Botswana Journal of Agriculture & Applied Sciences

Leading Agriculture through Science and Innovation

BOJAAS

Lime and foliar application of molybdenum affects nodulation, nutrient uptake and pod production in soybean grown in acid soils

Adjetey, J.A.[1*] and Ntombela, Z.[2]

[1]Department of Crop Science and Production, Botswana University of Agriculture and Natural Resources, Gaborone, Botswana.
[2]Discipline of Crop Science, University of KwaZulu-Natal, Scottsville 3209, Pietermaritzburg, South Africa.
*Corresponding author email: jaadjetey56@gmail.com; Tel: +2673650129; Fax: +2673928753

JA conceived project, supervised work, wrote manuscript. ZN carried out experiment, collected data, wrote dissertation.

ABSTRACT
Soil acidity is commonly ameliorated with lime while little attention is given to the application of molybdenum which is deficient in acid soils. Molybdenum is an important component of the nitrogenase enzyme essential for the symbiotic nitrogen fixing bacteria. An experiment was conducted in a controlled environment at the University of KwaZulu-Natal to evaluate the effect of lime and molybdenum on nodulation, growth and shoot nutrient content of soybean. The experiment was a randomised complete block design with three rates of lime applied at 0, 2650 and 4280 kg ha^{-1} and molybdenum as ammonium molybdate at 0 and 0.5 g L^{-1}. Lime significantly ($p<0.01$) increased the uptake of Ca and P but decreased that of Mn. Also, increasing lime levels positively influenced most of the important plant growth parameters measured in the study. Molybdenum application significantly increased nodule formation but did not influence the concentration and uptake of the nutrients measured. Lime application also improved soil Ca and Mg but reduced Mn and exchangeable acidity levels at the end of the study. The treatment combination of lime at 4280 kg ha^{-1} and ammonium molybdate at 0.5 g L^{-1} gave the best result in nearly all parameters tested and it can hence be concluded that this combination improves both the nutrient uptake and nodulation of soybean grown in soil with very high acid saturation.

Keywords: Acidity, lime, molybdenum, nodulation, soybean

INTRODUCTION

Soybean *(Glycine max* (L) Merr.) is one of the most important legumes in the world because of its high protein and oil contents, and hence its suitability for both human consumption and animal feed. Currently, South Africa uses about 1.3 million tons of oilseed meal for animal feed production annually, of which approximately 70% is soybean meal. However, only about 100 000-800 000 tons of soybean meal is produced annually and a large percentage is imported (Esterhuizen, 2010; Grain SA, 2016). The crop is able to nodulate and fixes atmospheric nitrogen in a symbiotic relationship with nitrogen fixing bacteria (*Bradyrhizobium japonicum).* Major environmental constraints to nodulation are high temperatures, low nutrient levels and high soil acidity (Hassen et al., 2014) and many areas in South Africa are characterised by such acid soils, which are deficient in Ca, Mg, Mo and P (Beukes, 1997). The consequences of these deficiencies are reduced nitrogen fixation and yields. When lime is applied it neutralises the toxic effects of H^+, Al^{3+} and Mn^{2+} in soils (Staley and Brauer, 2006; Wijanarko and Taufiq, 2016) and decreases the activities of iron and aluminium oxides which are good sinks of molybdenum in the soil (Mandal and Mandal, 1998) and therefore should make Mo more available. However, Mo availability is not always achieved in South Africa (Thibaud, 2005; Miles, 2013). Molybdenum deficiency in such situations results in poor yield and low quality of crops. Legumes are reportedly more sensitive to Mo deficiency than other crops (McBride, 2005) because it is a component of bacterial nitrogenase and it is important to plants that have a symbiotic relationship with nitrogen fixing bacteria (Parker and Harris, 1977; Adams 1997; Zhou et al., 2017). Plants deficient in molybdenum usually exhibit poor growth and low chlorophyll content (Marschner, 1995; Togay et al., 2008). Molybdenum application in deficient soils has reportedly improved nitrogen fixation by increasing nitrogenase activity and nodulation (Adams, 1997; Zhou et al., 2017).

Studies on plant responses to lime and Mo in relation to yield, growth and N_2 metabolism in legumes grown in acid soils have shown positive responses in *P. vulgaris* (Bambara and Ndakidemi, 2010) and peanuts (Quaggio et al., 2004). There are indications that in some areas in South Africa responses of soybean to liming are not very satisfactory. Consequently, some farmers apply Mo to seeds by immersing them in Mo solution before planting, as insurance against its deficiency in limed acid soils. The purpose of this study was to examine the responses of soybean to lime and foliar application of molybdenum. Foliar application was adopted to assess whether farmers could use it as a remedial tool when molybdenum deficiency is detected in the early stages of the growth of the crop.

