AMELIORATION OF Al, Mn and Fe TOXICITY IN RYGRASS AND CLOVER, and WHEAT and RICE, BY POLY-CARBOXYLIC ACIDS

Peter Bishop, P Jeyakumar and B F Quin

Advanced Agricultural Additives (NZ) Limited
PO Box 97 Tokomaru, Horowhenua.
email:bishopresearch@slingshot.co.nz

Abstract

The ability of the sodium salt of poly-carboxylic acid (AlpHa™) produced by Advanced Agricultural Additives (NZ) Ltd to alleviate the symptoms of aluminium (Al), manganese (Mn) and iron (Fe) phytotoxicity have been tested in pot and solution culture trials. In pot trials using acid soils (pH 4.2), AlpHa™ added with fine limestone achieved equivalent lime response of up to 2115 kg of lime flour per litre of AlpHa™ for ryegrass; less effect was found for white clover and wheat.

The mode of action of AlpHa™ was investigated in solution culture studies on ryegrass, rice and wheat with five levels of Mn and Fe, and six levels of Al. All metal levels were tested with three levels of AlpHa™ and replicated for five times.

The results showed that AlpHa™ reduced the phytotoxicity of (1) Al in annual ryegrass and rice, increasing both herbage and root growth, (2) Mn in annual ryegrass, wheat and rice with increases in herbage and root growth, and (3) Fe in annual ryegrass and wheat with increases in herbage and root growth. However no effect was seen in rice. This was probably due to the rapid drop in pH, which occurred in the paddy rice plant solutions as a result of oxidation of the Fe^{++} to Fe^{+++} and its precipitation.

The reduction in metal toxicity due to the addition of AlpHa™ means it can complement or replace lime as the current method of amelioration for metal phytotoxicity, particularly in situations where the cost of applying lime is a barrier to its use. AlpHa™ can easily be applied with fertiliser, with reduced amounts of lime, or separately.

In the New Zealand non-ploughed pastoral situation, our preliminary study indicated that even paddocks that have an adequate conventional soil test pH level, measured as the average over 15-20 soil cores from the top 7.5cm, are in fact a mosaic, vertically and spatially, of pH levels, and are likely to contain 10-25% of soil in the root-depth, principally old urine patches, that have soil pHs sufficiently low to be toxic to ryegrass in particular.

Introduction

In many countries of the world, agricultural production is limited by toxicity from metals such as aluminium, manganese and iron. The occurrence of metal toxicity in many cases can be overcome by the addition of lime (calcium carbonate), or slaked lime (calcium hydroxide) or other alkaline/liming materials to increase the soil pH and precipitate the toxic metal and thus increase plant growth. This phenomenon is commonly termed the “lime response”. However this may require the addition of several thousand kg of lime per hectare to elevate the effects of a few kg per hectare of soluble phytotoxic metals. An alternative to this is the addition of the patented neutralized poly-carboxylic acid AlpHa™ manufactured by Advanced Agricultural Additives (NZ) limited in New Zealand.

Therefore, the aim of this study is to test the effectiveness of AlpHa™ in comparison to lime applications in pot trials with perennial ryegrass, white clover and wheat, when grown in a very acidic soil (pH 4.2 to 4.5). In addition, solution culture studies have been conducted to determine the effect of AlpHa™ on the phytotoxic effects of Al, Fe and Mn on ryegrass, wheat and rice.

Plant response model to lime and comparison with AlpHa™

The plant dry matter yield response to lime was modelled using a Mitscherlich type equation (eq.1).

$$Y = Y_{max} - e^{(cLF - (Y_{max} - Y_0))} \quad \text{equation 1}$$

Where c is the exponential yield response constant, LF is the lime flour additions (kg LF/ha), with the response limited between the yield without lime flour addition (Y_0) and the maximum trial yield (Y_{max}) based in maximum experimental yield. This allows the yield effect due to AlpHa™ to be expressed in terms of lime response.

Methodology

The pot trials to evaluate the dry matter and grain yield of annual ryegrass, white clover and wheat were carried out on Dannevirke silt loam with an initial pH of 5.3 which was lowered to 4.2 to induce Al toxicity by acidification with fine elemental sulphur (<75 µm). The sulphur was added at a rate of 0.75g of fine sulphur per kg of dry soil and incubated for 4 weeks at approx. 70% field capacity prior to potting and planting of the trial plants. During the incubation period the pH (water 1:2 extraction) dropped to 4.2 ± 0.05. Based on previous soil solution measurements the Dannevirke soil at pH 4.2-4.5 has total water soluble Al in the range of 4.5 to 9.0 mg/l making this soil strongly Al phytotoxic.

500g grams of the moist acid soil were transferred to 11 cm diameter poly pots with a capacity of 800 ml. The ryegrass seedling “Moata” were transplanted into the pots (4 per pot) 7 days after germination, while the white clover “Bounty” and Wheat from Morton Smith-Dawe Ltd. were sown at 10 and 6 seeds per pot, on the surface and at 2 cm below the surface, respectively. The treatments of lime flour at rates of 0, 100, 500 and 1000 kg/ha were then applied to the surface of the soil in four replicates for each spices and rate, while the AlpHa™ was applied at a rate of 2 l/ha as a dilute solution (2.85 ml of a 1:1000 solution of AlpHa™ to water per pot) in four replicates to non-limed pots of the four species. A base nutrient solution (50 ml) was applied to give the following elemental inputs as kg/ha N200: P5: K30: Mg10: Zn5: Cu5: B0.5. The treated plants were then randomized and placed in a greenhouse on the 15/7/2012 and watered as required to maintain constant weight. After one week the clover and wheat seedlings were thinned to four plants per pot. The ryegrass was clipped to 3 cm and dry matter determined for each pot at 21, 41, 63, and 85 days. For the white clover and wheat, yields were determined at the end of the trial period. Statistical analysis was carried out on the results using a Student T-Test comparing the treatments with the nil-lime control.

