

COBALT AND NITROGEN
FIXATION IN *LUPINUS ANGUSTIFOLIUS* L.
I. GROWTH, NITROGEN CONCENTRATIONS
AND COBALT DISTRIBUTION

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(Accepted 24 November 1978)

SUMMARY

Cobalt application markedly increased the growth of and nitrogen concentrations in tops of *Lupinus angustifolius* irrespective of inoculation with an effective strain of *Rhizobium*. Cobalt-deficient plants produced a greater weight of lateral nodules and total nodule weight than cobalt-adequate plants. Cobalt-sufficient plants produced more crown nodule weight than cobalt-deficient plants.

Cobalt concentrations were higher in roots and nodules than in tops irrespective of cobalt application. In plant tops cobalt concentrations in young leaves were considerably lower than those in old leaves at both cobalt levels. Cobalt concentrations and contents increased in old leaves throughout the experiment.

Under cobalt deficiency cobalt appeared to be preferentially distributed to nodules. Six weeks after sowing cobalt contents of nodules of cobalt-deficient plants were similar to those in whole tops. By contrast at this time cobalt contents of nodules of cobalt-adequate plants were only approximately one-third those of whole tops.

INTRODUCTION

The mechanism by which cobalt deficiency limits growth is not completely understood. Cobalt is essential for symbiotic nitrogen fixation (Ahmed and Evans, 1960) and for rhizobial growth (Lowe and Evans, 1962; Cowles, Evans and Russell, 1969). Sweet lupins (*Lupinus angustifolius*) appear to be particularly sensitive to cobalt deficiency (Gladstones, Loneragan and Goodchild, 1977; Chatel *et al.*, 1978). In field-grown lupins cobalt deficiency decreased bacteroid number per g nodule (Chatel *et al.*, 1978). Additionally cobalt deficiency could be alleviated by rhizobial inoculation (Chatel *et al.*, 1978). However, in some instances cobalt deficiency decreased growth of lupins and increased nitrogen concentrations in tops leading to suggestions for roles for cobalt other than in nitrogen fixation (Gladstones *et al.*, 1977).

The distribution of cobalt within plants has generally been studied only at adequate cobalt supply and only using radioactive cobalt (e.g. Rinne and Langston, 1960; Handreck and Riceman, 1969). These studies suggest that cobalt is a mobile nutrient readily retranslocated from old leaves to new growth. In lucerne (*Medicago sativa*) cobalt concentrations and contents of leaves appeared to reach maximal values early and to decline rapidly with age (Handreck and Riceman, 1969). However, for other nutrients nutrient supply may markedly change the extent of retranslocation and nutrient distribution (see review by Loneragan, Snowball and Robson, 1976, for examples for copper and zinc).

Our experiment had two aims. Firstly we examined the effect of cobalt deficiency through time on nodulation, growth and nitrogen fixation by lupins either uninoculated or inoculated with an effective strain of *Rhizobium lupini*. This paper reports the effects of cobalt application on growth and nitrogen concentrations in tops. A subsequent paper will present information on nodule distribution, acetylene reduction activity, bacteroid number and on the concentrations of vitamin B₁₂ and leghaemoglobin in nodules (Dilworth, Robson and Chatel, 1979). Secondly we examined the effect of cobalt supply on the distribution of cobalt within lupin plants at several harvests using chemical analysis.

MATERIALS AND METHODS

The experimental design was a complete factorial of the following treatments – cobalt application (0 or 0.9 mg CoSO₄·7H₂O pot⁻¹) – inoculation (nil or inoculated with a dense suspension of *R. lupini* WU 425). There were 20 replicate pots of each treatment combination. Harvests were taken at 2, 4, 6, 8 and 11 weeks after sowing. At each of the first three harvests five replicate pots were harvested for each treatment combination. Two replicates were harvested at 8 weeks and three replicates were harvested at the final harvest.