MATERIALS AND METHODS

Growing conditions

The study was conducted in a growth room at the controlled environment facility at the University of KwaZulu-Natal. The conditions in the growth room were set as day/night temperatures of 28/20^{o}C, relative humidity of 60%, and 14 h day length with light sources of fluorescence (731.3 Wm^{-2}) and incandescent (113.4) lamps that gave a light intensity of 285.15 µmol $m^{-2}s^{-1}$.

Experimental design and treatments

The experiment consisted of a factorial combination of three levels of lime at 0, 2650 and 4280 kg ha^{-1} corresponding to 0, 8 and 16 g of lime per 5 kg of potted soil, and two levels of ammonium molybdenum at 0 and 0.5 g L^{-1}, laid in a randomized complete block design with three replicates, each comprising two pots. The lime levels corresponded to ameliorating soil acidity to 79%, 40% and 20% acid saturation, and are hereafter referred to as Lno, L40 and L20, respectively. The molybdenum levels are hereafter referred to as Mno and M1, respectively.

Soil preparation, analysis and planting

The Inanada soil form (Rhodic form) was obtained from Hilton in Pietermaritzburg (29º37'S, 30º23'E) and had no history of soybean production. The soil is described as an Acrisol with a luvic B horizon (Soil Classification Working Group, 1991). The soil was analysed at the soil testing service at Cedara for soil acidity and nutrient content, and limed three weeks before planting according to the treatments. Ambic-2-extractable P, K, Mn and KCl-extractable Ca, Mg and acidity levels were determined as described by Farina (1981) and Manson and Roberts (2000). The soil analysis results reported a deficiency of Mg, P and K (Table 2) and these nutrients were added to the pots to correspond to 60 kg Mg ha^{-1}, 90 kg K ha^{-1} and 20 kg P ha^{-1} using $MgSO_4$ and K_2HPO_4. Four inoculated seeds of soybean cultivar PAN 1652 from maturity group VI were planted and thinned to two plants per pot after emergence. Plants were sprayed with Mo at the V1 stage and kept well watered and weed-free by hand throughout the experiment.

Harvesting and sampling

Sixty days after sowing, the shoots were cut at the surface of the soil and the leaves were counted and leaf area was measured using a leaf area meter. Thereafter, the shoots were dried at 70^{o}C for 48 h and dry weight was recorded. The samples were then analyzed for N, P, K, Ca, Mg, Mn, and Al using standard procedures (Manson and Roberts, 2000). Samples were milled to pass through a 0.84 mm sieve and subsamples were dry-ashed overnight and taken up in 1 M HCl. Thereafter, P, K, Ca Mg, Al and Mn were determined using ICP spectrometry. Nitrogen was determined separately using the Automated Dumas dry combustion method (Matejovic, 1996) with a LECO CNS 2000 (Leco Corporation, Michigan, USA). Nutrient uptake was calculated as a product of shoot dry matter and nutrient concentration. Nodule number and mass, and root mass were taken after carefully washing roots in water. At the podding stage, the number of pods per plant was counted. Soil samples were then analysed for nutrient and acid saturation levels.

Statistical analysis

Data sets were analysed using GenStat 14th edition (VSN international 2011). The analysis of variance was used to determine the significant difference between the treatments and their interactions. Mean separation was done using LSD at 5% significance level.

RESULTS

Effect of lime and molybdenum on nodulation and root growth of soybean

Application of ammonium molybdate at 0.5 g L^{-1} increased the number and mass of soybean nodules significantly ($p<0.05$) compared to the 0 g L^{-1} application (Figure 1a and 1d). Liming the soil to 20% acid saturation had a significant ($p<0.05$) positive effect on nodulation (Figure 1b) but the differences between 20% and 40% acid saturation were not significant. Molybdenum addition resulted in visible increases in nodule mass from 0.23g to 0.47g, and increasing lime levels to L20 increased nodule mass from 0.278 to 0.465g. The interactions between lime and molybdenum were significant ($p<0.05$) with respect to nodule mass and number (Figure 1c and 1f). The root mass of plants grown under high acidity was similar to those grown in soils with lime application (Figure 2).

