops were affected by cultivar difference, being higher in Spear than Machete, and strongly reduced in the TAp samples, but there was no significant interaction of cultivar and patch effects. Magnesium and molybdenum concentrations in plant tops were both significantly decreased in the TAp plants, while nickel (Ni) concentration was affected by cultivar, being slightly higher in Machete than Spear wheat. There was no significant effect of cultivar or patch on the concentrations of Fe, Co, Zn, Ca, Na, K or P in the plant tops of samples from the Palmer site.

Correlations were calculated for the concentrations and the contents of Fe, Mn, B, Cu, Ni, Zn, Ca, P, and S in the plant tops with the percentage of infected roots (PIR) from

Table 7.3 Dry weights (mg) and elemental concentrations (mg / kg dry weight) of wheat plant tops of samples taken at Palmer, South Australia, October 1990. Wheat cultivars, Machete and Spear, were sampled from the same site. Disease status of the whole plant samples is shown as TAp (from a take-all or whitehead patch) or Ord (from an adjacent, symptom-free, 'ordinary' area in the crop). Values are the mean of three replicates and LSD values are given.

	Dry weight	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Machete	498	58.2	33.3	10.1	4.0	0.04	0.04	0.86	9.4	1274	1142	120	18179	1934	1393
Spear	461	63.4	34.9	14.8	5.2	0.11	0.05	0.75	10.9	1582	1252	458	19383	2014	1529
LSD		5.0	1.4	1.3	0.4	0.04	0.04	0.06	1.0	143	36	90	588	110	40
TAp	413	53.8	24.6	8.4	3.5	0.03	0.06	0.75	9.2	1557	966	392	18407	1810	1321
Ord	546	67.8	43.6	16.5	5.7	0.12	0.03	0.85	11.1	1299	1428	186	19155	2137	1601
LSD		7.3	3.4	1.6	0.6	0.02	0.04	0.17	1.2	170	134	189	535	131	105
TAp/Machete	421	50.7	18.1	8.2	3.1	0.02	0.07	0.83	8.3	1298	819	141	17278	1841	1319
TAp /Spear	405	57.0	31.1	8.6	4.0	0.05	0.06	0.67	10.1	1817	1114	644	19535	1780	1323
Ord/Machete	575	65.8	48.4	11.9	5.0	0.06	0.01	0.89	10.5	1249	1466	100	19079	2028	1466
Ord /Spear	517	69.9	38.8	21.0	6.4	0.18	0.04	0.82	11.7	1348	1390	272	19230	2247	1736
LSD		8.9	3.7	2.1	0.7	0.05	0.06	0.17	1.5	222	139	209	795	171	105

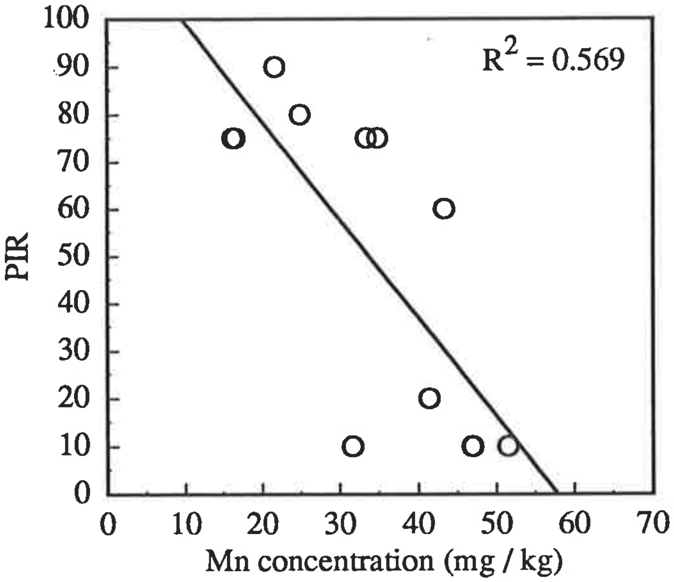

Fig. 7.2 Significant, negative correlation of percent of infected roots (PIR) of plants sampled from the Palmer site with the Mn concentration of the corresponding plant tops (mg Mn / kg dry weight). Plants were two wheat cultivars, sampled from both inside take-all patches and from the ordinary crop.

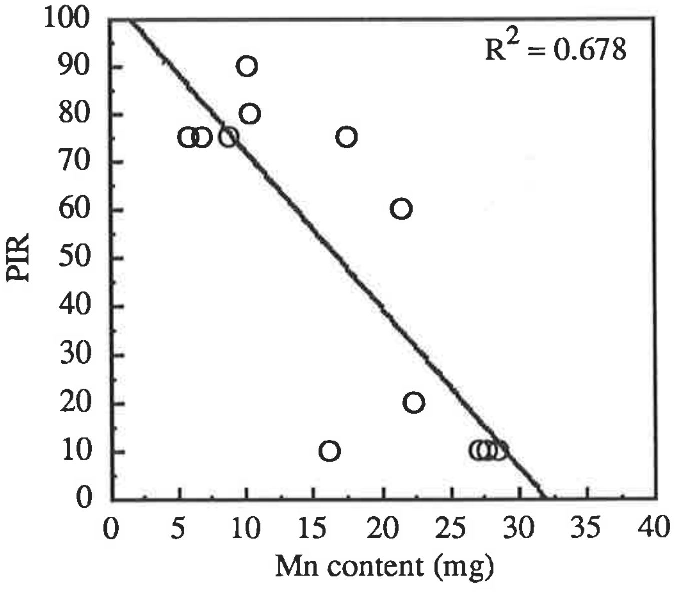

Fig. 7.3 Significant, negative correlation of percent of infected roots (PIR) of plants sampled from the Palmer site with the Mn content of the corresponding plant tops (mg Mn). Plants were two wheat cultivars, sampled from both inside take-all patches and from the ordinary crop.

