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Which stage of nodule initiation in *Lupinus angustifolius* L. is sensitive to iron deficiency?

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SUMMARY

Transfer experiments in solution culture were conducted to establish the stage of nodulation in lupins (*Lupinus angustifolius* L. cv. Yandee) most sensitive to iron deficiency. In all experiments, iron deficiency had a much greater effect on nodule number than on host plant growth.

Irrespective of the iron treatment of either the *Bradyrhizobium* or the lupin plant prior to inoculation, plants receiving 2·5 μM iron after inoculation successfully formed nodule initials and nodules while those receiving 0·05 μM iron almost completely failed to initiate nodules. Thus, the prevention of nodulation by iron deficiency is not a consequence of either an inadequate number of infective bradyrhizobia surviving in the solution or an alteration in the iron status of the host root.

Supply of 2·5 μM iron for 4 d or more after inoculation produced a similar number of nodule initials and nodules as did continuous supply of 2·5 μM iron. Delaying supply of 2·5 μM iron for 3 d or less after inoculation did not delay or prevent nodule initiation and formation. One-day exposure of plants to 2·5 μM iron on day 4 after inoculation induced the highest number of nodules of any 1 d treatment although this short exposure was not enough to allow the full complement of nodules to form. Hence, the impairment of the nodulation process by iron deficiency can be attributed to the prevention of a step at day 4, the stage just before nodule initials are formed.

A further study was conducted to examine the effect of iron on nodule formation by using a vertical split-root technique in which *Bradyrhizobium* sp. (*Lupinus*) was added to the upper compartment. Compared to plants receiving 0·05 μM iron in both compartments, plants receiving 0·05 μM in the upper and 5 μM iron in the lower compartments had only a slightly higher concentration of iron in the cortex of the upper part of the root. Furthermore, supplying 5 μM iron to the lower part of a root did not permit nodulation on the upper part of the root receiving 0·05 μM iron. Low concentration of iron in the cortex of roots may limit nodule formation.

Key words: *Lupinus angustifolius*, iron deficiency, nodule initiation, vertical split-root, iron translocation.

INTRODUCTION

In lupins, iron appears to be required in greater amounts for nodule formation than for host plant growth (Tang, Robson & Dilworth, 1990*a*) and the effect of iron on nodule formation is direct (Tang, Robson & Dilworth, 1990*b*). Iron deficiency markedly depressed the number of nodule initials and thereafter the number of visible nodules. Nodule initiation seems to be more sensitive than subsequent nodule development to iron deficiency. For iron-sufficient plants, appearance of nodule initials commenced on day 5 after inoculation and was completed within a further 3 d (Tang *et al.*, 1990*b*). The impairment of nodulation by iron deficiency in lupins must therefore occur at an early stage of nodule initiation.

In a horizontal split-root experiment, Tang *et al.* (1990*b*) found that nodule initials and nodules were formed only on that half of the root system exposed to a high concentration of iron. Iron was not translocated from the half of the root supplied with a high concentration of iron to the other half of the root, receiving a low concentration of iron. In addition, iron applied to leaves as foliar sprays was not translocated to the roots and nodules were not produced (Tang *et al.*, 1990*b*). These results did not specify whether iron within the roots or in the external solution is required for nodule formation.

The aim of the work described here was to

examine whether nodule initiation might be restricted by inadequate numbers of bradyrhizobia or uninfective forms resulting from a low concentration of iron in the plant growth medium or, alternatively, whether the iron status of the plant root at inoculation might limit nodule initiation. In addition, we report the effects of imposing a chosen level of iron at particular times during the nodulation process on the number and position of the subsequent nodules. Finally, we examine the hypothesis that the effect of iron on nodule formation is external, using a technique where upper and lower segments of a root system were supplied with different concentrations of iron.