The soil used was from Lancelin from an area where lupins had responded to cobalt in the field (Chatel *et al.*, 1978). Plants were grown in undrained 18 cm pots lined with plastic bags and containing 6 kg soil. Basal nutrients (KH₂PO₄, 408 mg pot⁻¹; K₂SO₄, 783 mg pot⁻¹; CaCl₂·2H₂O, 110 mg pot⁻¹; MgCl₂·6H₂O, 108 mg pot⁻¹; MnSO₄·H₂O, 91 mg pot⁻¹; CuSO₄·5H₂O, 23 mg pot⁻¹; ZnSO₄·7H₂O, 45 mg pot⁻¹; Na₂MoO₄·H₂O, 1.5 mg pot⁻¹) and treatments were applied in solution onto the soil surface and allowed to dry. All nutrients were mixed throughout the soil. Basal macro-nutrients were purified (Hewitt, 1966).

Fifteen seeds of *L. angustifolius* cv. Uniharvest grown without cobalt on a cobalt responsive soil were sown in each pot. Cobalt concentration in seed was 5 ng/g dry weight and each seed contained 0.8 ng cobalt. Each pot was thinned to eight plants prior to the first harvest. Where appropriate seeds were inoculated with 1 ml of a dense suspension of *R. lupini* strain WU 425 grown on a low-cobalt medium.

Root temperatures were maintained at 25 °C as this root temperature gave the largest response to cobalt application (McGrath, Robson and Dilworth, unpublished data). Soil was maintained at field capacity throughout the experiment.

At each harvest tops, roots and nodules were weighed after separation. Nodules were separated into two categories – crown (on the top 10 cm of the main root) and lateral (on lateral roots or below 10 cm on the main root). Whole plant tops were analysed for nitrogen using a Kjeldahl procedure (McKenzie and Wallace, 1954).

Plant tops from two replicates at each of the first three harvests were separated into plant parts (Table 2) prior to analysis for cobalt (Simmons, 1975).

RESULTS

Growth and nitrogen concentrations

Cobalt application increased ($P < 0.05$) dry wt of tops of both inoculated and uninoculated plants at 6, 8 and 11 weeks from sowing (Fig. 1). At the first two harvests (2 and 4 weeks from sowing) dry weight of tops was unaffected by either cobalt application or inoculation. In the inoculated treatment but not in the uninoculated treatment the differences between cobalt-sufficient and cobalt-deficient plants lessened between 8 and 11 weeks (Fig. 1) and at the 11-week harvest rhizobial inoculation alleviated cobalt deficiency to some extent.

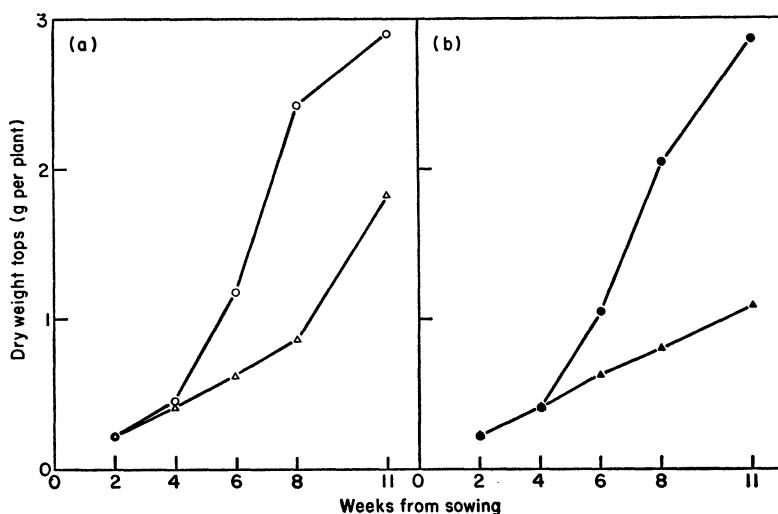


Fig. 1. Effect of cobalt application on the dry weight of tops of *Lupinus angustifolius* either: (a) inoculated with *Rhizobium lupini* or (b) not inoculated. ○, Inoculated, Co applied; △, inoculated, no cobalt applied; ●, not inoculated, Co applied; ▲, not inoculated, no cobalt applied. Main effect of cobalt significant ($P < 0.001$) at final three harvests. Main effect of inoculation significant ($P < 0.05$) at 8- and 11-week harvests. Interaction between cobalt and inoculation significant ($P < 0.05$) at 11-week harvest.

