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INVESTIGATIONS INTO *Gaeumannomyces graminis* var. *tritici* INFECTION OF MANGANESE-DEFICIENT WHEAT

by

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This thesis is presented for the degree of Doctor of Philosophy of the University of Adelaide.

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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.

NIGEL S. WILHELM

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS AND USAGES

2-79 - *Pseudomonas fluorescens* strain 2-79 (Weller and Cook 1983)

Ggt - *Gaeumannomyces graminis* var. *tritici*

Ggt - Experimental treatment using *Ggt* inoculum

mg kg^{-1} D.W. - nutrient concentration in plant tissue expressed on a dry weight basis

Mn - Manganese

Take-all - Disease caused by infection with *Ggt*

Wangary sand - 50/50 mix of top- and sub-soil of a highly calcareous, Mn-deficient aeolian sand from Wangary, Eyre Peninsula, South Australia . (Uc 1.11, Northcote 1979)

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THESIS SUMMARY

Investigations were conducted into the effect of manganese (Mn) deficiency on *Gaeumannomyces graminis* var. *tritici* (*Ggt*) infection of wheat. These experiments were conducted in the field, in pots and in petri-dishes. This is the first report of an application of Mn decreasing *Ggt* infection of wheat in the field in the Southern Hemisphere. Pot experiments were designed to test three alternative hypotheses previously proposed to explain decreases in *Ggt* infection with the addition of Mn to a Mn-deficient soil. The first of these hypotheses, that Mn was toxic to *Ggt*, was rejected. The second, that Mn caused changes in the rhizosphere microflora through altered wheat host exudates to the detriment of infection by *Ggt* , was also rejected. The third hypothesis, that Mn deficiency inhibited lignin synthesis in the host and thus decreased host resistance, was indirectly supported by many experiments and it is proposed that it is the most likely mechanism by which Mn deficiency increases *Ggt* infection of wheat. Further pot experiments demonstrated that wheat genotypes sensitive to Mn deficiency were also the least resistant to *Ggt* infection. A series of petri-dish experiments demonstrated that wheat root pieces, removed from plants grown without Mn, were invaded faster by *Ggt* on agar plates than root pieces taken from plants grown with adequate Mn. The addition of Mn to agar further decreased rates of penetration of *Ggt* into wheat root pieces. Wheat root pieces, removed from plants grown in Mn-deficient soil and infected with *Ggt*, were examined under a scanning electron microscope with energy dispersive X-ray microprobe techniques. These studies revealed extensive deposits of Mn-rich nodules coincident only with areas of the root where *Ggt* hyphae were present and Mn had been mixed through the soil. These Mn-rich nodules were also found in *Ggt* hyphae. Several *Ggt* isolates were tested for their ability to oxidize Mn^{2+} on agar and it was found that those isolates which most readily oxidized Mn were also those which were most virulent on the wheat root.

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THESIS INTRODUCTION

Take-all is a world-wide root disease which has plagued the Australian cereal grower since the early days of first settlement and take-all is still regarded as one of the major fungal diseases of wheat in southern Australia (MacNish 1980, Ballinger and Kollmorgen 1986).

Likewise, manganese (Mn) deficiency has a long recorded history in Australian agriculture. The first report of a micronutrient deficiency in Australia was the application of Mn correcting "grey speck" disease of oats in the south-east region of South Australia (Samuel and Piper 1928). Mn deficiency is now recognized as being a frequent problem in a wide range of crops on many soil types in Australia, and around the world.

Many factors that influence the level of take-all in the field also affect the release of soluble Mn^{2+} ions in the soil. For example, ammonium fertilizers and decreased soil pH, which reduce take-all, can also release Mn^{2+} whereas liming and increasing pH above 6.5 increases take-all and can decrease the level of soluble Mn^{2+} in the soil. In 1983, a causal relationship between severe take-all and Mn deficiency was discovered (Graham and Rovira 1984). Wheat plants growing under controlled environment conditions in a Mn-deficient soil had more take-all than plants which had been grown in the same soil to which Mn sulphate had been added.

The aim of this project was to investigate various aspects of the interaction between Mn nutrition of wheat and its susceptibility to *Ggt* infection in such a way that the underlying mechanism(s) would be uncovered. Initial directions for the project were provided by testing three hypotheses proposed by Graham and Rovira (1984);

1. Mn^{2+} may be directly toxic to the free inoculum of the fungus in the soil.
2. Mn may be acting through the physiology of the plant. Mn nutrition affects photosynthesis, which in turn controls the rate of exudation of soluble organic

compounds by roots. These exudates affect the rhizosphere microflora and, through it, the ectotrophic growth of the take-all fungus.

3. Lignin production is controlled by Mn-activated enzyme systems. Since ligneous materials are an acknowledged partial defence against take-all in the form of lignitubers, these structures may be more poorly developed in Mn-deficient plants.

A second aim of the project was to reproduce the interaction between Mn status of the wheat host and take-all in the field because at the time of the commencement of the project the phenomenon had only been reproduced in pots under controlled environment conditions.

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CHAPTER 1.

LITERATURE REVIEW

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CHAPTER 1. LITERATURE REVIEW

Take-all is a world-wide root disease which has plagued the Australian cereal grower since the early days of first settlement. In South Australia, Dr. Carl Muecke was moved to write an essay titled, "The Take-all - The Corn Disease of Australia" in 1870 in which he recorded the following pessimistic outlook;

> The hitherto fertile fields of South Australia have been visited by a demon-like enemy to agriculture, which is already beginning to fill the minds of farmers with despair. Small patches were observed here and there, where the crops died off not long after their appearance above the ground. These patches increased in number and size and were taken possession of by weeds. At first, very little notice was taken of those patches, but when they increased and spread year by year attention was attracted to them. The people, very appropriately, called the mysterious disease "take-all"; but no one hardly ever thought at that time that this pest would be able to destroy the prosperity of the whole district, and even to endanger the very existence of the colony as an agricultural country.

Nevertheless, agriculture in South Australia has persisted and remained viable but take-all is still regarded as one of the major fungal diseases of wheat in southern Australia (MacNish 1980, Ballinger and Kollmorgen 1986).

Likewise, manganese (Mn) deficiency has a long recorded history in Australian agriculture. The first report of a micronutrient deficiency in Australia was the application of Mn correcting "grey speck" disease of oats in the south-east region of South Australia (Samuel and Piper 1928). No grain was harvested from untreated plots of Algerian oats growing on a heavy black alkaline clay soil at Penola but the highest rate of Mn fertilizer yielded the equivalent of 6.1 tonnes per hectare. Mn deficiency is now recognized as being a

frequent problem in a wide range of crops on many soil types in Australia, and around the world.

Many factors that influence the level of take-all in the field also affect the release of soluble Mn^{2+} ions in the soil. For example, ammonium fertilizers and lowered soil pH, which decrease take-all, can also release Mn^{2+} whereas liming and increasing pH above 6.5 increase take-all and can decrease the level of soluble Mn^{2+} in the soil. In 1983, a causal relationship between severe take-all and Mn deficiency was discovered (Graham and Rovira 1984). Wheat plants growing under controlled environment conditions in a Mn-deficient soil had more take-all than plants which had been grown in the same soil to which Mn sulphate had been added.

The aim of this review is to integrate our knowledge of the take-all disease of wheat and Mn deficiency (especially in cereals) with the effect of the environment on each. The effect of the Mn on the resistance of plants to disease will also be discussed. Due to the wide scope and large volume of literature on the first two topics most emphasis will be placed on those aspects which are of particular relevance to this thesis.

1.1. TAKE-ALL

1.1.1. History and Distribution.

Historically, take-all disease of wheat was very much an Australian disease and was a recurring topic at local Agricultural Bureau meetings and in Department of Agriculture Journals from the turn of the century onwards. The disease was first recognized in South Australia, as early as 1852 (Anon. 1868, Butler 1961) but the correct identification of the causal organism took a further 40 years and occurred in France. Prillieux and Delacroix (1890) identified the fungal pathogen as an ascomycete, *Ophiobolus graminis* (Sacc.) but current nomenclature has re-classified the causal fungus of take-all of wheat as *Gaeumannomyces graminis* Arx and Olivier var. *tritici* Walker (*Ggt*) (cf. Walker 1981).

Take-all has a world-wide distribution (Sprague 1950, Garrett 1981) and occurs in all major wheat-growing countries of the world. Within Australia take-all occurs throughout the southern wheat belt, from Western Australia through South Australia, Victoria and most of New South Wales (Anon. 1984, MacNish 1980, Samuel 1924, Wong 1983).

1.1.2. Symptoms and impact of take-all on wheat yields.

McAlpine (1904) showed that the previously separate diseases known in Australia as `take-all' (patches, often circular, of severely stunted or dead seedlings) and `white-heads' or `hay-die' (apparently healthy plants approaching maturity senescing prematurely and producing dead, bleached inflorescences) were different stages of the same disease and were caused by *Ophiobolus graminis*.

Ggt-infected wheat plants exhibit characteristic root blackening (Clarkson and Polley 1981) and this blackening may extend up the basal stem of the plant and into the inner culm and leaf sheath surface if conditions are favourable for the disease (Butler 1961). The diseased root systems bear black necrotic lesions and are often reduced due to rotting near the crown. Perithecia of *Ggt* can sometimes be seen protruding through the basal leaf sheaths of infected, mature wheat plants (Weste 1972) although ascospores are of doubtful, and at best occasional, importance to the epidemiology of the disease (Garrett 1934c, Hornby 1981). As already mentioned, the disease can cause mature plants to senesce prematurely and heads are either completely empty of grain or the grain is severely shrivelled (McAlpine 1904, Green and Ivins 1984).

Identification of the presence of *Ggt* on plants has, until very recently, relied on accurate assessment of host symptoms and isolation of the causal fungus but the prospect of objectively quantifying initial *Ggt* infection by enzyme-linked immunosorbent assay techniques has now been proposed (El-Nashaar *et al.* 1986).

The importance of take-all of wheat to Australian and world agriculture in terms of unrealized cereal yields has been proclaimed for more than a century. An Agricultural Development and Advisory Service survey in the United Kingdom estimated the national annual average losses in yield of winter wheat due to take-all for the period 1975-80 at 2.1 % (equivalent to more than 25 million pounds in growers' returns) (Hornby 1985). In Western Australia take-all is estimated to cause losses of $20 million a year (MacNish 1980) while $50 million is lost annually to all cereal root diseases in South Australia (Stock Journal, Adelaide, Sept. 13, 1984). Take-all (Garrett 1934a) and cereal cyst nematode (O'Brien and Fisher 1981) are the two major root disease problems in South Australia. These estimates are mostly based on widespread, and sometimes casual, field observations but conclusive experimental data on yield losses attributable to take-all are now available. However, they are always restricted in terms of sites and years tested and sometimes further qualifications need to be made.

Soil fumigation has been used to estimate the size of yield losses to many diseases, including take-all. A maximum increase in yield of 37 % was attributed to control of take-all by Ebbels (1969) but this effect was partially confounded by the fumigation treatment controlling other diseases in the trial, incomplete control of take-all and also by the fumigant being 17.3 % nitrogen. This means that some of the yield increase must be partitioned into control of other diseases and to a fertilizer effect of the fumigant. Similarly, a fumigation trial at Rothamsted Experimental Station (Salt 1970) showed a 227 % yield increase with formalin. Formalin reduced take-all to negligible levels but also halved the incidence of cereal cyst nematode and decreased eyespot (*Cercosporella herpotrichoides*). Rovira (1976) found a yield decrease of 47 % in a fumigation trial at Roseworthy Agricultural College in South Australia which was ascribed to severe take-all alone.

Recent work investigating the effectiveness of fungicidal seed treatments to control take-all in the field have revealed large yield losses due to this disease. Take-all caused a 57 % yield loss in Kansas (Bockus 1983) and losses of up to 65 % were recorded from field

trials with natural disease levels in the Victorian wheat belt (Ballinger and Kollmorgen 1986, Sward and Kollmorgen 1986, Kollmorgen and Ballinger 1987). The fungicides had no effect on grain yields at Victorian sites which were not infested with take-all.

Regression approaches have also been used to quantify the effect of take-all incidence on wheat yields. Regressions between the percentage of plants with infected crowns and grain yields were calculated for a site at Turretfield in the lower North, and at Ceduna on the far West coast, of South Australia in 1969 (MacNish and Dodman 1973a). An increase in disease score from 10 to 50 % decreased yield from 21 to 12 g per plot at Turretfield and from 9 to 6 at Ceduna. The slope of the regression increased at Ceduna in 1970 (MacNish and Dodman 1973b). An almost identical regression line was derived at Avon in South Australia in 1982 (Rovira and Venn 1985). King (1984) found that *Ggt* incidence and soil nitrate at sowing explained up to 68 % of variation in wheat yields at Coonalpyn, South Australia.

A further approach has been to follow trends in yields with different rotations. Wheat following oats (a non-host crop for *Ggt*) with little take-all yielded 10.4 tonnes per hectare but wheat with a high incidence of take-all following barley only yielded 7.5 t/ha (Thorne *et al.* 1985). Extensive examination of the effects of various rotations on take-all and wheat yields over several years was conducted by a team of workers in the Victorian Mallee (Kollmorgen *et al.* 1987). They found increases in wheat yields with a break crop preceding wheat in the order of 100 % (compared to continuous wheat) and to a maximum of more than 300 %. Take-all reduction was perceived to be the dominant effect of the break crops on wheat yields.

However, significant decreases in take-all incidence have not always been associated with yield increases (e.g. Bateman 1986) and, conversely, wheat crops apparently free of disease may carry sufficient infection on its roots to decimate a following wheat crop if soil and seasonal conditions permit (Buddin and Garrett 1941).

1.1.3. The infection process and effects on host growth and physiology.

Ggt may survive as a saprophyte on infected dead plant remains in soil (MacNish 1973, Macnish and Dodman 1973c) or as separate hyphal strands (Warcup 1957). An attack is initiated by mycelium growing out from such plant and fungal debris in the close vicinity of, or in direct contact with roots or germinating seed of wheat or barley (Wildermuth *et al.* 1984). The contact between host roots and *Ggt* is established by trophic growth of the hyphae towards the roots (Brown and Hornby 1971). On reaching the roots, the fungus may start growth on all parts of the host below the soil surface (Fellows 1928, Weste 1972) although infections of root hairs rarely develop further (Brown and Hornby 1971). The mycelium of *Ggt* consists of black/brown thick-walled runner hyphae which form a net around the outside of the root and hyaline thin-walled hyphae which invade the root and host cells (Garrett 1934a).

Infection hyphae penetrate a host cell by enzymic dissolution of the cell wall (Davis 1925, Manners 1976) and ultimately kill the cell (Manners 1976, Kirk 1984). After penetration of the epidermal cell walls the infection hyphae grow in all directions, though mostly transversely (Davis 1925). The host cells rapidly respond to *Ggt* invasion by depositing lignin on the innermost surface of their walls, around the sites of penetration (Weste 1972). These deposits develop into closed tubular structures (lignitubers) as they encase the penetrating hypha. Lignitubers are rarely successful in completely halting the invasion of the penetrating hyphae (Skou 1981).

Impairment to host growth appears to begin only when hyphae have penetrated the stele and invaded the vascular tissue (Clarkson *et al.* 1975). The phloem is destroyed faster than the xylem (Clarkson *et al.* 1975) but the greatest damage occurs when the xylem tubes become occluded (Fellows 1928), not only by the thickening of the cell walls and the presence of hyphae, but also by mucilage deposited with the hyphae (the occlusion of xylem vessels was clearly captured in electron-micrographs in Foster *et al.* 1983). Later evidence

suggests that ion uptake is impaired before extensive plugging of the xylem vessels because disruption of the phloem leads to a restriction of assimilate supply to sites of active uptake (Clarkson *et al.* 1975, Fitt and Hornby 1978). Fellows (1928) found that the disease caused a non-uniform reduction of cellulose, which was replaced by lignin and a slight amount of suberin, but had no effect on hemicellulose, callose or pectic substances.

Under suitable conditions the disease progresses until all the below ground plant parts are infected including adventitious (crown) roots, the sub-crown internode and the crown itself (Fellows 1928). In the final phase of the attack the fungus reverts to the saprophytic state on the remains of the dead plant where it may produce perithecia (Skou 1981).

Virtually all aspects of vegetative growth are affected in plants which are infected by *Ggt* (Manners and Myers 1981). No toxins have been associated with any stage of infection by the take-all fungus (Weste 1972) and the hypothesis suggested by early workers (e.g. Davis 1925, Fellows 1928) that the pathogen damaged its host mainly by depriving the plant of most or all of its root system, has been confirmed in more recent investigations (e.g. Clarkson *et al.* 1975, Holden 1976, Deacon and Henry 1978, Kirk and Deacon 1986). Rovira (1979) reported that *Ggt*-infected wheat plants had lower contents of the major mineral elements per unit dry weight of shoots than healthy plants. The uptake and movement of inorganic nutrients in wheat plants infected by the take-all fungus have been investigated by Clarkson and co-workers (Clarkson *et al.* 1975) using radio-isotopic tracers of K, Ca, Fe and P. The uptake of all four elements was disrupted by lesions which had developed to the stage of phloem penetration and cessation of root elongation. Fitt and Hornby (1978) found K uptake was most sensitive to take-all and Ca and Mg less so. Disrupted water and ion uptake in the xylem also begins at the stage of phloem disintegration (Clarkson *et al.* 1975, Manners 1976), presumably through some feedback mechanism of decreased assimilate movement.

1.1.4. Management of take-all in Australia.

The control measures advocated by Australian researchers of take-all in the first quarter of this century were to burn stubble, fallow early, remove grassy pastures from rotation, prepare the seed-bed to a fine tilth, roll after seeding, use heavier rates of superphosphate and include oats (a non-host of *Ggt*) before wheat in the rotation (McAlpine 1904, Samuel 1923, 1924, Fish 1927). Except for the very recent prospect of chemical control with fungicidal seed dressings (Kollmorgen and Ballinger 1987) control options today are largely unchanged. The current recommendations to farmers in Western Australia are to reduce the grass component of pastures and use a cleaning crop (oats or any non-gramineae) if necessary, cultivate early to breakdown stubble and encourage good crop growth with reduced weed competition and adequate fertilizers, especially ammonia-N fertilizers (MacNish 1980) since the ammonium form of nitrogenous fertilizers will reduce take-all (Smiley and Cook 1973, MacNish and Speijers 1982). A similar policy applies in South Australia (Rovira and Ridge 1983, Anon. 1984) and Victoria (Price 1970), although the benefits of cultivation have been recently called into question (de Boer and Kollmorgen 1987) since reports have been made of less (Anon. 1984, Moore and Cook 1984, Rovira and Venn 1985), equal (Yarham 1979, Kollmorgen *et al.* 1987) and more (de Boer and Kollmorgen 1987, Rothrock 1987) take-all with conventional cultivation, compared to direct-drilling or reduced tillage techniques.

The farmer also has the option of sowing barley instead of wheat because barley tends to yield better than wheat where take-all is severe (Asher 1972, MacNish 1980).

1.1.5. Genotypic variation in susceptibility to take-all.

To date, no commercial cultivars of wheat with confirmed resistance to *Ggt* have been found (Butler 1961, Scott and Hollins 1985, Hollins *et al.* 1986, Wallwork 1987) although differences in root colonization by *Ggt* have been distinguished under closely controlled conditions (Penrose 1985) and preliminary field data from Western Australia

(Wilson and Parker 1987) and South Australia (Simon and Rovira 1985) suggest large differences in field performance between wheat genotypes where *Ggt* inoculum had been applied. In broad terms, wheat is highly susceptible to *Ggt* infection followed by barley and triticale, while rye is moderately resistant and oats highly resistant (Asher 1972, Scott and Hollins 1985, Hollins *et al.* 1986 and review by Scott 1981).

Ggt has been isolated from many species of grasses (c.f. Scott 1981), including many of agronomic importance to Australian agriculture, e.g. Barley grass (*Hordeum* spp.), Spear grass (*Bromus* spp.), Silver grass (*Festuca* (now *Vulpia*) *bromoides*) and Wimmera rye-grass (*Lolium rigidum*) (Samuel 1923, Griffiths 1933, Chambers and Flentje 1968, MacNish 1980).

Ggt does not attack non-graminaceous plants (Scott 1981).

1.1.6. Biological control of take-all.

Most soils contain micro-organisms which reduce the level of take-all, an effect termed `general suppression' (Garrett 1934a, Gerlagh 1968, Baker and Cook 1974, Cook and Rovira 1976). General suppression is increased by the addition of organic matter (Garrett 1934a) and may operate by reducing the impact of the take-all fungus on host growth. Rovira (1976) found that despite a lower incidence of *Ggt* on wheat roots from methyl-bromide fumigated plots (Warcup 1976) where the soil microflora had been depleted, take-all was more severe and the yield of wheat was reduced from the non-treated control.

The once common seedling blight phase of take-all has been largely replaced by the less acute haydie phase in southern Australia (Garrett 1934a, Cook and Rovira 1976) which suggests that some degree of general suppression now exists in these soils.

The studies of Henry (1932) and Garrett (1934a,b) on the interactions between soil micro-flora and take-all of wheat in relation to soil temperature, with natural and heat-sterilized soil, clearly established that soil microflora suppressed the disease and that this

suppression accounted for the reduction in take-all for wheat at soil temperatures above 20° C. Despite this early work establishing the biological nature of general suppression and the identification of many different soil microflora which are capable of reducing take-all since then, an hypothesis assigning relative importance to each of these and proposing modes and sites of action in general suppression of take-all is still forthcoming.

Numbers of mycophagous amoebae were higher in take-all suppressive soil from the Waite Agricultural Research Institute (Chakraborty and Warcup 1984). These mycophagous amoebae associated themselves with the take-all fungus in infected host residues, reduced the saprophytic survival of the fungus, lysed both pigmented and hyaline hyphae during pre-colonization and parasitic phases of the fungus and caused a reduction in disease severity (Chakraborty 1983).

Various soil-borne bacteria and actinomycetes have also been intimated in suppression of take-all (e.g. Garrett 1934a, Zogg and Jaggi 1974, Sivasithamparam and Parker 1978). Smiley (1978a,b) explained the reduction in take-all in a field soil with ammonium fertilizer in terms, not of soil or rhizosphere pH effects, but of small changes in the suppressive micro-flora of the soil which accompanied the use of NH_4^+-N. He found more antagonistic micro-flora, and a larger proportion of *Pseudomonas* spp. within this group, in NH_4^+-N treated soil and proposed these *Pseudomonas* spp. as the most likely antagonists acting in the soil.

A collaborative field programme at Roseworthy Agricultural College investigating the effects of N-fertilization and fumigation on take-all of wheat also yielded results which indicated that numbers of *Pseudomonas fluorescens* in the soil were important to the level of disease expressed (Cook and Rovira 1976, Ridge 1976, Rovira 1976, Warcup 1976,).

These studies on the role of *Pseudomonas* spp. in general suppression of take-all highlights the difficulties in assigning biological effects to separate groups of micro-

organisms. Antagonistic strains of *Pseudomonas fluorescens* have also been proposed as the biological entity causing specific suppression, which is explained below, so these two classes of suppression would appear to overlap and are not mutually exclusive.

Several *Bacillus* spp. have also been found to suppress take-all in pots and in the field (Campbell and Clor 1985, Capper and Campbell 1986).

A difficult technical problem in this field of research has been that `in vitro' tests of antagonism of micro-organisms against the take-all fungus have been largely unsuccessful in screening suitable biological control agents for disease suppression in soil (Baker 1968, Sivasithamparam and Parker 1978, Rovira and Wildermuth 1981, Weller 1985), although this seems to be true of many studies screening for biological control agents (Burr and Caesar 1984, Merriman 1987).

The cereal take-all fungi belong to a group which includes several species and varieties not, or only weakly, pathogenic to their cereal hosts. A whole field of research has developed around these hypovirulent `take-all-like' fungi because many have been shown to decrease take-all (see the review by Wong 1981 and papers by Wong 1975, Speakman and Lewis 1978, Speakman 1984 and Speakman and Kruger 1984). Wong (1975) showed that prior colonization of wheat roots by *Gaeumannomyces graminis* var. *graminis* (*Ggg*) reduced take-all infection. This effect could not be fully explained by physical exclusion of *Ggt* in the cortex by prior colonization with *Ggg* because spread up the stele by *Ggt* was also inhibited, although *Ggg* never entered the stele. Wong concluded that *Ggg* may have induced a specific response in the host. A very similar system was also described by Speakman and Lewis (1978) for *Phialophora radicicola* reduction of *Ggt* invasion into wheat roots.

Recent research has revealed that soil fungi, not closely related to *Ggt* may also provide protection against take-all of wheat. Herman (1985) isolated more antagonistic mycoflora from the rhizosphere of wheat roots from zero-tillage plots where take-all was

reduced. This antagonistic mycoflora included *Trichoderma*, *Fusarium*, *Aureobasidium* and *Spicaria* spp. *Microdochium bolleyi*, a weak pathogen, significantly reduced infection of wheat roots by *Ggt* (Kirk and Deacon 1987a,b) and a mechanism of competition for cortical cells was proposed.

`Take-all Decline' is a phenomenon reported to occur in Europe and north-western U.S.A. (Gerlagh 1968, Baker and Cook 1974, Shipton 1975, Cook and Rovira 1976) and is defined as the spontaneous reduction in take-all and increase in yield with continuous cropping of wheat and barley (Rovira and Wildermuth 1981). The suppression found in take-all decline has been termed `specific suppression' (Gerlagh 1968, Baker and Cook 1974) and its features, compared to general suppression are summarized in table 1.1, from Rovira and Wildermuth (1981), where T.A.D. is an abbreviation for `Take-all decline' and *transferable* means that a small volume (1-10 %) of suppressive soil mixed with a conducive (or non-suppressive) soil will induce suppression of take-all.

The importance of specific suppression of take-all has not been defined in Australia, possibly because continuous wheat rotations are rarely practiced and our high summer soil temperatures would be destructive to the non-sporing bacteria which are involved (Rovira and Wildermuth 1981, Rovira and Ridge 1983).

Table 1.1. Characteristics of specific versus general suppression of take-all (from Rovira and Wildermuth 1981).

I *Transferable* (specific), caused by
(*a*) continuous wheat with take-all (T.A.D. soil),
(*b*) additions of *Ggt* mycelia to soil,
(*c*) addition of other fungi to soil, e.g. *Gibberella zeae,Fusarium graminearum, Ggg* (Wildermuth 1977),
and with the following characteristics:
(*i*) Eliminated by moist heat - (60° c for 30 minutes),
(*ii*) eliminated by fumigation with methyl bromide and/or chloropicrin,
(*iii*) operates below 20° C and is masked by non-transferable suppression above 25° C.
(*iv*) operates primarily in the rhizosphere.

II *Non-transferable* (general), caused by
(*a*) higher microbial activity in soil associated with organic amendments and fertility build-up,
(*b*) NH_4^+-N uptake by roots,
(*c*) non-host plants,
(*d*) high soil temperatures,
The characteristics of non-transferable suppression are:
(*i*) not eliminated by 60° C (moist) for 30 minutes,
(*ii*) reduced but not eliminated by fumigation,
(*iii*) operates in soil at all temperatures but increases above 25° C,
(*iv*) operates primarily in the bulk soil.

The biological nature and heat susceptibility of specific suppression has been repeatedly shown (Gerlagh 1968, Brown *et al.* 1973, Shipton *et al.* 1973, Wildermuth 1977, Zogg and Amiet 1980) and was not due to changes in the chemical or physical properties of the soils (Zogg and Jaggi 1974). Wildermuth (1977) showed that the factor involved in specific suppression reduced the number of melanised *Ggt* hyphae in the rhizosphere of wheat roots and `germination' of inoculum particles and increased the lysis of hyaline hyphae (see also Wildermuth and Rovira 1977). Wildermuth concluded that the suppression factor operated in the rhizosphere and in bulk soil. Similarly, studies by Pope and Jackson (1973) on the effect of wheatfield soil on *Ggt* inocula showed that the trophic growth of *Ggt* towards wheat roots was inhibited in T.A.D. soil (a bulk soil site of action) and short

periods of contact of wheat roots with decline soil or its suspension reduced *Ggt* infection when they were subsequently grown in non-decline soil (a rhizosphere site of action). However, the estimated mean distances from root to inoculum particle for infection to occur were found to be the same in take-all suppressive or conducive soil (Wilkinson *et al.* 1982 Wilkinson *et al.* 1985) and the number of lesions produced per unit weight of colonized particles and the minimum inoculum particle size required for maximum infection were both similar in suppressive and conducive soils (Cook *et al.* 1986). The results of these latter two studies suggest that specific suppression does not operate in the bulk soil but occurs on or in the immediate vicinity of the root surface.

In the collaborative field programme at Roseworthy Agricultural College mentioned earlier, two of the fumigation treatments were methyl-bromide at 220 kg/ha and chloropicrin at the same rate. Similar incidences of *Ggt* were measured on wheat roots from these two treatments but there were far more white-heads in the methyl-bromide treated plots. This observation was partly explained by much higher numbers of fluorescent pseudomonads in general, and *Ggt* antagonistic strains in particular, from chloropicrin treated plots (Rovira 1976).

Rovira and Cook (1976) were the first to propose that fluorescent pseudomonads were the most likely agents responsible for specific suppression of take-all. Weller and Cook (1981) reported a higher proportion of fluorescent pseudomonads in take-all suppressive soil and within the fluorescent pseudomonads many more of the isolates from this soil inhibited *Ggt* than in conducive soils. These findings were followed up with successful testing of suppression of take-all in pot and field experiments with strains of fluorescent pseudomonads infiltrated into inoculum particles, which gave the best control, or applied to the seed (Wilkinson *et al.* 1982, Weller and Cook 1983). Soil drenches were not effective. One of their most successful strains was isolate 2-79 (NRRL B-15132) which is very antagonistic to *Ggt* `in vitro' and is a good competitor in the wheat rhizosphere (Weller and Cook 1983). Seed-inoculated 2-79 (NRRL B-15132) was detected down the entire

length of seminal roots of field-grown wheat and it still represented 25 % of the total fluorescent pseudomonad population on the roots 48 days after planting (Weller 1984).

Fluorescent pseudomonads are a group of soil-borne, oxidase and arginine dihydrolase positive, non-sporing, rod-shaped bacteria (Schroth and Hancock 1982) which are good colonizers of plant roots, especially of cereals (Kleeberger *et al.* 1983, Martin 1971). These bacteria have been extensively studied because of the strong antagonism they show to many plant pathogens and their ability to decrease disease. In addition, inoculation of roots of plants with fluorescent pseudomonads in the absence of any pathogens can have stimulatory, inhibitory, or no effect on plant growth (Elliott and Lynch 1984, Gardner *et al.* 1984, Lynch and Clark 1984, Olsen and Misaghi 1984, Elliott and Lynch 1985, Elad *et al.* 1987). The effects of fluorescent pseudomonads on many host/pathogen systems, apart from take-all of wheat, have already been studied, e.g. Fusarium wilt of carnation (Yuen *et al.* 1985) and of radish, flax and cucumber (Scher and Baker 1980,1982, Scher *et al.* 1984), common scab and *Erwinia* infection of potatoes (Geels and Schippers 1983a,b) and black root rot of tobacco (Ahl *et al.* 1986). The general field of beneficial plant bacteria was most recently reviewed by Burr and Caesar (1984).

Attention to the mechanism behind the reduction of disease by fluorescent pseudomonads has centred on their ability to produce siderophores (see review by Nielands and Leong 1986) although they can produce a wide range of antibiotic compounds (Burr and Caesar 1984, Ahl *et al.* 1986). Siderophores are low molecular weight (50-100 daltons) ferric-specific ligands whose biosynthesis is triggered by low iron conditions and their function is to scavenge Fe^{3+} (very insoluble under aerobic conditions) from the environment for the cell (Nielands 1981).

Although fluorescent pseudomonads now seem to be widely accepted as being important in suppression of take-all, the issue of the mechanism underlying this effect is far less settled. It has been hypothesized that siderophores function as biostatic compounds by

drastically reducing the amount of Fe^{3+} ions available to certain rhizosphere microflora (Kloepper *et al.* 1980, Misaghi *et al.* 1982, Scher and Baker 1982, Geels and Schippers 1983a,b, Hubbard *et al.* 1983, Elad and Baker 1985, Nielands and Leong 1986), including *Ggt* (Kloepper *et al.* 1980, Weller and Cook 1983).

Although disease reduction because of siderophore production by fluorescent pseudomonads almost certainly occurs under some conditions the universal applicability of this model is now coming under increasing pressure. Lockwood and Schippers (1984) concluded that their results provided no evidence for a primary role for siderophores in soil mycostasis but they conceded that a role for siderophores was not ruled out in the colonization of energy-rich substrates, including the rhizosphere. Weller and Cook (1983) qualified their conclusions by stating that siderophores were not the only possible mechanism because `in vitro' tests of fluorescent pseudomonad antagonists of *Ggt* showed that some inhibition was still present on Fe-rich media. Subsequently, 2-79 (NRRL B-15132) has been found to produce a phenazine-1-carboxylic acid which has strong antibiotic properties against *Ggt* and other organisms (Brisbane *et al.* 1987). Non-siderophore producing fluorescent pseudomonad antagonists of *Ggt* and antagonism on Fe-rich media have also been discovered by other workers (Hemming *et al.* 1982, Geels and Schippers 1983a, Gardner *et al.* 1984, Elad and Baker 1985, Wong and Baker 1985). In addition, there are the further complications that the siderophore may also inhibit Fe uptake into the host plant, which was found for peas and maize (Becker *et al.* 1985) and some very recent work showed that the siderophore was only toxic when it was bound to iron and it was proposed that siderophores were not toxic because they depleted iron but because they raised it to toxic levels at local sites (Ahl *et al.* 1986).

1.1.7. Influence of the environment, including host nutrition, on take-all severity.

Take-all of wheat and barley occurs world-wide in regions where soil pH is between approximately 6.0 and 8.5, where soils during much of the growing season are

between 5 and 15° C and near field capacity at the surface, and where the soils are relatively coarse-textured and non-compacted so that aeration is good (Cook 1981) or, at least, this is the widely accepted view.

Early research into take-all cites the disease as a severe problem only on coarse-textured alkaline soils (McAlpine 1904, Fish 1927, Griffiths 1933, Garrett 1934a, Price 1970) although severe take-all has also been recorded on soils of acid reaction (Rosen and Elliott 1923, Brenchley 1968, Yarham 1981, MacNish and Speijers 1982, Taylor *et al.* 1983, Murray *et al.* 1986, Kollmorgen pers. comm.). In many of these cases of severe take-all on acid soils, water-logging or very poor drainage were also involved.

Similar, apparently conflicting, reports have also been made regarding the effect of wet soils on the development and expression of take-all. The take-all fungus has a high water potential requirement for growth and cannot grow at water potentials below about -45 bars (Cook and Christensen 1976). Its growth is halved at water potentials as high as -20 bars and wheat grown under dryland conditions are commonly at potentials of -25 to -35 bars between tillering and heading stages (Papendick and Cook 1974). These findings are in keeping with field observations that take-all was always worst in years with a wet winter and/or spring (Griffiths 1933, Garrett 1934c, Price 1970, MacNish 1980), that take-all was worst on areas where drainage was very poor (Rosen and Elliott 1923, Brenchley 1968, Yarham 1981) and that disease on roots was worst in soil at 70-80 % of water-holding capacity (McKinney and Davis 1925). They contrast, however, with other observations that take-all is favoured by loose, coarse-textured seed-beds (McAlpine 1904, Samuel 1923, Griffiths 1933, Price 1970) and that the incidence of haydie is highest with a dry finish to the season (McKinney and Davis 1925, Price 1970, MacNish 1980, Trolldenier 1981). In addition, *Ggt* is sensitive to reduced oxygen tensions (Smith and Noble 1972, Ferraz 1973) so is not likely to grow well in very wet or water-logged soils despite its requirement for high water potentials. *Ggt* growth decreased very quickly at water contents greater than 44 % of water-holding capacity (Glenn *et al.* 1985) but McKinney and Davis (1925) found that

take-all was worst in soil at 70-80 % of water-holding capacity. In subsequent work, Glenn and co-workers (1987) concluded that oxygen, *per se*, was not likely to limit *Ggt* growth through soil but that the prevalence, size and distribution of gas-filled pore spaces were important in determining rates of spread of *Ggt* hyphae.

The apparent contradiction between take-all being favoured by wet winters and springs but haydie being worst under dry conditions is reconciled by reference to the nature of the damage that *Ggt* causes to the host. *Ggt* effectively truncates the wheat roots so in years when the season finishes early, heavily diseased plants (prevalent if conditions have been wet earlier in the season) do not have an effective root system to search for water deeper in the soil profile and die.

Whereas wet soil favours *Ggt* growth, the opposite condition is best for its long-term survival. The survival of the pathogen in host debris in soil has been demonstrated to be best when conditions were dry and cool (MacNish 1973, Wong 1984). The fungus also survived under conditions which were dry and hot or cool and moist but was quickly eliminated in hot, wet soils. *Ggt* can easily survive in host debris over Australian summers (MacNish and Dodman 1973c, Kollmorgen and Walsgott 1984). These results indicate that *Ggt* survival is favoured by conditions which restrict microbial activity.

Take-all has also been reported to be more prevalent in colder, duller seasons (Hornby and Henden 1986).

Ggt grows optimally `in vitro' in the temperature range 20-30° C (Davis 1925, Sivasithamparam and Parker 1981) but take-all is usually most severe at soil temperatures of 5-15° C. McKinney and Davis (1925) showed that disease was most severe at 12-16° C and in another study (Smiley *et al.* 1986) take-all was found on roots at 14 and 24° C, but not 29° C. The effect of increasing temperature on take-all is quite different in sterilized soil where increases over the range 12-27° C had either no effect (Henry 1932) or increased take-all (Garrett 1934b). The inhibitory effect of temperatures over approximately 20° C on *Ggt*

growth in non-sterile soil and take-all are due to increased microbial activity (general suppression) in the soil (Garrett 1934a, Cook and Rovira 1976, Grose *et al.* 1984).

Although heavy liming of soils will almost guarantee severe take-all (providing other conditions are favourable) (Rosen and Elliott 1923, Smiley and Cook 1973, Huber 1981, Murray *et al.* 1986) and ammonium-N fertilizers (in contrast to nitrate-N) usually decrease take-all (Smiley and Cook 1973, Smiley 1978a,b, Trolldenier 1981, MacNish and Speijers 1982) the complete interaction between soil pH and take-all is unclear. *Ggt* growth `in vitro' is relatively insensitive to pH with some growth recorded at the pH extremes of 3 and 10 (Davis 1925, Sivasithamparam and Parker 1981) and it grew equally well either side of the optimum range of 6-8. The concentration of H^+ ions, *per se*, does not seem to be an important factor determining take-all severity except perhaps at strongly acid soil pH's. Cook (1981) listed three mechanisms to explain the effect of soil pH on take-all. The first (originally proposed by Garrett 1936) was that a low pH shifts the $CO_2/HCO_3^-/CO_3^{2-}$ equilibrium to the gaseous and hence more inhibitory phase but work by Ferraz (1973) and Smith and Noble (1972) showed that *Ggt* growth in soil was not limited by CO_2. The second was that *Ggt* is more subject to antagonism from rhizosphere micro-organisms at lowered pH's. The inhibitory effects of ammonium-N on take-all were proposed to be two-fold (Smiley and Cook 1973); ammonium-N decreased the rhizosphere pH which directly inhibited *Ggt* at pH's less than 5.0 and at pH's above 5.0 the inhibition was biological. Later work by the first author in Australia indicated that increased antagonism of *Pseudomonas* spp. against *Ggt* was the likely mechanism for the inhibitory effect of ammonium-N on take-all (Smiley 1978a,b, 1979). A very recent biochemical study on *Pseudomonas fluorescens* 2-79 (NRRL B-15132) has isolated an antibiotic which is only active `in vitro' at pH's less than neutral (Brisbane *et al.* 1987). The third mechanism was that the reduced availability of certain plant nutrients, especially the trace minerals, in alkaline soils would result in nutritional deficiencies that favour take-all.

The complex interaction between nutrients and take-all has, so far, been responsible for several hundred scientific papers. The field has been recently reviewed by Huber (1981) excepting that little mention was made of the effects of trace minerals on *Ggt* growth or take-all severity.

Many papers in this area have concentrated on the effects of N,P and Ca (especially as lime) on take-all, probably reflecting their importance as fertilizers to lift cereal yields.

In general, severe take-all is restricted to plants of low nutritional status (Rosen and Elliott 1923, Butler 1961, Hornby and Goring 1972, Hornby 1985) and take-all normally increases the response of cereals to fertilizers because serious yield loss occurs on poorly-nourished plants while well-nourished plants suffer much less damage (Glynne 1953). This concept of `disease escape' (the plant is able to grow on by producing new roots to replace those destroyed by *Ggt*) by well-nourished plants has been widely published (e.g. Garrett 1948, Glynne 1953, Butler 1961, Asher 1972) but this does not necessarily mean that the roots of well-nourished plants are more resistant to those of poorly-nourished ones. It appears that the beneficial effects of P in reducing take-all are due to stimulated root development (Butler 1961). Many of the papers on nutrition and take-all, especially for N,P and Ca, did not assess levels of *Ggt* infection but only recorded plant growth which limits the conclusions that can be drawn. For instance, in one pot study which tested the response of *Ggt*-infected wheat to inadequate N,P or K fertilization, a greater yield depression occurred in treatments with no or low N, P or K fertilizer but assessment of infection on the roots was not made (Trolldenier 1985).

There are cases, however, where increased plant resistance to take-all with improved nutrition of certain elements has been demonstrated. Fertilization with ammonium-N decreases the number of infection sites, restricts lesion development or limits vascular damage and nitrate-N does the opposite (Butler 1961, Smiley and Cook 1973, Huber and Watson 1974, Smiley 1978a,b, Trolldenier 1981, MacNish and Speijers 1982).

Chloride fertilization may reduce *Ggt* infection and take-all severity (Taylor *et al.* 1983, Christensen and Brett 1985). It has been proposed that chloride may be effective because it reduces nitrification and maintains higher levels of ammonium-N in the soil (Christensen and Brett 1985) although Goos and co-workers (1987) reported that KCl fertilizer reduced common root rot of barley (caused by *Cochliobolus* (now *Bipolaris*) *sativus*) because it sharply decreased nitrate levels in the plant and soil effects were not important.

Ca, although generally applied as a fertilizer to neutralize soil pH (Rosen and Elliott 1923, Smiley 1978b, Murray *et al.* 1987) appears to favour take-all in addition to its effect on soil pH because take-all was increased after application of either calcium sulphate (neutral salt as gypsum) or calcium carbonate (lime) (Huber 1981).

A reduction in take-all by adding Mg to heavily leached soils in laboratory studies has been reported (Hornby and Goring 1972) and was most effective in combination with a mixture of ammonium-N and nitrate-N. Reis and co-workers (1982) also found Mg decreased take-all in sand culture.

The availability of most trace elements is very sensitive to changes in soil pH. With the single exception of molybdenum, the availability of the trace elements is decreased by increasing pH, with Mn and Fe the most sensitive, followed by B and Zn, and Cu the least sensitive (Mengel and Kirkby 1982). Until recently, the association of high pH soil with severe take-all and low availability of trace elements has received scant attention (Butler 1961, Huber 1981, Hornby 1985) but some studies have now been published which report reductions in take-all due to addition of trace elements.

Reis and co-workers (1982) reported that withholding Zn, Cu or Mn, but not Fe, from wheat plants growing in sand culture with complete nutrient solution increased the severity of take-all. The plants in the treatments where each trace element was withheld were the same size as those in the complete controls so the high levels of each of the trace elements

used may have been toxic to the pathogen rather than the withholding of each causing deficiency in the host and decreasing its resistance to attack. However, deficiency symptoms were noted in the nil treatments, and Zn and Cu, but not Mn, were also effective at decreasing take-all when applied to the foliage only, which suggests that, at least for Cu and Zn, the effect was on the host and not the pathogen. The effect of applying Cu and Zn in the field was also tested and both decreased the severity and incidence of take-all.

More conclusive evidence for Cu deficiency decreasing the resistance of wheat plants to *Ggt* infection was provided by pot studies conducted with Cu-deficient field soil (Wood and Robson 1984). They showed that the length and severity of proximal lesions (closest lesion to the crown) increased and the length of uninfected root between crown and proximal lesions decreased in Cu-deficient plants. Increasing the rate of soil-applied Cu above that needed to eliminate deficiency in the host did not further decrease disease.

Following the tentative evidence of Reis and co-workers (1982), Mn deficiency has been confirmed to markedly decrease the resistance of wheat plants to *Ggt* infection (Graham and Rovira 1984, Rovira *et al.* 1985). In pot studies conducted with Mn-deficient field soil, the number of seminal roots per plant with lesions, the number of lesions per plant and the total length of lesions per plant were reduced by up to 60 % with rates of soil-applied Mn which were sufficient to eliminate deficiency in the plants. An intermediate rate of Mn which resulted in marginally deficient plants slightly reduced take-all levels. The addition of Mn-EDTA to ammonium-N in the field was reported to further reduce take-all severity and increased wheat yields an additional 25 % (Huber and Mburu 1983). The application of ammonium-N alone decreased rhizosphere populations of Mn-oxidizing bacteria (Mn-oxides are unavailable to plants) and increased uptake of Mn by wheat so some of the benefits of ammonium-N on take-all may be due to improved Mn nutrition of the host.

The different effects of environmental factors on *Ggt* growth `in vitro' and in natural soil, and on the take-all disease, highlights our poor understanding of environmental

effects on the latter two processes and, in particular, our very limited knowledge of how environmental effects on the pathogen, the infection process and the host are integrated in the field. The effect of soil water is an obvious example; *Ggt* grows best in wet soil but is sensitive to poor aeration yet take-all can be severe on poorly drained sites and the nutrition of the host crop is almost certainly adversely affected by the poor drainage. The multitude of interactions in such a case have yet to be unravelled.

1.2. MANGANESE

1.2.1. Mobility and metabolic roles of manganese in the plant.

Mn is taken up by plants predominantly as the manganous ion and is transported through the xylem as a free divalent cation (Graham 1979). Mn is generally accepted to be immobile within plants and is not redistributed, even if the growing points suffer from deficiency (Single 1958, Henkens and Jongman 1965, Hocking 1984, Nable and Loneragan 1984a,b). However, there have been a number of reports which concluded that Mn was slightly mobile (e.g. Williams and Moore 1952, Single and Bird 1958, Vose 1963, Graham and Loneragan 1981, Hannam *et al.* 1985) although in these cases plants were either supplied with luxurious levels of Mn, were senescing, or the Mn was applied to the leaves. In most cases the amount of Mn retranslocated was small and not always physiologically significant.

Under adequate supply, Mn is distributed evenly throughout the plant (Vose 1963) but under a decreasing supply of Mn, roots can act as a reservoir for Mn for the shoots (Munns *et al.* 1963, Vose 1963), providing tissue concentrations of Mn do not become very low in the roots themselves (Single and Bird 1958). However, translocation from one half of the root system to the other was undetected in subterraneum clover growing in a split-root solution culture system (Nable and Loneragan 1984a,b).

The majority of Mn in leaves can be extracted with water and much less is removed by organic solvents (Single and Bird 1958, Hannam *et al.* 1984) which led Hannam *et al.* (1984) to the conclusion that much of the Mn in leaves was associated with structural components of the cells and may have been metabolically inactive. Under Mn toxic conditions, plants appear to compartmentalize Mn in a metabolically inactive form (Memon and Yatazawa 1984, Blamey *et al.* 1986), an effect which is visually striking in sunflowers because black/brown deposits of Mn appear at the base of,and in, the trichomes (Blamey *et al.* 1986).

Only one true `metallo-protein' of Mn has been isolated from higher plants (Dieckert and Rozacky 1969) but Williams (1982) classified super-oxide dismutase and acid phosphatase as metallo-proteins and various other enzymes as readily dissociable Mn (II) proteins. Nevertheless, the most common role for Mn in higher plants appears to be as a catalytic activator of enzymes and has been shown to be involved in many metabolic processes (Doby 1965). Magnesium can often substitute for Mn as an activator of many, but not all, of these enzymes (Mengel and Kirkby 1982).

The best documented, and most important, roles of Mn are in the photosynthetic process. Mn is intimately involved in oxygen evolution during photosynthesis (Kuwabara and Murata 1983, Nable *et al.* 1984, Kriedeman *et al.* 1985) and it has been proposed that Mn may act as an electron donor for photosystem II (the site of water splitting and oxygen evolution) in competition with H_2O (Handley *et al.* 1984). Mn is also important in the structural integrity of chloroplasts and the thylakoid membranes within (Possingham *et al.* 1964, Simpson and Robinson 1984), regulation of the biosynthesis of protein components of photosystem II specifically (Khmara 1984), biosynthesis of chlorophyll (Scarponi and Perucci 1984) and elimination of radical super-oxides generated by photosystem I (Handley *et al.* 1984, Palma *et al.* 1986, Leidi *et al.* 1987).

Mn also plays a stimulatory role in plants in the biosynthetic pathway of lignin (Gerretsen 1949, Engelsma 1972, Durst 1976, Autor 1982, Brown 1983, Brown *et al.* 1984). Phenylalanine ammonia-lyase (PAL) is a key enzyme in the lignin (and phenol) pathways (Gross 1980) and its activity is strongly enhanced by high concentrations of Mn^{2+} (Durst 1976, Engelsma 1972). Cinnamic acid-hydroxylase, another enzyme involved in the biosynthesis of lignin, is similarly affected by Mn^{2+} (Durst 1976). Engelsma (1972) suggested that the stimulatory effect of Mn^{2+} on PAL activity was due to the removal by Mn^{2+} of end-products of the PAL pathway. These end-products exhibit feedback inhibition on PAL activity so the Mn^{2+} effect on PAL activity may not be direct. In addition, Krishna and Bharti (1983) showed that there was a complex interplay between the hormone indole-acetic acid, endogenous levels of Mn^{2+} and levels of mono- and polyphenols. They concluded that Mn^{2+} may additionally regulate lignin and phenol production through indole-acetic acid mediated steps. Mn deficiency also affects the activity of several enzymes important in phenol synthesis (Carpena *et al.* 1977). These effects are of particular interest in this review because lignin production and deposition is an important defence response of plants to *Ggt* invasion (Skou 1981). Also, Mn has been found to accumulate in barley cell walls around penetration sites of *Erysiphe graminis* (powdery mildew) during the initial stages of the infection process (Kunoh *et al.* 1975, Kunoh and Ishizaki 1976). It was proposed that the specific deposition of elements like Ca, Si and Mn may have been part of the host resistance reaction.

Other metabolic roles for Mn which have been reported include the regulation and synthesis of plant hormones, especially indole acetic acid (Mengel and Kirkby 1982, Krishna and Bharti 1984, Mudliar and Bharti 1984), normal function of ribosomes (and hence protein synthesis) (Ma *et al.* 1985), DNA formation through control of biosynthesis of DNA precursors (Pflonzig and Auling 1987) and regulation of lipid and carbohydrates (Marschner 1986).

The functional requirements of plants for Mn, estimated by minimal tissue Mn concentrations needed for normal growth, appear to be very similar under varying environmental conditions for all genotypes (Mengel and Kirkby 1982, Marschner 1986). Generally, plants require internal concentrations of 15-20 mgkg^{-1} D.W. (Mengel and Kirkby 1982) to avoid growth depression due to Mn deficiency, although very different values have been published for some plant species (Labanauskas 1973). It is difficult to attach significance to reports of variations in critical Mn concentrations with genotype because factors such as choice of tissue, physiological age of tissue, plant age, nutrient interactions and culture medium may impact on the derivation of critical levels (Batey 1971).

However, within the temperate cereals, very little variation appears to exist in the critical Mn concentration. Analysis of young leaves, which are the best indicators of current Mn supply to the plant (Graham *et al.* 1985), revealed a critical Mn concentration of 11 (Graham *et al.* 1985) and 13 mg kg^{-1} (Ohki 1984) in two independent studies conducted in the field and in solution culture, respectively. A figure of 10 mg kg^{-1} was reported for oats in the field (Karamanos *et al.* 1984), 11 mg kg^{-1} for barley in the field (Hannam *et al.* 1987) and 8 mg kg^{-1} for barley (analysis of whole tops) in pots (Wilhelm *et al.* 1985). Little evidence has been reported for differences between critical Mn concentrations for cultivars within a cereal species (Graham 1984, Graham *et al.* 1985, Marcar and Graham 1986).

1.2.2. Geographical distribution of manganese deficiency.

Mn was first confirmed as an essential micronutrient for plants in 1922 (McHargue 1922) and was quickly followed by the first report of Mn deficiency in the field (Samuel and Piper 1928). They correctly identified that `road take-all' (not to be confused with the disease caused by *Ggt* infection) and `grey speck' disease of oats observed in the Penola and Mt. Gambier regions of South Australia were caused by Mn deficiency. Crops growing near the roads were worst because lime dust blew off the roads, increased the pH of the soils and decreased the availability of Mn.

Mn deficiency has been reported in many crop plants and on many different soil types around the world. Dudal (1976) identified eight world soil taxonomic groups which were susceptible to Mn deficiency for crops; arenosols (derived from quartz materials and low in most nutrients), chernozems (high in organic matter and calcium carbonate), gleysols (commonly waterlogged), histosols (high in organic matter and frequently water-logged), kastanozems (high in organic matter and high pH), podzols (heavily leached), rendzinas (high in calcium carbonate) and solonetz (high pH). The three features of either high soil pH, high levels of organic matter or excessive leaching seem to predispose these soil types to Mn deficiency for plants. Kubota and Allaway (1972) in U.S.A. and Batey (1971) in Great Britain reported similar findings. Batey (1971) estimated that 61,000-100,000 hectares may be susceptible to Mn deficiency in England.

In Australia, there are very few reports of Mn deficiency on water-logged or high organic matter soils, which probably reflects their scarcity and that most of our broad-acre agriculture is conducted in the lower rainfall areas where alkaline soils of low fertility predominate (Stephens and Donald 1958, Donald and Prescott 1975, King and Alston 1975). Western Australia tends to be an exception to this general rule because sites which cause Mn deficiency in cereal crops in that state are characteristically rich in ferruginous gravel, especially in the sub-surface layers, have surface sandy layers with a loose ashy or powdery structure and are slightly acidic (Ryan 1958, Smith and Toms 1958, Gartrell 1980). Over the period 1980/81 to 1984/85 an average of 2034 tonnes of manganese sulphate were incorporated into fertilizers in Australia (Anon. 1986). If it is assumed that all these fertilizers were used, and applied locally, at the rate of 5 kg of Mn/ha, then approximately 100,000 hectares of land were fertilized with Mn in each year for this period. This compares with the estimate of Donald and Prescott (1975) of 46,000 hectares each year for the period from 1967/68 to 1971/72 but contrasts with the 150,000 hectares treated with Mn in South Australia in 1984 reported by Graham (pers. comm.).

1.2.3. Yield reductions and symptoms of Mn deficiency on cereals.

Symptoms of Mn deficiency vary between plant species but affected crops generally are pale-green and growth is reduced (Batey 1971, Anon. 1979, Snowball and Robson 1983). The most common foliar symptom is the development of interveinal chlorosis which may develop into patches of necrosis as the deficiency develops (Samuel and Piper 1929, Toms 1958, Anon. 1979). Oats and wheat can also exhibit a collapsing and `kinking' of mature leaves (Gartrell 1980, Mengel and Kirkby 1982, Smith and Toms 1958). The leaves bend and collapse across a necrotic region in the middle of the leaf while the leaf extremities remain green. In barley, chlorosis in younger leaves may lead to development of grey-brown necrotic spots (Reuter *et al.* 1973a,b). Mn deficiency symptoms usually appear on the youngest tissues first (Samuel and Piper 1929, Snowball and Robson 1983, Davies *et al.* 1984) which reflects the poor mobility of Mn in the plant but observations have also been made of symptoms appearing first on older leaves and being worst on the oldest parts of individual leaves (Smith and Toms 1958, Batey 1971, Gartrell 1980).

Mn deficiency has also been reported to retard development and delay plant maturity (Anderson and Boswell 1968, Perry and Gartrell 1976, Wilhelm *et al.* 1985, Marcar 1986).

Mn deficiency has a reputation for being very transitory in the field and its severity can vary greatly within a single season and also between successive seasons (Batey 1971, Kubota and Allaway 1972, Graham *et al.* 1983). For instance, Graham *et al.* (1983) recorded the observation that during one of their field trials in South Australia, Mn deficiency was so severe that some untreated plots appeared to be dying mid-season. However, following a warm dry September and prolonged wet conditions in October these same plots recovered and produced reasonable yields, although yields were little more than half those of Mn-treated plots.

Although Mn deficiency has been reported from all around the world in many different crops (e.g. Batey 1971, Kubota and Allaway 1972, Durst 1976) and Mn deficiency

appears to be a major limiting factor on soybean yields in many areas of U.S.A. (Gettier *et al.* 1985, Mascagni and Cox 1985a, Ohki *et al.* 1987), the most spectacular effects of Mn deficiency on crop yields have been reported locally, from sites in South Australia. In trials conducted by Samuel and Piper (1928) with Algerian oats in the south-east of this state, plots which were not treated with Mn fertilizer were not harvested but the highest rate of Mn produced the equivalent of 6.1 tonnes per hectare. Graham *et al.* (1983) and Marcar (1986) have also reported little or no grain production in plots of wheat and barley grown on a severely Mn deficient site on the Eyre Peninsula of South Australia. Typically, cereal yields on the calcareous sands of South Australia without Mn fertilizer range from 40 to 75 % of those with Mn (Reuter *et al.* 1973b).

1.2.4. Availability of Mn in soils.

Mn is relatively more abundant in geological materials than the other micronutrients and this abundance is most pronounced in limestone-based materials (Krauskopf 1972). The high abundance of Mn in limestone is of particular interest because Mn deficiency is often a problem on soils rich in limestone (Stephens and Donald 1958, Leeper 1970, Batey 1971). Obviously, the total content of Mn in soil has little bearing on its availability to plants, unless there is an absolute lack of the element in the soil profile (e.g. arenosol soil-types, Dudal (1976)). Different soils have been found which were either toxic or deficient for Mn but still contained similar total amounts of Mn (Aubert and Pinta 1977).

The cycle of Mn through soils includes changes of valence between 2, 3 and 4 (Dion and Mann 1946, Leeper 1970, Cheng 1973, Mengel and Kirkby 1982). Leeper (1970) summarised the overall change in valence from 2 to 4, and vice versa, in two separate equations:

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

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

The reduced Mn(II) form is readily soluble and thus more mobile than the insoluble oxides of Mn(III) and Mn(IV). It is largely the oxidation/reduction cycling of Mn between the soluble reduced state and the insoluble oxidized states in soil that determines the availability of Mn to plants. Any factors which affect the redox balance in the soil will also influence the amount of plant-available Mn (Mengel and Kirkby 1982).

An additional factor in the cycling of Mn in soil is the impact of organic matter on Mn availability. Hodgson (1963) proposed that organic matter influenced Mn transformations in three ways ; 1. the production of complexing agents that effectively reduce the activity of the free ion in solution, 2. a decrease in the oxidation potential of the soil through increased microbial activity, and 3. a stimulation in microbial activity that results in incorporation of Mn in biological tissue. The occurrence of Mn deficiency on soils very high in organic matter (Batey 1971, Kubota and Allaway 1972, Dudal 1976) suggests that factors 1. and 3. may be more important than 2. but experiments have been reported where addition of organic matter has increased Mn availability (e.g. Christensen *et al.* 1950, Miller *et al.* 1985).

It is generally accepted that plants obtain Mn from soil as the Mn^{2+} ion (Geering *et al.* 1969) which means that plants have access to several `pools' of Mn in the soil ; 1. Mn^{2+} in soil solution, 2. Mn^{2+} in equilibrium with exchangeable Mn on colloidal surfaces (Reddy and Perkins 1976) and 3. any Mn^{2+} which can be readily reduced from manganese oxides (Leeper 1970, Mengel and Kirkby 1982, Jarvis 1984). In addition, plants can obtain Mn^{2+} directly from manganese oxides (Jones and Leeper 1951a, Uren 1969) by `contact reduction' of manganese oxides on the surface of the root (Uren 1981). The ability of different plant species to utilize this source of Mn appears to depend on the relative strength of the reductive capacity of their roots (Bromfield 1959, Uren 1981).

Returning to equations (1) and (2) of Leeper (1970), a simplified scenario was described by the author in which the supply of Mn^{2+} ions depends on the opposing rates of

reduction by organic compounds and oxidation to higher (valence) oxides by microbes. Leeper down-played the importance of chemical oxidation of Mn^{2+} and ignores the possibility of microbial reduction of manganese oxides. This view of microbial activity being a dominant effect on Mn oxidation in the soil has been expressed, or implied, by several other authors (Gerretsen 1937, Timonin 1946, Geering 1969, MacLachlan 1984).

The reduction of MnO_2 is strongly favoured by decreases in pH (Leeper 1970) and for every unit decrease in pH soluble Mn^{2+} increases 100-fold (Lindsay 1972). It follows that the few cases of Mn deficiency on acidic soils may be due to insufficient total levels of Mn in the soil profile (Smith and Toms 1958, Kubota and Allaway 1972) and in many of these cases water-logging is common which may further restrict nutrient uptake by limiting root growth (Batey 1971). Mn toxicity is a frequent problem on highly acidic soils (Kubota and Allaway 1972, Cregan 1979).

1.2.5. Role of micro-organisms in Mn cycling in soils.

Gerretsen (1937) was one of the first workers to publish experimental results which showed that the activity of soil micro-organisms was very important to the Mn balance in soil, although prior observations had been made that micro-organisms may be involved in the expression of Mn deficiency symptoms in plants (see Gerretsen 1937 and Timonin 1946). Gerretsen showed that classic `grey speck' disease symptoms of oats were due to low tissue levels of Mn but no symptoms appeared on plants which were grown in soil which had been sterilized with formalin and that these plants had higher tissue levels of Mn. Formalin, as a chemical, did not increase the water soluble or one-tenth nitric acid-extractable Mn fractions in the soil. Timonin (1946) concluded that a significant correlation existed between the severity of `grey speck' disease symptoms of oats and the population of Mn-oxidizing and cellulose-decomposing bacteria on the roots.

A range of micro-organisms have now been identified which are capable of oxidizing Mn^{2+} to insoluble Mn oxides (Gerretsen 1937 Leeper and Swaby 1940, Bromfield and Skerman 1950, Bromfield 1956, Zajic 1969, Bromfield 1979). Separate studies conducted on four different organisms ; three bacteria (Johnson and Stokes 1966, Bromfield and David 1976, Douka 1977) and one streptomycete (Bromfield 1979), each concluded that the oxidation of Mn^{2+} to MnO_2 occurred outside the cell. In the case of the streptomycete an extra-cellular non-dialysable substance was involved and for a *Pseudomonas* sp. (Douka 1977), a cell-free extract, which was heat sensitive and inhibited by mercuric chloride, could also oxidize Mn^{2+}.

Microbial oxidation of Mn^{2+} in well-aerated soils is generally most effective in the pH range of 6.0 to 7.5 (Bromfield 1956, Leeper 1970, Bromfield 1974) although the streptomycete previously mentioned could oxidize Mn `in vitro' at pH values less that 4.5, was optimized at 5.0 and did not occur in the range 6.0 to 7.5 (Bromfield 1979).

Bromfield (1959) reported an indirect effect of soil micro-organisms decreasing Mn availability to plants by decomposing MnO_2-reducing substances secreted by vetch and oat roots.

Soil pH values above 8.0 favour the auto-oxidation of Mn and suppresses the activity of micro-organisms (Leeper 1970). Approximately equal importance was assigned to chemical and microbial oxidation in a field soil which contained more than 80 % calcium carbonate (Reuter and Alston 1975).

Ross and Bartlett (1981) tested the effects of many of the chemical sterilants used by workers who claimed that micro-organisms oxidized most of the Mn^{2+} in soils (e.g. Gerretsen 1937 and Leeper and Swaby 1940 - chloroform, Jones 1957 and Sparrow and Uren 1987 - sodium azide and Reuter and Alston 1975 - mercuric chloride) and concluded that at least some of the apparent inhibition of microbial oxidation by sterilants was in fact the reduction of Mn oxides by these chemicals. There have also been several other reports

which showed that large amounts of Mn may be released into the soil by common soil sterilizing techniques, including U.V. and gamma irradiation (Dalton and Hurwitz 1948, Nelson 1977, Mantylahti and Ylaranta 1981).

In addition, the oxidation of Mn^{2+} by soil micro-organisms does not necessarily mean that the availability of Mn to plants will decrease. Bromfield (1958) reported that the Mn oxide produced by a *Corynebacterium* in liquid medium was completely available to oats grown in sand culture but less so when the oats were grown in a Mn-deficient soil.

Also, there have been several reports in the literature where sterilization of soil has decreased the uptake of Mn by plants (Lingle *et al.* 1961, Heilman 1967), the reverse of the studies summarized above. The uptake of Mn by barley in solution culture was reported to be stimulated by the presence of micro-organisms (of either casual or rhizosphere origins) (Barber and Lee 1974). Mycorrhizal infection of pine seedlings increased Mn uptake and decreased the accumulation of Mn in the roots (Miller and Rudolph 1986). It is possible that these beneficial micro-organisms may have been Mn reducers. Several different soil micro-organisms have been found to be capable of reduction of Mn oxides (Zajic 1969, Bromfield and David 1976, Gottfreund *et al.* 1985) but the list is not as long as the known Mn oxidizers. It has been proposed that Mn-reducing micro-organisms may use manganates as terminal electron acceptors in a process linked to oxidation of organic matter (Burbridge and Nealson 1985). Bromfield and David (1976) found that a Mn-oxidizing *Arthrobacter* sp. was also capable of reduction of MnO_2 to Mn^{2+} under poorly aerated conditions and they concluded that in soil the extent of oxidation or reduction of Mn near plant roots may not be due to the relative activities of specific oxidizing or reducing types of micro-organisms but may depend on small changes in the micro-environment of the rhizosphere which determine whether a given bacterium has oxidizing, non-oxidizing or reducing properties.

1.2.6. Genotypic variation in plant performance under low Mn conditions.

Wide differences have been reported in the growth of different plant species under the same conditions of low Mn supply (Jones and Leeper 1951a, Mulder and Gerretsen 1952, Ryan 1958, Toms 1958, Batey 1971, Borchmann *et al.* 1985, Marcar 1986). For instance, Jones (1957a) demonstrated that oats were severely Mn-deficient when grown in a calcareous soil in pots but rye showed only a small response to added Mn under the same conditions and vetch was unaffected by Mn fertilization.

Within the cereals, oats are the most sensitive to Mn deficiency, barley does better than wheat and rye often shows little or no response to the addition of Mn fertilizer (Gallagher and Walsh 1943, Ryan 1958, Smith and Toms 1958, Nyborg 1970, Marcar 1986). Borchmann *et al.* (1985) reported a different ranking of these cereals and classified winter rye as very sensitive to Mn deficiency (yielded only 13-14 % of Mn-sufficient control), wheat as sensitive (19-43 % of control) and 3 of the 4 tested triticale cultivars as very sensitive (4-7 % of control) and the other as sensitive (22 % of control). Graham *et al.* (1983) reported that Coorong triticale was sensitive to Mn deficiency in the field but Marcar (1986) concluded that the three triticale cultivars he tested, including Coorong, were reasonably insensitive.

Large differences have also been reported in the performance of different cultivars of the same cereal species under low Mn conditions. Graham *et al.* (1983) conducted a field study of the performance of 72 world barley genotypes on a severely Mn-deficient calcareous sand on the Eyre Peninsula of South Australia. They found a very large range in the relative yields of these genotypes compared to a `standard' cultivar (Weeah). The best performer in this study yielded 178 % of Weeah, while the worst yielded 0 %, and there was an almost continuous range of values in between these two extremes. Cultivar differences have also been observed in wheat (Nyborg 1970, Graham *et al.* 1985, Marcar 1986), triticale

(Borchmann *et al.* 1985) and oats (Gallagher and Walsh 1943, Timonin 1946, Ryan 1958, Nyborg 1970).

Graham (1978) in a review arguing the value of breeding for nutritional characters in cereals stated that micronutrient deficiencies were due to lack of availability and inefficient uptake by plants rather than lack of adequate quantities in soil profiles and Nyborg (1970) provided experimental evidence that the sensitivity of oats to Mn-deficient soils was due to their poor ability to take up Mn rather than a higher requirement for Mn. Similarly, Marcar (1986) concluded that the sensitivity of wheat, compared to barley, was due to a poorer ability to take up Mn from a Mn-deficient soil and no differences were found in the minimum tissue Mn concentration required for maximum growth between cultivars of wheat differing in their sensitivity to Mn deficiency (Graham *et al.* 1985, Marcar and Graham 1986).

1.2.7. Influence of environmental factors on Mn deficiency.

As already mentioned, Mn deficiency has a world-wide reputation for transitory appearances and disappearances in the field and these fluctuations are sometimes at odds with predicted changes in Mn availability due to shifted balances between opposing reactions of oxidation and reduction.

For example, Hannam (1984) observed that Mn deficiency in sweet lupins grown on neutral to acid sands in the south-eastern regions of South Australia, was more likely to occur if warm, dry conditions occurred during spring flowering, followed by substantial rainfall. From the redox balance one would predict that the substantial rainfall should have released more Mn^{2+} into the soil solution because of wetter, more reducing conditions and alleviated the deficiency. This pattern was observed for cereals growing on a calcareous sand on the Eyre Peninsula of South Australia (Graham *et al.* 1983).

