Microscopic evidence on how iron deficiency limits nodule initiation in *Lupinus angustifolius* L.

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**Summary**

Lupins (*Lupinus angustifolius* L. cv. Yandee), grown in solution culture, have been used to study the sites and process of infection by *Bradyrhizobium* sp. (*Lupinus*) and the impairment of nodulation by iron deficiency. Infection leading to nodulation occurred in an area of epidermal cells either lacking root hairs or with very young root hairs at the time of inoculation. Cells aged 13 h or over appeared not to be infected. Infection was initiated in the outer cortex. Rare, short infection threads were evident on day 4 after inoculation, 2 d after the initial division of cortical cells resulting from the bradyrhizobial inoculation. Bacteria had been released into the cytoplasm of cortical cells within 5 d after inoculation. Bacteroids multiplied in the cytoplasm, segregated passively and spread in the infection zones by repeated division of the invaded cells.

Under iron deficiency, initial cell division occurred in the outer cortex of host roots, as in iron-sufficient plants after inoculation. Iron deficiency then limited further division of cortical cells. Only a few surviving infection sites developed nodules with normal structure but development was much slower than in iron-sufficient plants.

Key words: Iron deficiency, *Bradyrhizobium* infection, *Lupinus angustifolius*, anatomy, ultrastructure.

**Introduction**

In many legumes, the first step in nodule formation involves rhizobial-induced deformation of host root hairs, the bacteria entering the roots by forming infection threads originating in the curled tips of root hairs. The infection threads, which contain bacteria and are enclosed by a cellulose wall, develop to the base of infected root hairs and then penetrate the root cortex. The rhizobial symbionts stimulate host cell division to form meristems and enter newly divided host cells through the growth and branching of the infection threads. Bacteria are released into cytoplasm through a degradation of the infection thread wall or folding of host plasmamembrane (Nutman, 1956; Dart, 1974, 1977; Dazzo, 1980; Meijer, 1982). In some tropical legumes, e.g. *Arachis hypogaea* (Chandler, 1978) and *Stylosanthes* spp. (Ranga Rao, 1977; Chandler, Date & Roughley, 1982) bradyrhizobia infection occurs at the junction of the lateral roots without the formation of infection threads either in the root hairs or in the nodules. The bradyrhizobia enter the cells through the structurally altered cell wall. In some woody legumes, epidermal infection appears likely. Sprent (1989) describes rhizobial penetration between epidermal cells in a manner similar to that for *Frankia* infection of the non-legume plant *Elaeagnus*. Rhizobial infection in *Mimosa scabrella* has been confirmed as being of this type (Faria, Hay & Sprent, 1988).

Little is known regarding the site of infection and the processes involved in nodule initiation in lupins, no infection threads having been observed in early studies either in root hairs (Dart, 1977; Quispel, 1983) or in nodules (Dart, 1977). However, in developing lupin nodules, infection threads have been subsequently found in nodule tissue, each with wall material continuous with the cell wall of the plant (Robertson et al., 1978).

In lupins, iron is required in a greater amount for nodule formation than for host plant growth (Tang, Robson & Dilworth, 1990a). Nodules were not formed on those roots directly exposed to a solution with deficient iron, irrespective of whether the shoot or the rest of the root system was iron-sufficient (Tang, Robson & Dilworth, 1990b, 1991). Iron
Infection site. Irrespective of the age of the seedling at the time of inoculation, the rate of root elongation of all plants was very similar (about 1.9 mm h⁻¹). However, the total number of nodules deficiency markedly decreased the number of nodule initials and thereafter the number of nodules. In iron-sufficient plants, the formation of nodule initials commenced on day 5 after inoculation and was completed within three days (Tang et al., 1990b). With transfer experiments, Tang et al. (1991) found that the impairment of the nodulation process by iron deficiency could be attributed to the prevention of a step at day 4 after inoculation, the stage just before nodule initials were formed. However, how iron deficiency interferes with the events of nodulation is not understood.

