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### Evaluation of *Austroanthonia* Accessions for Acid Tolerance and Growth Potential

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**Abstract:** Acid tolerance of *Austroanthonia* accessions was evaluated in two experiments. In the first, seeds of the 183 *Austroanthonia* accessions from 15 species, two *Austroanthonia* cultivars (Taranna and Bunderra) and three exotic grasses, were sown in pots containing a sandy loam soil at pH<sub>Ca</sub> 3.9, 4.4 and 5.3. Seedling emergence and survival were recorded at 44 and 170 Days After Sowing (DAS) and Dry Weight (DW) at 186 DAS. In the second experiment, seedlings of 20 of the *Austroanthonia* accessions and cvs used in experiment 1 were planted into a brown chromosol/brown kurosol soil located near Carcoar, New South Wales (33°37'S, 149°13'E, elevation 820 m), into areas with natural pH<sub>Ca</sub> values of 4.3 or 4.9. Plant growth was monitored over 16 months. Half the planting area was pre-sprayed with glyphosate to kill the resident pasture. In experiment 1, the lowest pH<sub>Ca</sub> value (3.9) severely decreased emergence, survival and DW. In experiment 2, the pH<sub>Ca</sub> value of 4.3 decreased survival and DW and there was an interaction with the herbicide pre-treatment. In both experiments, there were large differences between accessions in growth potential and acid tolerance. Relative acid tolerance rankings of accessions/cvs were similar in each experiment and DW seemed to be the most reliable index of acid tolerance. The diversity of acid tolerance and growth potential in *Austroanthonia* may be useful in studying the genetics of Al tolerance and in plant breeding and selection.

**Key words:** Acid tolerance indices, *Dactylis*, *Danthonia*, emergence and survival, *Phalaris*, *Vulpia*

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### INTRODUCTION

Excessive soil acidity is a major cause of the failure of exotic pasture species to persist in many parts of the world (Helyar, 1991; Sledge *et al.*, 2005; Islam *et al.*, 2006; Kariuki *et al.*, 2007; Narasimhamoorthy *et al.*, 2007). Liming effectively corrects acidity (Scott *et al.*, 2001); however, it has limitations such as cost and the lengthy delay between application and the correction of subsoil acidity (Helyar, 1991; Scott *et al.*, 2001; Islam *et al.*, 2006; Narasimhamoorthy *et al.*, 2007). Consequently, management of pastures on acid soils demands a system that might include liming, less acidifying farming systems and acid tolerant pasture plants (Helyar, 1991; Sledge *et al.*, 2005; Islam *et al.*, 2006; Wenzl *et al.*, 2006; Narasimhamoorthy *et al.*, 2007).

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On the Slopes and Tablelands of New South Wales (NSW), Australia, native grasses from a number of genera comprise 40-60% of pastures (Garden *et al.*, 2000). Prominent among them are species of the genus *Austrodanthonia*, formerly *Danthonia* (Linder, 1997) which are major components of extensive remnant native grasslands that still occupy more than two million hectares in southern temperate Australia (Garden *et al.*, 2001b). There are about 26 species of *Austrodanthonia* in Australia (Wheeler *et al.*, 2002), all of which are perennial grasses. The 19 species that occur on the Slopes and Tablelands of NSW grow on soils that are generally shallow, acidic and of relatively low fertility (Dowling *et al.*, 1996), in locations where low rainfall or temperature seasonally limit plant growth (Cashmore, 1932).

The *Austrodanthonia* genus contains nutritious forage plants (Archer and Robinson, 1988; Dowling *et al.*, 1996; Garden *et al.*, 2001b; Robinson and Archer, 1988; Wheeler *et al.*, 2002); with high frost tolerance and good winter growth (Wheeler *et al.*, 2002). Consequently, in the Tablelands environment, *Austrodanthonia* spp. may produce more Dry Weight (DW) per unit area than an introduced genus such as *Phalaris* (Archer and Robinson, 1988; Robinson and Archer, 1988). Some *Austrodanthonia* spp. may also tolerate acidic soils (Dowling *et al.*, 1996; Garden *et al.*, 2001a; Islam *et al.*, 2006); however, the inter- and intra-specific variation remains largely unknown (Islam *et al.*, 2006). By analogy with other grass genera, *Austrodanthonia* spp. are likely to exhibit considerable inter- and intra-specific variation in acid tolerance (Foy, 1984; Helyar and Conyers, 1994; Kariuki *et al.*, 2007).