Several workers have observed that Mn deficiency was often worst during prolonged cold, wet spells (Batey 1971, Kubota and Allaway 1972) and this observation

was reconciled with the chemical behaviour of Mn in soil (reduction to available Mn^{2+} should be favoured under these conditions) by proposing that root growth was so poor under these conditions that the plants failed to take up sufficient Mn for their purposes (Batey 1971). Conversely, Samuel and Piper (1928) reported that the general experience of farmers they had talked to was that Mn deficiency was worst in dry years. Warm spells following cold and wet conditions may worsen (Batey 1971) or alleviate (Toms 1958) Mn deficiency.

Extractable Mn increased sharply with very wet or water-logged conditions (Shuman 1980) and with increasing temperature (Shuman 1980, Reid and Racz 1985) but decreased if wet and dry cycles were used (Ryan and Hariq 1983). The fixation of Mn onto clay particles decreased under wet conditions but increased under cycling wet/dry conditions (Reddy and Perkins 1976). The chemical reduction of Mn also increased with increasing temperature (Sparrow and Uren 1987). Even altered light regimes have been reported to affect the availability of Mn in soil (Cheng and Pesant 1984) such that while alternating light and dark periods decreased available Mn both continuous light and continuous dark increased available Mn.

Decreasing availability of Mn and increased severity of Mn deficiency symptoms with increasing soil pH is easily the most frequently documented effect of the environment on Mn deficiency and the references which follow are merely a selection of the many accounts published (Samuel and Piper 1928, Batey 1971, Kubota and Allaway 1972, Mulder and Gerretsen 1952, Reuter *et al.* 1973a,b, Reuter and Alston 1975, Reddy and Perkins 1976, Osman *et al.* 1983, Ryan and Hariq 1983, Asher *et al.* 1984, Sparrow and Uren 1987). Most of these reports used lime as a treatment to increase soil pH but lime will decrease available Mn by precipitation of Mn carbonates and chemisorption of Mn^{2+} on its surfaces, in addition to its effects on soil pH (McBride 1979). However, there have also been reports of liming alkaline soils to pH values above 8.0 which alleviate Mn deficiency (Samuel and Piper 1929, Jones 1957a,b) and this observed effect was explained as

inhibition of the Mn-oxidizing soil micro-organisms at these extreme pH values (Leeper 1970).

1.2.8. Correcting Mn deficiency in the field.

Soil analysis of extractable Mn has generally been unsuccessful as a tool for either the diagnosis or prognosis of Mn deficiency (Leeper 1970, Cox and Kamprath 1972, Tiller 1983). Many factors contribute to the difficulty of using soil analysis for Mn and they include the need to account for individual requirements of various plant species and cultivars, the difficulty in predicting the effects of seasonal conditions on plant growth and on chemical activity in soil, and our limited knowledge of the types of Mn compounds available to plants.

Visual symptoms and plant analysis are the two techniques most widely used for the identification but only one example has been reported where the sophistication of plant analysis has reached the potential of true prognosis (Hannam *et al.* 1985).

Since Mn deficiency is most frequently a problem related to low availability and not inadequate quantities in the soil profile, amelioration with soil-applied fertilizers can be difficult. In an acidic soil, simple application of a manganese sulphate fertilizer at sowing eliminated Mn deficiency symptoms and increased oat yields (Smith and Toms 1958). However, on alkaline soils, repeated applications of Mn during the season may be necessary to maximize yields. Yields of barley growing on a highly calcareous sand on Yorke Peninsula of South Australia required 6 kg Mn/ha at sowing and 2 to 3 subsequent foliar sprays to attain maximum yields (Reuter *et al.* 1973a). Similar fertilizer regimes have been reported for wheat on the Eyre Peninsula (Graham pers. comm.), for oats in the south-eastern region (Samuel and Piper 1928) of South Australia and for soybeans in U.S.A. (Gettier *et al.* 1984, Mascagni and Cox 1985a).

Soil application of Mn is more effective when it is incorporated with an acidic fertilizer (Reuter *et al.* 1973b, Voth and Christensen 1980, Mascagni and Cox 1985a, Miner

et al. 1986) or is banded instead of broadcast (Reuter *et al.* 1973a, Mascagni and Cox 1985a). Mn fertilizers usually have limited or no residual benefits in the field (Davies *et al.* 1984, Gettier *et al.* 1984, Reuter 1975) due to their rapid conversion to unavailable forms (Reuter and Alston 1975, Marcar 1986).

Although manganese sulphate is the most common Mn fertilizer, other manganous compounds have also been used. Mn oxides, of differing chemical and physical composition, have been applied to soils with varying success but are generally much less available to plants than the equivalent rate of manganous sulphate (Jones and Leeper 1951a,b, Heintze 1956, Bromfield 1958, Bromfield 1959, Voth and Christensen 1980, Jauergut and Reisenauer 1982, Mascagni and Cox 1985b). The availability of Mn oxides seems to be a property of the size and crystallinity of the particles, rather than its chemical formula (Jones and Leeper 1951a) and the availability of an oxide in soil decreases with time as the oxide particles `age' (Jones and Leeper 1951b).

Mn silicate has been proposed as a Mn source which is stable over the pH range 4 to 12 and was more effective than manganese sulphate (Boxma and DeGroot 1985).

The problem of rapid transformation of soil-applied Mn into unavailable forms has often been overcome by foliar application (e.g. Reuter *et al.* 1973a, Hannam *et al.* 1984, Gettier *et al.* 1985). Repeated applications are sometimes necessary to protect subsequent growth from Mn deficiency because of the poor mobility of Mn in the plant. Various inorganic salts and chelate carriers of Mn have been used in foliar formulations but manganese sulphate is usually as effective (e.g. Ohki *et al.* 1987) but frequently cheaper.

Another approach which avoids the problem of transformation of soil-applied Mn is the soaking of seeds in Mn solutions prior to sowing. Roberts (1948) reported that a large part of the Mn requirement in the early stages of development of oats growing on a severely Mn-deficient alkaline peat could be provided by soaking the seed in a manganese sulphate solution prior to sowing. Pre-sowing soaking of wheat seed has been shown to increase

grain yields, both in the glasshouse (Khalid and Malik 1982) and in the field (Marcar and Graham 1986).

1.2.9. Manganese and plant resistance to disease.

The topic of the effect of mineral nutrition on plant diseases has received considerable attention, including at least ten reviews in the last twenty years (Black 1968, Borys 1968, Goss 1968, Krauss 1969, Trolldenier 1969, Huber and Watson 1974, Jenkyn and Bainbridge 1974, Huber 1980, Graham 1983) although only the review of Graham (1983) concentrated on the role of micronutrients. The effects of plant nutrients on disease may be attributed to effects on plant vigour that can influence the microclimate in a crop and so affect infections and sporulation of the pathogen, effects on cell walls and tissues as well as on the biochemical make-up of the host, the rate of growth of the host which may enable seedlings to escape infection in their most susceptible stage, and effects on the pathogen through alterations in the soil environment (Colhoun 1973).

Graham (1983) listed seven principles which summarized macronutrient effects on incidence of disease in higher plants and a further seven for micronutrient effects ;

Macronutrients.

1. A well nourished host is generally more tolerant of disease.
2. Some elements appear to affect disease severity simply through greater tolerance, for example, phosphorus and sulphur; while others including nitrogen and potassium clearly alter specific host-plant resistance mechanisms.
3. Nutrition is only one of many factors influencing resistance to disease.
4. Macronutrients increase resistance to disease, if at all, only in the deficiency range; supraoptimal amounts of nutrient do not provide

further protection, and may be detrimental in the case of nitrogen.

5. Yield responses to fertilizers often contain two components, due to overcoming both the deficiency *per se*, and changing the disease burden.

6. Nitrogen may either increase or decrease disease, depending on other interacting factors, but in either case will increase yield where the soil is deficient in the nutrient.

7. Nitrogen effects are associated with effects on the balance between the primary and secondary metabolic pathways in the host.

Micronutrients.

1. Correction of a micronutrient deficiency generally increases the tolerance and/or resistance of plants to pathogenic diseases.

2. Further protection in some conditions is conferred by a number of trace elements in concentrations above those needed for host plant growth in disease-free conditions.

3. In other situations, addition of a trace element may exacerbate the severity of disease. In these cases it would appear that interactions between trace elements or with nitrogen are involved, and element imbalances in the host are likely to be a predisposing factor.

4. The contribution of nutrition to disease resistance is usually only partial, there being many other factors.

5. Yield responses to an element often may contain two or even three components, due to overcoming the deficiency, changing the host plant's defence against disease, and having direct toxic effects on the pathogen.

6. Copper, boron and manganese all influence the synthesis of lignin and simple phenols; zinc, iron and nickel have generally

different effects, possibly related to phytoalexin synthesis; silicon and lithium appear to affect physical barriers to invasion. Silicon, nickel and lithium may be essential elements in the biochemical pathways of defence.

7. As in animals, iron may be a key element for which host and pathogen compete; an important factor in host-pathogen relationships is the iron:manganese ratio.

Plants low in Mn have been reported to be susceptible to, or the addition of Mn has decreased (micronutrient principles 1. and 2. of Graham (1983) make a distinction between these two effects) diseases caused by fungi (e.g. *Sclerotinia* infection of cucumbers (Abia *et al.* 1977), powdery mildew of wheat (Colquhoun 1940), take-all of wheat (Reis *et al.* 1982, Graham and Rovira 1984)), bacteria (e.g. *Pseudomonas* infection of citrus (Primavesi and Primavesi 1964), bacterial blight of rice (Philip and Devadath 1984)), nematodes (e.g. *Meloidogyne* spp. infection of tomatoes (Treskova 1961), cereal cyst nematode infection of barley (Wilhelm *et al.* 1985)) and an actinomycete (common scab of potatoes (Mortvedt *et al.* 1963, McGregor and Wilson 1966)).

The report of Mn influencing cereal cyst nematode infection of barley (Wilhelm *et al.* 1985) is a special case amongst these other examples of Mn affecting plant disease because it is the only study in which plant resistance was shown to be unchanged, while tolerance was markedly increased, by addition of Mn. Resistance is the ability of the plant to inhibit reproduction of the pathogen while tolerance is endurance of disease by the host without severe losses in yield or quality (modified from Schafer 1971). In this pot study the highest rate of nematodes decreased the height of Mn-deficient barley by 45 % but had no effect on the height of plants adequately supplied with Mn. Both Mn-adequate and Mn-deficient plants supported equal numbers of nematodes on their roots.

There has been only one report of Mn increasing disease. Wilks *et al.* (1983) reported that infection of ponderosa pine by the fungus *Ceratocystis wageneri* was favoured by soil conditions which were sufficiently wet to cause extensive reduction of Mn but not too wet to severely impede aeration. The fungus apparently was very well adapted to a Mn-rich environment and its growth `in vitro' was stimulated by Mn concentrations as high as 1000 mg kg^{-1}.

Application of Mn decreasing take-all of wheat and common scab of potatoes are the best documented examples from the above and there are important similarities and differences between them.

A measure of control has been gained over both these diseases by various agronomic practices and these practices also tend to increase the availability of Mn in soil. Decreasing soil pH, incorporating green manure crops, using ammonium-N instead of nitrate-N fertilizers and application of manganese, phosphorus or sulphur fertilizers all decrease take-all and common scab (Wenzl 1975, Asher and Shipton 1981, Trolldenier 1981, Graham 1983) and release Mn^{2+} in the soil (Leeper 1970).

The presence of high Mn levels has been shown to cause large reductions in common scab symptoms on potato tubers. Mortvedt *et al.* (1961) added *Streptomyces scabies* suspensions in Mn solutions of increasing strength to the tuber-setting zone of potato plants growing under controlled environment conditions. The roots of the plants were not exposed to the Mn solutions and were kept in complete nutrient solutions. Under these conditions, the disease was halved by a 2 mg kg^{-1} Mn solution and were completely eliminated by 20 mg kg^{-1} of Mn. These results suggest that high concentrations of Mn were toxic to the pathogen and did not improve the resistance of the plants because Mn levels in the plants were sufficient for growth without added Mn and never reached plant toxic levels where Mn was added. Similar conclusions were reached from field trials where Mn application did not increase plant growth in the absence of the pathogen but still reduced the

levels of common scab on tubers and increased the yield of infected plants (Mortvedt *et al.* 1963, McGregor and Wilson 1964). The growth of *Streptomyces scabies* `in vitro' is very sensitive to low levels of Mn^{2+} (Mortvedt *et al.* 1963). In addition, broadcast, rather than banded, Mn fertilizer was more effective at decreasing common scab (Mortvedt *et al.* 1963) yet banding Mn fertilizer is more effective at supplying Mn to the plant (Reuter *et al.* 1973a).

The studies reported by Reis *et al.* (1982) on the effect of Mn on take-all of wheat are also consistent with Mn being directly toxic to the pathogen. In their studies, take-all was decreased when Mn was increased in sand cultures from 1x to 4x that of Hoagland's solution. There was no change in plant growth in the absence of take-all with the high rate of Mn.

Separate studies on the effect of Mn on take-all of wheat (Graham and Rovira 1984, Rovira *et al.* 1985) showed that the resistance of wheat seedlings was altered by Mn nutrition because Mn-deficient plants were very susceptible to attack by *Ggt* and disease was much heavier on these plants. In the light of their results Graham and Rovira (1984) proposed three, not mutually exclusive, mechanisms to explain the role of Mn in increasing the resistance of wheat to *Ggt* and they are as follows;

1. Mn^{2+} may be directly toxic to the free inoculum of the fungus in the soil.

2. Mn may be acting through the physiology of the plant. Mn nutrition affects photosynthesis, which in turn, controls the rate of exudation of soluble organic compounds by roots. These exudates affect the rhizosphere microflora and, through it, the ectotrophic growth of the take-all fungus.

3. Lignin production is controlled by Mn-activated enzyme systems. Since ligneous materials are an acknowledged partial defence against take-all in the form of lignitubers, these structures may be more poorly developed in Mn-deficient plants.

Graham (1983) briefly reports a further experiment in which take-all was completely eliminated by concentrations of Mn high enough to cause cylinders of MnO_2 to be precipitated around the roots. These concentrations were not toxic to the wheat but were well above those needed for optimal growth rate in the absence of *Ggt*. These results imply that hypothesis 1. of Graham and Rovira (1984) may operate with high rates of Mn application but do not preclude the second two hypotheses from also being important. Reduction of take-all was also achieved at Mn rates one-tenth that which eliminated disease, and plants which were marginal for Mn (obtained with a rate of one-hundredth of that which eliminated disease) still had significantly less disease than severely Mn-deficient plants. These latter two results imply that Mn in the plant may be important in *Ggt* infection of wheat but do not distinguish between hypotheses 2. and 3. of Graham and Rovira (1984).

1.3. Conclusions

Take-all of wheat and barley and Mn deficiency are two problems which afflict broad-acre agriculture on a world-wide scale. Although both can cause almost total crop losses when conditions are favourable for their development, their effects on crop yields tend to be less obvious, and less severe, during years when seasonal conditions are more `average'.

It has been recognized for a long time that each of these problems are affected strongly by seasonal conditions and management practices and, as Graham (1983) has pointed out, factors which increase, or decrease, take-all also exacerbate, or alleviate, Mn deficiency.

Take-all and Mn deficiency both tend to be more severe as the pH of the soil increases from acidic to alkaline levels so cultural practices such as liming and using nitrate- rather than ammonia-N fertilizers will favour both problems. Similarly, soils of an alkaline

nature, and particularly those with high calcium carbonate levels, have well known reputations for producing severe Mn deficiency and bad take-all in cereal crops.

Take-all is generally worse on well aerated, loosely structured soils and these same conditions will also favour oxidation of Mn into unavailable forms. Conversely, there have also been reports of severe take-all on wheat seedlings during cold, wet spells and Mn deficiency can also appear under these conditions, presumably due to insufficient Mn uptake by the plant because of very slow root growth.

The similar pattern of environmental factors which encourage take-all and also favour Mn deficiency has been described in length by Graham (1983). Due to the closeness of fit between these two patterns Graham proposed that Mn is a common factor among the environmental effects on the severity of take-all and there is now some experimental evidence to show that Mn deficiency pre-disposes wheat to take-all.

The effect of Mn on take-all of wheat is one of the best documented examples of Mn influencing plant resistance to disease but experimental data on the topic is still very limited (only 4 published papers) and mechanisms to explain this effect have been proposed but not tested.

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CHAPTER 2.

EFFECT OF HIGH LEVELS OF SOIL MN ON GROWTH OF *GGT* AND ON THE DISEASE, TAKE-ALL

CHAPTER 2. EFFECT OF HIGH LEVELS OF SOIL MN ON GROWTH OF *GGT* AND ON THE DISEASE, TAKE-ALL

2.1. Introduction.

The simplest hypothesis proposed by Graham and Rovira (1984) to explain the reduction of take-all by Mn application to soil was that Mn^{2+} was directly toxic to the fungus in the soil. The results of Reis *et al.* (1982) and also some of those published by Graham (1983) are consistent with such an hypothesis, in that rates of Mn which did not further increase plant growth, gave additional control of take-all. Studies on the effects of Mn on common scab of potatoes imply that Mn may decrease this disease through such a mechanism (Mortvedt *et al.* 1963).

In order to investigate the validity of this hypothesis, growth of *Ggt* was first tested at increasing rates of $MnSO_4$ in two different agars. In this way the sensitivity to, and behaviour of, *Ggt* in the presence of high levels of Mn^{2+} could be readily observed.

Secondly, a `soil-sandwich' technique developed by Grose *et al.* (1984) to test the effect of temperature and moisture on *Ggt* mycelial growth through a soil matrix was modified to test the effect of high levels of Mn^{2+}.

Thirdly, the effect of increasing additions of Mn^{2+} to soil on the level of disease on wheat roots was compared to changes in mycelial growth of *Ggt* through soil over a similar range of Mn^{2+} concentrations. The data for the former was collated from $MnSO_4$ treatments of 14 pot experiments, 13 conducted during the course of this project and 1 from the published results of Graham and Rovira (1984). Data for the latter was taken from studies conducted with the modified `soil-sandwich' technique.

2.2. Materials and Methods.

2.2.1. Growth of *Ggt* on two agars at increasing rates of $MnSO_4$.

2.2.1.1. *PDA*. Three separate experiments were conducted to test the effect of increasing rates of $MnSO_4.4H_2O$ on the growth of *Ggt* on potato dextrose agar (PDA [1]).

2.2.1.1.1. Inoculum. A single isolate was used in all 3 experiments and this isolate was used throughout the project. The isolate (500) was supplied by Dr. A. Rovira [2] and was originally isolated by A. Simon from wheat roots collected at Coomandook, South Australia in 1976. All cultures used in this project were stored on full-strength PDA under sterile distilled double deionized water ($DDDH_2O$) and colonies for each experiment were prepared from stored cultures.

2.2.1.1.2. Mn. Mn^{2+} ion concentration of between 0 and 800 mg kg^{-1} were prepared by adding appropriate amounts of a $MnSO_4.4H_2O$ stock solution to weak PDA (approximately 40 % of manufacturer's recommendations) which had been made up with $DDDH_2O$. In experiment A, a series of 2-fold dilutions were prepared with an 8000 mg kg^{-1} Mn stock solution to give final concentrations in agar of 0, 25, 50, 100, 200, 400 and 800 mg kg^{-1} Mn. In experiments B and C a 500 mg kg^{-1} Mn stock was used to give final concentrations of 0, 10, 25, 50, 75, 100, 200, 400, 800 and 0, 25, 50, 100, 200, 400 mg kg^{-1} Mn, respectively. Four dishes were prepared for each Mn concentration in experiment A and three for B and C.

2.2.1.1.3. Procedure. Disposable petri-dishes (9 cm. diameter) were poured with 15 ml of agar (autoclaved at 120° C and 100 kPa for 15 minutes). Mycelial cubes (4 mm x 4 mm x 4 mm), removed from the colony edge of an actively growing culture on weak PDA (no added Mn), were used as inocula for test dishes. One mycelial cube was aseptically transferred to the centre of each dish and dishes were kept in the dark at 20° C for 5 days

1 Difco Laboratories, Detroit, Michigan, U.S.A.

(experiment A was conducted at room temperature at an average temperature of 20° C). Colony areas were calculated at regular intervals from the average of 2 measurements of colony diameter taken at right angles to each other. At the end of experiment A the pH of agar under *Ggt* colonies and also of sterile agar was estimated using Merck pH indicator test strips [3].

2.2.1.2. *KMB*. An experiment was conducted to test the effect of increasing rates of $MnSO_4.4H_2O$ on the growth of *Ggt* on solid King's medium B (King *et al.* 1954).

2.2.1.2.1. Inoculum. As for PDA.

2.2.1.2.2. Mn. Increasing concentrations of Mn^{2+} in solid King's medium B were prepared by adding appropriate amounts of a 250 mg kg^{-1} Mn (added as $MnSO_4.4H_2O$) stock solution to media prior to autoclaving for 15 minutes at 120° C and 100 kPa. Mn concentrations of 0,10, 50, 100, 200 mg kg^{-1} Mn were prepared.

2.2.1.2.3. Procedure. As for experiments A and B with PDA, except measurements were stopped after 4 days.

2.2.2. *Ggt* growth through soil at increasing rates of $MnSO_4$ (soil sandwich technique).

2.2.2.1. *Inoculum*. Inocula used in these experiments were *Ggt* 500 colonized whole oat seeds. Oat seeds were autoclaved with an equal volume of $DDDH_2O$ for 30 minutes at 120° C and 100 kPa in cotton wool-plugged wide-mouthed conical flasks and then autoclaved for a further 15 minutes one day later. Once the seeds had cooled they were inoculated with mycelial cubes of *Ggt* 500 taken from the edge of a colony growing on weak PDA (see 2.2.1.) and kept at room temperature for 4 weeks. Flasks were periodically shaken to help spread the fungus evenly through each flask. At the end of 4 weeks, flasks were emptied and the oat seed inocula dried in a sterile air stream. Five or six seeds were chosen at

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2 Div. of Soils, C.S.I.R.O., Glen Osmond, South Australia.
3 E. Merck, Darmstadt, F.R. Germany.

random from each and plated onto full-strength PDA. No contamination was found after 5 days at 20° C. Once dry, the oat seed inocula were transferred to paper bags and stored at room temperature.

2.2.2.2. *Soils*. Two soil types were used ; 1. An infertile, slightly acid to neutral siliceous sand (Uc 2.21, Northcote 1979) which had been collected from the A_2 horizon of a virgin site at Coonalpyn, in the upper south-eastern region of South Australia. This soil-type often results in Mn-deficient lupin crops in this area (Hannam *et al.* 1984). The sand was air-dried for storage in polythene bags but was not prepared for experiments in any other way. 2. A calcareous aeolian sand (Uc 1.11, Northcote 1979) collected at Wangary on the lower Eyre Peninsula of South Australia. This sand has a very high water-holding capacity compared to most sands, contains 80-90 % $CaCO_3$, has a pH of 8.5 (in $CaCl_2$) and a bulk density of only 0.6 g cm^{-3}. Soil was collected separately from depths at 0-10 and 10-20 cm, air-dried and sieved to pass a 2 mm mesh. The organic matter content was further reduced by carefully blowing surface organic matter away with a stream of air. The air-dried sand was placed in polythene bags and stored under cover. In all experiments conducted in this project with this sand an equal mixture, by weight, of top (0-10 cm) and sub (10-20 cm) soil was used.

2.2.2.3. *Mn*. Three separate experiments were conducted, each with 3 replicate dishes per treatment. Coonalpyn sand was used in two experiments (D and E). Mn (as $MnSO_4.4H_2O$) was added dry to the sand, mixed thoroughly, and then the sand was moistened to 10 % moisture content with $DDDH_2O$. The two experiments with Coonalpyn sand differed from each other only in the Mn treatments imposed; In experiment D Mn levels equivalent to 0, 50, 250, 2500 and 5000 mg kg^{-1} Mn^{2+} in air-dry sand were used but in experiment E rates of Mn were 0, 25, 100, 150 and 500 mg kg^{-1}. Wangary sand was used in one experiment (F) and 4 Mn treatments were imposed. Appropriate quantities of $DDDH_2O$ and a 2000 mg kg^{-1} Mn (added as $MnSO_4.4H_2O$) stock solution were added to the air-dry sand to produce a

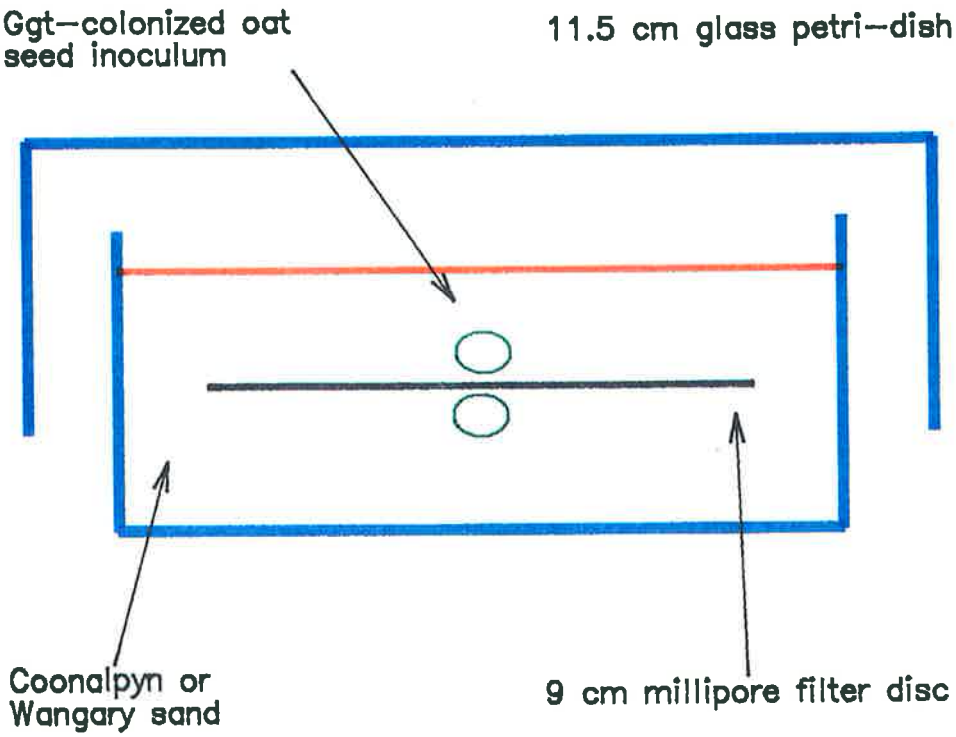
final moisture content of 25 % and Mn levels equivalent to 0, 50, 150, 250 or 500 mg kg^{-1} Mn in air-dry sand before mixing thoroughly.

2.2.2.4. *Procedure*. Figure 2.1 shows the relative placement in each 15 cm glass petri-dish of soil, a millipore filter [4] and the oat seed inoculum. Eighty grammes of wetted soil was added to each dish and compacted lightly with the base of a beaker. An oat seed inoculum was buried flush with the soil surface in the centre of each dish and covered with a filter, followed by another oat seed inoculum (again in the centre of each dish) and covered with a further 93 g of wetted soil. The top layer of soil was also compacted. Petri-dishes were enclosed in two individually sealed polythene bags with an additional dish containing absorbent cotton wool wetted with DDDH_2O. In this way water loss from dishes was minimized. Dishes were kept at 15° C for 15 days. This technique differed in two ways from the soil-sandwich technique of Grose *et al.* (1984). Firstly, an oat seed inoculum was used instead of wheat straw and two inocula were placed in each dish because preliminary experiments had shown that *Ggt* growth had not always occurred in all dishes. With two inocula there was a better chance of getting hyphal growth on at least one surface of the filter in every dish. Secondly, opaque, white millipore filters were used instead of transparent nuclepore filters because they were more readily available and cheaper. Also, it was found that after using millipore filters staining the mycelia was not necessary because the black runner hyphae stood out sharply against the white background of the filters.

2.2.2.5. *Growth measurements*. Each millipore filter was divided into 8 equal sectors drawn through the centre. The maximum radial growth of black runner hyphae and density of hyphae (scoring system of Grose *et al.* 1984) were recorded for each sector on both sides of each filter. Measurements on each surface were averaged over the 8 sectors. After *Ggt* growth had been measured, filters were flooded with 0.1 % benzidine in 1 % ethanol and the

4 Cat. no. SMWP 09025, 9 cm diameter, 5 um pore size, Millipore Corporation, Bedford, Massachusetts, U.S.A. 01730.

FIGURE 2.1. LATERAL VIEW OF APPARATUS USED IN SOIL SANDWICH TECHNIQUE TO MEASURE GGT GROWTH THROUGH SOIL.



rapid appearance of blue colour recorded. The appearance of blue with benzidine is a positive reaction for the presence of Mn oxides (Mellan 1941, Leeper 1970).

2.2.3. Effect of increasing rates of soil Mn on take-all disease and *Ggt* mycelial growth.

The effect of increasing soil Mn on symptoms of *Ggt* infection on wheat roots was assessed with the collective results of 14 pot experiments. Thirteen of these experiments were conducted during the course of this project and the details of the majority of these are in chapters 3, 4, 5, 6 and 7. Those that are not, have not been included in this thesis because they did not contribute anything further to the experiments that have been cited. The remaining experiment was published by Graham and Rovira (1984). Wangary sand and *Ggt* isolate 500 were used in all experiments but they differed in the rates of $MnSO_4$ used, the types of *Ggt* inoculum and resulting disease levels, the size of pots and in 3 of the experiments an additional treatment of suspensions of the biological control agent, *Pseudomonas fluorescens* 2-79 (NRRL B-15132), was imposed (Chapter 7). The average total length of take-all lesions per pot for each Mn rate was expressed as a percentage of the level in 0 Mn controls in each experiment. These results were plotted against the rate of Mn^{2+} added to the soil. The density of *Ggt* mycelia in Coonalpyn sand, similarly expressed, was plotted on the same axes.

2.3. Results.

2.3.1. Growth of *Ggt* on two agars at increasing rates of $MnSO_4$.

2.3.1.1. *PDA*. Figure 2.2 shows the effect of increasing Mn^{2+} concentration on the growth of *Ggt* 500 on PDA. Only data from day 5 have been presented because results from earlier assessments showed very similar effects of Mn^{2+}. This plot clearly shows that *Ggt* growth on this agar was very sensitive to Mn^{2+} and that colony area declined sharply in the concentration range of 70-100 mg kg^{-1}. Plate 2.1 shows representative dishes from each Mn rate, photographed on day 5.

Plate 2.1. Effect of increasing concentrations of manganous ions on the growth of *Ggt* 500 on weak potato dextrose agar after 5 days at 20° C.

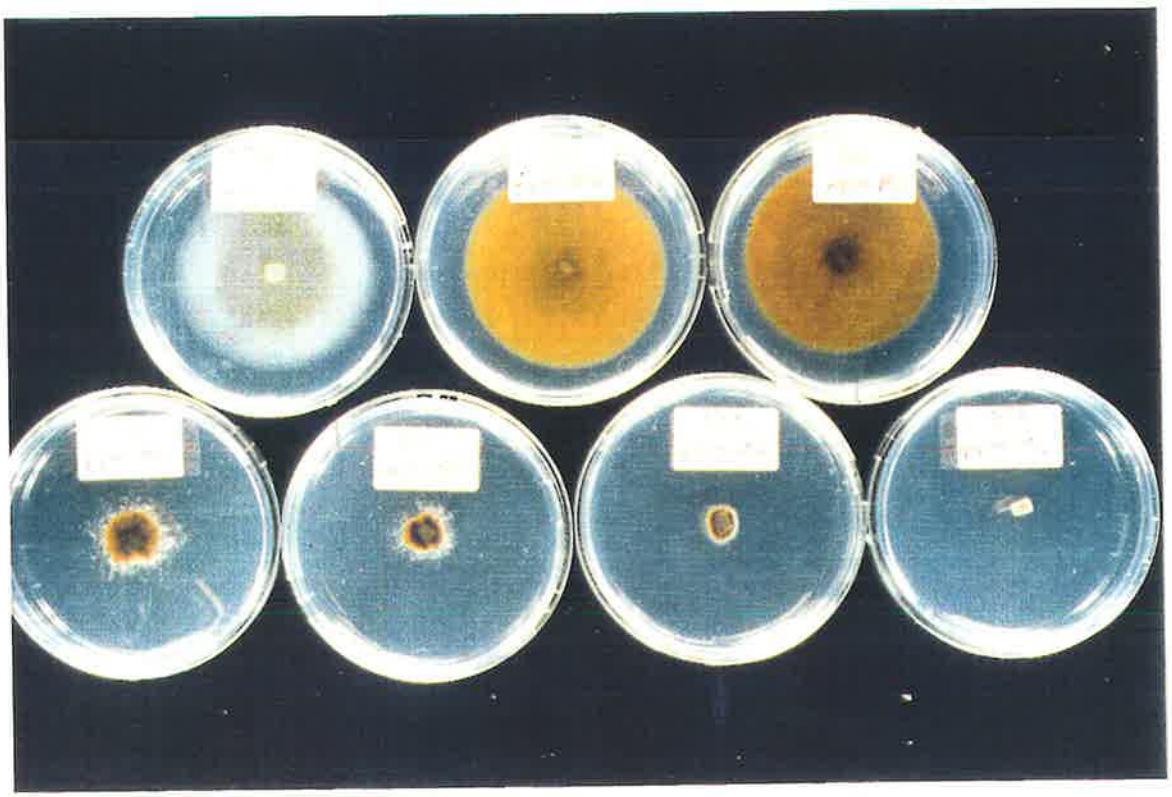
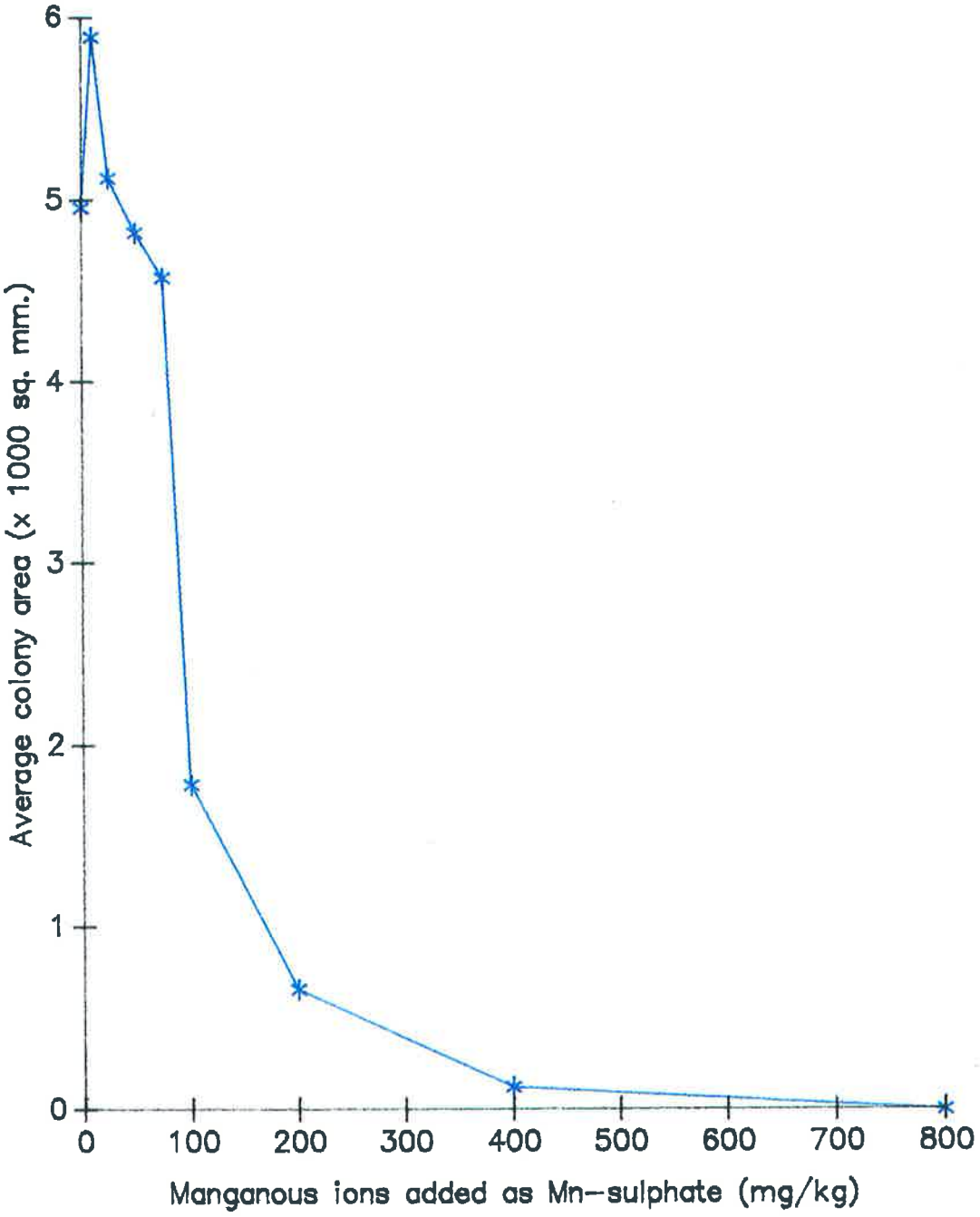


FIGURE 2.2. EFFECT OF INCREASING CONCENTRATION OF MANGANOUS IONS ON THE GROWTH OF GGT 500 ON WEAK PDA AFTER 5 DAYS AT 20o C.



There was a small stimulation of growth at the lowest rate of added Mn^{2+} which indicates that Mn levels in unamended PDA were too low for maximum growth of this fungus under the experimental conditions. Analysis of the PDA used for experiments A and B revealed that only 1 mg kg^{-1} of Mn was present which dilutes by more than 60 fold in the final preparation.

Ggt 500 was a very ready oxidizer of Mn^{2+}. Extensive brown/black deposits of Mn oxides were present in all dishes to which Mn had been added (see plate 2.1) and they appeared in mycelial cubes within 30 minutes of their transferral. Mn must have diffused into the mycelial cubes from agar in the dishes. These black/brown deposits were identified as Mn oxides because they stained blue in the presence of 0.1 % benzidine in 1 % ethanol. The Mn oxides were deposited as discrete particles in the agar and formed brown `halos' around all hyphae which grew within 1-2 mm of the agar surface. At rates of Mn equal to or greater than 100 mg $kg^{-1}$ the pattern of *Ggt* growth and Mn oxide deposition was altered. The fungus tended to grow as macrohyphae (`ropes' of many individual hyphae intertwined together) with clumps of hyphal proliferation at intervals along the macrohyphae. Mn oxidation was concentrated at the clumps of hyphal proliferation.

Estimation of agar pH showed that the pH beneath mature, Mn-oxidizing regions of a *Ggt* colony was between 6 and 7 but the pH of sterile agar and agar beneath the outer, non-oxidizing, edges of *Ggt* colonies was between 5 and 5.5. In an earlier experiment (not presented) dishes had become contaminated and many bacterial colonies which formed inhibited Mn oxidation by *Ggt* but had little effect on mycelial growth. The pH of agar beneath these bacterial colonies was less than 4.5.

2.3.1.2. *KMB*. *Ggt* 500 grew well on solid KMB at all Mn^{2+} concentrations (table 2.1). The slowest growth occurred in the 0 Mn control and all Mn concentrations stimulated growth although there was little increase with 10 mg kg^{-1} Mn.

Table 2.1. Effect of increasing concentrations of Mn^{2+} in KMB on growth of *Ggt* 500. Colony areas were calculated from 2 measurements of colony diameter, taken at right angles to each other, after 5 days at 20° C and were averaged over 3 replicate dishes. Values in parentheses are standard errors for each mean.

Mn added (mg kg^{-1})	0	10	50	100	200
Ggt colony area (mm^2)	2965 (254)	3288 (162)	3668 (36)	4072 (57)	3868 (103)

The only evidence for a toxic effect of Mn^{2+} occurred with 200 mg kg^{-1} Mn where colony area was smaller than at 100 mg kg^{-1}, but this growth was still faster than in the 0 Mn control.

Mn oxidation by *Ggt* occurred on this agar at all concentrations of added Mn but was restricted to a few isolated black/brown flecks on the colony surface which were acutely elliptic in shape. These flecks were confirmed to contain Mn oxides with benzidine. The largest of these flecks were 2-4 mm long and 1-1.5 mm wide.

2.3.2. *Ggt* growth through soil at increasing concentrations of Mn (soil sandwich technique).

2.3.2.1. *Coonalpyn sand.* Tables 2.2 and 2.3 show the effect of increasing Mn^{2+} concentration on growth of *Ggt* through Coonalpyn sand for experiments D and E, respectively. Due to a small difference in absolute radial growth rates between experiments D and E measurements were expressed as a percentage of the 0 Mn control in each experiment so that they could be compared over the whole range of Mn concentrations (see figure 2.3).

FIGURE 2.3. EFFECT OF INCREASING CONCENTRATION OF MANGANOUS IONS ON MYCELIAL GROWTH OF GGT 500 IN COONALPYN SAND.

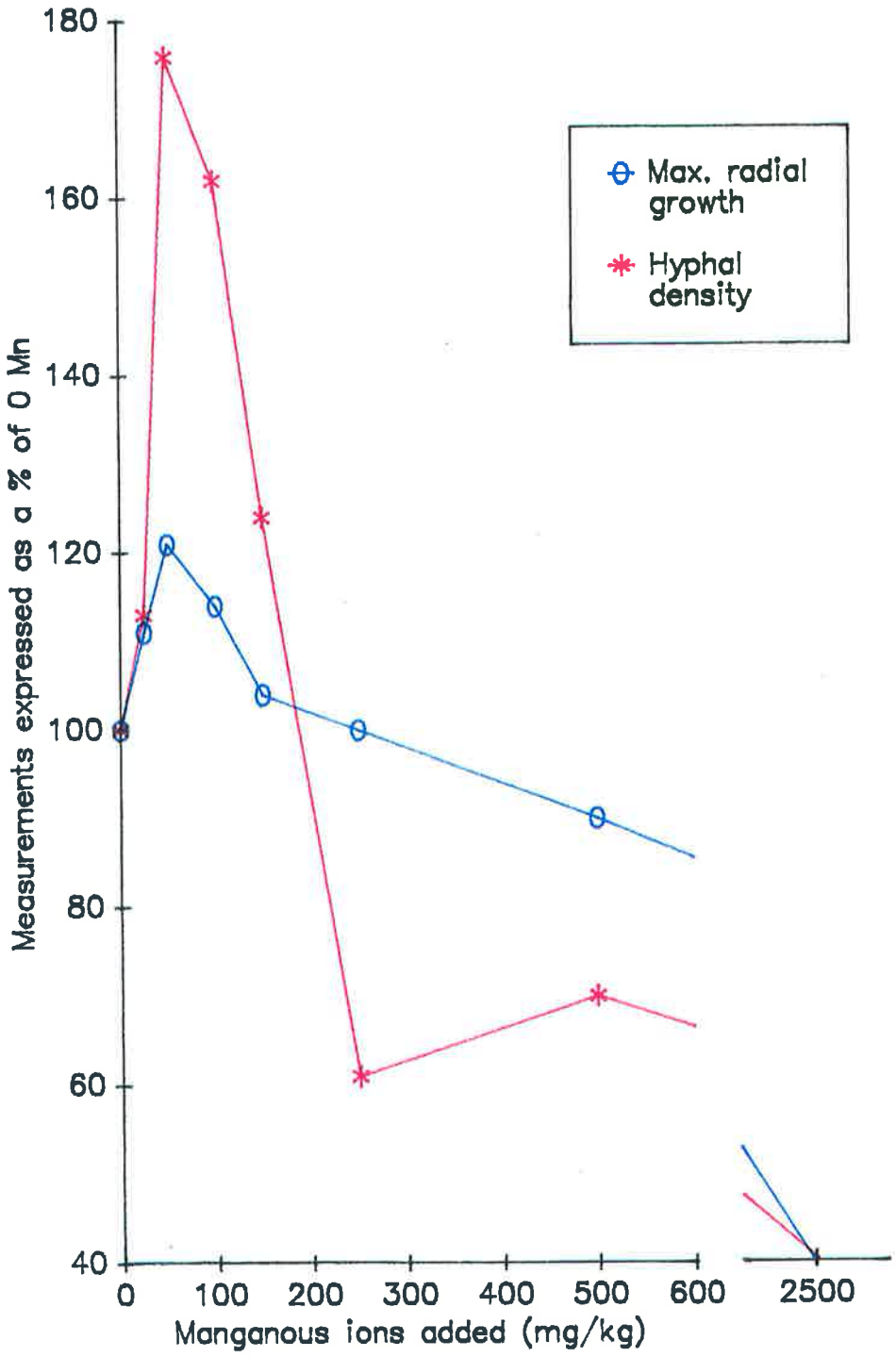


Table 2.2. Effect of increasing concentrations of Mn^{2+} in Coonalpyn sand on mycelial growth of *Ggt* 500, experiment D. Values in the table are the average of 3 replicate dishes, which in turn, were the average of growth in 8 equal sectors on the top surface of the filter in each dish. Values in parentheses are standard errors for each mean.

Mn added (mg kg^{-1})	0	50	250	2500	5000
Max. radial growth (mm^2)	31.4 (2.6)	38.0 (1.0)	31.3 (4.4)	0 (0)	0 (0)
Hyphal density[a]	2.48 (0.22)	4.35 (0.35)	1.50 (0.13)	0 (0)	0 (0)

[a] 0 (No growth) - 6 (Plate-like growth) (Grose *et al.* 1984)

Only growth measurements for top surfaces of filters in experiment D and for bottom surfaces in experiment E are presented because growth on the opposite surfaces was negligible. No explanation can be offered for this difference unless mycelia did not adhere well to one surface of the millipore filters and that the filters were placed upside-down in experiment E.

Table 2.3. Effect of increasing concentrations of Mn^{2+} in Coonalpyn sand on mycelial growth of *Ggt* 500, experiment E. Values in the table are the average of 3 replicate dishes, which in turn, were the average of growth in 8 equal sectors on the bottom surface of the filter in each dish. Values in parentheses are standard errors for each mean.

Mn added (mg kg^{-1})	0	25	100	150	500
Max. radial growth (mm^2)	26.3 (0.3)	29.3 (2.0)	30.0 (1.7)	27.3 (2.6)	23.7 (3.3)
Hyphal density[a]	2.4 (0.26)	2.73 (0.38)	3.93 (0.33)	3.00 (0.65)	1.70 (0.15)

[a] 0 (No growth) - 6 (Plate-like growth) (Grose *et al.* 1984)

Hyphal density was found to be a more sensitive indicator of Mn^{2+} effects on *Ggt* growth, rather than maximum radial growth. It showed that concentrations of more than 150 mg kg^{-1} of added Mn^{2+} were required before *Ggt* growth was decreased below levels in 0

Mn controls. Maximum radial growth was not similarly affected until concentrations exceeded 250 mg kg^{-1} of added Mn^{2+}.

Ggt growth was stimulated at low concentrations of Mn and hyphal density reached a maximum of nearly double that in 0 Mn controls at 50 mg kg^{-1} of added Mn.

Flooding filters with benzidine revealed that *Ggt* mycelia had oxidized observable amounts of Mn only in treatments which had increased hyphal density, i.e. between 25 and 150 mg kg^{-1} Mn. Plate 2.2.A shows the effect of flooding filters with benzidine at 0, 50 and 250 mg kg^{-1} of added Mn and Plate 2.2.B is a close-up of a *Ggt* mycelium grown at 100 mg kg^{-1} Mn and shows that deposition of Mn oxides closely followed individual hyphae. Mn oxidation by *Ggt* hyphae was inhibited at rates of Mn which decreased hyphal density and was not detected with the benzidine test.

2.3.2.2. *Wangary sand.* *Ggt* growth through Wangary sand at increasing rates of added Mn^{2+} is summarized in table 2.4. Wangary sand was found to be unsuitable for the soil-sandwich technique because it was very difficult to cleanly remove the filters from the sand layers and the *Ggt* mycelia frequently stuck to the sand rather than adhering to the filter surface. This meant that it was not possible to accurately measure *Ggt* growth in many of the dishes and the effect of increasing concentrations of Mn could not be adequately tested. However, a comparison of results from individual dishes from each Mn treatment where mycelial growth was extensive and reliably measured, suggested that there was little effect of Mn rates on *Ggt* mycelial growth and growth at 500 mg kg^{-1} was the same as growth at 0 Mn.

Plate 2.2A. Effect of 0, 50 and 250 mg kg^{-1} of added Mn^{2+} on mycelial growth of *Ggt* 500 in Coonalypyn sand. Right-hand millipore discs at each Mn rate were flooded with 0.1 % acidified benzidine (stains blue with Mn oxides) prior to being photographed.

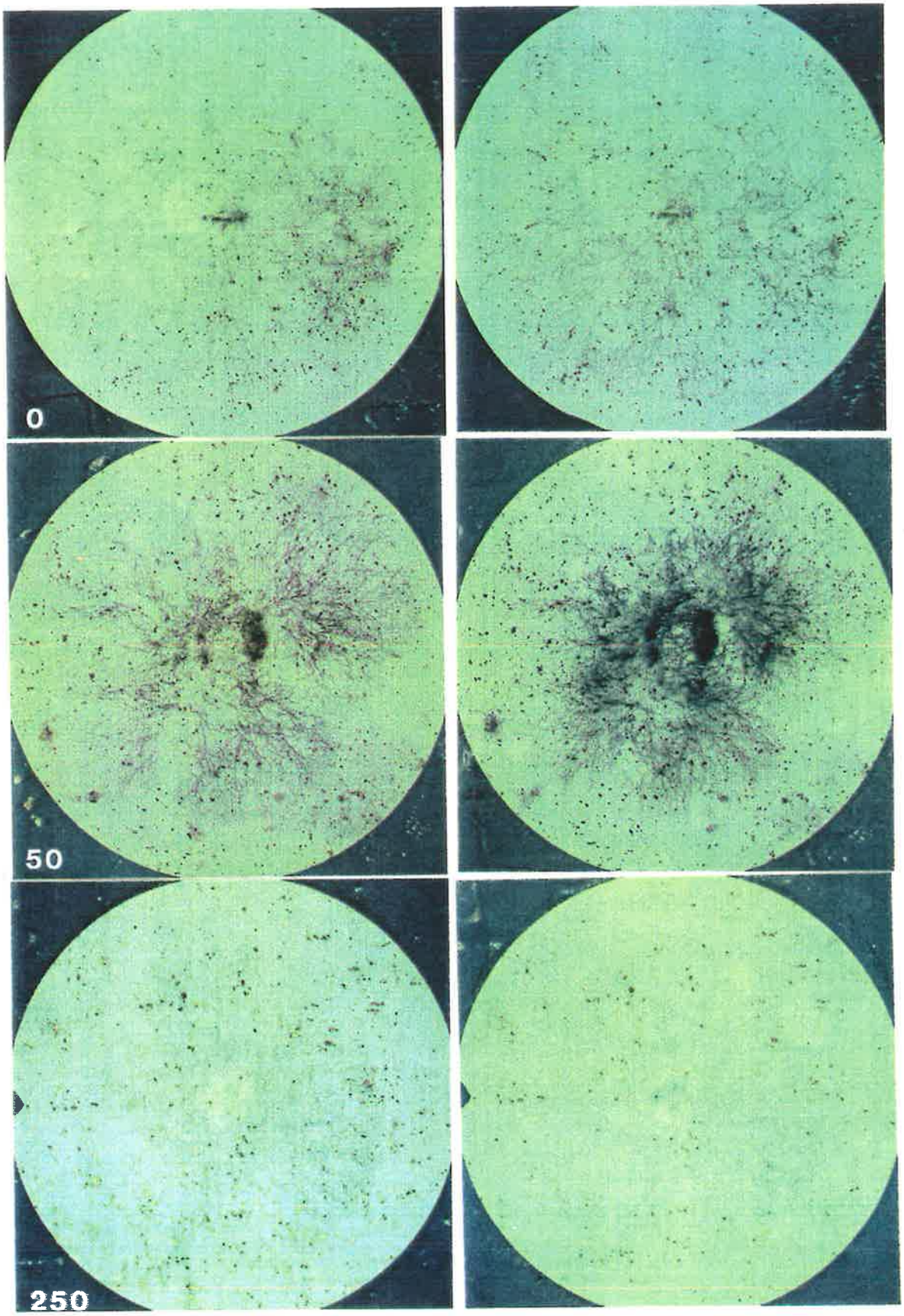


Plate 2.2B. Close-up of mycelial growth of *Ggt* 500 in Coonalpyn sand with 100 mg kg^{-1} of added Mn^{2+}, after flooding with 0.1% acidified benzidine. The darkly coloured hyphae in the top of the photograph were coated in Mn oxides.

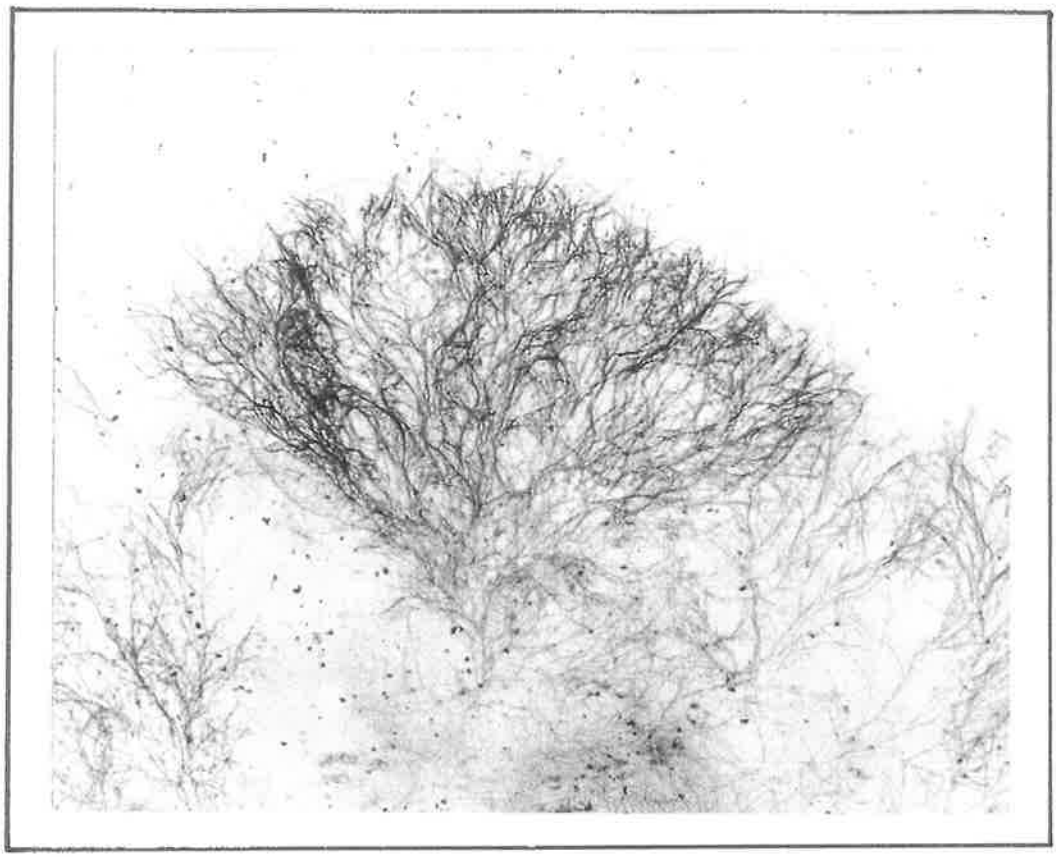


Table 2.4. Effect of increasing concentrations of Mn^{2+} in Wangary sand on mycelial growth of *Ggt* 500, experiment F. Values in the body of the table are the average of growth in 8 equal sectors in each of 3 replicate dishes. Values in parentheses are standard errors for each mean.

Mn added (mg kg^{-1})	0	50	150	250	500
Max. radial growth (mm^2)	28.9	20.9	10.6	1.9	10.9
	10.5	6.6	26.1	22.8	25.9
	0.0	16.5	9.5	8.6	21.9
(Average)	13.1	14.7	15.4	11.1	19.6
	(8.4)	(4.2)	(5.4)	(6.2)	(4.5)
Hyphal density[a]	2.81	1.56	0.63	0.13	0.81
	0.75	0.44	1.94	2.06	2.69
	0.00	1.38	0.63	1.31	1.56
(Average)	1.19	1.13	1.07	1.17	1.69
	(0.84)	(0.35)	(0.44)	(0.56)	(0.55)

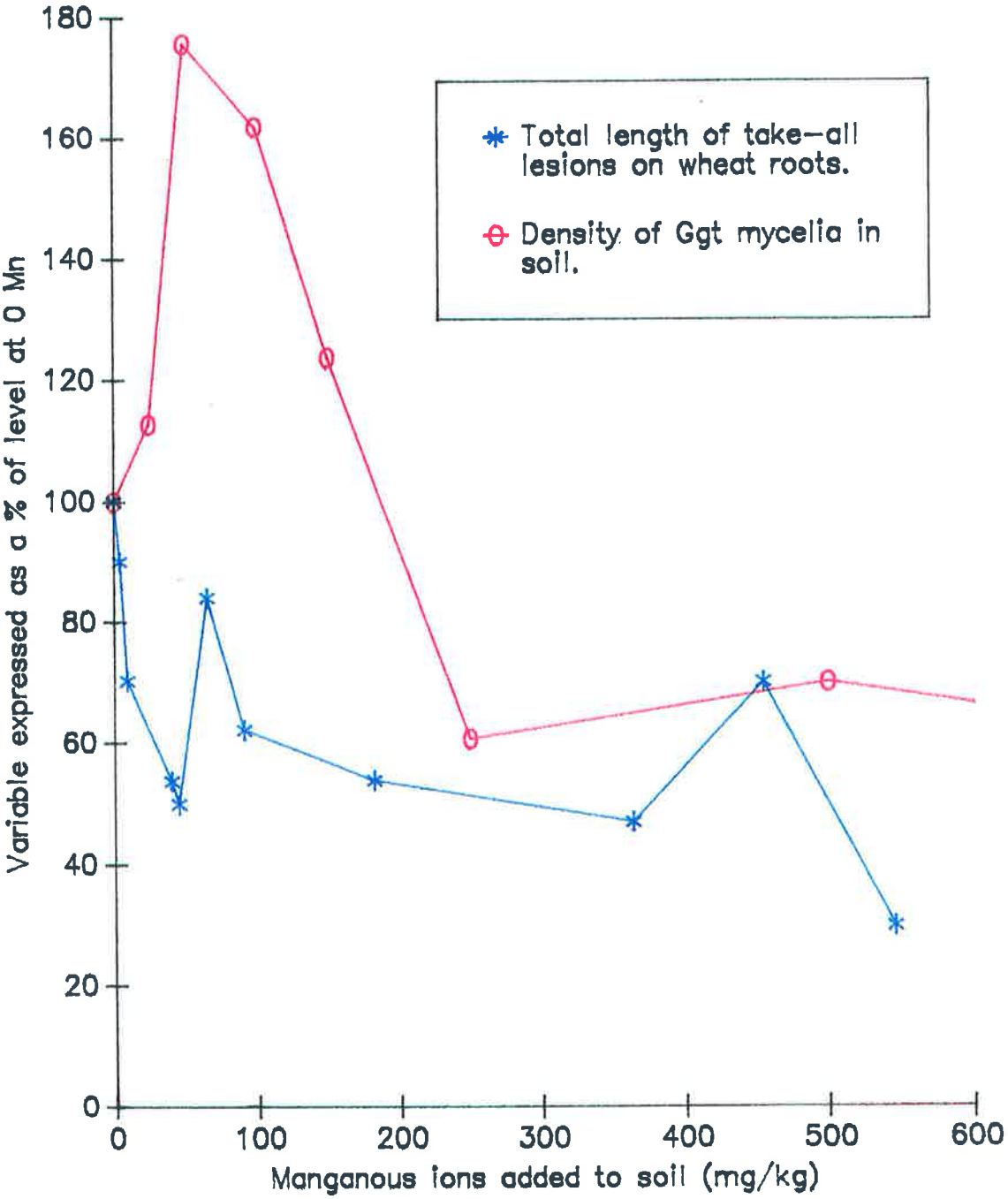
[a] 0 (No growth) - 6 (Plate-like growth) (Grose *et al.* 1984)

Very extensive Mn oxidation by *Ggt* hyphae was observed at 150, 250 and 500 mg kg^{-1} Mn. The growth of *Ggt* mycelia in Wangary sand appeared to differ from growth in Coonalpyn sand in 3 ways ; 1. There was no evidence of growth stimulation at low rates of Mn. 2. Mycelial growth at 500 mg kg^{-1} Mn was not different to growth at 0 Mn and 3. Mn oxidation by hyphae at 500 mg kg^{-1} Mn was very extensive. The highest maximum radial growth measured in Wangary sand compared well with average values obtained in 0 Mn controls in Coonalpyn sand.

2.3.3. Effect of increasing rates of soil Mn on take-all disease and *Ggt* mycelial growth.

Figure 2.4 shows a plot of the separate effects of increasing rates of Mn^{2+} added to soil on *Ggt* mycelial growth and on the level of symptoms of *Ggt* infection on wheat roots. This plot clearly shows that rates of soil-added Mn which were sufficient to halve take-all symptoms on wheat roots (40-91 mg kg^{-1} Mn) were not toxic to the pathogenic fungus in the soil but actually stimulated mycelial growth. At higher rates of soil-added Mn, where fungal growth was inhibited, there was little evidence of a further and associated decrease in take-all

FIGURE 2.4. EFFECT OF INCREASING MN CONCENTRATION ON GGT GROWTH OR TAKE-ALL OF WHEAT.
Saprophytic growth of Ggt through Coonalpyn sand. Wheat plants grown in Mn-deficient Wangary sand.



symptoms. Both of these results indicate that Mn effects on *Ggt* growth in soil can not explain the decrease in take-all symptoms on wheat roots with increasing rates of soil-applied Mn^{2+}.

Due to the difficulties encountered in using Wangary sand in the soil-sandwich technique, the comparisons made here between *Ggt* mycelial growth in soil and take-all symptoms on wheat roots involved different soil types. However, the limited evidence that was gained from using Wangary sand in the soil-sandwich technique suggested that *Ggt* growth was less sensitive to soil-applied Mn than in Coonalpyn sand which would further separate the effects of soil Mn on *Ggt* mycelial growth and on the level of take-all symptoms on wheat roots.

2.4. Discussion and Conclusions.

The hypothesis proposed by Graham and Rovira (1984) that Mn may decrease take-all by directly inhibiting *Ggt* growth was not supported by results obtained here. The collective results of 14 pot experiments showed that rates as low as 9 mg kg^{-1} of Mn^{2+} added to soil were sufficient to decrease take-all symptoms on wheat roots but more than 150 mg kg^{-1} Mn were required to cause inhibition of fungal growth and low rates of soil-applied Mn which decreased take-all symptoms actually stimulated fungal growth. In addition, at rates of soil Mn which were toxic to *Ggt* growth there was little evidence of an associated, further decrease in take-all symptoms which would be expected if the effect of Mn on take-all was to inhibit growth of the causal fungus through soil. Unfortunately, a comparison of the effect of Mn on *Ggt* growth through soil and on take-all symptoms could not be made in the same soil type because Wangary sand was found to be unsuitable for the soil sandwich technique. However, the few results that were obtained suggested that *Ggt* growth was even less sensitive to Mn in Wangary sand compared with Coonalpyn sand.

The effect of external concentrations of Mn^{2+} on *Ggt* growth on agar was not a reliable predictor of effects of Mn on *Ggt* growth through a soil matrix because results differed markedly between the two agars and neither agar closely reflected results obtained with the soil sandwich technique. *Ggt* growth was most sensitive to Mn on PDA and at 200 mg kg^{-1} Mn was less than 20 % of the 0 Mn control but on KMB growth was stimulated by this concentration of Mn. Even so, the sensitivity of *Ggt* on PDA could not explain the reduction in take-all symptoms on wheat roots with very low rates of Mn because take-all symptoms were decreased at Mn rates less than required to inhibit *Ggt* growth on PDA.

The mineral requirements of *Ggt* in culture are not known (Sivasithamparam and Parker 1981) but reports (Foster 1939, Perlman 1949) indicate Mn^{2+} optima for other fungi to range upward to a few parts per million. This fungal requirement for Mn^{2+} may explain the small stimulation of *Ggt* growth on PDA at low rates of Mn because the PDA used was found to contain a very low concentration of Mn (less than 0.02 mg kg^{-1} in final preparation) but does not explain the additional benefits of 50 and 100 mg kg^{-1} Mn in KMB, or of 50 mg kg^{-1} over 25 mg kg^{-1} in Coonalpyn sand.

It is difficult to compare the relative sensitivity of *Ggt* to Mn with other micro-organisms because of the widely different results obtained here and the different experimental conditions used in relevant papers. For instance, Wilks *et al.* (1983) referred to earlier work which showed that the growth of *Ceratocystis wageneri* (cause of black-stain root disease of ponderosa pine) was increased by 20 % in culture media containing 1000 mg kg^{-1} Mn. This fungus seemed to be very well adapted to high Mn environments and its tolerance to Mn was obviously much greater than *Ggt*, even allowing for different cultural conditions. On the other hand, six mycorrhizal fungi were reported to show at least 65 % growth on Melin-Morkran's medium at 500 mg kg^{-1} Mn, compared with the 0 Mn control (Thompson and Medve 1984) which is a level of sensitivity similar to that exhibited by *Ggt* in Coonalpyn sand and on KMB.

Ggt was found to be a powerful oxidizer of Mn^{2+} on PDA and in soil and limited oxidation was also observed on KMB. Estimation of the pH of PDA in the presence of *Ggt* colonies showed that where Mn oxidation was occurring the pH was between 6 and 7 but the pH of sterile PDA was 5.5. Bacterial contamination of some PDA cultures inhibited oxidation of Mn by *Ggt* and decreased the pH of the agar to less than 4.5 but did not affect the rate or pattern of mycelial growth. The oxidation of Mn is favoured by increasing pH (Leeper 1970), especially by rises in the range of slightly acid to neutral. These results suggest that the oxidation of Mn by *Ggt* was simply due to increased pH in the environment as a consequence of the metabolic activities of *Ggt*. However, Mn oxidation by *Ggt* on KMB, at an average pH of 7, was very limited so a simple pH effect may not be the only mechanism operating. A *Streptomyces* sp. has been reported to produce an extra-cellular non-dialysable substance which oxidized Mn^{2+} to MnO_2 (Bromfield 1979).

The oxidation of Mn^{2+} to insoluble oxides by *Ggt* may help protect the fungus from toxic levels of Mn^{2+} in its environment. This proposal is supported by the observation that the oxidation appeared to occur at a distance from the hyphal wall. Alternatively, the fungus may derive energy from the exergonic reaction (Leeper 1970) of Mn^{2+} oxidation, which has been suggested for other micro-organisms (Ghiorse 1984), although this is difficult to reconcile with oxidation occurring some distance from the fungal hyphae.

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CHAPTER 3.

DEVELOPMENT OF AN AGAR DISC INOCULUM TECHNIQUE FOR *GGT* IN POT STUDIES

CHAPTER 3. DEVELOPMENT OF AN AGAR DISC INOCULUM TECHNIQUE FOR *GGT* IN POT STUDIES

3.1. Introduction.

Two different *Ggt* inoculum techniques were reported in the pot experiments conducted by Graham and Rovira (1984) and Rovira *et al.* (1985). Ground oat kernel inoculum (0.25-0.5 mm particle size), mixed through dry soil, was used in the larger and former of these two experiments and *Ggt* colonized dead rye-grass seeds in the other. The first pot experiment conducted in this project confirmed the results of Graham and Rovira (1984), using the same techniques, inoculum and experimental design. The inoculum was the same preparation used by Graham and Rovira (1984), which had been subsequently stored at 4º C. The results reported here closely matched those of the original experiment. However, when a fresh batch of ground oat kernel inoculum was prepared, and a further experiment conducted, the levels of disease which resulted from the same rates of inoculum were much higher. It was unsatisfactory to continue with an inoculum technique which produced widely different disease levels between batches so two alternative inoculum techniques were tried; PDA discs removed from actively growing *Ggt* colonies and *Ggt* colonized sand grains.

Rovira (unpublished) has used the technique of placing *Ggt* colonized agar plugs at various depths in pots to screen biological controls agents against take-all. Agar discs placed immediately below germinating seeds have also been used to infect plants with *Ggt* in various other studies (Garrett 1934a, Chambers and Flentje 1967a,b, Brown *et al.* 1973). The effect of increasing strength of PDA and depth of burial of the agar plug on *Ggt* infection were investigated here.

MacNish *et al.* (1986) published a technique in which *Ggt* colonized sand grains were mixed through soil as inoculum. They found that the colonized sand grains were a low nutrient source, were of uniform size and easily mixed through soil and produced proportional infection per unit of inoculum over a wide range of densities. They proposed

the technique was amenable to test the effects of treatments like N sources, fungicides and biological control agents on the infection of wheat seedlings by *Ggt* so its suitability for these studies was also tested.

A suitable inoculum would be simple to prepare and easily applied so that many pots could be set-up at the same time. Also, it would need to produce similar disease levels between batches and regularly increasing disease levels with increasing rates.

3.2. Materials and Methods.

3.2.1. Confirmation of soil-applied Mn decreasing take-all of wheat in pots.

The experiment reported by Graham and Rovira (1984) was repeated to confirm the effect that soil Mn decreased take-all of wheat. The experiment was of factorial design with 3 rates of Mn (0, 1 and 10 Mn), 3 rates of *Ggt* inoculum (0, 0.05 and 0.1 Ggt) and 5 replicates.

3.2.1.1. *Inoculum*. The original *Ggt* 500 ground oat kernel inoculum of Graham and Rovira (1984) was used at the same rates of 0, 0.05 and 0.1 % w/w of air-dry soil. The complete details of the preparation of this inoculum were reported by Rovira *et al.* (1985). The pathogen was grown for 4 weeks at 25° C on autoclaved whole oats that had imbibed water for 24 hours before autoclaving. The oat kernels were ground and only the 0.25-0.5 mm fraction used. The inoculum was later stored at 4° C. Autoclaved inoculum at 0.1 % was used in 0 Ggt pots.

