Australian Grasslands Association
A partnership between the Grassland Society of Southern Australia and the Grassland Society of NSW

Australian Legume Symposium

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February 8 and 9, 2012
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Australian Legume Symposium

Proceedings of an Australian Grasslands Association Symposium

Melbourne Australia

February 8 and 9, 2012

Editor
Carol Harris

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The Grassland Society of Southern Australia Inc.
The Grassland Society of Southern Australia Inc. is the peak farmer organisation in southern Australia dedicated to the transfer of information and technology relating to temperate grasslands of clover and grass.

Formed in 1959, the Grassland Society of Southern Australia Inc. has branches in Victoria, South Australia, Tasmania and southern New South Wales. The Society welcomes new members (student, ordinary and corporate) with an interest in grassland farming. Pasture establishment, maintenance, utilisation, persistence and research are all key areas of interest for the 850 members of the Society, 50 per cent of whom are directly involved in livestock enterprises. The Society has a travel grant program, awards two student bursaries annually, runs an annual bus tour, publishes a bimonthly newsletter and holds and annual conference. Branches hold seminars and field days throughout the year.

The Grassland Society of New South Wales Inc.
The Grassland Society of NSW is the premier non-government organisation for transfer of information and technology relevant to pasture, grazing and land management in NSW. The Grassland Society of NSW was formed in March 1985 at a meeting of 28 interested people. The Society has 500 members and associates, 75 per cent of whom are farmers and graziers. The balance are agricultural scientists, advisers, consultants and executives or representatives of organizations concerned with fertilizers, seeds, chemicals and machinery.

The aims of the Society are to advance the investigation of problems affecting grassland husbandry and to encourage the adoption into practice of results of research and practical experience. The Grassland Society of NSW holds an annual conference to promote grassland farming and research publishes a quarterly newsletter and runs a number of field days, seminars and other activities through the regional branch network.

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Screening potential new perennial pasture legumes for tolerance to aluminium and manganese toxicities


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Abstract
There are currently few perennial legumes widely adapted to the high rainfall regions (600-800 mm) of southern Australia. There are several perennial legume species that have undergone development in recent years, which may be commercialised in Australia for use in these regions, including *Trifolium tumens*, *Lotus corniculatus*, *T. ambiguum*, *Bituminaria bituminosa* and *Cullen australasicum*. This study compared seedling tolerance of 23 perennial legume genotypes, as well as *Cichorium intybus*, to aluminium (Al) and manganese (Mn) stress, both of which commonly occur in acidic soils. Tolerance was assessed by observing seedling root and shoot growth after 23 days in hydroponic solution at 5 concentrations of Al and Mn, respectively. Results revealed that *Lotus pedunculatus* was the most tolerant to Al and *B. bituminosa* was most tolerant to Mn toxicities respectively. *C. australasicum* was the least tolerant species to toxicities of Al, and *Medicago sativa* was the least tolerant species to toxicities of Mn. Mn rankings for legumes from most tolerant to least based on the reduction of root length relative to control within each genotype is as follows; *L. pedunculatus*, *T. repens*, *T. tumens*, *L. corniculatus*, *B. bituminosa*, *T. ambiguum*, *T. pratense*, *M. sativa*, *T. fragiferium* and *C. australasicum*. Mn rankings using reduction in shoot weight relative to control are in decreasing order; *B. bituminosa* ssp. *abomarginata*, *L. corniculatus*, *T. ambiguum*, *C. australasicum*, *T. pratense*, *T. tumens*, *T. fragiferium*, *L. pedunculatus*, *T. repens* and *M. sativa*.

Keywords: Alfalfa, acid tolerance, lime

Introduction
Soil acidification within Australia is a significant problem with ~50% of agricultural soils having pHCa values ≤ 5.5 (de Caritat et al. 2011). The extent of the problem is such that it is estimated to affect an area 8-9 times that of dryland salinity (Australian Natural Resources Atlas, 2001). The largest affected areas occur in New South Wales (~37 M ha), Western Australia (~21 M ha), Victoria (~14 M ha) and Queensland (~11 m ha). Soils with pHCa values ≤ 4.8 are of the greatest concern within these areas (~ 12 to 24 M ha total) due to the adverse affects of aluminium (Al) and manganese (Mn) toxicities and deficiencies of calcium, magnesium, phosphorus and some trace elements associated with low pH (Australian Natural Resources Atlas, 2001). The use of species tolerant of soil acidity in conjunction with lime application has been shown to be the most effective method of dealing with soil acidity (Scott & Fisher 1989).

