Responses of accessions of *Austrodanthonia* spp. to factors associated with soil acidity

by

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Certificate of Originality

The work presented in this thesis is my own, except where otherwise acknowledged. No other person’s work has been used without due acknowledgment in the main text of the thesis. The investigations presented here have not been submitted for the award of any degree or diploma within the University of Sydney or elsewhere.

Mohammed Anowarul Islam

August 7, 2002

Dedication

I proudly dedicate this work to my beloved wife, Jesmin Banu and my lovely daughter Alhena Islam, whose strength and love sustain me; and to the memory of my parents, Abed and Anowara, who sacrificed their lives raising and educating their children, but did not have the chance to see the completion of the long desired work described in this thesis.

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Abstract

Pasture plants already adapted to acidic soil conditions are required as part of an integrated approach (with lime amelioration) to managing acid soils on the Tablelands of New South Wales, Australia. The objective of this thesis is to evaluate the usefulness of *Austrodanthonia* species for this purpose. The material evaluated in this study was collected during a previous survey of the distribution of *Austrodanthonia* on the Central, Southern and Monaro Tablelands of New South Wales. It was hypothesised that the genus *Austrodanthonia* has a wide range of tolerance to acid soils.

A series of experiments that provided information on the growth and physiology of *Austrodanthonia* in relation to soil acidity, with a view to the identification and eventual domestication of the most promising plant material have been conducted through pot, hydroponics and field investigations.

Firstly, soils were acidified or limed to obtain a range of soil pH and Al concentrations. This experiment showed that adding aluminium sulfate and calcium carbonate followed by washing excess salts with water is a simple, rapid and convenient method for adjusting soil pH for pot experiments. The pH of the amended soils remained relatively unchanged eight months after treatment. The experimental set-up also resulted in a wide range of soluble Al (2-52 mg/kg) across the soils.

The relative Al-tolerance of 183 accessions from 15 *Austrodanthonia* species was tested in a pot experiment using a range of soil pH. Emergence, survival and growth of all accessions were drastically reduced by high soil acidity (pH 3.9, $P < 0.001$). About 11% of plants emerged at pH 3.9, whereas at pH 4.4 and 5.3, ~72% of plants emerged.

Accessions exhibited large variation within and between species in their tolerance to soil acidity. From the species/accessions tested, 49 accessions from eight species were selected for further study (on the basis of being more acid tolerant).

Hydroponic experiments conducted in the glasshouse evaluated: (i) formulation of nutrient solution with a stable pH, (ii) effectiveness of the formulation using tap water and deionised water and (iii) estimation of free ion activities of Al and Mn in the nutrient solution and their effects on *Austrodanthonia* growth. These experiments showed that a NO_3-N/NH_4-N ratio of 9:4 is the most appropriate ratio to obtain a stable pH 4.0 without affecting plant growth; that there was little difference between tap water and deionised water on the ionic effects of Al and Mn, and plant-size did not play a role on accession survival and that accessions of *Austrodanthonia* could grow well within a wide range of pH (3.5-5.5), Al (50-250 µM) and Mn (100-2000 µM). Growth of *Austrodanthonia* accessions declined under high acidity (pH < 3.5) and Al (300 µM), but tolerated high concentrations of Mn (2000 µM).

Root-tips stained with hematoxylin grouped accessions in a similar way to the pot and hydroponic experiments for most of the accessions tested. The intensity of root staining with hematoxylin and the differential distribution of Al in the shoots and roots provided an indication that different tolerance mechanisms may be involved with *Austrodanthonia* accessions. It appears that both exclusion and internal mechanisms may operate for Al- and Mn-tolerance.

A field experiment was conducted at Carcoar ($33^0$37’S, $149^0$13’E, elevation 800 m) using gradients in soil pH and Al available on-site to grow selected accessions of *Austrodanthonia*. The accessions exhibited a range of responses to soil acidity. The

accession responses to acidity from the pot and hydroponic experiments were similar to those obtained in the field, especially where Al was present as a low Al-challenge.

Overall, this study shows that *Austrodanthonia* exhibits a wide range of acid tolerance between species and accessions within species. Among the species tested, *A. duttoniana* and *A. fulva* appeared to have the greatest commercial potential, because of their productivity and acid tolerance. The variability that exists in the accessions may be exploitable in breeding and selection programs for improved cultivars.

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Chapter One

General introduction

The soils of the Tablelands of New South Wales are shallow and of relatively low fertility. In these soils, pasture production is often limited by soil acidity (Simpson and Langford 1996) and the area of acidic soils is expanding (Fig. 1.1, Helyar *et al*. 1990; Fenton *et al*. 1996). Soil acidity changes the availability of some metal cations, in particular Al and Mn, resulting in concentrations that are toxic to many plants. Individual species of pasture plants, and cultivars within species, may differ widely in their tolerance to Al and Mn toxicity (Helyar and Conyers 1994). Farmers have tried to improve productivity by using introduced pasture species. In many cases, poor adaptation to these difficult soil conditions by the introduced species has resulted in low persistence and, may have contributed to further soil degradation. Some introduced pasture species may remain, with the balance of the pasture comprising both desirable (e.g. *Austrodanthonia* spp., *Microlaena stipoides, Elymus scaber*) and undesirable (e.g. *Aristida ramosa*) native perennial grasses, and annual grasses (e.g. *Vulpia* spp., *Bromus molliformis*) (Kemp and Dowling 1991). The deliberate introduction of exotic species may reduce species diversity in established pasture systems (Garden *et al*. 1996). A declining perennial base with a predominant annual population, especially after unsuccessful attempts at pasture improvement, would be further expected to continue the downward trend of soil pH (Helyar 1976; Duncan and Crocker 1998; Li *et al*. 2001).

Increased long-term productivity on acid soils can be achieved by raising soil pH using lime applications, making better use of the tolerant perennial species already

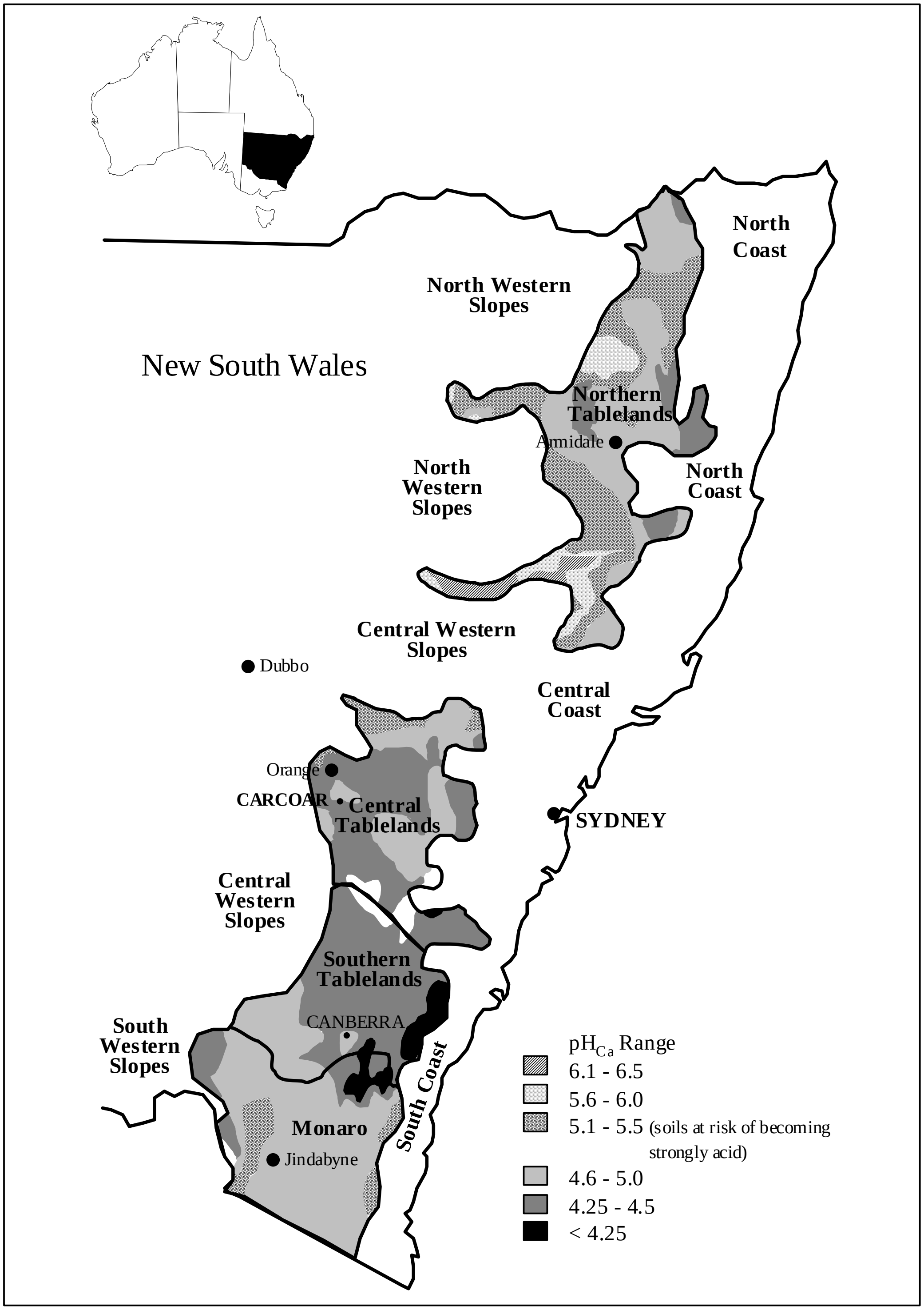

Fig. 1.1. Acid soils and soils at risk of becoming strongly acid in the Central, Monaro, Northern and Southern Tablelands of New South Wales.

Source: Helyar *et al*. (1990); Fenton *et al*. (1996).

present (Davidson 1987), or adopting both practices. Although liming is an effective method of correcting soil pH, the cost and other factors, such as sub-soil acidity, topography and nutrient availability may limit the benefit of liming (Cregan and Scott 1999). The Tablelands of NSW include large swathes of rolling to hilly country (Simpson and Langford 1996) that are difficult to access with ground equipment. This means that for a significant proportion of the tablelands, the potential productivity increases due to liming are not attainable. This creates a need to find alternative ways of dealing with acid soils as part of an integrated approach to the problem.

Use of plants adapted to acid soil conditions is one option that may overcome the problem. An important research activity relating to such an approach is to identify plant species and genotypes that are tolerant to high concentrations of soil Al and Mn (Foy 1988; Scott and Fisher 1989; Helyar and Conyers 1994). There is evidence in the literature that species and genotypes among the crops and pastures vary in their tolerance to acid soils. For example, Helyar and Conyers (1994) found *Microlaena stipoides*, *Themeda* spp., *Dactylis glomerata* cv Gr. Wana to be highly tolerant of soil acidity. Similarly, *Austrodanthonia* spp. are also generally considered as acid tolerant, though preliminary information suggests that there is a wide range of responses to pH, both inter-specific (Dowling *et al.* 1996) and intra-specific (Rubzen *et al.* 1996). If the accessions of these species which show tolerance to low soil pH could be identified and favourable agronomic characteristics retained, then introducing or increasing these preferred accessions on acid soils provides another avenue for addressing acidity on less accessible lands. Competitiveness would be one of these desired characteristics, since the introduction of any selected accession would be onto a sub-optimally prepared seedbed.

About 150 species of *Danthonia* are known in many areas of temperate and sub-tropical parts of the world (Wheeler *et al*. 1990) and many of the species are recognised as valuable fodder grasses (Abele 1959; Archer and Robinson 1988; Robinson and Archer 1988; Dowling *et al*. 1996; Mitchell 1996; Garden *et al*. 2001a). Following Linder's revision of the genus *Danthonia*, about 30 species of *Danthonia* including most Australian species were placed in the genus *Austrodanthonia* (Wheeler *et al*. 2002). In Australia, the *Austrodanthonia* (formerly *Danthonia*) genus (Linder 1997) is regarded as an excellent source of forage by landholders. However, landholders generally think of *Austrodanthonia* as a single species, when in fact there are about 26 species in Australia and 19 in NSW alone (Wheeler *et al*. 2002). These species may differ in tolerance and response to soil acidity and fertility (Dowling *et al.* 1996, Garden *et al.* 2001a). Two species prominent on the Northern Tablelands and North-West Slopes of NSW (*Austrodanthonia bipartita* (synonym *Danthonia linkii*) and *A. richardsonii*) have been subjected to a domestication program (Lodge 1996; Lodge and Sutherland 1996). Selected cultivars of these species are now commercially available for forage and also for land rehabilitation. However, in a survey of the Central, Southern and Monaro Tablelands (Dowling *et al.* 1996), these species were present on less than 4 % of the 126 sites sampled, and then only on soils of higher pH. The low natural frequency of these two species in the survey may mean that their respective domesticated cultivars will not as readily adapt to sites where they do not typically occur. Evaluation of these cultivars against other potentially useful *Austrodanthonia* species under conditions common to the tablelands may demonstrate their lack of suitability. The recent study of Garden *et al.* (2001a) suggests that there is a wide range of accessions of *Austrodanthonia* species, which allow them to adapt to a varying range of environmental conditions. It seems appropriate to explore this possibility further, especially where accessions of this genus have been selected for pastures (Lodge 1996).

Therefore it is hypothesised that performance and distribution of species and accessions of *Austrodanthonia* on acid soils may be related to their tolerance of acidity but little information is available on the adaptation of native species. Consequently, this project was undertaken to fulfil the following objectives:

1. to evaluate the relative influences of soil pH and soluble Al on growth of seedlings of 183 accessions of *Austrodanthonia*;
2. to examine the relative influence of acidity parameters on growth of a restricted range of *Austrodanthonia* accessions using nutrient culture techniques;
3. to identify *Austrodanthonia* species and accessions with a range of tolerance to pH, Al and Mn stresses;
4. to investigate, to a limited extent, what possible pH, Al and Mn tolerance mechanisms may be involved; and
5. to evaluate the tolerance of selected accessions under acid soil conditions in the field.

Although competitiveness is a desirable characteristic in pasture species, it is not addressed in this study.

Chapter Two

Review of literature

This review of literature is assembled under the following broad headings:

2.1 The soil acidity problem

2.2 Correcting soil acidity problems

2.3 Methods of studying plant tolerance to acidic soils

2.4 *Austrodanthonia* species

2.5 Conclusion and research opportunities

The review provides a detailed overview of the literature relevant to this study.

2.1 Soil acidity problem

Soil acidity is a serious agricultural and environmental problem (Cregan and Scott 1999) that limits the growth of pastures and crops in many parts of the world including Latin America, North America, Asia, Africa, Europe and Australia (McLean 1976; Adams 1979, 1981; Williams 1980; Clark 1982; Baligar *et al*. 1993; Bromfield *et al.* 1983a; Kamprath 1984; Helyar 1991; Rajaram *et al.* 1991; Eswaran *et al*. 1997; Duncan and Crocker 1998; Cregan and Scott 1999; Duncan 1999a; Tang *et al*. 2001).

Soil acidification is a natural process that is accelerated by the current production systems of pastures and crops. In southern temperate Australia, Williams and Donald (1957) had already observed a steady decline in the pH of soils under improved pastures. These pastures had been improved by applying superphosphate and growing subterranean clover (*Trifolium subterraneum* L.) as an N source. In Australia, excess acidity affects over 35 million ha of agricultural land and the area is expanding (Anon. 1995).

Cregan and Scott (1999) claim that soil acidity leads to severe environmental consequences e.g. increased turbidity of streams, siltation of dams and nitrate contamination of a rising water table, although the nature of events is not well documented. Thus, down-slope waterlogging and salinity may occur.

Plant stress caused by soil acidity can be an insidious problem as it may be expressed as symptoms of ordinary nutrient deficiency, drought effect, herbicide injury, low-temperature damage, or even plant disease (Foy 1984). Soil acidity can decrease crop yields (Kamprath 1984; Aniol 1991; Bona *et al.* 1991; Mahadevappa *et al.* 1991; Rajaram *et al.* 1991; Rai 1991; Helyar and Conyers 1994; Ritchie 1994; Carver and Ownby 1995; De la Fuente-Martinez and Herrera-Estrella 1999), seedling emergence and survival (Rubzen 1996; Voigt *et al.* 1999; Kelman *et al*. 1998; Islam *et al*. 2001), pasture establishment and persistence (Awad *et al.* 1976; Edmeades *et al.* 1991b; Keerthisinghe *et al.* 1991; Helyar and Conyers 1994; Dowling *et al.* 1996; Duncan and Crocker 1998; Garden *et al.* 2001a), legume nodulation (Rai and Prasad 1983; Cline *et al.* 1991; Shamsuddin *et al.* 1991; Kerridge 1991) and root development (Arnon and Johnson 1942; Islam *et al.* 1980; Bromfield *et al.* 1983a; Bruce *et al.* 1988; Caires and Rosolem 1991; Shamsuddin *et al.* 1991; Menzies *et al.* 1994; Voigt *et al.* 1999).

The detrimental effects of soil acidity normally occur when the soil pH falls below 4.5 measured in a 1:5 (w/v), soil: 0.01M $CaCl_2$ suspension (Arnon and Johnson 1942; Islam *et al.* 1980; Cregan *et al.* 1986; Shamsuddin *et al.* 1991) and are mainly due to toxicities of Al, Mn and to some extent of H^+ ions (Foy 1984, 1988,1996; Taylor and Foy 1985d; Kinraide and Parker 1987; Bruce *et al.* 1988; Marschner 1991; Ritchie 1994; De la Fuente-Martinez and Herrera-Estrella 1999). In addition, deficiencies of essential nutrient elements such as Ca, Mg, P and Mo may also be involved (Foy 1984; Kamprath and Foy 1985). The survival and the function of beneficial organisms such as rhizobia and micorrhizae may also be inhibited by soil acidity (Foy *et al.* 1978; Coventry and Evans 1989; Robson and Abbott 1989; Aarons and Graham 1991; Glenn and Dilworth 1991). Moreover, soil acidity may enhance the incidence and severity of several diseases. For instance, *Fusarium* wilts are increased by acidifying soil and decreased by lime application (Robson and Abbott 1989).

2.1.1 Extent and severity

In a recent review, De la Fuente-Martinez and Herrera-Estrella (1999) stated that approximately 43% of the world's tropical land area is classified as acidic, comprising about 68% of tropical America, 38% of tropical Asia, and 27% of tropical Africa. Acidic soils cover a total of 1660 million ha in 48 developing countries (Pandey *et al.* 1994), while the total area affected by soil acidity is about 4 billion ha (Rao *et al.* 1993; von Uexkull and Mutert 1995). Eswaran *et al.* (1997) have constructed maps showing the global distribution of acidic soils and sub-soils (Figs 2.1 and 2.2).

In Australia, more than 30 million ha of land are estimated to be affected by soil acidity (Helyar *et al.* 1990; Evans 1991). In a survey by Helyar *et al.* (1990), it was found that about 13.5 million ha of agricultural soil in New South Wales are seriously affected by soil acidification with a further 6 million ha susceptible to this problem. As a result, it has been estimated that the farmers' incomes are reduced by $90 million per year (Duncan 1998). The extent of acidic soils (0-10 cm zone) in

Australia, State by State, are summarised in Table 2.1.

Table 2.1. Extent of acidic soils in Australia (ha X 10^6) (After Anon. 1995)

State	Highly acidic (pH_{Ca} ≤4.8)	Moderately acidic (pH_{Ca} 4.9-5.5)	Slightly Acidic (pH_{Ca} 5.6-6.0)
New South Wales	13.5	5.7	5.1
Victoria	3.0	5.6	5.5
Western Australia	4.7	4.7	-
South Australia	2.8	-	-
Queensland	8.4	32.0	-
Tasmania	1.0	-	-

In addition, research indicates that the subsoil acidity is occurring either concurrently with, or subsequent to surface (0-10 cm) acidification. Subsoil acidity can cause yield loss due to damage to plant root systems resulting in an inability to absorb water and nutrients (Cregan and Scott 1999). It is also a very difficult and expensive procedure to ameliorate subsoil acidity in extensive agriculture (Helyar 1991; Tang *et al*. 2001) because of the inaccessibility of subsoil horizons for direct and effective liming treatments (Adams 1984).

2.1.2 Causes

Soil acidifies naturally as it weathers over millions of years (Williams and Donald 1957; Williams 1980; Helyar 1991). The process of soil acidification varies according to the rock from which it is derived, the length of time it has weathered and the local climate (Helyar and Porter 1989). Therefore, some soils are naturally very acidic while others are more alkaline. Generally the older and more weathered soils are more acidic than younger soils, and superimposing intensive agricultural production practices on natural ecosystems increases the rate of soil acidification (Helyar and Porter 1989; Ridley *et al*. 1990a, 1990b).

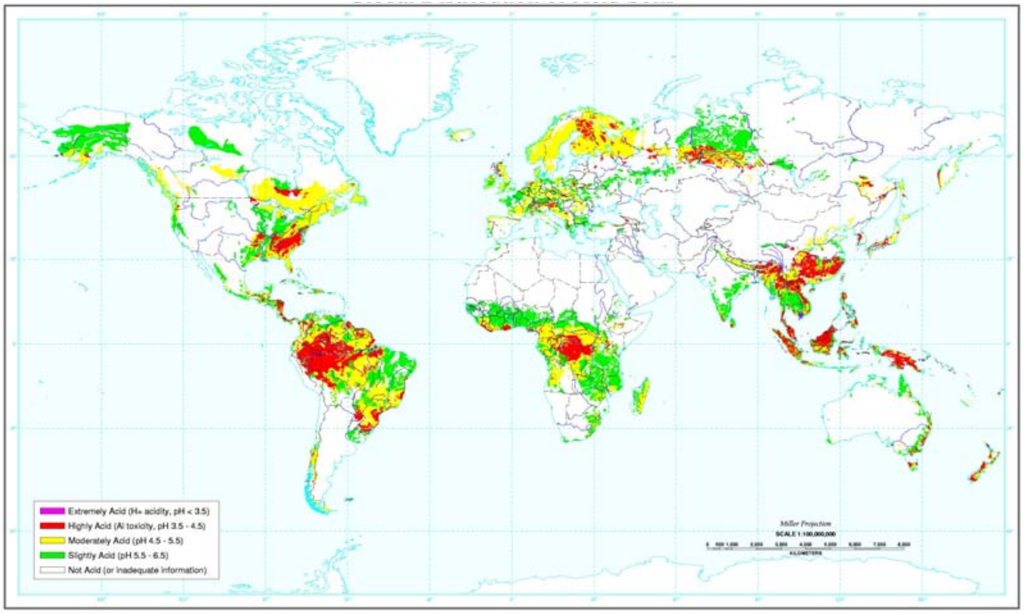

Fig. 2.1. Global distribution of acidic soils. Source: U.S. Dept. of Agriculture, Natural Resources Conservation Service, Soils Division, World Soil Resources.

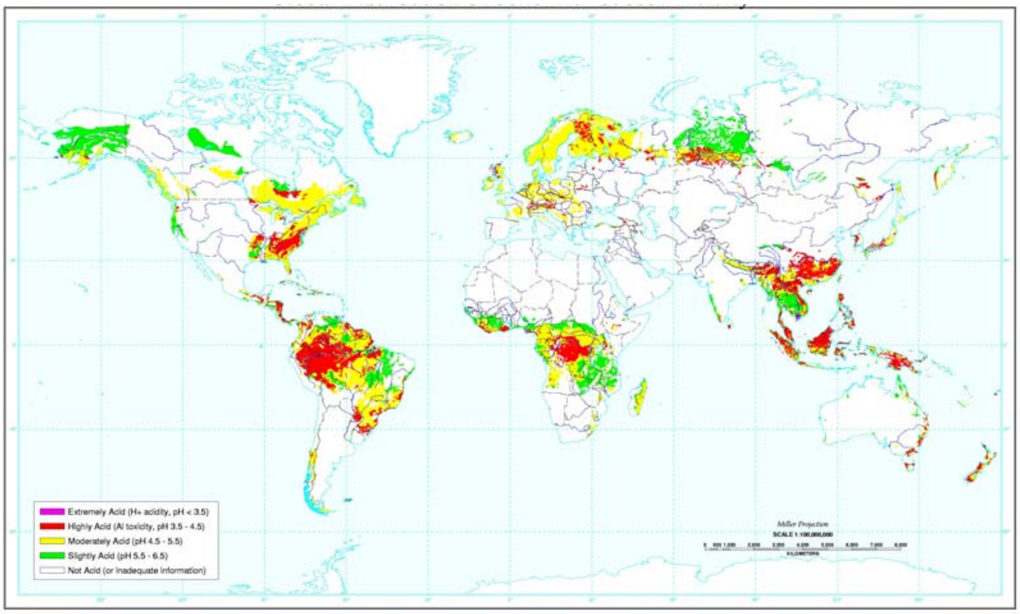

Fig. 2.2. Global distribution of soils with subsoil acidity. Source: U.S. Dept. of Agriculture, Natural Resources Conservation Service, Soils Division, World Soil Resources.

An understanding of the rates at which agricultural soils are acidifying may permit the prediction of when production will suffer, and how much alkali should be applied each year to stabilise the soil's acidity (Helyar and Porter 1989). Also, an understanding of the mechanisms of acidification may provide strategies for minimising the rates of acidification other than by applying lime.

Soils acidify if the rate of acid addition exceeds the capacity of the neutralising processes (Evans 1991). The mechanisms of acid addition in agricultural systems have been extensively explored and explained (Helyar 1976; Kennedy 1986; Helyar and Porter 1989), and mainly occur through carbon and nitrogen cycling or as a consequence of leaching of nitrate (Helyar and Porter 1989; Coventry and Slattery 1991; Tang *et al*. 1999). Soil acidification occurs through an extremely complex set of processes (Robarge and Johnson 1992), but the following discussion (Kennedy 1986) outlines the mechanisms by which soil acidity increases:

a. Ley farming and build-up of organic matter: organic matter derived from the lignin of plants contains a significant number of carboxylate groups. The increase of soil organic matter represents a transfer of weak acids to soil, tending to decrease pH. Ley farming systems also add N to infertile soils by the growth of nitrogen-fixing legumes (e.g. subterranean clover fertilised with superphosphate). Ley farming has been extensively practised in Australia and has resulted in an increase in organic matter of 2-6% over a period of 50 years, which is equivalent to about 100 keq H^+ ha^{-1} (Williams 1980). However, acidification occurs more quickly during the first few years, when organic matter content is increasing rapidly (Williams and Donald 1957; Williams 1980; Ridley *et al*. 1990a).

b. Soil acidification from nitrogenous fertilisers: the form of N fertiliser strongly influences the rates of acidification (Pierre 1928a). The most acidifying fertilisers are ammonium sulfate and monoammonium phosphate (MAP), followed by diammonium phosphate (DAP). Less acidifying are urea, ammonium nitrate and anhydrous ammonia; whereas, sodium- and calcium nitrate are non-acidifying. When ammonium fertilisers are added to soils, nitrification occurs and causes the soils to become acid. Some potential nitrifying reactions are:

Ammonium nitrate

$$NH_4NO_3 + 2O_2 \rightarrow 2\ NO_3^- + 2H^+ + H_2O \quad (1)$$

Urea

$$(NH_2)_2CO + 4O_2 \rightarrow 2NO_3^- + 2H^+ + CO_2 + H_2O \quad (2)$$

Anhydrous ammonia

$$NH_3 + 2O_2 \rightarrow NO_3^- + H^+ + H_2O \quad (3)$$

Ammonium phosphate

$$NH_4H_2PO_4 + 2O_2 \rightarrow NO_3^- + H_2PO_4^- + 2H^+ + H_2O \quad (4)$$

Ammonium sulfate

$$(NH_4)_2SO_4 + 4O_2 \rightarrow 2NO_3^- + SO_4^{2-} + 4H^+ + 2H_2O \quad (5)$$

Ammonium sulfate and ammonium phosphate invariably contribute significant acidity to soil (Eqns 4 and 5). Consumption of nitrate by plants is an alkaline process (because OH^- ions are released during uptake), whereas leaching or run-off of nitrate acidifies the soil (because H^+ ions produced during nitrification are left unneutralised). Also, plants take up much less of the sulfate and phosphate anions than ammonium or nitrate. Consequently, the acidity developed from the exuded protons is greater when fertilisers are applied as ammonium sulfate and ammonium phosphate.

c. Oxidation of reduced S compounds: reduced forms of sulfur have acidifying effects on the soil. The use of sulfur is normally recommended to correct the pH of highly alkaline soils by forming an acid on oxidation. The oxidation and mineralisation of organic matter from biota in ecosystems are involved in the production of sulphuric acid from reduced organic sulfur. In agricultural ecosystems, acids in the S cycle are sometimes important (e.g. acid sulfate soils, acid rain, elemental sulfur or finely divided sulfides in fertilisers). However, in many situations the effect of the S cycle is of minor significance, because the fluxes of S are small compared with those of N and C (Helyar and Porter 1989).

$$2FeS_2 + 7H_2O + 7/2O_2 \rightarrow 4SO_4^{2-} + 8H^+ + 2Fe(OH)_3 \qquad (6)$$

d. Leaching of anions:

Nitrate: following rainfall and irrigation, cations along with anions can be leached from the soil through the percolation of water. Bicarbonate ion (HCO_3^-) is primarily involved in neutral and alkaline soils. But at < pH 6, bicarbonate is no longer a significant constituent of the soil solution because it is converted to carbonic acid or carbon dioxide. Nitrate, sulfate and chloride are the other anions of significance. Protons produced by nitrification (Eqn 7), or any other means, displace exchangeable cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) from clay and other charged colloids, and the nitrate can be leached as the accompanying counter-ion. As a result, an increase in acidity of the surface soil occurs. The scope of acidification by this process is greater than where acidity is produced by the formation of carboxylic acids (see *a.* above). On the other hand, in anaerobic conditions, this leached nitrate may be denitrified (Eqns 8-9) to nitrogen gases in the subsoil, leaving the soil more alkaline. In most agricultural situations, this condition is likely to be transitory.

Nitrification

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ \qquad (7)$$

Denitrification

$2NO_3^- + 10H^+ \rightarrow N_2O + 5H_2O$ (8)

$N_2O + 2H^+ \rightarrow N_2 + H_2O$ (9)

Another possible source of acidification is the leaching of organic acid anions as counter-ions to metal ions. This may happen following the release of such materials by plants either by root excretion or by the breakdown of plant material at the end of the plant's life cycle. Once the anions are removed from the soil, the normal neutralisation process (Eqn 10) by oxidation of carboxylate compounds to carbon dioxide and water cannot occur, resulting in long-term acidification of soils.

$CH_3COO^- + H^+ + 2O_2 \rightarrow 2CO_2 + 2H_2O$ (10)

e. Other causes: when superphosphate is added to the soil solution, an acidic pH is developed through hydrolysis (Eqn 11). As a result, an insoluble calcium monohydrogenphosphate is formed. The net effect is an immediate acidification of soils when superphosphate is applied as a band application. However, this effect disappears over the long-term by diffusion and neutralising reactions, and is unlikely to contribute substantially to soil acidity.

$Ca(H_2PO_4)_2 + H_2O \rightarrow CaHPO_4 + H^+ + H_2PO_4^-$ (11)

The various sources of acid in the nutrient cycles (e.g. C, N, S and other nutrient cycles) contribute significantly to soil acidification where acid rain is not a measurable contributor (Helyar 1976; Helyar and Porter 1989), e.g. removal of plant and animal products, transfer of dung and urine to stock camps and the fixation of atmospheric N. For instance, the removal of one tonne of good-quality lucerne hay is about 20 times more acidifying to the soil than equivalent harvesting of cereal grain (Davidson 1987) and requires 70 kg of lime to neutralise the resulting acidity (Schumann 1999). Reduction of solid phase Mn and Fe oxides, the migration of reduced species and their oxidation, are outside the scope of this study but they are important processes in the dynamics of acid balance in soil profile.

The pH change caused by the acid depends on the pH buffer capacity of the soil (Helyar and Porter 1989):

$$\text{pH change} = (\text{moles } H^{+} \text{ added ha}^{-1})\ (\text{pHBC X W}) \qquad (12)$$

where pHBC is the pH buffer capacity (mol H^{+} kg^{-1} pH $unit^{-1}$) and W is the weight of the component of the ecosystem involved (kg ha^{-1}). Soil dominates the buffer capacity of most ecosystems because of its much higher mass. For example, 14000 t soil ha^{-1} and 0-50 t plant litter ha^{-1} may have the pH buffer capacities of about 100-200 and 0-16 kmol H^{+} ha^{-1} pH $unit^{-1}$ respectively.

2.1.3 Management options and strategies to minimise acidity

Pierre (1928a, 1928b) suggested using less acidifying fertilisers and Helyar (1976) suggested practising zero tillage to minimise the rate of nitrate leaching. To combat soil acidity, Davidson (1987) proposed three approaches: i) breeding more tolerant lines of crop and pasture plants or switching to more tolerant lines, ii) correcting the acidity by applying lime and iii) using non-acidifying farming systems. Although time consuming, selection and breeding of more tolerant lines are effective methods for combating the effects of soil acidity (Tang *et al*. 2001). With this approach, soil acidification may continue and eventually reduce crop yields until lime is applied in conjunction with the sowing of tolerant species (Davidson 1987; Scott and Fisher 1989). Liming can correct soil acidity but often is an expensive option (Helyar 1991; Tang *et al*. 2001). The success of liming is well documented (Martini *et al*. 1974; Coventry and Evans 1989; Conyers *et al*. 1991; Bolland *et al*. 2001; Li *et al*. 2001; Scott *et al*. 2001) but there are a number of limitations associated with lime applications e.g. cost, inaccessibility to some hilly areas with ground equipment, induced nutrient deficiencies (e.g. B, Zn), and subsoil acidity (acidity that develops below 10 cm depth).

In Australian agricultural systems, making a decision to use lime can be uneconomical, because the agricultural systems are complex, and the nature of the environment and commodity prices are unpredictable (Hochman *et al*. 1989). In addition to many positive impacts of liming in Australian agricultural soils, some negative effects (e.g. depressed plant performance, lack of growth responses, yield depression) following liming have also been reported (Cregan *et al*. 1989; Scott *et al*. 2001). Consequently, simply liming for maximum yields is not an adequate prescription for long-term management of acid soils (Helyar 1991). Improved non-acidifying farming systems are perhaps the most permanent options, but little research has been conducted and few practices have been developed (Davidson 1987).

According to Helyar and Porter (1989) and Helyar (1991), soil acidification can be minimised by: use of tolerant species; minimising soil nitrate accumulation (e.g. minimum tillage, perennial deep-rooted plants); avoiding over-fertilising with N or using less acidifying materials; maintaining more organic matter on the top soil (e.g. returning straw); feeding hay in the paddock rather than in concentrated feeding areas; minimising the camping behaviour of grazing animals; and finally, avoiding excessive grazing pressure to maintain a critical leaf area so that the capacity of the plant to utilise nitrate is maintained. This suggests that an integrated approach involving liming, management changes and introducing plant tolerance will probably be the best option for combating soil acidity.

There are some species, especially native perennial grasses (e.g. *Austrodanthonia*, *Microlaena* etc.) that naturally occur on acidic soils (Dowling *et al.*

1996). These acid tolerant perennial species may have a useful role in a multifaceted approach to the management of soil acidity. However, more information is needed about the competitiveness and adaptation of such species under acid soil conditions.

2.1.4 Plant growth in relation to soil acidity

On an acidic soil, plant growth can be limited by a variety of factors e.g. plant species and genotype, soil type and horizon, parent material, soil pH, concentration and species of Al, Mn ions, soil structure and aeration and climatic conditions (Clark 1982; Foy 1983; Ritchie 1989; Marschner 1991; Cregan and Scott 1999) and the interactions between these factors (Marschner 1991; Cregan and Scott 1999). Commonly, growth depression on acidic soils is due to deficiencies of P, Ca, Mg, and Mo (Foy 1983; Taylor and Foy 1985b; Ritchie 1989; Marschner 1991) or due to toxicities of Al, Mn and H^+ ions (Ritchie 1989; Marschner 1991; Cregan and Scott 1999). However, toxicities are the most common factor responsible for reduced plant growth (Ritchie 1989).

Hydrogen ion toxicity: nutrient uptake from the soil solution, and ultimately plant growth, is strongly influenced by soil pH (Arnon and Johnson 1942; Awad *et al.* 1976; Islam *et al.* 1980; Foy 1984; Edmeades *et al.* 1991b; Ila'ava *et al.* 2000a; Islam *et al.*2001; Kidd and Proctor 2001).

It is difficult to differentiate H^+ ion effects on plant growth from other factors when plants are grown in soil. Because at the levels of pH considered to be detrimental, Al and Mn may be soluble in toxic concentrations, and the availability of essential elements (e.g. Ca, Mg, P, and Mo) may be suboptimal (Foy 1984). In most soils where pH is 4.0-5.0, plant growth limitation is due mainly to Al^{3+} and Mn^{2+}

toxicities rather than H^+ ions (Foy 1984; Kidd and Proctor 2001).

Investigations of the responses of plants to low pH often use solution or sand culture techniques to reduce interactions. In very acidic conditions (pH < 4.0), generally the most affected plant parts are the roots in nutrient solution. The damaged roots become shorter, thickened, comparatively few in number, and discoloured brown or dull grey (Arnon and Johnson 1942; Islam *et al*. 1980). The lateral secondary roots may be seriously inhibited, and wilting of plants may appear when transpiration rates are high (e.g. during warm sunny weather). However, plant responses to pH can be variable. Canmore-Neumann *et al*. (1997) found that proliferation of root hairs of *Leucadendron* 'Safari Sunset' was arrested when pH was increased from 5.5 to 7.0. On the other hand, Ila'ava *et al*. (2000a) reported that the root growth of sweet potato (*Ipomoea batatas* L.) was either greatly reduced or inhibited at pH 3.5, while markedly increased at pH 4.0. Further increases in solution pH from 4.0 to 8.0 did not affect the root growth. Thawornwong and van Diest (1974) showed that a solution at pH 3.5 produced higher growth rate and dry matter yield of roots of lowland rice than at pH 6.0.

Extreme concentrations of H^+ ions in the solution bathing the roots can also cause damage to the root cell membrane (Foy 1984). Excess H^+ ions compete with other cations for the absorption sites on the root surface, and as a consequence, interfere with the uptake and transport of ions and, finally, cause the cell membrane to become leaky. As a result, roots may lose absorbed nutrients as well as organic compounds. Christiansen *et al*. (1970) reported that a solution pH < 4.0 greatly increased the loss of organic compounds from cotton radicles. Moore (1974) found that a substantial loss of Ca, Mg, K, and P occurred from the roots when the pH fell below 4.0. A similar result was also observed in barley roots by Hussain *et al*. (1954)

and the loss of nutrients was largely prevented by the presence of polyvalent cations (e.g. Ca^{2+}, Al^{3+}, La^{3+} and Ce^{3+}).

As excess H^+ ion affects nutrient uptake and retention by plant roots, it can also increase plant requirements for Ca, and perhaps other nutrients. For example, Lu and Sucoff (2001) found that seedlings of quaking aspen (*Populus tremuloides* Michx.) growing in a solution culture required a higher Ca concentration (250 µM) at pH 3.9 than at 5.0 (25 µM).

H^+ ions also influence ion uptake in other species, e.g. lowering of pH from 4.3 to 3.5 decreased the absorption of K, Ca and Mg in rice plants (Thawornwong and van Diest 1974). In another study, decreasing the solution pH from 5.0 to 3.9 reduced the root Mg about 42% in seedlings of aspen (Lu and Sucoff 2001). Similarly, large decreases have been observed in the rate of absorption of Mg, K (Islam *et al*. 1980; Kidd and Proctor 2001), Ca (Arnon and Johnson 1942; Islam *et al*. 1980; Kidd and Proctor 2001), Mn, Zn (Islam *et al*. 1980) and Cu (Bowen 1969).

Plant species and genotypes within species may widely differ in tolerance to excessive H^+ ion concentrations (Foy 1984). Growth of lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicum esculentum* Mill.) decreased when the pH was below 5 (i.e. 5 to 4), but the growth of bermudagrass (*Cynodon dactylon* L.) did not decrease (Arnon and Johnson 1942). Thawornwong and van Diest (1974) showed that rice roots were not affected by H^+ ion concentration down to pH 3.5 in the absence of Al.

Islam *et al*. (1980) grew six species i.e. ginger (*Zingiber officinale* Roscoe), cassava (*Manihot esculenta* Crantz, cv. Nina), maize (*Zea mays* cv. NK195), wheat (*Triticum aestivum* cv. Gatcher), french bean (*Phaseolus vulgaris* cv. Redland Pioneer) and tomato (*Lycopersicon esculentum* cv. Grosse Lisse) in continuously flowing nutrient culture at pH levels from 3.3 to 8.5. Ginger and cassava were the most tolerant of low pH. Roots of all species at pH 3.3 and of some species at pH 4.0, showed H^+-toxicity or injury symptoms (e.g. short, thickened, few in number and discoloured). Low pH also caused suboptimal uptake of Mg in all species, of N in tomato and cassava, and of Mn in maize. The order of ranking of the species for tolerance to H^+-toxicity (pH 3.3 and 4.0) was: ginger > cassava > tomato > french bean > wheat > maize. This ranking agreed with the ranking obtained for some of the same species under acidic field conditions (CIAT 1977).

Kidd and Proctor (2001) demonstrated the genotypic differences in plant growth response to increasing acidity in the grass *Holcus lanatus* L. (Yorkshire-fog) and the tree *Betula pendula* Roth. (silver birch). Genotypes from acid organic soils were H^+-tolerant, while those from acid mineral soils were Al^{3+}-tolerant but not necessarily H^+-tolerant. The possibility of plant adaptation to H^+-toxicity supports the idea that H^+-toxicity may be important in very acidic soils (e.g. pH $\leq$ 4.0) where Al and Mn are low (e.g. sandy and organic soils).

Aluminium toxicity: Al is the most abundant metal in the earth's crust (Driscoll and Schecher 1988; Martin 1988; Kochian 1995). In soils, it is found primarily in aluminosilicates or oxides. As soils become more acidic, Al^{3+} is released into the soil solution (Kinraide 1991; Kochian 1995; De la Fuente-Martinez and Herrera-Estrella 1999). Al-toxicity is one of the most important growth-limiting factors for plants in most strongly acidic surface soils (Foy 1974, 1983, 1984, 1988; Horst 1995; Kochian 1995; De la Fuente-Martinez and Herrera-Estrella 1999; Rout *et al*. 2001). Al-toxicity is usually severe in soils with a pH below 5.0, but it may occur at pH values as high as 5.5, especially in kaolinite soils (Foy 1984; Rout *et al*. 2001; Tyler and Olsson 2001). Strongly acidic subsoils cause poor root growth, which increases drought susceptibility and decreases the uptake of subsoil nutrients (Foy *et al*. 1978; Foy 1984, 1988; Foy *et al*. 1999; Rout *et al*. 2001).

Phytotoxic aluminium species: soluble Al can exist in many different ionic forms (species) in aqueous solutions (Kinraide 1991; Kochian 1995). As Al speciation is difficult, activities of individual ionic species in aqueous solution are calculated. The calculations assume equilibrium condition and use a set of thermodynamic constants for the reactions under study (Parker *et al*. 1995b; Bertsch and Parker 1996; Rengel 1996; De la Fuente-Martinez and Herrera-Estrella 1999). Evidence for transport of the various ionic species across the root-cell plasma membrane is not clear (Kinraide 1988; Kochian 1995; Rengel 1996).