3.2.1.2. *Soil*. Wangary sand was used (refer to section 2.2.2. for details of this soil) with basal nutrients added at the rate of 101 mg $Ca(NO_3)_2.4H_2O$, 15.8 mg KH_2PO_4, 12.5 mg K_2SO_4, 7.7 mg $MgSO_4.7H_2O$, 4.8 mg H_3BO_3, 2.9 mg $ZnSO_4.7H_2O$, 1.9 mg $FeSO_4.7H_2O$, 1.4 mg NaCl, 1.0 mg $CuSO_4.5H_2O$, 0.1 mg $CoSO_4.7H_2O$ and 0.1 mg $H_2MoO_4+H_2O$ per pot. Basal nutrients were added in solution and mixed through soil prior to sowing. KH_2PO_4 was added to soil in a separate solution to avoid precipitation of

$Ca_3(PO_4)_2$. Additional $DDDH_2O$ (adjusted to allow for Mn solutions) was added to each soil batch to ensure that all soil was prepared to 25 % water content.

3.2.1.3. *Mn.* Mn (as a $MnSO_4.4H_2O$ solution) was mixed through the soil at 3 different rates to give Mn treatments of 0, 1 and 10 mg of Mn per pot.

3.2.1.4. *Procedure.* Individual pots were filled with the equivalent of 240 g of air-dry soil, and compacted firmly. Five germinated wheat seeds, *Triticum aestivum* cv. Condor harvested at Karoonda in South Australia in 1983 (av. Mn concentration of 5 mg kg^{-1}), were sown 1 cm deep in each pot. Seeds were prepared for sowing by placing them in filter-paper lined glass petri-dishes, moistened with $DDDH_2O$, for 2 days at room temperature.

Pots were placed in randomized blocks at 15° C with a 12 hour light period (average photon flux density of 0.35 mEinsteins m^{-2} s^{-1}, supplied by a bank of fluorescent tubes) in every 24. Seeds which had not emerged 10 and 17 days after sowing were replaced with germinated seeds which had been kept at 4° C. Pots were watered to original weight every 2-3 days until harvest after 26 days.

Shoots were removed at the soil surface and fresh weight per pot recorded before drying at 70° C for 2 days. Roots were washed free of soil with reverse osmosis water (ROH_2O) and fresh weight recorded. The number of black stelar lesions, the total length of stelar lesions and the number of diseased seminal roots were recorded for each plant. After disease severity had been assessed the fresh weight of roots per pot was recorded (after free water was removed) before they were dried at 70° C for 2 days. Oven-dry weights for shoots and roots were also recorded prior to analysis of both tissues for Mn with a Philips SP9 atomic absorption spectrophotometer. Tissue was prepared for analysis by nitric/perchloric acid digestion.

Data were analyzed with ANOVA techniques appropriate to factorial designs.

3.2.2. Testing the effect of Mn on take-all of wheat in pots using a fresh preparation of ground oat kernel inoculum.

This experiment was conducted to test the effect of soil and foliar applied Mn on take-all of pot-grown wheat using a fresh preparation of ground oat kernel inoculum. The experiment was of factorial design with 2 rates of soil Mn (-, +soil Mn), 2 rates of foliar Mn (-, +folMn), 2 levels of *Ggt* inoculum (-, +Ggt) and 5 replicates.

3.2.2.1. *Inoculum*. Ground oat inoculum was prepared as in 3.2.1. except that the pathogen was incubated for 4 weeks at room temperature instead of 25° C. Live inoculum at the rate of 0.1 % w/w was used for +Ggt and autoclaved inoculum at the same rate for -Ggt.

3.2.2.2. *Soil*. Soil Mn was prepared as in 3.2.1. except $MnSO_4.H_2O$ was inadvertently used instead of $MnSO_4.4H_2O$ which resulted in a rate for +soil Mn of 13 mg of Mn per pot (rather than the 10 mg of Mn per pot used in 3.2.1.). Foliar Mn was applied to leaves 4, 7 and 12 days after sowing as a 1.5 % $MnSO_4.H_2O$ solution with wetting agent. $DDDH_2O$ with wetting agent was used for -folMn.

3.2.2.3. *Procedure*. As in 3.2.1. with the following alterations;

1. Seeds were germinated in filter paper lined petri-dishes, wetted with $DDDH_2O$, for 1 day at 4° C and a further day at 20° C. This technique was used in all subsequent pot experiments.
2. Pots were filled with less soil (200 g rather than 240 g of air-dry soil) and were not compacted.
3. Plants were grown at 15° C with a 10 hour light period (average photon flux density of 0.25 mEinsteins m^{-2} s^{1}, supplied by a bank of fluorescent tubes) in every 24.
4. Plants were harvested after 21 days.
5. Fresh weight of roots were recorded prior to disease assessment.

3.2.3. Development of PDA discs as an inoculum source for *Ggt*.

3.2.3.1. *Effect of concentration of PDA in agar discs on take-all of wheat in small pots.* This experiment was conducted to test the effect of increasing concentration of PDA in *Ggt* colonized agar discs on take-all of wheat seedlings grown in small pots. A factorial design was employed with 3 rates of soil applied Mn (0, 0.4 and 4 Mn), 3 rates of Ggt treatments (4 % sterile PDA, 1 % *Ggt* colonized PDA and 4 % *Ggt* colonized PDA), 2 rates of *Pseudomonas fluorescens* 2-79 RN_{10} (NRRL B-15132) (-, +2-79) and 5 replicates. The isolate of *Pseudomonas fluorescens* used in this experiment is a known antagonist against *Ggt* `in vitro' and has been shown to decrease take-all of wheat in the field (Weller and Cook 1983). This experiment is reported here because it illustrated the effects of PDA concentration on resulting take-all of wheat but the details of pots to which live suspensions of *Pseudomonas fluorescens* were added have been omitted because they are beyond the scope of this chapter. The complete details of studies conducted with this bacterium are presented in Chapter 9.

3.2.3.1.1. Inoculum. Petri-dishes were poured with approximately 17 ml of autoclaved media containing 1 g of `Difco' Bacto-agar and either 0.039 (1 %) or 0.156 g (4 %) of PDA in 100 ml of $DDDH_2O$. Dishes were inoculated with single mycelial cubes taken from the edge of an actively growing colony of *Ggt* 500 and placed at 20° C for 9 days. Extra dishes of 4 % PDA were made up and kept sterile for use in 0 Ggt pots.

3.2.3.1.2. Soil. Wangary sand was prepared as in 3.2.1. with basal nutrients added at the same rate on a per plant basis.

3.2.3.1.3. Mn. Mn was mixed through soil as in 3.2.1. to give either 0, 0.4 or 4 mg of Mn per pot (which were the same rates of Mn prepared in 3.2.1. on a per plant basis).

3.2.3.1.4. Procedure. `Pine-cell' [1] pots were used in this and all subsequent pot experiments. These pots have a 2.5 cm top diameter and are 16 cm long with a 1^{o} degree taper. They are made of opaque white polypropylene and are held in moulded trays of 200 pot capacity. All pots were washed in 10 % HNO_3 and rinsed in $DDDH_2O$ before first use. The drainage holes were taped over and each pot held the equivalent of 44 g of air-dry Wangary sand.

Pots were part-filled with 42.5 g of prepared soil, followed by an agar disc which had been cut to the internal diameter of the pots, a further 8.4 g of soil (resulting in a 2 cm deep layer), 2 germinated wheat seeds (as in 3.2.2. except seeds were surface-sterilized with 6 % NaOCl for 90 s prior to germination) and a final layer of 4.1 g of soil. *Ggt* colonized agar discs were cut from the outer margins of *Ggt* colonies which had reached the edge of petri-dishes by the day of sowing. Two ml of autoclaved *Pseudomonas fluorescens* suspension was added to the soil surface of each pot.

Pots were placed in a tray in randomized blocks and covered with a sheet of thin clear polythene (`Glad Wrap') to reduce drying of the soil surface, before being placed in controlled environment conditions of 15^{o} C with a 10 hour light period (average photon flux density of 0.2 mEinsteins m^{-2} s^{-1} supplied by a bank of fluorescent tubes) in every 24.

`Glad Wrap' was removed after 2 days and pots were watered to original weight every 3-4 days. Plants were harvested after 25 days and plants processed as in 3.2.1. Oven-dry shoots and roots were analyzed for Mn and 11 other essential elements with an ARL inductively coupled atomic emission spectrometer. Samples were prepared for analysis by nitric acid digestion.

Data were analyzed with ANOVA techniques appropriate to factorial designs in randomized blocks.

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1 Ray Leach 'Cone-tainer' Nursery, Oregon, U.S.A.

3.2.3.2. *Effect of depth of burial of agar disc on take-all of wheat in small pots.* Due to the limited success of producing reasonable differences in *Ggt* infection with changes in PDA concentration in agar discs (see section 3.3.3.1. below), the depth of burial of *Ggt* colonized agar discs of the same PDA concentration below germinating seeds was varied. The factorial experiment involved 2 rates of soil-applied Mn (0, 4 Mn), 4 Ggt treatments (*Ggt* colonized agar discs buried 2, 4 and 6 cm below seeds and a control of no agar disc) and 3 replicates.

3.2.3.2.1. Inoculum. *Ggt* colonized 4 % PDA discs prepared as in 3.2.3.1. Sterile 4 % PDA discs were used in -Ggt pots.

3.2.3.2.2. Soil. Wangary sand prepared as in 3.2.3.1.

3.2.3.2.3. Mn. Rates of 0 and 4 mg of Mn per pot were prepared as in 3.2.3.1.

3.2.3.2.4. Procedure. As in 3.2.3.1. with the following changes ;

1. Iron was added to soil as a separate solution. This meant that large volumes of basal nutrient (-Fe) and phosphorus stock solutions could be made up and used for many pot experiments and only fresh $FeSO_4.7H_2O$ solution was needed for each experiment.
2. Pots were part-filled with either 50.9 (0 Ggt control), 25.7 (6 cm burial of agar disc), 34.1 (4 cm burial) or 42.5 g (2 cm burial) of prepared soil and a further soil layer above agar discs of 0, 25.2, 16.8 or 8.4 g, respectively. All pots were filled with a final layer of 4.1 g of soil.
3. Condor seeds were harvested from Avon in the lower North region of South Australia in 1980 but were not surface-sterilized prior to germination.
4. All pots received 1 ml of $DDDH_2O$ to the soil surface and plants were grown at 15/10° C day/night temperatures with a 10 hour light period (average photon flux density of 0.7 mEinsteins m^{-2} s^{-1} supplied by a bank of mercury vapour lamps) in every 24.

3.2.4. Colonized sand grains as *Ggt* inoculum in small pots.

The suitability of *Ggt*-colonized sand grains as inoculum for investigations into the effect of Mn on take-all of wheat was tested. A factorial experiment was conducted in small pots with 3 rates of Mn (0, 4 and 16 Mn), 5 rates of sand grain *Ggt* inoculum (0, 32, 64, 128, 256 Ggt) and 4 replicates. Soil was moistened and incubated before sowing to increase the severity of Mn deficiency in plants, using a technique summarized in appendix A.

3.2.4.1. *Inoculum*. *Ggt* inoculum was prepared by growing *Ggt* 500 on a medium composed of equal volumes of clean bright gamma-irradiated millet seed, clean silica sand (washed with 5 % HCl and rinsed with ROH_2O and double sieved to collect <1.63 and >1.41 mm fraction) and $DDDH_2O$ (MacNish *et al.* 1986). The 3 components were placed in a tray, covered with foil and autoclaved for 60 minutes at 125° C and 155 kPa. The components were thoroughly mixed one day later and again autoclaved as before. The next day the mixture was added to 300 ml wide-mouthed flasks glass jars and autoclaved for 20 minutes. The medium was inoculated with mycelial cubes taken from the outer margins of an actively growing colony of *Ggt* 500 on weak PDA and incubated for 3 weeks at 25° C. Jars were shaken regularly. The contents were air-dried under a sterile air stream and uncontaminated cultures were split into colonized millet seed and surface colonized sand by passing over a 1.63 mm sieve. The colonized sand was cleaned of millet seed by winnowing. Sand inoculum was stored at 4° C until used. Live and autoclaved *Ggt* colonized sand were added to soil at rates to give 0 and 256 (0 Ggt), 32 and 224 (32 Ggt), 64 and 128 (64 Ggt), 128 and 128 (128 Ggt) and 256 and 0 (256 Ggt) grains per pot, respectively.

3.2.4.2. *Soil*. Wangary sand was incubated at 18 % water content for 2 weeks prior to sowing (see Appendix A for more details) at 15/10° C day/night temperatures. Basal nutrients were added to soil immediately prior to sowing at the same rates used in section 3.2.3.1. except boron was reduced by a factor of 10 because the rate of boron application in

previous experiments was unnecessarily high due to an arithmetic error. *Ggt* inoculum was also mixed through soil immediately prior to sowing.

3.2.4.3. *Mn*. Mn treatments were imposed prior to incubation of soil to give rates equivalent to 0, 4 or 16 mg of Mn per pot. $MnSO_4.4H_2O$ solutions were sprayed onto the soil surface with a hand-sprayer and thoroughly mixed. Mn was dissolved in sufficient $DDDH_2O$ to wet the soil to 18 % water content.

3.2.4.4. *Procedure*. The same procedure outlined in 3.2.3.1. was used, with the following alterations ;

1. All pots were part-filled with 50.9 g of prepared soil, followed by 2 germinated wheat seeds and a final layer of 4.1 g of soil.
2. Condor wheat seeds (harvested from a Mn-deficient site at Tooligie on the Eyre Peninsula of South Australia in 1984) were germinated for 2 days at 4° C and a further day at 20° C prior to sowing.
3. Growing conditions were the same except photon flux density was lower, at an average of 0.3 mEinsteins m^{-2} s^{-1} during the 10 hour light period.

3.3. Results.

3.3.1. Confirmation of soil-applied Mn decreasing take-all of wheat in pots.

3.3.1.1. *Ggt*. The addition of 10 mg of Mn per pot prior to sowing decreased *Ggt* infection of wheat seedlings (table 3.1). The total number of black stelar lesions and the total length of lesions per plant were both decreased by approximately 40 % with 10 Mn, regardless of the rate of *Ggt* inoculum. The percentage of diseased seminal roots was not as sensitive to Mn treatments and was decreased by only 24 % by 10 Mn. Plants had 4-5 seminal roots. *Ggt* infection was not decreased by 1 Mn.

Table 3.1. Effect of soil applied Mn on *Ggt* infection, growth and Mn nutrition of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the body of the table are the average of 25 plants. Disease measurements were analyzed without 0 Ggt treatments.

Mn added (mg/pot)	Rate of *Ggt* inoculum 0	0.05	0.1	Mean[a]
Number of black stelar lesions per plant				
0	0	4.6	8.6	6.6
1	0	5.4	8.6	7.0
10	0	2.2	5.4	3.8
Mean	0	4.0	7.6	
LSD[b] (P=0.05) Mn=1.2 ; Ggt=1.0 ; Mn*Ggt ns				
Total length of lesions per plant (mm)				
0	0	5.8	14.0	10.0
1	0	6.6	13.4	10.0
10	0	3.4	8.8	6.2
Mean	0	5.4	12.0	
LSD[b] (P=0.05) Mn=1.8 ; Ggt=1.4 ; Mn*Ggt ns				
Percentage of diseased seminal roots				
0	0	45	66	56
1	0	69	69	63
10	0	28	57	42
Mean	0	43	65	
LSD[b] (P=0.05) Mn=11 ; Ggt=9 ; Mn*Ggt ns				
Dry weight of shoots per plant (mg)				
0	19	18	17	18
1	23	18	20	20
10	30	26	26	27
Mean	24	21	21	
LSD (P=0.05) Mn,Ggt=2 ; Mn*Ggt ns				
Dry weight of roots per plant (mg)				
0	21	22	26	23
1	30	25	29	28
10	50	43	46	46
Mean	34	30	34	
LSD (P=0.05) Mn,Ggt=3 ; Mn*Ggt ns				
Mn concentration in whole shoots (mg kg^{-1} D.W.)				
0	9	9	10	9
1	10	11	11	11
10	38	39	38	38
Mean	19	19	20	
LSD (P=0.05) Mn=3 ; Ggt,Mn*Ggt ns				
Mn concentration in roots (mg kg^{-1} D.W.)				
0	19	20	26	22
1	24	23	25	24
10	56	60	56	57
Mean	33	34	36	
LSD (P=0.05) Mn=2 ; Ggt,Mn*Ggt ns				

[a] Means of Mn treatments for disease assessments were calculated without 0 Ggt treatments.
[b] Values separated by more than the appropriate LSD were statistically different.

Increasing the amount of live inoculum mixed through the soil increased symptoms of *Ggt* infection on seminal roots, e.g. an average of 5.4 and 12 mm of stelar lesions per plant were measured at 0.05 and 0.1 Ggt, respectively. No disease occurred in 0 Ggt pots.

3.3.1.2. *Plant growth.* Plants in 0 Mn pots were very pale green with interveinal chlorosis on young leaves but plants in 10 Mn pots were dark green. Plants from 1 Mn were intermediate. The average dry weight of shoots per plant was increased by more than 50 % by 10 Mn and the dry weight of roots nearly doubled (table 3.1). The dry weight of shoots was not increased by 1 Mn and there was only a small increase in the dry weight of roots (approximately 20 %). *Ggt* infection did not affect the response of plants to Mn.

Increasing *Ggt* inoculum rates did not cause any visual depression in plant growth and their effect on the dry weight of tissues was small (table 3.1). Both rates of *Ggt* inoculum decreased the average dry weight of shoots per plant by little more 10 % and only 0.05 Ggt decreased the dry weight of roots (also by approximately 10 %).

3.3.1.3. *Mn.* *Ggt* infection had no effect on plant Mn levels and the concentration of Mn in whole shoots averaged 9, 11 and 38 mg kg^{-1} (on an oven-dry weight basis) for 0, 1 and 10 Mn, respectively (table 3.1). A critical level of 18 mg kg^{-1} was calculated for wheat seedlings grown under controlled environment conditions during this project (see appendix B for details). Mn concentration in roots averaged 22 mg kg^{-1} at 0 Mn and was only increased by 10 Mn (to 57 mg kg^{-1}).

3.3.2. Testing the effect of Mn on take-all of wheat in pots using a fresh preparation of ground oat kernel inoculum.

3.3.2.1. *Ggt.* There was no effect of Mn treatments on *Ggt* infection (table 3.2). Live inoculum resulted in very high levels of disease with an average of 23 black stelar lesions and a total length of 92 mm per plant recorded for the whole experiment and 94 % of seminal roots diseased. All plants averaged 4 seminal roots. No disease was found in -Ggt pots.

3.3.2.2. *Plant growth.* Plants grown without added Mn were pale green without any obvious interveinal chlorosis but soil and foliar-applied Mn resulted in dark green plants. The average dry weight of shoots per plant was increased by nearly one-third by either soil- or foliar-applied Mn but there was no additional benefit from application of Mn to both soil and leaves (table 3.3). The dry weight of roots showed the same pattern of response but was increased by 50 % with the addition of Mn (table 3.4). *Ggt* infection did not affect the growth response of plants to Mn.

Table 3.2. Effect of soil- and foliar-applied Mn on *Ggt* infection of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the body of the table are the average of 25 plants. Disease assessments were analyzed for +Ggt treatments only.

	- soil Mn		+ soil Mn		
Ggt	-fol.Mn	+fol.Mn	-fol.Mn	+fol.Mn	Mean
Number of black stelar lesions per plant.					
-	0	0	0	0	0
+	23	23	24	22	23
Means for Mn treatments.		-soil Mn	23	-fol.Mn	23
(+ Ggt only)		+	23	+	23
LSD[a] (P=0.05) Soil Mn, Foliar Mn ns					
Total length of lesions per plant (mm).					
-	0	0	0	0	0
+	95	106	80	86	92
Means for Mn treatments.		-soil Mn	100	-fol.Mn	88
(+ Ggt only)		+	83	+	96
LSD (P=0.05) Soil Mn, Foliar Mn ns					
Percentage of diseased seminal roots.					
-	0	0	0	0	0
+	95	93	97	93	94
Means for Mn treatments.		-soil Mn	94	-fol.Mn	96
(+ Ggt only)		+	95	+	93
LSD (P=0.05) Soil Mn, Foliar Mn ns					

[a] Values separated by more than the appropriate LSD were statistically different.

Ggt infection decreased the average dry weight of shoots and roots per plant by 29 and 23 %, respectively, regardless of Mn treatments (tables 3.3,3.4).

Table 3.3. Effect of soil- and foliar-applied Mn and *Ggt* inoculum on shoot dry weight of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values (in mg per plant) in the 3-way table are the average of 25 plants.

	- soil Mn		+ soil Mn	
Ggt	-fol.Mn	+fol.Mn	-fol.Mn	+fol.Mn
-	20	26	26	25
+	13	18	18	18

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's at P=0.05 level).

		-	+	LSD[a]
	Ggt	24	17	1
	Soil Mn	19	22	1
	Foliar Mn	19	22	1
Soil Mn		-	+	
Ggt	-	23	25	
	+	16	18	ns
Foliar Mn		-	+	
Ggt	-	23	25	
	+	16	18	ns
Foliar Mn		-	+	
Soil Mn	-	17	22	
	+	22	22	2

[a] Values separated by more than the appropriate LSD were statistically different.

Table 3.4. Effect of soil- and foliar-applied Mn and *Ggt* inoculum on root dry weight of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values (in mg per plant) in the 3-way table are the average of 25 plants.

Ggt	- soil Mn -fol.Mn	- soil Mn +fol.Mn	+ soil Mn -fol.Mn	+ soil Mn +fol.Mn
-	10	14	14	14
+	7	10	10	11

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's at P=0.05 level).

		-	+	LSD[a]
	Ggt	13	10	2
	Soil Mn	10	12	2
	Foliar Mn	10	12	2
Soil Mn		-	+	
Ggt	-	12	14	
	+	9	11	ns
Foliar Mn		-	+	
Ggt	-	12	14	
	+	9	11	ns
Foliar Mn		-	+	
Soil Mn	-	8	12	
	+	12	12	1

[a] Values separated by more than the appropriate LSD were statistically different.

3.3.2.3. *Mn*. Disease-free plants which received no added Mn had an average Mn concentration in whole shoots of 11 mg kg^{-1}, well below the critical level of 18 mg kg^{-1} (see appendix B), and *Ggt* infection did not significantly decrease this value (table 3.5). However, where soil Mn had been applied, *Ggt* infection decreased whole shoot Mn concentration from 41 mg kg^{-1} to 34 mg kg^{-1}. Mn analysis of shoots of foliar Mn treated plants was confounded by painting Mn onto the leaves and they were not included in statistical analysis of data.

Table 3.5. Effect of soil- and foliar-applied Mn and *Ggt* inoculum on shoot Mn concentration of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values (in mg kg^{-1} D.W.) in the 3-way table are the average of 25 plants. Data were analyzed without foliar Mn treatments due to Mn painted onto leaves.

	- soil Mn		+ soil Mn	
Ggt	-fol.Mn	+fol.Mn	-fol.Mn	+fol.Mn
-	11	374	41	511
+	9	533	34	634

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's at P=0.05 level) for soil Mn and Ggt treatments only.

	-	+	LSD[a]
Ggt	26	21	2
Soil Mn	10	37	2
Soil Mn	-	+	
Ggt -	11	41	
+	9	34	3

[a] Values separated by more than the appropriate LSD were statistically different.

Mn concentration in roots was affected differently by Ggt and soil Mn treatments than shoots (table 3.6). Roots of disease-free plants without soil Mn contained 19 mg kg^{-1} which was not significantly different to the 29 mg kg^{-1} in *Ggt* infected plants. Soil Mn increased the concentration of Mn in roots of disease-free plants to 54 mg kg^{-1} but in *Ggt* infected plants this value was 110 mg kg^{-1}. The high concentration of Mn in roots of *Ggt* infected wheat plants supplied with soil Mn was not entirely due to the depression in root growth caused by *Ggt* infection because the dry weight of roots was decreased by only 23 % but the Mn concentration nearly doubled. This meant that the total Mn content of roots increased under these conditions but, at the same time, the Mn concentration in shoots

decreased. Foliar applied Mn did not increase the concentration of Mn in roots.

Table 3.6. Effect of soil- and foliar-applied Mn and *Ggt* inoculum on root Mn concentration of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values (in mg kg^{-1} D.W.) in the 3-way table are the average of 25 plants.

	- soil Mn		+ soil Mn	
Ggt	-fol.Mn	+fol.Mn	-fol.Mn	+fol.Mn
-	18	21	52	56
+	22	37	110	110

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's at P=0.05 level).

	-	+	LSD[a]
Ggt	37	70	13
Soil Mn	24	82	13
Foliar Mn	50	56	13

Soil Mn	-	+	
Ggt -	19	54	
+	29	110	19

Foliar Mn	-	+	
Ggt -	35	38	
+	66	74	ns

Foliar Mn	-	+	
Soil Mn -	20	29	
+	81	83	ns

[a] Values separated by more than the appropriate LSD were statistically different.

3.3.3. Development of PDA discs as an inoculum source for *Ggt*.

3.3.3.1. *Effect of concentration of PDA in agar discs on take-all of wheat in small pots.*

3.3.3.1.1. Ggt. Increasing the concentration of PDA from 1 to 4 % did not produce measurable differences in take-all symptoms on wheat roots and both concentrations of *Ggt*

colonized agar discs caused a high level of *Ggt* infection symptoms on wheat roots (an average of 44 mm of black stelar lesions per plant and 78 % of seminal roots diseased) (table 3.7). No disease was present in pots with sterile PDA discs.

The percentage of diseased seminal roots was decreased by 27 % with 4 Mn but 0.4 Mn had no effect on *Ggt* infection. The number and total length of lesions per plant were not affected by Mn treatments.

3.3.3.1.2. Plant growth. Plants with take-all were smaller than disease-free plants and diseased plants at 0 and 0.4 Mn were beginning to show signs of wilting in the week prior to harvest, which is a symptom of severe take-all (Clarkson and Polley 1981), despite all pots being maintained at the same water content. Plant growth was successively decreased by increasing rates of *Ggt* inoculum (table 3.7). The average dry weight of roots per plant showed a similar pattern but the decrease with 4 % PDA was not significantly greater than with 1 % PDA (table 3.7). Decreases in plant growth with increasing *Ggt* inoculum rates were not affected by Mn treatments.

Plants grown without added Mn were pale-green but there was little evidence of interveinal chlorosis. Both rates of added Mn resulted in dark green plants. Similar to previous pot experiments, root growth showed larger responses to added Mn than shoot growth but the differences here were even more marked (table 3.7). The average dry weight of shoots per plant increased by 33 % with 0.4 Mn and by 77 % with 4 Mn but the dry weight of roots increased by 80 and 220 %, respectively.

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Table 3.7. Effect of concentration of PDA in *Ggt* colonized agar discs on *Ggt* infection, growth and Mn nutrition of wheat seedlings grown at 3 rates of soil Mn in a Mn-deficient sand under controlled environment conditions. Values in the body of the table are the average of 10 plants. Disease assessments were analyzed without 0 Ggt treatments.

Mn added (mg/pot)	Concentration of PDA in agar discs: sterile-4 %	1 %	4 %	Mean[a]
Number of black stelar lesions per plant				
0	0	5.5	6.5	6.0
0.4	0	6.5	4.0	5.5
4	0	5.0	4.0	4.5
Mean	0	5.5	5.0	
LSD[b] (P=0.05) Mn, Ggt, Mn*Ggt ns				
Total length of lesions per pot (mm)				
0	0	46	50	48
0.4	0	50	40	46
4	0	43	34	38
Mean	0	47	42	
LSD[b] (P=0.05) Mn, Ggt, Mn*Ggt ns				
Percentage of diseased seminal roots				
0	0	78	95	86
0.4	0	91	77	84
4	0	62	62	62
Mean	0	77	78	
LSD[b] (P=0.05) Mn=12 ; Ggt, Mn*Ggt ns				
Dry weight of shoots per plant (mg)				
0	37	30	24	30
0.4	52	43	26	40
4	57	52	49	53
Mean	49	42	33	
LSD (P=0.05) Mn,Ggt=6 ; Mn*Ggt ns				
Dry weight of roots per plant (mg)				
0	14	9	6	10
0.4	29	15	10	18
4	3	30	28	32
Mean	27	18	15	
LSD (P=0.05) Mn,Ggt=5 ; Mn*Ggt ns				
Mn concentration in whole shoots (mg kg^{-1} D.W.)				
0	7.0	6.7	6.1	6.6
0.4	11.8	10.3	10.1	10.7
4	41.2	30.8	32.1	34.7
Mean	20.0	15.9	16.1	
LSD (P=0.05) Mn, Ggt=2.0, Mn*Ggt 3.5				
Mn concentration in roots (mg kg^{-1} D.W.)				
0	33	35	49	39
0.4	29	47	39	38
4	97	67	85	83
Mean	49	60	58	
LSD (P=0.05) Mn=12 ; Ggt,Mn*Ggt ns				

[a] Means of Mn treatments for disease assessments were calculated without 0 Ggt treatments.

[b] Values separated by more than the appropriate LSD were statistically different.

3.3.3.1.3. Mn. Mn concentration in whole shoots increased from an average of 6.6 mg kg^{-1} in plants grown without added Mn to 10.7 mg kg^{-1} with 0.4 Mn, regardless of Ggt treatments (table 3.7). However, the effect of 4 Mn on Mn concentration in shoots was modified by *Ggt* infection; 4 Mn increased the concentration of Mn in shoots to 41.2 mg kg^{-1} in disease-free plants but only to an average of 31.4 mg kg^{-1} in *Ggt* infected plants. *Ggt* infection did not affect root Mn concentrations and only 4 Mn increased levels above those at 0 Mn, from 39 mg kg^{-1} to 83 mg kg^{-1} (table 3.7).

3.3.3.2. *Effect of depth of burial of agar disc on take-all of wheat in small pots.* 3.3.3.2.1. Ggt. Increasing the depth of burial of *Ggt* colonized PDA agar discs below germinated wheat seeds decreased the levels of take-all on roots (table 3.8). With agar discs 2 cm below seeds 95 % of seminal roots were diseased but only 83 and 67 % were diseased with discs at depths of 4 and 6 cm, respectively.

There were more black stelar lesions per plant with burial depths of 2 and 4 cm than at 6 cm but the decreases in total length of lesions per plant with increasing depth of burial were not significant.

Mixing Mn through soil decreased *Ggt* infection of wheat roots at all disease levels (table 3.8). The number and total length of lesions per plant decreased by 25 and 26 % respectively with added Mn but a small decrease in percentage of diseased seminal roots with added Mn was not significant.

3.3.3.2.2. Plant growth. The older leaves of all plants with take-all showed signs of yellowing and necrosis at their distal ends by harvest, but it was worst on plants where PDA discs were only 2 cm below seeds (plate 3.1). The average dry weight of shoots per plant increased with increasing depth of burial of agar discs but there was no difference between plants from pots with an agar disc at 6 cm and disease-free plants (table 3.8).

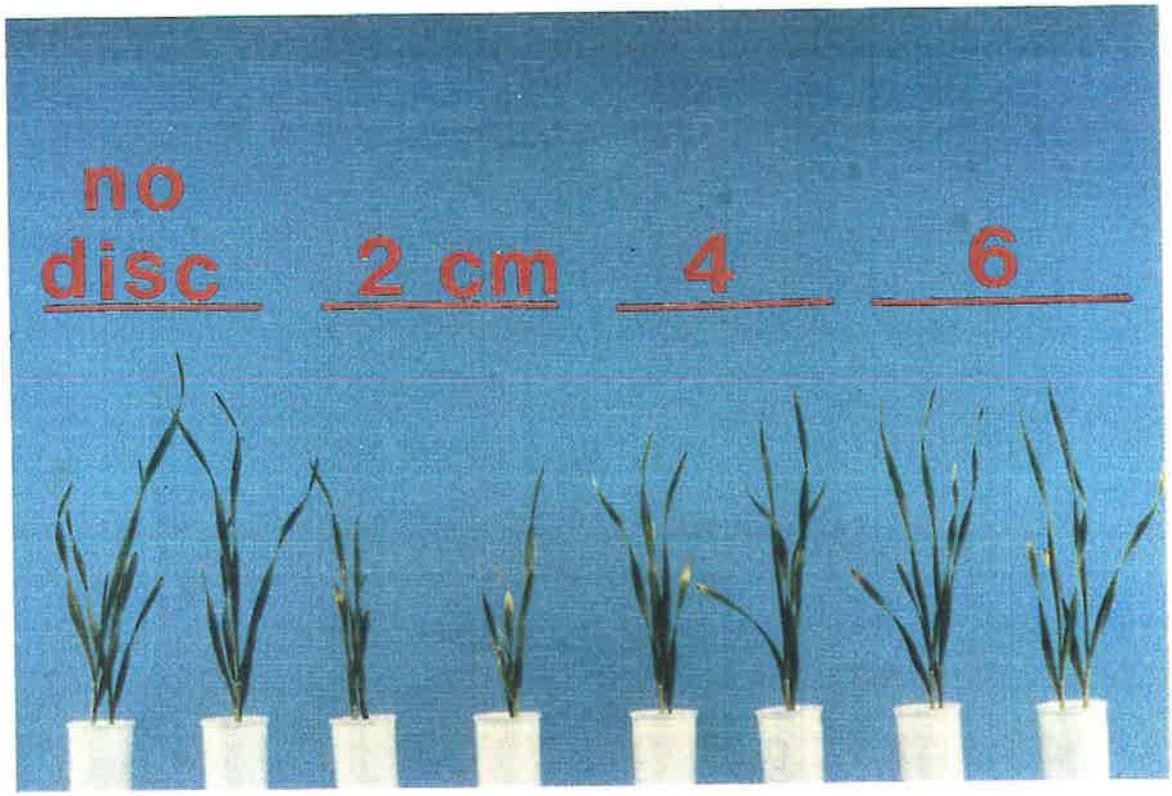
Table 3.8. **Effect of depth of burial of *Ggt* colonized agar discs on *Ggt* infection, growth and Mn nutrition of wheat seedlings grown at 2 rates of soil Mn in a Mn-deficient sand under controlled environment conditions. Values in the body of the table are the average of 6 plants. Disease measurements were analyzed without 0 Ggt treatments.**

Mn added (mg/pot)	Depth of burial of agar discs (cm below seeds)				
	no disc	6	4	2	Mean[a]
Number of black stelar lesions per plant					
0	0	6	9	9	8
4	0	5	6	8	6
Mean	0	5	8	9	
LSD[b] (P=0.05) Mn=1 ; Ggt=2 ; Mn*Ggt ns					
Total length of lesions per plant (mm)					
0	0	37	46	44	42
4	0	30	29	35	31
Mean	0	34	38	39	
LSD[b] (P=0.05) Mn=7 ; Ggt, Mn*Ggt ns					
Percentage of diseased seminal roots					
0	0	67	93	97	86
4	0	67	73	93	78
Mean	0	67	83	95	
LSD (P=0.05) Ggt=11 ; Mn, Mn*Ggt ns					
Dry weight of shoots per plant (mg)					
0	48	45	38	32	41
4	58	55	54	41	52
Mean	53	50	46	36	
LSD (P=0.05) Mn=5 ; Ggt=7 ; Mn*Ggt ns					
Dry weight of roots per plant (mg)					
0	55	51	42	27	44
4	68	63	62	36	57
Mean	61	57	52	32	
LSD (P=0.05) Mn=7, Ggt=10 ; Mn*Ggt ns					
Mn concentration in whole shoots (mg kg^{-1} D.W.)					
0	11.1	12.7	11.1	12.4	11.8
4	49.4	49.9	47.5	44.1	47.7
Mean3	0.3	31.3	29.3	28.2	
LSD (P=0.05) Mn=4.3, Ggt, Mn*Ggt ns					
Mn concentration in roots (mg kg^{-1} D.W.)					
0	19	17	16	22	18
4	135	127	141	191	148
Mean	77	72	78	107	
LSD (P=0.05) Mn=8 ; Ggt=11, Mn*Ggt 15					

[a] Means of Mn treatments for disease assessments were calculated without 0 Ggt treatments.

[b] Values separated by more than the appropriate LSD were statistically different.

Plate 3.1. Effect of depth of burial of *Ggt*-colonized agar discs and added Mn^{2+} on growth of wheat seedlings in a Mn-deficient sand under controlled environment conditions. The right-hand pot at each depth of disc burial had $MnSO_4$ mixed through the soil at a rate of 4 mg Mn per pot.



The average dry weight of roots per plant was halved by take-all from an agar disc 2 cm below seeds (compared to a 31 % decrease in dry weight of shoots) and decreased by 15 % with an agar disc at 4 cm (table 3.8). An agar disc at 6 cm below seeds did not decrease root growth. Soil Mn did not alter the effect of take-all on plant growth.

Plants without added Mn appeared to be the same size as plants grown with Mn but were a pale green colour. The dry weight of shoots and roots per plant showed a smaller response to soil Mn than the previous experiment and an increase of less than one-third was recorded for both shoots and roots (table 3.8).

3.3.3.2.3. Mn. Mn concentration in whole shoots of plants grown without Mn were not severely deficient (average of 11.8 mg kg^{-1}) which is consistent with the small growth response to added Mn (table 3.8). Soil Mn increased Mn concentrations to well above deficient (average of 47.7 mg kg^{-1}) and *Ggt* infection did not influence shoot Mn concentrations. Soil Mn increased root Mn concentrations to similar levels at all Ggt treatments except at 4 Mn with agar discs buried 2 cm below seeds where the concentration of Mn was higher than in all other treatments (table 3.8).

3.3.4. Colonized sand grains as *Ggt* inoculum.

3.3.4.1. *Ggt*. Increasing numbers of live *Ggt* colonized sand grains per pot increased *Ggt* infection symptoms on wheat roots (table 3.9). This effect was clearest in the percentage of diseased seminal roots which increased successively from 42 to 54, 70 and 83 % with 32, 64, 128 and 256 Ggt treatments, respectively. The number and and total length of lesions per plant also increased with Ggt rates but consecutive rates did not always produce significantly different levels of disease. No disease was present in pots which received only autoclaved sand grains.

Table 3.9. Effect of *Ggt* colonized sand grains on *Ggt* infection of wheat seedlings grown at 3 rates of soil Mn in a Mn-deficient sand under controlled environment conditions. Values in the body of the table are the average of 8 plants. Disease measurements were analyzed without 0 Ggt treatments.

Mn added (mg/pot)	No. of *Ggt* colonized sand grains per pot[a] 0	32	64	128	256	Mean[b]
Number of black stelar lesions per plant						
0	0	4.9[c]	4.5	5.5	7.5	5.5
		1.60[d]	*1.49*	*1.70*	*2.01*	*1.70*
4	0	4.7	5.3	5.6	8.6	5.8
		1.50	*1.67*	*1.72*	*2.15*	*1.76*
16	0	1.7	3.2	6.1	6.4	3.8
		0.50	*1.15*	*1.81*	*1.85*	*1.33*
Mean	0	3.3	4.2	5.7	7.4	
		1.02	*1.44*	*1.75*	*2.01*	
LSD[e,f] (P=0.05) Mn=*0.21* ; Ggt=*0.25* ; Mn*Ggt=*0.43*						
Total length of lesions per plant (mm)						
0	0	9.7	13.9	12.6	16.5	12.9
		2.27[c]	*2.63*	*2.54*	*2.81*	*2.56*
4	0	6.5	12.5	11.3	18.7	11.5
		1.87	*2.52*	*2.42*	*2.93*	*2.44*
16	0	1.7	3.7	12.3	10.9	5.3
		0.50	*1.30*	*2.51*	*2.39*	*1.68*
Mean	0	4.7	8.6	12.0	15.0	
		1.55	*2.15*	*2.49*	*2.71*	
LSD (P=0.05) Mn=*0.28*, Ggt=*0.33* ; Mn*Ggt=*0.57*						
Percentage of diseased seminal roots						
0	0	63	50	73	90	69
4	0	48	68	65	82	66
16	0	15	43	73	75	51
Mean	0	42	53	70	83	
LSD (P=0.05) Mn=10 ; Ggt=11 ; Mn*Ggt=19						

[a] Each pot contained the equivalent of 44 g of air-dry soil.
[b] Means of Mn treatments for disease assessments were calculated without 0 Ggt treatments.
[c] Geometric means calculated from natural log transformed data.
[d] Values in italics are natural log transformations.
[e] Values separated by more than the appropriate LSD were statistically different.
[f] LSD's apply to transformed data only.

Mixing a high rate of Mn through soil prior to incubation decreased *Ggt* infection at low rates of *Ggt* inoculum only (table 3.9). The percentage of diseased roots was decreased

by 16 Mn from 63 % to only 15 % at 32 Ggt but decreases at higher disease levels were not significant. The same pattern of disease reduction by 16 Mn was also present in the average number and length of lesions per plant, except that the total length of lesions was also decreased by 16 Mn at 64 Ggt. The effect of 16 Mn on *Ggt* infection was most dramatic at 32 Ggt where disease was decreased to an average of less than two 1 mm long lesions per plant, compared to an average of nearly five 2 mm long lesions per plant without added Mn. *Ggt* infection was not decreased by 4 Mn.

3.3.4.2. *Plant growth.* Although levels of *Ggt* infection measured here were not as high as recorded in the previous experiment, shoot symptoms of take-all were similar. Extensive yellow tipping on older leaves was present with 0 and 4 Mn at 128 and 256 Ggt but was only noted at 256 Ggt with 16 Mn. This interaction between *Ggt* inoculum rates and Mn rates was not present in shoot dry weights and was reversed in root dry weights (table 3.10). Only the highest *Ggt* inoculum rate decreased the average dry weight of shoots per plant (by 19 %) and this effect was independent of Mn rates. The average dry weight of roots per plant was not decreased by inoculum rates without Mn but at 4 Mn only the lowest inoculum rate did not decrease root growth. At 16 Mn only 256 Ggt significantly decreased dry root weights.

Plants grown without added Mn were small compared to plants at 4 and 16 Mn and they were also very pale green with extensive interveinal chlorosis on young leaves. The average dry weight of shoots per plant increased by 54 % with 4 Mn and dry weight of roots by more than 83 %. The highest rate of soil Mn did not further increase plant growth.

3.3.4.3. *Mn.* Incubation of Wangary sand at 18 % water content for 2 weeks prior to sowing resulted in wheat seedlings which were very Mn deficient (table 3.10). Mn concentration in whole shoots averaged 7.9 mg kg^{-1} without Mn but levels were increased to adequate levels by both Mn rates; 30 mg kg^{-1} with 4 Mn and 119 mg kg^{-1} with 16 Mn. A Mn concentration of 32 mg kg^{-1} at 4 Mn in disease-free plants compared to 41 and 49 mg

kg^{-1} in the two previous experiments in this chapter, which shows that Mn added prior to incubation was less available to wheat plants but the effect was only small. Root Mn concentrations were increased from 23 to 58 and 286 mg kg^{-1} with 4 and 16 Mn, respectively. *Ggt* infection did not significantly affect plant Mn levels, although root Mn concentrations were generally higher in *Ggt*-infected plants grown with added Mn which is an effect observed in previous experiments (see 3.3.3.1. and 3.3.3.2.).

Table 3.10. Effect of *Ggt* colonized sand grains on growth and Mn nutrition of wheat seedlings grown at 3 rates of soil Mn in a Mn-deficient sand under controlled environment conditions. Values in the body of the table are the average of 8 plants.

Mn added (mg/pot)	No. of *Ggt* colonized sand grains per pot[a] 0	32	64	128	256	Mean
Dry weight of shoots per plant (mg)						
0	24	26	32	31	27	28
4	49	45	42	40	37	43
16	52	47	46	49	37	46
Mean	42	39	40	40	34	
LSD[b] (P=0.05) Mn=4 ; Ggt, Mn*Ggt ns						
Dry weight of roots per plant (mg)						
0	13	17	21	20	19	18
4	42	38	33	29	25	33
16	44	36	37	40	28	37
Mean	33	30	30	30	24	
LSD (P=0.05) Mn=4 ; Ggt=5 ; Mn*Ggt=8						
Mn concentration in whole shoots (mg kg^{-1} D.W.)						
0	7.5	8.0	7.5	8.2	8.2	7.9
4	31.5	30.8	28.9	31.1	27.5	30.0
16	124.5	125.3	123.3	111.2	108.5	118.5
Mean	54.5	54.7	53.2	50.2	48.1	
LSD (P=0.05) Mn=4.4 ; Ggt, Mn*Ggt ns						
Mn concentration in roots (mg kg^{-1} D.W.)						
0	21	32	26	21	26	25
4	47	68	50	48	78	58
16	261	286	284	287	314	286
Mean	110	129	120	118	139	
LSD (P=0.05) Mn=15 ; Ggt, Mn*Ggt ns						

[a] Each pot contained the equivalent of 44 g of air-dry soil.

[b] Values separated by more than the appropriate LSD were statistically different.

3.4. Discussion and Conclusions.

The repeat of the experiment reported by Graham and Rovira (1984) confirmed that a rate of soil Mn which eliminated deficiency in wheat seedlings decreased take-all of wheat. However, results obtained here differed in that a low rate of soil Mn had no effect on disease levels. A low rate of soil Mn from the PDA concentration experiment produced similar plant Mn concentrations and also did not decrease take-all. Neither rate of Mn increased the concentration of Mn in wheat roots although shoot Mn concentrations were increased slightly but were still below adequate levels. Similarly, Mn applied to leaves was ineffective at decreasing *Ggt* infection on roots (although very heavy disease levels in this experiment made it difficult to assess lesion development) and did not increase root Mn concentration. Plant growth data indicated that foliar Mn increased the dry weight of shoots and roots of plants as effectively as soil Mn which suggests that foliar Mn eliminated Mn deficiency in shoots but does not ensure that the Mn nutrition of the roots was improved. Mn is poorly translocated in phloem (Single 1958, Nable and Loneragan 1984a,b) and the increase in root growth with foliar Mn may have been a secondary effect of improved nutrition of the shoots.

The results of these preliminary experiments suggest that rates of Mn which are high enough to eliminate deficiency in wheat seedlings and increase the concentration of Mn in roots above those in plants grown without Mn will decrease take-all. However, a low rate of Mn applied to soil in the sand grain experiment increased shoot Mn levels to well above critical for growth and increased root Mn concentrations but was not effective in decreasing take-all. This same rate of soil Mn was very effective in decreasing take-all in the depth of burial experiment, although the two Mn treatments are not directly comparable because in the sand grain experiment Mn was added prior to soil incubation. Further work needs to be undertaken to clarify the effect of low rates of Mn on susceptibility of wheat to take-all and to define minimum tissue concentrations of Mn in shoots and roots necessary for reduction of *Ggt* infection on wheat roots. The possibility exists that root or shoot Mn concentrations

which are marginal or just sufficient for growth of disease-free wheat seedlings may not be high enough for infected seedlings to avoid the increased susceptibility to *Ggt* infection which accompanies tissue levels of Mn below recognized critical values.

The increased susceptibility of Mn-deficient wheat seedlings to take-all was not due to an impaired ability of the host to take up Mn because reductions in take-all were recorded at disease levels which were too low to affect plant Mn concentrations. However, disease levels which severely inhibited plant growth reduced shoot Mn concentrations but increased root Mn concentrations. The increases in root Mn with *Ggt* infection were dramatic where high rates of soil Mn had been applied. The petri-dish experiments conducted in chapter 2 showed the strong Mn-oxidizing capabilities of *Ggt* 500 and the increased Mn concentrations in diseased wheat roots grown with high soil Mn rates may have been due to oxidation of Mn on root surfaces by *Ggt* hyphae although no visual deposits of Mn oxides were noted on roots from experiments reported in this chapter. Mn oxides were observed on roots of plants free of take-all in the soil incubation experiment (see appendix A) but they occurred at rates of soil applied Mn which were much higher than those used in this chapter. If the high Mn concentrations in roots of *Ggt*-infected plants grown at high soil Mn were due to deposits of Mn oxides, these oxides were apparently not available to plants because shoot Mn levels in these plants were depressed. Alternatively, the rates of contact reduction (Uren 1981) which occurred were insufficient for the plant's needs. More detailed studies on the location and appearance of high levels of Mn on wheat roots associated with *Ggt* infection are presented in chapter 10.

The technique of placing *Ggt*-inoculated PDA discs at increasing depths below wheat seeds proved to be the most reliable and flexible method of infecting wheat seedlings with take-all. Increasing the depth of soil between wheat seeds and 4 % PDA discs from 2 to 6 cm resulted in disease levels (assessed by effects on dry weight of shoots) which covered the range produced in all other pot experiments reported in this chapter and take-all

symptoms were decreased by Mn at all 3 depths of burial. Disease levels between experiments were compared by reductions in dry weight of shoots because this measurement was the most sensitive to increases in *Ggt* inoculum rates and separated *Ggt* treatments most clearly within each experiment.

Increasing the concentration of PDA in agar discs was not as effective because a four fold increase in PDA strength did not cause a measurable increase in symptoms on roots despite the dry weight of shoots being decreased by more than two fold at the same time. Although the inoculum potential of a pathogen is markedly influenced by its nutritional status (which will depend, among other things, on the physical and chemical nature of the substrate on which the inoculum is developed) (Garrett 1970) increasing PDA strength four fold was not sufficient to measurably increase take-all symptoms on wheat roots. In addition, *Ggt* infection symptoms were only slightly decreased by Mn in this experiment, even though the high rate of *Ggt* inoculum in this experiment was the same as that in the depth of burial experiment (and produced similar disease levels) where take-all symptoms were substantially decreased.

Inoculated sand grains were not suitable as *Ggt* inoculum because decreases in *Ggt* infection symptoms on roots with Mn could only be measured at low rates of disease, despite the high rates of inoculum causing disease levels which were similar to those produced in the depth of burial experiment.

As previously mentioned in the introduction to this chapter, ground oat inoculum was not suitable because of the inconsistency in disease levels produced between batches at the same rates. In the latter of the two experiments conducted with this inoculum the addition of Mn did not decrease take-all. The inability to measure a decrease in *Ggt* infection symptoms on wheat roots may have been due to the very heavy infection which occurred in this experiment. The roots of diseased plants were very stunted and beginning to rot by harvest and some root breakage occurred during harvest (especially on nil Mn plants). Both

of these factors made it very difficult to distinguish stelar lesions and disease assessments in terms of lesion development could not be accurately made.

Incubating moist Wangary sand was very effective at increasing the severity of Mn deficiency in wheat seedlings because rewetting a soil will decrease exchangeable Mn levels (Fujimoto and Sherman 1945, Shuman 1980). This technique was adopted for all further pot experiments. Mixing $MnSO_4$ through soil prior to incubation supplied levels of Mn to wheat seedlings that were more than adequate for growth and was also effective at decreasing take-all. Marcar (1986) showed that in Wangary sand, Mn added as Mn^{2+} was decreased to negligible levels within 20 days. This means that in pots where Mn was added to soil the take-all fungus should not have been exposed to Mn^{2+} levels which were much higher than in nil Mn. This further supports the conclusions of chapter 2 that rates of soil Mn which decreased take-all were not toxic to the fungus.

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CHAPTER 4.

RELATIVE SUSCEPTIBILITIES OF FOUR WHEAT GENOTYPES TO TAKE-ALL UNDER MANGANESE-DEFICIENT CONDITIONS

CHAPTER 4. RELATIVE SUSCEPTIBILITIES OF FOUR WHEAT GENOTYPES TO TAKE-ALL UNDER MANGANESE-DEFICIENT CONDITIONS.

4.1. Introduction.

Scott and Hollins (1985) concluded that after decades of screening for resistance to take-all in cereals there was little hope for dramatic progress in resistance breeding. However, three recent reports detail large differences between wheat genotypes in susceptibility to take-all.

Simon and Rovira (1985) screened 20 wheat genotypes from the Australian Wheat Collection, 4 from Centro Internacional de Mejoramiento de Maiz y Trigo and 4 Australian commercial cultivars for resistance to take-all in the field. Resistance scores varied from 110 (resistant) to 15 (susceptible) over all genotypes tested and scores for the Australian cultivars Halberd, Warigal, Kite and Condor were 72, 51, 31 and 15, respectively. Similarly, Wilson (pers. comm.) found the wheat cultivar Spear performed better than Condor in field plots inoculated with take-all while there was little difference in growth in uninoculated plots. Finally, Penrose (1985) reported that Kite was more resistant to take-all than Condor and, in turn, Condor was more resistant than RAC311 (later released as Bayonet) in sand culture.

Studies have also been conducted with Australian wheat cultivars to identify differences between genotypes in their ability to grow and yield well under Mn-deficient conditions (Marcar 1986, Graham 1987, Graham pers. comm.). These studies have been conducted both in the field and in pots under controlled environment conditions. The wheat cultivars mentioned above have been tested for their tolerance to Mn-deficient conditions (although not all in the same experiment) and can be ranked from least to most sensitive with moderate Mn deficiency as follows - Halberd, Kite, Condor and Bayonet (Graham 1987). Halberd was more sensitive than Warigal at a more severely deficient site (Graham 1987) and Spear was also found to be reasonably tolerant to Mn deficient conditions (Graham pers. comm.).

Comparison of the independent screenings of Australian wheat cultivars for tolerance to Mn deficiency and resistance to take-all show that, in general, those cultivars

which were tolerant of Mn deficiency were also resistant to take-all (relative to other cultivars) and vice versa.

A pot experiment was conducted under controlled environment conditions to test the relative susceptibilities of 3 Australian wheat cultivars and 1 breeders' line to take-all under Mn-deficient and -sufficient conditions. The experiment was factorially designed with 4 wheat genotypes (Bayonet, Condor, Spear, C8MM), 3 Mn rates (0, 4, 16 mg of Mn per pot), 2 Ggt levels (-,+Ggt) and 5 replicates.

4.2. Materials and Methods.

The basic techniques employed here were developed during experiments with small pots outlined in Chapter 3 but some minor refinements were included. Complete details for soil, Mn and *Ggt* techniques can be found in sections 3.2.1.1., 3.2.4. and 3.2.3.2., respectively.

4.2.1. Inoculum.

Petri-dishes were poured with approximately 17 ml of autoclaved media containing 1 g of `Difco' Bacto-agar and 0.156 g (4 %) of PDA in 100 ml of $DDDH_2O$. Dishes were inoculated with single mycelial cubes taken from the edge of an actively growing colony of *Ggt* 500 and placed at 20° C for 6 days. Extra dishes of 4 % PDA were made up and kept sterile for use in - Ggt pots.

4.2.2. Soil.

Wangary sand was incubated for 15 days at 18% water content prior to potting up (see appendix A for details).

4.2.3. Mn.

Mn was mixed through soil prior to incubation as a $MnSO_4.4H_2O$ solution at rates of 0 (0 Mn), 4 (4 Mn) and 16 (16 Mn) mg of Mn per pot.

4.2.4. Procedure.

Basal nutrients were mixed through soil at the same rates used in Chapter 3 with small pots.

Seeds for the 4 genotypes were collected from a trial grown on a Mn-deficient site at Tooligie on the Eyre Peninsula S.A., in 1985. The average Mn concentration in seed for each genotype was 7.3, 9.2, 8.6 and 12.7 mg kg^{-1} D.W. for Bayonet, Condor, Spear and C8MM, respectively. These seeds were surface-sterilized with 1% NaOCl for 2 minutes and rinsed with $DDDH_2O$ and germinated as before (see section 3.2.4.).

Pots were part-filled with 34.1 g of prepared soil, followed by an agar disc which had been cut to the internal diameter of the pots, a further 16.8 g of soil (resulting in a 4 cm deep layer), 2 germinated wheat seeds and a final layer of 4.1 g of soil. *Ggt* colonized agar discs were cut from the outer margins of *Ggt* colonies which had reached the edge of petri-dishes by the day of sowing. Each pot received 4 ml of $DDDH_2O$ to the soil surface to settle soil around the seeds.

Pots were placed in a tray in randomized blocks and covered with a sheet of thin clear polythene (`Glad Wrap'), to reduce drying of the soil surface, before being placed in controlled environment conditions of 15° C with a 10 hour light period (average photon flux density of 300 uEinsteins m^{-2} s^{-1} supplied by a bank of mercury vapour lamps) in every 24.

`Glad Wrap' was removed after 2 days and pots were watered to original weight every 3-4 days. Plants were harvested after 25 days. Shoots were removed at the soil surface and fresh weight per pot recorded before drying at 70° C for 2 days. Roots were washed free of soil and the number of black stelar lesions, the total length of stelar lesions and the number of diseased seminal roots recorded for each plant. After disease severity had been assessed, the total length of primary and secondary seminal roots per plant was estimated (Tennant 1975) and number and total length of nodal roots measured. The fresh weight of roots per pot was then recorded (after free water was removed) and the roots dried at 70° C for 2 days. Oven-dry weights for shoots and roots were recorded and shoots

analyzed for Mn and 11 other essential elements with an ARL inductively coupled atomic emission spectrometer. Samples were prepared for analysis by nitric acid digestion.

4.3. Results.

4.3.1. Ggt.

The effect of Mn on take-all (measured by the total length of take-all lesions per plant) depended on the wheat genotype involved (table 4.1). Without added Mn, Bayonet supported a high level of disease (an average of 25 mm of take-all lesions per plant), Condor and Spear moderate levels (17 and 19 mm of lesions, respectively) and C8MM only a low level (11 mm of lesions per pot). The total length of lesions per plant was decreased by 4 Mn for all genotypes except C8MM which had a low level of disease at all Mn levels. Disease was approximately halved by 4 Mn for Condor and Spear and by more than two-thirds for Bayonet. Disease levels were the same for all genotypes at 4 Mn and there was no additional effect from the use of 16 Mn.

The number of take-all lesions per plant and the percentage of infected seminal roots were not affected by treatments and averaged 11 and 76%, respectively, for +Ggt (data presented in tables C.1. and C.2. in Appendix C, respectively). No disease was detected in -Ggt pots.

Table 4.1. Effect of soil Mn on total length of black stelar lesions (mm per plant) on the roots of four wheat genotypes grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 10 plants. Analysis was conducted on +Ggt treatments only.

	0 Mn		4 Mn		16 Mn		
Genotype	-Ggt	+Ggt	-Ggt	+Ggt	-Ggt	+Ggt	Mean[a]
Bayonet	0	24.6	0	7.4	0	10.0	14.0
Condor	0	16.7	0	7.6	0	10.0	11.5
Spear	0	18.5	0	8.3	0	7.4	11.4
C8MM	0	11.2	0	11.4	0	10.1	10.9
Mean[a]		17.8		8.7		9.4	
LSD[b] (P=0.05) Mn=2.3 ; Genotype ns ; Mn*Genotype=4.5							

[a] Means calculated for +Ggt treatments only.
[b] Values separated by more than the appropriate LSD were statistically different.

4.3.2. Plant growth.

Plants with take-all were generally smaller at harvest and neither Mn rates nor genotypes appeared to influence the effect of take-all on shoot growth, except at 0 Mn where Bayonet appeared to suffer little effect of *Ggt* infection. However, there were large visual differences between genotypes grown without added Mn. Bayonet was very pale green with strong interveinal chlorosis and some necrosis on young leaves. Condor also had strong interveinal chlorosis on young leaves but Spear was a darker green with only moderate interveinal chlorosis. C8MM was a healthy green and interveinal chlorosis on young leaves was only just evident. At 4 and 16 Mn all genotypes grew well and were a dark green except Bayonet at 4 Mn which showed early signs of interveinal chlorosis on young leaves.

The average dry weight of shoots per plant was decreased by 27 % by take-all, regardless of genotype or Mn rate (table 4.2). However, genotypes and Mn rates interacted in such a way that there were large differences in shoot dry weights between genotypes at 0 Mn, which largely disappeared at 4 and 16 Mn. At 0 Mn, C8MM produced the highest shoot dry weights; 49, 60 and 91 % higher than Spear, Condor and Bayonet, respectively. Where Mn was added to pots, differences between genotypes were much smaller and the rankings of the genotypes changed, although C8MM still produced the highest dry weights.

Table 4.2. Effect of soil Mn and *Ggt* infection on shoot dry weight (mg per plant) of four wheat genotypes grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 10 plants.

Genotype	0 Mn -Ggt	0 Mn +Ggt	4 Mn -Ggt	4 Mn +Ggt	16 Mn -Ggt	16 Mn +Ggt
Bayonet	20.2[a]	19.6	62.8	41.5	66.7	51.6
	3.05[b]	*3.03*	*4.16*	*3.75*	*4.22*	*3.96*
Condor	24.5	21.0	68.6	40.1	58.9	47.9
	3.24	*3.09*	*4.24*	*3.72*	*4.09*	*3.89*
Spear	32.3	20.0	63.0	41.5	66.7	43.1
	3.50	*3.04*	*4.16*	*3.75*	*4.22*	*3.79*
C8MM	42.2	34.1	70.0	52.1	75.8	53.1
	3.77	*3.56*	*4.26*	*3.99*	*4.34*	*3.99*

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[c,d] at P=0.05 level).

					LSD
Mn	0	4	16		
	25.7	53.8	58.0		
	3.29	*4.00*	*4.06*		*0.07*
Genotype	Bayonet	Condor	Spear	C8MM	
	39.2	39.9	41.2	52.8	
	3.69	*3.71*	*3.74*	*3.99*	*0.08*
Ggt	-	+			
	50.3	36.7			
	3.94	*3.63*			*0.06*
Genotype	Bayonet	Condor	Spear	C8MM	
Mn					
0	19.9	22.7	25.4	37.9	
	3.04	*3.17*	*3.27*	*3.66*	*0.04*
4	51.1	52.5	51.1	61.0	
	3.95	*3.98*	*3.95*	*4.13*	
16	58.6	53.1	53.7	63.5	
	4.09	*3.99*	*4.00*	*4.17*	
Mn	0	4	16		
Ggt					
-	28.7	66.0	66.8		
	3.39	*4.21*	*4.22*		*ns*
+	23.1	43.8	48.8		
	3.18	*3.80*	*3.91*		
Genotype	Bayonet	Condor	Spear	C8MM	
Ggt					
-	44.1	46.4	51.4	60.7	
	3.81	*3.86*	*3.96*	*4.12*	*ns*
+	34.8	34.4	33.0	45.9	
	3.58	*3.57*	*3.53*	*3.85*	

[a] Geometric means calculated from natural log transformed data.

[b] Values in italics are natural log transformations, calculated to adjust for a skewed distribution.

[c] Values separated by more than the appropriate LSD were statistically different.

[d] LSD's apply to transformed data only.

Soil-applied Mn increased the dry weight of shoots for all genotypes but the size of the increase depended on the genotype. The shoot dry weight of C8MM increased by 61 % at 4 Mn and Spear, Condor and Bayonet by 101, 131 and 157 %, respectively. Only Spear and Bayonet increased further with 16 Mn but the increases in both cases were small; 5 and 15 %, respectively.

The effect of treatments on root growth was complex and increases in the average dry weight per plant with added Mn were influenced, separately, by both genotypes and take-all. In addition, the effect of *Ggt* infection on seminal and nodal root growth were both modified by genotype and Mn rate but in opposite directions.

Very large differences in average dry weight of roots per plant were measured at 0 Mn but all genotypes had similar root weights with added Mn (figure 4.1, table 4.3). This pattern was very similar to the one observed in shoot dry weights but differences between treatments were even more marked. At 0 Mn, the dry weight of roots of C8MM were 75, 141 and 270 % higher than Spear, Condor and Bayonet, respectively. The rankings of genotypes at 0 Mn were similar at 4 and 16 Mn but differences between genotypes were relatively small (maximum difference of only 33 %) and rarely statistically significant.

Addition of Mn increased the dry weight of roots but the size of the increase depended on the genotype. The low Mn treatment (4 Mn) increased root dry weight of C8MM, Spear, Condor and Bayonet by 100, 209, 328 and 456 %, respectively. Only Bayonet was increased further by 16 Mn.

Ggt infection decreased root dry weight at 4 and 16 Mn by 35 and 27 %, respectively, but no decrease occurred at 0 Mn. The increase in root dry weight with 4 Mn in -Ggt pots was nearly double that in +Ggt pots. There was no further increase with 16 Mn.

Table 4.3. Effect of soil Mn and *Ggt* infection on root dry weight (mg per plant) of four wheat genotypes grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 10 plants.

Genotype	0 Mn -Ggt	0 Mn +Ggt	4 Mn -Ggt	4 Mn +Ggt	16 Mn -Ggt	16 Mn +Ggt
Bayonet	5.1[a]	5.7	36.4	24.5	40.1	30.7
	1.80[b]	*1.90*	*3.62*	*3.24*	*3.72*	*3.49*
Condor	7.7	8.8	46.9	26.4	36.5	32.5
	2.17	*2.28*	*3.87*	*3.31*	*3.62*	*3.51*
Spear	12.4	10.7	43.6	28.8	46.3	27.7
	2.59	*2.46*	*3.80*	*3.40*	*3.86*	*3.36*
C8MM	19.6	20.1	47.3	33.5	52.1	34.7
	3.03	*3.05*	*3.88*	*3.54*	*3.97*	*3.58*

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[c,d] at P=0.05 level).

					LSD
Mn	0	4	16		
	10.1	34.9	37.0		
	2.41	*3.58*	*3.64*		*0.09*
Genotype	Bayonet	Condor	Spear	C8MM	
	21.8	32.4	24.6	18.3	
	3.13	*3.51*	*3.24*	*2.96*	*0.10*
Ggt	-	+			
	26.9	21.0			
	3.33	*3.09*			*0.07*
Genotype	Bayonet	Condor	Spear	C8MM	
Mn					
0	5.4	8.2	11.5	19.9	
	1.85	*2.22*	*2.53*	*3.04*	*0.17*
4	29.9	35.2	35.5	39.8	
	3.43	*3.59*	*3.60*	*3.71*	
16	35.7	34.4	35.8	42.6	
	3.60	*3.57*	*3.61*	*3.77*	
Mn	0	4	16		
Ggt					
-	10.0	43.4	43.4		
	2.40	*3.79*	*3.79*		*0.17*
+	10.3	28.1	31.5		
	2.42	*3.37*	*3.48*		
Genotype	Bayonet	Condor	Spear	C8MM	
Ggt					
-	20.1	24.0	29.4	36.5	
	3.05	*3.22*	*3.42*	*3.63*	*ns*
+	16.7	19.7	20.5	28.6	
	2.88	*3.03*	*3.07*	*3.39*	

[a] Geometric means calculated from natural log transformed data.

[b] Values in italics are natural log transformations, calculated to adjust for a skewed distribution.

Separate examination of the two root types (seminal and nodal) which contributed to total root weight per plant revealed that nodal roots responded very differently to *Ggt* infection than seminal roots. The total length of main axis and lateral seminal roots (figure 4.1, table 4.4) responded to treatments in a very similar fashion to dry weight roots (see figure 4.1) although effects of treatments on main axis seminal roots (data not presented) were usually small and often not significant. The similarity between treatment effects on seminal root lengths and total dry weight of roots probably reflected the major contribution that seminal roots made to the root systems of wheat seedlings at the early growth stage at which plants were harvested. The number of seminal roots per plant (data not presented) was not appreciably affected by treatments and averaged 5-6 roots per plant for the experiment.

The number and total length of nodal roots per plant were both increased by *Ggt* infection (figure 4.1 and tables 4.5,4.6, respectively). This effect is the reverse of that which occurred with seminal roots. In relative terms the stimulation of nodal root growth with *Ggt* infection was most dramatic without added Mn. For instance, the total length of nodal roots increased nearly eighteen-fold in +Ggt pots at 0 Mn but with added Mn the increase was just over two-fold. Un-infected Condor and Bayonet did not produce any nodal roots at 0 Mn. Effects of treatments on number and length of nodal roots were similar.

The interaction between Ggt, Mn rates and genotypes on total length of nodal roots also differed from that of the total length of seminal roots. At 0 Mn, there was no difference between genotypes in nodal root length.

FIGURE 4.1. EFFECT OF SOIL-APPLIED MN AND GGT ON ROOT GROWTH OF FOUR WHEAT GENOTYPES GROWN IN WANGARY SAND UNDER CONTROLLED ENVIRONMENT CONDITIONS. VALUES ARE THE AVERAGE OF 10 PLANTS.
Stippled bars were disease-free, slashed bars infected with Ggt.