Ryegrass pot trial results

The application of AlpHa™ and LF (100 to 1000 kg LF/ha) produced significant increases in herbage dry matter yields in the ryegrass compared to the 0 LF control (figure1, table1). This was observed following the second harvest when the pots were leached on a weekly basis to reduce excess salinity (up to 2 mS) produced by the acidification with elemental S which suppressed plant growth.

Table 1. Comparative dry matter yields from individual clips of annual ryegrass grown on acidic Dannevirke silt loam treated with lime flour (LF) and AlpHa™. T-Test P-values between control nil-lime and treatments.

	4/08/2012		24/08/2012		15/09/2012		7/10/2012	
	Dry Matter (g)	P value	Dry Matter (g)	P value	Dry Matter (g)	P value	Dry Matter (g)	P value
0 kg LF/ha	0.037		0.153		0.182		0.283	
100 kg LF/ha	0.046	0.294	0.179	0.239	0.310	0.009	0.308	0.657
500 kg LF/ha	0.046	0.240	0.187	0.153	0.365	0.002	0.410	0.089
1000 kg LF/ha	0.041	0.557	0.195	0.128	0.312	0.009	0.389	0.130
AlpHa™ 2l/ha	0.045	0.301	0.187	0.160	0.313	0.008	0.414	0.082

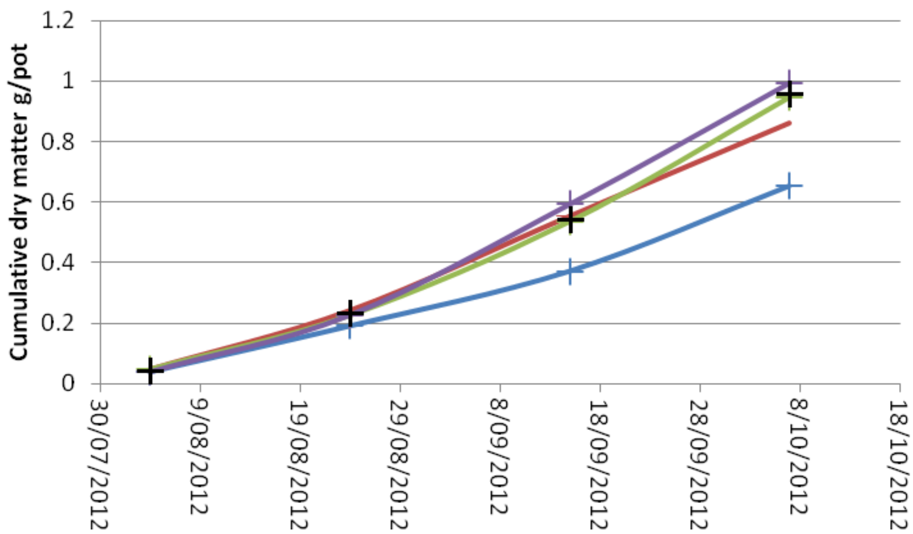

Figure 1. Cumulative dry matter response of annual ryegrass grown in acidic Dannevirke silt loam (initial pH 4.2) to additions of lime flour (100 kg/ha red line, 500kg/ha green line and 1000 kg/ha purple line) and AlpHa™ (black crosses) with a nil-lime control (blue line).

The response to both lime flour and AlpHa™ were similar for all lime application rates above 100 kg/ha making it difficult to obtain a good correlation between lime addition rate and plant growth particularly in the final harvest. The herbage response to LF was then modelled using eq.1 by plotting *Ln* $(Y_{max}-Y)$ against LF to obtain response constant c.

Equation 1 was then rearranged to give LF as a function of dry matter yield and thus allow the conversion of AlpHa™ dry matters to be converted into equivalent LF application rate (Table 2).

Table 2. Lime response equivalence for AlpHa™ based on individual response coefficient (c) values and averaged c values for annual ryegrass grown in acidic Dannevirke silt loam (initial pH 4.2).

	24/08/2012 Kg LF/l	15/09/2012 Kg LF/l	7/10/2012 Kg LF/l
AlpHa™ individual c values	269	542	2116
AlpHa™ average c value	223	306	1012

The lime response equivalence of AlpHa™ increased from 223 at harvest 2 to 2116 at harvest 4, apparently as the result of the liming effect reaching its maximum growth response between 100 to 500 kg LF/ha and the growth response to AlpHa™ being very close to the if not the maximum yield at the final harvest.

The cumulative response graph (Figure 2) evens out the response individual harvest results but still shows the major growth response of the annual ryegrass to the small addition of AlpHa™ under acidic soil conditions.