Cobalt application increased top growth more than root growth so that the proportion of total plant weight in the tops was greater in cobalt-treated plants at 4 and 6 weeks from sowing (Table 1). This effect was more marked in the inoculated treatment. However, at 8 weeks from sowing the distribution of fresh weight between tops and roots was similar in cobalt-sufficient and cobalt-deficient plants.

Cobalt application markedly increased nitrogen concentrations in tops at all harvests in both inoculated and uninoculated treatments (Fig. 2). Effects of cobalt in increasing nitrogen concentrations preceded effects of cobalt in increasing dry weight of tops. In the inoculated treatment nitrogen concentrations in the tops of cobalt treated plants declined to a similar level to those without cobalt at the final harvest.

In inoculated treatments cobalt application increased fresh weight of crown nodules

Table 1. *Effect of cobalt application on the proportion of total plant fresh weight as tops in Lupinus angustifolius either inoculated with Rhizobium lupini or not inoculated*

Treatment		Weeks from sowing			
Inoculation	Cobalt application	2	4	6	8
—	—	60	41	48	59
—	+	64	45	55	61
+	—	60	40	49	58
+	+	61	50	62	61

Main effect of cobalt significant ($P < 0.01$) at 4- and 6-week harvests. Main effect of inoculation significant ($P < 0.05$) at 6-week harvest. Interaction between cobalt and inoculation significant ($P < 0.05$) at 4-week harvest.

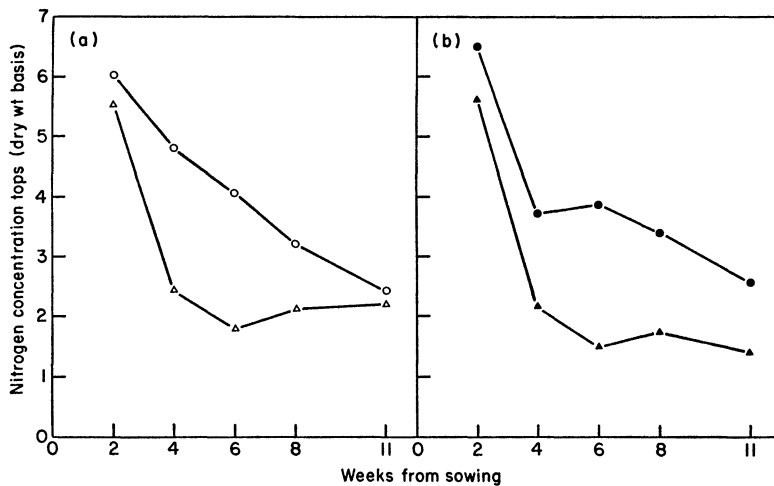


Fig. 2. Effect of cobalt application on nitrogen concentrations in tops of *Lupinus angustifolius* either (a) inoculated with *Rhizobium lupini* or (b) not inoculated. ○, Inoculated, cobalt applied; △, inoculated, no cobalt applied; ●, not inoculated, cobalt applied; ▲, not inoculated, no cobalt applied. Main effect of cobalt significant at 4 weeks ($P < 0.001$), at 6 weeks ($P < 0.001$), at 8 weeks ($P < 0.01$) and 11 weeks ($P < 0.05$) after sowing. Main effect of inoculation significant only at 4 weeks after sowing ($P < 0.05$).

but decreased fresh weight of lateral nodules (Fig. 3). Cobalt-deficient plants produced a greater total nodule weight due to this compensation.

Cobalt distribution within the plant

Inoculation did not markedly affect cobalt concentrations in the plant and only data from the inoculated treatment is presented.

Cobalt concentrations in roots were greater than those in nodules or whole tops at both cobalt levels (Fig. 4). Cobalt concentrations in nodules exceeded those in whole tops in both cobalt-deficient and cobalt-sufficient plants. Cobalt supply did, however, change the magnitude of the differences in cobalt concentration between plant parts. In cobalt-deficient plants the ratio of cobalt concentrations in tops to those in nodules and to those in roots was of the order 1:6:15. By contrast for cobalt-sufficient plants this ratio was of the order 1:3:25.