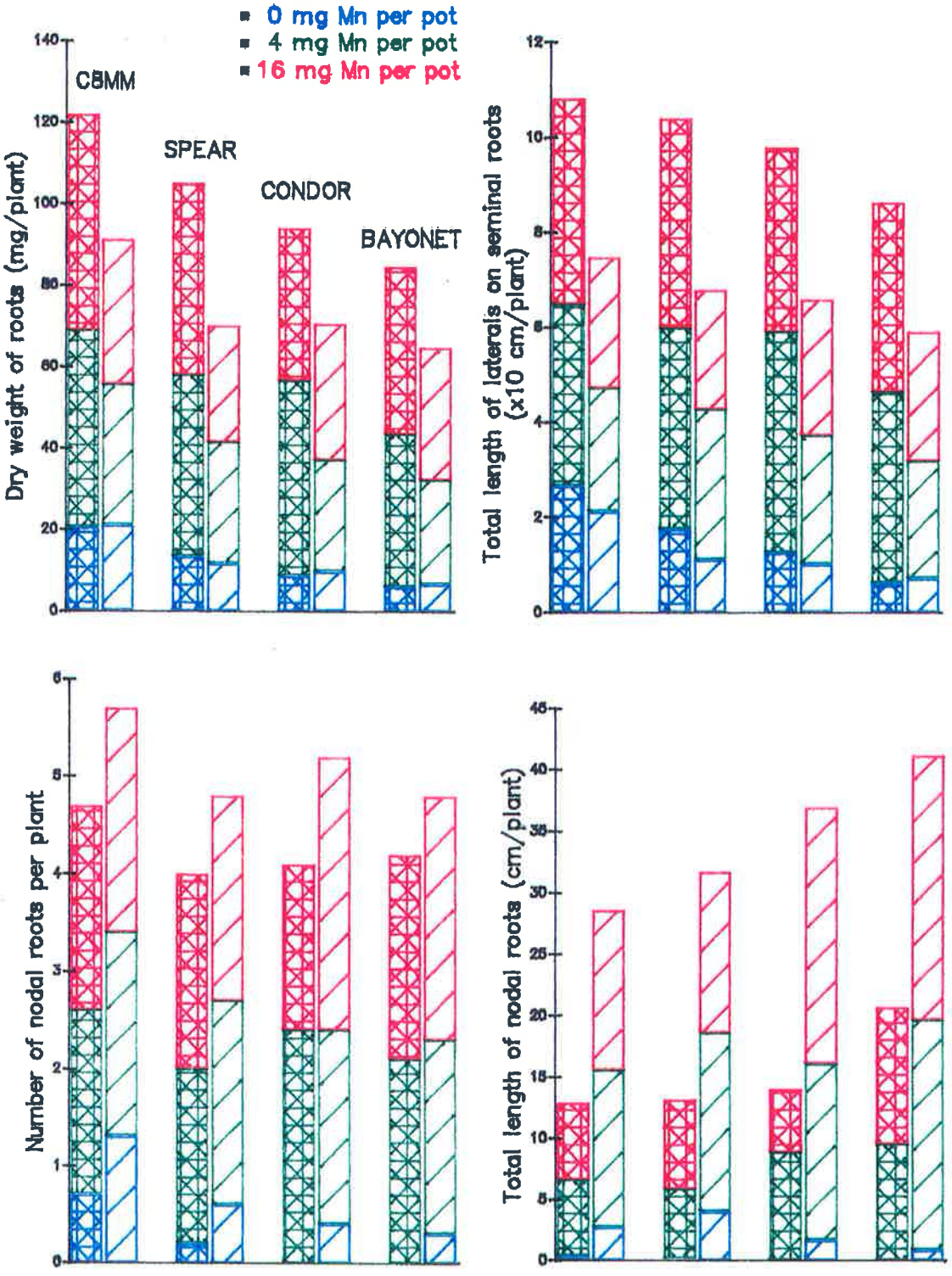

Table 4.4. Effect of soil Mn and *Ggt* infection on total length of lateral seminal roots (mm per plant) of four wheat genotypes grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 10 plants.

Genotype	0 Mn -Ggt	0 Mn +Ggt	4 Mn -Ggt	4 Mn +Ggt	16 Mn -Ggt	16 Mn +Ggt
Bayonet	124	143	805	494	796	545
Condor	251	203	930	541	776	572
Spear	350	222	846	632	884	504
C8MM	531	424	767	520	865	551

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

					LSD
Mn	0	4	16		
	281	692	687		35
Genotype	Bayonet	Condor	Spear	C8MM	
	484	546	573	610	40
Ggt	-	+			
	660	446			28
Genotype	Bayonet	Condor	Spear	C8MM	
Mn					
0	133	227	286	478	70
4	650	735	739	643	
16	670	674	694	708	
Mn	0	4	16		
Ggt					
-	314	837	830		49
+	248	547	543		
Genotype	Bayonet	Condor	Spear	C8MM	
Ggt					
-	575	652	693	721	ns
+	394	439	453	499	

[a] Values separated by more than the appropriate LSD were statistically different.

Table 4.5. Effect of soil Mn and *Ggt* infection on number of nodal roots per plant of four wheat genotypes grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 10 plants.

Genotype	0 Mn -Ggt	0 Mn +Ggt	4 Mn -Ggt	4 Mn +Ggt	16 Mn -Ggt	16 Mn +Ggt
Bayonet	0	0.3	2.1	2.0	2.1	2.5
Condor	0	0.4	2.4	2.0	1.7	2.8
Spear	0.2	0.6	1.8	2.1	2.0	2.1
C8MM	0.7	1.3	1.9	2.1	2.1	2.3

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

					LSD
Mn	0	4	16		
	0.4	2.1	2.2		0.3
Genotype	Bayonet	Condor	Spear	C8MM	
	1.5	1.6	1.5	1.7	ns
Ggt	-	+			
	1.4	1.7			0.1
Genotype	Bayonet	Condor	Spear	C8MM	
Mn					
0	0.2	0.2	0.4	1.0	0.3
4	2.1	2.2	2.0	2.0	
16	2.3	2.3	2.1	2.2	
Mn	0	4	16		
Ggt					
-	0.2	2.1	2.0		0.2
+	0.7	2.1	2.4		
Genotype	Bayonet	Condor	Spear	C8MM	
Ggt					
-	1.4	1.4	1.3	1.6	ns
+	1.6	1.7	1.6	1.9	

[a] Values separated by more than the appropriate LSD were statistically different.

Table 4.6. Effect of soil Mn and *Ggt* infection on total length of nodal roots (mm per plant) of four wheat genotypes grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 10 plants.

	0 Mn		4 Mn		16 Mn	
Genotype	-Ggt	+Ggt	-Ggt	+Ggt	-Ggt	+Ggt
Bayonet	0	8	95	188	112	215
Condor	0	16	88	144	51	209
Spear	2	40	57	146	73	131
C8MM	4	57	63	129	63	130

LSD[a] (P=0.05) Mn*Genotype*Ggt=62

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

					LSD
Mn	0	4	16		
	12	114	123		11
Genotype	Bayonet	Condor	Spear	C8MM	
	103	85	75	69	13
Ggt	-	+			
	51	115			9
Genotype	Bayonet	Condor	Spear	C8MM	
Mn					
0	4	8	21	15	22
4	141	116	102	96	
16	163	130	102	97	
Mn	0	4	16		
Ggt					
-	1	76	75		16
+	23	152	171		
Genotype	Bayonet	Condor	Spear	C8MM	
Ggt					
-	69	47	44	43	ns
+	137	123	106	95	

[a] Values separated by more than the appropriate LSD were statistically different.

Un-infected Bayonet had a higher total length of nodal roots than the other three genotypes at 16 Mn and a greater length than Spear or C8MM at 4 Mn. *Ggt*-infected Bayonet and Condor had higher total lengths of nodal roots than Spear and C8MM at 16 Mn while only Bayonet was higher at 4 Mn. There was a large increase in nodal root length with 4 Mn but 16 Mn did not cause any further increase except in *Ggt*-infected Condor.

4.3.3. Mn.

The concentration of Mn in whole shoots (oven dry weight basis) at harvest (table 4.7) averaged less than 5 mg kg^{-1} at 0 Mn for all genotypes and at both - and +Ggt, which is well below the critical level of 18 mg kg^{-1} for wheat seedlings grown in experiments of this type (see appendix B). *Ggt* infection had no effect on Mn concentration in whole shoots at 0 Mn nor at 16 Mn where shoot Mn concentrations averaged 42.3 mg kg^{-1}. However, at 4 Mn *Ggt* infection decreased shoot Mn concentration from an average of 19.9 mg kg^{-1} to 14.4 mg kg^{-1}. The average concentration of Mn in whole shoots for each genotype was not influenced by Mn rates or take-all and C8MM had higher shoot Mn concentrations than Spear or Condor which, in turn, had higher levels than Bayonet.

Table 4.7. Effect of soil Mn and *Ggt* infection on Mn concentration in whole shoots (mg kg^{-1} D.W.) of four wheat genotypes grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 10 plants.

Genotype	0 Mn -Ggt	0 Mn +Ggt	4 Mn -Ggt	4 Mn +Ggt	16 Mn -Ggt	16 Mn +Ggt
Bayonet	4.0[a]	3.6	16.2	13.1	37.6	37.4
	1.61[b]	*1.52*	*2.85*	*2.65*	*3.65*	*3.62*
Condor	4.6	5.0	18.7	13.8	44.0	37.6
	1.72	*1.79*	*3.03*	*2.69*	*3.80*	*3.65*
Spear	5.1	4.9	18.5	13.4	43.5	44.2
	1.80	*1.77*	*2.97*	*2.67*	*3.80*	*3.81*
C8MM	5.7	6.0	26.3	17.7	50.9	47.0
	1.89	*1.94*	*3.31*	*2.93*	*3.95*	*3.87*

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[c,d] at P=0.05 level).

					LSD
Mn	0	4	16		
	4.8	16.9	42.3		
	1.76	*2.89*	*3.77*		*0.07*
Genotype	Bayonet	Condor	Spear	C8MM	
	13.2	15.1	15.5	18.7	
	2.65	*2.78*	*2.80*	*2.98*	*0.08*
Ggt	-	+			
	16.6	14.5			
	2.87	*2.74*			*0.06*
Genotype	Bayonet	Condor	Spear	C8MM	
Mn					
0	4.8	4.8	6.0	5.8	
	1.57	*1.75*	*1.79*	*1.92*	*ns*
4	14.6	16.5	21.6	15.8	
	2.75	*2.86*	*2.82*	*3.12*	
16	36.9	40.6	43.5	49.0	
	3.64	*3.73*	*3.80*	*3.91*	
Mn	0	4	16		
Ggt					
-	4.80	19.9	43.7		
	1.76	*3.04*	*3.80*		*0.10*
+	4.8	14.4	41.0		
	1.76	*2.73*	*3.74*		
Genotype	Bayonet	Condor	Spear	C8MM	
Ggt					
-	13.9	17.3	16.4	20.1	
	2.70	*2.85*	*2.86*	*3.05*	*ns*
+	12.4	14.0	14.6	17.4	
	2.60	*2.71*	*2.75*	*2.91*	

[a] Geometric means calculated from natural log transformed data.

[b] Values in italics are natural log transformations, calculated to adjust for a skewed distribution.

[c] Values separated by more than the appropriate LSD were statistically different.

[d] LSD's apply to transformed data only.

4.4. Discussion.

The results of this experiment clearly showed that there was a strong and negative relationship between Mn efficiency and relative susceptibility to take-all. C8MM produced much more dry matter than Bayonet under conditions which were severely Mn deficient and accumulated a higher concentration of Mn in its shoots. Spear and Condor were intermediate between these two genotypes with Spear being more Mn-efficient than Condor. The ranking of these 4 genotypes for susceptibility to take-all under severely Mn-deficient conditions was in reverse order to Mn efficiency. The lowest total length of black stelar lesions per plant occurred on C8MM and the highest on Bayonet. The length of lesions on Spear and Condor was intermediate between C8MM and Bayonet and not significantly different from each other. All genotypes were equally susceptible where Mn was applied to soil.

The largest decrease in total length of lesions with Mn also occurred on Mn-inefficient Bayonet and decreases on more efficient Spear and Condor were smaller. There was no decrease in length of lesions with added Mn in C8MM although C8MM was Mn deficient by harvest. The high Mn seed content of C8MM may have been the reason for the absence of a decrease in total length of lesions with added Mn. Seed Mn is very important for early seedling growth (Marcar and Graham 1986) and the seed reserves of Mn of C8MM may have been sufficient for growth during the time when invasion by *Ggt* was occurring and Mn-deficiency did not begin until after roots were colonized. All seed was taken from the same field site and the high content of Mn in C8MM seed may have been due to better growth of C8MM under Mn-deficient conditions or it may have been due to a superior ability of C8MM to translocate Mn to its seed.

Penrose (1985) reported that invasion by *Ggt* into seminal roots of Condor was slower than RAC311 (Bayonet) and that fewer stelar lesions developed on Condor. Similar differences in resistance between Condor and Bayonet were only evident under Mn-deficient conditions in this experiment. Further work is warranted to investigate whether the differences in resistance discovered here under Mn-deficient conditions can be explained by differences in rates of invasion and colonization by *Ggt*. Studies were conducted to investigate *Ggt* invasion into Mn-deficient and -sufficient roots of Condor and are presented in chapter 7.

Disease levels produced in this experiment were sufficiently high that *Ggt* infection decreased shoot Mn concentrations at 4 Mn. Results of experiments reported in chapter 3 showed that high *Ggt* inoculum rates would decrease the shoot Mn content of infected plants but that this effect was not necessary for disease to be favoured under Mn-deficient conditions. *Ggt* inhibits host growth by reducing the size of the effective root system of infected plants. It does this by blocking and destroying root phloem bundles (which halts root elongation) and also xylem vessels (which restricts nutrient uptake and translocation to shoots (Fellows 1928 Weste 1972, Clarkson et al. 1975). Roots are effectively truncated at sites of well-developed stelar lesions (Clarkson et al. 1975). At 16 Mn, there was abundant Mn available to plants and the loss of root length caused by *Ggt* infection decreased shoot uptake of Mn to the same extent as shoot dry weight and shoot Mn concentration did not change.

However, at 4 Mn shoot Mn concentrations were raised to barely adequate levels and the loss of effective root length which accompanied *Ggt* infection reduced shoot uptake of Mn more than shoot dry weight. As a consequence shoot Mn concentrations were decreased to levels well below critical for growth. This induced Mn deficiency caused by *Ggt* infection resulted in additional host growth depression; *Ggt* infection decreased the dry weight of roots by 35 % at 4 Mn but by 27 % at 16 Mn. A similar interaction occurred in shoot dry weight but was not statistically significant.

As occurred at 16 Mn, the reduction in shoot dry weight with *Ggt* infection at 0 Mn matched those of Mn uptake and shoot Mn concentration was not decreased. The dry weight of roots per plant was not decreased by *Ggt* infection at 0 Mn which suggests that severe Mn deficiency was a more important factor limiting plant growth than take-all. Where Mn deficiency was relieved by the addition of soil Mn the effects of *Ggt* infection on plant growth increased.

Graham and Rovira (1984) reported that a low rate of Mn which increased shoot Mn concentrations in wheat seedlings but did not eliminate Mn deficiency was partially successful in decreasing *Ggt* infection. The rate of 4 Mn in this experiment did not completely eliminate Mn deficiency in Spear and Bayonet because further increases in growth occurred with 16 Mn in both these genotypes. This low rate of soil Mn decreased

Ggt infection as effectively as 16 Mn on all four genotypes but in one experiment reported in chapter 3 the same Mn rate did not decrease *Ggt* infection. The variable effects of low rates of Mn in decreasing *Ggt* infection highlights the subtle nature of the interaction between invasion of wheat roots by *Ggt* and rates of Mn which are marginal for growth of the plant host. Slight differences in Mn availability or timing and rates of *Ggt* invasion may be important in determining the progress of the disease.

Contrary to the depressive effects dry weight of roots and seminal root lengths, *Ggt* infection stimulated nodal root growth. Stimulated nodal root production, as a response to *Ggt* infection, has been proposed as a tolerance mechanism for cereals because it enables the infected plant to replace roots lost due to disease. Comparisons between genotypes for their relative abilities to produce nodal roots was not possible at 0 Mn because there were different disease levels on each genotype. However, at 4 and 16 Mn all disease levels were similar and under these conditions Bayonet generally produced higher total nodal root lengths than the other three genotypes (Bayonet also produced higher nodal root lengths than the other genotypes under disease-free conditions). Despite the higher nodal root lengths, shoot growth of Bayonet was still depressed by *Ggt* infection to the same extent as the other genotypes. Plants were probably harvested too early to allow differences in nodal root growth to affect plant growth because the nodal roots were only a minor component of root systems by harvest.

4.5. References.

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CHAPTER 5.

EFFECT OF MN ON THE GROWTH OF FOUR GGT ISOLATES AND ON THE DISEASE THEY CAUSE ON WHEAT ROOTS

CHAPTER 5. EFFECT OF MN ON THE GROWTH OF FOUR *GGT* ISOLATES AND ON THE DISEASE THEY CAUSE ON WHEAT ROOTS.

5.1. Introduction.

The papers of Graham and Rovira (1984) and Rovira *et al.* (1985) reported additions of Mn decreasing take-all caused by one isolate of *Ggt*. This same isolate (500) has been used in all the studies summarized in this thesis so far and experiments were conducted to test the sensitivity of three other field isolates of *Ggt* (and the disease they cause) to Mn.

In this chapter, three experiments are reported which tested the sensitivity of four *Ggt* isolates, including 500, to manganous ions on potato dextrose agar and the effect of addition of Mn to a Mn-deficient soil on disease caused by each isolate.

5.2. Materials and Methods.

5.2.1. Petri-dish experiment.

5.2.1.1. *Isolates*. Four different field isolates were provided by Dr. A. Rovira [1]. Isolate 500 has been previously described (see chapter 2). Isolates 47 and 43 were both isolated from wheat seedling roots grown on infected crowns from Avon, South Australia in 1978 and isolate 8 was collected from a wheat crown grown at Avon, South Australia in 1979. All cultures were stored on full-strength PDA under sterile $DDDH_2O$ and colonies for each experiment were prepared from stored cultures.

5.2.1.2. *Mn*. Mn^{2+} ion concentrations of 0, 25, 50, 100, 200, 400 and 800 mg kg^{-1} were prepared by adding appropriate amounts of a $MnSO_4.4H_2O$ stock solution to weak PDA [2] (approximately 40 % of manufacturer's recommendations) which had been made up with $DDDH_2O$. Three dishes were prepared for each Mn concentration.

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5.2.1.3. *Procedure*. Disposable petri-dishes (9 cm. diameter) were poured with 15 ml of agar (autoclaved at 120° C and 100 kPa for 15 minutes). Mycelial cubes (4 mm x 4 mm x 4 mm), removed from the colony edge of an actively growing culture on weak PDA (no added Mn), were used as inocula for test dishes. One mycelial cube was aseptically transferred to the centre of each dish and dishes were kept in the dark at 20° C for 5 days. Colony areas were calculated after 5 days from the average of 2 measurements of colony diameter taken at right angles to each other.

5.2.2. Pot experiment A.

This experiment was conducted under controlled environment conditions with a factorial combination of treatments; 4 *Ggt* isolates (500, 47, 43, 8), 2 Mn rates (0 and 4 mg of Mn per pot), and 4 replicates. The basic techniques employed here were developed during experiments with small pots outlined in Chapter 3 but some minor refinements were included. Complete details for soil, Mn and *Ggt* techniques can be found in sections 3.2.1.1., 3.2.4. and 3.2.3.2. of Chapter 3, respectively.

5.2.2.1. *Inoculum*. Petri-dishes were poured with approximately 17 ml of autoclaved media containing 1 g of `Difco' Bacto-agar and 0.156 g (4 %) of PDA in 100 ml of $DDDH_2O$. Dishes were inoculated with single mycelial cubes taken from the edge of actively growing colonies of each *Ggt* isolate and placed at 20° C for 7 days. Extra dishes of 4 % PDA were made up and kept sterile for use as controls for *Ggt* (for statistical analysis the *Ggt* controls were regarded as a fifth isolate).

5.2.2.2. *Soil*. Wangary sand collected in 1983 was again used. The soil was not incubated prior to sowing because the technique (see appendix A) had not been developed by the time of this experiment. The stored soil, however, was Mn-deficient.

5.2.2.3. *Mn*. Mn was mixed through soil as a $MnSO_4.4H_2O$ solution at either 0 (nil Mn) or 4 mg of Mn per pot (4 Mn).

5.2.2.4. *Procedure*. Basal nutrients were mixed through soil at sowing at the same rates used in chapter 3 with small pots.

Condor wheat seeds, from a trial grown on a Mn-deficient site at Tooligie on the Eyre Peninsula S.A. in 1984 (av. Mn concentration, 6.4 mg kg^{-1}), were surface-sterilized with 6 % NaOCl for 2 minutes, rinsed with $DDDH_2O$ and germinated as before (see section 3.2.4.).

All pots were part-filled with 42.5 g of prepared soil, followed by an agar disc which had been cut to the internal diameter of the pots, a further 8.4 g of soil (resulting in a 2 cm deep layer), 2 germinated wheat seeds and a final layer of 4.1 g of soil. *Ggt* colonized agar discs were cut from the outer margins of *Ggt* colonies. Each pot received 2 ml of $DDDH_2O$ to the soil surface to settle soil around the seeds. Four extra pots were filled with prepared soil (no added Mn) but without agar discs to test the effect of agar discs on seedling growth.

Pots were placed in a tray in randomized blocks and covered with a sheet of thin clear polythene (`Glad Wrap') to reduce drying of the soil surface, before being placed in controlled environment conditions of 15/10° C day/night temperatures with a 10 hour light period (average photon flux density of 0.7 mEinsteins m^{-2} s^{-1} supplied by a bank of mercury vapour lamps) in every 24.

`Glad Wrap' was removed after 3 days and pots were watered to original weight every 3-4 days. Plants were harvested after 25 days. Shoots were removed at the soil surface and fresh weight per pot recorded before drying at 70° C for 2 days. Roots were washed free of soil and the number of black stelar lesions, the total length of stelar lesions, the number of diseased seminal roots and the total length of nodal roots recorded for each plant. After disease severity had been assessed, the fresh weight of roots per pot was recorded (after free water was removed) and roots dried at 70° C for 2 days. Oven-dry weights for shoots and roots were recorded and both tissues analyzed for Mn and 10 other

essential elements with an ARL [2] inductively coupled plasma atomic emission spectrometer. Samples were prepared for analysis by nitric acid digestion.

5.2.3. Pot experiment B.

This experiment was conducted under controlled environment conditions with a factorial combination of treatments; 3 *Ggt* isolates (500, 47, 8), 3 Mn rates (0, 4 and 16 mg of Mn per pot), 2 Ggt levels (agar discs at 4 and 2 cm below the seeds) and 5 replicates.

5.2.3.1. *Inoculum*. Agar disc inocula for each *Ggt* isolate were prepared with the same techniques used in pot experiment A and *Ggt* controls were again regarded as an extra isolate for statistical analysis.

5.2.3.2. *Soil*. Wangary sand collected in 1983 was used but, unlike pot experiment A, the soil was incubated prior to potting up (see appendix A for details).

5.2.3.3. *Mn*. Mn was mixed through soil prior to incubation as a $MnSO_4.4H_2O$ solution at rates equivalent to either 0 (nil Mn), 4 (4 Mn) or 16 (16 Mn) mg of Mn per pot.

5.2.3.4. *Procedure*. Basal nutrients were mixed through soil prior to sowing at the same rates used in chapter 3 with small pots.

Condor wheat seeds from a trial grown on a Mn-deficient site at Tooligie on the Eyre Peninsula S.A., in 1985 (av. Mn concentration 9.2 mg kg^{-1}) were surface-sterilized with 6 % NaOCl for 2 minutes, rinsed with $DDDH_2O$ and germinated as before (see section 3.2.4.).

Pots were part-filled with either 34.1 (low Ggt) or 42.5 g (high Ggt) of prepared soil, followed by an agar disc which had been cut to the internal diameter of the pots and a further 16.8 or 8.4 g of soil, respectively. Two germinated wheat seeds were then added

2 Applied Research Laboratories, SA (distributed by Bausch and Lomb (Aust) Pty. Ltd., 47 Epping Rd., North Ryde, NSW.

and pots filled with a final layer of 4.1 g of soil. *Ggt* colonized agar discs were cut from the outer margins of *Ggt* colonies. Each pot received 3 ml of $DDDH_2O$ to the soil surface to settle soil around the seeds.

Pots were placed in a tray in randomized blocks and covered with a sheet of thin clear polythene (`Glad Wrap') to reduce drying of the soil surface, before being placed in controlled environment conditions of 15/10° C day/night temperatures with a 10 hour light period (average photon flux density of 0.3 mEinsteins m^{-2} s^{-1} supplied by a bank of mercury vapour lamps) in every 24.

Pots were maintained and plants processed at harvest as in experiment A except nodal root measurements were not taken and only shoots were analyzed for Mn and 10 other essential elements.

5.3. Results.

5.3.1. Petri-dish experiment.

The growth of isolate 500 was similar to that reported in chapter 2 (see section 2.3.1.1.). Increasing concentrations of Mn^{2+} in agar decreased the growth of 500 and at 100 mg kg^{-1} of Mn^{2+} colony area was only half that without added Mn (figure 5.1). Higher rates of Mn further reduced colony growth and no growth away from the mycelial cube was detected at 800 mg kg^{-1} of Mn^{2+}. Oxidation of Mn^{2+} to insoluble brown oxides occurred at all rates of added Mn except 800 mg kg-1 where growth was completely inhibited.

Isolates 47 and 8 behaved very similarly on PDA with increasing rates of added Mn (figure 5.1, plate 5.1). Only at 100 mg kg^{-1} of Mn^{2+} were their colony areas statistically different; the average colony area of isolate 47 was lowered at this rate of Mn by one replicate plate not growing as well as the other two. Both isolates grew slower on unamended PDA than 500, although the differences were not large, and neither was as

Plate 5.1. Effect of increasing concentrations of added Mn^{2+} on the growth of three *Ggt* isolates (47, 8, 43) on potato dextrose agar. Rates of added Mn were 0, 25, 50, 100, 200, 400 and 800 mg kg^{-1} Mn^{2+}, reading from left to right and top to bottom.

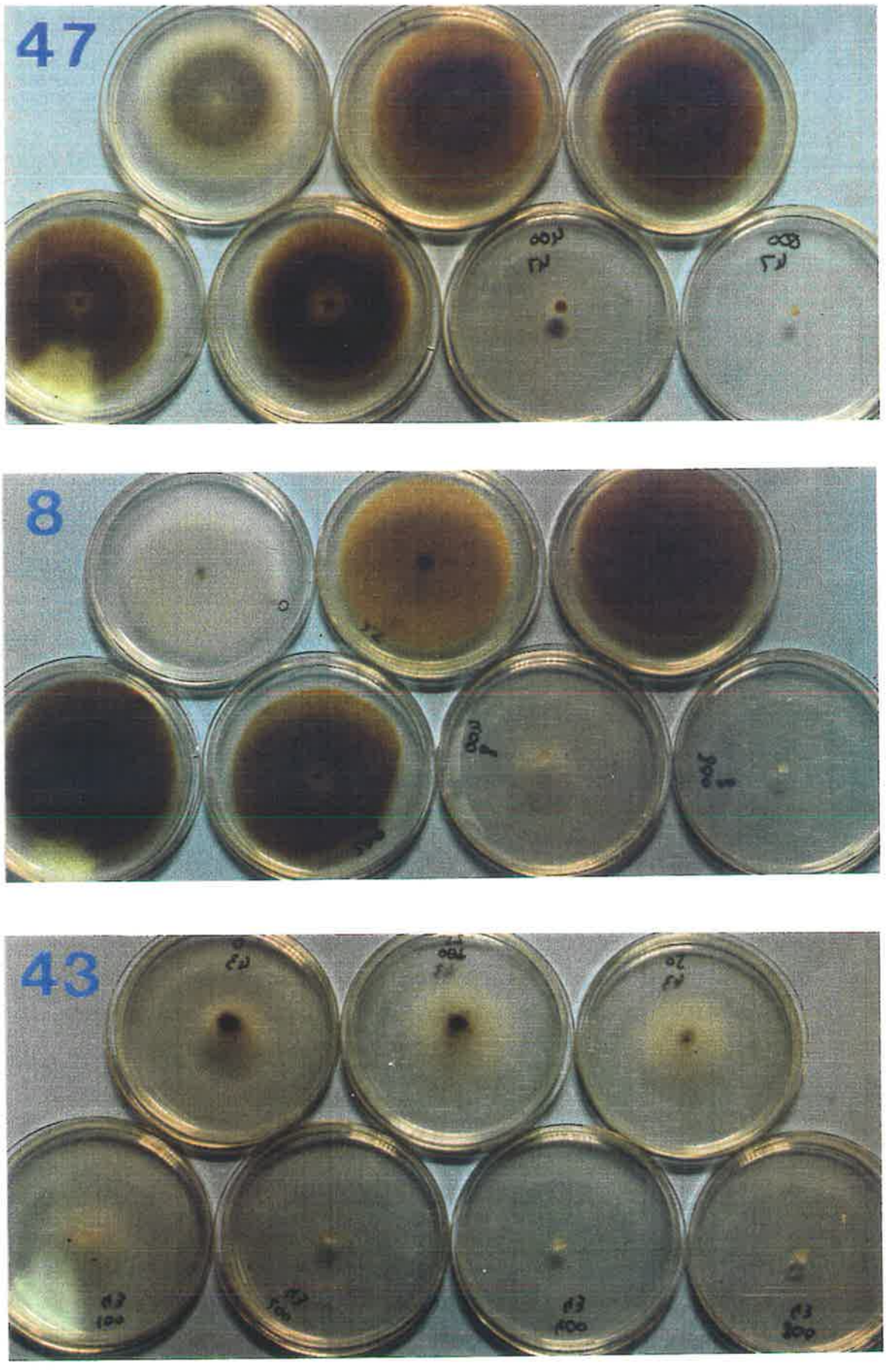
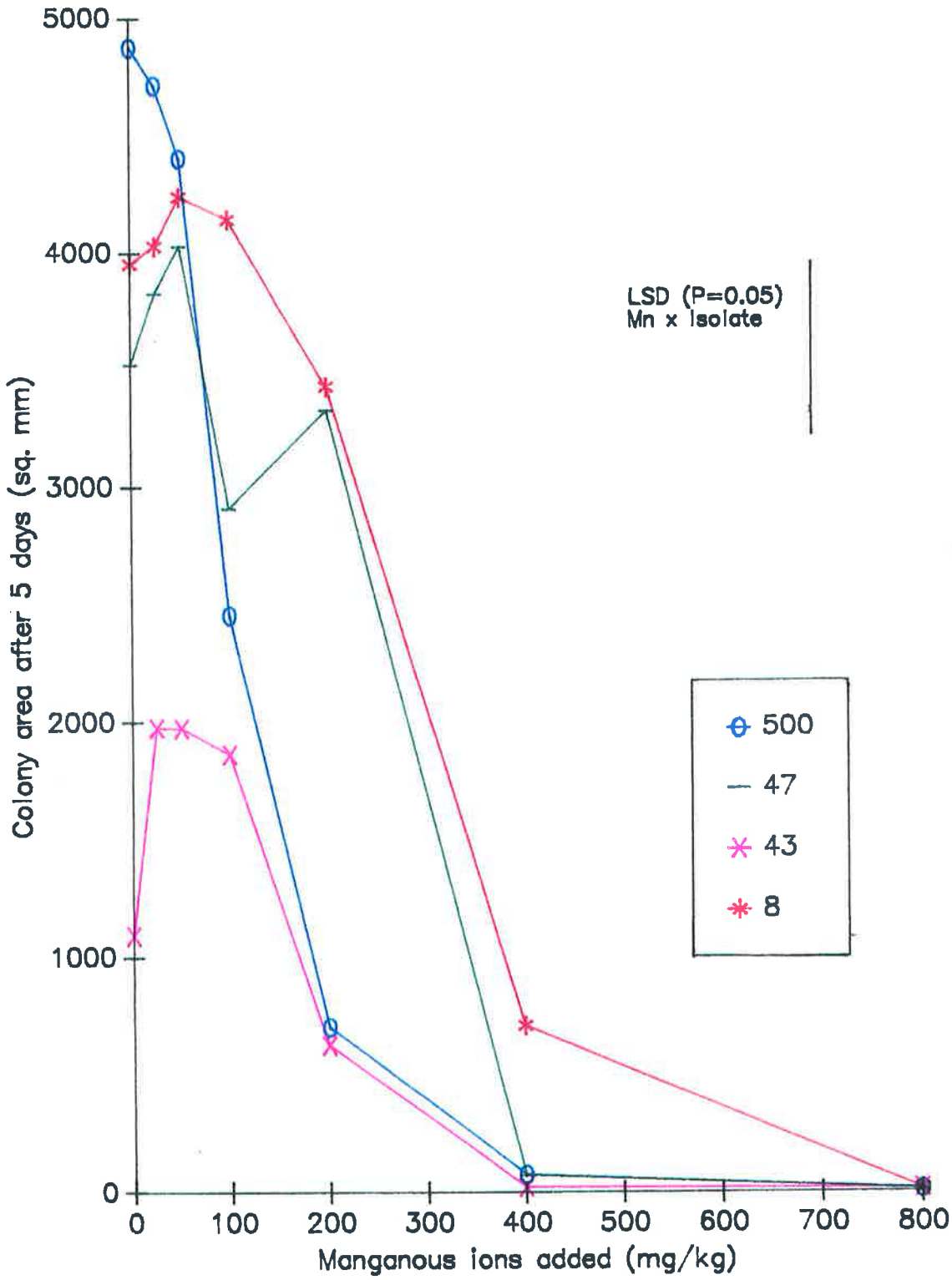


FIGURE 5.1. EFFECT OF INCREASING MANGANOUS ION CONCENTRATION ON GROWTH OF FOUR GGT ISOLATES ON PDA.



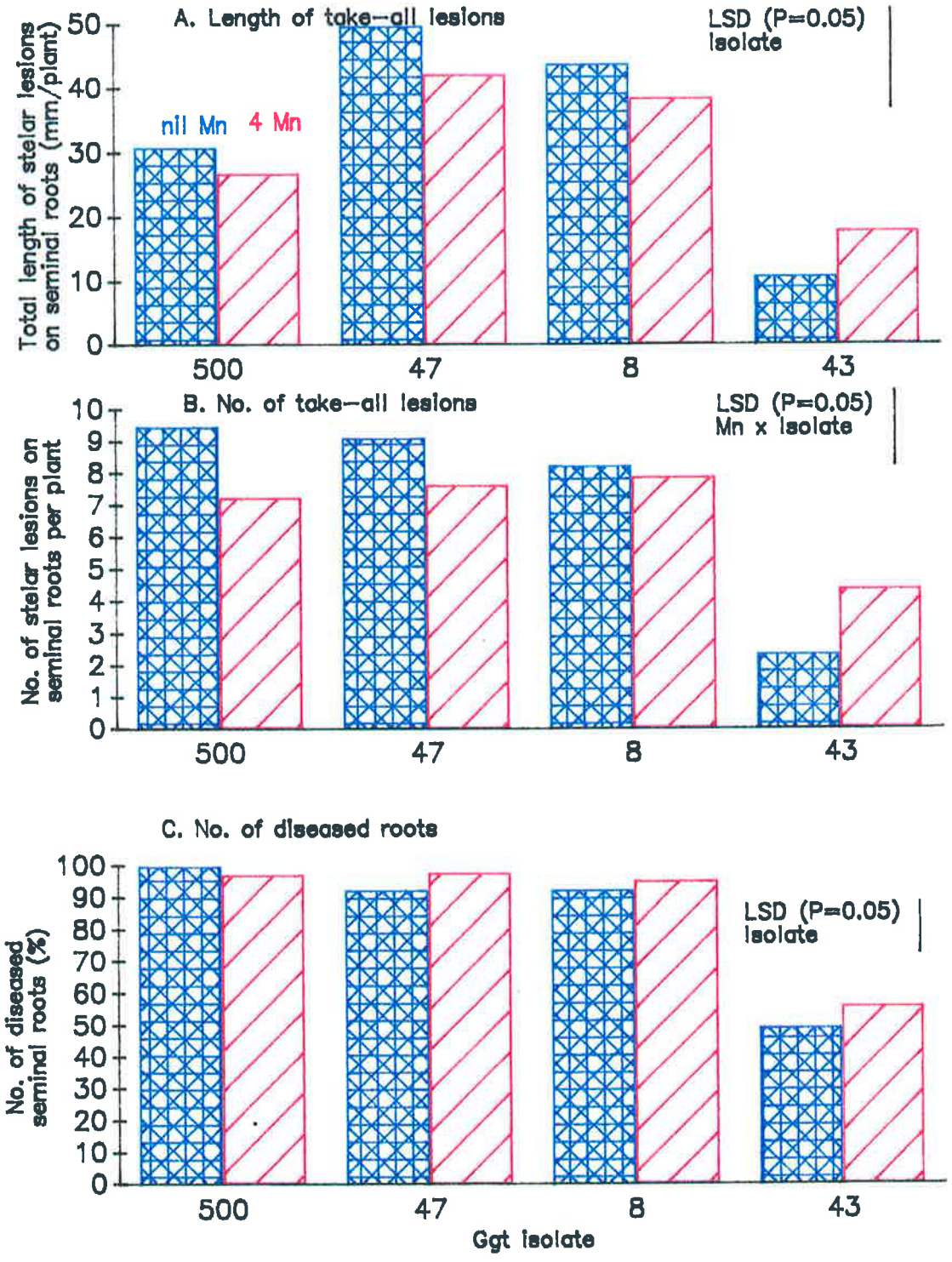
sensitive to increasing Mn^{2+} concentration as 500. Colony areas of isolates 47 and 8 were decreased by 400 and 800 mg kg^{-1} of added Mn only. Both 47 and 8 oxidized Mn^{2+} at all rates of added Mn except 800 mg kg^{-1} (plate 5.1). The pattern and extent of Mn oxidation was very similar to that observed on 500 colonies.

Isolate 43 grew slowly on PDA and its colony area on unamended PDA after 5 days was only 22 % of 500 (figure 5.1). The effect of increasing Mn^{2+} concentration on 43 was similar to that observed with 47 and 8 and only 400 and 800 mg kg^{-1} of Mn^{2+} significantly decreased colony area. Apart from its slow growth, 43 was also a very poor oxidizer of Mn^{2+} on PDA, compared to the other three isolates. Only two plates (at 25 mg kg^{-1} Mn) of 43 showed any visible Mn oxidation and the extent within these two colonies was patchy and infrequent. One feature of 43 colony growth which was similar to the other three isolates was a stimulation of growth at low Mn^{2+} concentrations. In the experiment reported in chapter 2 (see section 2.3.1.1.) 500 colony area was slightly increased at 10 mg kg^{-1} of Mn^{2+} but this effect was not observed here because of the higher rates of Mn used. The colony area of 43 was increased by nearly 75 % at 25, 50 and 100 mg kg-1 of added Mn (although the effect was not statistically significant at 100 mg kg^{-1} Mn). Isolates 47 and 8 also exhibited a stimulation of growth at low Mn rates but the effects were smaller and not significant.

5.3.2. Pot experiment A.

5.3.2.1. *Ggt*. Isolates 500, 47 and 8 caused similar and heavy disease levels on wheat roots but 43 produced only low levels of take-all (figure 5.2). Measurement of the number of black stelar lesions per plant showed that disease levels caused by the 4 *Ggt* isolates were affected differently by Mn (figure 5.2B). The number of lesions on plants infected by 500 decreased by 24 % with Mn but on plants infected by 43 the number of lesions was increased by 84 %. Decreases in the number of lesions with Mn for isolates 47 and 8 were not statistically significant (figure 5.2).

FIGURE 5.2. EFFECT OF SOIL-APPLIED MN AND INFECTION BY FOUR ISOLATES OF GGT ON TAKE-ALL SYMPTOMS OF WHEAT SEEDLINGS GROWN IN WANGARY SAND UNDER CONTROLLED ENVIRONMENT CONDITIONS.
Plants without Ggt inoculum were disease-free, values are the average of 8 plants.



Neither the total length of lesions per plant nor the percentage of diseased seminal roots were affected by Mn and averaged 14, 46, 41 and 29 mm and 53, 95, 94 and 99 %, respectively, for isolates 43, 47, 8 and 500 (figure 5.2A and C). The total length of lesions per plant produced by 500 was lower than levels produced by 47 or 8 but higher than for 43.

No disease was detected in pots to which sterile PDA discs had been added.

5.3.2.2. *Plant growth.* The different disease levels produced by the 4 *Ggt* isolates were not reflected in effects on plant growth, except for 43 which produced the lowest disease levels and did not decrease plant growth (figure 5.3). Increasing Mn rates did not influence the effect of *Ggt* infection on plant growth.

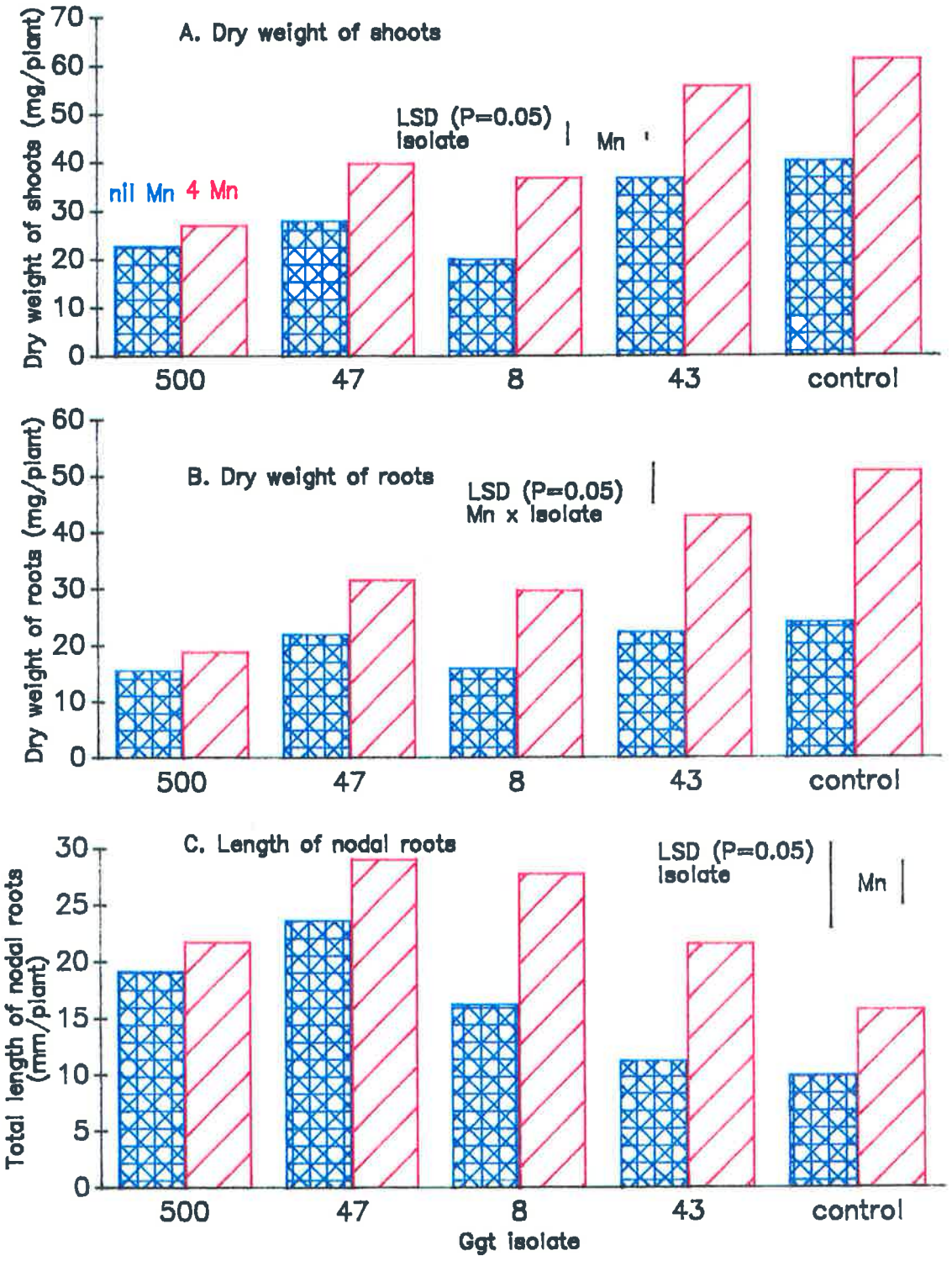
The average dry weight of shoots per plant was decreased to the greatest extent (51 %) by 500, although this isolate produced a lower total length of lesions and equal number of lesions and diseased roots to 47 and 8 (figure 5.3A). Isolate 47 decreased the dry weight of shoots by only 33 % while 8 caused a decrease intermediate to 500 and 47.

Added Mn caused a small increase in shoot dry weight (an average of 48 %) compared to experiments in which soil had been incubated prior to sowing (e.g. there was a 94 % increase in experiment B of this chapter) (figure 5.3A).

The average dry weight of roots per plant was less sensitive to infection by the 4 *Ggt* isolates than average dry weight of shoots without added Mn but more so at 4 Mn (figure 5.3B).

At nil Mn, effects of the 4 isolates were small. Neither 43 nor 47 decreased root dry weights and 8 and 500 caused a decrease of approximately one-third. However, at 4 Mn all 3 virulent isolates (isolates which decreased plant growth) caused large reductions in root dry weights; 63, 41 and 38 % for 500, 8 and 47, respectively.

FIGURE 5.3. EFFECT OF SOIL-APPLIED MN AND INFECTION BY FOUR GGT ISOLATES ON GROWTH OF WHEAT SEEDLINGS GROWN IN WANGARY SAND UNDER CONTROLLED ENVIRONMENT CONDITIONS. Values are the average of 8 plants.



The size of response of root dry weights to added Mn depended on *Ggt* infection (figure 5.3B). The average dry weight of roots of disease-free plants, and plants infected by 43, were approximately doubled by 4 Mn but responses in plant infected by the 3 virulent isolates were generally much smaller.

The total length of nodal roots per plant was increased by infection with the 3 virulent isolates, regardless of Mn rates. Infection by 43 did not increase nodal root length (figure 5.3C). The addition of Mn to the soil increased the total length of nodal roots by more than 40 %.

Growth of seedlings in the 4 extra pots set up without agar discs was not different to growth in pots at nil Mn with sterile agar discs.

5.3.2.3. *Mn.* Shoot Mn concentrations in plants grown without added Mn were very deficient (an average of 6.5 mg kg^{-1} over all Ggt treatments) and well below the critical level of 18 mg kg^{-1} (see appendix B). The addition of Mn increased shoot Mn levels in all plants to well above deficient but *Ggt* infection influenced the size of the increase (table 5.1). The concentration of Mn in plants infected by the 3 virulent isolates was lower than in plants infected by 43, or disease-free.

The effect of Ggt and Mn treatments on root Mn concentrations differed from the pattern observed in shoot levels (table 5.1). Without added Mn, infection by *Ggt* (by all 4 isolates) caused a small increase in root Mn concentrations, although the increase was not significant for 500. The largest increase occurred with 43, from 16.5 at nil Ggt to 23.2 mg kg^{-1}. Root Mn concentrations were high where Mn had been added (all plants had root concentrations higher than 100 mg kg^{-1}) but, as at nil Mn, levels in *Ggt*-infected plants were increased further. Unlike Mn concentrations in *Ggt*-infected plants at nil Mn, the highest concentration occurred with 500 (244 mg kg^{-1} from 144 at nil Ggt) and the lowest with 43

(180 mg kg^{-1}).

Table 5.1. Effect of soil Mn and *Ggt* isolates on the concentration of Mn in wheat seedlings (mg kg^{-1} D.W.) grown in a Mn-deficient sand under controlled environment conditions. Values in the body of the table are the average of 8 plants.

Mn added (mg/pot)	*Ggt* isolate 500	47	8	43	control	Mean
Mn concentration in whole shoots.						
nil	6.1	6.7	7.6	6.0	5.8	6.5
4	43.4	42.4	47.8	58.2	62.4	50.8
Mean	24.8	24.5	27.7	32.1	34.1	
LSD[a] (P=0.05)	Mn=2.6 ; Ggt=4.1 ; Mn*Ggt=5.8					
Mn concentration in roots.						
nil	18.3[b]	20.7	21.7	23.2	16.5	19.9
	2.96[c]	*3.08*	*3.12*	*3.19*	*2.86*	*3.04*
4	245	177	211	180	145	188
	5.51	*5.18*	*5.36*	*5.20*	*4.98*	*5.24*
Mean	68	61	68	65	50	
	4.23	*4.13*	*4.24*	*4.19*	*3.92*	
LSD[a,d] (P=0.05)	Mn=*0.08* ; Ggt=*0.12* ; Mn*Ggt=*0.17*					

[a] Values separated by more than the appropriate LSD were statistically different.
[b] Geometric means calculated from natural log transformed data.
[c] Values in italics are natural log transformations, calculated to adjust for a skewed distribution.
[d] LSD's apply to transformed data only.

5.3.3. Pot experiment B.

5.3.3.1. *Ggt*. The total length of black stelar lesions per plant was decreased by 45 % with the addition of 4 mg of Mn per pot, regardless of *Ggt* isolate type or depth of agar disc burial, and there was no further decrease with 16 mg of Mn per pot (table 5.2). The number of lesions per plant was not affected by Mn rates and effects of Mn on the percentage of diseased seminal roots were small and not consistent (tables 5.3,5.4). No disease was detected in pots with sterile agar discs.

Table 5.2. Effect of soil Mn, *Ggt* isolates and depth of burial of inoculated agar discs on total length of black stelar lesions on wheat seminal roots (mm per plant) grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants. Data were analyzed with Ggt control treatments removed.

Isolate	0 Mn		4 Mn		16 Mn	
	Depth of burial of agar discs					
	4 cm	2 cm	4 cm	2 cm	4 cm	2 cm
500	24.9	28.7	10.0	13.9	12.3	14.0
47	37.5	33.8	16.0	23.5	16.2	29.0
8	35.1	32.4	19.4	21.5	16.9	15.9
control	0	0	0	0	0	0

(3-way interaction not significant)

1 and 2-way treatment means[a] (with appropriate LSD's[b] at P=0.05 level).

				LSD
Mn	0	4	16	
	32.1	17.4	17.4	7.1
Isolate	500	47	8	
	17.3	26.0	23.6	7.1
Depth of disc	4 cm	2 cm		
	21.0	23.6		ns
Isolate	500	47	8	
Mn				
0	26.8	35.7	34.0	ns
4	12.0	19.8	20.5	
16	13.4	22.6	16.4	
Mn	0	4	16	
Depth of disc				
4 cm	32.7	15.4	15.1	ns
2 cm	31.6	19.7	19.7	
Isolate	500	47	8	
Depth of disc				
4 cm	15.7	23.2	23.9	ns
2 cm	18.9	28.8	23.3	

[a] Means calculated without control Ggt treatments.

[b] Values separated by more than the appropriate LSD were statistically different.

Table 5.3. Effect of soil Mn, *Ggt* isolates and depth of burial of inoculated agar discs on the number of black stelar lesions on wheat seminal roots (lesions per plant) grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants. Data were analyzed with Ggt control treatments removed.

Isolate	0 Mn		4 Mn		16 Mn	
	Depth of burial of agar discs					
	4 cm	2 cm	4 cm	2 cm	4 cm	2 cm
500	6.3	9.5	7.1	5.8	7.9	8.0
47	5.3	10.5	8.0	11.0	5.3	11.0
8	4.4	7.3	5.3	7.3	5.8	6.0
control	0	0	0	0	0	0

(3-way interaction not significant)

1 and 2-way treatment means[a] (with appropriate LSD's[b] at P=0.05 level).

				LSD
Mn	0	4	16	
	7.4	7.4	7.6	ns
Isolate	500	47	8	
	7.4	9.0	6.0	1.6
Depth of disc	4 cm	2 cm		
	6.5	8.5		1.3
Isolate	500	47	8	
Mn				
0	7.9	8.6	5.8	ns
4	6.4	8.5	6.3	
16	7.9	8.9	5.9	
Mn	0	4	16	
Depth of disc				
4 cm	5.8	6.8	6.8	ns
2 cm	9.1	8.0	8.3	
Isolate	500	47	8	
Depth of disc				
4 cm	7.1	7.2	5.1	2.3
2 cm	7.8	10.8	6.8	

[a] Means calculated without control Ggt treatments.

[b] Values separated by more than the appropriate LSD were statistically different.

Table 5.4. Effect of soil Mn, *Ggt* isolates and depth of burial of inoculated agar discs on percentage of diseased seminal roots on wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants. Data were analyzed with Ggt control treatments removed.

Isolate	0 Mn		4 Mn		16 Mn	
	Depth of burial of agar discs					
	4 cm	2 cm	4 cm	2 cm	4 cm	2 cm
500	90	98	89	74	95	93
47	93	96	95	98	90	98
8	81	88	80	98	86	82
control	0	0	0	0	0	0

(3-way interaction not significant)

1 and 2-way treatment means[a] (with appropriate LSD's[b] at P=0.05 level).

				LSD
Mn	0	4	16	
	91	89	91	ns
Isolate	500	47	8	
	90	95	86	5
Depth of disc	4 cm	2 cm		
	89	91		ns
Isolate	500	47	8	
Mn				
0	94	94	85	8
4	81	97	89	
16	94	94	84	
Mn	0	4	16	
Depth of disc				
4 cm	88	88	91	ns
2 cm	94	90	91	
Isolate	500	47	8	
Depth of disc				
4 cm	91	93	83	ns
2 cm	88	97	89	

[a] Means calculated without control Ggt treatments.
[b] Values separated by more than the appropriate LSD were statistically different.

Differences in symptom levels between infection by each of the 3 isolates were rarely large but 47 usually produced more symptoms than either 8 or 500. The extent of symptoms produced by infection with each isolate at a 2 cm depth of burial of agar discs

were similar to levels produced by the same isolates in experiment A (agar discs also at 2 cm).

Doubling the depth of burial of inoculated agar discs caused a small decrease in the number of lesions per plant but the total length of lesions and the percentage of diseased seminal roots were not affected.

When roots were washed free of soil at harvest the remnants of agar discs were still obvious. All inoculated agar discs from pots at 16 Mn were "stained" a dark brown colour. These discs stained dark blue with benzidine which indicated that Mn oxides were present. Sterile discs from 16 Mn were not brown and only stained very lightly with benzidine.

5.3.3.2. *Plant growth.* *Ggt* infection decreased the average dry weight of shoots per plant by nearly 40 % (averaged for both depths of burial of agar discs), regardless of Mn rates and isolate type (table 5.5). The only exception occurred with infection by 8 at 16 Mn which did not decrease shoot dry weight to the same extent as 47 or 500.

The addition of 4 mg of Mn per pot nearly doubled the dry weight of shoots per plant in disease-free plants or plants infected with either 47 or 500 (table 5.5). Shoot dry weights of plants infected by isolate 8 more than doubled with 4 Mn. The high rate of Mn caused a further increase in disease-free plants only but the increase was small (8 %).

Doubling the depth of inoculated agar disc burial increased the dry shoot weight of infected plants by between 22 and 39 % for all Mn rates, depending on the isolate. The increase was greatest with 47.

The only isolate to cause a significant decrease in the average dry weight of roots per plant at nil Mn was 8 (table 5.6). With added Mn, all 3 isolates caused reductions of between 41 and 44 % in dry weight of roots except for isolates 47 and 500 at 16 Mn which decreased root dry weights by more than 50 %.

Table 5.5. Effect of soil Mn, *Ggt* isolates and depth of burial of inoculated agar discs on dry weight of shoots (mg per plant) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

Isolate	0 Mn		4 Mn		16 Mn	
	Depth of burial of agar discs					
	4 cm	2 cm	4 cm	2 cm	4 cm	2 cm
500	18.0	15.8	32.8	30.6	35.0	24.0
47	18.8	13.9	36.1	26.4	33.0	23.1
8	14.6	13.0	36.6	25.5	34.8	28.4
control	24.6	25.6	45.8	47.3	51.4	50.0

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

					LSD
Mn	0	4	16		
	18.0	35.1	35.0		1.6
Isolate	500	47	8	control	
	26.0	25.2	25.5	40.8	1.9
Depth of disc	4 cm	2 cm			
	31.8	27.0			1.3
Isolate	500	47	8	control	
Mn					
0	16.9	16.3	13.8	25.1	3.2
4	31.7	31.3	31.1	46.5	
16	29.5	28.1	31.6	50.7	
Mn	0	4	16		
Depth of disc					
4 cm	19.0	37.8	38.5		2.3
2 cm	17.1	32.4	31.4		
Isolate	500	47	8	control	
Depth of disc					
4 cm	28.6	29.3	28.7	40.6	2.6
2 cm	23.5	21.1	22.3	41.0	

[a] Values separated by more than the appropriate LSD were statistically different.

Table 5.6. Effect of soil Mn, *Ggt* isolates and depth of burial of inoculated agar discs on dry weight of roots (mg per plant) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

Isolate	0 Mn		4 Mn		16 Mn	
	Depth of burial of agar discs					
	4 cm	2 cm	4 cm	2 cm	4 cm	2 cm
500	13.0	9.3	34.8	26.6	34.6	20.9
47	13.6	7.6	41.8	21.5	33.1	17.8
8	9.3	7.8	37.3	23.4	36.8	27.3
control	14.8	13.9	51.8	55.9	60.1	53.0

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

					LSD
Mn	0	4	16		
	11.1	36.6	35.5		2.0
Isolate	500	47	8	control	
	23.2	22.6	23.6	41.6	2.3
Depth of disc	4 cm	2 cm			
	31.7	23.7			1.6
Isolate	500	47	8	control	
Mn					
0	11.3	10.6	8.5	14.3	4.0
4	30.7	31.6	30.3	53.8	
16	27.8	25.4	32.0	56.6	
Mn	0	4	16		
Depth of disc					
4 cm	12.7	41.4	41.2		2.8
2 cm	9.6	31.8	29.7		
Isolate	500	47	8	control	
Depth of disc					
4 cm	27.5	29.5	27.8	42.2	3.2
2 cm	18.9	15.6	19.5	40.9	

[a] Values separated by more than the appropriate LSD were statistically different.

Adding 4 mg of Mn per pot increased the dry weight of roots of disease-free or 8-infected plants by nearly 300 % but the root dry weights of plants infected by 47 or 500

increased by less than 200 % (table 5.6). The addition of 16 mg of Mn per pot did not further increase root dry weights.

Increasing the depth of burial of inoculated agar discs from 2 to 4 cm increased root dry weights by between 43 and 89 % at all Mn rates, depending on the isolate. The increase was greatest with 47.

5.3.3.3. *Mn*. Shoot Mn concentrations at nil Mn were very deficient (all less than 7 mg kg^{-1}) and well below the critical level of 18 mg kg^{-1} (see appendix B) but all plants had adequate shoot Mn levels at 4 Mn (between 24 and 41 mg kg^{-1}) (table 5.7). Adding 16 mg of Mn per pot further increased shoot Mn concentrations to very high levels (all higher than 75 mg kg^{-1}).

At nil Mn *Ggt* infection did not change shoot Mn concentrations from the average of 5.5 mg kg^{-1} in disease-free plants but all 3 isolates decreased shoot Mn concentrations at 4 Mn; from 38 mg kg^{-1} in disease-free plants to 31 mg kg^{-1} in plants infected with 8 and to 25 mg kg^{-1} with isolates 47 and 500. At 16 Mn, 8 decreased shoot Mn concentrations from 121 to 104 mg kg^{-1} but 47 and 500 decreased levels to less than 91 mg kg^{-1}.

5.4. Discussion and Conclusions.

Mixing low rates of Mn through soil decreased total length of lesions on roots infected by all virulent *Ggt* isolates (virulent isolates produced black stelar lesions on infected host roots and depressed host growth). This is an important result because it demonstrates that the phenomenon of Mn decreasing take-all is not peculiar to infection by *Ggt* isolate 500 but suggests that infection by any virulent isolate should be decreased by applying Mn to a Mn-deficient soil.

In contrast to the depressive effect of soil Mn on infection by virulent isolates was the stimulation that Mn caused to infection by the weakly virulent isolate 43. This isolate

infected wheat roots and produced characteristic black stelar lesions but at levels which were so low they did not decrease host growth. The growth of 43 on PDA also contrasted with that of the 3 strongly virulent isolates (8, 47 and 500).

Table 5.7. Effect of soil Mn, *Ggt* isolates and depth of burial of inoculated agar discs on Mn concentration in whole shoots (mg kg^{-1} D.W.) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

Isolate	0 Mn		4 Mn		16 Mn	
	Depth of burial of agar discs					
	4 cm	2 cm	4 cm	2 cm	4 cm	2 cm
500	5.4	5.8	25.9	24.8	96.5	84.1
47	5.5	6.5	25.1	24.3	93.1	76.9
8	4.8	5.6	30.9	30.1	102.4	104.9
control	5.4	5.6	40.4	36.6	126.0	115.9

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

					LSD
Mn	0	4	16		
	5.6	29.8	100.0		3.0
Isolate	500	47	8	control	
	40.4	38.6	46.5	55.0	3.4
Depth of disc	4 cm	2 cm			
	46.8	43.4			2.4
Isolate	500	47	8	control	
Mn					
0	5.6	6.0	5.2	5.5	5.9
4	25.3	24.7	30.5	38.5	
16	90.3	85.0	103.7	120.9	
Mn	0	4	16		
Depth of disc					
4 cm	5.3	30.6	104.5		4.2
2 cm	5.9	28.9	95.4		
Isolate	500	47	8	control	
Depth of disc					
4 cm	42.6	41.2	46.0	57.3	ns
2 cm	38.2	35.9	46.9	52.7	

[a] Values separated by more than the appropriate LSD were statistically different.

Three features separated the behaviour of 43 on PDA from the other 3 isolates; very slow growth, a large stimulation in growth at low Mn concentrations and poor Mn-oxidizing ability. The first two features may help explain the increase in symptoms of infection by 43 with added Mn, given that infections by the 3 virulent isolates were decreased under the same conditions. The large stimulation in colony growth of 43 at low rates of Mn on PDA indicated that this isolate had a relatively high requirement for Mn. Infection of wheat roots at nil Mn by 43 may have been inhibited by slow hyphal growth due to insufficient levels of available Mn for normal hyphal growth. The rate of growth of runner hyphae down the outside of host roots is closely correlated with the length of infected roots (Garrett 1936, Wildermuth and Rovira 1977). Adding Mn to the soil eliminated deficiency in the host and perhaps also in 43 which may have increased the rate of hyphal growth of 43 (and consequently infection). This effect would not have been as important for the virulent isolates because their requirements for Mn on PDA were lower and they were fast growers at all Mn rates. Unfortunately, repeated attempts to investigate the effect of Mn on infection by 43 at different Mn rates and inoculum levels failed because the culture had lost virulence and no longer produced black stelar lesions on wheat roots. Repeated sub-culturing of *Ggt* isolates can cause a loss, or reduction, in virulence (Cunningham 1981).

The observation that the 3 virulent isolates were strong oxidizers of Mn on PDA and in soil (experiment B) but 43 was not suggests that Mn-oxidizing ability may be important in determining the virulence of individual isolates. Alternatively, the ability to oxidize Mn may simply be genetically linked, but otherwise unrelated, to virulence.

The sensitivity of isolates to toxic levels of Mn in PDA did not appear to be important to the extent of disease which infection by each isolate caused. Isolates 8, 43 and 47 had similar tolerances to increasingly toxic concentrations of Mn^{2+} in PDA but 500 was more sensitive. This pattern was not reflected in the virulence of each isolate in pot studies because infection by 47, 8 and 500 were decreased by soil-applied Mn to the same extent and

infection by 43 behaved very differently. This result supports the conclusions of chapter 2 that soil-applied Mn decreases take-all before levels toxic to the pathogen are reached. If toxic Mn concentrations were required to decrease disease then infection by 47 and 8 should not have been decreased by Mn to the same extent as 500.

Differences between the two pot studies in the size of decreases in take-all (for the virulent isolates) with added Mn, and their statistical significance, highlighted the experimental importance of producing very severe Mn deficiency in host plants. Pot experiments A and B were prepared and conducted in essentially the same way except for incubation of soil prior to sowing in experiment B. Adding Mn to soil in experiment A caused a decrease in symptoms on roots of approximately 20 % for infections by 8, 47 or 500 but this effect was only statistically significant for 500. However, in experiment B for the same isolates and at the same inoculum level, Mn decreased infection by 45 %. The important difference between the two experiments was that plants at nil Mn in B were more deficient than plants from the same treatment in A. Average Mn concentrations in shoots at nil Mn in both experiments were well below the critical level of 18 mg kg^{-1} (appendix B) and differences between experiments were small (5.8 and 5.5 mg kg^{-1} for disease-free plants in A and B, respectively). However, the response in plant growth to added Mn in B was at least double that in A.

5.5. References.

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CHAPTER 6.

EFFECTIVENESS OF DIFFERENT SOURCES OF MN IN DECREASING *GGT* INFECTION OF WHEAT SEEDLINGS

CHAPTER 6. EFFECTIVENESS OF DIFFERENT SOURCES OF MN IN DECREASING *GGT* INFECTION OF WHEAT SEEDLINGS.

6.1. Introduction.

The three previous reports of Mn decreasing take-all of wheat in pots (Reis *et al.* 1982, Graham and Rovira 1984, Rovira *et al.* 1985) used simple manganous ion salts as a source of Mn for host plants. These salts were dissolved in water and added to the rooting medium of the plants.

Rovira (pers. comm.) observed that the incidence of white-heads in a wheat crop was higher in strips where a foliar spray of Mn had missed. This suggests that Mn in the soil is not necessary for a decrease in disease to occur and that the chemical form of Mn is not important in this respect.

Two pot experiments were conducted to test the effectiveness of several sources of Mn in decreasing take-all of wheat seedlings grown under Mn-deficient conditions.

6.2. Materials and Methods.

The basic techniques employed here were developed during experiments with small pots outlined in chapter 3 but some minor changes were made. Complete details for soil, Mn and *Ggt* techniques can be found in sections 3.2.1.1., 3.2.4. and 3.2.3.2., respectively, of chapter 3.

6.2.1. Pot experiment A.

A factorial design was used with 7 sources of Mn, 3 rates of Ggt inoculum and 4 replicates, giving 84 pots laid out in randomized blocks.

6.2.1.1. *Inoculum.* Inocula of *Ggt* 500 on PDA were prepared as in chapter 5 (section 5.2.2.1.) and incubated for 9 days at 20° C. Extra dishes of 4 % PDA were also made up and kept sterile for use in nil Ggt pots.

6.2.1.2. *Soil.* Wangary sand collected in 1983 was incubated at 18 % water content for 16 days prior to potting up (see appendix A for details).

6.2.1.3. *Mn.* The seven sources of Mn (which were chosen in an attempt to produce similar shoot concentrations of Mn in plants) were as follows;

1. Nil Mn - no Mn was added to the pots.
2. Foliar (Fol/Mn) - plants were painted with a Mn solution (1.5 % w/v $MnSO_4.4H_2O$ + 2 drops of wetting agent/500 ml) 11 and 15 days after sowing.
3. Seed/Mn - seed was pre-soaked in 1.5 % w/v $MnSO_4.H_2O$ solution for 6 hours and air-dried before sowing (Marcar and Graham 1986). Seed for the other treatments was soaked in $DDDH_2O$ only.
4. Incubated MnO2 (I/MnO_2) - MnO_2 (BDH, analytical grade, majority of particles less than 0.01 mm in diameter) was mixed through soil prior to incubation at 17 g Mn kg^{-1} of air-dry soil.
5. Fresh MnO_2 (F/MnO_2) - MnO_2 was mixed through soil at sowing at 17 g Mn kg^{-1}.
6. Incubated $MnSO_4$ ($I/MnSO_4$) - dissolved $MnSO_4.4H_2O$ was mixed through soil prior to incubation at 0.36 g Mn kg^{-1}.
7. Fresh $MnSO_4$ ($F/MnSO_4$)- dissolved $MnSO_4.4H_2O$ was mixed through soil at sowing at 0.18 g Mn kg^{-1}.

The $F/MnSO_4$ treatment was half the rate of $I/MnSO_4$ because Mn was more available when applied at sowing and the two $MnSO_4$ rates were designed to produce similar levels of Mn in the shoots of the wheat plants (although this was not completely successful).

6.2.1.4. *Procedure.* Basal nutrients were mixed through soil immediately prior to sowing at the same rates used in chapter 3.

Condor wheat seeds, from a trial grown on a Mn-deficient site at Tooligie on the Eyre Peninsula S.A. in 1984 (av. Mn concentration 6.4 mg kg^{-1}), were surface-sterilized with 6 % NaOCl for 2 minutes, rinsed with DDDH_2O and germinated as before (see section 3.2.4.).

Pots were filled such that agar disc inocula were placed either 4 (High Ggt) or 5 cm (Low Ggt) below 2 germinating wheat seeds. Sterile agar discs were placed in pots at a depth of 4 cm below seeds as controls for Ggt treatments. All pots were covered with `Glad Wrap' for the first 3 days after sowing.

Pots were kept in a controlled-environment growth room at 15/10° c (day/night temperature), a 10 hour photoperiod and a photon flux density of approximately 0.3 mEinsteins m^{-2} s^{-1} for 25 days. Pots were watered to weight at regular intervals.

At harvest, plants were washed free of soil, scored for take-all (number of infected seminal roots, total number of black stelar lesions per plant and total length of lesions per plant) and weighed. Shoots and roots were dried at 80° C for 2 days, weighed, digested in nitric acid and analyzed for manganese and 10 other essential elements with an ARL inductively coupled plasma atomic emission spectrometer.

6.2.2. Pot experiment B.

A factorial design was used with 5 sources of Mn, 2 Mn rates, 3 rates of Ggt inoculum and 4 replicates, giving 120 pots laid out in randomized blocks.

6.2.2.1. *Inoculum.* As for experiment A except dishes were incubated for 6 days at 20° C. Extra dishes of 4 % PDA were also made up and kept sterile for use in nil Ggt pots.

6.2.2.2. *Soil.* Wangary sand collected in 1983 was incubated at 18 % water content for 15 days prior to potting up (see appendix A for details).

6.2.2.3. *Mn.* The five sources (and rates) of Mn (which were chosen in an attempt to produce similar shoot concentrations of Mn in plants) were as follows;

1.a Nil Mn - no Mn was added to the pots.

b Nil Mn - no Mn was added to the pots.

2.a A/MnO_2 - MnO_2 (BDH, analytical grade, majority of particles less than 0.01 mm in diameter) was mixed through soil at sowing at 17 g Mn kg^{-1} of air-dry soil.

b A/MnO_2 (BDH, analytical grade) was mixed through soil at sowing at 1.7 g Mn kg^{-1}.

3.a B/MnO_2 - MnO_2 (prepared according to Jauregui and Reisenauer 1982) was mixed through soil 3 days before sowing at 17 g Mn kg^{-1} of air-dry soil.

b B/MnO_2 (prepared according to Jauregui and Reisenauer 1982) was mixed through soil 3 days before sowing at 1.7 g Mn kg^{-1}.

