Acknowledgements

We thank the Grain Legumes Research Council for financial support, R. J. Gilkes for improving the manuscript, W. J. Simmons for helping interpreting the chemical analysis and R. John for statistical advice.

Drying of surface soil decreased *Lupinus angustifolius* root length and Mn uptake in a split root experiment

Abstract

In a glasshouse experiment, a split root experiment was used to determine the ability of lupins (*Lupinus angustifolius L.*) to take up Mn from dry soil either when young or at mid-flowering of the primary branches. Three soil watering regimes (maintained at field capacity, maintained below wilting point and alternating from field capacity to well below wilting point) were imposed after taproots had grown through topsoil and into a nutrient solution below. Four sequential harvests (11, 22, 37 and 49 days after planting) were taken to determine the effect of soil drying on lupin growth, Mn uptake and soil extractable Mn.

Soil drying, early in the lupin plants life, stopped the growth of lateral roots in the soil and slowed the growth of roots grown in sub-soil solution and of lupin tops. Soil drying decreased uptake of Mn in the tops to 13% of what was taken up under continuous wet soil conditions. Of the 13%, most (11%) was taken up while the soil was drying. Soil re-wetting enabled the plants to resume uptake of Mn and soil re-drying (just before anthesis) decreased the Mn concentration in the lupin stems to 4.8 ug/g, whereas stems of lupins grown in the wet and dry soils contained 10.3 and 3.3 ug/g respectively. Easily reducible and plant available soil Mn were not affected by soil wetting and drying treatments.

This work confirms that the uptake of Mn by lupin may be severely restricted by drying of surface soil at both the beginning and end of the lupin plants life. The decrease in root length restricted Mn uptake rather than the chemical form of Mn.

Keywords: lupins, manganese uptake, soil drying.

Introduction

In Western Australia lupins are mostly grown on sandy soils that have little clay (often < 6%) in the near surface soil horizon and consequently these soils have poor water holding capacities (Nelson and Delane 1990). Climatic conditions of southern Australia are typically Mediterranean and grain fill of crops occurs during spring when temperatures are increasing and soil is drying. At this stage of growth plants obtain water and some nutrient requirements from subsoil horizons. Farmers on sandplain soils near Esperance have experienced severe Mn deficiency in lupins despite applying 60 kg/ha of $MnSO_4$ to topsoil, being four times the recommended rate (Gartrell and Walton 1984).

Dry soil conditions are known to limit the uptake of nutrients due to increasing tortuousity for the diffusion of nutrients through soil pores. Reduced uptake of phosphorus by medics (*Medicago truncatula*) (Scott 1973) and copper by wheat (*Triticum aestivum*) (Grundon 1980) have been related to dry surface soil conditions.

"Split seed" or Mn deficiency in lupins has been shown to be more severe with later plantings (Perry and Gartrell 1976). This is perhaps due to the lupin pods filling later, under drier soil conditions, than earlier sown lupins. Mn can be moved from the stem and taproot to young growing tissue but not from other above ground plant material (Radjagukguk 1981; Hannam *et al.* 1985).

Deep placement of Mn has increased efficacy of Mn fertilisers for lupins during pod fill lupins obtained more Mn from moist subsoil than from the dryer surface soil (Crabtree 1998). This decreased Mn uptake by lupins from dry surface soils is despite extractable Mn (in phosphoric acid) being found to increase with soil drying in some situations (Leeper 1947; Hammes and Berger 1960). The hypothesis tested here is that surface soil drying will affect Mn uptake by lupins growing in a sandy acidic soil.

Materials and Methods

Pot design and plants

Lupins were grown in a horizontal split pot, where the roots were in a soil medium above a liquid medium. The plants grew through 1 kg of soil, in a upper pot, into a lower pot, containing 1.1 L of nutrient solution. Both pots were cylindrical and made of polythene. The upper pot was 86 mm in diameter and 94 mm high and the bottom pot was 107 mm in diameter and 250 mm high. The upper pot had an inverted cone attached to the bottom which had a 90 degree internal angle and the cone height was 35 mm. A 10 mm hole in the centre of the cone was sealed with Terrastat® putty and Vaseline® which allowed the lupin taproots to penetrate into the bottom pot without water being able to pass between the pots (technique developed by Loss et al 1993). The nutrient solution level was kept 5-15 mm below the seal.

Seven lupin seeds were planted per pot and the plants were thinned to 3 plants per pot by 11 days after sowing (DAS). All plants were grown in soil kept near field capacity (12%; w/w) until 11 DAS when all of the lupin taproots had just passed through the seal (5-15 mm long) and into the nutrient solution below. The plants were watered twice daily and the upper pots were weighed once per day. Seed weight ranged from 120 to 130 mg and seed Mn levels ranged from 8 to 10 μg Mn/g of seed.