In cobalt-deficient plants cobalt appeared to be preferentially accumulated in nodules. Six weeks after sowing cobalt content of nodules of cobalt-deficient plants was similar to that in whole tops (Fig. 5). By contrast, cobalt contents of nodules of cobalt-sufficient plants was only approximately one-third that of whole tops at this time. At earlier harvests the distribution of cobalt between tops and nodules was similar for cobalt-deficient and cobalt-adequate plants.

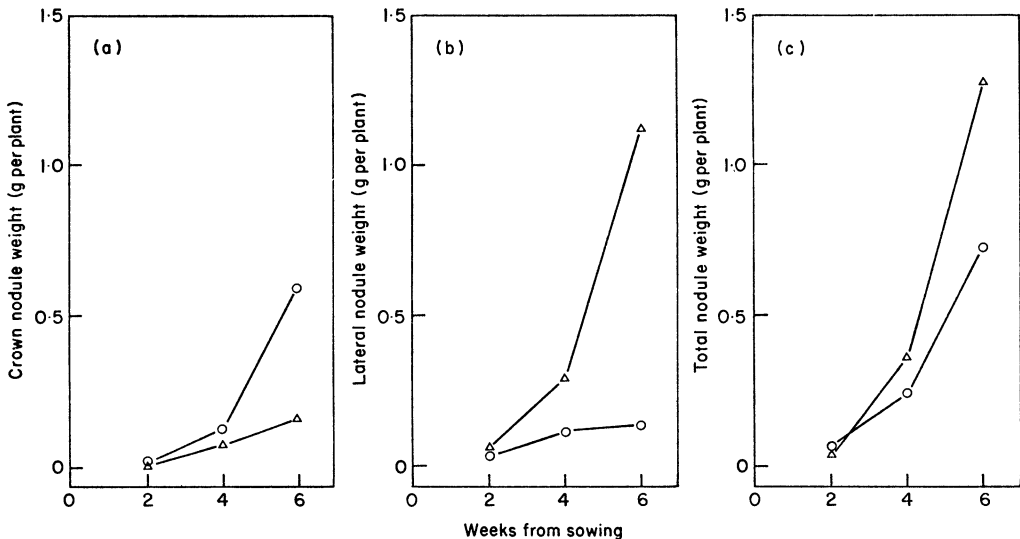


Fig. 3. The effect of cobalt application on the fresh weight of (a) crown, (b) lateral and (c) total nodules of *Lupinus angustifolius* inoculated with *Rhizobium lupini*. ○, Cobalt applied; △, no cobalt applied. At 6-week harvest significant effect of cobalt for total nodule weight ($P < 0.05$), for crown nodule weight ($P < 0.1$) and for lateral nodule weight ($P < 0.01$).

Within plant tops cobalt concentrations in old leaves were approximately double those in young leaves irrespective of cobalt supply (Table 2). Cobalt concentration in old leaves increased markedly with time. Cobalt concentrations in young leaves also increased but to a lesser extent. Cobalt concentrations in stems were generally even lower than concentrations in young leaves at both levels of cobalt supply.

Cobalt contents of old leaves increased with time irrespective of cobalt supply (Fig. 5). Two weeks after sowing cobalt content of the oldest pair of leaves exceeded the cobalt content of nodules at both levels of cobalt supply. The continued accumulation of cobalt in old leaves led to comparable cobalt contents in the oldest four leaves and in nodules under cobalt deficiency at four weeks after sowing.

DISCUSSION

Cobalt application increased the growth of lupins by increasing nitrogen fixation. This conclusion is based on two observations. Firstly cobalt application increased nitrogen concentrations in tops prior to effects on growth. Secondly cobalt-deficient plants when inoculated with *R. lupini* produced more nodule weight than cobalt-sufficient plants. This compensation in nodule production has been observed with other nutrient deficiencies known to be implicated in nitrogen fixation (Robson, 1978).