4.a MnO - MnO [1] (majority of particles less than 0.001 mm in diameter) was mixed through soil at sowing at 17 g Mn kg^{-1}.

b MnO - MnO [1] was mixed through soil at sowing at 1.7 g Mn kg^{-1}.

5.a Incubated $MnSO_4$ (I/$MnSO_4$) - dissolved $MnSO_4.4H_2O$ was mixed through soil prior to incubation at 0.36 g Mn kg^{-1}.

b Fresh $MnSO_4$ (F/$MnSO_4$)- dissolved $MnSO_4.4H_2O$ was mixed through soil immediately prior to sowing at 0.09 g Mn kg^{-1}.

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1 supplied by Top Australia Ltd., Port Adelaide (formerly Adelaide and Wallaroo Fertilizers Ltd.).

Delta-MnO_2 (B/MnO_2) was prepared by heating 750 ml of 0.4 M $KMnO_4$ to 90° C and gradually adding 120 ml of conc. HCl while stirring until all purple colour had disappeared. The suspension was cooled and filtered through No. 42 Whatman's filter paper with repeated rinses of DDDH_2O (pH adjusted to 8.5 with 0.5 M NaOH). MnO_2 was recovered off the filter paper and appropriate amounts of the slurry added to soil. A slurry was added to soil because it was a very finely divided product and hence more available source of Mn (Jones and Leeper 1951a). This treatment was added 3 days prior to sowing because the preparation was very acidic and may have caused a temporary increase in soil levels of Mn^{2+} (Leeper 1970).

The F/$MnSO_4$ treatment was one-quarter the rate of I/$MnSO_4$ because Mn was more available when applied at sowing and the two $MnSO_4$ rates were designed to produce similar levels of Mn in the shoots of the wheat plants.

6.2.2.4. *Procedure*. Basal nutrients were mixed through soil immediately prior to sowing at the same rates used in chapter 3 except magnesium was doubled because analysis of previous pot experiments had revealed low magnesium levels in shoots.

Condor wheat seeds, from a trial grown on a Mn-deficient site at Tooligie on the Eyre Peninsula S.A. in 1985, were surface-sterilized with 6 % NaOCl for 3 minutes, rinsed with DDDH_2O and germinated at 20° C for 2 days.

Pots were filled such that agar disc inocula were placed either 4 (Hi Ggt) or 6 cm (Low Ggt) below 2 germinating wheat seeds. Sterile agar discs were placed in pots at a depth of 4 cm below seeds as controls for Ggt treatments. All pots were covered with `Glad Wrap' for the first 3 days after sowing.

Pots were kept in a controlled-environment growth room at 15/10° c (day/night temperature), a 10 hour photoperiod and a photon flux density of approximately 0.3 mEinsteins m^{-2} s^{-1} for 25 days. Pots were watered to weight at regular intervals.

At harvest plants were washed free of soil, scored for take-all (number of infected seminal roots, total number of black stelar lesions per plant and total length of lesions per plant) and weighed. Shoots and roots were dried at 80° C for 2 days, weighed, digested in nitric acid and analyzed for manganese and 10 other essential elements with an ARL inductively coupled plasma atomic emission spectrometer.

6.3. Results.

6.3.1. Pot experiment A.

6.3.1.1. *Ggt.* I/$MnSO_4$ was the most effective treatment at decreasing take-all (figure 6.1). The total length of lesions, averaged over both depths of agar disc burial, was decreased by 68 % with I/$MnSO_4$ and by 59 % with F/$MnSO_4$, followed by seed/Mn (52 %) and Fol/Mn (28 %). The total number of lesions per plant and the percentage of diseased seminal roots were decreased from nil Mn by I/$MnSO_4$ alone but F/$MnSO_4$, I/$MnSO_4$ and seed/Mn had fewer lesions than F/MnO_2, and seed/Mn and I/$MnSO_4$ a lower percentage of diseased seminals. All plants had the same number of seminal roots. MnO_2 treatments did not decrease disease levels.

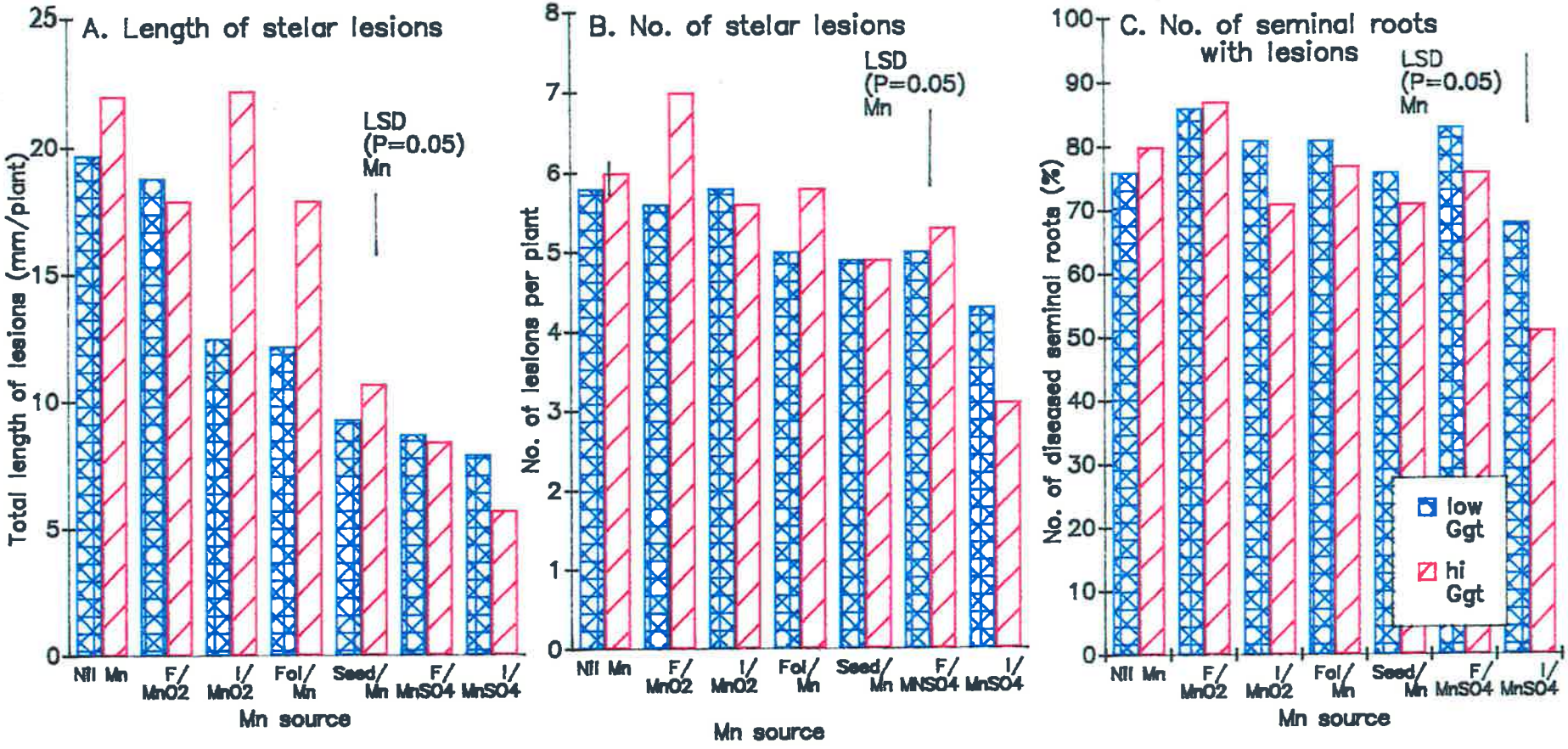
Increasing the depth of burial of inoculated agar discs from 4 to 5 cm did not decrease *Ggt* infection on wheat roots.

Washing plants free of soil at harvest revealed that roots which had been grown with I/$MnSO_4$ or F/$MnSO_4$, and infected by *Ggt*, were very brown where they were covered by runner hyphae. The brown areas on these roots, and the runner hyphae, stained dark blue in benzidine. There was very little brown staining on disease-free roots from either $MnSO_4$ treatment and roots from all other treatments were white. Placing roots from MnO_2 treatments in benzidine showed that MnO_2 particles remained on roots even after washing.

6.3.1.2. *Plant growth. Ggt* infection decreased the dry weight of shoots by nearly one-third, regardless of Mn treatment or depth of burial of inoculated agar discs. Plants grown

FIGURE 6.1. EFFECT OF MN SOURCE ON GGT INFECTION OF WHEAT SEEDLINGS GROWN IN WANGARY SAND UNDER CONTROLLED ENVIRONMENT CONDITIONS.

Plants from nil Ggt were disease-free. All values are the average of 8 plants.



without Mn were pale green and all plants supplied with Mn were dark green and had heavier shoots (figure 6.2A). Fol/Mn and seed/Mn increased the dry weight of shoots per plant by less than 40 % (averaged over all Ggt treatments) but some burning on leaves was noted in Fol/Mn which may have reduced plant response in this treatment. $MnSO_4$ and MnO_2 treatments increased shoot dry weights by more than 60 %.

The dry weight of roots per plant was decreased with *Ggt* infection by approximately 20 %, regardless of Mn treatment or depth of burial of inoculated agar discs (figure 6.2B). I/$MnSO_4$ increased the dry weight of roots by 82 % from nil Mn and all other Mn treatments increased root dry weights by between 36 (Fol/Mn) and 59 % (F/$MnSO_4$ and I/MnO_2).

6.3.1.3. *Mn*. *Ggt* infection did not change plant Mn levels. Plants grown without Mn had an average whole shoot Mn concentration of 8 mg kg^{-1} (table 6.1) which is well below the critical level of 18 mg kg^{-1} (see appendix B). All methods of supplying Mn (except Fol/Mn) increased the concentration of Mn in the plants to adequate or luxury levels. F/$MnSO_4$ was the most effective (an average of 73 mg kg^{-1} over all Ggt treatments), followed by seed/Mn and I/$MnSO_4$ (63 and 68 mg kg^{-1}, respectively), and the two MnO_2 treatments (40 mg kg^{-1}). Mn analysis of Fol/Mn shoots was confounded by painting Mn onto the leaves and was not included in analysis of the data.

Fol/Mn did not increase root Mn concentrations above those of nil Mn (40 mg kg^{-1} averaged over Ggt treatments) but seed/Mn increased Mn concentration to 67 mg kg^{-1} (table 6.1). $MnSO_4$ increased root Mn concentrations to 365 mg kg^{-1} and 446 mg kg^{-1}, respectively, when applied to the soil at sowing or 2 weeks prior to sowing. Mn analysis of roots from plants supplied with MnO_2 was confounded by surface contamination of the roots with MnO_2 particles and were not included in statistical analysis of the data.

FIGURE 6.2. EFFECT OF MN SOURCE AND GGT INFECTION ON GROWTH OF WHEAT SEEDLINGS GROWN IN WANGARY SAND UNDER CONTROLLED ENVIRONMENT CONDITIONS.
All values are the average of 8 plants.

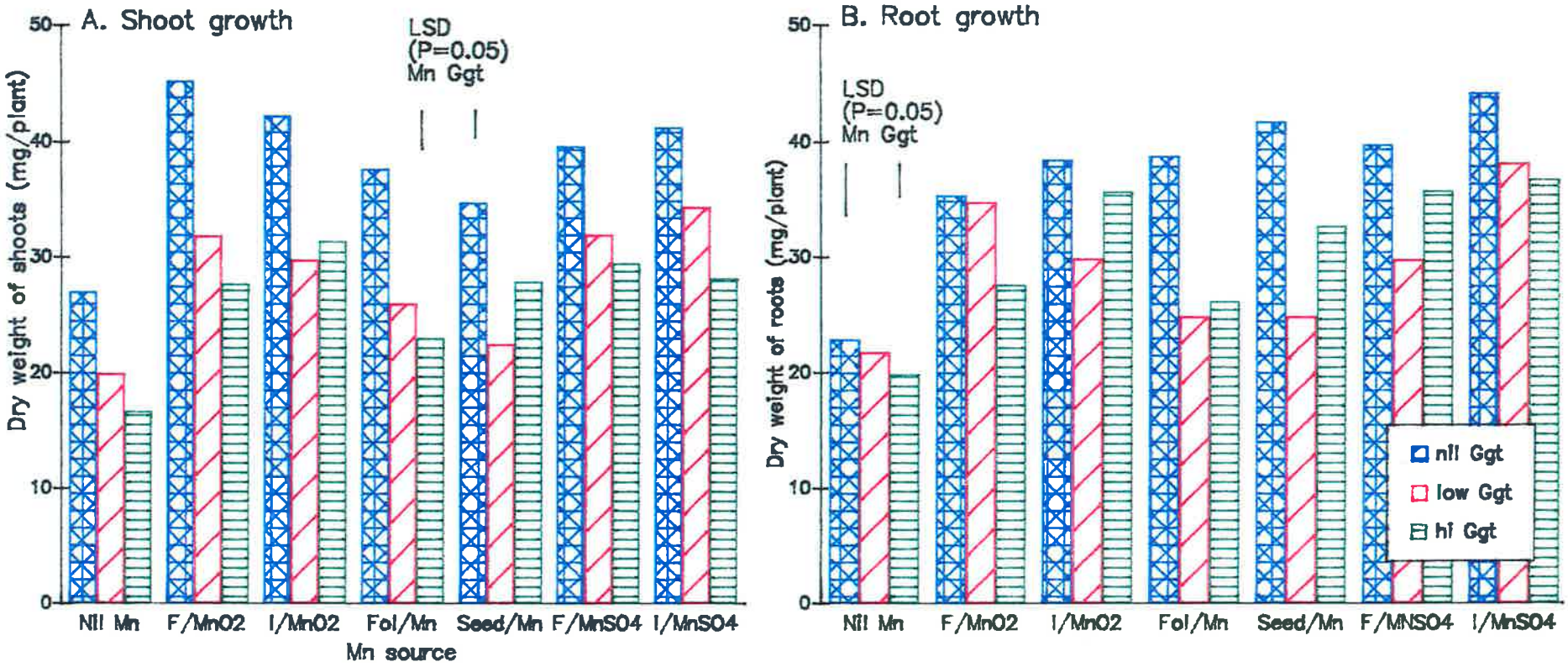


Table 6.1. Effects of Mn source and *Ggt* infection on concentration of Mn (mg kg^{-1} D.W.) in wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the body of each table are the average of 8 plants.

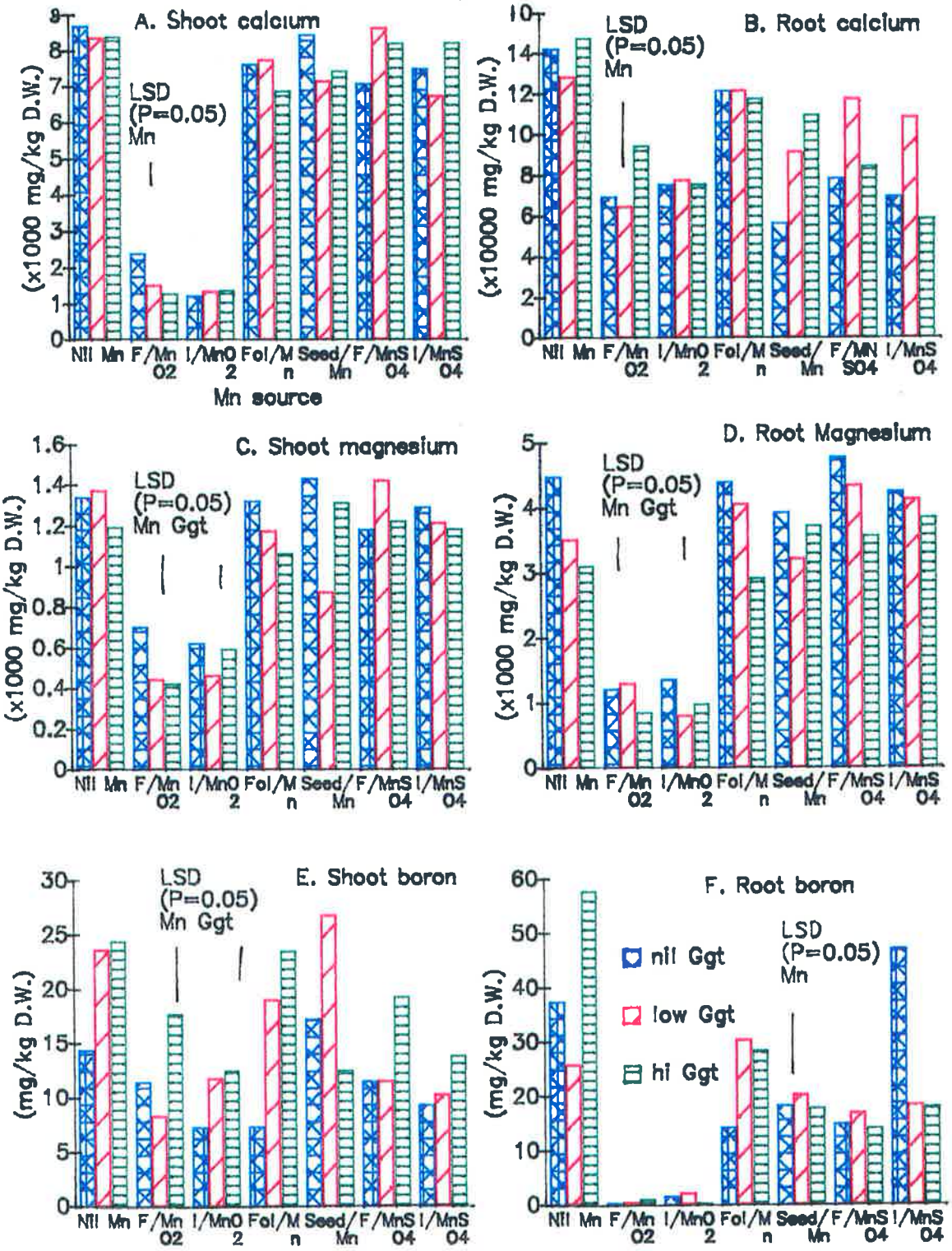
Ggt	Nil Mn	F/MnO_2	I/MnO_2	Fol/Mn	Seed/Mn	F/$MnSO_4$	I/$MnSO_4$	Mean
				Mn source				
Mn concentration in whole shoots								
Nil	8	44	42	2682[a]	65	76	65	50
Low	8	42	41	3280	61	76	63	48
High	7	34	37	4119	79	67	62	48
Mean	8	40	40	3360	68	73	63	
LSD[b] (P=0.05) : Mn=7 ; Ggt, Mn*Ggt ns								
Mn concentration in roots								
Nil	40[c]	11498[d]	12873	37	60	330	446	110
	3.7[e]	-	-	*3.6*	*4.1*	*5.8*	*6.1*	*4.7*
Low	45	4641	9642	40	81	403	403	122
	3.8	-	-	*3.7*	*4.4*	*6.0*	*6.0*	*4.8*
High	40	6508	9652	40	67	365	494	110
	3.7	-	-	*3.7*	*4.2*	*5.9*	*6.2*	*4.7*
Mean	40	7029	10624	40	67	365	446	
	3.7	-	-	*3.7*	*4.2*	*5.9*	*6.1*	
LSD[f] (P=0.05) : Mn=*0.2* ; Ggt, Mn*Ggt *ns*								

[a] Mn painted onto leaves and not included in analysis.
[b] Values separated by more than the appropriate LSD were statistically different.
[c] Geometric means calculated from natural log transformed data.
[d] MnO_2 particles stuck to roots and not included in analysis of data.
[e] Values in italics are natural log transformations.
[f] LSD's apply to transformed data only.

6.3.1.4. *Nutrient composition of plants*. The concentrations of iron, copper, molybdenum, zinc, potassium, phosphorus and sulphur were all at adequate or luxury levels for growth of wheat seedlings, regardless of effects of Mn or Ggt treatments (data not presented).

However, the use of MnO_2 as a Mn source caused a large reduction in the levels of calcium (Ca) in the shoots of treated plants (figure 6.3A). The Ca concentration in shoots of plants from MnO_2 treatments were below the 2-5000 mg kg^{-1} required for normal wheat growth (Reuter and Robinson 1986) but plants from all other Mn treatments had more than adequate shoot Ca concentrations. Concentrations of Ca in plant roots were very high for all

FIGURE 6.3. EFFECT OF MN SOURCE AND GGT INFECTION ON CONCENTRATION OF SIX ELEMENTS IN SHOOTS AND ROOTS OF WHEAT SEEDLINGS GROWN IN WANGARY SAND UNDER CONTROLLED ENVIRONMENT CONDITIONS.
All values are the average of 8 plants.



treatments but were inflated by contamination from the soil which was 80-90 % $CaCO_3$ (figure 6.3B). *Ggt* infection did not affect the concentration of Ca in plants.

Magnesium (Mg) levels in plants were affected differently by Mn sources (figures 6.3C,D). MnO_2 decreased Mg concentrations in shoots from marginal levels of between 1200 and 1300 mg kg^{-1} (Reuter and Robinson 1986) with nil Mn or Mn sources other than MnO_2, to less than 600 mg kg^{-1}. *Ggt* infection decreased shoot Mg concentrations by an average of 12 % over all Mn sources. The effects of Mn and Ggt treatments on root Mg concentrations were similar to shoots (figure 6.3D). The concentration of Mg in roots was increased slightly from an average of 3700 mg kg^{-1} at nil Mn to 4100 mg kg^{-1} with F/$MnSO_4$ but MnO_2 (applied at sowing or prior to incubation) decreased levels to less than 1150 mg kg^{-1}. Neither seed/Mn, Fol/Mn nor I/$MnSO_4$ changed root Mg concentrations from levels at nil Mn. Low Ggt decreased Mg concentrations in roots by 12 % (averaged over all Mn sources) and high Ggt caused a further decrease of 11 %.

Boron (B) concentrations in shoots averaged 21 mg kg^{-1} over Ggt treatments and were not decreased by Fol/Mn or seed/Mn (figure 6.3E). All four MnO_2 and $MnSO_4$ treatments decreased shoot B concentrations to a similar extent (to levels between 11 and 14 mg kg^{-1}) but levels were still adequate for growth (Reuter and Robinson 1986). Low Ggt increased shoot B concentrations from 11 mg kg^{-1} (average of all Mn sources) to 16 mg kg^{-1} but high Ggt did not cause a further increase. The pattern of effects of Mn and Ggt treatments on root B concentrations were very different to shoots (figure 6.3F). Both MnO_2 treatments decreased B concentrations in roots from an average of 41 mg kg^{-1} at nil Mn to less than 2 mg kg^{-1}. All other Mn treatments decreased B levels to concentrations between 28 and 16 mg kg^{-1}. *Ggt* infection did not affect root B concentrations.

6.3.2. Pot experiment B.

6.3.2.1. *Ggt*. All Mn sources decreased the total length of lesions per plant when applied at a low rate (table 6.2). There was an average (over both depths of agar disc burial) of 31.7

mm of lesions per plant in the nil Mn control but $MnSO_4$ and both MnO_2 treatments decreased the total length of lesions to between 9 and 12 mm per plant. MnO caused a further decrease to only 7 mm per plant. $MnSO_4$, B/MnO_2 and MnO applied at a high rate also caused large decreases in the total length of lesions per plant (from an average of 23.5 mm at nil Mn to 11.3 mm with B/MnO_2 and between 6 and 9 mm with MnO and $MnSO_4$). A/MnO_2 did not decrease the total length of lesions when applied at a high rate. Increasing the rate of application of $MnSO_4$, B/MnO_2 or MnO did not cause further decreases in the total length of lesions but a high rate of A/MnO_2 had nearly twice the total length of lesions of the low rate. These results compare favourably with pot experiment A, where $MnSO_4$ applied prior to incubation caused large decreases in take-all on roots but MnO_2 (same treatment as A/MnO_2) was ineffective.

The number of lesions per plant and the percentage of diseased seminal roots were not as sensitive to treatments as the total length of lesions but showed a similar pattern of response to different Mn sources applied at a low rate (tables 6.3,6.4). No Mn sources applied at a high rate decreased the number of lesions or the percentage of diseased roots but both MnO_2 treatments increased these two measurements of disease.

Decreasing the depth of burial of inoculated agar discs from 6 to 4 cm increased the total length of lesions and the number of lesions per plant by approximately 20 % but did not significantly increase the percentage of diseased roots. No disease was present in pots with sterile agar discs.

Table 6.2. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on the total length of black stelar lesions (mm per plant) on roots of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants. Data were analyzed without nil Ggt treatments.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	0	31.8[a]	29.6	0	19.8	27.8
		3.49[b]	*3.42*		*3.04*	*3.36*
A/MnO_2	0	10.0	14.1	0	23.8	20.7
		2.40	*2.72*		*3.21*	*3.08*
B/MnO_2	0	11.4	10.9	0	10.3	12.4
		2.52	*2.48*		*2.43*	*2.59*
MnO	0	6.8	7.3	0	6.1	7.0
		2.05	*2.12*		*1.96*	*2.08*
$MnSO_4$	0	8.1	11.3	0	5.6	11.8
		2.21	*2.51*		*1.89*	*2.55*

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[c,d] at P=0.05 level). Means were calculated with nil Ggt treatments removed.

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	26.9	16.3	11.2	6.8	8.9	
	3.33	*2.85*	*2.50*	*2.05*	*2.29*	*0.19*
Mn rate	low			high		
	12.3 *2.59*			12.7 *2.62*		*ns*
Ggt	low			high		
	11.4 *2.52*			13.7 *2.69*		*0.12*
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	31.7	11.9	11.2	7.0	9.6	
	3.46	*2.56*	*2.50*	*2.09*	*2.36*	*0.27*
high	23.5	22.2	11.3	6.5	8.2	
	3.20	*3.15*	*2.51*	*2.02*	*2.22*	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
low	25.1	15.5	10.9	6.4	6.8	
	3.26	*2.81*	*2.47*	*2.00*	*2.05*	*ns*
high	28.7	17.1	11.6	7.2	11.5	
	3.39	*2.90*	*2.54*	*2.10*	*2.53*	
Mn rate		low		high		
Ggt						
low		12.6 *2.53*		11.2 *2.50*		*ns*
high		13.1 *2.65*		14.3 *2.73*		

[a] Geometric means calculated from natural log transformed data.

[b] Values in italics are natural log transformations, applied to correct a skewed distribution.

[c] LSD's apply to transformed data only.

[d] Values separated by more than the appropriate LSD were statistically different.

Table 6.3. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on the number of black stelar lesions per plant on roots of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants. Data were analyzed without nil Ggt treatments.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	0	6.1	7.3	0	3.3	5.6
A/MnO_2	0	4.6	5.1	0	6.6	7.3
B/MnO_2	0	4.6	4.8	0	5.8	7.3
MnO	0	4.5	4.3	0	3.8	4.5
$MnSO_4$	0	4.1	5.0	0	3.8	6.3

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level). Means were calculated with nil Ggt treatments removed.

Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	LSD
	5.6	5.9	5.6	4.3	4.8	0.8
Mn rate	low		high			
	5.0		5.4			ns
Ggt	low		high			
	4.7		5.7			0.5
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	6.7	4.9	4.7	4.4	4.6	1.1
high	4.4	6.9	6.5	4.1	5.0	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
low	4.7	5.6	5.2	4.1	3.9	ns
high	6.4	6.2	6.0	4.4	5.6	
Mn rate	low		high			
Ggt						
low	4.8		4.6			ns
high	5.3		6.2			

[a] Values separated by more than the appropriate LSD were statistically different.

Table 6.4. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on the percentage of diseased seminal roots of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants. Data were analyzed without nil Ggt treatments.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	0	80	90	0	63	81
A/MnO_2	0	76	77	0	100	93
B/MnO_2	0	78	73	0	86	93
MnO	0	85	75	0	68	70
$MnSO_4$	0	69	74	0	68	93

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level). Means were calculated with nil Ggt treatments removed.

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	79	86	82	74	76	ns
Mn rate	low		high			
	78		81			ns
Ggt	low		high			
	77		82			ns
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	85	76	75	80	72	11
high	72	96	89	69	80	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
low	72	88	82	76	68	ns
high	85	85	83	73	83	
Mn rate	low		high			
Ggt						
low	77		77			ns
high	78		86			

[a] Values separated by more than the appropriate LSD were statistically different.

6.3.2.2. *Plant growth.* Plant growth showed strong positive responses to all Mn sources at low and high rates but the effect of *Ggt* infection on plant growth depended on the Mn source.

Shoot dry weights were decreased by between 20 and 30 % by low Ggt with all Mn sources except $MnSO_4$ and the nil Mn control, where decreases were not statistically significant (table 6.5). Hi Ggt only changed shoot dry weights from levels at low Ggt when MnO and $MnSO_4$ were used as Mn sources. Hi Ggt decreased shoot dry weight by 21 % with $MnSO_4$ but increased it to equal with the nil Ggt control with MnO. Increasing the rate of application of Mn sources from low to high did not further increase the dry weight of shoots per plant.

All Mn sources were equally effective at increasing the shoot dry weight of disease-free plants (increases of 95-104 %) from nil Mn but at low Ggt, $MnSO_4$ was more effective (increase of 124 %) than B/MnO_2 (increase of 74 %). A/MnO_2 and MnO showed increases at low Ggt intermediate to $MnSO_4$ and B/MnO_2. At high Ggt, MnO was the most effective Mn source (increase of 154 %) and A/MnO_2, B/MnO_2 and $MnSO_4$ increased shoot dry weights by 96-55 %.

Roots of plants removed from pots where B/MnO_2 had been used as a Mn source had scattered and occasional patches of a brown/purple substance stuck to them. They were not associated with *Ggt* hyphae and were identified as Mn oxides with benzidine (stained blue in the presence of Mn oxides). Roots from low MnO pots were white and agar discs were spotted dark brown (especially in *Ggt*-inoculated pots). At high MnO all roots had extensive deposits of black/brown Mn oxides and agar discs were also very dark with Mn deposits. No Mn oxides were visible in A/MnO_2 treatments and only inoculated agar discs were brown at a high rate of $MnSO_4$.

Table 6.5. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on the dry weight of shoots (mg per plant) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	34.4	24.5	26.0	23.2	21.3	20.3
A/MnO_2	51.3	41.8	39.8	63.2	47.8	38.5
B/MnO_2	51.8	35.5	33.8	60.3	43.8	38.0
MnO	62.2	43.3	59.7	55.4	46.3	57.9
$MnSO_4$	58.4	48.3	48.9	56.7	54.3	42.0

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	24.9	47.0	43.9	54.1	51.4	4.3
Mn rate	low	high				
	44.0	44.6				ns
Ggt	nil	low	high			
	51.7	40.7	40.5			3.3
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	28.3	44.3	40.4	55.0	51.9	ns
high	21.6	49.8	47.4	53.2	51.0	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
nil	28.8	57.2	56.0	58.8	57.5	7.4
low	22.9	44.8	39.7	44.8	51.3	
high	23.2	39.2	35.9	58.8	45.5	
Ggt	nil	low	high			
Mn rate						
low	51.6	38.7	41.6			ns
high	51.7	42.7	39.4			

[a] Values separated by more than the appropriate LSD were statistically different.

Table 6.6. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on the dry weight of roots (mg per plant) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	16.8	13.1	14.4	9.5	9.0	9.4
A/MnO_2	36.2	26.9	24.9	47.2	28.2	26.2
B/MnO_2	36.4	21.5	19.9	59.3	37.8	31.3
MnO	40.2	27.4	31.8	41.4	34.0	38.4
$MnSO_4$	45.7	30.0	30.0	41.9	41.7	27.5

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	12.0	31.6	34.4	35.5	36.1	3.5
Mn rate	low	high				
	27.7	32.2				2.2
Ggt	nil	low	high			
	37.4	27.0	25.4			2.7
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	14.8	29.3	25.9	33.1	35.2	5.0
high	9.3	33.8	42.8	37.9	37.0	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
nil	13.1	41.7	47.8	40.8	43.8	6.1
low	11.1	27.5	29.6	30.7	35.8	
high	11.9	25.5	25.6	35.1	28.8	
Ggt	nil	low	high			
Mn rate						
low	35.0	23.8	24.2			ns
high	39.9	30.1	26.6			

[a] Values separated by more than the appropriate LSD were statistically different.

The dry weight of roots per plant was more sensitive to treatments than dry weight of shoots and all Mn sources caused very large increases (the smallest increase was 115 % and the largest 265 %), especially at nil Ggt (table 6.6). Individual Mn sources were not

equally effective at increasing root dry weights but the rankings changed across Ggt treatments, except A/MnO_2 tended to be the least effective. Increasing the rate of application of Mn sources from low to high generally did not increase the dry weight of roots.

Low Ggt decreased the dry weight of roots per plant with all Mn sources except nil Mn (table 6.6). The largest decreases occurred with A/MnO_2 and B/MnO_2 (34 and 38 %, respectively) and the smallest with $MnSO_4$ (18%). The decrease at MnO was intermediate to the MnO_2 treatments and $MnSO_4$ at 25 %. High Ggt further decreased root dry weights only at $MnSO_4$ where an additional decrease from low Ggt of 20 % occurred.

6.3.2.3. *Mn*. Mn concentrations in shoots of plants grown without Mn were not affected by Ggt treatments and were very deficient (an average of 5.5 mg kg^{-1} for the experiment) (table 6.7). The critical level for Mn in shoots was 18 mg kg^{-1} (see appendix B). *Ggt* infection decreased shoot Mn concentrations only in plants supplied with a high rate of either A/MnO_2, MnO or $MnSO_4$ but the decreases were small and levels were still above adequate for growth.

MnO was the only Mn source which completely eliminated Mn deficiency in all plants when applied at a low rate (table 6.7). $MnSO_4$ and A/MnO_2 increased shoot Mn concentrations to above critical in disease-free plants but to only marginal in *Ggt*-infected plants. B/MnO_2 increased shoot Mn concentrations to between 14.6 and 15.7 mg kg^{-1}, which were still deficient (although an increase in shoot dry weight with a high rate of B/MnO_2 was not statistically significant).

High rates of all Mn sources increased shoot Mn concentrations to adequate levels (table 6.7). MnO increased shoot concentrations to above 250 mg kg^{-1} but the other three treatments increased levels to between 35 and 64 mg kg^{-1}.

Table 6.7. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on concentration of Mn in whole shoots (mg kg^{-1} D.W.) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	6.1	6.3	5.6	5.2	4.7	4.9
A/MnO_2	25.9	16.8	19.2	66.5	52.6	60.7
B/MnO_2	14.6	15.1	15.7	62.0	29.1	64.1
MnO	73.2	76.2	69.4	296.8	266.3	255.5
$MnSO_4$	23.5	19.4	16.3	49.1	42.8	34.9

LSD[a] (P=0.05) Mn source*Mn rate*Ggt=9.3

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	5.5	40.3	38.4	172.9	31.0	3.8
Mn rate	low	high				
	26.9	88.3				2.4
Ggt	nil	low	high			
	62.3	55.9	54.6			3.0
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	6.0	20.6	15.1	72.9	19.8	5.4
high	4.9	59.9	61.7	272.8	42.3	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
nil	5.7	46.2	38.3	185.0	36.3	6.6
low	5.5	34.7	37.1	171.2	31.1	
high	5.3	39.9	39.9	162.5	25.6	
Ggt	nil	low	high			
Mn rate						
low	28.7	26.7	25.2			4.2
high	95.9	85.1	84.0			

[a] Values separated by more than the appropriate LSD were statistically different.

$MnSO_4$ caused the smallest increases in shoot Mn concentrations.

A/MnO_2 and $MnSO_4$, applied at high rates, were the same treatments as F/MnO_2 and I/$MnSO_4$, respectively, in experiment A in this chapter. A/MnO_2 produced higher

concentrations of Mn in shoots than I/MnO_2 (67 compared to 40 mg kg^{-1} in disease-free plants) but A/$MnSO_4$ produced lower concentrations than I/$MnSO_4$ (49 compared to 68 mg kg^{-1}).

Root Mn concentrations were increased from an average of 27.9 mg kg^{-1} at nil Mn, regardless of Ggt treatments, to 78.3 and 226 mg kg^{-1}, respectively, with low and high rates of $MnSO_4$ (table 6.8). High Ggt caused a small decrease in root Mn concentrations where plants had been supplied with $MnSO_4$ but not in nil Mn treatments. Low Ggt did not affect root Mn concentrations. Mn analysis of roots from plants supplied with Mn oxides were confounded by surface contamination of the roots with oxide particles and were not included in statistical analysis of the data.

6.3.2.4. *Nutrient composition of plants*. The concentrations of iron, copper, molybdenum, zinc, potassium, phosphorus and sulphur were all at adequate or luxury levels for growth of wheat seedlings, regardless of effects of Mn or Ggt treatments (data not presented).

Ggt infection did not affect the concentration of Ca in shoots (table 6.9). All Mn sources decreased shoot concentrations of Ca but the size of the decrease depended on the rate of application and the Mn source (table 6.9). At a low rate of application $MnSO_4$, B/MnO_2 and MnO decreased shoot levels from 9939 mg kg^{-1} at nil Mn to between 7345 and 7862 mg kg^{-1} but A/MnO_2 decreased levels further to 6069 mg kg^{-1}. At a high rate of application A/MnO_2 caused a very large decrease in Ca in shoots (from 9756 mg kg^{-1} at nil Mn to 1467 mg kg^{-1}). This level of shoot Ca was below published critical levels for wheat seedlings (Reuter and Robinson 1986). B/MnO_2 decreased shoot concentrations to marginal levels (an average of 4601 mg kg^{-1}) but MnO and $MnSO_4$ caused only small decreases, to an average of 7203 and 8124 mg kg^{-1}, respectively. The effect of a high rate of A/MnO_2 on shoot Ca concentrations was the same as F/MnO_2 in experiment A. Concentrations of Ca in

Table 6.8. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on concentration of Mn in roots (mg kg^{-1} D.W.) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants. Only nil Mn and $MnSO_4$ treatments were statistically analyzed because roots from A/MnO_2, B/MnO_2 and MnO treatments were contaminated by oxide particles from the soil.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	22	22	19	23	48	32
A/MnO_2	687	394	386	5954	1878	1685
B/MnO_2	107	167	157	2192	1727	1289
MnO	631	650	623	7526	12091	10634
$MnSO_4$	89	77	68	245	243	191

(3-way interaction not significant)[a]

1 and 2-way treatment means for nil Mn and $MnSO_4$ treatments only (with appropriate LSD's[a,b] at P=0.05 level).

				LSD
Mn source	nil		$MnSO_4$	
	28		152	11
Mn rate	low		high	
	50		131	11
Ggt	nil	low	high	
	95	98	78	13
Mn source	nil		$MnSO_4$	
Mn rate				
low	21		78	15
high	35		226	
Mn source	nil		$MnSO_4$	
Ggt				
nil	23		167	19
low	35		160	
high	26		130	
Ggt	nil	low	high	
Mn rate				
low	56	50	44	ns
high	134	146	112	

[a] Only nil Mn and $MnSO_4$ treatments statistically analyzed.

[b] Values for nil Mn and $MnSO_4$ treatments separated by more than the appropriate LSD were statistically different.

plant roots were very high for all treatments because of contamination from the soil which was 80-90 % $CaCO_3$ and treatment effects were probably not very meaningful (table 6.10). However, *Ggt* infection tended to decrease root Ca concentrations and Mn sources (except A/MnO_2) to increase them.

Low Ggt caused a very small decrease in concentration of Mg in shoots (less than 7 %), regardless of Mn source (table 6.11). High Ggt did not further decrease shoot Mg concentrations. Shoot concentrations of Mg at nil Mn were decreased from 1960 mg kg^{-1} to 1167 mg kg^{-1} with a low rate of application of A/MnO_2 but the other three Mn sources caused even smaller decreases (shoot levels to between 1356 and 1425 mg kg^{-1}). $MnSO_4$ and MnO also caused small decreases in shoot Mg concentrations when applied at high rates but A/MnO_2 and B/MnO_2 both caused large decreases (from 1825 mg kg^{-1} at nil Mn to 547 and 754 mg kg^{-1}, respectively). The concentrations of Mg in shoots with a high rate of A/MnO_2 and B/MnO_2 may have been deficient for plant growth (Reuter and Robinson 1986).

Ggt infection decreased root Mg concentrations from 5852 mg kg^{-1} in disease-free plants at nil Mn to 4391 mg kg^{-1} at low Ggt and further to 3849 mg kg^{-1} at high Ggt (table 6.12). *Ggt* infection did not significantly decrease root Mg concentrations in plants which had been supplied with Mn. Root Mg concentrations were largely unaffected by Mn sources applied at a low rate although A/MnO_2 caused a small decrease and $MnSO_4$ a small increase. However, at a high rate of application A/MnO_2 caused a very large decrease from an average of 4649 mg kg^{-1} at nil Mn to 1073 mg kg^{-1}. B/MnO_2 decreased Mg concentrations to 1983 mg kg^{-1} but MnO did not alter levels and $MnSO_4$ caused an increase to 5428 mg kg^{-1}.

Ggt infection did not affect B concentrations in plants (tables 6.13,6.14). At a low rate of application, only $MnSO_4$ caused a significant decrease in shoot B concentrations from nil Mn but the effect was small (from an average of 42 mg kg^{-1} to 28 mg kg^{-1}) (table 6.13). All Mn sources at a high application rate decreased shoot B concentrations; from an average

Table 6.9. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on concentration of Ca in whole shoots (mg kg^{-1} D.W.) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	10202	10140	9474	10757	9434	9078
A/MnO_2	5742	6303	6160	1413	1389	1600
B/MnO_2	7273	7776	6986	4553	4605	4645
MnO	7048	7742	8088	6881	7467	7260
$MnSO_4$	7335	8301	7951	7673	8930	7768

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	9847	3768	5973	7414	7993	458
Mn rate	low	high				
	7768	6230				290
Ggt	nil	low	high			
	6888	7209	6901			ns
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	9939	6069	7345	7626	7862	648
high	9756	1467	4601	7203	8124	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
*nil	10480	3578	5913	5965	7504	ns
low	9787	3846	6190	7604	8615	
high	9276	3880	5815	7674	7859	
Ggt	nil	low	high			
Mn rate						
low	7520	8052	7732			
high	6255	6365	6070			

[a] Values separated by more than the appropriate LSD were statistically different.

Table 6.10. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on concentration of Ca in roots (mg kg^{-1} D.W.) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	36833	20450	30042	32193	30923	33723
A/MnO_2	54671	32371	25340	52812	15474	12477
B/MnO_2	75822	24696	21336	61656	40926	26473
MnO	58392	24047	44497	53207	32718	35939
$MnSO_4$	66256	42260	38974	58380	70137	24612

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	30694	32191	41818	41467	50103	8613
Mn rate	low	high				
	39732	38777				ns
Ggt	nil	low	high			
	55022	33400	29341			6672
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	29108	37460	40618	42312	49163	ns
high	32279	26921	43109	40622	51043	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
nil	34513	53741	68739	55799	62318	14918
low	25686	23923	32811	28382	56199	
high	31882	18908	23904	40218	31793	
Ggt	nil	low	high			
Mn rate						
low	58394	28765	32038			ns
high	51650	38036	26645			

[a] Values separated by more than the appropriate LSD were statistically different.

Table 6.11. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on concentration of Mg in whole shoots (mg kg^{-1} D.W.) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	1902	2056	1923	1861	1769	1845
A/MnO_2	1223	1195	1082	712	389	541
B/MnO_2	1416	1315	1338	812	688	763
MnO	1457	1267	1302	1607	1501	1432
$MnSO_4$	1512	1404	1358	1492	1496	1228

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	1893	857	1055	1427	1415	76
Mn rate	low	high				
	1450	1209				48
Ggt	nil	low	high			
	1399	1308	1281			59
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	1960	1167	1356	1342	1425	108
high	1825	547	754	1513	1406	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
nil	1881	967	1114	1532	1502	ns
low	1913	812	1050	1367	1293	
high	1884	812	1050	1367	1293	
Ggt	nil	low	high			
Mn rate						
low	1502	1447	1401			ns
high	1297	1168	1162			

[a] Values separated by more than the appropriate LSD were statistically different.

of 49 mg kg^{-1} at nil Mn to between 27 and 35 mg kg^{-1}, depending on Mn source. Root B concentrations were decreased by all Mn sources, regardless of type or application rate, from an average of 37 mg kg^{-1} at nil Mn to between 12 and 14 mg kg^{-1} (table 6.14).

A/MnO_2, applied at a high rate, was the same treatment as F/MnO_2 in experiment A in this chapter and caused very similar effects on the nutrient composition of plants as F/MnO_2. The only exception occurred with the concentration of B in roots. F/MnO_2 decreased root B concentrations to extremely low levels (much lower than caused by the other Mn sources in experiment A) but A/MnO_2 caused only a moderate decrease in root B concentrations (and only to the same extent as the other Mn sources in experiment B).

6.4. Discussion and conclusions.

Thirteen different sources of Mn were tested in two experiments for their ability to supply Mn to wheat seedlings in pots and to decrease take-all on wheat roots. These thirteen sources of Mn were $MnSO_4$ (either mixed through soil at sowing or at 2 rates prior to a 2 week incubation period, applied to leaves or as a seed "drench"), a commercial preparation of powdered MnO_2 (either mixed through soil at sowing or at 2 rates prior to a 2 week incubation period), a slurry of laboratory-prepared MnO_2 mixed through soil 3 days before sowing at 2 rates or as MnO mixed through soil at sowing at 2 rates.

Three Mn sources did not cause large decreases in the total length of black stelar lesions on wheat roots. These were $MnSO_4$ applied to leaves and commercially prepared MnO_2 mixed through soil at a high rate of application (17 mg Mn per kg of soil), either at sowing or prior to incubation. A low rate of application of the commercial MnO_2 did cause large decreases in take-all.

Twelve of the thirteen Mn sources supplied sufficient Mn to wheat seedlings for growth to be maximized (in terms of Mn nutrition). The exception was MnO_2 slurry applied at a low rate (1.7 mg Mn kg^{-1}) which resulted in Mn concentrations in shoots below the

Table 6.12. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on concentration of Mg in roots (mg kg^{-1} D.W.) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	5523	4598	4117	6182	4185	3581
A/MnO_2	3214	2942	2992	1315	968	934
B/MnO_2	4343	4729	4269	2289	1814	1844
MnO	4843	4733	4298	5125	4949	4853
$MnSO_4$	4926	5170	5528	5394	5494	5398

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	4698	2061	3215	4800	5318	293
Mn rate	low	high				
	4415	3622				186
Ggt	nil	low	high			
	4316	3958	3781			227
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	4746	3050	4447	4625	5208	415
high	4649	1073	1983	4976	5428	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
nil	5852	2265	3316	4984	5160	508
low	4391	1955	3272	4841	5332	
high	3849	1963	3057	4575	5463	
Ggt	nil	low	high			
Mn rate						
low	4570	4434	4241			ns
high	4061	3482	3322			

[a] Values separated by more than the appropriate LSD were statistically different.

Table 6.13. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on concentration of B in whole shoots (mg kg^{-1} D.W.) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	35.4	47.1	42.4	48.9	47.0	50.9
A/MnO_2	30.1	37.4	34.0	20.6	30.3	31.6
B/MnO_2	29.9	28.3	33.3	23.8	27.1	29.3
MnO	28.4	30.8	27.7	35.6	34.9	33.9
$MnSO_4$	24.6	28.5	30.0	27.4	33.6	27.5

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	45.3	30.7	28.6	31.9	28.6	9.3
Mn rate	low	high				
	32.5	33.5				ns
Ggt	nil	low	high			
	30.5	34.5	34.1			ns
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	41.6	33.8	30.5	29.0	27.7	13.1
high	48.9	27.5	26.7	34.8	29.5	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
nil	42.2	25.3	26.9	32.0	26.0	ns
low	47.0	33.9	27.7	32.8	31.1	
high	46.7	32.8	31.3	30.8	28.8	
Ggt	nil	low	high			
Mn rate						
low	29.7	34.4	33.5			ns
high	31.3	34.6	34.7			

[a] Values separated by more than the appropriate LSD were statistically different.

Table 6.14. Effects of type, and rate of application, of Mn sources and *Ggt* inoculum rates on concentration of B in roots (mg kg^{-1} D.W.) of wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 8 plants.

	Mn at low rate			Mn at high rate		
	nil Ggt	low Ggt	high Ggt	nil Ggt	low Ggt	high Ggt
Nil	20.1	31.0	31.4	60.8	39.6	37.2
A/MnO_2	10.3	12.2	16.7	10.9	11.5	15.1
B/MnO_2	12.0	22.0	20.1	11.2	8.3	11.9
MnO	11.4	8.9	16.0	12.1	11.5	8.8
$MnSO_4$	11.5	13.1	13.5	9.7	11.3	12.5

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

						LSD
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
	36.7	12.8	14.3	11.4	11.9	5.0
Mn rate	low	high				
	16.7	18.2				ns
Ggt	nil	low	high			
	17.0	17.0	18.3			ns
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Mn rate						
low	27.5	13.1	18.1	12.1	12.7	7.1
high	45.9	12.5	10.5	10.8	11.2	
Mn source	nil	A/MnO_2	B/MnO_2	MnO	$MnSO_4$	
Ggt						
nil	40.5	10.6	11.6	11.7	10.6	ns
low	35.3	11.9	15.2	10.2	12.2	
high	34.3	15.9	16.0	12.4	13.0	
Ggt	nil	low	high			
Mn rate						
low	13.1	17.5	19.6			ns
high	20.9	16.5	17.1			

[a] Values separated by more than the appropriate LSD were statistically different.

critical level (table 6.7) although only root growth, and not shoots, were increased by a high rate of application (17 mg Mn kg^{-1}). $MnSO_4$ applied prior to incubation at a low rate (0.09 mg Mn kg^{-1}) produced marginal Mn levels in shoots (table 6.7) but no growth increases

occurred with a high rate of application (0.36 mg Mn kg^{-1}). A high rate of commercial MnO_2 produced shoot Mn concentrations more than double the critical level (table 6.7).

$MnSO_4$ applied to the leaves of wheat seedlings had little effect on take-all. Reis *et al.* (1982) reported that foliar applications of Mn to wheat seedlings growing in a siliceous sand medium did not affect *Ggt* infection. Mn is poorly translocated in phloem (Nable and Loneragan 1985a,b) and foliar-applied $MnSO_4$ did not increase the concentration of Mn in the roots of treated plants and yet there was an increase in the dry weight of roots of treated plants. This increase in root dry weight may have been due to improved nutrition of shoots increasing the supply (or quality) of photosynthates to roots. However, the roots of treated plants were still as susceptible to *Ggt* infection as Mn-deficient plants. These results suggest that Mn is required in the tissues local to *Ggt* infection sites for decreases in disease to occur.

$MnSO_4$, applied as a seed "drench", was effective in decreasing take-all because it increased the shoot and root concentrations of Mn in treated plants to adequate levels for growth. This Mn source would not have increased soil Mn levels so precludes the possibility that Mn decreased take-all through toxic levels of Mn^{2+} in the soil (Graham and Rovira 1984).

Commercial MnO_2, mixed through soil at a high rate, increased shoot Mn levels (root Mn concentrations could not be measured) but did not decrease take-all. A low rate of application of the same substance also increased shoot Mn levels (although not to the same extent as the high rate) and decreased take-all. The high rate of application of commercial MnO_2 (not the low rate) caused some very large changes in the nutrient composition of treated plants. The concentration of Ca in the shoots of plants supplied with a high rate of commercial MnO_2 were reduced to deficient levels (root concentrations of Ca could not be accurately measured because of contamination from the soil which comprises 80-90 % $CaCO_3$). Although all Mn sources tended to decrease shoot Ca concentrations (especially the high rate of MnO_2 slurry) only a high rate of commercial MnO_2 decreased levels below

adequate for plant growth. A high rate of commercial MnO_2 may have increased the susceptibility of treated plants because it induced Ca deficiency. Ca has a critical metabolic role in carbohydrate removal, neutralization of cell acids, cell wall deposition, and formation of pectates in the middle lamella (Mengel and Kirkby 1982). Ca decreased infection by *Rhizoctonia* and *Pythium* (Huber 1980) but take-all of wheat and scab of potatoes increased after application of Ca (Huber 1980, Huber 1981).

In experiment A commercial MnO_2 decreased concentrations of B in roots of treated plants to almost undetectable levels but did not affect B levels in shoots differently to other Mn sources. However, this effect was not reproduced in experiment B so changes in root levels of B with commercial MnO_2 (at a high rate) does not offer any prospects for explaining the ineffectiveness of this Mn source in decreasing take-all.

A high rate of application of commercial MnO_2 caused very large decreases in plant (in both shoots and roots) concentrations of Mg to levels which may have been deficient for plant growth. A high rate of MnO_2 slurry caused similar changes but $MnSO_4$ treatments had little effect on plant Mg levels. A high rate of MnO_2 slurry caused large decreases in take-all so it seems that the very large decreases in Mg in plants treated with either MnO_2 source (at high rates) did not increase their susceptibility to take-all.

Mn is taken up by plants as Mn^{2+} and translocated through xylem as free divalent cations (Graham 1979). This suggests that commercial MnO_2 was affecting the nutrient composition of plants at sites external to roots and perhaps in the general soil matrix because the reduction of MnO_2 to Mn^{2+} must occur outside the roots. MnO_2 has a very high capacity to exchange and adsorb metal ions (Jarvis 1984b, Jorgensen and Jensen 1984) and high rates of commercial MnO_2 may have interacted differently (or to a larger extent) with Ca, Mg and B in the soil than MnO_2 slurry, MnO or $MnSO_4$. Mn oxides may also affect organic matter cycles in soil because of reactions with phenolic acids (Lehmann *et al.* 1987).

In section 2.3.3. of chapter 2, results were presented from all pot experiments which showed that no further decreases in take-all occurred once plant levels of Mn were adequate for growth. The results from the two pot experiments reported here also demonstrate the same relationship. Figure 6.3 shows that there was very little difference in the size of decreases in take-all caused by all Mn sources (except for commercial MnO_2 at a high rate) despite very large differences in shoot Mn concentrations between Mn sources.

Extensive oxidation by *Ggt* of Mn^{2+} to black/brown oxides in inoculated agar discs and on the surface of infected roots occurred in the presence of high rates of application of MnO and $MnSO_4$ (both treatments caused large decreases in take-all). No Mn oxide deposits of this type were observed in pots supplied with A/MnO_2 (no disease reduction occurred). However, extensive Mn deposition by *Ggt* hyphae was not necessary for decreases in take-all to occur because disease was also heavily decreased at low rates of MnO and $MnSO_4$ application, with seed-applied Mn and B/MnO_2. There was no evidence of Mn deposition by hyphae in these latter treatments.

MnO was the Mn oxide most available to plants. MnO produced higher shoot Mn concentrations than either commercial MnO_2 or MnO_2 slurry applied at the same rate of application. Mn is taken up by plants predominantly as Mn^{2+} (Graham 1979) and higher Mn oxides have to be reduced to soluble Mn^{2+} before uptake by plants can occur (Leeper 1970). The availability of Mn oxides is related to the crystallinity, particle size and ease of reduction of the oxide (Jones and Leeper 1951a,b). Most Mn oxides will be available to plants providing they occur as very small particles (which create large surface areas for root/oxide/soil contact) but highly crystalline oxides are generally less available. The surface of Mn oxides also appear to `age' in soils which will decrease their availability with time (Jones and Leeper 1951b). Particle size in the MnO product was much smaller than in commercial MnO_2 making MnO more plant-available. However, the MnO_2 slurry was a suspension of very fine particles and was still poorly available so MnO may have been more available to plants not only because of small particle size or lower crystallinity but also

because Mn was already reduced. MnO_2 slurry was less available to plants than commercial MnO_2 but this may have been due to poorer mixing of the slurry through soil than was achieved with dry, powdered commercial MnO_2. Incomplete mixing of MnO_2 slurry through soil would have decreased contact reduction (Godo and Reisenauer 1980, Uren 1981) through less frequent interceptions of oxide particles by roots. Obviously there is a need for further investigations into the chemical and physical properties of Mn oxides which confer availability to plants and how they impact on *Ggt* infection.

Additions of $MnSO_4$ to soil prior to incubation were readily available to plants and produced shoot concentrations of Mn comparable with the oxide treatments even though rates of application of $MnSO_4$ were much lower. Marcar (1986) showed that the recovery of Mn^{2+}, added to Wangary sand, decreased sharply with time and negligible levels of added Mn^{2+} were extracted with water from soil samples 10-20 days after addition. Mn^{2+} added to soil was probably present at sowing as a range of compounds; adsorbed Mn^{2+} on MnO_2 (Jauregui and Reisenauer 1982) or $CaCO_3$ surfaces (McBride 1979), $MnCO_3$ (McBride 1979), hydrous oxides (Jarvis 1984a) and oxides (Leeper 1970).

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CHAPTER 7.

THE EFFECT OF MANGANESE FERTILIZATION ON TAKE-ALL OF FIELD-GROWN WHEAT

CHAPTER 7. THE EFFECT OF MANGANESE FERTILIZATION ON TAKE-ALL OF FIELD-GROWN WHEAT.

7.1. Introduction.

Take-all is typically a greater problem on soils of moderate to high pH (Garrett 1936, Asher and Shipton 1981) and cold, dull conditions are also conducive to severe take-all (Hornby and Henden 1986). These soil types and conditions are also often associated with Mn deficiency (Leeper 1970, Graham 1983). NH_4^+-N, which favours the release of Mn from insoluble forms (Mulder and Gerretsen 1952) has been shown to decrease take-all (Huber *et al.* 1968, Smiley and Cook 1973, MacNish and Speijers 1982, Taylor *et al.* 1983), whereas NO_3^--N may increase the level of the disease. A causal relationship between Mn deficiency and severe take-all has been discovered under controlled environmental conditions in wheat seedlings, both previously (Graham and Rovira 1984) and during this thesis. The effect of Mn fertilization on take-all of wheat growing under Mn-deficient conditions in the field has not been tested in Australia. Decreases in take-all of field-grown wheat with soil-applied Mn fertilizers have been recently reported from Indiana (Huber and Wilhelm 1987) but foliar-applied Mn did not decrease take-all in field-grown wheat in Washington (Reis *et al.* 1982). The effect of Mn fertilizers on take-all of wheat was tested in two field experiments on the Eyre Peninsula of South Australia over three consecutive seasons. Both experiments were factorially designed with treatments applied in randomized blocks; the first and larger (experiment A) involved 4 soil-applied Mn sources, 3 Ggt inoculum rates, 5 replicates and 2 foliar Mn treatments applied to split-plots and the second and smaller (experiment B), 3 soil-applied Mn sources, 2 Ggt inoculum rates and 5 replicates.

7.2. Materials and Methods.

Both experiments were conducted at the same site (which had not been previously cropped) on the property of Mr. John MacDougall at Tooligie, Eyre Peninsula, South Australia. The soil was a brown calcareous sandy loam (Uc 2.11, Northcote 1979). Main

plot dimensions were 1.5 m wide (8 rows) x 6 m long for both experiments. The area has a Mediterranean climate and the property an average annual rainfall of 430 mm. Rainfall in 1984, 1985 and 1986 totalled 495, 316, 357 mm, respectively.

7.2.1. Experiment A.

7.2.1.1. *Inoculum.* *Ggt* 500 was added to plots as infected rye-grass seed propagules (supplied by Dr. A. Rovira) at rates of 2000 seeds m^{-2} (High Ggt). Low Ggt plots received 1000 infected and 1000 autoclaved seed propagules m^{-2}. Control plots received 2000 autoclaved rye-grass seed propagules m^{-2} (nil Ggt). *Ggt* inoculum was applied with the seed.

7.2.1.2. *Mn.* Soil-applied Mn was supplied to main plots in 3 different Mn compounds; $MnSO_4$, MnO or MnO_2. $MnSO_4$ was incorporated into superphosphate (Mn-super) at 40 kg Mn per tonne (giving 6.6 kg Mn ha^{-1}) and drilled with the seed. Granular MnO_2 [1] (particles between 2 and 10 mm in diameter) was broadcast onto main plots prior to sowing at 1.0 t ha^{-1} (giving 630 kg Mn ha^{-1}) and harrowed in. MnO [1] (majority of particles less than 0.001 mm in diameter) was similarly applied at 0.8 t ha^{-1} (also giving 630 kg Mn ha^{-1}). Mn was sprayed onto foliage of split-plots 61 days after sowing as `Mangasol'[R] [1] at 6 l ha^{-1} (giving 1 kg Mn ha^{-1}) (+FolMn); -FolMn split-plots were not sprayed.

7.2.1.3. *Procedure.* 7.2.1.3.1. 1984. All plots received basal fertilizers at 165 kg ha^{-1} of single superphosphate containing (per tonne) 5 kg copper, 5 kg zinc, 250 g molybdenum and 250 g cobalt, and 30 kg ha^{-1} of NH_4NO_3, drilled with the seed. The experiment was sown on 15 June with the wheat cultivar Condor at 60 kg ha^{-1}.

Twenty five plants were removed from every main plot 61 days after sowing (prior to foliar spray of Mn) when plants were at mid-tillering (Zadok's growth stage; 22-24, Zadoks *et al.* 1974) and assessed for shoot dry weight, tillers per plant and *Ggt* infection.

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1 supplied by Top Australia Ltd., Port Adelaide

The numbers of plants per metre of row were also counted in two 0.5 metre rows per plot at this time. The number of seminal roots with black stelar lesions on each plant was recorded and the results expressed as the percentage of diseased plants per plot and the percentage of diseased seminal roots per plant. Sections of roots with black stelar lesions were removed and plated onto PDA - *Ggt* was recovered from nearly all these root sections.

Twenty plants were removed from all split-plots 105 days after sowing (growth stage; 37-39). Take-all was scored on individual plants using the following method and results expressed as an average score per split-plot;

0 no disease observed
1 1-2 seminal roots with few black stelar lesions
2 up to 50 % of seminal roots with lesions and no or few lesions on crown roots
3 more than 50 % of all roots with lesions and crown roots with few lesions
4 as for 3 except crown roots with many lesions.

Youngest fully emerged leaf blades (YEBs) were removed from plants after disease assessment and oven-dried separately to whole shoots.

Split-plots were scored for colour and vigour 131 days after sowing.

Split-plots were machine-harvested for grain in late December.

Shoot tissues and whole grain samples were prepared for Mn analysis by nitric/perchloric acid digestion and measured with atomic absorption spectrophotometry.

7.2.3.1.2. 1985. The experiment was re-sown with 60 kg ha^{-1} of Condor on 20 June. Basal fertilizers were re-applied with the seed at the same rates as in 1984 except NH_4NO_3 was broadcast one week prior to sowing. $MnSO_4$ and MnO_2 were also re-applied

except MnO_2 was re-applied as a finely-ground product (particle diameter less than 2 mm). MnO was not re-applied nor was *Ggt* inoculum.

Plants were sampled from main plots 82 and 132 days after sowing, when growth stages were at mid-tillering (growth stage; 22-24) and ear emergence (growth stage; 51-53), respectively. Plots were also scored for colour and vigour at the first sampling.

Foliar Mn treatments were not applied.

Ggt infection at the first sampling was recorded as the number of seminal or crown roots with black stelar lesions per plant.

Plants were assessed at the second sampling for the presence of completely blackened sub-crown internodes, diseased seminal and diseased crown root systems. Plants were also scored for take-all on an individual basis using the method of Weller and Cook (1983). Main plots were machine-harvested for grain on 23 December.

Whole shoots from the first sampling and YEBs from the second were prepared for nutrient analysis by nitric acid digestion and measured with an ARL inductively coupled plasma atomic emission spectrometer. Grain was not analyzed for nutrient composition.

All data were analyzed with standard analysis of variance techniques appropriate to factorial experiments in randomized blocks, or factorial experiments with split-plots.

7.2.2. Experiment B.

7.2.2.1. *Inoculum.* *Ggt* 500 infected rye-grass seed propagules were again used as inoculum. Gamma-irradiated and autoclaved rye-grass seeds were inoculated with *Ggt* 500 and kept at 25° C for 24 days (cultures were regularly shaken to prevent hard coagulated lumps forming). After 25 days, infected seed propagules were dried in a sterile air stream.

Control plots received 2000 autoclaved rye-grass seed propagules m^{-2} (-Ggt) and +Ggt plots 2000 infected rye-grass seed propagules m^{-2}. *Ggt* inoculum was drilled with the seed.

7.2.1.2. *Mn*. Mn was supplied to plots in 2 different Mn compounds; $MnSO_4$ or MnO_2. $MnSO_4$ was incorporated into superphosphate at 40 kg Mn per tonne (giving 7.5 kg Mn ha^{-1}) and drilled with the seed. Finely-ground MnO_2 [1] (particle diameter less than 2 mm) was drilled into the soil with the seeding rig in a pass prior to seeding at 1.0 t ha^{-1} (giving 630 kg Mn ha^{-1}). MnO_2-treated plots received superphosphate at the same rate as in $MnSO_4$-treated plots.

7.2.1.3. *Procedure*. 7.2.1.3.1. 1985. All plots received basal fertilizers at 187 kg ha^{-1} of single superphosphate containing (per tonne) 5 kg copper, 5 kg zinc, 250 g molybdenum and 250 g cobalt, and 70 kg ha^{-1} NH_4NO_3, drilled with the seed. The experiment was sown on 20 June with the wheat cultivar Condor at 60 kg ha^{-1}.

Plants were sampled in the same way and at the same times as experiment A in 1985. The numbers of plants per metre of row were counted at the first sampling in two 0.5 metre rows per plot. Whole plots were machine-harvested for grain on 23 December.

7.2.1.3.2. 1986. The experiment was re-sown with 60 kg ha^{-1} of Bayonet (sufficient quantities of suitable Condor seed were not available) on 27 June. Basal fertilizers were re-applied at the same rates as in 1985 and $MnSO_4$ was also re-applied. MnO_2 was not re-applied nor was Ggt inoculum. The experiment was sampled at jointing (growth stage; 31) using the same procedure as in the second sampling in 1985 (except the number of blackened sub-crown internodes was not recorded) and machine-harvested for grain yields on 18 December.

Tissue preparation for nutrient analysis in both years was the same used in experiment A in 1985. Grain was not analyzed for nutrient composition in 1986. All data

were analyzed with standard analysis of variance techniques appropriate to a factorial experiment laid out in randomized blocks.

7.3. Results.

7.3.1. Experiment A.

7.3.1.1. *1984.* 7.3.1.1.1. Ggt. Although all three Mn sources tended to decrease *Ggt* infection on wheat roots these effects were not statistically significant (tables 7.1,7.2,7.3). The percentage of diseased plants in nil Mn plots was 13-24 % and 6-15 % higher (depending on the Mn source) than in Mn-treated plots at the first (table 7.1) and second samplings (tables 7.2,7.3), respectively. At the second sampling, a score of take-all on roots was 9-26 % higher in nil Mn plots than in soil Mn treated plots (Mn-super, MnO and MnO_2) (table 7.3).

Table 7.1. Experiment A. Effects of Mn source and *Ggt* inoculum on *Ggt* infection of wheat (growth stage[a]; 22-24) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source				
	Nil Mn	MnO_2	MnO	Mn-super	Mean
No. of diseased plants (%)					
Nil	20	18	24	25	22
Low	58	36	39	32	41
High	46	56	37	44	46
Mean	41	37	33	34	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					
No. of lesioned seminal roots on diseased plants (%)					
Nil	33	18	30	26	27
Low	34	28	26	30	30
High	34	36	32	37	35
Mean	34	27	29	31	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

Foliar-applied Mn had no effect on *Ggt* infection (tables 7.2,7.3) and MnO_2 always had higher disease levels than either MnO or Mn-super.

Table 7.2. Experiment A. Effects of Mn source and *Ggt* inoculum on the number of diseased wheat plants (%) (growth stage[a]; 37-39) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source							
	Nil Mn		MnO_2		MnO		Mn-super	
	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn
Nil	52	58	36	51	46	47	60	40
Low	96	83	74	83	81	72	74	66
High	73	68	74	87	65	74	72	61

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[b] at P=0.05 level).

					LSD
Mn source	nil	MnO_2	MnO	Mn-super	
	72	68	64	62	ns
Foliar Mn	-	+			
	67	66			ns
Ggt	nil	low	high		
	49	79	72		14
Mn source	nil	MnO_2	MnO	Mn-super	
Foliar Mn					
-	74	61	64	69	ns
+	70	74	64	56	
Mn source	nil	MnO_2	MnO	Mn-super	
Ggt					
nil	55	44	47	50	ns
low	90	79	77	70	
high	71	81	69	67	
Ggt	nil	low	high		
Foliar Mn					
-	49	81	71		ns
+	49	76	73		

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

Table 7.3. Experiment A. Effects of Mn source and *Ggt* inoculum on a take-all score[a] of roots of wheat plants (growth stage[b]; 37-39) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source							
	Nil Mn		MnO_2		MnO		Mn-super	
	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn
Nil	0.8	1.0	0.5	0.8	0.7	0.9	1.0	0.5
Low	1.6	1.5	1.2	1.3	1.5	1.1	1.6	0.8
High	1.0	1.2	1.2	1.6	1.3	1.0	1.1	0.8

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[c] at P=0.05 level).

					LSD
Mn source	nil	MnO_2	MnO	Mn-super	
	1.2	1.1	1.1	1.0	ns
Foliar Mn	-	+			
	1.1	1.0			ns
Ggt	nil	low	high		
	0.8	1.3	1.2		0.3
Mn source	nil	MnO_2	MnO	Mn-super	
Foliar Mn					
-	1.2	1.0	1.2	1.2	ns
+	1.2	1.2	1.0	0.7	
Mn source	nil	MnO_2	MnO	Mn-super	
Ggt					
nil	0.9	0.6	0.8	0.7	ns
low	1.6	1.3	1.3	1.2	
high	1.1	1.4	1.1	1.0	
Ggt	nil	low	high		
Foliar Mn					
-	0.7	1.5	1.2		ns
+	0.8	1.2	1.2		

[a] 0 (no disease observed) - 4 (seminal and crown roots with many black stelar lesions).
[b] Zadoks *et al.* 1974
[c] Values separated by more than the appropriate LSD were statistically different.