Experimental design

An incomplete factorial design was used which compared 3 soil watering regimes with 4 sequential plant harvests by 3 replicates. The pots were randomised and partly rotated within each replicate weekly. The watering regimes were; soil maintained near field capacity, soil dried after 11 DAS to below wilting point (1.4%; w/w), and soil dried from 11-22 DAS, re-wet to near field capacity from 22-37 DAS and re-dried to below wilting point from 37-49 DAS. Harvests were taken at days 11, 22, 37 and 49 DAS, the final harvest being when the primary inflorescences were at mid-flowering stage. The data were analysed using a one way analysis of variance.

Glasshouse conditions and nutrients added

The plants were grown in an evaporatively cooled glasshouse at the University of Western Australia from 9 November to 28 December 1990. The daily minimum and maximum air temperature ranged from 15-25 and 30-38^{o}C. Pots were immersed in a root cooling bath at 18^{o}C.

The soil used was topsoil sand from a virgin Lancelin brown sand site (Uc) with a pH of 5.3 (1:5 0.01 mol $CaCl_2$/L). This soil has been characterised by Brennan *et al.* (1980) as having 2% clay, 0.8% organic carbon and 0.7% free sesquioxides. The soil was dried for 36 hours then sieved to obtain the less than 2 mm fraction.

Basal nutrient solutions were pipetted onto the soil surface, dried and thoroughly mixed into the soil. The soil applied nutrients were (μg/g soil): H_3BO_3, 0.10; $CaCl_2.2H_2O$, 71.0; $CoSO_4.7H_2O$, 0.36; $CuSO_4.5H_2O$, 2.13; FeNaEDTA, 33.3; K_2SO_4, 71.0; $MgSO_4.7H_2O$, 19.9; $Na_2MoO_4.2H_2O$, 0.2; KH_2SO_4, 100 and $ZnSO_4.7H_2O$, 5.0. No Mn was applied to the soil as a preliminary experiment demonstrated this soil was able to supply sufficient Mn for adequate lupin growth.

The concentrations in the nutrient solution were (μM): H_3BO_3, 5.00; $CaSO_4$, 625; $CoSO_4.7H_2O$, 0.20; $CuSO_4.5H_2O$, 0.20; FeNaEDTA, 3.00; K_2SO_4, 600; $MgSO_4.7H_2O$, 20; $Na_2MoO_4.2H_2O$, 0.03; $NaNO_3$, 250; NaH_2PO_4, 20 and

$ZnSO_4.7H_2O$, 0.75. The nutrient solution was changed once or twice weekly and the pH was kept between 5.8 and 6.2 with 5 mM of MES buffer used to stabilize pH changes (as developed by Ewing and Robson 1991). Air was bubbled through the solution at 1 bubble/second this being sufficient aeration to not cause damage to the lupin taproots.

Measurements

Fresh weight of whole shoots was recorded at each harvest after which the shoots were separated into youngest open leaflet (YOL), stem and rest of shoots. The soil roots were washed from the soil with deionised water and along with the nutrient solution roots; blotted dry, weighed and then measured for root length. Both shoots and roots were oven dried at 70^{O}C in paper bags for 72 h then re-weighed. After oven drying the soil-roots were separated into laterals and taproots. The dry soil treatment resulted in sand granules being attached to the dead roots, consequently, estimates of sand mass mixed with the roots (by sub-sampling) were made for each sample and subtracted from the dry weight of the lateral roots grown in the soil.

During the course of the experiment the cotyledons and some leaves dropped from the plants, particularly the plants where the soil had dried. These leaves were collected, dried, weighed and their dry weights added to dry weights of the remainder of the plant.

An analysis of variance was done on the data. The root length and root weight in solution, at harvest on day 11, produced no data. Therefore only 8 treatments were included in the statistical analysis for these parameters.

Plant shoot fractions from 2 replicates of each treatment were analysed for Mn content. The plant samples were digested in a nitric acid:perchloric acid mixture (Johnson and Ulrich 1959) and Mn determined using atomic absorption spectrophotometry.