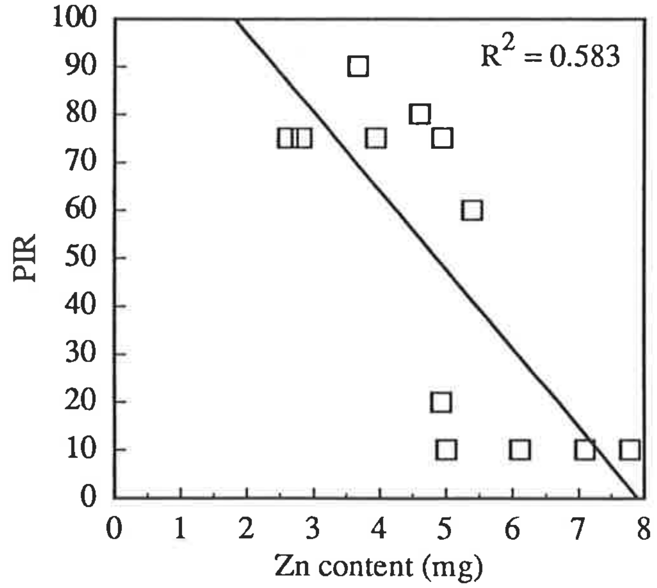

Fig. 7.4 Significant, negative correlation of percent of infected roots (PIR) of plants samples from the Palmer site with the Zn content of the corresponding plant tops (mg Zn). Plants were two wheat cultivars, sampled from both inside take-all patches and from the ordinary crop.

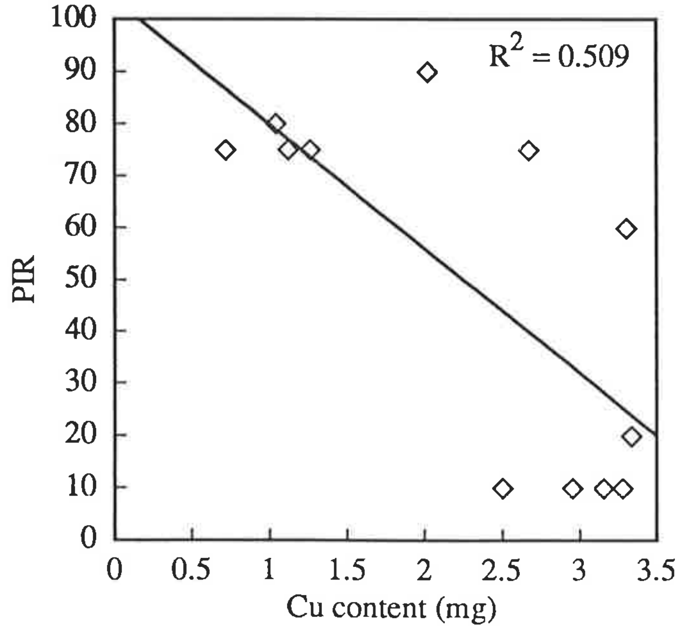

Fig. 7.5 Significant, negative correlation of percent of infected roots (PIR) of plants samples from the Palmer site with the Cu content of the corresponding plant tops (mg Cu). Plants were two wheat cultivars, sampled from both inside take-all patches and from the ordinary crop.

the matching root sample. Content of these elements in the plant tops was calculated as a product of dry weight and concentration. Using data from the Palmer site alone, a significant correlation for PIR with Mn concentrations ($R^2 = 57$) (Fig. 7.2), and for PIR with content of Mn ($R^2 = 0.68$) (Fig. 7.3), Zn ($R^2 = 0.58$) (Fig. 7.4) and Cu ($R^2 = 0.51$) (Fig. 7.5) in the corresponding plant tops.

Large differences in oxidation power between Ggt isolates from TAp and Ord plants were not found. Strong Mn oxidising isolates were taken from both TAp and Ord plant roots, although fewer Ggt isolates were obtained from Ord plant roots (Table 7.2).

7.4.4 Other Sites

Analysis of variance of the results showed no significant differences obtained between sites, and no significant interaction between any element and patch type (TAp or Ord) for these sites (Table 7.4). The clearest trends of interaction between patch and the nutrition of the plant top samples were for S and Mn concentrations, which decreased with increased PIR measures.

No correlation of PIR with concentration or content of Fe, Ni, Zn, Ca, P, and S in the plant tops was obtained. Mn, B and Cu concentrations and contents showed trends of negative interaction with increasing root infection (PIR).

The trends in the Mn concentrations in plant tops from these sites were similar, but not as consistent as those from the Palmer samples. One pair of samples from Karoonda had the expected higher level of Mn in the Ord than the TAp sample, while the second pair of samples had the reverse (Table 7.2). Samples from the sites Beer and Forrest also showed higher levels of Mn concentration in plant tops from TAp plants than from Ord plants.

The strength of Mn oxidising ability of the Ggt isolates taken from the plants from these sites was not clearly linked to TAp or Ord samples. However, the stronger oxidisers were isolated from plants which had relatively low levels of Mn in their tops (Table 7.2). Mn oxidising Ggt isolates were isolated from the roots of the Ord samples from Beer, Forrest and the second Karoonda pair, but not from the TAp samples at these sites. The corresponding tops of these samples had lower levels of Mn in the Ord samples than in the

Table 7.4 Dry weights (mg) and elemental concentrations (mg / kg dry weight) of wheat plant tops of samples taken from field sites in South Australia, other than Palmer, October 1990. Disease status of the whole plant samples is shown as TAp (from a take-all or whitehead patch) or Ord (from an adjacent, symptom-free, 'ordinary' area in the crop). Values are the mean of nine replicates, standard errors are given.