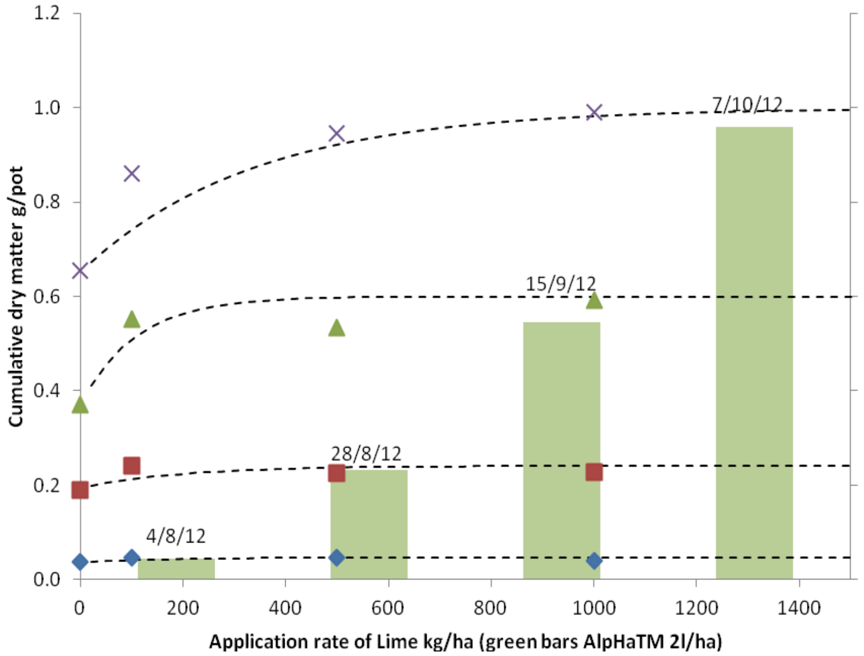

Figure 2. Cumulative growth response curves of annual ryegrass to the application of lime flour and AlpHa™ to acidic Dannevirke silt loam (initial pH 4.2). With the response to AlpHa™ (2 l/ha) being represented by green bars with harvests data points and Mitschlich equation fitted lines as per the dates indicated.

The results show that large savings in lime application may be achieved by the application of the polycarboxylic polymer (AlpHa^{TM}) to annual ryegrass grown on acidic allophanic soils.

Clover pot trial results

The application of AlpHa^{TM} and LF (100 to 1000 kg LF /ha) produced significant increases in herbage and root dry matter production in white clover grown on acidified Dannevirke silt loam (Figure 3).

The application of AlpHa^{TM} at 2 l/ha gave growth responses equivalent to 33 and 34 kg of lime flour per litre of polymer applied based on the Mitscherlich equations for the herbage and roots respectively.

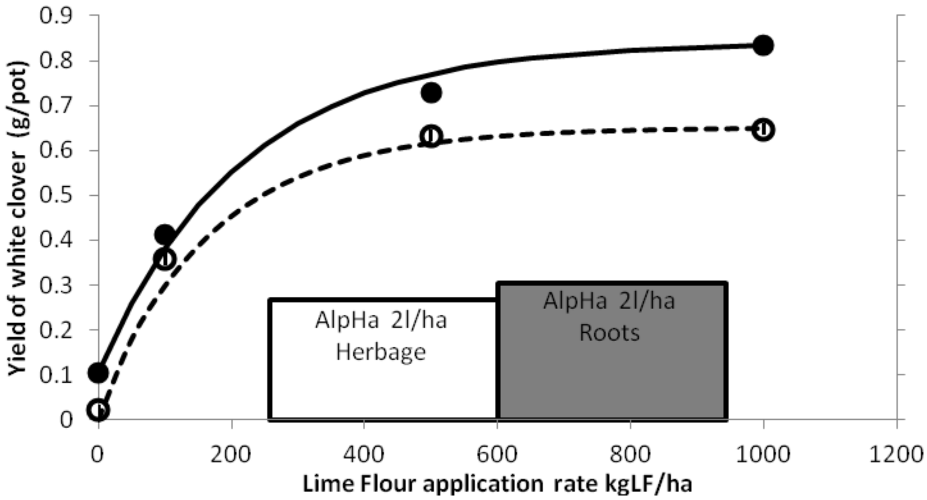

Figure 3. Dry mater yields for herbages (○) and roots (●) of white clover grown in acidic Dannevirke silt loam (initial pH 4.2) with added lime flour of 0, 100, 500 and 1000 kg/ha and AlpHa^{TM} at 2 l/ha as bars. Lines are Mitscherlich equations derived for herbage (dasher line) and roots (solid line).

Wheat pot trial results

The Wheat trial initially started with application rates of LF of 100, 500 and 1000 kg LF /ha however by the 6/8/2012 the observed poor plant development prompted an increase in LF levels to 1000, 1470 and 2660 kg LF /ha. This increase in application rate produced significant increases in both straw and grain yield at harvest on 1/1/2012 over the control. The observed higher sensitivity of the wheat to pH and metal toxicity render AlpHa^{TM} ineffectual with no increase in straw or grain yields over the control.