Acid tolerance of plants has been assessed using soils in the field or greenhouse, or using sand or solution culture (Scott and Fisher, 1989; Islam *et al.*, 2006; Wenzl *et al.*, 2006; Narasimhamoorthy *et al.*, 2007). Screening large numbers of plants for acid tolerance through their life cycle in the field is resource intensive due to the need to accommodate spatial heterogeneity of soil properties and seasonal variations in climate (Munns and James, 2003). The latter sources of variation can be minimised using pots in the greenhouse. However, greenhouse screening has enjoyed variable success (Foy, 1984; Helyar and Conyers, 1994; Narasimhamoorthy *et al.*, 2007), perhaps because tolerance may change with age (Hanson and Kamprath, 1979) and be affected by differences in growth rate (Helyar and Conyers, 1994; Hutton *et al.*, 1978).

The objectives of this study were to evaluate the relative acid tolerance and DW of 183 *Austrodanthonia* accessions collected from 126 sites on the NSW Tablelands (Dowling *et al.*, 1996) in a pot experiment. Then the performance of 20 of these accessions was evaluated over 16 months in the field. The results from both experiments were finally compared.

## MATERIALS AND METHODS

### ***Austrodanthonia* Accessions**

One hundred and eighty three accessions of *Austrodanthonia* had been collected from 126 sites on the Central, Southern and Monaro Tablelands of NSW, Australia during 1991/92 (Dowling *et al.*, 1996). The accessions were identified by a 7-character alphanumeric code that has been simplified here to a 3-digit ID (Table 1). We grew most of the specimens in pots for taxonomic identification and to increase the supply of seed. Caryopses were separated manually and any seeds that were obviously diseased, misshapen or small were discarded. Seeds were stored at 4°C to break dormancy and most accessions had 80-100% germination.

### **Experiment 1**

#### **Design**

The design was completely randomised, with three soil pH levels (3.9, 4.4 and 5.3), all 183 *Austrodanthonia* accessions and five additional grasses (Table 1). These were: *A. richardsonii*

Table 1: Genotypes used in experiments 1 and 2

Genotypes in experiment 1		<i>Austroanthonia</i> accessions/cvs common to experiments 1 and 2		
Name	No. of accessions/cultivars	ID <sup>a</sup>	Code <sup>a</sup>	Species
<i>Austroanthonia penicillata</i>	11	1	182251	<i>A. racemosa</i>
<i>A. pilosa</i>	36	2	182095	<i>A. racemosa</i>
<i>A. setacea</i>	6	3	182188	<i>A. racemosa</i>
<i>A. duttoniana</i>	16	4	182233	<i>A. racemosa</i>
<i>A. carphoides</i>	4	5	182288	<i>A. pilosa</i>
<i>A. fulva</i>	9	6	182087	<i>A. pilosa</i>
<i>A. eriantha</i>	18	8	182206	<i>A. fulva</i>
<i>A. racemosa</i>	69	9	182205	<i>A. fulva</i>
<i>A. laevis</i>	4	11	182131	<i>A. duttoniana</i>
<i>A. monticola</i>	1	12	182050	<i>A. duttoniana</i>
<i>A. richardsonii</i>	2	14	182081	<i>A. penicillata</i>
<i>A. bipartita</i>	1	15	182192	<i>A. penicillata</i>
<i>A. procera</i>	2	17	182031	<i>A. setacea</i>
<i>A. caespitosa</i>	3	21	182122	<i>A. richardsonii</i>
<i>A. tenuior</i>	1	23	182059b	<i>A. eriantha</i>
<i>A. richardsonii</i> cv. Taranna	1	25	182064	<i>A. duttoniana</i>
<i>A. bipartita</i> cv. Bunderra	1	30	182112	<i>A. pilosa</i>
<i>Dactylis glomerata</i> cv. Porto	1	31	182127	<i>A. pilosa</i>
<i>Phalaris aquatica</i> cv. Sirosa	1	32	cv. Taranna	<i>A. richardsonii</i>
<i>Vulpia myuros</i>	1	33	cv. Bunderra	<i>A. bipartita</i>

<sup>a</sup>Three-digit ID and the corresponding alphanumeric code used to identify the 183 *Austroanthonia* accessions collected by Dowling *et al.* (1996)

Table 2: Some properties of the surface soils used in experiments 1 and 2

Properties	Property values		
	Experiment 1 unamended soil	Experiment 2 <sup>a</sup>	
		High pH site	Low pH site
pH <sub>Ca</sub>	4.40	4.9 (4.7-5.3)	4.3 (4.2-4.5)
pH <sub>w</sub>	5.00	5.6 (5.3-6.0)	5.1 (4.9-5.3)
EC (dS m <sup>-1</sup> )	0.19	0.12 (0.08-0.16)	0.09 (0.08-0.10)
Bray-P (mg kg <sup>-1</sup> )	17.00	5.0 (2.1-9.3)	4.4 (2.3-7.0)
Al <sub>Ca</sub> (mg kg <sup>-1</sup> )	18.00	1.0 (0.50-1.90)	6.6 (3.1-9.1)
Mn <sub>Ca</sub> (mg kg <sup>-1</sup> )	5.00	17.0 (11-24)	17.0 (7-26)