MATERIALS AND METHODS

Transfer experiments

All experiments were conducted in a glasshouse, in solution culture with roots maintained at 20–22 °C. Uniform-sized seeds of *Lupinus angustifolius* L. cv. Yandee were germinated on a stainless-steel screen sitting over an aerated solution of 600 μM $CaCl_2$ and 2 μM H_3BO_3 for 4–7 d. Seedlings were then transplanted to 5 l pots containing continuously aerated nutrient solution. The composition of the basal nutrient solution was as previously described (Tang *et al.*, 1990*a*). Iron was applied as the ferric monosodium salt of ethylenediamine tetraacetic acid (Fe^{III}NaEDTA) at concentrations of 0·05 μM (inadequate) or 2·5 μm (adequate). A dense suspension of *Bradyrhizobium* sp. (*Lupinus*) WU 425 from a 5- or 6-d-old culture (Tang *et al.*, 1990*a*) was added to the solution at a rate of 3×10^5 cells ml^{-1}. The pH of the solution was adjusted daily to 5·5 with 0·1 M KOH. Nutrient solutions were changed every second day except where transfers were made. In all experiments day 0 is the day that plants first came in contact with *Bradyrhizobium*. Subsequent treatments of plants in individual experiments are summarized diagrammatically in Tables 1–5. Nodule initials were examined as described by Tang *et al.* (1990*a*).

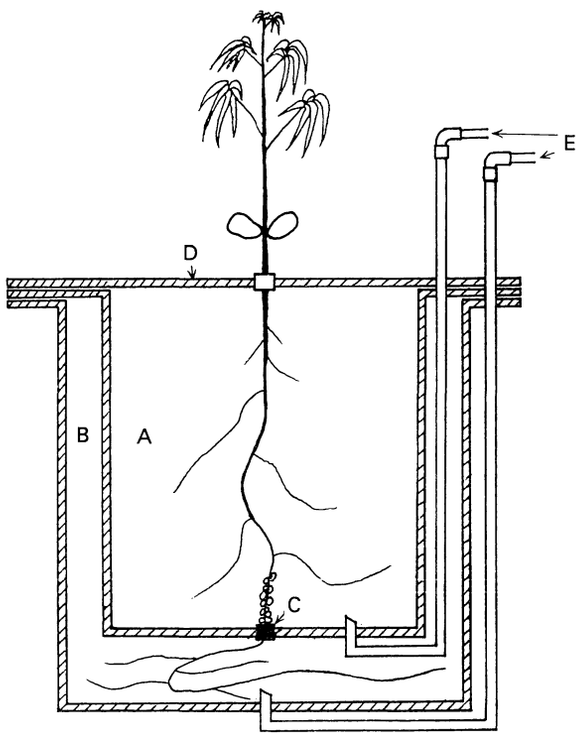


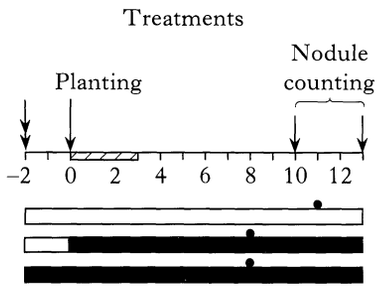
Figure 1. A vertical split-root system for growing *Lupinus angustifolius*. A, upper compartment; B, lower compartment; C, putty; D, lid; E, aeration system.

Vertical split-root experiment

Lupin seeds were germinated on a stainless steel screen over an aerated solution of 600 μM $CaCl_2$ and 2 μm H_3BO_3 for 5 d. Ten seedlings were then transferred to 5 l plastic pots containing basal nutrient solution and 0·05 μM iron. As the roots of lupins can be infected by bradyrhizobia only on a very small portion of the root just behind root tips, and the sensitive stage of nodule formation is at day 4 after inoculation, an inoculum of *Bradyrhizobium* was added to the solution for 2 d before iron treatments were applied. One seedling was finally transplanted into each split-root system which consists of two differently-sized cylindrical compartments (Fig. 1). The upper compartment was positioned in the lower compartment. The volumes of the upper and the lower compartments were 460 and 570 ml respectively. The plants were supported with a lid sitting over the upper compartment. The growing root was allowed to protrude by 2 cm through a hole at the bottom of the upper compartment into the solution of the lower compartment. The root parts in the two compartments were kept separate with Terostat putty (Teroson GmbH, Heidelberg, West Germany). The compartments were filled with basal nutrient solution and iron treatments were imposed at transplanting: 5 μM iron (as Fe^{III}NaEDTA) for an adequate level and 0·05 μM iron for a deficient level. A dense suspension of *Bradyrhizobium* WU425 (Tang *et al.*, 1990*a*) was added to the upper compartment of the system at a density of 3×10^5 cells ml^{-1}, providing the four treatments (Table 1), each of which had five replicates. The solution was left unchanged for 3 d after which it was renewed every third day without further addition of bradyrhizobia. The pH of the solution was adjusted to 5·5 with 0·1 M KOH twice a day throughout the experiment.

