The adaptation of some temperate agricultural, native and weed species to aluminium and manganese ions in acid soils

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Abstract

No study has defined how the comparative adaptation of agricultural and weed species to aluminium and manganese toxicity may influence plant competition in acid soils. Root growth data from glasshouse and laboratory investigations were used in a non-linear dose response model to define the relative tolerance of some common agricultural, native and weed species to toxic concentrations of aluminium and manganese ions. One ryegrass ecotype was highly tolerant to both toxicities, *Austrodanthonia linkii* was highly susceptible and several weed species were tolerant. In addition, a rapid bioassay procedure was developed and used to broaden the range of plant species/cultivars evaluated for plant tolerance to Ca^{2+}, H^+ and Al^{3+}. Another glasshouse experiment demonstrated that annual ryegrass (*Lolium rigidum*) ecotypes taken from soil types that were either acid (pH 3.7, 2.34 µg/mL Al^{3+}) or alkaline (pH 8.2, 0.01 µg/mL Al^{3+}) regulated the level of their competition with sensitive phalaris (cv. Sirolan, suppressed) or tolerant cocksfoot (cv. Porto, not suppressed). These results indicated the potential difficulty of establishing a sensitive plant type on acid soils if an adapted competitor also is present as a weed.

Keywords

pH, plant competition, replacement series.

Introduction

In south-eastern Australia, acid soils have been a focus for research since the 1980s (Scott et al. 2000) but the problem persists, particularly in non-arable soils under long-term pasture (Hackney et al. 2019a, Condon et al. 2020). Sub-optimal rates of fertilizer (Hackney et al. 2019a) and lime (Condon et al. 2020), pH stratification (Condon et al. 2020) and competition from adapted species (Hackney et al. 2019b) constrain pasture establishment and productivity. No study has defined the comparative adaptation of weed species in acid soils and their potential effect on the establishment of agricultural species. Glasshouse investigations were undertaken to evaluate the tolerance of representative weed and native species to acid soils, to screen ecotypes of weed and grass species to aluminium and manganese tolerance in acid soils, to define a dose response model for the response of the species to these constraints, to develop a simple bioassay for Al^{3+} toxicity using soil leachates, and to investigate the competitive performance of genotypes in the context of soil acidification.

Methods

Plant tolerance of aluminium and manganese toxicities. A factorial pot experiment with 10 species x 7 soil treatments was undertaken to assess the response to a range of soil pH, aluminium and manganese levels of several weed species collected as seed from field sites around Wagga, including silver grass (*Vulpia myuros*), annual ryegrass (*Lolium rigidum*), soft brome grass (*Bromus mollis*), wild oat (*Avena fatua*), Paterson's curse (*Echium plantagineum*) catsear (*Hypochoeris radicata*), wireweed (*Polygonum aviculare*) and sorrel (*Rumex acetosella*), plus two native wallaby grass species (*Austrodanthonia linkii* and *A. richardsonii*) sourced from NSW DPI at Tamworth. The soil treatments were created by adding either sulfuric acid (n = 1) or lime (n = 6) treatments to two contrasting acid soils, one a brown kandosol (Wrigley soil: pH_{Ca} 3.7) that had a high Al^{3+} content (2.34 µg/mL) but was low in reducible Mn (2.20 µg/mL), and the other a red chromosol (Dunn soil: pH_{Ca} 4.0) that was low in Al^{3+} (µg/mL 0.41) but high in Mn^{2+} (16.50 µg/mL). The addition of the acid treatment (0.01M H_2SO_4) and lime treatments (0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 t/ha) to soil extended the Wrigley soil treatments from pH_{Ca} 3.5, Al^{3+} 4.56 µg/mL and Mn^{2+} 2.80 µg/mL to pH_{Ca} 6.3, Al^{3+} 0.02 µg/mL and Mn^{2+} 0.10 µg/mL, and the Dunn soil treatments from pH_{Ca} 3.7, Al^{3+} 1.98 µg/mL and Mn^{2+} 45.20 µg/mL to pH_{Ca} 6.5, Al^{3+} 0.01 µg/mL and Mn^{2+} 0.30 µg/mL. Since the root is the part of the plant that is directly in contact with and acted upon by soil mineral toxicities, the root growth response data were primarily used in determining the relative tolerance of the above species to aluminium and manganese toxicities. In order to compare the results, the root growth response data were calculated as a percentage relative to the root growth at the highest lime treatment (8 t/ha) where soil Al^{3+} and Mn^{2+} concentrations were negligible and presumably non-toxic. Then, for each species, the means of the measured root data (% of control) at the various Al^{3+} and Mn^{2+} concentrations were the inputs in regression analyses using the following equation and the nonlinear regression program SHAZAM. The equation is: $W = W_0 + \left[\frac{S_m[X]^\alpha}{\beta+[X]^\alpha}\right] - \left[\frac{I_m[X]^\alpha}{\lambda+[X]^\alpha}\right]$ where W = weight of the plant roots (g/pot), W_0 = weight of plant roots at the control treatment, S_m = the maximum stimulation response at low Al^{3+} (or low Mn^{2+}), I_m = the maximum inhibition response at high Al^{3+} (or Mn^{2+}), X = aluminium (or manganese) concentration, α = the plant growth processes acted upon by Al^{3+} (or Mn^{2+}), β = a constant for the stimulation response of the plant and λ = a constant for the inhibition response of the plant. From these stimulation/inhibition curves, graphed as root dry weight (% of control) in response to Al^{3+} in the Wrigley soil or Mn^{2+} concentrations in the Dunn soil, were calculated the equivalent doses of aluminium (µM Al^{3+}, Wrigley soil) or manganese (µM Mn^{2+}, Dunn soil) to produce a 50% toxic response – $ED(Al)_{50}$ or $ED(Mn)_{50}$. These nonlinear regression analyses were applied to the treatment responses from the three replicates of the experiment to produce the ED_{50} results and ratings for each species, shown in Tables 1a and 1b. The shapes of these curves, which start at 100% of DM yield at the control before frequently rising above 100% at low doses of Al (or Mn) before declining at an increasing rate and then at a decreasing rate towards values of 0-50% at the highest concentrations of the toxic ions, are similar to the response of plants to allelochemicals in the work of An et al. (1997).