	Dry weight	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
TAp	1029	20.9	22.1	10.7	3.5	0.46	0.11	0.35	9.7	2190	1468	1077	12863	1647	1425
s.e.	13	3.5	6.9	1.6	0.5	0.10	0.04	0.11	1.3	386	124	320	1279	265	150
Ord	1057	27.2	21.3	8.8	3.1	0.70	0.05	0.23	9.6	1477	1314	714	10319	2021	1410
s.e.	11	5.1	4.6	2.5	0.4	0.14	0.01	0.05	1.3	299	88	277	1262	320	115

TAp samples. The Mn oxidising fungi in the roots might have affected the level of Mn available to the shoots and to the plant.

7.5 Discussion

Isolates of strong Mn oxidising ability were not consistently isolated from TAp plants, but the stronger Mn oxidising isolates were generally from the TAp samples, and roots with high PIR. However in some cases, notably the Beer and Forrest sites, this was reversed, and although the PIR was high in the TAp roots, the Mn oxidising fungi came from the Ord roots, and the Ord plant tops had lower Mn levels than the TAp tops.

There was a trend for the stronger Mn oxidising isolates of Ggt to be taken from plants with lower levels of Mn in plant tops, whether the plants came from take-all patches or not. Mn deficiency in crop plants grown in take-all patches has been previously recorded (Huber and McCay-Buis 1993). The depletion of plant available Mn by the oxidising fungus may cause this lowering of Mn in the plant tops, while also rendering the plants more susceptible to diseases.

It is possible that the isolates from the plant samples at these sites oxidised Mn best at temperatures other than 20°C as used in the laboratory. Rosemann et al. (1991) showed that Mn oxidation by Ggt isolates (in vitro) is subject to differences in temperature. Expression of take-all disease has been shown to be affected by temperature (Cook and Rovira 1976, Smiley et al. 1986). Alternatively, other microorganisms in the rhizosphere of the sample plants may have affected Mn availalbility to the plant roots, as well as the Ggt fungi. It must also be remembered that, since specific isolation media were not used, some *Gaeumannomyces* may have escaped isolation from the sampled plant roots.

The correlations of high disease levels (PIR) with low plant Mn concentrations is the most impressive in these field samples, particularly those from the Palmer site. The most significant differences in plant nutrition between TAp and Ord samples were shown by Mn, B, Cu and S concentrations in plant tops. Levels of these elements were consistently lower in plants from take-all patches, where root infection was higher. In addition, Zn content in plant tops was negatively correlated with root infection by Ggt. Disruption of the flow of nutrients occurs in plant roots infected with Ggt (Clarkson et al. 1975), but the effect of disease on specific nutrients is of particular interest. Previous workers have shown the importance of adequate Mn (Wilhelm et al. 1988, Huber and McCay-Buis 1993), Zn (Brennan 1992), Cu (Gardner and Flynn 1988, Brennan 1991) and S (Huber 1989) nutrition in reducing take-all in the field.

Additionally, in the Palmer samples, there was a significant interaction of patch and cultivar on Mn concentration in plant tops. Machete and Spear are both considered wheats of only moderate Mn efficiency. The middle order ranking of Mn efficiency in field and pot trials varies between experiments and is not entirely consistent from year to year in the field. However, in these Palmer samples, Spear not only had better overall nutrition (including Mn) than Machete but also suffered less take-all than Machete. It might be hypothesised that, under the conditions at Palmer, Spear was more nutrient efficient (specifically Mn efficient) than Machete and thus more tolerant of take-all disease.

The concept of breeding wheats for resistance to take-all is not regarded as worthwhile by some workers (Scott and Hollins 1985), since, in many of the screening trials carried out over the last fifty years, the repeatability of a genotype's resistance, or even consistent tolerance, is low. However, examination of amphiploid and chromosome substitution lines in winter wheats suggests some possibilities for resistance to take-all do exist (Conner et al. 1988). Additionally, Wilson et al. (1988) stated that repeatable differences between wheat cultivars exist, although they are very small,the cultivar Spear performing better than Condor and Bayonet, for three years out of four, in take-all trials. (NB Condor and Bayonet have extremely low Mn efficiency.) The screening trials mentioned previously have given little importance to the nutrient requirements and effects of the take-all fungus, Ggt, which may have a greater bearing on the 'variability' of the disease than previously thought.

A further piece of evidence in support of this hypothesis comes from observation of barley breeding trials at Wagga in N.S.W. (Read 1993). Although the take-all fungus is present in the soils around Wagga, the expression of the disease is not common. The soils are of slightly acidic pH, and not regarded as Mn deficient. Furthermore, barleys are both less susceptible to take-all and more Mn efficient than wheats. Yet, in the 1991 season, plots of the barley cultivar Skiff and breeding lines with Skiff parentage were decimated by take-all, while other cultivars and breeding lines grown in adjacent plots were much less affected by take-all. The cultivar Skiff is one of the least Mn efficient barley cultivars (Sparrow et al. 1983). Other parental cultivars in the breeding trials were Schooner, O'Connor and Forrest, which are more Mn efficient than Skiff. Schooner, related to Skiff but comparatively Mn efficient, suffered significantly less take-all disease.

Seasonal conditions (cool, but dry) which increase the likelihood of take-all appeared to have affected the barley genotypes in a way which differentiated between genotypes tolerant and intolerant of Mn deficiency. Increased oxidation of plant available Mn in the rhizosphere by microorganisms, including Ggt, would have lowered Mn availability, with the most Mn efficient barley lines suffering the least from this lack. Inoculum potential at the field site was probably fairly low, since the previous crop was not a take-all susceptible cereal.

The Mn efficient lines of barley effectively showed field resistance to take-all disease. Since, with its saprophytic habit and large host range, the Ggt fungus is permanently present in agricultural soil (Cotterill and Sivasithamparam 1989 a, Harvey 1994), seasonal effects which decrease plant Mn availability (and increase the likelihood of take-all) have a great effect upon the severity of take-all disease expression (Huber and Wilhelm 1988). The use of Mn efficient cereal genotypes, field resistant to take-all, could reduce the disastrous effect on cereal crops of seasons where weather conditions increase the likelihood of take-all disease, particularly in regions where soil Mn status is generally low.