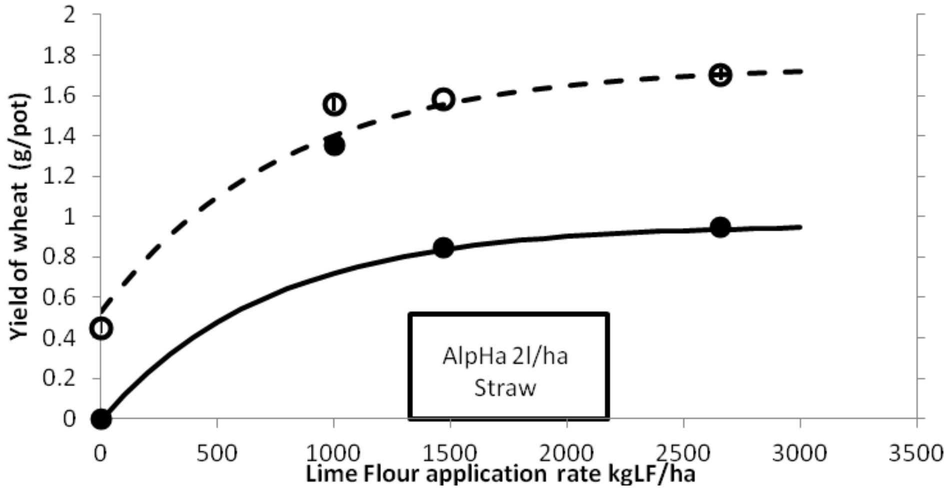

Figure 4. Yields for straw (○) and grain (●) of wheat grown in acidic Dannevirke silt loam (initial pH 4.2) with added lime flour of 0, 1000, 1470 and 2660 kg/ha and AlpHa™ at 2 l/ha as bar. Lines are Mitscherlich equations derived for straw (dashed line) and grain (solid line).

Discussion of pot trial results

The results of the pot trials have shown significant increase in annual ryegrass production by the application of AlpHa™ at 2 l/ha when grown under mild pH stress .The initial soil pH conditions produced a reduction in the cumulative yield of the annual ryegrass to 66% of the maximum yield obtained by the addition of lime flour at 1000 kg/ha, while AlpHa™ at 2 l/ha produced 97% of the maximum yield. The white clover and wheat showed high sensitivity to the low initial soil pH resulted in reductions in yields to 3% and 12% of the maximum herbage and root yields, respectively for white clover; and 26% of straw and 0% of grain maximum yields for wheat. Under these high stress conditions AlpHa™ at 2 l/ha produced little relief of the phytotoxic effects of the low soil pH compared to the addition of LF greater than 1000 kg/ha.

The results indicate that AlpHa™ is more suited for application when only mild phytotoxic stress is present as found in the annual ryegrass. This was further tested in solution culture trials to determine the effect of AlpHa™ and addition levels of 0.2, 2 and 20 µl/l on the phytotoxic effects of Al, Mn and Fe in annual ryegrass, wheat and rice.

Solution culture studies

A solution culture study has been carried out to confirm the effect of AlpHa™ on the phyto-toxic effects of Al, Mn and Fe on annual ryegrass, wheat and paddy rice. In these studies the growth of the plants were measured after exposure for six weeks to a range of metal and AlpHa™ concentrations.

Methodology

A glasshouse trial was conducted using paddy rice, wheat and annual ryegrass plants in solution culture media containing various levels of Al, Mn, Fe and AlpHa™. The solution culture media was the mixture of 'Solution A' (KNO_3 – 530 mg/L, $(NH_4)_2SO_4$ – 100 mg/L, $MgSO_4$ – 370 mg/L, $CaSO_4$ – 1290 mg/L, Na_2SO_4 – 480 mg/L, Na_2SiO_3 – 60 mg/L and NH_4NO_3 – 400 mg/L), 'Solution B' (KH_2PO_3 – 200 mg/L, H_3PO_3 – 270 mg/L, $ZnSO_4$ – 220 mg/L, $MnCl_2$ – 70 mg/L, $CuSO_4$ – 40 mg/L, NaMoO – 4 mg/L and $CoSO_4$ – 20 mg/L) and iron solution ($FeSO_4.7H_2O$ – 8340 mg/L) at the rate of 100A:1B:1Iron solution, respectively per litre of purified water.

The paddy rice seedlings were initially raised in a 5 ml vials containing nutrient solution for the first week prior to the application of the metal and/or polymer treatments. Following the application of the treatments the paddy rice seedlings remained in the 5 ml vials with treatment solutions being changed every three days. By 14 days the rice plant growth required the plants to be transferred to 50 ml containers with weekly solution changes.

The wheat seedlings were prepared as for the rice however their remained in the 5 ml vials for the full period of the experiment to avoid full submersion of the wheat roots in the treatment solution and increased aeration.

The annual ryegrass seedling were initially raised in solution for the first 7 days and then transferred to perforated PVEA foam supports sheets allowing five plants to be grown floating with an air gap between the treatment solution surface in a 250 ml reservoir. The treatment solutions were changed on a weekly basis.

Total metal concentrations (mg/L solution) in the treatments were for Al 0, 0.1, 0.2, 0.4, 0.8 and 1.6 ; for Mn 0.5, 1, 10, 20 and 40; and for Fe 1, 10, 20, 40, and 80. Each metal treatment was again treated with AlpHa™ at the rate of 0.0, 0.2, 2.0 and 20 µl/ ml. Therefore each metal level contained four levels of AlpHa™ (one was control without AlpHa™). The treatments were replicated five times. The containers were arranged in a Randomized Complete Block Design (RCBD) in a glasshouse. The glasshouse temperature was maintained at 11 ± 5^0C minimum (night) and 30 ± 6^0C maximum (day). After 42 days of plants growth the experiment was concluded, and plant shoots and roots from each container were progressively collected. pH and EC of each treatments solutions were measured periodically:

The pH values of Al and Mn treatments for all three plants did not change significantly (4.5±0.3). However, the pH values of treatments with Fe for rice plant were reduced during the first 4 days of the experiment (Figure 5). A significant reduction of pH from 4.42±0.08 to 2.1±0.04 observed in between solution changes.

