Availability and Management of Manganese and Water in Bauxite Residue Revegetation

Mark James Gherardi

B.Sc. (Hons)

This Thesis is Presented for the Degree of Doctor of Philosophy

at The University of Western Australia, July 2004

The University of Western Australia

Faculty of
Natural and
Agricultural Sciences

DECLARATIONS

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy at The University of Western Australia. I declare that, except where otherwise acknowledged, this thesis details my own account of my own research and has not been previously submitted for a degree at any other tertiary educational institution.

Mark James Gherardi

July 2004

A number of chapters in this thesis have been published or accepted for publication in peer-reviewed journals under joint authorship. The publications are listed below (over page) and indicated throughout. As PhD supervisor and as the co-author in these publications, I certify that the research detailed in this thesis was principally carried out by Mark James Gherardi and I fully sanction the presentation of the data herein.

Professor Zed Rengel

July 2004

PUBLICATIONS ARISING FROM THIS STUDY

Chapter II

Gherardi, M.J. and Rengel, Z. (2001) Bauxite residue has the capacity to rapidly decrease availability of added manganese. *Plant and Soil* **234**: 143-151.

Chapter III

Gherardi, M.J. and Rengel, Z. (2003) Genotypes of lucerne (*Medicago sativa* L.) show differential tolerance to manganese deficiency and toxicity when grown in bauxite residue sand. *Plant and Soil* **249**: 287-296.

Chapter IV

Gherardi, M.J. and Rengel, Z. (2003) Deep placement of manganese fertiliser improves sustainability of lucerne growing on bauxite residue: A glasshouse study. *Plant and Soil* ***257***: 85-95.

Chapter V

Gherardi, M.J. and Rengel, Z. (2003) Deep banding improves residual effectiveness of manganese fertiliser for bauxite residue revegetation. *Australian Journal of Soil Research* **41**: 1273-1282.

Chapter VI

Gherardi, M.J. and Rengel, Z. (In Press) The effect of manganese supply on exudation of carboxylates by roots of lucerne (*Medicago sativa* L.). *Plant and Soil* **260**: 271-282.

THESIS ABSTRACT

Industrial processing to refine alumina from bauxite ore produces millions of tonnes of refining residue each year in Australia. Revegetation of bauxite residue sand (BRS) is problematic for a number of reasons. Harsh chemical conditions caused by residual NaOH from ore digestion mean plants must overcome extremely high pH (initially ≥12), saline and sodic conditions. At such high pH, manganese (Mn) is rapidly oxidised from Mn^{2+} to Mn^{4+}. Plants can take up only Mn^{2+}. Thus, Mn deficiency is common in plants used for direct BRS revegetation, and broadcast Mn fertilisers have low residual value. Added to this, physical conditions of low water-holding capacity and a highly compactable structure make BRS unfavourable for productive plant growth without constant and large inputs of water as well as Mn. However, environmental regulations stipulate that the residue disposal area at Pinjarra, Western Australia, be revegetated to conform with surrounding land uses. The major land use of the area is pasture for grazing stock. Hence, pasture revegetation with minimum requirement for fertiliser and water application is desirable.

This thesis investigates a number of avenues with potential for maintaining a productive pasture system on BRS whilst reducing the current level of Mn fertiliser and irrigation input. Emphasis was placed on elucidation of chemical and physical factors affecting Mn availability to plants in BRS.

Transformation of added Mn from available to unavailable forms in BRS was studied using sequential chemical fractionation techniques. It was found that Mn transformation to unavailable oxides, carbonate-bound and other forms was rapid (<24 h) in fresh BRS. Prior to formation of oxides and carbonates, intermediate weakly-adsorbed Mn species were formed. Transformation was slower in four-year-old BRS, but oxidation of Mn was still strongly induced by the high pH and electrochemical conditions in the older BRS. Although pseudoequilibrium between available and unavailable forms is reached after addition of Mn is a readily soluble form (hydrated $MnSO_4$), reversion to high levels of readily soluble Mn is not favoured by the inherent chemical conditions and high pH-buffering capacity of BRS.

Lucerne (*Medicago sativa* L.) has been identified as a species suited to BRS revegetation, with its deep rooting habit and moderate tolerance for alkaline and saline conditions. Using 16 commercially available lucerne genotypes, plants were grown with low, adequate and high amounts of added Mn. Comparative growth at low and adequate Mn was used to rank the genotypes for tolerance to Mn deficiency in BRS. Further work on a genotype identified as Mn-deficiency tolerant (Salado) and a sensitive genotype (Sirosal) revealed significantly different critical Mn concentrations (18 and 23 µg/g shoot dry weight, respectively).

Glasshouse and field trials were used to evaluate deep banding of Mn fertiliser in comparison with broadcast application for lucerne productivity. In the glasshouse, 20 µg Mn/g BRS banded at 10-cm depth significantly increased lucerne growth compared with mixing Mn at the same rate throughout the profile. During early growth with Mn banded at 20-cm depth, symptoms of Mn deficiency were observed. However, recovery of new growth was observed after lucerne roots had proliferated at the depth of banding. Deep banding Mn appears to increase root growth proximal to the fertiliser band. With no plants present, deep-banded Mn is transformed to unavailable forms, and watering above field capacity causes little downward movement of Mn (<1 cm) away from the original banding depth.

In the field, banding 15 kg Mn/ha at 18-cm depth provided sufficient Mn supply to lucerne to maintain adequate shoot Mn concentrations over a 2.5-year period. In contrast, broadcast Mn was re-applied four times during the same period to maintain adequate Mn concentrations in lucerne and prevent yield decreases. Yield increases with an 80 % reduction in Mn fertiliser exemplify that residual value of applied Mn is greatly increased by deep banding. Root density at depth was greater in deep-banded treatments than broadcast treatments. The deep banding results also highlight the importance of roots and rhizosphere processes in affecting Mn availability and uptake from high pH substrates.

Low-molecular-weight carboxylates released by plant roots have significant potential to affect Mn availability in the rhizosphere, and possibly relative Mn-deficiency tolerance of plant genotypes. Growth of Salado (Mn-deficiency tolerant lucerne) and Sirosal (Mn-deficiency sensitive) in solution culture showed that a decreasing Mn supply causes an increase in carboxylate exudation. The increase was greater in Salado than Sirosal and also increased with plant age. Omitting Mn from nutrient solutions caused increased exudation of oxalate, tartarate, L-malate, lactate, citrate and succinate. Increased exudation by Salado compared with Sirosal was associated with increased production of roots with diameter <100 µm by Salado.

However, nutrient concentration and growth data suggested that high exudation is not the sole factor responsible for Mn-deficiency tolerance in lucerne genotypes.

Success of deep-banded Mn fertiliser as an application method is dependent on greater available water at depth than at the profile surface. The physical nature of BRS, however, means that the potential for wastage of irrigation water in BRS revegetation is high. Through modelling water retention and movement in BRS and incorporating interception and plant uptake parameters, irrigation regimes may be refined to maintain revegetation productivity with minimal resource input. Hydrological characterisation of the BRS profile was undertaken through a variety of *in-situ* and laboratory measurements. Parameters were utilised in the Soil Water Infiltration and Movement (*SWIMv1.1*) model to accurately represent both short and long-term profile water as measured by Time Domain Reflectometry in the Pinjarra BRS profile under a lucerne crop. When *SWIMv1.1* predicted water content at specified depth layers, greatest accuracy was seen in the near-surface layer, and modelling accuracy decreased with depth. The hydrological characterisation provides an accurate basis for further refinement of water balance prediction and optimising water usage for maintaining efficient lucerne pasture growth on BRS.

Although the inherent nature of BRS results in poor availability of Mn and water for plant growth, by incorporating methodologies developed for agriculture such as genotype selection, deep-banded fertiliser placement and crop-based water-use modelling, improved and sustained productivity of BRS revegetation should be achievable. The associated decrease in resource inputs, namely Mn fertiliser and irrigation water, will provide environmental and economic benefits.

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ACKNOWLEDGEMENTS

There are many people who deserve more thanks than I can possibly give for enabling me to start this PhD, finish this PhD and do all the little bits in-between for this PhD (as well as a number of accessory activities that did nothing to contribute content to the thesis, but were inexorably important for maintaining my sanity). This is my feeble attempt to thank all of you.

Firstly, the Australian Research Council and Alcoa World Alumina Australia Limited are gratefully acknowledged for their support through the Strategic Partnerships with Industry Research and Training scheme. Special thanks to Ian Lockley at Alcoa for assistance in the initial stages of the project, especially with the field trial and accessing of resources, until he was transferred to Kwinana, whereby Dr David Jasper followed by Dr Judy Eastham took over the reins. Thanks also to Frank Ripper and CSBP for the use of their fancy deep-fertiliser-placement machine.

To my terrifically busy, insomniac supervisor, Professor Zed Rengel, for taking on a Graduate Research Officer from the bowels of Murdoch University, virtually unseen, to the prestigious, hallowed halls of UWA Agriculture. The opportunity you have given me to expand my professional and personal development at UWA and beyond is one I shall never forget (and one I hope you do not regret). You always found time for me, even when you seemed to have none, and your guidance was always available when I sought it. Thank you.

All the students and staff at the Soil Science and Plant Nutrition Department deserve thanks to varying degrees. Lorraine Osborne for taking the brunt of the Lab Dragon duties when I whined about the disrespect macronutrient researchers showed for us “micronutrienteers”. To Pies McGann, Whizza Wherrett, Mieke Quaghebeur, Reza Valizadeh, Conrad Allmark, Vanessa Dunbabin and the rest of the 20 or so people who shared my noisy but decorative office at some stage, talking footy, politics, coffee and food, I have medals for you all. I shall hand them out…one day. To The Smirka and Ethel Braimbridge, who along with Pies and Whizza provided more comic relief in three years than a lifetime could require, along with the blissful beer at various pubs and barbeques, thanks fellas. Mark Whitten, Keith Smettem, Yvette Oliver, Rachel Poulter and the rest of you who made my PhD more than just a research project, thank you.

The other Leave it Alone Tuggers also merit a mention. That was it (ha!).

For the loaning of equipment and enabling me to undertake my ICP analyses with tremendous accuracy and at mates rates, thanks to Bernie Dell, Longbin Huang, Catherine Chamberlain and the Murdoch University Trace Element Research Laboratory.

The staff at ForestrySA, especially Sue Shaw, having taken me on as a Forest Research Officer (albeit away from my beloved Fremantle) well before my thesis was submitted, deserve huge thanks. Mount Gambier may be cold, wet and at times miserable, but you have been very supportive of me in completing my thesis. I hope I repay the faith you have placed in me by making a significant contribution to the ForestrySA Research Section.

Finally, to my family, and to my wonderful fiancéé Suzanne for your constant support, inspiration and encouragement. This thesis is as much a tribute to all of you as it is a demonstration to the world that I may actually have done some stuff, discovered a few things and learned a bit. You are the most important people in the world to me, wherever you are around this extraordinary planet, and to have your love is what sustains me.

To anyone else who may read this, and are as fascinated with all the weird and wonderful things that plants do just to exist, follow those thoughts. They just might lead you somewhere even more interesting than you have been before.

CHAPTER I

INTRODUCTION, LITERATURE REVIEW AND PROJECT AIMS

Plate 1.1. *Visitor information at the bauxite residue revegetation area, Rehab Road in Pinjarra.*

INTRODUCTION: BAUXITE RESIDUE STORAGE AND REVEGETATION

Extraction of alumina from refined bauxite ore in the Bayer process involves high temperature digestion in sodium hydroxide (NaOH) or caustic soda, followed by precipitation of an aluminium oxide. The by-product of this refining is a tailings residue primarily containing highly alkaline 'liquor', coarse sand and fine mud.

The majority of bauxite in Western Australia is sourced from the Darling Range. This bauxite is relatively low grade by world standards, with approximately 2.5 tonnes of bauxite residue produced for every tonne of alumina extracted. The residue is usually stored in impoundments close to the refinery.

Alcoa World Alumina Australia Limited is one of a number of alumina producers in Australia. From three Western Australian refineries alone, recent figures estimate Alcoa's annual production at around 7 million tonnes of alumina, generating just over 17 million tonnes of bauxite residue (Lockley 1999). The total land area covered by Alcoa bauxite residue deposits in Western Australia currently stands at over 1200 ha. It is anticipated that this area will have at least doubled in the next five decades. With such a large footprint on the landscape, and with refineries being located in relatively close proximity to major population centres of the state, successful and sustained revegetation of bauxite residue impoundments is paramount.

Current methods of bauxite residue deposition by Alcoa utilise a 'dry stacking' process (Figure 1.1). Dry stacking involves separation of the coarse bauxite residue sand (BRS) (>150 µm), and fine (red mud) fractions (approximately equivalent in volume), 'de-watering' to a slurry of 50 to 60 % solids by weight, pumping to the impoundments or 'mud lakes' and spreading in numerous alternating BRS and red mud layers (1 to 1.5-m deep each). Air drying, encouraged by mechanised tillage when the surface will support machinery traffic, occurs before subsequent layer depositions. Due to the greater porosity allowing water infiltration and subsequent leaching of dissolved salts and hydroxides, BRS is seen as preferable to red mud as a substrate for revegetation. Hence, a deep (1.5 to 5 m) layer of BRS is used as the final capping layer of an impoundment. The direct revegetation of this BRS layer is desired.

Despite washing and recent innovations to decrease the BRS pH (such as encouraging carbonic acid formation through CO_2 dissolution), residual NaOH in the BRS gives the substrate extreme alkalinity (initial pH often >12) and sodic soil properties. Coupled with physical characteristics such as poor structure and low water holding capacity, the conditions in BRS are such that nutrient availability to plants,

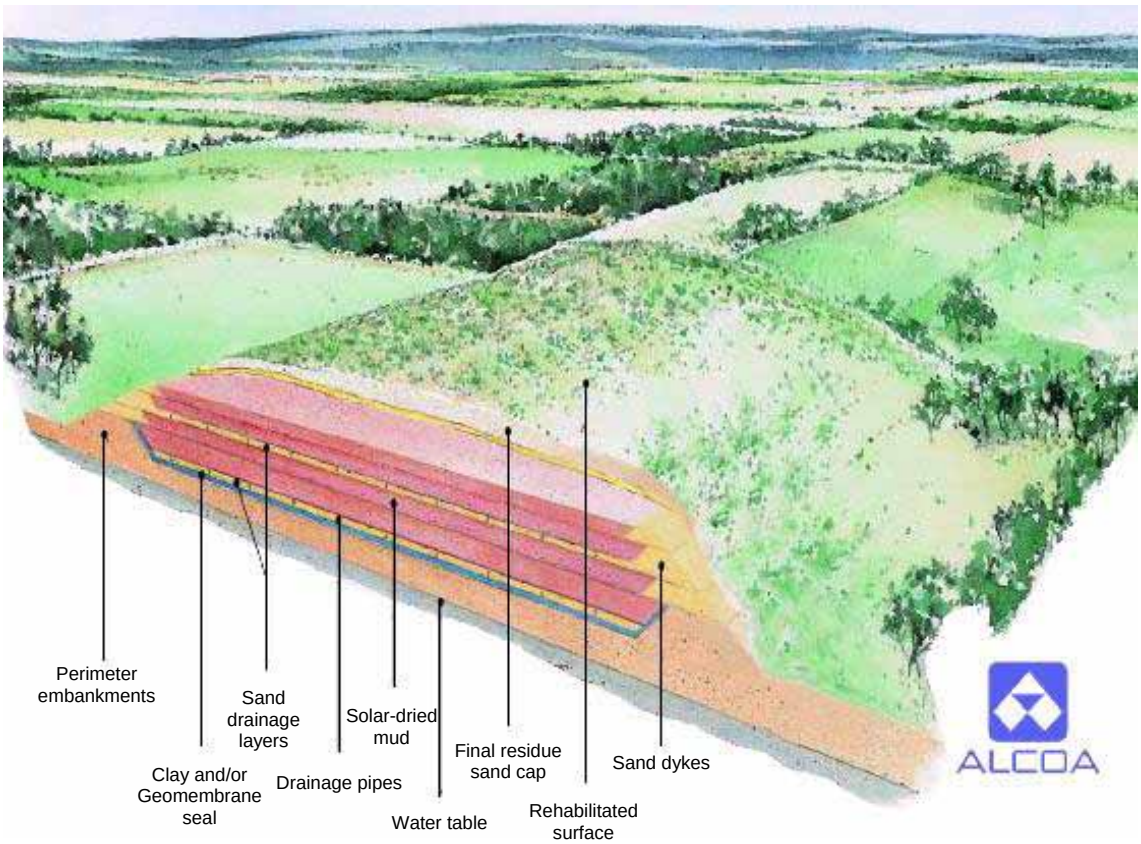

Figure 1.1. **Intermittent layers of bauxite residue sand and red mud through dry stacking in a residue disposal area leave a layer of residue sand as a final surface for revegetation (Alcoa World Alumina Australia, Environmental Department).**

especially that of micronutrients, is poor. Revegetation of BRS, therefore, is problematic. Plants used for direct BRS revegetation regularly show Mn deficiency (Gherardi and Rengel 2003a, 2003b, 2003c). Decreased Fe, Zn and Cu levels in plant tissues have also been found during periods of low rainfall and low irrigation (Alcoa World Alumina Australia, unpublished). Large broadcast fertiliser inputs, such as $MnSO_4$, have alleviated deficiency symptoms, but the residual value of these applications is low and further large inputs are continually required. Similarly, undesirably large irrigation water inputs have been required to sustain plant growth on the BRS deposits.

Clearly, continual excessive input of Mn (and other nutrients) and irrigation water is neither economically nor environmentally desirable. In working towards sustained and productive revegetation of BRS, research is required to investigate possible options for maximising productivity of BRS revegetation whilst minimising external nutrient and water inputs. This thesis details such an investigation, focussing on chemistry of Mn in BRS, the Mn nutrition of plants growing in BRS and storage and

movement of water inputs in a revegetated BRS system to provide a sound scientific basis for improved sustainability of BRS revegetation.

Preceding the experimental aims of the thesis, this chapter outlines scientific research and knowledge pertaining to processes that affect Mn availability in soils and soil-water-plant systems. The requirement for Mn in plant physiology, Mn uptake by plants, and the role of research into Mn and water availability for improving productivity and sustainability of revegetated bauxite residue are discussed. Due to the limited number of studies of plant nutrition on BRS, background is given on Mn availability and plant Mn nutrition in general, and with an emphasis on high pH soils.

MANGANESE: ESSENTIAL FOR PLANTS, ESSENTIAL FOR LIFE

Manganese has a unique and extensively studied chemistry; it can exist in the oxidation states 0, II, III, IV, VI and VII. In biological systems, oxidation states II and IV predominate, with III less common - often as a short-lived intermediate between the more common species. Hence, most biological functions of Mn stem from its potential as a sink and source of electrons in redox processes. Bartlett (1988) described Mn as "the paramount parking place for electrons," going so far as stating that the importance of Mn to life on earth takes precedence over O_2 because it is the provider of O_2, and even touting the possibility of Mn as the original medium of electron transfer giving rise to reduced substances in earth's primeval atmosphere. While Bartlett's statements may be rather speculative, Mn has been shown to play pivotal roles in a number of biological processes, including O_2 evolution in photosynthesis. Therefore, the importance of Mn in the evolution and development of the biology of the planet should not be underestimated.

Throughout the 1800s there was considerable debate among researchers over whether or not Mn be included in the growing list of essential plant elements, especially following the publication of Liebig's (1840) concerns that the mere presence of an element in plant tissue did not constitute proof of its essentiality for plant growth. Horstmar's (1851) work on oats in sand culture concluded that Mn did not seem essential unless Fe was in excess supply. Further progress was not made for half a century until workers in Japan and England (Azo 1902; Loew and Sawa 1902; Voelcker 1902) reported stimulated plant growth by Mn salts. A number of similar reports followed, as did claims of Mn having a catalytic role in vital enzymatic processes and in promoting photosynthesis, but the establishment of Mn as an essential element for growth of higher plants is attributed to the work of McHargue (1914; 1922) with

various plant species. In 1928 came the first of many recordings of Mn deficiency in Australia from observations of oats growing in peaty soil near Mount Gambier, South Australia (Samuel and Piper 1928).

The so-called "golden age" of plant nutrition followed these discoveries (Graham and Quirk 1988). The heightened research activity uncovered the vast complexity of Mn, its forms and relationships in soil and plant systems. Debate on a number of issues raised during this period continues presently, and will be touched upon in this chapter.

MANGANESE IN SOILS

Recent evidence indicates Mn to have an average total concentration of 900 mg/kg in the earth's crust (Barber 1995), making it the eleventh most abundant element. World–wide, natural soil Mn concentrations vary greatly, from traces in podzolic soils of Poland to 10 g/kg in the unleached soils of Chad (Swaine 1955). Most soils contain, on average, 200 to 300 mg total Mn/kg (Aubert and Pinta 1977). In Australia, Mn concentrations between 40 and 2500 mg/kg soil are commonly observed. Although these concentrations seem high, most Mn is "locked away" as a constituent of primary and secondary minerals, most commonly ferromagnesian silicates or as an isomorphic substituent for Fe and Mg in other minerals (Gilkes and McKenzie 1988). Concentrations of Mn found in soil solution, sorbed onto mineral and organic matter surfaces or incorporated into organisms are generally much lower, being in the range of 0.18 to 790 μ*M* (Barber 1995). Therefore, availability of Mn, rather than total Mn reserves, is the key to healthy plant Mn nutrition. Knowledge of total soil Mn concentrations is of little use or interest (Page 1962; Leeper 1970; Russell 1973; Rengel 2000). Manganese availability, in turn, is determined by various soil processes and reactions.

FORMS, REACTIONS AND AVAILABILITY

The chemistry of Mn and its compounds in soils is very complex. Extensive discussion of Mn chemistry is beyond the scope of this chapter (reader is referred to Bartlett 1988; Gilkes and McKenzie 1988; Norvell 1988 and references therein), but the widely-accepted chemical principles governing the availability of Mn in soil to plants are briefly discussed below.

There are numerous and diverse reactions in soils that involve Mn, including oxidation and reduction (redox), ion exchange, specific adsorption, and solubility equilibria (Norvell 1988). The chemistry of Mn in soils is complicated by the occurrence of the metal not only in the divalent state but also in oxidised forms. Manganese(II) is the only species expected in appreciable concentrations in solution (Morgan 1967; Lindsay 1979) and in association with exchange sites on soil surfaces, while in a variety of oxide-rich solid phases, Mn(III) and Mn(IV) predominate (Geering *et al.* 1969). Although Mn(III) has implicated roles as an intermediate in several redox reactions (Bartlett 1986), the high reactivities of Mn(III) and Mn(IV) and the low solubilities of their associated oxides preclude their persistence in soil solution. The pH, redox conditions and the characteristics of ligands and surfaces within a soil are factors which control the distribution of Mn between solution and solid phases, hence regulating Mn availability.

Historically, the forms of Mn in soils considered primarily for Mn supply to plant roots have been divided into three classes: exchangeable Mn; organically-bound Mn; and Mn oxides. Chemisorption of Mn on $CaCO_3$ surfaces will also control Mn solubility in calcareous soils (McBride 1979). These classes can be further sub-divided based on their release from soil by various extracting solutions (see Chapter II). The relative availability of the various soil-Mn fractions is dependent on soil chemical characteristics and the interactions between Mn and soil particle surfaces.

SURFACE REACTIONS, OXIDATION AND REDUCTION

Surfaces of mineral and organic material in soils can adsorb Mn^{2+}. Adsorbed Mn^{2+} can undergo secondary transformations and reactions such as oxidation, and Mn oxides themselves promote further oxidation of Mn^{2+} (Coughlin and Matsui 1976). The higher oxides of Mn [Mn(III) and Mn(IV)] cannot be taken up by plant roots without first undergoing transformation involving reduction processes. Therefore, processes that cause oxidation of Mn^{2+} in soils will decrease its availability. Prior to oxidation, various surface adsorption phenomena can hold Mn, at least temporarily, in forms that easily exchange with cations more specifically attracted to exchange sites (eg. Ca^{2+} and Mg^{2+}), or other more strongly bound forms (Curtin *et al.* 1980). Although not considered principal forms of soil Mn, adsorbed forms influence availability to plants as they are in pseudoequilibrium with aqueous Mn^{2+} (Warden and Reisenauer 1991).

Atoms of various elements present in the lattice structures of layer silicates impart a negative surface charge to which Mn^{2+} and other cations are attracted in a

diffuse layer. Electron spin resonance studies (McBride 1982) suggested, as expected, that electrostatic attraction of Mn^{2+} to such surfaces was of low specificity. As such, weakly adsorbed Mn is regularly involved in ion exchange with other metal cations in the soil solution. At high pH values (reportedly above pH 9 for Mn^{2+}, Lindsay 1979), however, metal hydrolysis is favoured and adsorption of metal cations is much stronger. Hence, through this innate Mn-fixing process, poor availability of Mn to plants is common in high-pH soils.

Similar to layer silicates, the adsorption of Mn^{2+} by surfaces of humic substances in soils appears to be of relatively low specificity. Negative charges on these surfaces are a consequence of the abundance of acidic functional groups, allowing Mn^{2+} and other cations to be held in exchangeable form (Norvell 1988). At common soil pH values, electrostatic attraction and weak outer sphere complexing are the dominant adsorption forces, but stronger inner sphere complex formation occurs to a greater extent at higher pH, especially when the ratio of humic substances to Mn^{2+} is high (Lakatos *et al.* 1977; McBride 1982).

Decreased uptake of Mn by plants, often leading to Mn deficiency, has been observed on many calcium-carbonate dominated (calcareous) soils around the world (see Reuter *et al.* 1988 and references therein). Calcium carbonate influences the system through its effects on pH, through specific surface chemisorption of Mn^{2+} (Bromfield and David 1978; McBride 1979) and through coprecipitation or formation of a manganocalcite (McBride 1979). Plant-available Mn is decreased by adsorption on, or precipitation with, carbonates (Reuter and Alston 1975; Jauregui and Reisenauer 1982).

Abiotic oxidation of Mn^{2+} is largely a surface-catalysed reaction with a first order dependence on Mn^{2+}, O_2, and active surfaces, and a second order dependence on OH^- (Morgan 1967; Coughlin and Matsui 1976). Oxides of Mn and Fe are among the most effective promoters of oxidation, but the reaction also appears to initiate on surfaces of $Mn(OH)_2$ and/or $MnCO_3$ formed at high pH and then to continue autocatalytically on the oxide products (Coughlin and Matsui 1976; Diem and Stumm 1984).

Biotic processes in soil also play a significant role in establishing equilibria between soil Mn forms and influencing redox reactions. Microbial reduction of Mn was demonstrated late in the 19th century, and microbial oxidation of Mn shortly after (Graham and Quirk 1988). It was noted that these reactions could occur at some distance from the organism, implicating the activity of one or more diffusible exudates (Beijerinck 1913). These processes are largely bacterial-dominated and will be discussed in further detail later.

Inorganic complexes

The importance of inorganic complexes of Mn^{2+} in Mn availability is uncertain. In soil solution, fully hydrated Mn^{2+} is by far the most dominant inorganic species, but SO_4^{2-}, HCO_3^- and Cl^- have been reported as the most significant inorganic complexing agents (Lindsay 1979). However, high activities of these species (0.005, 0.016 and 0.25 M for SO_4^{2-}, HCO_3^- and Cl^-, respectively, Norvell 1988) would be required in solution for the complexes to dominate over aqueous Mn^{2+}. Additionally, HCO_3^- will only be expected to play a substantial role in complexing Mn^{2+} under alkaline soil conditions. At high pH in soil, hydroxyl complexes may also be formed but in comparison to the thermodynamically favoured higher Mn oxides in these soils, such complexes seem only to play minor roles in decreasing Mn availability.

Organic complexes

Numerous organic compounds exist in soil, originating from plant, fungal and microbial sources. Many of these, including organic acids, amino acids, simple sugars, hydroxamate siderophores, phenols and other compounds can act as biochemical ligands to form complexes and chelates with Mn (Stevenson 1982). By altering the patterns of Mn-redox processes (contributing to enhanced Mn reduction, preventing Mn oxidation/reoxidation, Marschner 1988, 1995), these organic complexing and chelating agents influence available and unavailable soil-Mn pools. However, rapid microbial breakdown of many organic compounds results in their low concentrations in soils, and the low stability of Mn(II) complexes generally means other metal-ligand complexes (eg. Mg^{2+} and Ca^{2+}) are preferentially formed. Consequently, the importance of these organic ligands as complexes and chelates for improving Mn availability is limited to specific soil-root interface regions. Organic compounds of plant and microbial origin are further discussed later in this chapter.

EXTRACTING AND ESTIMATING SOIL MANGANESE

The estimation of available Mn in soils has been a contentious issue among soil scientists since the discovery of Mn as an essential element for plant growth. A wide variety of extractants have been used to estimate Mn in soil, from water alone, to aqueous solutions of complex organic chelating agents (Reisenauer 1988), and even Coca-Cola® (Schnug *et al.* 1996) as an alternative in places where chelating chemicals

are limited in supply and affordability. Clearly, the lack of standard procedures has led to the use of such a diverse range of extractants in the laboratory, with most of these extractants having been originally proposed for other purposes. The diversity of soils and the associated diversity in Mn behaviour within them has also contributed to the vast range of compounds and procedures cited in the literature. Even widely-accepted procedures such as DTPA extraction (Lindsay and Norvell 1978; Norvell 1984) have limited applicability in certain soils, and the relationship between DTPA-extractable Mn and plant growth is often tenuous (see Reisenauer 1988).

In order to properly understand how available Mn may be effectively manipulated by fertiliser addition, information about the various pools of soil Mn is required. A number of sequential extraction procedures have been proposed to estimate amounts of various fractions of micronutrients in soils (eg. Tessier *et al.* 1979; Shuman 1985; Han and Banin 1995), but few methods have been developed to specifically characterise soil Mn fractions (Warden and Reisenauer 1991; Tong *et al.* 1995). Each sequential phase in the extraction utilises an extractant determined as specific to the particular Mn fraction (eg. readily-soluble Mn, Mn oxides etc.). Extractants rely on simple dissolution, ion exchange between aqueous solution or chelating solutes and Mn adsorbed on soil particles, or acidic breakdown of precipitated Mn carbonates and and reduction of Mn oxides. Unintended release of Mn from different fractions, especially oxides, by a particular extractant is the most important potential error with sequential Mn fractionation techniques (Warden and Reisenauer 1991). To avoid over- or underestimation of any single fraction, the extractants, sequence and timing may require tailoring for the particular characteristics of each soil.

With the vast array of extracting solutions used for estimation of Mn forms in soil, and the general lack of standardised procedures, nomenclature and classification of Mn forms with respect to availability for plant uptake presents many challenges. By and large, Mn forms are defined rather arbitrarily by the operational extracting solution used. This is done out of convenience, and reflects the limitations of extractants to mimic the extraction capacity of plant roots, and the restricted understanding of Mn chemical behaviour in soils and its relationship to plant uptake ability. Availability of various Mn fractions will differ depending on the inherent characteristics of different soils, which can themselves differ with seasons. Additionally, levels of available Mn forms, such as readily-soluble Mn, are often not well related to plant uptake (Leeper 1947; Reisenauer 1988). One must be mindful of such limitations of soil extraction procedures when interpreting results and extending the inference to possible Mn fertiliser recommendations and plant responses.

EFFECTIVENESS OF MANGANESE FERTILISERS

Many compounds have been utilised as sources of Mn for plants. A scan of the literature reveals $MnSO_4$, $MnCl_2$, $MnCO_3$, MnO, MnO_2, Mn frits, and chelated Mn compounds have been added to soils as Mn fertiliser (Fitts *et al.* 1967; Mortvedt and Giordano 1975; Mascagni and Cox 1985; Norvell 1988; Walter 1988), but the sulphate form is most widely applied. Knowledge of the soil characteristics is vital in selection of the most appropriate Mn fertiliser source. Even so, the effectiveness of Mn fertiliser can vary greatly even on a small spatial scale in soils due to micro-environmental variations in pH, redox potential, particle size, particle surface characteristics, water content and other factors.

The aim of Mn fertilisers is to release Mn^{2+} which, in turn, is taken up by plants. However, depending on the soil conditions, most Mn^{2+} will undergo transformations and reactions with the soil and become distributed among the various fractions of soluble and solid phase Mn (Norvell 1988). In acidic soil, Mn^{2+} from fertiliser sources is often readily available in solution or readily exchangeable from weak adsorption sites on soil particles (see Martens and Westermann 1991), whereas as soil alkalinity increases, the tendency for strong adsorption and oxidation processes is great, holding much of the added Mn in forms unavailable for plant uptake. As such, the effectiveness of Mn fertilisers in alkaline soils is markedly decreased.

Addition of various soil amendments can be effective in acidifying alkaline soils to increase soluble and exchangeable Mn^{2+} fractions. Encouraging nitrification by using ammonium-based N sources can decrease soil pH and increase mobility and availability of Mn (Petrie and Jackson 1984). The acidifying power of 100 kg of ammonium nitrate theoretically equates to 60 kg of lime addition, and 100 kg ammonium sulphate is equivalent to 110 kg of lime. Incorporation of Mn in an acidifying macronutrient fertiliser mix will generally increase the effectiveness of the added Mn (Norvell 1988). Applications of strongly acidic fertilisers – monocalcium phosphate monohydrate (MCP), for example – can decrease soil pH levels below 2.0 near the site of application (Lindsay and Stephenson 1959). The use of MCP alone can be as effective in increasing plant uptake of Mn as adding Mn compounds to soil (Voth and Christenson 1980), as the mobilisation of previously unavailable Mn from the soil is more readily achieved at the sites where pH is decreased, and retarding the oxidation of Mn^{2+} slows the reversion of Mn to plant-unavailable forms.

The effectiveness of acidifying soil amendments is dependent on various soil conditions, and microbial activity is often a critical determinant. For example, elemental sulphur is reliant on soil bacteria to convert it to an acidifying form. Additionally, effective acidification by elemental sulphur requires: sufficient incorporation to ensure extensive soil contact; soil moisture; aerobic conditions; temperature conducive to bacterial growth; and sufficient time for reaction completion.

In soils with a predominantly sandy texture in upper horizons, low water holding capacity can have significant consequences for fertiliser addition. Availability of water is a critical determinant of nutrient uptake. Water provides not only the medium for dissolution of fertilisers and dissociation of ions, but also the vehicle for exchange between the soil particles and plant roots. Hence, nutrients added to topsoil will be unavailable to plants during dry periods (Pinkerton and Simpson 1986). For example, drying of topsoil restricted the root extension and uptake of Mn by lupin (Crabtree *et al.* 1998). Even short periods of drought can result in the appearance of Mn deficiency symptoms in crops (Cheng and Ouellette 1971). When highly-porous soils also possess specific chemical characteristics, such as high alkalinity encouraging rapid transformation of Mn to forms unavailable for plant uptake, traditional methods of broadcast Mn fertiliser application are often insufficient in preventing Mn deficiency in crops (Reuter *et al.* 1988). Any Mn fertiliser applied to the surface of such Mn-fixing soils tends to remain on the surface rather than to reach the root zone.

Fertiliser application methods alternative to broadcasting Mn include incorporation of broadcast Mn by tillage, foliar application, coating seed with Mn, banding Mn in drill lines with the seed, and banding Mn deep below the seed. Coating seed with Mn increased Mn uptake of wheat (Marschner *et al.* 1991) and improved Mn nutrition and yield of barley throughout a rotation (Longnecker *et al.* 1991), but little information is available for perennial crops. The other alternate methods also have merit, but on Mn-fixing soils as mentioned above, broadcast, incorporated and foliar applications tend to have a low residual value. Taking legumes, often considered sensitive to Mn deficiency, as an example, there are many reports of broadcast methods being ineffective in supplying Mn to plants (eg. lucerne, Gupta 1986; Heckman *et al.* 1993). In contrast, banding of Mn fertiliser deep below the seed provided an effective source of Mn to lupins for at least 15 years (Brennan 1993), successfully preventing the 'split-seed' disorder and increasing yield (Crabtree 1999; Brennan 2001a). Additionally, optimum rates of Mn application are generally much lower for banding than broadcasting (see Reuter *et al.* 1988; Martens and Westermann 1991 and references therein).

The success of deep-banded Mn as a fertiliser source stems from a general increase in moisture availability in the vicinity of the Mn band in comparison to the surface horizons, enabling nutrient dissolution, root elongation and rhizosphere activity. Increased ion concentrations and pH effects may be quite different near fertiliser bands than in the bulk soil (Norvell 1988). Fertiliser bands also minimise exposure and contact of Mn with reactive sites on soil particles (Reuter *et al.* 1988). Hence, the residual stability of Mn^{2+} in zones of high concentration is generally greater than that of widely dispersed Mn in broadcast-incorporated systems (Murphy and Walsh 1972; Mortvedt and Giordano 1975). Banding of Mn has, therefore, superseded broadcast methods for agriculture in many cases (Reuter *et al.* 1988), with the potential for decreasing the required inputs of Mn into the system and improving fertiliser efficiency. However, data regarding optimal placement depth of Mn are scarce. Optimal depth and Mn rate will invariably differ between species and with different soil types, thus there is a role for further investigations of deep-banded Mn in supplying adequate Mn for plant productivity.

MANGANESE AVAILABILITY IN THE RHIZOSPHERE

The rhizosphere is the zone of soil surrounding a root in which physical, chemical and biological properties have been altered due to the presence and activity of the root. Availability of nutrients and water and the microbial activity of the rhizosphere govern plant growth. Nutrient availability in the rhizosphere appears subject to control by a combination of factors such as soil properties, plant characteristics and interactions of plant roots with microbes and the surrounding soil (Bowen and Rovira 1991). Although precise mechanisms have yet to be elucidated, there is no doubt that chemistry and biology of the rhizosphere have a profound effect on plant availability and hence uptake of Mn (Rengel 2000).

CHEMISTRY OF THE RHIZOSPHERE

As mentioned above, the availability of Mn in soils for plant uptake is governed by processes of reduction and oxidation: the divalent or 'reduced' form (Mn^{2+}) is readily available to plants while oxidised Mn (predominantly Mn^{4+}) is not. In turn, the balance of soil reductive and oxidative processes is governed by a combination of chemical, microbial and plant factors in the soil, i.e. the rhizosphere. Areas within soils that have a partially restricted O_2 supply, through limited aeration or a predominating supply of

available electrons, show decreased redox potential. At such sites (eg. junctions between root and soil surfaces, microbial and soil surfaces, and aggregates and soil pores, Bartlett 1988) Mn can serve as an alternative electron acceptor in redox processes, being transformed to the reduced ionic species. Various organic constituents of roots and known root exudates have been proposed as the source of the required electrons (Reisenauer 1988; Uren and Reisenauer 1988).

In high-pH soils, the chemistry of Mn is not well understood, and various confounding factors have been surmised to describe the varying availability of Mn in these soils. Theoretically, in a well-aerated soil, a unit of pH increase should result in a 100-fold decrease in the Mn^{2+} concentration of the soil solution (Barber 1995). However, Neilsen *et al*. (1992) observed a lower 5- to 10-fold decrease in Mn^{2+} concentration with pH increase of 0.5 units. The difference could be attributed to reactions that change the solubility equilibria between the various Mn forms, such as complexation with organic compounds.

ROOT EXUDATES

Increases in micronutrient availability can stem from a lowering of soil pH by exudation of H^+ and organic acids (Jarvis and Hatch 1985; Dinkelaker *et al.* 1995). Although Mn-uptake processes in rice were greatly inhibited by increasing concentrations of H^+ ions in solution culture (van der Vorm and van Diest 1979), strong increases in plant Mn concentrations as soil pH decreases (see Marschner 1988) demonstrate that depressed uptake is more than compensated by the associated steep increases in Mn^{2+} concentration of soil solution (Sanders 1983). Hence, acidification of the rhizosphere can play an important role in the mobilisation of Mn. Changes in rhizosphere pH depend on the buffering capacity of the soil, initial or bulk soil pH, form of nitrogen supply, plant species and nutritional status of the plant (Riley and Barber 1971; Nye 1981; Marschner *et al.* 1986; Thompson *et al.* 1993).

Changes in pH and patterns of H^+ release alone do not account for levels of increased availability and uptake of Mn in the rhizosphere (Uren 1981), suggesting that other reducing agents play a major role. Root exudates comprise of both high- and low-molecular-weight solutes released or secreted by plant roots, and it is low-molecular-weight components, organic acids and phenolics that are most often implicated in increased solubility of nutrients (Marschner 1995). Marschner (1988) provides several simplified mechanisms for Mn mobilisation in the rhizosphere (Figure 1.2). However, precise modes of action and the nature of exudate components involved in Mn

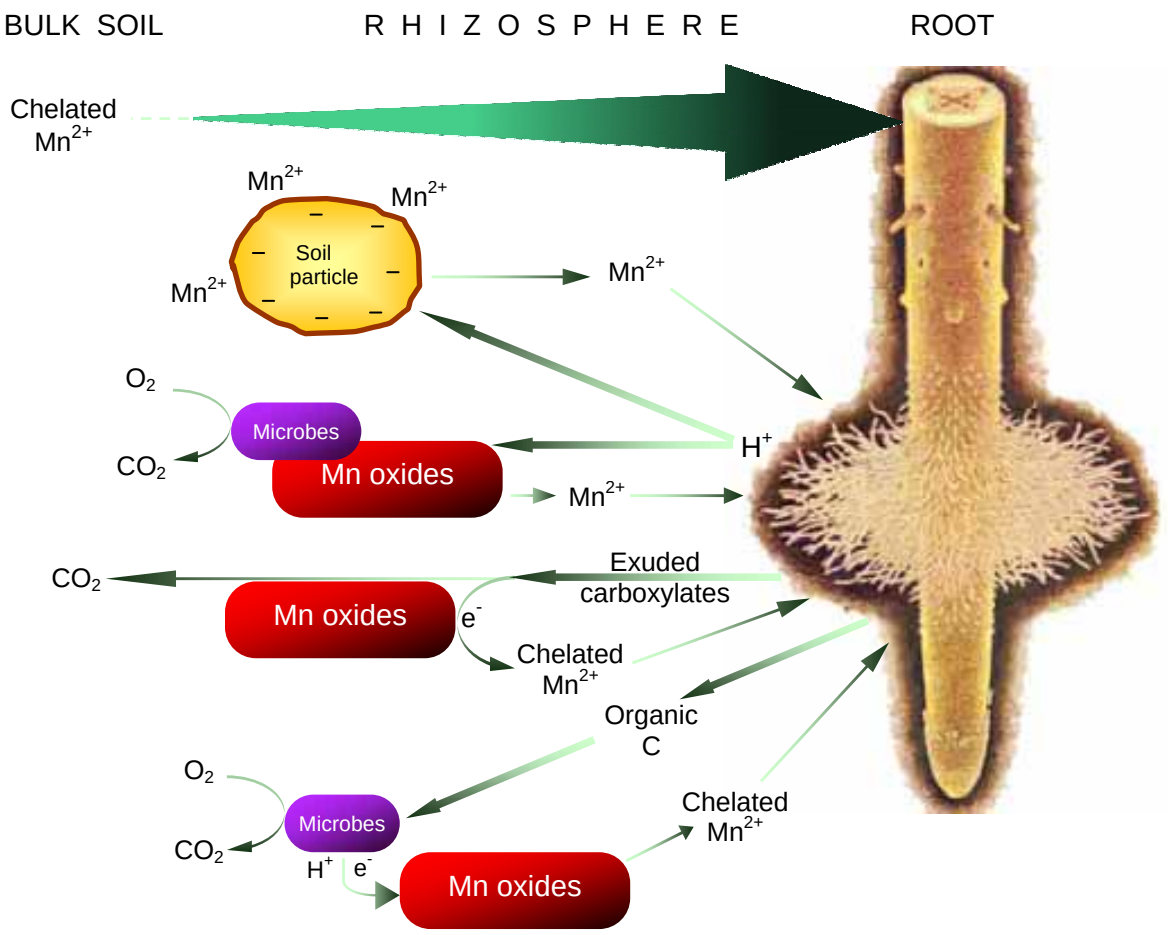

Figure 1.2 **Simplified theoretical mechanisms of mobilisation of manganese in the rhizosphere. Adapted from Marschner (1988).**

mobilisation are still unclear. Oxidation of malate, an important component of root exudates, followed by secondary hydrolysis and oxidation at the surface of MnO_2, released significant proportions of Mn^{2+} (Jauregui and Reisenauer 1982). Chelation of Mn^{2+} by organic acid anions – the conjugated bases of organic acids, prevents its reoxidation and increases the mobility of reduced Mn in the rhizosphere (Marschner 1995). Malate and citrate were suggested as reductants that mobilised Mn in the rhizosphere of wheat (Godo and Reisenauer 1980), but in high-pH soils, which commonly harbour Mn-deficient plants, their potential for forming stable complexes with micronutrients is significantly diminished (Jones and Darrah 1994).

Bromfield (1958a; 1958b) provided the first experimental evidence for exudate-mediated release of reduced Mn from MnO_2. Compounds in collected exudates from roots of wheat (Godo and Reisenauer 1980) and white lupin (Gardner *et al.* 1982) were found to cause reduction of Mn oxides. Dinkelaker *et al.* (1989) also found increased Mn availability in the rhizosphere of white lupin. However, there is often confusion as

to whether the compounds responsible for Mn solubilisation are of plant or microbial origin. Barber and Lee (1974) found that a low-molecular-weight substance, exuded from both root and microbial cells, mobilised Mn in the rhizosphere and/or promoted transport across the plasma membrane of root cells. Using axenic cultures of sunflower, Uren (1981) clearly established that roots alone could facilitate reduction of Mn oxides after roots of numerous other species had been shown to be capable of similar reduction under non-sterile conditions (eg. Jones and Leeper 1951; Bromfield 1958a, 1958b; Passioura and Leeper 1963). As early as 1934, Leeper coined the phrase "contact-reduction" to describe this root-mediated reduction of higher Mn oxides (Leeper 1934), but even though root exudates are involved, the mechanism still has yet to be fully elucidated. In addition, quantification of organic exudates is difficult due to crude collection techniques and rapid microbial degradation. Further development of collection and analysis techniques to determine the nature of root exudate components in relation to Mn uptake and form in the rhizosphere is warranted.

MICROORGANISMS

Discussion of availability of Mn in the rhizosphere is not complete without mention of soil microbes. Activity of rhizosphere organisms can be stimulated by compounds released by roots, from which they derive energy (Lynch and Whipps 1990). In fact, the term *rhizosphere* was first used to describe areas of increased microbial activity in the soil surrounding plant roots (Hiltner 1904). Owing mainly to the supply of organic carbon, bacterial population density in the rhizosphere can be 5- to 50-times higher than in the bulk soil (Marschner 1988). Experiments of Whipps and Lynch (1983) on wheat and barley revealed bacterial biomass sustained by carbon compounds from root exudates to be as much as 36 % of root dry weight.

Numerous genera of soil-borne bacteria and fungi have the capacity to oxidise or reduce Mn, or both, depending on the particular conditions in the soil environment, and hence play an important role in Mn nutrition of plants. The involvement of microbes in Mn oxidation and reduction processes in soils was established decades ago (see Ghiorse 1988 and references therein for a wider historical review). Chemical oxidation of Mn takes place in soils only at pH values above 8.5 to 9 – values uncommon in agricultural soils (Norvell 1988). Because the autooxidation of Mn by atmospheric O_2 cannot be demonstrated unless the pH is above 8 (Sung and Morgan 1981; Diem and Stumm 1984), the formation of Mn oxides in most soils has been considered to be carried out

only by specific compounds of microbial origin, and the activities of soil microorganisms have been studied in this regard.

Microorganisms which oxidise Mn (Bromfield 1979) will decrease its availability for plant uptake. Conversely, microorganisms which reduce oxidised Mn will increase its availability to plants, so that the ratio between Mn oxidisers and Mn reducers in the rhizosphere may ultimately determine the Mn status of the plant. Manganese-deficient soybean roots harboured up to 10-fold greater numbers of Mn-oxidising bacteria than roots of Mn-adequate plants in the same crop (Huber and McCay-Buis 1993). Fewer Mn-reducing bacteria were also found on Mn-deficient plant roots than Mn-adequate plant roots in the same study. Suppression of Mn-oxidising microorganisms in the rhizosphere by production of selectively toxic root exudates can lead to improved Mn availability and plant Mn nutrition (Timonin 1965), and is a possible mechanism for the tolerance of certain plants to potentially Mn-deficient soils.

The opportunistic use of Mn-reducing bacteria has resulted in improved Mn nutrition of plants. After inoculating wheat roots with *Pseudomonas flourescens* spp. in order to control the take-all fungus (*Gaeumannomyces graminis*), increased Mn levels were seen in plants (Marschner *et al.* 1991), and it was postulated that such applications may improve Mn uptake and growth in Mn-deficient soil. Similarly, inoculation of soil with *P. fluorescens* strain 2-79 increased Mn uptake by plants (wheat, Wilhelm *et al.* 1987; soybean, Huber and McCay-Buis 1993). The use of bacterial inoculants for decreasing the Mn deficiency potential of soils, however, does not appear to have become a popular management option.

There are varied and often contrasting reports as to the effect of mycorrhizal fungi on Mn uptake by plants, but mycorrhizal colonisation commonly will result in impaired, rather than improved, Mn nutrition (Posta *et al.* 1994; Abbott *et al.* 1995). Mycorrhizal fungi tend to promote populations of Mn-oxidising microorganisms in the rhizosphere (Kothari *et al.* 1990; 1991). Changes in the spectrum of rhizosphere microorganisms are attributed to altered patterns of root exudation upon mycorrhizal colonisation (Posta *et al.* 1994). Although lower uptake of Mn by mycorrhizal than non-mycorrhizal plants has been found, plant growth retardation due to Mn deficiency has never been attributed to mycorrhizae, and the associated enhanced uptake of other poorly-mobile soil nutrients, such as P, Zn and Cu, is overwhelmingly beneficial to plant growth.

MANGANESE UPTAKE AND DISTRIBUTION BY PLANTS

Research publications dealing with Mn uptake and transport processes, as identified more than fifteen years ago by Clarkson (1988) and more recently by Rengel (2000), have been extremely scarce since the mid-1970s. Although some recent research on Mn transporters in the plasma membrane has been published, hopes of a wide rejuvenation of Mn uptake kinetics work remain unfulfilled and many issues pertaining to plant Mn uptake kinetics remain unresolved.

UPTAKE BY PLANT ROOTS

Form of manganese supply for uptake by plant roots

It is generally accepted that the Mn supply at the root surface is predominantly divalent Mn (Mn^{2+}) ions (Clarkson 1988), but free Mn^{2+} ions are uncommon in soil solution. Manganese is thought to move to the root surface bound to organic compounds in the soil or soluble soil organic ligands (organic acids, amino acids, phenolic acids and simple sugars) sourced from root exudates or of microbial origin. The Mn is then thought to dissociate from these ligands prior to absorption (Stevenson and Ardakani 1972; Graham 1979), and is absorbed by plant roots as Mn^{2+}. It has been postulated that since both the free Mn^{2+} cation and chelated forms can co-exist in soil solution, both forms are readily taken up by plants (Barber 1995). However, the experimental evidence does not fully substantiate this theory. When supplied with free Mn^{2+} and MnEDTA, the free cation was absorbed at up to 50 times the rate of that supplied in chelated form (Barber and Lee 1974). When only MnEDTA was supplied in the same study, carbon from the ^{14}C-labelled EDTA was found inside the plant, but it was impossible to deduce whether transport across the plasma membrane occurred as a complex with Mn or independent of it.

Garcia and de la Puente (1977) have presented evidence that oat plants growing in a nutrient solution were able to absorb Mn as a trivalent cation (Mn^{3+}) via the formation of a soluble pyrophosphate complex, but dismutation of Mn(III) through its high reactivity, oxidative decomposition of associated ligands and the low solubility of Mn(III) oxides limits the persistence of Mn(III) complexes in soil solution (Dion and Mann 1946; Davies 1969). Thus, Mn(III) is unlikely to provide plants with a major Mn source for uptake in soil-plant systems.

Nonetheless, Norvell *et al.* (1993) clearly showed that a plasma-membrane-embedded ferric reductase in pea and soybean was able to reduce Mn^{3+} to Mn^{2+}. The research contradicts earlier theory that the need for such a reductase was unlikely since the root cell plasma membrane was bathed in Mn^{2+} (Clarkson 1988). The reductase activity is stimulated under Fe deficiency (Norvell *et al.* 1993) but not under Mn deficiency (Delhaize 1996; Cohen *et al.* 1997). Enhanced reductase activity in Fe-deficient bean plants also increased uptake of Mn (Fleming 1989).

Uptake and movement through the root

Roots generally do not possess a single zone through which most of the nutrients required by the plant are absorbed, although commonly as the distance from the root tip increases, the rate of ion uptake decreases (Clarkson and Hanson 1980). This may be due simply to older root parts existing in regions of soil that have already been explored and depleted of nutrients (Salisbury and Ross 1985). Younger meristematic cells at root apices tend to have a higher nutrient requirement for growth than differentiated cells in basal zones (Marschner 1995), but little is known about the locations along the root where micronutrients are absorbed. Evidence points to the apoplast and the symplast as pathways for movement of divalent metal ions across tissues (Clarkson 1991), but whether or not one is preferential to the other is yet to be established.

The apoplastic pathway encompasses a system of pores and wall surfaces outside the plasma membrane of the epidermal, cortical and stelar parenchyma cells, through which water and solutes move (Läuchli 1976). The pathway is interrupted by impermeable layers in the endodermis (the Casparian band) and, in some species, the hypodermis (Clarkson *et al.* 1978; 1987). In contrast, micronutrient uptake and transfer via the symplast is generally restricted to the peripheral layers, including the root hairs, which are metabolically active and have the capacity to actively absorb ions. However, there is some question over the ability of the symplast of roots to deliver adequate amounts of nutrients to the xylem (Clarkson 1991).

MANGANESE IN THE XYLEM AND PHLOEM

Manganese transport within plants occurs in both the phloem and xylem. Reported rates of 41 to 51 nmol Mn/g root dry weight per day in tomato xylem sap (Pich and Scholz 1996) compare with 1 to 3 nmol per day in phloem sap of castor bean (Schmidke and Stephan 1995). The literature shows varying reports of Mn concentrations in xylem and

phloem sap (see Rengel 2000) and no consistent relationship is seen between Mn concentrations in xylem and phloem within or among plant species. However, when Hocking *et al.* (1977) analysed both the xylem and the phloem sap from the same lupin plant, Mn concentrations in the phloem were significantly higher than in the xylem. Whether higher phloem than xylem Mn concentrations are specific to the lupin species tested by Hocking *et al.* (1977), or whether it is a consequence of greater accumulation in phloem due to the lower translocation rates, is as yet undetermined due to the rarity of such studies.

XYLEM

Transpiration causes movement of xylem sap from roots to shoots. Within this transpiration stream, Mn can move freely in varied forms and concentrations. Early suggestions of xylem sap containing Mn complexed with amino acids and carbohydrates (Höfner 1970), were not immediately confirmed by subsequent studies (Tiffin 1972; Graham 1979), leading to a consideration of Mn being the most dissociated micronutrient in xylem fluid (Tiffin 1972). However, the chemical composition of xylem sap suggested that Mn does bind weakly to low-molecular-weight organic acids in the xylem exudate of tomato and soybean (White *et al.* 1981a; 1981b; 1981c) and it was calculated that up to 40 % could be complexed with citrate and malate, while 60 % remained as Mn^{2+} ions. Although xylem sap properties (ligand concentrations and pH) were critically affected by collection technique, it was clear that Mn formed less stable complexes with xylem fluid components that did Cu, Fe, Ni or Zn (White *et al.* 1981a; 1981b).