7.6 Conclusion

These results are of value as a illustration in the field of results which have been seen in laboratory work and in previous field experiments. In a disease with such vagaries as take-all, samples taken from widespread sites and in a simple manner, show clearly the interaction of plant Mn nutrition with the take-all disease and its causative fungus, Ggt.

7.7 References

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Chapter 8

General discussion and conclusions.

8.1 Discussion

Reduction of take-all disease has been by methods of agronomic management and utilising disease escape. Management of soil pH and nutrition, crop rotation and use of break crops, the use of biological control agents, reduce the conditions in which the likelihood of take-all is increased and reduce the levels of Ggt inoculum in the soil. Reduction of inoculum levels generally increases the chance of disease escape (Yarham 1981). Disease escape can also occur through the use of genotypes which (although susceptible to disease) may not be infected because of their growth patterns. Late sowing of cereal crops, or use of early maturing varieties is likely to reduce the infection of seedlings plants by Ggt inoculum during warm autumn or cool spring weather (Garrett 1948). Escape of take-all has been shown in the late sowing of wheat in Indiana (Huber 1981).

The conetainer method used in experiments described in Chapters 2 and 4 does not permit roots to escape contact with the inoculum, thus any escape from infection is attributed to the resistance of the plant roots. None of the genotypes used showed consistent absolute resistance to Ggt infection. However, aspects of a genotype may meld to create general or field resistance: a useful tolerance of moderate levels of a pathogen which may involve many traits of the plants' genotype, rather than a specific resistance gene.

There is evidence in the literature (albeit often indirect) that general resistance to take-all disease is expressed in Mn efficient cereal genotypes (Wilson et al. 1988, Huber 1989, Wilhelm et al. 1990, Read 1993, this thesis).

The thrust of the work in this thesis has been to determine the extent and means by which genetic Mn efficiency reduces the susceptibility of wheat seedlings to the disease take-all. In the main, the original hypotheses have been supported, showing that Mn efficiency in wheat increases tolerance of take-all, and confers a useful form of resistance to take-all.

1) Mn efficient wheat genotypes are resistant to infection by the Ggt fungus.

Mn efficient wheats in Experiments 2.1, 2.2 and 4 showed less infection of roots by Ggt inoculum than inefficient wheat genotypes (in a system which did not permit roots to escape from contact with inoculum), under Mn deficient and Mn adequate conditions. Mn efficient wheats showed levels of infection which appeared to be independent of soil Mn treatment, similar infection levels in Mn deficient and Mn adequate treatments. The Mn inefficient wheats tended to show larger differences in infection levels; higher infection levels under Mn deficient conditions and lower infection under Mn adequate conditions. These results support those of Wilhelm et al. (1990).

The availability of Mn to plants from seed source nutrient has an effect on tolerance to Mn deficient conditions (Marcar and Graham 1986, Longnecker et al. 1991), and thus an effect on plant susceptibility to Ggt infection (Rosemann and Huber 1990). Seedling wheat plants with high seed source Mn did not show Mn deficiency symptoms when grown in Mn deficient soil. However, seed source nutrient effects did not entirely hide differences between the Mn efficiency of genotypes. Although high seed source Mn did decrease the Ggt infection of low Mn efficiency plants, low seed source plants of an efficient genotype (C8MM) showed significantly and consistently lower infection levels than inefficient genotypes.

From these experiments it was concluded that Mn efficiency increases the tolerance of take-all in wheat, and probably confers field resistance to the disease, at least in seedling plants.

Mn efficient genotypes are more tolerant of Mn deficiency, and at the level of requirement, pathways requiring the presence of Mn are less likely to be inhibited for want of the micro-element. The work by Wilhelm (1992) showed that the likely form of Mn involvement in tolerance was in the importance of Mn in lignin formation in roots.

2) Mn efficient genotypes have constitutively better lignin production under Mn stress, and under disease stress.

The production of lignin and phenolics plays a part in the resistance of cereals to Ggt infection. Poor production of lignin has been shown in Mn deficient wheat plants (Brown et al. 1984, Wilhelm 1992, Chapter 5). Some evidence was produced that Mn efficient wheat genotypes constitutively produced more lignin. Rengel et al. (1993) showed that under Mn deficient conditions, at sites of Ggt infection, the production of lignin is more stable in Mn efficient wheat genotypes than inefficient genotypes.

The accumulation of phenolics in Mn deficient wheat plants points to a limiting factor of Mn-dependent enzymes in the shikimate pathway. Mn-activated enzymes such as PAL and Mn-peroxidase are important in the formation of lignin precursor molecules and in the polymerisation of the precursors in the formation of the lignin molecule (Burnell 1988). Mn deficiency inhibits the action of these enzymes and decreases the rate of lignin production (Engelsema 1972, Halliwell 1978).

3) Action of the Ggt fungus may affect plant available Mn.

The availability of Mn to both plant host and Ggt fungus (in soil) has an effect upon the expression of take-all disease. Mn deficient conditions are known to increase Ggt infection in plant roots (Reis 1982, Graham and Rovira 1984, Wilhelm et al. 1988). Extreme Mn deficiency in soil was also shown to affect the saprophytic growth of Ggt (isolate 500), and through this effect, possibly its virulence as well.

However, it has also been shown that the Mn oxidation ability of Ggt isolates is positively correlated with the virulence of those isolates (Rosemann et al. 1991, Buchhorn and Graham 1992, this thesis).