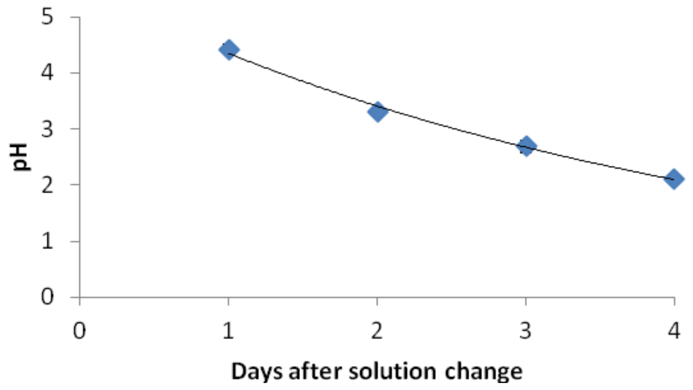

Figure 5. Change in pH of nutrient solution containing 10 to 80 mgFe/l and with rice seedlings present.

However, pH values for the treatment with Fe for wheat and rye grass were not significantly changed. The pH values were 4.4±0.4 and 4.5±0.2 for wheat and annual ryegrass, respectively. The significant reduction in solution pH associated with the rice plants in the iron containing solutions > 1 mg/l during the experimental period is most likely due to the oxygen exudations via the rice roots, which resulted in oxidation of the iron Fe^{++} to Fe^{+++}, which precipitated in a hydroxide/oxide form releasing H^+ ions and lowering the solution pH.

The analysis of the growth response curves of herbage was carried out using a common herbicide dose response equation (Brain and Cousens 1989) with the coefficients ‘a’ and ‘b’ being estimated using linear regression of the observed values of Y, Y_{max} and Y_{min} for the different concentrations of the phytotoxic metal (M) in the nutrient solution.

$$Y = \frac{Y_{max} - Y_{min}}{1 + a[M]^b} + Y_{min} \qquad \text{equation 2}$$

Results

(1) Annual ryegrass response to Aluminium, Manganese, Iron and AlpHa^{TM}

Annual ryegrass exhibited a range of phytotoxic responses to the addition of the different metals and was most sensitive to Al followed by Mn and then Fe (Figure 6). The addition of Al at any level > 0.1 mg/l resulted in significant reductions in herbage dry matter (DM) production from 71 mg to 31 mg/plant which remained relatively constant over the range of Al from 0.1 to 1.6 mgAl/l. The addition of AlpHa^{TM} at levels > 0.2 µl/l full compensated for
9
the added Al producing comparable results to the low level Mn (0.5 mg/l) and Fe (1 mg/l) herbage DM. More typical phytotoxic responses were observed for the Al root growth with increasing levels of AlpHa^{TM} increasing Y_{min} values.