It is generally accepted that Mn will move passively in the transpiration stream, but it has also been shown that Mn can be absorbed from the xylem fluid and temporarily stored in stem tissues to be later released and redistributed during grain development in both monocotyledonous and dicotyledonous species (Hannam *et al.* 1985b; Pearson and Rengel 1995). Such storage is considered to be an important strategy for providing Mn to developing plant parts during possible periods of low Mn uptake by roots, as remobilisation in the xylem is relatively easy (Rengel 2000). However, the mechanism of xylem remobilisation and Mn transport appears somewhat selective (eg. toward organs of grain development, but not toward leaves, though the xylem pathways stem from similar main vein origins) and is currently not understood.

PHLOEM

Whereas xylem sap-Mn moves freely and passively and is generally present as the divalent ion in equilibrium with unstable Mn–organic acid complexes depending on pH and sap composition, the movement of Mn in phloem sap is more complex. It seems that Mn accumulated in leaves cannot be remobilised whilst that in stem and root tissues can. In addition, Mn appears highly mobile in the phloem to seeds but immobile to roots (Loneragan 1988).

Similar to xylem sap, some Mn in phloem sap is bound to organic compounds, but the overwhelming majority is apparently present in ionic form (van Goor and Wiersma 1976). This occurs even with the higher pH and higher concentrations of amino acids and organic acids in phloem sap compared with xylem fluid. In castor bean plants, Mn in phloem sap was not complexed with proteinogenic amino acids (Schmidke and Stephan 1995), while some binding with peptides of molecular weight between 1000 and 5000 kDa has been found (van Goor and Wiersma 1976). Stephan and Scholz (1993) proposed a nonproteinogenic amino acid, nicotianamine, to be a chelator required for heavy metal transport in the phloem. In addition, nicotianamine is the precursor to numerous phytosiderophores (Stephan and Scholz 1993). The proposal was based on the nicotianamine concentration in phloem sap being in the same range as many micronutrient cations. Further research confirmed the ratio of nicotianamine to micronutrients in castor bean phloem to be constant, even after endosperm removal, while concentrations of other amino acids dropped dramatically (Schmidke and Stephan 1995). At the alkaline pH of phloem sap, the Mn–nicotianamine complex is relatively stable (Schmidke and Stephan 1995; Stephan *et al.* 1996). Phloem transport of Mn in such a complex can therefore be considered likely, although in tomato plants the importance of nicotianamine in Mn translocation appears to be low (Pich and Scholz 1996).

When applied to plant leaves, ^{54}Mn is translocated via the phloem. This has been demonstrated in numerous species (eg. bean, Bukovac and Wittwer 1957; oat, Vose 1963; soybean and tomato, Ohki 1976; maize, el-Baz *et al.* 1990). However, under controlled conditions, where plant tissues are not subject to nutrient leaching by rainfall, there is no experimental evidence of Mn that had accumulated in leaf blades being lost as the leaves aged. Such remobilisation would have to occur via the phloem.

There is some evidence that export of Mn can occur from stems and petioles. Manganese supplied to wheat roots accumulated in stems rather than leaves, from where it was later transported to developing grains (Pearson and Rengel 1995). It was

speculated that this remobilised Mn was transported by the xylem, but how Mn can be selectively transported in the xylem is not understood. In lupin, petiole–Mn decreased by nearly 50 % when deprived of Mn supply (Hannam *et al.* 1985b), but there is some conjecture as to whether this Mn was translocated via phloem to developing plant parts or simply moved into the nearby leaf blade.

The inability to export accumulated Mn from leaf blades means excess Mn in older leaves cannot be utilised in the development of new growth, and may explain why Mn deficiency symptoms are most prevalent in young shoots and leaves. In addition, studies (see Loneragan 1988) often show lower Mn concentrations in phloem than those required for adequate supply to developing seeds. Yet, at least for lupin, it has been reported that under low Mn availability situations, up to 90 % of Mn in seeds was supplied via the phloem (Hocking *et al.* 1977).

ACCUMULATION AND DISTRIBUTION OF MANGANESE IN PLANT TISSUES

The distribution of Mn among plant organs shows distinct trends. When Mn supply is adequate, Mn concentrations are generally: 1) higher in roots than in leaves; 2) higher in mature leaves than young leaves; 3) higher in leaves than in stems, reproductive organs and seeds (Nable 1983). As plants grow into Mn deficiency, root Mn concentrations can fall rapidly in many species (see Loneragan 1988). Onset and development of Mn deficiency in subterranean clover, for example, resulted in roots and young leaves with extremely low Mn concentrations, while concentrations in mature leaves remained high (Nable and Loneragan 1984). This is characteristic of a nutrient with low phloem mobility.

Work on oats in the 1960s showed three discernible fractions of Mn in roots of Mn-adequate plants (Munns *et al.* 1963a; 1963b). Two fractions were labile, one being rapidly exchangeable, the other being more slowly exchangeable but moving rapidly to the shoot. The labile fractions were generally isolated in younger parts of root systems such as root tips. The third fraction was non-labile, generally found in older root tissues, and did not appear to translocate to the shoot while external Mn supply was adequate. Vose (1963) proposed this non-labile fraction to constitute a storage reserve of Mn as it was mobilised and translocated to the shoot when external Mn supply was interrupted. Grain loading of Mn was also enhanced by remobilisation of Mn from roots (Pearson and Rengel 1994; 1995).

Autoradiographs of the leaves of Mn-adequate plants (flax, cabbage, lucerne, pea, soybean and tomato) grown in solution with ^{54}Mn revealed that Mn preferentially

accumulates in the marginal and interveinal tissues (Millikan 1951; Romney and Toth 1954). Further study showed that ^{54}Mn applied to a mature leaf was absorbed, moved with the transpiration stream, accumulated in leaf apices, and was not readily retranslocated (Bukovac and Wittwer 1957). Work by Jones (1970) with Mn-adequate maize leaves reaffirmed the earlier findings that Mn accumulates in the leaf apices and margins, with concentrations ranging from 49 µg/g at the leaf base to 124 µg/g at the tip, and 70 µg/g at the midrib to 272 µg/g at the leaf margin. Electron probe X-ray microanalysis by Memon *et al.* (1980; 1981a; 1981b) showed that within leaf tissue of Mn-adequate plants, Mn is more concentrated in peripheral cells than cells of the vascular bundle, and the tendency was for greater accumulation in cells of the epidermis than palisade and spongy parenchyma cells.

Studies regarding the distribution of Mn within higher plant cells have been primarily restricted to experiments involving high/toxic Mn supply or observations of Mn accumulating species (eg. Memon *et al.* 1980; 1981a; 1981b). Hence, definitive information in the distribution of Mn within plant cells is scarce. Limited evidence, such as that reported by Horst and Marschner (1978a; 1978b) on Mn-adequate bean leaves, suggests that appreciable Mn may be stored in the vacuoles of leaf cells. However, based on the ease with which Mn may be leached from leaves, Loneragan (1988) suggested that some of the Mn analysed in these studies may have originated from non-vacuolar sources. The presence of silicic acid in culture solution appeared to increase vacuolar Mn in bean leaves to greater levels than in plants grown without silicic acid (Horst and Marschner 1978a, 1978b). The necrotic spotting symptoms, but not the leaf chlorosis associated with Mn toxicity, are reduced in bean (Horst and Marschner 1978a) and barley (Horiguchi and Morita 1987) when Si is applied. In the Mn accumulator *Acanthopanax sciadophylloides*, chelation with oxalate appears to allow storage of large amounts of Mn in cell vacuoles (Memon and Yatazawa 1984).

KINETICS OF MANGANESE UPTAKE

IMPEDIMENTS TO UNDERSTANDING KINETICS OF MANGANESE UPTAKE

The technical inability to reliably measure Mn flux across membranes is the major obstacle to better understanding of Mn transport systems and uptake kinetics. The inherently low plant requirements that make Mn, by definition, a trace element, mean that uptake rates are low in comparison with macronutrients, therefore tracing membrane fluxes can be difficult. Additionally, binding of divalent cations in the cell wall exacerbates the problems of interpreting flux kinetics. Reid (2001) submits that the

results of many studies in the literature may be unreliable because of the failure to adequately demonstrate the degree to which residual binding in cell walls influences observed fluxes.

Rengel (2000) pointed out that the applicability of many experiments with potential to improve understanding of the kinetics of Mn uptake from soil was diminished by the use of unrealistically high Mn concentrations. In comparison with concentrations of Mn in soil solution as low as 0.002 µ*M* for an arable soil (Peters 1990, recalculated by Marschner 1995), experimental nutrient solutions regularly contain Mn concentrations around 1 µ*M*. Specific uptake studies have used Mn concentrations of 1 m*M* (Quiquampoix *et al.* 1993) and even 10 m*M* (Maas *et al.* 1969). Hence, extension of such results to uptake from soil may be considered somewhat dubious.

THE CURRENT STATE OF Mn^{2+} UPTAKE KINETICS KNOWLEDGE

An increase in solution concentration of Mn generally results in increased Mn uptake and the relationship typically obeys Michaelis-Menten kinetics, following a saturable curve (Maas *et al.* 1968; Bowen 1981; Landi and Fagioli 1983; Clarkson 1988). The development of chelate-buffered culture solutions (Chaney *et al.* 1989; Norvell and Welch 1993; Webb *et al.* 1993; Parker *et al.* 1995; Rengel and Graham 1995) meant that realistically low concentrations of Mn could be maintained in growth and uptake experiments. In such a system, ionic activity of Mn^{2+} is a more meaningful measure of available Mn than Mn concentration *per se*. Using barley, Webb *et al*. (1993) found a linear relationship between ionic activity of Mn^{2+} and Mn accumulation in the roots. This was seemingly in contrast to the non-linear responses to Mn concentration increases in the above-mentioned studies, other than at the low end of the wide concentration ranges used. However, the ionic activity/ion concentration ratio should, in itself, follow a saturable curve. Hence, expressing Mn uptake rates from older literature as a function of ionic activity rather than concentration would likely reveal a much more linear than saturable relationship.

The uptake of Mn by plant roots was long considered a bi-phasic process consisting of an initial rapid uptake phase followed by a slower, more constant uptake which may have some metabolic dependence. However, uptake and compartmentation studies using ^{54}Mn reveal a number of apparent fractions with different uptake half-times. Manganese content of the roots initially rises rapidly for 0.3 to 1 h, depending on the tissue examined, upon immersion in solutions containing Mn^{2+} (Clarkson 1988). This rapid uptake phase, most likely representing uptake into the apoplasm, was found

Table 1.1. **Estimates of kinetic parameters for low-affinity Mn^{2+} uptake in lower and higher plants (adapted from Rengel 2000). I_{max} = maximal net influx, K_m = Michaelis constant.**

Plant	Mn in culture solution (µ*M*)	I_{max} (µmol/g DW/h)	K_m (µ*M*)	Reference
Baker's yeast	5-200	[a]690 x 10^{-6}	62	(Gadd and Laurence 1996)
Chlorella algae	1-100	[a]1	760	(Garnham *et al.* 1992)
Maize roots	1.5-6	[bc]40-302	[c]4-11	(Landi and Fagioli 1983)
Barley roots	10-20000	720	400	(Maas *et al.* 1968)

[a] I_{max} expressed as µmol/h per 10^6 cells
[b] I_{max} expressed as µmol/g root dry weight/h, assuming authors' protein figures as 22.5 % of root dry weight.
[c] Range was genotype-dependent.

to have a half-time of 8 minutes (Page and Dainty 1964), but may itself involve an initial uptake phase with a half-time of <1 minute. A fraction detected with an uptake half-time between 0.5 and 2 h (Munns *et al.* 1963a) was characteristic of uptake into the cytoplasm. The nature of the slowest phase in the labelled ^{54}Mn experiments was typical of a fraction taken up into the vacuole with a half-time of 13 to 28 h (Munns *et al.* 1963a; Page and Dainty 1964).

Considering Michaelis-Menten kinetics, the hyperbolic or saturable relationship between uptake (influx) rate (I) and substrate concentration (C) has a component that should be almost linear when C is low. Estimates of K_m (C at 1/2 I_{max}) for Mn uptake by roots range between 4 and 400 µ*M* (Table 1.1). In comparison to soil solution concentrations, these K_m concentrations are relatively high. As a consequence, uptake of Mn from most soils should be linear (Barber 1995). Indeed, Halstead *et al.* (1968) measured total Mn accumulation by species with different root morphology (lettuce, tomato, wheat and soybean), and in each case there was a linear relationship with the calculated supply of Mn from the soil by mass flow and root interception.

TRANSPORT ACROSS CELL MEMBRANES

Whereas definitive information regarding kinetics and regulation of Mn uptake by higher plants is scarce, at least based on experiments using realistically low concentrations of Mn supply, less complex taxa (algae, yeasts etc.) have been widely studied. The transport of Mn across the plasma membrane in these species has often been studied in combination with transport to cell organelles. Transport of Mn into Golgi bodies in baker's yeast, for example, occurs via Ca^{2+}-ATPase (Dürr *et al.* 1998). Such model 'plant' systems are providing a basis for research into higher plant membrane transporters of Mn and their possible cloning.

Belouchi *et al.* (1997) suggested two putative transporter proteins of rice roots that may be involved in the uptake of Mn. The proteins, coded by the *OsNramp* genes, are highly likely to be Mn transporters as their amino acid sequences are homologous to those of *Nramp* proteins of mammals (coded by the *NRAMP2* gene) and baker's yeast (coded by *SMF1*). Plasma membrane proteins of the *SMF1* gene are involved in transport of Mn in baker's yeast (Supek *et al.* 1996; Pinner *et al.* 1997) and, along with the mammalian macrophage *NRAMP2* membrane protein, may play a role in Mn transport into cells for active oxygen scavenging (Portnoy *et al.* 2000). As yet, it is not clear whether *Nramp* genes are expressed in response to Mn deficiency in rice roots (Takahashi and Sugiura 2001). The refinement of molecular biology laboratory techniques is providing ever-increasing information regarding Mn-specific (eg. *LeGlp1* of tomato, Takahashi and Sugiura 2001) and non specific (eg. *IRT1* of *Arabidopsis*, Korshunova *et al.* 1999) transport proteins in the plasma membranes of plant root cells.

The specificity and selectivity of metal transporter proteins was studied by Rogers *et al.* (2000). In baker's yeast expressing *IRT1*, it was shown that single amino acid replacements could eliminate transport of both Fe and Mn, or Zn alone, or Fe, Mn, Zn and Cd together, whereas wild-type *IRT1* was capable of transporting ions of all four metals. These results imply, firstly, that genotypic differences in tolerance to nutrient deficiencies may result from only subtle changes in transport protein composition, and secondly, that manipulating transporters to alter nutrient uptake functions in plants may be simpler than previously anticipated.

There is significant evidence of an association between Mn and protons in cross-membrane transport, but the mechanisms appear to differ between specific membranes. Studies on mammalian (Gunshin *et al.* 1997), yeast (Chen *et al.* 1999), and *Arabidopsis* (Thomine *et al.* 2000) cells indirectly suggest that uptake of micronutrient cations occurs across the plasma membrane in symport with H^+. Within oat root cells, the more-specialised membrane surrounding the vacuoles, the tonoplast, appears to transport Mn from the cytoplasms by antiport with H^+ (Gonzalez *et al.* 1999).

REGULATION OF UPTAKE

Whilst uptake of the major plant nutrient ions is broadly regulated by growth and metabolism (Clarkson 1985), the divergence between critical functional requirement and actual content indicates that Mn uptake is not tightly controlled. Accordingly, there is no evidence of a direct energy expenditure in cellular uptake of Mn. Thermodynamically, the negatively charged cytoplasm provides a sufficient electrochemical potential gradient to strongly attract divalent cations from external

solution (Clarkson 1988). However, Tiffin (1967) reported an accumulation of Mn in the stem exudate of tomato plants up to 5 times the culture solution concentration. Such accumulation against a concentration gradient requires the involvement of metabolic processes at some point in the pathway between the culture solution and the xylem elements. Other studies have also associated metabolic processes with Mn uptake (eg. Maas *et al.* 1969; Ratkovic and Vucinic 1990; Garnham *et al.* 1992), but the association is most likely an indirect one whereby energy is required by H^+-ATPase to maintain the electrochemical potential gradient across the plasma membrane.

Although evidence for metabolic regulation of Mn uptake may be imprecise, there is a wealth of information regarding interactions between Mn and other ions influencing uptake. Experiments revealed that increasing activity of H^+ in culture solutions caused a decrease in Mn uptake by barrel medic (Robson and Loneragan 1970), maize (Ratkovic and Vucinic 1990) and barley (Malkanthi *et al.* 1995). Similarly, progressively decreasing H^+ by increasing pH of chelate-buffered nutrient solutions resulted in higher concentrations of Mn in barley plants (Huang *et al.* 1993). In soil, however, the reverse is often observed: As outlined above, decreasing H^+ activity by raising soil pH is generally associated with the transformation of Mn to forms unavailable for plant uptake, resulting in decreased Mn absorption.

Increasing concentrations of divalent cations, Mg for example, can affect Mn uptake. Blackwell *et al*. (1997) noted decreased Mn uptake by baker's yeast with increasing internal Mg^{2+} concentrations were likely due to Mg-related changes in membrane transporter activity. External Mg^{2+} also competitively inhibited plasma membrane transport of Mn in baker's yeast (Gadd and Laurence 1996) and a green algae (Garnham *et al.* 1992).

Interestingly, the most abundant divalent cation in nature, Ca^{2+}, does not appear to interact with Mn absorption to any great extent (Clarkson 1988). Such was the case in excised maize roots (Maas *et al.* 1969) and intact kidney bean plants (Taper and Leach 1957), and inhibition of Mn uptake was only found in barrel medic at high levels of Ca (Robson and Loneragan 1970). On the other hand, Mn^{2+} can affect uptake of Ca^{2+}: Ion channels such as the Ca^{2+}-selective ion channel in the wheat root-cell plasma membrane can facilitate uptake of Mn (Piñeros and Tester 1995). In maize roots, Mn^{2+} decreased Ca^{2+} flux across the plasma membrane via the Ca^{2+}-selective ion channel by as much as 70 % (Marshall *et al.* 1994) by taking up binding sites for Ca^{2+} on the apoplasmic side of the channel (Shumaker and Sze 1986).

Just as high levels of divalent cations can decrease absorption of Mn, micronutrient deficiencies can potentially facilitate uptake of other micronutrients,

including Mn (Kochian 1991). Zinc deficiency of wheat resulted in increased Mn, Fe and Cu absorption (Rengel and Graham 1995, 1996; Rengel *et al.* 1998). Compensatory absorption has also been reported between Mn and Cu or Zn (del Río *et al.* 1978; Harrison *et al.* 1983) and between Fe and Mn (Brown *et al.* 1984; Welch *et al.* 1993; Iturbe-Ormaetxe *et al.* 1995).

The Fe-Mn uptake interaction is one of the most spectacular in plant nutrition. Iron efficiency in plants is inherently related to two strategies: *Strategy I* (dicots and non-graminaceous monocots) whereby reducing capacity of root surface cell membranes and net excretion of H^+ and reducing and/or chelating compounds is enhanced under iron deficiency; and *Strategy II* (graminaceous species) whereby enhanced release of non-proteinogenic amino acids, so-called phytosiderophores occurs. Under iron deficiency, *Strategy I* plants show greatly increased Mn uptake, even to the point of Mn toxicity (soybean, Brown and Jones 1971; flax, Moraghan 1979). Since Fe deficiency and Mn deficiency often occur in similar soil conditions, selection of plant varieties for high iron efficiency may help prevent Mn deficiency in *Strategy I* plants. However, since *Strategy I* and *II* responses are repressed after resupply of Fe (Marschner 1995) the probability of continued high Mn accumulation by Fe-efficient genotypes in the absence of Fe deficiency is low.

THE ROLE OF MANGANESE IN PLANTS

The capacity of Mn in being able to undergo changes in oxidation state relatively easily means it is suited to roles in redox processes. Not surprisingly then, Mn plays key roles in processes such as electron transport in photosynthesis and detoxification of superoxide radicals generated in photorespiration. Perhaps more surprisingly, with the exception of the water-splitting enzyme in photosystem II and Mn-containing superoxide dismutase (Mn-SOD), Mn does not have a pivotal role as an integral component of plant enzymes. In contrast to metals such as Cu and Zn, generally Mn does not form metallo-proteins in the strict definition, but forms exchangeable metal complexes (similar to Mg and Ca) with proteins (Hughes and Williams 1988).

Although uncommon as a structural or functional protein component, Mn does serve as an activator of many enzymes (Table 1.2). Enzyme-mediated phosphorylations, decarboxylations, reductions and hydrolysis reactions may be activated by Mn (Burnell 1988). Plant Mn nutrition, therefore, affects a diverse range of metabolic processes,

Table 1.2. **Plant enzymes activated by Mn^{2+} (adapted from Burnell 1988).**

Class	Action	Enzyme	EC number	Reference
Oxidoreductases			**1**	
	Acting on CH-OH group of donors		1.1	
		NAD malic enzyme	1.1.1.39	(Wedding and Black 1983)
		NADP malic enzyme	1.1.1.40	(Johnson and Hatch 1970)
		NAD isocitrate dehydrogenase	1.1.1.41	(Coultate and Dennis 1969)
		NADP isocitrate dehydrogenase	1.1.1.42	(Maloney and Dennis 1978)
	Acting on aldehyde or oxo group of donors		1.2	
		Pyruvate oxidase	1.2.3.3	(Nason and McElroy 1963)
		Oxoglutarate dehydrogenase	1.2.4.2	(Amberger 1973)
	Acting on peroxide as acceptor		1.11	
		Mn-peroxidase	1.11.1.7	(Machlachlan and Waygood 1956)
	Acting on superoxide as acceptor		1.15	
		Mn-superoxide dismutase	1.15.1.1	(Michelson *et al.* 1977)
Transferases			**2**	
	Acetyltransferases		2.3	
		Citrate synthase	2.3.3.1	(Nason and McElroy 1963)
		γ-Glutamyl transferase	2.3.2.2	(Amberger 1973)
	Transferring alkyl or acyl (other than methyl) groups		2.5	
		3-deoxy-7-phosphoheptulonate synthase	2.5.1.54	(Ganson *et al.* 1986)
	Transferring phosphorus-containing groups		2.7	
		Hexokinase	2.7.1.1	(Nason and McElroy 1963)
		Glucokinase	2.7.1.2	(Nason and McElroy 1963)
		Phosphoglucokinase	2.7.1.10	(Amberger 1973)
		Adenosine kinase	2.7.1.20	(Nason and McElroy 1963)
		NAD kinase	2.7.1.23	(Nason and McElroy 1963)
		Dephospho-CoA kinase	2.7.1.24	(Nason and McElroy 1963)
		Pyruvate kinase	2.7.1.40	(Tomlinson and Turner 1973)
		Phosphoglycerate kinase	2.7.2.3	(Amberger 1973)
		Arginine kinase	2.7.3.3	(Nason and McElroy 1963)
		UDP-glucose pyrophosphorylase	2.7.7.9	(Amberger 1973)
Isomerases			**5**	
	Intramolecular transferases		5.4	
		Phosphoglucomutase	5.4.2.2	(Amberger 1973)
Hydrolases			**3**	
	Acting on ester bonds		3.1	
		Lecithinase A	3.1.1.4	(Nason and McElroy 1963)
		Deoxyribonuclease I	3.1.21.1	(Nason and McElroy 1963)
		Alkaline phosphatase	3.1.3.1	(Amberger 1973)
		Acid phosphatase	3.1.3.2	(Amberger 1973)
	Acting on carbon-nitrogen (other than peptide) bonds		3.5	
		Arginase	3.5.3.1	(Hellerman and Stock 1938)
		Allantoateamidohydrolase	3.5.3.9	(Winkler *et al.* 1985)
Lyases			**4**	
	Carbon-carbon lyases		4.1	
		Oxaloacetate decarboxylase	4.1.1.3	(Nason and McElroy 1963)
		PEP carboxylase	4.1.1.31	(O'Leary 1982)
		PEP carboxykinase	4.1.1.32	(Burnell 1986)
Ligases			**6**	
	Forming carbon-nitrogen bonds		6.3	
		Glutamine synthetase	6.3.1.2	(Haystead 1973)
	Forming carbon-carbon bonds		6.4	
		Pyruvate carboxylase	6.4.1.1	(Nason and McElroy 1963)

from respiration to the biosynthesis of amino acids and lignin. As an enzyme activator, Mn can often be replaced by other metal ions, especially Mg (Marschner 1995). While Mn may act *in vitro* as an enzyme activator, large concentration differences between Mn and Mg (50 to 100 times more Mg, Campbell and Nable 1988) in plants mean the physiological role of Mn as an activator *in vivo* is questionable in some cases (Römheld and Marschner 1991).

MANGANESE IN PHOTOSYNTHESIS

Perhaps the most important biochemical function of Mn, and the most extensively studied, is the role played in oxygen evolution in photosynthesis. Pirson (1937) first elucidated this particular role of Mn from studies on green algae. The precise chemical mechanism encompassing O_2 evolution by the Mn-containing complex of photosystem II is not entirely understood, and detailed discussion is beyond the scope of this chapter (For reviews, see Rutherford 1989; Yachandra *et al.* 1996)

Three distinct pools of Mn have been isolated in chloroplasts (Amesz 1983): The first is an easily removed fraction which appears to have no functional role in photosynthesis. The second is membrane-bound and involved in the water-splitting reaction (the Hill Reaction, Hill 1937; 1939 Figure 1.3). The third fraction is very strongly bound and appears to have a role in chloroplast ultrastructure and stacking of chloroplast lamellae.

The protein that catalyses the water-splitting reaction, a 33-kDa polypeptide often known as enzyme S, is located in the thylakoid membrane. Each molecule contains an active centre of 4 Mn atoms. In releasing one molecule of O_2, the Mn atoms are reduced in one or two steps, whereas the reoxidation of the Mn occurs sequentially in four steps (Rutherford 1989).

The process of photosynthetic O_2 evolution is perhaps the most sensitive to changes in Mn supply (Marschner 1995). Withdrawal of Mn supply to subterranean clover decreased O_2 evolution by more than 50 % within 6 d, even though effects on chorophyll content and leaf dry weight were minimal (Nable *et al.* 1984). However, restoration of O_2 evolution to pre-Mn-withdrawal levels occurred within 1 d of Mn resupply. Wheat plants have shown a similar response (Kriedemann *et al.* 1985).

Although mild or short-term Mn deficiency does not appear to affect chloroplast ultrastructure, severe deficiency can result in the structural breakdown of the lamellar system (Mercer *et al.* 1962). It was proposed that this alteration of the thylakoids was a result of the photooxidation (lipid peroxidation) and photodestruction of the lamellar

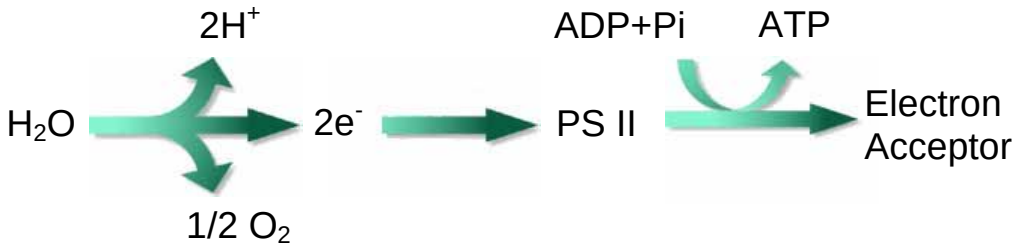

Figure 1.3. **Simplified representation of the Hill reaction in photosynthesis. Manganese is involved in the water-splitting step.**

system by free radicals (see below) generated in the water-splitting step of photosynthesis (Campbell and Nable 1988). However, more recent evidence (Polle *et al.* 1992) tends to refute this claim, indicating that inhibition of lipid and carotenoid biosynthesis may be the primary mode of structural disorganisation under Mn deficiency. The resupply of Mn can restore certain functional particles to thylakoid membranes (Simpson and Robinson 1984), but deficiency-induced ultrastructural alterations are generally very difficult to restore, or may be irreversible.

MANGANESE-CONTAINING SUPEROXIDE DISMUTASE

Superoxide free radicals (O_2^-) are generated in a wide range of metabolic reactions involving molecular O_2 (Burnell 1988), such as the photoreduction of O_2 by photosystem I (the Mehler Reaction). Secondary production of hydroxyl radicals and hydrogen peroxide will occur if superoxide accumulation is excessive (Winterbourn 1981). Accumulation of these reactive species can inhibit CO_2 fixation (Robinson *et al.* 1980), cause oxidation of fatty acids, and damage cellular membranes (Chia *et al.* 1981), protein amino acids and nucleic acids (Halliwell 1984).

As with other superoxide dismutases (Fe and Cu/Zn-SOD) under normal conditions, Mn-SOD plays an important role in protecting cells against deleterious effects of superoxide free radical accumulation. The activity of Mn-SOD is closely related to Mn supply (Garcia *et al.* 1981). Hence, cells of Mn-deficient plants may be subject to damage from superoxide free radicals and associated metabolites.

There is some conjecture as to the location of Mn-SOD within plants. Jackson *et al.* (1978) indicated more than 90 % of superoxide dismutase to be localised in the chloroplasts of green leaves. Numerous other studies of a range of species purport to show the presence of Mn-SOD in the chloroplasts, and an equal number have found it

absent from these organelles (see Burnell 1988; Marschner 1995 and references therein). The issue is not fully resolved yet, but there is growing acceptance among plant physiologists that presence or absence of Mn-SOD in the chloroplast may be species dependent.

OTHER PHYSIOLOGICAL REQUIREMENTS FOR MANGANESE

The direct requirement for Mn in photosynthesis and superoxide dismutase proteins means that decreases in O_2 evolution, photosynthesis in general and increased risk of photodestruction of cell components will occur rapidly at the onset of Mn deficiency. Additionally, various other physiological processes are affected by deficient Mn concentrations in plant tissues.

CARBOHYDRATE SYNTHESIS

Soluble carbohydrate levels are greatly decreased in Mn-deficient plants (Römheld and Marschner 1991). The effect is mainly caused by the inhibition of photosynthesis (Nable *et al.* 1984), and is particularly obvious in the roots: When bean plants were subjected to Mn deficiency, soluble carbohydrates in the roots, leaves and stems decreased by 90, 77 and 60 %, respectively (Vielemeyer *et al.* 1969).

Plants are classed as C_3 or C_4, depending on the initial carboxylation reaction used to incorporate inorganic C (CO_2) in photosynthesis. C_4 photosynthesis involves the incorporation of CO_2 into C_4 acids (oxaloacetate, malate, aspartate) in the mesophyll cells, transport of these acids to bundle sheath cells, and decarboxylation and the release of CO_2 which is then assimilated via the Calvin cycle. The C_3 pathway involves the Calvin cycle alone, in which CO_2 is incorporated into a 6-carbon compound that immediately splits into two molecules of the C_3 acid, 3-phosphoglycerate (the 6-carbon intermediate has never been isolated).

In C_4 plants, two major enzymes involved in the decarboxylation steps have an *absolute* requirement for Mn; nicotianamide adenine dinucleotide malic enzyme (NAD-ME) and phosphoenolpyruvate carboxykinase (PCK). On the other hand, two similar decarboxylating enzymes, nicotianamide adenine dinucleotide phosphate malic enzyme (NADP-ME) and phosphoenolpyruvate carboxylase (PEP carboxylase) can be activated by either Mn or Mg.

Enzymes requiring Mn for carbohydrate synthesis have yet to be clearly identified in C_3 plants. Nonetheless, despite the seeming disparity between C_3 and C_4

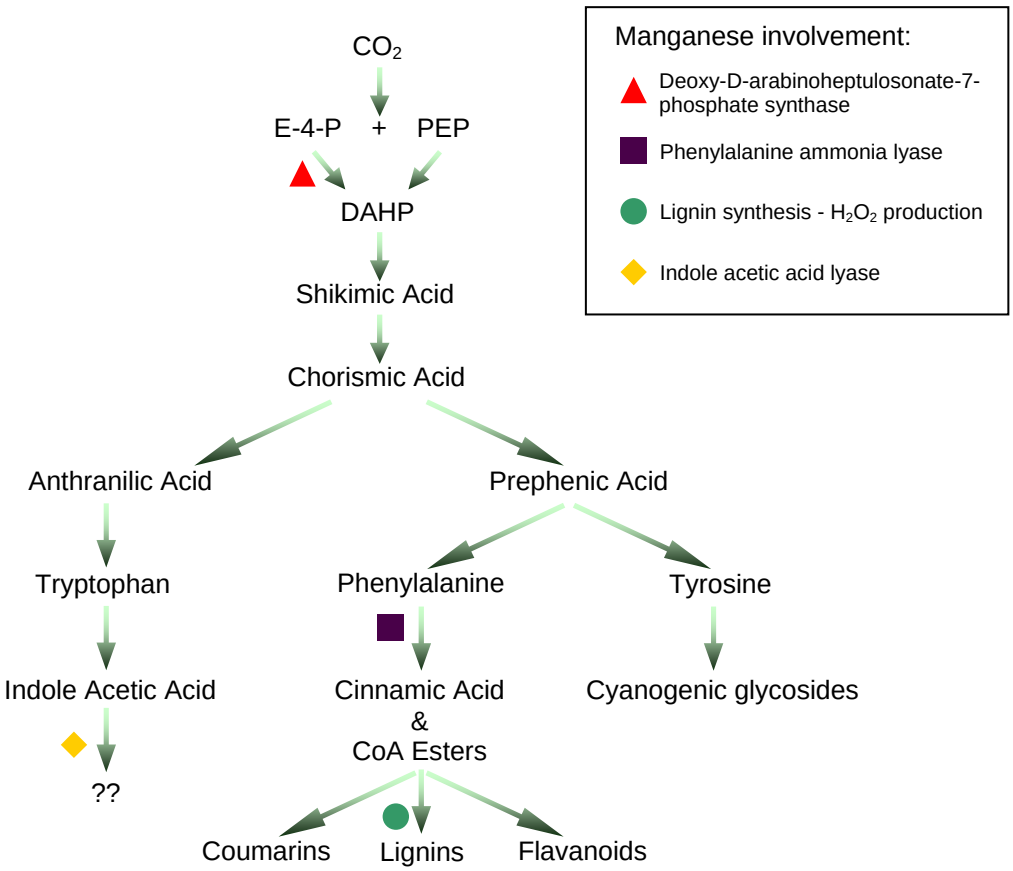

Figure 1.4. **Major steps in the shikimic acid pathway and the points of Mn involvement (adapted from Burnell 1988).**

plants in Mn requirements for photosynthesis, sensitivity to Mn deficiency is similar in C_3 and C_4 species (Römheld and Marschner 1991).

SYNTHESIS OF SECONDARY METABOLITES AND LIGNIN

As an important cofactor in many enzyme-regulated reactions (Table 1.2), Mn plays a key role in the biosynthesis of plant secondary metabolites (Burnell 1988; Marschner 1995). Manganese is involved at a number of stages in the shikimic acid pathway (Figure 1.4, Burnell 1988). Thus, plants suffering a deficiency of Mn will show decreased concentrations of metabolites associated with the shikimic acid pathway, such as aromatic amino acids, phenolics, coumarins, flavonoids and indole acetic acid (Brown *et al.* 1984; Burnell 1988; Römheld and Marschner 1991).

Manganese also has a direct involvement in the final step of lignin synthesis. Derivatives of cinnamic acid, hydroxylated cinnamyl alcohols, are the precursors of lignin (Gross 1980). Polymerisation of these units is the final step in the synthesis of

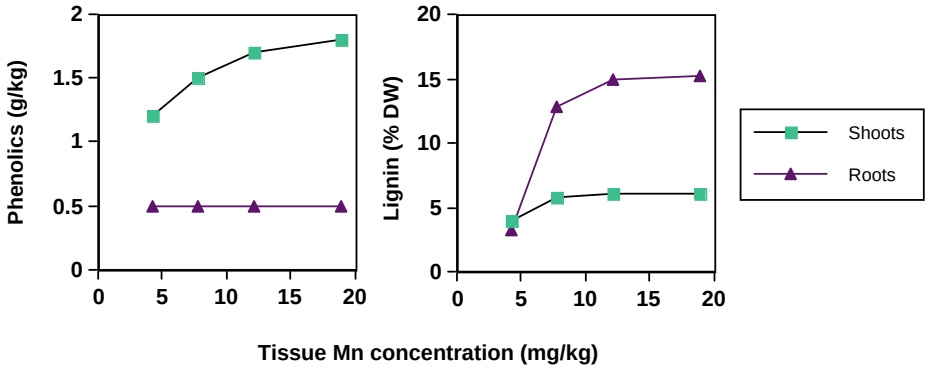

Figure 1.5. **Effect of tissue Mn concentrations on the content of total phenolics and lignin in wheat plants. (Based on data of Brown *et al.* 1984; as presented by Römheld and Marschner 1991).**

lignin, requiring phenolic compounds from a reaction requiring H_2O_2 (Stafford 1974a, 1974b). Production of H_2O_2 is stimulated by Mn^{2+} (Gross *et al.* 1977).

Lignin and the secondary metabolites generated in the shikimic acid pathway are the backbone of the plant defence system (Burnell 1988). Lower concentrations of phenolics, lignin (Figure 1.5) and flavonoids in Mn-deficient tissue have been implicated in decreased pathogen resistance of Mn-deficient plants.

MANGANESE DEFICIENCY IN PLANTS

When Mn levels within a plant are insufficient to sustain metabolism at rates required for growth and development, Mn deficiency and its associated symptoms will result. The vast majority of Mn in a plant originates from Mn^{2+} in the soil, therefore all factors that influence the concentration and availability of Mn in the rhizosphere and at the root surface will, in turn, influence the occurrence of Mn deficiency.

Since the publication of the first unequivocal evidence of the essentiality of Mn for plants (McHargue 1922), Mn deficiency has been discovered in most countries around the world, in plants from home gardens to agricultural crops and forestry plantations. After early observations of Mn deficiency in Australia demonstrated total yield loss in an oat crop (Samuel and Piper 1928), a wide range of important agricultural species have shown equally severe to very mild yield losses and symptoms of Mn deficiency (eg. see Reuter *et al.* 1988).

Symptoms of Mn deficiency in plants commonly become clearly visible only when growth rate and yield are severely depressed (Hannam and Ohki 1988). Foliar

symptoms are often similar to those of Fe, Mg and sometimes S deficiency, and hence Mn deficiency is commonly mis-diagnosed. Generally, young expanding leaf blades are the first and most severely affected plant parts (Reuter and Robinson 1997), showing diffuse interveinal chlorosis in contrast to the distinct and often reticulate nature of green veins as characterised by Fe deficiency. As severity of Mn deficiency increases, leaves can become entirely chlorotic and develop necrosis (Hannam and Ohki 1988). The loss of photosynthetic activity results in decreased growth rates and even plant death.

GENOTYPIC DIFFERENCES IN TOLERANCE TO MANGANESE DEFICIENCY

The ability of different species and genotypes to yield better than others on soils of poor Mn availability was recognised almost a century ago. A number of related definitions for the term "tolerant to Mn deficiency" have been used in plant nutrition studies. However, in an agronomic and operational sense, a species or genotype which shows a minimal decrease in yield when grown under Mn-deficient conditions, compared with Mn-sufficient conditions, is considered more tolerant to Mn deficiency than one which shows a larger yield decrease. The concept of tolerance to micronutrient deficiencies, often referred to as micronutrient efficiency, has long been studied by plant nutritionists as a means of improving crop yield in soils where micronutrient availability is poor. Tolerance to Mn deficiency appears to be a heritable trait under the control, at least partly, of major dominant genes (Graham 1988). Therefore, potential exists for exploiting these genes in plant breeding for Mn-deficiency tolerance (Graham 1988; Rengel *et al.* 1993; Rengel *et al.* 1994).

Owing to the economic importance of cereal crops, most of the research attention regarding genotypic tolerance to Mn deficiency has focussed on these species, especially wheat (see Rengel 2001). However, cereal crops are no more susceptible to Mn deficiency than other species, and there are a number of reports of genotypic variation in the ability of dicotyledons to tolerate soils of low Mn availability (eg. Graham 1988 and references therein; Graham *et al.* 1994; Krahmer and Sattelmacher 1995). Despite widespread and continual recognition of these genotypic differences, the underlying mechanisms are inadequately understood (Graham 1988; Huang *et al.* 1993, 1994; Rengel *et al.* 1994; Huang *et al.* 1996; Rengel 2000, 2001).

Studies based predominantly on cereal crop species suggest that, of the five mechanisms presented by Graham (1988), superior internal utilisation or lower functional requirement, a faster specific rate of Mn absorption, and better root geometry

are unlikely to be major contributing factors by which one genotype may better tolerate Mn-deficient conditions than another (Graham 1988; Rengel *et al.* 1994). Yet, genotypes tolerant to Mn deficiency are usually able to extract more Mn from such soils where Mn availability is restricted. The remaining possible strategies underlying Mn-deficiency tolerance are (1) better internal compartmentalisation and remobilisation of Mn (Huang *et al.* 1993), and (2) increased efflux of substances from roots which will ultimately result in increased mobilisation of insoluble and plant-unavailable Mn in the rhizosphere.

Evidence that differential genotypic tolerance to Mn deficiency is expressed by barley growing in soil, but not in solution culture was presented by Huang *et al.* (1994). It was reasonably concluded that since tolerance to Mn deficiency was not evident when Mn was supplied solely as aqueous Mn^{2+}, the mechanism of Mn-deficiency tolerance involves processes which enhance the availability and mobility of Mn to plant roots from less available forms. The implications of this work are that specific chemical reduction and subsequent mobilisation and/or microbial dynamics in the rhizosphere of genotypes tolerant to Mn deficiency are involved, but unequivocal experimental evidence on this topic, almost a decade later, is yet to be seen.

LUCERNE (*Medicago sativa* L.)

HISTORICAL

Lucerne, or alfalfa, has a long history of use in agriculture and is the oldest plant to be grown solely for forage. The number of names attributed to a given crop is often an indirect index of antiquity of cultivation. Even today, lucerne is known by different names in different countries and continents. Lucerne originated in the Iranian region. *Alfalfa*, as it is now most prevalently named in the Americas, is an Arabic word. According to Piper (1935) the ancient Iranian name could not be retained and was changed by the Arabs to *fesfisat— isfast— elkasab— alfasafat— alfalfa*.

Records show that the Hittites used lucerne for livestock feed over 3300 years ago (Evans and Richardson 1994a). By the fourth century BC, lucerne had arrived in Greece with invading armies and rapidly spread through the Roman Empire. It was acquired by the Chinese, along with some prize horses, from Russia in 126 BC. Records of its spread and use in Europe are scarce from the fall of the Roman Empire until the sixteenth century when, under the name alfalfa, cultivation spread from northern Africa

to Spain and then to all countries of the New World via Spanish and Portuguese ocean explorers. It has now gained ascendancy as the major forage in temperate areas of the world.

Australian conditions of temperature in agricultural regions during the growing season, water availability, and the comparatively lower area of irrigated land than is seen in the United States mean that continual intensive production of lucerne as a crop is not widespread. Consequently, the markets for cut lucerne and lucerne hay are not as extensively developed as in the United States. However, lucerne is gaining increasing acceptance in Australia as high-protein forage in dryland agriculture, especially during winter months when sown and native grass quality can be low.

Currently, lucerne is the focus of extensive research in Western Australia, and indeed the rest of Australia, as it shows promise as a productive crop with ameliorative characteristics. It is a deep-rooted, perennial species with the capacity to lower water tables and thus diminish a rise in dryland salinity (Latta and Blacklow 1997), while still remaining productive pasture for grazing and/or hay production.

MANGANESE DEFICIENCY OF LUCERNE

As is the case with many plant species and micronutrients, reports detailing investigations into Mn deficiency in lucerne are not bountiful. A review by Lanyon and Griffith (1988) on nutrition and fertiliser use in lucerne production does not mention Mn at all, and an American lucerne management guide (Undersander *et al.* 1991) states that Mn deficiency in lucerne is rare. This likely reflects the characteristics of the soils in which the lucerne is commonly grown rather than any inherent attribute of the species to resist Mn deficiency. Indeed, as early as 1948 an instance of Mn deficiency in lucerne was reported on a sandy loam in New Jersey with topsoil pH 7.2 and subsoil pH 6.9 (Evans and Purvis 1948). Lombin and Bates (1982) reported lucerne to be more sensitive to Mn deficiency than soybean and peanut grown in calcareous soil in the glasshouse.

Manganese-deficient lucerne plants have yellow to white leaves that show varying intensities of interveinal chlorosis, depending on the severity of the deficiency (Heckman *et al.* 1993). Thus, in addition to possible increases in forage yield, correction of Mn deficiency of lucerne may enhance the dark green leaf colour that is often associated with market quality.

CRITICAL MANGANESE DEFICIENCY CONCENTRATIONS

Studies determining critical Mn concentrations for deficiency diagnosis in lucerne are scarce, and suffer the same problems as other nutrients in lack of standardised procedures and use of a limited range of soil types, genotypes and plant ages. Heckman *et al*. (1993), using an unspecified lucerne genotype and a linear-plateau model (Anderson and Nelson 1975), found 21 µg Mn/g dry weight in the top 15 cm of shoots at early bloom stage as a critical value below which forage yield declined. This value is similar to the 20 µg/g critical value given by Kelling and Matocha (1990), but decisively lower than the 30 µg/g reported for lucerne growing in New Zealand (Reuter and Robinson 1997).

In general, genotypic differences in critical Mn concentrations are considered small (Graham 1988; Hannam and Ohki 1988). Similarly, the differences in Mn concentrations of shoots between plants showing Mn deficiency symptoms and those showing none can also be small (eg. Ohki 1976; Hannam *et al.* 1985a; 1988). This raises some question as to the applicability of critical Mn concentrations, calculated for a particular genotype under certain conditions, to the entire gamut of varieties within a species. James *et al*. (1995) quote a critical concentration range for the top half of lucerne shoots of 15 to 30 µg/g. Although a diagnosis of Mn deficiency in lucerne at 30 µg Mn/g may allow preventative actions to be taken to avoid the onset of deficiency symptoms, it may also cause unnecessary resource wastage if, say, the genotype will not show decreased yield unless Mn concentrations fall below 15 or 20 µg/g, while the soil is able to provide sufficient Mn to maintain plant concentrations above 25 µg/g. Hence, continual refinement of critical concentration ranges specific to genotypes and edaphic conditions is important.

LUCERNE FOR BAUXITE RESIDUE REVEGETATION

Lucerne has a number of characteristics that may be advantageous for its use in revegetating bauxite residue disposal areas, especially those where BRS forms the uppermost layer in the profile:

- The deep-rooted growth habit of lucerne may allow the plants to access moisture from where it is available deeper in the BRS profile. Surface layers are often dry, especially during spring and summer months, due to the low water-holding capacity of BRS with coarse particle sizes.

- Lucerne has a preference for alkaline soils, as acidity is detrimental to the symbiotic relationship with nitrogen fixing *Rhizobium* in lucerne root nodules.
- Lucerne has a moderate tolerance to saline and sodic substrates (Chaudhary *et al.* 1994). Good productivity has been achieved on salinised soil irrigated with moderately saline groundwater (Mehanni and Rengasamy 1990).
- Being a legume, lucerne is a highly acidifying species, with a single crop capable of producing acidity at an estimated rate equivalent to 60 kg $CaCO_3$/t vegetative yield/ha (Nyatsanga and Pierre 1973). The addition of H^+ and acidic exudates to BRS should provide some ameliorative aspects in decreasing the extreme alkalinity, encouraging microbial activity, and improving the conditions of BRS as a substrate for supporting plant growth.

With a number of Western Australian alumina refineries located in agricultural areas, revegetation of BRS deposits with perennial lucerne pasture will conform to the surrounding land-uses and provide a beneficial resource from what would otherwise be non-arable land.

THESIS OUTLINE AND AIMS

Alcoa World Alumina Australia Limited is attempting to establish perennial pastures on the bauxite residue disposal areas at Pinjarra, Western Australia, as stock grazing and dairy farms are the prevalent land-use of the surrounding area. Current practices in surface rehabilitation of bauxite residue deposits involve incorporation of soil amendments and identification of species that can tolerate the harsh chemical nature of BRS and red mud. Surface-applied amendments such as gypsum and poultry manure have been used to decrease the pH, increase organic matter and provide some plant nutrients. Leaching of the BRS by rainfall also assists in decreasing the pH and salinity. Inherent problems of poor water-holding capacity of BRS are compounded by non-uniform compaction down the profile. Thus, while surface layers can dry rapidly, roots of pasture species may be unable to explore the profile to any great depth, and regular irrigation was deemed necessary to achieve and maintain productive pasture. Shoots of lucerne planted directly on BRS show apparent symptoms of micronutrient deficiencies, especially Mn, and this has been validated by foliar analysis (Alcoa World Alumina Australia Limited, unpublished). As a result, long-term productivity of pasture on bauxite residue deposits is in doubt without frequent and large Mn additions. Investigating ways to decrease Mn fertiliser applications to BRS and maximising the value of applied Mn fetiliser is the central focus of this thesis.

Bauxite residue, especially the BRS fraction, is an ill-characterised substrate for plant growth. While a number of chemical analyses have been carried out for plant-available nutrients, there is limited information regarding micronutrients, and only standard chemical extractants (eg. DTPA, Lindsay and Norvell 1978; Norvell 1984) were used. Given the chemical nature of the BRS, standard chemical extractants may not provide sufficient information on concentrations of Mn forms that are useful for plants, nor how those and other Mn forms behave and interact within the substrate. Similar problems have been encountered with DTPA extraction of micronutrients in rehabilitated coal mines (Severson and Gough 1984). Hence, sequential extractants were used to determine the distribution of Mn among various chemical forms in BRS, and to characterise the dynamic interrelationships between these forms over time (Chapter II).

Lucerne has been identified as a species with characteristics suited to BRS revegetation (above), and thus is the focal plant species of this thesis. Lucerne was first trialed for BRS revegetation by Alcoa World Alumina Australia Limited in 1994. Since that time, numerous genotypes have been commercially released onto Western Australian markets. With the potential that some of these may be better able to grow and produce high yield under the harsh growth conditions imposed by a BRS substrate than others, a range of these genotypes was trialed in BRS with various rates of Mn addition (Chapter III). From this range of genotypes, varieties with high and low relative tolerance to Mn deficiency were further studied to determine their critical Mn deficiency concentrations when grown in BRS.

Current fertiliser practices by Alcoa World Alumina Australia Limited rely on broadcast applications to the surface of the BRS deposits. Gupta (1986) and Heckman *et al*. (1993) report that application of Mn in this manner is not effective in supplying Mn to a deep-rooted perennial such as lucerne. Regular and frequent Mn fertiliser applications on BRS sites at Pinjarra are not providing any residual Mn to lucerne. In contrast, a single banded application of Mn fertiliser deep in the soil profile provided an effective source of Mn to lupins for over 15 years (Brennan 1993). The potential for deep-banded Mn to provide an effective and long-lasting supply of Mn to lucerne was investigated in glasshouse (Chapter IV) and field (Chapter V) trials.

Differential genotypic tolerance to Mn deficiency has been linked to possibilities of differential production and release of carboxylates (organic acids and phenolics) into the rhizosphere (Huang *et al.* 1994). Certainly, substances produced by plant roots can reduce insoluble Mn oxides to forms available for plant uptake (Uren 1981). However, knowledge of the effect of Mn deficiency on carboxylate release is poor, and

information regarding exudation by Mn-deficient lucerne roots is practically non-existent. The suite of carboxylates released by lucerne genotypes with high and low relative tolerance to Mn deficiency was characterised under varying degrees of Mn stress at various times (Chapter VI).

Availability of water is important for dissolution of nutrient fertilisers and providing conditions for root-Mn-BRS interactions. Potential success of deep-banded Mn relies on increased water availability with profile depth. The low water-holding capacity of BRS means a reliance on irrigation for pasture establishment and maintenance through dry summer months. However, financial and environmental restrictions on groundwater usage mean that decreasing irrigation wastage will be beneficial in a BRS revegetation strategy. Successful modelling of water movement and retention in BRS under lucerne pasture may provide a basis for optimising an irrigation regime to minimise water waste. Hydrological characterisation of BRS from Pinjarra was undertaken using a variety of methods (Chapter VII). The parameters from this characterisation were integrated into the Soil Water Infiltration and Movement (SWIM) computer model based on Richard's equation (Richards 1931) in an attempt to accurately represent water content and flux in the BRS profile under the current rainfall+irrigation regime. Extending the predictive power of computer-assisted hydrological modelling may allow greater control over irrigation of BRS revegetated with lucerne in confidence that productivity may be maintained with the minimal possible requirement for irrigation inputs.

Successful and sustained productivity in revegetation of bauxite residue disposal areas will rely on improved knowledge of interactions between Mn, plants, BRS and water. By approaching these fields in a systematic way, and incorporating the findings into bauxite residue rehabilitation programs, environmental and economic benefits associated with decreased resource use and improved plant nutrition should follow.

CHAPTER II

THE CAPACITY OF BAUXITE RESIDUE SAND TO DECREASE AVAILABILITY OF MANGANESE TO PLANTS

Plate 2.1. *Bauxite residue dries within impoundments by air exposure assisted by surface cultivation.*

The major content of this chapter has been published in the journal Plant and Soil:

Gherardi, M.J. and Rengel, Z. (2001) Bauxite residue has the capacity to rapidly decrease the availability of added manganese. *Plant and Soil* **234:** 143-151.

CHAPTER SUMMARY

Bauxite residue sand (BRS), even though a poor substrate for plant growth because of very high pH, salinity and sodicity, is required to be revegetated. Manganese deficiency is observed in residue-grown plants because broadcast applications of manganese fertiliser to the surface of residue deposits have a low residual value. In a laboratory experiment, Mn (as $MnSO_4$) was added to fresh and 4-year-old BRS and a sequential fractionation procedure performed at 0, 1, 4, 8 and 24 h and 6, 14, 21, 43, 73, 103 and 130 d. Extraction with DTPA estimated plant-available Mn, while sequential fractionation with various extractants yielded the following fractions: readily soluble [$Ca(NO_3)_2$]; weakly adsorbed [CaDTPA-B_4O_7]; carbonate-bound [HNO_3]; and oxide-bound [$NH_2OH{\cdot}HCl$]. Residual Mn was calculated as a difference between the sum of all these forms and total Mn in BRS. Transformation of Mn from the initially dominant readily soluble form to the less-available forms was very rapid (<24 h). A change to fertilisation strategies will be required if better efficiency of Mn application and uptake is to be achieved for plants growing on bauxite residue.

INTRODUCTION

Revegetation of residues produced in alumina refining is an issue concerning alumina producers worldwide. The refining process, which involves aluminium extraction from bauxite ore through high-temperature digestion in NaOH, leaves two distinct residue fractions which are commonly separated before disposal. The fractions are often referred to as residue sand and red mud. The red colouration is due to high Fe levels in the primary ore minerals. Millions of tonnes of bauxite residue are generated each year in Western Australia alone. Current methods of residue disposal involve capping of alternating sand and mud layers with a deep layer (1.5 to 5 m) of the BRS which is to be revegetated. Incomplete removal of NaOH used in the ore processing means BRS is characterised by high alkalinity (pH 10-12 initially), high salinity and sodicity.

Pasture establishment is being investigated as a means of revegetating final surfaces of BRS disposal areas. Plants growing on BRS tend to have moderate to low nutrient concentrations, especially of micronutrients (Meecham and Bell 1977). Large nutrient additions are often required to enable establishment and continual productivity, but long-term sustainability of pasture production is in doubt. Manganese deficiency has been observed in residue-grown lucerne pasture. Manganese-deficient plants suffer

impaired growth as Mn is an essential nutrient for water splitting and oxygen evolution in photosynthesis (Rutherford 1989; Nitschke and Rutherford 1991; Yachandra *et al.* 1996) and for the activity of certain peroxidase, catalase and superoxide dismutase enzymes (Lall *et al.* 1998).