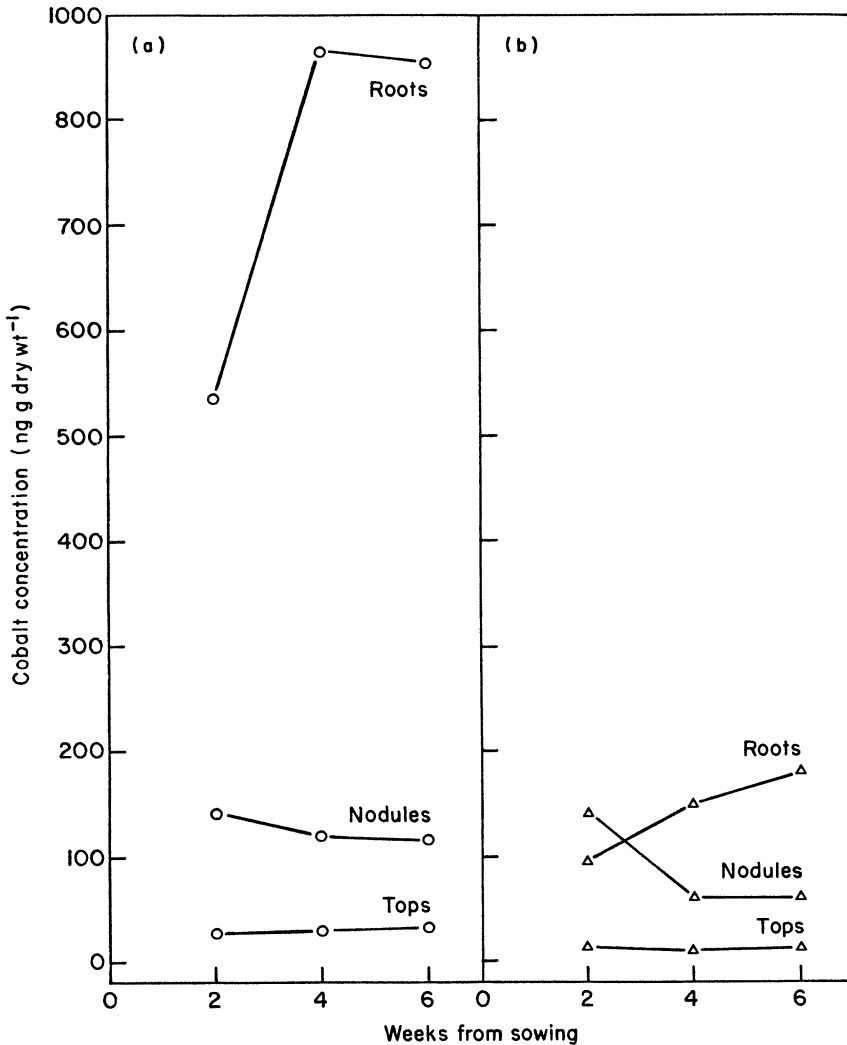


Fig. 4. The effect of cobalt application on cobalt concentrations in tops, nodules and roots of *Lupinus angustifolius* inoculated with *Rhizobium lupini*. ○, Cobalt applied; △, no cobalt applied.

The roles of cobalt in nodulation and nitrogen fixation by lupins will be considered in a subsequent paper (Dilworth *et al.*, 1979).

Cobalt concentrations in the tops and nodules of cobalt-deficient lupin plants (approximately 10 and 60 ng g⁻¹ respectively) were considerably lower than concentrations in tops (200 ng g⁻¹) and nodules (100 ng g⁻¹) of cobalt-deficient subterranean clover (Wilson and Hallsworth, 1965). In a field-study cobalt concentrations in the tops of cobalt-deficient subterranean clover plants were only 30 ng g⁻¹ (Ozanne, Greenwood and Shaw, 1963).