<sup>a</sup>Values are means with ranges in parentheses

(Cashmore) H.P. Linder cv. Taranna (T) and *A. bipartita* (Link) H.P. Linder cv. Bunderra (B) and three exotic grasses known to differ in acid tolerance; *Dactylis glomerata* L. cv. Porto (D), *Phalaris aquatica* L. cv. Sirosa (P) and *Vulpia myuros* (L.) Gmel. (V), all having > 95% germination. Twenty eight of the accessions and T, B, D, P and V were replicated three times, with the remaining 155 accessions being unreplicated due to limitation of seeds however repeated ten times within each treatment. At each pH there were an additional 12 pots without seeds to test the effect of plants on soil properties. The experiment was conducted outdoors at the Orange Agricultural Institute (33°21'S, 149°40'E, elevation 925 m), NSW, from April to October 1999 (mid-autumn to mid-spring).

### Soil

About 3 t of surface soil (0-20 cm) was collected from a grazing property at Binnaway (31°31'S, 149°17'E, elevation 460 m), NSW. Soil from this site had previously been used to rank the relative acid tolerance of crop and pasture species (Helyar and Conyers, 1994). The soil was a naturally acidic sandy loam (pH<sub>Ca</sub>~4.4) and selected properties are presented in Table 2 (Islam *et al.*, 2004).

The soil was air-dried, sieved through a 10 mm mesh and well mixed. Soil pH (pH<sub>Ca</sub>) was lowered or raised to pH<sub>Ca</sub> 3.9 and 5.3, thoroughly mixing either 380 g of Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>. 18H<sub>2</sub>O or 48 g of CaCO<sub>3</sub>

with each 100 kg of air-dry soil, incubating for three weeks and leaching to remove excess soluble salts (Islam *et al.*, 2004). Each pot (15 cm diam and 10 cm deep) contained 2.2 kg of air-dry soil. Soil was periodically sampled from pots with and without plants and analysed as follows.

Samples were air-dried at 40°C. Soil pH ( $\text{pH}_{\text{Ca}}$ ) was measured on 10 g of soil following a 1 h shake with 50 mL of 10 mM  $\text{CaCl}_2$ , end-over-end at 10 rpm (Rayment and Higginson, 1992). Al and Mn were measured in the supernatant of the  $\text{CaCl}_2$  extract using ICP (Rayment and Higginson, 1992). Electrical Conductivity (EC) and  $\text{pH}_{\text{w}}$  were determined in 1:5 soil:water extract (Rayment and Higginson, 1992). Available P was measured following the Bray-1 method (Bray and Kurtz, 1945).

Basal fertilisers per kg air-dry soil were: 4.5 mg Mg as  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 10.8 mg K as  $\text{K}_2\text{SO}_4$ , 2.2 mg Cu as  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 4 mg Zn as  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.6 mg B as  $\text{H}_3\text{BO}_3$  and 23.5 mg P as superphosphate (9.1% P). The superphosphate contained 200 mg Mo  $\text{kg}^{-1}$ . Ammonium nitrate was applied in solution in three equal amounts, to supply 18.8 mg N  $\text{kg}^{-1}$  air-dry soil at 35, 77 and 105 Days After Sowing (DAS).

### **Plant Culture and Observations**

Ten seeds of a single genotype were sown per pot. The surface of the soil was moistened and kept moist until emergence began, after which the soil was watered to field capacity and rewatered as required. Emergence was monitored at 44 DAS and survival at 170 DAS. At 186 DAS, the shoots of live plants were harvested and DW was measured after drying at 70°C.

### **Data Analysis**

A generalised linear mixed model analysis was used to model both emergence and survival. For both variables, the response at a given day (x) for a given pot was assumed to be a binomial variable. Binomial (10, p(x)) where p(x), the success probability, was modeled using the ASREML package (Gilmour *et al.*, 1999) as:

$$\text{logit}(p(x)) = \text{mean} + \text{treatment} + \text{species} + \text{treatment} \times \text{species} + \text{treatment} \times \text{accession}$$

Dry weight was subjected to variance components analysis and significance was tested by using the Wald statistic (Chi-square probability). When appropriate, data were log transformed to increase homogeneity of the variances. At  $\text{pH}_{\text{Ca}}$  3.9, most of the plants had died by the final harvest, so statistical analysis for DW was performed only for the data at  $\text{pH}_{\text{Ca}}$  4.4 and 5.3.