Al species that are relevant to phytotoxicity are both mononuclear and polynuclear (Kochian 1995). At pH < 5.0, Al^{3+} exists as the octahedral hexahydrate ($Al(H_2O)_6^{3+}$), which is conventionally called Al^{3+}. As the pH increases, $Al(H_2O)_6^{3+}$ undergoes successive deprotonations to form $Al(OH)^{2+}$ and $Al(OH)_2^{+}$. At near-neutral pH, the relatively insoluble $Al(OH)_3$ (gibbsite) is formed (Kinraide 1991; Kochian 1995; Sparks 1995). A number of polymeric Al species have also been proposed, but the most important one in a partially neutralised solution is triskaidekaaluminium ($AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$, and referred to as Al_{13}) (Kinraide 1991, Bertsch and Parker 1996). This polynuclear hydroxy-Al complex with high positive charge is more rhizotoxic than monomeric, less charged species (Parker *et al*. 1989, 1995a; Rengel 1996).

Mononuclear Al also forms low molecular weight complexes with a number of ligands on which the donor atom is oxygen, e.g. carboxylate, phosphate and sulfate groups (Kochian 1995). Also, Al can complex with inorganic ligands such as F to form non-toxic Al species (Kochian 1995; Parker *et al*. 1995a).

Interaction of Al-toxicity with other toxicity factors: although plant growth on acidic soils is mainly restricted by toxic levels of Al^{3+}, a combination of Al with other factors antagonistic to growth can further decrease productivity, e.g. low pH (Islam *et al*. 2001; Kidd and Proctor 2001), increased Mn concentration (Foy 1984; Kidd and Proctor 2001), deficiency of Ca, Mg and K, decreased P and Mo solubility (De la Fuente-Martinez and Herrera-Estrella 1999) and poor aeration (Foy 1984; Marschner 1991).

High organic matter content decreases Al toxicity (Adams and Moore 1983) and additions of organic amendments detoxify Al-toxic soils (Kinraide 1991). The effect may be twofold: the formation of organic complexes and non-specific attraction to exchange sites (Ritchie 1989).

Physiological effects of Al on plant growth:

Beneficial effects of Al: Al is not essential for plant growth, although low concentrations sometimes increase plant growth or produce some other beneficial effects (Foy 1974; Foy *et al*. 1978; Foy and Fleming 1978; Foy 1983, 1984). Species with positive responses to Al include rice (Howeler and Cadavid 1976), "BH 1146" wheat (Foy and Fleming 1978), betel palm (*Areca catechu* L.) (Kumar 1979), tropical legumes (Andrew *et al*. 1973), eucalyptus (*Eucalyptus* sp.) (Mullette 1975), tea (*Camillia sinensis* L.) (Matsumoto *et al*. 1976), peach (*Prunus persica* L.) (Edwards *et al*. 1976), sugarbeet (*Beta vulgaris* L.) (Keser *et al*. 1975) and maize inbreds (*Zea mays* L.) (Clark 1977). The beneficial effects of Al on plant growth are, however, less important than the detrimental effects.

Phytotoxic effects of Al: excess Al interferes with cell division in root tips and also in lateral roots; increases cell wall rigidity by cross-linking pectins; reduces DNA replication by increasing the rigidity of the DNA double helix; makes P unavailable by fixing it in soils and on plant root surfaces; reduces root respiration; interacts with enzymes governing sugar phosphorylation and the deposition of cell wall polysaccharides; reduces protein synthesis; and, interferes with uptake, transport and use of different essential nutrient elements (Ca, Mg, K, P and Fe), and water supply to plants (Foy 1974; 1983; 1984; Foy and Fleming 1978; Foy *et al*. 1978; McLean 1979; Matsumoto 1991, 2000; Rout *et al*. 2001). Helyar (1978) found that Al-toxicity was largely associated with P metabolism and with binding pectins in root cell walls, which stopped root elongation.

Al also alters root membrane structure and function (Foy 1983; 1984). Al can bind to either cell membranes proteins or lipids, depending on the pH and other conditions of the surrounding cells, and thus decrease the fluidity of lipids in the membranes (Vierstra and Haug 1978; Gomez-Lepe *et al*. 1979; Foy 1983, 1984). This allows nutrient loss through damaged membranes, decreased nutrient uptake (e.g. Ca Mg, P, K) and hence, inhibition of plant growth (Foy 1983, 1984).

Other effects of Al have also been reported, but it is unclear whether they are primary or secondary. Al decreases water use efficiency of plants (Kauffman and Gardner 1978) and increases the diffusive resistance of peach seedlings (Horton and Edwards 1976) perhaps through root damage. Excess Al lowers the chlorophyll level of plant cells (Sarkunan *et al*. 1984) and inhibits the flow rate of electrons (Wavare *et al*. 1983). The nodulation of legumes by Rhizobia may also be affected by Al (Foy 1983, 1984). For example, the nodulation of groundnut (*Arachis hypogaea* L.) decreased before any phytotoxicity to the host occurred (Pieri 1974). Nodule formation is more sensitive to Al than N fixation (Carvalho *et al*. 1982; Foy 1984).

Plant symptoms of Al-toxicity: the symptoms of Al-toxicity are not easily identifiable (Foy *et al*. 1978; Foy 1983, 1984; Taylor 1988b; Matsumoto 2000; Rout *et al*. 2001). The most dramatic effects are reduced growth both in root and shoot. In leaves of many plants, the symptoms may resemble phosphorus deficiency, including small leaf size, late maturation, purple coloration, and chlorosis and necrosis of leaf tips. Other symptoms may include petiole collapse, mottled chlorosis and necrosis, symptoms that are generally associated with Ca deficiency or transport problems within the plant (Foy 1983, 1984; Taylor 1988b; Rout *et al*. 2001). Excess Al may induce symptoms of Fe deficiency in rice, sorghum and wheat (Rout *et al*. 2001). In Al-injured plants, Al accumulates on or in the roots, often in association with P, but it does not generally accumulate in the tops of Al-sensitive plants (Foy 1974). Therefore, the primary cause of poor plant growth may be diagnosed incorrectly.

Lateral root initiation typically occurs near the apex of the main axis. Al-injury inhibits branching and the roots are characteristically stubby and brittle, brown in colour and occasionally necrotic (Foy 1984; Taylor 1988b). The root systems lack fine branching, are reduced in size and coralloid in appearance (Foy 1983, 1984; Taylor 1988b). Sasaki *et al*. (1996) observed that Al treatment markedly decreased cell length and increased the diameter of cells, in particular, the cells in the second and third layers of the cortex (Fig. 2.3). In general, younger plants are more susceptible to excess Al than older plants (Thawornwong and van Diest 1974).

Site of Al-toxicity: the primary site of the inhibition of root growth by Al is the root apex (Kochian 1995; Matsumoto 2000). Ryan *et al*. (1993) found that only 2-3 mm of maize root tip, including meristem and root cap, needed to be exposed in Al-containing solutions to cause root growth inhibition. They also observed that root growth rate was normal if the entire root, except the root apex, was exposed to Al.

The inhibition of root elongation may occur within minutes of treatment with µM concentrations of Al (Llugany *et al.*1995; Sasaki *et al.* 1997; Matsumoto 2000).

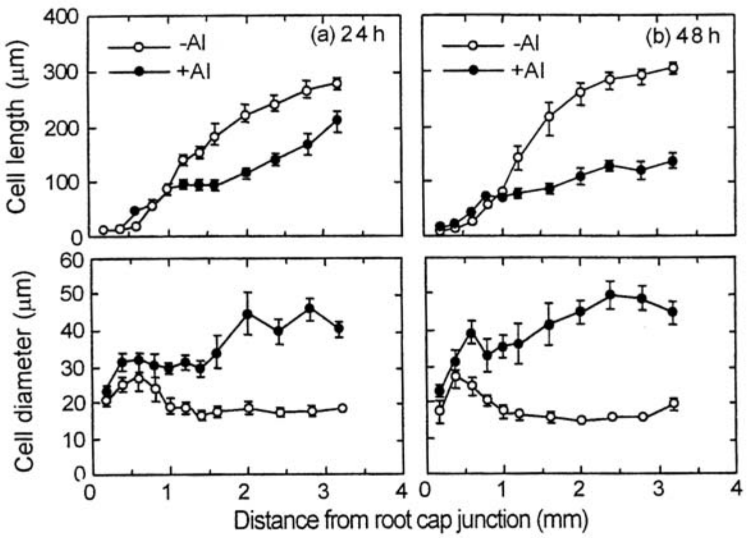

Fig. 2.3. Effects of Al on the lengths and diameter of roots cells in the second layer from the root surface in wheat (Atlas 66). Roots were treated with or without 20 µM Al for 24 h (a) or 48 h (b). Data are means (± SE) of results from 5 or 6 samples. *Source: Sasaki et al. (1996)*

The root apex, which includes root cap, meristem and elongation zone, accumulates more Al, and plays an important role in the Al-perception mechanism proposed by Bennet and Breen (1991a), and Matsumoto (2000). Bennet *et al.* (1987, 1991a) found rapid changes in the ultrastructure of the cells in the root cap of maize and suggested that Al might indirectly inhibit root growth through an unknown signal transduction pathway involving the root cap, apical meristem and hormones. However, the inhibition of root growth under Al-stress was the same for intact and decapped maize roots (Ryan *et al*. 1993). This argues against a major role for the root cap in either Al-toxicity or protection against Al-toxicity. Therefore, the cell elongation zone may be the major target for the inhibition of root elongation by Al stress (Ryan *et al*. 1993; Sivaguru and Horst 1998; Matsumoto 2000).

Manganese toxicity: in many acidic soils, Mn-toxicity is probably the second most important growth-limiting factor (Foy 1984; Cregan and Scott 1999). Mn-toxicity can occur in soils with pH 5.5 or below, when the soil contains sufficient Mn (Foy 1984). However, Mn-toxicity may occur at still higher pH values if the soils are poorly drained or compacted. These soil conditions favour both the production of divalent Mn, the form that plants absorb (Foy 1984), and inhibit its microbial oxidation to MnO_2 (Carver and Ownby 1995).

Factors affecting Mn-toxicity: in soils, Mn generally exists primarily in unavailable Mn^{3+} and Mn^{4+} forms, with the plant available form, Mn^{2+}, as a minor component (Cregan and Scott 1999). The availability and toxicity of Mn to plants, depends on total Mn content, pH, organic matter, aeration and microbial activity (Foy 1984; Carver and Ownby 1995), moisture content, temperature and redox potential (Ritchie 1989; Cregan and Scott 1999). Mn^{2+} behaves more or less similarly to other divalent cations, as it may be adsorbed onto the surface of hydrous oxides, clay particles and organic matter or exist as discrete manganese compounds.

Most soils of south-eastern Australia have sufficient quantities of reducible MnO_2 to allow sporadic out breaks of Mn toxicity (Cregan and Scott 1999; Scott *et al*.

2001). These are triggered by waterlogging and high temperature (Rufty *et al*. 1979). Environmental effects on the plants, e.g. high light intensity also affect the uptake of Mn (Horiguchi 1988).

Physiological and biochemical effects of Mn-toxicity: unlike Al, Mn is a micronutrient required for different biochemical processes. In green plants, Mn is important in photosynthetic O_2 evolution and cell division through its role in the activities of key enzymes, e.g. isocitric dehydrogenase and glutamine synthetase. Mn is also associated with P reactions (Foy 1984; Marschner 1986). Excess Mn concentrations may restrict plant growth by adversely influencing a number of physiological and biochemical processes (Foy 1984). For example, in ricebean (*Vigna umbellata*), net photosynthesis rate decreased with increasing concentration of Mn within 2 days of Mn supply in nutrient solution, and transpiration rate and stomatal conductance were affected slowly (Subrahmanyam and Rathore 2000).

Excess Mn also affects uptake of other nutrients. Marschner (1986) reported that a toxic level of Mn could influence the uptake and metabolism of Fe, Mg, Zn and Ca. Usually, Mn-toxicity is aggravated by Fe deficiency (Carver and Ownby 1995). The induction of nutrient deficiencies by Mn-toxicity occurs when plants lose control of their Mn-activated enzyme systems (Helyar 1978). Mn interferes with many other mineral nutrients, e.g. Si, Fe, Ca, P. Under certain conditions, supplementing these elements can alleviate Mn-toxicity (Vlamis and Williams 1967; Foy *et al*. 1978; Galvez *et al*. 1987; Alam *et al*. 2001).

Foy (1983, 1984) summarised the effects of excess Mn on plant growth as follows: destruction of auxin (IAA, indole-3-acetic acid) by increasing the activity of IAA-oxidase; a possible amino acid imbalance; decreased activities of catalase, ascorbic acid oxidase, glutathione oxidase, and cytochrome C oxidase; and lowered ATP contents.

Plant symptoms of Mn-toxicity: Mn^{2+} in the soil solution is readily taken up and translocated to the shoot (Carver and Ownby 1995); consequently, excess Mn usually affects plant tops severely (Foy *et al.* 1978; Foy 1983, 1984; Carver and Ownby 1995). Hence, Mn-toxicity produces more definitive symptoms in plant tops than does Al, and for a given species, the injury is approximately proportional to the concentration of Mn accumulated in the tops (Foy 1984). The older leaves of the plant typically exhibit toxicity symptoms first as they have accumulated Mn for a longer time; however, a sudden increment of available Mn can reverse this pattern.

Symptoms of Mn-toxicity vary among plant species (Foy *et al.* 1978). Foliar symptoms include marginal chlorosis and necrosis (alfalfa, rape, lettuce), puckering (cotton, snap bean) and necrotic spots (barley, soybean). Some crops also show specific physiological disorders due to excess Mn, such as ‘crinkle leaf’ of cotton, ‘stem streak necrosis’ of potato, ‘freckling’ on the unifoliate and first trifoliate leaves of cowpea and ‘internal bark necrosis’ of apple trees (Foy *et al.* 1978; Kang and Fox 1980; Foy 1983). Kitao *et al.* (2001) showed that increments of Mn up to 100 mg L^{-1} produced two distinct foliar symptoms in Japanese white birch (*Betula platyphylla* var. *japonica* Hara), namely: chlorosis of the entire leaf (resembling Fe deficiency), and brown speckles in the leaf marginal and interveinal area. They also observed a typical symptom of Mn-toxicity, called “cupping leaf” which occurred at extremely high Mn concentration (200 mg L^{-1}), and concluded that Mn preferentially accumulated into the leaf marginal and interveinal area where the brown speckles were observed. In case of severe Mn-toxicity, plant roots turn brown, but generally only after the tops have been severely injured (Foy *et al*. 1978; Foy 1983).

Aluminium-manganese interactions: very little information is available on combined Al + Mn interactions with other nutrients. Manganese-sensitive species, such as rape and other Brassica crops are more susceptible to Mn-toxicity when Al is also present (Hewitt 1948; Bromfield *et al*. 1983a). Bromfield and coworkers (1983a, 1983b) suggested that the increases of available Al (as increases in soil acidity) intensified the toxic effects of Mn on the crop growth (e.g. rape). However, they concluded that extractable Mn was not well correlated (r = 0.20) with Al, probably due to the wide variation in the concentrations of reactive reducible forms of Mn in the soils as compared to potentially available forms of Al.

Culvenor (1985) investigated the interaction of Mn-toxicity with increasing levels of Al in solution culture using two accessions of phalaris (*Phalaris aquatica* L.). His results showed that the presence of Al in the solution strongly decreased the uptake of Mn. The marked reduction in Mn uptake in the presence of Al suggests that, within limits, Al might counter the toxic effects of Mn. Protection by Al against Mn-toxicity was also observed in *Atriplex hastata* (Rees and Sidrak 1961). However, Zhang *et al*. (1999) found no association between Mn- and Al-tolerance in triticale. These apparently conflicting results illustrate our limited understanding of the combined effects of Al and excess Mn.

2.2 Correcting soil acidity problems

Acidification is the result of many processes and finding a solution to the problem has proven difficult. Researchers have tried to prevent, slow or reverse the process using a numerous techniques, e.g. alkaline ameliorants (lime); increased plant tolerance; use of lower input alternatives (lime pelleting of legume seeds); direct application of fertilisers to overcome nutrients deficiencies (Mo, Ca, Mg) from acidity; and avoidance of overliming. There is an awareness that over-liming may induce nutrient deficiencies (e.g. Zn, B, Cu, Mn and Fe) (Helyar 1991).

2.2.1 Liming

Liming is an ancient practice in agricultural soils. Intensive use of lime in the USA started in the 1930s under a subsidy payment program sponsored by the US Federal Government (Adams 1984). About a century ago, lime was considered too expensive to use in Australian agriculture (Cregan *et al.* 1989), but today, liming is recognised as essential for the correction of soil acidity and the improvement of crop production in acidic soils. The main function of liming acidic soils is to decrease Al and Mn-toxicity and more rarely to relieve Ca and Mg deficiencies (Kennedy 1986).

A liming material is defined as one whose Ca and Mg compounds are capable of neutralising soil acidity (Barber 1984). Liming materials include quick lime (CaO), hydrated lime ($Ca(OH)_2$), limestone or calcite ($CaCO_3$), magnesite ($MgCO_3$), dolomite ($CaCO_3.MgCO_3$), marl, shells and byproducts such as slag from blast furnaces, flue dust from cement factories, refuse or ash from sugar beet factories, paper mills and calcium carbide manufacture. The most common liming agent is limestone, ground to an average particle size of ≤ 0.3 mm to achieve rapid reaction (Barber 1984; Kennedy 1986). The effectiveness of liming obviously depends on application methods. Surface applications result in delayed beneficial effects so lime is usually ploughed in (Kennedy 1986).

The overall reaction of lime with an acid soil can be illustrated as (Tisdale *et* *al*. 1985; Sparks 1995):

$$2\text{Al-soil} + 3CaCO_3 + 3H_2O \rightarrow 3\text{Ca-soil} + 2Al(OH)_3 + 3CO_2 \qquad (13)$$

The ultimate products of the above reactions are exchangeable Ca^{2+} and insoluble Al-compounds ($Al(OH)_3$), alleviating both H^+ and Al-toxicity.

Effects of liming: there is a direct effect of using agricultural lime on the availability of Ca and Mg. When lime is added, the availability of Ca and Mg increases as these are usually present in the liming material. Alva *et al*. (1986) reported that, in the presence of Al, the maximum root growth of soybean (*Glycine max* L.) and subterranean clover occurred at the highest concentration of added Ca (15 mM). The positive effect of Ca concentration indicates the protective action of Ca against Al-toxicity on root growth. Kinraide and Parker (1987) suggested that the cation and Al compete with each other for external binding sites to cause amelioration. Ameliorative effects of Ca were also reported in soybean (*G. max* cv. Forrest) (Bruce *et al*. 1988) and in sweet potato (Ila'ava *et al*. 2000b).

pH has a great influence on microbial activity; consequently, it affects mineralisation of organic matter and the availabilities of N, P, S and some micronutrients to plants (Foy 1984). For example, ammonification (formation of NH_4^+ from the decomposition of organic matter) can apparently occur at a wide range of pH's (Chase *et al*. 1968; Alexander 1980), but is most rapid in the pH range 5.0 – 7.5 (Haynes and Swift 1989). The nitrification rate falls quickly with decreasing pH and stops at pH < 4.5 (Dancer *et al*. 1973; Alexander 1980). Liming acidic soils stimulates nitrification (Chase *et al*. 1968; Alexander 1980; Adams and Martin 1982; Haynes and Swift 1989).

Application of lime to acidic soils is generally credited with increasing the availability of P, but this is not always the case (Helyar *et al*. 1976). Murrmann and

Peech (1969) also reported that lime application decreased the amount of extractable phosphate in acid soils, whereas Martini *et al*. (1974) found no change with the increasing lime rates. Also, Hutton and Andrew (1978) reported that despite high liming rates, P availability was adequate for the growth of tropical pasture legumes. These different results can all be explained by taking into account the effect of liming on surface charge and the speciation of the phosphate ion (Barrow 1984; Anjos and Rowell 1987).

In general, the availability of micronutrients such as Fe, Zn, Mn, Cu, and B decreases with the increases of soil pH. Thus, overliming can result in micronutrient deficiencies in many acidic soils (Adams 1984; McLean and Brown 1984). Mo deficiency is likely with a soil pH < 5.5, that is highly weathered, low in organic matter and high in Fe oxides (Adams 1984). Liming soils to a pH of about 6.0 may correct the Mo deficiency.

Liming acidic soils indirectly increases the effective cation exchange capacity (ECEC) of soils that contain organic matter or variably charged clay minerals (Helling *et al*. 1964; McLean and Brown 1984; Thomas and Hargrove 1984). Pratt and Bair (1962) found that the ECEC of acidic soils increased slowly at pH values of around 5.0 but increased very rapidly at pH 8.0.

Lime requirement (LR): the amount of lime needed to change the pH of a soil varies widely between soils. LR has been estimated by many methods (McLean and Brown 1984) but no single method is universally successful because of the complex nature and varying buffering capacity of soils. The most common procedures are probably titration using a base (Adams 1984; Kennedy 1986) and the measurement of degree of saturation of the soil colloids with exchangeable Al (Kamprath 1970; Kennedy 1986;

Cregan *et al*. 1989).

Quality, effectiveness and economics of liming: lime ($CaCO_3$) is sparingly soluble, so the rate of the reaction represented in Eqn 13 can be increased by increasing the specific surface area of the lime (fine grinding) and by improving the distribution of lime through the soil (mixing) (Adams 1984; Barber 1984). Liming materials with particle size greater than 0.7 mm (i.e. sieve designation < 10-mesh) have little effect in alleviating soil acidity (Cregan *et al*. 1986).

One of the best measurements of the quality of liming materials is the neutralising value (NV) (Clements *et al*. 2000). The NV of a material is its ability to neutralise soil acidity. Pure limestone ($CaCO_3$) has a NV value of 100 (Weir 1987; Fenton *et al*. 1996; Clements *et al*. 2000). Dolomitic limestone has a higher NV (108) than calcitic limestone because of the lower atomic weight of Mg (Barber 1984).

Lime has a lower unit cost than chemical fertilisers, but much larger quantities of lime are needed to raise the pH. The cost of liming is relatively high and its use may be economically marginal in extensive Australian farming systems (Hochman *et al*. 1989); however, this situation is not sustainable in the long-term. To complement liming in the management of acid soils, there is a need to explore the selection, identification and use of acid-soil adapted species or accessions (Scott and Fisher 1989; Helyar 1991).

2.2.2 Tolerance of plants species and accessions to acidic soils

The introduction of species or accessions that are adapted to the acidic soil environment is an important strategy to manage soil acidity (Davidson 1987; Dowling *et al*. 1996). Some research has been conducted using perennial or summer-active grasses (e.g. *Austrodanthonia*, *Microlaena* etc.) as well as deep-rooted species (e.g.

chicory) (Davidson 1987; Helyar 1991); however, quantitative research on acid-adapted native perennial species is scarce.

2.2.3 Differences in tolerance among species/accessions

Plant species and genotypes may differ widely in their tolerance to mineral stresses (Foy 1974, 1983, 1984; Adams 1984; Taylor and Foy 1985a; Baligar *et al.* 1987, 1989, 2001; Baligar and Smedley 1989; Scott *et al.* 2001). There are distinguishable interspecific and intraspecific differences in tolerance to factors associated with soil acidity for a range of crops (Foy and Brown 1964; Foy *et al.* 1965, 1967, 1972, 1999; Foy 1983, 1996; Taylor and Foy 1985b, 1985c, 1985d, 1985e, 1985f; Cregan *et al.* 1986; Baligar *et al.* 1988; Scott and Fisher 1989; Bona *et al.* 1991; Mackay *et al.* 1991; Rao *et al.* 1993; Helyar and Conyers 1994; Toda *et al.* 1999; Yang *et al.* 2000; Caradus *et al.* 2001; Kim *et al.* 2001; Neto *et al.* 2001a, 2001b; Scott *et al.* 2001). Some of the differences in Al-tolerance are presented in Table 2.2 (Cregan *et al.* 1986; Duncan 1999b) and Table 2.3 (Helyar and Conyers 1994).

Cregan *et al.* (1986) grouped genotypes into four different categories of Al-tolerance on the basis of % Al saturation of ECEC above which yields were reduced. These include highly sensitive (1-5 % Al), sensitive (5-10 % Al), moderately sensitive (10-20 % Al) and highly tolerant (20-30 % Al). According to this grouping, cocksfoot, some oat cultivars and ryegrasses are highly tolerant compared to others, and there are differences in tolerance between cultivars within species such as ryegrass, oats and triticales (Table 2.2). Fenton *et al.* (1996) and Duncan (1999b) also reported similar groupings of Al sensitivity among different plant species.

Table 2.2. Critical levels and tolerance of some plant species to aluminium

Al-tolerance category	Plant species	Exchangeable Al above which yields are decreased (% Al saturation of CEC)
Highly sensitive	Lucerne, annual medics, barley, buffel grasses, tall wheat grass	1-5
Sensitive	Oilseed rape, phalaris seedlings, wheat, *Austrodanthonia bipartita* (Syn. *Danthonia linkii*), red grass (Wagga), red clover	5-10
Moderately sensitive	White lupins (*Lupinus albus*), some oats, white clover, subclover, tall fescue, Rhodes grass (Pioneer), ryegrasses, some triticales	10-20
Highly tolerant	Some triticales, narrow-leaf lupins, cocksfoot, some oats, cereal rye, *Austrodanthonia racemosa*, kikuyu, *Microlaena stipoides*, Consol lovegrass, *Themeda* spp.	20-30

Source: Cregan et al. (1986); Duncan (1999b)

Helyar and Conyers (1994) reported a wide range of Al- and Mn-tolerance among pasture plants. They found that some pasture cultivars (e.g. Rhodes grasses and Consol lovegrass) were extremely tolerant of both Al (Al_{Ca} 10 mg/L) and Mn (Mn_{Ca} 40 mg/L). They also found that yields of these cultivars were reduced by H^+ only at pH_{Ca} lower than 3.9. They ranked 150 cultivars in order of sensitivity to Al on a soil with high Al but little Mn. According to their ranking, *Austrodanthonia bipartita*, *Agropyron* spp., tall wheatgrass (cv. Tyrell), lucerne (cultivars Nova, Pioneer and Aurora), barrel medic (cultivars Parragio and Parabinga) and white clover (cv. Tamar) are highly sensitive; whereas, *Austrodanthonia richardsonii*, *A. racemosa*, *Microlaena stipoides*, cocksfoot (cultivars Porto and Grasslands Wana), perennial ryegrass (cultivars Yatsyn, Ellet and Concord), lovegrass (cv. Consol), oats (cultivars Carbeen, Echidna, Blackbutt and Mortlock) and kikuyu (cv. Noonan) are very highly tolerant of Al-toxicity (Table 2.3).

Table 2.3. Cultivars arranged in Al-tolerance classes based on estimates of Al concentration in the 1:5, soil: 0.01 M $CaCl_2$ extract that decreased yield to 50% of that without stress imposed by soil acidity

Tolerance class	Cultivars or species	Al_{Ca50} (mg/L)
Very Highly Sensitive (VHS)	*Agropyron* spp., tall wheatgrass (cv.Tyrell), *Austrodanthonia bipartita*, buffel grass (cv. Molopo), white clover (cv. Tamar), red clover (cv. Gr. Hamua), lucerne (cvs Nova, Sequal, Aurora etc.), barrel medic (cvs Parragio, Parabinga), wheat (cv. Worigal), barley (cvs Lara, Schooner)	0.21-0.93
Highly sensitive (HS)	Barrel medic (cv. Parragio), lucerne (cv. WL SS), balansa clover (cv. Paradona), red clover (cv. Redquin), yellow serradella (cv. Eigara), barley (cvs Schooner, O'Connor), wheat (cvs Grebe, Kiata, Vulcan, Matong)	0.94-1.41
Sensitive (S)	Red grass, buffel grass (cvs Biloela, Gayndah), *A. bipartita*, white clover (cvs Haifa, Hula), barrel medic (cv. Cyprus), several cultivars of lucerne, wheat and barley	1.43-2.45
Moderately sensitive (MS)	Phalaris (cvs Sirosa, Sirolan, Uneta, Holdfast), prairie grass (cv. Gr. Matua), Kikuyu (cv. Common), lucerne (cv. Trifecta), white clover (cvs Safari, Tamar), yellow serradella (cv. Tauro), oats (cv. Stout), triticale (cv. Currency), cultivars of wheat and barley	2.60-4.95
Tolerant (T)	Phalaris (cvs Uneta, Australian), Rhodes grass (cv. Pioneer), tall fescue (cv. Demeter), cocksfoot (cv. Currie), subclover (cvs Seaton Park., Goulburn, Junee, Clair, Nungarin), oats (cv. Coolabah), triticale (cv. Currency), barley (cv. Ulandra), cultivars of wheat	5.16-9.76
Highly Tolerant (HT)	Annual ryegrass (cv. Wimmera), perennial ryegrass (cvs Victorian, Kangaroo Valley), kikuyu (cv. Whittet), Rhodes grass (cvs Katambora, Callide), subclover (cvs Karidale, Junee, Woogenellup, Tricala, Dalkeith, Yarloop, Denmark etc.), white clover (cv. Haifa), oats (cv. Cooba), wheat (cv. Muir), cereal rye (cv. Ryesun)	10.15-19.85
Very Highly Tolerant (VHT)	Cocksfoot (cvs Porto, Gr. Wana), perennial ryegrass (cvs Yatsyn, Ellet, Brumby, Gr. Nui), Italian ryegrass (cv. Concord), triticale (cvs Currency, Tahara, Empat, 39E), yellow serradella (cvs Paros, Madeira, Avila), siratro (cv. Siratro), oats (cvs Coolabah, Yarran, Carbeen, Echidna, Blackbutt, Mortlock), cereal rye (cv. 30 B 761), *Austrodanthonia richardsonii*, *A. racemosa*, *Microlaena stipoides*, kikyyu (cv. Noonan), subclover (cv. Rosedale), lovegrass (cv. Consol)	> 30

Source: Helyar and Conyers (1994)

Mn-tolerance can differ between cultivars within plant species as well as between species (Kang and Fox 1980; Foy 1983,1984; Culvenor 1985; Helyar and Conyers 1994; Cregan *et al*. 1986; Fenton *et al*. 1996; Duncan 1999b; Zhang *et al*. 1999; Lidon 2001b). Table 2.4 lists plant species relative to their sensitivity to manganese toxicity (Cregan *et al*. 1986; Fenton *et al*. 1996).

Table 2.4. Tolerance to Mn and critical concentrations of Mn for crop and pasture plants

Tolerance to Mn*	Plant	Critical Mn concentrations** (mg/kg)
Highly sensitive	Lucerne, pigeon pea, barrel and burr medics	200-400
Sensitive	White and strawberry clover, chickpea, canola	400-700
Tolerant	Subterranean clover, cotton, cowpea, soybean, wheat (Matong, Vulcan, Lark, Dollarbird), barley (Yerong, Lara, Schooner), triticale (Empat, Muir, Tahara, O'Connor)	700-1000
Highly tolerant	Soybeans, oats, some wheat, barley, white lupins, *Lotononis* spp., cowpea, peanuts, potatoes, subclover	1000-1500
Extremely tolerant	Rice, cotton, sugar cane, tobacco, sunflower, most pasture grasses, oats, lettuce, triticale (Tiga and Currency), cereal rye, bananas	>1500

** These are general groupings only: varieties within species may vary widely in their susceptibility to Mn.*
*** The critical concentrations of Mn are the concentrations (in the youngest fully developed leaf) that cause a 10% decline in growth.*
Source: Cregan et al. (1986); Fenton et al. (1996)

It is clear from Tables 2.2, 2.3 and 2.4 that Al- and Mn-tolerance differs greatly between different crop and pasture species. However, some species are more sensitive to Al than to Mn and *vice versa*. For instance, white clover is tolerant of Al, but sensitive to Mn (Fenton *et al*. 1996), and Rhodes grass and Consol lovegrass are extremely tolerant of both Al and Mn (Helyar and Conyers 1994).

2.2.4 Mechanisms of adaptation/tolerance

Adaptation to H^+-toxicity: excess H^+ ions compete with other cations for absorption sites on the root, interfere with cation uptake and transport, and damage cell membranes (Christiansen *et al*. 1970; Moor 1974; Islam *et al*. 1980; Foy 1984; Lu and Sucoff 2001). Kidd and Proctor (2001) showed that plants growing on organic acidic soils were tolerant of H^+. They suggested that plant populations exposed to different soil characteristics were separately adapted to H^+ ion toxicity. Accordingly, it is thought that better-adapted genotypes may have greater efficiency to absorb ions and to protect the plasma membrane from the adverse effects of toxic concentration of H^+ ions.

Adaptation to Al-toxicity: the physiology of the mechanisms of Al-tolerance is still controversial (Foy 1984; Kochian 1995). Different genes control the differential tolerance of plant species and varieties through a variety of biochemical pathways (Foy *et al*. 1978; Foy 1983, 1984; Delhaize and Ryan 1995; Kochian 1995; Matsumoto 2000). Al-tolerant plants have the ability either to prevent excess absorption of Al or to detoxify Al once absorbed; consequently, no single mechanism of tolerance accounts for Al-tolerance (Taylor 1988a; Kochian 1995). Mechanisms of Al-tolerance can be categorised into two broad groups: (1) the exclusion of Al entry into the root apex and root hairs, i.e., apoplasmic, exclusion or external tolerance and (2) the sequestration of Al within the cells, i.e., symplasmic or internal tolerance (Taylor 1988a; Carver and Ownby 1995; Kochian 1995; De la Fuente-Martinez and Herrera-Estrella 1999).

(1) Exclusion mechanisms: many hypotheses have been proposed to explain Al-exclusion mechanisms. These include immobilisation of Al at the cell wall, selective permeability of the cell membrane, formation of a plant-induced pH barrier in the rhizosphere, and exudation of chelating ligands (Taylor 1988a). Likewise, Kochian

(1995) proposed four hypotheses *viz*: alteration of rhizosphere pH, low cell wall CEC, Al^{3+} efflux across the plasma membrane and Al-induced release of organic acids from the root apex. Some Al-tolerant cultivars of wheat, barley, rice, peas and corn increased the pH of nutrient solutions and, thus, decreased the solubility and toxicity of Al (Foy *et al*. 1978). On the other hand, Al-sensitive cultivars of the same species decreased pH or did not change pH of nutrient solutions for longer periods after exposure to high concentrations of Al (Foy *et al*. 1978). Differential pH changes were also observed both in thin layers of soils removed directly from plant roots and in bulk soils in pots.

Taylor and Foy (1985d, 1985e, 1985f) conducted a series of experiments that supported the hypothesis of 'Al-exclusion via alteration in rhizosphere pH'. All cultivars of wheat grown with or without Al in solution culture depressed the pH of nutrient solutions, presumably until NH_4^+ was depleted, at which point the pH increased. Cultivar tolerance (expressed as an index of both shoot and root tolerance) was negatively correlated with the pH depression in nutrient solution. Similarly Al-tolerance of *Arabidopsis thaliana* (mutant alr-104) was caused by an Al-induced increase in rhizosphere pH (Degenhardt *et al*. 1998). Most researches have measured the changes in pH of bulk solution in the region of matured roots, but not near the root apex (the primary site of Al-toxicity). For instance, Miyasaka *et al*. (1989) found no difference in the rhizosphere pH near the root apex (pH was measured using microelectrodes) of two cultivars of wheat ('Atlas 66, Al-tolerant' and 'Scout, Al-sensitive') during the initial hours of Al exposure or in the absence of Al. Foy *et al*. (1972) also reported that difference in tolerance of two snapbean varieties (*Phaseolus vulgaris* L.) was not related to differential pH changes in nutrient solutions.

Al-tolerance might be achieved by differential accumulation of Al at the cell wall and reduction of uptake into the symplasm (Taylor 1988a). However, the interaction of Al with cell wall constituents is not clear (Taylor 1988a; Carver and Ownby 1995). A chemical analysis of cell-wall polysaccharides from the roots of squash (*Cucurbita maxima* Duch.) revealed that Al increased pectin, hemicellulose and cellulose contents after 3 h immersion in 1 mM $AlCl_3$ (Le Van *et al*. 1994). Kochian (1995) suggested that cell walls of roots might be a site of Al^{3+} binding and immobilisation, due to negative charges lining with water-filled pores within the cell wall, which could prevent Al^{3+} from associating with the plasmalemma or entering the symplasm. However, there is no experimental evidence to support this model. On the other hand, plant tolerance of Al-toxicity can be favoured by low cation exchange capacity (CEC) of the cell wall (Taylor 1988a; Carver and Ownby 1995; Kochian 1995), which lowers binding of Al within the cell wall. Low root CEC can contribute to Al-tolerance in a number of ways (Taylor 1988a), e.g. preferential accumulation of monovalent cations; decreased amounts of Al on the exchange sites, which may be the first step in ion uptake; and, a relatively low uptake of cations relative to anions. The cell-wall CEC hypothesis is still unproven. If the CEC of the roots is attributed to the free carboxyl groups of pectins located in the cell walls, then root CEC should have little effect on the ion uptake into the cytoplasm (Taylor 1988a). Kinraide *et al*. (1992) concluded that root CEC did play a minor role in differential Al-tolerance in wheat cultivars.

The plasmalemma can act as a selective barrier to Al entry into the cytosol. The exclusion of Al from the symplasm is an energy dependent phenomenon (Taylor 1988a; Zhang and Taylor 1989, 1990; Kochian 1995). The difficulty with this hypothesis is in differentiating between uptake into the apoplasm and symplasm (i.e.

the difference in uptake between the cell wall and the plasmalemma) (Taylor 1988a). The best documented mechanism of Al-tolerance is exclusion based on the release of organic acids from the roots into the rhizosphere that complex Al (De la Fuente-Martinez and Herrera-Estrella 1999). Organic acids (e.g. citric and malic) are good chelates and can be synthesised in large amounts (Larsen *et al*. 1998). Miyasaka *et al*. (1991) found that the roots of an Al-tolerant cultivar of snapbean exposed to 148 µM Al, excreted 70 times as much citric acid as in the absence of Al, and 10 times as much citric acid as an Al-sensitive cultivar grown with or without Al. Kayama (2001) also found that *Miscanthus sinensis* (an acid tolerant perennial grass species) excreted twice as much citrate from its roots to form an Al-chelate, compared with *M. sacchariflorus* (an acid sensitive perennial).

Tolerance of Al due to the excretion of organic acids has been investigated intensively (Ownby and Popham 1989; Galvez *et al*. 1991; Haug and Shi 1991; Rincon and Gonzales 1992; Delhaize *et al*. 1993a, 1993b; Basu *et al*. 1994; Delhaize and Ryan 1995; Pellet *et al*. 1996; Cocker *et al*. 1998; Matsumoto *et al*. 1999; Matsumoto 2000;Yang *et al*. 2000; Kidd *et al*. 2001). For example, Delhaize *et al*. (1993a, 1993b) reported that Al-tolerant genotypes of wheat excreted 5- to 10-fold more malic acid than Al-sensitive genotypes and that the excretion could be detected after 15 min of exposure to Al. They also found that root apices (terminal 2-5 mm of root) were the primary source of the malic acid excretion, and that Al specifically stimulated malic acid excretion.

Some reports implicate condensed tannins, free proline and phenolic compounds in Al-tolerance (Galvez *et al*. 1991; Kidd *et al*. 2001; Ofei-Manu *et al*. 2001; Stoutjesdijk *et al*. 2001). For instance, Stoutjesdijk *et al*. (2001) grew *Lotus pedunculatus* Cav. (an Al-tolerant forage legume) in nutrient solutions (5-60 µM Al).

They showed that Al was deposited close to the root tips at all Al concentrations. Al was generally associated with osmium - binding vacuoles. As osmium has a high binding affinity for condensed tannins, they hypothesised that condensed tannins may bind and detoxify Al in the root apices. Ofei-Manu *et al*. (2001) showed that the Al-tolerance of ten species of common woody plants was positively related to the concentration of phenolic compounds in the roots, but not with the concentrations of exuded phenolic compounds. They suggested that a higher concentration of root phenolic compounds could bind strongly with Al and detoxify Al ions in the cytoplasm.

(2) Internal tolerance mechanisms: internal tolerance mechanisms include chelation in the cytosol, compartmentation in the vacuole, Al binding proteins and Al-tolerant enzymes (Taylor 1988a). Chelation of Al by organic ligands in the cytoplasm could efficiently reduce the activity of Al and thus, its phytotoxic effects (Taylor 1988a; Scott and Fisher 1989). In certain woody Al-accumulator species, Al in the cytosol is complexed by organic molecules without disrupting cell metabolism (Jones 1961; Jackson 1967). Once the Al is complexed, it might remain in the cytoplasm or be deposited elsewhere, e.g., in old xylem vessels or on cell walls (Helyar 1978). Some Al-accumulators, such as tea (*Camellia sinensis* L. Ktze) can tolerate very high Al concentrations (30,000 mg Al/kg) in the tops by using this mechanism (Matsumoto *et al*. 1976).

Al could be sequestered in the vacuole (Taylor 1988a), which is generally considered as a storage as well as a tolerance site of some heavy metals (e.g. Cd, Zn, Pb), supporting evidence about compartmentation of Al into other sites is inadequate.

Plant species can develop Al-tolerance through the synthesis of proteins that chelate Al and, hence, limit its toxicity within the symplast (Carver and Ownby

1995). Aniol (1984) showed that Al-tolerance of cultivars of wheat decreased when cycloheximide (an inhibitor of protein synthesis) was added. Such results imply that the induced detoxification effect is that of an Al-binding protein.

The differential distribution of Al between tops and roots has also been reported as a possible tolerance mechanism (Foy 1974, 1983, 1984, 1988; Foy *et al*. 1978). Al concentrations of Al-tolerant plants may not be consistently different from those of Al-sensitive, but the roots of Al-tolerant plants may contain less Al than the Al-sensitive roots. Al-tolerance is thus associated with low Al concentrations in the tops and entrapment of excess Al in the roots.

In summary, Al-tolerance mechanisms probably comprise a combination of exclusion and internal defences.

Adaptation to Mn-toxicity: Foy *et al*. (1978) and Foy (1983, 1984) suggested that Mn-tolerance is associated with the oxidising power of plant roots, the rate of uptake and translocation of Mn, Mn entrapment in non-metabolic centres, high internal tolerance to excess Mn, and the uptake and distribution of Si and Fe. Scott and Fisher (1989) surmised that plant tolerance to high concentrations of soil Mn may operate by exclusion, by restriction of Mn transport to plant tops, and by tolerance of shoots to high internal concentrations of Mn.

Waterlogging of soils promotes the reduction of MnO_2 to Mn^{2+} (Moraghan 1979) and, hence, plants that show tolerance of waterlogged conditions may tolerate high concentrations of Mn (Foy *et al*. 1978). Some species adapted to waterlogged conditions develop aerenchymatous vascular system in their roots. This system facilitates increased oxygen transportation and, thus, detoxifies excess Mn via transformation of Mn^{2+} to Mn^{4+} (Adams 1984; Marschner 1991). An example of this is rice, which is known to be extremely tolerant of excess Mn (Lidon 2001a, 2001b), and oxidises Mn on its roots (Foy *et al*. 1988). In addition, rice can contain 4000-5000 mg/kg Mn in the shoots without showing any toxicity symptoms (Hannam and Ohki 1988). Vlamis and Williams (1967) also reported that the old leaves of rice could contain 6000 to 7000 mg/kg of Mn with only a small yield depression.

Differential uptake of Mn is sometimes considered an important mechanism of Mn-tolerance. Culvenor (1985) found a lower Mn concentration in a Mn-tolerant cultivar of phalaris than a Mn-sensitive cultivar in both shoots and roots and attributed it to an exclusion mechanism.