Due mainly to its many oxidation states, Mn can be present in a number of forms in soils. However, Mn is only available for plant uptake as Mn^{2+}. Manganese deficiency in residue-grown plants is primarily a consequence of the highly alkaline nature of BRS (Fuller *et al.* 1982). High pH soil implies low levels of free protons and electrons (Bartlett 1988). This, accompanied by low levels of easily decomposed electron donors and the presence of electron-accepting species such as CO_3^{2-} and $COOH^-$ in BRS, strongly favours the oxidation of Mn. Hence, any Mn present in, or added to BRS, is expected to be rapidly transformed to plant-unavailable forms.

Aside from the extreme chemical parameters mentioned above, other factors such as poor water holding capacity and low microbial activity might also contribute to the sub-optimal nutrient status of residue-grown pasture. The extent to which nutrient fertiliser additions to bauxite residue are available to plants has not been investigated past the fact that nutrient concentrations in residue-grown plants tend to be low, but can be increased to some extent by addition of ameliorants such as gypsum ($CaSO_4$) and sewage sludge (Wong and Ho 1991, 1993, 1994). Gypsum can decrease the pH of BRS to 8.5 – 9.0 through the precipitation of hydroxides and carbonates by Ca^{2+} (Barrow 1982), but may also decrease Mn availability for plant uptake from the residue, producing deficient shoot Mn concentrations (Wong and Ho 1994). Sewage sludge amendments can also lower BRS pH slightly (Fuller and Richardson 1986), increase nutrient levels, organic matter and microbial activity, and likely initiate competitive exchange (via Ca^{2+} and other cations) that decreases sodium availability in bauxite residue (Wong and Ho 1994).

Bauxite residue sand is an ill-characterised substrate for plant growth and nutrient availability. In order to increase the availability and residual value of Mn fertiliser added to BRS for plant growth, it is first necessary to ascertain which forms of Mn are present after addition to the residue and whether these forms change in relative abundance over time. Subsequently, methods of manipulating the Mn pool to increase the plant-available component may be formulated. This paper outlines an investigation of the forms of Mn in BRS following addition of Mn in a readily soluble (plant-available) form.

MATERIALS AND METHODS

Bauxite residue sand was collected from 20 to 40-cm depth in the residue impoundment of Alcoa World Alumina Australia Limited's alumina refinery at Pinjarra, Western Australia. Both "fresh" (< 1 month since deposited) and "aged" (four-year-old) BRS were collected (selected properties are given in Table 2.1). Fresh BRS had received no inorganic or organic nutrient additions. Aged BRS, currently planted with native Australian vegetation species at Pinjarra, had received initial gypsum (50 t/ha) and poultry manure (50 m^3/ha) applications after deposition in the residue disposal area 4 years prior to collection: no applications of fertilisers nor other amendments were made. Bauxite residue sand was air-dried and sieved, and 4-kg lots were placed in polythene bags. Bags were placed in a 20 °C room overnight to allow temperature equilibration.

Manganese fertiliser (dry $MnSO_4.H_2O$ salt) was added to BRS at 0, 5 or 50 mg Mn/kg (Mn-0, Mn-5 and Mn-50) by placing each 4-kg BRS lot in a 5 L plastic container, adding $MnSO_4$ to the containers and mixing thoroughly in a mechanical tumble shaker (approximately 100 rpm) for 600 s. All equipment was acid-washed prior to use. Each treatment was replicated four times. The BRS was placed in clean polythene-lined containers (4 L volume), watered to 80 % field capacity with double de-ionised water (18 MΩ.cm resistivity) and maintained at this level throughout. Water was added 3 times per week and no more that 2 % of the initial water content was required for maintaining the water content. Containers were covered but not sealed to allow air exchange, and were housed in a temperature-controlled glasshouse (20 °C day, 15 °C night) in a randomised complete block arrangement. Samples were taken at 0, 1, 4, 8 and 24 h and 6, 14, 21, 43, 73, 103 and 130 d after Mn addition for extraction and analysis.

Samples were divided into two parts. One half was used for plant-available Mn analysis by DTPA extraction (Lindsay and Norvell 1978; Norvell 1984). The second half was subjected to Mn extraction by sequential chemical fractionation following the methods of Warden and Reisenauer (1991) and Tong *et al.* (1995). All extractions used a solution-to-soil ratio of 5:1 to allow adequate pH buffering. Briefly, 20 mL dilute calcium nitrate [0.05 *M* $Ca(NO_3)_2$] was added to 4.0 g (air-dry equivalent) of the BRS sample to extract the readily soluble Mn component. After 0.5 h shaking, the suspension was centrifuged (5000 x g for 300 s) and filtered (Whatman No. 42). Residual BRS was then shaken in 20 mL calcium diethylenetriamine pentaacetate – tetraborate (0.025 *M* CaDTPA in 0.025 *M* $Na_2B_4O_7$, pH 8.5) for 0.5 h, centrifuged and filtered to estimate the weakly adsorbed Mn fraction. Residual BRS was washed three times with dilute

Table 2.1. **Selected properties of fresh and aged (four-year-old) bauxite residue sand. Values are means ± sandard error (n=4).**

	Fresh residue sand		Four-year-old residue sand	
pH (DDI water 1:5)	10.8	± 0.1	8.6	± 0.3
EC (mS/m)	367	± 29	192	± 13
Organic C (g/kg)	0.009	± 0.001	0.071	± 0.012
Total N (g/kg)	0.003	± 0.001	0.009	± 0.002
Field capacity (%)	15	± 1	17	± 2

$Ca(NO_3)_2$ and shaken with 20 mL of nitric acid (0.5 *M* HNO_3) for 0.5 h, centrifuged and filtered to estimate the carbonate-bound Mn fraction. After a further two washes with dilute $Ca(NO_3)_2$, the residual BRS was shaken with 20 mL hydroxylamine hydrochloride (0.1 *M* $NH_2OH{\cdot}HCl$ in 0.01 *M* HNO_3) for 0.5 h, centrifuged and filtered to estimate the Mn oxide fraction.

Filtrates were analysed for Mn by atomic absorption spectroscopy (AAS). Analysis of variance (ANOVA) was performed by GENSTAT 5, and least significant difference ($LSD_{0.05}$) values were calculated where significant *F* values ($P \leq 0.05$) were found (GENSTAT Committee 1989). The rate of binding/formation of Mn as a particular fraction was calculated as follows: binding rate (R, in mg/kg/h) at any given time (x) after commencement of the incubation period was calculated as

$$R = \frac{(Mn\ at\ t = 0) - (Mn\ at\ t = x)}{x} \qquad \{2.1\}$$

with a negative binding rate taken as representing a rate of formation of the particular Mn fraction.

RESULTS

PLANT-AVAILABLE MANGANESE

Plant-available Mn, as estimated by DTPA extraction, decreased markedly with time (Figure 2.1A). The decrease was rapid after initial addition and then slowed over time. In the fresh BRS Mn-50 treatment, DTPA-extractable Mn decreased almost four-fold within 24 h. Although proportionately smaller, the decrease in available Mn for the fresh BRS Mn-5 treatment followed a trend similar to Mn-50. In both fresh BRS treatments, the rate of available Mn decrease slowed with time. After 14 d, plant-available Mn had reached very low levels and further decreases were minimal. Aged BRS showed a similar trend to fresh BRS but a decrease in available Mn occurred at a

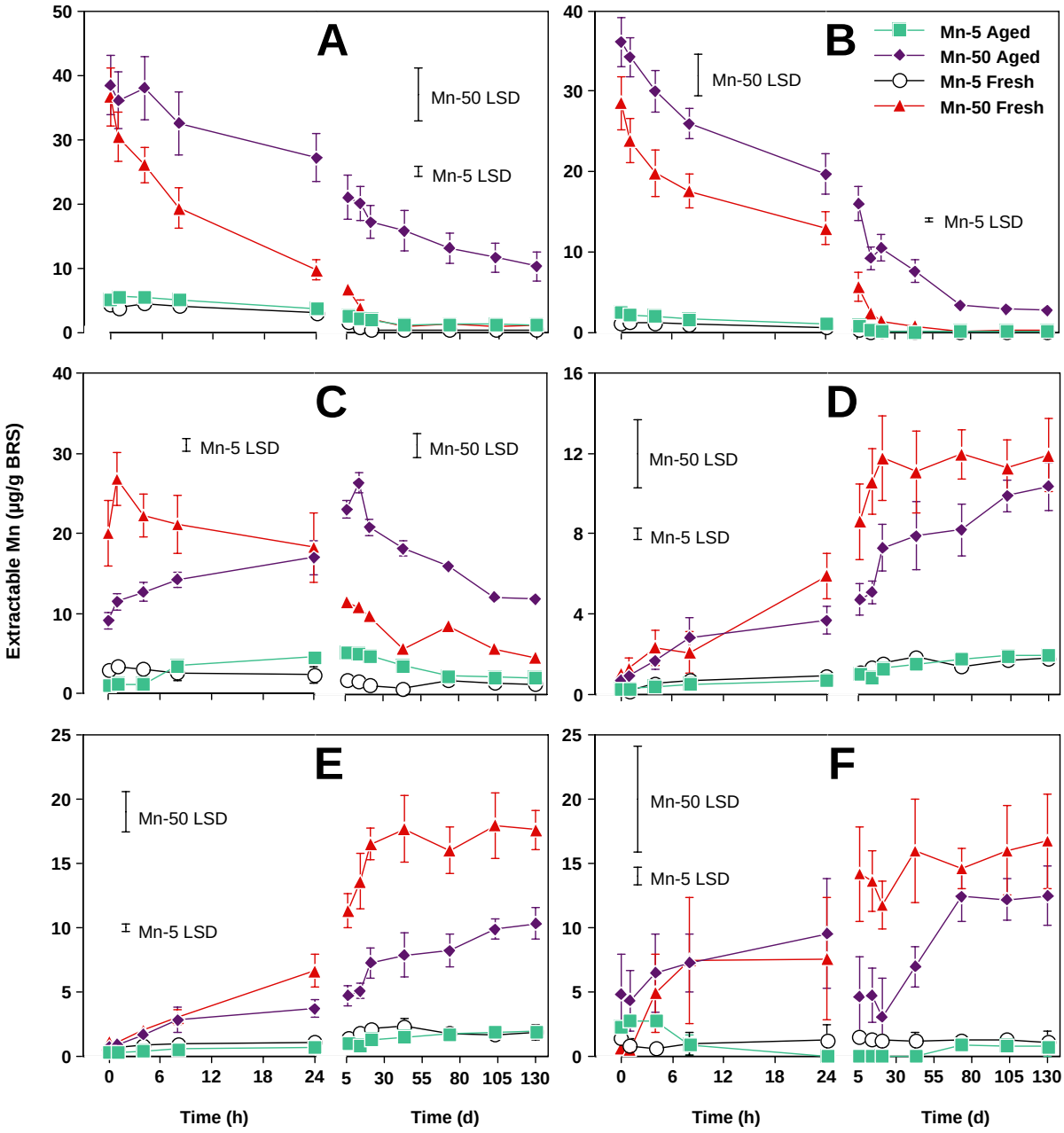

Figure 2.1. **Changes in extractable amounts of different manganese forms (± standard errors) after addition to bauxite residue sand in the reduced (Mn^{2+}) state. Fractions of manganese represented are A) plant available (DTPA extraction), B) readily soluble ($CaNO_3$ extractable), C) weakly adsorbed Mn (CaDTPA-$Na_2B_4O_7$ extractable), D) carbonate-bound Mn (HNO_3 extractable), E) oxide-bound Mn ($NH_2OH{\cdot}HCl$ extractable) and F) residual Mn (not extracted). Values are means with standard errors. The least significant difference (LSD) values for comparing old and fresh BRS of each Mn treatment are also shown.**

slower rate so that, especially in the Mn-50 treatment, plant-available Mn levels were still falling 130 d after commencement of incubation. In both Mn-5 and Mn-50 treatments, the rate of decrease in plant-available Mn was significantly faster in fresh compared to aged BRS ($P \leq 0.001$ for both Mn treatments).

SEQUENTIAL FRACTIONATION

Trends in relative levels of each of the extracted Mn fractions (Figure 2.2) show the binding of plant-available Mn to occur in fairly distinct phases in BRS. Initially, readily soluble Mn was the major Mn form in both aged and fresh BRS, but weakly adsorbed Mn rapidly became the predominate species. In fresh BRS, weakly adsorbed Mn became the dominant Mn fraction in 1 h. In aged BRS, the initial transformation was slower than in fresh BRS, but weakly adsorbed Mn still became the dominant fraction within 24 h. As the incubation period progressed further, the carbonate, oxide and residual Mn fractions increased while the weakly adsorbed Mn proportion decreased. After 80 d, proportions of all Mn fractions appeared to have reached equilibrium and remained stable until the end of the experiment. At the end of incubation, oxide Mn was present in greater proportion than other forms. In all Mn fractions, transformations (increases or decreases in extracted amounts) occurred at a faster rate in fresh BRS than in aged BRS. Over the longer term, however, extracted amounts from fresh and aged BRS began to converge (Figure 2.1) and at 130 d, only weakly adsorbed Mn was significantly higher in aged BRS ($P=0.002$) while oxide-bound Mn was significantly higher in fresh BRS ($P \leq 0.001$). No other fractions showed significantly different levels between fresh and aged BRS after 130 d.

Extractable amounts of readily soluble Mn decreased markedly with time, eventually reaching levels close to AAS detection limits in some samples (data not shown). Transformation of readily soluble Mn into other forms (Figure 2.3) started at a high rate then decreased in a curvilinear trend over time. Initially, Mn binding was occurring more than twice as fast in fresh BRS as in aged BRS. After the initial difference (up to 8 h), fresh and aged BRS showed similar rates of Mn binding. Thus, although amounts of readily soluble Mn extracted were significantly different ($P \leq 0.001$) between fresh and aged BRS, the overall pattern of Mn binding showed no significant difference ($P=0.408$).

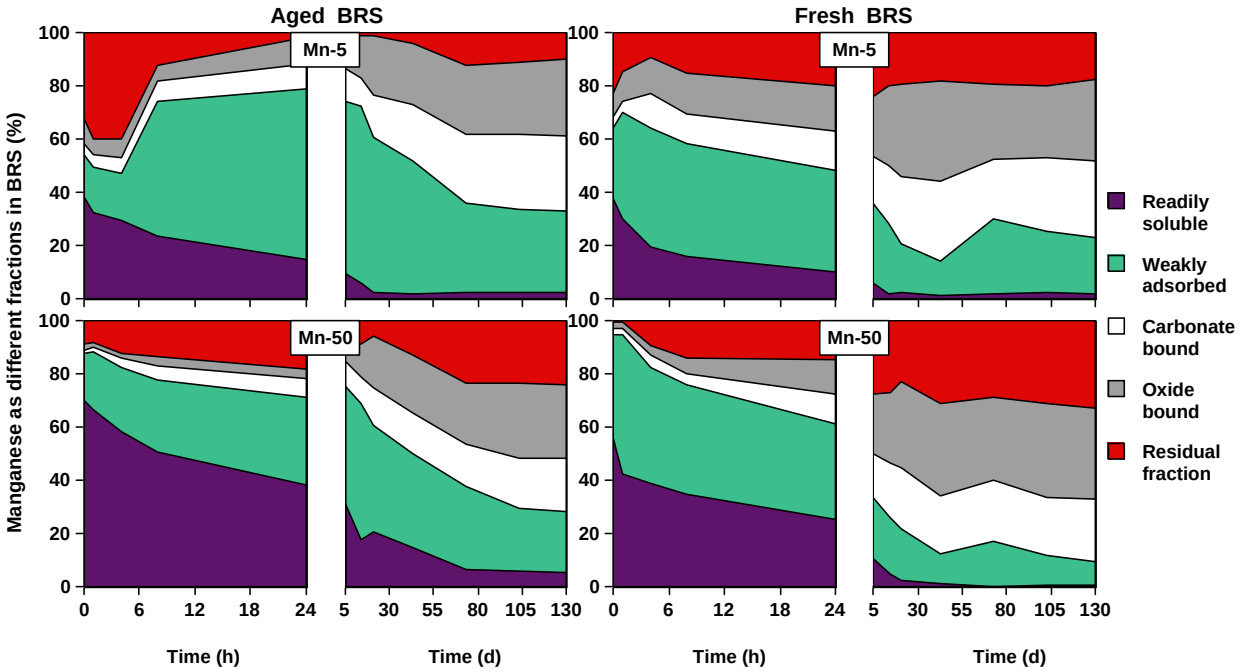

Figure 2.2 **Relative proportions of manganese forms extracted over time from bauxite residue sand of different ages.**

Weakly adsorbed Mn levels rapidly increased over the early incubation stages. After reaching extractable concentrations of approximately 26 (Mn-50) and 5 (Mn-5) µg/g BRS, extracted amounts of weakly adsorbed Mn decreased over the remainder of the experiment. The similar but slower trends in extractable Mn for fresh and aged BRS was more evident in the weakly adsorbed fraction than in any other. Weakly adsorbed Mn in fresh BRS was decreasing within 4 h, whereas extractable amounts from aged BRS did not fall until after 14 d. This difference in behaviour of fresh and aged BRS was indicated by ANOVA to be significant ($P \leq 0.001$). The rate of early formation of weakly adsorbed Mn was inversely proportional to that of binding of readily soluble Mn, but the subsequent desorption rate after reaching maximum extractable levels was similar to readily soluble Mn in both the pattern and the proportion.

The pattern and rate of binding of Mn by oxides and carbonates were similar (Figure 2.1D and 2.1E), being most rapid during initial incubation stages and reaching a fairly stable equilibrium at 21 d. In actual extracted levels of carbonate- and oxide-bound Mn, only oxide Mn from treatment Mn-50 showed a significant overall difference (P=0.002) between fresh and aged BRS, even though higher amounts and proportions of both forms were observed in fresh BRS at all treatment levels.

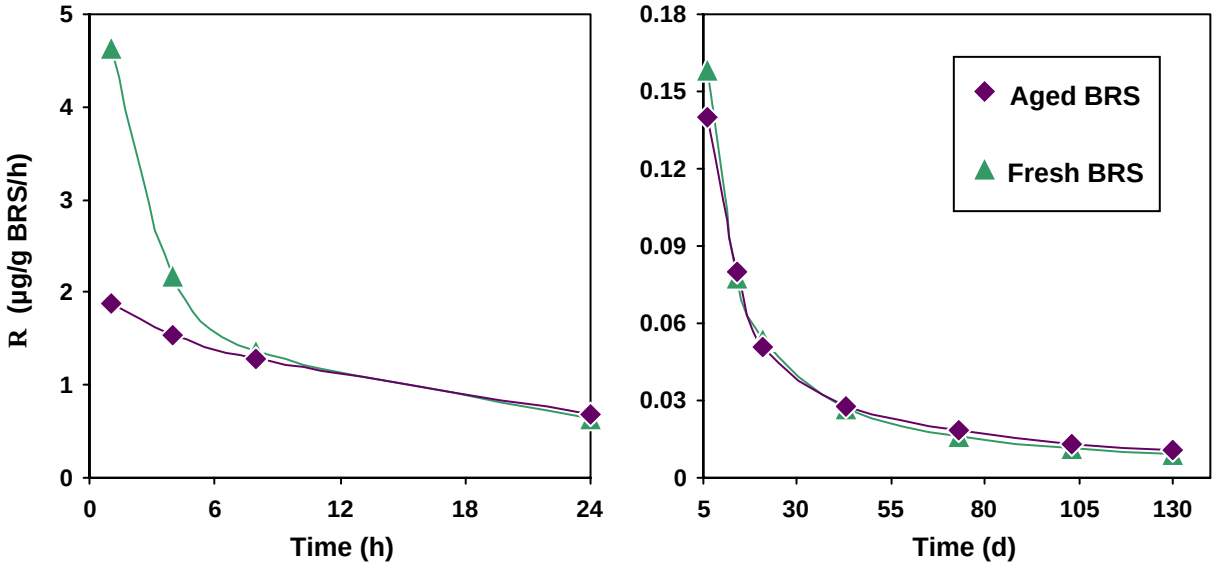

Figure 2.3. **The change in rate of transformation of readily soluble manganese over the incubation period. Only Mn-50 treatment is shown for clarity.**

RECOVERY OF MANGANESE

Recovery of added Mn was slightly greater from aged BRS than fresh BRS (approximately 90 and 80 % respectively, Table 2.2). The remaining percentage represents the residual Mn not extracted by the various chosen solutions and therefore cannot be assigned to any one particular form. Residual Mn was determined by calculation, and its formation showed a similar trend to oxide and carbonate Mn. At the end of the incubation period, residual Mn concentrations had stabilised at levels akin to those of oxide-bound Mn.

Table 2.2. **Recovery of Mn by sequential fractionation after $MnSO_4$ addition to fresh and aged bauxite residue sand.**

	Fresh residue sand		Four-year-old residue sand	
Added Mn (µg/g)	Total[a] extracted (µg/g)	Recovery (%)	Total[a] extracted (µg/g)	Recovery (%)
0	1.2		1.9	
5	5.1	78	6.3	88
50	41.1	80	44.5	85

[a] Total of means for each fraction extracted over all sampling times (mean readily soluble Mn + mean weakly adsorbed Mn + mean carbonate Mn + mean oxide Mn).

Plate 2.2. *Bauxite residue sand and red mud is pumped and spread across the residue impoundments at Pinjarra.*

Plate 2.3. *Bulldozers cultivate the surface to assist in drying of the residue layers. However, excessive heavy vehicle traffic can cause sub-surface compaction. Crystallisation of dissolved salts is often seen on the surface of recently-dried residue.*

DISCUSSION

Amounts of DTPA-extracted Mn from soils are not often highly correlated with plant uptake and tissue concentrations (Reisenauer 1988), especially in mine spoils (Severson and Gough 1984). However, the rapid decrease in amounts of plant-available Mn (DTPA extraction) observed in the present experiment reinforced the visual observations and determination by foliar analysis of Mn deficiency in lucerne pasture growing on BRS (Alcoa World Alumina Australia Limited, unpublished). Mn deficiency in lucerne is prevalent despite broadcast Mn fertiliser additions being an integral part of pasture management in the residue disposal area. The ability of high pH soils to bind Mn in plant-unavailable forms has been well documented (see Barber 1995) but the unique nature and lack of characterisation of BRS as a substrate for plant growth provides a fresh opportunity to study the reactions and interactions involved in Mn transformations. Mention of "unavailable" Mn forms in the present discussion refers to forms not available for plant uptake in their present state (ie. not Mn^{2+}). It is recognised that soil Mn oxides [Mn(III) and Mn(IV)] may provide a source of Mn to plants (Uren 1981; Walter 1988), but reduction reactions must take place before uptake will occur.

With the exception of readily soluble Mn, the behaviour of all Mn fractions over time was similar to those of a previous study where Mn was added to a high pH calcareous soil (Tong *et al.* 1995), with the exception of readily soluble Mn. Tong *et al.* (1995) found readily soluble Mn to be below inductively coupled plasma spectrometer detection limits, but no extractions were made inside 1-d incubation. Considering the chemical characteristics of BRS, Mn transformation to unavailable forms was expected to be as rapid, if not more so. Hence, a number of extractions were scheduled within 24 h of Mn addition. Analyses revealed that readily soluble Mn persisted in appreciable amounts throughout the entire incubation period in the aged BRS Mn-50 treatment and up to 14 d in all other treatments.

Sequential extraction showed the transformation of Mn from available to unavailable species to occur in stages. The initial Mn transformation appears to be from the readily soluble to the weakly adsorbed form, evidenced by the respective rapid decrease and increase in their proportions of the total Mn pool (Figure 2.2). Later, when weakly adsorbed Mn had become the dominant Mn fraction, the formation of Mn carbonates and oxides began to dominate transformations until an apparent equilibrium was reached. This is in agreement with the study of Mn distribution by Goldberg and Smith (1984) where, in a number of soils, levels of soluble Mn decreased rapidly, in the

initial phase, due to exchange, then over the longer term by processes such as oxidation, precipitation and diffusion into less accessible positions in mineral structures.

Tong *et al.* (1995) expressed the change of available Mn to fixed Mn in a simple equation. The same may be attempted for the different fractions in the present study. Though the three pathways given (Figure 2.4) all have merit and represent essentially the same reactions, pathway (1) is rather simplistic. Pathway (2) gives a good representation of the fractionation results, but it is most likely that the carbonate and oxide fractions are in pseudoequilibrium with the more readily soluble forms (Reisenauer 1988), so that pathway (3) may be the most accurate representation. The extent to which the equilibrium will allow transformations back to more soluble forms is governed by factors such as pH and electron activity (Norvell 1988). In BRS, Mn will therefore be strongly held in fractions such as oxides, with little reversion to readily soluble forms without the alteration of conditions in the system.

Nomenclature and classification of relative availability of Mn forms has long been a challenging issue among soil scientists and plant nutritionists due to the vast array of extractants, lack of standard procedures and the variable nature of Mn forms under different conditions. Generally, forms are defined operationally out of convenience. Limitations in the ability of extractants to mimic plant roots mean that interpreting data requires a large degree of inference. This is true for the sequential fractionation method used here. Readily soluble Mn is available, but its level at any one time may not be well related to plant uptake (Leeper 1947). On the other hand, using the definition above and due to limited solubility, oxide and carbonate Mn are considered unavailable for plant uptake in such a state, yet equilibrium, desorption, reduction and dissolution processes can provide plant-available Mn originating from these sources (Leeper 1947; Uren 1981). It is unlikely, however, that these processes will be highly active in BRS as they are a function of pH (Reisenauer 1988), and they are suppressed as pH increases.

Weakly adsorbed Mn is held on soil surfaces by forces ranging from weak electrostatic to strong ligand-exchange bonds, and is considered to be in pseudoequilibrium with the readily soluble form (Warden and Reisenauer 1991). Since it is not a principle form of soil Mn, weakly adsorbed Mn is more difficult to classify with respect to availability for plant uptake. Adsorption of metal cations is enhanced by alkalinity increasing to above pH 6 (Loganathan *et al.* 1977; McBride 1982), hence decreasing Mn availability (Reisenauer 1988). The initial increase in weakly adsorbed Mn in the present study, encouraged by the inherent chemistry of BRS, appears to occur

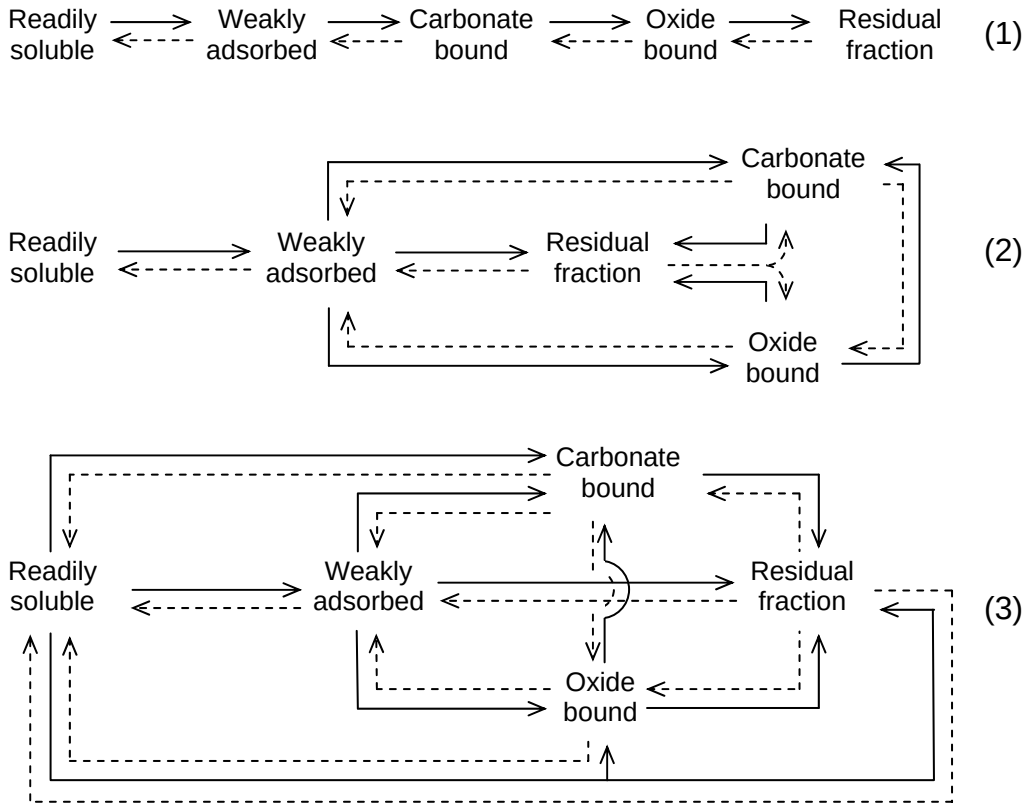

Figure 2.4. **Three theoretical chemical pathways for the transformation of Mn^{2+} added to bauxite residue sand. Pathway (1) shows a relationship between all the Mn fractions but in very simplistic terms. Pathway (2) represents the reactions apparent from the present results, with the initial transformation from readily soluble to weakly adsorbed Mn followed by an interactive equilibrium between the more complexed forms. Pathway (3) infers that each Mn fraction is in pseudoequilibrium with every other fraction through direct and indirect reactive mechanisms. Solid lines represent predominant reaction directions observed in the present study. Dashed lines represent "reverse" reactions allowing equilibrium formation.**

at the expense of some of the available (readily soluble) Mn, yet weakly adsorbed Mn may be much more easily transformed back into solution than oxide and carbonate Mn. It may therefore be seen as "partially available." The residual fraction also poses a problem as the data would suggest it be classified as unavailable, even though it may only prove so for the currently chosen extractants and conditions. Understanding the extensive limitations of soil extraction procedures means that subsequent classification of Mn forms as available, unavailable or partially available is relative to the particular set of conditions and must only be used as a rough guide in generalisations and applying

classifications to soil types. In the present experiment, distribution of the majority of added Mn, when equilibrium was reached, was in fractions considered to be of low availability to plants (Sims and Patrick 1978; Goldberg and Smith 1984).

Manganese carbonate and Mn(II) chemisorbed on soil carbonates are reported to influence Mn solubility in soils (Reisenauer 1988). The present results confirm this since carbonate Mn levels over time were inversely proportional to readily soluble Mn. The extractant for carbonate Mn, however, does have the ability to break down Mn oxides. Unintended dissolution of Mn oxide is the most important potential error associated with sequential chemical fractionation of soil Mn (Warden and Reisenauer 1991). The difficulties in estimating carbonate Mn as opposed to oxide Mn have been discussed elsewhere (Chao 1972; Warden and Reisenauer 1991; Han and Banin 1995; Tong *et al.* 1995). Although no analysis was carried out in the present experiment, the carbonate content of bauxite residue is considered high (Fuller and Richardson 1986; Wong and Ho 1994). Consequently, the potential for Mn reaction with carbonates in the system is substantial. Although a portion of the fraction designated as carbonate Mn may in fact be derived from the oxide Mn pool, it is still seen as being in a form unavailable for uptake by plants, and the high pH of BRS will act to prevent its transformation to the more soluble forms.

Microbes have the capacity to cause oxidation and/or reduction of Mn in soils. Hence, irrespective of experimental methods, it is very difficult to determine whether Mn transformations are chemical or bacterial in origin, and the current study is no exception. From the present results, it may be speculated that considering its alacrity, the initial Mn transformation from readily soluble to weakly adsorbed was most likely chemical in origin, at least primarily. Chemical oxidation of Mn^{2+} takes place only in soils above pH 8.5 – 9 (Reisenauer 1988), otherwise it is largely a microbial process. Bacterial populations in BRS are likely to be extremely small initially, yet the facilitation of Mn oxidation by bacteria may have been responsible for Mn oxide becoming the dominant form later in the incubation period (>21 d) after bacterial populations reached sufficient numbers to have a major influence over oxidation of Mn. Further work is warranted to elucidate the role of bacterial populations in controlling Mn transformations in BRS, and the microbial influence over equilibria between the various Mn fractions before management methods to increase Mn availability can be applied with confidence.

An interesting feature of the results was the more sluggish nature of Mn-transforming processes in the aged BRS than the fresh. If aged BRS has a pH that would be considered very high in agricultural terms, the fresh BRS pH must be

considered extreme. This 2.2 pH unit difference (Table 2.1) must contribute somewhat to the faster reaction in fresh BRS. Gotoh and Patrick (1972) demonstrated a faster rate of reaction of Mn from soluble to higher oxide forms in soils of higher pH. In addition, studies have shown that when oxides of Fe and Mn are present in soil, both rate and amount of Mn adsorption increase dramatically with increasing pH (McKenzie 1980, 1981). It has been suggested that Mn^{2+} concentration in soil solution should decrease 100-fold per unit pH increase (Barber 1995), but Neilsen *et al*. (1992), considering a variety of factors which alter solution equilibria, revealed that in reality, decreases in amounts of Mn^{2+} with pH increases are not so severe (5- to 10-fold decreases per 0.5 pH unit increase).

The presence of plants in any system will have a large effect on the balance between Mn forms. The presence of plant roots implies the presence of a rhizosphere, which will induce a vastly different chemistry and biology to the surrounding soil. Root exudates can reverse Mn oxidation processes directly through the process known as contact reduction (Leeper 1934; Uren 1981). Exudates can also influence rhizosphere bacterial populations by providing carbon sources, energy and nutrients for microbes as well as other compounds that promote or retard bacterial growth (Lynch and Whipps 1990). The collection of aged BRS from a vegetated area means it had been subject to rhizosphere processes for a number of years prior to the study. It is not known how long effects of root exudates will persist if plants are removed from a system, but it is unlikely to be prolonged since microbial metabolism of exuded substances can be very rapid. However, the rhizosphere processes may have set up a “basal” microbial population with a balance between Mn oxidisers and Mn reducers that may in turn cause the slower fixation of Mn in the aged BRS. The importance of rhizosphere microbial population dynamics influencing plant Mn nutrition, especially in relation to Mn nutrition, has been intimated elsewhere by a number of researchers (see Rengel *et al.* 1994 and references therein). Further research on the influence of rhizosphere and root exudates over Mn forms and equilibria is currently in progress and may provide a tool to manage nutrient availability on degraded soils and substrates such as BRS.

CONCLUSION

Manganese added to bauxite residue is transformed to plant-unavailable species very rapidly. Initially present in a readily available form in solution, it is quickly adsorbed to the BRS and then transformed less rapidly to oxide, carbonate and other forms. The occurrence and recurrence of Mn deficiency symptoms in BRS-grown plants suggests

that the fixation of Mn occurs faster than plants may be able to take it up. Current broadcast fertilisation methods would therefore seem an ineffective practice for improving Mn nutrition in both the short and long term since Mn will tend to be trapped at the surface of the residue profile where it can only be accessed by a few plant roots and only if moisture conditions are favourable. Given the present results, it would seem prudent to investigate alternate methods of Mn fertilisation for residue-grown plants, such as deep placement. Although binding will undoubtedly still take place, the position of the Mn in the profile may allow "reverse" transformations through rhizosphere processes to take place when plant roots reach the fertiliser layer.

The response and differential genotypic tolerance to growth under Mn-deficient conditions in BRS may also influence rhizosphere processes and, in turn, Mn transformations and equilibria between the various Mn forms. Using lucerne as a model species, genotypic response to varying amounts of Mn addition to BRS is detailed in Chapter III.

CHAPTER III

DIFFERENTIAL TOLERANCE TO MANGANESE DEFICIENCY BY LUCERNE GENOTYPES GROWING IN BAUXITE RESIDUE SAND

Plate 3.1. *Response of Salado lucerne to increasing manganese addition (L to R) in bauxite residue sand.*

The major content of this chapter has been published in the journal Plant and Soil:

Gherardi, M.J. and Rengel, Z. (2003) Genotypes of lucerne (*Medicago sativa* L.) show differential tolerance to manganese deficiency and toxicity when grown in bauxite residue sand. *Plant and Soil* **249**: 287-296.

CHAPTER SUMMARY

The physical and chemical characteristics of bauxite residue sand (BRS) decrease the availability of a number of nutrients to plants, especially manganese (Mn). Lucerne (*Medicago sativa* L.) has been chosen as a BRS revegetation species because of its deep-rooting habit and tendency for tolerating moderately alkaline and saline soils, but it is still prone to Mn deficiency stress. Sixteen commercially-available lucerne genotypes were grown in BRS at 5, 50 and 500 µg Mn/g BRS in a glasshouse. Manganese deficiency and toxicity symptoms were observed at 5 and 500 µg/g treatments, respectively. Symptom expression varied in severity among genotypes. Relative Mn-deficiency tolerance was defined by shoot dry weight at 5 µg/g as a percentage of shoot dry weight at 50 µg/g. Salado, a Mn deficiency-tolerant genotype, and Sirosal, a Mn deficiency-intolerant genotype were then grown at 0, 10, 20, 50, 100, 200 and 800 µg Mn/g BRS and found to have critical shoot Mn concentrations of 17.7 (Salado) and 22.6 µg/g (Sirosal). The use of genotypes with high relative Mn-deficiency tolerance is recommended to help improve sustainability of BRS revegetation and to improve productivity on Mn-fixing agricultural soils.

INTRODUCTION

The refining of bauxite ore to produce alumina results in a residue by-product containing alkaline liquor, coarse sand and fine mud. In south-western Australia, only one third of the ore is recoverable as alumina. Consequently, for every tonne of alumina processed, two tonnes of residue are produced. At Pinjarra, Western Australia, residue is stored in large impoundments where alternating layers of BRS and mud are finally capped with a 1.5- to 5-m-deep layer of BRS.

Revegetation of the Pinjarra residue impoundments to conform to the surrounding land-use of grazing pasture is problematic due to the harsh nature of the residue sand for plant growth. Extreme pH (10 - 12 initially) and salinity, accompanied by poor structure and water holding capacity provide conditions where availability of nutrients is markedly diminished. Poor availability of Mn is of primary concern because BRS conditions strongly favour the oxidation of manganese (Mn) to Mn^{3+} and Mn^{4+} (Chapter II, see also Gherardi and Rengel 2001). However, Mn is only available for plant uptake in the divalent form (Mn^{2+}).

Lucerne is extensively studied in Australia because it is a deep-rooted, perennial species with the capacity to lower water tables where subsequent leaching may ameliorate soil salinity (Latta and Blacklow 1997). These characteristics, along with a preference for alkaline soils, a moderate tolerance to saline and sodic substrates (Chaudhary *et al.* 1994) and the ability for good productivity on salinised soil irrigated with moderately saline groundwater (Mehanni and Rengasamy 1990), may give lucerne advantages over annual pasture species for revegetation of bauxite residue. However, lucerne planted on BRS deposits has suffered from Mn deficiency even with frequent Mn applications (Alcoa World Alumina Australia Limited, unpublished).

The low residual value of Mn fertilisers on Mn-deficient soils led to the alternative approach of identifying and breeding plant cultivars with a greater tolerance to Mn-deficient conditions (Graham 1988). Genotypic variability in lucerne does influence nutritional traits such as uptake and transport of potassium and phosphorus (James *et al.* 1995), but work with respect to micronutrient efficiency and lucerne genotypes has received little research attention.

Genotypic variation in the ability to tolerate soils of low Mn availability has been observed in a number of crop species (eg. Graham 1988; Graham *et al.* 1994; Krahmer and Sattelmacher 1995; Tong *et al.* 1997; Khabaz–Saberi *et al.* 1999). The literature also reveals a number of cases where lucerne genotypes have shown different concentrations of Mn in response to various soil, environmental stress, macronutrient and micronutrient treatments (Buss *et al.* 1975; Hill and Jung 1975; Lombin and Bates 1982; Dionne and Pesant 1985; James *et al.* 1995; Esechie and Rodriguez 1998; Townsend *et al.* 1998). These, however, are rare exceptions as most studies on nutritional variation of lucerne genotypes tend to disregard Mn, and even fewer studies have been carried out to identify Mn-efficient lucerne genotypes (Krahmer and Sattelmacher 1995).

Just as tolerance to Mn deficiency is related to substrate conditions and genotype, so too are critical Mn concentrations for deficiency diagnosis. Using the top 15 cm of vegetative growth, critical Mn concentrations in lucerne range from 30 µg/g in a New Zealand soil (Reuter and Robinson 1997) to around 20 µg/g in a US loamy sand and a silt loam with pH close to 7 (Heckman *et al.* 1993). Critical diagnostic concentrations can be very useful tools for formulating short-term and long-term nutrient fertilisation strategies, and in providing a reference point for monitoring of crop nutritional status. Establishing a critical Mn concentration for deficiency diagnosis of lucerne grown in BRS will provide a more specific basis for recommendation of Mn

application to enable better establishment and continual productivity of lucerne as a species for BRS revegetation.

Lucerne was first trialled by Alcoa World Alumina Australia for BRS revegetation in 1994, but the growth of lucerne in response to Mn applications in a BRS substrate has, so far, been poorly characterised. Since that time, a number of new cultivars have been released for commercial use. The present study involved testing of a range of commercially-available lucerne cultivars for growth and Mn uptake in BRS. Determination of genotypes tolerant to low Mn conditions may allow more sustainable BRS revegetation through a decreased requirement for fertiliser applications, and may also aid in improving production on other soils of poor Mn status. Subsequently, an investigation into critical Mn-deficiency concentrations of genotypes with different abilities to tolerate Mn deficiency was carried out to provide a concentration range which will aid in the future diagnosis of Mn deficiency.

MATERIALS AND METHODS

EXPERIMENT 1: GROWTH OF COMMERCIAL LUCERNE GENOTYPES AND TOLERANCE TO MN DEFICIENCY IN BAUXITE RESIDUE SAND

Lucerne was grown in pots of BRS in a temperature-regulated glasshouse (20 °C day, 15 °C night). Four-year-old BRS (for properties see Gherardi and Rengel 2001 and Chapter II), collected from 20 to 40-cm depth in the residue impoundment at Alcoa World Alumina Australia's refinery at Pinjarra, Western Australia, was used. Residue sand had been under irrigation, supporting limited native vegetation growth, for two years. Gypsum (50 t/ha) and poultry manure (50 m^3/ha) had been applied before planting in the impoundment, but no Mn fertiliser had been added. Collected BRS was air-dried, sieved and 3 kg placed into polythene-lined PVC pots (35 cm height, 9 cm diameter). Basal nutrients were added to the pots in the following concentrations (µg/g BRS): KH_2PO_4 90; K_2SO_4 140; $CaCl_2.2H_2O$ 150; $MgSO_4.7H_2O$ 20; $ZnSO_4.7H_2O$ 9; $CuSO_4.5H_2O$ 2; H_3BO_3 0.7; $CoSO_4.7H_2O$ 0.4; $Na_2MoO_4.2H_2O$ 0.2. Manganese (as $MnSO_4.H_2O$) was added to pots at rates of 5, 50 and 500 µg/g BRS (referred to hereafter as Mn-5, Mn-50 and Mn-500, respectively). All nutrients were added as solutions; soil was allowed to dry at 29 °C for one day, and thoroughly mixed. Pots were watered to approximate field capacity with double de-ionised water (DDI, 17.5 MΩ.cm resistivity) and maintained at approximate field capacity throughout.

Table 3.1. **Manganese content of seed of lucerne genotypes sown in bauxite residue sand to assess growth and Mn uptake and utilisation efficiency. Values are means ± standard errors (n=3).**

Genotype	Mn content (ng/seed)		Genotype	Mn content (ng/seed)	
Aquarius	232	± 12	Quadrella	220	± 29
Aurora	249	± 8	Salado	233	± 16
CUF 101	239	± 16	Sceptre	248	± 13
Eureka	262	± 21	Sequel	251	± 31
Genesis	265	± 14	Sequel HR	257	± 12
Hallmark	255	± 9	Siriver	253	± 20
Hunterfield	261	± 24	Sirosal	263	± 8
Hunter River	243	± 18	Trifecta	229	± 27

Seeds of 16 commercially-available lucerne cultivars (Table 3.1) were soaked overnight in 2 *mM* $CaSO_4$. Seeds were then inoculated with slurry of *Rhizobium meliloti* peat inoculant (Nitrogerm®) group AL (WSM826), and sown at 1.5-cm depth. Average Mn content of lucerne seed was 248 ng/seed and variation among cultivars was minimal (Standard error = 11 ng/seed). Seedling emergence occurred between 3 and 6 d after sowing, and plants were thinned to 3 per pot at d 12.

At 49 d after sowing, plants were cut just above the sand surface, rinsed three times with DDI, and oven dried at 70 °C. Sand was washed carefully from the roots which were then oven dried at 70 °C. Whole shoot and root material was ground, digested in HNO_3 at 140 °C, and analysed by ICPAES for Mn, Cu, Fe, Zn and P concentrations (Zarcinas *et al.* 1987).

All treatments were replicated three times within a completely randomised block. All data were subjected to analysis of variance (ANOVA). Where non-homogeneity of variances was present, data were subjected to natural log transformation prior to ANOVA. Since significance of treatment effects and interactions was not altered by natural log transformation, only the untransformed results are presented. Relative Mn-deficiency tolerance of each lucerne cultivar was defined as the shoot dry weight at Mn-5 as a percentage of shoot dry weight at Mn-50.

EXPERIMENT 2: DETERMINATION OF CRITICAL MANGANESE CONCENTRATION OF LUCERNE GENOTYPES AND GROWTH RESPONSES TO MANGANESE ADDITION WHEN GROWING ON BAUXITE RESIDUE SAND

From experiment one, a Mn deficiency-tolerant genotype (Salado) and a Mn deficiency-intolerant genotype (Sirosal) were selected for a second pot experiment. Glasshouse

conditions, BRS, pots, watering, inoculant and basal nutrients were used as described in experiment one. Manganese treatments of 0, 10, 20, 50, 100, 200 and 800 µg/g BRS (Mn-0 to Mn-800, respectively) were applied to the pots in triplicate.

Salado and Sirosal seeds were soaked overnight in 2 mM $CaSO_4$ which produced approximately 70 and 40 % radicle emergence respectively. Seeds were inoculated with *R. meliloti* and planted at 1.5-cm depth. Seedlings were thinned to 3 per pot 10-d after sowing.

Plants were harvested after 56 days, rinsed, dried, digested and analysed as per experiment one. After removing data representing extreme Mn toxicity, the Mitscherlich model as used by Ware *et al.* (1982) was fitted to describe the curvilinear relationship between shoot dry weight and Mn concentration. Critical Mn concentration was derived as the value corresponding to 90 % of the maximum shoot dry weight predicted by the model.

RESULTS

EXPERIMENT 1: GROWTH OF COMMERCIAL LUCERNE GENOTYPES AND TOLERANCE TO MN DEFICIENCY IN BAUXITE RESIDUE SAND

Lucerne growth and Mn-induced symptoms

Symptoms of Mn deficiency first became visible in Mn-5 plants of Genesis, Hunterfield and Sirosal 3 to 4 weeks after seedling emergence. Symptoms of Mn deficiency varied in severity among genotypes. A loss of green lustre and a yellowing of growing tips were the first indicators of Mn deficiency. This further developed to interveinal chlorosis, producing a yellow striped effect on the expanding leaves which, in some instances, led to a white/bleached appearance followed by necrosis and premature senescence of the affected leaves. Leaves of Mn-deficient plants were smaller and new leaf development was retarded compared with leaves of Mn-sufficient plants. At harvest (7 weeks) all cultivars were showing Mn deficiency symptoms in Mn-5 treatment plants, while all Mn-50 plants appeared healthy and showed significantly increased growth (Figure 3.1).

Manganese addition at 500 µg/g BRS significantly depressed growth of shoots and roots of all genotypes other than Salado, in comparison to Mn-50 treatment. Only Salado, Genesis, and Quadrella showed a high relative tolerance to Mn toxicity, producing significantly greater biomass at Mn-500 than at Mn-5. Comparatively,

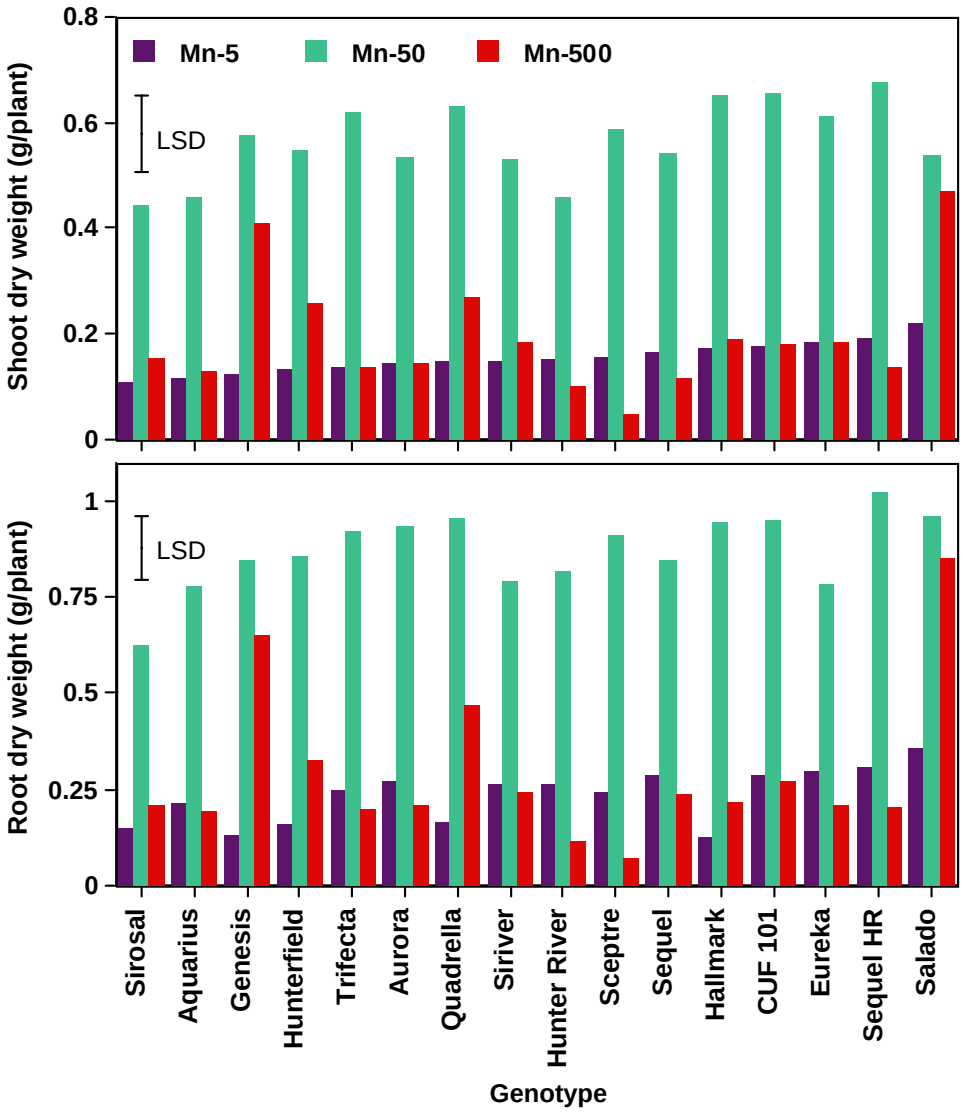

Figure 3.1. **Dry matter response of lucerne genotypes to low, adequate and high Mn (experiment 1). Least significant differences (LSD) for the genotype by Mn treatment interaction are shown.**

Sceptre and Hunter River were very sensitive to Mn toxicity and produced little biomass at Mn-500. This toxicity effect was accompanied by a range of visible Mn toxicity symptoms, including decreased leaf size, yellow and often brown, speckled discolouration of older leaves leading to necrotic patches. As with deficiency, toxicity symptoms also varied in severity among genotypes.

Analysis of variance revealed a significant interaction of genotype and Mn rate to affect biomass production of both roots ($P \leq 0.001$) and shoots ($P = 0.009$). Overall, in terms of biomass production, Salado was the best performing genotype with the smallest depression in growth at both Mn-5 and Mn-500 compared with Mn-50.

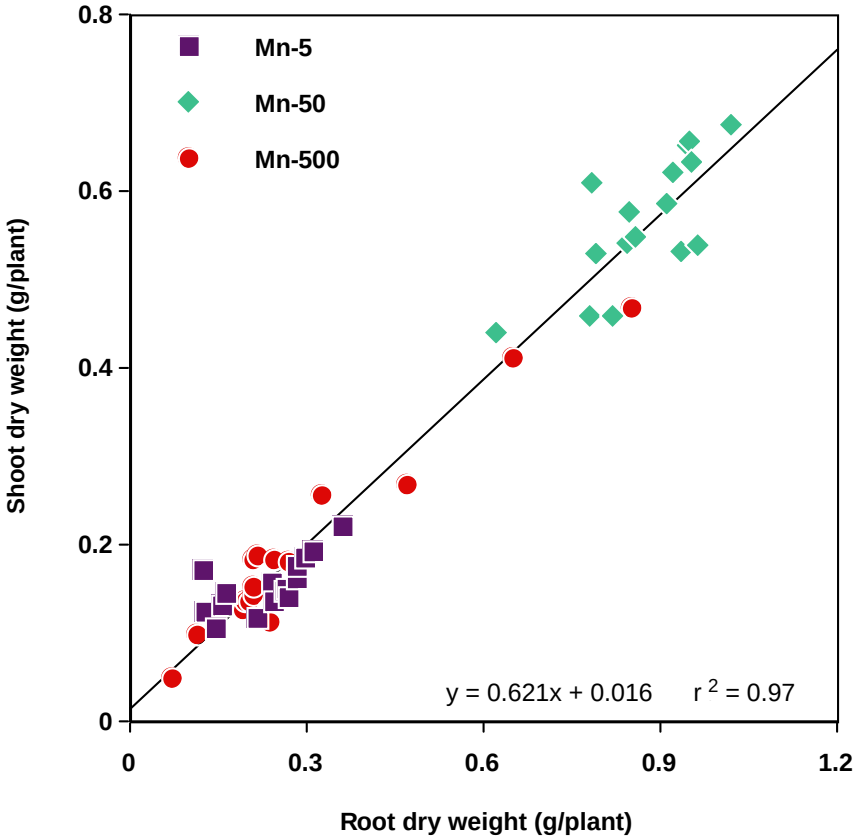

Figure 3.2. **Consistency of lucerne root:shoot dry weight at low, adequate and high Mn (experiment 1).**

In all plants, root growth was greater than shoot growth. However, the correlation between root mass and shoot mass was very high across all treatments (Figure 3.2) and neither Mn rate nor different cultivars had a significant effect on the ratio of root:shoot dry weight (mean = 1.635 ± 0.0245 standard error.).

Nutrient concentration and Mn distribution

Increasing Mn application rate significantly ($P \leq 0.001$) increased the concentration of Mn in lucerne roots and shoots (Table 3.2), but differences among genotypes were not significant. Root Mn concentrations were 2 to 3 times those of shoots of corresponding plants. The concentration of Mn in lucerne shoots of Mn-5 treatment plants (mean = 5.6 µg/g ± 1.2 standard error) was well below previously established guideline ranges for Mn deficiency diagnosis (Heckman *et al.* 1993; Reuter and Robinson 1997). Under the guidelines of Reuter and Robinson (1997), Mn concentrations of Mn-50 and Mn-500 plants can be classified as adequate and high/toxic, respectively.