Plants were harvested 15 d after iron treatments had been applied. To remove dust, whole shoots were rinsed in deionized water, washed in 0·1 M HCl and again rinsed in deionized water. Roots were rinsed in deionized water twice. Plant materials were separated into the youngest fully expanded leaf blade (YEB), shoots, cortex of the taproots, stele of the

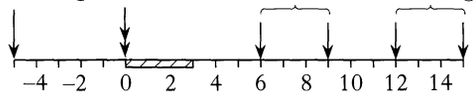
Table 1. *Effects of iron applied to bradyrhizobial suspensions in solution culture before and after introduction of* Lupinus angustifolius. *Preinoculation treatments were imposed on the fully grown inoculum of* Bradyrhizobium *WU 425 in the nutrient solution for 2 d*

Treatments	Nodules plant^{-1}		Top fresh weight (g plant^{-1})	Root fresh weight (g plant^{-1})
	Day 10	Day 13	Day 13	Day 13
				
	0b	0·2b	1·75a	2·23b
	11a	36a	1·99a	2·53a
	12a	39a	1·79a	2·57a

The horizontal scale is time in days. Symbols on the horizontal scale are: ▨, the period of plant contact with bradyrhizobium; □, 0·05 μM iron in solution; ■, 2·5 μM iron in solution; ↓, addition of *Bradyrhizobium* to the solution; ●, the time of nodule appearance.

Nodule number was analysed as $\log_{10}$. Values in each column followed by the same letter are not different at $P = 0{\cdot}05$.

Table 2. *Effect of adequate supply of iron, before and after inoculation, on nodule formation on* Lupinus angustifolius. *Preinoculation treatments were imposed on 5-d-old seedling for 5 d*

Treatments	Nodule initials plant^{-1}		Nodules plant^{-1}		Fresh weight of shoot (g plant^{-1})		Fresh weight of root (g plant^{-1})	
	Day 6	Day 9	Day 12	Day 15	Day 12	Day 15	Day 12	Day 15
								
	0b	1c	1b	4c	1·62a	1·95a	1·40a	2·51a
	3a	4b	4b	13b	1·68a	2·00a	1·64a	2·35a
	3a	16a	27a	71a	1·77a	2·01a	1·76a	2·43a
	4a	18a	27a	76a	1·76a	2·14a	1·67a	2·69a

The horizontal scale is time in days. Symbols on the horizontal scale are: ▨, the period of inoculation with *Bradyrhizobium*; □, 0·05 μM iron in solution; ■, 2·5 μM iron in solution; ↓, addition of *Bradyrhizobium* to the solution; ●, the time of nodule appearance.

Number of nodule initials and nodules was analysed as $\log_{10}$ and data in each column with a common letter are not significantly different at $P = 0{\cdot}05$.

taproots, lateral roots and nodules. All plant tissues were oven-dried at 68 °C.

Total iron concentration in tissues were measured using atomic absorption spectrophotometry after digesting plant materials in a 4:1 mixture (by volume) of concentrated nitric and perchloric acids (Johnson & Ulrich, 1959).

RESULTS

General effects of iron-deficiency

In all transfer experiments, plants grown in solution containing 0·05 μM iron showed foliar symptoms of iron deficiency about 2 wk after germination. The symptoms of iron deficiency were delayed for 3–6 d in the case of plants transferred from 2·5 μM iron to 0·05 μM (treatments described in Tables 2, 3 and 5), but no symptoms were observed for plants continuously given 2·5 μM iron. Slight nitrogen deficiency occurred in all plants during the late stages of all experiments.