Effect of lime and molybdenum on shoot growth of soybean

Shoot dry matter doubled ($p<0.001$) with lime application to 20% acid saturation compared to no lime application (Figure 3a). Also, application of Mo increased ($p<0.01$) shoot dry mass (Figure 3b). Increasing lime levels from Lno to L20 increased leaf area and leaf number significantly ($p<0.05$, Figure 3c and 3d) while Mo did not affect these parameters ($p>0.05$).

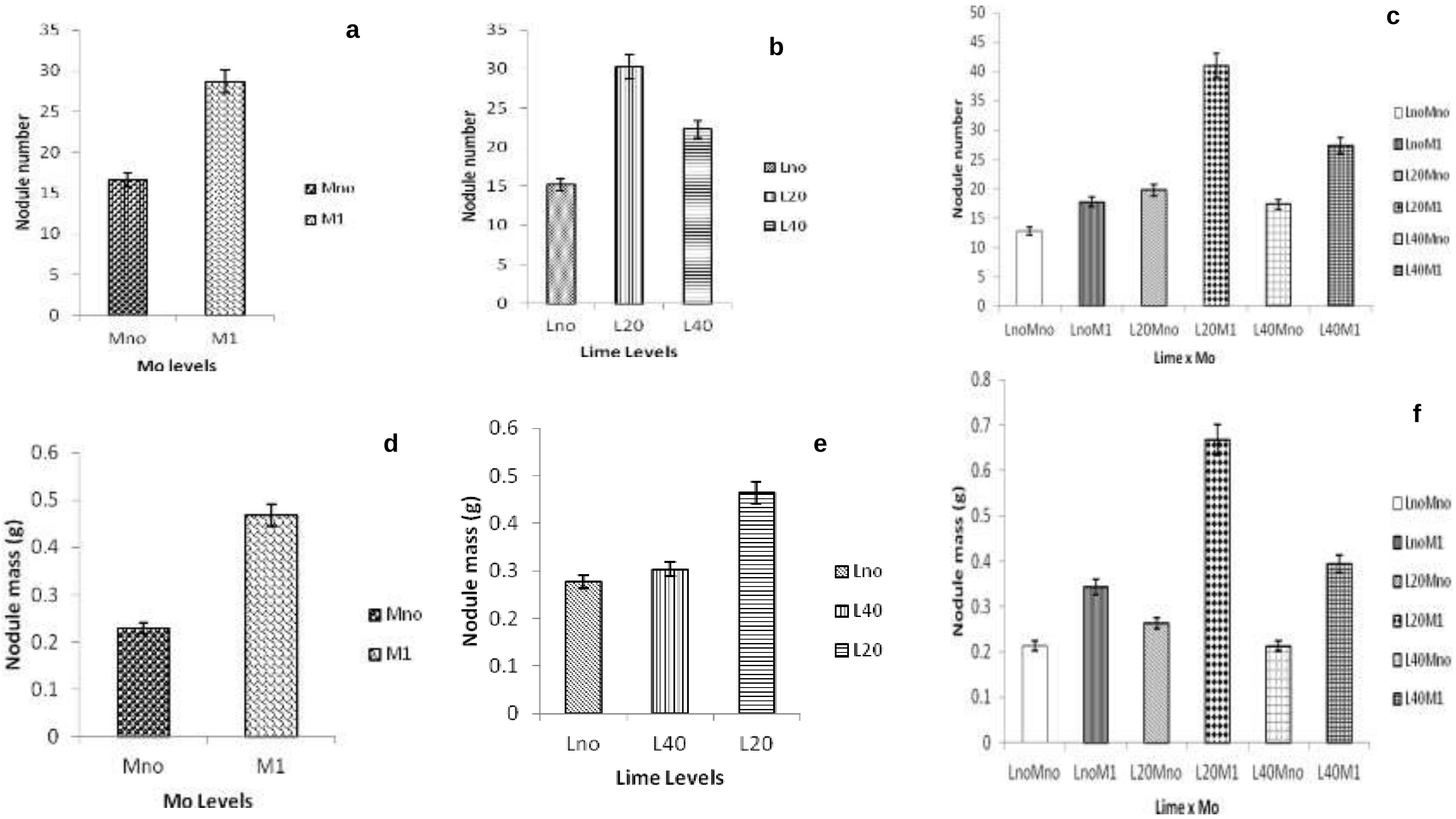

Figure 1. Response of nodulation to lime and molybdenum application (Lno = control, L40 = liming to 40% acid saturation, L20 = liming to 20% acid saturation)

Figure 2. Roots of plants that grew on soil unamended (Lno) and amended with lime at L40 and L20