Restricted movement of Mn from roots to shoots has also been suggested as a Mn-tolerance mechanism (Scott and Fisher 1989). Culvenor (1985) reported that the Mn-tolerant wheat cultivar (Egret) contained about 3 times greater Mn concentration in the roots than in the shoots with high Mn conditions.

Tolerance of high levels of Mn in the tops of plants is referred to as an internal tolerance mechanism (Scott and Fisher 1989). This mechanism permits plant species to survive and grow vigorously under conditions of high tissue Mn. Gupta *et al*. (1970) found that carrots could grow without showing any yield loss with a Mn tissue concentration up to 2600 mg/kg. Culvenor (1985) also reported that phalaris tolerated Mn concentrations of 700-1000 mg/kg in the older leaves without significant yield loss.

The basis of tissue tolerance to excess Mn may be attributed to - the formation of metabolically inactive organic Mn-complexes; binding to cell walls and/or deposition in vacuoles; or tolerance of some vital enzyme systems to high concentrations of ionic Mn (Scott and Fisher 1989). The tolerance of higher Mn tissue concentration may also be achieved by preventing localised high Mn concentrations by distributing the Mn homogeneously throughout the leaves. Memon *et al*. (1980)

reported that the highest concentrations of Mn occurred in the epidermis, collenchyma and bundle sheath cells in a Mn-accumulator plant species (*Acanthopanax sciadophylloides*). This localisation may be a tolerance mechanism, which keeps Mn away from the key metabolic sites (Foy 1983, 1984). In contrast, Horst (1983) suggested that addition of Si to the nutrient solution might distribute Mn homogeneously in the leaf-tissues of cowpea, reducing the localised symptoms of brown spots (a typical Mn-toxicity symptom). Alam *et al*. (2001) also noted that brown spots on barley leaves could be minimised by increasing the supply of Fe.

2.3 Methods of studying plant tolerance to acidic soils

Screening of plant tolerance to acidic soil environments has been tested in soils either in the field or glasshouse, and in sand or solution culture systems (Scott and Fisher 1989). In addition, rapid screening techniques, such as hematoxylin staining, have been used (Polle *et al*. 1978; Crawford and Wilkens 1998; Giaveno and Filho 2000).

2.3.1 Field screening

Most acidic soils may be deficient in macro- and micro-nutrients and have excess Al and Mn. Therefore in field screening, care must be taken to isolate the factors associated with soil acidity. In the field, addition of lime and sulfur and/or sulfuric acids are normally used to achieve a pH gradient (Kang and Fox 1980; Howeler 1991). Cultivars are then grown to assess their relative growth over this pH gradient. This technique has been used for rice (Mahadevappa *et al*. 1991), tomato (Coltman and Kuo 1991), wheat (Scott and Fisher 1989) and barley (Gallardo *et al*. 1999) to evaluate cultivars susceptibility to acidity.

The advantage of this technique is that it screens cultivars under natural soil and climatic conditions, and over the entire growth cycle. The disadvantages include: the time taken, the requirement for a large uniform area, and the effect of uncontrolled environmental hazards such as diseases and insects, or damage due to lodging, birds and wild animals on the results (Howeler 1991).

2.3.2 Glasshouse screening using soils

Screening plants in the glasshouse using soil is an alternative to field screening (Scott and Fisher 1989). Foy (1976) discussed the principles of using soils as screening media for Al and Mn-tolerance. The problems associated with this technique are to characterise reproducible range of single stress of either Al- or Mn-toxicity, or both without causing other nutritional deficiencies (e.g., Ca, P, Mo and Mg). Success using this approach has been varied (Foy 1984; Ring *et al*. 1993; Helyar and Conyers 1994).

2.3.3 Screening using nutrient solutions

The culture of plants experimentally in soil-less media has become one of the most useful approaches for the study of physiological phenomena with particular emphasis on root activity and function (Hoagland and Arnon 1938, 1950; Asher and Edwards 1983; Parker and Norvell 1999). Plants are generally cultured with their roots immersed completely in the solution, or grown an inert medium such as sand or gravel through which the nutrient solution is perfused; or mist or aeroponic culture in which, the roots are suspended in moist air and sprayed with fine droplets of nutrient solution (Parker and Norvell 1999).

The main advantages of nutrient solution techniques are that they provide a well-defined, homogeneous medium with exact control of the stress factor (e.g. Al and Mn) as well as pH and other nutrients (Howeler 1991; Parker and Norvell 1999) and permit ready examination and recovery of the roots (Parker and Norvell 1999). Several factors that need to be considered to ensure a successful solution culture experiment (Scott and Fisher 1989) include pH control; adequate concentrations of P, Ca, Mg and Fe; and temperature. The disadvantages are that the method does not take into account the effects of other soil factors like diffusion and vesicular arbuscular mycorrhiza (Howeler 1991). Therefore, the results obtained in nutrient solutions do not always correspond with those obtained in soils.

Despite differences, studies conducted in both nutrient culture and soil have shown similar cultivar rankings for acid tolerance of wheat (Foy *et al*. 1965), barley (Macleod and Jackson 1967), cowpea (Horst 1983), phalaris and cocksfoot (Culvenor *et al*. 1986b) and sorghum (Furlani *et al*. 1991).

2.3.4 Rapid screening methods

The rapid methods used to test Al-tolerance include the Al-pulse technique (Moore *et al*. 1976); hematoxylin stain (Polle *et al*. 1978) and peroxidase stain (Scott and Fisher 1989). These techniques may take only 2-3 days but suffer from the disadvantage that the expression of tolerance at the seedling stage may differ from that at other growth stages (Hanson and Kamprath 1979).

Al-pulse: Moore *et al*. (1976) first used this method to screen wheat cultivars. They placed pregerminated seeds of each cultivar in various nutrient solutions with increasing concentrations of Al for 42 hours. After this period, the roots were carefully washed and seedlings were placed in a non-Al-toxic nutrient solution for another 72 hours. After that, they observed the new root growth to determine the lowest Al concentration that caused irreversible inhibition of cell division. A similar technique was also used to screen rice germplasm for Al-tolerance (Martinez 1976).

Hematoxylin stain: this technique is widely used for the visualisation and localisation of Al in root tissues. It is a useful approach to detect Al accumulation in the root tips by the formation of an intense blue coloration. The reaction occurs through the oxidation of hematoxylin to hematyn in the presence of $NaIO_3$. The hematyn produces nucleic acid coloration with Al (Polle *et al*. 1978). The biological basis of this overall reaction is that, in Al-sensitive cultivars, hematoxylin forms complexes with Al, which precipitate with phosphate in intercellular spaces (Ownby 1993). Using this technique, Polle *et al*. (1978) successfully grouped wheat cultivars for Al-tolerance. Wallace *et al*. (1982) and Carver *et al*. (1988) confirmed the reliability of the hematoxylin technique for wheat cultivars. A few standard cultivars should also be evaluated in each batch to standardise the tolerance scoring. Hematoxylin staining has been used to screen cultivars of perennial ryegrass (*Lolium perenne*) (Bennet 1995), native perennial grasses (*Austrodanthonia bipartita* and *Microlaena stipoides*) (Crawford and Wilkens 1998), barley (Bona and Carver 1998), maize (Cancado *et al*. 1999; Giaveno and Filho 2000) and sorghum (Yoshida and Yoshida 2000).

Peroxidase stain: Scott and Fisher (1989) developed a modified version of the hematoxylin stain test (Polle *et al*. 1978) and an enhancement of the approach described by Moore *et al*. (1976). In this approach, roots of germinated seedlings are exposed to Al solution for 48 hours with the aim of causing irreversible damage (i.e. death) of root apices in sensitive cultivars. At the completion of the Al exposure, the roots are exposed to a peroxidase stain, then floated on a recovery solution (nil Al) for three days. The measurement of tolerance is then obtained by visual scoring. Death of root apex occurs in the sensitive cultivar (as shown by the stained root tip) but not in the tolerant cultivar.

To investigate Al-tolerance, efforts have been made to conduct studies at the cellular level. Kochian and Shaff (1991) used a highly sensitive extracellular vibrating microelectrode system to map the ion-current patterns surrounding root apices. Bennet and Breen (1991b) and Rengel *et al*. (1995) demonstrated an ultrastructural investigation of the stages involved in recovery in Al-free solution showing that the resumption of root elongation rates during recovery coincided with the presence of a morphologically distinctive secretory activity in the peripheral cells of the root cap. They used this signal to detect root cap changes due to Al-toxicity. Furthermore, Delhaize *et al*. (1991) used two-dimensional gel electrophoresis to measure proteins, and Stass and Horst (1995) used the cell suspension-culture to estimate callose formation on the root apices as indicators of Al effects. However, the above techniques of rapid screening are still in their early stages of development.

2.4 ***Austrodanthonia*** **species**

There is a long-held general view that Australian native grasses are not well adapted for grazing and that they are inferior to exotic species (Whalley 1970). Donald (1970) and Wolfe (1972) concluded that native grasses are incapable of high levels of production in the temperate zone of Australia. However, the comparisons on which these conclusions were based may have been flawed. The exotic species were heavily fertilised whereas the native pastures received no or little fertiliser. In most cases, native pastures were set-stocked for longer periods than the introduced pastures, with no attempt being made to remove dry herbage accumulated after summer before the commencement of grazing (Johnston *et al*. 1999). In addition, a longer-term comparison may have indicated a tendency for the exotic sown pastures to decline in persistence (Kemp and Dowling 1991; Garden *et al*. 1996; Garden *et al*. 2000; Lodge 2000; Johnston *et al.* 2001; Mitchell *et al*. 2001). Finally, Jones (1996) has suggested that many comparisons of native and exotic grasses have compared plants of different ages, with recently sown exotic plants being compared to native plants which were several years old.

There is, however, increasing evidence of the value of native grasses for grazing. Robinson and Archer (1988) and Archer and Robinson (1988) conducted experiments comparing the productivity and forage quality of native grasses from the New England tablelands of New South Wales and introduced species, with the same rate of fertiliser and irrigation. They found that the average seasonal growth of two highly regarded native temperate C_3 grasses (*Microlaena stipoides* (Labill.) R. Br. and *Austrodanthonia bipartita* (Link) H.P. Linder (Syn. *Danthonia linkii* var. *linkii* Kunth)) and two common native C_4 grasses (*Bothriochloa macra* (Steud.) S.T. Blake and *Themeda australis* (R. Br.) Stapf) relative to the introduced perennial *Phalaris aquatica*, were more productive than phalaris from late spring to autumn, whereas phalaris was more productive during winter only. They also demonstrated that the year-long green C_3 grasses *A. bipartita* and *M. stipoides* were in general more nutritious than C_4 grasses, and that the yield of the introduced *Phalaris aquatica* declined during the 3-years of the study. Similar results were reported by Robinson and Whalley (1991) and Robinson (1993).

There is increasing interest in the development of Australian perennial native grasses for agricultural purposes, particularly to decrease the degradation of hill-slopes and to lower the rates of soil acidification and dryland salinisation. Therefore, it is important that the pasture systems should contain a combination of C_3 and C_4

species, which are capable of using water and providing ground cover at critical times of the year, e.g. summer (Johnston 1996; Johnston *et al.* 1999). The role and potential of native grasses were assessed by Dowling and Garden (1991), Reid (1995), and Lodge and Sutherland (1996). Johnston *et al.* (1999) assembled a wide range of potentially useful species and genera, and selected five potential cultivars for release (Mitchell *et al.* 2001). Because of such releases, there is an urgent need to evaluate inter- and intra-specific differences in traits of native grasses including: persistence, productivity, palatability, forage quality, tolerance of drought and soil acidity, as well as the potential to decrease soil erosion and water movement (Garden *et al.* 1996; Johnston *et al.* 1999). Tolerance of acidity is likely to be an important attribute for such grasses, as many of the areas where they are likely to be grown have acidic soils (Simpson and Langford 1996).

Among the perennial native grasses, *Austrodanthonia* (wallaby grass) is widely regarded as a productive genus (Archer and Robinson 1988; Robinson and Archer 1988; Dowling *et al.* 1996; Mitchell 1996; Garden *et al.* 2001b). *Austrodanthonia* spp. were originally part of the *Danthonia* genus, which had about 150 species worldwide (Wheeler *et al.* 1990). Distribution is extensive, although mainly in the Southern Hemisphere (Cashmore 1932). The original *Danthonia* genus was cosmopolitan and was found in the temperate areas of Australia, New Zealand, South Africa, South America, North America, Europe and Asia (Breakwell 1923; Cashmore 1932). Recently, the whole *Danthonia* genus was revised and most of the 33 Australian species were moved to a new genus, *Austrodanthonia* (Linder and Verboom 1996; Linder 1997). *Austrodanthonia* spp. are well adapted to conditions of low fertility, and are common in areas where soil fertility, rainfall or temperature limit productivity (Cashmore 1932). In New South Wales, the genus is common on the slopes and tablelands (Garden *et al.* 2001b) and species are considered to be important grasses, due to their high tolerance of frost and good winter growth - a valuable attribute for the sheep and cattle industries (Breakwell 1923).

Austrodanthonia spp. are fine-leaved grasses with a tussocky growth habit. They are characterised by the presence of white hairs on the floral parts, resulting in the common names of silver-top, white-top or fluffy-top (Cashmore 1932). The grass has long hairs on the ligule, and the lamina is folded in the bud with parallel-thickened lines on the leaf blade, characteristics that assist in identification of the genus (Mitchell 1996). The presence and pattern of hairs on the lemma assist in identification to species level (Wheeler *et al.* 1990). The *Danthonia/Austrodanthonia* genera are polyploid in nature worldwide (i.e. somatic chromosomes 2n = 12, 24, 36, 48, 72, 96 and 120) (Abele 1959). In a cytological study on genus *Austrodanthonia* (Australian 28 species), Abele (1959) recorded somatic chromosomes counts of 24, 42, 48, 72 and 96. Among the species tested, two species displayed intraspecific polyploidy, *viz*. *A. caespitosa* (2n = 24, 48 and 72) and *Notodanthonia longifolia* (Syn. *D. longifolia*, 2n = 24 and 48). Only one species had 2n = 96 chromosomes (*A. procera*) and no Australian species had 2n = 12 or 36 chromosomes. Increasing levels of polyploidy were associated with increased hairiness of the lemma, which could be a useful trait in species identification (Abele 1959). The taxonomy of *Austrodanthonia* is problematic, but new methods may improve identification (Garden *et al*. 1996).

Being native to Australia, *Austrodanthonia* spp. are well adapted to Australian conditions. They have the ability to tolerate drought (Breakwell 1923; Rivelli *et al*. 2001) and acidic soils (Robinson *et al*. 1993; Dowling *et al*. 1996; Garden *et al*. 2001a). They persist well under high grazing pressure (Cashmore 1932; Robinson and

Dowling 1976) and respond favourably to irrigation (Breakwell 1923; Robinson and Archer 1988; Archer and Robinson 1988) and added fertilisers (Hodgkinson 1976; Robinson 1976; Robinson and Archer 1988; Archer and Robinson 1988; Pinkerton and Randall 1994; Simpson and Langford 1996; Bolger and Garden 1999a, 1999b). Digestibility of *Austrodanthonia* is comparable to some introduced pasture species such as phalaris and fescue (e.g. 45-74%) (Archer and Robinson 1988), and crude protein content may also be high (10-17%, Lodge and Whalley 1989). While *Austrodanthonia* spp. clearly grow under low pH conditions (Dowling *et al.* 1996; Garden *et al.* 2001a), there is a lack of knowledge of inter- and intra-specific tolerance to low soil pH and associated high availability of Al and Mn.

There may be many valuable species of *Austrodanthonia*, but very little is known about their individual attributes. When this information is available, improved types of *Austrodanthonia* may be obtained by selection and breeding. In this way, highly productive and acid tolerant pasture species may be developed.

2.5 Conclusion and research opportunities

Soil acidity is a major concern, particularly in New South Wales. Although, toxic concentrations of Al, Mn and, to some extent H^+, are the main factors causing depressed plant growth and yield, some nutrient deficiencies such as Ca, Mg, P and Mo may also occur with decreasing soil pH. The agricultural practices imposed on Australian soils, which are generally acidic and poorly buffered against pH change, have enhanced the natural rate of decline in soil pH.

Liming is justifiably claimed to be the most effective way to deal with soil acidity, but problems linked with liming (e.g. subsoil acidity, inaccessibility of land to ground equipment and cost) make this method unsuitable in many instances.

Therefore, there is an urgent need to find complementary ways to deal with soil acidity. Introducing acid tolerant genotypes in the affected areas may be one option. This can be achieved by developing acid tolerant (especially Al- and Mn-tolerant) cultivars through long-term breeding programs. However, the mechanisms of plant adaptation to acidic soil environments are not yet clearly understood, and improved knowledge of this aspect will be necessary to improve plant-breeding programs.

Another approach to dealing with acidic soils is to use cultivars or species that are already present in these different and marginal environments. There are several native species that could reverse or slow the rate of soil acidification. Acid tolerant perennial grass species (e.g. *Austrodanthonia*, *Microlaena*) may be useful in such situations. In addition, some acid tolerant grasses may be used as a genetic resource for breeding more valuable crop and pasture species that are tolerant of toxic concentrations of Al, Mn and H^+. However, there is a scarcity of information on genotypic tolerances of native species to acidic soil conditions. Therefore, there is a need to conduct studies aimed at gathering information on the tolerance of some native species and their accessions to factors associated with soil acidity. Consequently, this project aimed to obtain this information for a range of species and accessions of the genus *Austrodanthonia*.

Chapter Three

Principles and practice of adjusting soil pH for pot experiments*

3.1 Introduction

Usually the pH of acidic soil is raised by adding lime. The amount of lime required (LR) to obtain a target pH depends on the increase in pH required (i.e. target pH minus the present pH), the pH buffering capacity, and the texture of the soil (Martini *et al*. 1974; Kamprath 1984; Fenton *et al*. 1996; Clements *et al*. 2000). The LR of an acid soil can be determined by different titration and buffer methods (Chapter 2, Section 2.2.1). However, there is debate about the accuracy of these methods (Conyers *et al*. 2000; Tsakelidou 2000), and no one procedure is preferable for all soils due to enormous variations in soil properties (Jackson and Reisenauer 1984; McLean and Brown 1984; Thomas and Hargrove 1984; Tsakelidou 2000). Reliable as well as rapid prediction of the LR for agricultural acid soils is important (in conjunction with measures of extractable and exchangeable Al) for the interpretation of plant responses to soil acidity and its correction by liming. Although many techniques have been used to measure soil titration curves, limited information is available about the suitability of the methods used (Barrow and Cox 1990). Thus a simple, rapid and accurate method for predicting LR is needed, especially for routine use in pot experiments.

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* Part of the work presented in Chapter 3 has appeared in a refereed conference proceedings: Islam MA, Milham PJ, Conyers MK, Dowling PM, Jacobs BC, Garden DL (2000) A method of acidifying soil for pot trials. In 'Proceedings of the 4th International Conference on Soil Dynamics'. pp. 169-173. (University of South Australia: Adelaide, South Australia)

There is also no consensus on the best method for acidification. Methods used include direct acidification by mineral acids (e.g. HNO_3, HCl or H_2SO_4) or hydrolysis of salts of trivalent metal ions (e.g. Al and Fe), and indirect, biologically mediated acidification (e.g. through oxidation of NH_4 or S) (Kennedy 1986; Brady 1990). The biological reactions are slower than the reactions with mineral acids, but the acids may cause some artefacts, e.g. salinity and non-equilibrium conditions for Al, Fe and Mn ions (Helyar and Porter 1989). Hydrolysis of trivalent metal ions such as Al and Fe is rapid and produces compounds which may occur naturally in soils. Moreover, the use of Al rather than Fe III salts should avoid extraneous redox effects (Kennedy 1986).

This study assesses simple methods of adjusting soil pH for pot experiments using a sandy loam soil. The procedures developed here are applied in Chapter 4.

3.2 Materials and methods

Soils

A bulk sample (3000 kg) of the A-horizon (0-20 cm) of an acid sandy loam was collected from a commercial grazing farm near Binnaway, NSW ($31^0$31’S, $149^0$17’E, elevation 460 m). The soil was naturally acidic and was similar to the soil used by Helyar and Conyers (1994). Some physical and chemical properties of the soil are presented in Table 3.1. The collected soil was air-dried, crushed and sieved through a 10 mm mesh to remove stones and plant debris. Batches of about 100 kg of the soil were mixed using a cement mixer.

Methods

a. *Acidification*: to obtain the desired pH_{Ca} (3.89), a sub-sample was drawn from the mixed soil, crushed to pass a 2 mm mesh and oven-dried (70^o C). Aliquots of the

Table 3.1. Some properties of the surface soil (0-20 cm) from Binnaway

Properties	Units	Analytical results
Physical[a]		
Coarse sand (0.2-2.0 mm)	(%, w/w)	52.5
Fine sand (0.02- 0.2 mm)	(%, w/w)	26.9
Silt (0.002-0.02 mm)	(%, w/w)	6.0
Clay (< 0.002 mm)	(%, w/w)	14.0
Texture[b]		Sandy loam
Chemical		
pH_{Ca}[c]		4.35
Organic matter[d]	(%, w/w)	2.1
Exchangeable cations[e]	(cmol(+) kg^{-1})[f]	
Ca		0.3
Mg		0.1
Na		0.1
K		0.1
Al		0.2
Mn		0.02
Ca + Mg + Na + K + Al + Mn = ECEC		0.8
% Ca[g]		37.5
% Mg[g]		12.5
% Na[g]		12.5
% K[g]		12.5
% Al[g]		25.0

[a] *(Loveday 1973)*
[b] *Textural class was ascertained using USDA textural triangle*
[c] *The soil pH was determined in a 1:5 (w/v) soil:10 mM $CaCl_2$ (Rayment and Higginson 1992)*
[d] *Walkley and Black (1934)*
[e] *Gillman and Sumpter (1986)*
[f] *Previously meq/100 g (Fenton et al. 1996)*
[g] *= (Exchangeable cation of each element x 100) / ECEC*

ground, dry soil (10 g) were weighed into plastic bottles (200 mL capacity) and each of the following amounts of aluminium sulfate ($Al_2(SO_4)_3 . 18H_2O$): 0, 10, 20, 40, 60 and 80 (mg) was allocated randomly to six bottles. At each treatment level, calcium chloride (10 mM, 50 mL) was added to three bottles and water (50 mL) to another three, that is, the design was completely randomised and replicated three times. The bottles were capped and shaken end-over-end at 30 rpm for 72 h at room temperature

(22°C). Aliquots of the suspensions (10 mL) were withdrawn after 16, 32, 48 and 72 h. Electrical conductivity (EC) was measured using the suspensions in water. pH of the suspensions was measured in 10 mM $CaCl_2$ (Milham 1987). Soluble Al was measured colorimetrically at 575 nm λ in the supernatant of the 10 mM $CaCl_2$ suspensions after reaction with pyrocatechol violet (Conyers *et al.* 1991).

A non-linear equation was fitted to the relation between aluminium sulfate addition and pH_{Ca}. This equation was used to estimate the quantity of aluminium sulfate required to achieve a pH_{Ca} of 3.89 and the estimated quantity was added to 2 kg of soil (three replicates). The moist soil was wet to field capacity, mixed and placed in pots where it was allowed to stand for 48 h. Soil was then leached to reduce EC. A linear equation was fitted between the volume and EC of the leachate. This equation was used to predict the volume of leachate required to reduce EC to ≤ 0.4 dS/m.

Soil was loaded into the cement mixer in 100 kg batches, then the estimated amount of aluminium sulfate (380 g) was added and mixed for about 7 min. Water was added to field capacity, determined under natural drainage for 48h (McIntyre 1974), and the wet soil was mixed for another 7 min. Wet soil was tipped into plastic-lined wooden trays (1 m x 1 m x 0.1 m deep) and the trays were stacked in a shed (day/night temperature 25/12°C) until the soil had dried (~3weeks). The dry soil was recrushed (< 2 mm), mixed by coning and quartering, then placed into bottom-drained plastic pots (2 kg/pot). The EC of the soil was adjusted by leaching with the estimated amount of water. During the next six months, three pots of acidified soil and three of the control were subjected to three wetting /drying cycles in the laboratory, and 260 pots of each treatment were placed outdoors (Fig. 3.1), where *Austrodanthonia* spp. were grown in most of the pots. On five occasions, i.e. 0, 1, 2, 4 and 6 months after potting, the soils in the same three pots from each pH and storage treatment were sampled. The samples were dried and analysed for pH_{Ca}, EC and soluble Al following the same procedures described before.

Fig. 3.1. Pots with amended soil kept outdoors; *Austrodanthonia* spp. were grown in most of the pots.

b. The LR: lime requirement was measured using $CaCO_3$ where the aim was to obtain a target pH_{Ca} of 5.2. Aliquots of 10 g dry soil were weighed into plastic bottles (200 mL capacity) and base ($CaCO_3$, 93% finer than 250 µm) was added at the following rates: 0, 2.5, 5.0, 10.0, 15.0, 17.5, 20.0, 50.0 and 100.0 (mg). Calcium chloride (10 mM, 50 mL) was added to each bottle. The treatments were assigned randomly and replicated three times. The design was completely randomised. The bottles were capped tightly and shaken end-over-end at 10 rpm for 96 h at room temperature (22°C). Aliquots (10 mL each) of the suspensions were withdrawn after 24, 48, 72 and 96 h. Measurements and all other practices (except leaching of soil) are described in method a (see above).

A nonlinear equation was fitted to the relation between calcium carbonate addition and pH_{Ca}. This equation was then used to estimate the quantity of calcium carbonate needed to achieve a target pH_{Ca} of 5.2. The soil was loaded into the cement mixer in 100 kg batches, then the estimated amount of calcium carbonate (48 g) was added and mixed with the soils as described for acidifying soils in the previous section.

Data analysis

Relations between pairs of variables were analysed using non-linear and linear regression (Genstat 5 release 4.2). The values in parentheses that follow the coefficients in the equations are the standard errors of the coefficients.

3.3 Results and discussion

Increments of aluminium sulfate caused a progressive decline in pH_{Ca} towards a limiting value of ~3.6 (Eqn 3.1, Fig. 3.2):

$$pH_{Ca} = 3.65\ (\pm\ 0.02) + 0.70\ (\pm\ 0.03) \times [0.96\ (\pm\ 0.004)]^{\text{aluminium sulfate}} \quad (3.1)$$

The pH_{Ca} limit occurs due to the pK_a value (Perrin 1982) for the initial hydrolysis of aluminium ion in aqueous solution as follows:

$$Al^{3+} + HOH = [Al\,(OH)]^{2+} + H^{+} \quad (pK_a \approx 5.0) \qquad (3.2)$$

From Eqn 3.1, the quantity of aluminium sulfate required to adjust 10 g of soil to pH 3.89 is 40 mg (oven-dry basis) or 380 g /100 kg soil (assuming soils with 5% moisture). Addition of this concentration of aluminium sulfate, followed by thorough mixing resulted in a pH_{Ca} of 3.86.

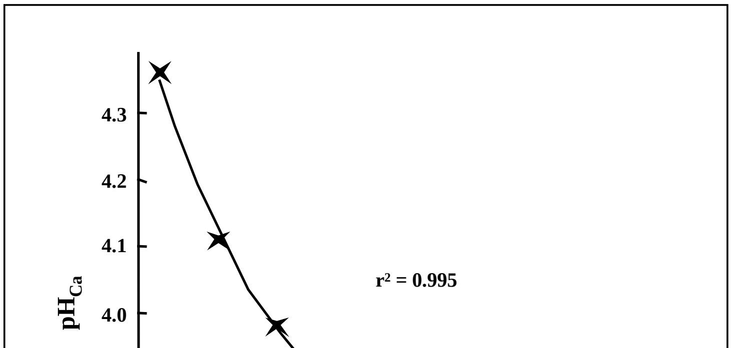

Fig. 3.2. The pH of 1:5 (w:v) suspensions of Binnaway soil in 10 mM $CaCl_2$, with various additions of aluminium sulfate. The fitted line is defined by Eqn 3.1.

The amended soil had an EC of ~ 0.61 dS/m (1:5, w:v), i.e. it was too saline for the unimpeded growth of most plants (Ayers 1977). Leaching was used to reduce the EC. The relationship between the volume of water leached through the amended soil (pH_{Ca} 3.86) and the EC of a 1:5 (w:v) suspension is given by Eqn 3.3:

$$EC = 0.603\ (\pm\ 0.002) - 0.180\ (\pm\ 0.001) \times (\text{L water/kg soil}) \qquad (3.3)$$

A linear equation was used because the curvature was slight ($r^2 = 0.994$, Fig. 3.3).

The desired EC was ≤ 0.4 dS/m. Based on Eqn 3.3, a conservative value of 0.37 dS/m was chosen, corresponding to 1.3 L of water/kg soil (Fig. 3.3). The pH_{Ca} of the leached soil was 3.94, i.e., slightly above the target pH.

Incremental additions of calcium carbonate resulted in a gradual increase in pH_{Ca} towards an equilibrium value of ~ 6.5 (Eqn 3.4, Fig. 3.4).

$$pH_{Ca} = 6.78\ (\pm\ 0.05) - 2.52\ (\pm\ 0.07) \times [0.91\ (\pm\ 0.006)]^{\text{calcium carbonate}} \qquad (3.4)$$

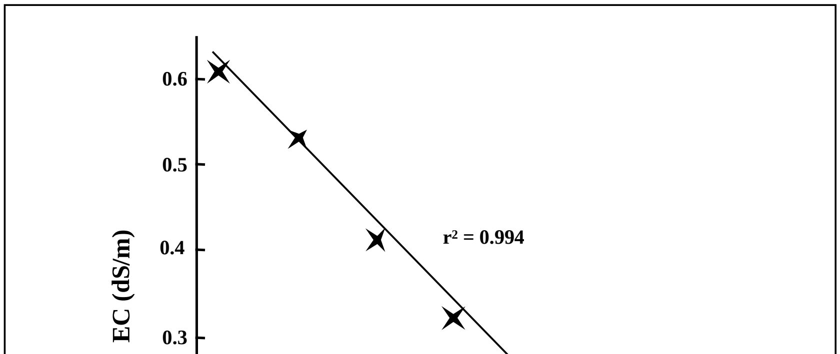

Fig. 3.3. The EC of 1:5 (w:v) suspensions of acidified Binnaway soil in water, as affected by leaching of the soil with water. The fitted line is defined by Eqn 3.3.

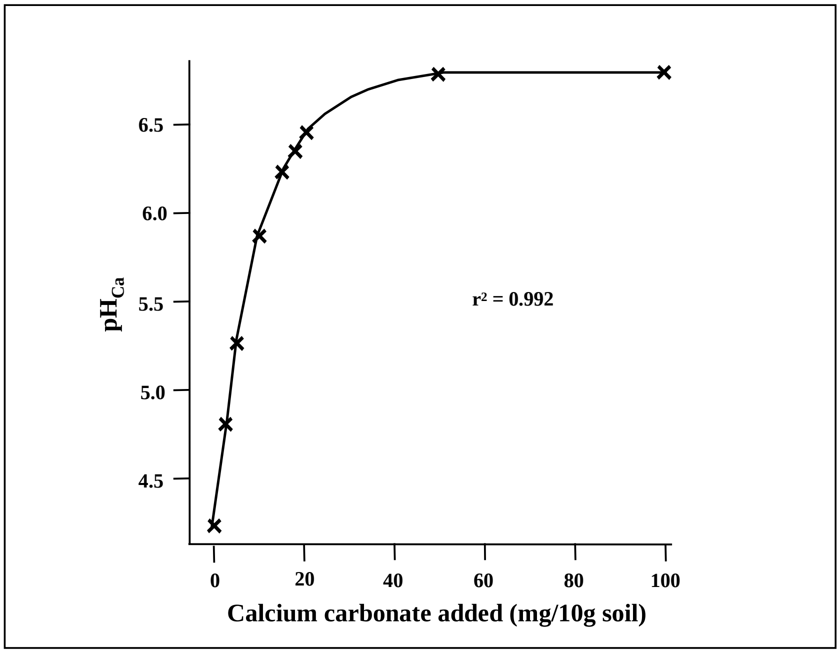

Fig. 3.4. The pH of 1:5 (w:v) suspensions of Binnaway soil in 10 mM $CaCl_2$, with various additions of calcium carbonate. The fitted line is defined by Eqn 3.4.

The pH limit occurs due to the pK_a value (Perrin 1982) for the carbonic acid in the aqueous solution as follows:

$$CO_3^{2-} + H^+ \rightleftarrows HCO_3^- \qquad (3.5)$$

$$HCO_3^- + H^+ \rightleftarrows H_2CO_3\ (pK_a = 6.35) \qquad (3.6)$$

$$H_2CO_3 \rightleftarrows H_2O + CO_2\uparrow \qquad (3.7)$$

The equilibrium condition was virtually observed after 48 h of continuous shaking (Table 3.2). The main reactions that buffer the soil pH in the range of pH_{Ca} 3.5 to 6.5 are considered to be the neutralising of Al^{3+} and of H^+ dissociated from pH dependent cation exchange sites (Ritchie 1989; Cregan *et al.* 1989; Conyers *et al.* 1995; Helyar *et al.* 1995; Conyers *et al.* 2000). In acidic soils, the concentration of the H^+ in the solution is related to the hydrolysis of Al^{3+} (Eqn 3.2) or hydroxy-Al or hydroxy-Fe ions (Tisdale and Nelson 1975). As increasing amounts of base (calcium carbonate) are added to the soil system, the hydrolysis reaction continues with more and more of the adsorbed Al being neutralised and replaced on the soil colloid with the cation of the added base (Ca). As a result, a gradual increase in the soil pH occurs.

The quantity of calcium carbonate required to adjust 10 g of soil to pH_{Ca} 5.2 was estimated from Eqn 3.4. The amount was 5 mg (oven-dry basis) i.e. 48 g/100 kg soil (soils with 5% moisture). Adding this amount (48 g) of calcium carbonate, followed by thorough mixing resulted in a pH_{Ca} of 5.16 (Table 3.3). The lower than expected pH was probably due to the less intimate contact of the soil with calcium carbonate (Dunn 1943).

Table 3.2. pH_{Ca} of the soil shaken with different concentrations of calcium carbonate after different intervals

Data are means of three replications and the values in parentheses are standard errors

Calcium carbonate additions (mg/10 g soil)	Shaking interval (h)			
	24	48	72	96
0	4.38 (± 0.015)	4.37 (± 0.015)	4.36 (± 0.009)	4.35 (± 0.025)
2.5	4.73 (± 0.003)	4.70 (± 0.028)	4.67 (± 0.038)	4.66 (± 0.039)
5.0	5.20 (± 0.003)	5.19 (± 0.000)	5.19 (± 0.003)	5.21 (± 0.027)
10.0	5.78 (± 0.065)	5.88 (± 0.031)	5.89 (± 0.028)	5.89 (± 0.023)
15.0	6.17 (± 0.006)	6.27 (± 0.006)	6.31 (± 0.021)	6.36 (± 0.007)
17.5	6.30 (± 0.003)	6.38 (± 0.022)	6.41 (± 0.042)	6.50 (± 0.006)
20.0	6.36 (± 0.034)	6.46 (± 0.046)	6.54 (± 0.038)	6.54 (± 0.033)
50.0	6.68 (± 0.026)	6.71 (± 0.015)	6.75 (± 0.018)	6.75 (± 0.017)
100.0	6.71 (± 0.018)	6.77 (± 0.007)	6.79 (± 0.003)	6.80 (± 0.003)

A wide range of soluble Al concentrations (1:5, w:v, 10 mM $CaCl_2$) is accessible using the preceding techniques. For example, the acidified, leached soil had a pH_{Ca} value of 3.94 which corresponded to a soluble Al concentration of 52 mg/kg soil. The natural soil had a pH_{Ca} of 4.4 and a soluble Al concentration of 4.2 mg/kg soil, and the limed soil had a pH_{Ca} of 5.2 and a soluble Al concentration of 1.8 mg/kg soil (Fig. 3.5). These results are consistent with previous work, which shows that increasing the pH of an acidic soil decreases Al solubility by the formation of polymers (White 1980) and the neutralisation of Al (Kamprath 1970).

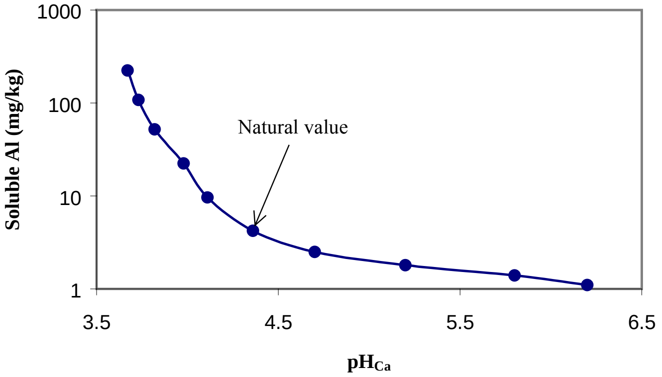

Fig. 3.5. Relation between soluble Al ($\log_{10}$ scale) and pH_{Ca} in soil amended with different concentrations of aluminium sulfate (Fig. 3.2) and calcium carbonate (Fig. 3.4). The line was fitted by inspection.

In the 6 months following the treatments, the pH_{Ca} of the soils in pots gradually increased, whether the soil was subjected to wetting/drying cycles in the laboratory or was stored outdoors, with or without plants (Table 3.3). The rise in pH_{Ca} for soils stored in the laboratory can be attributed to gradual chemical equilibration (Eqn 3.5 - 3.7). The larger rise in pH_{Ca} for soil that was outdoors, without plants, includes the additional effect of leaching due to rainfall (~ 390 mm). Finally, where plants were grown, the rise in pH_{Ca} was greatest, presumably due to the added effect of selective ion uptake and the excretion of organic acids by the plants (White 1980; Kennedy 1986; De la Fuenta-Martinez and Herrera-Estrella 1999).

Table 3.3. Temporal changes in soil pH under different conditions

Data are means of three replications

Treatments	Conditions	pH_{Ca} (months after treatment)				
		0	1	2	4	6

Control	Wetting/drying in the laboratory:	4.36	4.44	4.53	4.60	4.70
	Outdoors: no plants	4.37	4.45	4.60	4.70	4.70
	Outdoors: with plants	4.37	4.54	4.55	4.70	4.73
	Standard errors	0.004	0.004	0.002	0.000	0.004
Acidified	Wetting/drying in the laboratory:	3.94	3.98	4.00	4.03	4.10
	Outdoors: no plants	3.94	3.99	4.06	4.10	4.18
	Outdoors: with plants	3.94	3.99	4.07	4.19	4.20
	Standard errors	0.002	0.002	0.004	0.005	0.001
Limed	Wetting/drying in the laboratory:	5.16	5.18	5.20	5.21	5.23
	Outdoors: no plants	5.16	5.16	5.18	5.20	5.30
	Outdoors: with plants	5.16	5.17	5.19	5.20	5.33
	Standard errors	0.011	0.005	0.002	0.005	0.004

From this study, it can be concluded that adding aluminium sulfate and calcium carbonate followed by leaching excess salts with water are simple, rapid and convenient methods for adjusting soil pH for pot experiments. The variation in pH_{Ca} and EC between individual pots of both acidified and limed soil is negligible (range 0.05 pH and 0.02 dS/m), and the pH_{Ca} remains relatively stable for at least six months of use as a plant growth medium. A wide range of soluble Al is also achievable. Further experiments are needed to test this approach on a range of soils.

Chapter Four

Screening of *Austrodanthonia* for Al-tolerance and vigour*

4.1 Introduction

The *Austrodanthonia* genus (~33 species) is generally considered to be acid tolerant (Robinson *et al*. 1993; Garden *et al*. 2001a) and to have the potential for vigorous growth (Robinson and Archer 1988). However, preliminary data indicate that there may be a wide range of genotypic variability in both characteristics (Dowling *et al.* 1996; Rubzen *et al.* 1996; Garden *et al*. 2001b).

This study was undertaken to select *Austrodanthonia* species/ accessions with a range of Al-tolerance and vigour for further investigation. The experiment screened 183 accessions from 15 species of *Austrodanthonia*, previously collected from 126 sites on the Central, Southern and Monaro Tablelands of NSW (Garden *et al.* 1993). The screening was conducted in pots using a soil that, when acidic, presents an Al-challenge that is relatively uncomplicated by other factors (Helyar and Conyers 1994).

4.2 Materials and methods

Seed collection and preparation

The various accessions of *Austrodanthonia* had been collected from 126 sites on the Central, Southern and Monaro Tablelands of NSW during 1991/92 (Garden *et*

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*Part of the work presented in Chapter 4 has appeared in refereed publications: Islam MA, Dowling PM, Jacobs BC, Milham PJ, Garden DL, Conyers MK, van de Ven R (2001) Effect of soil pH on emergence and survival of *Austrodanthonia* spp. In 'Proceedings of the XIX International Grassland Congress'. pp. 204-205. (Brazilian Society of Animal Husbandry: Sao Paulo, Brazil);

Islam MA (2000) Acid tolerance and fertility responses of important native grasses for acid soils. In 'Acid Soil Action Detailed Reports of Southern Region Projects'. (Eds B Scott, B Schumann, and G Fenton) pp. 25-26. (NSW Agriculture: Wagga Wagga).

al. 1993). The classification of the accessions and their locality of collection are shown in Appendix 1. Seed from most of the specimens was grown in pots for purposes of identification and to increase the supply of seed for further experiments. For the experiment described in this chapter, caryopses were separated manually, and disease-free seeds of similar size and shape were collected and stored in a refrigerator to break any dormancy (Bradbeer 1988). Before planting, germination tests of selected accessions were carried out in the laboratory. Most of the accessions had high germination counts (80-100%).

Soil collection, preparation and pH adjustment

Top soil (0-20 cm) was collected from a commercial grazing property at Binnaway, NSW (31°31’S, 149°17’E, elevation 460 m). This soil was naturally acidic (pH_{Ca} 4.35), high in exchangeable Al (14 mg/kg), and relatively low in exchangeable Mn (<5 mg/kg). (The exchangeable Al and Mn values correspond with those in Table 3.1 for which the units are cmol(+) kg^{-1}). Details of the physical and chemical characteristics of the soil are presented in Chapter 3 (Table 3.1). Samples of this soil had previously been used to characterise the relative Al-tolerance of a range of crop and pasture species (Helyar and Conyers 1994). The soil was initially air-dried and sieved through a 10 mm mesh.

A series of trials was carried out in the laboratory to adjust the soil to pH_{Ca} values of 5.3, 4.4 and 3.9 to provide an increasing Al-challenge (Fig. 3.5), with an acceptable EC (< 0.4 dS/m, Fig. 3.3). The Al sulfate and lime additions were 3.8 and 0.48 g/kg soil, on an oven-dry basis, respectively. Unamended soil had a pH_{Ca} of 4.4, and pH_{Ca} 5.3 was the Al-control treatment (Fig. 3.5). Basal fertilisers were added (Helyar and Conyers 1994) as follows (mg element per kg air-dry soil): 4.47 mg Mg as $MgSO_4.7H_2O$, 10.76 mg K as K_2SO_4, 2.17 mg Cu as $CuSO_4.5H_2O$, 4 mg Zn as $ZnSO_4.7H_2O$, 0.6 mg B as H_3BO_3 and 23.53 mg P as fertiliser grade $Ca(H_2PO_4)_2$ (superphosphate). The superphosphate contained 200 mg Mo/kg. Nitrogen was applied in three split dressings of 18.82 mg N/kg soil as ammonium nitrate.