## **Experiment 2**

### **Site and Soils**

The field site was located at Carcoar, NSW (33°37'S, 149°13'E, elevation 820 m) and has been described elsewhere (Michalk *et al.*, 2003). Briefly, it comprised a highly variable, undulating landscape, where the soil was of low fertility and classified as a light textured brown chromosol/kurosol (Isbell, 1996) or yellow/red podsolic. The average  $\text{pH}_{\text{Ca}}$  of the surface soil was 4.5 and the average annual rainfall 871 mm. Climatic conditions during the experimental period are presented in Table 3. Key soil properties were measured as described for experiment 1 (Table 2).

The plant community on-site was extremely diverse, with over 100 species being identified (Michalk *et al.*, 2003). The dominant plant species included: *Austrodanthonia* spp. (wallaby grasses), *Themeda australis* R. Br. (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 3: Monthly means of soil water deficit (0-20 cm), air temperature, sub-surface (5 cm) soil temperature and rainfall at the field site, Carcoar, NSW during experiment 2

Time	Soil water deficit (mm)	Air temperature (°C)	Sub-surface soil temperature (°C)	Rainfall (mm)
<b>2000</b>				
Oct	-9.13	11.75	14.22	81.00
Nov	-8.42	16.00	19.16	105.60
Dec	-27.48	19.54	23.54	14.00
<b>2001</b>				
Jan	-28.48	22.71	26.88	20.40
Feb	-34.86	21.47	24.66	33.40
Mar	-33.86	16.30	20.19	61.80
Apr	-30.90	13.90	15.19	70.80
May	-16.13	8.73	10.10	13.40
Jun	-7.84	7.42	7.75	47.40
Jul	-7.23	6.53	7.14	52.40
Aug	-0.31	6.43	7.03	78.20
Sep	-0.39	10.98	12.17	57.80
Oct	-7.33	11.27	14.44	70.00
Nov	-12.33	14.46	18.34	47.00
Dec	-27.55	18.45	21.92	22.80
<b>2002</b>				
Jan	-20.09	19.92	25.54	47.40
Feb	-23.01	18.13	22.60	129.40
Mar	-24.04	17.59	20.19	11.40

### Design

Plots were nested in six unfertilised naturalised pasture paddocks that were part of a sustainable grazing systems experiment (Michalk *et al.*, 2003). Seedlings of 20 accessions/cvs from nine *Austrodanthonia* spp., that were expected from the results of experiment 1 to range widely in acid tolerance and DW, were planted at two naturally occurring levels of pH<sub>Ca</sub> (4.3±0.2 and 4.9±0.4, Table 2) within each of the six paddocks. These pH values are referred to as 4.3 and 4.9. Plots were selected to minimise the effect of other variables e.g., botanical composition, aspect and slope. Within each paddock there were four sub-plots, each 1×2 m: two plots on areas of lower pH<sub>Ca</sub> (4.3) and two on higher pH<sub>Ca</sub> (4.9). Each sub-plot was divided into two sub-sub-plots, one of which was treated with the herbicide, glyphosate, at 350 g a.i. ha<sup>-1</sup>, two weeks before the seedlings were transplanted. The herbicide was applied to test for the effects of the established resident pasture species on survival and growth of the transplanted seedlings. The experiment was laid out in a factorial (split-split) design with selected accessions/cvs repeated from two to five times within each sub-sub-plot, depending on availability. The experiment was conducted from November 2000 (late spring) to March 2002 (early autumn).

### Seedling Preparation, Transplanting and Maintenance

Disease-free seeds (naked caryopses) of similar size and shape, of the 20 *Austrodanthonia* accessions/cvs grown in experiment 1, were collected and stored in a refrigerator to break dormancy before planting. A single seed was sown into pasteurised soil in each paper pot (3 cm diam × 7 cm tall). The pots were placed in a greenhouse and were watered regularly for nine weeks. Seedlings were trimmed to a height of ~12 cm one day before transplanting.

In the field, seedlings were transplanted with a spacing of ~10 cm within and between rows. Planting cavities were created by the removal of soil cores. Plots were hand watered twice a week until the seedlings established.

### Observations

At transplanting and at 14, 33, 67 and 116 Days After Transplanting (DAT), a growth score was given to all seedlings. Growth was scored from 0 to 10: 0 = dead and scores greater than 3 indicated

that plants had grown since transplanting. The growth score at 116 DAT was used to estimate DW using the following procedure: 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°C for 48 h before weighing. The DW data were regressed against growth scores to predict DW for all plants (Helyar and Conyers, 1994). Survival was estimated at 116 DAT and the final at 477 DAT.