Oxidation of Mn by Ggt isolates may be sufficient to cause localised deficiency of Mn in rhizosphere, affecting the availability of Mn to host plant and perhaps even leading to (subclinical) Mn deficiency in the plant (Chapter 6, Chapter 7). Ggt attacked plants have lower levels of Mn content in shoots, in both field and in pot experiments (Chapter 7, Chapter 4). Subclinical Mn deficiency has been shown to affect the process of lignification in wheat roots (Webb and Dell 1990). Mn deficiency in the rhizosphere, caused by Ggt, may be sufficient to affect the lignification response to fungal infection. Specifically, the cell-wall bound Mn-peroxidase, important in the deposition of lignin, may be deprived of Mn and inhibit plant response to infection. Constitutive lignification and the lignification response to root infection by Ggt in Mn deficient wheat plants is poor (Brown et al 1984, Wilhelm 1992, Rengel et al. 1993, Chapter 5). Susceptibility to infection by Ggt is thus greatest in hosts with less tolerance of Mn deficient conditions: plants of low Mn efficiency. Similarity in the rankings of cereals for Mn efficiency, and for resistance to take-all infection was noted in Chapter 1 (section 1.5.4, Table 1.3).

It is suggested that the reason strong resistance to take-all disease does not exist in cereals is that the disease was not a strong selection pressure in the evolution of cereals, being subject to natural biological controls (Scott 1981, Hornby and Bateman 1991). If a pathogenic action of Ggt does result in localised Mn deficiency in the rhizosphere of host plants, this is a selection pressure which is unlikely to engender strong genetic resistance in plants. The resistance to take-all in oats (through the production of avenacins) is of interest, since oats are among the least Mn efficient cereals, and thus more susceptible to Mn deficency in the rhizosphere.

Whilst determination of the mechanism of Mn efficiency in cereals is still to come, it is easily seen that the Mn efficient genotypes are more likely to withstand any localised deficiency caused by the Ggt fungus. Genes which may affect Mn efficiency may also vary the effectiveness of resistance to the localised Mn deficiency and thus to Ggt infection. However, variation in the virulence and Mn oxidising ability of the Ggt fungus will also affect infection of plant roots.

Genetic study of isolates of *Gaeumannomyces graminis* fungi has shown great variability within the species and its varieties (Harvey 1994), with populations of Ggt isolates showing the greatest genetic diversity. Although inter-population and inter-varietal gene transfer appears to be rare, *Gaeumannomyces graminis* has an ability to maintain this large genetic variability, which would permit swift adaptation to new conditions by the fungus. Harvey (1994) also suggests that host-mediated selection greatly affects genetic drift within the fungal populations. Host-specific adaptations of *Gaeumannomyces graminis* isolates have been suggested previously (Dewan and Sivasithamparam 1990). Host-specific adaptation of *Gaeumannomyces graminis* populations to wheat cropping may affect the process of take-all decline. Attenuation of pathogenicity (and Mn oxidation ability) in cultured isolates of Ggt may indicate a lack of host-mediated selection, whilst conservation of pathogenicity occurs in the field.

The Mn oxidation ability of a Ggt isolate is linked with the isolate's pathogenicity (Buchhorn and Graham 1992, this thesis). The resistance to Ggt infection by plant roots in linked with the Mn efficiency of the plant host (Wilhelm et al. 1990, this thesis). Wheat genotypes of great Mn efficiency should thus be useful as take-all tolerant cultivars. However, the highly variable nature of the *Gaeumannomyces* populations allows for great variability, and advantage in the competition for available Mn between Mn oxidising Ggt and Mn efficient cereals is likely to lie with the fungus. The possibility that the fungus uses or creates Mn deficiency in the rhizosphere as a unique pathogenic action against plant host is surely only rivalled by those pathogenic soil bacteria which utilise siderophores to induce Fe deficiency in their competitors. Use of biological control agents, such as rhizobacteria which reduce Mn oxides, may decrease the effect of strong Mn oxidation by Ggt fungi (Marschner et al. 1991, Huber and McCay-Buis 1993). Would extensive use of Mn efficient cultivars cause evolutionary pressure to increase the Mn oxidation power of the Ggt fungus ?

8.2 Conclusion

The use of Mn efficient cereals in combating take-all will not be the whole means of reducing the impact of this disease upon crop yields, but may play a major part in creating a better system for decreasing the effects of take-all disease.

Usefulness of Mn efficiency as a tool for breeding resistance to take-all is dependent upon the range of Mn efficiency traits available to wheat breeders, and upon the flexible nature of the Ggt fungus. Additionally, the maintenance of overall soil nutrition, and the use of break crops and biological control agents to reduce inoculum levels are all still a necessary part of management of cereal crops in order to minimise the likelihood of take-all disease. However, with all these strategies in place, the use of Mn efficient wheats (and cereals) can only improve the chances of reduced yield losses due to take-all.

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Appendix 1 Elemental concentrations (mg / kg) of the seed of 22 wheat genotypes, used in Experiment 2.2, described in Chapter 2. Genotypes are listed in alphabetical order, as in Table 2.3.

Genotype	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Aroona	10.9	23.1	4.5	5.3	0.17	0.01	0.01	27.5	475	1256	32	5306	3545	2165
Bayonet	11.2	32.9	6.5	5.3	0.20	0.03	0.92	32.3	453	1596	130	5517	3581	1857
Bindawarra	9.1	33.0	4.2	6.4	0.28	0.14	0.67	34.4	568	1781	112	5948	4854	1975
Blade	7.4	46.6	2.2	4.9	0.04	0.19	0.18	13.9	358	1352	28	3946	2949	1682
Bodallin	35.8	29.1	2.4	4.7	1.71	0.02	0.15	53.6	906	1410	51	5747	3669	1906
C8MM	10.16	38.2	5.2	5.1	0.16	0.22	0.46	29.6	383	1576	41	4688	3652	1906
C8MM*MM*MMC	15.5	24.8	4.1	3.6	0.26	0.07	0.61	22.1	378	1483	64	5174	3221	1740
Cook	14.8	36.8	4.2	4.0	0.28	0.24	0.58	24.9	430	1663	158	4014	3461	1862
Halberd	16.1	23.3	1.1	1.9	0.60	0.34	0.16	17.8	485	1267	130	3432	2649	1407
Kite	12.1	25.2	5.7	4.8	0.24	0.25	0.81	25.7	393	1590	83	4677	3335	1870
Kulin	44.3	32.6	2.0	6.2	1.70	0.04	0.25	74.5	736	1310	72	5811	3773	2276
Machete	8.5	29.6	5.1	4.0	0.24	0.07	0.72	24.4	364	1594	111	4314	3627	1486
Millewa	9.1	32.6	5.2	4.8	0.16	0.11	0.57	28.9	502	1657	129	4801	3752	1676
Molineux	14.52	24.0	1.5	3.3	0.75	0.32	0.47	13.8	526	1341	66	4481	3843	1611

Appendix 1 continued.