Before sowing, soil samples were collected and air-dried at 40°C. pH_{Ca} was measured on the 1:5, soil: 10 mM $CaCl_2$ suspension following 1 h shake, end-over-end at 10 rpm (Rayment and Higginson 1992). Soluble Al and Mn were measured in the supernatant: Al using the pyrocatechol violet method (Conyers *et al.* 1991) and Mn using atomic absorption spectroscopy. Electrical conductivity (EC) was determined in 1:5 soil: water extract (Rayment and Higginson 1992). Pots (15 cm by 10 cm) were then filled with 2.2 kg air dry soil and leached with 2.5 L of water to remove the excess salt (EC > 0.6 ds/m). This leaching operation was particularly necessary for the aluminium sulfate treatment (Chapter 3).

Sowing of seeds, maintenance of the experiment, data collection and plant harvesting

Ten seeds were placed in each pot and the surface of the soils was kept moist until seedlings began to emerge. The soil was then watered to field capacity, which was determined as described previously (Section 3.2), and rewatered as required for the duration of the experiment. Seedling emergence and survival were monitored until 170 days after sowing (DAS). Leaf length (leaf base to tip) and leaf breadth (mid way between base and tip) were measured on one median size plant in each pot for the 28 replicated accessions of *Austrodanthonia* at 107 DAS. Tillers were also counted on the same day. For dry matter (DM) measurement, the aboveground (~ 5 mm from the soil surface) parts of all live plants were harvested at 186 DAS and dried to a constant weight in a dehydrator at 70° C.

Location, design and data analysis

The study was conducted at Orange Agricultural Institute (33°21’S, 149°40’E, elevation 925 m) during the months of April to October 1999. 183 accessions from 15 *Austrodanthonia* species, two commercial cultivars of *Austrodanthonia* (cvs. Taranna and Bunderra) and three other species as comparisons (V*ulpia myuros* collected near Orange, NSW, *Dactylis glomerata* cv. Porto and *Phalaris aquatica* cv. Sirosa) were grown at three levels of soil acidity (pH_{Ca} 3.9, 4.4 and 5.3) in pots.

The experiment was a randomised complete block design with 33 genotypes replicated three times, with the remainding unreplicated. Of the 33 genotypes, 28 were accessions of *Austrodanthonia* and the remainder were the five additional genotypes used for comparison. The main reasons for reduced replication were a shortage of seed of many accessions and the need to reduce the size of the experiment to a manageable number of pots. Thus the experiment consisted of 762 pots of which 254 were allocated to each pH level. An additional 36 pots (12 for each pH level) without seeds were allocated randomly for soil analysis. The experiment was sown on 16th April 1999, and the final harvest completed on 19th October 1999.

A two-way analysis of variance (ANOVA) was performed using GenStat (Release 4.2) on seedling emergence and survival, to test the effect of pH treatment at different days after sowing (DAS) among the genera, species and accessions. The interactions between treatment (pH), species and accessions were also tested. Differences between means were assessed for significance by using least significant difference (LSD), where the probability of the F test was at the 5% level (Gomez and Gomez 1984; Collins and Seeney 1999).

Growth data (leaf length, leaf breadth, tiller number and DM) were analysed by REML variance components analysis (mixed model analysis) and significance was tested by the Wald statistic (Chi-square probability). At pH_{Ca} 3.9, most of the plants had died by the final harvest, so statistical analysis was performed only for the data at pH_{Ca} 4.4 and 5.3. Analysis of tiller number is not presented because there were no treatment effects. Where appropriate, data were log transformed to homogenise variance. Where the transformation did not affect the analysis, non-transformed data were analysed (e.g. Table 4.1). The relationship between the plant parameters was measured by general regression analysis (e.g. leaf length and leaf breadth by simple linear regression; DM with leaf length and leaf breadth by multiple regression).

4.3 Results

Emergence and survival

Emergence of all four genera had ceased by 44 DAS as illustrated for the 183 accessions of *Austrodanthonia* (Fig. 4.1). At 44 DAS, cumulative emergence of *Austrodanthonia* reached ~11% at pH_{Ca} 3.9, and ~66% and ~72% at pH_{Ca} 4.4 and 5.3 respectively (Fig. 4.1). The trend was similar for the other three genera, and emergence at pH_{Ca} 3.9 increased in the order: *Austrodanthonia* <*Dactylis* <*Phalaris* <*Vulpia* (Fig. 4.2).

By the final seedling count at 170 DAS, the *Austrodanthonia* seedlings surviving at pH_{Ca} 3.9 numbered <1% of the seeds sown, whereas at pH_{Ca} 4.4 and 5.3, 20-30% survived (Fig. 4.3). The trend was similar for the other three genera; however, no *Dactylis* or *Phalaris* seedlings survived at pH_{Ca} 3.9, whereas *Vulpia* survived well at all three values of pH_{Ca} (Fig. 4.4).

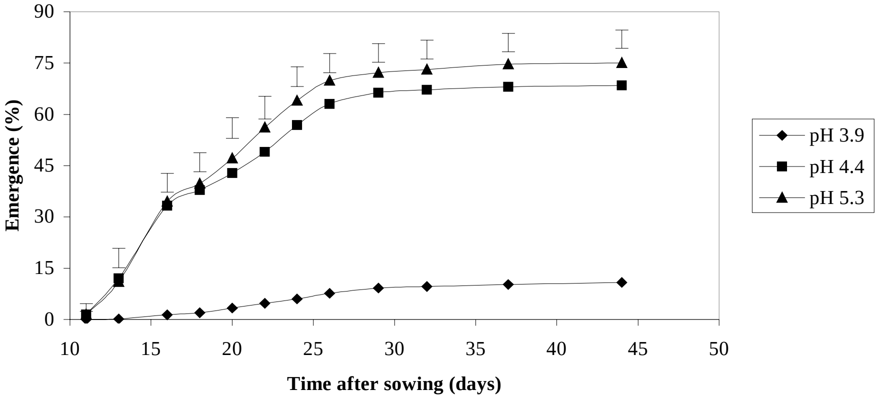


Fig. 4.1. Cumulative seedling emergence of *Austrodanthonia* at 3 levels of soil pH_{Ca}. Data points are the means for 183 accessions. Percent emergence is based on the number of seeds sown. Lengths of the bars indicate LSD ($P = 0.05$).

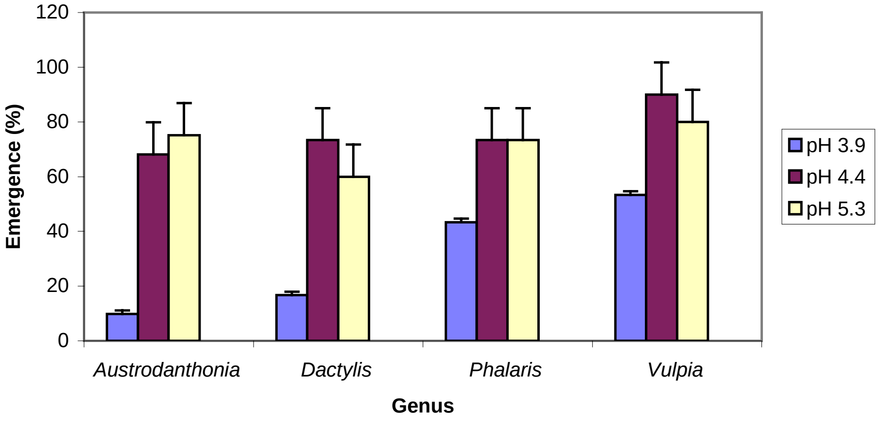


Fig. 4.2. Main effect of pH_{Ca} on the emergence of different genera at 44 DAS. Lengths of bars indicate standard errors.

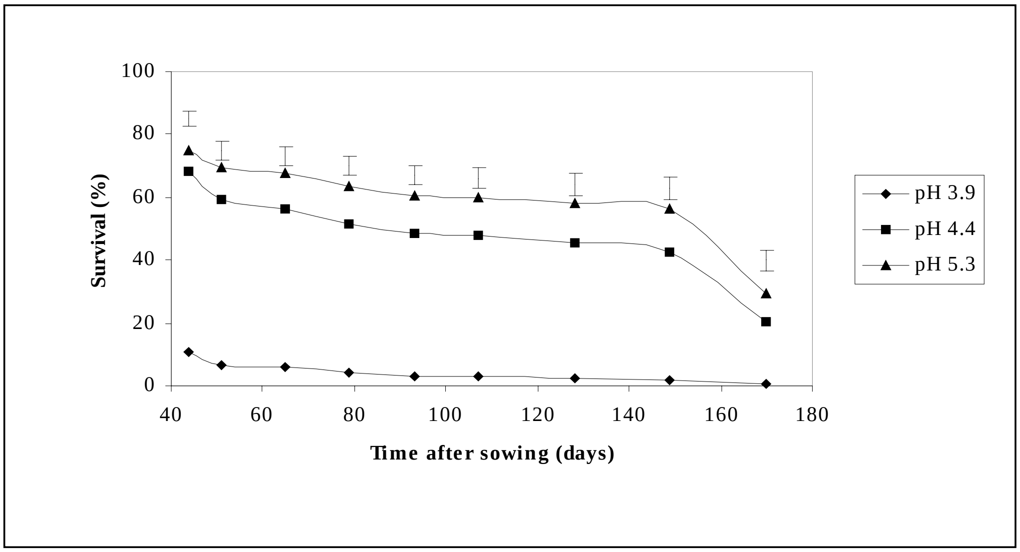


Fig. 4.3. Effect of soil pH_{Ca} on survival of *Austrodanthonia* from 44 to 170 DAS. Data points are the means for 183 accessions. Percent survival is based on the number of seeds sown. Lengths of bars indicate LSD (P = 0.05).

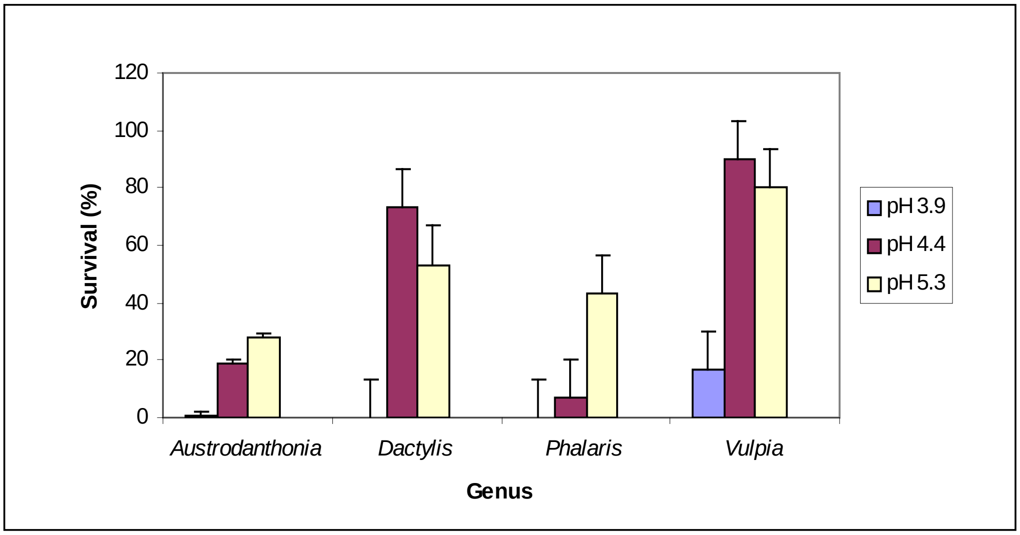


Fig. 4.4. Main effect of pH_{Ca} on the survival of different genera at 170 DAS. Lengths of bars indicate standard errors.

There was a large range in emergence and survival among the 15 *Austrodanthonia* spp. For example, the range of emergence was 0-22% at pH_{Ca} 3.9, 30-85% at pH_{Ca} 4.4, and raising pH_{Ca} to 5.3 had little further effect (Table 4.1). There was a similar range of pH_{Ca} effects on survival; however for some spp., survival continued to increase as pH_{Ca} was increased from 4.4 to 5.3 (Table 4.1).

Table 4.1. Effect of pH_{Ca} on emergence and survival of *Austrodanthonia* spp. Values in parentheses are standard errors

Species	No. of accessions used	Emergence (%) (44 DAS)			Survival (%) (170 DAS)		
		pH 3.9	pH 4.4	pH 5.3	pH 3.9	pH 4.4	pH 5.3
A. penicillata	11	21.8 (4.4)	72.9 (4.4)	77.6 (4.4)	3.5 (5.5)	15.9 (5.5)	44.1 (5.5)
A. pilosa	36	20.7 (2.8)	60.9 (2.8)	66.4 (2.8)	0.2 (3.5)	13.1 (3.5)	21.9 (3.5)
A. setacea	6	18.8 (6.3)	53.8 (6.3)	73.8 (6.3)	0	13.8 (8.1)	16.3 (8.1)
A. duttoniana	16	14.0 (4.0)	76.0 (4.0)	84.5 (4.0)	0	31.0 (5.1)	18.5 (5.1)
A. carphoides	4	10.0 (6.3)	75.0 (6.3)	78.8 (6.3)	0	38.8 (8.1)	18.8 (8.1)
A. fulva	9	7.7 (4.9)	85.4 (4.9)	81.5 (4.9)	0.8 (6.3)	37.7 (6.3)	46.9 (6.3)
A. eriantha	18	7.1 (3.7)	54.2 (3.7)	62.5 (3.7)	0.4 (4.7)	12.5 (4.7)	22.5 (4.7)
A. racemosa	69	4.5 (2.1)	77.3 (2.1)	87.6 (2.1)	0.1 (2.6)	20.7 (2.6)	35.3 (2.6)
A. laevis	4	3.8 (6.3)	53.8 (6.3)	58.8 (6.3)	0	1.3 (8.1)	15.0 (8.1)
A. monticola	1	3.3 (10.4)	73.3 (10.4)	70.0 (10.4)	0	26.7 (13.2)	13.3 (13.2)
A. richardsonii	2	1.4 (6.8)	58.6 (6.8)	68.6 (6.8)	0	15.7 (8.6)	27.1 (8.6)
A. bipartita	1	0	70.0 (7.3)	73.3 (7.3)	0	13.3 (9.3)	26.7 (9.3)
A. procera	2	0	57.5 (8.9)	52.5 (8.9)	0	15.0 (11.4)	32.5 (11.4)
A. caespitosa	3	0	45.7 (6.8)	55.7 (6.8)	0	7.1 (8.6)	18.6 (8.6)
A. tenuior	1	0	30.0 (10.4)	20.0 (10.4)	0	0	0

For individual genotypes, cumulative emergence at 44 DAS interacted with pH treatment ($P < 0.001$). This is illustrated in Fig. 4.5, where emergence is plotted for pairs of pH combinations. On each plot, the two solid lines represent the mean emergence at each pH. In such a plot the quadrant on the top right contains genotypes with above average emergence at both pH_{Ca} values and *vice versa* for the bottom left. *Austrodanthonia* cvs. Taranna and Bunderra tend to appear towards the bottom left, and *Phalaris* and silvergrass to the top right, with cocksfoot lying between the groups.

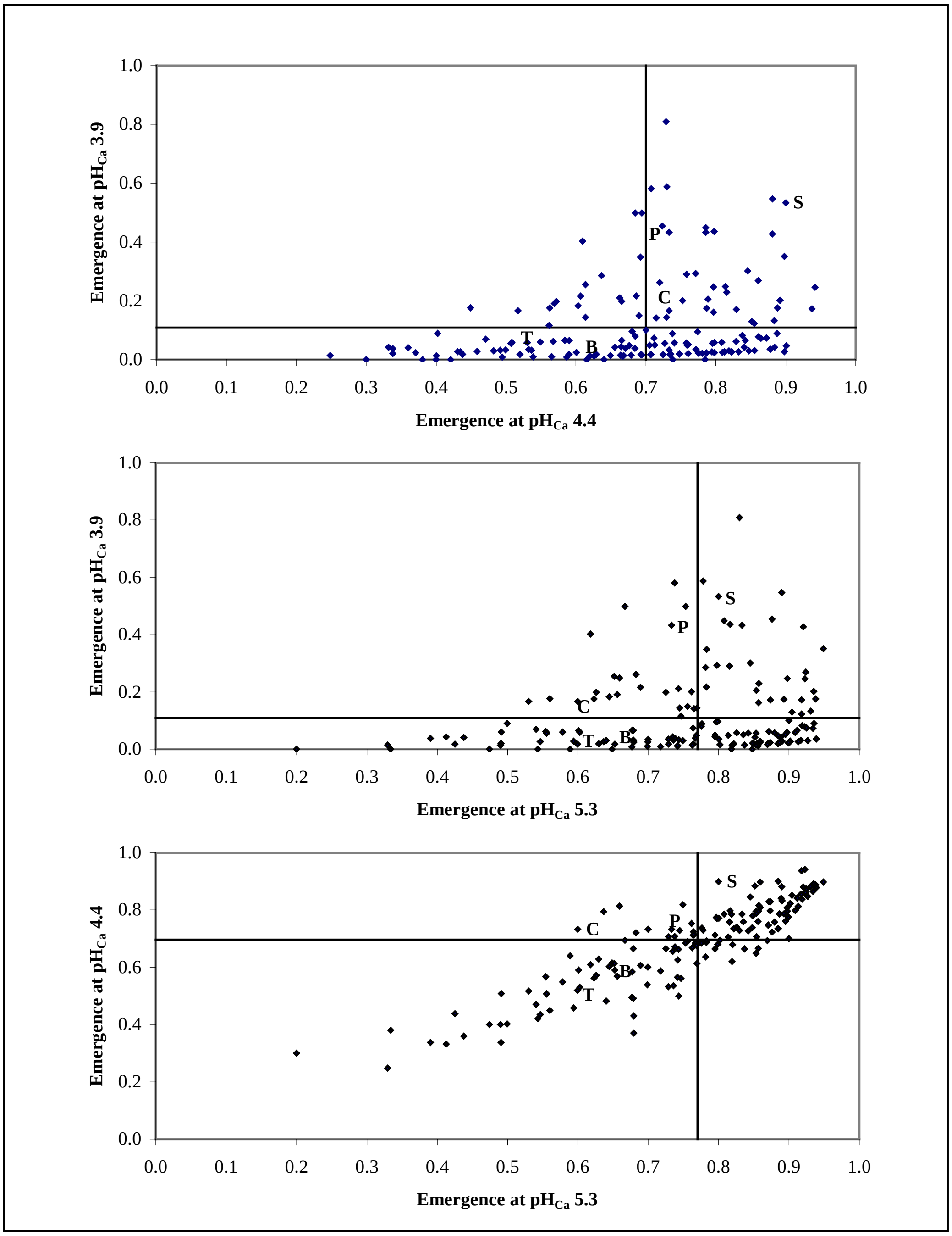


Fig. 4.5. Effect of pH_{Ca} and accessions on cumulative emergence at 44 DAS. Axes are for emergence at the respective pH_{Ca} values. Solid lines represent mean emergence at the corresponding pH_{Ca}. Dots are the emerged proportion of seeds for 183 accessions of *Austrodanthonia*. B, *A. bipartita* cv. Bunderra; T, *A. richardsonii* cv. Taranna; C, cocksfoot *D. glomerata* cv. Porto; P, *P. aquatica* cv. Sirosa; S, silvergrass *V. myuros*.

Growth parameters

At pH_{Ca} 3.9, <1% of seedlings survived to 170 DAS, consequently results are presented only for pH_{Ca} values of 4.4 and 5.3. Data for tiller numbers are not presented because pH treatment effects were not significant ($P > 0.05$).

Leaf length and leaf breadth: for the 28 *Austrodanthonia* accessions that had replicate pots, there was a positive linear relationship ($r^2 = 0.76$) between leaf length (LL) and leaf breadth (LB) that was not affected by the pH treatments ($P > 0.05$):

$$\text{LL (mm)} = 1.64\ (\pm\ 0.367) + 6.293\ (\pm\ 0.264)\ \text{LB (mm)} \qquad (4.1)$$

Dry matter: the variables pH, and *Austrodanthonia* species and accessions had large effects on DM production (Table 4.2, Figs 4.6 and 4.7, Appendix 2); however, for the data as a whole, the interactions of pH by species and by accession were not significant ($P > 0.05$, Table 4.2).

For the 30 replicated *Austrodanthonia* accessions/cultivated varieties, there was a week relation of DM with leaf L and B (Eqn 4.2, $r^2 = 0.41$), that was unaffected by pH ($P > 0.05$, Table 4.3):

$$\text{DM (mg/plant)} = 3.339\ (\pm\ 0.646) - 1.287\ (\pm\ 0.34)\ \text{LL (mm)}$$
$$+\ 3.773\ (\pm\ 0.526)\ \text{LB (mm)} \qquad (4.2)$$

Table 4.2. REML variance component analysis for variate DM, at 186 DAS for all *Austrodanthonia* accessions. Prior to analysis, data were transformed ($\log_e$)

Term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob.
Main effects				
pH	12.79	1	12.79	<0.001
Species (S)	31.23	14	2.23	0.005
Accessions (Ac)	245.80	184	1.34	<0.002
Ac within S	214.57	170	1.262	0.012
Interaction				
pH x S	11.00	14	0.79	0.686
pH x Ac	212.49	184	1.15	0.074

Table 4.3. Accumulated ANOVA for DM, leaf L and B for all 30 replicated *Austrodanthonia* accessions and cultivated varieties

Prior to analysis, data were transformed ($\log_e$)

Term	d.f.	s.s.	m.s.	v.r.	F prob.
Leaf length (LL)	1	394.813	394.813	73.46	< 0.001
Leaf breadth (LB)	1	274.636	274.636	51.10	< 0.001
pH	1	2.054	2.054	0.38	0.537
LL x pH	1	2.037	2.037	0.38	0.539
LB x pH	1	4.022	4.022	0.75	0.388
Residual	174	935.160	5.374		
Total	179	1612.722	9.010		

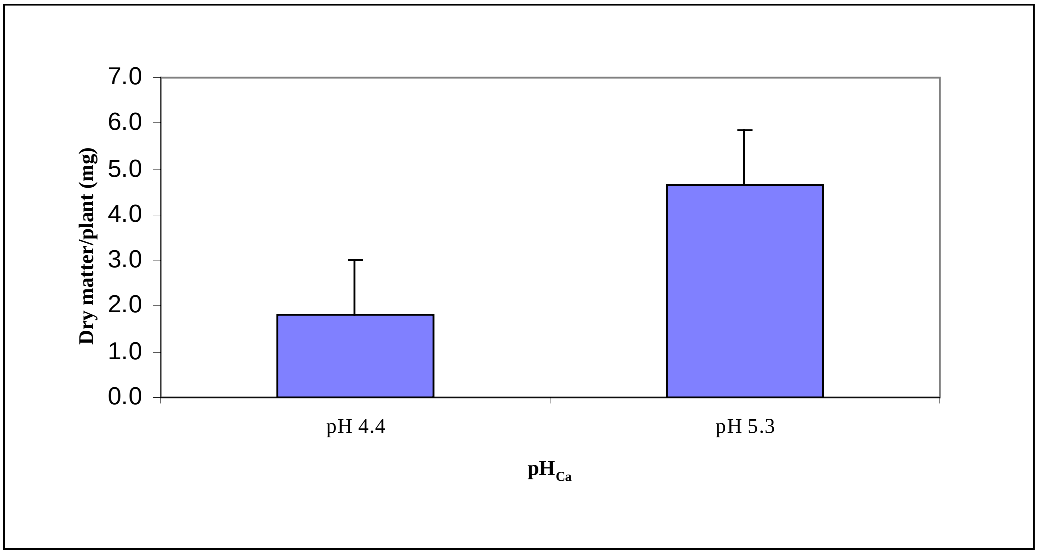


Fig. 4.6. Main effect of pH$_{Ca}$ on top DM of seedlings of *Austrodanthonia* that survived to the harvest at 186 DAS. Data are overall means (back-transformed values). Bars associated with each column represent the standard errors.

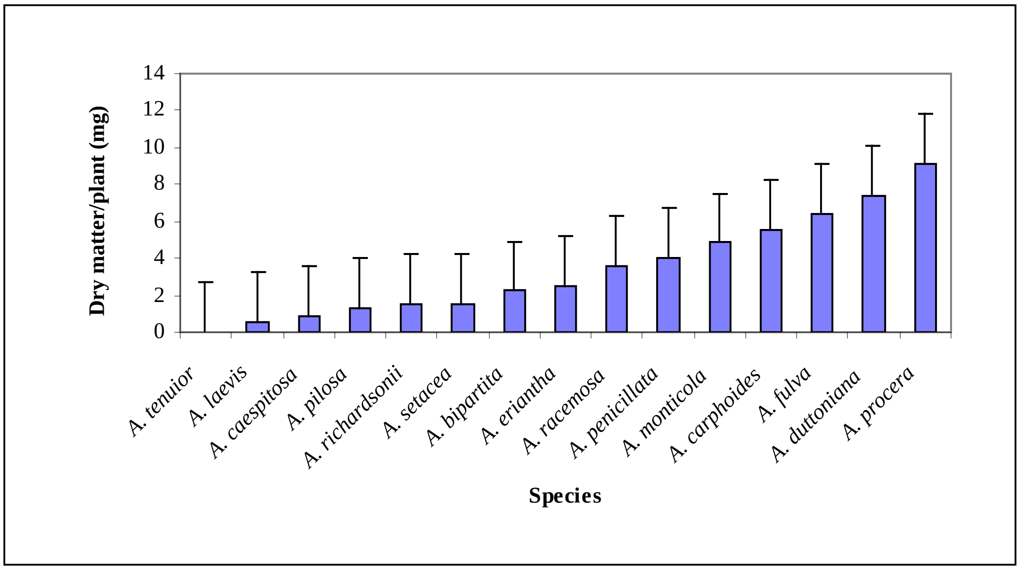


Fig. 4.7. DM of tops of different *Austrodanthonia* spp. harvested at 186 DAS. Data are means for pH_{Ca} 4.4 and 5.3 (back-transformed values). Bars associated with each column represent the standard errors.

4.4 Discussion

Emergence, survival and growth

Emergence and survival decreased in the order pH_{Ca} 5.3 >4.4 >>3.9 (Figs 4.2, 4.4). These effects were not due to toxicities of Mn or H^+, because of the nature of the soil (Helyar and Conyers 1994; Cregan and Scott 1999). The preceding three pH_{Ca} values were associated respectively with Al_{Ca} concentrations of 2, 4 and 52 mg/kg soil (Chapter 3, Fig. 3.5). Therefore, it can be argued that the pH effects were caused by differences in the concentrations of soluble Al. The sudden drop in survival of *Austrodanthonia* accessions after 149 DAS (Fig. 4.2) occurred during a cold snap (average 5.3°C). During this period the soil froze at night when the temperatures fell as low as -1.2°C. Nevertheless, 20% of plants survived at pH_{Ca} 4.4 and 30% at pH_{Ca} 5.3, confirming that *Austrodanthonia* spp. have some frost tolerant (Mitchell 1996).

The species that emerged well at pH_{Ca} 3.9 also tended to survive and grow well across the higher pH's (Table 4.1). The very Al-tolerant group of *Austrodanthonia* species included *A. penicillata*, *A. pilosa*, *A. fulva*, *A. eriantha* and *A. racemosa*. The most Al-sensitive included *A. bipartita*, *A. procera*, *A. caespitosa* and *A. tenuior*. The remaining species, *A. setacea*, *A. duttoniana*, *A. carphoides*, *A. laevis*, *A. monticola* and *A. richardsonii*, fell between these two distinct groups, although some of the species emerged well but had lower persistence, even at the higher pH level (e.g. *A. laevis* and *A. setacea*). As expected, *Vulpia* (cf. Rossiter 1966; Dowling 1996; Wallace 1997) and *Dactylis* fell into the very Al-tolerant group and *Phalaris* into the sensitive group (Figs 4.2 and 4.4, cf. Helyar and Conyers 1994; Rubzen *et al*. 1996). Somewhat surprisingly, the two commercially selected cultivars of *Austrodanthonia* (Bunderra and Taranna) also fell into the Al-sensitive group (Fig. 4.5). The wide range of Al-tolerance exhibited by *Austrodanthonia* species and accessions within species is consistent with findings under controlled conditions (Helyar and Conyers 1994; Rubzen *et al*. 1996) and the natural distribution of species/accessions in the field (Dowling *et al*. 1996; Garden *et al*. 2001a). It is also consistent with the results of studies on other genera (Foy *et al*. 1988; Edmeades *et al*. 1991b; Helyar and Conyers 1994).

The experiment also provided data on the yield potential of a wide range of *Austrodanthonia* species and accessions. The high yield potential group of species included *A. duttoniana*, *A. fulva*, *A. procera*, *A. carphoides* and *A. monticola* (Fig. 4.7). While in case of accessions, the highest dry matter yield was obtained from accession 182287 (*A. duttoniana*, 124 mg/plant), followed by accessions 182179a (*A. fulva*, 105 mg/plant), 182114 (*A. racemosa*, 100 mg/plant) and 182229 (*A. pilosa*, 93 mg/plant), but there was no difference between them ($P > 0.05$, Appendix 2). The top

67 accessions that were not different from each other included: species (number of accessions) - *A. duttoniana* (eight), *A. fulva* (six), *A. racemosa* (25), *A. pilosa* (eight), *A. eriantha* (eight), *A. penicillata* (five), *A. richardsonii* (one), *A. procera* (one), *A. setacea* (one), *A. carphoides* (two), *A. bipartita* (one) and *A. monticola* (one). Two agro-accessions of *Austrodanthonia* (Bunderra and Taranna) were similar to other low yielding accessions apparently showing their comparatively low yield potential. Thus there is a wide range of yield potential between species (Fig. 4.7) and accessions within species (Appendix 3) of *Austrodanthonia*. This finding supports the earlier reports of a wide range of variation in dry matter production of *Austrodanthonia* species (Eddy and Garden 1996; Rubzen *et al*. 1996).

A positive relationship between leaf length and leaf breadth (Eqn 4.1) indicates that accessions with wider leaves can produce longer leaves and ultimately influence dry matter production. Wider leaves have been associated with forage quality (Whittet 1964) and may also have a role to play in physiological processes and vegetative development by having a greater number of vascular bundles (Chastain and Young III 1998).

Selection of accessions for further study

One of the main objectives of this study was to select promising accessions of *Austrodanthonia* for further investigation for acid tolerance. It was observed that accessions that emerged and survived well at low pH typically also did so at higher pH values, and there was a positive association between emergence and survival. As the maximum cumulative emergence occurred at 44 DAS and there was a similar pattern of emergence at pH_{Ca} 4.4 and 5.3, emergence at lower pH_{Ca} (3.9 and 4.4) at the final day of counting (i.e. 44 DAS) was chosen as the date for selecting accessions.

Classification-I: to select superior accessions that performed well with respect to emergence at the lower pH values, the following selection criteria were used (Fig. 4.8): a) all accessions that had emergence at pH_{Ca} 3.9 that exceeded mean emergence by more than *v* times the standard deviation of the mean emergence at this pH (*v* had to be determined; the greater the value of *v*, the fewer accessions selected and *vice versa*); b) all accessions that had emergence at pH_{Ca} 4.4 that exceeded mean emergence by more than *v* times the standard deviation of the mean emergence at this pH; and c) all accessions in the upper right hand half of the region containing accessions performing better than the mean for pH_{Ca} 3.9 or pH_{Ca} 4.4, that had not been selected on either of the previous two criteria.

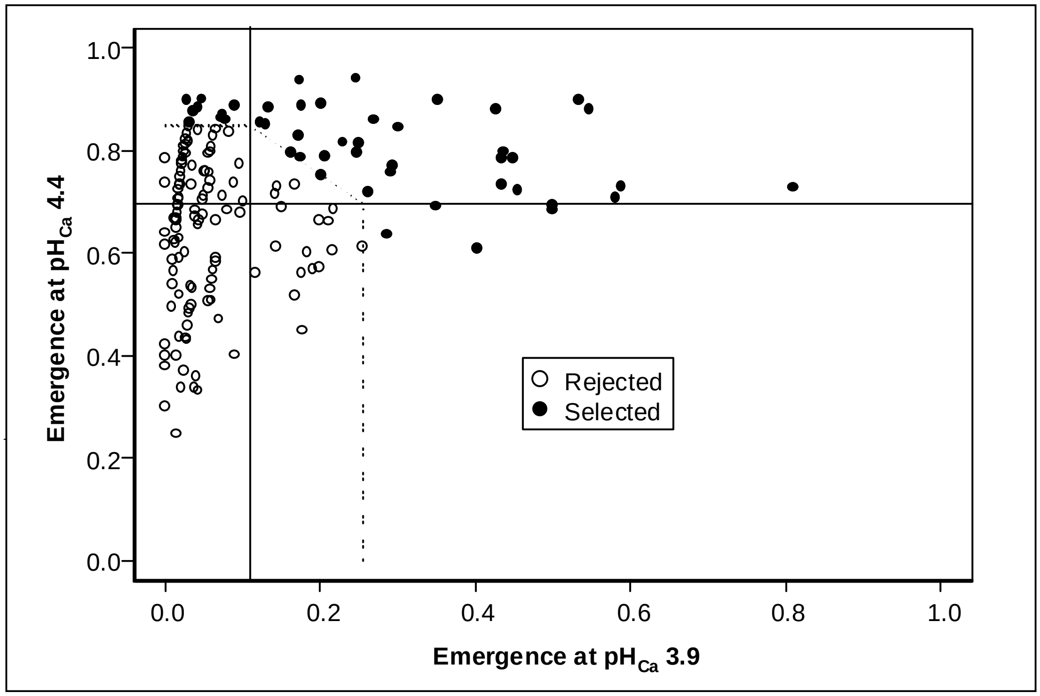


Fig. 4.8. Selection of accessions on the basis of emergence at the two lower pH's. Axes are for emergence at the respective pH's. Solid lines represent mean emergence at the corresponding pH. Dots are the proportion emergence of 183 accessions of

Austrodanthonia. Solid dots are the accessions selected for further study. The dotted line is the boundary between selected and non-selected accessions.

The value chosen for v was 1.0358, and delineated those accessions with superior emergence at pH_{Ca} 3.9 and 4.4, in addition to reducing the number of selected accessions to manageable levels. Thus 49 accessions of *Austrodanthonia* spp. were in the selected group. Details of the accessions are shown in Appendix 3 and their likely tolerance in relation to collection site is discussed later (see Chapter 6).

To relate emergence of selected accessions with their persistence, the cumulative emergence and survival data at pH_{Ca} 4.4 at 44 DAS and 149 DAS, respectively, were plotted against each other. It is clear that accessions with higher emergence persisted better than the accessions with lower emergence (Fig. 4.9). Of the 49 selected accessions, ~ 84 % fell in the high performing region.

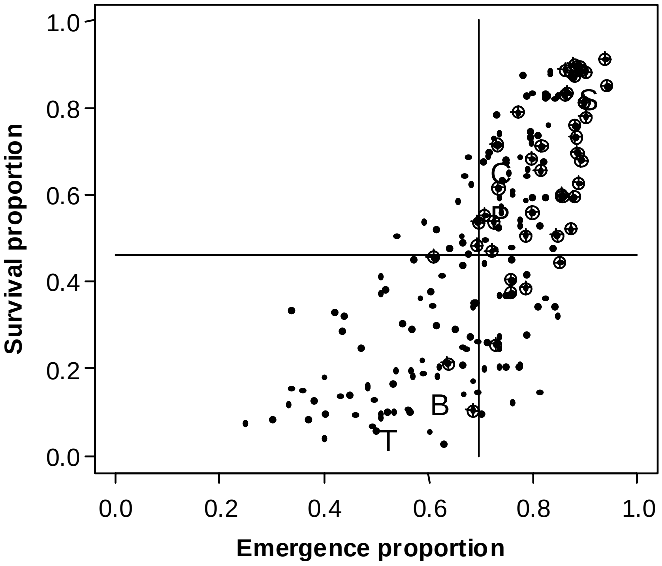


Fig. 4.9. Emergence (44 DAS) versus survival (149 DAS) of different accessions of *Austrodanthonia* spp. Solid lines represent mean emergence and mean survival at pH_{Ca} 4.4. Dots with circles are the selected accessions. B, *A. bipartita* cv. Bunderra; T, *A. richardsonii* cv. Taranna; C, cocksfoot, *D. glomerata* cv. Porto; P, phalaris, *P. aquatica* cv. Sirosa; S, silvergrass, *V. myuros*.

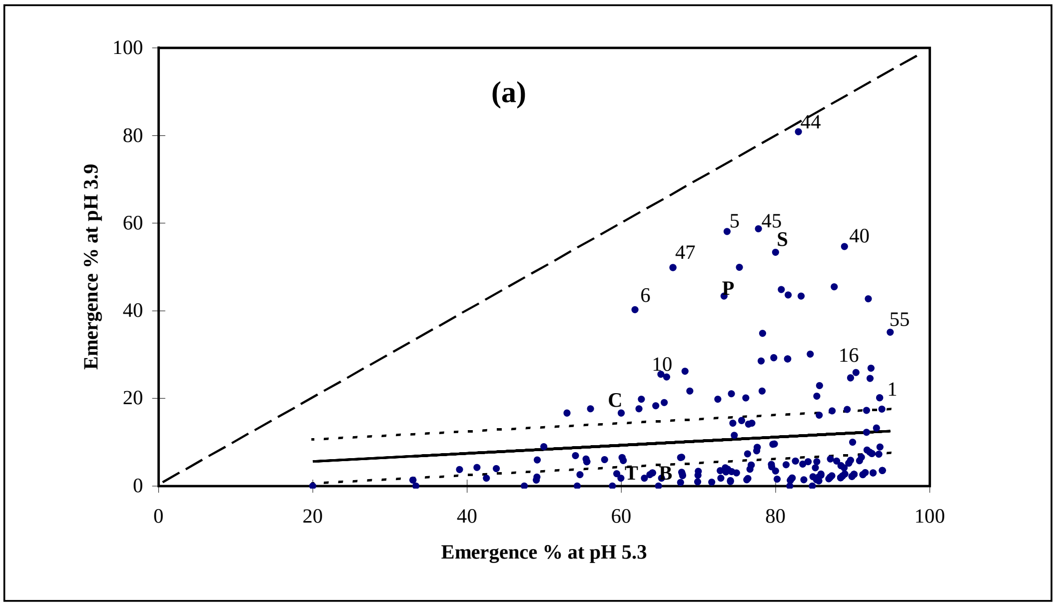
Inherent vigour versus tolerance

Logically, if an accession has tolerance, it has vigour, but not necessarily *vice versa*. According to the Chambers Dictionary of Science and Technology (Collocott and Dobson 1974), “tolerance” can be defined as the ability of a plant to endure adverse environmental conditions, especially drought and shading; while “growth” - an irreversible change in an organism accompanied by the utilisation of material, and resulting in increased volume, dry weight or protein content. Similarly, Oxford dictionary (Crowther 1995) describes “growth” as the action, process or manner of growing; vegetative development; increase. On the other hand, A Dictionary of Ecology Evaluation and Systematics (Lincoln *et al*. 1998) defines “vigour” as the intensity of growth or general metabolic activity of an organism, population or community. Therefore, following the above selection procedures (classification-I), it is possible that tolerance of the selected accessions be confused with inherent vigour, although vigour is one of the important agronomic attributes to be associated with high yield (Hutton *et al*. 1978). Also there have been difficulties of setting criteria for selection of accessions for further study (see pages 80-82).

In the present study, the higher emergence at the control pH (pH 5.3 – where Al is low) in Fig 4.5 indicates that the accessions differ markedly in vigour. Progressively lower emergence as the pH decreased could be attributed, at least partially, to increasing levels of Al (Figs 4.5 and 4.10), indicating that some sort of differential Al-tolerance was operating among the accessions. However, to have more confidence in the ‘real’ effect of Al on accession emergence, the values need to be adjusted to take into account the inherent vigour associated with each accession. *Classification-II*: an approach described by Hutton (Hutton *et al*. 1978) can be used to correct for inherent vigour by regressing emergence values at a high Al challenge (especially pH 3.9) against the corresponding control (pH 5.3), and observing the deviation from the regression. This is shown in Figs 4.10a and 4.10b. It was observed that accessions are more scattered with a high Al challenge than with a low Al challenge (Fig. 4.10b). Some accessions are well above or below the regression lines in both cases. An arbitrary deviation line (i.e. ± 5% of the fitted lines) was allocated to each Figure to differentiate an accession's 'tolerance', and the following criteria were used to group the accessions: a) tolerant – accessions above the + 5% lines (i.e. accessions with emergence exceeding the predicted values by >5%); intolerant – accessions below the 5% lines (i.e. accessions with emergence less than the predicted values by at least 5%); and c) neutral – accessions with values within the 5% lines.

A summary of tolerance classifications of the previously selected accessions (49) and some accessions that were used in subsequent experiments are shown in Table 4.4. Many of the accessions (13) are classified as 'tolerant' in both methods of classification. The second approach (classification-II) resulted in a decreased number of accessions classified as tolerant (34 – pH 3.9, 16 – pH 4.4) compared with 49 in classification-I (Table 4.4), suggesting that this approach has indeed constrained the number of accessions that might be regarded as tolerant. This approach also provided agreement across classifications for tolerant, intolerant and neutral on 48 of the possible 65 accessions listed, again suggesting an improved system for classification for tolerance.

It is surprisingly noticed that both classifications classed *Phalaris* as Al-tolerant (Figs 4.5, 4.9 and 4.10). The unexpected result might be associated with seed vigour as *Phalaris* had larger sized seeds than other genera used in the present study.



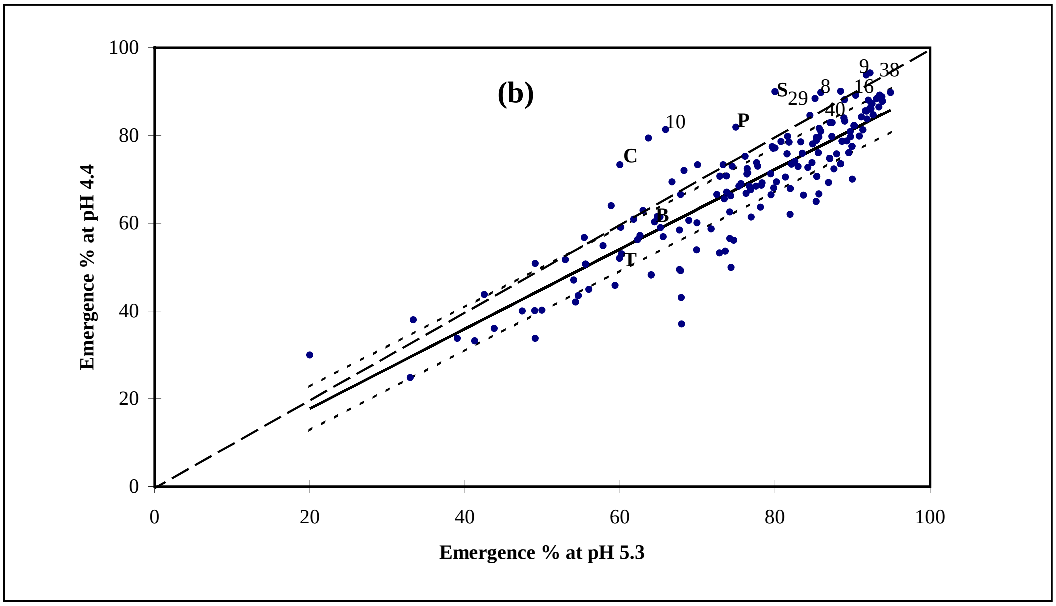


Fig. 4.10. Relationships between emergence percentage at pH 5.3 and pH 3.9 (a), equation of line: Y = 0.093X + 3.74; and at pH 5.3 and pH 4.4 (b), equation of line: Y = 0.909X – 0.428. Dotted lines represent ± 5% of the fitted lines. B, *A. bipartita* cv. Bunderra; T, *A. richardsonii* cv. Taranna; C, cocksfoot, *D. glomerata* cv. Porto; P, phalaris, *P. aquatica* cv. Sirosa; S, silvergrass, *V. myuros*. The numbers within the Figure represent some selected accessions (see text and Table 4.4 for detailed explanation). The dashed lines (1:1) represent equivalent emergence for each pH comparison.