The present study had three aims: (i) to identify the location of the infection site in lupins by marking the positions of the smallest emergent root hairs and the root tips at the time of inoculation and examining the distribution of nodules, (ii) to examine the time course of the infection process using light and transmission electron microscopy and (iii) to study how iron deficiency impairs the process of nodulation in Lupinus angustifolius L.

### MATERIALS AND METHODS

#### Infection site

Seeds of narrow-leafed lupin (Lupinus angustifolius L. cv. Yandee) were germinated on a stainless steel screen over an aerated solution of 600 µM CaCl₂ and 2 µM H₂BO₃ for 3, 5 or 8 d. Five seedlings were transferred to 5-l plastic pots containing an aerated nitrogen-free nutrient solution with concentrations (µM) of KH₂PO₄, 20; K₂SO₄, 600; MgSO₄, 200; CaCl₂, 600; Fe⁺³NaEDTA, 2.5; H₃BO₃, 5; Na₃MoO₄, 0.03; ZnSO₄, 0.75; MnSO₄, 1.0; CoSO₄, 0.2; and CuSO₄, 0.2, and roots were maintained at 20–22 °C. At the time of transplanting, the positions of the smallest emergent root hairs and the root tips of the plants were determined by inspection through a dissecting microscope and marked with a waterproof pen on pieces of plastic which were attached to the plants. The length of the taproot and the distance between the smallest emergent root hairs and the root tips were recorded (Fig. 1). A suspension of *Bradyrhizobium* sp. (*Lupinus*) WU 425 (Tang et al., 1990a) was then added to the solution in order to provide 3 x 10⁵ cells ml⁻¹. The solution was left unchanged for 3 d and then replaced with a similar solution with added bradyrhizobia. After another 2 d, plants were placed in solutions without added bradyrhizobia and the solution changed every second day. The pH of the solution was adjusted daily to 5.5 with 0.1 M KOH.

Twelve days after inoculation, plants were harvested and nodule distribution on the taproot was recorded. No lateral nodules were formed at this stage. The rate of root elongation was calculated from the increment of root length during the period of the experiment.

### Microscopy

Lupin seeds were germinated for 5 d as above. Five seedlings were then transferred to 5-l plastic pots containing an iron-free nutrient solution otherwise identical to the above experiment and roots were maintained at 20–22 °C. Iron was added as Fe⁺³NaEDTA at concentrations of 0.05, 0.25 or 2.5 µM. A dense suspension of *Bradyrhizobium* sp. (*Lupinus*) WU 425 was added to the solution. After 24 h, this was replaced by solution without added rhizobia, which was thereafter changed daily. A 0.1-cm segment of root behind the mark was sampled for microscopy, and the appearance of nodules was recorded.

Small pieces of harvested root tissue were fixed in 2.5 % (v/v) glutaraldehyde in 0.025 M phosphate buffer (pH 7) and post-fixed in 1 % (w/v) osmium tetroxide for 2 h at room temperature. The specimens were dehydrated in increasing acetone concentrations and embedded in Spurr’s epoxy resin (Spurr, 1969). Cross sections of root or nodule tissues 1–2 µm thick were stained with 0.1 M KOH.

Serial sections were also undertaken where enlarged root hairs were associated with the infection centre. Ultrathin sections of selected areas were mounted on 75/300 mesh naked grids, stained with saturated aqueous uranyl acetate and lead citrate (Reynolds, 1963), and examined under a Zeiss research light microscope.