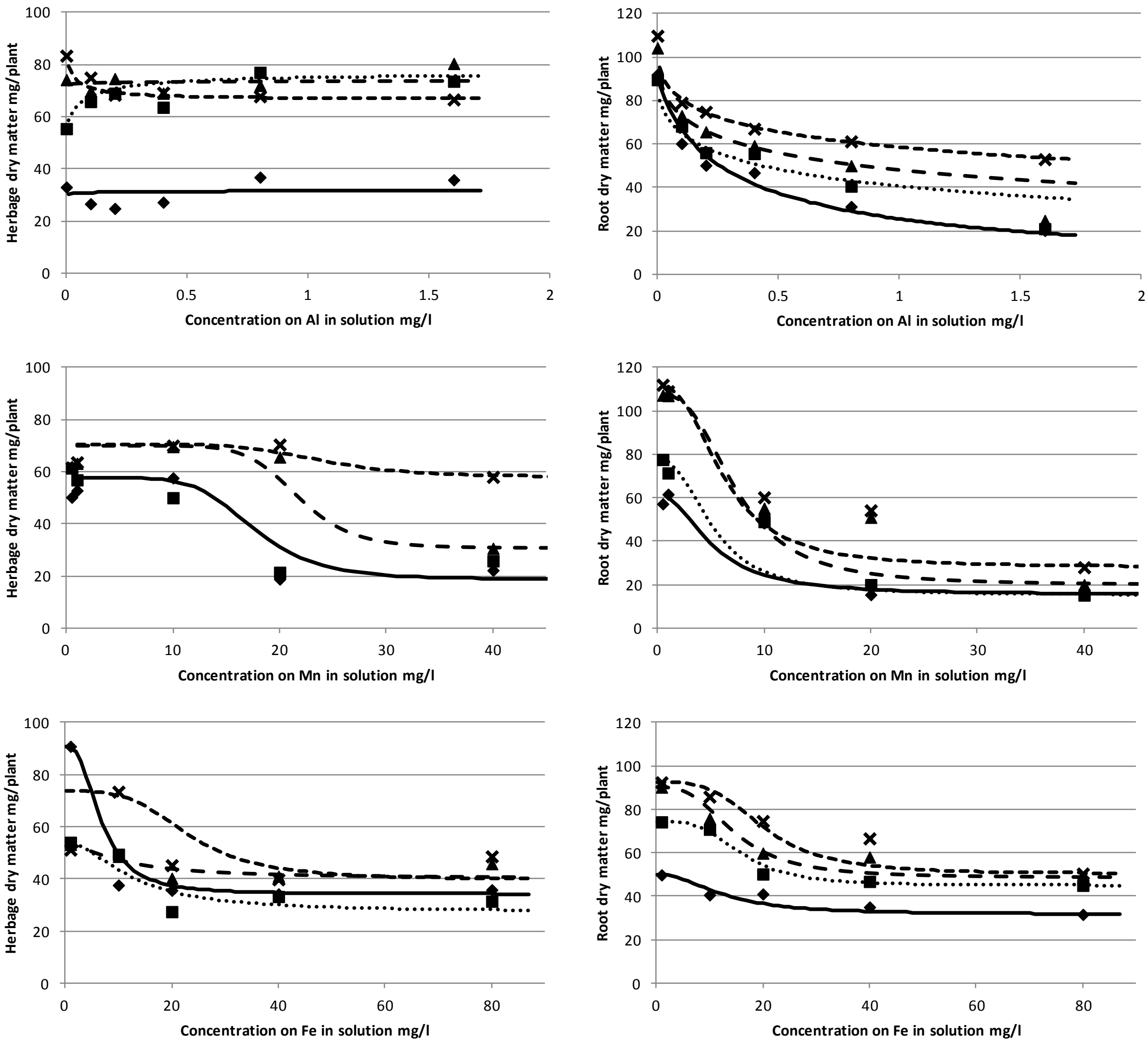

Figure 6. Growth response curves for herbage and roots of annual ryegrass grown in nutrient solutions containing additional Al, Mn and Fe with AlpHa^{TM} added at 0(♦), 0.2(■), 2(▲) and 20(×) ul/l. lines are lest squares fitted herbicide dose response (equation 2) for each metal and AlpHa^{TM} combination

In terms of Mn phytotoxicty of annual ryegrass a little effect on herbage production was observed up to 10 mgMn/l for the non-AlpHa^{TM} or 0.2 µl/l AlpHa^{TM} treatments; above this critical level herbage growth was strongly affected. The addition of AlpHa^{TM} at 2.0 µl/l increased this critical level to 20 mgMn/l while 20 µl/l of AlpHa^{TM} further increased the critical level beyond 80 mgMn/l. Mn at levels > 1 mgMn/l strongly affected the growth of roots in both non-AlpHa^{TM} and AlpHa^{TM} treatments, however the rate of the phytotoxic response was slowed by AlpHa at levels >0.2µl/l.

Iron > 1 mgFe/l produced a strong phytotoxic affect on herbage production of the annual ryegrass in all treatment except the 20 µl/l of AlpHa^{TM} which increased the critical level to 10 mgFe/l. The smaller phytotoxic effects of Fe on root growth of annual ryegrass were observed at levels above 1 mgFe/l for the non-AlpHa^{TM} treatment; however the addition of AlpHa at all levels appears to have increased the critical level to 10 mgFe/l, slowed the rate of effect, and increased the Y_{min} values.

(2) Response of wheat and rice growth to Al, Mn, Fe and AlpHa™

Wheat exhibited strong phytotoxic response to all three metals Al, Mn and Fe (Figure 7), with the addition of AlpHa™ > 2µl/l producing relief to wheat root growth affected by Mn up to 10 mgMn/l. Rice however, exhibited strong herbage phytotoxic responses to Fe only (Figure 8), but here AlpHa™ had no effect on herbage response. However, root growth of rice was reduced by all three metals; AlpHa™ reduced the severity of all three, and particularly Al.