Genotype	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Red Fife	60.7	42.4	2.0	6.9	1.20	0.03	0.47	90.7	760	1582	37	4731	4043	2711
Richelle Hative	68.6	40.5	2.9	8.2	2.32	0.01	0.18	91.7	748	1607	35	5599	4591	2761
Schomburgk	12.6	23.1	3.3	4.9	0.09	0.19	0.26	21.9	432	1555	121	5250	3617	1768
Seite Cierros	67.6	39.7	1.8	7.8	1.68	0.02	0.39	91.4	745	1571	33	4804	4625	2319
Songlen	12.0	26.7	4.3	4.0	0.27	0.08	0.51	41.0	396	1740	169	4984	4098	2044
Spear	10.2	38.6	3.5	4.6	0.24	0.30	0.82	41.1	485	1605	132	5311	4123	2061
Tatiara	15.6	24.9	4.1	5.9	0.27	0.04	0.87	26.0	423	1692	95	5520	3148	1683
Warigal	7.6	36.7	1.9	7.7	2.38	0.02	0.21	106.5	770	1505	57	7053	5375	2941
mean	21.6	32.0	3.5	5.2	0.69	0.12	0.47	42.4	533	1533	86	5050	3797	1987
s.e. (±)	4.3	1.5	0.3	0.3	0.17	0.02	0.06	6.1	32	32	10	168	136	86

Appendix 2 Elemental concentrations of seed (mg / kg) used in the experiment described in Chapter 4. Seed of three genotypes of wheat, C8MM, Spear and Bayonet, was obtained from sources differing in Mn availability, as shown in Table 4.1.

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
C8MM Wangary	9.0	3.7	1.5	2.0	0.39	0.33	0.34	17.6	564	1357	159	3506	2498	1497
C8MM Lameroo	10.2	38.2	5.2	5.1	0.16	0.22	0.46	29.6	383	1576	41	4688	3652	1906
Spear Tooligie	12.7	8.6	1.7	3.2	0.77	0.31	0.64	21.3	472	1648	127	4255	3017	1662
Spear Wangary	20.2	3.9	1.5	2.4	0.74	0.30	0.22	23.1	584	1405	162	4311	3123	1761
Spear Lameroo	10.9	37.5	3.5	4.6	0.25	0.21	0.82	41.2	456	1647	133	5375	4332	2043
Bayonet Wangary	23.1	3.2	2.1	2.2	0.35	0.29	0.27	23.2	590	1356	171	4384	3239	1600
Bayonet Lameroo	25.2	33.0	4.9	5.3	0.30	0.13	0.92	30.3	444	1596	130	5517	3581	1857
Bayonet Glasshouse	58.8	25.2	2.2	7.4	2.17	0.00	0.14	69.2	705	1326	47	5467	3586	2457

Appendix 3 Elemental concentrations of shoot tissue from plants grown in the experiment described in Chapter 4. Three genotypes of wheat , C8MM, Spear and Bayonet, were grown under 10 soil Mn treatments (Mn 0 - Mn 9), from four seed sources (WAngary - low Mn, Tooligie - low Mn, Lameroo - high Mn, Glasshouse - high Mn). Only +Ggt treatments are shown here. Values are the mean of three replicates.

C8MM, Wangary-source, +Ggt

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Mn 0	57.80	27.35	16.46	11.43	2.74	0.52	6.08	32.90	6818	1398	358	44787	1508	2426
Mn1	50.78	38.56	17.55	5.52	1.23	0.16	0.81	35.37	6297	1188	426	36712	1529	2141
Mn 2	56.77	39.23	15.33	6.52	1.38	0.17	0.86	32.84	7342	1495	327	44686	1343	2291
Mn 3	51.59	41.83	12.75	4.66	0.93	0.10	0.61	24.02	6036	1300	330	38625	1220	2026
Mn 4	48.61	61.74	17.32	6.64	1.58	0.20	1.62	46.29	6448	1452	416	39276	1223	2107
Mn 5	48.01	134.00	16.58	5.81	1.47	0.20	0.69	45.53	6640	1348	328	35600	1318	2050
Mn 6	56.32	81.76	18.76	6.20	1.17	0.16	0.52	39.33	7264	1321	379	41706	1650	2277
Mn 7	44.57	91.20	16.79	5.81	1.12	0.26	1.55	35.54	6188	1231	323	33437	1432	1922
Mn 8	63.77	128.07	21.30	7.17	2.06	0.24	1.26	50.57	7578	1595	306	40116	1336	2415
Mn 9	60.48	103.12	18.81	6.40	2.11	0.18	1.63	36.50	6802	1682	432	45044	1483	2510

Appendix 3 continued.