### RESULTS

#### Iron-adequate plants

Localization of infection site. Irrespective of the age of the seedling at the time of inoculation, the rate of root elongation of all plants was very similar (about 1.9 mm h⁻¹). However, the total number of nodules

### Table 1. The rate of root elongation and nodulation of seedlings of *Lupinus angustifolius* inoculated with *Bradyrhizobium* 3, 5 and 8 d after germination

<table>
<thead>
<tr>
<th>Age of seedling at the time of inoculation (d)</th>
<th>Rate of root elongation* (mm h⁻¹)</th>
<th>Number of nodules† (no. plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.89 ± 0.05</td>
<td>64 ± 4</td>
</tr>
<tr>
<td>5</td>
<td>1.86 ± 0.009</td>
<td>85 ± 7</td>
</tr>
<tr>
<td>8</td>
<td>1.86 ± 0.06</td>
<td>134 ± 6</td>
</tr>
</tbody>
</table>

* The average rate between the times of inoculation and harvest, means of three replicates ± standard error.
† Nodules counted 12 d after inoculation; means of 15 plants ± standard error.
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The development of the infection from these cells may take place by proliferation of this initial. The infected cell may divide repeatedly to extend the infection centre (Fig. 10); the bradyrhizobia seemed to be largely spread by repeated plant cell division. Bacteria became distributed to the inner cortex (Fig. 3). By day 5, cell division was evident in the inner cortex as well. A small cluster of newly divided cells in the outer cortex had filled with cytoplasm, as indicated by dark staining (Fig. 4); this was the meristem of a nodule initial and was distinguishable after roots were cleared and stained with Brilliant Green (Tang et al., 1990a). Bacteroids were visible in these cells (micrograph not shown).

By days 6 to 8, repeated cell division had occurred in both the inner and outer cortex and the meristems of nodule initials had enlarged. Near the meristems, the cortical cells had divided further, distorting the alignment of their periclinal walls (Figs 5, 6). The meristem of nodule initials had distended the epidermis to form visible nodules after day 9. A distinct nodule cortex formed over the nodule meristem and the vascular bundle connecting the nodule vascular system with the root stele had begun to differentiate (Fig. 7). Some nodules consisted of more than one bacteroid zone, each of which was separated by rows of nodule cortical cells. Uninvaded cells in bacteroid zones were seldom observed (micrographs not shown).

The clusters of dividing cortical cells were associated with enlarged root hairs in some cases, but curled root hairs were rarely observed. Infection threads could not be seen within the infection centre by light microscopy, nor in the root hairs in serial sections.

Ultrastructure. Structures resembling infection threads were relatively rare and short; they were usually found between the epidermal cells and the outermost layer of cortical cells at early stages, and between infected cells of the nodule meristem and neighbouring uninfected nodule cortical cells at later stages. Infection thread-like structures were formed by day 4 after inoculation. An infection thread-like structure was observed beneath an epidermal cell of a young nodule when it was just visible on the root surface on day 9 after inoculation (Figs 8, 9). The wall of the infection thread-like structure was thicker than that of host cells. The wall material appeared continuous with the wall of cortical cells and seemed to have a similar electron density. The infection thread-like structure did not appear to cross cells or to produce branches.

Individual bacteroids were observed in the cytoplasm of infected cells as early as day 5 (data not shown), just 1 d after infection thread-like structures were first evident. If bacteria are released from such threads into the cytoplasm, the process must occur very shortly after threads penetrate a cortical cell.

The development of the infection from these cells may take place by proliferation of this initial infection. The infected cell may divide repeatedly to extend the infection centre (Fig. 10); the bradyrhizobia seemed to be largely spread by repeated plant cell division. Bacteria became distributed
Figures 2–7. Light micrographs of cross sections of the roots of seedlings of *Lupinus angustifolius* receiving 2.5 μM iron. (Bar = 150 μm for Figs 2–6 and 300 μm for Fig. 7).