Soil Mn was extracted using 2 extractants for each pot at each harvest and 3 samples were taken prior to commencement of the experiment The soil was teased from the roots and 2 lots of 5-g weights (dry weight equivalents) were sub-sampled from the soil from each pot and put into 25 mL vials. Extractant solution was then added to provide a 1:5 soil:solution ratio and shaken on an end over end shaker. As a measure of plant available Mn; a solution of 0.033*M* H_3PO_4 was added and shaken for 3 h (Salcedo and Warncke 1979) and as a measure of easily reducible Mn; a solution of 1 M NH_4OAc containing 0.2% hydroquinone at pH 7 (Hammes and Berger 1960) was shaken for 10 minutes. The vials were then centrifuged at 894 G for 10 minutes, filtered with 0.45 μm Whatman No. 4 paper and solutions were then analysed for Mn using atomic absorption spectrophotometry.

Results

Plant growth

The split pot system separated the two media with the plug between the soil and solution effectively preventing transfer of solution to the soil and *vice versa*. The lupin plants constantly grew through time when watered to field capacity (Table 1). In contrast, topsoil drying slowed the growth of the shoots and roots in the topsoil and solution. Re-wetting the soil increased the rate of growth, and subsequent drying again slowed plant growth.

The roots in the solution grew well despite topsoil drying. Root growth was still occurring toward the end of the experiment although it was more due to the

thickening of the roots rather than to elongation. The apparent root growth in the topsoil while drying was due to the taproot thickening and not to growth of lateral roots, as is demonstrated by the root length data. Sand particles adhered to the lateral roots which died soon after drying was imposed. The dry weight and length of lupin roots increased with soil re-wetting ($P<0.05$) and soil re-drying stopped further growth of roots in the soil.

Table 1. Treatment, lupin age at each harvest, dry weights of tops and roots, root length and hydroquinone (Hq) and phosphoric acid (P) extractable Mn.

Mn uptake

Mn uptake for the wet soil increased with time (Table 2). However, soil drying from day 11 stopped Mn uptake, despite some (although not significant) uptake occurring as the soil dried from day 11-22. Mn movement into YOLs was slow for the plants that were grown in dry soil and the movement was from the stem, taproot and perishing lateral roots and not from the soil. Mn uptake from the drying soil treatments was 31% of Mn uptake from the wet soil during the same period (day 11-22). Re-wetting the soil tripled Mn uptake during the re-wet phase. However, once re-dried Mn uptake ceased again.

Increasing Mn uptake was associated with increased length of the lateral roots in the soil ($P<0.05$). Also, increasing dry weight (DW) of lateral roots grown in the soil, increased with the uptake of Mn by shoots of lupins ($P<0.001$). The relationship being:

DW of lateral roots (g/pot) = {Mn in shoots (μg/pot) - 19.2}/263 $R^2 = 0.94$

Table 2. Content (μgMn/pot) and concentration (μgMn/g) of Mn in lupin shoots, stem, rest, YOLs (youngest open leaflets) and seed in glasshouse experiment.

Soil extractable Mn

Easily reducible (extractable) Mn did not alter with time for either dry soil or soil at field capacity (Table 1). However, two wetting and drying cycles did decrease the amount of easily reducible Mn. The phosphoric acid extractable Mn, which more closely reflects plant available Mn (Ritchie, 1989), showed no significant changes with soil moisture regime.

Discussion

Lupin lateral roots, grown in the soil, died as the soil dried to below wilting point. This decreased the growth of lupin shoots and the roots grown in the solution. The loss of lateral roots in the dried soil rendered the plants unable to extract Mn, and other nutrients, from the soil. During initial soil drying, the Mn content of shoots increased from 17 to 35 μgMn/pot. Much more Mn was taken up during the second drying (increased from 109 to 147 μgMn/pot). For both treatments the Mn content in lupin shoots was much less than for plants that had grown in permanently wet soil (313 μg/pot). Final Mn concentration in the whole shoots and seed was marginal for wet soil and severely deficient for the dry soil.

Root exudation can increase with soil moisture stress (Svenningsson *et al.,* 1990) and some plants are able to exude water from their roots into soil at night. Root exudates can reduce and complex Mn and make it more available for uptake (Godo and Reisenauer 1980). These exudates also provide microbial substrate which could aid microorganisms in reducing Mn. However, acid soils, as used here, inhibit Mn reducing microorganisms (Leeper and Swaby 1940).

In this study, plentiful moisture was available to the roots growing in the solution, but this water was not used by the lupins to improve their ability to extract Mn from the dry topsoil. This result is consistent with field responses on the Esperance sandplain where a usually sufficient Mn applications in the topsoil resulted in severe Mn deficiency despite water being available at depth (Crabtree 1998, Gartrell and Walton 1984).

These results are also consistent with field observations where surface soil drying has inhibited the uptake of phosphorus by medics (Scott 1973) and copper by wheat (Grundon 1980). It also agrees with greenhouse experiments with phosphorus by corn (*Glycine max* L. Merr.) (Marais and Wiersma 1975) where uptake was limited by soil drying.