Effect of lime and molybdenum on shoot nutrient levels

Lime significantly improved ($p<0.001$) both Ca concentration and uptake in soybean shoot with little difference between L20 and L40 (Table 1). Molybdenum addition did not influence Ca concentration in the shoot ($p>0.05$). Phosphorus concentration was increased significantly ($p<0.05$) by liming but not by Mo application. Shoot manganese concentration decreased significantly ($p<0.001$) with lime application. Neither lime nor Mo had a significant influence on shoot Al concentration ($p>0.05$)**.** Magnesium is generally increased by liming, however, in this study neither lime nor molybdenum had a positive effect on Mg concentration and uptake probably due to the $MgSO_4$ applied across treatments. There was only a small but non-significant increase in shoot nitrogen concentration and uptake with lime ($p>0.05$) while Mo had little effect.

The effect of lime and molybdenum on number of pods per plant

Both lime and molybdenum significantly ($p<0.05$) increased the pod yield (Figure 4a, b). Molybdenum application increased the pod number from 21 to 24 (Figure 4a) and more pods were observed when the soil was ameliorated to 20% acid saturation (Figure 4b). The combination L20M1 gave the best pod yield while LnoMno gave the poorest yield (Figure 4c).

Effect of lime on nutrient level in the soil at the end of the study

At the end of the study lime increased soil Ca, Mg, and K but decreased Mn and exchangeable acidity levels (Table 2).