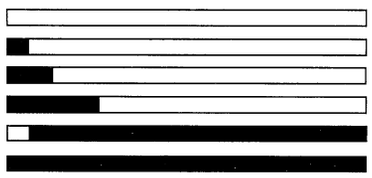
The fresh weight of shoots was not affected by iron treatments in any experiment (Tables 1–5). The fresh weight of roots exposed to 0·05 μM iron or exposed to 2·5 μM iron for only 1 d was less than that of those grown continuously with 2·5 μM iron at the latest harvest but not at earlier harvests (Tables 1–5).

Under iron-sufficient conditions, nodules appeared 8–9 d after inoculation. Nodules were formed on a narrow region of roots just behind the root tips at the time of inoculation. Under iron-deficient conditions, many brown points were clearly observed on the narrow region of roots just behind the root tips at the time of inoculation.

Treatment of Bradyrhizobium *with iron prior to inoculation.*

Whether or not *Bradyrhizobium* cells were supplied with 2·5 μM iron in solution for 2 d before plants were introduced, the lupins subsequently receiving 2·5 μM iron produced over 10 and 30 nodules each at

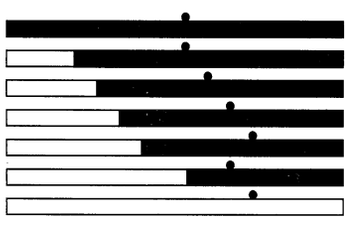
Table 3. *Effect of withdrawal of adequate iron or delayed supply of adequate iron on the number of nodule initials and nodules, and growth of* Lupinus angustifolius

Treatments: Planting; Counting nodule initials; Nodule counting (0 2 4 6 8 10 12 14 16)	Nodule initials plant^{-1}	Nodules plant^{-1}		Fresh weight of shoot (g plant^{-1})		Fresh weight of root (g plant^{-1})	
	Day 8	Day 12	Day 16	Day 12	Day 16	Day 12	Day 16
	2b	1b	4b	1·47a	2·03a	1·51a	2·23ab
	3b	2b	4b	1·54a	1·77a	1·63a	2·14b
	2b	2b	5b	1·57a	1·74a	1·58a	2·00b
	17a	12a	26a	1·54a	1·70a	1·55a	2·25ab
	21a	20a	45a	1·59a	2·08a	1·53a	2·80a
	21a	26a	38a	1·71a	1·95a	1·79a	2·55a

The horizontal scale is time in days. Symbols on the horizontal scale are: ▨, the period of inoculation with *Bradyrhizobium*; □, 0·05 μM iron in solution; ■, 2·5 μM iron in solution; ↓, addition of *Bradyrhizobium* to the solution.

Number of nodule initials and nodules was analysed as $\log_{10}$, and data with a common letter are not different at $P = 0{\cdot}05$.

Table 4. *Effects of delayed supply of adequate iron on nodulation and growth of* Lupinus angustifolius *at day 15 after inoculation*

Treatments: Planting; Nodule counting (0 2 4 6 8 10 12 14)	Nodules plant^{-1}	Nodule position* (cm)	Top fresh weight (g plant^{-1})	Root fresh weight (g plant^{-1})
	35a	11·8c	2·18a	3·47a
	38a	12·5c	1·97a	3·26a
	37a	16·5b	2·07a	3·36a
	24a	18·3ab	2·20a	3·44a
	9b	21·3a	2·15a	3·56a
	1c	na	1·96a	3·07a
	0·5c	na	1·80a	2·85a

The horizontal scale is time in days. Symbols on the horizontal scale are: ▨, the period of inoculation with *Bradyrhizobium*; □, 0·05 μM iron in solution; ■, 2·5 μM iron in solution: ↓, addition of *Bradyrhizobium* to the solution; ●, the time of nodule appearance.

* Nodule position refers to the distance between hypocotyl of roots and the site of earliest nodules formed on the roots; Data were the average of 10 plants.

na, not applicable since plants in these two treatments almost completely failed to nodulate.