**Figure 2.** 2 d after inoculation. Cells of the outer cortex have divided into two daughter cells. Single arrows indicate the wall of the original cortical cell and arrowheads indicate the new wall. Nuclei are divided by the new wall (double arrows). **Figure 3.** 4 d after inoculation. The inner cortex starts to show cytoplasmic staining and cell division. Cells of outer layers of cortex divide repeatedly and contain cytoplasm and enlarged nuclei. An enlarged root hair is associated. **Figure 4.** 5 d after inoculation. Cell division has occurred in the inner cortex. Oblique divisions of cortical cells have occurred (arrows). The newly divided cells of the outer cortex have become isodiametric, obscuring the parental cell profiles, and have stained darkly, indicating that they have become enriched in cytoplasm. An enlarged root hair is associated (R). The meristem of a nodule initial (arrowhead) has formed. Part of another centre of cortical cell division is shown nearby. **Figure 5.** 7 d after inoculation. Cells are dividing further and the darkly stained area (arrowhead) is expanded. Anticlinal cell division has begun (arrows). **Figure 6.** 8 d after inoculation. Activity of the meristem (arrowhead) of a nodule
within the progeny of these cells as cell division proceeded. Bacterial cell division could occur within the peribacteroid membrane, resulting in two or more bacterial cells within one envelope (Fig. 11). As a result of further bacterial multiplication, the cytoplasm of infected cells became more fully occupied by bacteria (Fig. 12).

**Iron-deficient plants**

**Plant growth and nodulation.** Plants grown in 0.05 μM iron showed chlorosis of young leaves from day 5 after treatments were imposed, while those grown in 0.25 μM iron were slightly pale after day 11. The fresh weight of shoots was not significantly (P > 0.05) affected by the iron treatments for the 17 d of iron deficiency. Roots exposed to 2.5 μM iron had more than 40 nodules per plant at the latest harvest, while those grown in 0.05 and 0.25 μM iron had only 2 and 5 nodules, respectively (Table 2). Nodules appeared 9 d after inoculation on roots supplied with 2.5 μM iron, but nodule appearance on roots supplied with 0.05 or 0.25 μM iron was delayed for a further 2 or 3 d. Many small brown spots, possibly aborted infection sites, were observed on the surface of roots grown in 0.05 and 0.25 μM iron, but not on roots grown in 2.5 μM iron.

Differences in events in the process of nodulation in iron-deficient and iron-sufficient plants are summarized in Table 2.

**Anatomy.** Under iron deficiency, cortical cell division occurred as early as 2 d after inoculation, and followed a generally similar pattern to that of iron-sufficient plants until day 4. The root cortical cells of plants given 0.05 or 0.25 μM iron did not divide further after day 5. Cell division ceased in the outer cortex (Figs 13, 14), with nodule meristems and visible nodules rarely being formed in root tissues of these two treatments. The initial division of root cortical cells in these plants eventually ceased and showed up as brown points on the root surface.

**Ultrastructure.** Until day 4 after inoculation, the ultrastructure of target cells was generally similar to that of plants grown in iron-sufficient solutions. In iron-deficient plants no infection centre was formed, though infection thread-like structures might have been formed occasionally (Fig. 15), similar to those in iron-sufficient plants at early stages. Bradyrhizobia might also be released into the cytoplasm (Fig. 16), but infection thread-like structures and release of bacteria were observed in only a few sections. Moreover, these bradyrhizobia did not appear to divide further. The cortical cell wall and thread wall became thickened. In contrast, under iron-sufficient conditions, bradyrhizobia actively multiplied and normal organelles were present (Figs 11, 12).

Nodules, which were occasionally formed from infection sites that survived in roots exposed to 0.05 or 0.25 μM iron, showed delayed development, and the numbers of bradyrhizobia per cell were much decreased at the earlier stages (day 14) compared with those in roots exposed to 2.5 μM iron (Figs 17, 18). Subsequently, the number of bradyrhizobia in the nodules of iron-deficient plants reached levels similar to those in cells of iron-sufficient plants at a late stage. The development process of these infection sites that survived in iron-deficient roots was generally similar to that in iron-sufficient roots. The ultrastructure of bacteroids in nodules was not markedly affected by iron deficiency (micrographs not shown).