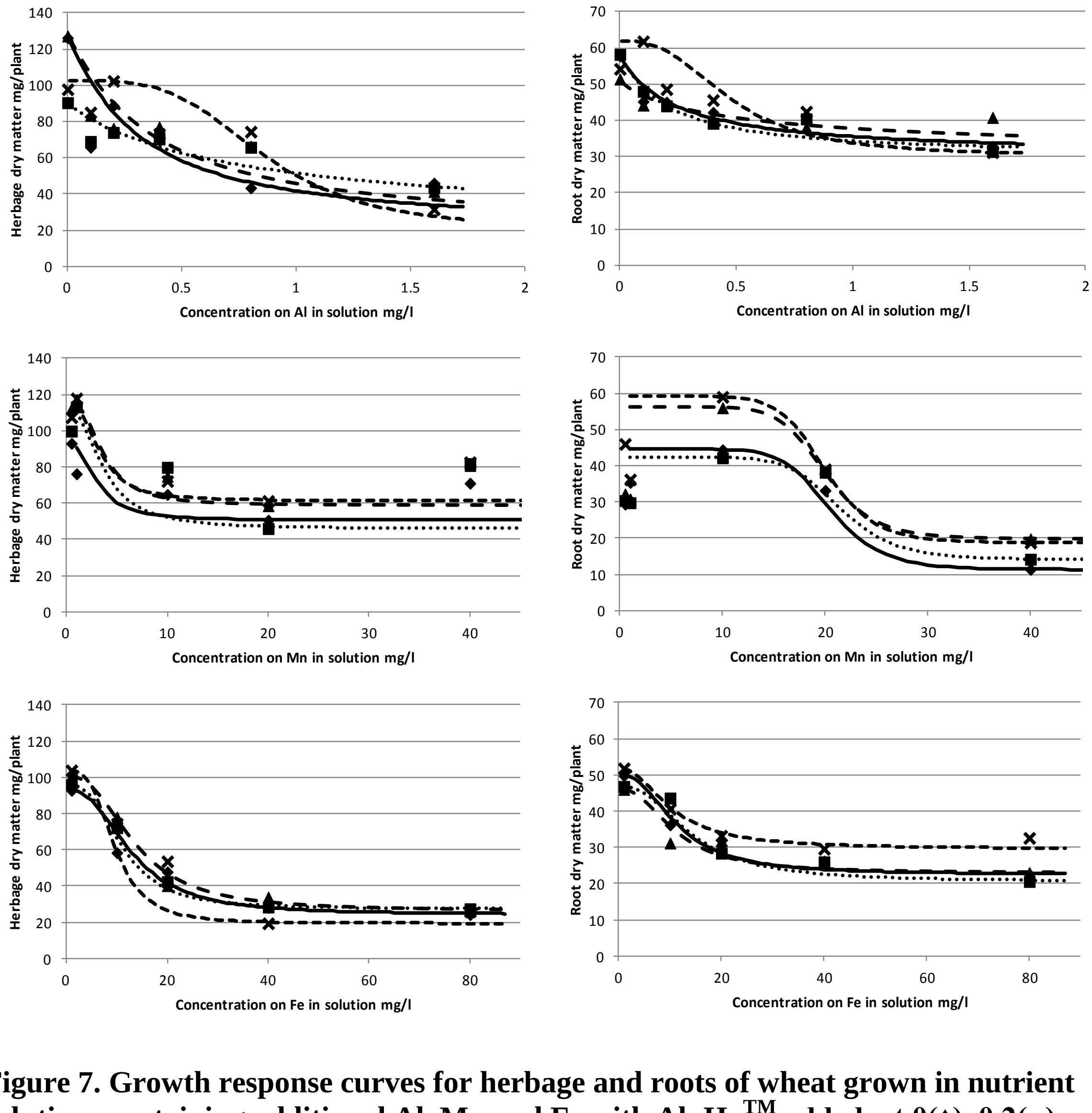

Figure 7. Growth response curves for herbage and roots of wheat grown in nutrient solutions containing additional Al, Mn and Fe with AlpHa™ added at 0(♦), 0.2(■), 2(▲) and 20(×) ul/l. lines are lest squares fitted herbicide dose response (equation 2) for each metal and AlpHa™.