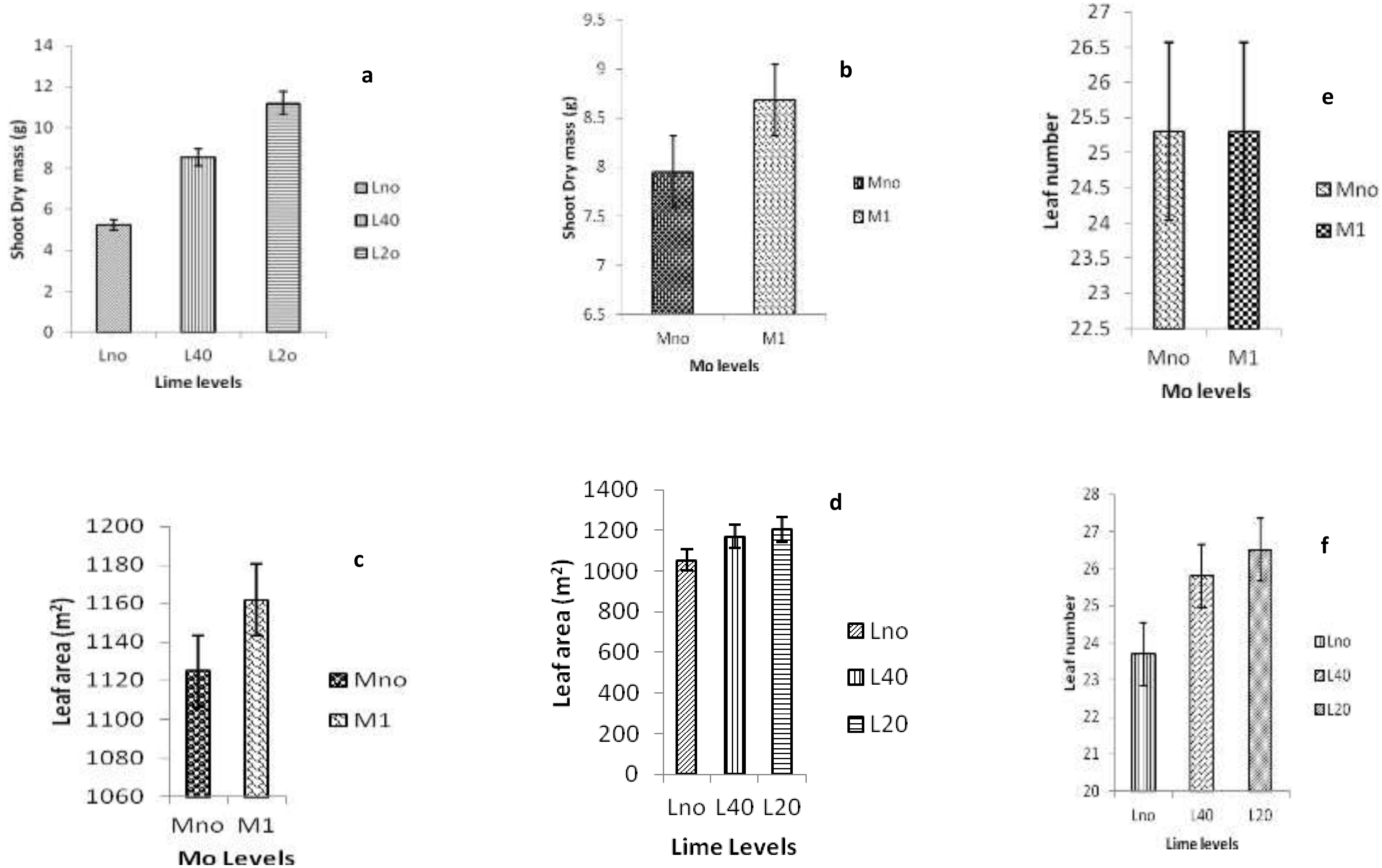

Figure 3. The effect of lime and molybdenum on shoot growth and dry matter, leaf area and leaf number of soybean

Table 1. The nutrient concentration in soybean shoots in response to varying lime and molybdenum treatments at flowering stage

	Nutrient concentration (%)						Nutrient content (g)					
Treatment	**Ca**	**Mn**	**P**	**Al**	**N**	**Mg**	**Ca**	**Mn**	**P**	**Al**	**N**	**Mg**
Lime												
Lno	0.595	0.0326	0.1367	0.0230	2.390	0.3067	0.0484	0.00262	0.01120	0.00174	0.1897	0.02513
L40	1.197	0.0145	0.1633	0.0208	2.473	0.2900	0.0979	0.00127	0.01361	0.00160	0.2074	0.02424
L20	1.222	0.0080	0.1700	0.0213	2.488	0.2733	0.1043	0.00070	0.01448	0.00179	0.2113	0.02327
Molybdenum												
Mo	1.033	0.0199	0.1667	0.0187	2.509	0.2978	0.0867	0.00161	0.01379	0.00150	0.2070	0.02476
M1	0.976	0.0169	0.1467	0.0248	2.392	0.2822	0.0803	0.00145	0.01241	0.00192	0.1986	0.02367
P value												
Lime	<0.001	<0.001	0.035	NS	NS	NS	<0.001	<0.001	0.008	NS	NS	NS
Mo	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
L x Mo	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV(%)	2.9	10.2	1.1	22.2	3.9	1.5	14.1	28.5	1.2	16.7	12.5	8.7
LSD	0.0911	0.00739	0.02540	–	–	–	0.02141	0.000792	0.002660	–	–	–

Table 2. Soil nutrient levels at the commencement of the study and its termination at podding, after application of different combinations of lime and molybdenum.

	Nutrient levels					
Treatment	**Ca (mg L^{-1})**	**Mg (mg L^{-1})**	**P (mg L^{-1})**	**K (mg L^{-1})**	**Mn (mg L^{-1})**	**Exchange Acidity (cmol L^{-1})**
At commencement of study						
(None)	98.5	20.5	17.0	39.0	8.5	2.88
At termination of study						
LnoMn0	257	40	23	32	6	2.43
LnoM1	275	45	24	43	6	1.93
L40Mn0	752	74	32	60	5	0.75
L40M1	712	68	18	36	4	0.77
L20Mn0	913	71	19	39	3	0.37
L20M1	870	84	34	48	4	0.60