Nodule number (day 15 after inoculation) was analysed as $\log_{10}$. Values in each column followed the same letter are not different at $P = 0{\cdot}05$.

days 10 and 13, respectively. However, lupins failed to nodulate if they received 0·05 μM iron throughout (Table 1).

Treatment of plants with iron prior to inoculation

Plants continuously given 2·5 μM iron, and plants given 0·05 μM iron before but 2·5 μM iron after inoculation, produced a similar number of nodule initials and nodules. These numbers were much greater than those on plants either receiving 0·05 μM iron continuously or receiving 2·5 μM iron before but 0·05 μM iron after inoculation. Plants receiving 2·5 μM iron before but 0·05 μM iron after inoculation had slightly more nodules than those continuously receiving 0·05 μM iron (Table 2). However, on plants given 0·05 μM iron after 2·5 μM iron, nodules were distributed in a very narrow region of the taproot, with 90% of nodules within 4 cm of the topmost nodule, while plants receiving 2·5 μM iron after inoculation had 50% of their nodules more than 4 cm below the topmost nodule.

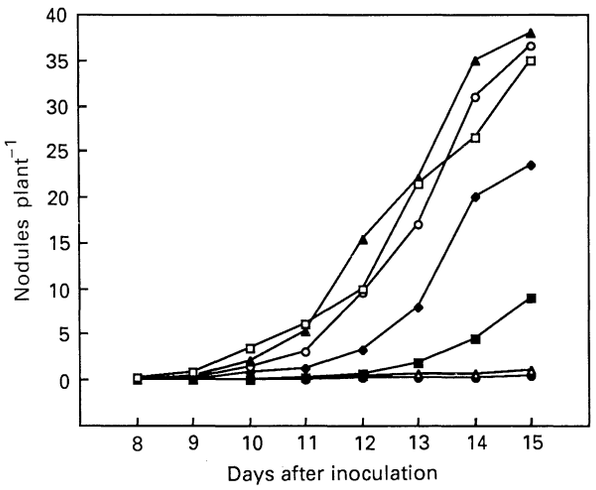


Figure 2 Effects on the number of nodules on *Lupinus angustiofolius* of delaying supply of 2·5 μM iron for 0 (□), 3 (▲), 4 (○), 5 (◆), 6 (■) or 8 (△) d after inoculation. Control plants received a continuous supply of 0·05 μM iron (●).

Withdrawal of iron or delayed supply of adequate iron

Plants given 2·5 μM iron for 4 d following inoculation produced a similar number of nodule initials and nodules to those receiving 2·5 μM iron continuously. However, supplying 2·5 μM iron for only 1 or 2 d markedly decreased the number of nodule initials and nodules; plants almost failed to nodulate, and were similar to those given 0·05 μM iron continuously. Delaying the supply of 2·5 μM iron for 1 d after inoculation did not decrease the number of nodule initials and nodules (Table 3).

Plants supplied with continuous 2·5 μM iron produced nodules 8 d after inoculations and produced more than 30 nodules (Fig. 2, Table 4). However, delaying supply of 2·5 μM iron for 4, 5 and 6 d following inoculation delayed the subsequent appearance of nodules by 1, 2 and 3 d respectively. Delaying supply of 2·5 μM iron for 8 d almost completely prevented nodule formation; plants behaved similarly to those receiving a continuous supply of 0·05 μM iron. In contrast, delaying supply of 2·5 μM iron for 3 d did not delay nodule appearance and did not decrease nodule number (Fig. 2). Nodule number was decreased when supply of 2·5 μM iron was delayed for 5 d or more, and nodules occurred lower on the taproot when supply of 2·5 μM iron was delayed for 4 d or more following inoculation (Table 4).

Short exposures to adequate iron

Where plants were given 2·5 μM iron only for 1 d rather than continuously, nodule appearance was delayed and nodule formation was almost prevented. An exception to this behaviour was seen in plants exposed to 2·5 μM iron for 24 h on day 4 after inoculation. In these plants nodule appearance was not delayed and they induced more nodules than plants receiving 2·5 μM iron on any other single day. However, the number of nodules was still less than that on plants receiving a continuous supply of 2·5 μM iron (Table 5).