Table 4.4. ***Austrodanthonia*** **accessions, their tolerance classifications and an indication as to where utilised in subsequent experiments**

Common ID[a]	Accessions ID	Species	Relative tolerance classifications			Referred to in subsequent experiments (Exp)/ chapters (Ch)[d]
			Classification-I[b]	Classification-II[c]		
				pH 3.9	pH 4.4	
1	182251	*A. racemosa*	Tolerant	Neutral	Neutral	Exp 5.2, Ch 6, Ch 7
2	182095	*A. racemosa*	Tolerant	Intolerant	Neutral	Exp 5.2, Ch 6, Ch 7
3	182188	*A. racemosa*	Intolerant	Intolerant	Intolerant	Exp 5.2, Ch 6, Ch 7
4	182233	*A. racemosa*	Intolerant	Intolerant	Neutral	Exp 5.2, Ch 6, Ch 7
5	182288	*A. pilosa*	Tolerant	Tolerant	Neutral	Exp 5.2, Ch 6, Ch 7
6	182087	*A. pilosa*	Tolerant	Tolerant	Tolerant	Exp 5.2, Ch 6, Ch 7
7	182267	*A. pilosa*	Intolerant	Intolerant	Neutral	Exp 5.4, Ch 6,
8	182206	*A. fulva*	Tolerant	Intolerant	Tolerant	Exp 5.2, Ch 6, Ch 7
9	182205	*A. fulva*	Tolerant	Tolerant	Tolerant	Exp 5.2, Ch 6, Ch 7
10	182256	*A. fulva*	Tolerant	Tolerant	Tolerant	Ch 6
11	182131	*A. duttoniana*	Tolerant	Tolerant	Neutral	Exp 5.2, Ch 6, Ch 7
12	182050	*A. duttoniana*	Tolerant	Tolerant	Neutral	Exp 5.2, Ch 6, Ch 7
13	182106	*A. duttoniana*	Intolerant	Neutral	Neutral	Exp 5.3, Ch 6
14	182081	*A. penicillata*	Tolerant	Tolerant	Tolerant	Exp 5.2, Ch 6, Ch 7
15	182192	*A. penicillata*	Tolerant	Tolerant	Neutral	Exp 5.2, Ch 6, Ch 7
16	182300	*A. duttoniana*	Tolerant	Tolerant	Tolerant	Ch 6
17	182031	*A. setacea*	Tolerant	Tolerant	Intolerant	Ch 6, Ch 7
18	182075	*A. setacea*	Intolerant	Intolerant	Intolerant	Ch 6
19	182024	*A. caespitosa*	Intolerant	Intolerant	Intolerant	Ch 6
20	182220	*A. laevis*	Intolerant	Intolerant	Neutral	Ch 6
21	182122	*A. richardsonii*	Intolerant	Intolerant	Neutral	Ch 6, Ch 7
22	182088	*A. monticola*	Intolerant	Intolerant	Neutral	Ch 6
23	182059b	*A. eriantha*	Tolerant	Tolerant	Neutral	Ch 6, Ch 7
24	182028	*A. auriculata*	Not used before			Ch 6
25	182064	*A. duttoniana*	Intolerant	Intolerant	Neutral	Exp 5.1, 5.5, Ch 7
26	182294	*A. racemosa*	Intolerant	Intolerant	Intolerant	Exp 5.4
27	182265	*A. penicillata*	Tolerant	Tolerant	Tolerant	Exp 5.4
28	182293	*A. duttoniana*	Tolerant	Tolerant	Neutral	Exp 5.5
29	182221	*A. fulva*	Tolerant	Intolerant	Tolerant	Exp 5.5
30	182112	*A. pilosa*	Intolerant	Intolerant	Neutral	Ch 7
31	182127	*A. pilosa*	Intolerant	Intolerant	Neutral	Ch 7
32	Taranna	*A. richardsonii*	Intolerant	Intolerant	Neutral	Ch 7
33	Bunderra	*A. bipartita*	Intolerant	Intolerant	Neutral	Ch 7
34	182145	*A. carphoides*	Tolerant	Tolerant	Neutral	-
35	182239	*A. duttoniana*	Tolerant	Tolerant	Neutral	-
36	182245	*A. duttoniana*	Tolerant	Tolerant	Neutral	-
37	182351	*A. duttoniana*	Tolerant	Neutral	Neutral	-
38	182179a	*A. fulva*	Tolerant	Tolerant	Tolerant	-
39	182407	*A. fulva*	Tolerant	Intolerant	Tolerant	-
40	182153	*A. penicillata*	Tolerant	Tolerant	Tolerant	-
41	182208	*A. penicillata*	Tolerant	Tolerant	Tolerant	-
42	182328	*A. penicillata*	Tolerant	Tolerant	Neutral	-
43	182214	*A. penicillata*	Tolerant	Tolerant	Tolerant	-
44	182266	*A. pilosa*	Tolerant	Tolerant	Neutral	-
45	182090	*A. pilosa*	Tolerant	Tolerant	Neutral	-
46	182280	*A. pilosa*	Tolerant	Tolerant	Neutral	-
47	182218	*A. pilosa*	Tolerant	Tolerant	Tolerant	-
48	182224	*A. pilosa*	Tolerant	Tolerant	Tolerant	-

Common ID[a]	Accessions ID	Species	Relative tolerance classifications			Referred to in subsequent experiments (Exp)/ chapters (Ch)[d]
			Classification-I[b]	Classification-II[c]		
				pH 3.9	pH 4.4	
49	182304	*A. pilosa*	Tolerant	Tolerant	Neutral	-
50	182163	*A. pilosa*	Tolerant	Tolerant	Tolerant	-
51	182237	*A. pilosa*	Tolerant	Tolerant	Neutral	-
52	182161	*A. pilosa*	Tolerant	Tolerant	Neutral	-
53	182110	*A. pilosa*	Tolerant	Tolerant	Neutral	-
54	Unknown	*A. pilosa*	Tolerant	Neutral	Neutral	-
55	182262	*A. racemosa*	Tolerant	Tolerant	Neutral	-
56	182152	*A. racemosa*	Tolerant	Tolerant	Neutral	-
57	182234	*A. racemosa*	Tolerant	Tolerant	Neutral	-
58	182171	*A. racemosa*	Tolerant	Neutral	Neutral	-
59	182282	*A. racemosa*	Tolerant	Neutral	Neutral	-
60	182299	*A. racemosa*	Tolerant	Neutral	Neutral	-
61	182007	*A. racemosa*	Tolerant	Neutral	Neutral	-
62	182108	*A. racemosa*	Tolerant	Intolerant	Neutral	-
63	182000	*A. racemosa*	Tolerant	Intolerant	Neutral	-
64	182146	*A. racemosa*	Tolerant	Intolerant	Neutral	-
65	182157	*A. racemosa*	Tolerant	Intolerant	Neutral	-

[a] For simplicity, a common identification (ID) number for the accessions is given for later use
[b] Based on mean emergence at pH 3.9 and 4.4, Fig. 4.8
[c] Based on Hutton et al. (1978) approach, see text and Fig. 4.10 for detailed explanations
[d] Accessions used in the mentioned experiments/chapters
-Not used in subsequent experiments

Finally, whether the selection was based on mean emergence at pH 3.9 and 4.4 (classification-I) or Hutton approach (classification-II), substantially, nearly same groups of Al-tolerant or Al-intolerant accessions were identified. Of the 49 accessions previously selected (classification-I), only 12 accessions appeared to be neutral or intolerant using classification-II (Table 4.4) and this is not unexpected. Further investigations could confirm these traits.

In conclusion, *Austrodanthonia* exhibits a wide range of Al-tolerance/vigour within species (accessions) and between species. This variability may be exploitable in the breeding and selection of improved cultivars.

Chapter Five

Hydroponics for *Austrodanthonia*

Hydroponic experiments offer an opportunity to separate factors that are difficult or impossible to separate when growing plants in soils. The use of solutions with very high ionic strengths and concentrations of ameliorative nutrients (e.g. P, S and Ca) may cloak the phytotoxic effects of factors associated with soil acidity, such as Al^{3+}, Mn^{2+} and H^+ (Thawornwong and Diest 1974; Edmeades *et al.* 1991a; Gallardo *et al.* 1999). Therefore, proper formulation of the solution is important for hydroponic culture.

Accurate control of pH and nutrient ion concentrations in the root environment is essential for studying fundamental relationships involving nutrient concentration, uptake and plant growth. Rapid depletion of nutrient solutions in contact with the root can cause deficiency symptoms of elements, pH change or unfavourable conditions in the solutions (Asher *et al.* 1965; Asher and Loneragan 1967; Asher and Ozanne 1967; Blamey *et al.* 1991). The form of N also has a large influence on growth media (e.g. pH) and growth of plants. Although N assimilation is mainly associated with reduction of NO_3^- to NH_4^+, many plants show growth inhibition when NH_4^+ is supplied as the prime source of N (Magalhäes and Huber 1989; Raab and Terry 1994; Logan *et al.* 2000; Walch-Liu *et al.* 2000). Growth inhibition might occur due to the contribution of various factors, such as NH_4^+ - induced disorders in pH regulation and toxic effects of free ammonia (Nelson and Hsieh 1971; McElhannon and Mills 1977; Goyal *et al.* 1982; Claussen and Lenz 1995); deficiency of mineral nutrients, such as

K^+, Ca^{2+} and Mg^{2+} (~20-50% decrease in response to NH_4^+ application); increase of Cl^- (~102 mM) (Walch-Liu *et al*. 2000); and carbohydrate limitation due to excessive consumption of soluble sugars for NH_4^+ assimilation (Breteler 1973; Walch-Liu *et al*. 2000).

The procedure for testing *Austrodanthonia* tolerance to acidity using hydroponics has been divided into four parts: 1) formulation of the nutrient solution and making the solution pH stable; 2) optimising the formulation to match the nutrient requirements of *Austrodanthonia*; 3) comparing the effectiveness of the formulation using tap water and deionised water; and 4) estimating the free ion activities of Al and Mn in the nutrient solution and their effects on plant growth.

To fulfil the objectives of the above procedures, a range of growth parameters were assessed. These include: plant height, shoot length, tillers/plant, leaves/tiller, root length, root/plant, leaf length, leaf breadth and dry matter.

5.1 Experiment 1. Pilot study of the effect of different ratios of NO_3^- and NH_4^+ on the stability of pH of nutrient solutions

The aims of this experiment are to optimise the formulation of a generic hydroponic solution to the particular requirements of *Austrodanthonia* spp.

Materials and methods

Design

This experiment was conducted in a glasshouse at Orange Agricultural Institute, Orange from April 20 to June 15, 2000 under a day/night temperature regime of 25-15°C. Polystyrene boxes (10 L capacity, 380 x 290 x 160 mm) were used in the experiment. Each box was filled with 8.5 litres of nutrient solution (Table 5.1). *A. duttoniana* (accession 182064) was selected as a test species, as it had been found to be one of the more Al-sensitive accessions (Chapter 4). The experiment was laid out as a completely randomised design consisting of one accession and four treatments of NO_3^- / NH_4^+ ratios. Each treatment was allocated in one box and six single tillers were planted per box. There was no replication. Thus the experiment consisted of four boxes with 24 tillers. Rooted tillers were assigned randomly to each polystyrene box and each tiller was numbered. The boxes were then re-randomised on the bench in the glasshouse every week for eight weeks so that all the boxes would receive similar environmental conditions throughout the experimental period.

Preparation of tillers

Plants of the selected accession were grown out in pots prior to the study. Healthy, disease free and similar sized tillers with roots were separated carefully from the potted plants. The tillers were washed firstly using tap water at least three times to remove soil particles and any other foreign material, then washed with deionised water three times and shoots and roots trimmed off so that the plant heights were ~12 cm and the root lengths were ~2 cm. The tillers were immediately transferred to the control experimental solution for a week to allow recovery from injury during the separation process, before treatments were applied.

Preparation of nutrient solutions with varying ratios of NO_3^- and NH_4^+

Four ratios of NO_3^--N and NH_4^+-N were made by decreasing NO_3^- and increasing NH_4^+ salts (Table 5.2). The modified formulation of Taylor and Foy (1985d) was used as ratio 1 (R1). For ratio 2 (R2), 0.3 mM of K^+ as KNO_3 was substituted by 0.3 mM K^+ as K_2SO_4 for decreasing NO_3^--N; and 0.3 mM of NH_4^+ as NH_4Cl was added to increase NH_4^+-N. Similarly, in ratio 3 (R3), 0.9 mM of K^+ as

KNO_3 was substituted by 0.9 mM of K^+ as K_2SO_4 and an extra 0.9 mM of NH_4^+ was added as NH_4Cl. However, in ratio 4 (R4), in addition to the replacement of 0.5 mM K^+ as K_2SO_4, 0.4 mM of Mg^{2+} as $Mg(NO_3)_2.6H_2O$ was completely replaced by 0.4 mM of Mg^{2+} as $MgSO_4.7H_2O$, and additional 1.2 mM of NH_4^+ was added as NH_4Cl.

Table 5.1. Composition of nutrient culture solution used in the experiment

Element	Concentration		Chemical used
Major	mM	mg/L	
NO_3^--N	3.70	51.8	Ca, K and Mg nitrates
NH_4^+-N	0.30	4.2	$(NH_4)_2SO_4$
Ca	1.00	40.0	$Ca(NO_3)_2.4H_2O$
K	1.10	43.0	KNO_3
Mg	0.40	9.6	$Mg(NO_3)_2.6H_2O$
SO_4^{2-}-S	0.15	4.8	$(NH_4)_2SO_4$
HPO_4^{2-}-P	0.10	3.1	KH_2PO_4
Trace	µM	µg/L	
Cl	58.5	2076.7	NaCl
Na	58.5	1345.5	NaCl
Fe	17.9	998.8	FeNa-EDTA
B	6.6	71.3	H_3BO_3
Mn	2.4	131.8	$MnSO_4.H_2O$
Zn	0.6	39.2	$ZnSO_4.7H_2O$
Cu	0.2	12.7	$CuSO_4.5H_2O$
Mo	0.1	9.6	$Na_2MoO_4.2H_2O$

(After Taylor and Foy 1985d)

Table 5.2. Composition of nutrient solutions with varying levels of NO_3^--N and NH_4^+-N concentrations

Ratios	NO_3^--N		NH_4^+-N	
(NO_3^--N: NH_4^+-N)	mM	mg/L	mM	mg/L
Ratio 1 (R1) - 12:1	3.7	51.8	0.3	4.2
Ratio 2 (R2) - 11:2	3.4	47.6	0.6	8.4
Ratio 3 (R3) - 9:4	2.8	39.2	1.2	16.8
Ratio 4 (R4) - 7:6	2.5	35.0	1.5	21.0

Planting procedure

Six uniform tillers from the pretreatment solutions (7-day-old after separation from pots) were transferred and inserted through the holes of the box covers in such a way that the roots came into direct contact with the solutions. The tillers were mounted in cotton wool within rubber tubes. The nutrient solutions were continuously aerated.

pH measurement, solution renewal, data collection and plant harvesting

The pH of the solutions was measured daily (initially twice a day) (Parker and Norvell 1999) using an automatic portable digital pH meter with a research grade calomel glass combination electrode (pH 330 / SET - 1, Germany). Hydroponic solutions were renewed when rapid pH changes occurred. After 8 weeks, the plants were harvested, and maximum root and leaf lengths, plant heights and tiller numbers were recorded. The harvested plants were then divided into roots and shoots, rinsed four times in deionised water, dried to constant weight at 60^{o} C and weighed (Taylor and Foy 1985d). Due to the limited weight of plant material, whole shoots (leaf + stem) from each treatment were ground using a stainless-steel ring and puck grinder (Janke & Kunkel GMBH Co. Brazil) and used for mineral analyses. At the time of harvesting, a sample of the hydroponic solution was analysed.

Nutrient analysis

For both plants and hydroponic solutions, nitrate was determined colorimetrically using an automated cadmium reduction method (Huffman and Barbarick 1981); ammonium by using an automated indophenol blue reaction (Pym and Milham 1976); Ca, Mg, K, Na, P, S, B, Al, Fe, Cu, Zn and Mn by ICP (Inductively Coupled Plasma spectrometry, Zarcinas *et al*. 1987); and, Cl and F by titrimetric methods (Best 1929).

Statistical analysis

Statistical analyses of the experimental data were performed using the computer statistical program Genstat 5 (Release 4.2, PC/Windows 95). The regression analysis was used to examine the treatment effects on pH of nutrient solutions. As there was only one replication with six tillers in each treatment (i.e. samples), the analysis was limited to calculating standard errors for the samples within treatments.

Results

pH of nutrient solution

A ratio of 12:1, NO_3^-: NH_4^+ tended to increase solution pH relative to all other ratios (Fig. 5.1), and there was a sudden decrease in pH of all ratios after 18 days of treatment (Fig. 5.2). Although all the ratios behaved in a similar fashion, the relationship between ratio and time was different on at least one occasion (e.g. eleven days after transplanting, Fig. 5.2).

Growth response

Although plant height, shoot length and tiller number of *Austrodanthonia duttoniana* responded similarly to different nitrogen ratios, there was an effect of treatment on root length and dry matter (DM) (Table 5.3). Greater root length was obtained in ratio 1 than the other ratios, and ratios 2-4 were the same (Appendix 4). There was a similar trend for root DM production. Shoot yield was in the order: ratio $4 \geq 3 \geq 1 \geq 2$. For total DM, ratio 2 had the lowest value. While shoot: root ratios were in the order: ratio $4=3>2>1$.

Solution composition and tissue analysis

Chemical analysis of the nutrient solutions indicated that the formulations used for plant growth were relatively stable. There were no marked changes in nutrient composition even at the time of final harvest (Appendix 5). The concentrations of NO_3^--N were relatively unchanged (Appendix 5) probably due to a conversion of NH_4^+-N to NO_3^--N.

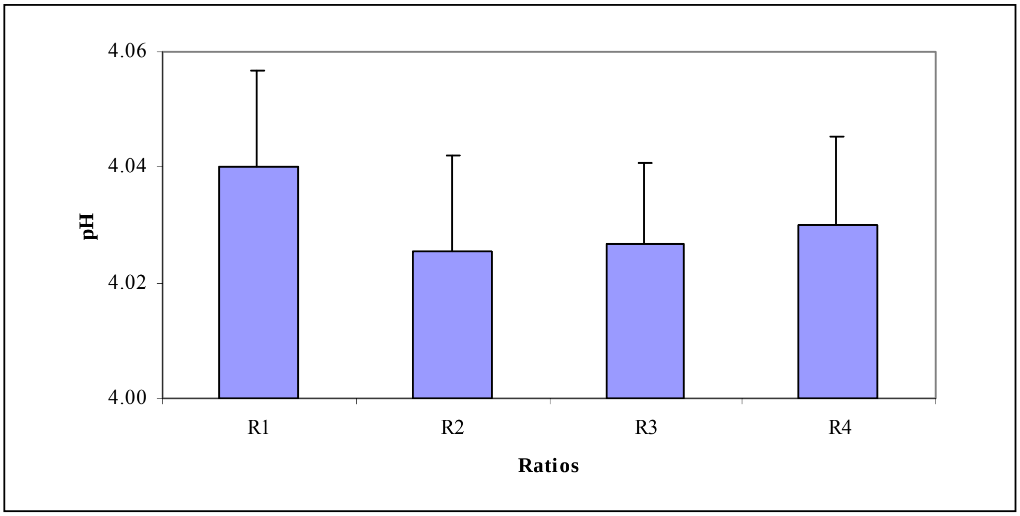


Fig. 5.1. Effect of different nitrogen ratios on pH of nutrient solutions. Vertical bars indicate standard errors. R is NO_3^-: NH_4^+ ratio, 1 = 12:1; 2 = 11:2; 3 = 9:4; 4 = 7:6.

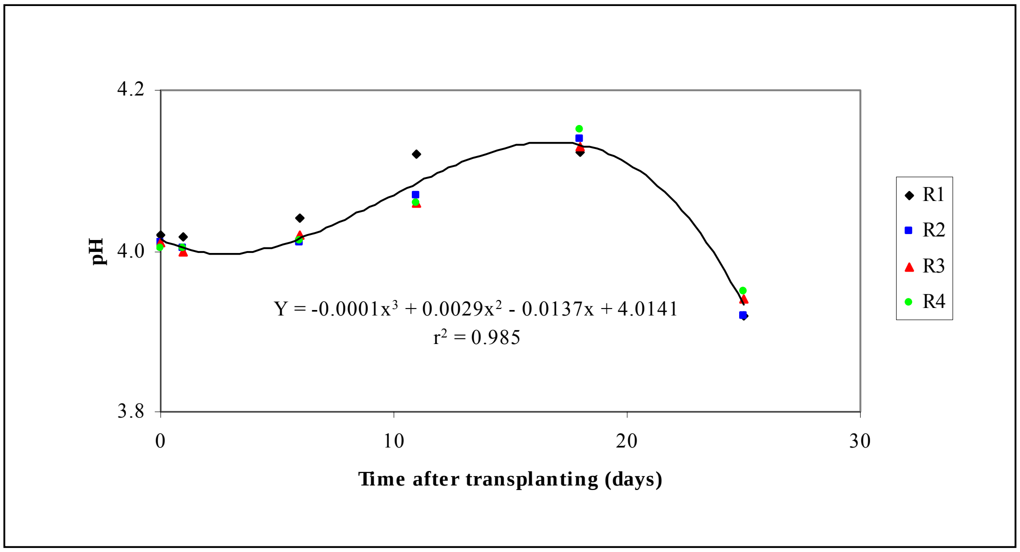


Fig. 5.2. pH of nutrient solutions with different ratios at different days after transplanting. Solid line represents a single trend line (polynomial) covering all ratios. R is NO_3^-: NH_4^+ ratio, 1 = 12:1; 2 = 11:2; 3 = 9:4; 4 = 7:6.

Tissue analysis for different elements did not show any marked change between the different N-ratios, except for the concentrations of K and Mn, which tended to decline with increased addition of NH_4^+-N (Appendix 6). Increasing addition of NH_4^+-N to the solution tended to increase NH_4^+-N concentration in plants.

Table 5.3. Growth response and DM yield of *A. duttoniana* in relation to different nitrogen ratios of growth media

Data are means for six plants. Values within parentheses are standard errors of means

NO_3-N: NH_4-N Ratio	Root length	Shoot length	Plant height	Tillers/ plant	DM yield (g/plant)			Shoot: root ratio
		cm			Shoot	Root	Total	
R1	48.8(±3.1)	45.4(±2.6)	36.4(±2.1)	13.5(±1.5)	0.46(±0.01)	0.12(±0.01)	0.58(±0.02)	3.74(±0.11)
R2	34.4(±3.5)	44.6(±4.2)	35.9(±3.2)	10.8(±2.2)	0.44(±0.02)	0.08(±0.01)	0.51(±0.02)	5.71(±0.12)
R3	33.7(±2.8)	43.2(±1.7)	33.7(±1.6)	14.0(±2.7)	0.49(±0.01)	0.07(±0.01)	0.56(±0.01)	6.82(±0.51)
R4	33.6(±3.7)	46.2(±2.3)	36.2(±1.8)	10.5(±1.6)	0.50(±0.01)	0.08(±0.01)	0.57(±0.02)	6.70(±0.27)

Discussion

pH of nutrient solution

The pH associated with different ratios (Fig. 5.1), and the sudden decline in pH after 18 days of treatment regardless of N-ratio (Fig. 5.2), may have been caused by unequal absorption of anions and cations from the root environment. As large amounts of N are required compared with other mineral nutrients, the form in which N is applied tends to exert a major influence on the direction of pH change. Therefore, absorption of NO_3^- commonly leads to an increase in the pH, whereas absorption of NH_4^+ leads to a decrease in pH (Asher and Edwards 1983). Trelease and Trelease (1935) found that varying ratios of NO_3^-/NH_4^+ could cause the pH to increase, decrease, or remain about constant for wheat. The pH of a nutrient solution for non-nodulated jack beans (*Canavalia ensiformis*) decreased markedly with time even when all nitrogen was supplied in the form of NO_3^- indicating that species differences in nutrient absorption and assimilation might be an important factor in changing the solution pH (Asher and Edwards 1983; Galvez and Clark 1991). The results of the current experiment indicate that changes in solution pH are most likely caused by differential uptake of nitrate and ammonium by plants. Release of hydroxyl- or bicarbonate-ions in exchange for NO_3^- can cause the pH of the solution to rise, whereas increased release of H^+ in exchange for NH_4^+ can decrease the solution pH. This result was confirmed by tissue analysis, where increased concentration of NH_4^+ was observed with increasing addition of NH_4^+ (Appendix 6).

Growth response

Increasing the concentration of NH_4^+ in solution can increase or decrease DM production and DM production is dependent on the NO_3^-: NH_4^+ ratio and the type of plant (McElhannon and Mills 1977; Galvez and Clark 1991; Bar-Tal *et al*. 2001b; Flores *et al*. 2001). In the present study, R1 (i.e. NO_3^-: NH_4^+, 12:1) produced longer root lengths (Table 5.3, Appendix 4) and higher DM yield of root and total (i.e. root + shoot) than the other ratios (Table 5.3). R3 and R4 (i.e. NO_3^-: NH_4^+, 9:4 and 7:6) showed little difference in any growth parameters. McElhannon and Mills (1977) found no differences in shoot and root dry weights of lima bean (*Phaseolus limensis* L.) treated with 75/25 and 50/50 of NO_3^-: NH_4^+. Plants can take up N as NO_3^- and NH_4^+, but NO_3^- is the most predominant form. For N assimilation, NO_3^- must be reduced to NH_4^+. When NH_4^+ is provided at a high rate, its uptake may exceed the assimilation rate or change the ionic equilibria, leading to toxicity (Flores *et al*. 2001). This may explain, why ratios 3 and 4 generally produced higher shoot biomass than the other ratios (Table 5.3).

Solution composition and tissue analysis

In water culture systems, the composition of the nutrient solution is usually unbuffered, and large changes in solution composition may occur within a relatively short time (Asher and Edwards 1983). As the nutrient solutions were renewed at the time of rapid pH change (e.g. at day 26), the formulation used in this experiment offered a relatively stable composition of nutrient elements (Appendix 5). Concentrations of most nutrients in the shoots of *Austrodanthonia* plants were not affected by different ratios of NO_3^-: NH_4^+ (Appendix 6). A slight decreasing trend of K and Mn with increasing NO_3^- to NH_4^+ ratio may be due to the decreasing trends in the concentrations of K and Mn in the nutrient solutions, and/or the inhibitory effect of increased NH_4^+ on ion uptake (Scott and Fisher 1989; Walch-Liu *et al*. 2000; Bar-Tal *et al*. 2001a; Flores *et al*. 2001). Increasing tissue-NH_4^+-N concentration with an increasing NH_4^+ to NO_3^- ratio, further supports these results.

Overall, the results of the experiment show that different ratios of NO_3^-: NH_4^+ can influence the pH of the nutrient solution as well as the growth of *Austrodanthonia* spp. However, on the basis of pH stability and DM production, the ratio of NO_3^-: NH_4^+, 9:4 (ratio 3) was chosen for further study.

5.2 Experiment 2. Effect of pH on different accessions of *Austrodanthonia* in nutrient solution culture

The hypothesis advanced was that H^+ did not retard growth of *Austrodanthonia*.

Materials and methods

Design

This experiment was carried out in a glasshouse during the period 5 July to 24 August 2000 at temperatures (day/night) ranging from 25-15°C. The methods of preparation were similar to the previous experiment. The NO_3/NH_4 ratio was 9: 4 (Table 5.2). Tillers of 12 accessions from 5 species of *Austrodanthonia* (*A. racemosa*, *A. duttoniana*, *A. fulva*, *A. penicillata*, and *A. pilosa*) which included a range of tolerances to acidity (Chapter 4), and most likely, Al-tolerance were used (Table 5.4). The experiment was a randomised complete block design consisting of 12 accessions, each repeated two times in each box (treatment) and with five treatments of pH (3.0, 3.5, 4.0, 4.5 and 5.5).

All treatments were replicated four times so that altogether, 20 boxes were arranged randomly on the bench of the glasshouse (Appendix 7). Five levels of pH were achieved by initially using 0.5M H_2SO_4 to pH ~5.7, and to lower levels by 1M HCl to avoid any extraneous effects of chloride toxicity (Hoagland and Arnon 1938, 1950; Taylor and Foy 1985d; Gallardo *et al.* 1999). Twenty-four tillers were planted in each box following the same procedures as for experiment 1. Boxes were re-randomised weekly. The pH's of the solutions were measured daily and adjusted using 1M HCl or 0.5M KOH. The solutions were renewed when any sharp change in solution pH occurred (cf. Taylor and Foy 1985d).

Table 5.4. The accessions of *Austrodanthonia* spp. used in the experiment

Species	Common ID*	Accession ID
A. racemosa	2	182095
A. racemosa	1	182251
A. racemosa	4	182233
A. racemosa	3	182188
A. duttoniana	12	182050
A. duttoniana	11	182131
A. fulva	8	182206
A. fulva	9	182205
A. penicillata	14	182081
A. penicillata	15	182192
A. pilosa	6	182087
A. pilosa	5	182288

** For details, see Table 4.4*

Harvesting, root staining and data handling

After eight weeks of treatment, the plants were harvested and the following measurements were recorded: 1) tillers per plant (only new tillers), 2) leaves per tiller (fully expanded), 3) length of each leaf, 4) width of each leaf, 5) number of roots (roots that arose only from the base of each tiller).

The harvested plants were then divided into shoots (leaf+stem+sheath) and roots. Each part of the shoot was rinsed with deionised water at least three times, blotted and dried to constant weight at 60^{o} C. Total leaf area was determined by summing the individual leaf areas (leaf area = length x width of leaf at mid-length). Due to the limited mass of some accessions, mineral analyses were restricted to shoots of selected accessions from each treatment. Minerals were determined as in experiment 1.

The roots of each accession, after washing with deionised water, were placed in a solution of deionised water (40 mL) and staining solution (0.5 mL) made from methyl violet stain (1g) in ethyl alcohol (100 mL). The roots were then stored in a cool room for at least 48 h at 4^{o} C. The total length and average width of roots were measured (Fig. 5.3) using an image scanner (Delta-T Scanner Mark 2, Cambridge, UK). Following the measurement of root dimensions, root-weights were determined after drying the roots for at least 24 h at 60^{o} C.

Statistical analyses

The effects of pH on tillers/plant, leaves/tiller, leaf area, number of roots, and DM of shoots and roots, were analysed using ANOVA. The length and width of roots and chemical composition of shoots were analysed by REML variance components analysis (mixed model) and significance was tested by the Wald statistic (Chi-square probability). Data were square-root or log transformed whenever necessary to obtain homogeneity of variance.

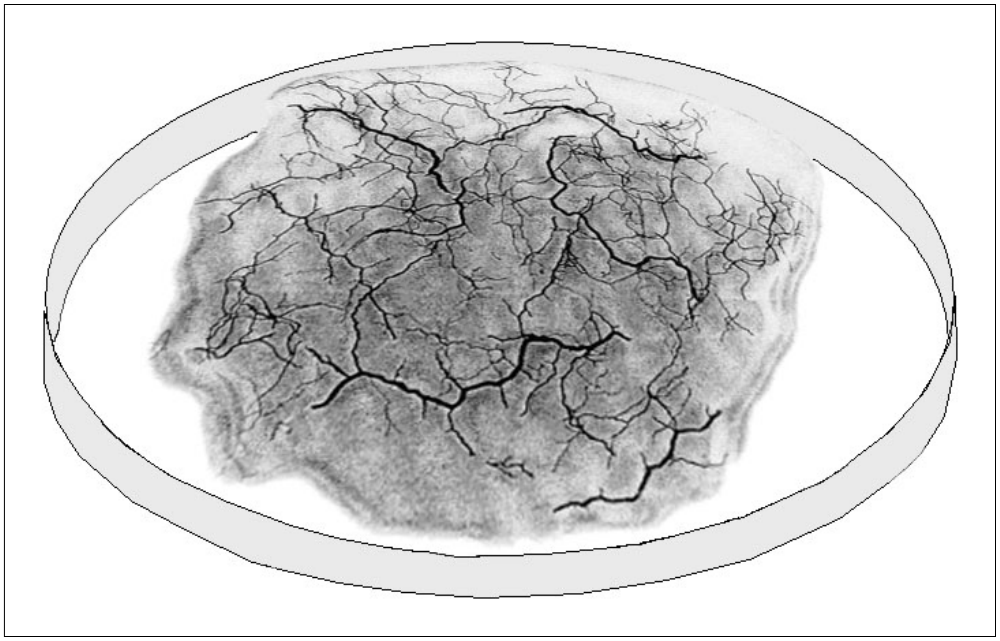


Fig. 5.3. Petri dish with stained roots ready for image scanning.

Results

Effect of pH on growth

Shoot and root: pH had a large effect on growth of *Austrodanthonia* accessions. The effect was more prominent on root growth than shoot growth (Table 5.5, Fig. 5.4). pH 3.0 produced the lowest number of roots and total root length per plant, and there were less differences between the other pH's (i.e. pH 3.5-5.5). For all parameters, pH 3.5 generally resulted in superior values. Similar results were also found in tillers per plant, leaves per tiller and leaf area per plant. There was no effect of pH on average width of root.

Table 5.5. Main effect of pH on shoot and root growth of 12 *Austrodanthonia* accessions with four replications

Values have been back transformed. Within columns, means followed by the same letters are not different ($P > 0.05$)

pH	Tillers* /plant	Leaves* /tiller	Leaf* area (cm^2/plant)	Roots* /plant	Total$^{\#}$ root length (cm/plant)	Average$^{\#}$ root width (cm)
3.0	1.59^{b}	0.71^{ab}	1.97^{b}	0.30^{c}	1.29^{c}	0.054^{a}
3.5	1.75^{a}	0.96^{a}	7.24^{a}	1.92^{a}	18.50^{ab}	0.069^{a}
4.0	1.04^{b}	0.57^{bc}	3.32^{b}	1.04^{b}	33.67^{a}	0.069^{a}
4.5	0.96^{b}	0.43^{c}	2.79^{b}	1.13^{b}	24.70^{ab}	0.067^{a}
5.5	0.84^{b}	0.40^{c}	2.33^{b}	0.78^{b}	14.49^{b}	0.062^{a}

** Square root transformed; $^{\#}$ log transformed*

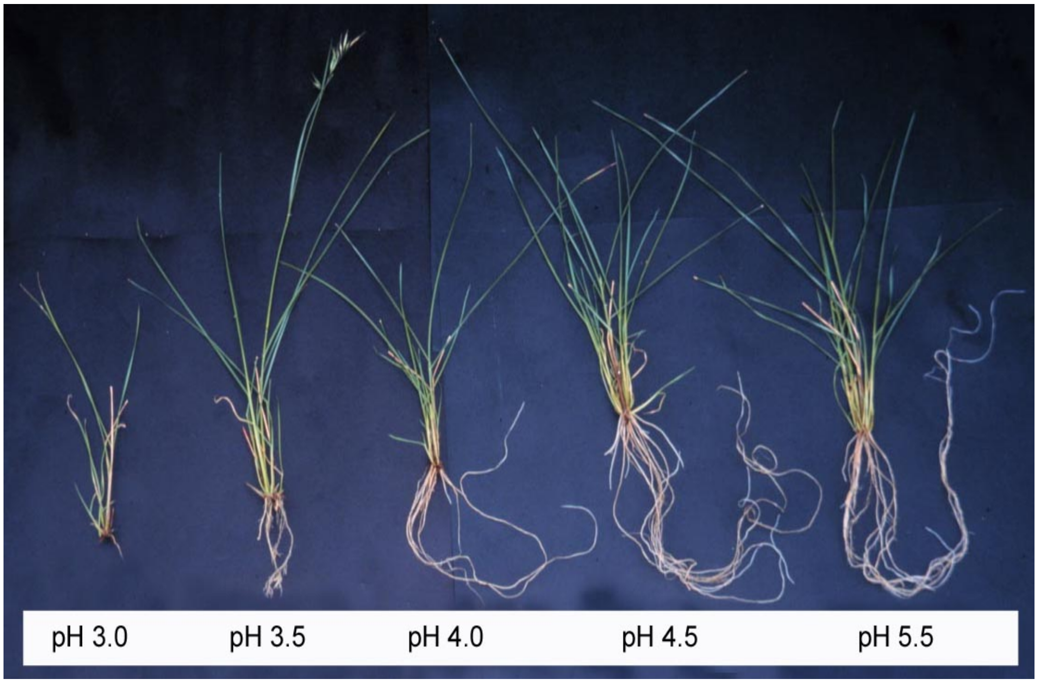


Fig. 5.4. Shoot and root growth of *A. duttoniana* (accession 182050) as affected by different solution pH (at the time of harvest).

Growth parameters of the 12 accessions of *Austrodanthonia* spp. differed widely. (Table 5.6). Among the accessions tested, accession 11 (i.e. 182131, *A. duttoniana*) had clearly superior plant vigour, followed by accessions 12 (182050, *A. duttoniana*), 8 (182206, *A. fulva*), 9 (182205, *A. fulva*) and 5 (182288, *A. pilosa*) where differences were smaller. There were two distinct groups of accessions – high performing (accessions 12, 11, 8, 9 and 5) and low performing (accessions 1-4, *A. racemosa*; 14-15, *A. penicillata*; 6, *A. pilosa*). Accessions with thicker roots had greater root length.

Table 5.6. Mean shoot and root growth response of 12 *Austrodanthonia* accessions (Table 5.4) to five pH treatments

Values have been back transformed. Within columns, means followed by the same letters are not different (P > 0.05)

Accessions	Tillers* /plant	Leaves* /tiller	Leaf* area (cm^2/plant)	Roots* /plant	Total$^{\#}$ root length (cm/plant)	Average$^{\#}$ root width (cm)
2	0.80^{de}	0.45^{cd}	0.80^{d}	0.17^{e}	5.46^{cd}	0.047^{d}
1	0.46^{ef}	0.55^{bcd}	0.80^{d}	0.39^{e}	8.33^{bc}	0.052^{bcd}
4	0.27^{ef}	0.31^{de}	0.41^{d}	0.11^{e}	10.48^{bc}	0.040^{d}
3	0.32^{ef}	0.86^{bc}	0.64^{d}	0.03^{e}	0.82^{d}	0.050^{d}
12	3.25^{b}	0.94^{b}	12.87^{b}	3.36^{b}	68.18^{a}	0.094^{a}
11	8.31^{a}	2.16^{a}	35.03^{a}	10.02^{a}	76.62^{a}	0.089^{ab}
8	1.75^{c}	0.87^{b}	6.09^{c}	1.63^{cd}	28.51^{ab}	0.074^{abcd}
9	1.75^{c}	0.85^{bc}	8.51^{bc}	2.44^{bc}	32.11^{a}	0.089^{ab}
14	0.06^{f}	0.04^{f}	0.07^{d}	0.05^{e}	6.41^{bcd}	0.069^{abcd}
15	0.15^{f}	0.23^{def}	0.31^{d}	0.04^{e}	7.13^{bc}	0.045^{d}
6	0.16^{f}	0.11^{ef}	0.24^{d}	0.18^{e}	8.55^{bc}	0.045^{d}
5	1.36^{cd}	0.87^{b}	4.60^{c}	1.24^{d}	21.39^{abc}	0.079^{abc}

* Square root transformed; $^{\#}$ log transformed

Table 5.7. Growth response of *Austrodanthonia* accessions (Table 5.4) as affected by pH

Values are means of four replications and have been back transformed (square root). Within columns, means followed by the same letters are not different ($P > 0.05$)

Accessions	Leaf area (cm^2/plant)					Roots /plant				
	pH									
	3.0	3.5	4.0	4.5	5.5	3.0	3.5	4.0	4.5	5.5
2	1.05^{bc}	1.59^{cde}	0.43^{de}	0.36^{cd}	0.86^{d}	0.20^{b}	0.49^{cde}	0.002^{d}	0.11^{d}	0.15^{d}
1	0.56^{c}	0.33^{e}	2.27^{cde}	0.66^{cd}	0.66^{d}	0.18^{b}	0.25^{de}	1.09^{cd}	0.36^{cd}	0.26^{cd}
4	0.28^{c}	0.39^{e}	1.17^{cde}	0.36^{cd}	0.11^{d}	0.18^{b}	0.002^{e}	0.07^{d}	0.38^{cd}	0.002^{d}
3	0.57^{c}	1.09^{e}	0.36^{de}	0.70^{cd}	0.56^{d}	0.002^{b}	0.11^{e}	0.002^{d}	0.07^{d}	0.002^{d}
12	7.76^{ab}	32.56^{b}	6.23^{bc}	10.35^{b}	13.88^{b}	0.61^{ab}	7.40^{b}	2.34^{bc}	3.76^{b}	4.68^{b}
11	18.36^{a}	62.33^{a}	46.56^{a}	30.30^{a}	25.82^{a}	2.70^{a}	14.81^{a}	13.35^{a}	11.93^{a}	10.34^{a}
8	2.99^{bc}	9.25^{c}	16.72^{b}	5.30^{bc}	1.34^{cd}	0.45^{b}	2.57^{c}	4.19^{b}	1.83^{bc}	0.45^{cd}
9	2.11^{bc}	39.64^{ab}	4.65^{cd}	3.59^{bd}	7.61^{bc}	0.25^{b}	10.37^{ab}	2.16^{bc}	1.38^{bcd}	1.93^{bc}
14	0.002^{c}	0.58^{e}	0.002^{e}	0.002^{d}	0.002^{d}	0.002^{b}	0.33^{de}	0.002^{d}	0.002^{d}	0.002^{d}
15	1.06^{bc}	0.64^{e}	0.08^{de}	0.002^{d}	0.16^{d}	0.07^{b}	0.002^{e}	0.002^{d}	0.002^{d}	0.15^{d}
6	0.13^{c}	1.42^{de}	0.002^{e}	0.22^{cd}	0.002^{d}	0.11^{b}	0.99^{cde}	0.002^{d}	0.15^{cd}	0.002^{d}
5	2.75^{bc}	8.48^{cd}	2.12^{cde}	9.67^{b}	2.47^{cd}	0.31^{b}	1.92^{cd}	0.75^{cd}	3.68^{b}	0.63^{cd}

Austrodanthonia accessions were affected by pH only for leaf area and the number of roots per plant (Table 5.7). Accession 11 performed very well relative to the other accessions, irrespective of pH treatments. At all pH values, this accession had the greatest leaf area and root number among the accessions (except for accession 9, at pH 3.5 - leaf area/plant) indicating its high tolerance to all pH values. The performance of other accessions was consistent with the results presented earlier (Tables 5.5 and 5.6).

DM: pH 3.0 severely decreased DM weights of both shoots and roots (Fig. 5.5). Root weights were not affected by pH's ≥ 4.0, whereas shoots and total DM yield were not affected by pH's ≥ 3.5.