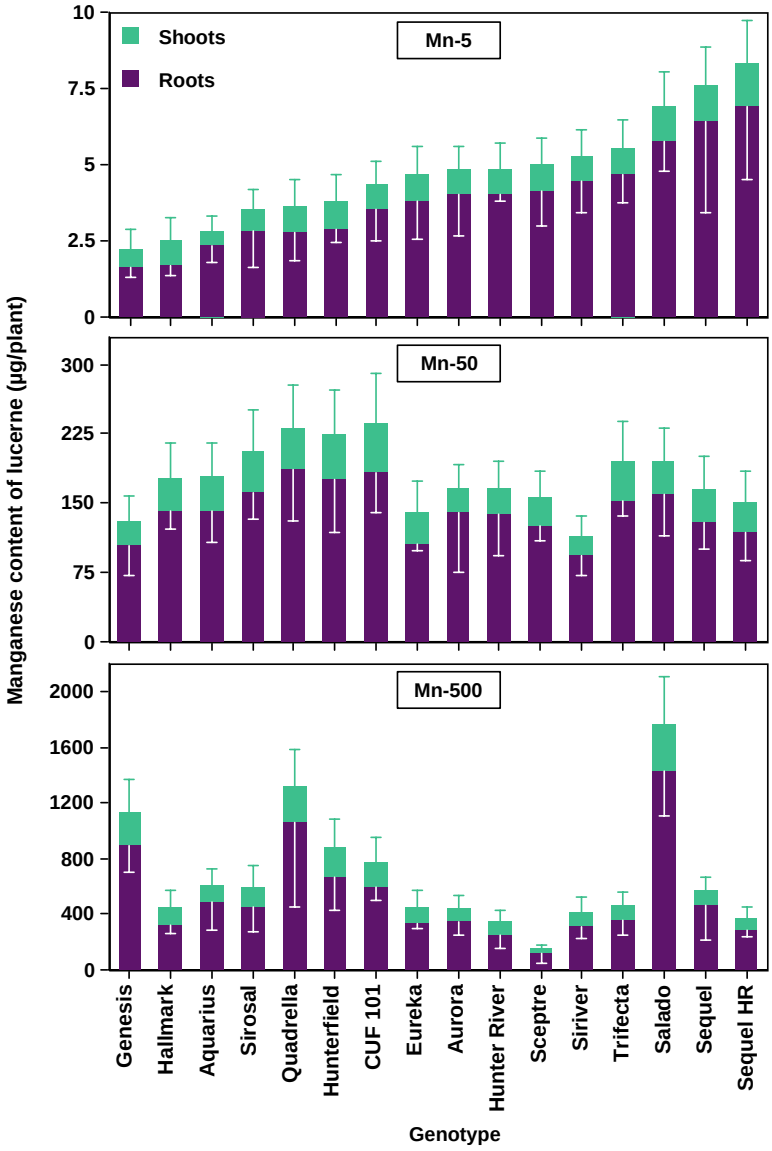

Figure 3.3. **Distribution of Mn between roots and shoots of lucerne genotypes (experiment 1). Bars represent standard errors (positive = shoot SE, negative = root SE).**

Table 3.2. **Concentration of Mn ± S.E. in lucerne shoots and roots (µg/g dw) at low, adequate and high Mn treatments. Data were averaged over all genotypes because the genotype effect and the interaction including the genotype effect were shown to be non-significant.**

	Mn concentration (µg/g dw)			
Mn treatment (µg/g BRS)	Shoots		Roots	
5	6	±0.3	16	±0.8
50	66	±4	164	±8
500	753	±37	1822	±84
$LSD_{(P=0.05)}$	*61*		*137*	

Manganese content increased with Mn application rate. The overwhelming majority was retained in the roots of all lucerne genotypes (Figure 3.3), but as Mn application increased, so did the proportion of Mn translocated to the shoots (Mn-5: 19.6 % ± 1.2, Mn-50: 20.4 % ± 0.5 and Mn-500; 22.6 % ± 0.7). Relative proportions of Mn in roots and shoots showed little variation among genotypes under the same Mn treatment, even though Mn contents did vary. Although not the case for shoots (P=0.062), differences in root (P≤0.001) and whole plant Mn content (P≤0.001) were significant due to two-way interaction between lucerne genotypes and Mn treatment rate.

Lucerne tissue concentrations of Cu, Fe, Zn and P were adequate for healthy plant growth (data not shown). Plants at Mn-5 treatment showed a degree of elevation in all four elements compared with higher Mn treatments, but in no case was this difference significant.

Tolerance to Mn deficiency

Based on the ratio of shoot dry matter in Mn-5 treatment to shoot dry matter in Mn-50 treatment, relative Mn-deficiency tolerance showed wide variation across the lucerne genotypes tested (Figure 3.4). Deficiency tolerance scores ranged from 22.5 % in Trifecta to 41.3 % in Salado. In selecting a Mn deficiency-tolerant genotype for further examination in experiment two, Salado was the stand-out choice producing the highest growth at low Mn application rate and with low internal Mn concentration (Figure 3.5). For Mn deficiency-intolerant genotype selection, although Trifecta had the lowest relative Mn-deficiency tolerance percentage, Sirosal was chosen as it produced the lowest shoot biomass of all varieties at Mn-5 and Mn-50, whilst having one of the highest shoot Mn concentrations. The combination of biomass production and shoot Mn concentrations revealed Sirosal to have a greater demand for Mn than other cultivars, whereas Salado had a comparatively lower demand.

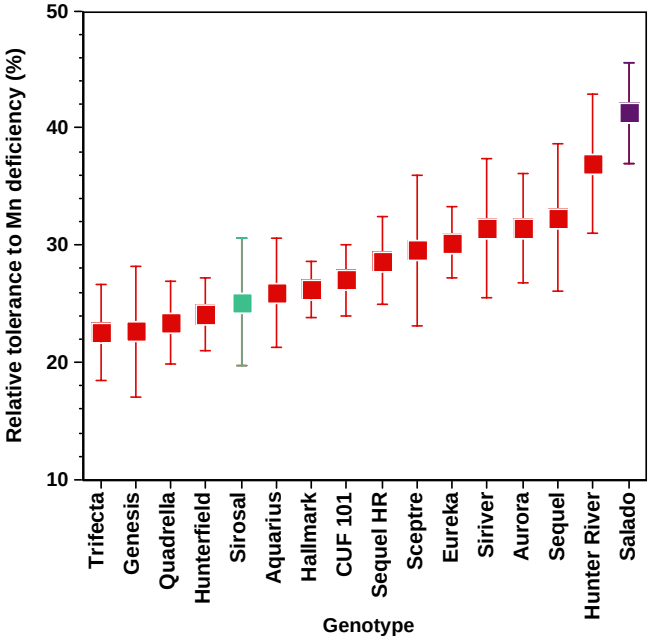

Figure 3.4. **Relative Mn deficiency tolerance of 16 lucerne genotypes growing on bauxite residue sand. Bars represent ± standard errors.**

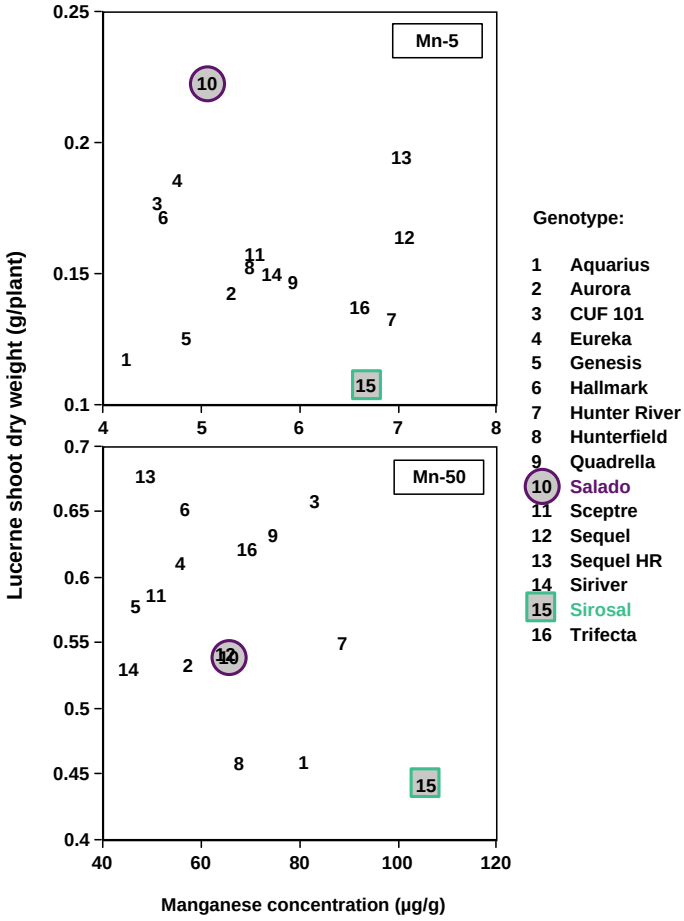

Figure 3.5. **Differentiation of lucerne genotypes based on the relationship between Mn concentration and biomass (experiment 1). Genotypes used in experiment 2 are highlighted.**

EXPERIMENT 2: DETERMINATION OF CRITICAL MANGANESE CONCENTRATION OF LUCERNE GENOTYPES AND GROWTH RESPONSES TO MANGANESE ADDITION WHEN GROWING ON BAUXITE RESIDUE SAND

Growth and biomass production

Manganese deficiency symptoms, as described in experiment one, were observed in both Salado and Sirosal plants at Mn-0 and some Sirosal plants at Mn-10. Severe Mn toxicity symptoms were observed in plants of both genotypes at Mn-800 to the extent that growth rate was severely depressed and no new shoots were produced by Sirosal after d 31. The growth trend response to increasing Mn application was similar in Salado and Sirosal, with large biomass increases being associated with small initial Mn rate increases up to 20 - 50 µg/g and thereafter a gradual decrease in biomass with further increases in Mn rate. Optimal application rate of Mn for shoot growth in BRS was about 20 µg/g. Apart from Mn-0 plants, Salado mean shoot mass and mean root mass were significantly higher than those of Sirosal ($P \leq 0.001$ for both shoot and root dry weight) in all Mn treatments (Figure 3.6). Maximum shoot mass was attained at 20 µg Mn/g BRS, whereas maximum root mass was recorded at 50 µg/g.

Manganese concentrations

In both Salado and Sirosal, addition of Mn increased Mn concentration in shoots and roots (Figure 3.7). External Mn rate increases up to 200 µg/g increased tissue concentrations exponentially, whereas further Mn rate increases resulted in proportionately lower tissue concentration increases. Differences in shoot Mn concentration were significant between the two genotypes ($P=0.015$) and Mn concentration increases with increased Mn treatment were also significant ($P \leq 0.001$). Similarly, ANOVA showed significant genotypic differences and Mn treatment-induced root Mn concentration differences. A significant interaction ($P=0.017$) between genotype and Mn treatment was also observed.

Determining critical Mn concentration

The plotted relationship between shoot dry weight and shoot Mn concentration was distinctly tri-phased. Salado and Sirosal shoots with Mn concentrations close to zero produced very little biomass, but small increases in shoot Mn concentration resulted in a

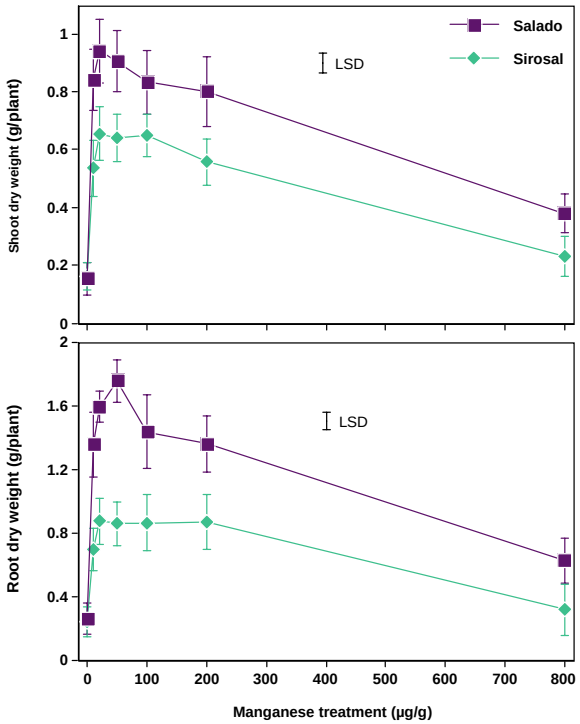

Figure 3.6. **Biomass response (± standard error) of Mn-deficiency tolerant (Salado) and Mn-deficiency intolerant (Sirosal) lucerne to increasing rate of Mn application to bauxite residue sand (experiment 2). Least significant differences for comparing genotypes are shown.**

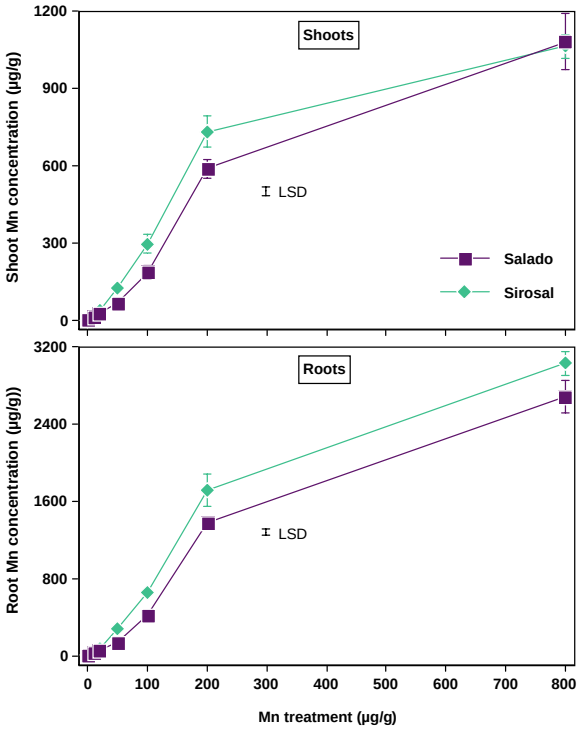

Figure 3.7. **Patterns of increasing Mn concentration (± standard error) in shoots and roots of Mn-deficiency tolerant (Salado) and Mn-deficiency intolerant (Sirosal) lucerne with increasing Mn application to bauxite residue sand (experiment 2). Least significant differences between genotypes are also shown.**

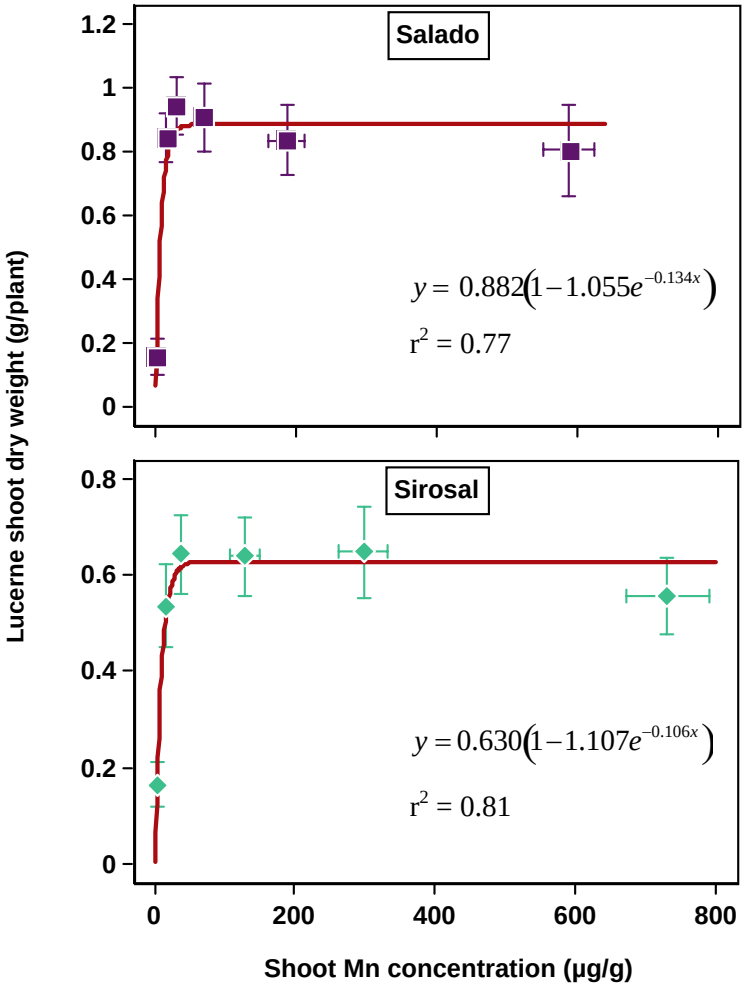

Figure 3.8. **The relationship between Mn concentration and dry weight in shoots of Salado and Sirosal lucerne. Only mean values are plotted for clarity and bars represent ± standard errors. Curves represent the fitted Mitscherlich model.**

substantial dry matter increase. A point was reached where further increasing shoot Mn concentration resulted in negligible differences in shoot yield, until at high shoot Mn concentration, shoot dry weight was depressed by Mn toxicity

Removing toxicity data of the highest Mn treatment (800 µg/g BRS) allowed the two-phased Mitscherlich descriptive model to be fitted to the data (Figure 3.8). The Mitscherlich model was able to explain around 80 % of the data variation in the shoot dry weight-shoot Mn concentration relationship.

Critical shoot Mn concentration for lucerne growth was calculated at 90 % of the maximum shoot dry weight predicted by the Mitscherlich model. The critical value calculated for Salado, 17.7 µg Mn/g dry weight (Mitscherlich $r^2 = 0.77$), was well below that calculated for Sirosal, 22.6 µg/g (Mitscherlich $r^2 = 0.81$).

Plate 3.2. *Lucerne foliage from successive branchlets (left to right: oldest to youngest) of Mn-deficient (top) and Mn-adequate (bottom) plants.*

Plate 3.3. *Interveinal chlorosis of new shoots and severe growth retardation in lucerne growing in BRS with manganese added at 5 µg/g BRS.*

DISCUSSION

Under the Mn-fixing conditions in BRS, lucerne plants not receiving substantial supply of Mn during the vegetative stage exhibit severe Mn deficiency symptoms, a depression in biomass production, decreased Mn uptake and decreased translocation from roots to shoots. As is the case of Zn deficiency (Grewal and Williams 1999), lucerne genotypes differ markedly in their response to Mn deficiency. A range of deficiency symptoms was evident depending on the level of Mn treatment and the genotype affected. The Mn deficiency symptoms observed in BRS-grown lucerne are similar to those described by Heckman *et al.* (1993) and by Lombin and Bates (1982) for lucerne grown in calcareous soils.

The capacity of BRS to bind Mn in forms unavailable for plant uptake is immense (Gherardi and Rengel 2001). Considering this, the appearance of Mn toxicity symptoms at Mn-500 was somewhat surprising. However, Mn toxicity was confirmed at high application rates in the second experiment. The brown speckled and necrotic patches on leaves affected by Mn toxicity are most likely due to the deposition and accumulation of oxidised Mn as documented by Horiguchi (1987). Increasing Mn application rate does decrease the transformation to unavailable forms in BRS, and the age and history of the BRS also affect Mn transformation rates (Gherardi and Rengel 2001). It may be expected that increasing equilibration time between Mn application and sowing will decrease the extent of Mn toxicity. A fresh BRS substrate may have a similar effect, but plants will then be faced with harsher pH and salinity levels.

Salado was developed as a saline-tolerant lucerne variety (Downes 2000), and is marketed for its abilities in production in agricultural areas affected by salinity. Bauxite residue sand resembles a highly salinised soil in many ways. The ability of Salado to produce reasonable growth at Mn levels that are deficient or toxic to other genotypes was highly evident. It may be speculated that the same mechanisms that are advantageous to salinity tolerance also benefit lucerne genotypes in tolerating high concentrations of other mineral nutrients. This is supported by the relatively high growth of Genesis, classed as a moderately saline-tolerant genotype, at high Mn.

The better growth of Salado at Mn-5 may also bear some relationship to its degree of salinity tolerance in allowing root growth in a substrate where other genotypes struggle, though the relatively poor performance of Genesis at Mn-5 suggests that this is not necessarily true as a general rule. High root density is beneficial in allowing roots to explore as much of the substrate as possible in order for exudation and other nutrient

uptake processes to maximise a plant's nutrient acquisition ability, especially in Mn-fixing substrates such as BRS. Lucerne genotypes selected for increased lateral and fibrous root growth ability show increased herbage yields (Lamb *et al.* 2000) and these are heritable traits. Ability for roots to exude compounds which allow or improve uptake from Mn-fixing soils is one proposed mechanism of tolerance to Mn deficiency in plants (Marschner 1988, 1995). Further investigation of exudation by genotypes with high relative Mn-deficiency tolerance, such as Salado lucerne, is warranted to determine the effectiveness of root exudates in mobilising Mn from high-pH substrates.

Although comparative shoot weight production at deficient (Mn-5) and sufficient (Mn-50) supply as a measure of relative tolerance to Mn deficiency showed strong variation across lucerne genotypes (Figure 3.4), the use of such an index alone should not govern the selection of a particular genotype for use in BRS revegetation. Salado was the genotype most tolerant to Mn deficiency (41.3 %) of those tested here, and showed the highest biomass production at Mn-5, whilst ranking low- to mid-range in internal Mn concentration at the same treatment level and hence can be strongly recommended for use in BRS revegetation. However, Hunter River, which ranked second highest in relative Mn-deficiency tolerance (37.0 %), also ranked 9th lowest in shoot biomass production at Mn-5 and 3rd lowest at Mn-50. On the other hand, Sequel HR, ranking only 8th best in relative Mn-deficiency tolerance (28.7 %) was second only to Salado in shoot and root biomass production at Mn-5, and second to none at Mn-50. On the basis of such data, Sequel HR appears a better choice than Hunter River as a lucerne genotype for BRS revegetation, even though it showed almost 10 % lower tolerance to Mn deficiency. Careful consideration of aspects such as water-use efficiency, productivity and fertiliser-use efficiency during successive growth/harvest periods should also be taken before recommendation of lucerne varieties for revegetation purposes. Field-based assessment of genotypes identified as tolerant to Mn deficiency in the present glasshouse study is also required.

Differential genotypic expression of Mn deficiency is related to plant Mn concentrations in cereal crop species (Rengel *et al.* 1993; Huang *et al.* 1994; Tong *et al.* 1997). In contrast, the lack of significant Mn concentration differences across genotypes in the present study indicates that relative tolerance is not dependent on shoot Mn concentration. This tends to suggest that lucerne genotypes have different abilities for internal Mn utilisation and/or there is variation among lucerne genotypes in minimum functional Mn requirement. Similar results have been published for lucerne and Zn-deficiency tolerance (Grewal and Williams 1999).

Graham (1988) presented some evidence, based predominantly on cereal studies, that internal Mn requirement is not an important mechanism for deficiency tolerance. The present results confound this conclusion as Salado (41.3 %) was almost twice as tolerant to Mn deficiency as Sirosal (25.1 %), producing significantly higher growth while showing lower shoot and root Mn concentrations and significantly lower critical Mn concentration (Figure 3.8). This indicates that Mn deficiency-tolerant lucerne, or at least the genotype Salado, can produce more growth per given amount of Mn absorbed by the root system through more effective internal utilisation, and hence that internal utilisation may be a more important mechanism for tolerance to Mn deficiency than previously thought.

Difficulties can arise when fitting statistical models to plant growth data. In the present case, variance in both shoot biomass and shoot Mn concentration increased with increasing Mn rate applied. When these data were combined to form the plant growth by Mn concentration relationship (Figure 3.8), the variances combine to reduce the goodness-of-fit of the model at high Mn concentrations. This was not unexpected for lucerne as selection and breeding has not, in the past, been aimed at Mn fertiliser responsiveness. Critical nutrient concentrations may only be established after several criteria have been met (Smith and Loneragan 1997). In the present study, a full response of plant growth was obtained at adequate levels of other nutrients. Secondly, a positive relationship between shoot Mn concentration and shoot growth was shown to exist. The third criterion is that the tissue sampled has a relatively stable Mn concentration at the specified harvest time. The minimal amount of variance expressed in replicate plants at low Mn treatments, allowed this final criterion to be satisfied within reason and acceptable r^2 values were obtained.

Determination of critical values for Mn deficiency in lucerne has not been carried out for a wide range of soil types. James *et al.* (1995) quote critical shoot Mn concentration range of 15 to 30 µg/g based predominantly on studies in non-alkaline soils. The critical shoot Mn concentrations calculated for Salado (18 µg/g) and Sirosal (23 µg/g) lucerne grown in highly alkaline BRS lie well within this range, and they closely resemble the limits of critical concentrations of a wider range of plant species and varieties (Hannam and Ohki 1988) and lie either side of the critical shoot concentration of 21 µg/g determined previously for and unspecified lucerne variety (Heckman *et al.* 1993). The difference in critical shoot Mn concentration for Salado and Sirosal in the present results reinforces the specificity of response of different genotypes to a given nutrient and the necessity of refining critical values to be more applicable to specific plant varieties in specific situations. The values presented here may be used as a

guide for Mn deficiency diagnosis of Salado and Sirosal lucerne grown on alkaline substrates.

CONCLUSION

Without generous additions of Mn, lucerne growing in BRS shows Mn deficiency. Methods of decreasing the need for Mn applications are being sought so as to improve the sustainability of bauxite residue revegetation. Although the mechanisms are yet to be determined, genotypes with high relative deficiency tolerance, such as Salado lucerne, show promise in being better able to utilise Mn under conditions of low Mn availability in BRS. It would seem advantageous to focus on such varieties for both future revegetation programs and wider agricultural and plant production systems where Mn-fixing soils are prevalent. Lucerne genotypes identified in this study could also be used in breeding programs aimed at improving lucerne tolerance to Mn deficiency.

CHAPTER IV

IMPROVED PRODUCTIVITY OF LUCERNE GROWING ON BAUXITE RESIDUE IN THE GLASSHOUSE BY DEEP PLACEMENT OF MANGANESE FERTILISER

Plate 4.1. *Lucerne in pots in the glasshouse with bauxite residue sand and deep-banded manganese.*

The major content of this chapter has been published in the journal Plant and Soil:

Gherardi, M.J. and Rengel, Z. (2003) Deep placement of manganese fertiliser improves sustainability of lucerne growing on bauxite residue: A glasshouse study. *Plant and Soil* **257**: 85-95.

CHAPTER SUMMARY

Successful revegetation of bauxite residue sand (BRS) requires large inputs of nutrients such as manganese (Mn), yet Mn deficiency is still encountered, raising doubts about sustained productivity of revegetated BRS disposal areas. The application of deep banding of Mn, a measure common in agriculture, was examined as a method for improving productivity when lucerne (*Medicago sativa* L.) is used as a species for BRS revegetation. In pots containing BRS, Mn was banded at 2.5-, 10- and 20-cm depths at rates of 10, 20 and 50 µg/g BRS. Two lucerne genotypes used were Salado, a Mn-deficiency-tolerant variety, and Sirosal, a Mn-deficiency-sensitive variety. Banding at 10-cm depth produced the best shoot growth of Sirosal at each Mn rate. Greatest shoot growth in Salado was found at 2.5-, 10- and 20-cm depths for 10, 20 and 50 µg Mn/g BRS, respectively. Deep banding 20 µg Mn/g BRS at 10-cm depth significantly increased lucerne growth compared with mixing through the profile. Banding at 20 cm produced Mn deficiency symptoms in lucerne during early growth, but symptoms were alleviated when sufficient amounts of roots proliferated in the banding zone. Dissolution and movement of Mn away from the fertiliser band were also investigated. In pots without plants, water throughput from watering twice weekly to 110 % field capacity had no effect on the amount of extractable Mn at distances more than 1 cm away from the original Mn band position. Whilst not only providing a more effective supply of Mn for BRS revegetation over one growth period, deep-banding of adequate rates of Mn may also result in a longer residual value, decreasing the need for frequent broadcast applications.

INTRODUCTION

Broadcast spreading of fertiliser granules over the soil surface at and after seeding is common practice in agricultural cropping and pasture systems. While such top-dressing may be the most cost-effective method on many soils, this may not be the case in soils where nutrient deficiencies occur as a result of the inherent characteristics of the soil.

Bauxite residue sand (BRS), a by-product of the alumina refining process, is an example of a soil-like system with immense capacity to induce nutrient deficiencies. The innate ability of BRS to rapidly decrease availability of a nutrient such as manganese (Mn) has been recently documented (Gherardi and Rengel 2001, 2003a).

The mobility of Mn in soil is generally low, especially in high-pH substrates (Reuter and Alston 1975) where rapid adsorption to substrate particles is promoted, with subsequent chemical and biological processes further transforming Mn to plant-unavailable forms. The Mn-fixing ability of BRS is chiefly related to its high pH promoting oxidation from Mn^{2+} to Mn^{4+}. Bauxite residue sand is dominated by coarse quartz silicate material, but oxides of iron and aluminium are also present in significant proportions (Roach 1992), adding to the specific adsorption ability of BRS. In addition, physical characteristics such as very low water-holding capacity and a highly compactable, porous structure result in rapid drying of surface layers after rainfall or irrigation events, and can inhibit root exploration of top horizons.

Lucerne has been chosen as a species for revegetation directly onto bauxite residue disposal areas due to its rooting habit in being able to access water from deep below the surface (Latta and Blacklow 1997). Additionally, lucerne shows moderate tolerance to saline and sodic substrates (Chaudhary *et al.* 1994). However, although BRS lucerne fields have been broadcast-fertilised with a full complement of macro- and micronutrients, symptoms of nutrient deficiencies have been observed, and foliar analyses have indicated Mn deficiency to be of primary concern (Alcoa World Alumina Australia Limited, unpublished data). The large amounts of broadcast fertiliser required to maintain sufficient nutrient concentrations in residue-grown lucerne are casting doubt over sustainable productivity of the revegetated system. Therefore, methods of decreasing the frequency of required Mn fertiliser additions and improving efficiency of Mn uptake from the BRS system are being sought. To this end, the method of deep banding Mn fertiliser may be beneficial.

Drying of topsoil has been found to restrict root uptake of various nutrients applied to the surface in a number of species. Manganese uptake by lupin was restricted by soil drying (Crabtree *et al.* 1998). Decreased uptake of phosphorus was observed in lucerne (Simpson and Lipsett 1973), wheat (Piper and de Vries 1964), barrel medic (Scott 1973) and lupin (Jarvis and Bolland 1991) in dry soil. Advantages of deep-banded fertiliser stem primarily from the increased moisture availability at depth encouraging nutrient dissolution, root elongation and activity. However, uptake of deep-placed Zn by wheat was greater than uptake of Zn at the surface even when surface soil was kept moist (Nable and Webb 1993), indicating a higher probability of root and rhizosphere interaction with the regions of increased nutrient concentration in a deep-banded system. This would enable more efficient mobilisation of nutrients in the active root zone.

Poor utilisation of broadcast Mn has been reported for various crops in agricultural production in conjunction with low Mn soils or soil treatments that increase soil pH (eg. soybean, Wilson *et al.* 1981; lupin, Hannam *et al.* 1985b; lucerne, timothy, wheat and oats, Gupta 1986). Broadcast applications of Mn salts almost invariably fail to correct Mn deficiency in deep-rooted perennial crops (Reuter *et al.* 1988), and have been superseded for agricultural production by banding in many cases. Seasonal crops require higher rates of broadcast Mn than banded applications to correct Mn deficiency (Mortvedt and Giordano 1975; Randall *et al.* 1975; Mascagni and Cox 1984).

In an effort to investigate possible effectiveness of supplying Mn to lucerne in BRS by banding, a glasshouse experiment was undertaken with various rates and depths of Mn banding for lucerne growth to a single harvest. The degree of passive Mn movement away from the Mn band with water drainage was also investigated. It was hypothesised that deep banding of Mn in BRS may be a more effective method of supplying Mn to BRS-grown lucerne and, if so, may improve the quality and effectiveness of BRS revegetation. This study provides an example of how technologies developed for agricultural production can be integrated into programs for revegetation and rehabilitation of processing wastes and degraded lands.

MATERIALS AND METHODS

RESIDUE SAND AND BASAL NUTRIENTS

Residue sand was obtained from 50- to 100-cm depth in Pinjarra residue disposal site of Alcoa World Alumina Australia. The collection site was supporting 4-year-old native vegetation, and had received gypsum (50 t/ha) and poultry manure (50 m^3/ha) prior to native vegetation seeding. No inorganic nutrient fertilisers had been previously applied. Gypsum treatments and leaching from 4 years of winter rainfall and summer irrigation had moderated the initial extreme alkalinity (pH 10-12) to pH 8.8.

Basal nutrients were added to the BRS as solutions to give final concentrations as follows (µg/g BRS): KH_2PO_4 90; K_2SO_4 140; $CaCl_2.2H_2O$ 150; $MgSO_4.7H_2O$ 20; $ZnSO_4.7H_2O$ 9; $CuSO_4.5H_2O$ 2; H_3BO_3 0.7; $CoSO_4.7H_2O$ 0.4; $Na_2MoO_4.2H_2O$ 0.2. Double-deionised water (DDI, 18 MΩ.cm resistivity) was used throughout. The BRS was left to dry, followed by transfer to 5-L plastic containers and thorough mixing by a mechanical tumble shaker (approximately 100 rpm) for 600 s.

LUCERNE RESPONSE TO MANGANESE PLACEMENT

The BRS fertilised with basal nutrients was placed into polythene-lined pots (40-cm height, 9-cm diameter, vertically cut to allow separation) up to 4.5, 12 and 22 cm from the top of the pot and watered to 80 % field capacity. Manganese as $MnSO_4.H_2O$ salt was placed in a single band (1 cm wide) across the diameter of each pot. Manganese rates of 10, 20 and 50 µg/g BRS were used (hereafter referred to as Mn-10, Mn-20 and Mn-50, respectively). Pots were filled with the remainder of BRS fertilised with the basal nutrients. At the top of each pot, 2 cm was left unfilled for watering, producing Mn bands at 2.5-, 10- and 20-cm depths. Bauxite residue sand above the Mn band was then watered to 80 % field capacity. A non-banded Mn treatment (Mn mixed thoroughly through pot profile by mechanical shaking as described above) of 20 µg Mn/g BRS was also set up for each cultivar.

Two lucerne cultivars with contrasting growth abilities in Mn-deficient BRS were used: Salado with high Mn efficiency and tolerance to Mn-deficient conditions, and Sirosal with low Mn efficiency (Gherardi and Rengel 2003a). Seeds were pre-germinated in aerated 2m*M* $CaSO_4$ solution for approximately 40 h, inoculated with *Rhizobium meliloti* (group AL, WSM 826) and placed in a line directly above the Mn fertiliser band, approximately 0.5 cm below the surface. Pots were maintained at 80 % field capacity throughout by daily watering to weight.

Seven days after emergence, plants were thinned to three per pot. Plants were grown for a further 7 weeks in an evaporatively-cooled glasshouse (mean maximum and minimum daily temperatures 28 °C and 16 °C, respectively). Shoots were cut just above the sand surface and rinsed three times with DDI. Each shoot was cut in half (the top part being classed as young shoots and the lower part as old shoots) and oven dried at 70 °C. Shoot material was ground, digested in HNO_3 at 140 °C, and analysed by an inductively-coupled plasma spectrometer for Mn, Cu, Fe, Zn, P and K concentrations (Zarcinas *et al.* 1987).

At harvest, the BRS column from each pot, and roots contained within, were divided into four sections (0-5, 5-15, 15-25 and 25-38 cm depth). Roots from each section were carefully washed clean with water. Root lengths were analysed by WinRHIZO® 3.9 (Régent Instruments Inc., Québec) scanning software, using a 300 dpi resolution and Lagarde's adaptative threshold for pale roots.

The experiment was set up in a randomised complete block design with factorial arrangement of treatments (2 genotypes by 3 Mn rates by 3 banding depths plus 1 non-banded treatment) within three replicate blocks. All results were subjected to analysis of

variance (ANOVA) by GENSTAT 5 (GENSTAT Committee 1989). Where necessary, data were natural log transformed to preserve homogeneity of variance; however, because this did not affect the significance of treatment effects, only the untransformed results are presented. To maintain orthogonality in ANOVA, only treatments containing banded Mn were included in analyses for interactive effects of Mn rate and banding depth. Where significant *F* values ($P \leq 0.05$) were found, least significant differences (LSD) were also calculated.

FORMS AND VERTICAL MOVEMENT OF BANDED MANGANESE

To investigate movement of banded Mn in the profile, identical pots were filled with BRS fertilised with basal nutrients as described for the experiment above, but with open draining bases and without plants. In half of 42 pots, Mn as $MnSO_4.H_2O$ was banded at 50 µg/g BRS 10 cm below the sand surface as described above. The remainder were control pots containing no Mn. Pots were placed in a temperature-controlled glasshouse (20 °C day, 12 °C night) and watered twice weekly (for a total of 5 weeks) to 110 % field capacity by hand-spraying the BRS surface with DDI.

On each of days 0, 1, 7, 14, 21, 28 and 35, BRS was sampled from banded and control treatments. Samples were taken from depths of 9, 10, 11, 12 and 15 cm by removing half of the pot wall (to expose the soil column) and pressing square cuvettes (approximately 1 x 1 cm internal cross-section) into the soil column, parallel to and vertically aligned with the Mn band, such that half the sample would be taken above the designated depth and half below (i.e. the 10-cm sample actually represented the layer 9.5 to 10.5 cm deep). Sub-samples of the BRS (4.0 g air-dry equivalent) from each pot were subjected to sequential chemical fractionation as outlined in Gherardi and Rengel (2001) to determine concentrations of plant-available Mn [DTPA-extractable], readily soluble Mn [$Ca(NO_3)_2$], weakly adsorbed Mn [CaDTPA-B_4O_7], carbonate-bound Mn [HNO_3], and oxide-bound Mn [$NH_2OH.HCl$].

RESULTS

SYMPTOMS OF MANGANESE DEFICIENCY DISORDERS

The number of leaves, leaf size and overall plant size were decreased in plants treated with Mn-10 at all banding depths. During early growth, this was also observed in plants where Mn-20 and Mn-50 were placed at 20-cm depth. The affected leaves of these plants were much paler than healthy plant foliage and often showed interveinal

chlorosis of varying intensity, giving affected leaves a lime green colour with distinct yellow stripes. Reduced internodal distances were observed in plants with retarded leaf development, contributing to the overall decrease in plant size. After 4 to 5 weeks of growth, newly developing leaves of most plants from Mn-20 and Mn-50 treatments at 20-cm banding depth showed none of the symptoms of Mn deficiency described above, whereas older leaves remained under-developed and continued to show Mn deficiency symptoms.

Plants of genotype Sirosal were generally more susceptible to Mn deficiency than those of Salado. In addition, shallow-banded (2.5 cm) Mn-50 retarded the stem growth of Sirosal for the first 2 weeks. Leaves of Salado plants subjected to the same treatment showed no distinct symptoms of a nutrient disorder, although cotyledons did develop some brown/purple discolouration.

SHOOT AND ROOT GROWTH

Lucerne shoot production trends with increasing depth of Mn placement differed with Mn rate applied. General trends for banded treatments were as follows: (i) for Mn-10, increasing depth of placement from 2.5 to 10 or 20 cm decreased shoot production; (ii) for Mn-20, Salado produced more shoot biomass with Mn at 10-cm depth than at 2.5 or 20 cm, whereas Sirosal shoot mass at both 2.5- and 10-cm banding depth was greater than at 20 cm; and (iii) for Mn-50, shoot growth was similar across all banding depths except for depressed growth of Sirosal at 2.5 cm (Figure 4.1).

Shoot biomass responses to Mn and depth treatments were similar in both lucerne genotypes, but Salado showed higher overall shoot dry weights than Sirosal ($P \leq 0.001$). A significant interaction ($P \leq 0.001$) between Mn rate and placement depth was also observed. At harvest, the highest shoot biomass production for Salado was 1.8 g/plant at Mn-20 banded 10 cm deep.

Shoot production of both genotypes in the banded treatments at Mn-20 was equivalent to, or greater than, the treatment in which this rate of Mn was mixed throughout the profile (i.e. not banded). Significantly greater shoot biomass was produced in the 10-cm banding treatment than the non-banded treatment.

The response of lucerne root growth to Mn rate and depth treatments was similar to that of shoots (Figure 4.2). Analysis of variance indicated that Salado produced significantly greater length of roots than Sirosal ($P \leq 0.001$), and a significant interaction

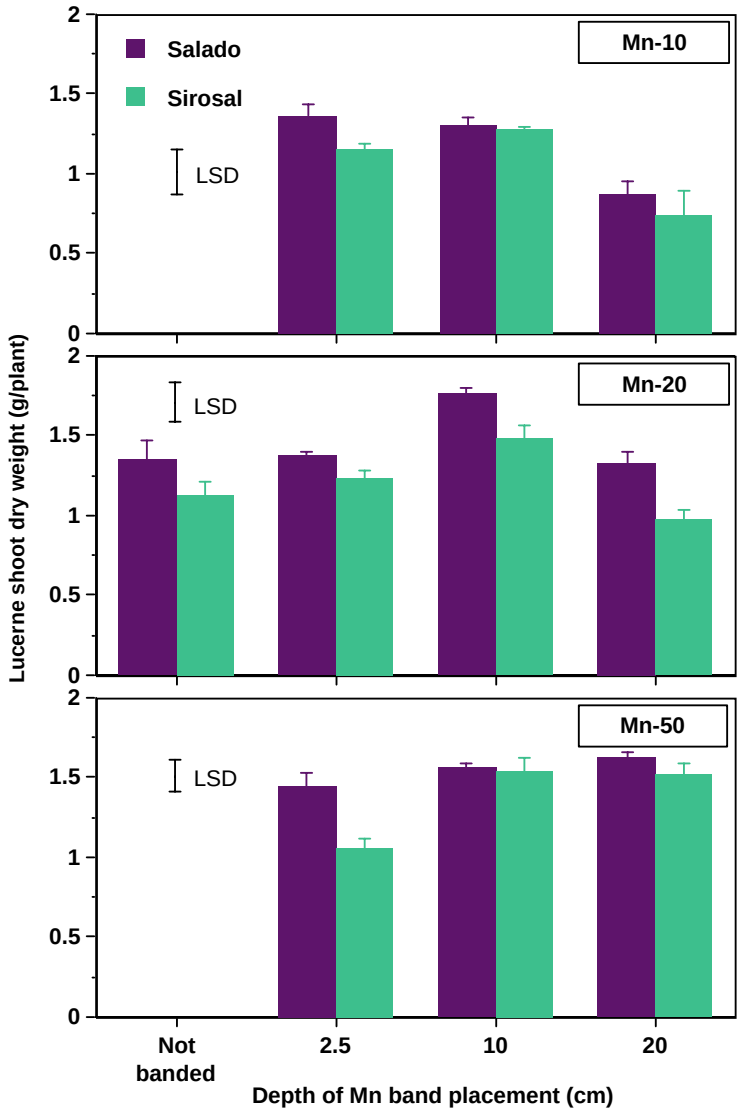

Figure 4.1. **Lucerne shoot weight response to manganese placement at different rates and depths. Bars represent standard errors of means. Least significant differences for comparing depth treatments at each Mn rate are shown.**

between Mn rate and depth of band placement (P=0.015) was found. However, the trends in the root length response to increasing Mn rate and placement depth were similar for both genotypes. As with shoots, greatest root length was found for Salado at Mn-20 and for Sirosal at Mn-50 when Mn fertiliser was banded at 10-cm depth.

Measurements of root length density (Figure 4.3) revealed that the majority of roots of both cultivars were found in the upper substrate layers. Generally, root length density close to the surface decreased with increasing depth of Mn placement, while in most 10-cm depth treatments and some 20-cm depth treatments root length density deeper than 5 cm in the BRS profile was greater than in treatments with banding at 2.5 cm and in non-banded treatments. This effect was most marked in plants of both genotypes grown in the Mn-50 treatment.

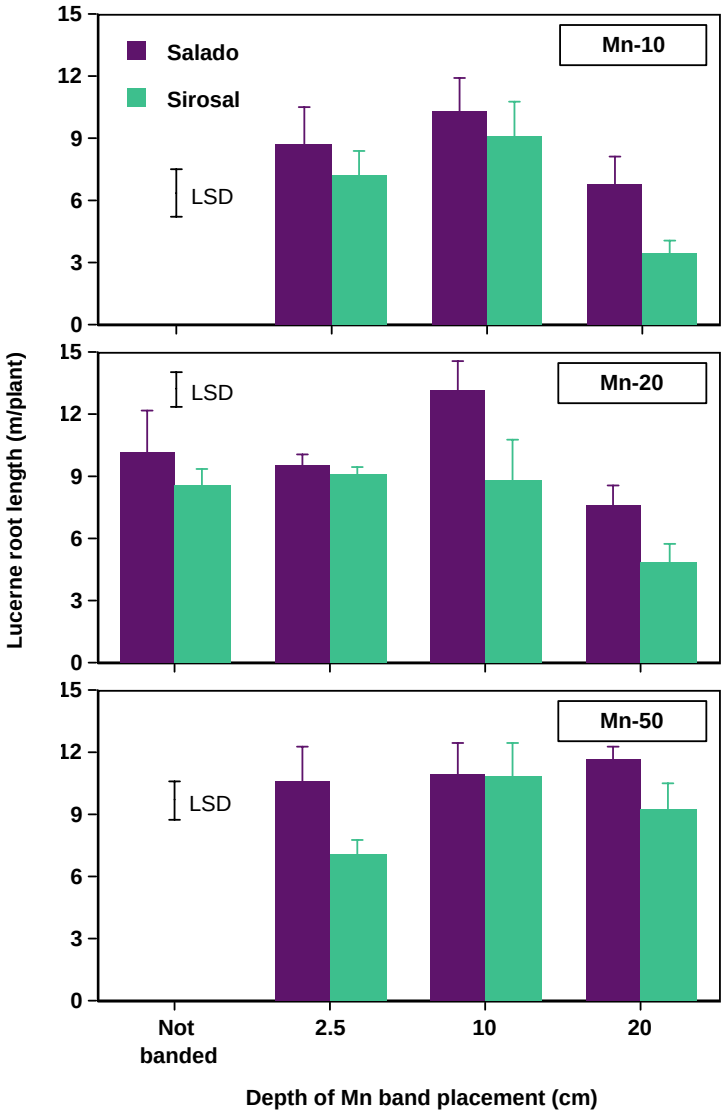

Figure 4.2. **Lucerne root length response to manganese placement at different rates and depths. Bars represent standard errors of means. Least significant differences for comparing depth treatments at each Mn rate are shown.**

Depth of Mn band placement was a key factor influencing root distribution through the BRS profile, although a significant (P=0.019) three-way interaction between depth of banding, genotype and Mn rate resulted in differences in root length densities. The interaction may have arisen from the majority of roots being found in the uppermost pot section in all treatments and a non-proportional decrease in root length density in the two genotypes with depth at Mn-50. Least significant difference values indicated that root length densities in most individual pot segments containing a Mn band were equivalent to, or significantly greater than in, similar depth segments not containing the Mn band (i.e. sections from pots of treatments with different banding depths).

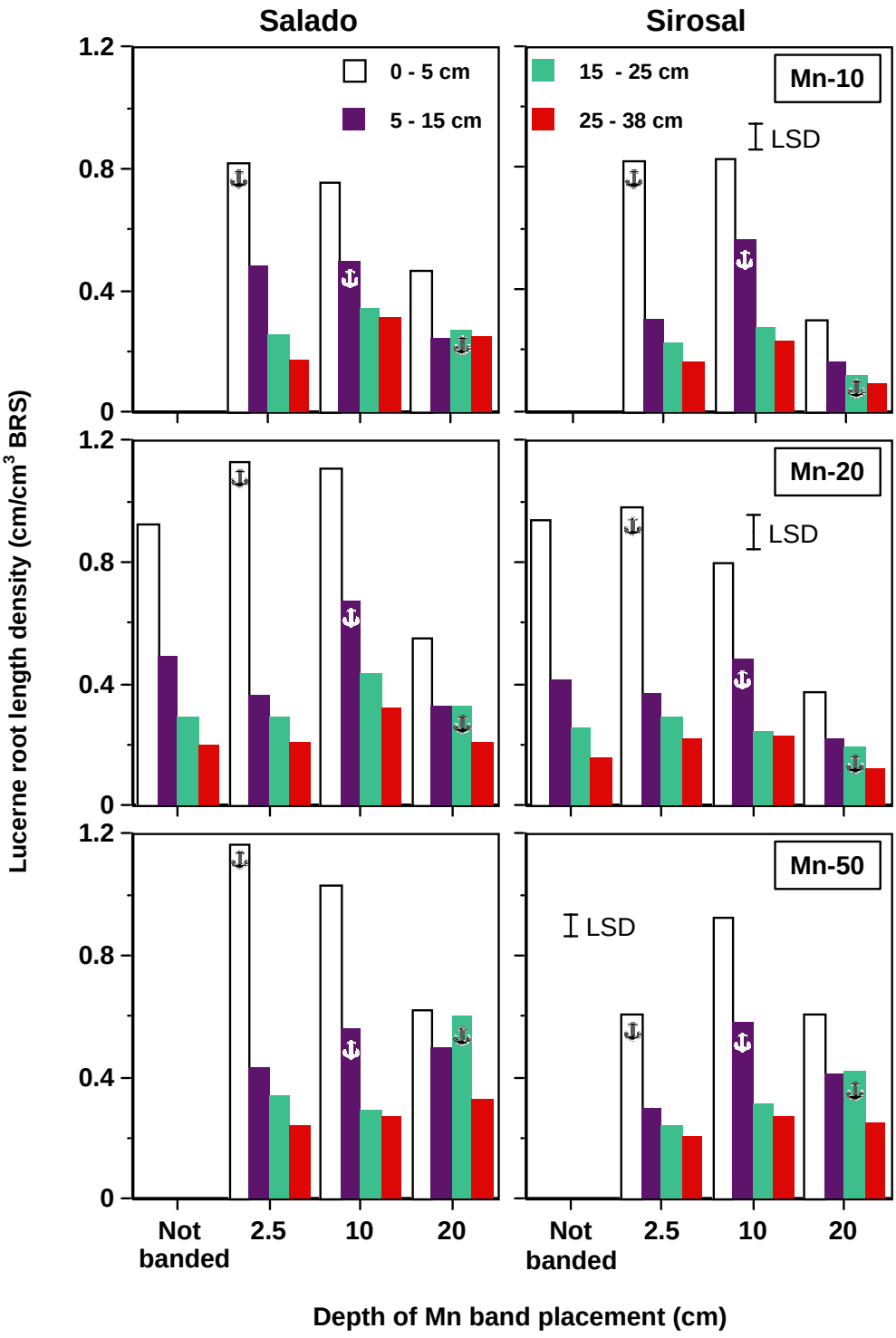

Figure 4.3. **Root length density of lucerne in different sections of the pot profile. Anchors (⚓) indicate pot section containing the manganese band. Least significant differences for the three-way interaction (genotype x banding depth x pot section) for each manganese rate are also shown.**

MANGANESE CONCENTRATIONS IN LUCERNE FOLIAGE

As the depth of Mn banding increased, Mn concentrations in the older shoots decreased significantly (Figure 4.4). Although similar trends were observed, variation in the Mn concentrations of young shoots was of lesser magnitude with increasing depth, and differences were generally not significant, when compared with the old shoots. However, except for plants in the Mn-10 treatment, all young shoots were well above critical Mn deficiency levels. In the case of the deepest banding (20 cm) at Mn-20 and Mn-50, the Mn concentration of young shoots was close to double that of older shoots, whereas at Mn-10, Mn concentrations were lower in the young than in old shoots.

With few exceptions, Sirosal plants showed higher shoot Mn concentrations than Salado in corresponding treatments. All other nutrient concentrations were within adequate ranges (Reuter and Robinson 1997) for healthy plant growth (data not shown), and there was little difference between the two cultivars.

MANGANESE MOVEMENT DOWN THE BAUXITE RESIDUE SAND PROFILE

Watering pots without plants to 110 % field capacity induced some drainage. However, although average water addition was 8 mL/pot/d, equivalent to approximately 4.5 mm precipitation required twice weekly to maintain the BRS profiles at 110% field capacity, movement of Mn away from the band was minimal. Significant changes in Mn concentration in bauxite residue over the experimental period were only evident in the BRS layers at (10 cm) or directly below (11 cm) the Mn fertiliser band (Table 4.1). At 10-cm depth, the initial high concentration of plant-available Mn rapidly decreased, reaching a low equilibrium concentration between 1 and 7 d, after which further decreases were not significant. Readily soluble and weakly adsorbed Mn concentrations followed the same trend, although the maximum extracted value for weakly adsorbed Mn was found after 24 h rather than at time zero.

Plant-available Mn, the readily-soluble Mn and weakly-adsorbed Mn fractions followed similar trends at 11-cm depth as at 10 cm (the actual depth of Mn banding), but the magnitude of the concentration changes across sampling times was much smaller. Highest concentrations for these three Mn forms at 11-cm depth were found after 1 d; significant ($P \leq 0.05$) decreases in extractable concentrations proceeded beyond 7 d.

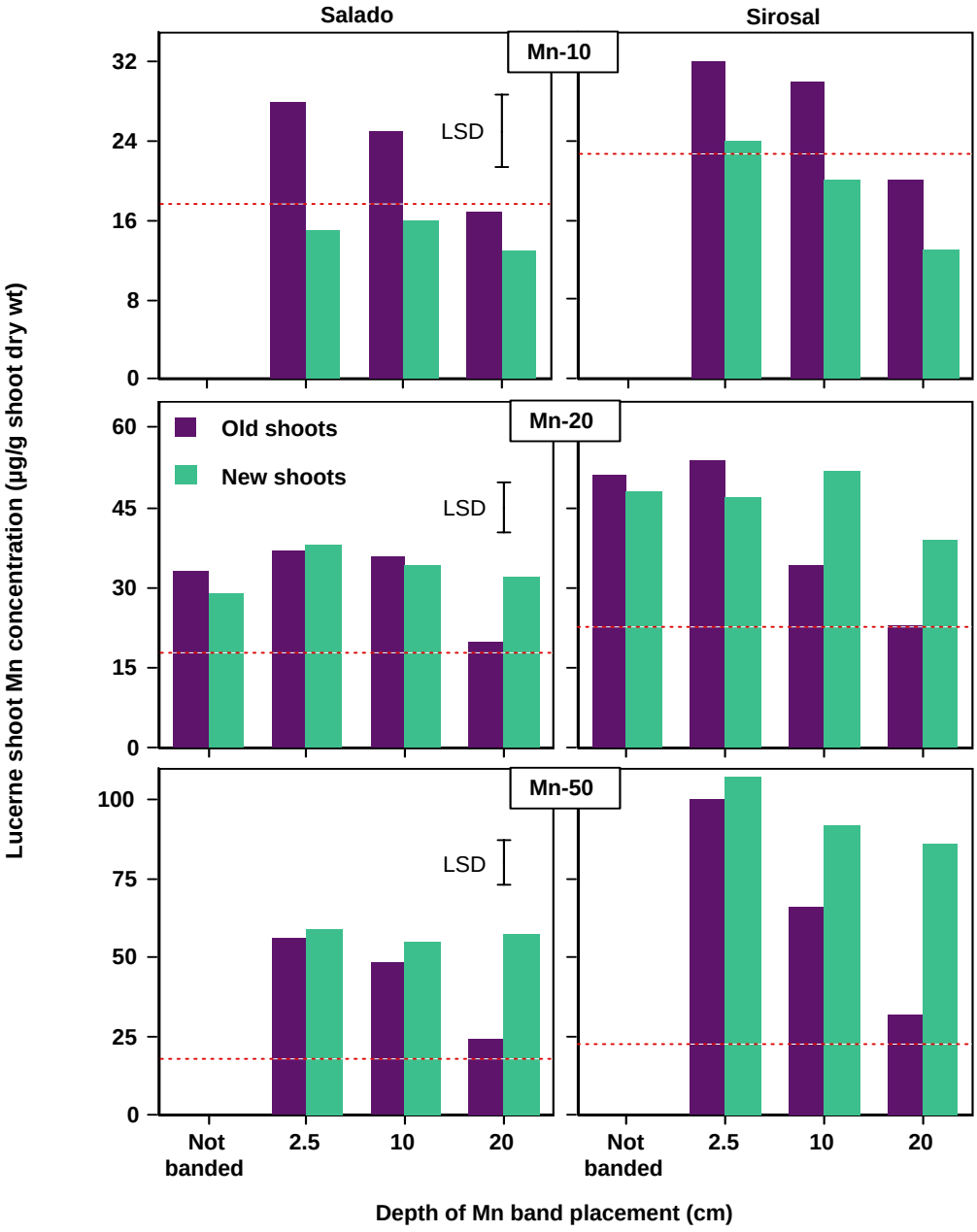

Figure 4.4. **Manganese concentration of lucerne shoots after deep banding of manganese in bauxite residue sand. Least significant differences for comparing depth treatments at each Mn rate are shown. Dashed horizontal lines represent critical concentrations for deficiency diagnosis (Gherardi and Rengel, 2002).**

At both 10- and 11-cm depths, carbonate-bound and oxide-bound Mn concentrations increased over time. These increases occurred at a similar rate, and in similar proportion, to concentration changes of other Mn forms. Once again, the magnitude of the concentration changes was smaller at 11-cm depth than at 10 cm. After 7 d, no significant increases in concentrations of carbonate-bound and oxide-bound Mn were found.