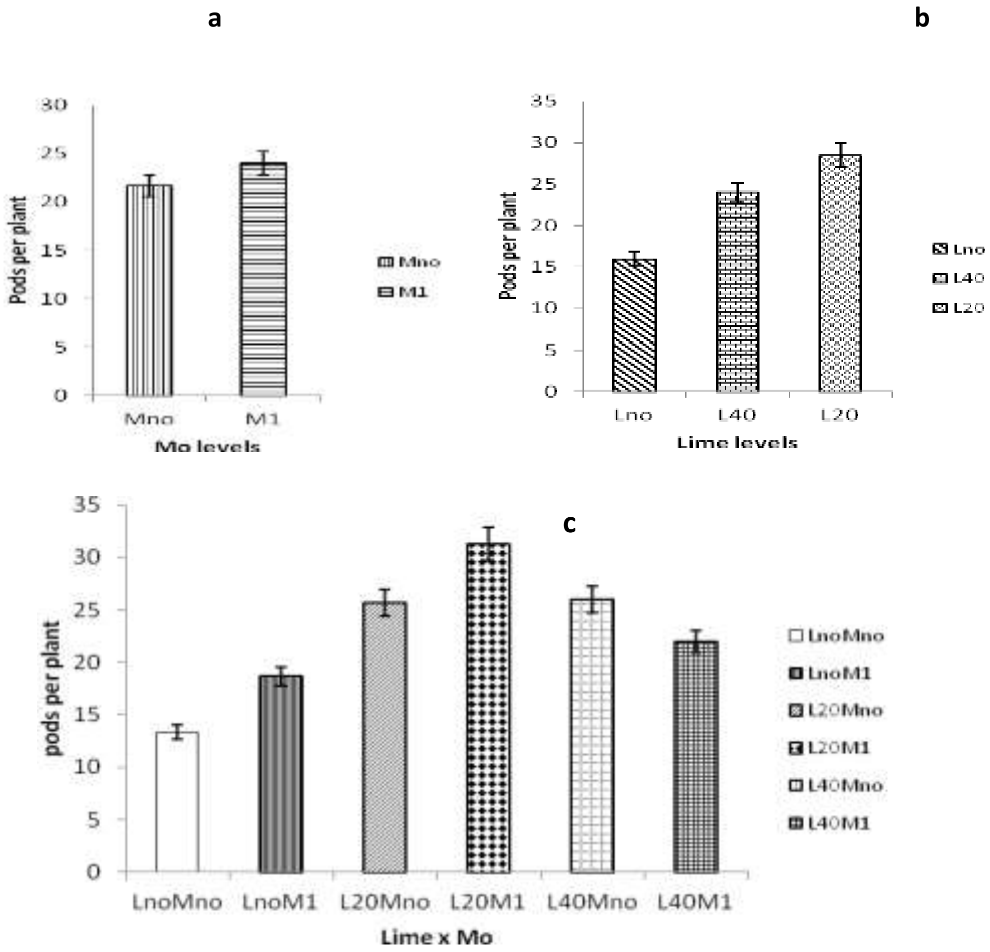

Figure 4. The effect of lime and molybdenum on the number of pods produced per plant

DISCUSSION

The increases in nodule number and mass with Mo rate are consistent with those of Hashimoto and Yamasaki (1976) for soybean and Zhou et al. (2017) for alfalfa. In terms of nodule production, the current results suggest that soybean responded best to a combination of liming to 20% acid saturation and application of ammonium molybdenum at 0.5 g L^{-1}. The responses of rooting to liming suggests an ability of the cultivar used in the study to tolerate acidity to some extent, as most crops show reduced root growth at acid saturations greater than 50%. In a comparable study, Caires et al. (2008) reported that low soil pH did not influence root growth of soybean plants; and more recently Duressa et al. (2011) have reported that some soybean cultivars tolerate acidity via complexing and extrusion of Al by Al-induced root citrate secretion. Anetor and Akinrinde (2006) reported that increasing lime levels increased dry matter yield of shoots.

The improvement of Ca uptake with lime is consistent with findings of Caires et al. (2008) who reported both linear and quadratic relationships between lime application and Ca uptake. The lack of difference between ameliorating the soil to 40% and 20% acid saturation in this study implies that there is no need to ameliorate the soil to 20% acid saturation if soybean is able to absorb adequate Ca from the soil at 40% acid saturation. The reduction in Mn uptake with lime is similar to reports by Caires et al. (2008) that lime detoxified Mn effectively, accompanied by an increased shoot dry matter yield of soybean and attributed this to competition of Ca^{2+} with Mn^{2+} for uptake with higher Ca reducing Mn uptake, and consequently high plant Ca increasing plant tolerance of Mn. According to Marschner (1995) a balance between Ca and Mn is required for a normal plant growth with soybean requiring a Ca/Mn ratio of 50:1 or more for normal growth. This ratio was achieved by liming in this study.

The fact that liming had no effect on shoot Al concentration in this study raises a question about the tolerance mechanism of soybean to Al toxicity. Aluminium tolerance mechanisms are broadly distinguished as those that prevent Al uptake or that bind to Al internally, converting it to a less toxic species once inside root cells. The first mechanism has gained a strong support for Al tolerance in many plant species including wheat, snap beans and soybean (Yang et al., 2000). This mechanism involves the excretion of organic acids to the rhizosphere in order to detoxify Al before it is absorbed by the roots.

The results from the current study suggest that soybean possibly excludes Al since plants in different treatments showed little difference in tissue Al concentration as well as root growth. Lime and Mo did not affect shoot N, an observation that was unexpected as the liming would normally improve nitrogen concentration in shoots. Rosolem and Caires (1998) reported that nitrogen uptake was increased by Mo and lime in peanut and that different stages of growth responded differently to lime and molybdenum in relation of N concentration in shoots, and that at 25 days after plant emergence there was no significant effect of lime on N concentration in leaves whereas at 75 days after plant emergence lime significantly increased N concentration in the leaves. This could also be the case in the current study since the N uptake was not examined at later stages of growth.