There was no interaction between pH and accession, although accessions differed in DM yields. Of the accessions tested, accession 11 had the highest root, shoot and total DM weights (Fig. 5.6). The next best performing accessions were 12, 9, 8 and 5. The rest of the accessions fell into a low yielding group, between which there were no differences (Fig. 5.6).

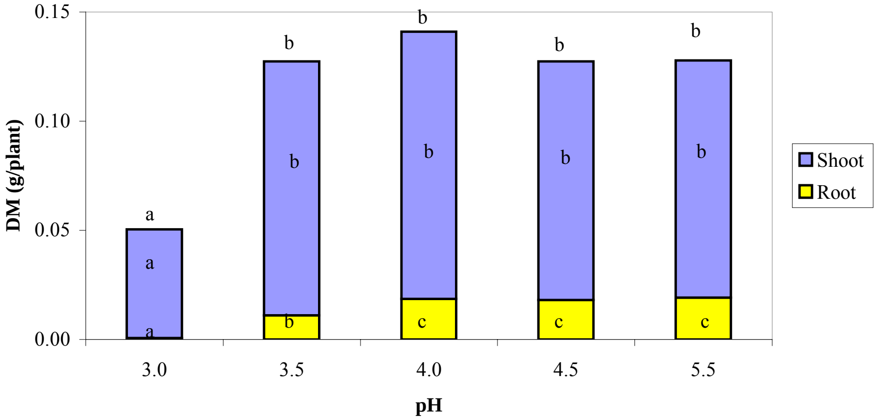


Fig. 5.5. Main effect of pH on DM yield of *Austrodanthonia* accessions. Data are means of twelve accessions (back-transformed values) with four replications. Columns associated with the same letters are not different (P > 0.05). Letters above each column are for total DM.

Composition of the solution and plant tissues

Chemical analysis of the nutrient solutions after the final harvest showed that the composition was stable. Few variations were observed across the pH treatments (Appendix 8). The highest concentration of NH_4^+-N remained at pH 3.0, while there was a continuous decline in Fe concentrations with each increment of pH > 3.5.

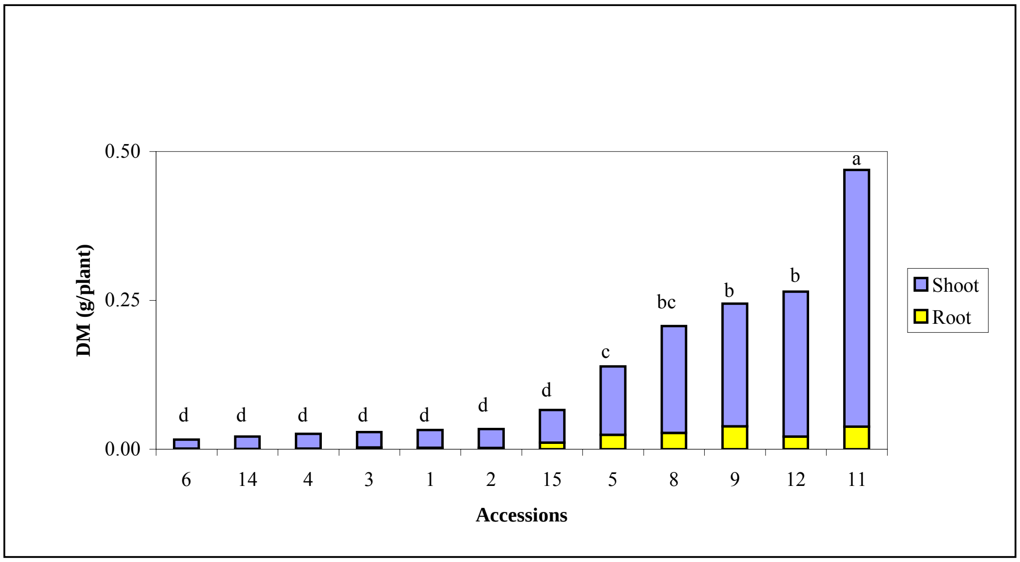


Fig. 5.6. DM of different *Austrodanthonia* accessions (Table 5.4). Data in the histogram are means for five pH values (back-transformed). Columns associated with the same letters are not different (P > 0.05).

pH had no effect on the concentrations of K, Na, Ca, Mg, S, Mn, Fe, Cu, Zn and B in the shoots, but markedly affected the concentration of P in the shoots. The lowest concentration of P (0.29 %, dry weight basis) was observed at pH 3.0 and this P concentration differed from the concentration at all the other pH values.

There were large variations between accessions in element concentration in their shoots, particularly for P, Ca, Fe and B (Table 5.8); however, there was no interaction between pH and accession (P = 0.05) and is not presented.

Table 5.8. Mean element concentrations in whole shoots of ten accessions of *Austrodanthonia* (Table 5.4) grown hydroponically

Data are means for the five pH levels

Accessions	Concentration in shoots (dry weight basis)										
	(%)						(mg/kg)				
	P	K	Na	Ca	Mg	S	Mn	Fe	Cu	Zn	B
2	0.30	1.87	0.29	0.67	0.29	0.38	61.85	161.00	9.47	61.36	12.11
1	0.27	1.81	0.30	0.47	0.29	0.36	58.12	163.10	14.25	62.07	7.20
4	0.31	1.85	0.32	0.44	0.32	0.41	69.87	209.70	12.10	78.28	8.09
3	0.24	1.93	0.27	0.64	0.27	0.35	44.65	244.00	11.92	60.56	13.80
12	0.50	2.59	0.23	0.36	0.23	0.51	44.37	120.70	10.11	46.09	8.19
11	0.45	2.36	0.24	0.34	0.22	0.44	42.60	131.40	7.96	39.70	7.50
8	0.38	2.34	0.23	0.46	0.23	0.45	40.84	143.10	10.48	55.30	8.02
9	0.35	2.31	0.26	0.35	0.24	0.45	29.94	116.80	8.14	53.00	6.50
15	0.31	2.45	0.35	0.70	0.35	0.29	67.28	246.60	12.23	72.74	5.05
5	0.44	2.22	0.22	0.43	0.21	0.42	47.85	130.50	9.87	50.40	4.67
LSD (P = 0.05)	0.10	0.37	0.08	0.18	0.08	0.13	20.66	52.08	7.93	22.52	1.71

Discussion

Effect of pH on growth

Shoot and root: increasing the pH from 4.0 to 5.5 had no effect on either shoot or root growth of *Austrodanthonia* accessions (Table 5.5). pH 3.5 resulted in more growth of shoots and roots than the other pHs, probably because a greater number of roots was produced at this pH. The damaging effects of extremely low solution pH on root growth were observed in all accessions at pH 3.0 (Fig. 5.4). These results are in agreement with previous solution culture studies involving other species (Arnon and Johnson 1942; Islam *et al*. 1980; Ila'ava *et al*. 2000a; Lu and Sucoff 2001). For example, Arnon and Johnson (1942) found that roots of Bermuda grass, tomato and lettuce seedlings were unable to grow in the solution culture maintained at a pH of 3.0. Similarly, Lu and Sucoff (2001) observed that root and shoot growth of Quaking aspen (*Populus tremuloides* Michx.) seedlings were completely inhibited in solutions maintained at pH 3.3. The poor growth was probably due to the effect of low pH on impaired ion transport or root-membrane damage (Islam *et al*. 1980).

Other solution culture studies have shown that plant species and cultivars differ widely in their tolerance to low solution pH. For example, Edmeades *et al*. (1991b) showed that, with the exception of phalaris, temperate grasses grow well at pH values down to at least pH 4.5. Islam *et al*. (1980) found that ginger and cassava were more tolerant species than wheat and maize at pH 3.3-4.0. Accessions of *Austrodanthonia* showed a differential growth response in relation to solution pH (Table 5.7, Fig. 5.6). Some of the accessions (e.g. accession 12, 11, 8, 9 and 5) even performed better in low pH (e.g. pH 3.5) than at higher pH values. Thus the results indicate there is a wide range of genetic variation of *Austrodanthonia* accessions in response to low solution pH.

DM: Arnon and Johnson (1942) concluded that Bermuda grass, tomato and lettuce could tolerate fluctuations in pH between 4 and 8, provided that an adequate supply of all nutrient elements was maintained. At pH 4.0, Bermuda grass grew well; the yields of tomato and lettuce were decreased to about 35% of the maximum; but no growth was obtained at pH 3.0 for any species. Thawornwong and van Diest (1974) showed that DM yields (shoots and root) of rice seedlings were not affected by pH down to 3.5. Root yields of *Austrodanthonia* accessions were affected at pH < 4.0, and shoot and total yields at pH 3.0 (Fig. 5.5). Such limited DM yield of shoots and roots at pH 3.0 may be due to restricted absorption of nutrients by the roots (Arnon and Johnson 1942).

Although there was no interaction between pH and accession, accessions varied in producing DM across the pH treatments. Some of the accessions produced extremely high yields, and there was variation among the accessions within the same species, e.g., accessions 12 and 11 of *A. duttoniana* (Fig. 5.6). These results are supported by the previous growth response data (Tables 5.6 and 5.7). The variation in response by accessions is most likely due to a difference in genetic potential of the plant species (Arnon and Johnson 1942; Moore 1974; Islam *et al.* 1980; Foy 1984).

Solution composition and tissue analysis

Composition of the nutrient solution was relatively stable, as the solutions were renewed before any sharp changes of pH occurred (Appendix 8). Presence of the highest amount of NH_4^+-N at pH 3.0 was probably due to a low conversion rate of NH_4^+-N to NO_3^--N (cf. Flores *et al.* 2001). Increasing pH from 3.0 to 5.5 decreased Fe concentrations in the solutions and this happened possibly because of the precipitation or binding effect of FeNa-EDTA (source of Fe) at the higher pH (Islam *et al.* 1980).

Several short-term studies have shown that absorption of cations is often decreased by decreasing pH (Thawornwong and van Diest 1974; Islam *et al.* 1980; Ila'ava *et al.* 2000a; Lu and Sucoff 2001; Kidd and Proctor 2001). In the present experiment, tissue analysis could not explain the differential tolerance of *Austrodanthonia* accessions to low solution pH. Only tissue P concentration was affected by solution pH. The lowest concentration of tissue P (0.29%) was obtained at pH 3.0 indicating a possible cause of growth reduction in solutions with low pH. Lu and Sucoff (2001) demonstrated that shoots of Quaking aspen showed typical P deficiency symptoms (purple colouration; leaf P concentration 0.16-0.20%) when grown in solution at pH ≤ 3.5. A large variation in element concentrations was noticed in the shoots of different selected accessions (Table 5.8). However, nutrient concentrations in the shoots of *Austrodanthonia* accessions were generally indicative of adequate levels for plant growth (based on other perennial pasture species, Pinkerton *et al*. 1997). As far as is known, there are no detailed data for deficient, sufficient and toxic concentrations of elements in *Austrodanthonia* spp.

This experiment shows that the adverse effect of pH is found only at extreme acidity. Growth was reduced drastically at pH 3.0. Hutton approach was applied following the same principles as in Chapter 4 i.e. DM at pH 5.5 was regressed against DM at high H^+ challenge (pH 3.5; Fig. 5.7). All the accessions were closely associated with the fitted lines indicating again that pH 3.5 did not affect *Austrodanthonia* growth.

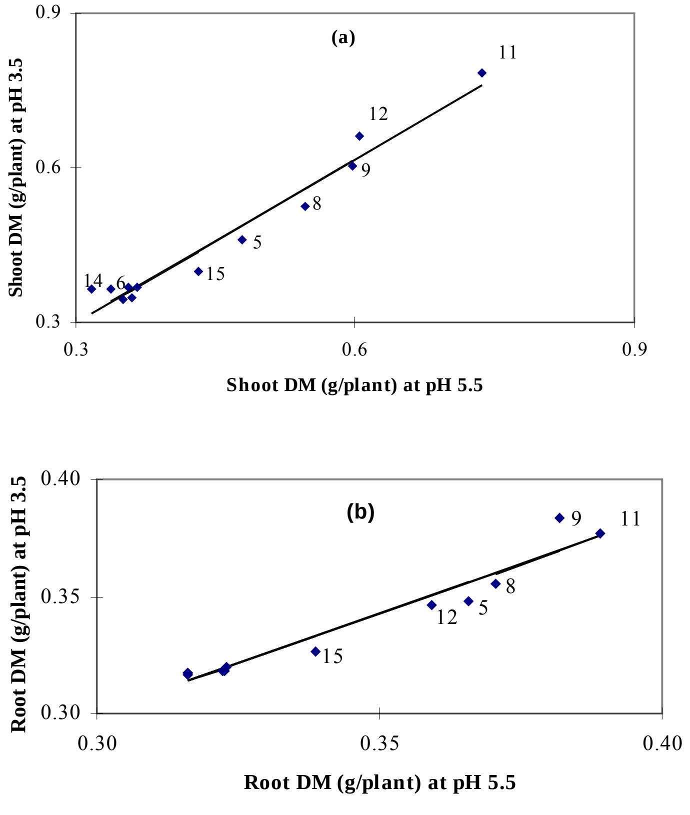


Fig. 5.7. Relationships between shoot DM at pH 5.3 and pH 3.5 (a), equation of line: Y = 1.058X - 0.017, n = 12, r^2 = 0.98; and root DM at pH 5.3 and pH 3.5 (b), equation of line: Y = 0.852X + 0.044, n = 12, r^2 = 0.97. Dots are data points for 12 accessions (Table 5.4; numbers displayed in the figures are few example accessions).

Thus, the *Austrodanthonia* accessions tested grow well between pH 3.5 and 5.5 when provided with an adequate supply of nutrients. The lowest pH_{Ca} value of soils on the Tablelands of NSW is ~ 3.9 (Fig. 1.1), so the concentration of H^+ in these soils is unlikely to affect the growth of any of the *Austrodanthonia* accessions tested.

5.3 Experiment 3. Pilot evaluation of the effect of Al at pH 4.0 on growth of a selected accession of *Austrodanthonia duttoniana* in hydroponic solution

The aims of the experiment were to optimise the range of Al concentration for the later experiment (Chapter 6) and to investigate whether it was necessary to use deionised water or whether tap water would suffice.

Materials and methods

To model a dose-response effect of Al on *Austrodanthonia* spp. and to estimate the free ion activities of Al in nutrient solutions, a glasshouse experiment was conducted during the period 10 October to 5 December 2000. The experiment was carried out following exactly the same methods and using the same materials used in experiments 1 and 2. One of the accessions, the response of which seemed to be Al-intolerant to neutral (*A. duttoniana*, accession 182106) from the previous pot experiment (Table 4.4) was used.

A completely randomised design with one accession repeated five times, two sources of water (deionised and tap water) and five levels of Al (0, 50, 100, 200 and 250 μM Al) was used. Each of the 10 treatments (i.e. 2 X 5) was allocated in one box and five tillers were planted per box. There was no replication. The following abbreviations are used: deionised water = DW; tap water = TW; and Al 0, Al 50, Al 100, Al 200 and Al 250 instead of 0-250 μM Al. Aluminium was added in the form of aluminium sulfate to avoid the potentially toxic levels of chloride ions associated with the addition of aluminium chloride ($AlCl_3$) (Crawford and Wilkens 1998). The treatments of Al were prepared by adding Al from a stock solution of 100 mM Al of $Al_2(SO_4)_3.18H_2O$ to the nutrient solutions to provide total Al concentrations in the range 0-250 μM. The pH of the treatment-solutions was adjusted to a final value of 4.0 using 1M HCl. The levels of Al were selected to provide a range of toxic effects based on the previous work with cultivars of barley (*Hordeum vulgare* L.) (Gallardo *et al.*1999). The pH of the solutions was checked daily, adjusted using 1M HCl or 0.5M KOH, and renewed every alternative week when a sharp rise of pH occurred. Growth and visible symptoms were observed and recorded daily. Growth (shoots and roots) measurements, sample collection and processing, and data handling were the same as in experiment 2. Data were analysed following the same principles of the previous experiment 1. As single replication was used, analysis was restricted to calculating standard errors for the samples within treatments. The chemical speciation program, GEOCHEM-PC version 2.0 (Parker *et al.* 1995b), was used to predict the concentrations of various Al species in the solutions.

Results

Effect of Al, DW and TW

Symptoms: no distinct visible symptoms were observed in the shoots of *A. duttoniana* during the experiment. Comparatively short, thick and deformed roots were observed with increasing Al treatments. At the high Al concentrations (e.g. 200, 250 μM), in both sources of water, root tips and lateral roots became thickened and turned light brown. The whole root system became stubby in appearance and lacked fine branching (Fig. 5.8).

Growth response

Shoot and root: sources of water (DW and TW) appeared to have no effects on growth of this *Austrodanthonia* accession (Fig. 5.8). Increasing Al concentrations appeared to depress most of the indices of growth (e.g. tillers/plant, total leaves/plant, leaf breadth, leaf area/plant and total root length/plant), but to have no effect on average leaves/tiller, maximum leaf length/plant, root number/plant and average root width/plant (Table 5.9). The damaging effects appeared to increase progressively with increasing Al dose. Total root length appeared the most sensitive index of Al toxicity.

Dry matter (DM): sources of water again appeared to have no effects on DM production and a continuously decreasing trend was observed with increasing doses of Al (Fig. 5.9).

Table 5.9. Effect of Al on different growth parameters of *A. duttoniana* (accession 182106)

Data values are means of two sources of water. Values in parentheses are standard errors of means

Treatment	Tillers /plant	Average leaves /tiller	Total leaves /plant	Leaf breadth (cm)	Max. leaf length (cm/plant)	Leaf area (cm^2/plant)	Roots /plant	Total root length (cm/plant)	Average root width (cm)
Al 0	12.4(±1.1)	1.71(±0.1)	17.9(±1.8)	0.29(±0.01)	23.55(±1.4)	57.9(±8.3)	21.5(±1.3)	344.7(±33.1)	0.14(±0.01)
Al 50	9.9(±1.1)	1.85(±0.1)	14.5(±2.1)	0.25(±0.02)	20.99(±1.9)	44.6(±9.3)	16.1(±1.7)	236.8(±23.9)	0.16(±0.01)
Al 100	11.0(±1.2)	1.61(±0.1)	13.2(±1.4)	0.25(±0.01)	19.87(±2.0)	40.3(±7.1)	20.4(±2.8)	120.9(±11.9)	0.16(±0.01)
Al 200	7.9(±0.5)	1.74(±0.1)	10.0(±1.0)	0.22(±0.01)	18.93(±1.1)	26.5(±3.4)	16.1(±1.3)	51.0(±5.1)	0.17(±0.01)
Al 250	7.3(±1.2)	1.56(±0.1)	8.3(±1.5)	0.21(±0.01)	17.60(±2.7)	23.4(±5.5)	16.1(±3.1)	40.3(±4.1)	0.17(±0.01)

Fig. 5.8. Effect of Al on growth of *A. duttoniana* (accession 182106). DW = deionised water; TW = tap water.

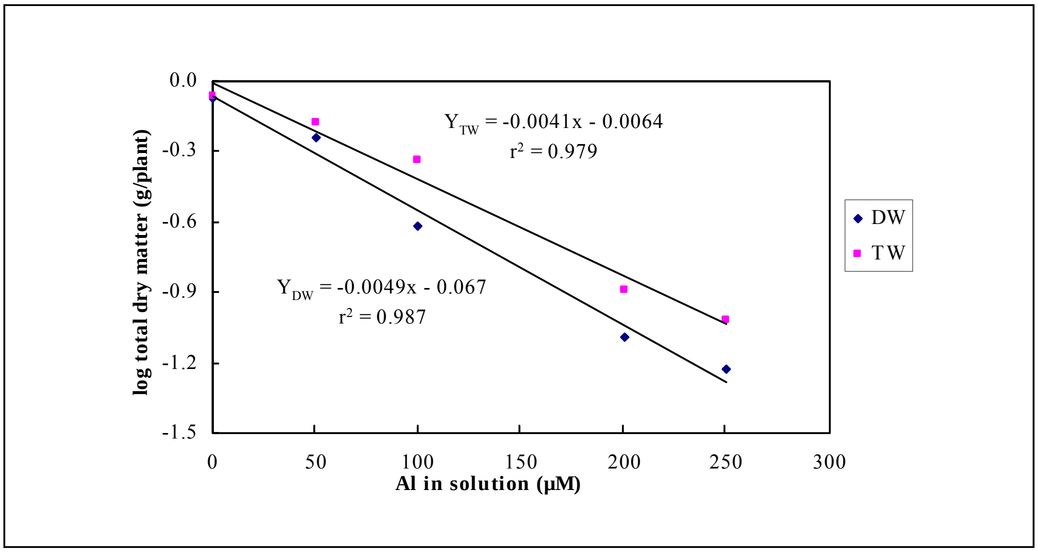


Fig. 5.9. Total DM yield of *A. duttoniana* (accession 182106) as affected by water sources at different levels of Al. Data are means for five plants (natural log transformed). Co-variance analysis shows no difference between the slopes of two fitted lines (P = 0.16). DW = deionised water; TW = tap water.

As with shoot and root, DM (all components) also appeared to be decreased by increasing Al concentrations (Table 5.10). The damaging effect appeared to start at Al 100 and was severe at Al 250. High shoot to root ratios at Al 200 (19.1) and Al 250 (19.8) were indicative of more extensive damage in the roots than in the shoots.

Table 5.10. Growth response (DM) of *A. duttoniana* accession to different Al concentrations

Data are overall means for two sources of water. Values in parentheses are standard errors of means

Treatment	DM (g/plant)				Shoot: root ratio
	Leaf	Shoot	Root	Total*	
Al 0	0.30(±0.04)	0.84(±0.08)	0.092(±0.010)	0.93(±0.08)	10.6(±2.1)
Al 50	0.27(±0.04)	0.73(±0.09)	0.081(±0.013)	0.81(±0.10)	9.9(±0.9)
Al 100	0.23(±0.03)	0.57(±0.09)	0.053(±0.008)	0.63(±0.10)	11.6(±1.3)
Al 200	0.14(±0.01)	0.35(±0.02)	0.021(±0.003)	0.37(±0.03)	19.1(±1.9)

Al 250	0.10(±0.01)	0.31(±0.04)	0.018(±0.003)	0.33(±0.04)	19.8(±2.3)

** Total = shoot + root*

Solution composition, Al speciation and tissue analysis

The composition of nutrient solution at the conclusion of the experiment provided little concern about nutrient supply (Appendix 9). Higher concentrations of Na, Ca, Mg, Cl, S and F were found in TW than DW, and increasing levels of Al were found as Al treatment progressively increased. Al activity and the concentration of free Al also increased with increasing Al levels (Appendix 10). For example, about 51% (in DW) and 38% (in TW) of the total Al concentration was present as free metal at the highest Al treatment (i.e. 250 µM), while the remaining Al was complexed mainly with the SO_4^{2-}, F^- and PO_4^{3-} present in the solutions. A larger proportion of the Al was complexed with F at lower than at higher treatment levels of Al. These speciation calculations assume that root exudates did not complex Al. Tissue analysis of shoots showed little variation among element concentrations (except Al) in both DW and TW (Appendix 11). Increasing Al levels increased tissue Al concentrations and Al accumulation was much higher in DW than TW.

Discussion

Effect of Al on plant growth

The symptoms of Al toxicity are not easy to identify. Al toxicity in shoots is often characterised by symptoms resembling P deficiency (e.g. purpling of stems) or Ca deficiency (e.g. cupping or rolling of young leaves) (Foy 1974, 1983, 1984, Matsumoto 2000; Rout *et al*. 2001). The primary site for Al toxicity is the root. Foy (1983, 1984) and Taylor (1988b) reported that Al-affected roots are characteristically stubby, brittle and brown in colour. They also reported that, due to excess Al, the elongation of the main root axis is inhibited, roots become thickened and necrotic, and the whole root system appears to lack fine branching. As a result, affected roots absorb nutrients less efficiently and cause poor growth and plant persistence. In the present experiment, no distinctive symptoms were visible in the shoot but symptoms of Al toxicity were evident in the roots (Fig. 5.8). The root was affected more than the shoot as Al increased in both DW and TW, and thicker roots were obtained at high Al levels compared with low Al (Table 5.9, Fig. 5.8). Taylor and Foy (1985d) demonstrated that growth of winter wheat was depressed in all Al-sensitive cultivars at 74 µM Al. They did not observe any growth depression in the Al-tolerant cultivar (Atlas-66).

Hoagland and Arnon (1938, 1950) suggested that waters suitable for irrigation or drinking can be used in the solution-culture provided an adequate supply of all nutrient elements along with a suitable pH is maintained. In the present study, DW and TW did not affect plant growth across the Al treatments, although a slightly lower yield was always obtained from DW (Figs 5.8 and 5.9). This may have been due to the difference in free Al activity (Fig. 5.10). DW contained about 50% free Al^{3+} whereas, TW contained 5-38% free Al^{3+} depending on Al treatment (Appendix 10).

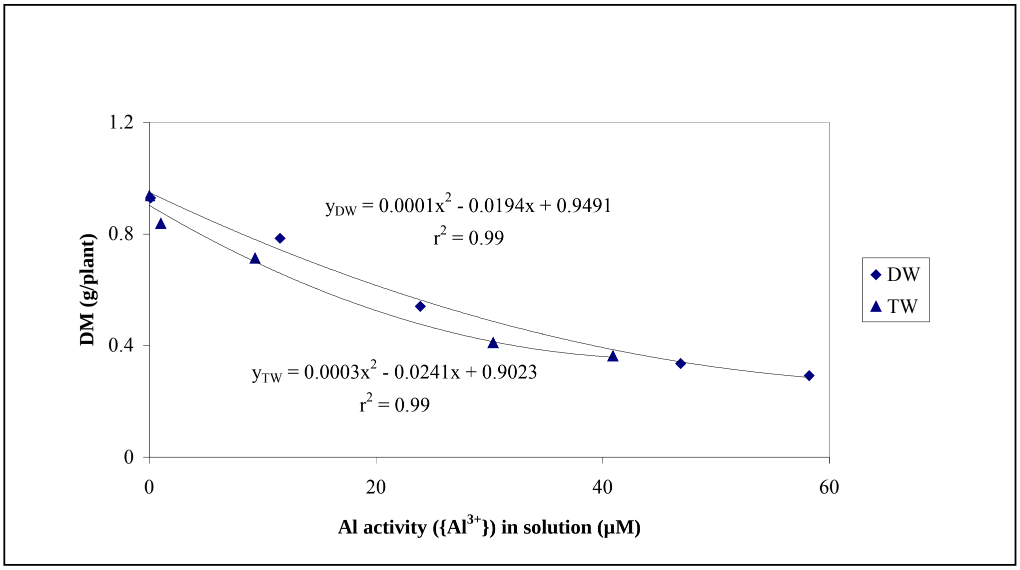


Fig. 5.10. Total DM yield of *A. duttoniana* (accession 182106) as affected by free Al activity in nutrient solutions of DW (deionised water) and TW (tap water) at pH 4.0. The fitted lines explain the relationship between DM production and free Al activity. Data points are means of five plants.

Reductions in the growth parameter (i.e. DM production) from increasing Al have been reported (MacLeod and Jackson 1967; Taylor 1988b; Cocker *et al*. 1997; Zsoldos *et al*. 2000; Liang *et al*. 2001). For example, Zsoldos *et al*. (2000) demonstrated yield depression of durum wheat from 10 µM Al^{3+}. Liang *et al*. (2001) showed that increasing Al-addition from 0 to 150 µM at pH 4.2 decreased yield of barley by about 33%. A similar result occurred in the present study. Increasing addition of Al in the nutrient solution from 50-250 µM at pH 4.0 decreased total DM yield from 13-65% compared to the control (Al 0) treatment (Table 5.10). Shoot DM followed the same trend, but in the case of root DM, about an 80% yield decrease (compared to the control) was found at Al 250, indicating that the root is the primary site of the Al effect (Foy 1984; Kochian 1995; Matsumoto 2000).

Chemical composition of solution and tissue

The chemical analysis of nutrient solutions and speciation estimates using GEOCHEM showed similar elemental compositions both in DW and TW, especially for Al (Appendices 9 and 10). Higher concentrations of Na, Ca, Mg, Cl, S and F were recorded in the nutrient solutions made using TW than DW, because the TW initially contained these elements at higher concentrations; however, these differences were not reflected in the tissue analysis (Appendix 11). A decreasing trend of NO_3^--N with increasing Al concentrations in solution was observed in both TW and DW. A similar trend was also found in P and K concentrations. Al accumulation in the shoots increased with increasing concentration of Al in solution irrespective of water sources, resulting in toxic levels of Al that ultimately reduced plant growth (Foy *et al.* 1978; Foy 1984). The above results are consistent with previous work, e.g. Cocker *et al.* (1997), Kidd and Proctor (2000), Zsoldos *et al.* (2000) and Liang *et al.* (2001) who showed decreased concentrations of P, K, N and Ca in plant tissue with increasing additions of Al. Decreased concentrations of these elements were probably due to the interference of excess Al with nutrient absorption and ion transport (Foy 1974, 1983, 1984; Foy and Fleming 1978; Foy *et al.* 1978; Matsumoto 1991, 2000; Rout *et al.* 2001) through root membrane damage (Vierstra and Haug 1978; Gomez-Lepe *et al.* 1979) and nutrient loss through the damaged membranes (Foy 1983, 1984).

Increasing Al appeared to reduce growth of *Austrodanthonia* accession 182106 at pH 4.0. The effect was larger on root growth than shoot growth and most severe at ≥ 200 μM Al. Although no difference was found between DW and TW, the toxic effect of Al appeared more obvious in DW than TW.

5.4 Experiment 4. Pilot evaluation of the effect of the plant size on survival and growth of *Austrodanthonia* spp.

The aim of the experiment was to evaluate the effect of initial plant size (number of tillers) on survival and growth of *Austrodanthonia* spp.

Materials and methods

The aim of the experiment was to investigate the influence of size of rooted tillers of different species on growth and survival, and to examine the relative performance of a range of *Austrodanthonia* species in their ability to grow in hydroponic culture. This experiment was undertaken under controlled conditions in a glasshouse from 8 May to 3 July 2001. The day/night temperature ranged from 25 to 15°C. The experiment was conducted following the same procedures as in experiments 1 and 2. Three of the most common species of *Austrodanthonia* (*A. racemosa**, accession 182294; *A. pilosa*, accession 182267; and *A. penicillata*, accession 182265) were used for this experiment. In experiment 2, these species did not grow well under hydroponic conditions using single rooted tillers. Because of this, there was a need to evaluate the effect of the initial size of rooted tillers on subsequent growth in hydroponic culture.

A completely randomised design with three species of *Austrodanthonia* repeated four times was used. There was no replication. Three sizes of rooted tillers were used (i.e. single, two and three tillers per hole in the nutrient solution boxes). The tillers were separated carefully so that each specimen had one, two or three tillers joined at the base. These were washed in deionised water, then planted in the box of

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*The most widely distributed species, *Austrodanthonia racemosa* (Dowling *et al*. 1996; Garden *et al.* 2001a) has a smaller tiller size and may be less likely to survive than other species.

nutrient solution. Maintenance of the experiment, sampling and harvesting, and data analysis were carried out in the same way as experiment 1.

Results and discussion

Plant size (number of tillers) appeared to have no effect on survival of any of the three species used in the experiment. All plants survived regardless of initial plant size. Plant size appeared to affect only tillers/plant, and shoot and total DM yield as might be expected (Table 5.11, Appendix 12). There appeared no difference between plant sizes of one and two in producing tillers/plant. Plant sizes two and three were the similar for shoot and total DM.

Across the three species, plant size appeared to have a similar effect on subsequent plant growth. However, there appeared to have been large interspecific differences in all growth parameters (Table 5.11). *A. racemosa* and *A. pilosa* always produced higher biomass as well as having greater values for most of the other variables than *A. penicillata*, as suggested by Garden *et al*. (2001a). Greater leaf area means a greater potential to accumulate biomass (Pearse 1948), so a greater growth response, as initial tiller number increased, was not unexpected (Table 5.11).

Table 5.11. Effect of plant size and species on growth of *Austrodanthonia* in nutrient solution

Data are means of four plants. Values in parentheses are standard errors of means

Treatment	Shoot length (cm)	Root length (cm)	Tillers/ plant	Roots /plant	DM (g/plant)		
					Root	Shoot	Total
Tiller size							
Single	262(±39)	262(±53)	8.25(±1.76)	6.00(±1.60)	0.06(±0.01)	0.28(±0.06)	0.33(±0.07)

Two	282(±17)	316(±46)	10.50(±1.56)	7.50(±1.76)	0.08(±0.02)	0.36(±0.05)	0.43(±0.06)
Three	300(±23)	313(±41)	15.08(±1.64)	5.83(±0.97)	0.08(±0.02)	0.50(±0.07)	0.57(±0.07)
Species							
A. racemosa	315(±17)	211(±25)	16.00(±1.73)	10.92(±1.67)	0.04(±0.01)	0.46(±0.07)	0.50(±0.08)
A. penicillata	212(±33)	288(±59)	7.83(±1.57)	3.75(±0.80)	0.06(±0.02)	0.25(±0.05)	0.31(±0.07)
A. pilosa	316(±19)	392(±35)	10.00(±1.80)	4.67(±0.75)	0.11(±0.02)	0.42(±0.05)	0.53(±0.07)

Overall, the results indicate that plant size appeared to have no effect on survival of *Austrodanthonia* spp. but appeared to influence growth response. The poor growth of the species in experiment 2 was probably due to the use of less vigorous plant material or greater injury during transplanting.

5.5 Experiment 5. Preliminary evaluation of the effect of Mn on accessions of *Austrodanthonia* spp. at a constant pH

The aim of the experiment was to investigate that Mn is not toxic to *Austrodanthonia* spp.

Materials and methods

The aim was to establish a dose-response effect of Mn using A*ustrodanthonia* spp. The experiment was carried out in a glasshouse using the same methods as described previously, during the period 8 May to 3 July 2001. A completely randomised design with three accessions (*A. duttoniana*, accessions 182064; 182293; and *A. fulva*, accession 182221) repeated six times at five levels of Mn was used. There was no replication. The experiment commenced with five levels of Mn treatments: control (2.4), 10, 50, 100 and 500 µM. Mn was added as $MnSO_4.H_2O$. The pH was adjusted to 4.0 using 1M HCl.

As far as is known, there is no published data on Mn toxicity to *Austrodanthonia* spp. in nutrient solutions. Growth of many plant species decreases in the range of 10-50 mg Mn/L (Helyar and Conyers 1994; Rubzen 1996). After four weeks, no visual symptoms of Mn toxicity were found even at the highest dose of Mn (i.e. 500 μM). The Mn doses were then increased to include rates of 2.4, 100, 500, 1000 and 2000 μM.

The pH adjustment, solution renewal, growth measurements, sample collection, and tissue and data analyses were the same as for experiment 1.

Results

Growth response

Increasing Mn from 2.4 μM (control) to 100 μM appeared to increase growth of *Austrodanthonia* (Table 5.12); however, in most cases, growth was similar in the range 100-2000 μM Mn. Accessions appeared to differ in relation to all growth components except roots/plant (Table 5.12). Accessions 182064 (*A. duttoniana*) and 182221 (*A. fulva*) were similar, with apparently higher growth than accession 182293 (*A. duttoniana*).

Accessions responded differentially to Mn treatments for all growth components (Table 5.13) except for tiller number and roots/plant, and these data are not presented. The results reflected the similar trends of main effects of Mn and accessions (Table 5.12). In general, growth parameter values tended to increase for accessions 182064 and 182293 up to 500 μM Mn, and decline thereafter. Accession 182221 responded exceptionally well, with increasing doses of Mn in solution (Table 5.13).

Table 5.12. Main effects of Mn and accessions on the growth of *Austrodanthonia*

Data are means for six plants. Values in parentheses are standard errors of means

Treatment Mn (µM)	Shoot length (cm)	Root length (cm)	Tillers/ plant	Roots /plant	DM (g/plant)		
					Root	Shoot	Total
Control (2.4)	365.8(±19.2)	375.0(±41.9)	8.11(±0.42)	6.50(±0.52)	0.08(±0.01)	0.42(±0.03)	0.50(±0.04)
100	448.3(±21.3)	398.3(±33.7)	11.22(±0.74)	10.00(±0.52)	0.12(±0.01)	0.61(±0.03)	0.73(±0.04)
500	450.3(±15.1)	509.7(±38.5)	9.22(±0.52)	9.22(±0.81)	0.14(±0.01)	0.64(±0.04)	0.78(±0.05)
1000	428.6(±21.1)	373.6(±23.9)	9.83(±0.74)	8.89(±0.79)	0.14(±0.01)	0.64(±0.04)	0.78(±0.05)
2000	439.9(±28.0)	482.2(±42.3)	8.67(±0.91)	7.33(±0.86)	0.15(±0.02)	0.63(±0.07)	0.78(±0.09)
Accessions							
182064	481.7(±8.6)	587.2(±17)	8.30(±0.43)	9.30(±0.54)	0.15(±0.01)	0.61(±0.02)	0.76(±0.03)
182293	338.3(±8.9)	285.3(±14.5)	9.70(±0.43)	8.67(±0.48)	0.07(±0.01)	0.51(±0.03)	0.58(±0.03)
182221	459.8(±18.4)	410.8(±24.8)	10.23(±0.72)	7.80(±0.73)	0.15(±0.01)	0.65(±0.05)	0.80(±0.07)

Solution composition and tissue analysis

The concentrations of the major nutrients declined at the highest Mn treatment (i.e. 2000 µM, Appendix 13). GEOCHEM speciation calculations showed that on an average, about 94% free Mn was present in the solutions while the balance formed a complex with SO_4^{2-} (Appendix 14). It was assumed that root exudates did not complex Mn. Tissue analysis of the shoots and roots of *Austrodanthonia* accessions did not show large variations in the concentrations of elements other than Mn (Appendix 15). Although exposure to higher Mn levels increased Mn concentrations both in shoot- and root-tissues, accessions 182064 and 182293 accumulated more Mn in shoots than accession 182221. In roots, accession 182221 had higher concentrations of Mn than the other two accessions at the higher Mn treatments (i.e. 1000 and 2000 µM) (Appendix 15).

Table 5.13. Plant growth of *Austrodanthonia* as affected by Mn and accessions

Data are means for six plants. Values in parentheses are standard errors of means

Treatment Mn (µM)	Accessions	Shoot length (cm)	Root length (cm)	DM (g/plant)		
				Root	Shoot	Total

Control (2.4)	182064	460.0(±16.5)	604.2(±18.1)	0.13(±0.01)	0.55(±0.03)	0.68(±0.04)
	182293	301.7(±9.5)	261.7(±21.2)	0.06(±0.01)	0.44(±0.01)	0.50(±0.02)
	182221	335.8(±25.2)	259.2(±37.1)	0.06(±0.01)	0.26(±0.01)	0.33(±0.02)
100	182064	504.2(±24.6)	556.7(±20.5)	0.15(±0.01)	0.68(±0.02)	0.83(±0.02)
	182293	353.3(±15.7)	245.8(±16.1)	0.06(±0.01)	0.49(±0.04)	0.55(±0.04)
	182221	487.5(±32.3)	392.5(±34.8)	0.14(±0.02)	0.66(±0.07)	0.80(±0.09)
500	182064	476.7(±11.2)	685.0(±25.2)	0.18(±0.01)	0.65(±0.05)	0.83(±0.07)
	182293	381.7(±19.4)	357.5(±53.5)	0.09(±0.03)	0.63(±0.10)	0.72(±0.12)
	182221	492.5(±19.6)	486.7(±26.8)	0.15(±0.02)	0.64(±0.06)	0.79(±0.07)
1000	182064	475.8(±15.9)	461.7(±29.4)	0.16(±0.02)	0.63(±0.07)	0.79(±0.09)
	182293	322.5(±23.6)	269.2(±30.4)	0.07(±0.01)	0.52(±0.04)	0.59(±0.05)
	182221	487.5(±17.9)	390.0(±15.1)	0.19(±0.02)	0.75(±0.05)	0.94(±0.06)
2000	182064	491.7(±25.8)	628.3(±22.5)	0.15(±0.01)	0.51(±0.05)	0.67(±0.06)
	182293	332.2(±16.0)	292.5(±11.9)	0.07(±0.01)	0.46(±0.05)	0.53(±0.05)
	182221	495.8(±59.9)	525.8(±75.8)	0.22(±0.05)	0.92(±0.16)	1.14(±0.20)

Discussion

Shoots of plants are normally affected more severely by excess Mn than roots (Foy *et al*. 1978; Foy 1983, 1984), because Mn is an essential element readily taken up by plants and transported to the shoot (Carver and Ownby 1995). Therefore, Mn produces definitive symptoms in plant shoots (reduced growth, chlorosis, necrosis or necrotic spotting on leaves - Foy *et al*. 1978; Kang and Fox 1980; Kitao *et al*. 2001). Symptoms vary between species (Foy *et al*. 1978). Excess Mn can produce decreased root growth, e.g. Lidon (2002) reported that toxic concentrations of Mn (32 mg/L) severely inhibited the production of root hairs in rice. Alam *et al*. (2001) also found 61% root growth inhibition in barley from excess Mn addition. No specific symptoms for Mn occurred in shoots of *Austrodanthonia* accessions but browning of roots was observed in accession 182221 (*A. fulva*) at the higher Mn treatments (i.e. 1000 and 2000 µM) (Appendix 16). The brown colour was probably due to the accumulation of oxidised Mn on the root surface and/or oxidised phenolics in the root cortex (Foy *et al*. 1988; Alam *et al*. 2001; Iwasaki *et al*. 2002).

Mn-tolerance of higher plants varies between species and genotype (Foy *et al*. 1978; Horst 1983; Iwasaki *et al*. 2002). Thus some species show extreme tolerance to Mn (e.g. rice, Lidon 2001a, 2001b, 2002; sugar cane, cereal rye and oats, Cregan *et al*. 1986; Fenton *et al*. 1996). This study also indicated high tolerance of *Austrodanthonia* accessions to excess Mn. Increasing Mn concentrations from 100- 2000 µM did not adversely affect the growth of any accessions (Table 5.13). Accession 182221 responded exceptionally well even at the highest Mn treatment (Table 5.13).

To correct for inherent vigour, the Hutton approach is not appropriate for only three data points at each treatment. So percentage change (increase or decrease) over control Mn treatment for growth (DM) was used (Fig. 5.11). It was observed again that all accessions responded well, and that one accession (182221) responded extremely well to a high Mn-challenge, indicating its superiority of tolerance compared with other accessions.

GEOCHEM analysis showed that solutions contained about 94% free Mn^{2+} (Appendix 14). Hence, the tolerance of accessions is associated with genotypic differences (cf. Foy *et al*. 1978). The DM response to Mn activities in solution showed that accession 182221 had greater tolerance than other two accessions (Fig. 5.12).

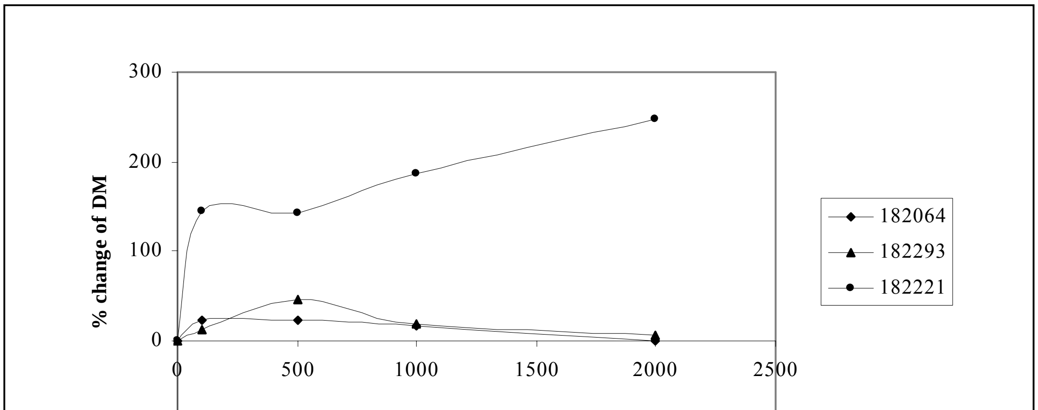

Fig. 5.11. Percentage change of total DM (over control Mn treatment) of *Austrodanthonia* accessions as affected by Mn concentrations in nutrient solutions at pH 4.0.