There are currently few perennial legumes broadly adapted to the 600-800 mm rainfall zone of south-eastern Australia - most of which is on acid soils. Lucerne (*Medicago sativa*), is limited in this zone by soil acidity and winter waterlogging (Dear & Ewing, 2008). Periodic drought in this region limits the use of white clover (*Trifolium repens*) and subsequently often acts as an annual. In recent years there has been a significant push to develop new pasture species that fill this gap in the Australian landscape. New cultivars of *Lotus corniculatus*, *T. tumens*, and *T. ambiguum* are likely to reach the Australian market within the next 1-3 years. *Bituminaria bituminosa* and *Cullen australasicum* are species previously identified as being of interest but are less developed and less certain to be commercialised in the immediate future. The objective of this study was to assess the relative tolerance to Al or Mn toxicities for a range of newly developed perennial legume species, cultivars and accessions.

Methods and Materials
Description of germplasm
Seedlings of 23 perennial legume genotypes and one *Cichorium intybus* cultivar, Puna, were assessed in this study (Table 1). Cultivars were sourced from commercial suppliers and cultivars currently being commercialised of *T. tumens* and *T. ambiguum* were supplied by Tas Global Seeds (Launceston, TAS). Accessions of *B. bituminosa* and *L. corniculatus* were provided by Dr D. Real (Dept. Agriculture and Food, Western Australia) and one of the authors (G. A. Sandral); accessions of *C. australasicum* were sourced from the South Australian Genetic Resource Conservatorium (S. J. Hughes).

Experiment 1 – aluminium screening
The Al screening experiment was a split plot design with three replicates. Main plots were each a 45 L container of nutrient solution (see below) containing one of 5 concentrations of Al: 0, 25, 50, 75 and 100 µM. Within each container (main plot), a grouping of 10 seedlings of each genotype was grown in individual modified eppendorf tubes, representing the ‘subplot’ in this experimental design. Replicates were separated in time due to space constraints with approx 5 days between the harvest of one replicate and the start of the next. Relative rankings of Al tolerance (Table 1) were determined by the percentage decline in root length of the highest concentration of Al relative to the control for each of the genotypes.

Experiment 2 – manganese screening
The Mn screening was conducted as above for the Al screening using a re-randomised split plot design. There were 5 concentrations of Mn, 0, 0.25, 0.5, 0.75 and 1 mM each in a 45 L container. Relative rankings of Mn tolerance (Table 1) were determined by the...
percentage decline in shoot growth of the highest Mn concentration relative to the control for each of the genotypes.

Nutrient Solution
All experiments were conducted in a temperature controlled laboratory set at 20°C for two weeks to help control algal growth, then at 23°C for the final week to increase growth of the seedlings for harvest. Light was artificially provided above the plants at an average photosynthetic photon flux density of 340 ± 70 µmol s⁻¹ m⁻² on a 16/8 hr day/night cycle. Plants were held in individual tubes placed in screens suspended above 45 litre containers containing 44 litres of aerated basal nutrient solution. The concentration of nutrients in the basal nutrient solution was (µM): 500 Ca; 2000 N (300 NH₄, 1700 NO₃); 500 K; 201.1 SO₄; 200 Mg; 50 PO₄; 23 B; 10 Fe; 9 Mn; 0.8 Zn; 0.3 Cu; and 0.1 Mo. Deionised water was added to the containers when necessary to account for evaporation and transpiration losses. The pH of the solutions were maintained at pH 4.5 in CaCl₂ over the course of the experiment and adjusted as necessary using 1 M HCl. The respective Al and Mn treatments were added to the nutrient solutions at the beginning of the experiment as Al₂(SO₄)₃ or MnCl₂ solutions. The nutrient solution and treatments were completely replaced after 16 days.

Experimentation
Seeds were scarified and had a staggered planting within their tubes in the nutrient solution culture according to their speed of germination. Slower germinating species (T. tumens, T. fragiferum, T. pratense, T. repens, T. ambiguum, L. corniculatus, L. pedunculatus) were planted on day 1 of the experiment, medium germinating species (M. sativa, C. intybus) were planted on day 3 and fast germinators (B. bituminosa, C. australasicum) were planted on day 5. At day 23 all seedlings were harvested with seedlings being removed from the eppendorf tubes, individual root lengths measured, then all plants separated into roots and shoots. Individual roots and shoots from each subplot were bulked, dried at 70°C for 24 hrs and weighed.