### Data Analysis

A similar approach to that used in experiment 1 was applied here to growth score, survival and predicted DW. Growth score and survival were analysed on a logit scale as binary variables while DW analysis was performed on the  $\log_e (Y + 0.25)$  scale, where Y denoted growth score, ignoring plants that had not survived following the ASREML procedure (Gilmour *et al.*, 1999). Significance was tested using the Wald statistic (Chi-square probability).

### Relative Acidity Tolerance in Experiments 1 and 2

In experiment 1, relative tolerance was calculated using emergence (44 DAS) and survival (170 DAS) for pH<sub>Ca</sub> 3.9 and 4.4 and DW at 186 DAS for pH<sub>Ca</sub> 4.4 and 5.3, using the method of Hutton *et al.* (1978). In experiment 2, the same method was applied to survival and DW data at 116 DAT from the minus herbicide plots at pH<sub>Ca</sub> 4.3 and 4.9. An arbitrary deviation line (i.e., ±5% of the fitted lines) was used to rank accession's relative acid tolerance as high, intermediate and low (Islam, 2003).

## RESULTS

### Experiment 1

#### Treatments

The values of pH<sub>Ca</sub> changed by no more than 0.05 units during the experiment and were unaffected by the presence of plants (Islam *et al.*, 2004). The pH<sub>Ca</sub> values of 5.3, 4.4 and 3.9 corresponded with soluble Al<sub>Ca</sub> concentrations of ~2, ~18 and ~52 mg kg<sup>-1</sup> soil (Islam *et al.*, 2004), therefore the treatments provided a wide range of acidity (i.e., Al) challenge.

#### Emergence, Survival and Growth

At pH<sub>Ca</sub> 3.9, the emergence of all four genera was decreased relative to that at pH<sub>Ca</sub> 5.3 (Table 4) and survival at 170 DAS (Table 4) was: *Vulpia* (~17%) > *Austroanthonia* (~0.41%) >> *Dactylis* ≈ *Phalaris* (0%) i.e., few plants survived so DW was not measured. At pH<sub>Ca</sub> 4.4, emergence and survival were generally similar to that at pH<sub>Ca</sub> 5.3, with the exception that the survival of *Phalaris* was at least halved (Table 4 and 5).

Table 4: Effect of soil pH on emergence at 44 DAS and survival at 170 DAS of different genera in experiment 1 at pH<sub>Ca</sub> values of 3.9, 4.4 and 5.3. Means followed by standard errors in parentheses

Genus	pH <sub>Ca</sub>	Emergence (%) at 44 DAS	Survival (%) at 170 DAS
<i>Austroanthonia</i>	3.9	9.84 (5.44)	0.41 (1.16)
	4.4	68.08 (8.51)	18.73 (7.12)
	5.3	75.14 (7.89)	28.12 (8.21)
<i>Dactylis</i>	3.9	16.67 (6.80)	0.00
	4.4	73.33 (8.07)	73.33 (8.07)
	5.3	60.00 (8.94)	53.33 (9.11)
<i>Phalaris</i>	3.9	43.33 (9.05)	0.00
	4.4	73.33 (8.07)	6.67 (4.55)
	5.3	73.33 (8.07)	43.33 (9.05)
<i>Vulpia</i>	3.9	53.33 (9.11)	16.67 (6.80)
	4.4	90.00 (5.48)	90.00 (5.48)
	5.3	80.00 (7.30)	80.00 (7.30)

Table 5: Effect of pH<sub>Ca</sub> on shoot DW in experiment 1 of 15 *Austroanthonia* spp. and of the 27 accessions of *A. racemosa*, which survived at both pH<sub>Ca</sub> values of 4.4 and 5.3 at the day of harvesting (186 DAS)

Species	DW (mg plant <sup>-1</sup> )		<i>A. racemosa</i> accessions (ID) <sup>a</sup>	DW (mg plant <sup>-1</sup> )	
	pH <sub>Ca</sub> 4.4	pH <sub>Ca</sub> 5.3		pH <sub>Ca</sub> 4.4	pH <sub>Ca</sub> 5.3
<i>A. tenuior</i>	0.00	0.00	62	4.00	17.20
<i>A. laevis</i>	2.25	61.68	73	8.00	29.00
<i>A. setacea</i>	10.56	17.50	96	12.00	25.00
<i>A. bipartita</i>	14.33	41.70	91	13.00	72.43
<i>A. pilosa</i>	15.87	31.95	76	14.00	37.39
<i>A. penicillata</i>	18.00	32.58	71	15.00	26.56
<i>A. procera</i>	18.47	30.78	109	18.67	61.38
<i>A. richardsonii</i>	24.67	46.84	57	19.00	65.50
<i>A. racemosa</i>	27.49	34.06	63	19.25	46.63
<i>A. eriantha</i>	30.80	31.30	60	21.67	46.67
<i>A. fulva</i>	31.73	56.24	95	28.86	46.25
<i>A. duttoniana</i>	39.45	33.51	1	30.38	81.56
<i>A. monticola</i>	41.50	30.75	93	30.60	69.80
<i>A. caespitosa</i>	41.61	28.17	59	31.83	62.41
<i>A. carphoides</i>	60.23	42.63	56	34.00	93.50
			88	36.00	85.33
			106	39.33	51.14
			82	51.50	40.00
			86	65.00	40.50
			99	68.22	42.00
			2	69.00	39.00
			94	77.00	67.70
			110	84.00	51.60
			97	87.50	45.60
			105	102.00	12.25
			58	113.00	37.78
			69	153.22	65.10
Standard errors range	0.10-28.71	0.13-30.78		0.07-4.58	0.05-3.71