Table 4.1. **Concentrations of various manganese forms over time, at different depths, after banding 50 µg Mn/g BRS at 10 cm deep in a bauxite residue sand profile.**

		Manganese extracted in each fraction (µg/g BRS)				
Depth (cm)	Time (d)	Available Mn [DTPA]	Readily-soluble Mn [$Ca(NO_3)_2$]	Weakly-adsorbed [CaDTPA-B_4O_7]	Carbonate-Mn [HNO_3]	Oxide-Mn [NH_2OH•HCl]
9	0	0.21	0.34	0.27	0.28	0.26
	1	0.35	0.32	0.28	0.34	0.19
	7	0.24	0.46	0.36	0.31	0.42
	14	0.36	0.47	0.34	0.33	0.43
	21	0.38	0.40	0.29	0.36	0.41
	28	0.41	0.43	0.35	0.34	0.42
	35	0.37	0.37	0.31	0.27	0.44
	$LSD_{0.05}$	*0.16*	*0.21*	*0.19*	*0.19*	*0.24*
10	0	90	67	16	11	5.7
	1	49	25	31	22	28
	7	23	8.5	4.5	30	32
	14	28	6.1	2.9	37	40
	21	19	4.0	3.2	40	43
	28	16	3.2	2.4	34	56
	35	14	3.6	2.9	41	60
	$LSD_{0.05}$	*17*	*12*	*11*	*16*	*13*
11	0	0.54	0.34	0.24	0.41	2.4
	1	8.3	6.2	5.4	5.1	6.4
	7	6.8	5.5	2.5	5.7	7.2
	14	5.3	2.1	1.2	7.8	8.1
	21	5.1	1.3	0.95	7.7	8.7
	28	2.3	0.95	0.51	8.2	7.8
	35	1.1	0.73	0.45	8.2	8.5
	$LSD_{0.05}$	*1.4*	*0.56*	*2.1*	*3.9*	*1.7*
12	0	0.41	0.31	0.30	0.77	0.61
	1	0.42	0.36	0.23	0.66	0.69
	7	0.46	0.41	0.30	0.85	0.70
	14	0.40	0.41	0.25	0.67	0.63
	21	0.46	0.34	0.24	0.81	0.72
	28	0.37	0.28	0.32	0.90	0.86
	35	0.38	0.26	0.29	0.93	0.88
	$LSD_{0.05}$	*NS*	*NS*	*NS*	*NS*	*0.26*
15	0	0.30	0.16	0.30	0.23	0.31
	1	0.36	0.13	0.24	0.19	0.35
	7	0.39	0.21	0.31	0.20	0.33
	14	0.30	0.17	0.25	0.27	0.35
	21	0.36	0.18	0.33	0.32	0.45
	28	0.27	0.14	0.30	0.29	0.37
	35	0.28	0.21	0.32	0.31	0.41
	$LSD_{0.05}$	*0.10*	*NS*	*NS*	*NS*	*NS*
Control ranges[a]		0.21 to 0.61	0.15 to 0.40	0.19 to 0.57	0.17 to 0.75	0.20 to 0.68

[a] Maximum and minimum means of control (–Mn) treatment for all days and sampling depths.

No difference in concentrations extracted from control pots was significant at the 5 % level.

Plate 4.2. *Growth of Salado lucerne at week 6 in bauxite residue sand with Mn banded at 20-cm depth (left to right: 10, 20 and 50 µg Mn/g BRS).*

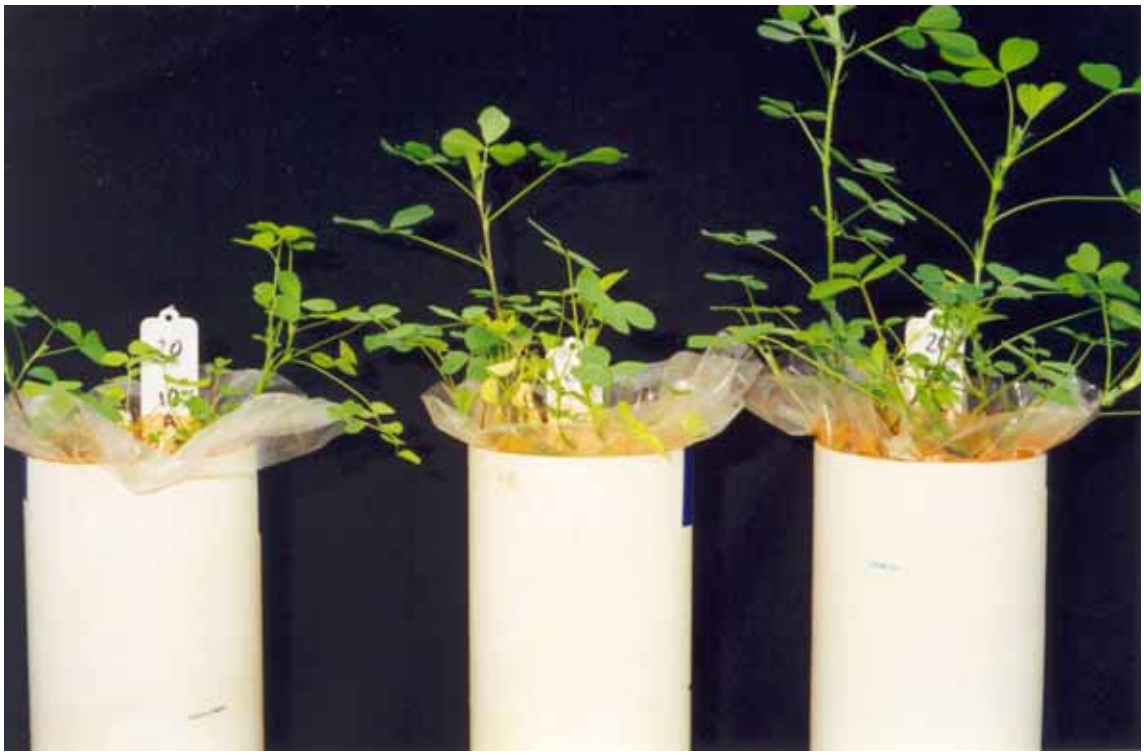

Plate 4.3. *Growth of Sirosal lucerne at week 5 in bauxite residue sand with Mn banded at 20-cm depth (left to right: 10, 20 and 50 µg Mn/g BRS).*

Plate 4.4. *Recovery of new lucerne foliage (left: Salado, right: Sirosal) after roots access fertiliser band, while old leaves still show deficiency symptoms (50 µg Mn/g BRS banded at 20-cm depth).*

DISCUSSION

Deep banding 20 µg Mn/g BRS at 10-cm depth was more beneficial to lucerne growth than incorporating the same amount of Mn throughout the profile in the present study. Earlier work found optimal lucerne growth in BRS to be achieved at, or close to, a rate of 20 µg Mn/g BRS when mixed (not banded) throughout the substrate (Gherardi and Rengel 2003a, Chapter III). Deep banding even half this amount at 10-cm depth resulted in lucerne growth equivalent to that achieved by the 20 µg Mn/g BRS treatment mixed through the BRS column. Similarly, a number of studies have shown that higher Mn rates are required to correct Mn deficiency by broadcast than by band application (see Martens and Westermann 1991 and references therein). The results demonstrate that deep banding of Mn fertiliser, at the correct rate and depth, has the potential to decrease the requirement for Mn inputs into a system with such high capacity for fixing Mn in forms unavailable for plant uptake.

The non-banded treatment represented a traditional broadcast-incorporated fertiliser application. Often less than 1 % of Mn applied to soil (broadcast) is absorbed by plants regardless of its form (Shorrocks 1984); hence deep banding and foliar Mn applications have been recommended for alleviating Mn deficiency (Gupta 1986). The

nature of perennial lucerne in producing several crops per year will increase the expense of foliar application. Additionally, the potential for crop damage by chemical burning, the need for monitoring to ensure correct timing of spraying and the lack of residual value of foliar applied Mn to subsequent crops, make Mn banding a more attractive option.

Banding micronutrient fertilisers is becoming a popular method of application in agriculture. Up to three-fold increases in soybean seed yield were observed after furrow-banding Mn on a pH 6.5 soil compared with broadcast application (Alley *et al.* 1978). In sandy Western Australian soils, drilling Mn with seed eliminated the split-seed disorder in the subsequent lupin crop (Gartrell and Walton 1984). In a similar soil, deep placement of Mn increased grain yield of lupin, with the deepest treatments (30 cm) producing the greatest biomass, grain yield and highest grain quality (Crabtree 1999). The present study shows promise in that adaptation of the fertiliser banding technology, developed for agriculture, may assist revegetation as well as economic and environmentally-conscious productivity on mine-processing wastes and other soils.

Combining banded Mn with other fertilisers such as phosphorus (P) may further improve the Mn effectiveness. Proliferation of roots has been observed where P was placed (Baeumer and Bakermans 1973; Drew and Saker 1978), and this may increase plant capacity for Mn uptake if both nutrients are placed together. Banding Mn in combination with acidifying diammonium phosphate was three times as effective as broadcasting in increasing the leaf Mn concentration of maize (Mascagni and Cox 1984). With respect to BRS, studies have found the buffering capacity of the substrate to be so high that pH will rise to Mn-deficiency-inducing levels again within a short time of concentrated acid addition (DeSantis 1997; Somes *et al.* 1998). This may negate any beneficial effect of acidifying compounds on increasing availability of Mn in BRS.

The Salado lucerne genotype has been found to produce greater biomass than that of Sirosal over a wide range of Mn fertiliser rates in BRS (Gherardi and Rengel 2003a). The present results confirm this finding, and demonstrate that differential genotypic response to Mn fertiliser also occurs with deep-banded Mn applications. The direct translation of root length differences to shoot weight differences also confirm the consistent positive relationship between root and shoot biomass previously observed in these two genotypes and 14 others (Gherardi and Rengel 2003a). Interestingly, differences in relative Mn efficiency, or tolerance to Mn deficiency of the two genotypes, had little effect on the magnitude of response to the rate of application or depth of Mn placement. Additionally, the shoot Mn concentration data suggest that the Mn-solubilising ability of Sirosal is commensurate with Salado. Thus, advantageous

mechanisms for tolerance to Mn deficiency involving substances exuded from roots may be of lesser importance in a banded system, provided that sufficient root growth can reach the luxury supply at the fertiliser band. Should this occur, genotypic growth differences will only be apparent if internal utilisation efficiencies or functional growth requirements differ.

Other than Salado at Mn-10, both shoot and root growth of lucerne benefited from Mn banding at 10-cm depth, and even deeper if the Mn rate was increased. Because root measurements were only made after harvest, it is likely that the increases in plant root biomass largely occurred after some roots were able to enter the zone around the Mn band where increased concentrations of Mn existed. Evidence for this was seen in the recovery of new shoots in treatments with the deepest banding that previously showed Mn deficiency. Reuter *et al*. (1988) suggested that roots close to a Mn band may have adequate Mn supply, but the rest of the root system may grow poorly due to a lack of Mn, and the severity of Mn deficiency in a plant may change as the proportion of roots in the Mn-fertilised zone increases or decreases with time. Manganese concentrations of new shoots in the present study reveal that in treatments with the deepest banding, once adequate root proliferation occurs at or near a Mn band, the increased uptake and translocation to new growth will alleviate Mn deficiency symptoms and encourage healthy plant growth.

Early Mn deficiency in lucerne at the 20-cm banding depth emphasises the importance of developing strategies to ensure optimum banding depth is achieved. If Mn bands are too deep, seed reserves of Mn may be inadequate to sustain root growth enough to adequately explore the profile for the fertiliser band. Seed-Mn reserves will differ between species and genotypes, seed sources and seed sizes, but band positioning is vital, and the necessity may call for a combination with broadcast or incorporated Mn applications to assist early growth if deeper band placement is sought.

Bauxite residue sand has a very low water-holding capacity. Drainage and evaporation from highly porous substrates mean that soil moisture levels often will decrease with increasing proximity to the surface. In acidic soils, it has been shown that an increase in Mn extractability occurs as soil dries (Ritchie 1989), but this is not always the case (Crabtree 1999). Air-drying may also increase availability of Mn in alkaline, calcareous soils (Legget and Argyle 1983). In contrast, the results suggest that the air-drying of BRS does not significantly alter its ability to fix Mn in plant-unavailable forms. Combined with the innate decrease in the ability of plants to access Mn from drier soils, this further accounts for the low residual value of broadcast applications to BRS.

Although some water movement through the highly porous BRS profile was occurring following water additions above field capacity, the movement of Mn down the profile was minimal, being detected by changes in extractable concentrations of the various Mn forms only 1 cm below the original Mn band position, but not 2 cm below. The mobility of Mn in soil and soil-like systems is largely determined by the inherent characteristics of the system, such as pH, redox conditions and mineral composition. Hence, availability of Mn sources within the actively absorbing root zone will determine the efficiency with which roots gain Mn (Reuter *et al.* 1988). The minimal movement of Mn through BRS in the present study, even from a highly concentrated band, is undoubtedly due to the binding and transformation of Mn to forms resistant to dissolution (Gherardi and Rengel 2001), restricting Mn movement by diffusion or associated with water movement.

The changes in extractable amounts of different Mn species over time in the present study compare well with the previous results (Gherardi and Rengel 2001) where initial transformation to plant-unavailable forms was extremely rapid when Mn was mixed thoroughly through BRS. Despite the expectation that such transformation would preclude most, if not all, of the banded Mn from being taken up by lucerne plants, Mn uptake was initiated when roots reached the regions of the Mn band, thus stimulating further root growth. It is likely that substances exuded from roots of the two lucerne genotypes were responsible for reducing the Mn to a solubilised form that could be taken up. Root exudate components can act in a number of ways. Low-molecular-weight organic acids can release Mn^{2+} from plant-unavailable MnO_2 (Jauregui and Reisenauer 1982) and chelate Mn^{2+} preventing its reoxidisation and increasing the mobility of reduced Mn in the rhizosphere (Marschner 1995), while phenolics can contribute to enhanced Mn reduction (Marschner 1988). It appears that root exudates are most effective when released into zones of high overall Mn concentration, such as a banded region, where they will be more effective in shifting the Mn equilibrium to plant-available forms. This is speculative, as the present experimental results do not provide unequivocal evidence. Yet, the relative increases in root length density observed in proximity to Mn bands, placed at easily-accessible depths, may reflect a plant response in attempting to capture maximum resources with a minimal investment in energy and organic matter.

Deep banding of Mn has a number of potential benefits. The results demonstrate improved accessibility of Mn to young lucerne plants growing in BRS, in-turn decreasing or correcting the onset of Mn deficiency and improving growth. However, the correct rate and depth of placement must be utilised to prevent growth being

adversely affected as in the case of 10 and 20 µg Mn/g BRS banded at 20-cm depth. Further testing in a field situation, where control over variability in watering, physical conditions in the BRS profile and the neatness and depth of large-scale banding is limited to a degree, will determine the feasibility of deep banding as a technique for improving BRS revegetation success. The likelihood of increased residual effectiveness of Mn banding over broadcast treatments, due to slow Mn dissolution and depletion from banded regions by water movement, also requires investigation. A positive result may improve the sustainability of BRS revegetation by maintaining healthy lucerne Mn nutrition with decreased need for fertiliser and water inputs.

CONCLUSION

Deep banding shows promise in supplying Mn to lucerne better than broadcast-incorporated applications on BRS when the whole profile is maintained at an adequate moisture level. Banding too deep in the profile of a Mn-fixing substrate can induce Mn deficiency in plants, and combination treatments with incorporated Mn in surface horizons may be required if banding at 20 cm or deeper is sought. The stimulation of root growth by Mn banding allows more effective uptake and improved Mn nutrition. Diffusive movement of Mn away from the band is minimal; therefore, bands have the potential to persist and remain an effective source of Mn for BRS revegetation for long time periods. Further validation in the field is warranted to assess the feasibility of deep Mn banding on an operational scale.

CHAPTER V

DEEP-BANDED MANGANESE FOR LUCERNE IN BAUXITE RESIDUE SAND: RESIDUAL EFFECTIVENESS IN THE FIELD

Plate 5.1. *Deep banding of manganese in lucerne pasture at the Pinjarra revegetated bauxite residue site*

The major content of this chapter has been published in Australian Journal of Soil Research:

Gherardi, M.J. and Rengel, Z. (2003) Deep banding improves residual effectiveness of manganese fertiliser for bauxite residue revegetation. *Australian Journal of Soil Research* **41: 1273-1282**.

CHAPTER SUMMARY

Revegetation of high-pH substrates such as bauxite residue sand (BRS) can be problematic since poor availability of nutrients like manganese (Mn) requires extensive and frequent fertiliser additions to maintain plant productivity. Recent glasshouse studies have shown improved growth and Mn nutrition of deep-rooted, perennial lucerne (*Medicago sativa* L.) growing in BRS when Mn was banded deep below the surface. Deep banding of Mn fertiliser shows promise in having long residual effectiveness in BRS. A field study involving deep-banded Mn in a BRS disposal area was undertaken. Manganese fertiliser, banded at a rate of 15 kg Mn/ha approximately 18-cm deep, provided effective supply of Mn to lucerne for at least 2.5 years, while 5 separate surface-broadcast applications (15 kg Mn/ha each) were required over the same period to maintain similar lucerne productivity. Shoot and root yields and Mn concentrations in shoots were consistently greater in the banded compared with broadcast treatments. Root extension deeper than 30 to 40 cm was largely inhibited by a physical compaction layer and an increase in pH with depth. Lucerne root length density was increased significantly by deep banding of Mn. In conclusion, deep banding of Mn fertiliser can improve sustainability of revegetated BRS areas and, potentially, productivity of other high pH substrates, by decreasing the frequency and amounts of Mn fertiliser additions required for healthy plant growth.

INTRODUCTION

Placing fertilisers deep in soil profiles can improve plant growth and uptake of mineral nutrients. This is considered to be due to the higher moisture content at depth allowing dissolution of nutrients from the fertiliser source, and access by roots for longer periods than in drier topsoil, as surface soil drying hampers the uptake of a range of nutrients by different plants (eg. Scott 1973; Simpson and Lipsett 1973; Grundon 1980; Jarvis and Bolland 1991; Crabtree *et al.* 1998; Crabtree 1999). In glasshouse trials, depth and extension of roots can be limited and, to some extent, directed by the size and shape of the pot. For a single harvest, this will not usually be a limiting growth factor for most plants if watering is adequate to maintain soil profile moisture. In field-grown perennials such as lucerne, however, likelihood of exposure to dry topsoil layers is great, especially during summer months in the Mediterranean type of climate

experienced in the south-west of Western Australia. On the other hand, rooting depth will only be limited by physical compaction layers below the surface, or barriers created by adverse chemical conditions such as pH extremes and salinity.

Bauxite residue sand (BRS), a coarse fraction of the by-products of alumina refining, shows extreme pH, salinity and sodicity due to incomplete removal of sodium hydroxide used in the refining process. Tens of millions of tonnes of bauxite residue are produced annually in Australia. Recent yearly estimates from three Western Australian refineries alone were over 17 million tonnes of residue for 7 million tonnes of alumina produced (Lockley 1999). Commonly in residue storage, the coarse BRS and fine (red mud) fractions are layered intermittently before a final 1.5 to 5 m layer of BRS is placed at the top of the impoundment. This layer needs to be revegetated to satisfy environmental policy requirements.

At Pinjarra, Western Australia, BRS revegetation through perennial lucerne pasture establishment is sought, as lucerne has characteristics which may enable it to tolerate the harsh BRS environment (Gherardi and Rengel 2003a), and a pasture system will be compatible with the land-uses surrounding the residue impoundments. However, Mn deficiency symptoms have been observed in lucerne growing on BRS (Gherardi and Rengel 2001, 2003a). In the four years following initial revegetation of a residue impoundment at Pinjarra, foliar analyses of lucerne have found that broadcast applications of Mn fertiliser can increase Mn concentrations in the shoots, but these concentrations peak at 3 to 4 months after application (Alcoa World Alumina Australia Limited, unpublished data) and then drop to below critical deficiency levels (Reuter and Robinson 1997) so that further large applications of Mn fertiliser are required.

Glasshouse studies have shown deep-banded Mn to be beneficial to lucerne when growing in BRS, even when plants were not subjected to moisture stress (Gherardi and Rengel 2003b, Chapter IV). In the same study, water movement (drainage) was found to have little effect on shifting Mn away from the original band position, indicating a strong likelihood that Mn bands may persist for extended time periods in BRS. It is likely that deep banding of Mn in a true BRS impoundment will also be effective in supplying Mn for lucerne growth. Additionally, the promise of longer residual value of deep-placed Mn over broadcast applications may improve sustainability of a revegetated BRS system by decreasing the need for frequent fertiliser applications while still providing an effective Mn supply.

MATERIALS AND METHODS

In August 1998, an area of irrigated lucerne growing on bauxite residue at the Pinjarra bauxite residue disposal site (32.64°S, 115.94°E) was cleared of all vegetation by glyphosate (360 g/L at 2.0 L/ha) and scarification. The field had a history of broadcast Mn fertiliser applications since its commissioning for revegetation in early 1994. Regular superphosphate, potassium, copper and zinc fertiliser had also been applied.

After clearing, Mn as $MnSO_4.H_2O$ salt was banded deep in the residue profile, at rates equivalent to broadcasting 0 and 15 kg Mn/ha, using newly developed drilling machinery (CSBP Futurefarm Research) and the surface was raked flat. On plots with no Mn placed at depth, $MnSO_4.H_2O$ (15 kg/ha) was applied to the surface, evenly broadcast throughout each plot by hand. Banded plots contained 6 lengthwise Mn bands at approximately 0.25-m intervals. All plots measured 2.1 m wide by 20 m long. A 0.6-m buffer was left between each plot. Plots were replicated 4 times within a randomised complete block design.

During the passes to band Mn fertiliser, urea granules (10 kg/ha) were also deep-banded in all plots (+Mn and –Mn at depth). The coarse urea granules aided consistent flow and delivery of the fine $MnSO_4.H_2O$ salt through the banding equipment and also allowed physical assessment of the depth of fertiliser placement: After banding, bauxite residue was carefully excavated at 8 randomly selected positions in each plot until the band of urea granules was detected, and the depth below the surface was measured and taken as Mn banding depth. A banding depth of around 18 cm was achieved (see Figure 5.1).

The pH of the BRS profile was measured (1:5, BRS:deionised H_2O) in augered BRS samples taken just prior to fertiliser banding at 10 cm intervals to a depth of 140 cm (below which the water table was reached).

The field was sown with a mixture of 3 lucerne cultivars, Aquarius, Genesis and Sceptre, each at 10 kg seed/ha for a total of 30 kg/ha. Seeds were first inoculated with commercial *Rhizobium meliloti* peat inoculant (group AL, WSM 826, Nitrogerm®). All plots received topdressing of Superphosphate-Cu-Zn at 100 kg/ha. This todpressing was repeated after every second shoot harvest (approximately 3-monthly), and 100 kg K/ha (as K_2SO_4) was included in every second application. During the first three weeks after sowing, irrigation (approximately 5 mm) was applied every 2 days after the last rainfall or irrigation event to maintain high surface moisture for germination. Thereafter,

irrigation was applied (up to 20 mm, twice-weekly) during periods of low rainfall (October to April, Figure 5.2) to prevent excessive drought stress.

The first lucerne harvest occurred in December 1998 (3 months after seeding). Thereafter, lucerne was harvested approximately every 1.5 months by cutting 5 to 10 cm above the ground level. Prior to each harvest 40 cm by 40 cm subplots were harvested by hand at 5 random positions within each replicate treatment plot. Subplot samples were bulked, oven dried at 70°C and weighed in order to estimate plot yield. The remainder of harvested shoots was removed from the site or, on approximately every fourth harvest, returned to the site as mulch. A portion of dried shoot material (upper-15 cm of shoots) from each plot was ground, digested in HNO_3 at 140°C, and analysed by ICPAES for P, K, S, Ca, Mg, Fe, Mn, Cu, Zn, Na, and B concentrations (Zarcinas *et al.* 1987). Only Mn concentration was analysed after the first 6 harvests. If analyses revealed shoot Mn concentrations near or below 21 μg/g dry weight, $MnSO_4.H_2O$ was re-applied to the plot by hand broadcasting at 15 kg Mn/ha.

In February 2001, a 220-cm deep trench was dug across all plots, and BRS samples were taken by horizontal insertion of a 10 cm length of steel box section (cross-sectional measurement: 4 cm height by 8 cm width) into the trench walls successively down to 76-cm depth. Measurements of soil penetrability were taken in triplicate using a pocket penetrometer at 10-cm intervals, and BRS samples were also taken for pH measurement as described above. The BRS was carefully washed from the lucerne roots in each sample by water over a 2-mm mesh. Root lengths were measured by WinRHIZO® 3.9 (Régent Instruments Inc., Québec) scanning software, using a 300 dpi resolution and Lagarde's adaptative threshold for pale roots.

All data were subjected to analysis of variance (ANOVA) using GENSTAT 5. Least significant difference values (LSD P=0.05) were calculated when F values from ANOVA revealed significant differences ($P \leq 0.05$) between variates (GENSTAT Committee 1989).

RESULTS

The fertiliser banding depth achieved was relatively consistent across all plots (Figure 5.1). Average depth of fertiliser placement was 18 cm. The target banding depth of 20 cm was not achieved entirely because of an underlying layer of compacted BRS that prevented tine penetration. This compacted layer was also associated with a substantial increase in pH (Figure 5.3). In February 2001, pH levels were generally lower than at the commencement of the study in September 1998, with the greatest decrease of 0.55

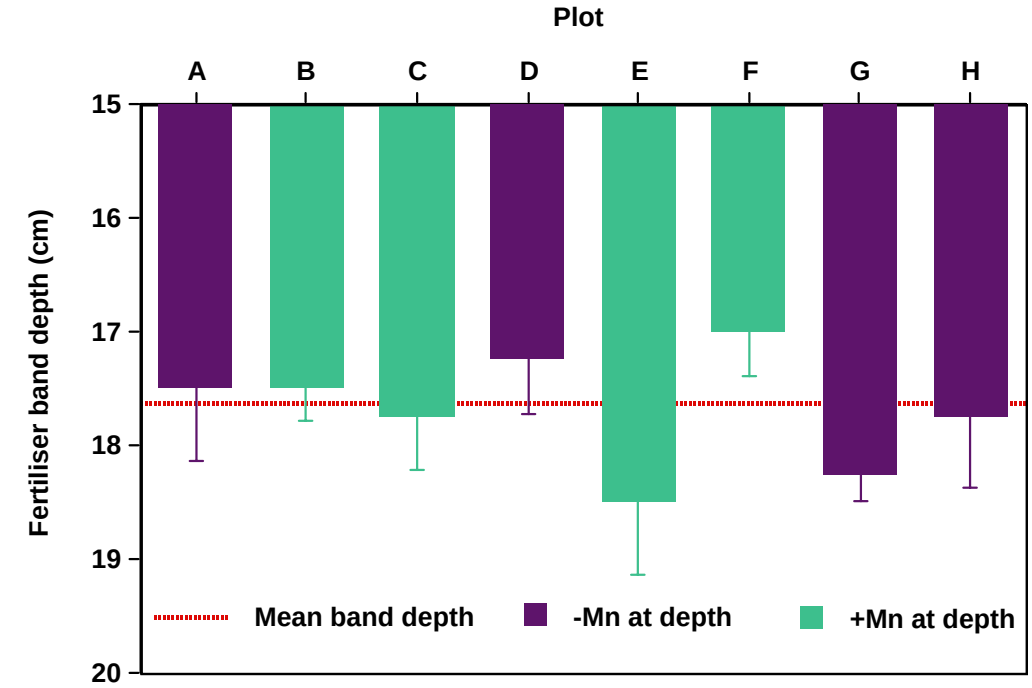

Figure 5.1. **Variation in depth of fertiliser placement in a bauxite residue profile. Bars represent standard errors of means (n=8).**

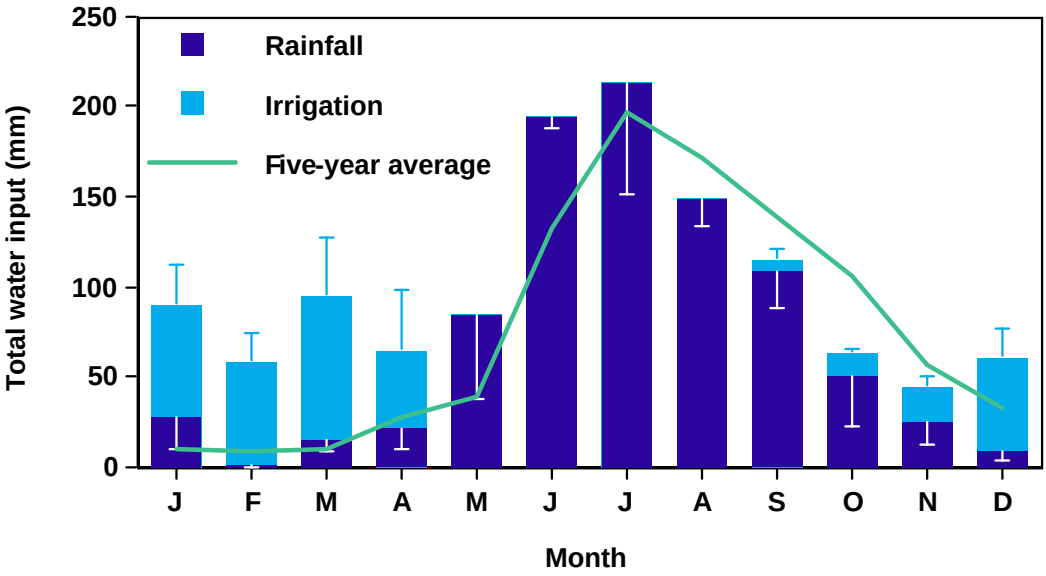

Figure 5.2. **Monthly averages of rainfall and irrigation inputs at the Pinjarra bauxite residue disposal site over the experimental period. The line represents a five-year average, of rainfall alone, prior to commencement of the field trial. Positive bars represent standard errors of irrigation means, negative bars are standard errors of rainfall means.**

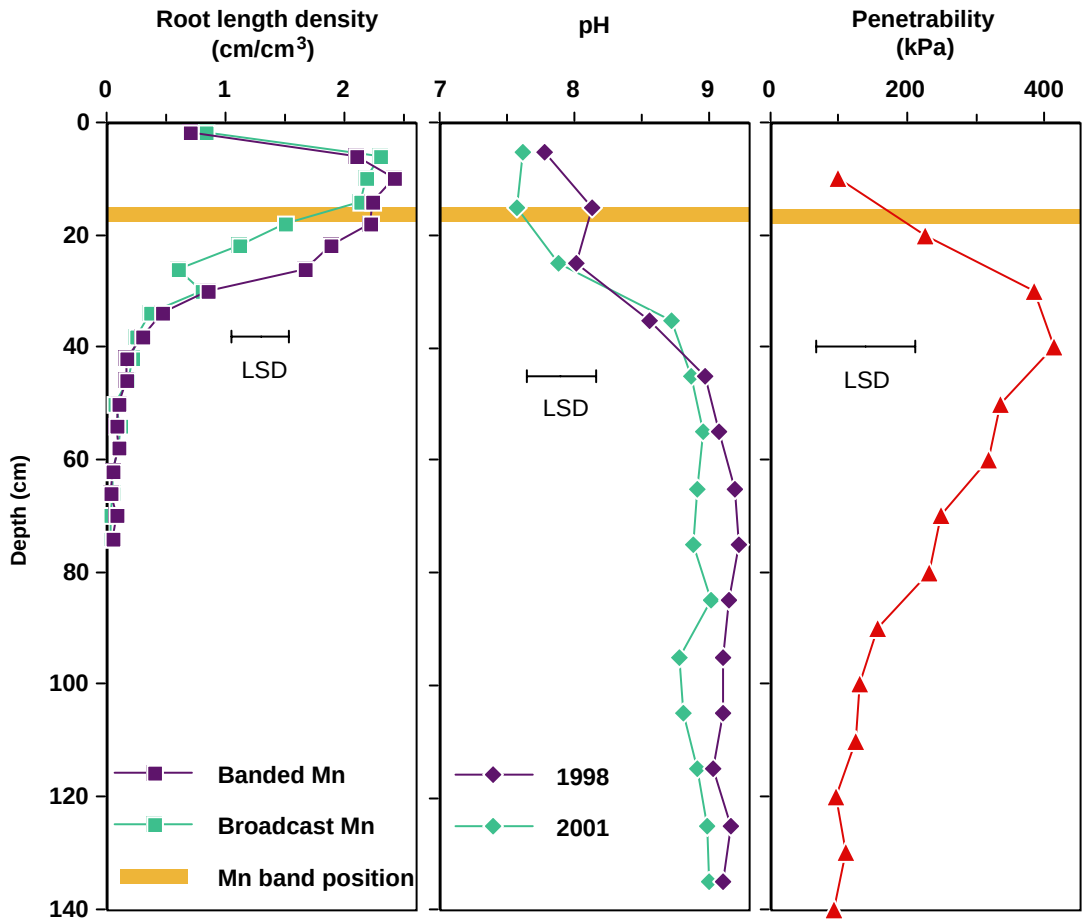

Figure 5.3. **Changes in root length density, pH and penetrability of bauxite residue sand with depth and the relative placement of the Mn fertiliser band. Bars represent least significant differences (*P*=0.05).**

pH units at 15 cm depth, but no differences were observed between Mn-banded and Mn-broadcast plots.

Root distribution was greatly restricted to profile depths between the surface and 30 cm. Although tap-rooted form was predominant, many of these roots extended laterally at between 20 and 30 cm depth, with few roots achieving deeper vertical penetration. A significant overall difference in lucerne root length density was found in response to the two Mn application methods (P=0.009) due to more roots being found in the 18 to 26 cm deep layer of the profile of banded-Mn plots.

Overall, the concentration of Mn in lucerne shoots was significantly higher ($P \leq 0.001$) in plots receiving deep-banded Mn treatments than those receiving broadcast Mn. Deep-banded Mn was sufficient to maintain lucerne shoot Mn concentrations at or above 25 µg/g dry weight for the duration of the study (Figure 5.4). In contrast, the

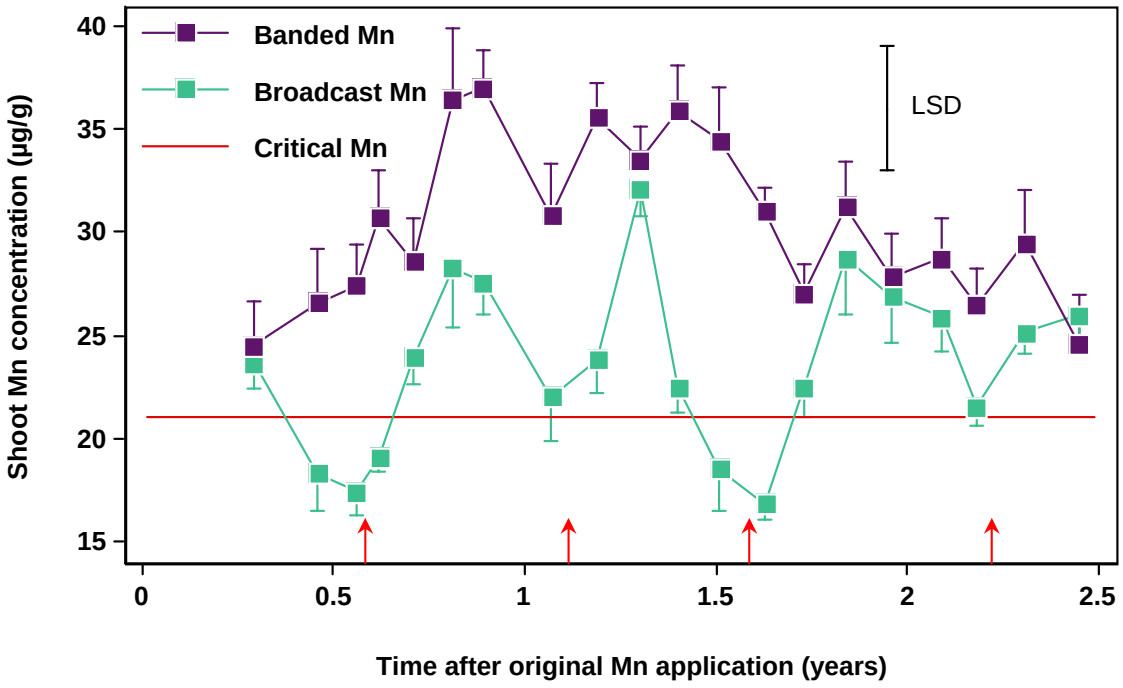

Figure 5.4. **Fluctuations of lucerne shoot Mn concentration after application of deep-banded and broadcast Mn treatments in the field. Arrows indicate re-application of broadcast Mn at 15 kg/ha. Bars represent ± standard errors of means (n=4) and least significant difference (*P*=0.05) is indicated.**

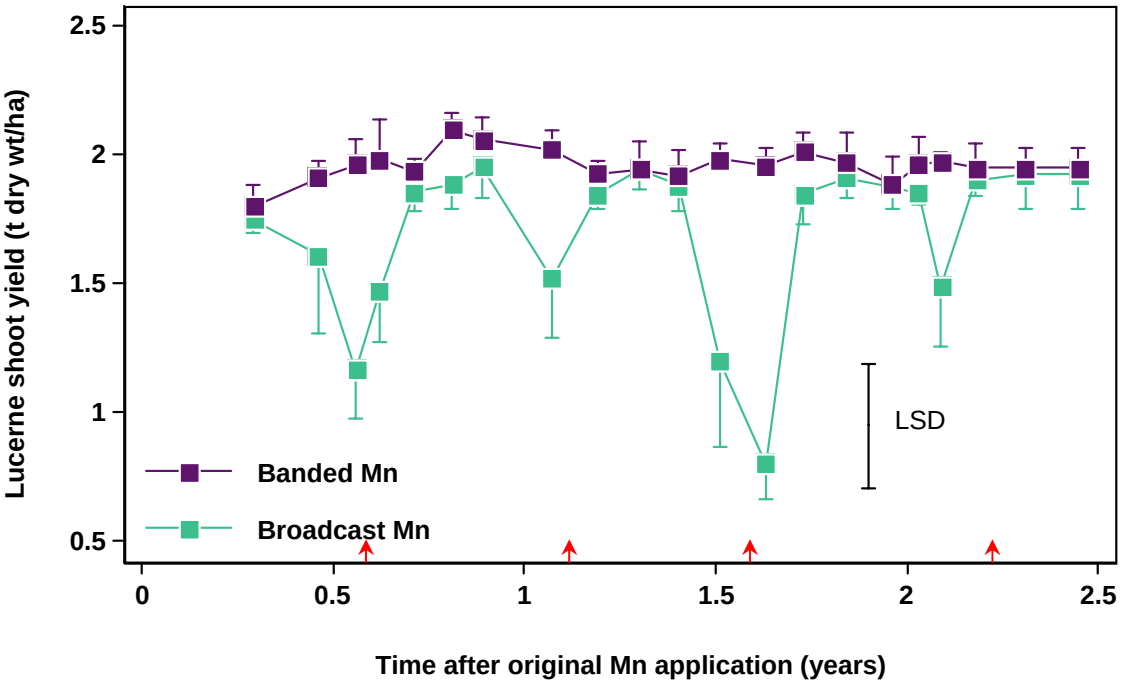

Figure 5.5. **Lucerne hay (shoot dry weight) yields after application of deep-banded and broadcast Mn treatments in the field. Arrows indicate re-application of broadcast Mn at 15 kg/ha. Bars represent ± standard errors of means (n=4) and least significant difference (*P*=0.05) is indicated.**

initial broadcast application of Mn was only able to supply sufficient Mn to lucerne for 4 - 6 months. After this time, shoot Mn concentrations fell below critical deficiency levels (Heckman *et al.* 1993), and hence, re-application of broadcast Mn was required 4 more times during the period of the trial to maintain lucerne growth. Concentrations of other analysed nutrients in lucerne shoots were adequate (Reuter and Robinson 1997) for the first six harvests (data not shown), so were not analysed thereafter.

Cumulatively, plots with Mn banded at depth produced greater yields than plots receiving broadcast Mn (Figure 5.5). Mean harvest yields were 2.0 t dry weight/ha in deep-banded-Mn plots compared with 1.7 t dry weight/ha in broadcast-Mn plots. The mean yield difference between application methods was significant (P=0.003). The relationship between lucerne yield and shoot Mn concentration was strong (Figure 5.6). A Mitscherlich curve was fitted to the yield versus shoot Mn concentration data (r^2 = 0.72). The yield was decreased greatly when shoot manganese fell below 22 μg/g (point of 90 % of maximum yield). At times, yield decreases in the broadcast Mn treatments were visible to the naked eye, and often associated with Mn deficiency symptoms such as yellow discolouration of young foliage. Significant yield decline in the broadcast Mn treatments occurred four times during the study, corresponding with significant decreases in shoot Mn concentration. The yield and Mn concentration decreases in February-March 1999 and March-April 2000 were greater in magnitude than those in August-September 1999 and September 2000.

DISCUSSION

Deep placement of Mn in BRS was beneficial to lucerne growth in a number of ways. Most evident was the capacity of deep-banded Mn at 15 kg/ha to supply adequate levels of Mn for lucerne growth for more than two years without further Mn addition, whereas broadcast Mn required five applications of 15 kg Mn/ha over the same time period.

Advantages in improved efficiency and extended residual value of deep-banded fertilisers have been seen for different fertilisers in various crops. When placed at 5 to 9 cm depth, superphosphate increased effectiveness of P supply to wheat by 20 % over drilling with seed, and when placed at 5 to 13 cm depth increased effectiveness for lupin grain production by 30 to 60 % over a year later (Jarvis and Bolland 1990). A single application of banded Mn fertiliser in naturally Mn-deficient, slightly acidic, deep grey sand in Western Australia provided an effective source of Mn to lupin for over 15 years (Brennan 1993).

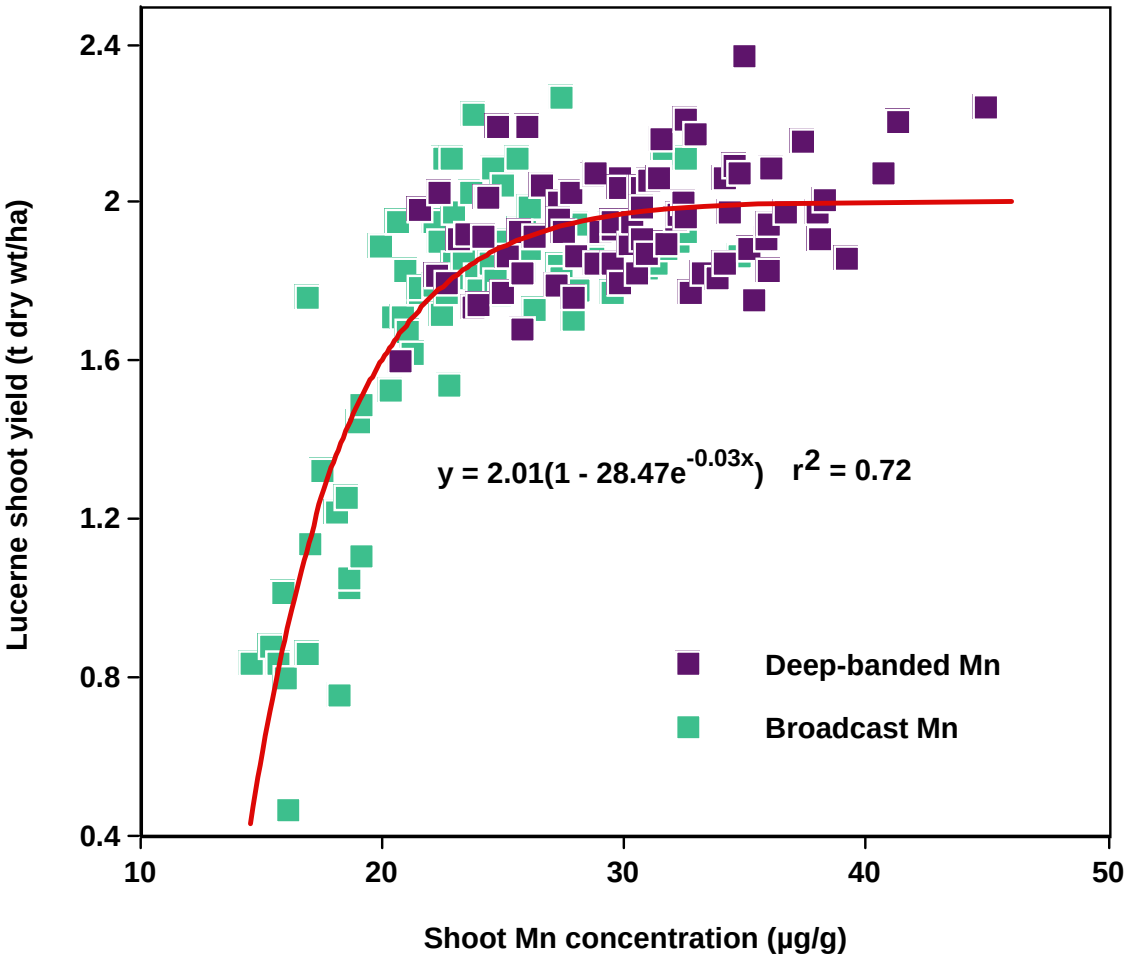

Figure 5.6. **The influence of shoot Mn concentration on yield of lucerne grown on a bauxite residue deposition site. A Mitscherlich function was fitted to the data.**

Plate 5.2. *Equipment used for deep banding of Mn fertiliser at the Pinjarra site.*

Plate 5.3. *Example of differences in lucerne growth in plots with broadcast (1 and 2) and deep-banded (3 and 4) manganese.*

Plate 5.4. *Horizontal proliferation of lucerne roots in the bauxite residue profile at Pinjarra.*

Plate 5.5. *Further evidence of horizontal and restricted root penetration by compaction layers and chemical barriers. Note also water table seepage in pit at 1.4-m depth (right) during winter 1998.*

Although current fertiliser practice at revegetated Pinjarra bauxite residue disposal areas involves regular broadcast applications of Mn fertiliser, Mn deficiency has commonly been observed in BRS-grown lucerne (Gherardi and Rengel 2001, 2003a), and shoot yields between 1994 and the commencement of the present study were consistently low, often <1 t/ha at each harvest (Alcoa World Alumina Australia Limited, unpublished data). Residual value of Mn fertiliser is different for different application methods (Brennan 1993, 1999) and different soil characteristics (Brennan 2001b). As a result of the improved Mn status of lucerne for a longer residual period by deep placement of Mn in the present trial, shoot yield improved by 15 % on average and symptoms attributable to Mn deficiency were not observed. Similarly, deep-banding of Mn increased shoot biomass and grain yield of lupin sown in an agricultural soil (Crabtree 1999; Brennan 2001a). In agronomic terms, lucerne yields in the present study on bauxite residue sand were not large; however, the significant productivity increases combined with improved Mn status of the BRS-grown plants resulting from deep placement of Mn make it an attractive option for future revegetation operations.

The lucerne shoot Mn concentration below which yield was significantly depressed (22 µg/g) corresponds with a critical deficiency concentrations of 21 µg/g reported elsewhere (Heckman *et al.* 1993). Recent work has shown critical concentrations for diagnosis of Mn deficiency in lucerne to range between 18 and 27 µg/g depending on the genotypic tolerance to Mn deficiency (Gherardi and Rengel 2003a). A similar range, 15 to 30 µg/g is quoted by James *et al.* (1995) based on studies from predominantly non-alkaline soils. The 22 µg/g concentration lies in the centre of these ranges and, considering a range of genotypes were planted, is a representative critical concentration for Mn deficiency diagnosis in BRS-grown lucerne.

A number of reasons have been touted for the success of deep-placed fertiliser bands. The influence of increasing moisture with soil depth (where the fertiliser band is placed), and the persistence of this moisture for extended periods of time in comparison to surface horizons in free draining soil is one such theory. Root growth and activity increases with soil moisture, following a typical optimum curve (Marschner 1995), and water is the required medium for nutrient ion transfer from substrate surfaces to roots, as well as providing optimal conditions for microorganisms associated with improving availability of immobile nutrients such as Mn, P, etc. Increasing soil moisture in a calcareous soil resulted in increased Mn concentrations in both soil solution and plants (Misra and Tyler 1999). Similarly, drying of surface soil in a field restricted uptake of surface-applied phosphorus by lucerne (Simpson and Lipsett 1973). In addition, Mn uptake from surface soil by lupin in a pot experiment decreased as the soil dried (Crabtree *et al.* 1998). In the present study, lucerne yield and Mn concentration decreases were greater over hot late summer/early autumn months than over relatively cold late winter/early spring months. Consistently higher soil moisture in surface horizons of BRS impoundments would be expected during winter and spring months. The low water holding capacity of BRS, coupled with high evaporation rates in southern Australia during months of low rainfall, results in rapid drying of surface BRS horizons. Hence, despite the use of irrigation, the surface of BRS disposal areas remains dry for significant periods, whereas persistence of moisture in subsurface layers should be greater. The seasonal dynamics of water content and movement in the profile of BRS deposits revegetated with lucerne are currently being investigated. With restrictions on volume of groundwater used for irrigation becoming more stringent in Western Australia, and indeed many areas world-wide, the comparable value of deep-banded fertiliser systems over broadcast-incorporated applications will likely become more evident.

Resultant higher concentrations of Mn in a soil substrate, relative to soil volume, in the vicinity of a Mn band may play a role in improving plant growth in substrates with a predisposition for Mn fixing. Norvell (1988) suggested that reactions in the bulk soil that decrease Mn availability may be quite different near fertiliser bands due to chemical effects of increased ion concentrations or pH. It is also recognised that concentrated zones of Mn may be more conducive to continued Mn^{2+} stability (Murphy and Walsh 1972; Mortvedt and Giordano 1975). When Mn is added to BRS, a number of chemical transformations occur until pools of various Mn species reach an equilibrium favouring plant-unavailable forms (Gherardi and Rengel 2001). Placing Mn fertiliser in a band should minimise exposure to reactive sites in the soil, with equilibrium processes ensuring greater levels of plant-available Mn within these zones relative to widely dispersed Mn in a broadcast system.

The distribution and proliferation of roots can be affected by uneven nutrient distribution in soils. Root growth is stimulated by localised supply of nutrients or in a nutrient rich zone (Robinson 1994). In a glasshouse study, lucerne root growth increased in proximity to the BRS zone containing a Mn band (Gherardi and Rengel 2003b). Likewise in the present study, the density of lucerne roots immediately below Mn band depth was greater in banded than in broadcast treatments. However, in contrast to the glasshouse study, no Mn deficiency symptoms were observed in deep-banded Mn plots during early lucerne growth in the field. The physical disturbance from tines on the banding machinery may have re-positioned some of the surface-broadcast Mn fertiliser from previous applications into deeper layers with more moisture. This Mn was previously held at the BRS surface in unavailable forms. In layers with higher available water content and greater root density, rhizosphere activity may have transformed the Mn to forms available for uptake. In addition, the BRS disturbance likely provided easily root-penetrable channels between the surface and the Mn fertiliser bands.

Roots of lucerne plants reportedly have the ability to grow deep into soil profiles and to penetrate dense or compacted soil due to the plants' perennial nature and the tap-rooted form (Cresswell and Kirkegaard 1995). In contrast, earlier work of Carlson (1925) showed soil compaction to cause overall decrease in lucerne root length and a development of root branching. Lucerne roots in the present study were largely restricted in penetration depth to less than 30 cm. Such horizontal proliferation of roots close to the surface of the BRS profile rather than at depth was considered to result from the compacted layer of BRS below 30 cm. There is some suggestion that heavy vehicle traffic during construction may be responsible for the compacted layer (Alcoa World

Alumina Australia Ltd., unpublished). If such traffic is unavoidable in future construction of bauxite residue deposition sites, deep ripping to open the BRS profile structure below 30-cm depth may allow improved root growth. This, in turn, may also improve plant access to moisture stored deep in the profile.

In addition to compaction, a chemical (eg. pH) barrier may also have contributed to decreased vertical root penetration. It is, however, difficult to ascertain whether pH had a more significant effect on root growth or vice-versa, but one can speculate. The association of a drop in pH over the study period with the zone of greatest root density indicates that plant growth may be ameliorating the extreme alkalinity found in BRS deposits. Plant roots, legumes in particular, have a strong capacity to acidify soil due to an imbalance of cation and anion uptake (see Tang and Rengel 2003 and references therein) and the release of numerous acidifying compounds into the rhizosphere. Certain root-exuded compounds also improve Mn solubility and availability through pH decreases, reduction of oxidised Mn (Mn^{4+}) and chelation to increase mobility and prevent re-oxidisation of Mn^{2+} (Marschner 1995). Such mechanisms for improving plant-available Mn are likely to be favoured by placing Mn in the active root zone and likely contribute to the increased efficiency of deep banding as a Mn fertiliser application method.

CONCLUSION

In substrates with high capacity for Mn fixation, the need for efficient methods of supplying nutrients to plants is paramount to maintaining productivity. Sustainability of revegetated BRS may be vastly improved by deep banding of Mn. Banding Mn at depth should decrease both the frequency and total volume of Mn fertiliser inputs required to maintain productive growth and Mn status of plants in revegetated areas.

CHAPTER VI

THE EFFECT OF MANGANESE SUPPLY ON EXUDATION OF CARBOXYLATES BY LUCERNE ROOTS

Plate 6.1. *Growth of lucerne under various manganese treatments in nutrient solution in the glasshouse.*

The major content of this chapter has been published in the journal Plant and Soil:

Gherardi, M.J. and Rengel, Z. (2004) The effect of manganese supply on exudation of carboxylates by roots of lucerne (*Medicago sativa* L.). *Plant and Soil* **260**: 271-282.

CHAPTER SUMMARY

Some low-molecular-weight carboxylates commonly found in plant root exudates have the potential to increase the availability of Mn in the rhizosphere. Release of various compounds into the rhizosphere by plant roots may also be a mechanism by which certain species and genotypes are able to tolerate conditions of low Mn availability better than others. Lucerne (*Medicago sativa* L.) plants of Salado, a genotype tolerant to Mn deficiency, and Sirosal, an intolerant genotype, were grown in solution culture with 0, 5 or 500 n*M* Mn (Mn-0, Mn-5 and Mn-500). Exudates of whole root systems were collected at 14, 24 and 36 d and analysed by HPLC. Oxalate, tartarate, L-malate, lactate, malonate, maleate, citrate and succinate were detected and quantified in exudates under all Mn treatments. Malonate, citrate and succinate accounted for the majority of carboxylates in the exudates. Exudation increased with plant age, but amounts of individual carboxylates remained constant in proportion to the total amount exuded. A significant increase in exudation of all carboxylates other than malonate and maleate resulted from omission of Mn from nutrient solutions. Salado exuded more oxalate, tartarate, L-malate, lactate, citrate and succinate than Sirosal at Mn-0, and more citrate and succinate than Sirosal at Mn-5. Genotypic differences in carboxylate exudation under Mn-0 were associated with the proportion of roots with diameter <100 µm. Plant Mn concentrations and growth rates suggested carboxylate exudation differences were not the sole factor responsible for differential tolerance to Mn deficiency in the lucerne genotypes.