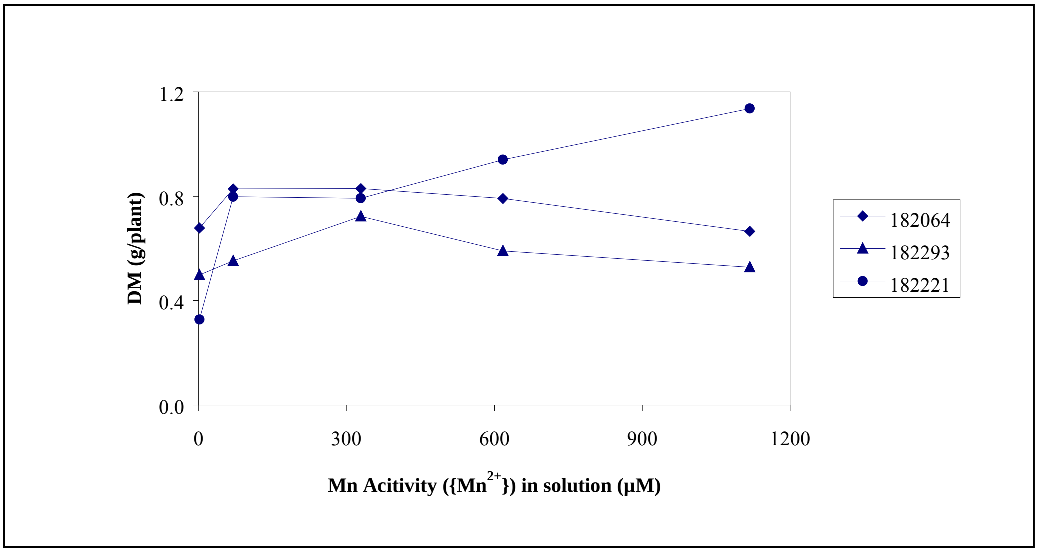

Fig. 5.12. Total DM yield of *Austrodanthonia* accessions as affected by Mn activity in nutrient solutions at pH 4.0.

Toxic levels of Mn could influence the uptake and metabolism of Fe, Ca, Zn and Mg (Marschner 1986) and usually Mn toxicity is exacerbated by Fe deficiency (Carver and Ownby 1995; Alam *et al*. 2001). Comparisons of the mineral concentrations of both shoots and roots (bulked samples) of the present study did not indicate any such deficiency or malfunctioning from excess Mn in the solutions

(Appendix 15). Increasing shoot Mn concentrations were associated with increased DM production of accession 182221, but with decreased DM production for accessions 182064 and 182293 at tissue Mn concentrations > 1130 mg/kg (Fig. 5.13). The concentrations of Mn in the shoots of accession 182221 were much lower (at all Mn-treatments); but were higher in roots (at 1000 and 2000 µM Mn treatments) than accessions 182064 and 182293 (Appendix 15) indicating that an exclusion mechanism of tolerance from shoots might be involved with this accession (Foy *et al*. 1978; Foy 1983,1984; Scott and Fisher 1989).

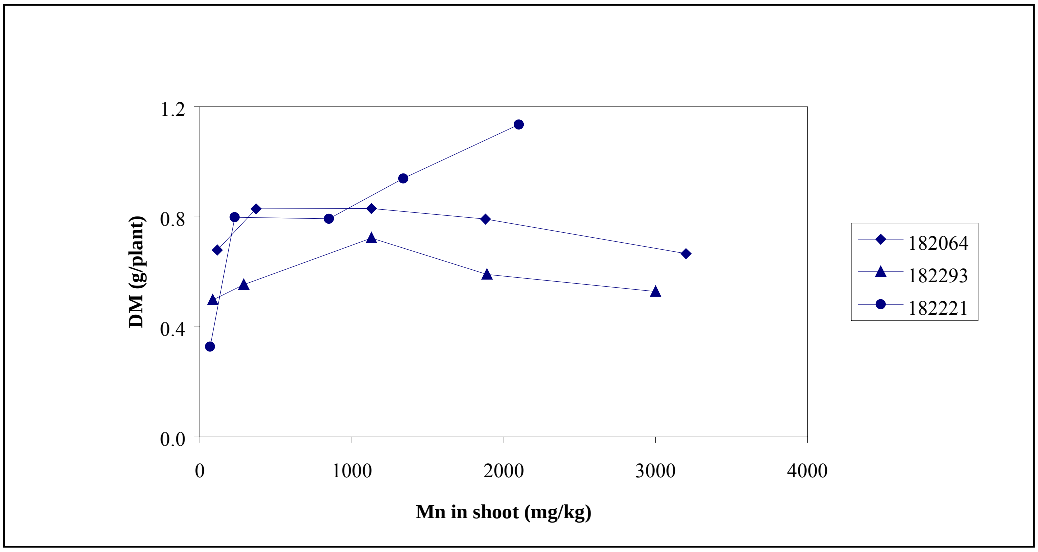

Fig. 5.13. Total DM yield of *Austrodanthonia* accessions as affected by tissue Mn concentrations.

The overall results from the present study thus indicate that the accessions of *Austrodanthonia* tested are highly tolerant to excess Mn. There was variation in tolerance between species and also accessions within species. Among the accessions used, accession 182221 was extremely tolerant to excess Mn. Therefore, *Austrodanthonia* accessions tested are unlikely to be affected by Mn-toxicity when grown in the field on the Tablelands of NSW, as the soils in these acidic regions are unlikely to contain such high Mn concentrations.

Chapter Six

Screening of *Austrodanthonia* accessions for Al-tolerance using hydroponics and hematoxylin staining

6.1 Introduction

The objectives of this study were to determine the degree of Al-tolerance of 24 *Austrodanthonia* accessions selected from a previous experiment (Chapter 4), by using a nutrient solution screening method and to relate this ranking to the results of staining root-tips with hematoxylin. Some limited mechanistic information on Al-tolerance was also collected by measuring the accumulation of Al in the shoots and roots.

6.2 Materials and methods

Nutrient solution culture: this experiment was conducted in a glasshouse following exactly the same methods and using the same materials used in the previous experiments 1 and 2 of Chapter 5, during the period 24 July to 28 September 2001. Tillers of 24 accessions from 12 species (Table 6.1) were used, based on Al-tolerance rankings developed in Chapter 4. The experiment was laid out as a randomised complete block design consisting of 24 accessions, each repeated two times in each box (treatment), with seven concentrations of Al (0, 50, 100, 150, 200, 250 and 300 μM). Concentrations of N, Ca, K, Mg, B, Mn, Cu and Zn in the medium were increased by 20, 100, 20, 100, 20, 50, 50 and 20 % respectively relative to those used in Chapter 5 to ensure sufficiency.

Table 6.1. The accessions of *Austrodanthonia* spp. used in the experiment

Species	Common ID*	Accession ID
A. racemosa	1	182251
A. racemosa	2	182095
A. racemosa	3	182188
A. racemosa	4	182233
A. pilosa	5	182288
A. pilosa	6	182087
A. pilosa	7	182267
A. fulva	8	182206
A. fulva	9	182205
A. fulva	10	182256
A. duttoniana	11	182131
A. duttoniana	12	182050
A. duttoniana	13	182106
A. penicillata	14	182081
A. penicillata	15	182192
A. duttoniana	16	182300
A. setacea	17	182031
A. setacea	18	182075
A. caespitosa	19	182024
A. laevis	20	182220
A. richardsonii	21	182122
A. monticola	22	182088
A. eriantha	23	182059b
A. auriculata	24	182028

** For details, see Table 4.4*

All treatments were replicated three times. The pH was adjusted to 4.0 using 1M HCl. Solutions were renewed every alternative week when a sharp rise of pH occurred. The day before harvest, all nutrient solutions were renewed, but free of Al. Thus the roots of all plants were kept in Al-free solutions for about 24 h prior to harvest to minimise Al presence on the surface of the roots (Tice *et al*. 1992). The plants were then harvested and divided into shoots and roots. Each part was rinsed with deionised water at least three times, blotted, dried to a constant weight at 60^{o} C and weighed separately. The dried material for the plants with insufficient dry matter (DM) was bulked on a treatment basis for chemical analysis (Appendix 17).

Yield data were analysed by ANOVA and ASREML (Gilmour *et al*. 1999). Of particular interest however is the relative change in DM as Al increases in the nutrient solutions. Relative DM was calculated by dividing mean DM at each Al-treatment by mean DM at the nil Al treatment. When appropriate, data were square root or log transformed to achieve normal distribution and homogeneity of variances. The relationship between DM and Al in the solutions was determined using a smoothing spline technique (Verbyla *et al*. 1999). The standard error of the differences and degrees of freedom (df) from ANOVA were used to define the distribution parameters of the spline.

The Al activities in the nutrient solutions were determined as previously (Experiment 5.3). Nutrient solutions contained about 55% free Al^{3+} across all Al treatments (Table 6.2) and free Al^{3+} is considered to be the main rhizotoxic species of Al (Kinraide 1991). The majority of the remaining Al was in the forms of non-toxic Al-sulfate and Al-PO_4 species (cf. Kochian 1995; Parker *et al*. 1995a; Crawford and Wilkins 1998).

Hematoxylin staining of root-tips: a modified method of Polle *et al*. (1978) was used for visual detection of Al in the roots. Two weeks prior to harvest, growing root-tips (~ 15 mm) were cut out from each plant of selected Al-treatments (i.e. 0, 200 and 300 μM Al). The higher Al-treatments (200 and 300 μM Al) were chosen because of their

Table 6.2. Activity ({Al^{3+}}) and concentration ([Al^{3+}]) of free Al^{3+}, and percent of Al as free metal, and Al complexed with ligands in nutrient solutions (pH 4.0) with different Al treatments - based on GEOCHEM

All Al concentrations are in µM

Al Treatment	{Al^{3+}}	Free [Al^{3+}]	Al as free metal (%)	Al complexed with (%)				
				SO_4	F	PO_4	EDTA	OH^-
50	10.23	26.06	52.12	10.28	1.98	32.24	0.10	3.28
100	21.00	53.96	53.96	13.14	0.99	28.45	0.07	3.40
150	31.89	82.65	54.99	16.04	0.66	24.80	0.05	3.45
200	42.56	111.30	55.51	18.36	0.50	22.18	0.04	3.41
250	52.54	138.50	55.40	20.83	0.40	19.94	0.04	3.40
300	54.29	143.50	55.53	22.74	0.33	17.96	0.03	3.40

severe effects on the root growth of *Austrodanthonia* (see Chapter 5, experiment 5.3). The cut root-tips were placed in aerated deionised water for 30 min. Root-tips were then stained with 0.2% hematoxylin (w/v) for 60 min at room temperature. Stained roots were rinsed with flowing deionised water for about one minute and kept in deionised water until photographed. The stain was freshly prepared 1 h before use by placing 0.2 g of hematoxylin and 0.02 g $NaIO_3$ in 100 mL of deionised water and stirring rapidly for about 1 h to dissolve and partially oxidise the hematoxylin. After photography, the root-tips were placed in nutrient solutions without Al for 24 h (Crawford and Wilkens 1998) and photographed again.

6.3 Results

Both shoot and root DM were depressed with increasing levels of Al ($P < 0.001$, Fig. 6.1). The damaging effect on total DM was evident at concentrations ≥100 µM Al. Toxicity symptoms were most evident on the roots, which were relatively short, thick and had numerous undeveloped laterals (Fig. 6.2). No distinctive symptoms were observed in the shoots.

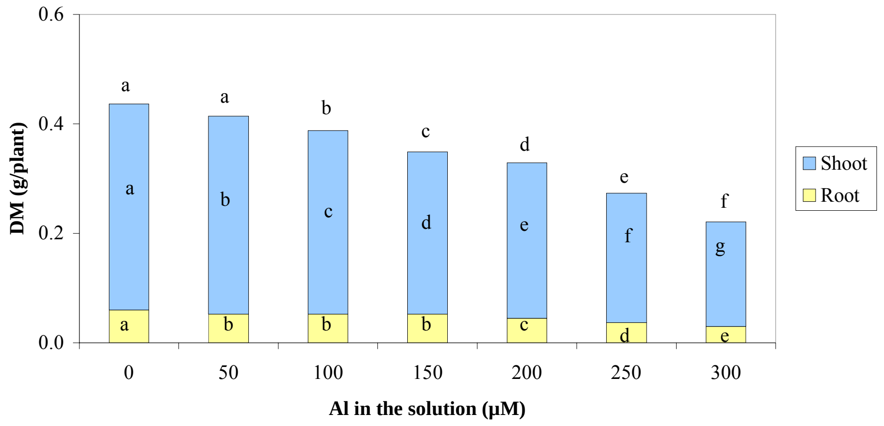

Fig. 6.1. Main effect of Al on DM yield of *Austrodanthonia* accessions. Data are means for 24 accessions (back-transformed values) with three replications. Columns associated with the same letters are not different ($P > 0.05$). Letters on top of each column are for total DM.

There was an interaction between accessions and Al treatments and was evident by showing differential responses of accessions to Al. Some of the 24 accessions of *Austrodanthonia* differed in their tolerance to Al, as shown by the relative changes in DM (Figs 6.3 and 6.4). The effects can be visualised for selected accessions in Fig. 6.5. Increasing Al in solution decreased the growth of some of the accessions. Other accessions showed no change or exhibited increased growth up to certain Al-concentrations, and then gradually declined. Some accessions (e.g. 2, 6, 10, 15 and 16) showed extreme tolerance to excess Al in the case of relative root DM (Fig. 6.4).

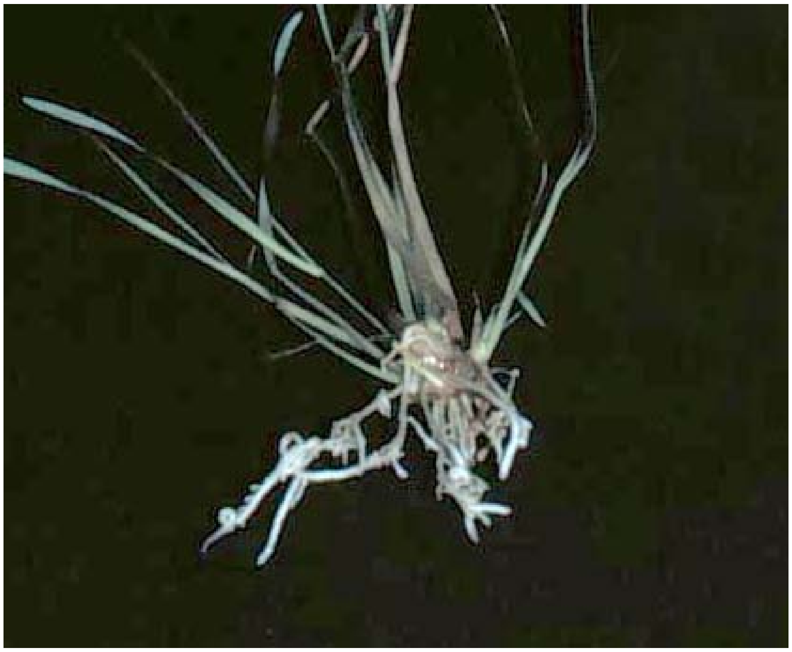

Fig. 6.2. Root growth of one of the more sensitive accessions - accession 13 (182106, *A. duttoniana*, Table 6.1) as affected by 300 µM Al in the nutrient solution.

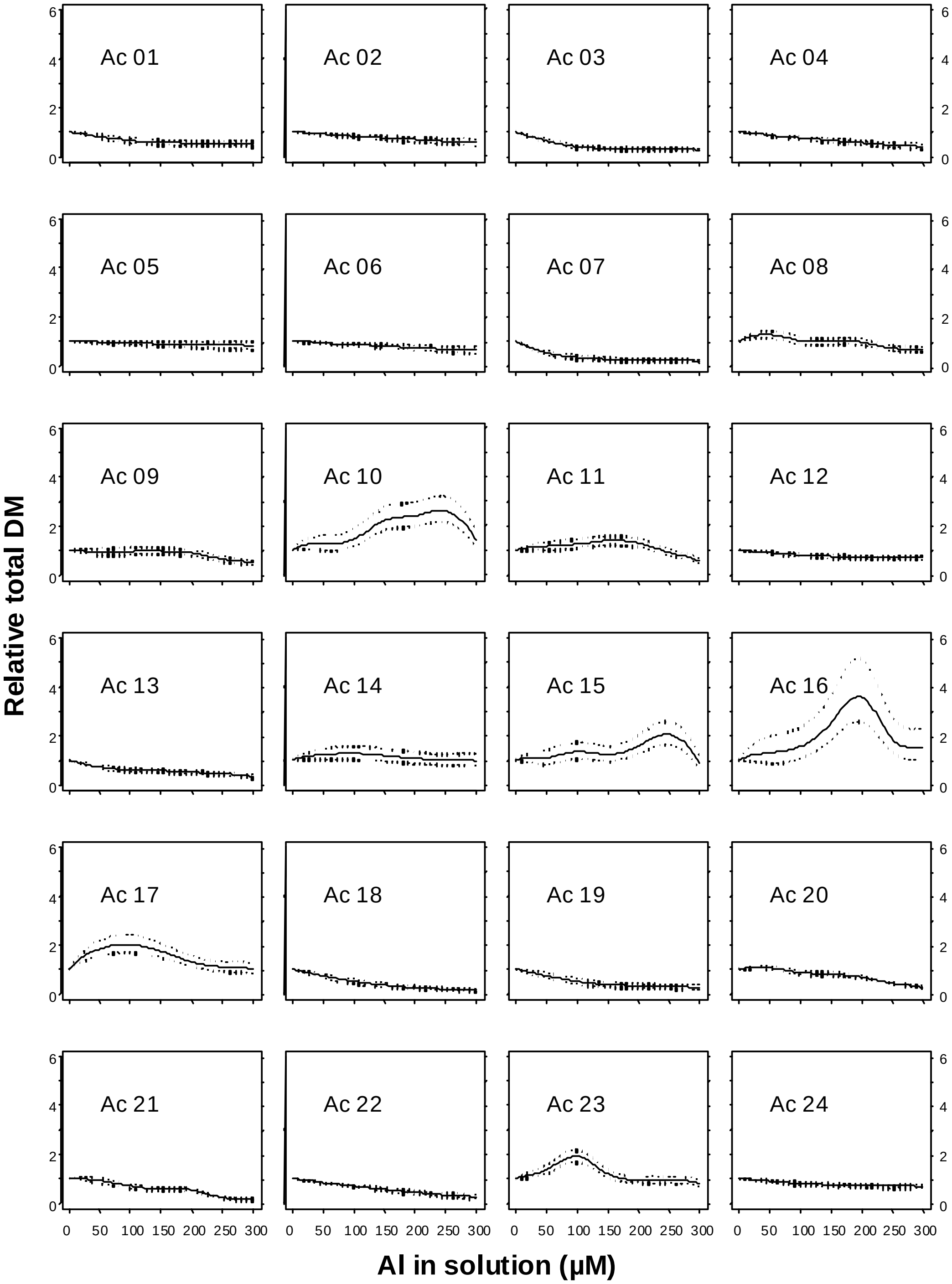

Fig. 6.3. The effect of Al on relative growth (see page 130 for explanation) of 24 accessions of *Austrodanthonia* spp. Ac = accession (Table 6.1). Dotted lines represent the 95% confidence interval.

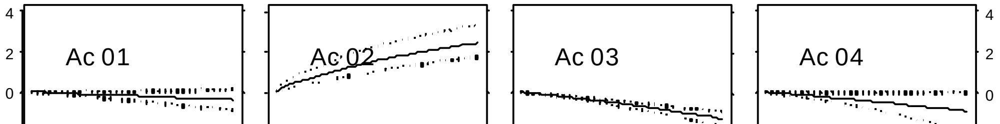

Fig. 6.4. The effect of Al on relative root-growth (see page 130 for explanation) of 24 accessions of *Austrodanthonia* spp. Ac = accession (Table 6.1). Dotted lines represent the 95% confidence interval.

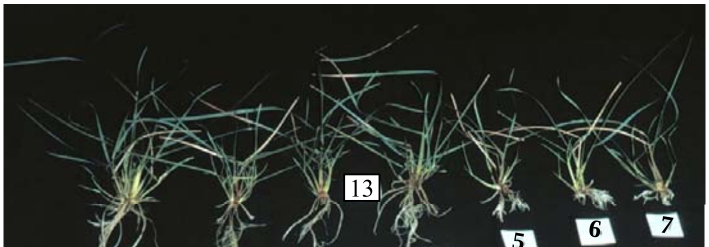

Fig. 6.5. Relative growth of three accessions of *Austrodanthonia* spp. grown with Al in nutrient solutions. The numbers 12, 13 and 21 represent accessions (Table 6.1). 1-7 are Al-treatments of 0, 50, 100, 150, 200, 250 and 300 μM, respectively.

To assess the relative Al-tolerance by each accession, a score for each accession was determined on the basis of total DM, over the range 0-300 μM Al. The score used was a value obtained from the area between the fitted curve for relative total DM (Fig. 6.3) and the line “relative total DM = 1” (for the definition of relative total DM see Section 6.2) across all Al treatments. The “relative total DM = 1” line corresponds to the response when an accession is not affected by Al treatments. The score was approximated using the mean deviation of the “relative DM - 1” evaluated at 1001 equally spaced (rectangles) values for Al on the interval 0-300 (cf. Frank and Sprecher 1975). Table 6. 3 shows the values so obtained. Figure 6.6 plots the accession rank against the tolerance score, indicating at each plot point the particular accession. Among the accessions used, accessions 16, 10, 17 and 15 were the top-ranked, whereas accessions 21, 13, 22, 19, 3, 18 and 7 appeared as lower-ranked, showing their higher sensitivity to excess Al (Fig. 6.6).

Table 6.3. Tolerance scores of 24 accessions used in this experiment

Accessions	Scores
1	-0.35
2	-0.23
3	-0.56
4	-0.33
5	-0.10
6	-0.19
7	-0.64
8	-0.03
9	-0.14
10	0.90
11	0.15
12	-0.21
13	-0.42
14	0.13
15	0.41
16	1.02
17	0.52
18	-0.56
19	-0.53
20	-0.25
21	-0.42
22	-0.42
23	0.21
24	-0.22

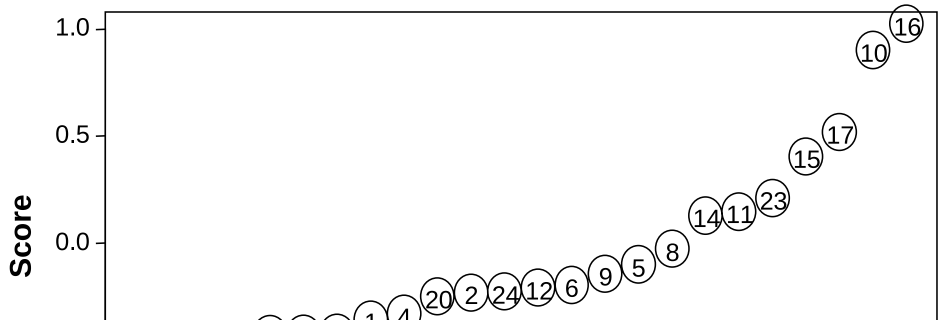

Fig. 6.6. Ranking of 24 accessions of *Austrodanthonia* spp. for Al-tolerance based on relative total DM. Circled numbers represent accessions (Table 6.1).

Alternatively, using the Hutton approach is an attempt to separate vigour from Al-tolerance, total DM at the highest Al-challenge (300 μM) was regressed against total DM in the nil Al treatment (Fig. 6.7). It was observed that many of the accessions are well scattered from the fitted line, indicating again that some sort of Al-tolerance might be operating among the accessions. Of the accessions used, some accessions (e.g. 24, 12, 8, 23, 17, 10 and 14) were the top-ranked, whereas some accessions (e.g. 20, 13, 21, 3, 7, 18 and 22) appeared as lower-ranked, consistently showing their higher sensitivity to excess Al (Figs 6.6 and 6.7).

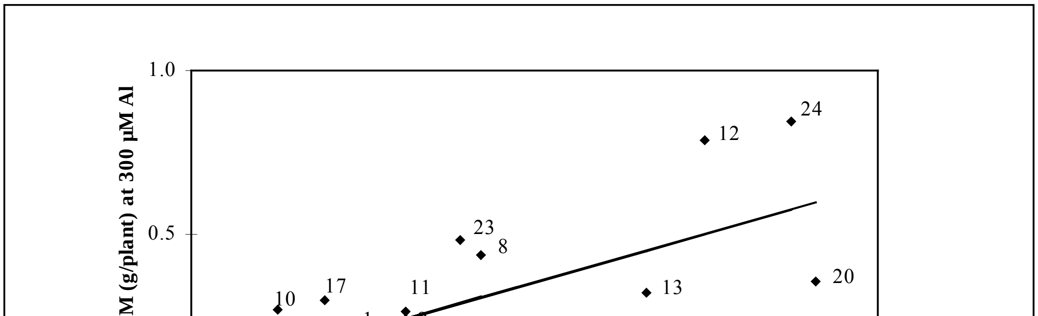

Fig. 6.7. Relationship between total DM at nil and 300 μM Al. Equation of line: $Y = 0.398X + 0.052$, $r^2 = 0.74$. Dots are data points for 24 accessions (Table 6.1; numbers displayed in the figures are few example accessions).

Staining of root-tips with hematoxylin showed that accessions exposed to Al treatments stained most intensely at the root apex, particularly behind the root cap (Fig. 6.8). Accessions at nil Al-treatment did not take up any stain. The intensity of stain increased with increasing Al treatment level. Root caps and meristematic zones of some accessions and in some cases whole root-tips (e.g. accessions 3, 4, 13,19, 20, 21 and 22) stained more intensely than the others, indicating greater accumulation of Al in these tissues.

Staining of root-tips that were allowed to recover in Al-free nutrient solution for 24 h demonstrated a striking decrease in the uptake of hematoxylin in all accessions (Fig. 6.8). Some accessions (e.g. 13, 18, 19, 20, 22, 23 and 24) had darker intensities of stain and thus indicated that shoots of these accessions might have higher Al accumulations than those less-intensively stained.

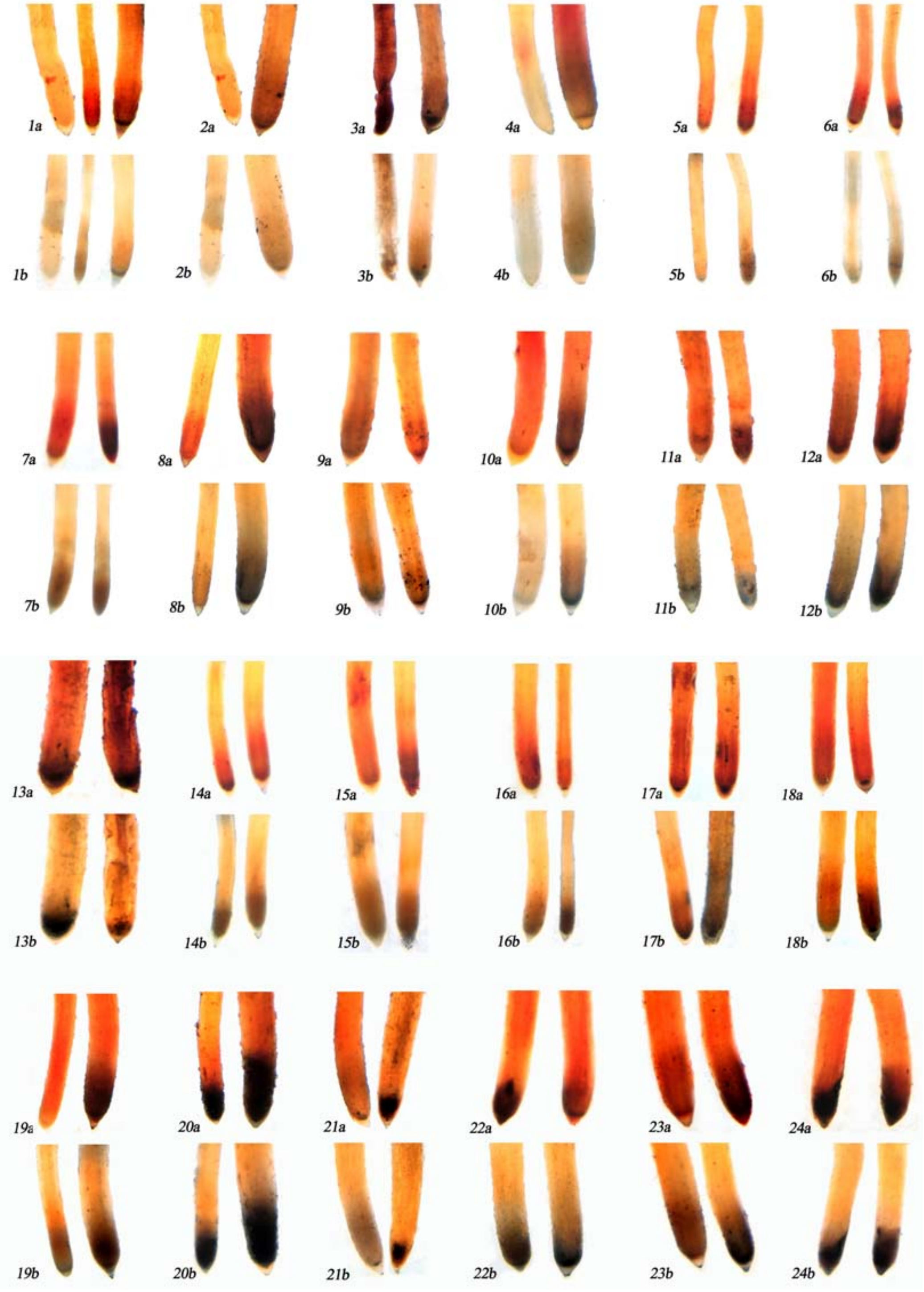

Fig. 6.8. Hematoxylin stained root-tips of 24 *Austrodanthonia* accessions (Table 6.1). The numbers indicate accessions. From left to right: accession 1; 0, 200 and 300 µM Al in the nutrient solution, all other accessions; 200 and 300 µM Al. a) 1 h stained with 0.2% hematoxylin (w/v), b) 24 h recovery in Al-free nutrient solution at pH 4.0. 10x magnification.

Tissue analysis of the shoots and roots of the 24 *Austrodanthonia* accessions did not show large variations in the concentrations of elements (Appendix 17) other than Al (Appendix 18). Of more importance however is the Al concentration in shoots and roots (Appendix 18), which was increased as Al in the nutrient solutions increased. Some accessions varied in their Al-accumulation both in shoots and roots, but roots always had a greater accumulation than the shoots. However, a few accessions e.g. 8, 9, 10, 11, 12 and 17, had less Al accumulation in the shoots than the other accessions indicating their tolerance to excess Al through some sort of exclusion mechanism.

6.4 Discussion

Al reduced both shoot and root DM of many of the accessions (Figs 6.1, 6.3 and 6.4). Although the root is the primary site of Al toxicity (Taylor and Foy 1985d; Scott and Fisher 1989; Delhaize *et al*. 1991; Pellet *et al*. 1996; Gallardo *et al*. 1999), the affected root systems might exhibit a thickened appearance and consequently, the dry weight of the whole root might remain relatively unchanged (Gallardo *et al*. 1999). In the present study, comparatively thick and short root systems were observed with increasing Al in the solutions (Figs 6.2 and 6.5) and the root weights at 50, 100 and 150 µM Al-treatments did not differ (Fig. 6.1).

In terms of relative total DM, accessions (Ac) 10 (182256, *A. fulva*), 11 (182131, *A. duttoniana*), 14 (182081, *A. penicillata*), 15 (182192, *A. penicillata*), 16 (182300, *A. duttoniana*), 17 (182031, *A. setacea*) and 23 (182059b, *A. eriantha*) were more tolerant of soluble Al species than other accessions (Fig. 6.3). Accessions 10 and 15 exhibited a high tolerance, showing progressive increments of relative total

DM up to 250 µM Al (equivalent to 53 µM Al^{3+} activity, Table 6.2). Accession 16 had the highest relative yield at 200 µM Al; the yield dropped thereafter but was still greater than the yield at nil Al. A sharp decrease in relative DM of some accessions, e.g. 3 (182188, *A. racemosa*), 4 (182233, *A. racemosa*), 7 (182267, *A. pilosa*), 13 (182106, *A. duttoniana*), 18 (182075, *A. setacea*), 19 (182024, *A. caespitosa*), 21 (182122, *A. richardsonii*) and 22 (182088, *A. monticola*) indicated their high sensitivity to excess Al. A similar pattern was also found for relative root DM (Fig. 6.4). These results, to some extent, agree with known tolerance categories for pasture species, which place *Agropyron* spp., tall wheatgrass and buffel grass in the "very highly sensitive" class, and cocksfoot, kikuyu and *Microlaena stipoides* in the "very highly tolerant" class (Helyar and Conyers 1994). The high Al-tolerance of some accessions (10, 11, 14, 15, 16, 17 and 23) is confirmed in this study, with the response by these accessions being similar to Al-tolerant cultivars of wheat (Taylor and Foy 1985d) and forage legumes (Baligar *et al*. 2001) where similar increases in shoot and root growth were demonstrated.

Despite the differences in accession sensitivities, most accessions exhibited a high Al-tolerance (to about 100 µM, Fig. 6.3; equivalent to 21 µM Al^{3+} activity, Table 6.2). This is significant because studies have shown that micromolar activities of Al^{3+} can decrease growth in some introduced perennial pasture grasses. Cultivars of ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* Shreb.) and phalaris (*Phalaris aquatica* L.) were sensitive to Al^{3+} activities < 5 µM (Edmeades *et al*. 1991b) but were ranked as tolerant by Helyar and Conyers (1994). Thus these accessions could be ranked as: highly tolerant (Ac's 10, 11, 14, 15, 16, 17 and 23); tolerant (1. 2, 4, 5, 6, 8, 9, 12, 20 and 24); and sensitive (3, 7, 13, 18, 19, 21 and 22)

(Fig. 6.6). When the tolerance rankings were related to the absolute yield of accessions at nil Al treatment in solution, the correlation indicated that plant vigour was a minor component of the tolerance score (Fig. 6.9). Some accessions had high vigour but appeared to have intermediate tolerance (e.g. accessions 20, 24, 12, 8) or low tolerance (e.g. accessions 21,13, 3). A slightly different pattern of Al-tolerance was observed when using the Hutton approach (Fig. 6.7), where there was an indication that accessions 12 and 24 were substantially Al-tolerant. This ranking is mostly consistent with the previous pot experiment (Chapter 4) indicating that accessions that can tolerate low soil pH may also tolerate excess Al in the solution culture.

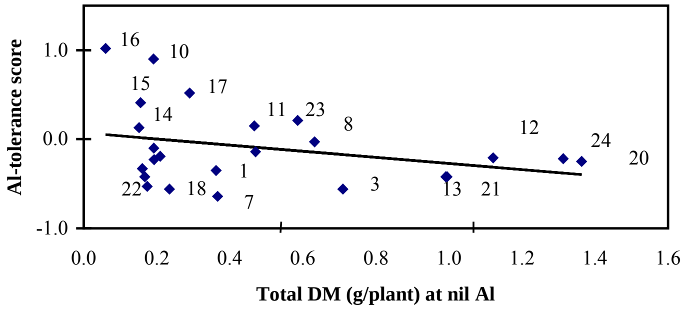

Fig. 6.9. Relationship between total DM at nil Al and Al-tolerance score (Table 6.3), equation of line: $Y = 0.071 - 0.343X$, $r^2 = 0.31$. Dots are data points for 24 accessions (Table 6.1; numbers displayed in the figures are few example accessions).

These results could be interpreted on the basis of spatial distribution (i.e. collection locations) of the respective accessions. A graphical representation of the relationships between the 24 accessions of *Austrodanthonia* and four components, namely pH, Bray-P (BP), Al-response (AR) and quadratic response (QR), is provided by biplot analysis (Fig. 6.10). Prior to the study, a field survey was conducted during 1990/1991 on the distribution of *Austrodanthonia* accessions on the Tablelands of NSW (Dowling *et al*. 1996), and soil data (e.g. pH, Bray-P) were recorded (Appendix 1). AR was estimated by inspection of relative tolerance of accessions to Al from their relative response in Fig. 6.3, thus increasing values indicate their increasing tolerance and *vice-versa*. QR was obtained from an earlier experiment (Chapter 4). Values of QR were determined by inspection of comparative emergence responses by accessions from their position in Fig. 4.8. The most responsive accessions appeared in the upper right hand quadrat and the least responsive in the lower left hand quadrat in the Figure. Accordingly a relative rank was given to individual accession following the same principles used for AR. Thus the directional vectors represent the four attributes (pH, BP, AR, QR). The solid lines in the biplot indicated the correlation structure among the attributes. Most of the tolerant accessions (Fig. 6.6) were associated with Factor 1, indicating that there is an association between the Al-response (AR) and the quadratic response (QR). The most sensitive accessions (Fig. 6.6) were more closely associated with high pH plus fertile soils (as indicated by high Bray-P values) from where these accessions were collected. The results thus indicate that greater tolerance of the accessions to Al may reflect the soil conditions from where the accessions were collected (Edmeades *et al*. 1991b).

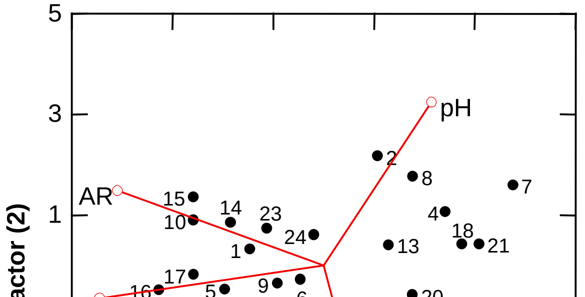

Fig. 6.10. Biplot displaying vectors 1 and 2 using values from the field and this experiment for 24 accessions of *Austrodanthonia* (see text for explanation). Numbers indicate accessions (Table 6.1). AR = Al-response (Chapter 6), BP = Bray-P and pH (values at the sites from where each accession was collected, Appendix 1), QR = quadratic response indicating the degree of tolerance by each accession of soil acidity in terms of emerging seedlings (Chapter 4).

The hematoxylin staining method is simple and rapid for the visual detection of Al in the root tissues on the basis of the ability of hematoxylin to form a red-purple complex with Al (Polle *et al*. 1978; Crawford and Wilkens 1998). Root-tips stained with hematoxylin showed differences in stain intensities among the accessions (Fig. 6.8). Consistently greater stain intensities (both after 1 h stained and 24 h in Al-free solution) in the root-caps and root-tips of accessions 3, 4, 7, 13, 19, 18, 20, 21 and 22 indicate their higher sensitivity to soluble Al than the other accessions, and is the result of greater accumulation and/or binding of Al by the root-tissues. The sensitivity of these accessions was also determined by relative DM (Figs 6.3 and 6.4).

In theory, Al-sensitive plant species may accumulate and/or bind Al in the roots at high rates, and Al-tolerant cultivars exclude Al from their root apices. Polle *et al*. (1978) followed by Wallace *et al*. (1982) used the hematoxylin staining technique to screen Al-tolerant wheat cultivars. Al-sensitive cultivars showed more intense staining than Al-tolerant cultivars across the vertical axes of the root-tips. Studies by Delhaize *et al*. (1993a), Bennet (1995), Crawford and Wilkens (1998), Giaveno and Filho (2000) and Yoshida and Yoshida (2000) have also demonstrated that the root-tips of Al-sensitive cultivars stain more intensely with hematoxylin than Al-tolerant cultivars.

Close examination of hematoxylin stained root-tips of some tolerant accessions (8, 10, 11, 12, 15, 16, 17, 23 and 24) indicated that root-tips, particularly the root apex cells, might take up Al to a similar extent as the more sensitive accessions (Fig. 6.8). Since Al is not excluded from these tissues, it seems unlikely that the root-cap is directly associated with Al-tolerance mechanisms, and therefore, that internal tolerance processes are involved in these accessions. This result agrees with the finding of Ryan *et al*. (1993), who demonstrated that de-capped roots of maize were equally sensitive to Al as those with intact root-caps and thus argues against the root-cap playing a role in Al-tolerance mechanism.

Further evidence of an Al-tolerance mechanism is demonstrated by comparing the relative Al accumulation in the shoots and roots. Accessions with densely stained root-tips showed greater Al accumulation than the accessions with less-densely stained root-tips. The most sensitive accessions accumulated much higher Al both in shoots and roots than the tolerant accessions, and the accumulation increased by increasing Al concentration in solution (Appendix 18). The most tolerant accessions also had similar higher Al accumulation at the highest Al-treatment (i.e. 300 μM) and thus exhibited reduced growth at this treatment (Figs 6.3 and 6.4). Some of the tolerant accessions (e.g. 1, 2, 5, 6, 14 and 16) had high Al accumulation, especially at concentrations ≥150 μM Al, supporting the earlier suggestion that an internal tolerance mechanism might be involved. Once Al enters the plant cells, mechanisms other than exclusion or binding may be activated. Scott and Fisher (1989) suggested that cytoplasmic Al can complex with organic molecules without disrupting the cell metabolism. As a result, the Al-toxicity reaction is inactivated and Al may be deposited in the xylem vessels or cell walls (Helyar 1978).

Although in a few instances, the results from hematoxylin staining technique (Fig. 6.8) and tissue analysis (Appendix 18) were inconsistent, the staining technique had merit. It aligned with analytical data and provided with rapid indication of Al-tolerance. The chemical data were obtained from whole roots whereas the data from the staining technique were only from root-tips. Therefore, it is likely that there should be little variation between these two approaches.

The overall relative Al-tolerance ranking is summarised in Table 6.4. It is clear from the Table that all three approaches have ranked Al-sensitive accessions in similar ways, however, for the Al-tolerant accessions, a slightly different patterns of ranking is observed. For instance, rankings that obtained from relative total DM (Fig. 6.6) classed accessions 4, 12, 20 and 24 as intermediate tolerant, whereas the Hutton approach (Fig. 6.7) classed the same accessions as highly tolerant (accessions 12 and 24) and low tolerant (accessions 4 and 20), indicating again about the difficulties in proper ranking for Al-tolerance. Thus, the data from more closely observation suggest that the Hutton approach is only that, an approach to isolating inherent vigour from true tolerance; it is not infallible in all situations.

Table 6.4. A summary table showing the relative Al-tolerance rankings for 24 accessions (Table 6.1) obtained from three different approaches. H = high; I = intermediate; L = low

Accessions	Relative Al-tolerance rankings		
	Based on relative total DM (Fig. 6.6)	Based on the Hutton approach (Fig. 6.7)	Based on hematoxylin staining technique (Fig. 6.8)
1	I	I	H-I
2	I	I	H-I
3	L	L	L
4	I	L	L
5	I	I	H-I
6	I	I	I-L
7	L	L	L
8	I	H	I-L
9	I	I	I-L
10	H	H	I-L
11	H	I	I-L
12	I	H	I-L
13	L	L	L
14	H	I	I-L
15	H	I	I-L
16	H	I	I-L
17	H	H	I-L
18	L	L	L
19	L	L	L
20	I	L	L
21	L	L	L
22	L	L	L
23	H	H	L
24	I	H	L

However, these studies collectively show that there is a wide range of Al-tolerance, from highly sensitive to highly tolerant in *Austrodanthonia* accessions. For sensitive accessions, the Al-tolerance ranking obtained from the nutrient culture was similar to that from the hematoxylin staining technique; although, for tolerant accessions, that accumulated Al in the roots, very different rankings were given by the two techniques.