<sup>a</sup>ID corresponds to the accession code of Dowling *et al.* (1996)

At pH<sub>Ca</sub> 5.3 emergence of all genotypes was ~70% and survival ranged from ~25% for *Austroanthonia* to ~75% for *Vulpia*. There were also large differences in DW between genera and between species of *Austroanthonia* (Islam, 2003) and within single species, as illustrated for the 27 accessions of *A. racemosa* which survived at both pH<sub>Ca</sub> values of 4.4 and 5.3 (Table 5).

## Experiment 2

### General Observations

Survival declined independent of the treatments during the first 33 DAT, which was a hot, dry period (Table 3); furthermore, fewer plants survived in the plus than the minus herbicide plots e.g., by 116 DAT median survival was ~35% and ~80%, respectively (Fig. 1), with a similar difference by 477 DAT (~25% vs ~70%). The relationship between DW and the growth score was sufficiently accurate to justify the presentation of estimated DW values instead of growth scores (n = 46, r<sup>2</sup> = 0.99):

$$DW (g \text{ plant}^{-1}) = e^{(0.354 \times \text{growth score} - 1.631)} - 0.25$$

Finally, the acidity challenge at pH<sub>Ca</sub> 4.9 and 4.3 differed widely i.e., the concentrations of Al<sub>Ca</sub> were ~1 and ~7 mg kg<sup>-1</sup> soil (Table 2).

### pH Effects on Survival and DW

Survival was decreased at pH<sub>Ca</sub> 4.3 regardless of the herbicide treatment (Fig. 1); however, applying herbicide prevented the expression of an effect of pH<sub>Ca</sub> on DW (data not shown) that was highly significant in its absence and differed between accessions/cvs (Table 6 and Fig. 2).



Table 6: Effect of pH<sub>C<sub>a</sub></sub> on shoot DW at 116 DAT for the minus herbicide plots in experiment 2 at pH<sub>C<sub>a</sub></sub> values of 4.3 and 4.9

Accessions (ID) <sup>a</sup>	DW (g plant <sup>-1</sup> )	
	pH <sub>C<sub>a</sub></sub> 4.3	pH <sub>C<sub>a</sub></sub> 4.9
33	0.01	0.12
25	0.02	0.18
30	0.02	0.14
3	0.03	0.21
15	0.03	0.14
5	0.04	0.19
4	0.04	0.18
21	0.04	0.15
14	0.04	0.12
1	0.05	0.30
2	0.05	0.19
12	0.05	0.25
9	0.06	0.29
17	0.06	0.28
23	0.07	0.28
8	0.07	0.21
31	0.07	0.16
32	0.07	0.21
6	0.08	0.26
11	0.12	0.27
Average standard error	0.03	0.03

<sup>a</sup>ID corresponds to the accession code of Dowling *et al.* (1996)

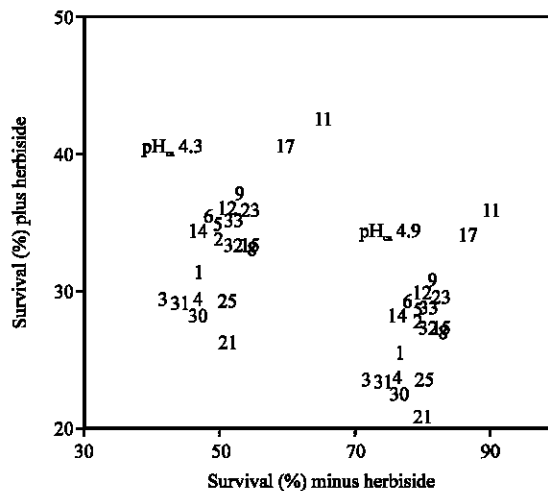


Fig. 1: Survival of 20 *Austroanthonia* accessions/cvs at 116 DAT as affected by herbicide and pH<sub>C<sub>a</sub></sub> (4.3 and 4.9) in experiment 2. Survival is expressed as a percentage of the number of seedlings planted. Numbers displayed are the ID numbers of the accessions/cvs (Table 1). Values broadly separate into two groupings based on soil pH<sub>C<sub>a</sub></sub> - left hand side (4.3); right hand side (4.9)