Results
Experiment 1
There was a significant (P <0.001) difference in the percentage reduction in root growth at the highest Al concentration level (100 µM), relative to the control (0 µM Al) between species (Table 1). The two genotypes of C. australasicum were the most sensitive to Al toxicity. The 10 genotypes of B. bituminosa had differential tolerances to Al with the percentage reduction in root length ranging from 72.1 to 86.5 % ranking from the middle to the bottom cultivars. Three genotypes of L. corniculatus (LC07AUF, LC07AUYF and LC07AT) were more tolerant of Al than B. bituminosa. The genotype LC07AS was not as tolerant as the other genotypes of L. corniculatus and 7 genotypes of B. bituminosa. Using the most tolerant line of each genotype, the rankings of for Al stress in decreasing order were; L. pedunculatus, T. repens, T. tumens, C. intybus, L. corniculatus, B. bituminosa, T. ambiguum, T. pratense, M. sativa, T. fragiferum and C. australasicum.

Experiment 2
There was significant interaction (P <0.001) in the percentage reduction in shoot weight at the highest Mn concentration (1 mM), relative to the control (9 µM Mn) between species (Table 1). B. bituminosa was the most tolerant of Mn toxicity. Differential tolerances to high Mn was more evident within the genotypes of C. australasicum, and L. corniculatus than for Al tolerances. C. australasicum line SA4966 was more tolerant to high Mn than SA42965 (73.0 and 86.4 % respectively). L. corniculatus line LC07AS (50.5 %) was also more tolerant than the other 3 genotypes of that species (64.9, 77.9 and 78.5 %). Using the most tolerant genotypes the rankings of most to least tolerant for Mn stress were; B. bituminosa, L. corniculatus, T. ambiguum, T. pratense, C. australasicum, T. tumens, T. fragiferum, L. pedunculatus, T. repens, C. intybus and M. sativa.

Discussion
Aluminium tolerance in L. pedunculatus and T. repens is well documented, and these two ranked as the most tolerant in the Al screening. M. sativa was sensitive to Al and along with T. fragiferum was the most sensitive of the released cultivars. Relative to L. pedunculatus and M. sativa, C. intybus was tolerant, but T. pratense was sensitive to Al. Of the new cultivars, T. ambiguum and three genotypes of L. corniculatus were the most tolerant of Al while B. bituminosa and L. corniculatus (LC07AS) had an intermediate tolerance of Al. C. australasicum was sensitive to Al and ranked last of all genotypes. There would seem to be some potential for increasing Al tolerance of B. bituminosa through selective breeding due to large differences in root growth between individuals within, though some of this affect may be attributable to differences in vigour.

The sensitivity of M. sativa to acidity was shown in the Mn experiment where it ranked last in terms of tolerance to high Mn. The other released cultivars including L. pedunculatus, T. fragiferum, T. pratense, T. repens, and C. intybus had an intermediate or sensitive response to high Mn. B. bituminosa was very tolerant to high Mn relative to M. sativa. There was less variation within the accessions tested for B. bituminosa suggesting reduced opportunity to select for tolerance to Mn toxicity than Al toxicity. The soon to be released T. ambiguum, T. tumens and three L. corniculatus genotypes had an intermediate tolerance to high Mn. L. corniculatus (LC07AS) had a higher Mn tolerance ranking and was as tolerant as the B. bituminosa genotypes.

In many environments of the high rainfall zone the dual stresses of Al and Mn exist. These stresses can reduce seedling numbers of sown species (Hayes et al. 2012), reduce plant growth (mainly Mn) and restrict root exploration (mainly Al) effectively reducing available soil water. The combined impacts of these effects with defoliation from grazing livestock can result in the complete disappearance of sown sensitive genotypes. It is therefore important for broad scale adaptation to acid soils that genotypes have good tolerance to both Al and Mn stress. Of the new genotypes screened, B.
bituminosa and L. corniculatus were the most tolerant to toxicities of both Al and Mn. For tolerance to Al and Mn the most consistent accessions for B. bituminosa were 6, 10, and 27 while for L. corniculatus, LC07AT was the most consistent compared to M. sativa which was sensitive to both Al and Mn. T. tumens, C. intybus and T. repens had a high tolerance to Al but were sensitive to high Mn. M. sativa, C. australasicum and T. fragiferium were consistently sensitive to toxicities of Al and Mn and will require a wider search of the germplasm to determine if there are tolerant genotypes available.

References

Table 1: Rankings and percentage of reduction of root length due to Al at 100µM or reduction of shoot weight due to Mn toxicities at 1 mM, respectively, in nutrient solution culture relative to the its corresponding control for each cultivar/accession

<table>
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<tr>
<th>Species</th>
<th>Cultivar/accession</th>
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<th>Root length % reduction due to Al</th>
<th>Mn tolerance ranking</th>
<th>Shoot weight % reduction due to Mn</th>
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