Iron supply to different parts of the split-root system

Slight chlorosis of young leaves was observed in plants receiving 0·05 μM iron in both compartments on day 6 after treatments had been imposed. In these plants, this symptom persisted throughout the experiment, but did not appear for any other treatment. The fresh weights of shoots and roots, the number of lateral roots and the lengths of taproots were unaffected by the iron treatments (Table 6).

Table 5. *Effects of short exposure to adequate iron on nodulation and growth of* Lupinus angustifolius *inoculated with* Bradyrhizobium

Treatments	Nodules plant^{-1}			Top fresh weight (g plant^{-1})	Root fresh weight (g plant^{-1})
Planting / Nodule counting	Day 9	Day 12	Day 15	Day 15	Day 15
0 2 4 6 8 10 12 14					
	0·0 b	0·2 c	0·5 c	1·80 a	2·85 c
	0·0 b	0·4 b	0·7 c	1·97 a	2·92 b c
	0·4 a	1·2 b	2·2 b	1·95 a	2·85 c
	0·0 b	0·4 b	1·6 b	2·03 a	3·05 b c
	0·0 b	0·4 b	1·2 b	1·92 a	2·97 b c
	0·0 b	0·1 c	0·5 c	2·03 a	3·13 b
	0·8 a	9·0 a	35·0 a	2·18 a	3·47 a

The horizontal scale is time in days. Symbols on the horizontal scale are: ▨, the period of inoculation with *Bradyrhizobium*; □, 0·05 μM iron in solution; ■, 2·5 μM iron in solution: ↓, addition of *Bradyrhizobium* to the solution; ●, the time of nodule appearance.

Nodule number was analysed as $\log_{10}$. Values in each column followed by the same letter are not different at $P = 0{\cdot}05$.

Table 6. *Effect of iron supply on growth and nodulation of* Lupinus angustifolius *in the vertical split-root system*

Concentrations of iron in solution (μM)		Upper compartment (UC)				Lower compartment (LC)			
UC	LC	Fresh weight of shoots (g plant^{-1})	Nodules plant^{-1}	Lateral roots plant^{-1}	Fresh weight of roots* (g plant^{-1})	Nodules plant^{-1}	Length of taproot (cm)	Lateral roots plant^{-1}	Fresh weight of roots (g plant^{-1})
0·05	0·05	2·37	0·2 (0·06)†	33	1·23	1·0 (0·20)	46	75	1·24
0·05	5	2·35	0·2 (0·06)	27	1·14	8·6 (0·73)	46	71	1·81
5	0·05	2·29	19·2 (1·23)	20	1·21	2·2 (0·37)	48	71	1·34
5	5	2·38	20·6 (1·32)	28	1·17	9·8 (0·99)	50	65	1·28
Significance at $P = 0·05$		n.s.	(0·27)	n.s.	n.s.	(0·59)	n.s.	n.s.	n.s.

* Fresh weight of roots includes nodules where applicable.
† Figures in parenthesis are $\log_{10}(x+1)$ transformation.
n.s., not significant.

Roots directly exposed to an adequate level of iron (5 μM) in solution in the upper compartment had large number of nodules, while those grown in the solution with a deficient level (0·05 μM) almost failed to nodulate, irrespective of the concentration of iron in the solution surrounding the other part of the root system (Table 6). Because the lower 2 cm segment of the root had been pretreated with bradyrhizobia, nodules were also formed on the lower part of the roots exposed to 5 μM iron.

Plants grown in 0·05 μM iron in both compartments had an iron concentration in YEB below the critical level of 65 μg (g d wt)$^{-1}$ required for chlorophyll synthesis (Tang *et al.*, 1990*a*). Plants grown in the other three treatments had iron concentrations in YEB above this critical level. However, plants treated with 5 μM iron in the lower or both compartments had greater iron concentrations in YEB than those in plants treated with 5 μM iron in the upper compartment only. Concentrations of iron in whole shoots followed a similar trend to those in YEB (Table 7).