C8MM, Lameroo-source, +Ggt

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Mn 0	53.75	28.16	15.20	6.67	2.38	0.23	1.03	44.45	6376	1295	282	40611	1753	2453
Mn1	62.62	42.67	12.37	6.60	1.56	0.18	0.82	55.11	7910	1572	377	46158	1806	2966
Mn 2	53.19	40.96	14.60	6.16	1.80	0.18	0.98	41.29	6638	1292	332	40083	1424	2338
Mn 3	57.05	63.44	20.95	6.52	1.23	0.23	0.78	42.52	7514	1548	335	42978	2246	2571
Mn 4	51.79	77.14	16.02	6.02	0.85	0.16	0.80	41.17	7162	1213	320	39013	1708	2299
Mn 5	59.34	129.87	20.98	7.10	1.49	0.26	0.89	60.65	8223	1494	370	39584	1732	2524
Mn 6	50.66	83.08	20.06	5.51	0.87	0.15	0.34	38.33	6936	1234	293	35651	1673	2293
Mn 7	56.76	112.19	21.49	6.07	1.21	0.06	0.62	51.62	6981	1354	421	38859	1892	2392
Mn 8	60.67	136.40	19.72	6.96	1.76	0.17	0.92	60.69	7855	1531	315	39476	1646	2538
Mn 9	75.20	172.97	18.42	7.52	2.87	0.40	1.13	63.33	7893	1690	285	45398	1883	2927

Appendix 3 continued.

Spear, Tooligie-source, +Ggt

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Mn 0	70.67	5.43	21.00	9.00	10.63	0.94	2.90	47.33	10103	2113	729	63680	3470	3378
Mn1	96.33	13.67	15.63	8.03	5.20	2.22	3.17	51.33	8212	1678	1172	48794	3130	2979
Mn 2	64.67	21.00	12.33	6.23	4.03	0.46	1.61	33.67	7788	1380	393	48054	1935	2840
Mn 3	67.00	38.33	14.13	7.00	3.20	0.27	1.01	36.33	8695	1456	523	55770	2421	3297
Mn 4	67.33	50.00	15.57	5.93	2.70	0.90	1.84	39.50	7703	1010	684	39913	1836	3062
Mn 5	74.00	65.67	19.13	5.30	3.30	0.27	2.40	41.67	7437	891	473	51675	2041	3151
Mn 6	73.33	87.33	15.57	8.70	2.93	0.43	2.68	45.33	8770	1655	472	57434	2833	3591
Mn 7	67.67	149.00	12.60	8.33	3.37	0.64	1.10	47.33	8297	1301	398	52936	1904	3270
Mn 8	65.00	96.33	13.97	5.27	3.20	0.61	1.70	32.00	8349	1533	473	44961	1587	2855
Mn 9	71.33	191.67	13.67	5.10	3.53	1.23	3.65	44.33	7646	1326	462	48905	2143	2898

Appendix 3 continued.

Spear, Wangary-source, +Ggt

	Fe	**Mn**	**B**	**Cu**	**Mo**	**Co**	**Ni**	**Zn**	**Ca**	**Mg**	**Na**	**K**	**P**	**S**
Mn 0	129.67	6.47	39.33	18.53	11.33	10.24	20.50	94.00	11019	2852	890	69296	4813	4266
Mn1	64.67	23.00	12.43	6.97	4.47	0.60	2.13	43.67	8593	1653	761	53244	2181	3198
Mn 2	61.00	22.33	10.43	6.37	3.97	0.43	1.40	36.33	8154	1479	545	46188	2450	2909
Mn 3	75.00	44.67	11.13	9.77	3.57	1.00	3.10	48.33	9847	1729	492	54732	2379	3370
Mn 4	49.33	56.33	17.00	7.87	2.63	1.67	4.17	33.33	7170	1541	609	41235	1894	2479
Mn 5	72.67	76.67	15.83	8.23	2.40	0.66	3.10	35.00	9540	1550	666	54778	2185	3505
Mn 6	76.33	114.67	16.00	10.03	3.07	1.15	2.13	36.00	10456	1809	374	57772	2151	3706
Mn 7	69.33	140.00	17.37	10.30	3.17	1.24	3.27	40.67	9918	1738	535	54846	2305	3657
Mn 8	70.33	145.33	16.37	9.03	3.30	0.99	2.67	45.33	10235	1718	565	54354	2135	3431
Mn 9	64.67	155.33	18.00	7.93	3.17	1.42	2.60	63.33	8710	1556	512	51379	1919	2939

Appendix 3 continued.

Spear, Lameroo-source, +Ggt

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Mn 0	70.67	8.47	21.67	8.97	8.23	1.97	4.20	27.33	7860	1510	646	50763	2277	2595
Mn1	59.67	33.33	19.67	9.17	4.53	2.15	4.87	37.00	8459	1720	839	56795	2176	2937
Mn 2	62.67	58.00	15.33	9.53	3.17	1.33	3.27	36.00	8935	1610	555	51581	2409	3381
Mn 3	74.00	58.67	16.33	9.37	3.00	0.97	4.20	39.33	9513	1682	519	57405	2714	3726
Mn 4	64.33	76.00	15.67	38.07	2.19	2.21	5.17	55.67	8520	1586	536	54943	2526	3257
Mn 5	63.33	93.33	16.33	10.07	3.40	1.14	3.30	48.67	10195	1859	705	51942	2423	3578
Mn 6	78.33	116.67	14.00	12.00	3.67	1.30	3.33	42.67	10190	1903	309	56909	2339	3786
Mn 7	77.33	144.00	14.00	11.00	3.30	1.29	3.20	43.33	10174	1797	418	60116	2573	3794
Mn 8	61.33	142.33	17.33	10.00	2.60	1.82	4.00	36.00	9536	1620	512	44896	1791	2993
Mn 9	75.67	196.33	19.67	10.33	2.87	1.26	4.13	40.33	10348	1974	609	59226	2780	3809

Appendix 3 continued.