## DISCUSSION

### Emergence, Survival and DW as Indices of Acid Tolerance

In experiment 1, pH<sub>C<sub>a</sub></sub> values of 5.3, 4.4 and 3.9 were associated with soluble Al<sub>C<sub>a</sub></sub> concentrations of 2, 18 and 52 mg kg<sup>-1</sup> soil (Islam *et al.*, 2004) and the concentrations of Mn<sub>C<sub>a</sub></sub> (Table 2) were not excessive (Helyar and Conyers, 1994). Therefore, it can be argued that the pH effects on emergence

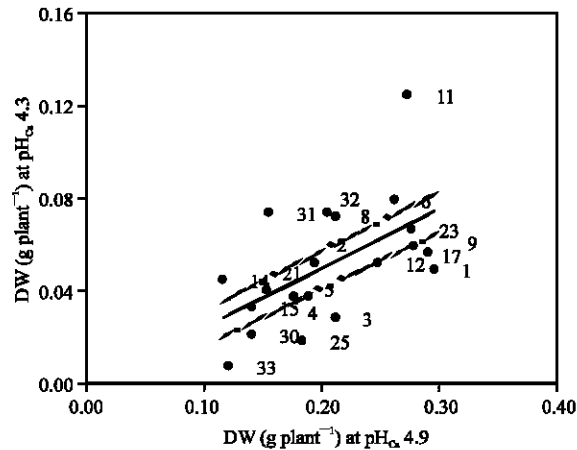


Fig. 2: Relationship between DW of 20 *Austroanthonia* accessions/cvs at pH<sub>Ca</sub> 4.9 and 4.3 in the minus herbicide plots at 116 DAT in experiment 2. The plotted numbers are accessions/cvs ID (Table 1). Equation of line:  $Y = 0.254X (\pm 0.087) - 0.001 (\pm 0.018)$ ,  $R^2 = 0.57$ ,  $p = 0.009$ . Dotted lines represent  $\pm 5\%$  of the fitted line. Note the different scales on the axes

Table 7: Relative acidity (AI) tolerance of the 18 accessions and two cultivars of *Austroanthonia* common to experiments 1 and 2 (H = High; I = Intermediate; L = Low). Relative acidity tolerance was ranked based on the properties listed, according to Hutton *et al.* (1978)

ID <sup>a</sup>	Experiment 1			Experiment 2	
	Emergence	Survival	DW	Survival	DW
1	I	H	L	I	L
2	L	H	H	I	I
3	L	L	L	L	L
4	L	I	L	L	I
5	H	I	H	I	I
6	H	H	L	I	H
8	L	H	I	I	H
9	H	H	I	I	I
11	H	H	H	H	H
12	H	I	H	I	I
14	H	H	H	I	H
15	H	L	H	I	I
17	H	I	H	H	L
21	L	L	L	L	I
23	H	I	L	I	I
25	L	L	L	L	L
30	L	L	L	L	L
31	L	H	L	L	H
32	L	L	L	I	H
33	L	L	L	I	L

<sup>a</sup>ID corresponds to the accession code of Dowling *et al.* (1996)

and survival (Table 4) primarily resulted from interactions between the concentrations of soluble AI and the relative AI tolerance of the genotypes. A similar argument can be made for the effects of pH<sub>Ca</sub> in experiment 2.

It is evident that pH<sub>Ca</sub> affected seedling emergence, survival and DW (Table 4-6 and Fig. 1-2). Relative tolerance rankings for all three measures were assessed in experiment 1 for all 188 genotypes and for survival and DW for the 20 genotypes in experiment 2. All the rankings from experiment 1 are available (Islam, 2003); however, we here present only the rankings for the 20 *Austroanthonia* accessions/cvs common to both experiments (Table 7).