The concentrations of iron in the cortex of taproots, lateral roots or whole roots of the upper part exposed to 5 μM iron were more than twice as high as those in the same tissues exposed to 0·05 μM iron. The upper part of the root of plants given 0·05 μM iron in the upper compartment and 5 μM in the lower compartment had a slightly higher concentration of iron in the cortex than that of plants given 0·05 μM iron in both compartments. The concentrations of iron in the stele of the upper part of the roots were higher when the lower or both compartments received 5 μM iron than when the upper compartment received 5 μM or when both compartments received 0·05 μM iron. Iron concentrations in the cortex were much higher than those in the stele for all treatments (Table 7).

DISCUSSION

The nodulation process of *L. angustifolius* is apparently most sensitive to iron deficiency 4 d after inoculation with *Bradyrhizobium*, just before nodule initials are beginning to be formed (Tang *et al.*, 1990*b*). It is possible therefore that impairment of nodulation by iron deficiency is due either to a limiting effect on the stimulation of cell division in host roots by *Bradyrhizobium*, which normally establishes the meristem of nodule initials, or to inhibition of the proliferation of *Bradyrhizobium* within the roots. However, this has not been firmly established.

The limitation of nodule formation does not seem to be caused by a limited number of *Bradyrhizobium* in iron-deficient solution. Bradyrhizobia supplied at a rate of 3×10^5 cells ml^{-1}, incubated for 2 d with either inadequate iron or adequate iron, could successfully nodulate lupins, as long as adequate iron

Table 7. *Effect of iron supply on iron concentrations ($\mu g\ g^{-1}$ d. wt) in the youngest fully expanded leaf blades (YEB), shoots, roots and nodules of* Lupinus angustifolius *grown in the vertical split-root system*

Concentrations of iron in culture solution (μM)				Upper compartment (UC)					Lower compartment (LC)		
UC	LC	YEB	Shoots	Root cortex	Root stele	Lateral root	Whole root	Nodules	3-cm segment*	Root below 3-cm segment	Whole root
0·05	0·05	55	41	144	52	115	95	†	164	130	138
0·05	5	150	83	181	69	148	116	†	280	243	255
5	0·05	80	54	429	59	411	228	302	172	130	140
5	5	148	96	479	66	457	251	378	365	364	366
LSD ($P = 0·05$)		29	13	92	9	167	32		62	87	75

* 3 cm segment of roots from the putty that separated two compartments.
† Insufficient sample.

was applied after inoculation (Table 1). Although iron deficiency might have affected the growth or survival of the introduced bradyrhizobia, the numbers of surviving cells were still adequate for successful nodulation. In field conditions, O'Hara *et al.* (1988) found that the numbers of peanut *Bradyrhizobium* sp. in the rhizosphere of peanuts were not limited in an iron-stress soil in which seedlings developed symptoms of iron deficiency.

Internal iron status of host roots may not be an important factor in limiting nodule formation. Pretreating plants with adequate iron for 5 d allowed the formation of only 15–22 % of the nodule initials and nodules found on plants given adequate iron after inoculation. The occurrence of slightly more nodule initials and nodules on the pretreated plants than on those receiving inadequate iron could be due to the presence of iron remaining in the apoplast after iron was withdrawn from the solution.

Lupins could nodulate successfully if adequate iron was applied for 4 d or more after inoculation. This period of adequate iron supply seems to be enough to establish nodule initials. Nodules developed from these nodule initials even if no more iron was supplied. In a previous study, Tang *et al.*, (1990*b*) found that the response of nodule number to iron supply had the same pattern as that of nodule initials and suggested that nodules could develop successfully from nodule initials in the roots under conditions of both iron-deficiency and iron-sufficiency.

Delaying supply of adequate iron for 3 d or less after inoculation neither decreased nodule number nor delayed nodule appearance, possibly indicating that iron deficiency did not limit root colonization and/or infection by *Bradyrhizobium*. In the present study, many brown points were observed on the taproot of iron-deficient plants. If these are infection sites that die from iron deficiency, the formation of nodule initials could eventually be prevented.