Despite the trial site not having been previously sown to cereals, take-all was present in nil Ggt plots. Twenty-two percent of plants were diseased in nil Ggt plots at the first sampling and 27 % of their seminal roots had black stelar lesions (table 7.1). A low rate

of *Ggt* inoculum increased the percentage of diseased plants at the first sampling to an average of 41 %. The percentage of diseased seminal roots was not increased by Ggt inoculum. The high rate of *Ggt* inoculum did not further increase disease.

The incidence of *Ggt* infection had increased by the second sampling and 49 % of plants in nil Ggt plots were diseased (diseased plants had lesions on their crown or seminal root systems) (table 7.2). A low rate of *Ggt* inoculum increased the percentage of diseased plants to 79 % but a high rate of inoculum did not further increase disease. A score of disease increased from an average of 0.77 in nil Ggt plots to 1.32 at low Ggt but was not increased further in high Ggt plots (table 7.3).

7.3.1.1.2. Plant growth. Vegetative growth and the number of plants per metre of row were not affected by Ggt treatments (tables 7.4,7.5,7.6).

Mn-super was the only Mn source to increase plant growth. The dry weight of shoots and number of tillers per plant increased by 49 and 40 % in Mn-super plots, respectively, at the first sampling (table 7.4) and were 40 and 23 % higher at the second (tables 7.5,7.6). However, plots treated with either MnO or Mn-super were scored as being darker green than nil Mn and MnO2 plots 131 days after sowing, although only Mn-super plots were scored as being more vigorous (table 7.7). Foliar Mn may have been less effective because rain fell on the night and day following application.

7.3.1.1.3. Grain yields. A high rate of *Ggt* inoculum decreased grain yields by 18 % and a low rate by 12 % although the latter effect was not quite significant at the P=0.05 level (table 7.8).

Grain yields averaged 1.02 t ha^{-1} in nil Mn plots and Mn-super increased grain yields by 27 % (table 7.8). Mn-super was the only Mn source to increase yields.

Table 7.4. Experiment A. Effects of Mn source and *Ggt* inoculum on growth and Mn concentration of wheat (growth stage[a]; 22-24) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source				
	Nil Mn	MnO_2	MnO	Mn-super	Mean
No. of plants per metre of row					
Nil	51	53	49	54	52
Low	49	49	50	54	51
High	49	51	51	54	51
Mean	50	51	50	54	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					
Dry weight of shoots (mg plant^{-1})					
Nil	94	105	102	141	111
Low	104	104	118	155	120
High	110	104	122	163	125
Mean	102	105	114	153	
LSD[b] (P=0.05) : Mn=25 ; Ggt, Mn*Ggt ns					
No. of tillers per plant					
Nil	1.7	1.5	1.6	2.1	1.7
Low	1.4	1.6	2.1	2.2	1.8
High	1.5	1.6	1.7	2.1	1.7
Mean	1.5	1.6	1.8	2.1	
LSD[b] (P=0.05) : Mn=0.2 ; Ggt, Mn*Ggt ns					
Mn concentration in whole shoots (mg kg^{-1} D.W.)					
Nil	14.6	12.1	31.4	24.3	20.6
Low	11.9	12.1	30.3	23.2	19.4
High	11.6	12.1	35.3	28.2	21.8
Mean	12.7	12.1	32.3	25.3	
LSD[b] (P=0.05) : Mn=2.8 ; Ggt, Mn*Ggt ns					

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

Table 7.5. Experiment A. Effects of Mn source and *Ggt* inoculum on the dry weight of shoots (g plant^{-1}) of wheat plants (growth stage[a]; 37-39) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source							
	Nil Mn		MnO_2		MnO		Mn-super	
	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn
Nil	0.96	1.02	1.05	1.24	1.21	1.11	1.78	1.27
Low	1.03	1.21	1.03	1.14	1.31	1.22	1.41	1.54
High	0.94	1.12	0.95	1.15	1.13	0.99	1.47	1.29

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[b] at P=0.05 level).

					LSD
Mn source	nil	MnO_2	MnO	Mn-super	
	1.05	1.10	1.16	1.46	0.19
Foliar Mn	-	+			
	1.19	1.19			ns
Ggt	nil	low	high		
	1.21	1.24	1.13		ns
Mn source	nil	MnO_2	MnO	Mn-super	
Foliar Mn					
-	0.98	1.01	1.22	1.55	0.17
+	1.12	1.18	1.11	1.37	
Mn source	nil	MnO_2	MnO	Mn-super	
Ggt					
nil	0.99	1.15	1.16	1.52	ns
low	1.12	1.09	1.27	1.48	
high	1.03	1.05	1.06	1.38	
Ggt	nil	low	high		
Foliar Mn					
-	1.25	1.20	1.12		ns
+	1.16	1.28	1.14		

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

Table 7.6. Experiment A. Effects of Mn source and *Ggt* inoculum on the number of tillers (per plant) on wheat plants (growth stage[a]; 37-39) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source							
	Nil Mn		MnO_2		MnO		Mn-super	
	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn
Nil	1.89	1.88	1.73	1.94	1.79	1.86	2.38	2.21
Low	1.66	1.94	1.66	2.07	2.34	2.25	2.15	2.23
High	1.63	1.88	1.83	2.10	1.94	1.71	2.23	2.12

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[b] at P=0.05 level).

					LSD
Mn source	nil	MnO_2	MnO	Mn-super	
	1.80	1.89	1.98	2.22	0.28
Foliar Mn	-	+			
	2.02	1.93			ns
Ggt	nil	low	high		
	1.95	2.04	1.93		ns
Mn source	nil	MnO_2	MnO	Mn-super	
Foliar Mn					
-	1.70	1.74	2.02	2.26	ns
+	1.91	2.04	1.94	2.19	
Mn source	nil	MnO_2	MnO	Mn-super	
Ggt					
nil	1.85	1.84	1.83	2.30	ns
low	1.80	1.86	2.30	2.19	
high	1.76	1.96	1.82	2.18	
Ggt	nil	low	high		
Foliar Mn					
-	1.93	1.95	1.91		ns
+	1.97	2.12	1.95		

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

Table 7.7. Experiment A. Effects of Mn source and *Ggt* inoculum on colour[a] and vigour[b] scores of wheat plants, 131 days after sowing, grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots. Foliar Mn had no visible effect and only main plots of the experiment were scored (i.e. foliar Mn treatments were ignored).

Ggt	Mn source				
	Nil Mn	MnO_2	MnO	Mn-super	Mean
Score of plot colour					
Nil	3.6	3.8	4.0	5.0	4.1
Low	3.2	3.2	4.0	4.8	3.8
High	3.0	2.8	4.2	5.0	3.8
Mean	3.3	3.3	4.1	4.9	
LSD[c] (P=0.05) : Mn=0.3 ; Ggt, Mn*Ggt ns					
Score of plot vigour					
Nil	3.4	3.6	2.8	4.6	3.6
Low	4.0	3.0	3.6	4.4	3.8
High	3.2	3.2	3.4	4.8	3.7
Mean	3.5	3.3	3.3	4.6	
LSD[c] (P=0.05) : Mn=0.4 ; Ggt, Mn*Ggt ns					

[a] 0 (plants yellow) - 5 (plants dark green).
[b] 0 (plants limp and small) - 5 (plants erect and large).
[c] Values separated by more than the appropriate LSD were statistically different.

7.3.1.1.4. Mn. Increasing rates of *Ggt* inoculum had no effect on Mn levels in plants at either sampling (tables 7.4,7.9,7.10). The concentration of Mn in whole shoots (D.W. basis) averaged 12.7 mg kg^{-1} in nil Mn plots at the first sampling and was not increased by MnO2 (table 7.4). Mn-super increased Mn concentrations in shoots to 25.3 mg kg^{-1} which is well above the critical level of 12 mg kg^{-1} for field-grown wheat (Graham *et al.* 1985). MnO had the largest effect on shoot Mn concentrations at the first sampling with an average of 32 mg kg^{-1} in this treatment.

The concentration of Mn in whole shoots in nil Mn plots at the second sampling was the same as at the first sampling and concentrations were not increased by MnO_2 (table 7.9). Shoot concentrations were higher in Mn-super and MnO plots and averaged 16.6 and 21.1 mg kg^{-1}, respectively. Foliar Mn did not increase the concentration of Mn in YEBs at

the second sampling and MnO was the only soil-applied Mn treatment to increase YEB concentrations in foliar-Mn treated split-plots (table 7.10). Mn concentrations in YEBs in split-plots not treated with foliar Mn were slightly increased by all soil-applied Mn sources.

Table 7.8. Experiment A. Effects of Mn source and *Ggt* inoculum on grain yields (t ha^{-1}) of wheat plants grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source							
	Nil Mn		MnO_2		MnO		Mn-super	
	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn
Nil	1.00	1.17	1.23	1.17	1.36	1.02	1.36	1.35
Low	0.86	1.16	0.97	0.84	1.09	1.08	1.19	1.38
High	0.99	0.96	0.89	0.79	0.84	0.95	1.41	1.10

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

					LSD
Mn source	nil	MnO_2	MnO	Mn-super	
	1.02	0.98	1.06	1.30	0.16
Foliar Mn	-	+			
	1.10	1.08			ns
Ggt	nil	low	high		
	1.21	1.07	0.99		0.14
Mn source	nil	MnO_2	MnO	Mn-super	
Foliar Mn					
-	0.95	1.03	1.10	1.32	ns
+	1.09	0.93	1.02	1.27	
Mn source	nil	MnO_2	MnO	Mn-super	
Ggt					
nil	1.09	1.20	1.19	1.35	ns
low	1.01	0.91	1.08	1.28	
high	0.97	0.84	0.89	1.25	
Ggt	nil	low	high		
Foliar Mn					
-	1.24	1.03	1.03		ns
+	1.18	1.11	0.95		

[a] Values separated by more than the appropriate LSD were statistically different.

Table 7.9. Experiment A. Effects of Mn source and *Ggt* inoculum on Mn concentration in whole shoots (mg kg^{-1} D.W.) of wheat plants (growth stage[a]; 37-39) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source							
	Nil Mn		MnO_2		MnO		Mn-super	
	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn
Nil	11.9	14.0	13.5	12.3	20.4	19.0	16.7	16.1
Low	12.5	12.5	13.7	13.1	20.9	24.1	17.2	15.6
High	12.9	12.8	13.9	12.4	20.5	21.5	18.0	16.3

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[b] at P=0.05 level).

					LSD
Mn source	nil	MnO_2	MnO	Mn-super	
	12.8	13.2	21.1	16.6	2.2
Foliar Mn	-	+			
	15.8	16.0			ns
Ggt	nil	low	high		
	15.5	16.2	16.0		ns
Mn source	nil	MnO_2	MnO	Mn-super	
Foliar Mn					
-	12.4	13.7	20.6	17.3	ns
+	13.1	12.6	21.5	16.0	
Mn source	nil	MnO_2	MnO	Mn-super	
Ggt					
nil	12.9	12.9	19.7	16.4	ns
low	12.5	13.4	22.5	16.4	
high	12.9	13.1	21.9	17.1	
Ggt	nil	low	high		
Foliar Mn					
-	15.6	16.1	16.3		ns
+	15.3	16.3	15.7		

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

Table 7.10. Experiment A. Effects of Mn source and *Ggt* inoculum on Mn concentration in YEBs (mg kg^{-1} D.W.) of wheat plants (growth stage[a]; 37-39) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source							
	Nil Mn		MnO_2		MnO		Mn-super	
	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn
Nil	15.3	16.5	17.3	14.7	18.2	18.7	19.1	17.6
Low	17.4	16.8	18.0	15.4	18.5	20.7	18.4	17.5
High	17.3	18.1	19.7	16.5	18.6	17.0	20.2	18.6

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's[b] at P=0.05 level).

					LSD
Mn source	nil	MnO_2	MnO	Mn-super	
	16.9	16.9	18.6	18.6	ns
Foliar Mn	-	+			
	17.3	18.2			ns
Ggt	nil	low	high		
	17.2	17.8	18.2		ns
Mn source	nil	MnO_2	MnO	Mn-super	
Foliar Mn					
-	16.7	18.3	18.4	19.2	1.5
+	17.1	15.5	18.8	17.9	
Mn source	nil	MnO_2	MnO	Mn-super	
Ggt					
nil	15.9	16.0	18.5	18.4	ns
low	17.1	16.7	19.6	17.9	
high	17.7	18.1	17.8	19.4	
Ggt	nil	low	high		
Foliar Mn					
-	17.5	18.1	18.9		ns
+	16.9	17.6	17.5		

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

Mn concentrations in grain in nil Mn, nil Ggt plots averaged 11.5 mg kg^{-1} (table 7.11). Grain Mn concentrations were not consistently affected by Mn or Ggt treatments although concentrations tended to be slightly higher in Ggt-inoculated plots and plots treated with MnO or Mn-super at sowing (table 7.11).

Table 7.11. Experiment A. Effects of Mn source and *Ggt* inoculum on Mn concentration in grain (mg kg^{-1} D.W.) produced by wheat grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1984. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source							
	Nil Mn		MnO_2		MnO		Mn-super	
	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn	-FolMn	+FolMn
Nil	11.5	15.3	13.2	12.9	12.9	13.4	14.9	12.2
Low	14.6	11.3	14.7	13.8	15.2	15.4	15.1	14.9
High	14.7	13.6	13.1	14.8	14.7	13.9	15.9	14.1

LSD (P=0.05) : Mn source*Foliar Mn*Ggt=2.1

1 and 2-way treatment means (with appropriate LSD's[a] at P=0.05 level).

					LSD
Mn source	nil	MnO_2	MnO	Mn-super	
	13.5	13.7	14.2	14.5	ns
Foliar Mn	-	+			
	13.8	14.2			ns
Ggt	nil	low	high		
	13.3	14.4	14.3		ns
Mn source	nil	MnO_2	MnO	Mn-super	
Foliar Mn					
-	13.6	13.7	14.3	15.3	ns
+	13.4	13.8	14.2	13.7	
Mn source	nil	MnO_2	MnO	Mn-super	
Ggt					
nil	13.4	13.0	13.1	13.5	ns
low	13.0	14.2	15.3	15.0	
high	14.2	13.9	14.3	15.0	
Ggt	nil	low	high		
Foliar Mn					
-	13.1	14.9	14.6		ns
+	13.5	13.9	14.1		

[a] Values separated by more than the appropriate LSD were statistically different.

7.3.1.2. *1985*. 7.3.1.2.1. Ggt. The percentage of diseased plants at the first sampling was decreased by more than 20 % by all Mn sources (table 7.12). The percentage of seminal roots with black stelar lesions was decreased by more than 25 % by all Mn sources but the variability in this measurement of *Ggt* infection resulted in these effects not being statistically significant (table 7.12). *Ggt* infection of crown roots was not affected by Mn treatments (table 7.12).

Table 7.12. Experiment A. Effects of Mn source and *Ggt* inoculum on *Ggt* infection of wheat (growth stage[a]; 22-24) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source				
	Nil Mn	MnO_2	MnO	Mn-super	Mean
No. of diseased plants (%)					
Nil	81	56	60	73	67
Low	74	58	72	62	67
High	83	66	56	50	64
Mean	79	60	62	62	
LSD[b] (P=0.10) ; Mn=16 ; Ggt, Mn*Ggt ns					
No. of lesioned seminal roots on diseased plants (%)					
Nil	48	24	34	29	34
Low	27	26	30	34	29
High	36	31	25	17	27
Mean	37	27	30	27	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					
No. of lesioned crown roots on diseased plants (%)					
Nil	47	34	44	47	43
Low	44	28	58	47	44
High	48	40	29	38	39
Mean	34	27	29	31	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

The decreases in *Ggt* infection with Mn sources measured at the first sampling were not present at the second sampling and *Ggt* infection was not affected by any Mn source at

this time (table 7.13).

Table 7.13. Experiment A. Effects of Mn source and *Ggt* inoculum on *Ggt* infection of wheat (growth stage[a]; 51-53) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source				
	Nil Mn	MnO_2	MnO	Mn-super	Mean
No. of diseased plants (%)					
Nil	84	82	87	99	88
Low	96	70	99	83	87
High	84	94	92	88	89
Mean	88	82	93	90	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					
Percentage of plants with blackened sub-crown internodes					
Nil	21	32	24	39	29
Low	23	16	29	28	24
High	23	23	40	18	26
Mean	34	27	29	31	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					
Percentage of plants with diseased crown root systems					
Nil	80	76	85	97	84
Low	76	82	96	81	84
High	80	88	89	83	85
Mean	79	82	90	87	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					
Take-all score[c]					
Nil	2.0	1.7	2.0	2.7	2.1
Low	2.0	1.8	2.5	2.0	2.1
High	2.0	2.0	2.1	2.1	2.0
Mean	2.0	1.8	2.2	2.3	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

[c] method of Weller and Cook (1983): 0=healthy, 5=dead.

Increasing rates of *Ggt* inoculum (applied in 1984) did not increase disease in 1985 (tables 7.12,7.13). Sevent-nine percent of plants were diseased in nil Mn plots at the first sampling which was higher than at the first sampling in 1984 (although the two samplings

were not taken at precisely the same time after sowing). Disease incidence had increased by the second sampling and 88 % of plants were diseased at this time.

7.3.1.2.2. Plant growth. Increasing rates of 1984-applied Ggt inoculum had no effect on plant growth except that high Ggt decreased a colour score in MnO-treated plots at the first sampling (table 7.14).

MnO_2 did not improve plant growth at either sampling, despite finely-ground MnO_2 being re-applied in 1985 (tables 7.14,7.15). MnO (applied in 1984) and Mn-super (re-applied in 1985) were equally effective at increasing plant growth at the first sampling. Plant growth was both a darker green and more vigorous in MnO and Mn-super at the first sampling (table 7.14). The dry weight of shoots per plant at the first sampling was increased by approximately 30 % with MnO and Mn-super but the number of tillers per plant was not affected by Mn treatments (table 7.14). Plant growth was not affected by Mn treatments at the second sampling (table 7.15).

7.3.1.2.3. Grain yields. Grain yields were not affected by Ggt treatments and the effects of Mn sources was not statistically significant even though yields were nearly doubled by Mn-super in high Ggt plots, increased by 22 % in low Ggt plots but not changed in nil Ggt plots (table 7.15).

7.3.1.2.4. Mn. Increasing rates of Ggt inoculum had no effect on Mn concentrations in whole shoots at the first sampling or in YEBs at the second sampling (table 7.16).

Mn concentrations in whole shoots at the first sampling averaged 12.3 mg kg^{-1} which was increased to 15.8 mg kg^{-1} by Mn-super and to 23.5 mg kg^{-1} by MnO (table 7.16). MnO_2 did not increase plant Mn concentrations at the first sampling. Mn concentrations in YEBs at the second sampling increased from an average of 17.7 mg kg^{-1} in nil Mn plots to 25.3 mg kg^{-1} in MnO plots (table 7.16). MnO_2 and Mn-super did not

increase Mn concentrations in YEBs.

Table 7.14. Experiment A. Effects of Mn source and *Ggt* inoculum on growth of wheat (growth stage[a]; 22-24) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source				
	Nil Mn	MnO_2	MnO	Mn-super	Mean
Score of plot colour[b]					
Nil	3.2	3.1	4.0	3.9	3.6
Low	3.7	3.0	4.0	4.0	3.7
High	3.1	3.4	3.3	4.1	3.5
Mean	3.3	3.2	3.8	4.0	
LSD[c] (P=0.05) : Mn=0.3 ; Ggt ns ; Mn*Ggt=0.5					
Score of plot vigour[d]					
Nil	2.9	3.2	3.5	3.6	3.3
Low	3.1	3.1	3.8	3.4	3.4
High	3.0	3.4	3.0	4.1	3.4
Mean	3.0	3.2	3.4	3.7	
LSD[c] (P=0.05) : Mn=0.4 ; Ggt, Mn*Ggt ns					
Dry weight of shoots (mg $plant^{-1}$)					
Nil	123	142	201	185	163
Low	154	128	208	175	167
High	137	167	144	171	155
Mean	138	146	184	177	
LSD[c] (P=0.05) : Mn=30 ; Ggt, Mn*Ggt ns					
No. of tillers per plant					
Nil	1.5	1.7	1.7	1.7	1.7
Low	1.5	1.3	1.8	1.9	1.6
High	1.4	1.6	1.5	1.6	1.5
Mean	1.5	1.5	1.7	1.7	
LSD[c] (P=0.05) : Mn, Ggt, Mn*Ggt ns					

[a] Zadoks *et al.* 1974

[b] 0 (plants yellow) - 5 (plants dark green).

[c] Values separated by more than the appropriate LSD were statistically different.

[d] 0 (plants limp and small) - 5 (plants erect and large).

Table 7.15. Experiment A. Effects of Mn source and *Ggt* inoculum on growth (growth stage[a]; 51-53) and grain yields of wheat grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source				
	Nil Mn	MnO_2	MnO	Mn-super	Mean
Dry weight of shoots (g plant^{-1})					
Nil	1.2	1.1	1.5	1.3	1.3
Low	1.6	1.3	1.6	1.3	1.4
High	1.2	1.3	1.4	1.6	1.4
Mean	1.4	1.2	1.5	1.4	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					
No. of tillers per plant					
Nil	1.2	1.1	1.2	1.2	1.2
Low	1.3	1.2	1.3	1.2	1.2
High	1.2	1.2	1.2	1.4	1.2
Mean	1.2	1.1	1.2	1.3	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					
Grain yield (t ha^{-1})					
Nil	0.73	0.67	0.76	0.70	0.72
Low	0.77	0.57	0.72	0.94	0.75
High	0.59	0.74	0.71	1.01	0.76
Mean	0.70	0.66	0.73	0.89	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

7.3.1.2.5. Nutrient composition of plants. The concentrations of iron, boron, copper, molybdenum, zinc, calcium, magnesium, potassium, phosphorus and sulphur in plants were adequate for wheat growth at both samplings and any effects of Mn or Ggt treatments were small (data not presented).

7.3.2. Experiment B.

7.3.2.1. *1985*. 7.3.2.1.1. Ggt. The percentage of seminal roots with black stelar lesions on diseased plants was highest in MnO_2 plots and 21 % lower in Mn-super plots at the first sampling (table 7.17). Levels were intermediate in nil Mn plots. All other estimates of

disease incidence and severity were not affected by Mn treatments at this time.

Table 7.16. Experiment A. Effects of Mn source and *Ggt* inoculum on Mn concentrations (mg kg^{-1} D.W.) in wheat grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source				
	Nil Mn	MnO_2	MnO	Mn-super	Mean
Mn concentration in shoots at the first sampling[a]					
Nil	14.9	12.0	25.6	14.4	16.7
Low	11.6	11.3	23.0	15.4	15.3
High	10.3	11.2	21.8	17.6	15.2
Mean	12.3	11.5	23.5	15.8	
LSD[b] (P=0.05) : Mn=1.8 ; Ggt, Mn*Ggt ns					
Mn concentration in YEBs at the second sampling[c]					
Nil	18.5	18.1	28.4	19.1	21.0
Low	20.2	16.1	24.0	15.8	19.4
High	14.3	23.9	23.6	26.2	22.0
Mean	17.7	19.4	25.3	20.3	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns					

[a] Mid-tillering (growth stage; 22-24, Zadoks *et al.* 1974).
[b] Values separated by more than the appropriate LSD were statistically different.
[c] Ear-emergence (growth stage; 51-53, Zadoks *et al.* 1974).

Ggt infection was decreased by Mn-super at the second sampling. The percentage of plants with blackened sub-crown internodes (a symptom of severe *Ggt* infection) was decreased by 34 % with Mn-super (table 7.18). Mn-super also decreased an average disease score of 2.2 in nil Mn plots to 1.7. Similarly, the percentage of plants with diseased crown roots was decreased by 17 % with Mn-super. The percentage of diseased plants (diseased plants had lesions on their crown or seminal root systems) was not significantly decreased by Mn-super. MnO_2 did not decrease Ggt infection.

Table 7.17. Experiment B. Effects of Mn sources and *Ggt* inoculum on *Ggt* infection of wheat (growth stage[a]; 22-24) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, S.A. in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source			
	Nil Mn	MnO_2	Mn-super	Mean
No. of diseased plants (%)				
-	20	29	19	23
+	99	99	99	99
Mean	59	64	59	
LSD[b] (P=0.05) : Mn ns ; Ggt=9 ; Mn*Ggt ns				
No. of seminal roots with lesions (%)				
-	6	10	6	8
+	83	90	73	82
Mean	45	50	40	
LSD[b] (P=0.05) : Mn=7 ; Ggt=5 ; Mn*Ggt ns				
No. of crown roots per plant with lesions				
-	0.04	0.13	0.10	0.90
+	0.94	1.27	0.90	1.03
Mean	0.49	0.70	0.50	
LSD[b] (P=0.05) : Mn ns ; Ggt=0.24 ; Mn*Ggt ns				
Take-all score[c]				
-	0.2	0.5	0.3	0.3
+	3.2	3.6	3.3	3.4
Mean	1.7	2.1	1.8	
LSD[b] (P=0.05) : Mn ns ; Ggt=0.3 ; Mn*Ggt ns				

[a] Zadoks *et al.* 1974.
[b] Values separated by more than the appropriate LSD were statistically different.
[c] Method of Weller and Cook (1983): 0=healthy, 5=dead.

The addition of live Ggt inoculum to plots caused a very high level of *Ggt* infection. For example, the percentage of diseased plants at the first sampling increased from 23 % in -Ggt plots to 99 % in +Ggt plots (table 7.17). These results also show that there was a high background of `native' *Ggt* at this site. The percentage of diseased plants had increased to 56 % in -Ggt plots by the second sampling but levels in +Ggt plots remained the same (table 7.18). The percentage of plants with diseased crown roots at the first sampling averaged 7 % and 61 % in -Ggt and +Ggt plots, respectively, (table 7.17) but had increased to 36 % and

94 % by the second sampling (table 7.18).

Table 7.18. Experiment B. Effects of Mn sources and *Ggt* inoculum on *Ggt* infection of wheat (growth stage[a]; 51-53) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, S.A. in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source			
	Nil Mn	MnO_2	Mn-super	Mean
No. of diseased plants (%)				
-	51	59	59	56
+	100	96	98	98
Mean	75	77	78	
LSD[b] (P=0.05) : Mn ns ; Ggt=11 ; Mn*Ggt ns				
No. of plants with blackened sub-crown internodes (%)				
-	4	7	5	5
+	62	71	38	57
Mean	33	39	22	
LSD[b] (P=0.05) : Mn=9 ; Ggt=7 ; Mn*Ggt=12				
No. of crown roots with lesions (%)				
-	36	48	23	36
+	98	96	88	94
Mean	67	72	56	
LSD[b] (P=0.10) : Mn=10 (P=0.05) : Ggt=10 ; Mn*Ggt ns				
Take-all score[c]				
-	0.9	1.1	0.6	0.8
+	3.5	3.2	2.9	3.2
Mean	2.2	2.1	1.7	
LSD[b] (P=0.10) : Mn=0.3 ; (P=0.05) : Ggt=0.3 ; Mn*Ggt ns				

[a] Zadoks *et al.* 1974.
[b] Values separated by more than the appropriate LSD were statistically different.
[c] Method of Weller and Cook (1983): 0=healthy, 5=dead.

7.3.2.1.2. Plant growth. Scores of plot colour and vigour at the first sampling were more than halved by live *Ggt* inoculum (table 7.19). Inoculation of plots with *Ggt* decreased plant numbers by 38 % and decreased early plant growth (table 7.19). The dry weight of shoots was decreased by 57 % in +Ggt plots at the first sampling (table 7.19) and by 50 % at the second (table 7.20). The number of tillers per plant at the first sampling and number of fertile tillers at the second sampling were decreased by 33 % and 24 %,

respectively, by live *Ggt* inoculum (tables 7.19,7.20).

Table 7.19. Experiment B. Effects of Mn sources and *Ggt* inoculum on growth of wheat (growth stage[a]; 22-24) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, S.A. in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source			
	Nil Mn	MnO_2	Mn-super	Mean
Score of plot colour[b]				
-	3.0	3.0	4.4	3.5
+	1.4	1.2	2.2	1.6
Mean	2.2	2.1	3.3	
LSD[c] (P=0.05) : Mn=0.5 ; Ggt=0.4 ; Mn*Ggt ns				
Score of plot vigour[d]				
-	3.4	3.2	4.0	3.5
+	1.3	1.2	1.9	1.5
Mean	2.4	2.2	3.0	
LSD[c] (P=0.05) : Mn=0.3 ; Ggt=0.3 ; Mn*Ggt ns				
No. of plants per metre of row				
-	29	29	26	28
+	16	18	18	17
Mean	22	23	22	
LSD[c] (P=0.05) : Mn ns ; Ggt=3 ; Mn*Ggt ns				
Dry weight of shoots (mg plant^{-1})				
-	186	185	230	201
+	73	69	115	86
Mean	130	127	173	
LSD[c] (P=0.05) : Mn=30 ; Ggt=24 ; Mn*Ggt ns				
No. of tillers per plant				
-	1.7	1.7	2.2	1.4
+	1.1	1.1	1.5	1.2
Mean	1.4	1.4	1.8	
LSD[c] (P=0.05) : Mn=0.2 ; Ggt=0.1 ; Mn*Ggt ns				

[a] Zadoks *et al.* 1974

[b] 0 (plants yellow) - 5 (plants dark green).

[c] Values separated by more than the appropriate LSD were statistically different.

[d] 0 (plants limp and small) - 5 (plants erect and large).

Table 7.20. Experiment B. Effects of Mn sources and *Ggt* inoculum on growth (growth stage[a]; 51-53) and grain yield of wheat grown on a Mn-deficient site at Tooligie, Eyre Peninsula, S.A. in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source			
	Nil Mn	MnO_2	Mn-super	Mean
Dry weight of shoots (g plant^{-1})				
-	1.5	1.3	2.1	1.6
+	0.7	0.6	0.8	0.7
Mean	1.1	0.9	1.4	
LSD[b] (P=0.05) : Mn=0.2 ; Ggt=0.2 ; Mn*Ggt ns				
No. of fertile tillers per plant				
-	1.2	1.1	1.4	1.2
+	0.9	0.9	1.0	0.9
Mean	1.0	1.0	1.2	
LSD[b] (P=0.05) : Mn=0.1 ; Ggt=0.1 ; Mn*Ggt ns				
Grain yield (kg ha^{-1})				
-	775	765	951	830
	6.63[c]	*6.62*	*6.80*	*6.68*
+	78	95	240	138
	4.12	*4.36*	*5.43*	*4.64*
Mean	427	430	596	
	5.37	*5.49*	*6.11*	
LSD[b,d] (P=0.05) : Mn=*0.4* ; Ggt=*0.3* ; (P=0.10) Mn*Ggt=*0.4*				

[a] Zadoks *et al.* 1974
[b] Values separated by more than the appropriate LSD were statistically different.
[c] natural log transformed (skewed distribution).
[d] LSD's apply to transformed data only.

Mn-super increased colour and vigour scores of plots at the first sampling by 50 % and 26 %, respectively (table 7.19). The dry weight of shoots of plants which received Mn-super was 33 % higher at the first sampling and 24 % higher at the second (tables 7.19,7.20). Mn-super increased the number of tillers per plant at the first sampling by 31 % (table 7.19) and the number of fertile tillers at the second sampling by 13 % (table 7.20). MnO_2 had no effect on plant growth.

7.3.2.1.3. Grain yields. Grain yields in the experiment were very low and were severely depressed in +Ggt plots, averaging only 12 % of -Ggt plots, or 0.14 t ha^{-1} (table 7.20). Grain yields of +Ggt plots were increased more than 3-fold with Mn-super but in -

Ggt plots Mn treatments did not significantly increase yields. MnO_2 did not increase grain yields.

7.3.2.1.4. Mn. Mn concentrations in shoots were not affected by Ggt treatments at either sampling (table 7.21). The average Mn concentration of whole shoots in nil Mn plots was 11.6 mg kg^{-1} at the first sampling, which is marginal for Mn (Graham *et al.* 1985). MnO_2 did not affect concentrations of Mn in shoots but Mn-super increased concentrations to 22 mg kg^{-1}.

MnO_2 and Mn-super increased Mn concentrations in whole shoots at the second sampling from 9.5 mg kg^{-1} (in nil Mn plots) to 12.2 and 12.9 mg kg^{-1}, respectively. The concentration of Mn in YEBs at the second sampling was not affected by Mn treatments and averaged 20 mg kg^{-1} for the experiment. Mn concentrations in grain were increased by Mn-super to 15.5 mg kg^{-1} from an average of 12.9 mg kg^{-1} in nil Mn plots. MnO_2 and live *Ggt* inoculum did not affect Mn concentrations in grain.

7.3.2.1.5. Nutrient composition of plants. The concentrations of iron, copper, molybdenum, calcium, magnesium, potassium and sulphur in shoots of plants were adequate for wheat growth at both samplings and any effects of Mn or Ggt treatments were small (data not presented). However, the concentrations of boron in whole shoots at the first sampling were decreased by MnO_2 from an average in nil Mn plots of 6.2 mg kg^{-1} to 4.4 mg kg^{-1} (table 7.22), which may be inadequate for wheat growth (Reuter and Robinson 1986). Both zinc and phosphorus were below adequate levels for growth at the second sampling (table 7.22) (Reuter and Robinson 1986). Zinc concentrations in whole shoots at the second sampling were not affected by Mn or Ggt treatments and averaged 8.6 mg kg^{-1} for the experiment but YEB concentrations were decreased from 11.1 mg kg^{-1} in nil Mn plots to 9.0 mg kg^{-1} in Mn-super plots. Live *Ggt* inoculum had no effect on zinc concentrations in plants. Phosphorus concentrations in whole shoots at the second sampling were marginal in -Ggt plots (average of 1159 mg kg^{-1}) but were increased to an average of 1502 mg kg^{-1} in

+Ggt plots.

Table 7.21. Experiment B. Effects of Mn source and *Ggt* inoculum on Mn concentrations (mg kg^{-1} D.W.) in wheat grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source			
	Nil Mn	MnO_2	Mn-super	Mean
Mn concentration in whole shoots at the first sampling[a]				
-	11.4	12.0	22.5	15.3
+	11.7	12.0	21.4	15.0
Mean	11.6	12.0	22.0	
LSD[b] (P=0.05) : Mn=2.2 ; Ggt, Mn*Ggt ns				
Mn concentration in whole shoots at the second sampling[c]				
-	9.4	11.9	12.5	11.3
+	9.7	12.5	13.3	11.8
Mean	9.5	12.2	12.9	
LSD[b] (P=0.05) : Mn=2.2 ; Ggt, Mn*Ggt ns				
Mn concentration in YEBs at the second sampling[c]				
-	19.1	20.1	23.1	20.7
+	16.1	19.4	22.1	19.2
Mean	17.6	19.7	22.6	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns				
Mn concentration in grain				
-	13.3	12.9	15.7	13.9
+	12.5	13.9	15.3	13.9
Mean	12.9	13.4	15.5	
LSD[b] (P=0.05) : Mn=1.7 ; Ggt, Mn*Ggt ns				

[a] mid-tillering (growth stage; 22-24, Zadoks *et al.* 1974).

[b] Values separated by more than the appropriate LSD were statistically different.

[c] ear-emergence (growth stage; 51-53, Zadoks *et al.* 1974).

Table 7.22. Experiment B. Effects of Mn source and *Ggt* inoculum on nutrient composition (mg kg^{-1} D.W.) of wheat plants grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source			
	Nil Mn	MnO_2	Mn-super	Mean
Boron concentration in whole shoots at the first sampling[a]				
-	6.6	4.6	5.5	5.5
+	5.9	4.2	5.1	5.0
Mean	6.2	4.4	5.3	
LSD[b] (P=0.10) : Mn=1.0 ; Ggt, Mn*Ggt ns				
Zinc concentration in whole shoots at the second sampling[c]				
-	6.6	7.9	9.7	8.1
+	10.1	9.6	7.4	9.1
Mean	8.4	8.8	8.6	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns				
Zinc concentration in YEBs at the second sampling[c]				
-	10.9	12.0	9.0	10.7
+	11.3	10.7	8.9	10.3
Mean	11.1	11.4	9.0	
LSD[b] (P=0.10) : Mn=1.5 ; Ggt, Mn*Ggt ns				
Phosphorus concentration in whole shoots at the second sampling[c]				
-	965	1358	1153	1159
+	1516	1639	1350	1502
Mean	1240	1499	1252	
LSD[b] (P=0.05) : Mn ns ; Ggt=206 ; Mn*Ggt ns				
Phosphorus concentration in YEBs at the second sampling[c]				
-	1207	1454	1253	1305
+	1413	1417	1179	1336
Mean	1310	1435	1216	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns				

[a] mid-tillering (growth stage; 22-24, Zadoks *et al.* 1974).
[b] Values separated by more than the appropriate LSD were statistically different.
[c] ear-emergence (growth stage; 51-53, Zadoks *et al.* 1974).

YEB concentrations of phosphorus at the same time were not affected by Mn or Ggt treatments and averaged 1321 mg kg^{-1} for the experiment.

Nutrient composition of grain was generally not affected by Mn or Ggt treatments (data not presented).

7.3.2.2. *1986*. 7.3.2.2.1. Ggt. There was a 39 % decrease in the number of diseased plants and a 50 % decrease in average disease score with Mn-super (table 7.23). The percentage of diseased seminal and diseased crown roots per plant were decreased by more than half with Mn-super.

Table 7.23. Experiment B. Effects of Mn sources and *Ggt* inoculum on *Ggt* infection of wheat (growth stage[a]; 31) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, S.A. in 1986. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source			
	Nil Mn	MnO_2	Mn-super	Mean
No. of diseased plants (%)				
-	33	38	26	32
+	46	44	22	37
Mean	39	41	24	
LSD[b] (P=0.05) : Mn=12 ; Ggt ns, Mn*Ggt ns				
No. of seminal roots with lesions (%)				
-	13	13	8	11
+	20	24	6	17
Mean	16	18	7	
LSD[b] (P=0.05) : Mn=7 ; Ggt, Mn*Ggt ns				
No. of crown roots with lesions (%)				
-	6	4	3	4
+	6	7	2	5
Mean	6	5	2	
LSD[b] (P=0.10) : Mn=2 ; Ggt, Mn*Ggt ns				
Take-all score[c]				
-	0.5	0.5	0.3	0.4
+	0.7	0.9	0.3	0.6
Mean	0.6	0.7	0.3	
LSD[b] (P=0.05) : Mn=0.3 ; Ggt, Mn*Ggt ns				

[a] Zadoks *et al.* 1974.
[b] Values separated by more than the appropriate LSD were statistically different.
[c] Method of Weller and Cook (1983): 0=healthy, 5=dead.

There was no measurable difference in *Ggt* infection between + and -Ggt plots at the time of sampling and the average percentage of diseased plants for the whole experiment was 35 % (compared to 61 % at the first sampling in 1985) (table 7.23). This apparent

reduction in the prevalence of take-all in 1986 may have been due to an increase in the suppressiveness of the soil, which is an effect commonly observed with cereal monoculture (Garrett 1934, Cook and Rovira 1976). However, this effect was not apparent in the second year of experiment A.

7.3.2.2.2. Plant growth. The dry weight of shoots per plant was not affected by live *Ggt* inoculum (applied in 1985) or Mn sources at the time of sampling but the number of tillers per plant was increased by 39 % in Mn-super plots without live *Ggt* inoculum (table 7.24).

7.3.2.2.3. Grain yields. The yields of grain in 1986 were as low as in 1985 and were decreased by an average of 15 % in +Ggt plots (table 7.24). Mn-super increased grain yields by an average of 29%. MnO_2 did not increase grain yields.

7.3.2.2.4. Mn. Only Mn-super increased the concentration of Mn in whole shoots and YEBs (table 7.25). Mn concentrations in plants from nil Mn and MnO_2 treatments were less than the critical level for Mn (Graham *et al.* 1985). Live *Ggt* inoculum had no effect on plant Mn concentrations (table 7.25).

7.3.2.2.5. Nutrient composition of plants. The concentrations of iron, copper, calcium, magnesium, potassium and sulphur in whole shoots and YEBs were adequate for wheat growth when plants were sampled and were not affected by Mn or Ggt treatments (data not presented). Molybdenum concentrations in shoots and YEBs were lower in MnO2 and Mn-super plots but were still at luxury levels for growth (data not presented).

Table 7.24. Experiment B. Effect of Mn sources and *Ggt* inoculum on growth (growth stage[a]; 31) and grain yield of wheat grown on a Mn-deficient site at Tooligie, Eyre Peninsula, S.A. in 1986. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source			
	Nil Mn	MnO_2	Mn-super	Mean
Dry weight of shoots (g plant^{-1})				
-	0.3	0.3	0.5	0.4
+	0.4	0.3	0.3	0.4
Mean	0.3	0.3	0.4	
LSD[b] (P=0.05) : Mn, Ggt, Mn*Ggt ns				
No. of tillers per plant				
-	1.9	1.6	2.6	2.0
+	1.9	1.9	1.9	1.9
Mean	1.9	1.8	2.3	
LSD[b] (P=0.05) : Mn=0.3 ; Ggt ns ; Mn*Ggt=0.4				
Grain yield (kg ha^{-1})				
-	508	452	678	546
+	428	437	528	464
Mean	468	445	603	
LSD[b] (P=0.05) : Mn=73 ; Ggt=60 ; Mn*Ggt ns				

[a] Zadoks *et al.* 1974

[b] Values separated by more than the appropriate LSD were statistically different.

Table 7.25. Experiment B. Effects of Mn source and *Ggt* inoculum on Mn concentrations (mg kg^{-1} D.W.) in wheat (growth stage[a]; 31) grown on a Mn-deficient site at Tooligie, Eyre Peninsula, South Australia in 1985. Values in the body of each table are the average of 5 replicate plots.

Ggt	Mn source			
	Nil Mn	MnO_2	Mn-super	Mean
Mn concentration in whole shoots				
-	8.7	8.9	12.1	9.8
+	9.1	9.2	12.9	10.4
Mean	8.7	9.1	12.5	
LSD[b] (P=0.05) : Mn=1.2 ; Ggt, Mn*Ggt ns				
Mn concentration in YEBs				
-	9.9	10.4	12.9	11.1
+	10.4	10.6	14.2	11.7
Mean	10.2	10.5	13.5	
LSD[b] (P=0.05) : Mn=1.6 ; Ggt, Mn*Ggt ns				

[a] Zadoks *et al.* 1974.

[b] Values separated by more than the appropriate LSD were statistically different.

7.4. Discussion and Conclusions.

The correction of Mn deficiency in wheat growing on a Mn-deficient soil decreased take-all (depending on the Mn source) in two of the three years tested and similar trends were present in the third year. $MnSO_4$-supplemented superphosphate (Mn-super) and MnO were equally effective at decreasing take-all but MnO_2 and foliar Mn did not decrease disease levels and were also ineffective at supplying Mn to plants. Mn-super and MnO decreased the incidence and the severity of take-all of wheat.

Huber and Dorich (1987) found that the effectiveness of Mn application at decreasing take-all was related to its ability to resist biological oxidation in soil. Thus, broadcast application had been the poorest, band application fair and seed treatment best. Similar results were recorded in the experiments reported here because MnO, which was broadcast onto plots at nearly ten times the rate of Mn as Mn-super, was no more effective at decreasing take-all than Mn-super, which was applied to the seed rows (band application). However, MnO was more effective at supplying Mn to wheat plants than Mn-super.

Reis *et al.* (1982) reported that foliar application of Mn did not decrease take-all of wheat in the field. Foliar application was also ineffective at decreasing take-all in 1984 although it was also ineffective at improving the Mn nutrition and growth of treated plants so may not have been a fair test of this Mn source.

The use of Mn fertilizers on wheat crops growing on soils of marginal or deficient Mn status has three possible beneficial effects: firstly, improvement of the Mn status of the crop resulting in better growth and increased grain yields; secondly, a decrease in Ggt infection with a consequent increase in grain yield; and lastly a reduction in inoculum levels for the following season. Mn fertilizer decreased the number of take-all infected plants. It is the crowns and roots of these plants which are the inocula for the following season so improved Mn nutrition of wheat crops may decrease disease levels in subsequent crops (in the same way as suggested by Moore and Cook (1984) for tillage effects). A large increase

in yield with Mn-super in live *Ggt* inoculated plots was confirmed in 1985 in experiment B and the interaction was also present in experiment A in that year (although not statistically significant).

Insoluble Mn higher oxides have to be reduced before they become available to plants (Leeper 1970). They were tested as residual Mn sources because Mn-super has to be re-applied every year, and under severe deficiency conditions, additional foliar sprays may be necessary to maximize yields in cereals (eg., Reuter et. al 1973). The philosophy behind their use was that mixing very large quantities of finely-ground Mn-oxides through the soil may encourage the growth of naturally occurring Mn-reducing soil microbial populations (Bromfield and David 1976, Zajic 1969) because of the large amounts of substrate present. This, in turn, would slowly release available Mn^{2+} to the plants. This effect should persist for many seasons. However, in light of the poor ability of MnO_2 to decrease take-all in experiment B, their use as residual Mn sources for cereal crops needs careful evaluation.

The availability of Mn oxides is related to the crystallinity, particle size and ease of reduction of the oxide (Jones and Leeper 1951). MnO_2 was ineffectual at supplying Mn to plants even when supplied as a more finely granulated product. However, MnO released more Mn to plants than Mn-super (including comparisons where MnO had been applied in the previous season) and also decreased take-all and would appear to be a more suitable product for further testing as a residual Mn source than MnO_2. Finely powdered MnO_2 at low rates of incorporation supplied large amounts of Mn to plants and decreased take-all in pot experiments (see chapter 6) which is in contrast to the results achieved in these field experiments. The differences may have been due to the poorer incorporation of MnO_2 and greater dilution of MnO_2 through the soil profile in the field. Better incorporation of Mn oxides through soil may have increased contact reduction (Uren 1981) through more frequent interceptions of oxide particles by roots. Also, the MnO_2 used in the pot experiments was a fine powder but the oxide used in the field experiment was mostly

granular which may partly explain why the MnO_2 was more available in pots (Jones and Leeper 1951).

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CHAPTER 8.

INVASION BY *GGT* OF EXCISED WHEAT ROOT PIECES PRE-CULTURED WITH AND WITHOUT MN

CHAPTER 8. INVASION BY *GGT* OF EXCISED WHEAT ROOT PIECES PRE-CULTURED WITH AND WITHOUT MN.

8.1. Introduction.

Deacon and Lewis (1986) published a technique where rates of invasion by *Ggt* into excised wheat root sections were measured. They tested several *Ggt* isolates of different virulence (established in pot experiments) for their ability to invade wheat root sections and found a high correlation between the extent of depression in host growth and the speed at which the *Ggt* isolate invaded excised wheat root sections.

In all experiments previously reported, both in this thesis and in the literature (e.g. Reis *et al.* 1982, Graham and Rovira 1984), the effects of Mn on take-all of wheat have been measured indirectly by following the development of symptoms on the host rather than measuring the rate of invasion by *Ggt*. The technique of Deacon and Lewis (1986) was adapted to directly measure the effect of Mn on the invasion by the take-all fungus into the host. Wheat plants (or excised wheat root pieces) were pre-cultured with and without Mn before root sections were removed from the pre-cultured plants (or root pieces) and exposed to *Ggt* colonies. Wheat plants were pre-cultured under either sterile or non-sterile conditions.

8.2. Materials and Methods.

8.2.1. Pre-culturing of wheat plants under sterile conditions and invasion of excised root sections by *Ggt*.

Wheat plants were grown aseptically on mineral-supplemented agar with and without Mn for several weeks before root sections were removed and placed on the margins of actively growing *Ggt* colonies on PDA.

8.2.1.1. *Basal nutrients for wheat pre-culture.* A basal nutrient solution containing 5 mM KNO_3, 2.5 mM $Ca(NO)_3.4H_2O$, 1mM $MgSO_4$, 0.5 mM$(NH_4)_3PO_4$, 0.03 mM FeEDTA, 0.015 mM H_3BO_3, 0.01 mM NaCl, 0.0025mM $ZnSO_4.7H_2O$, 0.0005 mM $CuSO_4.H_2O$,

0.0002 mM $CoSO_4.7H_2O$ and 0.0001 mM $H_2MoO_4+H_2O$ was prepared after macronutrient stock solutions were passed through a controlled pore-glass CPG/8-hydroxyquinoline column to remove micronutrient contaminates (Eskew *et al.* 1984). The pH of the solution was adjusted to 5.0 with dilute HCl.

8.2.1.2. *Mn*. Mn was added to wheat pre-culture as a solution of $MnSO_4.H_2O$ to give a final concentration of 1 mM.

8.2.1.3. *Procedure*. Six acid-washed one-litre `Schott' bottles were part-filled (approximately 300 ml of solution per bottle) with either - or +Mn basal nutrient solution. Difco `Bacto-agar' was added to every bottle at 0.5 % of the nutrient solution. The mouths of bottles were plugged with non-absorbent cotton wool and bottles were autoclaved for 15 min at 120° C and 100 kPa.

Condor wheat seed (from a Mn-deficient field site) was soaked in $DDDH_2O$ for 5 hrs and then surface-sterilized by immersion in $DDDH_2O$ at 53° C for 10 min, followed by saturated calcium hypochlorite solution (7 % calcium hypochlorite mixed in $DDDH_2O$ and filtered) for 45 min at room temperature on a `wrist-shaker'. The seeds were blotted dry on sterile filter paper and germinated on 50 % PDA at 20° C. Sterile germinating seeds were aseptically transferred to the surface of agar in `Schott' bottles (2 per bottle) after 4 days and bottles were transferred to controlled environment conditions of 15° C constant temperature and a 10 hr photoperiod in every 24 hrs (approximate photon flux density of 0.3 mEinsteins m^{-2} s^{-1}). No attempt was made to keep light out of the agar.

Iron (at the same rate used initially) was added to all bottles, and Mn to +Mn bottles, after 41 days and plants were removed after 55 days. Shoots were removed from plants and oven-dried at 80° C before digestion in nitric acid. Digested samples were analyzed for nutrient content with an ARL inductively coupled plasma atomic emission spectrometer.

A 2 cm section was aseptically removed from behind the tip of every seminal root and transferred onto the outer margin of an actively growing *Ggt* 500 colony on full strength PDA. Roots from +Mn and -Mn pre-treatments (at least three sections per treatment) were placed on separate plates and removed after 6, 12, 18, 24 and 48 hrs at 20° C. Root sections were preserved in 70 % methanol.

Transverse sections were cut by hand from the middle of preserved root pieces and flooded with 1 % phloroglucinol in 70 % ethanol for 5 min. Excess stain was poured off before sections were flooded with conc. HCl and observed at 2-400 x magnification for depth of penetration using the scoring method of Deacon and Lewis (1986). Root sections were considered to have six or seven sectors, each containing one of the radial arms of the usually heptarch stele. The cortex of wheat seminal root axes usually has six cell layers, invasion of which was scored from 1 to 6; a score of 7 was given for invasion of the endodermis, 8 for the pericycle and 9 for the phloem or xylem.

8.2.2. Tissue culture of wheat root sections in a sterile liquid medium and subsequent invasion by *Ggt*.

In this experiment excised wheat root sections were grown in sterile White's medium (White 1956) (amended with IAA) before being placed on *Ggt* colonies on PDA.

8.2.2.1. *Mn*. Mn was either withheld from complete White's medium (-Mn) or added as a solution of $MnSO_4.4H_2O$ to give a final concentration of 1 mM.

8.2.2.2. *Procedure*. Condor seed was soaked in $DDDH_2O$ for 5 hrs and surface-sterilized as before (see section 8.2.1.3.) except the hot water treatment was not included. Seeds were thoroughly rinsed in sterile $DDDH_2O$, blotted dry on sterile filter paper and germinated on 50 % PDA at 20° C. Two cm sections were aseptically removed from behind the root tips of sterile, strongly germinating seeds and transferred to sterile White's medium in 100 ml conical flasks (mouths plugged with non-absorbent cotton wool), five root sections per 50 ml of media. FeEDTA was substituted for $FeSO_4$ in the preparation of the liquid medium

and stock solutions of the macronutrient salts (except NaH_2PO_4) were stripped of micronutrient contaminates as before (see section 8.2.1.3.). Indole-3-acetic acid (10^{-5} mM) was added to liquid cultures to stimulate root growth (Roberts and Street 1955).

Flasks were incubated at 25° C for 34 days on an orbital shaker.

After 34 days in liquid culture root sections were aseptically transferred to the margins of actively growing *Ggt* 500 colonies on full strength PDA. Roots were removed after 18 hrs at 20° C and preserved in 70 % methanol. All root sections were stained with phloroglucinol and scored for extent of *Ggt* invasion as before (see section 8.2.1.3.).

8.2.3. Pre-culturing of wheat plants under non-sterile conditions and invasion of excised root sections by *Ggt*.

Wheat plants were grown in non-sterile solution culture with and without Mn for several weeks before root sections were removed and placed on the margins of actively growing *Ggt* colonies on PDA.

8.2.3.1. *Mn*. Mn was added to solution culture as a $MnSO_4.H_2O$ solution to give a final concentration of 1 mM Mn.

8.2.3.2. *Procedure*. Condor seeds were surface sterilized in 12.5 % sodium hypochlorite solution for 2.5 min, rinsed in $DDDH_2O$, and germinated with $DDDH_2O$ at 20° C. After 2 days, vigourously germinating seeds, visually free of microbial contamination, were transferred onto stainless steel grids above aerated sterile 0.5 mM $CaSO_4$ solution. These grids were placed under mercury vapour lamps with a 12 hr photoperiod (0.3 mEinsteins m^{-2} s^{-1}) and ambient temperatures of 20-30° C. No attempt was made to keep germinating seeds sterile past this stage.

Vigourous seedlings were transferred after a further 7 days into 5 l pots containing nutrient solution of the same composition (without agar) used previously (see section

8.2.1.1.). Seven seedlings were transferred into each pot (seeds were removed) and 4 pots were set-up, 2 with and 2 without Mn. Pots were aerated and kept under the same conditions as used previously (see section 8.2.1.3.).

Fresh nutrients were added to each pot after 7, 14 and 21 days culture at the same rates used initially and pH returned to 5.0 with dilute HCl.

After 24 days, sections 2 cm long were removed from behind the root tips of plants grown with and without Mn and half the sections were placed on the edge of actively growing colonies of *Ggt* 500 on full strength PDA. The remaining root sections were placed on the edge of *Ggt* 500 colonies growing on full strength PDA to which 50 mg kg^{-1} Mn had been added (as $MnSO_4.4H_2O$). Some root sections were also placed on - and +Mn PDA without *Ggt* colonies. Plates were kept at 20° C and root sections removed after 30 hrs and preserved in 70 % methanol.

Fresh weights of plants were recorded when root sections were removed. Shoots and roots were dried at 80° C and analyzed for nutrient composition as before (see section 8.2.1.3.).

8.3. Results.

8.3.1. Pre-culturing of wheat plants under sterile conditions and invasion of excised root sections by *Ggt*.

8.3.1.1. *Ggt invasion of excised root sections.* Root sections removed from plants pre-cultured without Mn (-Mn roots) were invaded faster by *Ggt* 500 (table 8.1). This effect was present at all incubation times except when invasion by *Ggt* was so early (after 6 hours) that it could not be accurately scored and when invasion was so advanced that all root sections were completely invaded by *Ggt* (after 48 hours). *Ggt* penetration rates through the cortex of -Mn roots averaged almost one cell layer ahead of +Mn pre-cultured root sections

(+Mn roots). All -Mn root steles were invaded after 18 hours but it took 48 hours for all +Mn root steles to be invaded (table 8.1).

The endodermis and xylem of root sections were the most clearly and consistently lignified tissues although lignification was also seen in other tissues in various sections and times. There was no difference in extent or pattern of lignification between -Mn and +Mn roots.

Table 8.1. The effect of pre-culture of wheat plants with and without Mn under sterile conditions on the subsequent invasion of excised root sections by *Ggt* on PDA. Values in the table are the average of at least 3 root sections.

Time of exposure of roots to *Ggt* (hrs)	Pre-culture of wheat plants -Mn		+Mn	
Depth of penetration[a]; 1 (exodermis) - 9 (xylem or phloem)				
6	0.18	*0.10*[b]	0.34	*0.13*
12	5.94	*0.41*	5.00	*0.21*
18	7.05	*0.27*	6.00	*0.11*
24	7.38	*0.29*	6.30	*0.52*
48	9.00	*0.00*	9.00	*0.00*
Percentage of root sections with invaded steles[c]				
6	0		0	
12	50		0	
18	100		30	
24	83		80	
48	100		100	

[a] Scoring method of Deacon and Lewis 1986.
[b] Values in italics are standard errors of means.
[c] Stele includes endodermis and pericycle.

While *Ggt* hyphae seemed to move easily and quickly through the epidermis and cortex of wheat root pieces they were frequently observed to `queue up' outside the endodermis, giving the impression that the endodermis was a barrier to deeper penetration. When the endodermis was finally invaded it was always at a point nearest to xylem bundles.

8.3.1.2. *Plant growth.* The culture of wheat on nutrient agar (with or without Mn) under axenic culture produced small plants which had no tillers or nodal roots. Seminal roots on these plants had little branching. There were no visual differences between plants from -Mn and +Mn pre-culture. The concentration of Mn in shoots of plants pre-cultured without Mn averaged 5.3 mg kg^{-1} D.W. which is well below the critical level of 20 mg kg^{-1} established for wheat plants grown in non-sterile solution culture (Graham and Loneragan 1981). Shoot Mn concentration in +Mn cultured plants averaged 92.7 mg kg^{-1}.

8.3.2. Tissue culture of wheat root sections in a sterile liquid medium and subsequent invasion by *Ggt*.

8.3.1.1. *Ggt invasion of root sections.* Hyphae of *Ggt* 500 had penetrated into root pieces pre-cultured without Mn to an average depth of nearly 6 cortical cell layers, or nearly to the endodermis, after only 18 hours (table 8.2).

Table 8.2. The effect of pre-culture of wheat root pieces without Mn under sterile conditions on their subsequent invasion by *Ggt* on PDA. Root pieces were placed on *Ggt* colonies for 18 hours. Values in the table are the average of at least 4 root sections.

Pre-culture of wheat root pieces	Depth of penetration[a]		Root pieces with invaded steles[b] (%)
-Mn	5.86	*0.10*[c]	40
+Mn	1.72	*0.63*	0

[a] Scoring method of Deacon and Lewis (1986); 1 (exodermis) - 9 (xylem or phloem).
[b] Stele includes endodermis and pericycle.
[c] Values in italics are standard errors of means.

Depth of invasion into +Mn pre-cultured root pieces averaged less than 2 cortical cell layers over the same time interval. *Ggt* hyphae had penetrated into the steles of 40 % of -Mn roots but no +Mn roots had steles penetrated by *Ggt* (table 8.2).

8.3.2.2. *Growth of root pieces.* Tissue culture of excised wheat root pieces in White's medium amended with indole-3-acetic acid did not produce rapid growth of roots. After 34

days of culture, root pieces had elongated by only 2-3 mm and had produced lateral roots only 1-2 mm in length. There were no differences between -Mn and +Mn pre-culture in root growth or development.

8.3.3. Pre-culturing of wheat plants under non-sterile conditions and invasion of excised root sections by *Ggt*.

8.3.3.1. *Ggt invasion of excised root sections*. Root sections from plants pre-cultured without Mn were invaded faster by *Ggt* (table 8.3, plate 8.1). After 30 hours on -Mn PDA, *Ggt* had invaded -Mn roots to the depth of vascular tissue but +Mn roots were, on average, only invaded to the depth of the inner cell layers of the cortex. Less than 40 % of +Mn roots had steles invaded by *Ggt* compared to 100 % in -Mn roots.

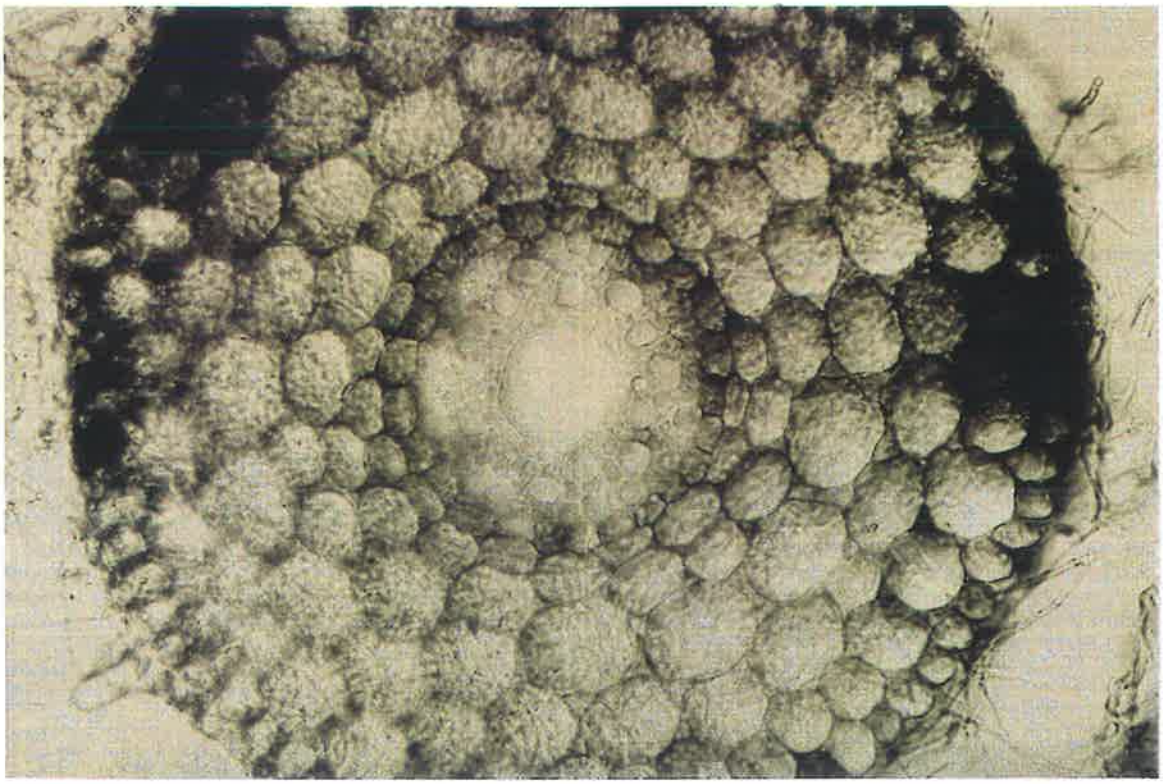
Growth rates and invasion into root sections by *Ggt* on +Mn PDA were slower than on -Mn PDA but the presence of Mn in agar did not fully substitute for a pre-culture of plants with Mn. Invasion by *Ggt* into -Mn roots on +Mn PDA was faster than into +Mn roots (table 8.3). Nearly three-quarters of -Mn roots had steles invaded by *Ggt* after 30 hours but no +Mn roots had been invaded to this depth after the same time.

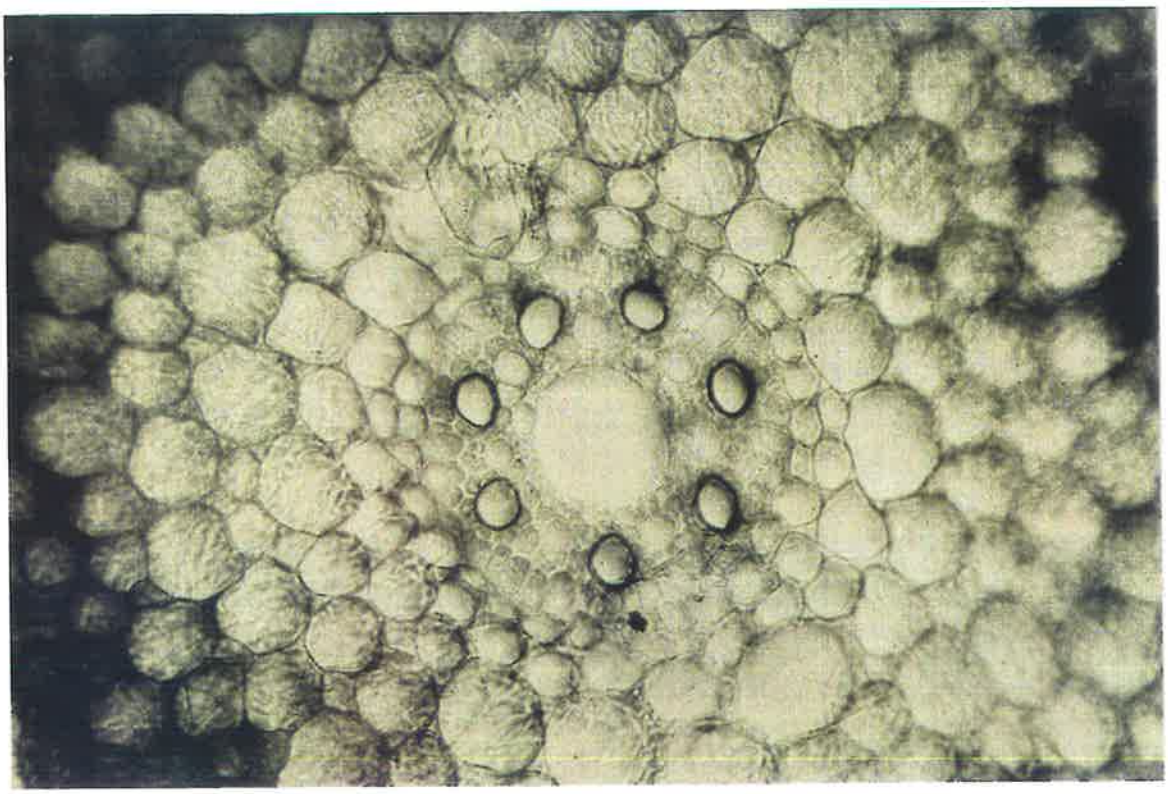
Table 8.3. The effect of pre-culture of wheat plants without Mn under non-sterile conditions on the subsequent invasion of excised root pieces by *Ggt* on PDA (with or without 50 mg kg^{-1} Mn). Root pieces were placed on *Ggt* colonies for 30 hours. Values in the table are the average of at least 6 root sections.

Mn in PDA	Pre-culture of wheat root pieces	Depth of penetration[a]		Root pieces with invaded steles[b] (%)
-	-Mn	8.71	*0.43*[c]	100
-	+Mn	5.87	*0.19*	38
+	-Mn	6.04	*0.36*	71
+	+Mn	2.17	*0.21*	0

[a] Scoring method of Deacon and Lewis (1986); 1 (exodermis) - 9 (xylem or phloem).
[b] Stele includes endodermis and pericycle.
[c] Values in italics are standard errors of means.

Plate 8.1. Effect of pre-culture of wheat plants without Mn on the subsequent invasion of excised root pieces by *Ggt*. Plants were cultured under non-sterile conditions - the upper micrograph is a section from a root piece without Mn pre-culture, the lower micrograph of a section from pre-culture with 50 mg kg^{-1} Mn.





Phloroglucinol staining of invaded root sections revealed extensive lignification of the exodermis and outer cortical cell layers and often also the endodermis and xylem in +Mn roots. Lignification in invaded -Mn roots was usually restricted to the epidermis and xylem and sometimes the exodermis. Addition of Mn to PDA had no observable effect on lignification patterns in wheat root sections. Similar patterns of lignification were observed in root pieces which were placed on sterile PDA and were not invaded by *Ggt*.

Ggt invasion rates into non-sterile root pieces were similar to invasion rates into sterile root pieces.

8.3.3.2. *Plant growth.* After 24 days of growth in solution culture without Mn wheat plants had leaves which were weak, floppy and pale green; features which are typical of Mn deficiency (Snowball and Robson 1983). Plants from +Mn culture had erect dark-green leaves. The fresh weight of shoots and roots of plants from +Mn culture averaged 8.7 and 34.2 g per plant, respectively, compared to 1.1 and 4.3 g per plant for -Mn plants. The concentration of Mn in shoots of -Mn plants were deficient (an average of 5.6 mg kg^{-1} D.W.) but levels in +Mn plants were adequate for growth (an average of 39 mg kg^{-1}). The concentration of Mn in roots of +Mn plants was 47 mg kg^{-1} (samples of -Mn roots were lost during preparation for analysis).

8.4. Discussion.

Invasion by *Ggt* hyphae into wheat root pieces, and colonization of their steles, was very rapid in root pieces which had been pre-cultured without Mn. Root pieces from all methods of pre-culture which supplied Mn; sterile or non-sterile, whole plants or separate root pieces, were more resistant to invasion by *Ggt* hyphae and invasion through the cortex and colonization of the stele was slower. The presence of high levels of Mn in the agar did not fully substitute for a pre-culture of roots (or intact plants) with Mn.

Invasion by *Ggt* hyphae into host roots does not appear to impair host growth until both the stele and vascular tissue are penetrated (Clarkson *et al.* 1975). The phloem is destroyed faster than the xylem (Clarkson *et al.* 1975) and the disruption of the phloem leads to a restriction of assimilate supply to sites of active ion uptake (Clarkson *et al.* 1975, Fitt and Hornby 1978). Occlusion of xylem tubes by *Ggt* hyphae will decrease translocation of ions (Fellows 1928). If the results obtained here in petri-dish studies reflect the effect of Mn nutrition of whole plants on *Ggt* invasion of intact roots (as Deacon and Lewis 1986 found for different *Ggt* isolates) then wheat plants grown under Mn deficient conditions would be invaded quicker, have more and larger stelar lesions and suffer larger decreases in growth than plants grown under Mn adequate conditions. Mn deficiency of wheat plants has been shown to increase the number and length of black stelar lesions caused by *Ggt* (Graham and Rovira 1984 and in this thesis).