**DISCUSSION**

**Infection site**

Infection in lupins seemed to be localized in a zone similar to that in soybean and the apical infection in clover but, unlike clover, the development of lateral infections in the mature zone of roots does not appear to occur in lupins. In soybean, Bhuveeswari, Turgeon & Bauer (1980) found that nodules developed most frequently in the region from 20 to 80% of the distance between the root tips and the smallest emergent root hairs from the root tip marks. From observation of infection thread formulation of clover, Nutman (1956) concluded that apical infections initiated at the tips of root hairs occurred only in the zone of the developing root hairs. However, lateral infections initiated at a branch near the base of a root hair were observed in the zone of mature fully elongated root hairs.
Figures 8-12. For legends see opposite.
Table 2. Comparison of events in the nodulation process of iron-sufficient and iron-deficient lupin roots

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Iron-sufficient</th>
<th>Iron-deficient</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Abort infected sites</td>
<td>Surviving infected sites</td>
</tr>
<tr>
<td>2-3</td>
<td>Initial cell division of outer cortex of roots</td>
<td>As in iron-sufficient plants</td>
</tr>
<tr>
<td>4</td>
<td>Infection threads observed</td>
<td>No infection threads observed</td>
</tr>
<tr>
<td>5</td>
<td>Bacteria released into cytoplasm of cortical cells. Meristems of nodule initials formed, resulting in nodule initiation. Cell division in both outer and inner cortex</td>
<td>No bacteria observed in cytoplasm of cortical cells. Meristems of nodule initials not formed</td>
</tr>
<tr>
<td>7</td>
<td>Expansion of infection centres. Further division of cortical and bacterial cells in the centres</td>
<td>Majority of infection sites not developed. Brown points on the root surface</td>
</tr>
<tr>
<td>8</td>
<td>Cell division extended to stele, ending the stage of nodule initiation</td>
<td>Very short infection threads and bacterial release into the cytoplasm</td>
</tr>
<tr>
<td>9</td>
<td>Nodules visible on root surface</td>
<td>Organelles in initially divided cortical cells disappearing</td>
</tr>
<tr>
<td>11</td>
<td>Further development of nodules. Bacteroids multiply and occupy the cytoplasm of infected cells</td>
<td>Majority of infection sites aborted</td>
</tr>
<tr>
<td>14</td>
<td>Maximum bacteroid number in nodules reached</td>
<td>Further expansion of infection centres</td>
</tr>
<tr>
<td>17</td>
<td>Development of nodules ceased</td>
<td>Nodules visible on the roots. Vascular bundle differentiated</td>
</tr>
<tr>
<td></td>
<td>Over 40 nodules per plant formed</td>
<td>Further development of nodules</td>
</tr>
<tr>
<td></td>
<td>Maximum bacteroid number in nodules reached. Ultrastructure similar to that in nodules on iron-sufficient plants</td>
<td>Only 2 or 5 nodules formed on plants receiving 0.05 or 0.25 μM iron respectively</td>
</tr>
</tbody>
</table>

Many studies of infection in legumes have provided evidence that rhizobia normally enter the root through infection threads formed in root hairs. However, our light microscope examination of serial sections of the infected region of Lupinus roots did not show infection threads in root hairs, consistent with the observations of Dart (1977) and Quispel (1983) for this genus. In contrast in soybean, Bhuvaneswari et al. (1980) observed that infection threads were developed in short emergent root hairs and thus suggested that emergent root hairs were the infectible cells.

**Infection process**

The bradyrhizobial inoculation process in lupin roots seems to differ from that for many other legumes.

The entry of bradyrhizobia into lupin roots may be similar to that for *Frankia* infection in the non-legume (actinorhizal) plant *Elaeagnus angustifolia* (Miller & Baker, 1985) or for *Rhizobium* infection in *Mimosa scabra* (Faria et al., 1988). The present study suggests that infection threads are very scarce or absent and may not be of significance in nodulation in lupins; bradyrhizobia may therefore have to enter the lupin roots through gaps between epidermal cells and then penetrate cells of the root cortex through their walls. Further study would be required to confirm the mechanism of infection in lupin.