Nodule formation was severely restricted where adequate iron was provided for only a 1 d period, implying that the iron-dependent steps in the process of nodule initiation require more than 1 d. Nevertheless, plants treated in this way gave a clear indication of the stage in the process of nodule initiation that is most sensitive to iron deficiency. One-day supply of adequate iron on day 4 after inoculation yielded the highest number of nodules as compared to that on any other day. The results further confirm the earlier findings that the most sensitive stage is at day 4 (Table 3, Table 4).

In the vertical split-root system, nodules were produced only on that part of the root where adequate iron was present, regardless of whether or not the other part of the root or the shoot received sufficient iron. The results are in agreement with the finding that the limitation of nodulation in lupins by iron deficiency is independent of iron status in the shoot or growth of the host (Tang *et al.*, 1990*b*). Using the vertical split-root technique, we expected that iron would be translocated from the lower part of the root exposed to a high concentration of iron to the upper part of the root exposed to a low concentration of iron, and high concentrations of iron in the upper part of the root would be obtained. However, our study showed that the concentration of iron in the upper part of the root given a low concentration of iron in solution only slightly increased when the lower part of the root was given a high concentration of iron. This increased iron in the upper part of the root is insufficient for successful nodule formation (Tang *et al.*, 1990*a*). It cannot therefore categorically be concluded that the effect of iron on nodule formation in lupins is external. In a horizontal split-root experiment, Tang *et al.* (1990*b*) found that iron was not translocated from the iron-adequate half of the root to the iron-inadequate half and that nodules were only formed on that half of the root of lupins where adequate iron was applied. Foliar application of iron did not increase the concentration or content of iron in the roots (Tang *et al.*, 1990*b*). However, in corn plants, a pronounced translocation of iron towards root tips occurs in the

phloem (Kashirad, Marschner & Richter, 1973), and foliar-applied iron is also translocated to roots in peanut (O'Hara *et al.*, 1988) and other plants (Brown, Yamaguchli & Leal-Diaz, 1965). The mobility of iron in phloem may vary with plant species. One way to study the effects of internal iron on nodulation in lupins may be to use seeds with different iron concentrations.

The process of nodulation in many legumes includes the curling of root hairs and the formation of infection threads through root hairs. However, in lupin plants these phenomena have not yet been observed (Dart, 1977; Quispel, 1983), and the process of lupin nodulation remains unclear. How iron deficiency interferes with events of the sensitive stage is therefore not understood.

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REFERENCES

Brown, A. L., Yamaguchli, S. & Leal-Diaz, J. (1965). Evidence for translocation of iron in plants. *Plant Physiology* **40**, 35–38.

Dart, P. J. (1977) Infection and development of leguminous nodules In: *A Treatise on Dinitrogen Fixation* (Ed. by R. W. F. Hardy & W. S. Silver), pp. 367–472. John Wiley & Sons, New York.

Johnson, C. M. & Ulrich, A. (1959). Analytical methods for use in plant analysis. *California Agricultural Experiment Station Bulletin* No. 766.

Kashirad, A., Marschner, H. & Richter, C. (1973). Absorption and translocation of ^{59}Fe from various parts of the corn root. *Zeitschrift für Pflanzenernahrung und Bodenkunde* **134**, 136–147.

O'Hara, G. W., Dilworth, M. J., Boonkerd, N. & Parkpian, P. (1988). Iron deficiency specifically limits nodule development in peanut inoculated with *Bradyrhizobium* sp. *New Phytologist* **108**, 51–57.

Quispel, A. (1983). Dinitrogen-fixing symbiosis with legumes, non-legume angiosperms and associative symbioses. In: *Encyclopedia of Plant Physiology* (new series), vol. 15A (Ed. by A. Läuchli & R. L. Bieleski), pp. 286–329. Springer-Verlag.

Tang, C., Robson, A. D. & Dilworth, M. J. (1990*a*). The role of iron in nodulation and nitrogen fixation in *Lupinus angustifolius L. New Phytologist* **114**, 173–182.

Tang, C., Robson, A. D. & Dilworth, M. J. (1990*b*). A split-root experiment shows that iron is required for nodule initiation in *Lupinus angustifolius* L. *New Phytologist* **115**, 61–67.