While the application of Mo *per se* did not result in significant changes in some of the parameters examined, the combined effect of the increases recorded in nodule mass and shoot dry mass among others, may have cumulatively contributed to the increased pod yield. Lime was reported to have significantly increased the yield and components of soybean (Bekere et al., 2013; Suryantini, 2014; Wijanarko and Taufiq, 2016) and those of groundnut (Shezi, 2011). The results of the current study indicate that there was only a slight difference in the number of pods produced between L40 and L20. Thus in economic terms, liming to L20 may not be desirable.

Results reported by Hashimoto and Yamasaki (1976) for soybean and Rosolem and Caires (1998) for peanuts demonstrated increased growth, and development and yield in response to Mo application. In the current study, lime and molybdenum interaction significantly ($p<0.01$) influenced the number of pods produced by soybean. Although treatment L20M1 was the best combination, from the economic point of view and considering the price of liming the soil, liming to L40 would be enough to give reasonable yields. Lime improved the soil condition at the end of the study in line with reports by Andrade et al. (2002), who reported that liming had a significant influence on soil pH, Ca levels and exchangeable Al and suggested that the decrease in exchangeable Al were due to increases in exchangeable Ca and Mg concentrations.

CONCLUSIONS

Foliar application of Mo significantly improved nodule number of soybean. Therefore it was beneficial for soybean to be supplemented with Mo through this method, even when the soil was limed. Lime was more important in improving nutrient uptake than Mo but the combination of the two factors provides a suitable balance for nutrient uptake and nodulation necessary for improved soybean production. Therefore in areas of very high acid saturation one can consider liming to acid saturation slightly higher than 20% taking into consideration the cost of liming. Foliar spray of Mo is more of a direct way of making Mo available to the plant; therefore in cases of slow reacting lime materials, one can consider using this form of micronutrient fertilization in order to enhance nodulation.

ACKNOWLEDGEMENTS

The authors are thankful to the University of KwaZulu-Natal for support with the study.

CONFLICT OF INTEREST

None.

REFERENCES

Adams, J.F. (1997). Yield response to molybdenum by field and horticultural crops. In: Molybdenum in agriculture. Pp 182–201, Gupta, U.C (Ed). Cambridge University Press, UK.

Andrade, D.S., Murphy, J.P. and Giller, K.E. (2002). Effect of liming and legume/cereal cropping in populations of indigenous rhizobia in acid Brazilian Oxisols. *Soil Biology and Biochemistry* 34: 477–485.

Anetor, M.O. and Akinrinde, E.A. (2006). Response of soybean [*Glycine max* (L.) Merrill] to lime and phosphorus fertilizer treatments on an acidic Alfisol of Nigeria. *Journal of Nutrition* 5: 286–293.

Bambara, S. and Ndakidemi, P.A. (2010). The potential role of lime and molybdenum on the growth, nitrogen fixation and assimilation of metabolites in nodulated legumes: A special reference to *Phaseolus vulgaris*. *African Journal of Biotechnology* 8 (17): 2482–2489.

Bereke, W., Kebede, T. and Dawud, J. (2013). Growth and nodulation responses of soybean (*Glycine max* L) to lime, *Bradyrhizobium japonicum* and nitrogen fertilizer in acid soil at Melko, South Western Ethiopia. *International Journal of Soil Science* 8: 25–31.

Beukes, D.J. (1997). Perspective on the soil acidity problem in South Africa. *Proceedings Soil Acidity Initiative Symposium*, Emerlo, South Africa, 8–10.

Caires, E.P., Barth, G., Garbuio, J. and Churka, S. (2008). Soil acidity, liming and soybean performance under no-till. *Scientia Agricola* 65(5): 532–540.

Duressa, D., Soliman, K., Taylor, R. and Senwo, Z. (2011). Proteomic analysis of soybean roots under aluminium stress. *International Journal of Plant*

Genomics http://dx.doi.org/DOI:10.1155/2011/282531 (downloaded 6 May 2017).

Esterhuizen, D. (2010). Soybean production in South Africa could reach 1.62 million tons by 2020. Global agriculture information network. http://gain.fas.asda.gov (downloaded 12 May 2012).

Farina, M.P.W. (1981). The Hunter system of soil analysis. *Fertilizer Society of South Africa Journal* 1: 39–41.

Grain SA (2016). South Africa's soybean industry: A brief overview (grain market). www.grainnsa.co.za/south-africa-s-soybean-industry-a brief-overview (downloaded 19 May 2017).