Chapter Seven

Evaluation of acid tolerance of *Austrodanthonia* accessions under field conditions

7.1 Introduction

Considerable effort has been directed towards screening acid tolerant cultivars using laboratory and glasshouse based techniques (Gallardo *et al*. 1999), but the major concern is that rankings for acid tolerance in pot or solution culture may not correspond with field performance (Scott and Fisher 1989).

The objective of the present study was to determine the effect of pH and competition on survival and persistence of selected accessions of *Austrodanthonia* under natural field conditions over two growing seasons. The hypotheses tested are: *Austrodanthonia* accessions will survive and compete effectively with established plant populations irrespective of pH.

7.2 Materials and methods

Site characteristics

The experimental site (Sustainable Grazing Systems, SGS; $33^0 37$’S, $149^0 13$’E, elevation 800 m) is situated on a commercial grazing property near Carcoar, about 30 km south of Orange. The site represents much of the Tablelands, comprising a highly variable, undulating landscape, with light textured red and yellow podsolic soils of low fertility. The average pH_{Ca} of the surface soil is 4.5 and the average annual rainfall is 871 mm (King and Kemp 2001). Some soil characteristics and climatic conditions during the experimental period are presented in Table 7.1 and Fig. 7.1.

The plant community on-site is extremely diverse, with over 100 species being identified (King and Kemp 2001). The dominant species include: *Austrodanthonia* spp. (wallaby grasses), *Themeda triandra* (kangaroo grass), *Bothriochloa* spp. (red grasses), annual grasses (e.g. *Vulpia* spp., *Bromus* spp.), annual legumes and broadleaf weeds (e.g. Paterson's curse, thistles and flatweeds).

Table 7.1. Some properties of the surface soil (0-20 cm) of the experimental sub-plots from the SGS site, Carcoar

Properties	Units	Analytical results
pH_{Ca}[a]		4.12 - 5.25
pH_{w}[b]		4.78 - 5.97
EC[c]	dS/m	0.07 - 0.19
Bray-P[e]	mg/kg	2.05 - 9.25
Al[f]	mg/kg	1.0 - 9.6
Mn[f]	mg/kg	8.0 - 32.0

[a] *Determined in a 1:5 (w/v) soil: 10 mM $CaCl_2$ (Rayment and Higginson 1992)*
[b] *Determined in a 1:5 (w/v) soil: water (Rayment and Higginson 1992)*
[c] *Electrical conductivity (EC) was determined in a 1:5 (w/v) soil: water (Rayment and Higginson 1992)*
[e] *Bray - 1 (Bray and Kurtz 1945)*
[f] *Measured using ICP in the supernatant of the 10 mM $CaCl_2$ extract (Rayment and Higginson 1992)*

Treatments imposed

This experiment was conducted during the period November 2000 to March 2002, utilising the six unfertilised naturalised pasture plots at the SGS site, comprising three continuously grazed and three actively managed (grazed within pre-

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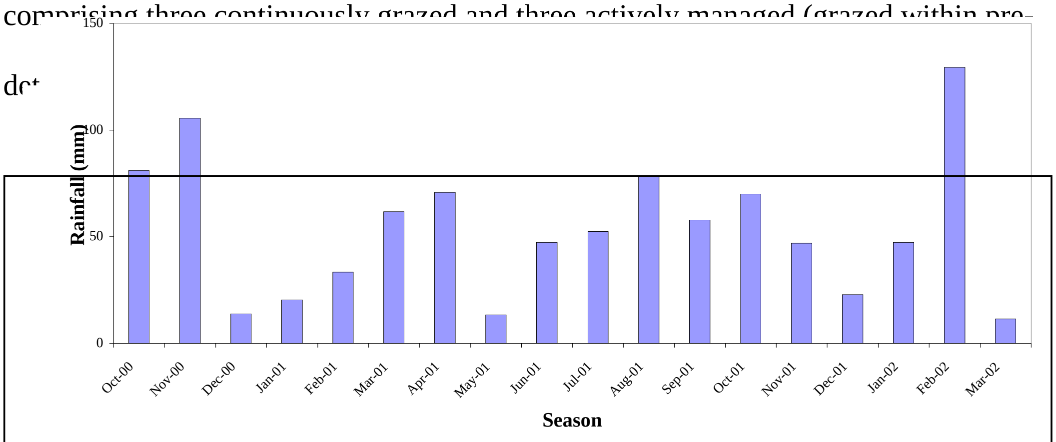

Fig. 7.1. Seasonal trends of soil water deficit (SWD, 0-20 cm), temperature and rainfall at the SGS site, Carcoar between October 2000 and March 2002. Values are monthly means. AT = air temperature, SST = surface soil temperature, SbST = sub-surface (5 cm) soil temperature.

limits of available forage) plots (Appendix 19). Seedlings of 20 accessions from nine species of *Austrodanthonia* (Table 7.2), ranging from Al-tolerant to Al-sensitive (Table 4.4) were planted at two levels of pH_{Ca} (low ~ 4.4 and high ~ 5.3). Within each plot (Appendix 19), four sub-plots, each 1 m x 1 m, were set up in such a way that two sub-plots were located on lower pH areas (~ 4.4) and two on higher pH areas (~ 5.3). As pH was the factor of primary interest, the sub-plots had similar botanical composition and other extraneous factors (e.g. aspect, slope). Each sub-plot was divided into two sub-sub-plots, one of which was treated with the herbicide glyphosate at 350g a.i./ha, before the seedlings were transplanted, and the other, untreated. The reason for herbicide application was to rest for the effect of intraspecific competition from the resident species on survival and growth of the introduced *Austrodanthonia* accessions.

Table 7.2. The accessions of *Austrodanthonia* spp. used in the experiment

Species	Common ID	Accession ID
A. racemosa	1	182251
A. racemosa	2	182095
A. racemosa	3	182188
A. racemosa	4	182233
A. pilosa	5	182288
A. pilosa	6	182087
A. fulva	8	182206
A. fulva	9	182205
A. duttoniana	11	182131
A. duttoniana	12	182050
A. penicillata	14	182081
A. penicillata	15	182192
A. setacea	17	182031
A. richardsonii	21	182122
A. eriantha	23	182059b

A. duttoniana	25	182064
A. pilosa	30	182112
A. pilosa	31	182127
A. richardsonii	32	Taranna*
A. bipartita	33	Bunderra*

** Domesticated cultivars; Lodge (1993a, 1993b), Lodge and Schipp (1993), Lodge (1996)*

Seedling raising and preparation

Disease-free seeds (naked caryopses) of similar size and shape, of the selected 20 *Austrodanthonia* accessions (Table 7.2) were collected and stored in a refrigerator to break dormancy before planting (cf. Bradbeer 1988). Seeds were then placed in paper pots (FH315, Japan) containing pasteurised soil (Appendix 20), with each pot having a single seed. The pots were placed in a glasshouse (Appendix 20) and were watered regularly.

Experimental layout

The experiment was laid out in a factorial design with selected accessions repeated from two to five times within each sub-sub-plot. The experiment consisted of 24 sub-plots (48 sub-sub-plots) of which 12 sub-plots (24 sub-sub-plots) were allocated to each pH level.

Transplanting of seedlings, maintenance of experiment and data collection

One day before transplanting, seedlings were trimmed to similar height (~12 cm). Nine weeks old seedlings were randomly planted approximately 10 cm apart in a grid pattern, into cavities created by removal of soil cores (Appendix 21). A few seedlings were pulled out by livestock during grazing. These were replaced and the plots were subsequently fenced. All sub-sub-plots were hand watered twice a week until seedlings established.

A growth score of each seedling was recorded at days 14, 33, 67 and 116 after transplanting. At transplanting, a growth score of 3 was given to all seedlings. Subsequently, a score from 0 to 10 was given to plants: 0 = dead, and higher scores indicated progressively healthier plants. Plants (if any) that were removed by rabbits were treated as missing values.

A ranking score of 1 to 10 was used to estimate dry matter (DM) at 116 days after transplanting (DAT). Five plants from each of the scores (except score 10, which described only one plant) were randomly selected, cut to the crown and oven-dried separately at 70^0C for 48 h before weighing (Helyar and Conyers 1994). These oven-dry weights were regressed against DM estimates and used to predict DM for all plants.

Survival was estimated at 116 DAT. The fencing was removed at 208 DAT, while the final survival was recorded at 477 DAT (i.e. on 12 March 2002).

Data analysis

An ANOVA was performed on growth score, survival and predicted DM to test the effect of pH and other components at different DAT. After removing all non-significant terms (e.g. management or stocking rate, main plot, management x pH and management x herbicide), the ASREML procedure was applied to log transformed data (Gilmour *et al*. 1999). As growth score over time was measured on a semi-quantitative scale (0-10), mean growth score at each sampling time for all plants of the same accession was used to analyse growth response. The significance was tested using the Wald statistic (Chi-square probability). An exponential regression between DM and growth score (1 to 10) was initially performed (Gomez and Gomez 1984).

7.3 Results

Plant survival

At 116 DAT, where herbicide had been applied, the percent of plants surviving over all accessions was unaffected by soil pH (Table 7.3). In contrast, where no herbicide was applied, 74% of plants survived at high pH and 47% at low pH (Table 7.4). A similar result was also observed at 477 DAT (Table 7.4). Survival of each accession, when plotted with and without herbicide at two levels of pH (Fig. 7.2), showed that survival of accessions 11 (*A. duttoniana*, 182131), 17 (*A. setacea*, 182031), and 9 (*A. fulva*, 182205) was highest irrespective of pH/herbicide combinations. While accessions 21 (*A. richardsonii*, 182122), 30 (*A. pilosa*, 182112), 25 (*A. duttoniana*, 182064), 31 (*A. pilosa*, 182127), 4 (*A. racemosa*, 182233) and 3 (*A. racemosa*, 182188) consistently had lower survival. The association between accessions was surprisingly similar for both pH levels (Fig. 7.2).

Table 7.3. ANOVA for survival of *Austrodanthonia* accessions at 116 DAT

Term	df	Sum of squares	Wald statistic	Probability (Chi-square)
Herbicide	1	90.87	77.84	< 0.001
Minus herbicide x pH	1	44.99	38.54	< 0.001
Plus herbicide x pH	1	1.19	1.02	0.31
Residual		1.17		

Table 7.4. Proportional survival of *Austrodanthonia* accessions at two levels of pH with plus- and minus-herbicide combinations over two growing seasons

Values are back transformed and overall means of 20 accessions. Means followed by the same letter are not different ($P > 0.05$). Values following the means are standard errors

Date	Soil pH	Minus herbicide	Plus herbicide
116 DAT (26/03/01)	High pH (~5.3)	0.74^c (0.04)	0.29^a (0.04)
	Low pH (~4.4)	0.47^b (0.05)	0.35^a (0.04)
477 DAT (12/03/02)	High pH (~5.3)	0.71^c (0.04)	0.19^a (0.04)
	Low pH (~4.4)	0.37^b (0.05)	0.25^a (0.04)

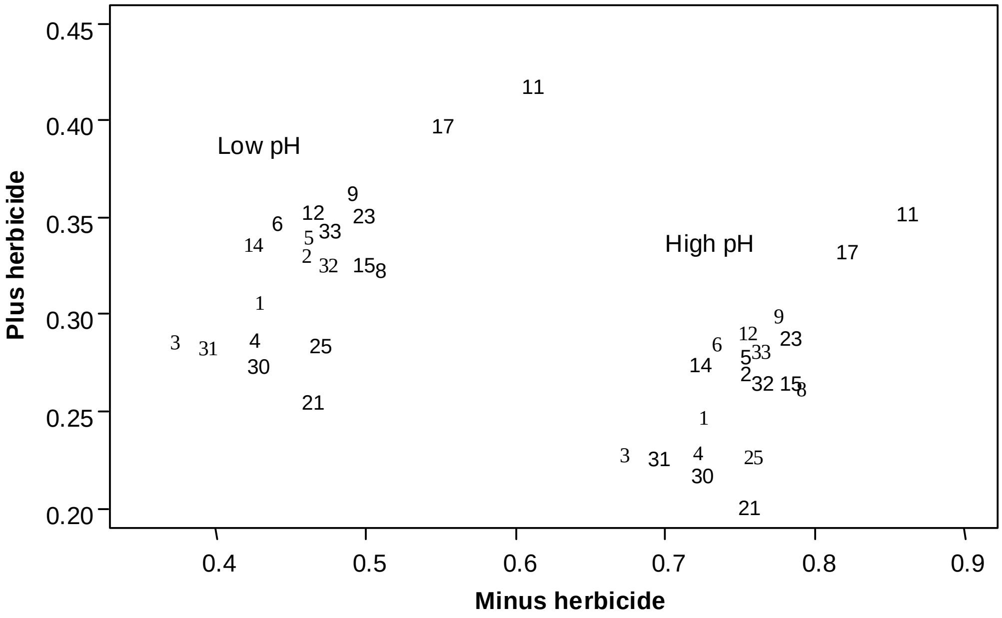

Fig. 7.2. Survival of 20 *Austrodanthonia* accessions at two levels of pH (high, ~5.3; low, ~4.4) with plus and minus herbicide combinations. Axes are proportional survival, expressed as a proportion of numbers planted. The values within the figure indicate the accessions used in the experiment (Table 7.2).

Growth score

The estimated mean growth scores over time with four pH/herbicide combinations showed that the growth score of all accessions declined until 33 DAT and then gradually increased (Fig. 7.3). High pH (~5.3) without herbicide produced the highest growth score at all the times.

The deviation of individual growth scores from the mean for 20 *Austrodanthonia* accessions within each pH/herbicide combination showed a similar trend over time (Fig. 7.4). Comparison of growth response of each accession via regression parameters (Table 7.5) exhibited similar results to those obtained for survival (Fig. 7.2).

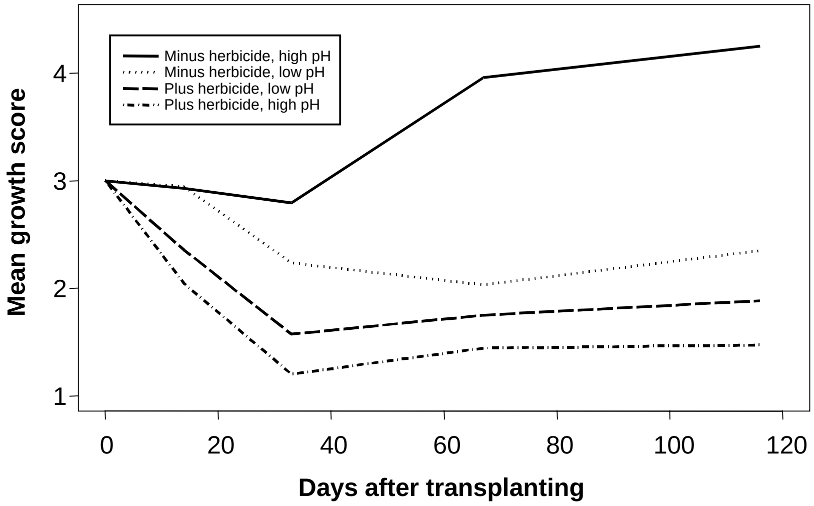

Fig. 7.3. Growth response of a group of 20 *Austrodanthonia* accessions scored at different days after transplanting (DAT). Plants were grown with four pH/herbicide combinations. High pH, ~5.3; low pH, ~4.4.

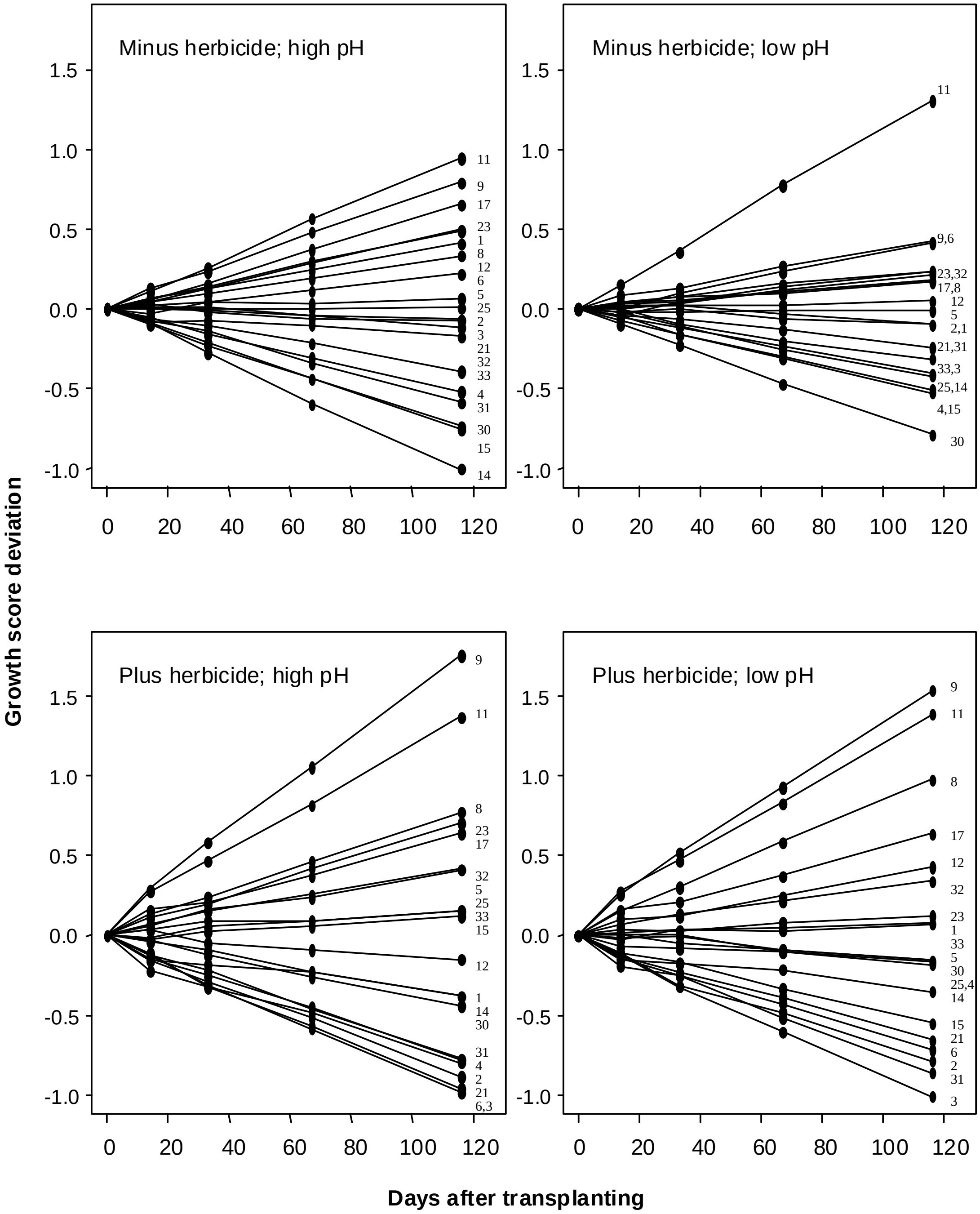

Fig. 7.4. Deviation of individual growth scores from mean score (Fig. 7.3) for 20 *Austrodanthonia* accessions (Table 7.2). Scores were assessed at different times after

transplanting, for plants grown with four pH/herbicide combinations (see text and Table 7.5 for detailed explanation). High pH, ~5.3; low pH, ~4.4.

Table 7.5. Growth rate (change in score over time) of 20 *Austrodanthonia* accessions (Ac) ranked in descending order, using regression data plotted in Fig. 7.4. High pH (~5.3); low pH (~4.4)

Minus herbicide				Plus herbicide			
High pH		Low pH		High pH		Low pH	
Ac	Slope	Ac	Slope	Ac	Slope	Ac	Slope
11	0.0082	11	0.0113	9	0.0154	9	0.0134
9	0.0070	9	0.0038	11	0.0120	11	0.0122
17	0.0056	6	0.0034	8	0.0067	8	0.0085
23	0.0043	23	0.0021	23	0.0061	17	0.0056
1	0.0042	32	0.0020	17	0.0056	12	0.0037
8	0.0036	17	0.0017	32	0.0037	32	0.0030
12	0.0028	8	0.0016	5	0.0036	23	0.0011
6	0.0017	12	0.0015	25	0.0014	33	0.0006
5	0.0006	5	0.0015	33	0.0013	1	0.0006
25	0.0001	2	0.0004	15	0.0010	5	-0.0013
2	-0.0006	1	-0.0002	12	-0.0013	30	-0.0014
3	-0.0007	21	-0.0007	1	-0.0033	25	-0.0014
21	-0.0009	31	-0.0008	14	-0.0035	4	-0.0016
32	-0.0016	33	-0.0020	30	-0.0038	14	-0.0033
33	-0.0033	3	-0.0028	31	-0.0067	15	-0.0049
4	-0.0045	25	-0.0035	4	-0.0068	21	-0.0057
31	-0.0050	14	-0.0036	2	-0.0070	6	-0.0062
30	-0.0063	4	-0.0044	21	-0.0077	2	-0.0069
15	-0.0065	15	-0.0045	6	-0.0084	31	-0.0074
14	-0.0086	30	-0.0068	3	-0.0085	3	-0.0087

The average standard error of each slope estimate is 0.003 and the standard error of the differences is 0.004

Dry matter (DM)

An exponential curve was fitted to the visual growth score for all live plants at 116 days after transplanting to predict DM (Fig. 7.5). Results obtained (Appendix 22) showed that DM was exponentially related to the visual score, thus validating the previous scoring system (Fig. 7.3, Table 7.3). Plants produced higher DM in sub-plots where herbicide was applied than the sub-plots without herbicide (Table 7.6). This is expected because of less inter- and intra-specific competition. There was wide variation in DM production across accessions (Table 7.7).

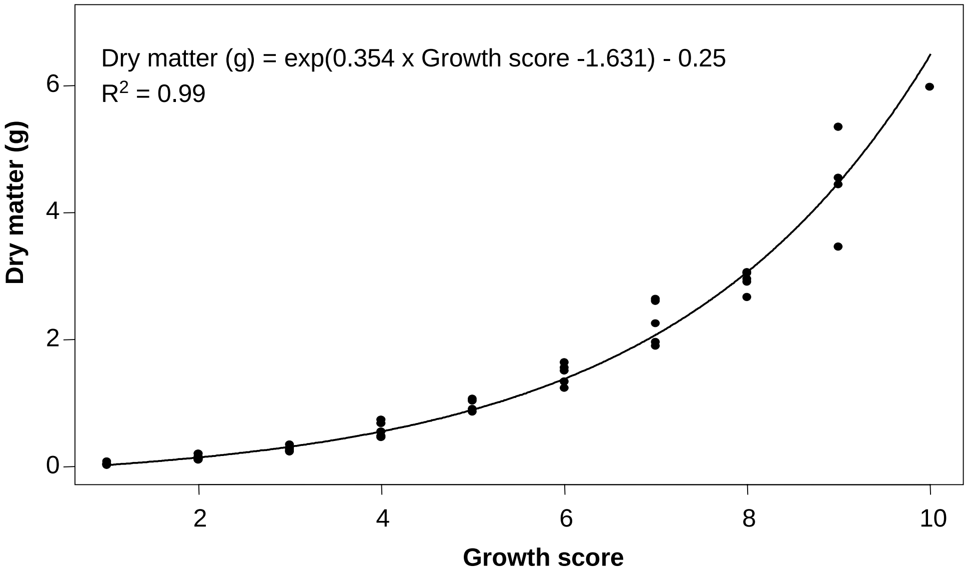

Fig. 7.5. Calibration curve to predict dry matter of *Austrodanthonia* accessions at 116 days after transplanting (for explanation, see Materials and methods).

Table 7.6. Predicted dry matter yield of *Austrodanthonia* accessions at two levels of pH with plus- and minus-herbicide combinations at 116 days after transplanting

Values are back-transformed, overall means (g/plant) of 20 accessions. Means followed by the same letter are not different ($P > 0.05$). Values following the means are standard errors

Soil pH	Minus herbicide	Plus herbicide
High pH (~5.3)	0.28^{ac} (0.04)	0.37^{a} (0.05)
Low pH (~4.4)	0.19^{b} (0.03)	0.36^{a} (0.05)

Table 7.7. Predicted mean DM yield (g/plant) of 20 *Austrodanthonia* accessions using the regression equation illustrated in Fig. 7.5 at 116 days after transplanting

Values are back transformed

Accessions	Minus herbicide	Plus herbicide
23	0.28	0.43
9	0.27	0.50
1	0.26	0.42
31	0.26	0.38
4	0.26	0.39
6	0.25	0.39
17	0.25	0.39
12	0.25	0.35
11	0.24	0.36
8	0.24	0.44
14	0.23	0.52
32	0.23	0.38
5	0.22	0.36
2	0.22	0.37
3	0.22	0.25
25	0.21	0.34
15	0.21	0.36
30	0.20	0.28
21	0.19	0.21
33	0.15	0.28
Average standard errors	0.03	0.05

7.4 Discussion

pH in the plots where herbicide was not applied caused large effects on plant growth and survival over time (Fig. 7.3, Tables 7.3, 7.4 and 7.6, Appendix 22). Plant survival after 116 days was much higher at the high pH (74%) than at the low pH (47%) and the trend was consistent in the following growing season (Table 7.4). It has been noticed that *Austrodanthonia* species and accessions differ widely in their response to soil acidity (Robinson *et al*. 1993; Dowling *et al*. 1996; Garden *et al*. 2001a). Some of the accessions responded extremely well irrespective of pH/herbicide combinations (Figs 7.2 and 7.4, Table 7.5) indicating their range of differential responses to soil acidity. For example, accessions 11, 17, 9 and 23 appeared at the top of the group, whereas accessions 21, 30, 25, 4, 31 and 3 were lower. These accessions responded similarly in the previous pot and hydroponic experiments (Chapters 4 and 6). Although the response of most of the accessions was similar to that in the experiments reported in Chapters 4 and 6, accessions 32 (Taranna) and 33 (Bunderra) previously ranked as less responsive, were in the intermediate responsive group (Fig. 7.2 and Table 7.5). This is most likely because at the time of registering these two cultivars, Lodge (1993a, 1993b) indicated that Taranna and Bunderra are moderately responsive to soil acidity.

An attempt was made to separate plant vigour from Al- tolerance. Using the approach proposed by Hutton *et al*. (1978) is not appropriate because of small number of treatments (low and high pH) being compared with the predicted DM. Alternatively, in an attempt to resolve plant vigour and Al-tolerance, percentage change (increases/decreases) in survival at low pH compared with the high pH was used. This percentage change was plotted as a function of growth rate score at the high pH (Fig. 7.6). It was observed that the accessions are closely associated with the fitted line, indicating that plant vigour of individual accessions might play might play an important role in response to soil acidity. This is most likely because the Al-challenge at pH ~ 4.4 was low (10 mg/kg vs 1 mg/kg at the higher pH, Table 7.1), and the differences in response by the accessions would not be expected to be great. A similar Al-challenge was mounted in the earlier pot experiment where at similar levels of pH (4.4 and 5.3), observed differences were unlikely to be attributable to Al-tolerance (Fig. 4.10b). Therefore, it would be difficult to separate the effects of plant vigour and Al-tolerance in the soils utilised in this field experiment.

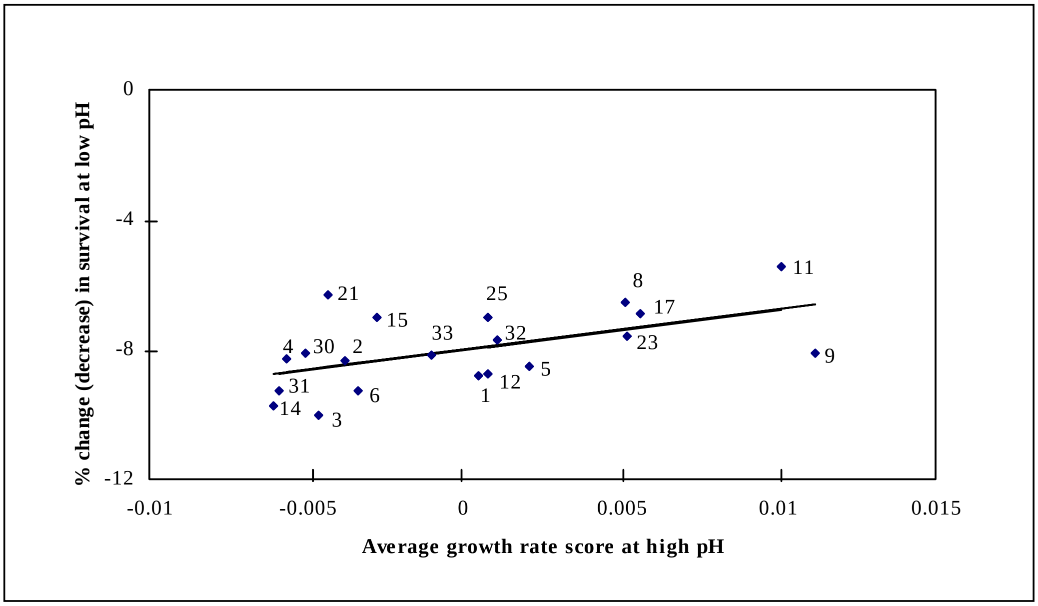

Fig. 7.6. Relationship between growth rate score at high pH (~5.3) and percentage change (decreases) in survival at low pH (~4.4). Growth rate score (change in score over time) meaned over plus and minus herbicide (Table 7.5). Percentage change in survival at low pH was calculated over high pH, and meaned over plus and minus herbicide (Fig. 7.2). Numbers displayed in the figure are accessions (Table 7.2).

Weather conditions are crucial for plant establishment, growth and production. Robinson and Archer (1988) demonstrated that a supplementary water supply is useful to minimise initial water stress. Also they showed that temperature was an important factor affecting plant growth. In the present study, the initial decline of growth score (Fig. 7.3) was probably due to severe moisture stress as well as high temperatures, especially during December 2000 and January 2001 (Fig. 7.1). These effects would be much larger where herbicide had been applied, reducing the bulk of the resident vegetation, and allowing the soil temperature to increase, creating a hostile environment for seedling growth. Under these conditions, the main limitation to plant growth was most likely soil moisture, and any pH effect would not be adequately expressed. Although an initial supplementary irrigation was applied to each of the sub-plots (equivalent to ~ 10 mm, twice a week), this was not adequate to keep seedlings alive especially in the bare sub-plots. Thus, no effect of pH was observed in plant growth and survival where herbicide had been applied (Fig. 7.3, Tables 7.3, 7.4 and 7.6, Appendix 22). On the plots where herbicide had been applied, about 68% of plants had died by 477 DAT, a result most likely caused by exposure and the effect of moisture stress and hot weather conditions.

The pasture management practices used on the site were irrelevant as the sub-plots were protected from grazing (at least for the first 208 days) by fences. However, the favourable growth and survival of the accessions where herbicide was not applied indicates that once they had established, *Austrodanthonia* accessions competed effectively with other species. Therefore, establishment of these perennial grasses in low-fertility diverse plant communities could have potential for competing with less desirable species such as annual grasses (King and Kemp 2001).

The present results demonstrate that there is a wide range of variation among *Austrodanthonia* species and accessions in their growth response to soil acidity, extending the earlier findings of Helyar and Conyers (1994), Dowling *et al*. (1996) and Rubzen *et al*. (1996). The range of differential responses exhibited is also consistent with the suggestion that *Austrodanthonia* populations have a broad genetic base, which would enable them to adapt to a wide range of environments (Abele 1959; Scott and Whalley 1984). Therefore, there is potential to use *Austrodanthonia* accessions to increase productivity in areas where edaphic factors limit productivity in pasture systems.

Chapter Eight

General discussion

This thesis was based on the premise that the genus *Austrodanthonia* contains a wide diversity of tolerance to soil acidity. This hypothesis was tested by growing plants in acid soils in pots (Chapter 4), by challenging the plants with excess H^+, Al or Mn in hydroponic culture (Chapters 5 and 6), and by growing plants in the field using natural gradients in soil pH and Al (Chapter 7).

The experiment reported in Chapter 5 showed that of the 12 accessions from five species of *Austrodanthonia* tested, all were so tolerant of H^+ that some of them could grow well even at pH 3.5 (Figs 5.5 and 5.7, Table 5.7). Therefore, *Austrodanthonia* is not likely to be adversely affected by H^+ when grown in the soils of the Slopes and Tablelands of New South Wales, all of which have values of $pH_{Ca} > 3.9$ (Fig. 1.1). This confirms the finding of Helyar and Conyers (1994) who showed a similar response for a very highly tolerant species, Consol lovegrass (*Eragrostis curvula*).

The 183 accessions (15 species) of *Austrodanthonia* screened for Al-tolerance, in a pot experiment using soil with a modified pH, that presented a minimal Mn toxicity challenge (Chapters 3 and 4), exhibited a wide range of Al-tolerance (Table 4.4). For a subset of 24 accessions that included 12 *Austrodanthonia* species, a similarly wide relative Al-tolerance ranking was confirmed at pH 4.0, using Al additions in hydroponics (Chapter 6).

The relative Al-tolerance ranking of *Austrodanthonia* accessions was generally similar between experiments (Fig. 8.1). Had tolerance relied mainly on Al exclusion from the root due to changes in the rhizosphere pH (Foy *et al*. 1978; Taylor 1988a; Kochian 1995), or the excretion of organic acids (Taylor 1988a, Miyasaka *et al*. 1991; Kochian 1995; Larsen *et al*. 1998; De la Fuente-Martinez and Herrera-Estrella 1999; Kayama 2001), the mixing that occurs in hydroponic media would be expected to seriously degrade the efficacy of such mechanisms. This was not the case, therefore it seems likely that the exhibited Al-tolerance did not depend heavily on these particular exclusion mechanisms; however, this observation does not preclude the exclusion of Al by other mechanisms.

The wide range of relative Al-tolerance of the subset of 24 accessions (12 species) of *Austrodanthonia* (Fig. 8.1), is consistent with the natural distribution of *Austrodanthonia* accessions in relation to their associated soil properties (Fig. 6.10), and with inferences drawn from results of field surveys (Scott and Whalley 1982; Dowling *et al*. 1996; Garden *et al*. 2000; Garden *et al*. 2001b). This outcome substantially extends the findings of Helyar and Conyers (1994), that ranked the Al-tolerance of *A. bipartita* and *A. richardsonii* as highly sensitive and highly tolerant, based on just three *Austrodanthonia* species.

This study has identified 49 accessions of *Austrodanthonia* from 15 species, most of which are vigorous, highly productive and acid tolerant. These traits may be exploitable in breeding programs and selection of improved cultivars. Further commercial potential may emerge given detailed screening of more accessions for Al- and Mn-tolerance. Hydroponics could be an efficient method for this screening.

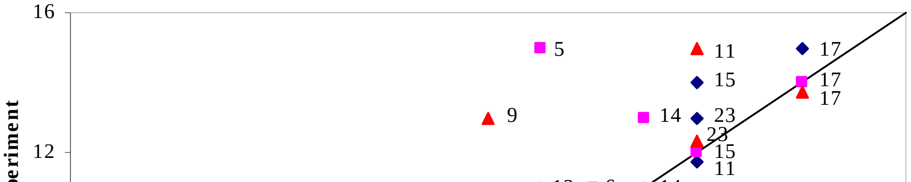

Fig. 8.1. Comparison of tolerance rankings for 15 *Austrodanthonia* accessions. Tolerance was independently assessed in three different experiments, conducted respectively in pots (squares), hydroponics (diamonds) and the field (triangles). Rankings obtained in the separate experiments are plotted on the Y-axis against the respective median rankings on the X-axis. Clustering of the pot and field scores away from the 1:1 line would indicate a systematic difference between the rankings for plants grown in soil or hydroponics. The numbers in the Figure represent accessions (Table 4.4).

Relative Al-tolerance of the accessions grown in hydroponics (Chapter 6) was also ranked using hematoxylin staining of root-tips. There was a wide range in the intensity of staining (Fig. 6.8); but an objective method of ranking the intensity could not be found, despite the high quality colour images. Consequently the stained root-tips were categorised by inspection into two groups, i.e. intensely and weakly stained. These groups were generally consistent with the Al-tolerance ranking based on growth in hydroponics for 15 of the 24 accessions tested (Chapter 6); however, there were some notable exceptions. Accessions 8, 10, 11, 12, 15, 16, 17, 23 and 24 were Al-tolerant (Figs 6.6 and 6.7), yet chemical analysis showed that they contained high concentrations of Al (Appendix 18), and the root-tips stained with hematoxylin (Fig. 6.8). Thus, it is argued that these nine accessions demonstrated an internal tolerance mechanism for Al. Had hematoxylin been the sole method used to identify Al-tolerance, these accessions would have been wrongly allocated to the Al-sensitive group. This misallocation may also occur in genera other than *Austrodanthonia*. Therefore, hematoxylin staining cannot be recommended as a definitive method of screening for Al-tolerance without further investigation.

Relative Mn-tolerance was examined for only three accessions of *Austrodanthonia* and the experiment was conducted using hydroponics. All three accessions were highly tolerant of Mn and one was extremely tolerant (Figs 5.11, 5.12 and 5.13). The extreme tolerance was based at least partly on an exclusion mechanism (Foy *et al*. 1978; Foy 1983,1984; Scott and Fisher 1989), as evidenced by precipitation of Mn-oxides on the surface of the roots (Appendix 16) and lower Mn concentrations in the tops (Appendix 15).

Because of the small amount of data on Mn-tolerance, it was not possible to demonstrate whether Al and Mn are linked; but by analogy with other, more intensively studied species, this is unlikely (Foy *et al*. 1978; Foy 1984; Culvenor 1985; Culvenor *et al*. 1986a; Edmeades *et al*. 1991b). It appears that *Austrodanthonia* exhibits Al- and Mn-tolerance based on both exclusion and internal mechanisms. The mechanisms of Al- and Mn-tolerance in *Austrodanthonia* require more detailed study.

The field experiment (Chapter 7) determined the relative responses of 20 of the selected *Austrodanthonia* accessions in an acidic environment under natural conditions. This experiment also showed that *Austrodanthonia* accessions had a range of responses to soil acidity (Figs 7.2 and 7.4) which was consistent with earlier studies under controlled environments (Fig. 8.1). The soils of the field experiment (Chapter 7) contained much lower Al concentrations than the pot experiment (Chapter 4), and as a consequence, it was more difficult to separate out the vigour effect from the Al-tolerance effect (Fig. 7.6). Thus a more detailed study with a high Al-challenge in natural field conditions is required. Once established, *Austrodanthonia* accessions competed effectively with other less desirable species present in the system (King and Kemp 2001). Although the field experiment was confined to only 20 of the 183 accessions screened in the pot experiment (Chapter 4), the results suggest that highly responsive accessions of *Austrodanthonia* could improve the productivity of pasture systems on soils on the Central Tablelands of NSW and perhaps other locations, where edaphic factors might limit productivity.

There is genetic variation in the ability of plants to tolerate soil acidity (e.g. Scott and Whalley 1984; Foy *et al*. 1988; Scott and Fisher 1989). In different soils, the responses by plants to soil acidity depends on differences in the solubility of Al and Mn with soil pH, and the differences in tolerance to Al, Mn and H^+. Plant tolerance to Al, Mn and H^+ ions, appears to be independently inherited and to vary both between species and between accessions within species (Baligar *et al*. 1987; Baligar *et al*. 1988; Foy *et al*. 1988; Baligar and Smedley 1989; Baligar *et al*. 1989; Scott and Fisher 1989; Edmeades *et al*. 1991a, 1991b; Howeler 1991). The mechanisms that plants have developed to tolerate and survive could include exclusion of the toxic ions, or detoxification of the ions once they have been absorbed (Foy 1984; Foy *et al*. 1988; Scott and Fisher 1989; De la Fuente-Martinez and Herrera-Estrella 1999). No systematic investigation of Al- and Mn-tolerance mechanisms was undertaken in this study; nonetheless, some evidence for the operation of both exclusion and internal mechanisms was obtained (Chapters 5 and 6), indicating that sufficient genetic diversity exists among *Austrodanthonia* accessions, allowing them to adapt to a wide range of stress conditions. Further research aimed at understanding the tolerance mechanisms may provide insights into plant interactions in diverse ecosystems.

Similar dose-response relations to varying concentrations of Al and Mn have been used to characterise plant growth responses to potentially toxic elements. Such responses have been demonstrated with Al (Foy 1974; Foy *et al*. 1978; Foy and Fleming 1978; Foy 1983, 1984; Baligar and Smedley 1989; Baligar *et al*. 2001) and Mn (Helyar 1978; Foy 1984; Marschner 1986; Carver and Ownby 1995; Cregan and Scott 1999). For example, growth stimulation from low concentrations of Al has been reported in rice (Howeler and Cadavid 1976) and "BH 1146" wheat (Foy and Fleming 1978), while high concentrations of Al either inhibited growth for Al-sensitive cultivars or increased growth for Al-tolerant cultivars in many plant species (Foy 1983, 1984; Taylor and Foy 1985d; Baligar *et al*. 1988; Baligar *et al*. 1993; Helyar and Conyers 1994; Baligar *et al*. 2001). The plant growth stimulation/inhibition responses may primarily be due to the physiological effects of the toxic element on the counterbalancing growth processes within the plant (Helyar 1978; Marschner 1986).

Although substantial advances have been made in the present investigations towards identifying acid tolerant *Austrodanthonia* accessions, this study has some shortcomings. For example, selection of the accessions for further investigation (Chapter 4) was made on the basis of superior emergence at pH_{Ca} 3.9 and 4.4. There was difficulty in separating the vigour and Al-tolerance effects even after following the Hutton approach (Hutton *et al*. 1978), especially where Al was present in the soils as a low Al-challenge, and some inconsistencies occurred e.g., *Phalaris* being classed as tolerant (Fig. 4.10). In order to reduce the number of pots to manageable levels, not all accessions x pH combinations were replicated. In addition, it was not possible to include all the accessions selected from this experiment in all the other experiments. Thus, an accession with superior agronomic potential might have been omitted from the selection, and the order of rankings between accessions may change when larger numbers of accessions are included (Gallardo *et al*. 1999).

This overall study on *Austrodanthonia* provides new and useful information on differential responses to H^+, Al and Mn, and complements existing information for other species (Foy and Fleming 1978; Scott and Whalley 1984; Cregan *et al*. 1986; Foy *et al*. 1988; Baligar and Smedley 1989; Edmeades *et al*. 1991a, 1991b; Helyar and Conyers 1994; Fenton *et al*. 1996; Baligar *et al*. 2001). The ramifications of the findings are important for further work related to plant breeding, liming schedules and the development of an integrated approach to the management of acid soil environments on the Tablelands of New South Wales.

To summarise, the genus *Austrodanthonia* is highly H^+-tolerant, and within the genus, there is a wide range of tolerance to Al and Mn. *A. duttoniana* and *A. fulva* appeared to be superior to the other 13 species tested, because of their outstanding productivity on a per plant basis, and their acid tolerance. Comparative productivity with other species on an area (t/ha) basis and a field investigation with high Al-challenge remain to be ascertained.