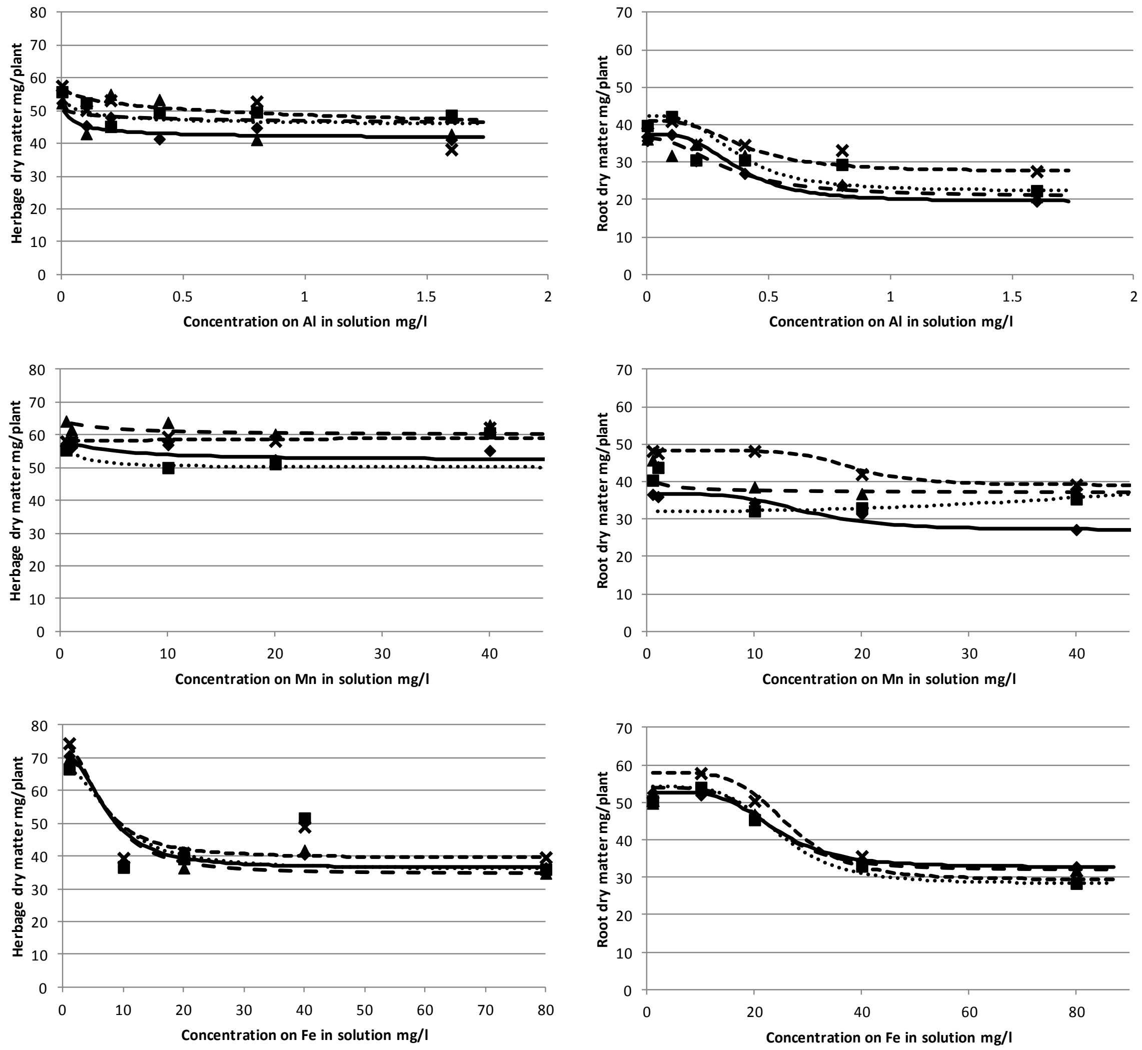

Figure 8. Growth response curves for herbage and roots of rice grown in nutrient solutions containing additional Al, Mn and Fe with AlpHa™ added at 0(♦), 0.2(■), 2(▲) and 20(×) ul/l. lines are lest squares fitted herbicide dose response (equation 2) for the metal and AlpHa™ levels.

The overall results of both pot trial and solution culture, and recommendations

The results showed that AlpHa™ reduced the phytotoxicity of (1) Al in annual ryegrass and rice, increasing both herbage and root growth, (2) Mn in annual ryegrass, wheat and paddy rice, with increases in herbage and root growth, and (3) Fe in annual ryegrass, with increases in both herbage and root growth. However, no effect was observed in rice, probably due to the rapid decline in pH, an effect which occurred only in the rice solutions, as a result of oxidation of ferrous to ferric iron and its resulting precipitation.

These reductions in metal toxicity in the presence of the poly-carboxylic acids in AlpHa™ explain its demonstrated ability to complement or replace lime as a method for the amelioration of metal toxicity in soil. It may well be more cost-effective in many situations, particularly where micro-variability in soil pH is high.

To investigate this aspect, 46 soil pH measurements were made on 12 cores at 5 cm intervals to depths of 15-20 cm along a 1.5 m line, across grazed pasture on a dairy farm, through both old and new urine patches using a Veris® direct soil pH probe. The results show a mean soil pH of 5.29 with a standard variation (SD) of 0.85. This compared to a mean of 5.49 with an SD of 0.76 as measured conventionally in water 1:2 in the laboratory. An average pH of 5.5 would not cause immediate concern to many farmers, but analysis of the individual cores showed fully 35% had a pH of less than 5.0, and 17% less than 4.5 (Figure 9). The graph (Figure 9) indicates the presence of pH populations; the lower population is likely to reflect the end effects of urine deposition.

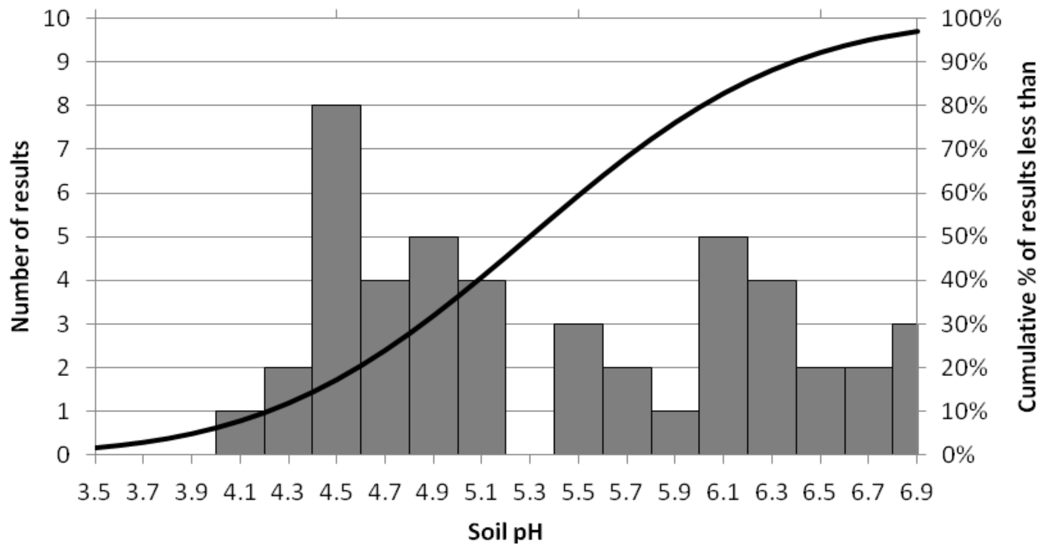

Figure 9. Distribution curve of soil pH along a 1.5m dairy pasture transect

These results suggest that there is likely to be considerable Al phyto-toxicity present in pastoral soils with pH levels in the 'optimum' range on a paddock basis, particularly where lime application is infrequent, as is generally the case on pastoral farms in New Zealand. The incorporation of AlpHa™ in fertiliser applications is likely to ameliorate these adverse effects. Funding is being sought for a three-year, split-paddock comparison of lime and AlpHa™ under grazed conditions.

References

Brain, P. and R. Cousens (1989). An equation to describe dose responses where there is stimulation of growth at low doses. Weed Research 29 (2): 93-96.