Root bioassays. There is a need for a simple bioassay, a direct measurement of the response of a plant species to a toxicity factor such as the aluminium ion. A soil leachate extraction method was developed and leachates from three replicates of six amendment treatments (acid treatment, nil, 0.75, 1.5, 3.0 and 6.0 t/ha of lime) were used in a micro-hydroponic cell system to measure, over 8 days, the root growth of seedlings sown and held in macro-cuvettes sited in 250 mL cups holding the leached solutions. After refinement, the micro-hydroponic system was routinely used to determine the tolerance of species to Ca^{2+}, H^+ and Al^{3+} ions in several experiments. Reported here are the responses to the Al^{3+} concentrations in the soil leachates for eight cultivars of subterranean clover (*T. subterraneum*), as well as for the above two ecotypes of annual ryegrass and the cultivars of cocksfoot (Porto) and phalaris (Sirolan) used in the competition experiment.

Plant competition experiment. The effects of differential tolerance to Al^{3+} on competitive relationships in binary perennial grass/annual ryegrass mixtures were evaluated over 36 weeks in a pot experiment. The grasses in the binary mixtures were either phalaris (cv. Sirolan, sensitive to Al^{3+}) or cocksfoot (cv. Porto, v tolerant) with either of two ryegrass ecotypes, one (A-ryegrass) collected from an acid soil site (Wrigley soil) where Al^{3+} was present at potentially toxic levels, and another (B-ryegrass) from a site with an alkaline soil (pH_{Ca} 8.2, a third ryegrass site) that contained no free aluminium (0.01 µg/mL). The plants were harvested at 12, 24 and 36 weeks, during which competition intensified – only the results from third harvest (growth from week 25 to week 36) are considered here.

Results

Plant tolerance of aluminium and manganese toxicities – pot trial. The Analyses of Variance performed on the top and root dry weight yields recorded for each species on each treatment on each soil (Wrigley, Dunn) revealed differences that were all highly significant ($P<0.01$) in terms of the main effects (rate treatments, species, soil), the two-factor interactions and the three-factor interaction. The ED50 values were used to classify each of the 10 species in terms of their tolerance of aluminium and manganese toxicities (see Tables 1a and 1b, respectively). The ryegrass ecotype (collected from the Wrigley site) was highly tolerant to both toxicities and *Austrodanthonia linkii* highly susceptible.