When the acidity tolerance was ranked on emergence at  $\text{pH}_{\text{Ca}}$  3.9 and 4.4 in experiment 1, for the 20 accessions/cvs common to both experiments, the rankings were almost consistent except for accessions 2, 15 and 31 (Table 7). With the extension of the criteria to DW, rankings were inconsistent for a further few accessions (e.g., 1 and 6). Within experiment 2 there were inconsistencies between the tolerance rankings from survival and DW for accessions 17 and 31 (Table 7). Factors in addition to acidity tolerance may influence emergence, survival and DW within an experiment, so such differences were not surprising. However, the differences in the rankings based on DW for accessions 6, 17 and 31 that occurred between the experiments were unexpected because DW appropriately ranked the acidity tolerances of *Vulpia* (Dowling, 1996), *Dactylis* and *Phalaris* (Helyar and Conyers, 1994) in experiment 1 and DW is considered the most reliable index of acidity tolerance (Helyar and Conyers, 1994; Islam *et al.*, 2006). Nevertheless the rankings for some accessions may have been influenced by the climatic differences between the experiments i.e., moisture stress and high temperatures (Robinson and Archer, 1988), especially during the establishment of experiment 2 (Table 3). The experiments also differed in the severity of the acidity (Al) challenge at  $\text{pH}_{\text{Ca}}$  values of 4.4 and 4.3 and there were systematic differences in other soil properties such as  $\text{Mn}_{\text{Ca}}$  and available P (Table 2).

It has been reported that *Austroanthonia* spp. and accessions may differ widely in their tolerance of soil acidity (Dowling *et al.*, 1996; Garden *et al.*, 2001a; Islam *et al.*, 2006) and large differences were evident in our data (Table 4-7 and Fig. 2). For example, Dowling *et al.* (1996) suggested that accessions from species of *Austroanthonia* namely, *A. pilosa*, *A. eriantha*, *A. duttoniana*, *A. setacea* and *A. monticola* were more likely to be found on acidic soils. *A. carphoides* appeared to be pH independent, *A. auriculata* was uncommon and *A. racemosa* preferred higher pH soils. Further, Garden *et al.* (2001a) found all the above species on an acidic soil ( $\text{pH}_{\text{Ca}}$  4.1-4.3) on the southern Tablelands of NSW, of which, *A. racemosa*, *A. carphoides* and *A. auriculata* were the most common species.

Among the 15 *Austroanthonia* spp. represented in this study, eight exhibited the greatest acid tolerance based on DW, two (e.g., *A. tenuior* and *A. laevis*) being the least acid tolerance while the remaining species fell between the two preceding groups (Table 5) indicating a range of inter-specific differences. However, there were insufficient accessions from species other than *A. racemosa*, *A. pilosa*, *A. eriantha*, *A. duttoniana* and *A. penicillata* (Table 1) to be confident that the inter-specific differences observed are representative of the diversity in the populations. Large intra-specific differences were also clearly apparent (Table 5-6 and Fig. 2). The two commercially selected cultivars of *Austroanthonia* (Taranna, ID 32 and Bunderra, ID 33) fell into the least tolerant group in experiment 1 and the most tolerant group in experiment 2 (Table 7). Possible reasons for this difference have been presented earlier. Given that these cultivars originated from material collected on the slopes of NSW (Lodge, 1993a and b) where the soils are variably mildly acidic, one might have expected that they would not be as acid tolerant as accessions from more acidic sites. The wide range of inter- and intra-specific tolerance within the *Austroanthonia* genus is consistent with findings for other genera (Edmeades *et al.*, 1991; Helyar and Conyers, 1994) and the natural distribution of *Austroanthonia* in the field (Dowling *et al.*, 1996; Garden *et al.*, 2001a).

#### **Genotypic Differences in DW at $\text{pH}_{\text{Ca}}$ 5.3 and 4.9**

The argument has already been made that at  $\text{pH}_{\text{Ca}}$  values of 5.3 (experiment 1) and 4.9 (experiment 2), the acidity (Al) challenge was negligible in both experiments. Consequently we postulate that the differences between genotypes in DW at the higher  $\text{pH}_{\text{Ca}}$  values within an experiment are an expression of differences in growth potential mediated by whatever constraints on growth pertained during that experiment, such as moisture stress and high temperatures.

### **Accessions With Superior Growth Potential And Acid Tolerance**

The genetic potential to produce DW was not necessarily linked to acid tolerance (Fig. 2); however, in the field, accessions 11 and 6 scored highly for both attributes: by comparison, the commercial releases of *Austrodanthonia*, cvs Taranna (ID 32) and Bunderra (ID 33), scored highly for at most, one of these attributes.

Thus there is a wide range of inter- and intra-specific yield potential and acid tolerance in the *Austrodanthonia* genus, consistent with earlier reports (Dowling, 1996; Helyar and Conyers, 1994; Islam *et al.*, 2006), which is in agreement with the suggestion that *Austrodanthonia* populations have a broad genetic base, that would enable them to adapt to a wide range of environments (Scott and Whalley, 1984; Islam *et al.*, 2006). These perennial grasses may compete with less desirable species such as annual grasses in diverse plant communities on low-fertility sites (Michalk *et al.*, 2003). Therefore *Austrodanthonia* accessions might be used to study the genetics of Al tolerance and as the genetic source for improved grasses for use as pastures on areas with acidic soils.

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