Bayonet, Wangary -source, +Ggt

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Mn 0	174.42	6.01	22.79	13.42	8.72	1.93	4.44	50.81	9201	2871	855	65745	4680	4187
Mn1	125.49	15.12	13.96	8.06	4.42	0.14	1.70	33.69	9522	2123	1010	54100	1970	2980
Mn 2	111.52	32.88	12.18	6.83	3.75	0.10	1.80	42.91	8737	1760	705	54213	2171	3100
Mn 3	122.90	48.26	17.24	9.57	3.83	1.21	2.81	41.55	9722	2109	1155	54657	2026	3327
Mn 4	165.02	86.35	22.58	13.04	5.09	1.19	5.05	60.67	11295	2490	885	50996	2895	4196
Mn 5	137.21	95.02	20.82	9.75	1.97	0.16	1.15	49.42	10294	2321	533	63618	2535	3679
Mn 6	133.65	90.82	17.10	7.51	2.57	0.38	2.04	44.82	7473	1606	523	48596	1916	2938
Mn 7	188.40	120.37	17.70	9.04	3.23	0.40	1.35	49.28	8865	1843	422	53711	2269	3349
Mn 8	161.18	107.25	18.27	10.48	3.77	0.32	2.81	48.68	9765	2267	724	59988	2380	3579
Mn 9	191.56	186.19	24.45	10.18	3.77	0.31	0.94	48.64	11019	2186	337	53073	2146	3778

Appendix 3 continued.

Bayonet, Lameroo-source, +Ggt

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Mn 0	130.66	9.65	25.39	6.39	8.20	0.00	6.11	31.58	12722	2103	1695	65013	2573	2735
Mn1	111.21	19.92	20.25	8.45	5.74	0.60	2.41	35.12	10451	2271	1224	60377	2257	3372
Mn 2	100.64	32.16	19.66	5.30	3.50	0.47	1.45	28.81	7911	1920	680	53661	2049	2825
Mn 3	101.11	37.30	16.11	6.58	2.54	0.30	1.18	33.84	8508	1996	779	47982	1839	2502
Mn 4	134.26	51.54	19.83	7.15	2.51	1.44	2.58	33.10	9773	2245	1394	51574	2122	3134
Mn 5	115.07	67.72	15.06	6.77	1.41	0.30	0.97	35.37	9285	1952	666	54065	2199	2937
Mn 6	157.22	77.11	16.37	7.19	2.36	0.09	2.81	34.37	8885	1984	1129	49803	1873	3079
Mn 7	151.75	99.82	18.10	7.26	1.62	0.10	1.61	36.45	8976	1878	469	54340	2165	2991
Mn 8	224.84	161.06	22.59	9.58	3.04	0.26	1.63	50.38	11286	2342	595	59233	2310	3708
Mn 9	234.27	157.55	21.52	7.55	2.55	0.17	1.11	44.32	9537	2245	877	57499	2098	3516

Appendix 3 continued.

Bayonet, Glasshouse source, +Ggt

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Mn 0	127.77	10.13	17.40	9.64	8.21	0.15	2.35	38.26	8753	2054	815	56660	2744	3395
Mn1	112.47	20.23	14.72	9.31	5.58	0.12	1.36	36.92	10199	2076	524	54797	2167	3544
Mn 2	115.80	25.77	17.47	7.86	3.59	1.41	1.04	31.85	9787	2050	739	46955	2046	2934
Mn 3	108.79	42.52	14.04	8.29	3.29	0.05	1.43	37.61	9455	1926	496	47211	1833	3173
Mn 4	123.83	55.56	20.67	9.00	3.02	0.49	1.12	36.31	9860	2080	425	52291	2302	3399
Mn 5	124.37	86.58	19.56	8.24	3.99	0.26	2.69	36.36	9777	2134	551	49452	1984	3119
Mn 6	147.09	98.75	19.54	10.41	3.50	0.46	1.15	46.83	10294	2262	377	57514	2283	3788
Mn 7	209.64	102.13	16.63	11.84	4.95	0.15	1.42	57.25	8559	3631	909	44931	7702	4424
Mn 8	184.45	155.54	19.40	8.92	3.70	0.05	1.62	46.19	9292	2172	368	53848	2338	3617
Mn 9	196.39	181.83	23.69	8.97	3.40	0.37	0.79	44.74	9337	2112	356	52746	1858	3479

Appendix 4 Elemental concentrations of shoots from plants grown in Experiment 5.1. Two wheat genotypes, Aroona and Bayonet, were grown in two soils, Laffer sand and Wangary soil. Four soil Mn treatments were used in Laffer sand, two in Wangary soil. Values are the mean of five replicates. Levels for Mo, Co and Ni were not recorded.

Laffer sand

	Fe	Mn	B	Cu	Zn	Ca	Mg	Na	K	P	S
Aroona											
Mn 4	82.6	79.4	28.9	1.3	40.3	22415	1824	174	46633	2729	3305
Mn 2	78.3	68.3	30.1	9.0	39.6	22485	1842	146	42884	2503	3197
Mn 1	84.2	53.8	28.6	10.6	42.3	22659	1905	169	49495	2631	3429
Mn 0	83.3	13.3	28.1	11.8	39.8	26453	2077	182	56241	3340	3786
Bayonet											
Mn 4	85.1	77.8	24.2	11.2	33.1	18850	1897	162	43863	3022	3469
Mn 2	91.6	69.2	31.1	11.8	31.0	22252	2082	185	50709	3076	3745
Mn 1	87.5	34.9	27.1	11.7	36.8	25483	2078	212	53128	3191	3677
Mn 0	85.6	17.4	29.0	12.1	41.6	20121	2183	233	59907	3283	3894

Appendix 4 continued.

Wangary soil

	Fe	Mn	B	Cu	Zn	Ca	Mg	Na	K	P	S
Aroona											
+Mn	97.9	23.0	24.6	13.3	78.69	11318	2368	465	55607	3268	4156
-Mn	60.2	59.2	13.9	7.6	56.63	6059	1316	280	46898	1990	2651
Bayonet											
+Mn	79.9	35.9	17.6	9.1	51.73	9376	1867	360	66410	2767	3380
-Mn	67.1	66.9	15.1	8.5	50.00	9179	1425	238	45412	1961	2488