INTRODUCTION

Chemical conditions in soil often result in Mn being present in forms that are unavailable for plant uptake. This is especially true for soils of high pH where equilibrium conditions favour the oxidation of plant-available Mn^{2+} to unavailable Mn^{4+}. Plant yield on many soils is, therefore, limited by poor Mn availability, rather than a low Mn content in the soil (Welch *et al.* 1991).

Uren (1981) clearly established that substances originating from sunflower roots could reduce insoluble Mn(IV) oxides. The mechanism of direct dissolution of Mn oxides by plant roots is yet to be fully elucidated, but was surmised decades earlier by Leeper (Leeper 1934) to be dependent on the process he termed “contact reduction”. Further suggestive evidence that substances from plant roots were responsible for this

reduction was provided by Jones and Leeper (1951) and Bromfield (1958a; 1958b). Linehan et al. (1985) reported a fifteen-fold increase in concentration of Mn in soil solution during early stages of barley development. It is now widely accepted that plants are able to increase the availability of nutrients by exuding various organic substances into the rhizosphere. Exuded compounds can alter micronutrient availability both directly, by influencing the solubility and equilibria of nutrient chemical forms, and indirectly, by altering the composition and prevalence of rhizosphere microflora populations capable of nutrient chemical transformations (Uren 1981; Uren and Reisenauer 1988; Darrah 1993). Up to 30% of total C assimilated by plants may be released into the rhizosphere as exudates of varying form (Hinsinger 1998). It is also likely that the ability to exude such substances is a key strategy in an ability of plants to tolerate the Mn-fixing conditions in alkaline substrates (Marschner 1995).

Since availability of Mn to plants is governed by redox processes, plant- or microbe-derived organic compounds in the rhizosphere may increase availability of Mn by providing a source of electrons for Mn reduction. However, the precise modes of action and nature of exudate components involved in Mn mobilisation (reduction) are still unclear. Exudation of hydrogen ions and organic acids has been shown to lower the pH of the rhizosphere in alkaline substrates (Jarvis and Hatch 1985; Dinkelaker *et al.* 1995), in turn altering availability and plant uptake of micronutrients. Chelation of Mn^{2+} by carboxylate anions (the conjugated bases of carboxylic acids) suppresses its reoxidation and increases the mobility of the reduced Mn in the rhizosphere (Marschner 1995). Characterisation of exudate components may, therefore, shed more light onto the Mn solubilising ability of plants and processes involved in Mn acquisition from soil.

In aqueous systems, various small organic compounds will reduce higher Mn oxides (Stone and Morgan 1984). The release of Mn^{2+} from insoluble MnO_2 by two common carboxylate components of root exudates, namely citrate and malate, was clearly demonstrated by Godo and Reisenauer (1980). Increased availability of Mn in soil due to these carboxylates has been confirmed by many workers over the past two decades (eg. Jauregui and Reisenauer 1982; Dinkelaker *et al.* 1989; Fan *et al.* 2001; White and Kottler 2002). Other carboxylates added to soil have also influenced Mn availability. In studies of various soils, the following decreasing orders of solubilising ability were observed: citrate > malate > oxalate > fumarate (Msaky and Msanya 1986); and citrate > oxalate > acetate (Ström 1997).

While the potential of carboxylates in increasing availability of Mn in soil under controlled conditions is well recognised, in less-controlled and natural systems the value of carboxylates has been questioned (Jones and Darrah 1994; Jones *et al.* 1994;

Hinsinger 1998; Jones 1998; Rengel 2000). Coupled with a tendency for rapid metabolism by rhizosphere microflora (Darrah 1991; Jones *et al.* 1996), decreases in both specificity and stability of complex formation between carboxylates and Mn would be expected in soils with elevated pH where Mn deficiency commonly occurs. However, in attempting to characterize the sequence of reactions between malate and MnO_2, Jaregui and Reisenaer (1982) found that although chelation of Mn and oxaloacetate (oxidised malate) was somewhat favoured by acidic conditions, adsorption was still significant over a considerable pH range. Chelation of Mn^{2+} is important in preventing its reoxidation and increasing the mobility of reduced Mn in the rhizosphere (Marschner 1995).

Genotypic differences in tolerance to Mn-deficient conditions have been recognised for decades. Genotypes tolerant to Mn deficiency tend to have an ability to extract greater amounts of Mn from a soil compared to less-tolerant ones (Graham 1988; Rengel *et al.* 1994). Evidence that differential tolerance to Mn deficiency is manifested in soil, but not in solution culture (Huang *et al.* 1994), highlights the possible role of specific rhizosphere processes and dynamics of microbial rhizosphere populations in this expression. The putative mechanisms of tolerance to Mn deficiency may rely on greater excretion of various substances into the rhizosphere by plant roots (Rengel 2000). Root exudation and associated tolerance to nutrient deficiencies may, in turn, be governed by root morphology such as the case of cluster-root forming species showing greater tolerance to low available P soils (Marschner 1995). Root structure associated with tolerance to Mn deficiency has not been widely explored.

Literature regarding the effect of Mn deficiency on carboxylate release by roots is scarce (Jones 1998). Even less is known about the suite of carboxylates released under conditions of limited Mn availability by important pasture species, such as lucerne, which have wide-ranging uses from agriculture to the revegetation of mine-processing wastes (Gherardi and Rengel 2003a, 2003b, 2003c). In fact, literature regarding exudates of lucerne roots in general is limited to only a few studies (Lipton *et al.* 1987; Masaoka *et al.* 1993). Thus, a study of carboxylate release by lucerne roots in solution culture was undertaken. Lucerne genotypes differing in tolerance to Mn deficiency were grown under various Mn treatments ranging from deficiency to sufficiency. Growth patterns were observed and exudation of carboxylates by root systems was analysed to determine how the genotypes respond to low Mn supply and whether observed responses may explain differential Mn-deficiency tolerance in lucerne.

MATERIALS AND METHODS

GROWTH CONDITIONS

Seeds of a lucerne genotype tolerant to Mn deficiency (Salado), and a less-tolerant genotype (Sirosal) were germinated on paper towels moistened with 2 m*M* $CaSO_4$. Seedlings were inoculated with *Rhizobium meliloti* group AL (WSM826) immediately following germination. When the primary root had reached 5 to 6 cm in length, seedlings were transferred to 5-L polythene-lined pots containing nutrient solution (µ*M*): KH_2PO_4, 15; K_2HPO_4, 15; K_2SO_4, 500; $CaCl_2$, 500; $MgSO_4$, 100; Fe-EDTA, 20; H_3BO_3, 2.5; $ZnSO_4$, 0.375; $CuSO_4$, 0.1; Na_2MoO_4, 0.015; $CoSO_4$, 0.005; and, initially, $MnSO_4$, 0.25. Thirty seedlings were placed in each pot. Nutrient stock solutions contained analytical grade salts only. Iron-EDTA stock was made by combining equimolar solutions of analytical grade Na-EDTA and $FeSO_4$ and mixing overnight by aeration. Double de-ionised water (DDI, >18 MΩ.cm resistivity) was used throughout.

In the culture pots, nutrient solutions were set at pH 6.0 with dilute KOH, and continually aerated to ensure homogeneity and prevent anoxia. Culture solutions were buffered, initially, using 2-m*M* (N-morpholino)ethanesulphonic acid (MES). After one week, basal nutrient concentrations were doubled and Mn treatments of 0, 5, and 500 n*M* (Mn-0, Mn-5 and Mn-500) were applied. After a further week, MES was increased to 4 m*M* to ensure maintenance of buffering capacity with large plants. Culture solutions were completely replaced twice per week for the first two weeks of Mn treatment, and every second day thereafter. Each treatment was replicated four times for each lucerne genotype. Pots were housed in a temperature-controlled glasshouse (20 °C day, 12 °C night).

EXUDATE COLLECTION

Exudates were collected 14, 24 and 36 d after commencement of Mn treatments. At approximately 1 h after sunrise on each collection day, pots were transferred to a 20 °C room under artificial light (880 to 1020 µmol m^{-2} s^{-1} PAR). Collection method was modified from Neumann *et al*. (1999). Briefly, after approximately 2 h of adjustment to the artificial light source, all plants from each replicate pot were transferred to a collection vessel (150 mL volume). Roots were carefully washed through three successive 20-L DDI baths, allowed to stand for 1 min in 1 L of DDI containing a dissolved Puritabs® bactericide (sodium dichloroisocyanurate 17 mg, DermaTech,

Seven Hills, NSW, Australia) followed by a rinse in sterile DDI. Sterilisation of DDI was achieved by boiling for 10 min.

Approximately 60 mL sterile DDI (pH 6.0) was added to each collection vessel, enough to cover all lucerne roots. Collection solutions were constantly aerated to ensure adequate mixing, and pH was maintained at 6.0 by dropwise addition of 0.01 *M* KOH at approximately 6 min intervals to prevent possible effects of increasing external H^+ concentrations on membrane permeability and exudate release rate. After 1 h, exudate collection solution volumes were measured, filtered (Millex®–GS 22 μm, sterile filters), separated into approximately 5 mL volumes and immediately placed on ice and transferred to a –22 °C freezer for temporary storage.

PLANT SAMPLING AND ANALYSIS

After exudate collections, ten plants of each treatment replicate were harvested and separated into root and shoot material. Remaining plants were transferred back to 5-L pots in the glasshouse to continue growth under Mn treatments. Sampled roots were stored in water at <4 °C until root length could be measured by WINRHIZO® 3.9 (Régent Instruments Inc, Quebec) scanning software, using a 300 dpi resolution and Lagarde's adaptative threshold for pale roots. WINRHIZO® was used to determine the length of roots in successive 0.1 mm diameter classes. Roots and shoots were then dried in a 70 °C oven and weighed. Shoots and roots were ground, digested in HNO_3 at 140 °C and analysed for Mn, Cu, Fe, Zn, P and Mg concentrations by ICPAES (Zarcinas *et al.* 1987).

ORGANIC ACID ANALYSIS

Solution samples were defrosted at room temperature and transferred to 1.5 mL glass cuvettes. Within 12 h, samples were analysed by HPLC (Waters/Millipore, Milford, MA, USA) using a reversed phase C–18 column, 150 mm length, 2.1 mm internal diameter, with a 5-μm particle size (Alltech Associates Inc., Deerfield, IL, USA) at 25 °C. Sample injections (0.1 mL) were eluted isocratically with a mobile phase of 0.05 *M* KH_2PO_4, pH 2.5, at a flow rate of 1 mL/min and detected by absorption at 215, 254 and 300 nm using a multiple wavelength spectrophotometer (490 UV-Vis, Waters/Millipore).

Absorption peaks of exudate solutions were compared to mixed standard solutions containing (in order of retention time) oxalic, tartaric, formic, L-malic, lactic,

malonic, maleic, citric, succinic and fumaric acids. Standards were prepared individually in distilled DDI from free acids (Sigma-Aldrich, Sydney, Australia), and various aliquots were mixed and diluted in distilled DDI. Good separation of carboxylates was achieved from the mixed standards, with retention times ranging from about 2.5 to 16 minutes. Corresponding absorption peaks, confirmed by standard additions to exudate solution sub-samples analysed separately, allowed identification of exuded carboxylates, and linear calibration curves of absorption at 215 nm versus standard concentrations were used to quantify the identified carboxylates. Millenium software (1997, Waters/Millipore) was used for chromatogram absorption peak integration.

STATISTICAL ANALYSIS

All data were subjected to analysis of variance (ANOVA). Where necessary, data were transformed by natural logarithm ($\log_e$) prior to ANOVA to ensure normality and homogeneity of variance. Least significant difference (LSD) was used for all pairwise comparisons where significant *F*-probabilities ($P \leq 0.05$) were found.

RESULTS

LUCERNE GROWTH

Growth of lucerne was significantly compromised at Mn-0 and Mn-5 in comparison to Mn-500 (Figure 6.1). Within 6-d of Mn treatment application, symptoms of Mn deficiency were clearly evident in new foliage of Mn-0 plants of both genotypes, and became progressively more evident through the treatment period. Symptoms included decreased leaf size and growth rate, pale-green new growth developing to bright yellow interveinal chlorosis with age, slender and more flexible stems with irregular undulations, and weak petioles easily damaged by handling.

Growth rates of lucerne in the Mn-0 treatment slowed after 24 d. Shoot growth rate slowed more than root growth rate, and Sirosal growth suffered more than Salado. However, under Mn-5 and Mn-500, lucerne growth rate did not decrease throughout the treatment period.

In general, Salado showed slightly greater shoot growth across all Mn treatments, but the difference was significant only at Mn-5 ($P<0.001$). Plants at the Mn-5 rate were visibly smaller than at Mn-500, but showed interveinal chlorosis and stem

Table 6.1. **Effect of external Mn concentration on total root length (m/plant) of lucerne genotypes. Values are means ± standard error. Anchors (⚓) represent significant genotype differences ($P \leq 0.05$) within a Mn treatment rate at the specified harvest day.**

	0 n*M* Mn				5-n*M* Mn				500-n*M* Mn			
Day	Salado		Sirosal		Salado		Sirosal		Salado		Sirosal	
14	10.5	±2.1	8.1	±1.9	18.5	±3.2	11.9	±2.0	22.5	±4.5	21.2	±3.2
24	27.7⚓	±4.3	17.2⚓	±3.1	39.4	±3.9	27.0	±4.3	43.5	±7.7	40.0	±8.5
36	37.2⚓	±5.0	25.1⚓	±4.1	55.7⚓	±5.1	41.2⚓	±6.1	78.3	±12.8	67.4	±11.3

deformation to a much lesser extent than Mn-0 plants. Shoot growth increased with increasing Mn rate, and growth differences were significant between all Mn rates at each sampling date for both genotypes.

Root growth trends were the same as for shoot growth, with the exception of additional significant differences ($P<0.001$) in root biomass production between genotypes when supplied with no Mn. Sections of roots near the root tip of plants with no Mn supply had a dull, cream colouration in comparison to the fresh white/translucent colour of those under Mn-500 treatment. Root biomass across treatments had similar responses and significant differences to shoots. Nodulation of roots was unaffected by Mn treatment; the fewer root nodules observed at low Mn rates were rather a reflection of decreased overall size of root systems than any inhibition of *R. meliloti* caused by low Mn.

Root length (Table 6.1) mirrored root biomass growth trends (Figure 6.1). With no Mn supply, Salado plants had significantly greater ($P \leq 0.05$) length of very fine roots (diameter < 200 µm) than Sirosal. The difference in measured length of fine roots between genotypes with no Mn was more than two-fold at 36 d, amounting to proportions of total plant root length of 37 % and 20 % for Salado and Sirosal, respectively (Figure 6.2). Length of roots in each diameter class >200 µm without Mn supplied, and their relative contribution to total plant root length, was the same for the two genotypes. Other than very fine roots of Salado, root length in all diameter classes increased significantly ($P \leq 0.05$) with an increase in Mn rate from 0 to 500 n*M* in the culture solution (data not shown).

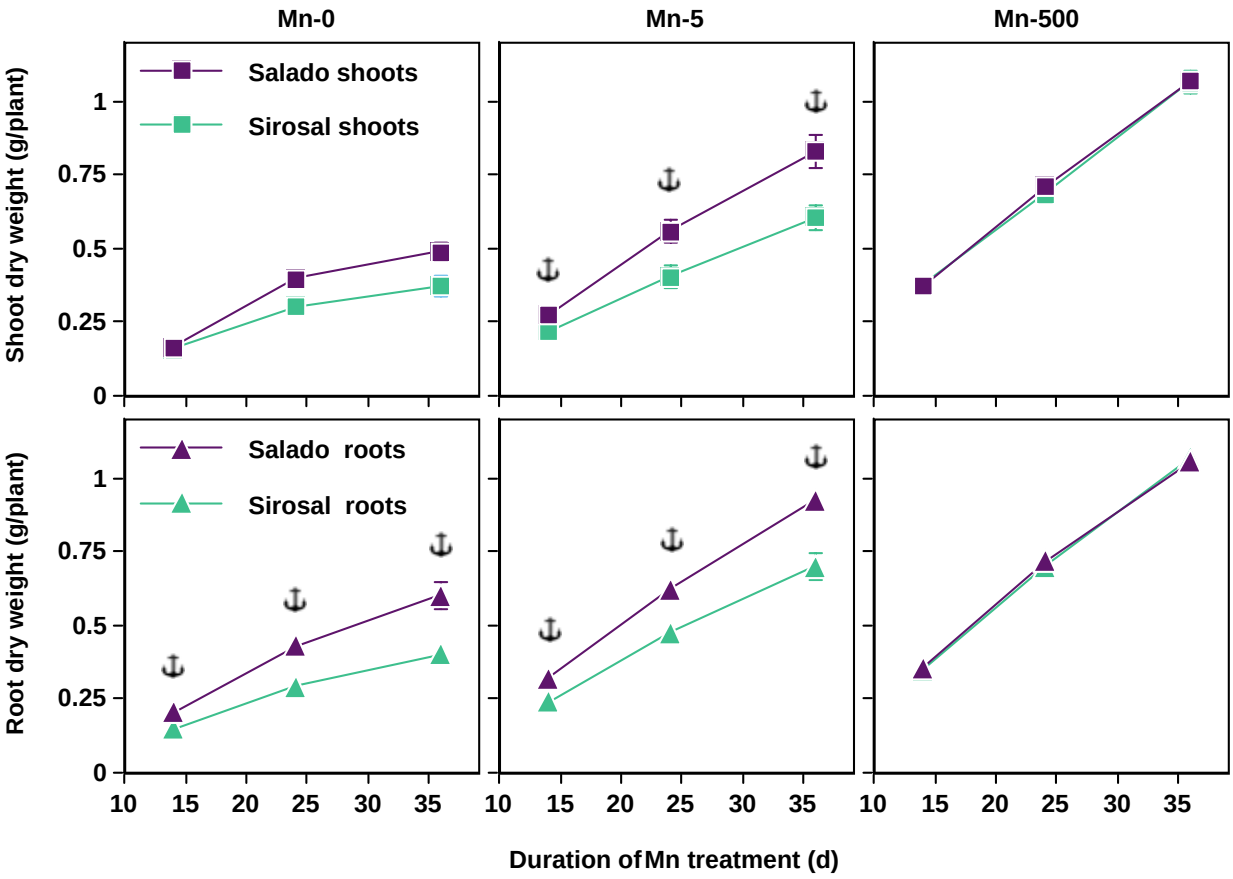

Figure 6.1. **Growth of lucerne in solution culture under different Mn treatment rates. Anchors (⚓) represent significant differences between genotypes ($P \leq 0.05$). Bars represent standard error.**

NUTRIENT CONCENTRATIONS

Concentrations of Mn in the shoots and roots were highly correlated with external Mn supply in the culture solutions (Tables 6.2 and 6.3), without discernible difference between genotypes. A decrease in Mn concentration occurred in Salado under no Mn and both genotypes under Mn-5 treatments over the treatment period. In contrast, Mn concentrations in shoots at Mn-500 increased over time. Concentrations of Mn in whole root systems of Mn-0 and Mn-5 plants were slightly lower than in the shoots, whereas under Mn-500 treatment root concentrations were higher than shoot concentrations. Accumulation of Mn was equivalent in shoots and roots of plants under no Mn and Mn-5 treatments. At Mn-500, however, root-to-shoot Mn translocation was much greater than in Mn-0 and Mn-5 treatments, with greater accumulation of Mn in shoots than in roots.

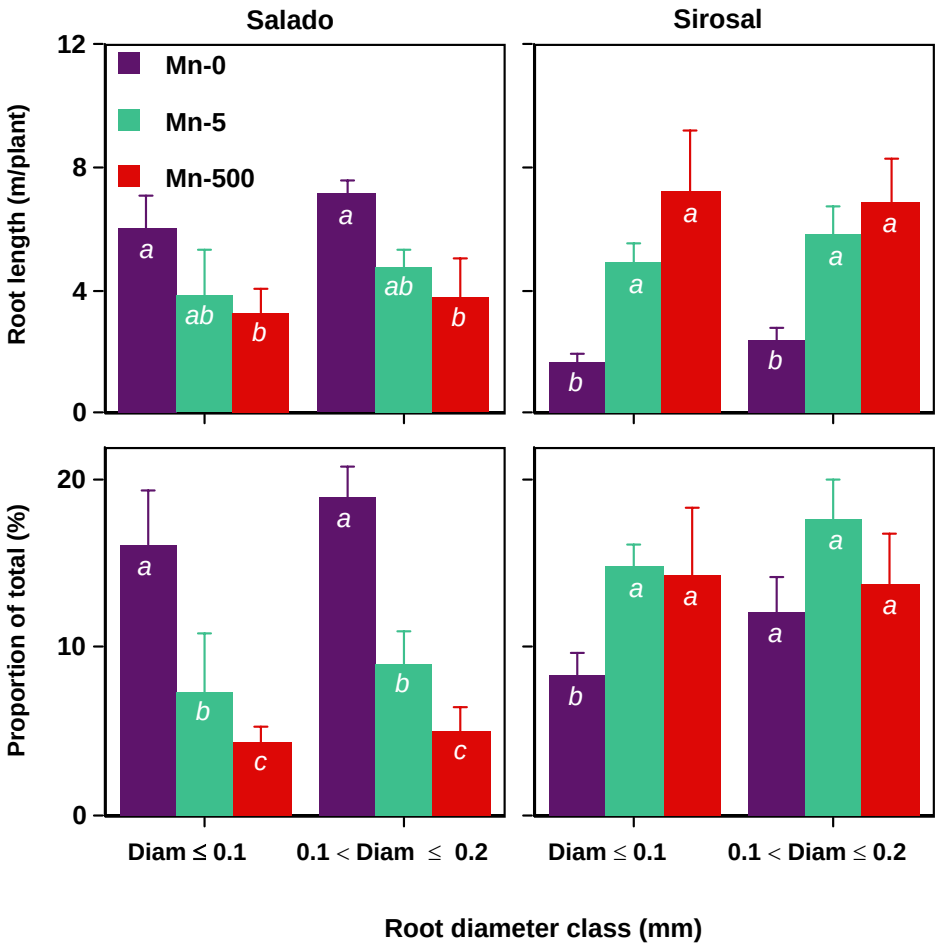

Figure 6.2. **Effect of Mn treatment on fine root production (<200 µm diameter) by lucerne genotypes in solution culture after 36 d. Different letter represent a significant difference between Mn treatments within diameter classes (*P*≤0.05). Bars represent standard error.**

Table 6.2. **Manganese concentrations and accumulation in shoots of lucerne genotypes. Values are means ± standard error. Anchors (⚓) represent significant differences among harvest days within the specified genotype's Mn treatment.**

	Salado	Mn treament (n*M*)		Sirosal	Mn treament (n*M*)	
Day	0	5	500	0	5	500
	Mn concentration (µg/g dry weight)					
14	11⚓ ±2.6	25⚓ ±2.1	53⚓ ±11	14 ±5.0	27⚓ ±5.5	82⚓ ±18
24	8.0 ±2.1	19 ±3.3	81 ±19	11 ±3.1	21 ±1.5	94 ±14
36	6.1⚓ ±2.1	17⚓ ±3.7	99⚓ ±12	8.8 ±1.3	18⚓ ±2.9	130⚓ ±21
	Mn content (µg/whole shoots)					
14	1.8 ±0.42	6.9⚓ ±1.7	20⚓ ±3.5	2.2 ±0.79	5.8⚓ ±0.87	31⚓ ±2.9
24	3.1 ±1.1	11 ±1.5	57⚓ ±7.5	3.3 ±0.88	8.5 ±1.3	64⚓ ±4.7
36	3.0 ±1.2	14⚓ ±3.1	105⚓ ±17	3.3 ±1.0	11⚓ ±1.8	139⚓ ±16

Table 6.3. **Manganese concentrations and accumulation in roots of lucerne genotypes. Values are means ± standard error. Anchors (⚓) represent significant differences among harvest days for the specified genotype's Mn treatment.**

	Salado	Mn treament (n*M*)		Sirosal	Mn treament (n*M*)	
Day	0	5	500	0	5	500
	Mn concentration (µg/g dry weight)					
14	9.4⚓ ±2.2	22⚓ ±3.1	84⚓ ±12	9.8 ±1.8	21 ±2.2	121⚓ ±16
24	6.1 ±2.0	16 ±3.5	118 ±14	7.3 ±1.8	18 ±3.5	152 ±15
36	3.7⚓ ±1.8	12⚓ ±3.1	153⚓ ±24	5.4 ±3.6	14 ±5.0	204⚓ ±28
	Mn content (µg/whole root system)					
14	1.9 ±0.71	7.0 ±2.0	30⚓ ±6.1	1.4 ±0.43	5.0⚓ ±0.7	42⚓ ±8.2
24	2.6 ±1.2	10 ±2.6	85⚓ ±11	2.1 ±1.2	8.5 ±1.5	106⚓ ±10
36	2.2 ±0.70	11 ±1.7	162⚓ ±27	2.2 ±1.0	9.8⚓ ±1.5	218⚓ ±26

CARBOXYLATE EXUDATION

Of the carboxylates included in the standards, oxalate, tartarate, L-malate, lactate, malonate, maleate, citrate and succinate were detected in exudate solutions from plants of all treatments, and peaks were readily quantifiable. Malonate, citrate and succinate accounted for the majority of total identifiable carboxylate exudation (Figure 6.3). Oxalate, tartarate, L-malate and lactate were present in smaller concentrations. Maleate was present only in trace amounts. Chromatograms (Figure 6.4) showed various other absorption peaks, some with retention times similar to formate and fumarate standards, but noise and interference from additional substances in the exudate solutions, identified by different relative absorption patterns at the different wavelengths, showed these peaks to be highly impure. Hence, no identification of these two carboxylates could be made with confidence. Although increases in carboxylate exudation by plants were observed over time, changes in relative contributions of individual carboxylates to total carboxylate exudation were not significant, and only the individual carboxylate data from the 36-d collection are presented.

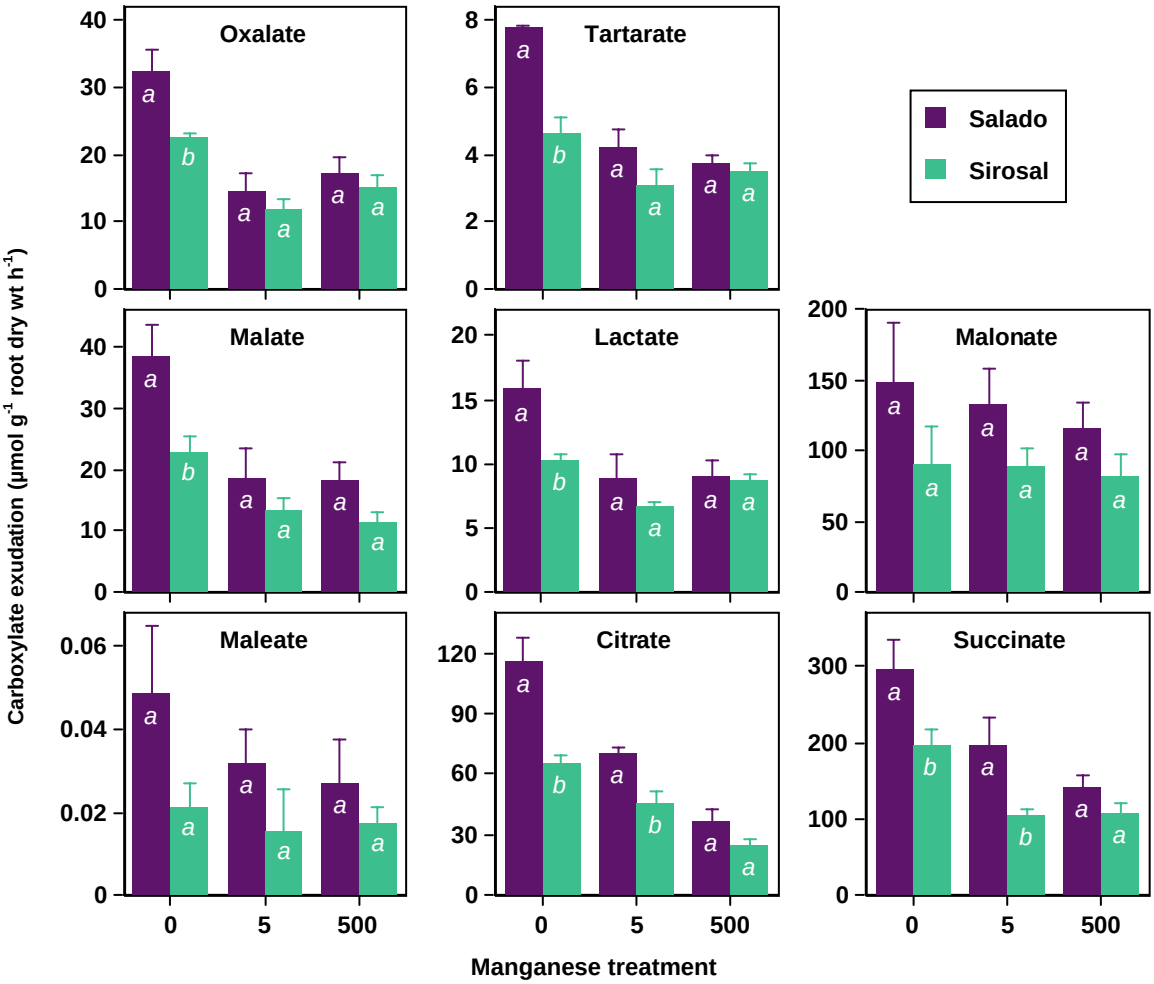

Figure 6.3. **Carboxylates identified by HPLC in root exudates of lucerne after 36 d under different Mn treatments. Different letters represent significant treatment differences between genotypes ($P \leq 0.05$). Bars represent standard error.**

CHANGES IN EXUDATION WITH GENOTYPES AND MANGANESE TREATMENT

For each carboxylate, other than malonate and maleate, the main effects of Mn treatment rate and genotype significantly affected exudation ($P \leq 0.05$). A significant interaction between Mn rate and genotype was seen for tartarate ($P=0.015$) and citrate ($P=0.042$) exudation due to large genotypic differences in the Mn-0 treatment. As a general observation, measurable amounts of each carboxylate exuded per unit root mass decreased with increasing rate of Mn treatment. Certainly, Salado lucerne exuded significantly greater amounts of oxalate, tartarate, L-malate, lactate, citrate and succinate per unit root mass with no Mn supplied than at Mn-5 or Mn-500. In addition,

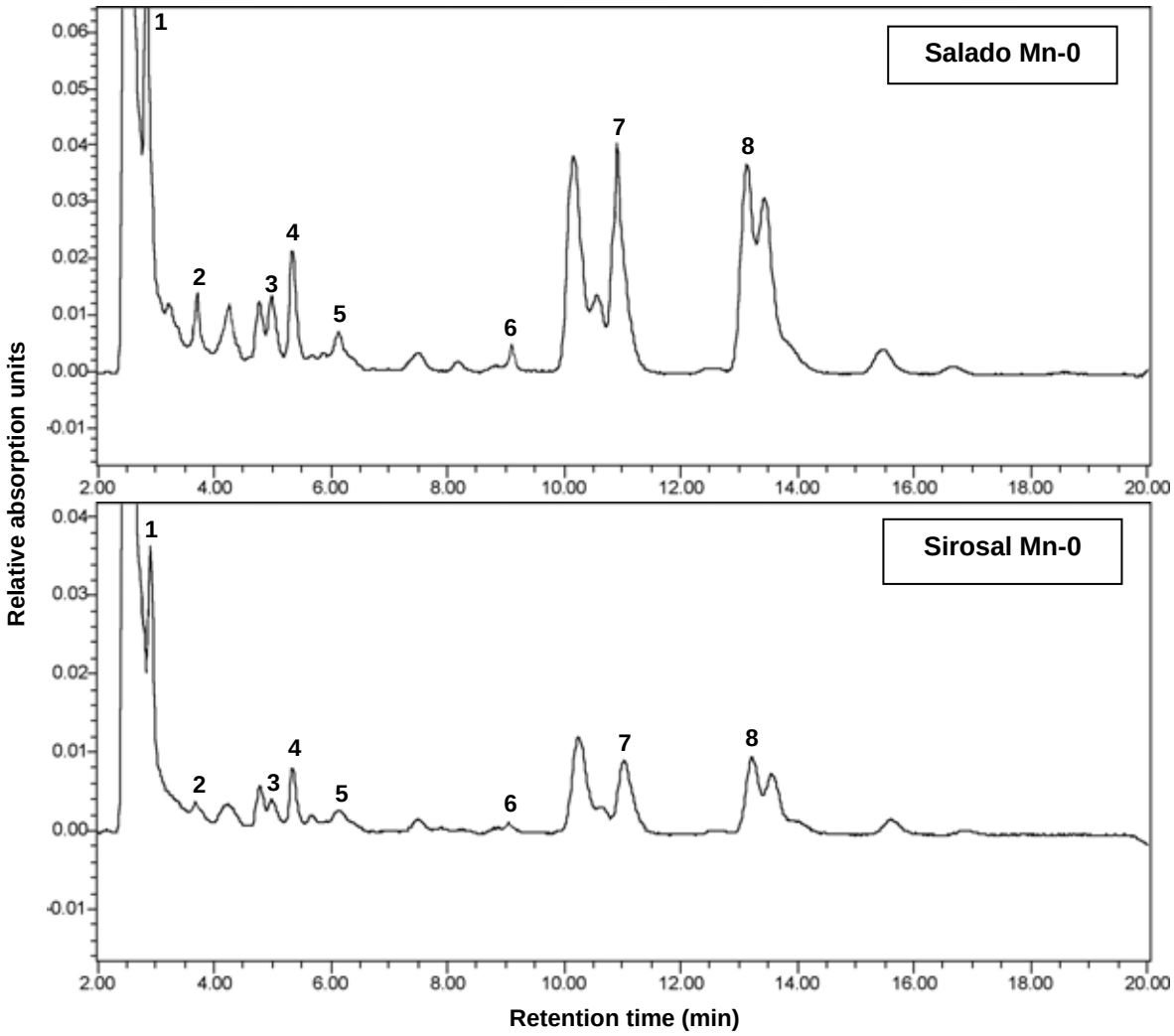

Figure 6.4. **Example of chromatograms (λ=215 nm) for Salado and Sirosal root exudates after growth under Mn-0 treatment. Numbered carboxylate absorption peaks are: 1 Oxalate; 2 Tartarate; 3 L-malate; 4 Lactate; 5 Malonate; 6 Maleate; 7 Citrate; and 8 Succinate.**

citrate and succinate exudation by Salado was greater at Mn-5 than at Mn-500. Similarly, Sirosal exuded significantly more oxalate per unit root mass at Mn-0 treatment than at Mn-5 or Mn-500, and significantly more citrate per unit root mass at Mn-0 treatment than at Mn-500.

Carboxylate exudation by Salado lucerne was generally greater than by Sirosal in plants grown at low Mn rates. With no Mn supplied, significantly more oxalate, tartarate, L-malate, lactate, citrate and succinate ($P \leq 0.05$) was exuded per unit root mass

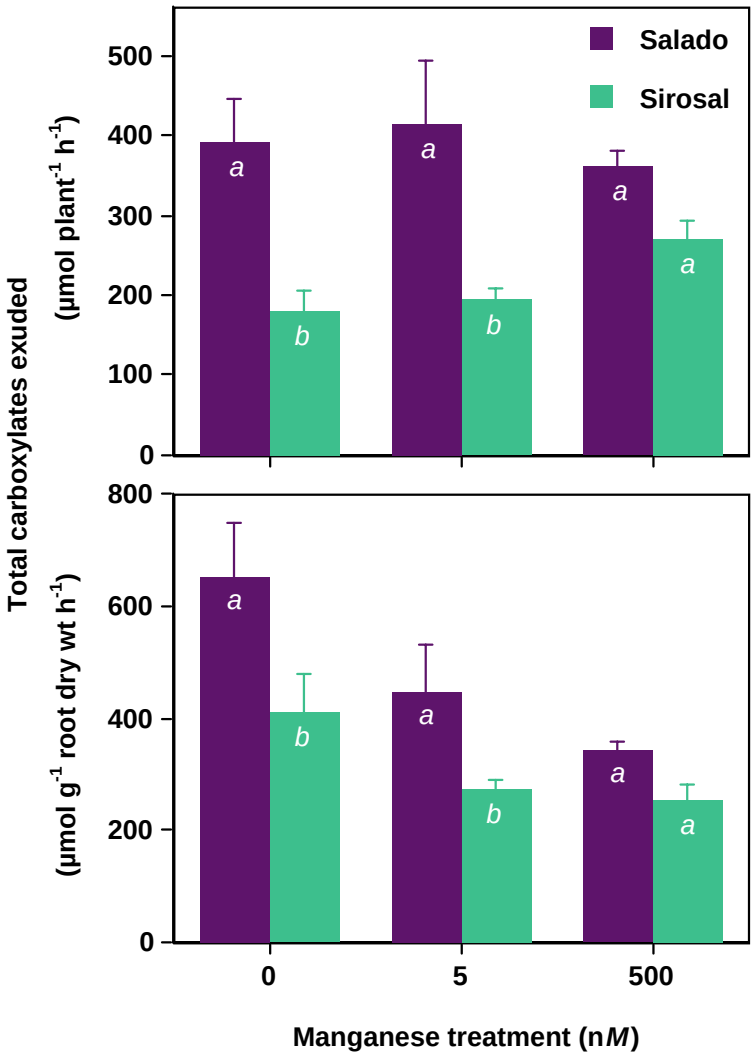

Figure 6.5. **Total carboxylate exudation rates by lucerne genotypes after 36 d under Mn treatments. Exudation is expressed per whole plant root system (top) and per unit root biomass (bottom). Different letters represent significant genotypic differences in each treatment ($P \leq 0.05$). Bars represent standard error.**

by Salado than Sirosal. In contrast, at Mn-5 exudation of only citrate and succinate was greater in Salado than Sirosal ($P \leq 0.05$), and at Mn-500 no discernible differences between genotypes were found for any of the detected carboxylates.

Total carboxylate exudation per unit root mass significantly decreased ($P=0.008$) with increasing Mn supply, but exudation per whole plant did not change with Mn treatment. The genotype effect was also significant for both exudation on a whole plant ($P=0.007$) and per unit biomass basis ($P=0.005$) due to the greater exudation from Salado compared to Sirosal under Mn-0 and Mn-5 treatments (Figure 6.5).

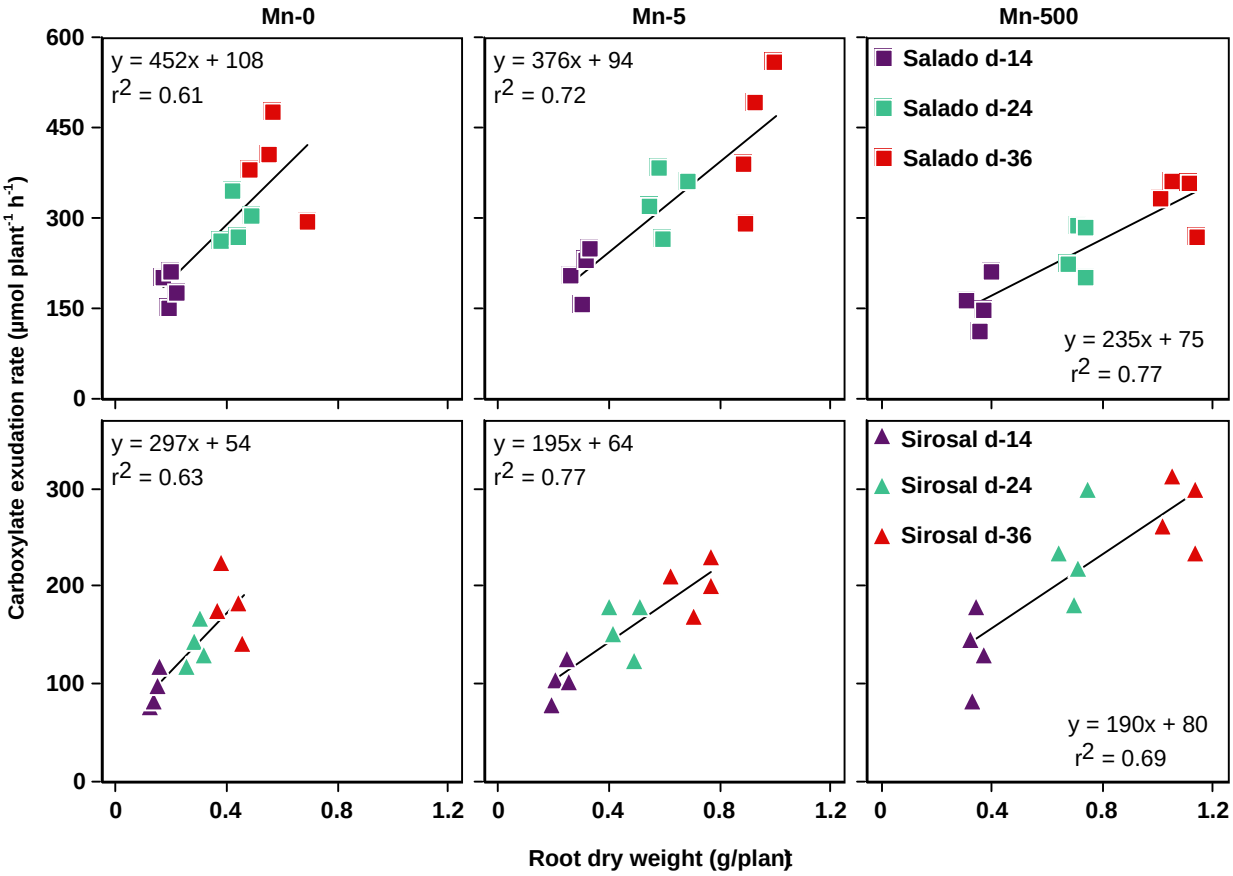

Figure 6.6. **The relationship between lucerne root biomass and rate of total carboxylate exudation. All regression coefficients are significant at *P*≤0.05.**

Successive exudate collections revealed the amount of carboxylates exuded by whole lucerne root systems increasing over time at all Mn treatments. Whole-plant exudation rate was directly proportional to the total plant root mass at collection time, and the relationship was linear at each Mn treatment rate (Figure 6.6). The slopes of linear relationships indicate that increases in carboxylate exudation per unit increase in root mass were much greater at low Mn rates than at adequate Mn rates.

Plate 6.2. *Equipment for root exudate collection (14 d). Vessels were covered in black plastic to minimise light on roots. Aeration hoses are visible.*

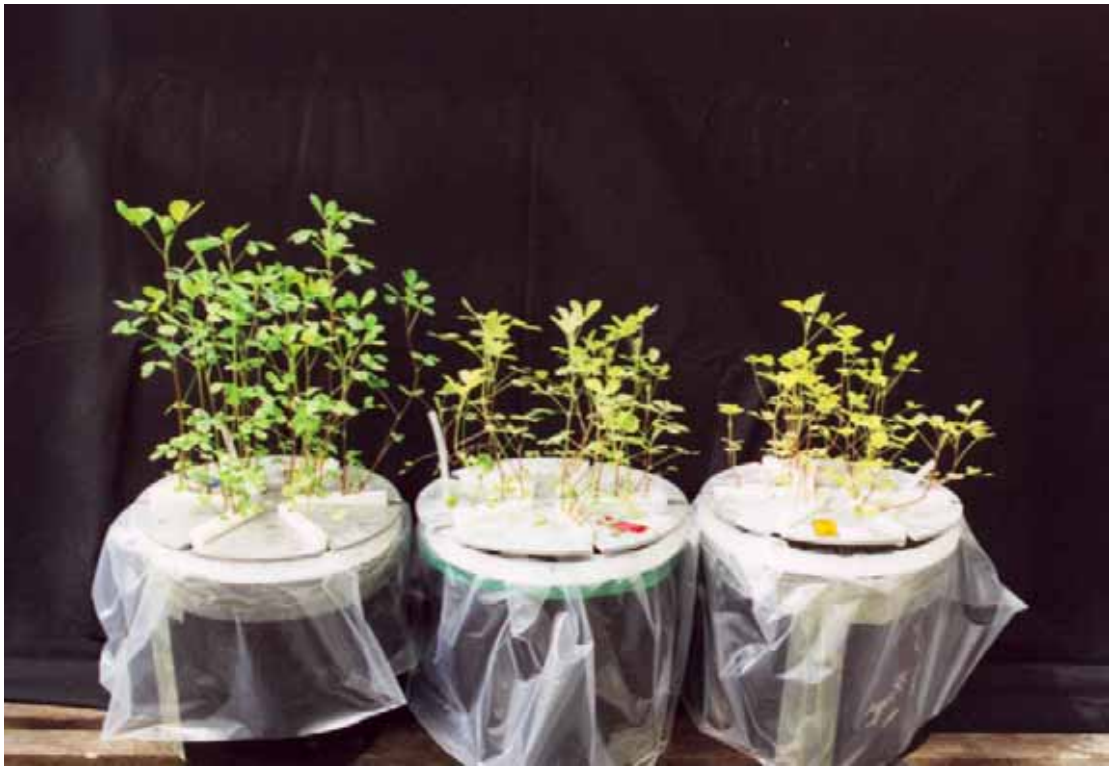

Plate 6.3. *Treatment differences in Salado shoot growth after 24 d. Left to right: 500-, 5- and 0-nM manganese added.*

Plate 6.4. *Shoot and root growth of lucerne after 36 d under 500-nM (left) and 5-nM (right) manganese treatment.*

DISCUSSION

Decreasing Mn supply to lucerne roots caused significant decrease in plant growth, and nutrient analysis confirmed the observed symptoms to be a result of severe Mn deficiency. Manganese concentrations at 14 d in shoots of Mn-0 plants (Table 6.2) were well below published critical range for Mn deficiency diagnosis (21 µg/g, Heckman *et al.* 1993; 20-30 µg/g, Reuter and Robinson 1997; 18-23 µg/g, Gherardi and Rengel 2003a). Plants grown at Mn-5 had Mn concentration in shoots at or below critical deficiency levels from d 24 onward. The described Mn deficiency symptoms match those of previous experiments where lucerne was grown in Mn-deficient conditions in soil (Lombin and Bates 1982; Heckman *et al.* 1993) and soil-like systems (mineral processing residue, Gherardi and Rengel 2003a, 2003b). All other analysed nutrients (data not shown) were at adequate concentrations in lucerne shoots (Reuter and Robinson 1997), and there was little difference between genotypes. The nutrient concentration data suggested that decreased Mn supply resulted in increases in shoot concentrations of Cu, Fe and Zn, but this was not consistent at all harvest dates.

Of interest was the continual slowing of growth rate in plants in the Mn-0 treatment, while the growth rate of plants under Mn-5 treatment showed no significant decrease to 36 d, even though Mn concentrations in the shoots of Mn-5 plants were deficient after 24 d. The data of Rengel et al. (1993; 1994) indicated that considerable plant growth can be achieved at Mn tissue concentrations that are considered very

deficient. However, the low Mn concentrations in the lucerne shoots are considered unlikely to sustain such a growth rate for a long duration, and it is probable that growth rate under Mn-5 treatment would have slowed if the treatment/growth period were lengthened.

Recent reviews highlight the fact that effects of Mn deficiency on carboxylate release by roots have not been quantified to date (Jones 1998; Jones *et al.* 2003). The consistent trends in carboxylate exudation across three separate sampling occasions suggest that decreasing Mn supply induced carboxylate release. In terms of the amount of carboxylates exuded per unit root mass at 36 d, exudation by lucerne plants supplied with no Mn in the present study was almost twice that of plants supplied with adequate Mn (Mn-500, Figure 6.5).

Of the identified carboxylates produced at greater rates under deficient than adequate Mn conditions, citrate, malate and, to a lesser degree, oxalate have been implicated in releasing Mn in plant-available forms from previously unavailable Mn oxides through a combination of reduction and complexation processes (Jauregui and Reisenauer 1982; Msaky and Msanya 1986; Ström 1997; Fan *et al.* 2001). Succinate and malonate also formed a high proportion of the total carboxylate exudation of both lucerne genotypes, while concentrations of lactate and tartarate were much lower. The exudation of malonate in significant quantities is considered uncommon in plants other than the Proteaceae (Roelofs *et al.* 2001). However, at least two other legumes, chickpea (Ohwaki and Hirata 1992) and pigeonpea (Otani *et al.* 1996), have shown malonate as a substantial proportion of exuded carboxylates. Decisive roles of malonate and these other carboxylates in Mn nutrition of plants are yet to be uncovered. While there is an expectation that they will affect mobilisation of Mn to some degree, such mobilisation can happen via different mechanisms. The di- and tri-carboxylic acids, for example, appear to have a stronger solubilising power of Mn in soil than mono-carboxylic acids (Ström 1997).

Microbial degradation of exuded organic compounds has the potential to cause significant variation in quantities of root exudates measured. The use of the Puritabs® treatment was an attempt at minimising the microbial populations on the roots without causing root-cell damage. Such measures are not widely reported in published methodologies for root exudate collection. No microbial counts were taken, however, so microbial presence in the collection vessels cannot be discounted and the quantities of carboxylates measured may indeed be affected by microbial degradation to some extent. Nonetheless, carboxylates escaping microbial decomposition (measured amounts) will be potentially available to directly affect rhizosphere Mn availability. Carboxylates and

other compounds originating from roots may also promote or retard the growth of microbes (Timonin 1946) that consume other exudate components with potential for Mn mobilisation (organic acids, phenolics, non-proteinogenic amino acids etc.). Such microbes may even also affect Mn availability (Tinker 1984). Although the relationship among root exudates, microbial population dynamics and Mn availability is complex and difficult to define, it is an area worthy of future research to improve understanding of rhizosphere processes that help plants to cope with conditions conducive to Mn deficiency such as alkaline soils.

Salado has been shown to be more Mn-efficient, or tolerant to Mn-deficient conditions, than Sirosal as defined by relative growth and yield ability under deficient and adequate Mn supply (Gherardi and Rengel 2003a). Increasingly, evidence is suggesting that tolerance to Mn deficiency is implicitly connected to a species' or genotype's ability to increase root exudation under Mn-deficient conditions (Rengel 1999, 2000). Indeed, under conditions with no added Mn, roots of Salado released greater amounts of each carboxylate than those of Sirosal, though the spectrum was the same (Figure 6.6).

Previous work has shown Mn deficiency to cause decreased production of non-structural carbohydrates in roots (Vielemeyer *et al.* 1969; Marcar and Graham 1987). In light of this, increases in carboxylate exudation under Mn deficiency, and the differing levels of increased exudation by genotypes tolerant and intolerant to Mn deficiency, are interesting, albeit a physiological basis for the differences is open to speculation. If increased exudation of particular carboxylates does represent an adaptation resulting in increasing availability of rhizosphere Mn, the carboxylate source and inducing mechanism remain unclear as low Mn supply simultaneously increased exudation of almost all carboxylates. Equally, the significance of the carboxylates for Mn availability and plant uptake remains undefined. Generally, Mn deficiency occurs where plants are growing on soils of high pH. Even carboxylates such as citrate and malate, known to increase availability of Mn in some soils, show decreased specificity in forming stable complexes at high pH (Jones and Darrah 1994; Jones *et al.* 1994). It must, however, be emphasized that extension of the present nutrient solution results to explaining responses and processes in soil must be done with caution as the nutrient solution system is simplistic. Although no or low amounts of Mn were supplied, no Mn was present in unavailable form and the dynamics of available/unavailable equilibrium processes were not represented.

Extending the agronomic definition of tolerance to Mn deficiency (relative yield in deficient and adequate Mn conditions) to the present results, Salado is able to express

greater tolerance to Mn deficiency than Sirosal in solution culture as well as in soil-like systems (eg. mineral processing residue, Gherardi and Rengel 2003a, 2003b). Similar expression of tolerance to Mn deficiency in solution culture was reported for cultivars of soybean (Ohki *et al.* 1980), another leguminous species. In cereals, there are conflicting reports. One recent study has shown barley expressed tolerance to Mn deficiency in soil but not in solution culture (Huang *et al.* 1994). In comparison, earlier solution culture studies reported expression of tolerance to Mn deficiency in maize (Landi and Fagioli 1983) and oats (Munns *et al.* 1963b; Brown and Jones 1974), but there is some doubt as to whether precipitation of Mn may have affected interpretation of these early results. Following findings of Huang et al. (1994), plants which express tolerance to Mn deficiency in soil but not in solution culture most likely do so via mechanisms which enhance availability of Mn in the rhizosphere. Hence, expression of Mn-deficiency tolerance by Salado in solution culture suggests that the genotype's influence over rhizosphere chemistry is not solely responsible for the observed Mn-deficiency tolerance in soil and soil-like systems. Efficient internal utilisation of Mn by Salado appears to be involved, and has been suggested previously (Gherardi and Rengel 2003a).

Other plant factors may also influence genotypic tolerance to Mn-deficient conditions. As a rule, acquisition of nutrients by the roots plays an important role (Gutschick 1993). Root morphology, along with any morphological relationship with root exudation, will have a large bearing on nutrient acquisition, but previous low Mn studies have focussed on macro-geometry or total root mass production rather than microscopic morphological features (Graham 1988). Additionally, it is difficult to distinguish whether root geometry and morphology is a determining factor in tolerance to Mn deficiency or a product of it. What is clear from the present results is that at low Mn supply, both the measured root length and, to a greater extent, the relative proportion of total root length made up by roots with fine diameter increased significantly in Salado and decreased for Sirosal (Figure 6.2). The majority of root exudates are released from the region of the root near the sub-apical meristem cells (Uren and Reisenauer 1988). It is possible that the observed increase in fine root production by Salado at low Mn rates is associated with increased root branching and rootlet production near this meristem region. In turn, this may explain increases in carboxylate exudation per unit root mass in Salado under Mn deficiency. Hence, the ability to produce fine roots and rootlets under conditions of low available Mn, and subsequent carboxylate exudation from these regions, may play a part in differential tolerance to Mn deficiency in lucerne genotypes. However, further validation in soil

systems is warranted.

CONCLUSION

A decrease in Mn supply to lucerne roots increased the exudation of carboxylates by those roots. Different rates of exudation of carboxylates may play a role in differential genotypic tolerance to Mn deficiency in lucerne. If so, total amounts of carboxylates released by a root seem more important than a different spectrum. High exudation under Mn deficiency also appears related to production of fine roots in deficiency-tolerant lucerne genotypes. There remains a need for more work in order to understand the driving mechanism behind changes in root exudation induced by low Mn, and the role of rhizosphere carboxylates in accessing unavailable Mn from soils.

CHAPTER VII

AVAILABILITY OF WATER IN REVEGETATED BAUXITE RESIDUE SAND: HYDROLOGICAL CHARACTERISTICS AND MODELLING WATER BALANCE UNDER LUCERNE PASTURE

Plate 7.1. *Irrigation of lucerne revegetation at the Pinjarra bauxite residue disposal area.*

CHAPTER SUMMARY

Plant response to Mn fertiliser on highly-porous bauxite residue sand (BRS) is largely dependent on water availability, as has been demonstrated by the effectiveness of deep banding. However, high porosity also means that irrigation is required to support BRS revegetation during summer months, and there is high potential for wastage of water by excessive irrigation that rapidly drains past the root zone. Optimising irrigation inputs to the revegetated BRS system for minimal waste will improve the economic and environmental viability of the stored by-product of mine processing. An extensive hydrological characterisation of a BRS profile was undertaken using a variety of *in-situ* and laboratory methods. Accurate parameterisation of water retention curves (WRCs) by *WIN-RETC*, using the fitted van Genuchten equation and $m=1-2/n$ restriction, was achieved for 10, 30 and 80-cm depths in the BRS profile (WRC $r^2>0.99$ for each depth). Surface and subsurface saturated hydraulic conductivities were high, ranging from 40 to 60 cm/h. Using the calculated and modelled parameters and Time Domain Reflectometry (TDR) measurements of water content in the field, a computer-assisted modelling program, *SWIMv1.1*, was able to model volumetric water content and total profile water to 135-cm depth with high precision over summer and winter monthly simulations and a long-term (810 d) simulation. Modelling accuracy decreased with increasing profile depth. Interception of irrigation by a lucerne (*Medicago sativa* L.) canopy was significantly influenced water reaching the BRS, especially when the canopy was initially dry. *SWIMv1.1* outputs suggest summer irrigation at rates used in the 2000-2001 summer (lower than previous two summers) will adequately sustain a lucerne crop. Further refinement and incorporation of specific crop-based (lucerne) modules may allow tailored summer irrigation regimes to be optimised for long-term efficiency of BRS revegetation.