Lignification in root pieces from non-sterile pre-culture where Mn was supplied was more extensive and this may have been responsible for the slower invasion rates by *Ggt*. Host cells rapidly respond to *Ggt* invasion by depositing lignin on the innermost surface of their walls around the sites of penetration (Weste 1972). Some of these deposits develop into closed tubular structures (lignitubers) as they encase the penetrating hypha. Lignitubers are rarely successful in completely halting the invasion of the penetrating hyphae (Skou 1981) but they may cause important delays. Lignitubers were rarely seen in these studies. The more extensive lignification in invaded wheat root pieces cultured with Mn was also present in root pieces not exposed to *Ggt*. This suggests that differences in *Ggt* invasion rates between -Mn and +Mn pre-culture were due to pre-formed lignin defence structures but does not exclude the possibility that -Mn roots may have been unable to respond to the presence of *Ggt* with elicited defence products (Skou 1981). Lignin production is controlled by Mn-activated enzyme systems (Gross 1980).

Phloroglucinol staining may not have revealed all lignin deposits in wheat roots because not all lignins will stain with this particular dye (Skou 1981). Lignins produced in response to pathogenic invasion, in particular, stain poorly with phloroglucinol.

This technique of scoring invasion by *Ggt* into excised root pieces appears to be very suitable for studies into the importance of the prior nutrition of root pieces to their resistance to invasion by *Ggt*. The technique is quick, does not require specialized apparatus and many root pieces can be exposed to *Ggt* because the root pieces can be preserved and scored for depth of penetration when convenient. There is also the dual feature of being able to vary both the pre-culture of the plants from which the root pieces are removed and also the conditions under which the root pieces are exposed to *Ggt*..

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CHAPTER 9.

EFFECT OF MN ON THE GROWTH OF A SOIL-BORNE PSEUDOMONAD AND ITS ANTAGONISM AGAINST *GGT*

CHAPTER 9. EFFECT OF MN ON THE GROWTH OF A SOIL-BORNE PSEUDOMONAD AND ITS ANTAGONISM AGAINST *GGT*.

9.1. Introduction.

Many soil-borne bacteria and actinomycetes have been proposed as causal agents of suppression of take-all (e.g. Garrett 1934, Zogg and Jaggi 1974, Sivasithamparam and Parker 1978). For instance, Smiley (1978a,b) explained the reduction in take-all in a field soil with ammonium fertilizer in terms, not of soil or rhizosphere pH effects, but of small changes in the suppressive micro-flora of the soil which accompanied the use of NH_4^+-N. He found more antagonistic micro-flora, and a larger proportion of *Pseudomonas* spp. within this group, in NH_4^+-N treated soil and proposed these *Pseudomonas* spp. as the most likely antagonists acting in the soil. The rhizosphere pseudomonad, *Pseudomonas fluorescens*, is now a well-documented antagonist of *Ggt* and has been used to decrease take-all in the field (Weller and Cook 1983, Weller 1984, Wong and Baker 1985, Weller *et al.* 1985, Huber and Wilhelm 1988). The mode of action of antagonism in the field is not known but *P. fluorescens* produces a suite of antibiotic compounds (Ahl *et al.* 1986, Brisbane *et al.* 1987), including siderophores which are only produced under low Fe conditions (Kloepper *et al.* 1980). Huber (1988) found that *P. fluorescens* was either antagonistic or beneficial to take-all of wheat in the field, depending on the N fertilizer used; antagonistic if NH_4^+-N and beneficial if NO_3^--N. Huber and Dorich (1988) proposed that fluorescent pseudomonads may exert biological control over take-all by increasing the availability of Mn in the rhizosphere of wheat plants through the reduction of insoluble Mn oxides.

Micro-organisms have long been recognized as important in the soil chemistry of Mn (Timonin 1946, Gerretsen 1937, Leeper 1970). Many soil-borne micro-organisms have been identified which are capable of oxidizing Mn^{2+} to insoluble Mn oxides (Gerretsen 1937, Leeper and Swaby 1940, Bromfield and Skerman 1950, Bromfield 1956, Zajic 1969, Bromfield 1979) including various *Pseudomonas* spp. (Huber 1988).

Fewer studies have been conducted to identify micro-organisms capable of reduction of Mn oxides (Zajic 1969, Bromfield and David 1976, Gottfreund *et al.* 1985). Bromfield and David (1976) found that a Mn-oxidizing *Arthrobacter* sp. was capable of reduction of MnO_2 to Mn^{2+} under poorly aerated conditions which suggests that other Mn-oxidizers may also be capable of Mn-reduction under certain conditions.

Experiments are reported here which tested the effect of Mn on the `in vitro' growth and siderophore production of *Pseudomonas fluorescens* 2-79 $RN_{10}$ (Weller and Cook 1983) (2-79). In addition, experiments were conducted `in vitro' and in pots to test whether the antagonism by this bacterium against *Ggt* would still operate when Mn was added to the system. The hypothesis of Huber and Dorich (1988) predicts that it would not because they propose that antagonism is dependent on the bacteria relieving Mn deficiency in the host by reducing Mn oxides in the soil. In the presence of adequate soil Mn, the bacteria should not further increase resistance of the host to take-all.

9.2. Materials and Methods.

9.2.1. Effect of Mn on the growth of 2-79 on PDA.

The effect of increasing concentrations of Mn^{2+} on the growth and behaviour of 2-79 on PDA was tested and the behaviour of 2-79 on PDA in the presence of MnO_2 was also observed.

9.2.1.1. *Mn^{2+}*. Bacterial cells from a colony of 2-79 on a full-strength PDA (39 g in 1000 ml) slope were suspended in 100 ml of sterile $DDDH_2O$. A 0.5 ml aliquot of this suspension was diluted into a further 100 ml of sterile $DDDH_2O$ and 0.5 ml of the final suspension was transferred to each of 3 sterile 9 cm petri-dishes. Each dish was part-filled with 15 ml of cooling, weak PDA (17 g PDA in 1000 ml), 3 dishes per Mn rate. Dishes were incubated at 20° C for 10 days.

Mn^{2+} was added to weak PDA as a sterile stock solution of $MnSO_4.4H_2O$ to give final concentrations of 0, 10, 50, 100 or 200 mg kg^{-1} of Mn^{2+}.

Bacterial growth after 10 days was estimated as the number of colonies in each dish within 3 fields of a dissecting microscope at 10 x magnification.

9.2.1.2. *MnO_2*. Petri-dishes of weak PDA, to which MnO_2 [1] had been added at 10 mg g^{-1}, were streaked with 2-79 from a full-strength PDA slope. Dishes were incubated at 20° C and observed at regular intervals for disappearance of black MnO_2.

9.2.2. Effect of Mn on siderophore production by 2-79 in KMB.

The effect of Mn on siderophore production by 2-79 in liquid King's medium B (King *et al.* 1954) (KMB) was tested. KMB is an iron-free medium on which *Pseudomonas fluorescens* freely produces siderophores. Cultures of 2-79 were grown on KMB with Mn present initially (T0) or added after 4 days (T4) and compared with cultures grown without Mn (nil Mn).

The liquid media was prepared with the following reagents;

50 ml of media.

1 g `Difco' Proteose Peptone No. 3

10 ml, 7.5 g kg^{-1} of $MgSO_4.7H_2O$ stock solution

10 ml, 7.5 g kg^{-1} of KH_2PO_4 and 40 ml kg^{-1} of glycerol stock solution

30 ml $DDDH_2O$

Mn was added aseptically to 50 ml of media initially (T0) by replacing 10 ml of $DDDH_2O$ with 10 ml of a 2 mg kg^{-1} of $MnSO_4.4H_2O$ stock solution, giving 100 mg kg^{-1} of Mn in media. Mn in treatment T4 was added aseptically to cultures as 10 ml of a 2 mg kg^{-1} of $MnSO_4.4H_2O$ stock solution (10 ml of $DDDH_2O$ was added to other treatments). Ten ml

1 BDH, analytical grade, majority of particles less than 0.01 mm in diameter.

of $MnSO_4.4H_2O$ stock solution (10 ml of $DDDH_2O$ was added to other treatments). Ten ml of a 0.15 mg kg^{-1} of $FeCl_3$ stock solution was added to one flask of each treatment after 8 days.

Erlenmeyer flasks (125 ml capacity, 2 per treatment) were part-filled with 50 ml of media and autoclaved for 15 min at 120° C and 100 kPa. Flasks were inoculated with 2-79 from a solid KMB culture with 100 mg kg^{-1} of added Mn and incubated on a horizontal shaker at 25° C.

Siderophore production was visually estimated at regular intervals by observing flasks under ultra-violet (UV) light for blue-green fluorescence (King *et al.* 1954, Philson and Llinas 1982).

9.2.3. Effect of Mn on the antagonism of 2-79 against *Ggt* on PDA.

Ggt 500 was grown on PDA with increasing rates of Mn in the presence or absence of 2-79 to assess the effect of Mn on the level of antagonism by the bacteria against *Ggt* `in vitro'.

Weak PDA was prepared with increasing rates of Mn; 0 (0 Mn), 10 (10 Mn), 50 (50 Mn) and 100 mg kg^{-1} of Mn^{2+} (100 Mn) (see section 9.2.1.1.) and six 9 cm disposable petri-dishes were part-filled with autoclaved agar from each Mn rate.

Four drops of a turbid suspension of 2-79 were added to the outside margin of 3 dishes from each Mn rate and run around the dish such that the suspension formed a complete ring. Dishes were incubated at 20° C.

One day after the addition of 2-79 a single weak PDA block (4 x 4 x 4 mm) of *Ggt* 500 was placed in the centre of every dish. Dishes were returned to 20° C. *Ggt* colony areas were calculated from the average of 2 measurements of colony diameter taken at right angles to each other after 3 and 5 days.

9.2.4. Effect of Mn on the control by 2-79 of take-all of wheat grown in a Mn-deficient sand. Bacteria added to pots in a suspension of $DDDH_2O$.

This pot experiment was factorially designed with 3 rates of soil applied Mn (0, 8 and 24 Mn), 3 rates of Ggt (sterile 20 % PDA, 4 % *Ggt*-colonized PDA and 20 % *Ggt*-colonized PDA), 3 rates of 2-79 (autoclaved suspension of bacteria, 1 % suspension of live bacteria, full-strength suspension of bacteria) and 4 replicates. Most of the materials and procedures used in this experiment have already been presented in pot experiments outlined in earlier chapters so only a brief summary will be included here.

9.2.4.1. *Inoculum.*; *Ggt* 500 colonies were prepared on petri-dishes of 4 and 20 % PDA (0.156 g and 0.78 g of PDA in 100 ml, respectively) and incubated at 20° C for 7 days. Extra sterile 20 % PDA dishes were prepared for use in nil Ggt controls.

9.2.4.2. *2-79.* Cultures of 2-79 were grown at 20° C on liquid KMB (with 10 ml of 0.15 mg kg^{-1} of $FeCl_3$ solution per 50 ml of media) for 4 days prior to sowing. A suspension of bacteria was prepared in $DDDH_2O$ to give a concentration of approximately 10^7 cells ml^{-1} (suspension appeared turbid). Some of this full-strength suspension (high 2-79) was autoclaved (-2-79) and some diluted 100 fold (low 2-79).

9.2.4.3. *Soil.* Wangary sand (see section 3.2.1.1.) was incubated for 15 days prior to sowing (see Appendix A). Normal rates of basal nutrients were added at sowing (see section 3.2.3.1.).

9.2.4.4. *Mn.* Mn was mixed through soil prior to incubation to give either 0, 8 or 24 mg of Mn^{2+} per pot.

9.2.4.5. *Procedure.* Pots were part-filled with soil such that agar plugs were placed 4 cm below germinating wheat seeds (see section 3.2.3.2.). One ml of bacterial suspension was added to seeds before they were covered with a final layer of soil. Pots were placed in randomized blocks under controlled environment conditions of 15° C with a 10 hour light

period (average photon flux density of 0.3 mEinsteins m^{-2} s^{-1} supplied by a bank of mercury vapour lamps) in every 24. The same bacterial treatments were re-applied to the surface of pots 10 days after sowing (1 ml of $DDDH_2O$ was added to -2-79 pots instead of an autoclaved suspension). Pots were watered to original weight at regular intervals. Plants were harvested after 25 days and assessed for growth, *Ggt* infection and Mn nutrition.

9.2.5. Effect of Mn on the control by 2-79 of take-all of wheat grown in a Mn-deficient sand. Bacteria added to pots in liquid media.

This pot experiment was factorially designed with 3 rates of soil applied Mn (0, 0.4 and 4 Mn), 3 rates of Ggt (sterile 4 % PDA, 1 % *Ggt*-colonized PDA and 4 % *Ggt*-colonized PDA), 2 rates of 2-79 (-,+ 2-79) and 5 replicates. This experiment was first reported in chapter 3 (+2-79 treatments removed) and most of the details of the materials and procedures used in this experiment can be found in section 3.2.3.1. of that chapter so, again, only a brief summary will be included here.

9.2.5.1. *Inoculum*. Weak PDA plugs of *Ggt* 500 were placed 2 cm below wheat seeds in pots.

9.2.5.2. *2-79*. Cultures of 2-79 were grown in liquid KMB (with 10 ml of 0.15 mg kg^{-1} of $FeCl_3$ solution per 50 ml of media) for 3 days at 25° C on a horizontal shaker. At sowing, 2 ml of live bacterial culture were added to the soil surface of +2-79 pots and 2 ml of autoclaved culture to -2-79 pots.

9.2.5.3. *Soil*. Wangary sand was used with normal rates of basal nutrients but without prior incubation.

9.2.5.4. *Mn*. Mn was mixed through soil to give either 0, 0.4 or 4 mg of Mn per pot.

9.2.5.5. *Procedure*. Pots were placed in randomized blocks under controlled environment conditions of 15° C with a 10 hour light period (average photon flux density of 0.2

mEinsteins m^{-2} s^{-1} supplied by a bank of fluorescent tubes) in every 24. Plants were harvested after 25 days and assessed for growth, *Ggt* infection and Mn nutrition.

9.3. Results.

9.3.1. Effect of Mn on the growth of 2-79 on PDA.

9.3.1.1. Mn^{2+}. The growth of 2-79 was inhibited by very low concentrations of Mn^{2+} in PDA (table 9.1). The number of 2-79 colonies at 10 mg kg^{-1} of Mn was less than half the number at 0 Mn and none were found on dishes with concentrations of Mn^{2+} in agar of more than 100 mg kg^{-1}.

Table 9.1. Effect of Mn on the growth of *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) on potato-dextrose agar after 10 days at 20° C.

Mn added to PDA (mg Mn^{2+} kg^{-1})	No. of colony forming units (units $field^{-1}$)[a]		(% of units at 0 Mn)
0	157	*23.0*[b]	100
10	63	*3.1*	40
50	10	*1.1*	6
100	2	*0.1*	1
200	0	*0.0*	0

[a] Av. number of colonies in 3 fields of a dissecting microscope at 10 x magnification. Each value is the average of 3 replicate dishes.
[b] Standard error of mean.

Colonies of 2-79 were smooth in appearance and cream in colour, regardless of Mn treatment. No oxidation of Mn^{2+} by 2-79 was observed but dark brown elongated crystals were found within colonies which had been grown for several weeks on agar supplemented with Mn. No crystals were found in colonies grown on PDA without added Mn. These

crystals did not contain Mn oxides because they did not stain blue with acidified benzidine in ethanol.

9.3.1.2. *MnO_2*. MnO_2 in PDA was rapidly reduced by 2-79. Within 2 days of incubation at 20° C clear haloes were present around the streaked surface colonies of 2-79. The clear haloes were created by the reduction of black insoluble MnO_2 to soluble Mn^{2+}. Dishes were completely clear after 2 weeks.

Despite vigorous shaking of the autoclaved PDA before dishes were poured, the majority of MnO_2 particles still settled to the bottom of dishes before the agar had set. Bacteria were streaked onto the surface of solidified agar which means that reduction by 2-79 of the majority of MnO_2 particles occurred at a distance equal to the depth of the agar, or 3-4 mm.

9.3.2. Effect of Mn on siderophore production by 2-79 in KMB.

Flasks were viewed after 3 days of incubation and nil Mn cultures fluoresced strongly under UV light but T0 cultures exhibited even greater siderophore production and fluoresced very strongly. The T0 cultures also appeared a brighter yellow/green under natural light. Siderophores produced by *P. fluorescens* on KMB are yellow/green in colour (King *et al.* 1954).

Cultures were again observed for UV fluorescence on day 4, 4 hours after the addition of Mn to T4 flasks. Brightest fluorescence was in T0 flasks but fluorescence in nil Mn flasks was also strong. There was almost no UV fluorescence in T4 flasks. A similar pattern was also observed after 7 days incubation.

The addition of Fe^{3+} to flasks after 8 days resulted in an immediate brick red colour in all cultures. Ferri-siderophores (siderophores complexed with Fe^{3+}) are brick red in colour (Nielands 1984). UV fluorescence after 9 days was absent in all cultures to which Fe^{3+} had been added except T0 which exhibited very weak fluorescence. The addition of

extra Fe^{3+} to the T0 culture which had already received Fe did not further diminish UV fluorescence. The pattern of UV fluorescence in cultures which received no Fe^{3+} was the same as observed after 7 days.

9.3.3. Effect of Mn on the antagonism of 2-79 against *Ggt* on PDA.

The presence of Mn^{2+} at levels which were toxic to the growth of *Ggt* 500 increased the antagonism by 2-79 against *Ggt* 500. Both 50 and 100 Mn were toxic to the growth of *Ggt* (see growth after 3 days in table 9.2) and *Ggt* colony area in the presence of 2-79 was only 37 and 27 %, respectively, of the area in the absence of 2-79. This compared to 43 % at 0 Mn. The extent of inhibition of *Ggt* colony area at 10 Mn was similar to 0 Mn after 3 days and after 5 days was slightly lower than at 0 Mn (table 9.2).

Table 9.2. Effect of Mn on the antagonism of *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) against *Ggt* 500 on potato-dextrose agar. Cultures were incubated at 20° C.

Mn added to PDA (mg Mn^{2+} kg^{-1})	Average colony area of *Ggt* 500 (mm^2)			
	3 days		5 days	
	- 2-79	+ 2-79	- 2-79	+ 2-79
0	1800	772	5116	930
10	1963	792	5586	1091
50	1602	588	4964	709
100	1544	416	3303	511
3 days - LSD (P=0.05) Mn = 98 ; 2-79 = 69 ; Mn x 2-79 = 138				
5 days -	194	137	274	
Colony area of *Ggt* 500 in presence of 2-79 as a percentage of area without 2-79				
0		43		18
10		40		20
50		37		14
100		27		15

Colony area of *Ggt* after 5 days in the absence of 2-79 was significantly larger at 10 Mn than at 0 Mn and antagonism by 2-79 was least at this rate of Mn. In general, the patterns of inhibition of *Ggt* growth by 2-79 exhibited after 3 days growth were still present after 5 days growth except the levels of inhibition were much higher.

The presence of 2-79 on Mn-supplemented PDA also inhibited Mn oxidation by *Ggt*. In the absence of 2-79, oxidation of Mn by *Ggt* colonies occurred throughout the extent of their mycelia but in the presence of 2-79, no oxidation occurred in the outer margins of *Ggt* mycelia.

9.3.4. Effect of Mn on the control by 2-79 of take-all of wheat grown in a Mn-deficient sand. Bacteria added to pots in a suspension of $DDDH_2O$.

9.3.4.1. *Ggt*. The addition of live 2-79 had no effect on *Ggt* infection except at a low rate of inoculation in 24 Mn pots (tables 9.3, 9.4 and 9.5). The total length of stelar lesions per plant was decreased by nearly three-quarters with low 2-79 in 24 Mn pots (table 9.3). Total length of lesions at 24 Mn and a high rate of 2-79 inoculation was larger than without live 2-79 but not statistically different. A similar pattern was present in the average number of lesions per plant (table 9.4) and percentage of diseased seminal roots (table 9.5) although in the former case the interaction between Mn and 2-79 inoculation was not significant.

The mixing of a high rate of Mn (24 Mn) through soil caused large decreases in *Ggt* infection (tables 9.3, 9.4 and 9.5). The total length of lesions was decreased by 84 % with 24 Mn (averaged across all 2-79 treatments), average number of lesions by 60 % and the percentage of diseased seminal roots by more than half. The low rate of Mn (8 Mn) addition had no effect on *Ggt* infection except for a 39 % decrease in the total length of lesions in pots with high 2-79.

Table 9.3. **Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the total length of black stelar lesions on wheat seminal roots (mm per plant). Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 8 plants. Statistical analysis was conducted without nil Ggt controls.**

Mn added (mg/pot)	Concentration of PDA in agar discs: sterile-20 %			4 %			20 %		
	Rate of inoculation with 2-79: nil	low	high	nil	low	high	nil	low	high
0	0	0	0	15.7[a]	16.3	17.4	17.7	17.9	24.1
				3.5[b]	*3.5*	*3.6*	*3.6*	*3.6*	*3.9*
8	0	0	0	11.1	13.4	12.0	14.5	13.3	13.2
				3.1	*3.3*	*3.2*	*3.4*	*3.3*	*3.3*
24	0	0	0	4.4	0.7	3.0	4.4	2.0	5.2
				2.3	*0.9*	*1.9*	*2.3*	*1.6*	*2.4*

(3-way interaction not significant)

1 and 2-way treatment means, calculated with nil Ggt control removed (with appropriate LSD's[c,d] at P=0.05 level).

Mn	0	8	24	LSD[a]
	18.0 *3.6*	12.8 *3.3*	2.9 *1.9*	*0.2*
Ggt	sterile-20 %	4 %	20 %	
	0	7.9 *2.8*	10.1 *3.1*	*0.2*
	nil	low	high	
2-79	9.8 *3.0*	7.0 *2.7*	10.2 *3.1*	*0.2*
Mn	0	8	24	
Ggt				
sterile-20 %	0	0	0	
4 %	16.4	12.1	2.2	
	3.5	*3.2*	*1.7*	*ns*
20 %	19.7	13.6	3.6	
	3.7	*3.3*	*2.1*	
Mn	0	8	24	
2-79				
nil	16.6	12.7	4.4	
	3.5	*3.3*	*2.3*	*0.4*
low	17.1	13.3	1.2	
	3.6	*3.3*	*1.2*	
high	20.5	12.6	4.0	
	3.7	*3.3*	*2.2*	
Ggt	sterile-20 %	4 %	20 %	
2-79				
nil	0	9.3	10.5	
		3.0	*3.1*	*ns*
low	0	6.0	8.1	
		2.6	*2.8*	
high	0	8.7	12.0	
		2.9	*3.2*	

[a] Geometric means calculated from natural log transformed data.

[b] Values in italics are $\log_e$ transformations (on per pot basis), calculated to adjust for a skewed distribution.

[c] Values separated by more than the appropriate LSD were statistically different.

[d] LSD's apply to transformed data only.

Table 9.4. Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the number of black stelar lesions on wheat roots (lesions per plant). Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 8 plants. Statistical analysis was conducted without nil Ggt controls.

Mn added (mg/pot)	Concentration of PDA in agar discs: sterile-20 %			4 %			20 %		
	Rate of inoculation with 2-79: nil	low	high	nil	low	high	nil	low	high
0	0	0	0	5.6	4.9	6.6	7.1	8.3	8.0
8	0	0	0	6.0	6.9	6.4	7.5	6.8	7.9
24	0	0	0	3.4	1.3	2.9	3.1	2.3	3.3

(3-way interaction not significant)

1 and 2-way treatment means, calculated with nil Ggt control removed (with appropriate LSD's at P=0.05 level).

	0	8	24	LSD[a]
Mn	6.8	6.9	2.7	0.7
	sterile-20 %	4 %	20 %	
Ggt	0	4.9	6.0	0.6
	nil	low	high	
2-79	5.5	5.0	5.8	ns
Mn	0	8	24	
Ggt				
sterile-20 %	0	0	0	ns
4 %	5.7	6.4	2.5	
20 %	7.8	7.4	2.9	
Mn	0	8	24	
2-79				
nil	6.4	6.8	3.3	ns
low	6.6	6.8	1.8	
high	7.3	7.1	3.0	
Ggt	sterile-20 %	4 %	20 %	
2-79				
nil	0	5.0	5.9	ns
low	0	4.3	5.8	
high	0	5.3	6.4	

[a] Values separated by more than the appropriate LSD were statistically different.

Table 9.5. Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the percentage of infected seminal wheat roots. Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 8 plants. Statistical analysis was conducted without nil Ggt controls.

Mn added (mg/pot)	Concentration of PDA in agar discs: sterile-20 %			4 %			20 %		
	Rate of inoculation with 2-79: nil	low	high	nil	low	high	nil	low	high
0	0	0	0	79	77	86	96	100	91
8	0	0	0	77	93	83	90	87	92
24	0	0	0	50	17	42	58	41	46

(3-way interaction not significant)

1 and 2-way treatment means, calculated with nil Ggt control removed (with appropriate LSD's at P=0.05 level).

	0	8	24	LSD[a]
Mn	88	87	42	7
	sterile-20 %	4 %	20 %	
Ggt	0	67	78	6
	nil	low	high	
2-79	75	69	73	ns
Mn	0	8	24	
Ggt				
sterile-20 %	0	0	0	ns
4 %	80	84	36	
20 %	96	90	48	
Mn	0	8	24	
2-79				
nil	87	83	54	13
low	88	90	29	
high	89	88	44	
Ggt	sterile-20 %	4 %	20 %	
2-79				
nil	0	68	81	ns
low	0	62	76	
high	0	70	76	

[a] Values separated by more than the appropriate LSD were statistically different.

Increasing the concentration of PDA in plugs from 4 to 20 % caused a small increase in symptoms of *Ggt* infection (tables 9.3, 9.4 and 9.5). No disease was present in pots inoculated with sterile agar plugs.

9.3.4.2. *Plant growth.* Inoculation of pots with live 2-79 had no effect on plant growth (tables 9.6 and 9.7).

Plants grown without added Mn were very Mn deficient and large increases in plant growth occurred with the addition of Mn (tables 9.6 and 9.7). The high rate of Mn addition generally did not further increase plant growth. Increases in plant growth with added Mn were greatest in disease-free plants; the average dry weight of roots per plant nearly doubled with 8 Mn but the increase in high Ggt pots was only 51 % (table 9.7). This same pattern was also present in the dry weight of shoots per plant except the difference between response to Mn in diseased and disease-free plants was not as large (table 9.6).

The low rate of *Ggt* inoculum (4 % PDA) decreased the dry weight of shoots by approximately one-third, regardless of Mn treatment (table 9.6). The high rate of *Ggt* inoculum did not further decrease shoot dry weights except in 24 Mn pots (a further 21 % decrease). A similar effect of *Ggt* inoculum was measured in the dry weight of roots except there was no decrease with *Ggt* inoculum in nil Mn pots (table 9.7).

9.3.4.3. *Mn.* Inoculation of pots with live 2-79 had no effect on the concentration of Mn in plants (tables 9.8 and 9.9).

Whole shoot Mn concentrations in plants grown without Mn were well below the critical level of 18 mg kg^{-1} (see Appendix B) required for normal growth in these pot experiments (table 9.8). Shoot concentrations of Mn were increased to adequate levels by 8 Mn and further increased to very high levels by 24 Mn (table 9.8).

Table 9.6. Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the average dry weight of shoots of wheat seedlings (mg per plant). Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 8 plants.

Mn added (mg/pot)	Concentration of PDA in agar discs: sterile-20 %			4 %			20 %		
	Rate of inoculation with 2-79: nil	low	high	nil	low	high	nil	low	high
0	34	33	34	25	20	24	22	22	21
8	57	49	51	33	32	33	27	31	34
24	58	49	45	35	33	44	32	23	34

(3-way interaction not significant)

1 and 2-way treatment means, calculated with nil Ggt control removed (with appropriate LSD's at P=0.05 level).

	0	8	24	LSD[a]
Mn	26	39	39	3
	sterile-20 %	4 %	20 %	
Ggt	46	31	27	3
	nil	low	high	
2-79	36	32	36	ns
Mn / Ggt	0	8	24	
sterile-20 %	34	52	51	4
4 %	23	33	37	
20 %	22	31	30	
Mn / 2-79	0	8	24	
nil	27	39	41	ns
low	25	37	35	
high	26	39	41	
Ggt / 2-79	sterile-20 %	4 %	20 %	
nil	50	31	27	ns
low	44	28	25	
high	43	34	29	

[a] Values separated by more than the appropriate LSD were statistically different.

Table 9.7. Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the average dry weight of roots of wheat seedlings (mg per plant). Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 8 plants.

Mn added (mg/pot)	Concentration of PDA in agar discs: sterile-20 %			4 %			20 %		
	Rate of inoculation with 2-79: nil	low	high	nil	low	high	nil	low	high
0	18	19	22	20	15	18	14	17	17
8	48	43	39	28	29	26	21	24	28
24	45	38	30	29	28	33	25	18	25

(3-way interaction not significant)

1 and 2-way treatment means, calculated with nil Ggt control removed (with appropriate LSD's at P=0.05 level).

Mn	0	8	24	LSD[a]
	18	32	30	3
Ggt	sterile-20 %	4 %	20 %	
	46	31	27	3
2-79	nil	low	high	
	36	32	36	ns
Mn	0	8	24	
Ggt				
sterile-20 %	19	43	38	5
4 %	18	28	30	
20 %	16	24	23	
Mn	0	8	24	
2-79				
nil	17	32	33	ns
low	17	32	28	
high	19	31	29	
Ggt	sterile-20 %	4 %	20 %	
2-79				
nil	37	26	20	ns
low	33	24	20	
high	30	26	23	

[a] Values separated by more than the appropriate LSD were statistically different.

Table 9.8. **Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the concentration of Mn in whole shoots of wheat seedlings (mg kg^{-1} D.W.). Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 8 plants.**

Mn added (mg/pot)	Concentration of PDA in agar discs: sterile-20 %			4 %			20 %		
	Rate of inoculation with 2-79: nil	low	high	nil	low	high	nil	low	high
0	8[a]	8	7	8	8	8	9	9	8
	2.16[b]	*2.21*	*2.11*	*2.20*	*2.23*	*2.20*	*2.28*	*2.30*	*2.22*
8	64	63	68	67	61	57	52	53	49
	4.17	*4.16*	*4.24*	*4.21*	*4.13*	*4.06*	*3.96*	*3.99*	*3.92*
24	227	256	257	252	257	236	228	226	221
	5.43	*5.55*	*5.55*	*5.53*	*5.55*	*5.47*	*5.43*	*5.43*	*5.40*

(3-way interaction not significant)

1 and 2-way treatment means, calculated with nil Ggt control removed (with appropriate LSD's[c,d] at P=0.05 level).

				LSD[a]
Mn	0	8	24	
	8 *2.21*	59 *4.09*	240 *5.48*	*0.05*
Ggt	sterile-20 %	4 %	20 %	
	51 *3.95*	51 *3.95*	49 *3.88*	*0.05*
2-79	nil	low	high	
	50 *3.93*	51 *3.95*	49 *3.91*	*ns*
Mn	0	8	24	
Ggt sterile-20 %	8	65	247	
	2.16	*4.19*	*5.51*	*0.09*
4 %	8	61	248	
	2.21	*4.13*	*5.52*	
20 %	9	51	225	
	2.27	*3.96*	*5.42*	
Mn	0	8	24	
2-79 nil	8	60	236	
	2.21	*4.12*	*5.47*	ns
low	8	59	246	
	2.25	*4.09*	*5.51*	
high	8	58	238	
	2.18	4.07	5.48	
Ggt	sterile-20 %	4 %	20 %	
2-79 nil	50	53	48	
	3.92	*3.98*	*3.89*	ns
low	52	52	49	
	3.97	*3.97*	*3.91*	
high	52	49	48	
	3.97	*3.91*	*3.85*	

[a] Geometric means calculated from natural log transformed data.

[b] Values in italics are natural log transformations (on per pot basis), calculated to adjust for a skewed distribution.

[c] Values separated by more than the appropriate LSD were statistically different.

[d] LSD's apply to transformed data only.

Table 9.9. Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the concentration of Mn in roots of wheat seedlings (mg kg^{-1} D.W.). Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 8 plants.

Mn added (mg/pot)	Concentration of PDA in agar discs: sterile-20 %			4 %			20 %		
	Rate of inoculation with 2-79: nil	low	high	nil	low	high	nil	low	high
0	35[a]	31	32	36	36	33	27	31	30
	3.58[b]	*3.46*	*3.49*	*3.61*	*3.61*	*3.53*	*3.34*	*3.45*	*3.71*
8	153	175	173	255	268	289	377	296	301
	5.04	*5.17*	*5.16*	*5.55*	*5.60*	*5.67*	*5.94*	*5.69*	*5.71*
24	1194	1237	1260	2423	2360	2093	2245	4212	2866
	7.09	*7.12*	*7.14*	*7.79*	*7.77*	*7.65*	*7.72*	*8.35*	*7.96*

(3-way interaction not significant)

1 and 2-way treatment means, calculated with nil Ggt control removed (with appropriate LSD's[c,d] at P=0.05 level).

				LSD[a]
	0	8	24	
Mn	32 *3.50*	244 *5.50*	2038 *7.62*	*0.10*
	sterile-20 %	4 %	20 %	
Ggt	189 *5.25*	281 *5.64*	308 *5.73*	*0.10*
	nil	low	high	
2-79	247 *5.52*	264 *5.58*	250 *5.53*	*ns*
Mn	0	8	24	
Ggt sterile-20 %	32	167	1231	
	3.51	*5.12*	*7.12*	*0.18*
4 %	35	271	2288	
	3.58	*5.60*	*7.74*	
20 %	29	322	3004	
	3.41	*5.78*	*8.01*	
Mn	0	8	24	
2-79 nil	32	245	1866	
	5.23	*5.65*	*5.66*	ns
low	32	240	2309	
	3.51	*5.49*	*7.75*	
high	33	247	1964	
	3.48	*5.51*	*7.58*	
Ggt	sterile-20 %	4 %	20 %	
2-79 nil	187	283	287	
	5.23	*5.65*	*5.66*	ns
low	190	286	339	
	5.25	*5.66*	*5.83*	
high	192	274	298	
	5.26	*5.62*	*5.70*	

[a] Geometric means calculated from natural log transformed data.
[b] Values in italics are natural log transformations (on per pot basis), calculated to adjust for a skewed distribution.
[c] Values separated by more than the appropriate LSD were statistically different.
[d] LSD's apply to transformed data only.

The effect of a high rate of *Ggt* inoculum on shoot Mn concentrations varied according to the Mn treatment (table 9.8). In nil Mn pots shoot Mn concentration was increased slightly by high *Ggt* inoculum but at 8 and 24 Mn shoot concentrations decreased slightly. Low Ggt did not affect shoot Mn concentrations.

The concentration of Mn in roots of disease-free plants increased five-fold with 8 Mn from an average of 33 mg kg^{-1} in nil Mn and by a further 7 fold with 24 Mn (table 9.9). *Ggt* infection had no effect on root Mn concentrations in nil Mn pots but in pots where Mn had been added root Mn concentrations were progressively increased by low and high *Ggt* inoculum rates. In 24 Mn pots with a high rate of *Ggt* inoculum root Mn concentration was nearly three times the level in disease-free plants.

9.3.5. Effect of Mn on the control by 2-79 of take-all of wheat grown in a Mn-deficient sand. Bacteria added to pots in liquid media.

9.3.5.1. *Ggt.* The addition of live 2-79 at sowing completely prevented the production of take-all symptoms on wheat roots, regardless of Mn or Ggt treatments, although extensive browning on these roots made it quite difficult to identify take-all lesions (table 9.10).

The percentage of diseased seminal roots was decreased by 27 % with 4 Mn in -2-79 pots but 0.4 Mn had no effect on *Ggt* infection (table 9.10).

The number and total length of lesions per plant were also decreased by 4 Mn but the effects were not statistically significant.

Increasing the concentration of PDA from 1 to 4 % in -2-79 pots did not cause a measurable increase in take-all symptoms on wheat roots and both concentrations of *Ggt* colonized agar discs caused a high level of *Ggt* infection (table 9.10). No disease was present in pots with sterile PDA discs.

Table 9.10. Effect of Mn nutrition and inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) on *Ggt* 500 infection of wheat roots. Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 10 plants. Disease assessments were analyzed without nil Ggt and +2-79 treatments.

Mn added (mg/pot)	Concentration of PDA in agar discs: sterile-4 %	1 %	4 %	Mean[a]
Total length of black stelar lesions per plant (mm)				
-2-79				
0	0	45.6	50.4	48.0
0.4	0	50.5	40.0	45.3
4	0	42.7	33.7	38.2
Mean	0	46.3	41.4	
+2-79				
0	0	0	0	0
0.4	0	0	0	0
4	0	0	0	0
Mean	0	0	0	
LSD[b] (P=0.05) Mn, Ggt, Mn x Ggt ns				
Number of black stelar lesions per plant				
-2-79				
0	0	5.3	6.3	5.8
0.4	0	6.6	4.8	5.7
4	0	5.2	3.8	4.5
Mean	0	5.7	5.0	
+2-79				
0	0	0	0	0
0.4	0	0	0	0
4	0	0	0	0
Mean	0	0	0	
LSD[b] (P=0.05) Mn, Ggt, Mn x Ggt ns				
Percentage of infected seminal roots.				
-2-79				
0	0	78	95	86
0.4	0	91	77	84
4	0	62	62	62
Mean	0	77	78	
+2-79				
0	0	0	0	0
0.4	0	0	0	0
4	0	0	0	0
Mean	0	0	0	
LSD[b] (P=0.05) Mn=12 ; Ggt, Mn x Ggt ns				

[a] Means calculated without nil Ggt treatments.

[b] Values separated by more than the appropriate LSD were statistically different.

9.3.5.2. *Plant growth.* The growth of plants was slightly decreased by inoculation with live 2-79. The average dry weight of shoots per plant was decreased by 22 % with live 2-79 (table 9.11) but the decrease in dry weight of roots was only 5 % (table 9.12). The effect of 2-79 on plant growth was the same across all Mn and Ggt treatments.

Plant growth was successively decreased by increasing rates of *Ggt* inoculum, regardless of 2-79 or Mn treatments, despite no lesions being recorded on 2-79 inoculated roots (tables 9.11 and 9.12). The dry weight of shoots per plant was decreased by 12 % with low Ggt and by a further 18 % with high Ggt (table 9.11). Root growth was more sensitive and was decreased by 25 and a further 13 % with low and high Ggt, respectively.

Addition of Mn to soil increased the growth of plants. The average dry weight of shoots per plant increased by 28 % with 0.4 Mn and by a further 31 % with 4 Mn (table 9.11).

The dry weight of roots increased with 0.4 and 4 Mn by 52 % and a further 69 %, respectively (table 9.12). Ggt and 2-79 treatments did not significantly affect the response in plant growth to added Mn, although responses in +2-79 pots tended to be smaller.

9.3.5.3. *Mn.* The concentration of Mn in whole shoots was increased by inoculation with live 2-79 at all Mn treatments (table 9.13). Shoot Mn concentrations without added Mn were well below the critical level of 18 mg kg^{-1} (see appendix B) in -2-79 pots and levels were still deficient with 0.4 Mn.

Shoot concentrations of Mn were increased to adequate levels with 4 Mn. In +2-79 pots shoot Mn concentrations were marginally deficient at nil Mn but were increased to adequate and luxury levels at 0.4 and 4 Mn, respectively. Increasing rates of *Ggt* inoculum had only small and inconsistent effects on shoot Mn concentrations.

Table 9.11. Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the average dry weight of shoots of wheat seedlings (mg per plant). Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 10 plants.

Mn added (mg/pot)	Concentration of PDA in agar discs					
	sterile-4 %		1 %		4 %	
	-2-79	+2-79	-2-79	+2-79	-2-79	+2-79
0	37.0	28.2	29.5	24.3	23.3	23.2
0.4	51.3	35.6	42.3	30.8	25.3	25.9
4	56.9	42.3	52.1	41.6	49.2	33.6

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's at P=0.05 level).

Mn	0	0.4	4	LSD[a]
	27.6	35.2	46.0	3.9
Ggt	sterile-4 %	1 %	4 %	
	41.9	36.8	30.1	3.9
2-79	-	+		
	40.8	31.7		3.2
Mn	0	0.4	4	
Ggt				
sterile-4 %	32.6	43.5	49.6	ns
1 %	26.9	36.6	46.9	
4 %	23.3	26.6	41.4	
Mn	0	0.4	4	
2-79				
-	30.0	39.7	52.8	ns
+	25.3	30.8	39.2	
Ggt	sterile-4 %	1 %	4 %	
2-79				
-	48.4	41.3	32.6	ns
+	35.4	32.3	27.6	

[a] Values separated by more than the appropriate LSD were statistically different.

Table 9.12. Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the average dry weight of roots of wheat seedlings (mg per plant). Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 10 plants.

Mn added (mg/pot)	Concentration of PDA in agar discs					
	sterile-4 %		1 %		4 %	
	-2-79	+2-79	-2-79	+2-79	-2-79	+2-79
0	14.1	15.3	9.2	11.4	6.2	12.5
0.4	29.0	20.0	14.7	15.1	10.1	15.2
4	37.9	29.6	29.4	28.8	27.9	22.9

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD's at P=0.05 level).

Mn	0	0.4	4	LSD[a]
	11.5	17.4	29.3	1.6
Ggt	sterile-4 %	1 %	4 %	
	24.2	18.1	15.8	1.6
2-79	-	+		
	19.9	18.9		ns
Mn	0	0.4	4	
Ggt				
sterile-4 %	14.7	24.5	14.9	ns
1 %	10.3	14.9	29.1	
4 %	9.4	14.7	25.4	
Mn	0	0.4	4	
2-79				
-	9.9	18.0	31.8	ns
+	13.1	16.8	26.8	
Ggt	sterile-4 %	1 %	4 %	
2-79				
-	27.0	17.8	14.8	ns
+	21.3	18.5	16.9	

[a] Values separated by more than the appropriate LSD were statistically different.

Table 9.13. Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the concentration of Mn in whole shoots of wheat seedlings (mg kg^{-1} D.W.). Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 10 plants.

Mn added (mg/pot)	Concentration of PDA in agar discs					
	sterile-4 %		1 %		4 %	
	-2-79	+2-79	-2-79	+2-79	-2-79	+2-79
0	7.0	16.4	6.7	14.9	6.1	18.6
0.4	11.8	29.4	10.3	22.5	10.1	27.2
4	41.2	56.6	30.8	77.1	32.1	54.0

LSD[a] (P=0.05) Mn x Ggt x 2-79=6.7

1 and 2-way treatment means (with appropriate LSD's at P=0.05 level).

Mn	0	0.4	4	LSD[a]
	11.6	18.5	48.6	2.8
Ggt	sterile-4 %	1 %	4 %	
	27.1	27.0	24.7	ns
2-79	-	+		
	17.3	35.2		2.3
Mn	0	0.4	4	
Ggt				
sterile-4 %	11.7	20.6	48.9	4.8
1 %	10.8	16.4	53.9	
4 %	12.4	18.7	43.1	
Mn	0	0.4	4	
2-79				
-	6.6	10.7	34.7	3.9
+	16.7	26.4	62.6	
Ggt				
sterile-4 %	1 %	4 %		
2-79				
-	20.0	15.9	16.1	ns
+	34.2	38.2	33.3	

[a] Values separated by more than the appropriate LSD were statistically different.

Similar treatment effects were found in root Mn concentrations except live 2-79 did not increase concentrations at nil Mn and *Ggt* infection increased root Mn concentrations at 4 Mn in +2-79 pots (table 9.14).

9.4. Discussion.

Huber and Dorich (1988) proposed that fluorescent pseudomonads may exert biological control over take-all by increasing the availability of Mn in the rhizosphere of wheat plants through the reduction of insoluble Mn oxides. The pot experiments reported here showed that 2-79 will increase the availability of Mn to plants and some of the results are consistent with 2-79 antagonism acting through increased Mn availability.

In the second pot experiment, adding live 2-79 had a dramatic effect on the Mn nutrition of the wheat host. The addition of live 2-79 to pots, in the absence of Mn additions to the soil, increased shoot Mn concentrations of wheat plants from deficiency to sufficiency, confirming that fluorescent pseudomonads can increase the availability of Mn to plants.

The addition of autoclaved bacterial media had no apparent effect on Mn nutrition of the host, at least when compared to the experiment where bacteria were added as a water suspension. Ghiorse (1988) concluded at the end of an extensive and recent review on the biology of Mn transforming microorganisms that the relative importance of microbial activity versus abiotic reactions in Mn transformations in soil still is not certain. However, the results of this pot experiment suggest that microorganisms have the potential to cause major shifts in Mn availability in soil, to the extent that plant nutrition is dramatically altered.

No black stelar lesions on wheat roots were seen in any pot (of the second experiment) which had received live 2-79, regardless of Mn treatment, so the effect of added Mn on the level of antagonism by 2-79 against *Ggt* was not tested. Although no take-all symptoms could be distinguished on wheat roots with live 2-79, the growth of these plants was decreased compared to the nil Ggt controls which indicated that *Ggt* infection had

Table 9.14. Effect of Mn nutrition, inoculation with *Pseudomonas fluorescens* 2-79 RN_{10} (Weller and Cook 1983) and *Ggt* 500 infection on the concentration of Mn in roots of wheat seedlings (mg kg^{-1} D.W.). Plants were grown in Mn-deficient sand in pots for 25 days. Values in the body of the main table are the average of 10 plants.

Mn added (mg/pot)	Concentration of PDA in agar discs					
	sterile-4 %		1 %		4 %	
	-2-79	+2-79	-2-79	+2-79	-2-79	+2-79
0	33	33	35	44	49	38
0.4	29	53	47	61	39	71
4	84	184	97	228	67	247
LSD[a] (P=0.05)	Mn *Ggt *2-79=26					

1 and 2-way treatment means (with appropriate LSD's at P=0.05 level).

Mn	0	0.4	4	LSD[a]
	39	50	151	11
Ggt	sterile-4 %	1 %	4 %	
	69	85	85	11
2-79	-	+		
	53	106		9
Mn Ggt	0	0.4	4	
sterile-4 %	33	41	134	ns
1 %	39	54	162	
4 %	44	55	157	
Mn 2-79	0	0.4	4	
-	39	38	83	15
+	38	61	220	
Ggt 2-79	sterile-4 %	1 %	4 %	
-	49	60	52	ns
+	90	111	119	

[a] Values separated by more than the appropriate LSD were statistically different.

occurred and was restricting normal host development. The extensive browning of roots which accompanied the addition of live 2-79 may have prevented the detection of black stelar lesions on roots or the presence of live 2-79 may have inhibited the development of characteristic lesions without affecting the impact of *Ggt* infection on host growth.

In the absence of identifiable stelar lesions, host growth was the only other estimate of severity of *Ggt* infection, even though it is not always the most reliable method of measuring the extent of *Ggt* infection (Clarkson and Polley 1981 and chapter 3 of this thesis). If fluorescent pseudomonads decrease take-all through increased Mn availability (Huber and Dorich 1988) then adding live 2-79 to pots inoculated with *Ggt* and which also received high rates of Mn to the soil should not have further increased host growth because the bacteria would not exert an effect on *Ggt* infection over and above that of added Mn. However, if 2-79 decrease *Ggt* infection by some other means, adding live 2-79 to pots inoculated with *Ggt* and which also received high rates of Mn to the soil would have further increased host growth because the disease would have been suppressed by both the bacteria and the addition of Mn. An assessment of host growth in this way is complicated by live 2-79 suspensions being phytotoxic, especially to shoot growth. Even though there was no statistical interaction between Mn and 2-79, trends in *Ggt*-inoculated pots were consistent with 2-79 acting against take-all by increasing Mn availability. Root growth was increased by 2-79 in *Ggt*-inoculated pots with either no or a low rate of Mn addition (improved Mn nutrition and consequently, decreased take-all) but, at the high rate of Mn addition, 2-79 decreased root growth which suggests that in these pots there was no antagonism by 2-79 against *Ggt*. Similar trends were also present in shoot growth, although in this case, apparent decreases in take-all at nil and low Mn only partially compensated for the phytotoxicity of the live 2-79 suspensions.

In the first pot experiment there were no reliable and measurable effects of live 2-79 on either Mn nutrition or growth of the wheat host, or on the extent of *Ggt* infection on

wheat roots. However, the technique of suspending 2-79 in $DDDH_2O$ prior to addition to the soil may have decreased the viability of the bacteria due to hypertonic osmotic stress. This possibility was avoided in the second pot experiment by adding live 2-79 to the soil in culture media but introduced the complication of adding nutrients and bacterial metabolites from the media to pots.

In subsequent attempts to repeat this experiment (not reported), live 2-79 were suspended in weak salt solutions (e.g. 1.5 g $MgSO_4.7H_2O$ and 0.283 g NaCl in 1000 ml of $DDDH_2O$) to prevent osmotic shock but avoid the addition of nutrients and metabolites in the culture media. No browning of wheat roots was observed in these experiments but, again, live 2-79 had no measurable effect on *Ggt* infection or on Mn nutrition or growth of the wheat host.

`In vitro' experiments with *Ggt* 500 in the presence of 2-79 and added Mn showed that antagonism by 2-79 against *Ggt* was enhanced when Mn was added to the system at levels toxic to the *Ggt*, despite these Mn rates being very toxic to the growth of 2-79. As previously mentioned, fluorescent pseudomonads produce a suite of antibiotic compounds (Ahl *et al.* 1986, Brisbane *et al.* 1987), including siderophores (Kloepper *et al.* 1980) and `in vitro' antagonism has been shown repeatedly. However, the report of Brisbane *et al.* 1987 is one of the few which has investigated conditions which are important to take-all incidence in the field (in this case, pH) in petri-dishes with antagonistic pseudomonads. They identified an antibiotic compound (phenazine-1-carboxylic acid) produced by 2-79 which was antagonistic against *Ggt* but was only active at pH values less than 7, which suggests that the hypothesis of Huber and Dorich 1988 may be an over-simplification of the mode of action of 2-79 against *Ggt*, although the experiments reported in this chapter show that Mn is obviously very important in the level of antagonism expressed. However, results established in petri-dishes on antagonism against *Ggt* have often shown poor correlation with pot and field trials assessing take-all on the wheat host (Weller and Cook 1983).

The `in vitro' experiments reported in this chapter did not shed much light on the specific role that Mn was playing in enhancing antagonism by 2-79. The simplest explanation was that 2-79 produced compounds that inhibited the ability of *Ggt* to oxidize $Mn^{2+}$ to insoluble Mn oxides, thereby `preserving' the toxic levels of added Mn. The results presented in section 9.3.1. of this chapter show that 2-79 can reduce insoluble Mn oxides `in vitro' at a distance from their colonies (which must be due to the production of diffusible compounds). However, if this were the case, antagonism should not have been present in PDA without added Mn because the concentration of Mn in PDA is too low to be toxic to the fungus. The alternative is that Mn was performing a more complex role in the biochemical activities of 2-79, e.g. acting as an elicitator, constituent, activator, or co-factor of an antibiotic compound (or its pre-cursors).

No green/yellow areas were produced by 2-79 colonies on PDA so it was assumed that the antagonism by 2-79 was not due to the production of siderophores, although siderophore production by 2-79 was enhanced on Mn-rich KMB (see section 9.3.2.). However, if Mn was added to 2-79 colonies which had already produced siderophores, UV fluorescence was quenched which suggested that the activity of the siderophores would have been diminished, even though Mn^{2+} has a very poor affinity for the Fe^{3+} binding site (Philson and Llinas 1982, Elad and Baker 1985). Adding Fe^{3+} to such a culture did not completely quench fluorescence which suggests that Mn^{2+} was competing for binding sites on the siderophores by preventing the complete conversion of siderophores to ferri-siderophores.

In summary, 2-79 will increase the availability of Mn to wheat plants and, although not tested rigorously, results from pot experiments were consistent with the hypothesis that antagonism by 2-79 against *Ggt* is due to increased availability of Mn to the wheat host. However, experiments conducted `in vitro' suggested that 2-79 has the ability to antagonize

Ggt by an additional mechanism whose effectiveness is enhanced by high levels of Mn but there was no evidence that this mechanism operated in the `in vivo' system used here.

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CHAPTER 10.

MN OXIDATION BY *GGT* HYPHAE ON WHEAT ROOTS GROWING IN A MN-DEFICIENT CALCAREOUS SAND - OBSERVATIONS UNDER THE DISSECTING AND SCANNING ELECTRON MICROSCOPES

CHAPTER 10. MN OXIDATION BY *GGT* HYPHAE ON WHEAT ROOTS GROWING IN A MN-DEFICIENT CALCAREOUS SAND - OBSERVATIONS UNDER THE DISSECTING AND SCANNING ELECTRON MICROSCOPES.

10.1. Introduction.

Ggt-infected wheat roots grown in Wangary sand to which high rates of Mn had been added were coated in dark brown deposits (see chapter 3 of this thesis). These deposits stained blue in acidified benzidine which is characteristic for a reaction with Mn oxides. Following these observations, more detailed examinations of wheat roots from pot experiments with Ggt and Mn treatments were made. Plants were taken from pot experiments typical of those described in earlier chapters of this thesis. Whole wheat roots were viewed under the dissecting microscope and sections of *Ggt*-infected roots were examined under a scanning electron microscope and analyzed with energy dispersive x-ray analysis microprobe techniques (EDXA).

10.2. Materials and Methods.

10.2.1. Examination of whole wheat roots under the dissecting microscope.

Plants were taken from individual treatments of a pot experiment in which Mn and Ggt treatments were combined factorially. The treatments included for examination were 0 and 24 mg of added Mn per pot, both with and without PDA plugs of *Ggt* 500 inoculum. The *Ggt* inoculum produced a high level of infection.

Fresh wheat roots from plants which had been washed free from the soil, were examined under a dissecting microscope at 40 x magnification with incident light and a white background. Micrographs were taken with a 35 mm camera loaded with black and white film. After examination, representative roots from each treatment were flooded with 0.1 % benzidine in 1 % ethanol and the development of any blue areas noted.

10.2.2. Examination of wheat root sections under the scanning electron microscope with EDXA.

10.2.2.1. *Oven-dried roots.* One centimetre long sections of main axis seminal roots were removed from oven-dried root systems of wheat plants in the same treatments as 10.2.1. Root segments were removed at the level of the agar plug of *Ggt* inoculum. Segments were coated with carbon under vacuum to an approximate depth of 80 nm and examined in a `Cambridge Systems' scanning electron microscope. Full spectral analyses of sample composition at points within the field of view (0.001 mm^3) were conducted with EDXA microprobe techniques, although only the major (K-alpha) peaks (expressed as the ratio of peak height to background "noise") of selected elements are displayed in the accompanying figures. Micrographs of the image in view were produced from 2 different signals. Micrographs produced from secondary electrons reveal surface detail of the sample because secondary electrons penetrate to a maximum depth of only 10 nm. Micrographs produced from reflected or back-scattered electrons reveal sub-surface detail as they penetrate the sample to a maximum depth of 10 um. A small section of *Ggt*-inoculated agar plug from the 24 Mn treatment was also examined.

The principle of electron probe X-ray microanalysis is based on the incident electron beam from the electron microscope producing characteristic X-rays from each element in the sample. These X-rays can be detected and the strength of each X-ray signal is proportional to the concentration of element in the sample.

10.2.2.2. *Freeze-dried roots.* Hand-cut longitudinal sections of fresh main axis seminal roots were kept in DDDH$_2$O before being transferred to iso-pentane and snap-frozen. Sections were then freeze-dried at -70° C for 24 hours before returning to room temperature. Sections were removed from roots of plants from a treatment with 24 mg Mn added per pot and agar plugs of *Ggt* 500 inoculum which produced severe disease.

Freeze-dried samples were coated with carbon under vacuum to an approximate depth of 40nm and examined in a `Cambridge Systems' scanning electron microscope. Full spectral analyses of sample composition at points within the field of view (0.001 mm^3) and digitized distribution maps of Mn were conducted with EDXA microprobe techniques.

10.3. Results.

10.3.1. Examination of whole wheat roots under the dissecting microscope.

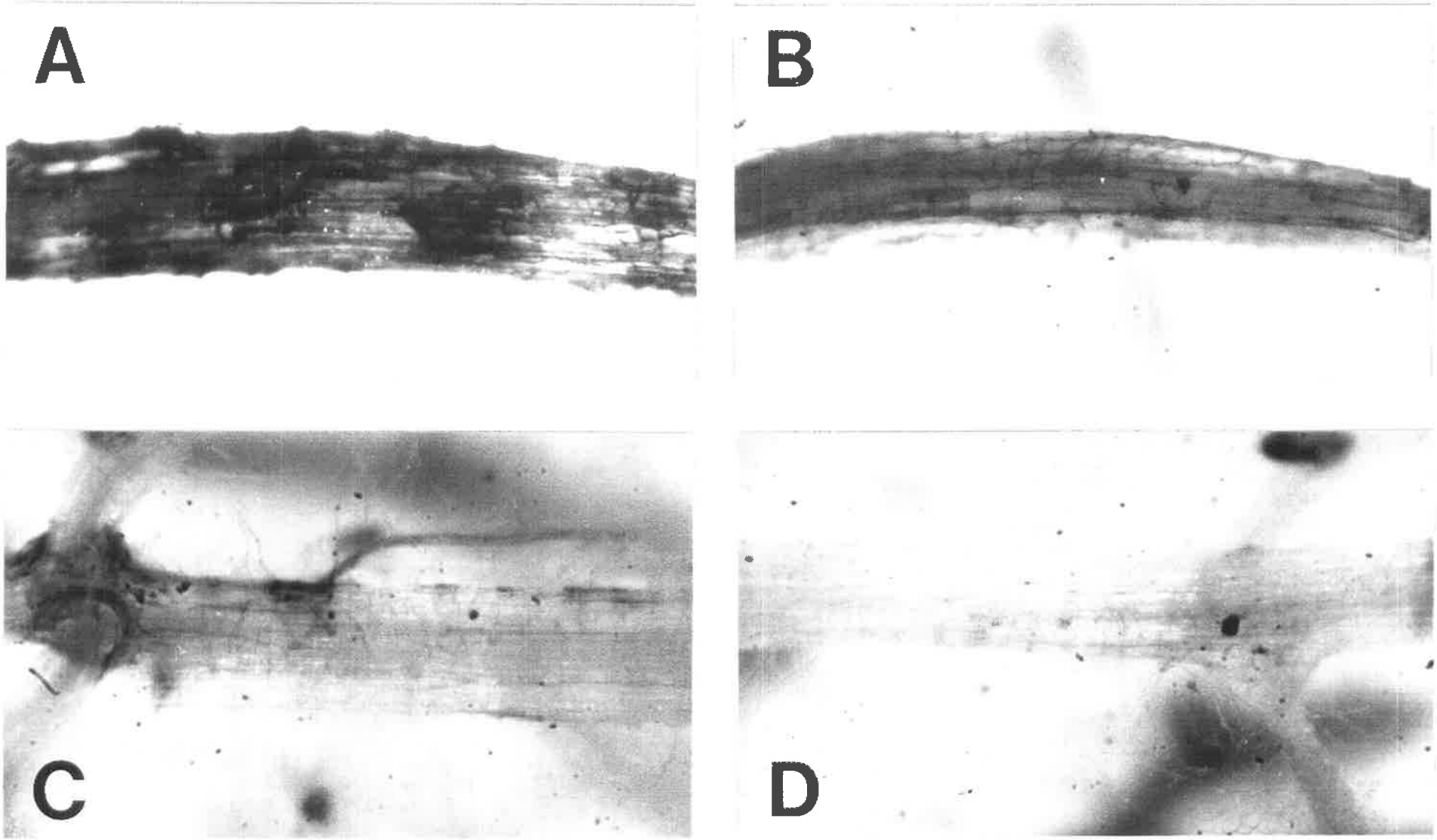
The 4 micrographs on plate 10.1 are representative for roots from each of the 4 treatments examined (A-24 Mn, +Ggt; B-nil Mn, +Ggt; C-24 Mn, -Ggt; D-nil Mn, -Ggt).

Ggt-infected roots from 24 Mn, in contrast with the 3 other treatments, appeared very dark with the naked eye. Examination under the dissecting microscope revealed that roots were coated in extensive black/brown deposits wherever the root was covered with runner hyphae (micrograph A) and well beyond the areas where black stelar lesions had developed. Runner hyphae also appeared to be covered in the same black/brown deposits but not all dark areas on the roots were closely associated with runner hyphae. Both the dark areas on the roots and the runner hyphae stained blue with benzidine, suggesting that the black/brown deposits contained Mn oxides. Areas of roots beyond the network of runner hyphae appeared white.

Ggt-infected roots from nil Mn were very white but were covered in a fine network of black runner hyphae. The runner hyphae can be easily seen on micrograph B. No areas stained blue with benzidine.

Disease-free roots from 24 Mn appeared white with the naked eye but examination under the dissecting microscope revealed small brown areas scattered across the surface of the root and where secondary roots had emerged (micrograph C). These areas stained blue

Plate 10.1. Micrographs of *Ggt*-infected or disease-free wheat roots grown in a Mn-deficient sand with and without Mn. A - 24 mg of added Mn per pot, *Ggt*-infected ; B - no added Mn, *Ggt*-infected ; C - 24 mg of added Mn per pot, disease-free ; D - no added Mn, disease-free.



with benzidine, indicating the presence of Mn oxides. Disease-free roots from nil Mn pots were very white and no areas stained blue with benzidine (micrograph D).

10.3.2. Examination of wheat root sections under the scanning electron microscope with EDXA.

10.3.2.1. *Oven-dried roots*. Although oven-dried roots were severely collapsed following placement in vacuum, the surface detail of cells and intercellular spaces were still visible and runner hyphae on the outside of roots could also be distinguished (plate 10.2).

Examination of *Ggt*-infected wheat roots from 24 Mn revealed very high concentrations of Mn in localized areas. These areas only occurred where the root surface was covered in a network of runner hyphae. The 2 micrographs on plate 10.3 show the surface of a root from within such an area. Micrograph B was produced by reflected electrons and clearly reveals small bright globular "nodules" below the surface of the root which were barely visible on the micrograph showing surface detail only (micrograph A). Since brightness of the image is related to the atomic weight of the atoms in the sample, the globular nodules must have been rich in an element with a high molecular weight, possibly Mn. Analysis of this area (arrow 1 on micrograph A) by X-ray microprobe confirmed a very high concentration of Mn and a summary of the spectrum is shown in figure 10.1A. The concentration of Mn in this area was much higher than in an area of the same root which had no obvious nodules or surface particles (see the spectrum in figure 10.1B, taken at arrow 2 in micrograph a of plate 10.3). The concentrations of Fe in Mn-rich areas were only slightly elevated from "background" levels, indicating that the nodules contained little Fe-oxide. The spectrum taken at arrow 2 (figure 10.1B) is typical for organic matter although calcium is abnormally high due to the Wangary sand in which the plants were grown (80-90 % $CaCO_3$). The high concentrations of potassium and chlorine were due to KCl crystals which were frequently found on the root surface. Analysis of some particles stuck to the surface of the root (taken at arrow 3 of micrograph a of plate 10.3) revealed very high concentrations of Mn in this area (figure 10.1C) as well as elevated levels of Ca and Fe. These particles were

Plate 10.2. Scanning electron micrograph of *Ggt*-infected wheat roots grown in a Mn-deficient sand with 24 mg of added Mn per pot. Image generated by secondary electrons. Note the long, looping runner hypha of *Ggt* in the centre of the frame which has pulled away from the root surface during sample preparation.

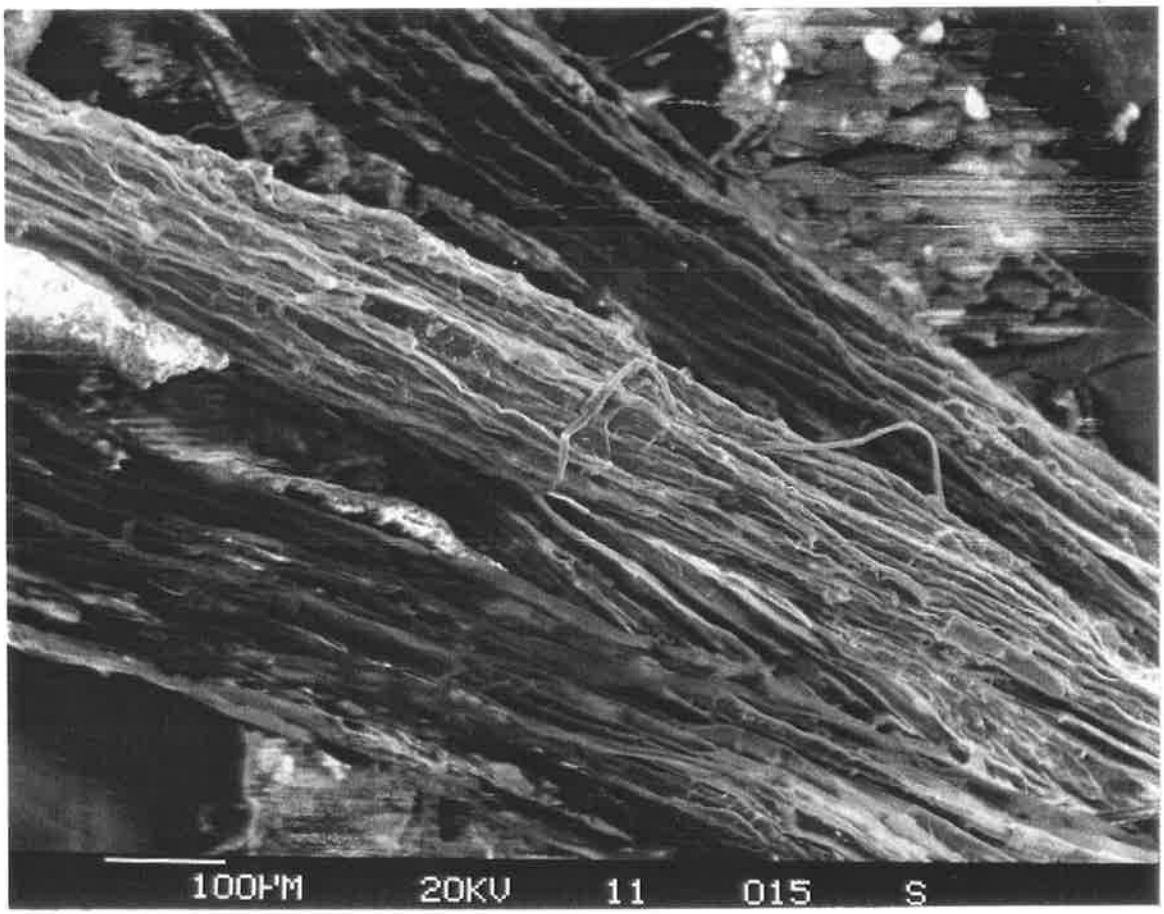


Plate 10.3. Close-up detail of the surface of a *Ggt*-infected wheat root grown in a Mn-deficient sand with 24 mg of Mn added per pot. A - Image generated by secondary electrons (surface detail only). B - Image generated by reflected electrons (reveals sub-surface detail) ; full spectral analysès of the sample at each of the three arrows in A are presented in figure 10.1. Note the bright sub-surface nodules highlighted in B.

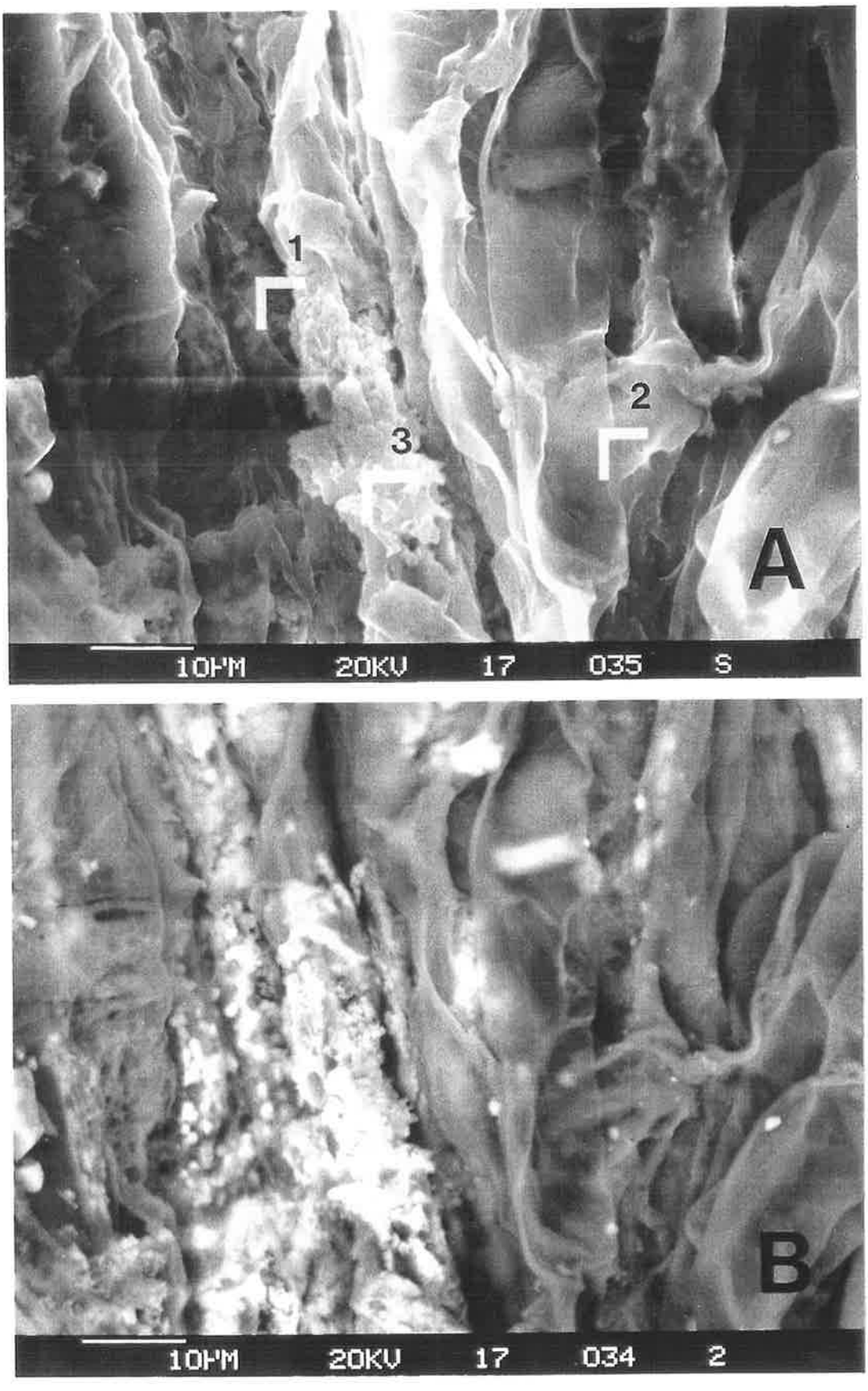


FIGURE 10.1. ENERGY DISPERSIVE X-RAY MICRO-ANALYSIS OF GGT-INFECTED WHEAT ROOTS FROM MN-ENRICHED WANGARY SAND (see plate 10.3. for micrographs of samples).