Hashimoto, K. and Yamasaki, S. (1976). Effects of molybdenum application on the yield nitrogen nutrition of soybeans. *Soil Science and Plant Nutrition* 22(4): 435–443.

Hassen, A.L., Bopape, F.L., Rong, I.H. and Seane, G. (2014). Nodulation efficacy of *Bradyrhizobium japonicum* inoculant strain WB74 on soybean (*Glycine max*) is affected by several limiting factors. *African Journal of Crop Science* 8: 2069–2076.

Mandal, P.S. and Mandal, L.N. (1998). Effect of molybdenum, phosphorus and lime application to acid soils on dry matter yield and molybdenum nutrition of lentil. *Journal of Plant Nutrition* 21: 139–147.

Manson, A.D. and Roberts, V.G. (2000). Analytical methods used by the soil fertility and analytical services section. KwaZulu-Natal Department of Agriculture and Environmental Affairs, Pietermaritzburg, South Africa. KwaZulu-Natal Agri-report No. N/A/2001/04.

Marschner, H. (1995). Mineral Nutrition of Higher Plants. 2nd Edition. Academic Press, New York. Pp889.

Matejovic, I. (1996). The application of Dumas method for determination of carbon, nitrogen, and sulphur in plant samples. *Rostlinna Vyroba* 42: 313–316.

McBride, M.B. (2005). Molybdenum and copper uptake by forage grasses and legumes grown on a metal-contaminated sludge site. *Communications in Soil Science and Plant Analysis* 36: 2489–2501.

Miles, N. (2013). Soil acidity and its management in crop production. www.grainsa.co.za/soil_acidity_and its_management_in_crop_production (downloaded 5 September 2017).

Parker, M.B. and Harris, H.B. (1977). Yield and leaf nitrogen of nodulating and non-nodulating soybeans as affected by nitrogen and molybdenum. *Agronomy Journal* 69: 551–554.

Quaggio, J.A., Gallo, P.B., Owino-Gerroh, C., Abreu, M.F. and Cantarella, H. (2004). Peanut response to lime and molybdenum application in low pH soils. *Revista Brasileira de Ciência do Solo* 28: 659–664.

Rosolem, C.A. and Caires, E.F. (1998). Yield and nitrogen uptake of peanuts as affected by lime, cobalt, and molybdenum. *Journal of Plant Nutrition* 21: 827–835.

Shezi, H.N. (2011). Screening groundnut (*Arachis hypogaea* L) genotypes for tolerance to soil acidity. MSc thesis, University of KwaZulu-Natal, South Africa.

Soil Classification Working Group (1991). Soil classification – A taxonomic system for South Africa. Memoirs of the agricultural natural resources of South Africa No. 15, Department of Agricultural Development, Pretoria, South Africa.

Staley, T.E. and Brauer, D.K. (2006). Survival of a genetically modified root colonizing Pseudomonad and Rhizobium strain in an acidic soil. *Journal of Soil Science* 70: 1906–1913.

Suryantini, B. (2014). Effect of lime, organic matter and inorganic fertilizer on nodulation and yield of soybean (*Glycine max*) varieties in Ultisol soils. *Journal of Experimental Biology and Agricultural Sciences* 2(1): 78–83.

Thibaud, G.R. (2005). Molybdenum relationship in soil and plants. KwaZulu-Natal Department of Agriculture and Environmental Affairs, Pietermaritzburg, South Africa. http://www.12asa.org/Download/zn_cnf_06/.mo. (downloaded 6 September 2017).

Togay, Y., Togay, N. and Dogan, Y. (2008). Research on the effect of phosphorus and molybdenum applications on yield and yield parameters in lentil *(Lens culinaris* Medic.) *African Journal of Biotechnology* 7: 1256–1260.

Wijanarko, A. and Taufiq, A. (2016). Effect of lime application on soil properties and soybean yield on tidal land. *AGRIVITA Journal of Agricultural Science* 38(1): 14–23.

Yang, Z.M., Sivaguru, M., Horst, W.J. and Hideaki, M. (2000). Aluminium tolerance is achieved by exudation of citric acid from roots of soybean. *Journal of Plant Physiology* 110: 72–77.

Zhou, J., Deng, B., Zhang, Y., Cobb, A.B. and Zhang, Z. (2017). Molybdate in rhizobial seed-coat formulations improves the production and nodulation of alfalfa. PLOS ONE 12(1):e0170179. https://dio.org/10.1371/journal.phone.0170179 (downloaded 6 September 2017).