In contrast, Thorup (1969) found that roots of tomato plants (*Lycopersicon esculentum*) grew into dry soil, transporting some water into this dry soil which enabled uptake of phosphorus. Similarly, Nambiar (1977) found that ryegrass (*Lolium multiflorum* Lam.) roots grew in dry soil and may have absorbed Mn from dry soil.

It would appear that tomatoes have the ability to initiate roots in dry soil (Thorup 1969), whereas ryegrass roots may have the ability to persist in dry soil (Nambiar 1977). However, both of these observations were from studies where the roots were sealed or closed to evaporational loss. Lupins grown in this study were open to evaporative loss from the sandy soil, and perished rendering them unable to take up nutrients from the dry soil.

It is clear that different plant species differ in their ability to sustain root growth and function in dry soil. Work with stoloniferous bermudagrass (*Cynodon dactylon* L.) clearly demonstrates its ability to transfer moisture from wet to dry soil, even in an open system (VanBavel and Baker 1985). In this work, where there are high evaporative losses, *Lupinus angustifolius* did not demonstrate a capacity to transfer water into lateral roots in the dry soil layer, such movement was not measured, but the roots did dehydrate quickly.

Under field conditions it is therefore likely that surface soil drying in spring will severely limit the uptake of Mn by lupins. Since soil drying caused the death of lupin roots in dry soil in this experiment it is likely that all nutrients in the dry topsoil, will be unavailable to lupins under these drying conditions.

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Table 1. Treatment, lupin age at each harvest, dry weights of tops and roots, root length and hydroquinone (Hq) and phosphoric acid (P) extractable Mn.

Harvest No.	Soil Water	Lupin age (days)	Root length (m)		Dry weight (g/pot)				Soil extr. Mn (ug/g)[B]	
					Shoots	Roots from		Total		
			Soil	tion		Soil	Solution		Hq	Phos
0	Dry	0	-	-	-	-	-	-	0.34	0.61
1	Wet	11	10.5	0.0	0.39	0.04	0.00	0.43	0.52	1.01
2	Wet	22	24.8	5.9	1.31	0.39	0.13	1.83	0.42	0.72
2	Dry	22	5.6[A]	1.3	0.71	0.05	0.05	0.81	0.45	0.80
3	Wet	37	30.2	17.9	2.74	0.75	0.47	3.96	0.35	0.77
3	Dry	37	5.6[A]	9.3	1.36	0.06	0.17	1.59	0.44	0.98
3	Dw[C]	37	15.7	7.3	1.49	0.28	0.19	1.96	0.30	0.65
4	Wet	49	25.7	22.1	4.17	0.99	0.68	5.85	0.46	0.83
4	Dry	49	6.5[A]	16.3	2.25	0.07	0.58	2.90	0.55	0.92
4	Dwd[C]	49	18.3	17.7	2.73	0.33	0.53	3.59	0.28	0.59
	l.s.d.	(P=0.05)	8.2	3.6	0.44	0.12	0.17	0.73	0.14	ns
	l.s.d.	(P=0.10)	6.7	3.0	0.36	0.11	0.14	0.61	0.11	ns

[A] Difficult to assess root length in dry soil, see text.

[B] Extracted with Hq (0.2% hydroquinone in 1M NH_4OAc) or Phos (0.033M H_3PO_4).

[C] Dw or Dwd refer to dry:wet or dry:wet:dry.

Table 2. Content (μgMn/pot) and concentration (μgMn/g) of Mn in lupin shoots, stem, rest, YOLs (youngest open leaflets) and seed in glasshouse experiment.

Harvest No.	Soil water	Lupin Age (days)	Mn content in plant shoots (μgMn pot^{-1})					Mn concentration in shoots (μgMn g^{-1} plant)				
			Total	Stem	Rest	YOLs	Seed	Total	Stem	Rest	YOLs	Seed
1	Wet	11	17	0.6	16	1.0	-	57	19	62	48	-
2	Wet	22	73	3.4	66	3.9	-	56	16	65	54	-
2	Dry	22	35	0.7	33	1.3	-	47	7	54	35	-
3	Wet	37	209	7.7	198	3.6	-	75	15	90	66	-
3	Dry	37	35	1.1	33	0.5	-	26	4	32	9	-
3	Dw[A]	37	109	2.8	103	3.1	-	69	11	81	66	-
4	Wet	49	313	8.6	293	3.0	8.1	70	10	98	71	12

4	Dry	49	42	1.5	40	0.3	0.2	19	3	24	8	5
4	Dwd[A]	49	147	2.8	141	1.4	0.9	53	5	69	40	9
l.s.d. (P=0.05)			36	1.6	37	1.6	2.9	15	2.7	17	22	1.5
l.s.d. (P=0.10)			30	1.3	30	1.3	2.5	13	2.2	14	18	1.2

[A] Dw or Dwd refer to dry:wet or dry:wet:dry.