In other nitrogen-fixing associations concentrations in nodules in cobalt-deficient plants ranged from 20 ng g⁻¹ (*Casuarina cunninghamiana*: Hewitt and Bond, 1966) to 90 ng g⁻¹ (*Alnus glutinosa*: Bond and Hewitt, 1962) to 170 ng g⁻¹ (*Myrica gale*:

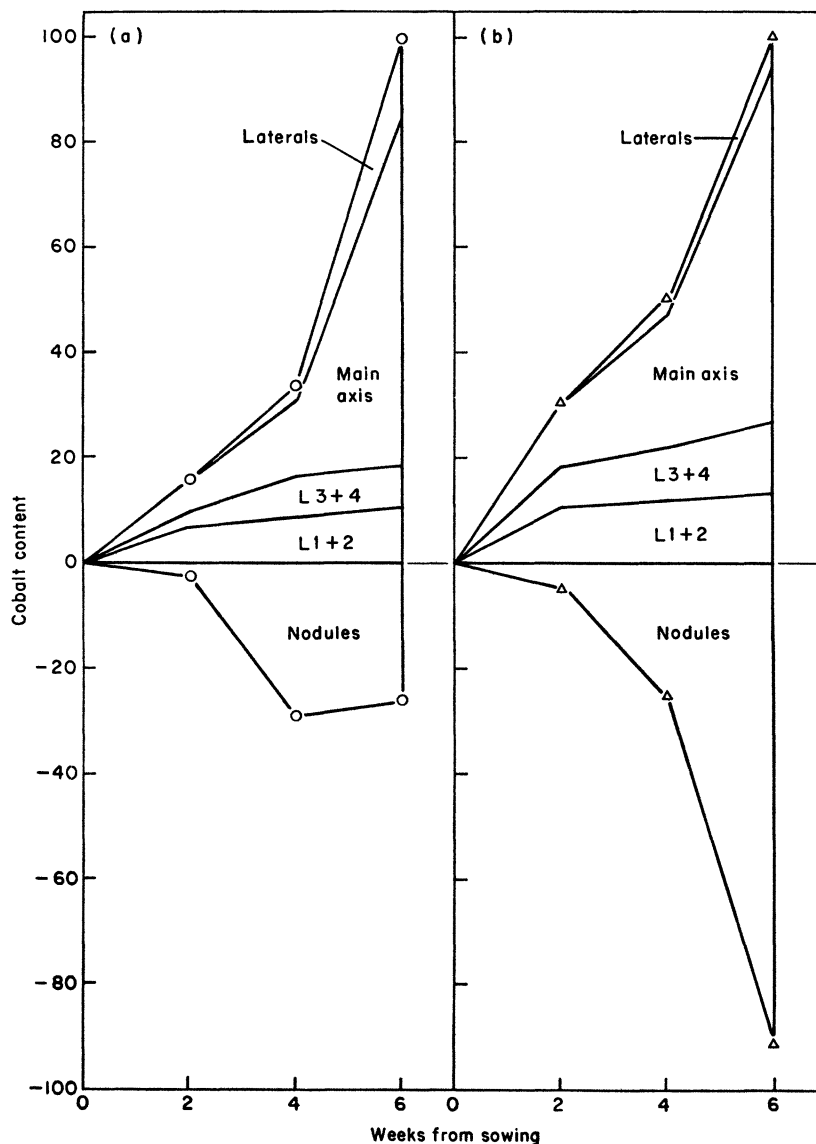


Fig. 5. Effect of cobalt application on cobalt distribution within *Lupinus angustifolius* inoculated with *Rhizobium lupini*. Cobalt content in whole tops at 6 weeks after sowing set at 100. (a) Cobalt applied (100 = 40.8 ng per plant); (b) no cobalt applied (100 = 8.2 ng per plant).

Hewitt and Bond, 1966). As in our experiment these data are derived from experiments with only two levels of cobalt application. Studies with more levels of cobalt application are required to determine accurately the cobalt concentration in nodules at which nitrogen fixation is limited.