Root bioassays. The results from the root bioassays undertaken with soil leachates in micro-hydroponic cells showed a wide range of $ED\ (Al)_{50}$ values and response curve patterns of the subterranean clover cultivars and the ryegrass ecotypes to various levels of soil leachates. The subclover cultivars Dalkeith ($ED(Al)_{50}$ = 199.3 µM) and Clare (172.5 µM) were highly tolerant to aluminium toxicity, Trikkala was tolerant (121.6 µM) while Junee, Seaton Park, Karridale and Daliak were sensitive (70.9, 60.2, 56.0 and 50.4 µM respectively) and Woogenellup (39.8 µM) was least tolerant of Al toxicity. Differential levels of tolerance were also evident between the two ecotypes of annual ryegrass and the perennial grasses – the tolerant A-ryegrass from the acid Wrigley soil recorded an $ED(Al)_{50}$ value of 187.5 µM, the susceptible B-ryegrass from the alkaline soil was 20.7 µM, the tolerant Porto cocksfoot was 272.2 µM) and the sensitive Sirolan phalaris was 28.5 µM.

Table 1. The aluminium (a) and manganese (b) tolerance ratings of ecotypes of ten species, based on the $ED(Al)_{50}$ of the root yields of the species in the Wrigley soil and the $ED(Mn)_{50}$ of the root yields in the Dunn soil.

	Species	$ED(Al)_{50}$* µg/mL	S.E.	Al tolerance ratings^
a)	*Lolium rigidum*	3.68 a^{+}	±0.30	Highly tolerant (HT)
	Rumex acetosella	2.68 b	±0.29	Tolerant (T)
	Avena fatua	2.61 b	±0.09	Tolerant (T)
	Vulpia myuros	2.20 b	±0.05	Tolerant (T)
	Hypochoeris radicata	2.13 b	±0,19	Tolerant (T)
	A'danthonia richardsonii	2.04 bc	±0.17	Tolerant (T)
	Bromus mollis	1.53 cd	±0.18	Sensitive (S)
	Echium plantagineum	1.20 d	±0.13	Sensitive (S)
	Polygonum aviculare	1.05 d	±0.11	Sensitive (S)
	A'danthonia linkii	0.43 e	±0.16	Highly sensitive (HS)
b)	**Species**	**$ED(Mn)_{50}$*** µg/mL	S.E.	**Mn tolerance ratings^**
	Bromus mollis	256.2 a^{+}	±7.5	Highly tolerant (HT)
	Avena fatua	144.6 b	±4.3	Highly tolerant (HT)
	Lolium rigidum	131.1 b	±9.4	Highly tolerant (HT)
	Vulpia myuros	52.6 c	±9.0	Sensitive (S)
	Echium plantagineum	40.6 cd	±8.1	Sensitive (S)
	Hypochoeris radicata	33.4 cde	±3.7	Sensitive (S)
	Rumex acetosella	31.6 de	±6.7	Sensitive (S)
	A'danthonia richardsonii	29.2 de	±4.1	Sensitive (S)
	Polygonum aviculare	19.3 c	±5.1	Highly sensitive (HS)
	A'danthonia linkii	15.8 c	±4.7	Highly sensitive (HS)

***Equivalent dose of soil Al^{3+} or Mn^{2+} (µg/mL) to produce a 50% toxic response*

$^{+}$*Values with the same letter are not significantly different (P<0.05)*

$^{\wedge}$*Tolerance ratings are based on the ED_{50} ratings in µM of Al^{3+} or µg/mL Mn^{2+} <25=HS, 25-74=S, 75-125=T, >125=HT*

Plant competition experiment. In pots that contained alkaline soil (no free aluminium), the A and B ryegrasses suppressed phalaris or cocksfoot, but in acid soil the competitive outcomes were determined by the level of tolerance of each of the grasses to aluminium (Table 2). The A-ryegrass ecotype suppressed the sensitive phalaris but it was slightly dominated by the highly aluminium-tolerant cocksfoot cultivar. These results indicate that any difficulty in establishing a sensitive plant type on acid soils may be exacerbated by the presence of an adapted competitor.

Table 2. Relative changes in the yield of the components* of 50:50 binary mixtures^ (replacement series design) compared with the yield of each component in monoculture.

Soil treatment in pot	Binary mixtures	
Acid soil (pH 3.7, 2.3 µg/mL Al^{3+})	A-ryegrass/Phalaris +87% > -82%	B-ryegrass/Phalaris -50% < +39%
	A-ryegrass/Cocksfoot -37% < +40%	B-ryegrass/Cocksfoot -85% < +86%
Alkaline soil (pH 8.2, 0.01 µg/mL Al^{3+})	A-ryegrass/Phalaris +73% > -70%	B-ryegrass/Phalaris +69% > -65%
	A-ryegrass/Cocksfoot +68% > -77%	B-ryegrass/Cocksfoot +72% > -67%

*Regrowth was measured from the second to the third harvest, i.e., from 24 to 36 weeks after sowing.