Response from W.L. Crabtree regarding submitted AJAR paper AR98015

Discussion sheet

Referee 1's comments:

I have included more words in the first paragraph (see italics below) of the discussion to address the concern that perhaps "Mn alone is the issue of importance":

"The loss of lateral roots in the dried soil rendered the plants unable to extract Mn, *and other nutrients,* from the soil."

I believe I have already referred to other nutrients and other species and drying relationships in the discussion. See lines 237-255. I hope this adequately answers this issue.

Referee 3's comments:

This referee has been thorough in his/her review, and has addressed a number of important issues that will greatly improve the paper and have been incorporated, with one exception. The referee provides comments in 6 paragraphs and I will deal with them in order. The 6 minor comments I agree with completely and I have made appropriate changes.

Para 1: This is the only comment made that I will challenge. Alan Robson read the draft paper on two occasions and has made useful comments. Gerry Ritchie read a very early draft when the work was first done. Professor Bob Gilkes (UWA) is now helping me finalize this paper. Bob tells me that both authors addresses should stay as Department of Soil Science and Plant Nutrition – where the work was done, also Alan is still attached to this section of the University. I have added G Ritchie's current address.

Para 2: Yes, I agree with giving credit where it is due with respect to Loss and Ewing, I have made appropriate reference to these authors and their pioneering work in respective fields.

Para 3: I agree that the drying conditions of the experiment were harsh and the lateral roots in the dried soil, died prior to each harvest. However, I believe this is a new finding in that "the lupin roots, in the surface soil, dehydrated despite adequate moisture being available to the lower roots. Adequate moisture at depth and dry surface soils does occur in field-lupin crops in Western Australian sandy surfaced soils." And "Other workers, with other species, have shown that plants can move moisture from one section of their root system to another and remain active as discussed in the paper (lines 253-56)."

Para 4: I have clarified this apparent discrepancy by adding text to the: *Pot design and plants* sub-section of the Materials and Methods. The comment: "no roots in the solution when harvested at day 11" is due to the amount of roots penetrating into the solution being too small to be measured. Lupin roots emerged through the cone, time-wise, very consistently and pruning seedlings back from 7 seeds planted to 3 seedlings emerged did ensure uniformity. The length of the roots that emerged were 5-15 mm. This root emergence was also the trigger to commence the drying procedure.

Para 5: The zero data set for root length and root weight in solution was not included in the ANOVA, so the referee's concern is unfounded, this is now mentioned in the text. Log transformations would make the data set harder for non-researchers to interpret and would then be required for the whole data set, I presume.

Para 6: I have addressed this concern by including the following statement at the end of the first paragraph in the discussion section; "Final Mn concentration in the whole shoots and seed was marginal for wet soil and severely deficient for the dry soil." The leaf drop was due to moisture stress and natural loss of the cotyledons, and not due to Mn deficiency. Mn deficiency predominantly only occurs in the lupin seed of domesticated L. angustifolius lupins due to the initial selection procedure induced by J Gladstone when he was selecting for other desirable (sweet and non shattering) traits.

Jenny Fegent, Managing Editor

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Dear Jenny,

In reference to your letter dated 26 March 1998, regarding a paper I submitted called "Drying of surface soil decreased *Lupinus angustifolius* root length and Mn uptake in a split root experiment." AR98015.

I have carefully considered the referee's comments and have made appropriate adjustments to, hopefully, rectify deficiencies observed by the referees. I have rebutted only one suggestion made by one referee, as explained in the attached discussion sheet.

I have included all your comments on the manuscript. I have also found 4 other errors which I had previously missed. These occur on the following lines of old manuscript:

1. line 122, "soils" now reads soil:
2. line 237, "consistant" now reads consistent;
3. line 292, "af" now reads at, and
4. line 221-222 where lsd's at 5 and 10% were around the wrong way.

After talking with my supervisor, I have now provided 2 addresses at the front of the paper for myself and G Rithie. One where, and with whom, we did the work, and a current address. I hope this is the correct procedure.

I again enclose 3 copies of the modified paper and also a copy of it in electronic form.

Yours sincerely.

Bill Crabtree
20th April 1998