The decreasing gradient in cobalt concentration from roots to nodules to shoots observed in our experiment irrespective of cobalt supply agrees generally with data for soybean (Ahmed and Evans, 1960), for subterranean clover (Wilson and Hallsworth,

1965) and for some non-leguminous symbiotic nitrogen fixing angiosperms (Bond and Hewitt, 1962; Hewitt and Bond, 1966). However the relative cobalt concentrations in roots and nodules differ among symbioses studied. Under cobalt deficiency, cobalt concentrations in nodules were less than those in roots of subterranean clover (Wilson and Hallsworth, 1965), for *Casuarina cunninghamiana* (Hewitt and Bond, 1966) and for *Myrica gale* (Hewitt and Bond, 1966) but not in *Alnus glutinosa* (Bond and Hewitt, 1962). However, in cobalt-adequate plants cobalt concentrations in nodules are less than those in roots only for *Alnus glutinosa* (Bond and Hewitt, 1962) and *Myrica gale* (Hewitt and Bond, 1966) but not in subterranean clover (Wilson and Hallsworth, 1965) and *Casuarina cunninghamiana* (Hewitt and Bond, 1966).

Table 2. *Effect of cobalt application on cobalt concentrations (ng per g dry wt) within plant tops of Lupinus angustifolius inoculated with Rhizobium lupini*

Weeks from sowing	- Co			+ Co		
	2	4	6	2	4	6
Plant part						
Leaves 1 + 2	11	15	18	36	63	83
Leaves 3 + 4	13	16	25	24	59	67
Leaves 5 + 6	—*	nd†	18	—*	nd	61
Rest main axis leaves	np†	8	10	np	26	38
Laterals	np	10	9	np	35	30
New growth	8	7	11	16	23	36
Stem	4	4	12	12	8	12

* At first harvest new growth was leaves 5 + 6.

† np, Not present.

‡ nd, Not determined.

The differences in cobalt concentrations among tops, roots and nodules in lupins (this study; Chatel *et al.*, 1978) are more marked than those for most other symbioses. In particular cobalt concentrations in lupin nodules are much lower than those in roots for both cobalt-deficient and cobalt-adequate plants.

Since concentrations in lateral nodules appear to be greater than those in top nodules (Chatel *et al.*, 1978; Dilworth *et al.*, 1979) and more similar to root concentrations, differences among symbioses in the magnitude of the difference between root and nodule concentrations may reflect differences in the proportion of lateral to tap nodules. For lupins, however, cobalt concentration in lateral nodules were generally less than those in roots.

In lupins cobalt concentrations and contents in leaves increased with age. Additionally cobalt concentrations in old leaves were much greater than those in young leaves. These findings contrast with observations obtained with radioactive cobalt in pasture plants (lucerne, subterranean clover) given adequate cobalt (Handreck and Riceman, 1969). In many plant species, but perhaps not in lupins, cobalt appears to be readily mobile in the phloem. Cobalt painted on leaves moved to root tips (Gustafson, 1956) and nodules (Wilson and Hallsworth, 1965; Small *et al.*, 1967). However, with lupins cobalt continued to accumulate in old leaves although nodules were cobalt-deficient. The difference between lupins and other plant species may be a characteristic of that species. Manganese is poorly retranslocated in lupins (Hocking *et al.*, 1977) but may be readily translocated in other species (Williams and Moore, 1952).

However, in subterranean clover we have also observed a similar gradient in cobalt concentration from old to young leaves (McGrath, Robson and Dilworth, unpublished).

Distribution patterns obtained with ^{60}Co may reflect the distribution of recently added radioactive cobalt rather than that of total cobalt. In the study of Handreck and Riceman (1969) radioactive cobalt was added 22 days after sowing, 10 days before distribution was assessed. As macro nutrient salts were not purified cobalt would have been present prior to the addition of the labelled cobalt. Handreck and Riceman do not state the number of leaves present nor the cobalt concentrations of leaves at the time of addition of cobalt.

Continuity of supply may also affect distribution patterns. If initial concentrations of cobalt in solution are depleted by plant uptake cobalt concentrations in old leaves may exceed those in young leaves. A different pattern may be obtained if the supply is maintained. With lupins the distribution of cobalt within plant tops differed little in cobalt-deficient and cobalt-sufficient plants.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Mr K. Snowball and Miss D. Overhue for technical assistance and to the W.A. State Wheat Research Committee and to the Wheat Industry Research Council for financial support.

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