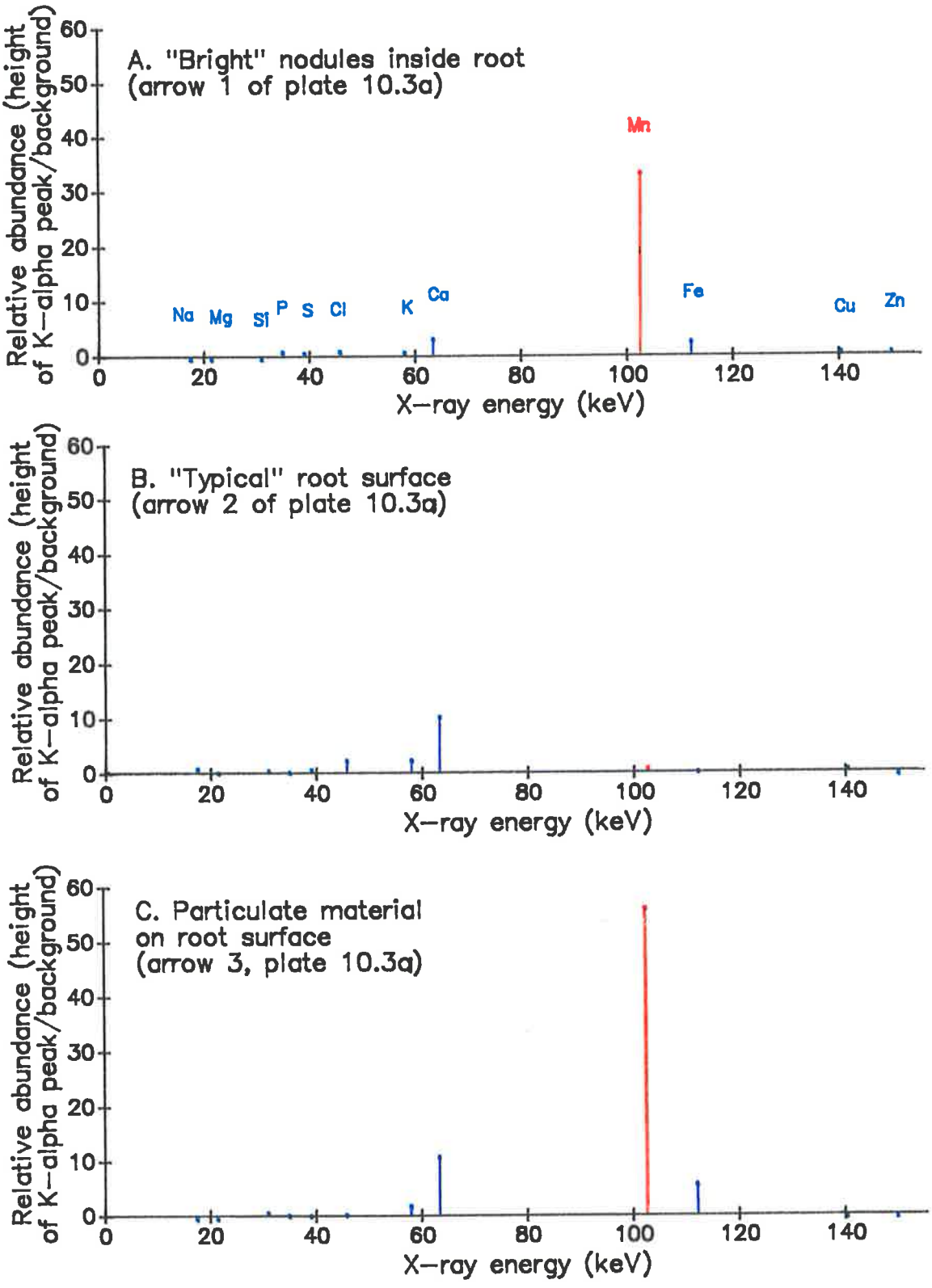
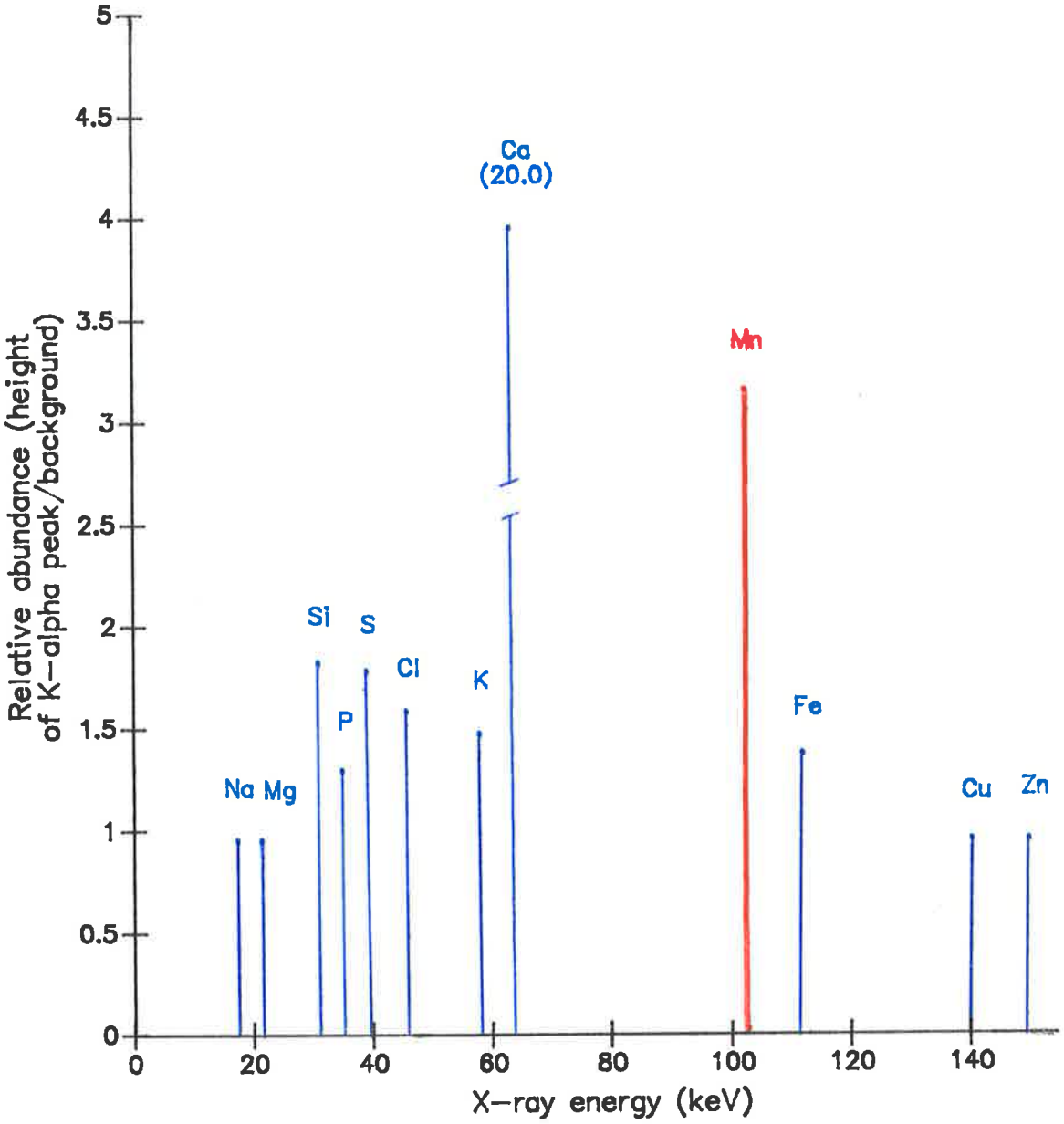


FIGURE 10.2. ENERGY DISPERSIVE X-RAY MICRO-ANALYSIS OF GGT-INOCULATED AGAR PLUG FROM MN-ENRICHED WANGARY SAND. (see plate 10.4. for micrograph of this sample).
Calcium levels were raised by soil contamination (soil was 80-90% calcium carbonate).



probably a mixture of Mn and Fe oxides which had formed around some $CaCO_3$ in the soil. Mn^{2+} will readily oxidize and/or precipitate as $MnCO_3$ on $CaCO_3$ surfaces (Leeper 1970, McBride 1979).

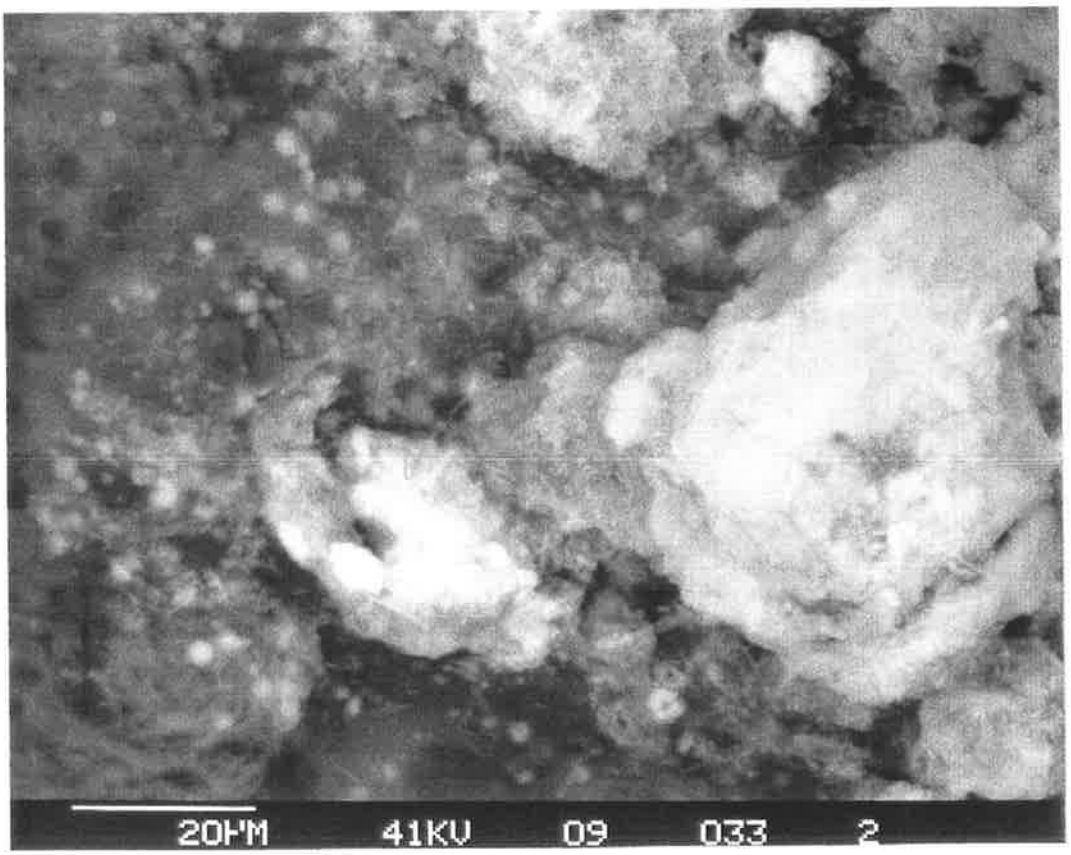
Localised areas of high concentrations of Mn were found in disease-free roots from 24 Mn but the areas were infrequent, concentrations of Mn were never more than 2-3 times higher than background levels and no discrete particles could be found which were rich in Mn.

No areas of high concentrations of Mn were found on roots from nil Mn pots, regardless of whether they were disease-free or infected with *Ggt*. Spectral analysis of the root surface produced a pattern of elemental composition typical of that shown in figure 10.1B although Mn concentrations were generally lower.

Examination of the agar plug of *Ggt* inoculum which had been removed from a 24 Mn pot revealed the presence of small bright globular nodules (plate 10.4) within the agar. These nodules were approximately 0.001-0.002 mm in diameter and usually congregated in areas 0.01-0.02 mm wide. The spectrum in figure 10.2, taken from a typical area of high density of nodules in the agar, clearly shows that these areas had very high concentrations of Mn. The appearance of these nodules compare well with the pattern of Mn oxidation which was observed to occur with *Ggt* on PDA in petri-dishes; Mn was deposited in agar as discrete brown spots in a halo around each hypha (see chapter 2 of this thesis).

10.3.2.2. *Freeze-dried roots*. Following the identification of Mn-rich nodules in *Ggt*-infected wheat roots from soil with high rates of added Mn in oven-dried samples, similar roots were freeze-dried and examined. Freeze-drying preserved the structure of the roots and fungal hyphae and the micrograph in plate 10.5 of a longitudinal section of a root clearly shows the distribution of bright nodules within a *Ggt*-infected root. The digitized map of Mn in the image of plate 10.5 confirms that the nodule-rich areas in the root had very high concentrations of Mn (figure 10.3). The nodules appeared to be situated in the cell walls of

Plate 10.4. Scanning electron micrograph of a *Ggt*-inoculated agar plug, taken from a Mn-deficient sand to which 24 mg of Mn per pot had been added, which highlights the presence of small, bright nodules. Image generated by secondary electrons.



the root although this may have been an artifact of the freeze-drying process which causes cell contents to dehydrate against cell walls. The density of nodules was extremely high in the epidermis and outer 2 cortical cell layers but were far less common in cell layers more removed from the root surface. No nodules were found in the stele.

Examination of individual runner hyphae of *Ggt* revealed they also contained bright globular nodules (plate 10.6) and microprobe analysis of a hypha typical of those shown in plate 10.5 confirmed that these nodules also had high concentrations of Mn (figure 10.4). The nodules appeared to occur within hyphal cells, possibly embedded in the cell walls.

10.4. Discussion.

Investigations with benzidine stain and scanning electron microscope with EDXA microprobe confirmed that the extensive black/brown deposits on *Ggt*-infected roots from Mn-enriched Wangary sand contained high concentrations of Mn oxides. These Mn oxides were deposited on and in the infected roots as globular nodules and were only present where the root was encircled by a network of runner hyphae, even though nodules were not always closely associated with hyphae within these areas. The nodules of Mn oxides were also found in the runner hyphae themselves and in the agar plugs of *Ggt* inoculum. No invasive hyaline hyphae of *Ggt* were found in the root sections examined with the electron microscope so it is not known whether they also contained nodules of Mn oxides.

The effect of these extensive deposits of Mn oxides on the invasion process by *Ggt* and on the nutrition of the wheat host (or fungal hyphae) is not known. Levan and Riha 1986 conducted studies with several conifer species on the precipitation of black oxide coatings on flooded roots. They were investigating the hypothesis that the deposition of oxide sheaths on roots that were subjected to flooding alleviated Mn toxicity by immobilizing reduced Mn in the root cortex (Takijima 1965).

Plate 10.5. Scanning electron micrographs of a longitudinal section of *Ggt*-infected wheat root grown in a Mn-deficient sand with 24 mg of Mn added per pot. A - Image generated by secondary electrons (surface detail only). B - Image generated by reflected electrons (sub-surface detail revealed). Note the presence of small, bright nodules in the outer cortical cell layers of the wheat root.

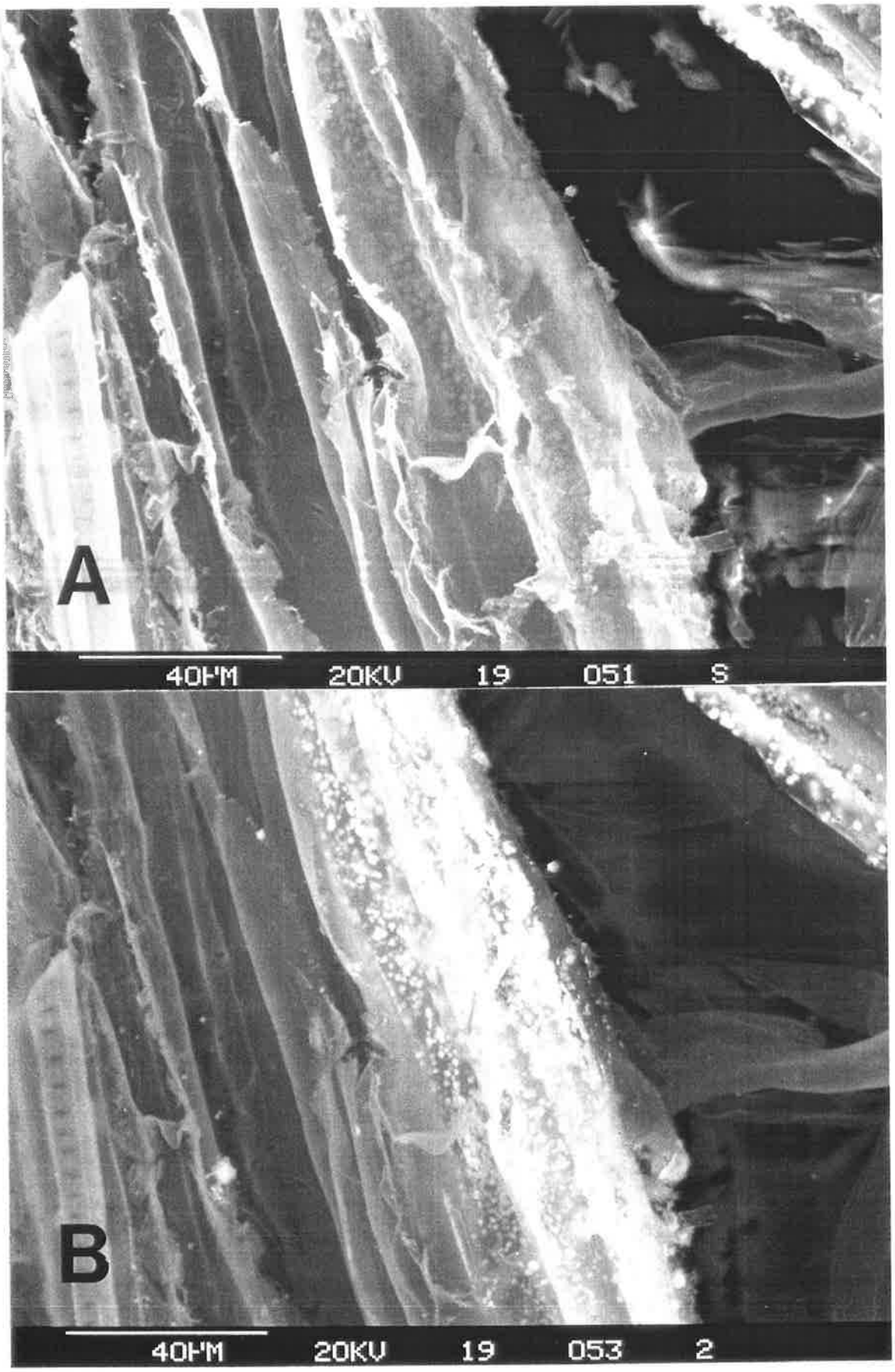


FIGURE 10.3. DIGITIZED X-RAY DISTRIBUTION MAP OF MN IN PLATE 10.5. (LEFT-HAND BOX) WITH A SECONDARY ELECTRON IMAGE TO CONFIRM LOCATION OF DIGITIZED MAP (RIGHT-HAND BOX). THE SCALE BAR IN THE RIGHT-HAND BOX REPRESENTS 1000.00 um.

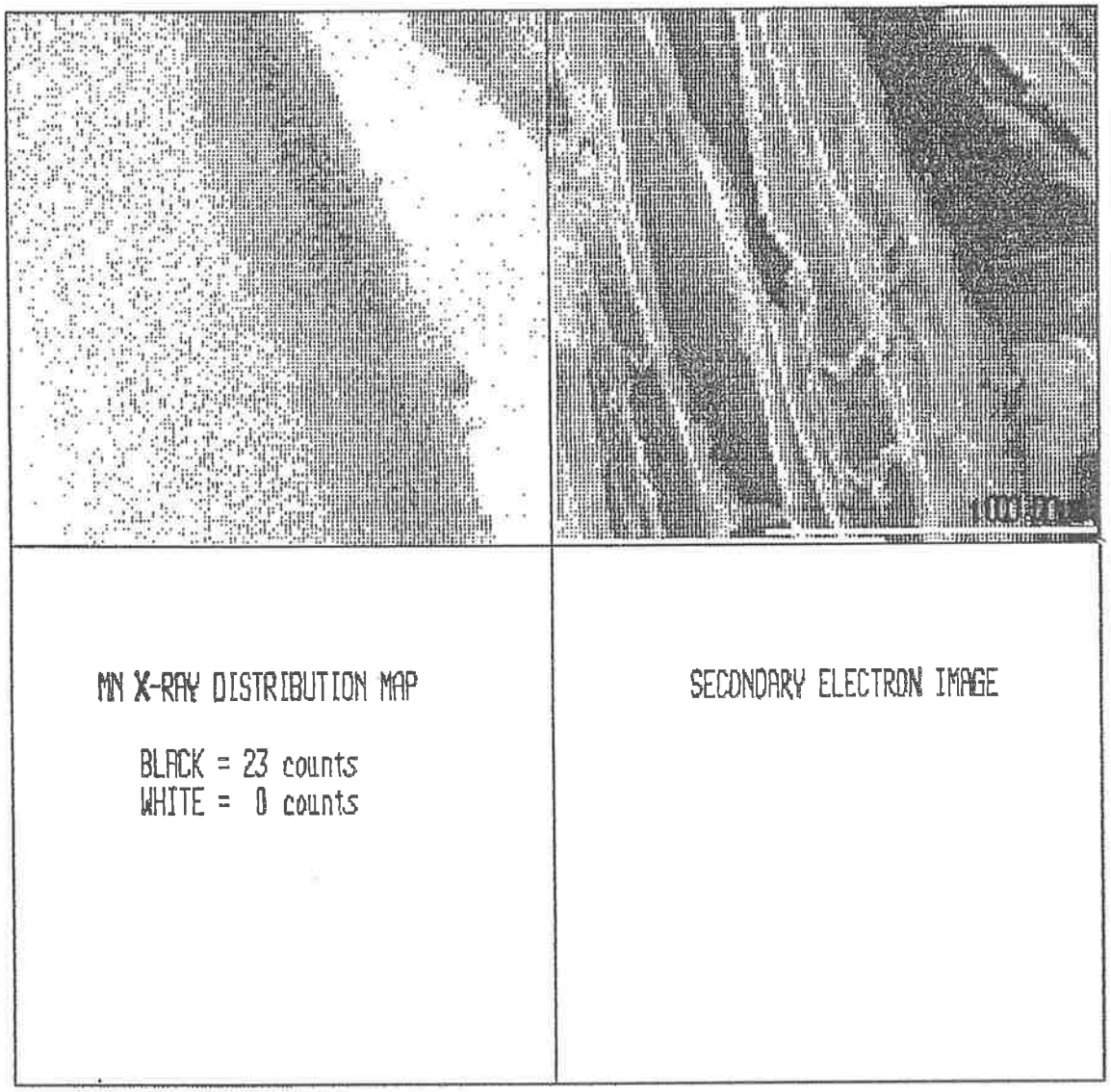
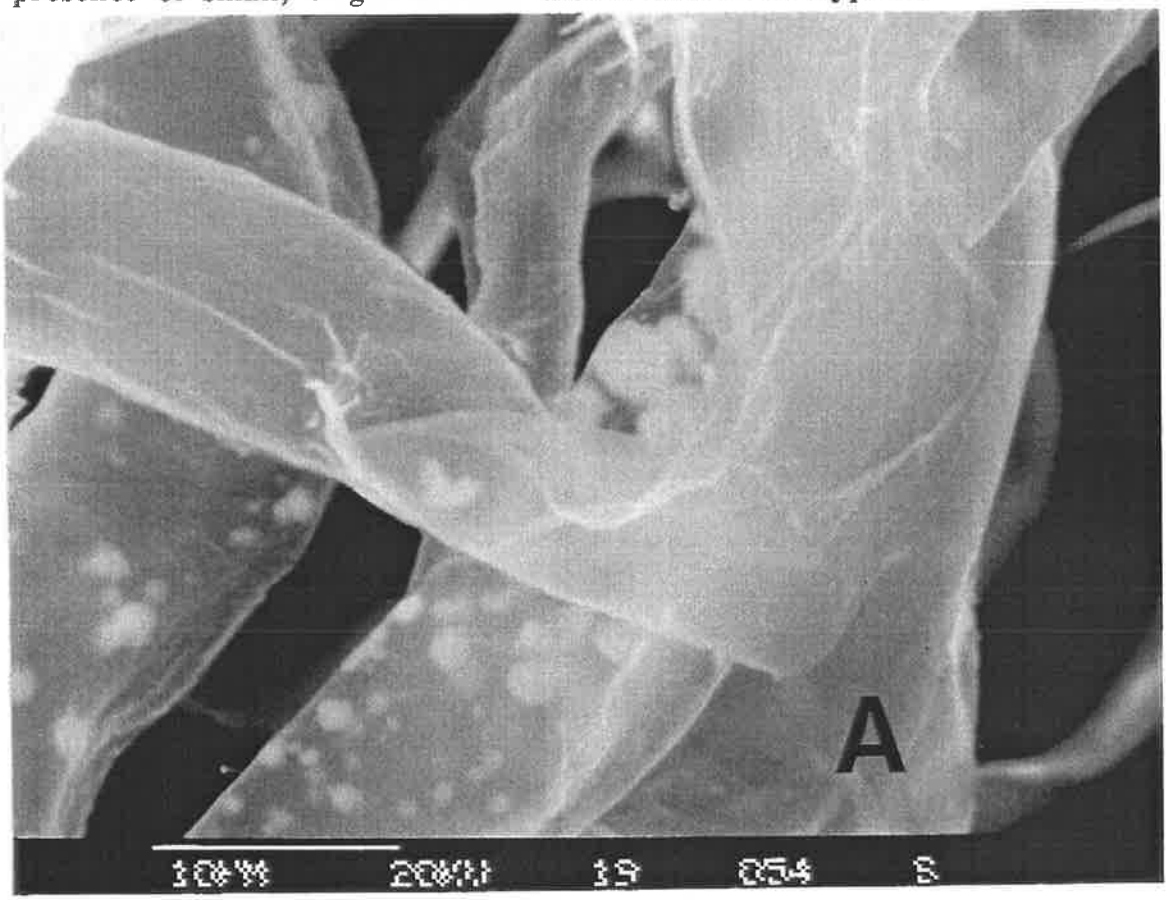


Plate 10.6. Scanning electron micrographs of *Ggt* runner hyphae taken from a Mn-deficient sand to which 24 mg of Mn had been added per pot. A - Image generated by secondary electrons (surface detail only). B - Image generated by reflected electrons (sub-surface detail revealed). Note the presence of small, bright nodules within individual hyphae.



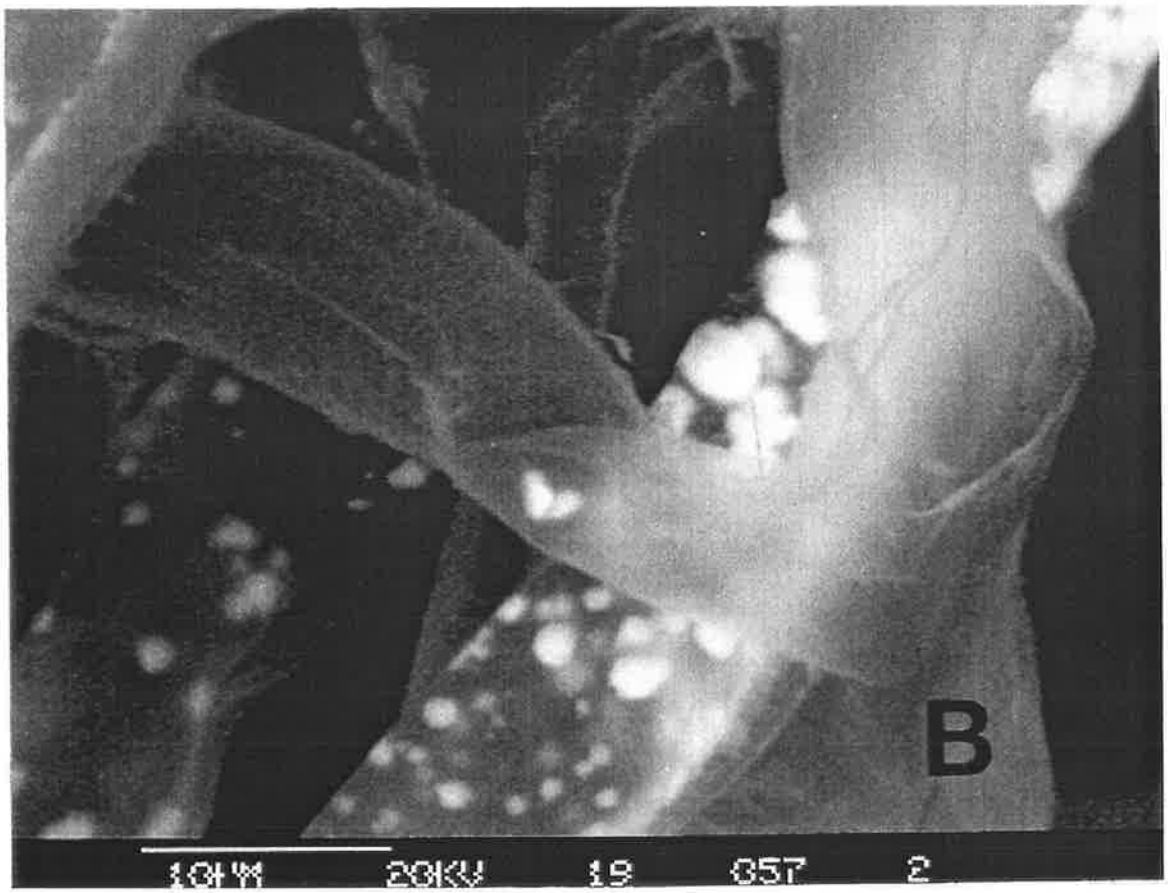
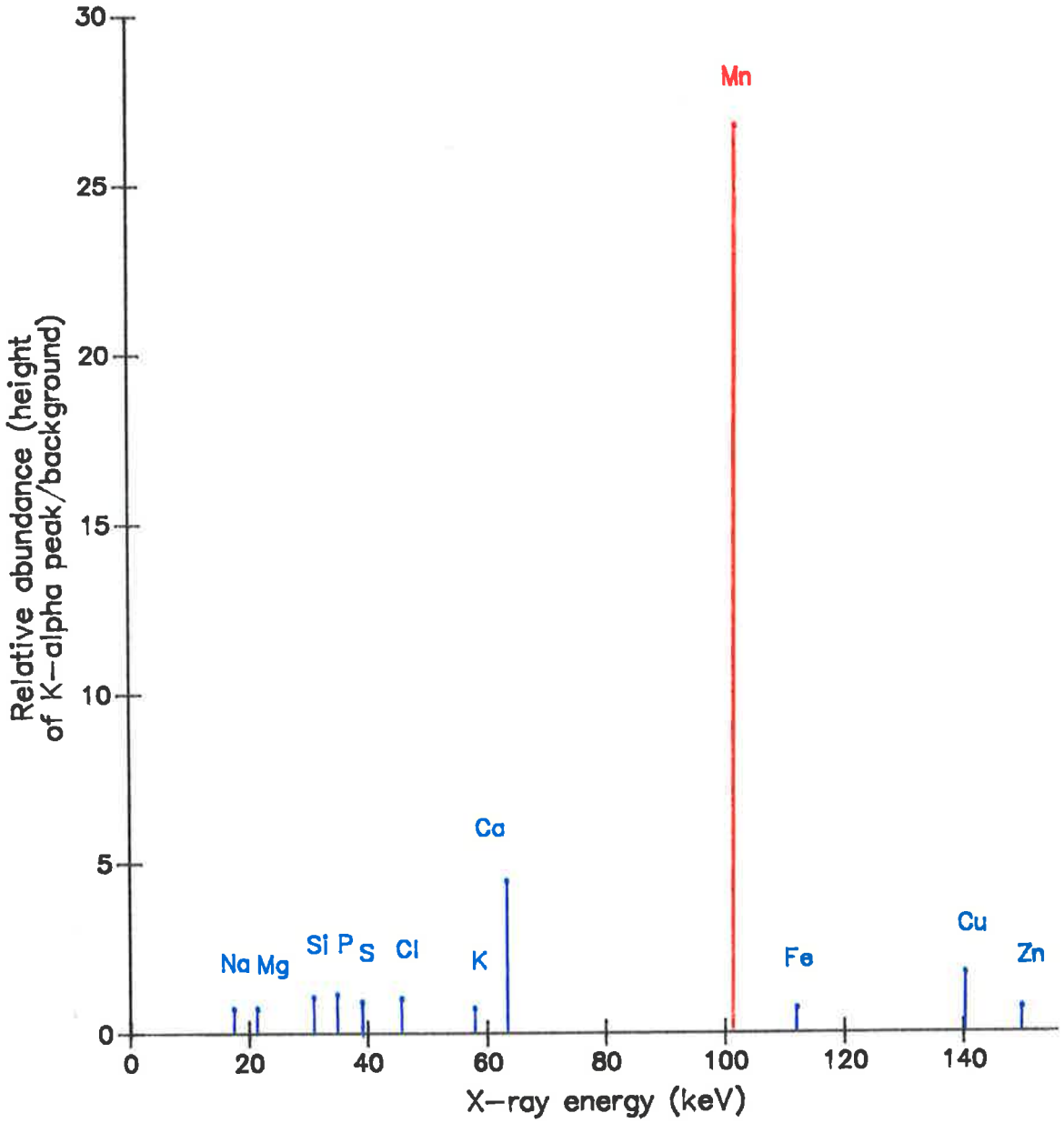


FIGURE 10.4. ENERGY DISPERSIVE X-RAY MICRO-ANALYSIS OF GGT RUNNER HYPHAE FROM MN-ENRICHED WANGARY SAND. (see plate 10.6. for micrograph of this sample).



They found that black coatings on flooded roots consisted of both Mn and Fe oxides (up to 33 % Mn, by weight) and that these deposits extended into the inner layers of the cortex and, occasionally, into the primary xylem vessels of the stele. Very little Fe was found in the deposits of *Ggt*-infected wheat roots and nodules were never found in the stele. No reference to the morphology of the oxide deposits in conifer roots was made except to note that the precipitates were found in cell walls.

From the nutrient composition of conifers from flooded treatments, Levan and Riha (1986) found that, even if the oxide deposits were serving some protective function, xylem sap concentrations of Mn were not maintained at low levels. They proposed two mechanisms by which the uptake of reduced Mn by the plants may be decreased with deposition of oxides in the roots. The first was that the physical presence of the oxide deposits may decrease the surface area available for nutrient uptake. This could have a detrimental effect by also interfering with the uptake of other essential nutrients which were not in toxic supply (Howeler 1973). Secondly, the uptake of Mn may be decreased simply through its removal from soil solution as oxide precipitates.

Nutrient analysis of *Ggt*-infected wheat plants with extensive Mn oxide deposits on their roots generally revealed little to suggest that the presence of Mn oxides was exerting a dramatic effect on host nutrition, including Mn. Shoot concentrations of Mn, Ca, Mg, B (see sections 6.3.1.4. and 6.3.2.4. in chapter 6 of this thesis), Fe, Cu, Mo and Co (not reported) in *Ggt*-infected wheat plants from Mn-enriched Wangary sand were the same as disease-free plants from the same Mn treatment. Concentrations of P, K and Zn were slightly lower in *Ggt*-infected plants but it is not possible to separate the effect of the presence of the Mn oxide nodules from the effects of *Ggt* infection on host growth, especially root pruning.

However, these results do not preclude the possibility of changes in nutrient availability occurring in the root, rhizosphere and/or infection court due to the presence of

Mn oxide particles. These changes may be small in terms of nutrition of the host but may be very important to the infection process in the micro-environments of the rhizosphere and infection court. Mn oxides are highly effective adsorbers of many metals, and they participate in a variety of redox reactions, including oxidation of Mn^{2+} and Fe^{2+} (Norvell 1988). In addition, Jarvis (1984) found that the availability of Co in soils was strongly influenced by the level of Mn oxides.

Mn oxide deposits may also be part of an elicitated response by the wheat host against invasion by *Ggt*. Kunoh *et al*. 1975 found that high levels of Ca, Mn and Si were deposited around penetration sites in barley leaves caused by *Erysiphe graminis hordei* (powdery mildew). They postulated that the elements migrated in the epidermal cytoplasm to the infection site and their accumulation was part of the host resistance reaction. However, since *Ggt* can oxidise Mn to insoluble oxides in the absence of the host (in petri-dishes and in soil, see chapter 2 of this thesis) it seems more likely that the presence of Mn oxide nodules in host roots was due to the activities of the pathogen, and not the host.

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CHAPTER 11.

GENERAL DISCUSSION AND CONCLUSIONS

CHAPTER 11. GENERAL DISCUSSION AND CONCLUSIONS.

Graham and Rovira (1984) proposed three mechanisms to explain the decreased susceptibility of wheat seedlings to *Ggt* infection when Mn deficiency was corrected;

1. Mn^{2+} may be directly toxic to the free inoculum of the fungus in the soil.

2. Mn may be acting through the physiology of the plant. They proposed that Mn nutrition affects photosynthesis, which in turn, controls the rate of exudation of soluble organic compounds by roots. These exudates affect the rhizosphere microflora and, through it, the ectotrophic growth of the take-all fungus.

3. Lignin production is controlled by Mn-activated enzyme systems. Since ligneous materials are an acknowledged partial defence against take-all in the form of lignitubers, these structures may be more poorly developed in Mn-deficient plants.

Results presented in this thesis are in direct support of the third hypothesis. They also demonstrate that the first hypothesis should be rejected and that the second hypothesis plays a secondary role only, if it operates at all.

In addition, the results are consistent with a refinement of the third hypothesis which states that decreased activity of a Mn-activated, cell wall peroxidase, which mediates lignin deposition in cell walls, occupies a central role in pre-disposing Mn-deficient wheat plants to take-all.

Results are also presented which document the first report from the Southern Hemisphere of an application of Mn fertilizer decreasing take-all in the field.

The four topics of the relative importance of the 3 hypotheses of Graham and Rovira (1984) and the implications of results from field experiments are examined in detail in the following sections.

11a. Mn deficiency decreases the resistance of wheat plants to *Ggt* by inhibiting lignin production in the host.

The results of the virulence and Mn oxidizing study of 4 *Ggt* isolates (see chapter 5) became the fore-runner for a more extensive study of 14 isolates (Buchhorn and Graham 1989). (Buchhorn and Graham 1989) produced a correlation between virulence of *Ggt* isolates and Mn-oxidizing ability and found that nearly 60 % of the variation in length of black stelar lesions caused by 14 different isolates was explained by Mn-oxidizing ability on PDA. Observations reported in this thesis (see chapters 5 and 10) showed that Mn oxidation by virulent *Ggt* isolates in PDA and in soil occurred at a distance from the hyphae and formed discrete "nodules" of Mn oxide. The combination of high rates of soil-applied Mn and *Ggt* infection resulted in extensive deposition of Mn-oxide "nodules" in the interstices of epidermal and superficial cortical cells of wheat roots but nodules were never found in root segments free of *Ggt*. Since virulence appears to be strongly linked to Mn-oxidizing ability and virulent *Ggt* isolates cause extensive deposition of Mn-oxides in the infection court of the outer cell layers of wheat roots it seems reasonable to propose that Mn oxidation is a direct response by virulent *Ggt* isolates to weaken lignin production at the site of invasion into the wheat host. These Mn-oxide nodules have the potential to specifically disrupt the activity of Mn-activated cell wall peroxidases involved in lignin deposition by depriving the enzyme of its essential Mn^{2+} co-factor (Takijima 1965, Jauregui and Reisenauer 1982, Gilkes and McKenzie 1988), physically blocking nutrient exchange and lignin deposition in cell walls (Howeler 1973, Levan and Riha 1986) and/or acting as catalysts for oxidation/reduction processes (Norvell 1988). Cell wall peroxidase enzymes may require high local concentrations of Mn for full activity because foliar-applied Mn was not effective

at decreasing *Ggt* infection or increasing root Mn concentrations despite producing large increases in shoot and root growth.

Investigations into *Ggt* invasion of excised wheat root pieces on agar suggested that barriers to fungal invasion existed in Mn-adequate root pieces prior to exposure to the pathogen and that resistance of both Mn-deficient and -adequate root pieces could be further increased by a current external supply of Mn, although external Mn was not as effective as Mn pre-culture. Staining of root pieces for lignin revealed that Mn-adequate roots had more extensive lignification throughout the root section which suggests lignin as a prime candidate for the pre-formed barriers to *Ggt* invasion apparently existing in Mn-adequate roots. Pre-formed lignin and the production of lignitubers are important in decreasing the rate of invasion of *Ggt* into roots, even if they can not halt it completely (Weste 1972, Skou 1981). An external supply of Mn in agar further delayed rates of *Ggt* invasion into Mn-adequate roots which suggests that Mn may also have secondary roles in slowing *Ggt* invasion into wheat roots. The nature of this role may be enhanced peroxidase activity, protection of pectolytic and proteolytic exoenzymes required by *Ggt* for pathogenesis (Sadasivan 1965, Huber and Keeler 1977), or a completely different mechanism which has not yet been identified. More evidence in line with pre-formed defence barriers in Mn-adequate plants is provided from pot experiments where moderate rates of Mn decreased *Ggt* infection and corrected Mn deficiency in the host but did not result in the production of nodules of Mn oxide in infected tissues (see chapter 3).

There is already evidence which suggests that lignins produced in response to disease may differ from that normally produced (Skou 1981) and Brown and co-workers (1984) have shown that lignin production in non-diseased wheat roots is severely inhibited by Mn deficiency. To further test the validity of this hypothesis, studies into the biochemical composition of *Ggt*-infected, Mn-deficient and -adequate wheat plants need to be conducted. These studies should concentrate on the amounts, types and localities of pre-cursors, intermediates and end-products of the lignin pathway, especially at local sites of *Ggt*

invasion into roots. High magnification, electron microscopic examination of the invasion process by *Ggt* into Mn-deficient and -adequate roots would be an important adjunct to the biochemical studies, especially since markers can be used to "map" high concentrations of particular compounds. The aim of these studies would be to demonstrate that Mn-deficient roots are more easily invaded by *Ggt* because they have lower lignin levels in tissue and their ability to respond to invasion with increased lignin deposition at the site of invasion is inhibited. Use of sterile, excised root pieces rather than whole plants growing in soil would avoid complications with soil and microbial factors.

Studies also need to be conducted to confirm the role of Mn oxidation on virulence of *Ggt*, perhaps through comparisons of Mn oxidizing and non-oxidizing isolates. The impact of Mn oxide particles on metabolism of *Ggt* hyphae and the lignin metabolism of wheat roots warrants investigation, even though high levels of Mn oxides are not required for take-all to be decreased.

Removal of Mn^{2+} from the infection court by oxidation would also protect the activity of fungal pectolytic and proteolytic exoenzymes required by *Ggt* for pathogenesis (Sadasivan 1965, Huber and Keeler 1977). However, this would appear to occupy a secondary role in plant resistance because high levels of Mn were not required in the external environment for disease reduction to occur (cf. soaking seed in Mn prior to sowing decreased *Ggt* infection in pot experiments (see chapter 6) and pre-culture of plants with Mn increased the resistance of excised root pieces on low Mn agar (see chapter 8).

In pot experiments presented here, low rates of soil-applied Mn, which did not fully correct Mn deficiency in the wheat host, caused little or no depression in take-all symptoms. This may be an example of the oxidative capabilities of *Ggt* being able to successfully overcome marginally increased plant resistance which should follow partial relief of Mn deficiency in the host. At high rates of Mn application the oxidative capabilities of the pathogen are overwhelmed. Graham and Rovira (1984) found that low Mn rates caused

partial relief from Mn deficiency in the host and also partially decreased *Ggt* infection which suggests that small differences in Mn availability or timing and rates of *Ggt* invasion (for instance from inocula of different potentials) may be critical for determining progress of the disease. Also, results from a subsequent study in a Mn-sufficient soil suggest that there may be a higher critical level for Mn for resistance to take-all than for growth (Webb and Graham 1989).

The nature of antagonism by *Pseudomonas fluorescens* 2-79 (Weller and Cook 1983) against *Ggt* is also consistent with the Mn/lignin/peroxidase hypothesis as a component of virulence because *Pseudomonas* spp. will enhance peroxidase activity (Anderson and Guerra 1985), is a strong reducer of Mn oxide and that antagonism in agar was enhanced in the presence of added Mn (see chapter 9). In addition, coating wheat seeds with fluorescent pseudomonads capable of Mn reduction decreased take-all in the field, providing additional treatments were also applied to prolong Mn availability, e.g. nitrification inhibitors (Huber *et al.* 1986, Huber and Dorich 1988). However, the interaction between 2-79, Mn and *Ggt* could not be fully reproduced in the pot experiments attempted here.

11b. Mn does not need to be toxic to decrease take-all of wheat.

Results from many studies conducted during the course of this project provide extensive evidence that the first hypothesis of Graham and Rovira (1984) should be rejected. The most conclusive evidence was collected during studies which investigated the effects of pre-culture with Mn on the resistance of excised wheat root pieces to invasion by *Ggt* (see chapter 9). The results from these experiments clearly demonstrated that the resistance of wheat roots to invasion by *Ggt* was increased if plants had been grown under Mn adequate conditions, compared to those grown under Mn-deficient conditions. In these experiments, Mn treatments were imposed prior to excision of root pieces so that the infection process by *Ggt* occurred in a very low Mn environment, regardless of pre-culture treatments of roots. The concentration of Mn in unamended potato-dextrose agar (PDA) was several orders of

magnitude less than that toxic for the fungus. In addition, total concentrations of Mn in Mn pre-cultured root pieces were well below toxic levels for the fungus and concentrations of free Mn^{2+} in solution in roots should have been less than 30 % of the total (Munns *et al.* 1963).

Further, corroborating evidence for the rejection of the first hypothesis was provided by a series of pot experiments, not all of which were specifically designed to test the validity of this particular hypothesis. The first hypothesis predicts that increasing rates of soil-applied Mn would progressively decrease the extent of *Ggt* infection on wheat roots, regardless of the nutritional status of the wheat host. However, collation of results from 14 pot experiments with different rates of soil-applied Mn and comparison with effects of soil-applied Mn on saprophytic growth of *Ggt* hyphae through soil revealed that rates of soil-applied Mn which were not toxic to growth of *Ggt* mycelia through soil decreased *Ggt* infection of wheat roots, providing they fully corrected Mn deficiency in the wheat host (see chapter 2). In fact, decreases in *Ggt* infection on wheat roots occurred with rates of soil-applied Mn which may have enhanced saprophytic growth of the take-all fungus in soil.

In addition, Mn did not have to be applied to soil to be effective at decreasing *Ggt* infection on wheat roots. All Mn sources which fully corrected Mn deficiency in the wheat host (with one exception) also decreased *Ggt* infection. The most important source of Mn in this context was soaking seed in a Mn solution prior to sowing. This technique will eliminate Mn deficiency in the plant (see chapter 6 and Roberts 1948, Khalid and Malik 1982, Marcar and Graham 1986) but should have little impact on soil Mn. Soaking seed in Mn solution prior to sowing decreased *Ggt* infection on wheat roots. However, foliar application of Mn was not effective at decreasing *Ggt* infection in the same experiment because it did not increase root concentrations of Mn in the wheat host. The only exception to Mn sources which fully corrected Mn deficiency and also decreased *Ggt* infection was a high rate of commercially-prepared MnO_2. This product at high rates of application appeared to be ineffective at decreasing *Ggt* infection because it caused large changes in the nutrient

composition of treated plants. The only change which was consistent in both pot experiments was a decrease in calcium concentrations in shoots of treated plants to below deficiency levels. Since calcium nutrition has been implicated in the resistance of plants to several diseases (Huber 1980, 1981) it seems reasonable to assume that the ineffectiveness of commercial MnO_2 at decreasing *Ggt* infection was due to induced calcium deficiency in the host. However, high rates of commercial MnO_2 also produced marked changes in boron and magnesium nutrition of treated plants but these effects were not consistent in both pot experiments so they have been regarded as less likely to explain the ineffectiveness of MnO_2 at decreasing *Ggt* infection (although they should not be dismissed completely). Use of commercial MnO_2 has the potential to be a very useful experimental tool because it allows the Mn status of the host to be manipulated without altering the host's resistance to *Ggt*; confirmation (or otherwise) of the mechanism(s) causing ineffectiveness may provide further insights into the overall role of Mn in resistance of wheat to take-all.

Results from investigations into the effects of soil-applied Mn on infection by four *Ggt* isolates are also inconsistent with the first hypothesis of Graham and Rovira (1984). If Mn increased the resistance of wheat plants to take-all because it was toxic to the fungus in the soil, there should be a positive relationship between sensitivity of the isolate to Mn^{2+} on agar and the extent of depression of *Ggt* infection by soil-applied Mn. However, ranking of the four isolates for sensitivity to Mn^{2+} on agar did not relate to extent of symptom depression on the host by soil-applied Mn. The fungal property which did relate with extent of symptom depression on the host by soil-applied Mn was ability to cause extensive oxidation of Mn on PDA, the importance of which was discussed above.

11c. Inhibition of take-all of wheat by Mn via changes in rhizosphere microflora is of secondary importance.

Changes in rhizosphere microflora as a flow-on effect from correction of Mn deficiency in the wheat host does not have a primary role in explaining the increased

susceptibility of Mn-deficient wheat plants to *Ggt*. The finding that sterile excised wheat root pieces, pre-cultured with Mn, were more resistant to invasion by *Ggt* than sterile root pieces cultured without Mn (see chapter 8) precludes the possibility that rhizosphere microflora changes following Mn addition are required for increased resistance to occur. Also, the Mn-preculture effect occurred on top of the presence of bacteria from non-sterile wheat roots. Nevertheless, Mn fertilization and application of soil-borne microflora have interacted to produce decreases in take-all of wheat larger than those achieved with the individual treatments (Huber *et al.* 1983, Huber *et al.* 1986) which suggests that changes in rhizosphere microflora may play a secondary, additional role. Mn enhancement of antagonism by *Pseudomonas fluorescens* 2-79 against *Ggt* on agar, perhaps due to their role in Mn redox reactions, is also important in this context (see chapter 9).

None of the results presented in this thesis test the simpler hypothesis that Mn may act through the physiology of the plant and cause changes in root exudates which directly inhibit the development of *Ggt* infection. This mechanism could still operate in sterile excised root pieces on agar because root pieces continue to grow on agar (Deacon and Lewis 1986) and, presumably, produce exudates.

11d. Treating Mn-deficient wheat with Mn fertilizer will control take-all in the field.

Drilling Mn sulphate-supplemented fertilizer with the seed corrected Mn deficiency in wheat and also decreased take-all in the field (see chapter 7). This is the first report in Australia (and in the Southern Hemisphere) of Mn fertilizer decreasing take-all in the field, although such decreases have been reported from Indiana in U.S.A (Huber and Wilhelm 1988).

The combination of these results with those of chapter 4 (which showed that "Mn-efficient" wheat genotypes were less susceptible to *Ggt* infection in Mn-deficient soil) offers the potential of presenting a fertilizer package to cereal farmers in southern Australia with

Mn-deficient soil to help control take-all. At present, the only management options that farmers have to control take-all is to include non-host crops in the rotation, ensure that the non-host crops remain free of grass weeds which will carry the disease and provide adequate fertilizer to help the crop grow away from *Ggt* damage (Price 1970, Macnish 1980, Rovira and Ridge 1983). However, the combination of selecting a wheat variety which performs well on Mn-deficient soil (compared to a sensitive variety) and inclusion of Mn fertilizer at seeding should provide the farmer with an improvement in the Mn status of the crop resulting in better growth and increased grain yield, a decrease in *Ggt* infection with a consequent increase in grain yield, and lastly a reduction in *Ggt* inoculum levels for following seasons. A longer term prospect to control take-all more effectively is applying Mn on the seed and also inoculating with Mn-reducing micro-organisms antagonistic to *Ggt* (e.g. *Pseudomonas fluorescens* 2-79). These separate techniques have been successful in field trials in Indiana (Huber and Wilhelm 1988).

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APPENDIX A.

INCUBATION OF MOIST WANGARY SAND TO INCREASE SEVERITY OF MN DEFICIENCY

APPENDIX A. INCUBATION OF MOIST WANGARY SAND TO INCREASE SEVERITY OF MN DEFICIENCY.

.A.1. Introduction.

Results from the first four experiments presented in chapter 3 (which are in chronological order) showed that the severity of Mn deficiency which developed in wheat seedlings varied considerably, despite very similar growing conditions and the same soil collection being used for all four experiments. The average Mn concentration in whole shoots and average increase in shoot dry weight with Mn in the first experiment (results reported in section 3.3.1.) were 9.1 mg kg^{-1} and 51 %, respectively. For the experiments similarly summarized in sections 3.3.2., 3.3.3.1. and 3.3.3.2., the respective values were 11 and 32, 6.6 and 76, and 11.8 and 28. Storage of air-dry soil and increasing temperature will increase exchangeable Mn levels (Fujimoto and Sherman 1945, Boken 1952, Shuman 1980, Reid and Ruiz 1985) so a general increase in available Mn with time, superimposed by short term fluctuations caused by temperature changes (soil was stored in an un-insulated shed) may explain the inconsistency in severity of Mn deficiency observed in pot experiments.

Marcar (1986) showed that the recovery of Mn^{2+} added to Wangary sand, decreased sharply with time and negligible levels of added Mn^{2+} were extracted from soil samples 10-20 days after addition. This meant that the standard technique of adding relatively low rates of $MnSO_4$ to soil immediately prior to sowing resulted in developing wheat seedlings facing a rapidly diminishing supply of Mn^{2+} and may have even resulted in Mn-deficient conditions in the latter stages of the experiments.

The two problems of fluctuating severity of Mn deficiency and diminishing supplies of Mn^{2+} were addressed with the development of a technique in which soil was moistened and incubated several weeks prior to sowing because re-wetting a soil will cause a gradual decrease in exchangeable Mn levels (Fujimoto and Sherman 1945, Shuman 1980). High rates of $MnSO_4$ were also added to soil prior to `incubation' in an attempt to increase the

exchangeable and easily reducible pools of soil Mn to levels that were adequate for growth of wheat seedlings. In this way, the rapidly diminishing supply of Mn^{2+} originally used as a Mn source for plants would be replaced by a reservoir of exchangeable and easily reducible Mn which would persist for the entire length of each experiment.

A factorially designed experiment was conducted in small pots with 3 rates of Mn (0, 40 and 400 Mn), 2 levels of soil moisture during incubation (0 and 20 % water content) and 3 replicates.

A.2. Materials and Methods.

A.2.1. Soil and Mn.

Mn was added to Wangary sand (see section 2.2.2. for full details of this soil) as a fine spray of $MnSO_4.4H_2O$ solution and the soil shaken thoroughly to ensure even mixing of Mn through soil. Mn was dissolved in sufficient quantities of $DDDH_2O$ to give a moisture content in soil of 20 % and final rates of Mn were equivalent to 0, 40 and 400 mg of Mn per pot (50 g of air-dry soil per pot).

A.2.2. Incubation.

Two days after Man was applied, soil for each Mn treatment was equally divided and one half allowed to air dry (dry incubation) and the other half sealed in a polyethylene bag (moist incubation). All soil was stored at 15/10° C day/night temperatures for a further 25 days.

A.2.3. Procedure.

Basal nutrients were added to soil as in 3.2.3.1. with extra $DDDH_2O$ added to soil incubated dry to increase the soil water content to 25 % (level used in previous pot experiments). The water content of moist incubated soil was 38 % after the addition of basal nutrients, which made the soil quite wet and it packed down in pots more tightly than dry

incubated soil. The weight of prepared soil per pot was adjusted so that pots were filled with either dry or moist incubated soil of the same air-dry weight. Pots were part-filled with 47.9 g of dry-incubated soil (52.6 g of moist-incubated soil), followed by 2 germinating wheat seeds (as in 3.2.3.1.) and a final layer of 2.1 (or 2.5) g of soil. Growing conditions were the same as in 3.2.3.1. All pots were maintained at 25 % water content by watering to weight every 3-4 days. Plants were harvested after 25 days and fresh (free water removed from roots) and dry weights of shoots and roots per pot recorded before analysis of whole shoots for Mn and 11 other essential elements with an ARL inductively coupled atomic emission spectrometer. Shoot and root tissues were prepared for analysis with nitric acid digestion. Data were analyzed with ANOVA techniques appropriate to the factorial design.

A.3. Results.

A.3.1. Plant growth.

Plants from 0 Mn were pale green if they had been grown in dry-incubated soil but were very pale green with extensive interveinal chlorosis on floppy young leaves if they had been grown in moist-incubated soil. All plants grown with added Mn were dark green and healthy except for plants in 400 Mn in moist-incubated soil, which appeared smaller than other Mn treated plants. The highest rate of Mn resulted in extensive black/brown external coatings (identified as Mn oxides with benzidine) on the roots of plants grown in dry-incubated soil but this coating was patchy on roots grown in moist-incubated soil. There was some brown discolouration due to Mn oxide deposits on roots of plants grown with 40 Mn in dry-incubated soil but it was concentrated on seminal roots within 3 cm of the seed and was only faint on roots grown in moist-incubated soil at the same Mn rate.

The average dry weight of shoots per plant was the same at both levels of added Mn, regardless of incubation treatments, and much higher than at 0 Mn (table A.1).

Table A.1. Effect of incubating Wangary sand moist for 27 days prior to sowing on growth and Mn nutrition of wheat seedlings grown under controlled environment conditions. Mn was added to soil prior to incubation. Values in the body of the table are the average of 6 plants.

Mn added (mg/pot)	Incubation treatment Dry	Moist	Mean
Dry weight of shoots per plant (mg)			
0	42	31	37
40	60	64	62
400	62	59	61
Mean	55	52	
LSD[a] (P=0.05) Mn=4 ; Incubation ns ; Mn*Incubation=6			
Dry weight of roots per plant (mg)			
0	51	24	37
40	93	75	84
400	61	44	53
Mean	68	48	
LSD[a] (P=0.05) Mn=4 ; Incubation=8 ; Mn*Incubation ns			
Mn concentration in whole shoots (mg kg^{-1} D.W.)			
0	12.7[b]	3.7	7.0
	2.62[c]	*1.55*	*2.09*
40	529.0	172.8	302.7
	6.27	*5.16*	*5.72*
400	3316.6	2405.7	2823.3
	8.11	*7.79*	*7.95*
Mean	287.6	124.5	
	5.67	*4.83*	
LSD[a,d] (P=0.05) Mn=*0.26* ; Incubation=*0.24* ; Mn*Incubation=*0.37*			

[a] Values separated by more than the appropriate LSD were statistically different.
[b] Geometric means calculated from natural log transformed data.
[c] Values in italics are natural log transformations, calculated to adjust for a skewed distribution.
[d] LSD's apply to transformed data only.

However, shoot dry weights were lower from pots of moist-incubated soil without added Mn than from dry-incubated soil and shoot dry weight doubled with 40 Mn in moist-incubated soil but increased by only 43 % in dry-incubated soil. Root dry weights per plant were, on average, 30 % smaller with moist-incubated rather than dry-incubated soil, regardless of Mn treatments (table A.1). The observation that plants were smaller at 400 Mn

than at 40 Mn was not reflected in dry weights of shoots but root dry weights showed a clear growth depression with 400 Mn. Dry weight of roots at 0 Mn more than doubled with 40 Mn but 400 Mn caused an average decrease of 37 % from levels at 40 Mn. Growth depressions in roots with 0 and 400 Mn were most pronounced in moist-incubated soil but the interaction between Mn and incubation treatments was not significant.

A.3.2. Mn.

The severe Mn deficiency symptoms and poor growth observed in plants grown in moist-incubated soil without added Mn were reflected in shoot Mn concentrations (table A.1). Average concentration of Mn in whole shoots in this treatment was only 3.7 mg kg^{-1}, compared to a level of 12.7 mg kg^{-1} in plants grown in dry-incubated soil, which was still below the critical level of 18 mg kg^{-1} established for these pot experiments (see Appendix B). Mixing Mn through soil prior to incubation at the rate of 40 mg per pot increased plant Mn levels to luxury levels but the increase was much greater in dry-incubated soil. Shoot Mn concentrations in plants grown with 400 Mn were well above published toxic levels for wheat (Reuter and Robinson 1986) and averaged 2823 mg kg^{-1} for both incubation treatments. However, the effect of these very high Mn concentrations on plant growth was only significant for roots.

A.4. Discussion.

Moistening Wangary sand for several weeks prior to sowing was very successful in increasing the severity of Mn deficiency in wheat seedlings subsequently grown in the prepared soil. The mixing of high rates of $MnSO_4$ through soil prior to `incubation' supplied abundant Mn to wheat seedlings and, in combination with moist incubation, proved to be a reliable technique of eliminating Mn deficiency in wheat seedlings in a very deficient soil. The addition of basal nutrients at sowing overcame any changes in availability of other essential plant nutrients which may have occurred with incubation.

The technique that was adopted for subsequent pot experiments was to wet the soil to 18 % water content (which allowed for the addition of basal nutrients at sowing and pots to be filled with soil at 25 % water content as in previous experiments) and incubation for a period of 2 weeks. Reasonably low rates of $MnSO_4.4H_2O$ were subsequently used because the high rates used here supplied luxury to toxic levels of Mn to wheat seedlings.

A.5. References.

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APPENDIX B.

A CRITICAL LEVEL OF MN FOR GROWTH OF WHEAT SEEDLINGS IN EXPERIMENTS WITH SMALL POTS

APPENDIX B. A CRITICAL LEVEL OF MN FOR GROWTH OF WHEAT SEEDLINGS IN EXPERIMENTS WITH SMALL POTS.

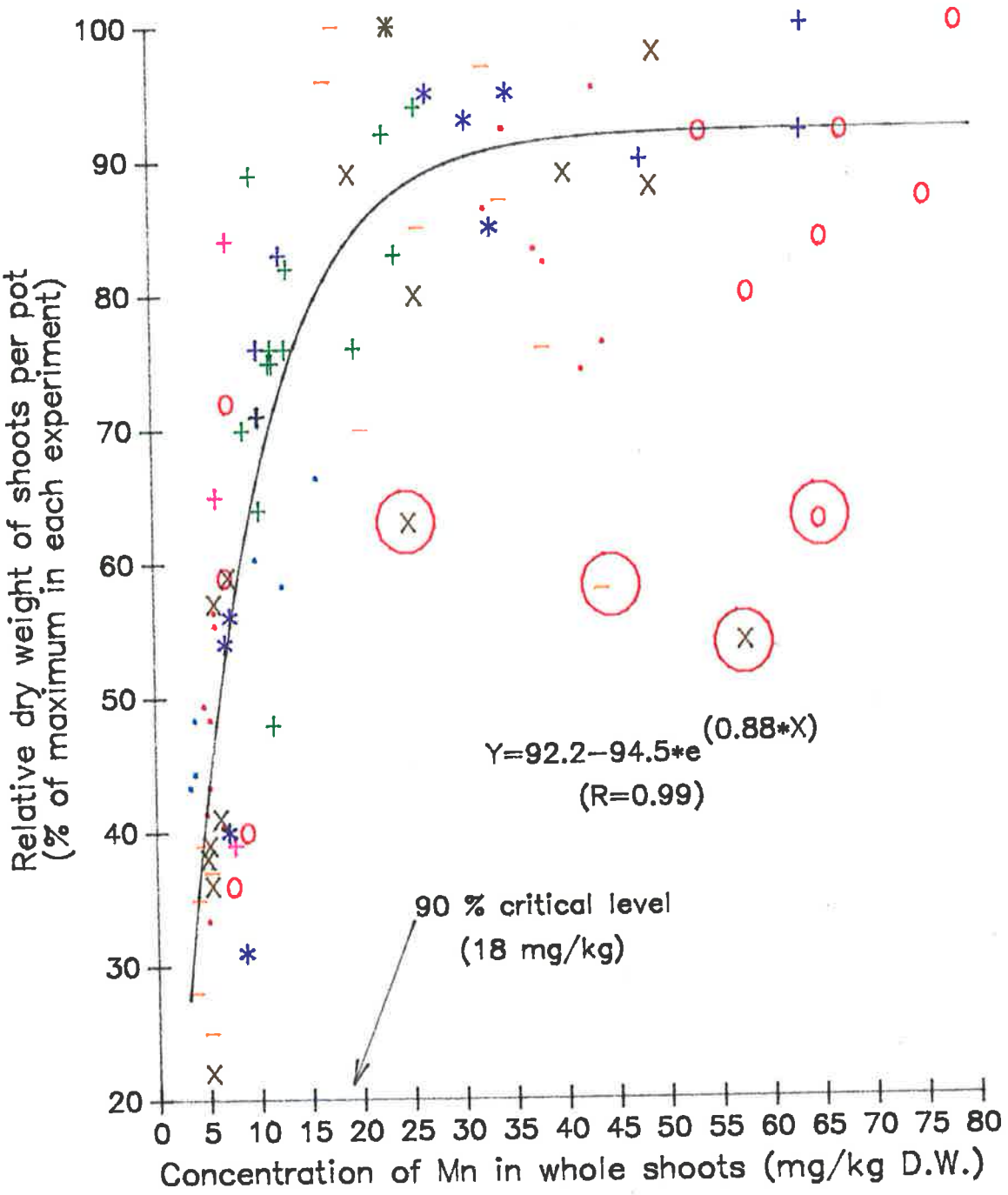
Figure B.1 shows a Mn critical level curve for growth of wheat seedlings under controlled environment conditions. The curve was compiled from the growth and whole shoot Mn concentration data of wheat seedlings grown in nine small pot experiments during the course of this project. These nine experiments were representative of all experiments conducted with small pots in this project but only the data from plants free of take-all were included. Each point is the average dry weight of whole shoots per pot for one Mn treatment, expressed as a percentage of the Mn treatment which maximized growth in the same experiment. The curve was calculated using the Mitscherlich plant growth model (Ware and Ohki 1982) and produced a critical level for Mn at 90 % of maximum yield of 18 mg kg^{-1} D.W. The four ringed points on the figure were not included in the calculation of the critical curve but data points with Mn concentrations between 80 and 650 mg kg^{-1} were, although they have not been plotted.

B.1. References.

Ware, G.O. and Ohki, K. (1982). The Mitscherlich plant growth model for determining critical nutrient deficiency levels. *Agron. J.* **74**, 88-91.

FIGURE B.1. EFFECT OF WHOLE SHOOT CONCENTRATIONS OF MN ON GROWTH OF WHEAT SEEDLINGS IN SMALL POTS OF WANGARY SAND. (The four ringed points were not included for the calculation of the curve (Ware and Ohki 1982)).

Individual experiments are represented as points of the same shape and colour



APPENDIX C.

TOTAL NUMBER OF STELAR LESIONS ON WHEAT ROOTS AND PERCENTAGE OF DISEASED ROOTS FROM CHAPTER 4, SECTION 4.3.1.

APPENDIX C. TOTAL NUMBER OF STELAR LESIONS ON WHEAT ROOTS AND PERCENTAGE OF DISEASED ROOTS FROM CHAPTER 4, SECTION 4.3.1.

Table C.1. Effect of soil Mn on the number of black stelar lesions per plant on the roots of four wheat genotypes grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 10 plants. Analysis was conducted on +Ggt treatments only.

	0 Mn		4 Mn		16 Mn		
Genotype	-Ggt	+Ggt	-Ggt	+Ggt	-Ggt	+Ggt	Mean[a]
Bayonet	0	8.8	0	8.6	0	11.2	9.6
Condor	0	11.2	0	9.6	0	10.6	10.6
Spear	0	10.0	0	10.2	0	10.2	10.0
C8MM	0	13.2	0	13.0	0	10.2	11.8
Mean[a]		10.6		10.4		10.6	

LSD (P=0.05) Mn, Genotype, Mn*Genotype ns

[a] Means calculated for +Ggt treatments only.

Table C.2. Effect of soil Mn on the percentage of diseased seminal roots of four wheat genotypes grown in a Mn-deficient sand under controlled environment conditions. Values in the 3-way table are the average of 10 plants. Analysis was conducted on +Ggt treatments only.

	0 Mn		4 Mn		16 Mn		
Genotype	-Ggt	+Ggt	-Ggt	+Ggt	-Ggt	+Ggt	Mean[a]
Bayonet	0	74	0	72	0	88	78
Condor	0	78	0	80	0	84	81
Spear	0	78	0	82	0	84	81
C8MM	0	82	0	94	0	84	87
Mean[a]		78		82		85	

LSD (P=0.05) Mn, Genotype, Mn*Genotype ns

[a] Means calculated for +Ggt treatments only.