INTRODUCTION

Perhaps the most important factors for healthy plant growth are nutrients and water, and in many cases the supply of these to plants can be altered by human intervention. The physical and chemical characteristics of BRS, as mentioned earlier, are such that both water and nutrient availability are severely limited for plant growth. The coarse residue sand has a low available water holding capacity, so that uptake of nutrients is greatly

limited by the short residual period of available moisture in the root zone after rain or irrigation events.

An important reason for the success of deep-banded fertiliser applications is the positioning of fertiliser in the profile where soil moisture levels are higher than at the soil surface, and remain so for longer during low-rainfall periods. In addition to providing the fundamental conditions for nutrient dissolution, water is the vehicle by which nutrients move to plant roots and by which compounds exuded into the rhizosphere by plants and microorganisms are able to mobilise nutrients in unavailable forms and make them available to plants. In contrast to deep-banded applications, nutrients added to topsoil will be unavailable to plants during dry periods (Pinkerton and Simpson 1986). Such an increase in residual effectiveness of deep-banded Mn has been shown for lucerne growing on BRS at Pinjarra (Chapter V, see also Gherardi and Rengel 2003c), and similarly for other species growing on sandy soils (eg. lupin, Brennan 1993; Crabtree 1999).

While maintenance of adequate available moisture is paramount for both vegetation growth and nutrient dissolution, the macroporous nature of coarse BRS means that there is a high potential for irrigation wastage. Excessive water is poorly retained in the BRS profile after application and rapidly drains past the root zone. Improving the environmental and economic viability of bauxite residue revegetation, therefore, will require the refinement of irrigation schedules in addition to more efficient employment of fertiliser applications (Chapter IV, Chapter V, see also Gherardi and Rengel 2003b; 2003c).

Knowledge of the water movement and retention in a soil is a useful tool in the formulation of strategies to optimise plant growth. Through a combination of laboratory and field measurements and the use of computer-assisted modelling, good representations of the water content through the Pinjarra BRS profile may be obtained. Models may also provide an accurate tool for predicting water balance of the area. The revegetated bauxite residue disposal area at Pinjarra, however, has not been comprehensively characterised with respect to water retention and movement through the profile. Such a characterisation requires measurements and analyses in a number of stages. Since the subject matter is dissimilar to that of the main literature review of this thesis (Chapter I), a brief review of water retention and movement theory is included below for completeness, followed by the development and application of that theory for a comprehensive hydrological characterisation of BRS to assist in predictive modelling applications.

THE MOVEMENT OF WATER IN THE SOIL

In a one-dimensional saturated system, water flow through a porous media in response to an imposed hydraulic gradient $\partial H/\partial x$ is described by Darcy's law (Darcy 1856):

$$q = -K\frac{\partial H}{\partial x} \qquad \{7.1\}$$

where q is the flow rate or area flux (volume flux divided by the cross-sectional area), K is the hydraulic conductivity, and H is the hydraulic head at a soil depth, x.

When a fluid of constant density is flowing, conservation of volume must be observed in a system where flow decreases the hydraulic head, which in turn decreases the flow rate. In other words, the water input into a system at a given time and the water moving through the system must balance. This is described by the continuity equation:

$$\frac{\partial \theta}{\partial t} = \frac{\partial q}{\partial x} + S \qquad \{7.2\}$$

where θ is the volumetric water content, t is time and S is a source or sink strength that accounts for plant water uptake.

If Darcy's law {7.1} and the continuity equation {7.2} are combined, the resultant equation can generally describe the one-dimensional flow of water in a soil. This is known as Richards' equation:

$$\frac{\partial \theta}{\partial t} = \frac{\partial q}{\partial x}\left(K\frac{\partial H}{\partial x} + S\right) \qquad \{7.3\}$$

Richards' equation (Richards 1931) is the basis of the numerical model *SWIM* (Soil Water Infiltration and Movement, Verburg *et al.* 1996) which can describe water flow and solute transport through a soil matrix. Using *SWIM*, prediction of water content profiles over time in bauxite residue disposal areas should be possible. There are also simplistic modules in *SWIMv1.1* to account for plant uptake and transpiration effects. Overwhelmingly, however, the successful application of models such as *SWIM* in predicting water balance is dependent on reliable estimation of the basic physical properties governing water movement and storage in the substrate (van Genuchten *et al.* 1991).

Assuming that soil structure is rigid and that air pressure is always atmospheric in the soil (ie. air movement is not restricted), H will therefore be the sum of the water pressure head or matric potential, ψ, and the gravitational potential, z:

$$\frac{\partial \theta}{\partial t} = \frac{\partial q}{\partial x}K\left(\frac{\partial \psi}{\partial x} + \frac{\partial z}{\partial x}\right) + S \qquad \{7.4\}$$

Writing Richards' equation as such {7.4}, it is seen that in order to describe water movement in a soil, or BRS in the present case, two hydrological characteristics must be defined:

(1) The relationship between matric potential and the water content of BRS [$\theta(\psi)$], described by the water retention curve (WRC).

(2) The relationship between hydraulic conductivity and water content in BRS [$K(\theta)$].

WATER RETENTION CURVES: THE RELATIONSHIP BETWEEN MATRIC POTENTIAL AND WATER CONTENT OF A SOIL

The relationship between the soil-water content, θ, and matric potential (the soil-water pressure head), ψ, is a fundamental part of the characterisation of the hydraulic properties of a soil (Klute 1986). The relationship is identified in the literature by various names, including *water retention function* or curve, *moisture characteristic* curve, and the *capillary pressure-saturation* curve. The dynamics of the WRC of a substrate are governed by the relationship between the ψ and the capillary pore radii as described by the Laplace capillarity equation:

$$\psi = \frac{2\gamma \cos\sigma}{\rho g R} \qquad \{7.5\}$$

where γ is the surface tension, σ the contact angle between water and the capillary wall, ρ the density of water, g the acceleration due to gravity and R is the equivalent pore radius. At 20 °C, this simplifies to

$$\psi = \frac{0.15}{R} \qquad \{7.6\}$$

Hence, the WRC of a soil will be primarily dependent upon the texture or particle size distribution of the soil (Salter and Williams 1965), and the structure or arrangement of the particles (Croney and Coleman 1954). Large pores will drain first at a low soil-water pressure head, whereas smaller pores will drain later at a higher soil-water pressure head. The number of pores of each particular size will determine the shape of the WRC. In sandy soils with a pore-size distribution biased toward larger-sized pores, water can be removed at lower soil-water pressure heads, producing a shallow slope to the WRC (Figure 7.1). Clay soils have much smaller pores. This is synonymous with greater capillary adsorption forces, so that a higher pressure head, or greater suction, is required to remove water from these pores, and the resultant WRC is steeper than for sandy soils.

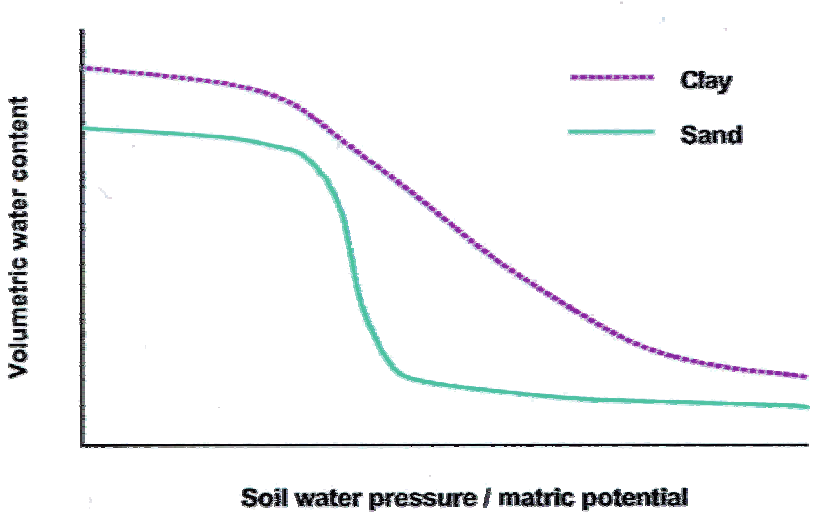

Figure 7.1. **Representative (theoretical) difference between water retention curves of clay soils with low porosity and highly porous, sandy soils.**

DESCRIBING WATER RETENTION CURVES

Empirical description of the soil-water retention curve has been achieved by several models. Brooks and Corey (1964) proposed one such function:

$$\theta = \theta_s (\alpha\psi)^{-\lambda} \qquad \text{when} \quad \psi > \frac{1}{\alpha}$$

$$\text{and} \qquad \theta = \theta_s \qquad \text{when} \quad \psi < \frac{1}{\alpha} \qquad \{7.7\}$$

where θ_s is the saturated water content, α is an empirical parameter whose inverse is known as the air entry value (h_g), and λ is a pore-size distribution parameter that affects the slope of the retention function.

Since the water content of a soil does not always become zero at a very high suction, a parameter for residual water may be required in the water retention function. Residual water is defined by Luckner *et al*. (1989) as water in the soil which does not contribute to liquid flow due to blockage of the flow paths or strong adsorption onto the solid phase. A term for residual water, θ_r, was added to the original Brooks and Corey water retention function {7.7} and the resultant equation {7.8} (Brooks and Corey 1964) has become one of the most popular functions for describing the WRC of a soil:

$$\theta = \theta_r + (\theta_s - \theta_r)(\alpha\psi)^{-\lambda} \qquad \text{when } \psi > \frac{1}{\alpha}$$

$$\text{and} \quad \theta = \theta_s \qquad \text{when } \psi < \frac{1}{\alpha} \qquad \{7.8\}$$

The Brooks-Corey equation has been shown to produce relatively accurate results for coarse textured soils characterised by relatively narrow pore-size or particle-size distributions (large λ values) (van Genuchten *et al.* 1991).

Some discontinuity is observed in the Brooks-Corey equation because of the inherent assumption that when the soil-water pressure head (ψ) is less than the air entry value ($1/\alpha$), the water content is always at saturation. The soil is not always at saturation below $1/\alpha$, hence $\theta \neq \theta_s$ below $1/\alpha$. In order to overcome the discontinuity near saturation, van Genuchten (1980) fitted the WRC to an empirical sigmoid shape which is continuous from saturation to residual water content:

$$\theta = \theta_r + \frac{\theta_s - \theta_r}{\left[1 + (\alpha\psi)^n\right]^m} \qquad \text{when } \psi > 0$$

$$\text{and} \quad \theta = \theta_s \qquad \text{when } \psi < 0 \qquad \{7.9\}$$

where α becomes a scaling parameter, and n and m are constants affecting the slope and symmetry of the retention curve, respectively.

Both the Brooks-Corey equation {7.8} and van Genuchten equation {7.9} accurately describe WRCs at low water contents. However, in the region of the water retention curve near saturation, deviations in the fit of the Brooks-Corey model can occur (van Genuchten 1980; Mehta *et al.* 1994; Jarvis and Messing 1995). Although this inherent error may cause problems for water retention curve estimation, the Brooks-Corey equation remains a simple model and is still able to accurately describe a large number of soils. Adding more parameters as in the van Genuchten equation {7.9} may improve the fit of the WRC to observed data but may not always be required (Smettem and Gregory 1996).

THE HYDRAULIC CONDUCTIVITY - WATER CONTENT [$K(\theta)$] RELATIONSHIP

Whereas water retention characteristics are an expression of the ability of a soil to store water, the hydraulic conductivity (K) is a measure of its ability to transmit water. As water content decreases from saturation, large pores, the most effective in conducting water, are the first to drain. Also, the tortuosity (the ratio of the actual flow path to the apparent or "straight" flow path through a soil matrix, Hillel 1971) of the flow paths may increase, and the average properties (density and viscosity) of the soil solution may

change (Klute and Dirksen 1986). Hence, a decrease in water content will be associated with a decrease in K, and K may be regarded as a function of the soil-water content as defined by Darcy's law {7.1}.

In order to apply Richards' equation of water transport, $K(\theta)$ must be defined. For a particular matrix, $K(\theta)$ can be estimated by measuring infiltration under saturated and unsaturated conditions both in the laboratory or *in-situ* (for examples and detailed methodologies see Amoozegar and Warrick 1986; Green *et al.* 1986; Klute and Dirksen 1986), or may be predicted from water retention curves by a number of functions (eg. Brooks and Corey 1964; Campbell 1974; van Genuchten 1980). In general, water balance models based on Richards' equation (eg. *SWIM*) determine the $K(\theta)$ statistically based on a function of pore radius distribution modelled from water retention parameters using measured water retention data (Zhang and van Genuchten 1994).

In-depth description of model determination of $K(\theta)$ from water retention data by water balance models is beyond the scope of this chapter, but it is important to note the key requirement for a reference point by which the $K(\theta)$ relationship may be scaled. A commonly-used scaling point is the saturated hydraulic conductivity (K_{sat}) of the soil. Two relatively simple *in-situ* methods for K_{sat} determination by infiltration are a ponded-ring technique and a bore-hole permeameter technique.

PONDED RING METHOD FOR ESTIMATING SATURATED HYDRAULIC CONDUCTIVITY AT THE PROFILE SURFACE

The study of infiltration in the field may be carried out by numerous techniques involving a ring or cylinder infiltrometer (Amoozegar and Warrick 1986; Bouwer 1986; Green *et al.* 1986). The ring infiltrometer is a simple device consisting of a metal ring which is driven a small distance into the soil (Figure 7.2). The area above the soil surface inside the ring can then be flooded with water and the infiltration of the water into the soil can be measured. Flooding and measurements are generally continued until the infiltration rate becomes essentially constant, ie. errors due to capillary forces and lateral divergence of flow have become minimal and infiltration rate is governed by K_{sat}, which can then be calculated.

According to Bouwer (1986), it is rare that the wetted zone directly under the ponded ring will be, strictly speaking, completely saturated. Hence, it was generally accepted that the K of the wetted zone, calculated by applying the Darcy equation to the flow system presented by Green and Ampt (1911), will be slightly less than the

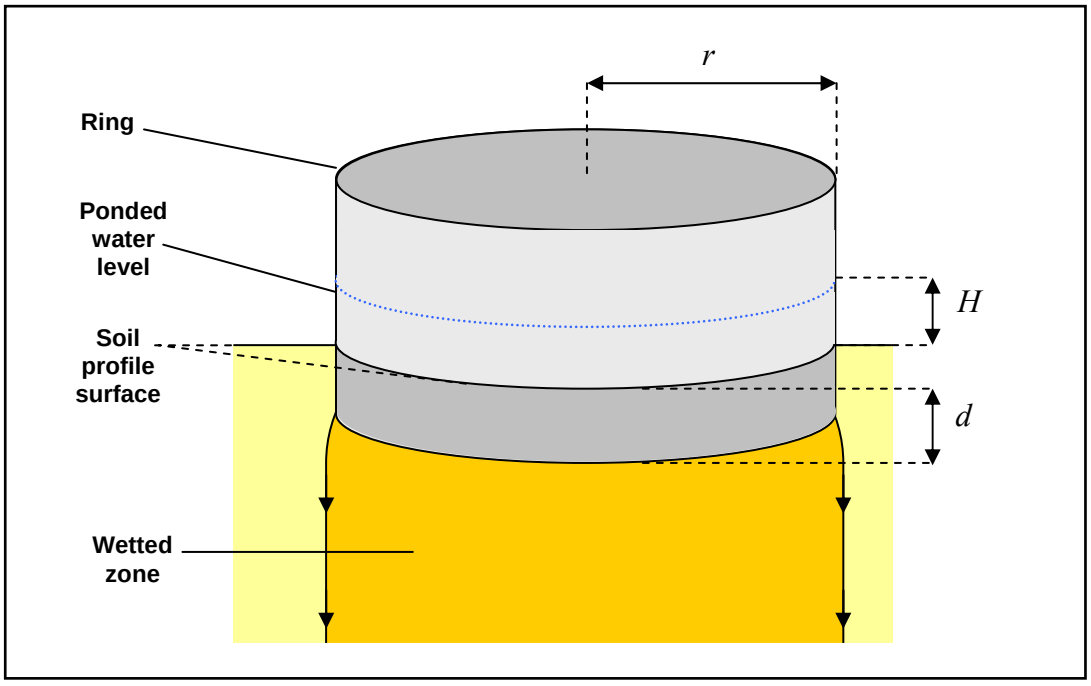

Figure 7.2. **The ponded ring infiltrometer for "near-surface" measurement of infiltration and hydraulic conductivity.**

saturated hydraulic conductivity (K_{sat}). The term 'field saturation' is often used to describe this condition. Developments by Reynolds and Elrick (1990) provided a new analysis of ponded infiltration that employed a numerically determined shape factor (G) as well as taking soil hydraulic properties, the ring radius (r), the depth of ring insertion into the substrate (d) and the depth of ponding (H) into account:

$$Q_s = \frac{r}{G(KH + m) + \pi r^2 K} \qquad \{7.10\}$$

where Q_s is the volume flux and ϕ_m is the matrix flux potential (defined below). The shape factor is defined as:

$$G = 0.316\left(\frac{d}{r}\right) + 0.814 \qquad \{7.11\}$$

and

$$\phi_m = \frac{b\chi^2}{\Delta\theta} \qquad \{7.12\}$$

where b = 0.55, χ is sorptivity, a measure of the capillary properties of the soil that affect unsaturated flow, and $\Delta\theta$ the change in soil-water content ($\theta_{sat} - \theta_{init}$) (White and Sully 1987).

The large particle size and the essentially macro-porous nature of the BRS profile lead to a dominance of gravity flow at early times after ponding. Continuous flooding of a ring infiltrometer should produce a wetted zone just below the surface that is very

close to BRS saturation. Hence, by using the analysis of Reynolds and Elrick (1990), the estimated K should be a valid estimate of K_{sat} at the surface of the BRS profile.

BORE-HOLE PERMEAMETER METHOD FOR SUB-SURFACE HYDRAULIC CONDUCTIVITY

The bore-hole permeameter method, also known as the well permeameter and auger-hole pump-in method, is one of a number of similar methods for determining K at sub-surface depths in the soil profile above, or in the absence of, a water table by maintaining a constant head (H) in a small auger hole. Commercial equipment is available for the bore-hole method, known as the "Guelph Permeameter" after development of the method by Reynolds *et al.* (1983) at Guelph, Canada. A fixed air inlet tube in the permeameter ensures H remains constant even though the reservoir water level drops as infiltration progresses.

A hole of radius r is augered in the soil to a sufficient depth in order that H/r will be at least 10 when water is added. The height of the air inlet tube base above the base of the auger hole when the permeameter is held in position defines H (Figure 7.3). Initial infiltration into the "dry" soil will be rapid as it is dominated by capillary forces. Once capillaries are filled and the wetting front begins to move away from the hole, continual monitoring of infiltration rate should reveal the flow rate becoming constant. This means the soil surrounding the hole can be considered as saturated, meaning that the rate of flow from the bore-hole (Q) is controlled by K_{sat}.

When the H/r ratio is sufficiently high, K can be expressed in the following terms:

$$K_{sat} = \frac{Q\left\{ \ln\left[(H/r) + \sqrt{H^2/r^2 + 1} \right] - 1 \right\}}{2\pi H^2} \qquad \{7.13\}$$

or

$$K_{sat} = \frac{Q\left[\sinh^{-1}(H/r) - 1 \right]}{2\pi H^2} \qquad \{7.14\}$$

Talsma and Hallam (1980) used this equation {7.14} when investigating K in forest soils by bore-hole methods. By numerical modelling, Reynolds *et al*. (1983) were able to replace $[\sinh^{-1}(H/r) - 1]$ with a parameter C:

$$K_{sat} = \frac{CQ}{2\pi H^2} \qquad \{7.15\}$$

The C parameter is essentially a geometrical shaping factor, and may also be estimated by analytical methods (the Glover and half-source improvement to the Glover solution, Reynolds *et al.* 1983), but over a wide range of H/r ratios, these give less accurate estimations of C than numerical modelling.

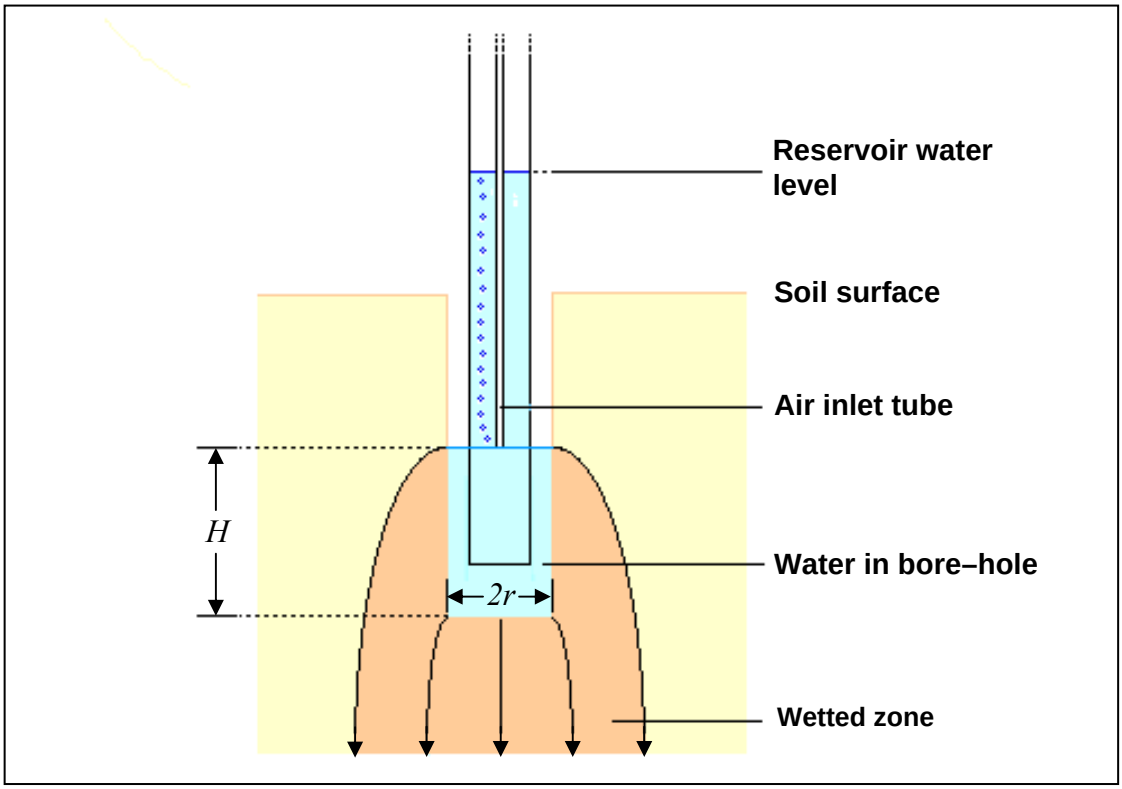

Figure 7.3. **The bore-hole permeameter for measuring sub-surface saturated hydraulic conductivity above a water table.**

The equation of Reynolds *et al*. (1983) {7.15} involves a number of assumptions about the nature of water flow out of a bore hole. In fact, in comparison to equation {7.13}, the numerical solution of Reynolds *et al*. (1983) produced a 68 and 65 % increase in K values for H/r values of 5 and 10 respectively (Amoozegar and Warrick 1986). The theory of water flow was therefore extended to include the effect of unsaturated flow (capillary properties) on the measured K by incorporating the sorptivity term χ:

$$K_{sat} = \frac{CQ}{\left(2\pi H^2 + \pi r^2 + \frac{2\pi H^2}{\chi}\right)} \qquad \{7.16\}$$

SIMULATION OF WATER MOVEMENT AND RETENTION BY SWIMV1.1

Based on Richards' equation, *SWIMv1.1* is a computer-assisted modelling program that can simulate one-dimensional transport of water and solutes. *SWIMv1.1* relies on measured or estimated soil hydraulic properties mentioned above in order to solve Richards' equation to estimate water flux in a soil profile.

Various interfaces are available for *SWIMv1.1*. One interface version, integrated into a series of EXCEL spreadsheets for input and output, has been described in depth by Oliver (2002). Briefly, inputs required for a model simulation run include times (start, end and output interval), surface conditions affecting infiltration and runoff, rainfall, evaporation, soil hydraulic properties, surface and lower boundary conditions, and vegetation parameters if plant uptake and transpiration must be taken into account (eg. in a vegetated system). *SWIMv1.1* outputs are converted into three spreadsheets which detail: (1) water content at each depth at each specified time interval; (2) soil-water pressure at each depth for each time interval; and (3) components of the water balance for each time interval.

For *SWIMv1.1* inputs, parameters required to describe soil hydraulic properties will differ slightly depending on the associated models chosen to represent water retention and hydraulic conductivity during a simulation run. However, the accuracy of *SWIMv1.1* output appears to be more sensitive to accuracy in measurement and estimation of the parameters than the choice of models (Oliver 2002). Additionally, the number of depth increments chosen to represent a profile must suit the experiment. Although *SWIMv1.1* always preserves mass balance, increasing the number of described soil depth increments can increase the output accuracy of estimation of water content versus profile depth.

Accurate prediction of soil-water balance by models must be verified by field data if application of the model to a set of substrate hydraulic parameters is to be relied upon as a predictive tool in soil-water management. However, methodology to evaluate the predictive capacity of water balance models is limited (Addiscott and Wagenet 1985; Willmott *et al.* 1985; Loague and Green 1991) and is still subject to debate.

Comparisons between predicted (P_i) and observed (O_i) data, or between alternative models, can be achieved by simple regression analysis (Addiscott and Whitmore 1987) or be based on statistical criteria and graphical displays (Loague and Green 1991). Statistical tests such as the significant *t*-test (Jacovides and Kontoyiannis 1995) can be rather stringent, with few models giving statistically significant results when more than 10-20 data are included.

Indices that provide a relative statistic based on the difference between predicted and observed values, residual error analyses, are commonly used to evaluate model performance. Maximum error (*ME*), modelling efficiency (*EF*) and relative average error (*D*) are examples (Willmott 1981; Loague and Green 1991) of residual error analyses. Wu *et al.* (1996) successfully used derivations of these indices to describe model performance:

$$ME = \text{Max}\left|P_i - O_i\right|_{i=1}^{f} \quad \{7.17\}$$

$$EF = \left[\sum_{i=1}^{f}(O_i - O_i)^2 - \sum_{i=1}^{f}(P_i - O_i)^2 - \right] / \sum_{i=1}^{f}\left(O_i - \overline{O}\right)^2 \quad \{7.18\}$$

$$D = 1 - \left[\sum_{i=1}^{f}(P_i - O_i)^2 / \sum_{i=1}^{f}\left(\left|P_i - \overline{O}\right| + \left|O_i - \overline{O}\right|\right)^2\right] \quad \{7.19\}$$

where $\overline{O}$ is the average of f observed values. The maximum possible value for *EF* or *D* is one. Negative *EF* and low *D* index values generally indicate low accuracy in representation of field soil-water balance. Wu *et al.* (1996) also formulated a calculation for comparing the difference (%) between simulated and observed water contents

$$\text{Difference (\%)} = [(P_i - O_i) / O_i]100 \quad \{7.20\}$$

Including the difference (%) index in an evaluation of model performance is useful in that there may be only a short time-frame within a simulation which causes low *EF* or *D* index values even if, for the most part, simulation was an accurate representation of observed field data.

APPLYING HYDROLOGICAL CHARACTERISATION THEORY FOR BENEFIT IN BAUXITE RESIDUE REVEGETATION

In the following study, hydrological characterisation of a BRS profile vegetated with irrigated lucerne was undertaken. By computer-assisted modelling, using well-defined parameters for BRS water storage and movement and a better understanding of factors that influence water input to the system such as canopy interception, a basis may be provided for formulating irrigation schedules to minimise use of excessive water whilst maintaining healthy growth of lucerne vegetation. The study of hydrological balance and water content in the profile below the surface layers may also provide further insight into the success of deep-banded Mn fertiliser treatments in providing longer residual value than surface broadcast applications.

MATERIALS AND METHODS

WATER RETENTION CURVES

Bauxite residue sand core samples were taken from depths of 10, 30 and 80 cm adjacent to the experimental lucerne trial at the Pinjarra residue disposal area (Chapter V). Four replicate cores were taken at each depth. The intact cores, 3 cm tall by 3 cm internal

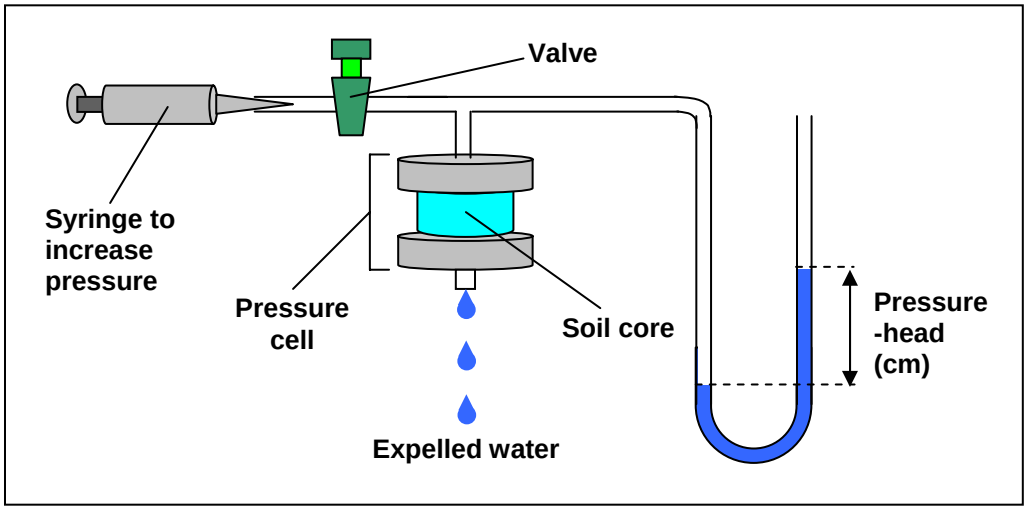

Figure 7.4. **Tempe® pressure cell arrangement to measure water retention from saturation to 100 cm soil-water pressure.**

diameter, were transported to the laboratory, saturated by capillary rise with de-aired water, sealed in Tempe® pressure cells (Soilmoisture Equipment Corp., Santa Barbara) and weighed. The Tempe® cell arrangement shown in Figure 7.4 was used to measure water retention through desorption. Cores were subjected to pressure heads equivalent to 1.5, 10, 20, 40 and 80 kPa sequentially. When no further water was expelled from the cores at a particular pressure head, the cores were re-weighed before being placed under the next pressure head in the sequence to allow calculation of retained water.

For pressure heads above 100 kPa, the intact BRS cores were divided equally into three parts by carefully pushing the BRS from the 3-cm-tall ring into stack of 1-cm-tall rings and slicing horizontally between the rings. The sub-cores were weighed and placed on pre-saturated, pressure-rated ceramic plates inside high-pressure chambers (Figure 7.5). Pressure heads equivalent to 1,000, 10,000 and 15,000 kPa were imposed via regulated N_2 gas from a size D cylinder. After 2 weeks, the gas was slowly released from the pressure chambers and the core sections weighed, oven-dried, and re-weighed to determine retained water at each pressure and allow bulk density determination.

Using WIN-RETC (van Genuchten *et al.* 1991), the van Genuchten WRC equation {7.9} was fitted to the observed water contents (both individually for each core sample and means) at the specified suction pressures, under the $m = 1 - 2/n$ restriction, to produce WRCs and parameter estimations for each sample depth. Differences in the WRC parameters between depth samples were compared by ANOVA using GENSTAT 5 (GENSTAT Committee 1989).

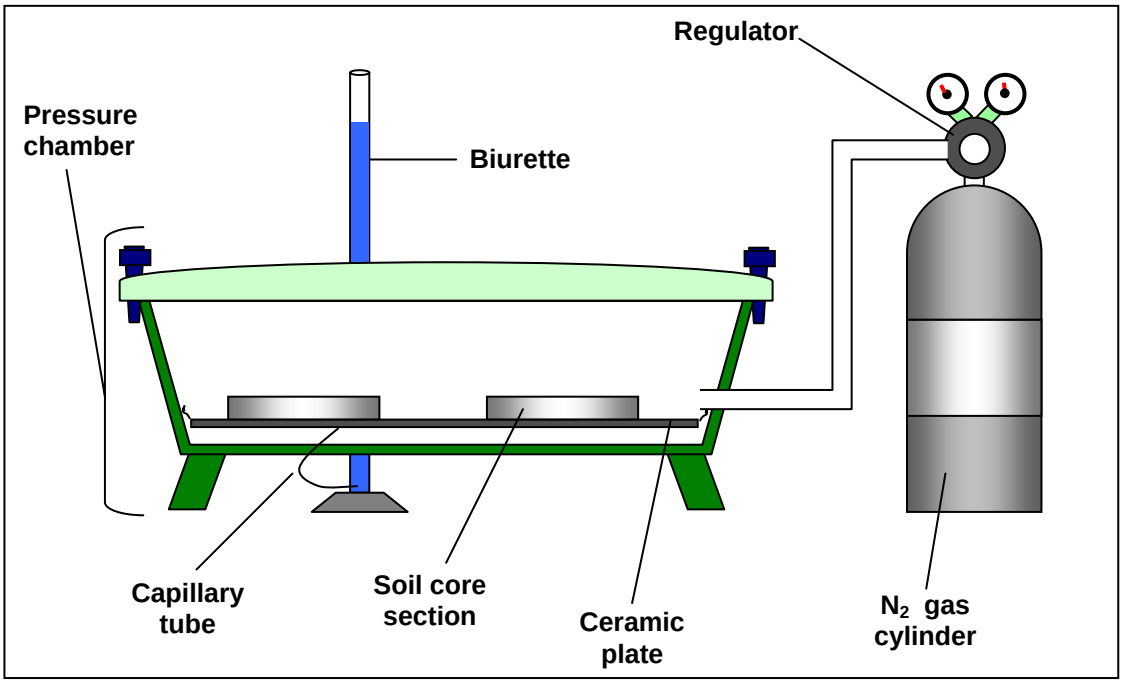

Figure 7.5. **Arrangement of soil core sections in high pressure chambers.**

HYDRAULIC CONDUCTIVITY MEASUREMENTS

The ponded ring method (Reynolds and Elrick 1990, see above), replicated 3 times, was used to determine surface *K* at the Pinjarra BRS disposal site. Subsurface *K* was also determined *in-situ* at depths of approximately 30 and 60 cm by the bore-hole permeameter method. In order to smooth the variation in observed readings caused by rapid drainage and vigorous bubbling, 9 separate bore-hole replications were used for each depth.

Since the BRS profile had a largely macroporous nature, and gravity flow was dominant almost immediately after ponding, the sorptivity term *s* was removed from calculations of *K* using ponded ring measurements, and the equation {7.15} of Reynolds *et al*. (1983) was used to derive subsurface *K* values.

FIELD MEASUREMENT OF VOLUMETRIC WATER CONTENT

Water content of BRS in the field was measured by Time Domain Reflectometry (TDR). In December 1998, a pit was dug at the border of the lucerne plots in the BRS impoundment at Pinjarra (see Chapter V for detailed field site description). Probes

(Buriable Waveguide 6005L2, Soilmoisture Equipment Corp, Santa Barbara, CA, USA) were installed horizontally, in triplicate, at 30, 60 and 110 cm below the surface. Readings were taken manually during regular (maximum 6-weekly) site visits until March 2001. Surface (0-10 cm) readings were also taken in triplicate at each visit by vertical insertion of parallel 10-cm-length probes.

MODELLING WATER MOVEMENT WATER CONTENT OF THE BRS PROFILE OVER TIME

Using the BRS hydraulic properties determined above, *SWIMv1.1* was used to model water content over time in the Pinjarra BRS profile. Single-month outputs using a 6-hourly timestep were made for the months of February and July during the lucerne and deep-banded Mn field trial (September 1998 to March 2001, Chapter V). Discrete layer water contents from the outputs were used to calculate total profile water to a depth of 135 cm. A long-term simulation spanning 810 d from December 1998 to March 2001 was also carried out, with an output timestep of 24 h. Initial conditions and input parameter settings are described below.

Soil parameters

Since using buried TDR probe depths as mid points in discrete layers allowed total profile water estimation to 135 cm, the simulated profile was also set to 135 cm. Specified upper and lower boundary conditions were set to surface conductance and free drainage, respectively. *SWIMv1.1* input tables were set out to require BRS hydraulic properties θ_s, θ_r, h_g (where $h_g = 1/\alpha$), m (where $m = 1\text{-}2/n$), and K_{sat} after WRC and K_{sat} determinations as described above. Hydraulic properties were specified for a range of depth layers between 0 and 135 cm (see Table 7.4).

Vegetation parameters

Representative lucerne root data (Tables 7.1 and 7.2) was based on root length density (RLD) versus depth measurements (Chapter V). Mean RLD of all lucerne plots (+Mn and –Mn at depth, Chapter V) was used. Considering the perennial nature of lucerne crops, root density and distribution was set as constant throughout all simulation runs.

Table 7.1. **Vegetation parameters for lucerne in bauxite residue sand used in *SWIMv1.1* simulations.**

Minimum xylem potential (cm)	-15,000
Depth constant for roots (cm)	74
Maximum effective root length density (cm/cm^3)	2
Maximum fraction of PET	0.9
Fraction of PET at day x	0.9
Day x	1
Fraction of PET at day y	0.9
Day y	2

PET: Potential evapotranspiration

Table 7.2. **Specified root length density input in *SWIMv1.1* simulations.**

Depth (cm)	RLD (cm/cm^3)	Depth (cm)	RLD (cm/cm^3)
0	0.775	38	0.28
2	0.775	42	0.20
6	2.21	46	0.18
10	2.31	50	0.09
14	2.19	54	0.11
18	1.87	58	0.10
22	1.50	62	0.05
26	1.14	66	0.05
30	0.83	70	0.06
34	0.42	74	0.05

Data are RLD means of +Mn and –Mn plots at each depth determined in Chapter V.

Surface conditions

Parameters for surface conductance and runoff (Table 7.3) were set to represent no surface sealing or runoff occurrence during the simulation.

Initial water content

Initial water content inputs (Table 7.4) for *SWIMv1.1* simulations were taken from TDR measurements in the BRS profile on the start date for the simulation.

Precipitation and evaporation

Daily rainfall and pan evaporation data were obtained from an automated weather station approximately 3 km south-west of the Pinjarra trial site (Commonwealth Bureau of Meteorology, Perth). Evaporation in *SWIMv1.1* input files was taken as 80 % pan evaporation values. Irrigation was either calculated using monitored pump pressure and time of irrigation application, or measured using precipitation gauges in the trial plot area (see below).

Table 7.3. **Surface conductance and runoff parameters for *SWIMv1.1.*** [a]

Conductance	Initial soil surface conductance (/h)	4
	Minimum soil surface conductance (/h)	0.02
	Precipitation constant (cm)	2.5
	Effectiveness parameter	0.184
Runoff	Initial soil surface storage (cm)	2
	Minimum soil surface storage (cm)	1
	Precipitation constant (cm)	5
	Runoff rate factor ([cm/h]/cm^P) [b]	2
	Runoff rate power (P)	2
	Initial surface water depth (cm)	0

[a] No surface sealing or runoff represented in BRS using these values.
[b] Runoff calculated using a simple power function. Detailed description is given by Ross (1990).

Table 7.4. **Initial water content (θ_{init}) input for *SWIMv1.1* simulations.**

	Simulation start date					
Depth	1998	1999		2000		2001
cm	10 Dec	1 Feb	1 Jul	1 Feb	1 Jul	1 Feb
0[a]	0.045	0.037	0.125	0.036	0.101	0.036
10[a]	0.045	0.037	0.125	0.036	0.101	0.036
15	0.050	0.042	0.135	0.040	0.120	0.040
20	0.055	0.048	0.145	0.044	0.130	0.044
25	0.060	0.054	0.160	0.047	0.140	0.047
30[a]	0.065	0.060	0.177	0.049	0.159	0.049
35	0.075	0.065	0.160	0.050	0.150	0.049
40	0.087	0.070	0.140	0.055	0.140	0.050
50	0.099	0.075	0.130	0.060	0.120	0.055
60[a]	0.110	0.084	0.116	0.071	0.115	0.062
70	0.130	0.095	0.150	0.090	0.140	0.080
80	0.150	0.105	0.180	0.110	0.170	0.100
90	0.170	0.135	0.200	0.130	0.220	0.120
100	0.190	0.17	0.229	0.150	0.270	0.140
110[a]	0.206	0.197	0.229	0.185	0.270	0.158
135	0.206	0.197	0.229	0.185	0.270	0.158

[a] Measured by TDR. Remainder are estimates.

Model goodness-of-fit

Simulation data for volumetric water content at the surface, 30, 60 and 110-cm depths and total profile water to 135 cm were compared with observed field data from TDR measurements following the methods of Wu *et al.* (1996, equations 7.17, 7.18, 7.19 and 7.20.)

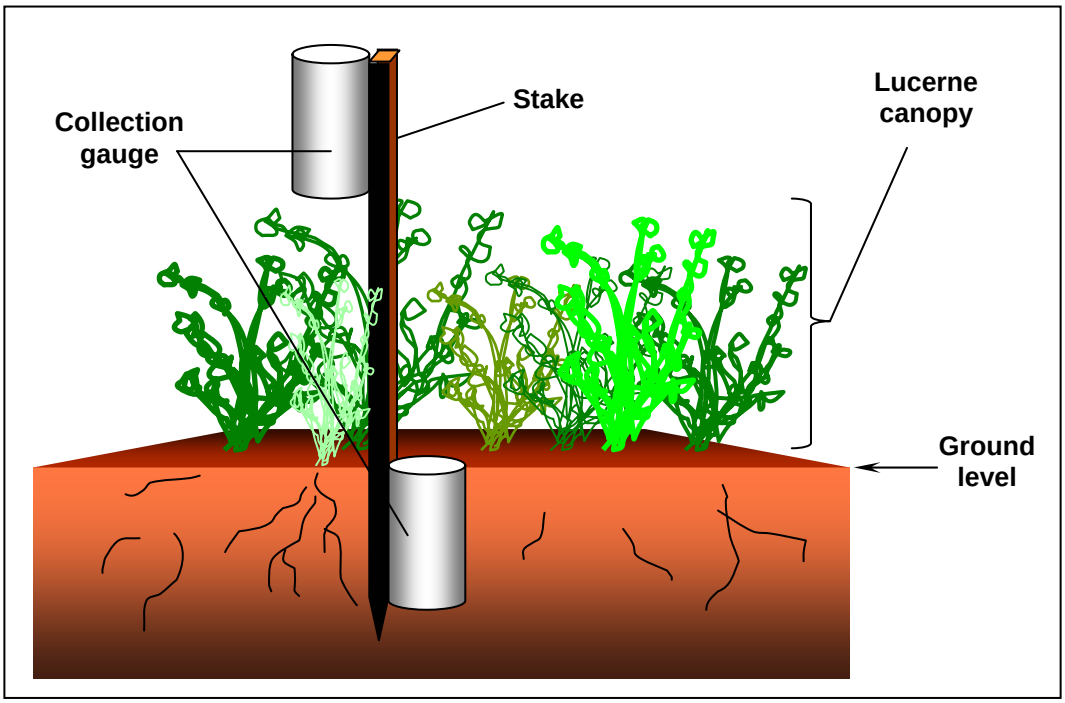

Figure 7.6. **Configuration of vessels to measure interception of irrigation water by lucerne foliage.**

ASSESSMENT OF WATER INTERCEPTION BY THE LUCERNE CANOPY

Lucerne vegetation at the Pinjarra site was harvested approximately every six weeks (see Chapter V). An assessment of canopy interception of irrigation water was carried out over a period of 3 successive harvests during the months of low rainfall (November to February). Precipitation gauges were positioned at 10 points in the lucerne field, with a ground-level and an above-foliage-level gauge at each point (Figure 7.6). Irrigation (average 9 mm/h) was run for 20 min onto the dry lucerne canopy and gauge readings taken. Immediately following readings, irrigation was run for a further 20 min and gauge readings taken again to compare canopy interception between 'dry' and 'saturated' canopies. Wet/dry foliage interception experiments were repeated at weekly intervals from 2 to 6 weeks after each harvest. Following each interception experiment, 5 randomly placed quadrats (40 cm by 40 cm) were harvested from the lucerne field and biomass (oven-dried at 70 °C) was measured.

RESULTS

WATER RETENTION CURVES

The WRCs show the characteristic slope of a highly-porous, sand-dominated substrate (Figure 7.7). Little variation in water retention between different depth BRS samples was found over most of the range of matric potential. Some variation was observed at low matric potential, noted by a significantly greater θ_s value at 10-cm depth than at 30 and 80 cm. No differences were found in θ_r or α with depth, and ANOVA of the slope factor n indicated no significant differences in WRC slope between different BRS depths. The van Genuchten WRC model showed strong goodness-of-fit to the observed data. Modelled parameters are given in Table 7.5.

HYDRAULIC CONDUCTIVITIES

Hydraulic conductivity was greater at 60-cm deep in the BRS profile than at the profile surface and 30-cm depth (Table 7.6). The lowest K was observed at 30-cm depth, but the calculated value was comparable with that of the surface layer.

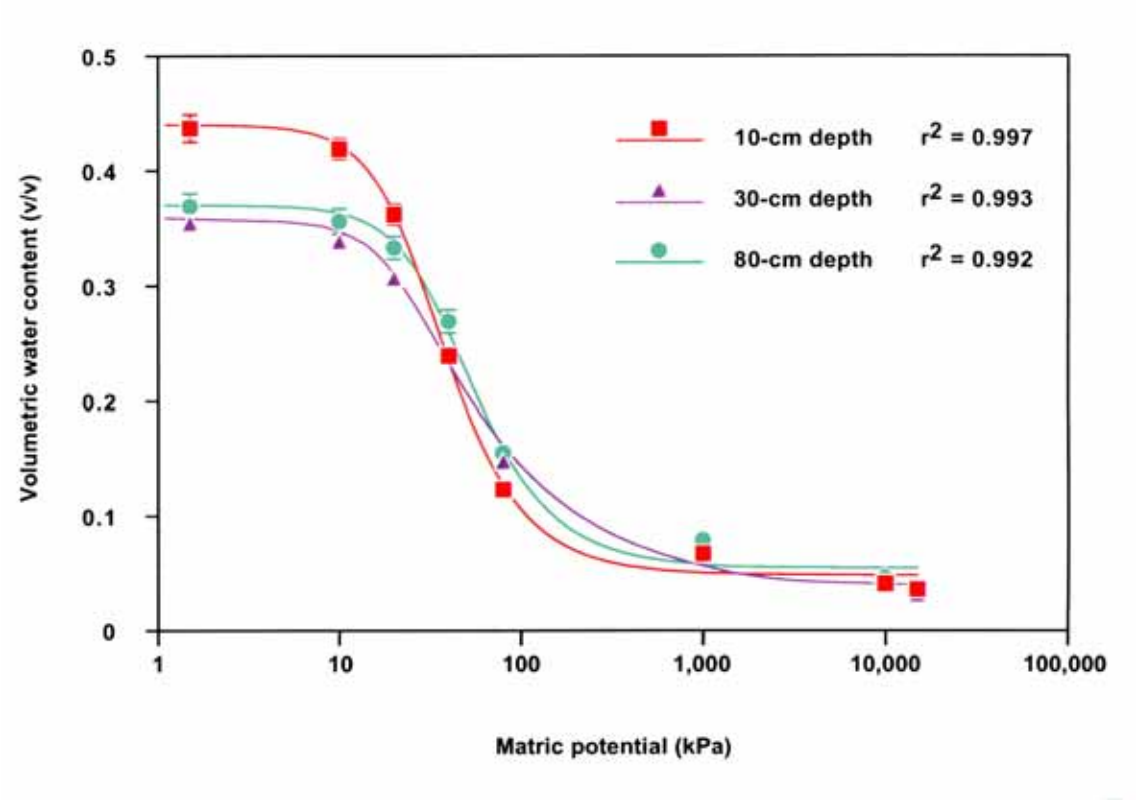

Figure 7.7. **Observed water retention data and fitted water retention curves for bauxite residue sand sampled from different profile depths. Water retention curve parameters are given in Table 7.5.**

Table 7.5. **Parameters for *WIN-RETC*-fitted water retention curves and bulk densities at different profile depths. Values are means ± standard errors, and goodness-of-fit statistics relate to retention curves fitted to mean observed data. Anchor(⚓) highlights a significant difference ($P \leq 0.05$) from other depths.**

Parameter	Depth (cm)					
	10		30		80	
Saturated water content (θ_s)	0.44⚓	± 0.006	0.36	± 0.007	0.37	± 0.006
Residual water content (θ_r)	0.048	± 0.001	0.035	± 0.004	0.054	± 0.003
Air entry index (α)	0.036	± 0.004	0.048	± 0.007	0.026	± 0.010
Slope parameter (n)	2.47	± 0.081	2.69	± 0.152	2.40	± 0.113
Goodness-of-fit:						
r^2	0.997		0.993		0.992	
Error (SSQ x10^{-4})[a]	6.2		7.5		9.6	
Bulk density (g/cm^3)	1.28	± 0.04	1.71	± 0.09	1.36	± 0.03

[a] Residual sum of squares

Table 7.6. **Hydraulic conductivities (K_{sat}) at different depths in the bauxite residue sand profile at Pinjarra, Western Australia. Values are means ± standard errors.**

Depth		K_{sat} (cm/h)		Replicate measurements
(cm)	Method	Mean ± SE	Geometric Mean	
Surface	Ponded ring	39.8 ± 1.3	39.6	3
32 ± 1.3	Borehole	38.1 ± 2.5	37.4	9
61 ± 1.2	Borehole	59.7 ± 3.6	58.9	9

MODELLING WATER MOVEMENT AND RETENTION IN BRS

Month-long simulations during summer and winter

Month-long simulations for each February and July during the field trial period indicated that initial specified model conditions were valid and *SWIMv1.1* outputs gave a reasonably accurate representation of volumetric water content over these months (Figures 7.8 and 7.9). Visually, deviations of simulated volumetric water content from the observed TDR data tended to increase with increasing depth. This was confirmed by modelling efficiency (*EF*) statistics for volumetric water content in each monthly simulation being farthest from one (optimum fit) at either 60- or 110-cm depths (Table 7.7). In February simulations, drainage and/or evapotranspiration decreased volumetric water content at a faster rate than was observed from measurements by TDR, while in July simulations, modelled decreases in volumetric water content after rainfall events were generally slower than in the observed data.

Table 7.7. **Residual error goodness-of-fit indices for modelled volumetric water content at specified layers and for total profile water to 135 cm in month-long simulations.**

		Index		
Month	Depth (cm)	*ME*[a]	*EF*[b]	*D*[c]
July 1999	0-10	-0.04	0.50	0.74
	30	0.03	-2.46	0.60
	60	0.05	-0.55	0.59
	110	-0.03	-4.50	0.21
	Profile water	-18.33	0.43	0.85
July 2000	0-10	0.03	0.64	0.86
	30	0.04	-1.53	0.63
	60	0.06	-2.18	0.45
	110	-0.06	-3.26	0.26
	Profile water	18.64	0.71	0.92
February 1999	0-10	0.03	-0.02	0.87
	30	0.01	-3.71	0.10
	60	0.04	-2.41	-0.03
	110	-0.02	0.72	0.89
	Profile water	13.79	0.47	0.82
February 2000	0-10	0.01	-2.67	0.37
	30	0.01	-0.08	0.07
	60	0.03	-5.57	0.23
	110	-0.02	-0.27	0.78
	Profile water	-14.41	0.54	0.86
February 2001	0-10	-0.03	0.38	0.68
	30	0.01	-0.58	0.60
	60	0.03	-12.94	0.11
	110	-0.02	-0.47	0.77
	Profile water	-4.81	0.86	0.96

[a] Maximum error
[b] Modelling efficiency See equations {7.17} {7.18} and {7.19}.
[c] Relative average error.

Percentage differences in model assessment showed *SWIMv1.1* simulations, on average, to slightly overestimate volumetric water content at surface, 30- and 60-cm depths, and underestimate it at 110-cm depth (Table 7.8). Fluctuation between over- and underestimation through the monthly periods was greatest in the surface layer and decreased with increasing layer depth.

Total profile water to 135 cm was well described by *SWIMv1.1* over the February and July monthly timeframes (Figures 7.10 and 7.11). Values for *EF* index were positive and reasonably close to one for all months. Increases in profile water were associated with irrigation and rainfall events. Applied irrigation was decreased in February of consecutive years from 1999 to 2001 and a slight decrease in total profile water to 135 cm followed a similar pattern.