$^{\wedge}$Companion grasses were:	cocksfoot (cv. Porto):	$ED(Al)_{50}$ value = 272 µM, highly tolerant
	phalaris (cv. Sirosa):	$ED(Al)_{50}$ value = 28 µM, sensitive
$^{\wedge}$Ryegrasses were:	A-ryegrass:	$ED(Al)_{50}$ value = 188 µM, highly tolerant
	B-ryegrass:	$ED(Al)_{50}$ value = 21 µM, highly susceptible

Discussion

There are two findings from this investigation that are important in terms of potential competition between plant types. The first finding was the demonstration that weed species, renowned for their ability to compete with crop and pasture species in the mixed farming systems of south-eastern Australia, differ in their adaptation to the aluminium and manganese toxicities that impede root development in acid soils. In particular, there were strong differences between the three ecotypes of annual ryegrass, one from the Wrigley soil type that was tolerant of the Al^{3+} ion, one from the Dunn soil that was tolerant to high concentrations of Mn^{2+}, and a third ecotype from an alkaline soil with little tolerance to either toxic ion. The free-seeding, cross-pollinating annual ryegrass is notorious for developing tolerance/resistance in croplands and pastures in response to a stressor such as the repeated applications of herbicides with the same mode of action. Presumably, most of the other weed species evaluated, which are notable for their tolerance of competition (C), stress (S) and/or disturbance (D, ruderal species) factors (Grime 1977, Wolfe and Dear 2001), also may demonstrate different levels of tolerance to these ions, depending on the level and duration of exposure of ecotypes to these soil constraints. In the plant tolerance pot experiment, the concentrations of aluminium and manganese in the tops of the weed species were assayed (results not shown). The dicot species accumulated more aluminium than did the grasses, indicating aluminium exclusion as a possible tolerance mechanism. However, except for ryegrass, there was no correlation between the Al^{3+} content of the soil and the Al^{3+} concentration in the tops of grasses, while sorrel in particular followed an exponential pattern of Al^{3+} accumulation in the tops. Sorrel may have a tolerance mechanism that detoxifies aluminium at the root (root exudates?) or inside the plant. Another tolerance mechanism indicated by the analyses of manganese in the tops was competition between Al^{3+} and Mn^{2+} for reactive sites on the plant roots – sorrel tolerated 871 µg/g of aluminium in the tissues on the Wrigley soil (Al) but had a greater reduction in yield on the Dunn soil (Mn) when it had only 259 µg/g of aluminium but 6,247 µg/g of manganese in the tissues.

The second important finding was the importance of adaptation between ecotypes of annual ryegrass to the aluminium ion toxicity in determining the outcome of plant competition when establishing pasture (and crop) species. The suppression of Sirolan phalaris by an adapted Al^{3+} annual ryegrass ecotype illustrates the potential difficulty of establishing pasture species in acid soil situations if the resident weed population is well adapted to the soil constraints of these situations. A primary solution to this problem is to ensure that the potential stressor, in this case aluminium toxicity, is removed by liming the soil to a topsoil pH_{Ca} (>5.2) so that Al^{3+} is diminished to non-toxic levels (Scott et al. 2000). A secondary approach is to use pasture species that are tolerant of the stressor. Phalaris (sensitive to aluminium toxicity) and subterranean clover (manganese toxicity) have been modified by selection for tolerance (Scott et al. 2000).

Conclusions.

From the results, it seems feasible to select for tolerance to aluminium toxicity in the species but liming the soil to eliminate Al^{3+} is the practical management option. Poor adaptation to soil acidity may be a factor determining the weak persistence of perennial species in pasture situations, such as Sirolan phalaris on acidic soils (Scott et al. 2000) or legumes in acidic, low-rainfall sites on sedimentary soils in the Monaro region, where many legumes and acid-sensitive species such as wallaby grasses have declined in frequency (Wolfe and Dear 2001), and 'improved' pastures have reverted back to the original native spear grass (*Austrostipa scabra*) (Hackney et al. 2019). However, caution is needed in extrapolating the results of a single representative of most of the species in this study to ecotypes of the same species in other localities or situations.

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