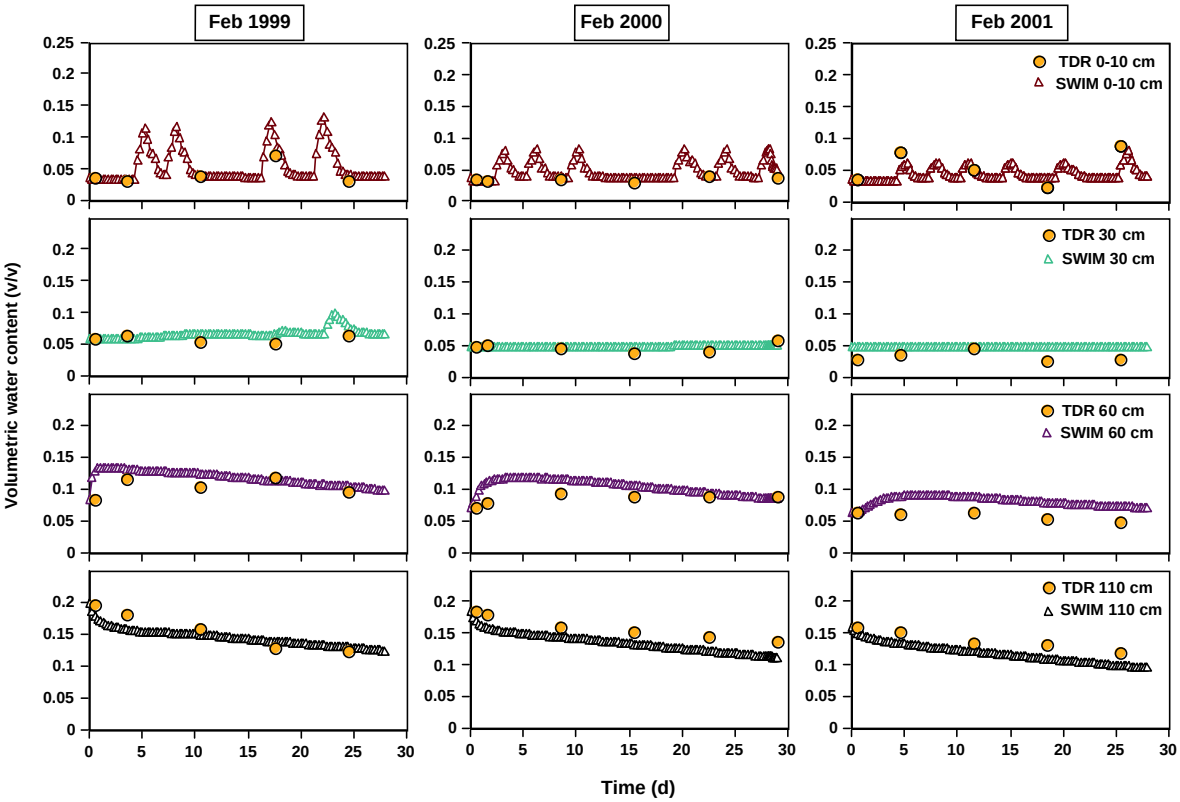

Figure 7.8. **Simulated and measured water content over month of February in successive years (1999 to 2001).**

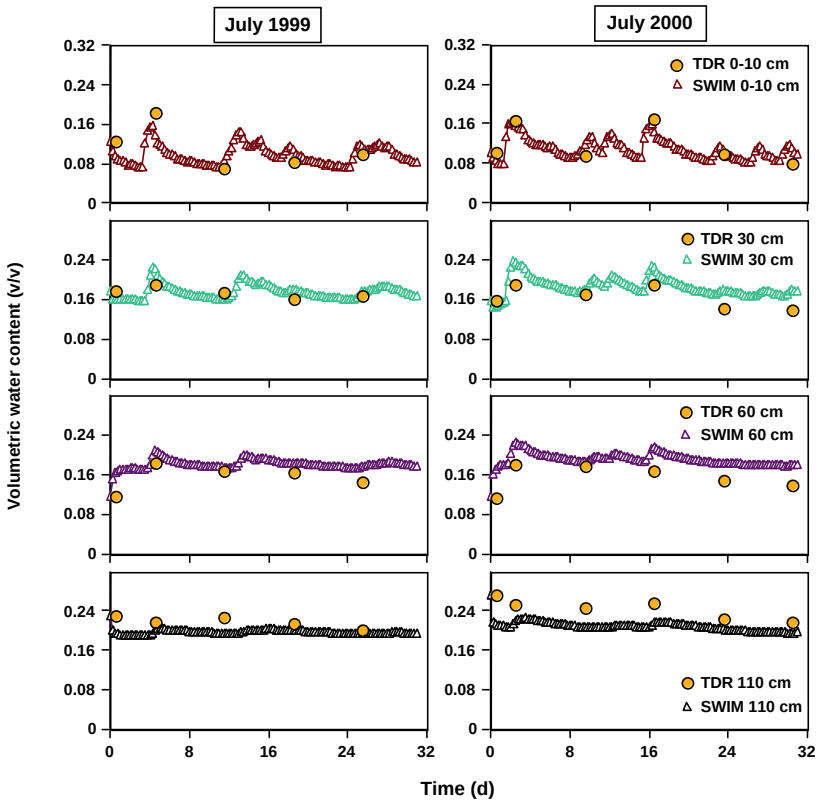

Figure 7.9. **Simulated and measured water content over month of July in successive years (1999 to 2000)**

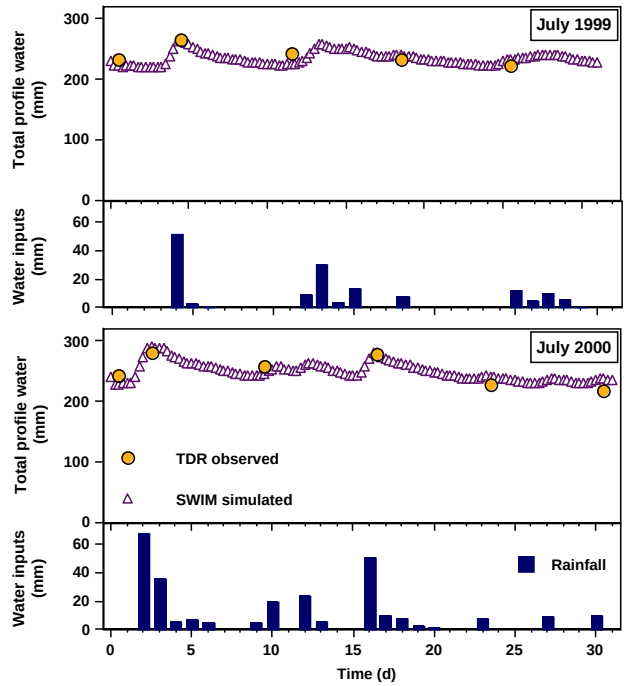

Figure 7.10. **Simulated and observed total profile water to 135 cm for July 1999 and 2000. Rainfall inputs are also indicated.**

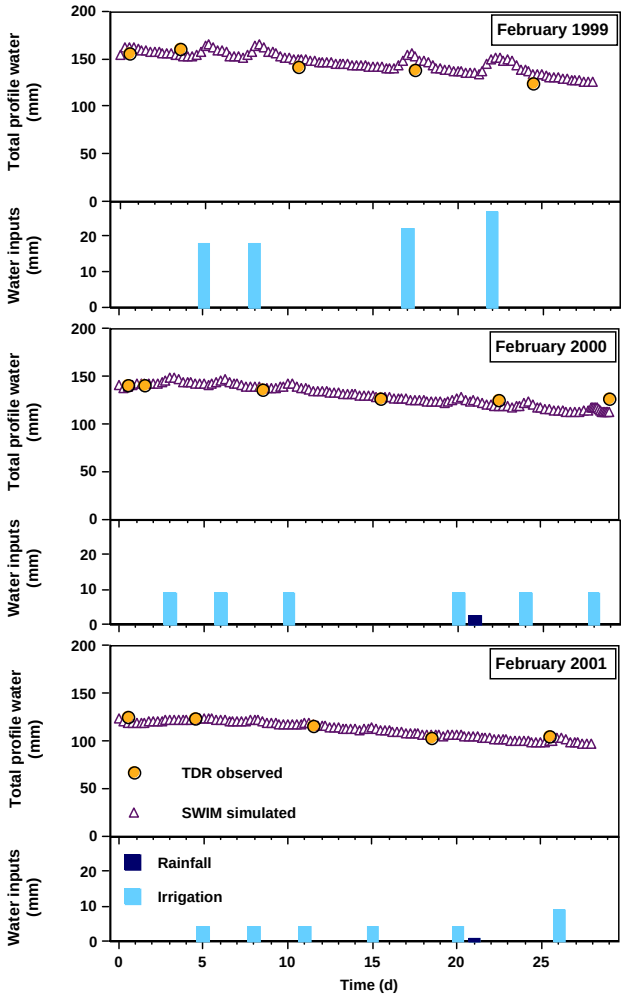

Figure 7.11. **Simulated and observed total profile water to 135 cm for February 1999, 2000 and 2001. Total water input (irrigation and rainfall) is indicated.**

Table 7.8. **Differences (%) between observed and predicted (*SWIMv1.1*) volumetric water contents and total profile water to 135 cm. Positive values are overestimation by model compared to TDR observations, negative values are underestimation.**

		Layer depth (cm)				Profile
Month	Day	0-10	30	60	110	water
July 1999	1	-23	-9	41	-15	-5
	5	-25	16	14	-7	0
	12	21	-6	4	-14	-8
	19	28	12	12	-7	3
	26	14	6	22	-4	5
July 2000	1	-16	-9	52	-21	-6
	3	-6	23	24	-12	3
	10	27	4	6	-15	-5
	17	-16	19	29	-16	0
	24	9	27	26	-10	6
	31	39	29	30	-10	9
February 1999	1	-7	1	53	-10	5
	4	8	-7	13	-13	-5
	11	5	25	22	-6	5
	18	46	26	-5	8	10
	25	31	23	9	5	8
February 2000	1	-7	-1	25	-9	-2
	2	2	-4	40	-11	1
	9	12	6	24	-10	2
	16	29	32	20	-12	2
	23	-3	21	6	-15	-5
	29	38	-12	-4	-18	-11
February 2001	1	-8	0	1	-5	-4
	5	-34	25	45	-12	-1
	12	3	-8	39	-9	1
	19	58	19	45	-16	3
	26	-34	16	54	-18	-5

Long-term simulation

In the 810-d simulation, volumetric water content was well described by *SWIMv1.1* at all depth layers other than 110 cm (Figure 7.12). Similar to the monthly simulations, drainage and/or evapotranspiration caused deviations between modelled and observed volumetric water content at times. Although the simulation followed the observed data trend in a winter wetting and summer drying pattern at all depths, volumetric water content was consistently underestimated by *SWIMv1.1* at 110-cm depth (Figure 7.13) resulting in substantially poorer *EF* values (Table 7.9).

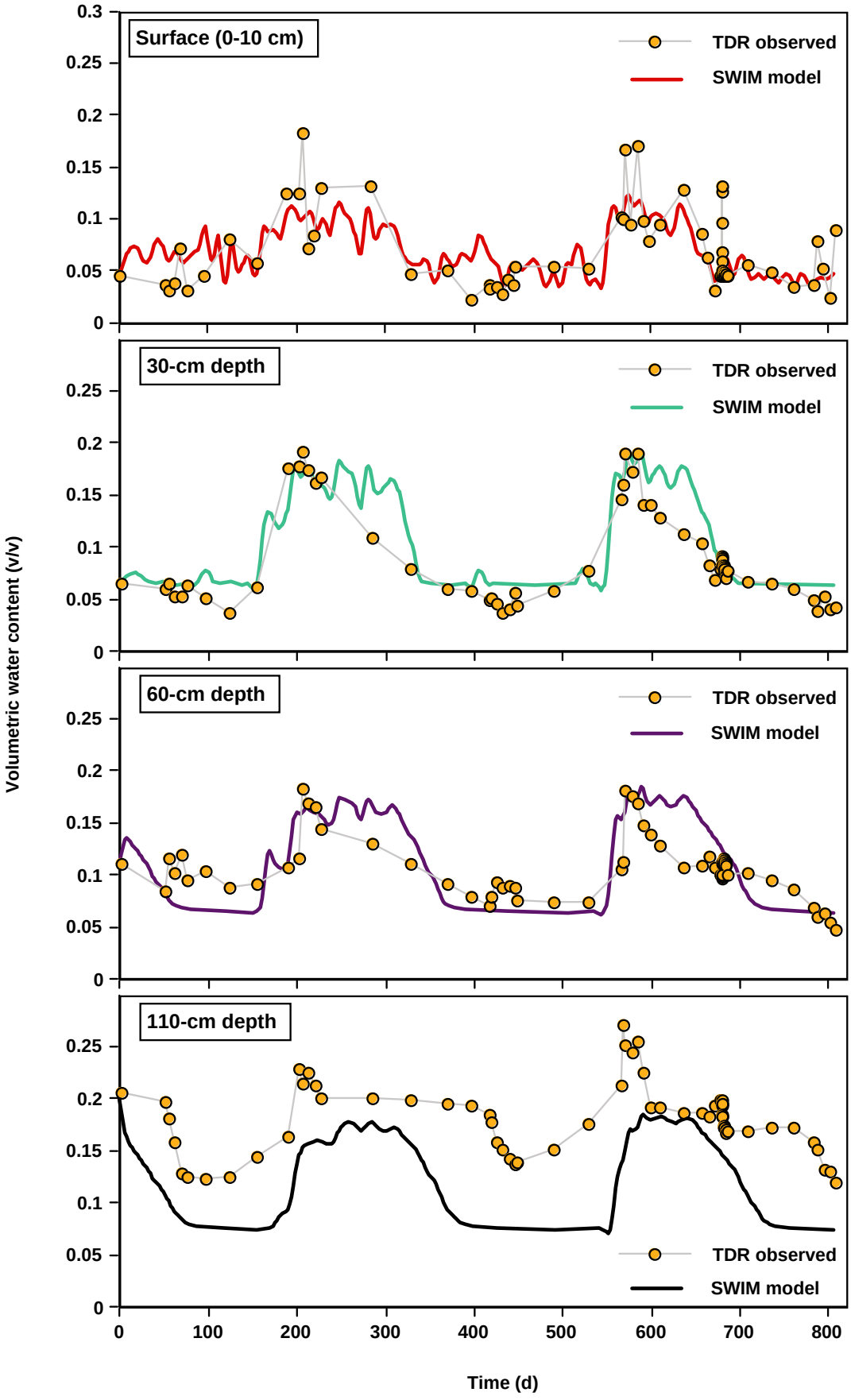

Figure 7.12. **Modelled and observed volumetric water content of different BRS profile layers through the entire 810-d time period of the deep-banded Mn field trial.**

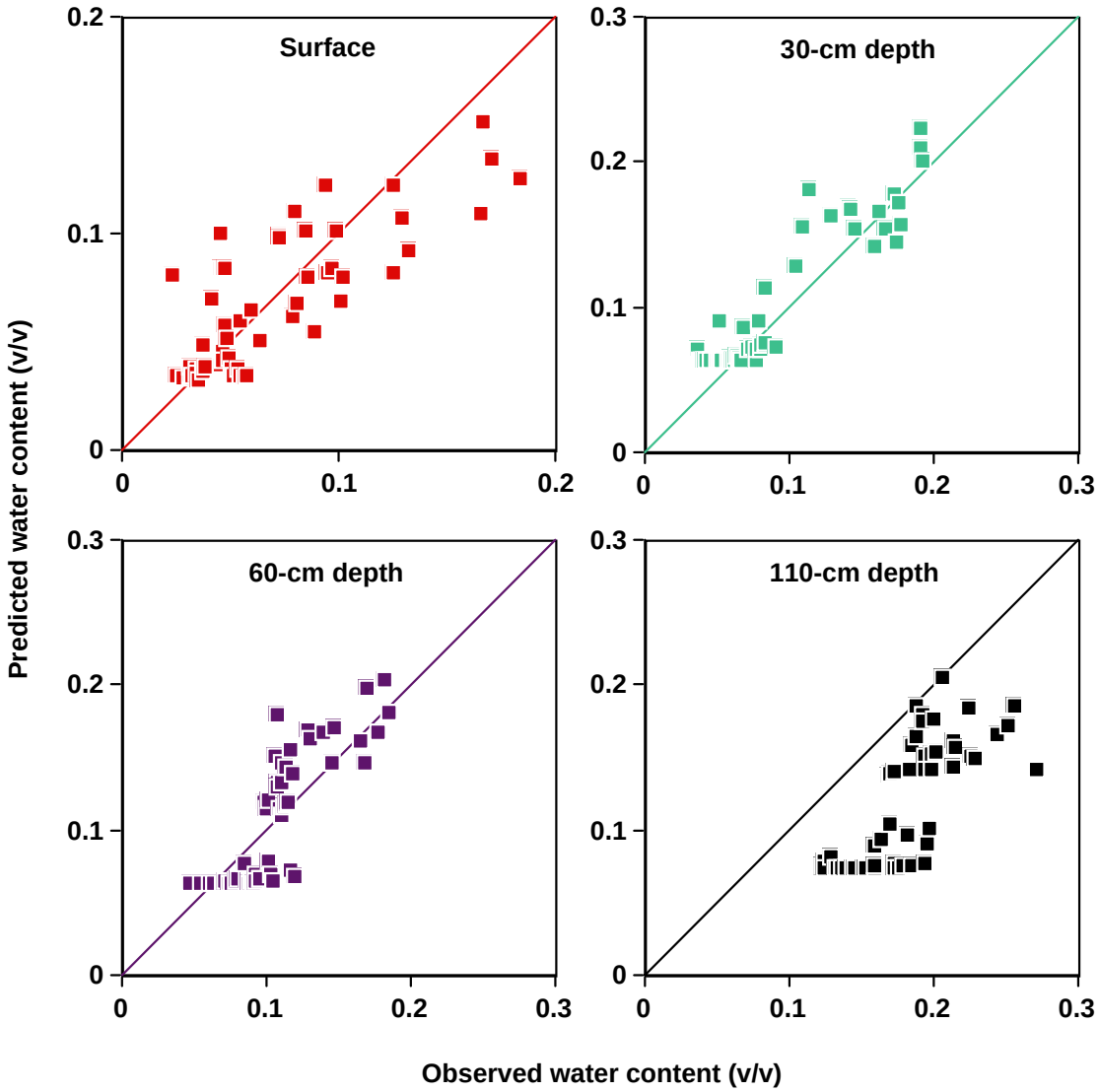

Figure 7.13. **Predicted versus observed volumetric water contents in different BRS profile layers throughout the field trial. Lines indicate 1:1 ratio.**

Table 7.9. **Residual error goodness-of-fit indices for modelled volumetric water content at specified layers and for total profile water to 135 cm for an 810-d simulation.**

	Index		
Depth (m)	*ME*[a]	*EF*[b]	*D*[c]
0-10	0.06	0.61	0.86
30	0.01	0.91	0.98
60	0.07	0.60	0.89
110	0.06	0.13	0.82
Profile water	56.38	0.41	0.88

[a] Maximum error
[b] Modelling efficiency
[c] Relative average error

See equations {7.17} {7.18} and {7.19}

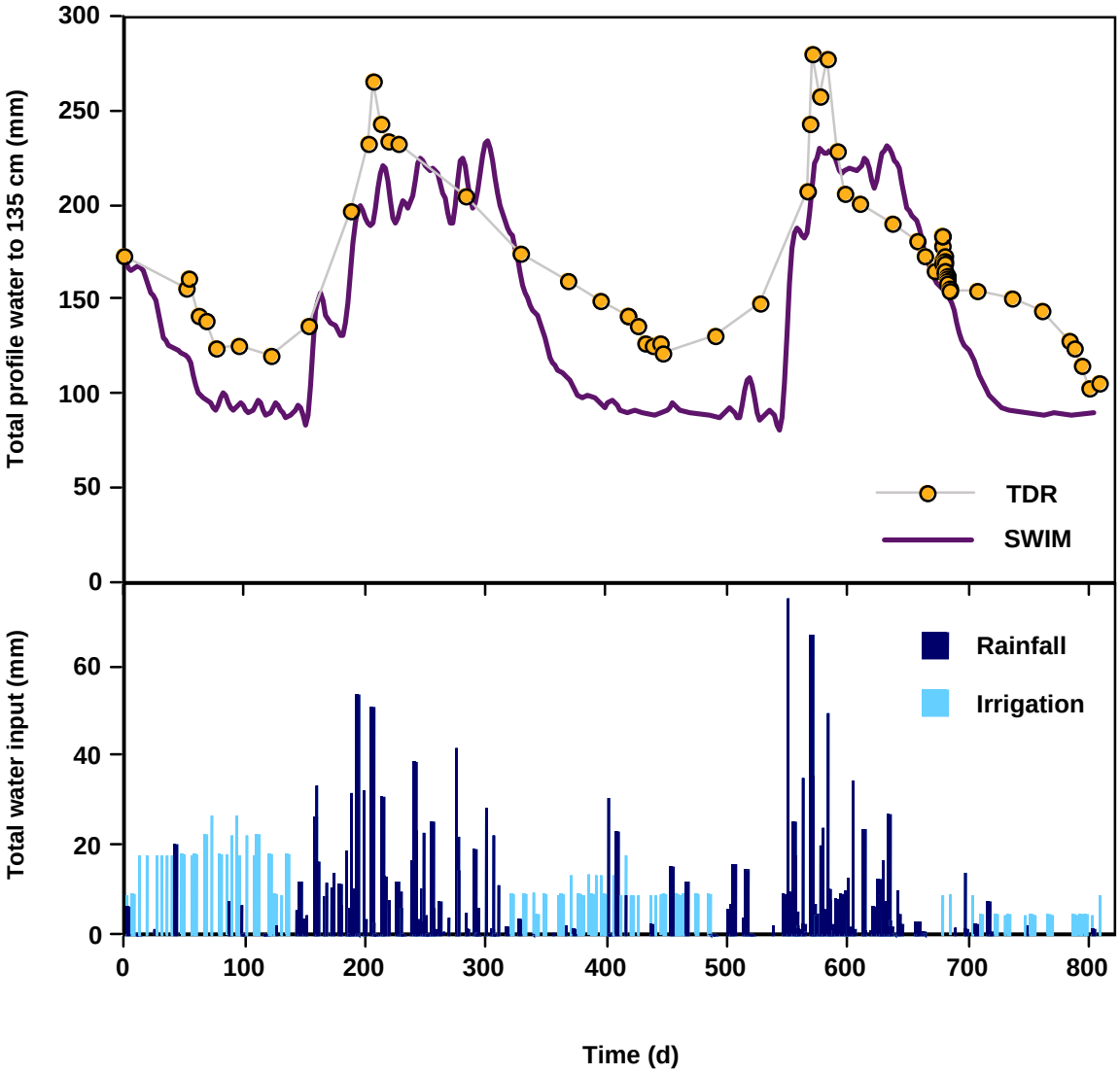

Figure 7.14. **Total profile water to 135 cm through the entire 810-d time period of the deep-banded Mn field trial.**

Underestimation at 110-cm depth influenced the long-term simulation of total profile water to 135 cm (Figures 7.14 and 7.15), but overall prediction by *SWIMv1.1* was reasonably reliable. Significant increases in total profile water followed season-breaking rains, and were maintained until the end of the rainfall period. Modelled profile water did not show notable evidence of a decrease in the minimum amount of stored water (around 90 mm) over consecutive low-rainfall periods as was seen in the monthly February simulations and TDR observed data. However, the minimum profile water was reached earlier in consecutive years (Figure 7.16). Additionally, fluctuations in predicted profile water decreased markedly with decreasing volumes of regular irrigation inputs over the consecutive low-rainfall periods (Figures 7.14 and 7.16).

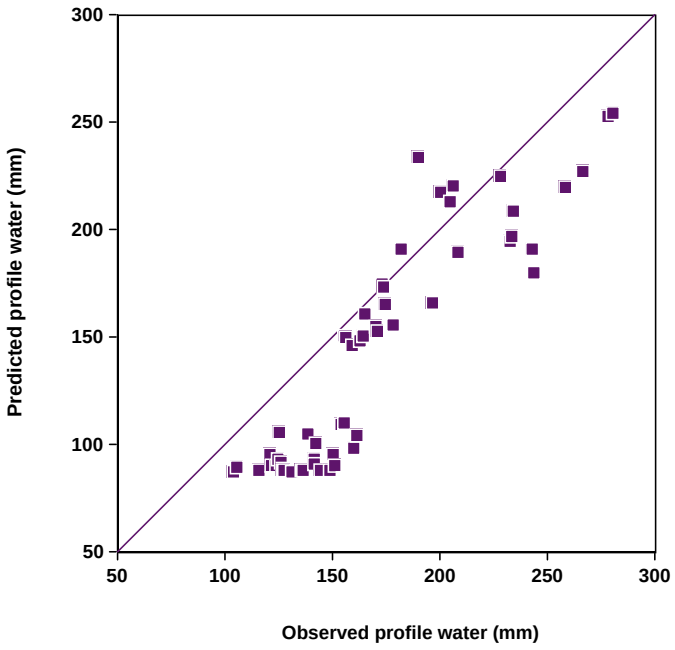

Figure 15. **Predicted versus observed data for total profile water to 135 cm throughout the field trial. Line indicates 1:1 ratio.**

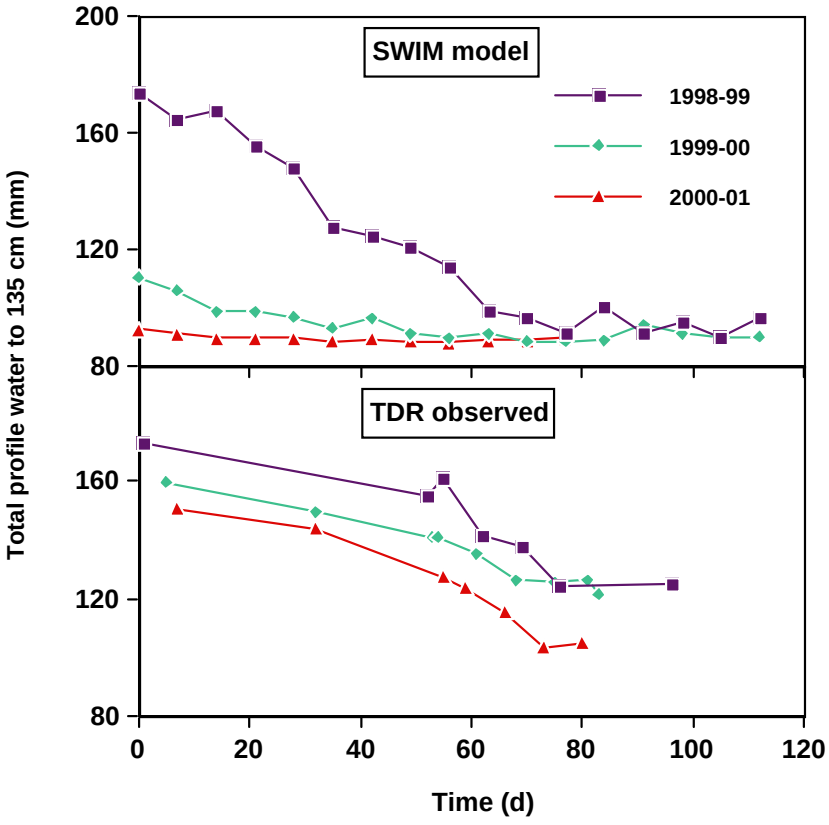

Figure 7.16. **Comparison of successive December to March periods of long-term simulation outputs.**

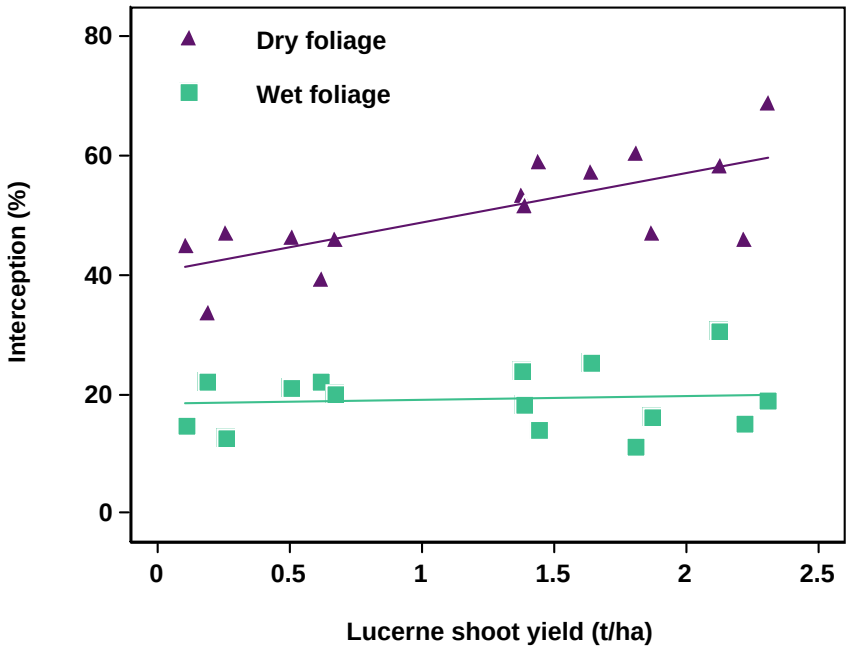

Figure 7.17. **Irrigation interception by dry and wet (20-min irrigated) lucerne canopies in relation to shoot yield. Values are means of fortnightly readings over 5 successive growth-harvest periods.**

CANOPY INTERCEPTION

A significant proportion of water input by sprinkler irrigation was intercepted by lucerne foliage (Figure 7.17). Dry foliage intercepted a greater percentage of irrigation water than that which was wet after having just received approximately 3 mm irrigation in 20 min. From 2 weeks to 6 weeks since the previous harvest, mean estimated lucerne yield increased from 0.2 to 2.3 t dry wt/ha and the percentage of irrigation water intercepted by dry foliage in 20 min increased from 43 to 62 %. In contrast, interception by wet foliage remained almost constant at approximately 20 % despite the increased biomass over time.

Plate 7.2. *Digging machinery assisted in access to the profile for taking soil cores and insertion of TDR probes.*

Plate 7.3. *Buriable TDR probes inserted in triplicate at each depth (30-cm depth shown). Note prevalence of pale green, Mn-deficient lucerne foliage.*

DISCUSSION

The van Genuchten model WRC parameters under the m=1-2/n restriction gave an excellent representation of observed draining water retention curve. The parameters demonstrate that, essentially, water retention of BRS is the same throughout the profile. Such consistency is expected in a profile where the matrix is constructed from a uniform substrate, as the slope of the WRC reflects the textural character of the material (Smettem *et al.* 1999). Likewise, strong similarities in water retention between profile layers at high matric potential reflect the identical base material of the different layers. At the high suction range, water is held by adsorption forces (Hillel 1971), and hence retention is a factor of BRS particle properties rather than profile structure and compaction.

Variation observed and modelled at the wet end of the WRCs from different depth layers is attributable to variations in bulk density. The bulk density of the surface layer was lower than in sub-surface layers (Table 7.5.). This is also reflected in the compaction/penetrability measurements at the site (Chapter V, Figure 5.3). Decreased bulk density and compaction is synonymous with increased total porosity, resulting in increased water-saturable volume of a profile layer. Cultivation during the deep placement of Mn fertiliser bands and sowing likely contributed to the open structure of the surface layer. In contrast, heavy vehicle traffic, especially during construction of the BRS deposit is the probable cause of compaction in the layer around 30-cm depth.

Saturated hydraulic conductivity at all depths was high, reflecting the highly porous nature of the profile. Whereas bulk density and compaction affected water retention somewhat, there was little tenable relationship to measured K_{sat} (Table 7.6). While the lowest numeric value of K_{sat} was observed at 30-cm depth, it was comparable to K_{sat} at the profile surface.

Water balance modelling was undertaken initially over one-month periods to determine the validity of the defined BRS hydraulic parameters. Additionally, the capability of *SWIMv1.1* to use these parameters in accurately simulating water content and flux in layers of the Pinjarra BRS profile was assessed. Although reasonable prediction was achieved in comparison to observed θ at the distinct profile layers (Figures 7.8 and 7.9), goodness-of-fit indices for sub-surface layers were generally poorer than those of the modelled surface layer (Table 7.7). Sub-surface volumetric water content is dependent on the θ, $\theta(\psi)$, K, $K(\theta)$ and evapotranspiration in the profile

above it. Therefore, errors in model estimation will compound as depth increases, as seen from the poorest model fit generally being found at the 110-cm depth layer.

Parameterisation of the θ, $\theta(\psi)$, K and $K(\theta)$ at more depths in the profile may improve accuracy in model simulation of volumetric water content, but will also be more time consuming. However, in the present case, and in soil-plant systems generally, the surface layer (0-10 cm) is perhaps the most important layer to accurately model as the majority of roots are present in this layer (Chapter IV Figure 4.3, Chapter V Figure 5.3). This applies especially in the BRS case because of difficulties encountered by roots in penetrating deep into the profile. Hence, determining availability of water and nutrients in this layer is important since it is the region of the majority of plant uptake. Prediction of both summer and winter volumetric water content in this surface BRS layer by *SWIMv1.1* was relatively accurate, and use of the BRS hydraulic parameters should provide a sufficiently robust model for predicting near-surface water content under various irrigation regimes.

The long-term simulations showed that total water in the profile was underestimated by *SWIMv1.1*, especially during summer months. That profile water over high rainfall winter months was accurately represented indicates that the model incorporation of evapotranspiration is the source of deviation from observed values over summer rather than an overestimation of drainage that is dependent on BRS hydrological characteristics. Spatial variations in lucerne plant density at the Pinjarra site, decreased growth activity as a result of Mn-deficiency through summer in –Mn plots (Chapter V) and the limited ability of *SWIMv1.1* to simulate water uptake by plants may all have contributed to the underestimation of water in BRS. Nonetheless, both volumetric water content and total profile water trends were adequately predicted by SWIM over the trial period, and the presented hydrological characterisation could be applied in future prediction of water balance at the site.

Interestingly, decreasing the irrigation input during low-rainfall periods over consecutive years did not impact on the predicted minimum profile water (Figure 7.16). In contrast, the measured minimum profile water level did decrease over consecutive summers, but was still greater than the predicted minimum in each case. It is assumed that the minimum profile water level in model simulations corresponds with the point where further uptake by lucerne roots is precluded (ie. permanent wilting point). The fact that the measured BRS water did not reach this point under any of the three summer irrigation regimes suggests that the lowest irrigation rates used during the 2000-2001 summer should be sufficient to sustain lucerne growth, provided adequate winter rain is received. However, based solely on the present results, formulating a summer irrigation

regime without excess water being applied would be difficult without additional specific crop-based modelling. The potential for the water balance predictions and hydrological characterisation use in determining optimal irrigation regimes for lucerne growth on BRS will be discussed in the subsequent chapter.

It was anticipated that a degree of error in prediction of water content in BRS would stem from interception by the lucerne canopy. Indeed, the irrigation intercepted by the dry lucerne canopy dramatically affected water reaching the BRS profile surface (Figure 7.17), with the percentage intercepted increasing with increasing plant growth. Conversely, a wet lucerne canopy had little effect on irrigation reaching the BRS profile. Canopy interception has received little attention as a source of error in soil water balance studies of cropping systems. Reasons for this include the historical emphasis in agriculture on annual crops that are planted during periods of high rainfall and luxury water supply as opposed to perennial systems, and the considerable difficulties in integrating interception information into a modelling regime for predicting water balance. The latter reason is perhaps the greatest barrier. A preliminary attempt at incorporating canopy interception in water balance modelling of the Pinjarra BRS system was made during the present study, but the interception dynamics and interrelationship with other factors could not be appropriately described by the simple interception study and did not improve the fit or modelling efficiency by *SWIMv1.1*.

Canopy interception depends on many factors including foliage morphology, canopy structure, biomass and density (Figure 7.17), environmental considerations (eg. wind), water droplet size and intensity, and the effect of canopy-intercepted water on plant transpiration and stomatal activity, as well as micro-environmental changes in relative humidity affecting evaporation at the profile surface. Consideration of canopy interception and further development in assessing the role of canopy interception in affecting profile water, especially in perennial systems, may allow increased precision in prognostic simulations of water balance and availability for vegetation on BRS and soil in general.

In addition to the lack of characterisation of canopy interception, *SWIM* lacks incorporation of a detailed crop model to simulate effects of infiltration on crop growth. Additional models such as *APSIM* (Agricultural Production Systems Simulator, McCown *et al.* 1996), in combination with a *SWIM* module for water balance simulation, have given reliable predictions of crop growth in Australian conditions (Connolly *et al.* 2001; Connolly *et al.* 2002). However, the most important factors in accurate simulation remain the detail with which the physical system is represented and accurate parameterisation of the model by simple methods of determining soil

hydrological characteristics. The present results demonstrate that parameterisation by independent, point measurement (eg. ponded rings, bore-hole permeameters and pressure plate-derived WRCs) allowed sufficiently accurate hydrological characterisation of BRS for *SWIMv1.1* to predict water balance with reasonable precision. Further precision may be gained through developing methods to better incorporate crop growth effects, and possible investigation of any effects of macroporosity, consolidation and/or crust dynamics in BRS that were not undertaken in this study.

CONCLUSION

The BRS profile at Pinjarra was comprehensively characterised using independent, point measurements in the field and laboratory. Using the determined BRS hydrological characteristics, numerical modelling by SWIMv1.1 provided a reasonable prediction of the water balance over short-term and long-term simulations, although underestimation was observed during low rainfall periods. Errors in water balance estimation are more likely to be associated with limited ability of *SWIMv1.1* to simulate plant factors in the BRS and hence produce overestimation of evapotranspiration. Further refinement and additional modules representing plant growth and water uptake may improve summer water balance prediction. The hydrological characterisation presented here provides an accurate starting point for optimising water usage schedules to maintain perennial pasture growth on a revegetated BRS site.

CHAPTER VIII

GENERAL DISCUSSION

Plate 8.1. *Well-nodulated root system of lucerne grown in solution culture with 500-nM manganese.*

GENERAL DISCUSSION

In simple terms, the theme of this thesis was an exploration of the three points of a complex relationship that I have affectionately termed a bizarre love triangle: manganese; plants; and water. When BRS is added as a substrate, the relationships form a tetrahedron (Figure 8.1) with each point directly connected to all other points in the framework. Although the points and sides of the tetrahedron were considered separately in the different chapters, only when considered as a whole will they lead to potential improved and sustained productivity in revegetation of BRS deposits. It is useful, then, to discuss some general conclusions here, as well as further work that could stem from the ideas and findings presented in this thesis.

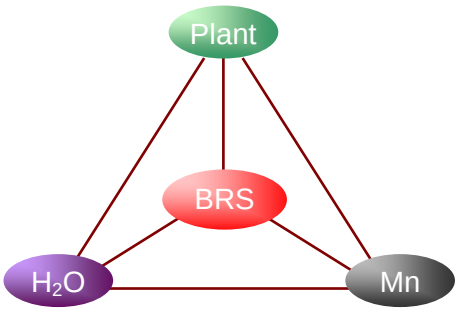

Figure 8.1. **Simplified graphical representation of the relationships explored in this thesis.**

In soil, Mn supply to plants is governed by (i) physical/positional availability, and (ii) chemical availability. However, these factors are a product of numerous and often complex interactions between plants, soil solid phase, nutrient ions, non-nutrient ions, water and microorganisms within the soil. This project has demonstrated that the case is the same for soil-like systems, such as BRS, with extreme physical and chemical conditions resulting in exceedingly poor Mn supply to plants used for revegetation.

Understanding the processes governing nutrient availability for plant uptake has wide-ranging environmental and agricultural implications. The current scenario of high inputs of Mn fertiliser and water with low residual value in revegetated BRS at Pinjarra is clearly undesirable. In the present study, the immense capacity for the rapid transformation of Mn to forms unavailable for plant uptake was demonstrated (Chapter II Gherardi and Rengel 2001). From this study, there is little doubt that such rapid transformations are largely responsible for the poor residual value observed after

broadcast Mn fertiliser applications to BRS revegetation areas in the past, but the relative extent to which the transformations may be attributed to chemical and/or biological processes is not entirely clear.

The pH of newly produced BRS (around 10 to12) is well above the minimum where chemical oxidation of Mn^{2+} will occur (8.5 to 9, Reisenauer 1988). There is little doubt that this extreme pH represents the greatest hurdle for plant growth and Mn nutrition in BRS. Further investigation of methods to decrease the initial pH of BRS should be encouraged as transformations of Mn^{2+} to unavailable forms were much slower in the aged BRS with lower pH compare to the fresh substrate (Chapter II, see also Gherardi and Rengel 2001). Novel methods for pH neutralisation such as leaching with seawater show some promise (Somes *et al.* 1998), but questions remain as to the effect of residual salinity on plant growth.

This thesis placed an emphasis on elucidation of chemical and physical factors affecting Mn availability, with particular reference to BRS revegetation, but the importance of microbial ecology in relation to plant-available Mn has also been alluded to throughout. Although in-depth exploration of this area was beyond the scope of the present project, experimental inoculation of BRS with genera of Mn-oxidising and Mn-reducing microbes would make for interesting and perhaps worthwhile study. Potential benefits of soil and plant inoculation with Mn-reducing bacteria have been demonstrated elsewhere in agriculture (Wilhelm *et al.* 1987; Marschner *et al.* 1991; Huber and McCay-Buis 1993). Microbial remediation of bauxite residue was considered highly feasible by Mussell *et al.* (1993), but the theoretical basis of their report focussed on pH decrease through bacterial acid production alone. While such a pH decrease may indirectly improve Mn availability, the direct action of microbes and their excretions may be of equal or greater importance to Mn nutrition of revegetated BRS. The potentially positive effect of adding Mn reducers to BRS would hinge on repeated applications of Mn fertiliser in the first stage to create appreciable levels of Mn oxide that these microbes can act upon. Prior to additions of Mn fertiliser to freshly produced BRS, little Mn oxide will exist and hence little effect of Mn reducers can be expected. No publication of further studies stemming from the feasibility report (Mussel *et al.* 1993) are apparent in the literature, exposing the opportunity for a comprehensive microbiological study of pH and nutrient availability in BRS.

Ameliorative additions may, with further research and refinement, produce more effective methods to make BRS more hospitable as a substrate for plant growth, but the fact remains that Mn addition will still be required. The challenge, then, is to gain as much value as possible from those applications of Mn. This thesis took a number of

approaches in addressing this challenge. The first approach was selection of suitable genotypes of plants to be used for revegetation.

As a species, lucerne has a number of characteristics that make it a suitable choice for revegetation of BRS (see Chapter I). By screening a number of genotypes for their ability to grow with low and adequate Mn additions, a wide range of growth rates were observed. The use of genotypes tolerant to Mn deficiency is a technique that has enjoyed success in agriculture on soils where Mn deficiency is prevalent (eg. Bansal *et al.* 1991; Graham *et al.* 1994; Krahmer and Sattelmacher 1995; Khabaz–Saberi *et al.* 1999). There should be no reason why similar selection of Mn-efficient lucerne genotypes would not be advantageous in BRS revegetation. Genotypes such as Salado should maintain greater productivity per unit of Mn fertiliser addition than Mn-inefficient genotypes, but field-based assessment over several harvests is warranted. Field assessment will ascertain whether the tolerance to Mn deficiency observed in the glasshouse (Chapter III) will also be maintained under conditions of greater substrate volume for root expansion and infrequent irrigation/rainfall regimes, especially over successive harvests in this perennial species.

The use of genotypes tolerant to Mn deficiency such as Salado in breeding programs may lead to wider spread of Mn efficiency in lucerne germplasm, as the trait appears to be heritable with major dominant genes involved (Graham 1988). Another field with significant potential for improving Mn-deficiency tolerance in plants is genetic manipulation. Recent advances in engineering lucerne plants for enhanced synthesis and secretion of organic acids from roots have proven highly promising in conferring tolerance of Al toxicity in acid soil (Tesfaye *et al.* 2001). Not only does the chemistry of the rhizosphere change through enhanced exudation, but ribosomal DNA studies have also found qualitative changes in the abundance of bacterial phylogenetic groups between rhizosphere soils of transgenic and untransformed lucerne (Samac *et al.* 2002). The same study also found nitric acid extractable Mn to increase significantly in the rhizosphere of transgenic lucerne plants, along with P, K, Zn and Cu, compared to the rhizosphere of untransformed lucerne plants. Considering the importance of organic acid exudation and rhizosphere biology to Mn nutrition of plants, selection and engineering of plants for increased root exudation activity is worthy of further research attention.

A popularly touted reason for differential genotypic tolerance to Mn deficiency is the amount of carboxylate exudation, in particular low-molecular-weight organic acids and phenolics, from roots of a genotype under conditions of low available Mn (Marschner 1988; Huang *et al.* 1994; Marschner 1995; Rengel 2000, 2001; Dakora and

Phillips 2002). Carboxylate exudation by lucerne was investigated with different levels of Mn addition (Chapter VI). Carboxylate exudation increased with decreasing Mn supply, and the lucerne genotype tolerant to Mn deficiency (Salado) released more carboxylates at low Mn supply than the sensitive genotype (Sirosal). This suggests a role for differential exudate production in determining differential tolerance to Mn deficiency in Salado and Sirosal lucerne. However, Mn uptake and plant concentration results (Chapters III, IV and VI) confound this conclusion somewhat. In BRS, similar or higher Mn concentrations were found in Sirosal compared to Salado (Figures 3.7 and 4.4), though this may be at least partly due to a dilution effect in Salado because of greater growth. In nutrient solution, increased exudation and fine root production by Salado did not improve Mn uptake compared to Sirosal (Tables 6.2 and 6.3) because no Mn was present in an unavailable form. Yet, Salado growth was consistently greater than that of Sirosal under low Mn conditions in BRS and nutrient solution, and Salado showed a significantly lower critical Mn concentration than Sirosal. Differential efficiency of internal Mn utilisation by lucerne may therefore be as important, or indeed more important, than carboxylate release as a determinant of tolerance to Mn deficiency. This appears to contrast evidence arising from studies of other species (predominantly cereals, see Graham 1988) where internal requirement or utilisation efficiency did not influence, or was not a consequence of, tolerance to Mn deficiency because tolerant varieties showed higher critical Mn concentrations. Similar to the present results for Mn-deficiency tolerance, Grewal and Williams (1999) found differential Zn-deficiency tolerance in lucerne genotypes to also have some relationship with internal requirement and/or utilisation efficiency. Internal micronutrient utilisation studies of lucerne genotypes may provide further insight into the unique nature of this species.

Comprehensive conclusions about the role of root exudates in Mn availability, uptake and the Mn nutrition and Mn status of plants will only be confidently realised from studies in soils. There are, however, a number of confounding issues in soil-based exudation studies that make such experiments and results difficult to interpret. Soil microbes may rapidly consume, metabolise or degrade exuded compounds (Bromfield and David 1976; Whipps and Lynch 1983; Tinker 1984; Ghiorse 1988; Lynch and Whipps 1990; Dakora and Phillips 2002) making them undetectable in the rhizosphere. Microbes themselves exude compounds into the rhizosphere that affect plant uptake of Mn (Barber and Lee 1974). Physical rupturing and lysis of root cells through sampling technique and sloughing off due to contact with soil particles may occur, increasing the apparent release of compounds (Rovira 1969). The success of alternate sampling

techniques (eg. applied suction to extract soil solution, centrifugation, ion-exchange resins etc.) is dependent on a number of factors including rapid microbial metabolism, chemical reactions and transformations, formation of complexes and the strength of adsorption on soil particulates. Unless these problems can be sufficiently overcome, the theory of mechanisms for root exudate-mediated increases in soil-Mn availability will continue to rely on extrapolation from simplified axenic or semi-axenic systems such as nutrient solution experiments.

The approach of considering placement of fertiliser in a specific position as often used in agricultural systems is another concept that could well be integrated into BRS revegetation to beneficial effect. Advantages of banding Mn fertiliser in the soil profile have been demonstrated in a wide range of crops and soils (eg. Mortvedt and Giordano 1975; Randall *et al.* 1975; Alley *et al.* 1978; Mascagni and Cox 1984; Brennan 1993, 1999; Crabtree 1999; Brennan 2001a). It was hypothesised that similar advantages may be conveyed to revegetated BRS by deep banding Mn within the BRS profile. The glasshouse study (Chapter IV) demonstrated that under non-limiting irrigation conditions, deep banding of Mn in BRS was more effective than a representative broadcast-incorporated system up to a single lucerne harvest. In the field (Chapter V), the deep banding result from the glasshouse study was unequivocally confirmed; over a 2.5-year period an average 15 % increase in lucerne productivity was achieved by deep-banding over broadcast Mn application. Perhaps more intriguing was the fact that this productivity increase was achieved with an 80 % decrease in total Mn applied over the same period. Even with a moderate increase in application cost for a deep-banding procedure compared to a single broadcast one, the long residual effectiveness of deep-banded Mn will be more economically and environmentally favourable in terms of productivity per unit resource input. Improving residual effectiveness of Mn fertiliser was one of the key aims of the study. Given the present results, broad-scale adoption of deep banding of Mn in BRS revegetation programs is strongly recommended.

Deep banding of Mn for BRS revegetation may be further refined by field investigation of optimal banding depth. Benefits of deep-placed fertiliser bands may be offset by the lower number of roots generally found at depth (eg. Barber and McKay 1986; Gäth *et al.* 1989) or the inability of a species to effectively reach the zone of banding. For growth of lucerne to a first harvest in the glasshouse (Chapter IV), banding at 10-cm depth was more beneficial than the placement at 20-cm depth due to early Mn deficiency prior to roots reaching the deeper-placed Mn band. As lucerne seed is small, seed-Mn reserves are small (see Chapter III, Table 3.1). In substrates that provide

adverse conditions for root growth these reserves may be insufficient to produce good early root growth and thus sustain adequate profile exploration to reach the fertiliser band. In the field, however, Mn banded near 20-cm depth resulted in no early Mn deficiency in lucerne. This was attributed to years of broadcast and incorporated Mn in the near-surface layer being repositioned in a channel above the Mn band during a pass of the deep banding machinery tines, combined with provision of an easily root-penetrable channel by the cultivation. Such guidance of root growth is seen in species other than lucerne. For example, rip lines have been found to encourage deep root proliferation in native vegetation areas of revegetated BRS at Pinjarra (Plate 8.2). This suggests that an initial broadcast-incorporated Mn application may be beneficial in combination with Mn band placement deeper than 10 cm. The broadcast-incorporated Mn will provide Mn supply for early growth, while the deep-banded Mn will provide residual Mn supply over the longer term after sufficient root proliferation occurs at the banding depth.

In addition to combination banded+broadcast Mn applications, incorporation of alternate products in a deep-placed band may also be beneficial in revegetating BRS areas. Positive responses of root proliferation and nutritional status have been found when P was banded in soil (Baeumer and Bakermans 1973; Drew and Saker 1978). Although Mn itself encourages root proliferation close to the banding zone (Chapters IV and V), P may have an additive effect. The effect of increased root proliferation in improving Mn uptake will stem from increased exudation of compounds that lead to greater availability of Mn to plants (Chapter VI) and, subsequently, greater interception of the available Mn. Addition of ammonium-based fertilisers to a Mn band may also increase Mn availability through acidification as has been demonstrated elsewhere (eg. Mascagni and Cox 1984; Modaihsh *et al.* 1989; Moraghan and Mascagni 1991). The ability of pH of BRS to 'bounce back' after a drop due to addition of concentrated acid (DeSantis 1997; Somes *et al.* 1998) may counteract the effect of an acidifying supplement. Nonetheless, deep-banding of other fertiliser sources and acidifying products either separate from, or in combination with, Mn should be trialled because the residual effectiveness of the acidifying agent may increase in the same manner as banded Mn fertiliser alone (Chapter V).

Optimal field banding depth will also be influenced by dynamics of soil-water or, in the present case, BRS-water content. Water provides the medium for nutrient dissolution, ion transfer and microbial growth. In the glasshouse, under non-limiting water conditions, Mn uptake from bands at 2.5- and 10-cm depths was adequate for

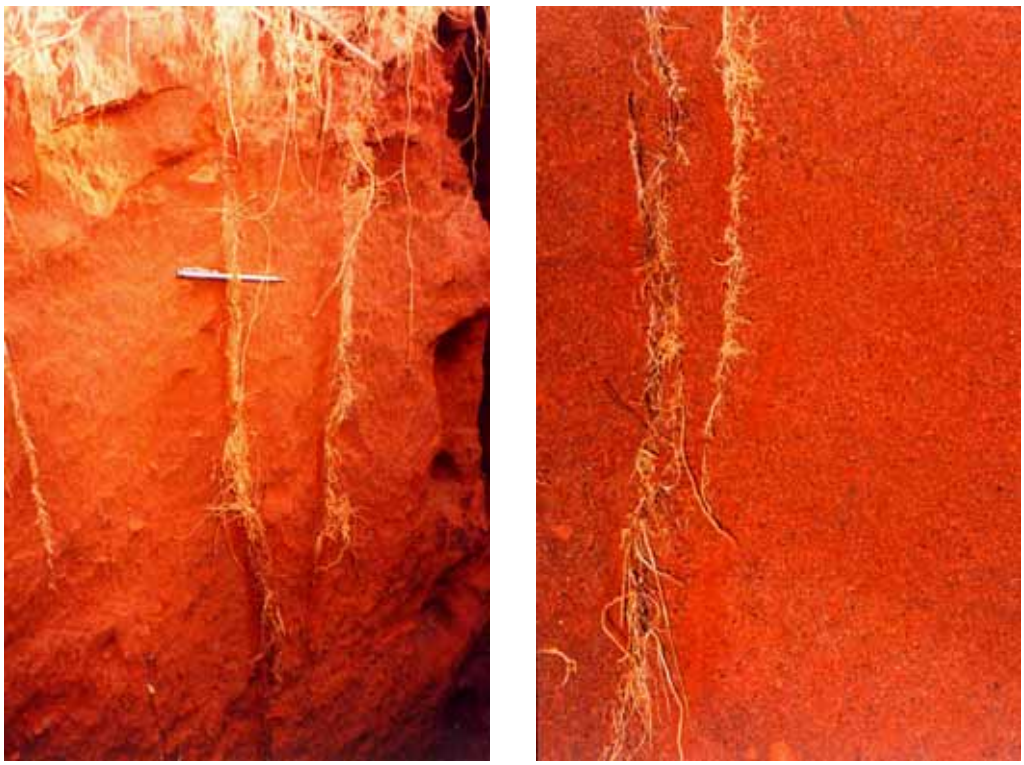

Plate 8.2 *The effect of rip lines on native vegetation root growth in revegetated bauxite residue deposits.*

healthy lucerne growth (Chapter IV). However, observation and modelling of the Pinjarra field site (Chapter VII) showed greater fluctuation in water content of the 0 – 10 cm layer and generally lower volumetric water content than in deeper profile layers (eg. Figure 7.12). In addition to increased water content, horizontal proliferation and localised aggregation of root growth above a compacted layer at about 30-cm depth (Figure 5.3) likely contributed to a banding depth of close to 20 cm being so successful at Pinjarra. Similar subsurface compaction layers are commonly found in BRS areas (Alcoa World Alumina Australia, unpublished). Without ripping below 30-cm depth, extensive growth of roots beyond this depth will not occur and banding below about 20 cm will not be possible, hence 20 cm may in fact be the optimal banding depth for Mn in the revegetated BRS system.

While increased water content and, therefore, available water was found with increasing depth in the Pinjarra BRS profile (Chapter VII), the highly porous substrate structure remains subject to rapid drainage. Hence, the margin between optimal irrigation for lucerne productivity on BRS and either excessive watering that will be wasted as through-drainage or inadequate water to maintain productive growth is small. Gradual restriction of groundwater use for irrigation means that optimising irrigation regimes for minimal wastage should be sought in BRS revegetation. With such small

error margins, predictive modelling of water retention and movement in the BRS profile will need to be extremely reliable if they are to provide a basis for formulating irrigation regimes in BRS systems.

The difference between modelled and observed total profile water during low rainfall months in the log-term simulation (Chapter VII) suggests that a decrease in irrigation during these months may be feasible, especially closely following the high rainfall period. However, a closer look at the distinct layer simulations (Figure 7.12) reveals that upper layers regularly approach or reach dryness where water uptake by roots is theoretically precluded (permanent wilting point), suggesting greatly decreased productivity without further significant irrigation input. This exemplifies the need for specific crop-based modelling in formulating irrigation regimes as mentioned in Chapter VII. Models that take into account additional factors such as the degree of drought tolerance of lucerne and responses in root growth to gradients of water availability through the profile (ie. increased water availability at depth encouraging deeper root growth, Janson 1975; Hoffman *et al.* 2001) will provide greater confidence in recommendations for irrigation management. At present, methods for parameterising and applying detailed infiltration models in a cropping system framework are not well developed, and further combination of crop growth and water movement models (eg. the approach of Connolly *et al.* 2001; Connolly *et al.* 2002) is encouraged.

It is clear from the lucerne growth data (Chapter V) and irrigation data (Chapter VII) that a decrease in irrigation over low-rainfall periods of consecutive years did not result in a significant yield decrease in lucerne. Until further refinement from water-balance modelling predictions is available, maintaining irrigation at the levels used in summer 2000-2001 should be encouraged. This will result in significant savings in water usage compared to previous summer regimes without compromising growth of lucerne on the revegetated BRS areas. In addition, decreased plant density will allow further decreases in irrigation requirement through lower transpiration and summer water demand. The current practice of sowing lucerne seed at 30 kg/ha is well above recommended rates for both irrigated and dryland lucerne (irrigated 3-10 kg/ha, dryland 12-15 kg/ha, Evans and Richardson 1994b).

CONCLUSION

Manganese availability to plants growing in BRS is poor, even in aged BRS which has had ameliorative additions and several years of leaching rains and irrigation. However, this thesis has identified a number of steps that may be taken to improve the long-term

productivity of BRS revegetation whilst providing associated environmental and economic benefits. The use of acidifying, deep-rooted perennial legumes such as lucerne should be continued, but genotype selection for tolerance to Mn deficient conditions in BRS and high rates of carboxylate exudation should be encouraged. Incorporating deep-banded Mn into the BRS fertiliser regime will decrease the need for frequent and large broadcast Mn applications that, as seen previously, are neither cost-effective nor beneficial to lucerne productivity in the long-term. Optimising irrigation for minimal wastage of groundwater resources through the use of predictive models will also enhance the short- and long-term environmental effectiveness of a revegetated BRS system. The goal of effective and sustained pasture revegetation of BRS with minimal resource input is dependent on a complex series of factors and relationships. By improved management of manganese and water following the methods investigated in the present thesis, this goal may be achieved.

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