The bradyrhizobial stimulus to division of target cells in root tissues was active prior to bacterial invasion. The enlarged nucleus and condensed cytoplasm were observed 2 d after inoculation,

**Figures 8-12.** Electron transmission micrographs of cross sections from selected areas of infected roots receiving 2.5 μM iron.

**Figure 8.** Root tissue containing infection threads beneath an epidermal cell (single asterisk). Bacteria are surrounded by wall material continuous with the host cell wall. The double asterisk indicates a cortical cell (bar = 4 μm). **Figure 9.** Higher magnification of the outlined area in Fig. 8 illustrating nodule bacteria in the infection thread (bar = 1 μm). **Figure 10.** Portion of infection centre at day 8. An infected cell is dividing. Newly-formed wall material (arrows) is associated with the vesicles (bar = 4 μm). **Figure 11.** Two (single arrows) and several (double arrows) bacteroids are within the same peribacteroid membrane (bar = 1 μm). **Figure 12.** Bacteroids have occupied almost the whole cytoplasm of a cell in the infected zone of a nodule, 14 d after inoculation. At the periphery of the cell, amyloplasts (A) and other organelles are visible (bar = 2 μm).
Figures 13–18. For legends see opposite.
whereas the formation of infection threads and bradyrhizobial invasion into cortical cells occurred at days 4 and 5 respectively. In lucerne, a sulphated acylated oligosaccharide signal appears to be involved in cortical cell proliferation (Lerouge et al., 1990); a similar extracellular signal may operate following addition of *Bradyrhizobium* to lupin roots and bacterial proliferation in the rhizosphere. In contrast to some other legumes (Libbenga & Harkes, 1973), no threads developed into the inner cortex of lupin roots. The bradyrhizobia in lupin infection threads are usually surrounded by a thin layer of what may be rhizobial polysaccharide (Robertson et al., 1978). In pea and some other legumes, however, the development of root nodules begins with the induction of cell division in inner cortical cells at some distance from the advancing infection thread (Libbenga & Harkes, 1973).

The expansion of infection zones in lupin roots appears to be similar to that in *A. hypogaea* (Chandler, 1978) and *Stylosanthes* (Chandler et al., 1982), with the invaded cells dividing repeatedly to form a nodule. Uninfected cells were seldom observed in the infection zones in lucips whereas uninfected cells are often found in nodules on other legumes, e.g. pea (Newcomb, 1976), mung bean (Newcomb & McIntyre, 1981) or soybean (Meijer, 1982) in which the infection zone is expanded by branching of infection threads.

**Effect of iron deficiency**

Initial division of cortical cells resulting from inoculation with bradyrhizobia does not seem to be limited by iron deficiency since it occurred in both iron-deficient and iron-sufficient plants as early as 2 d after inoculation. There was little effect on the division of cortical cells from different iron treatments until 4 d after inoculation. However, after this time, initially-stimulated cortical cells did not divide further to form the meristem of nodule initials if iron supply was limited. The total number of aborted infection sites and visible nodules in iron-deficient roots was similar to the number of visible nodules in iron-sufficient roots (Tang, 1991). The present study suggests that iron deficiency inhibits the division of cortical cells and the establishment of the meristems of nodule initials, perhaps by decreasing the proliferation of bradyrhizobia inside the roots of *L. angustifolius*.

While very small numbers of infection thread-like structures were formed in iron-deficient root tissues, the structure did not extend. Although, in some circumstances, bradyrhizobia may be released into the cytoplasm under iron-deficient conditions, their further proliferation may also be greatly inhibited. The evidence from anatomical observations thus supports the previous finding from transfer experiments that iron deficiency impaired a step at the stage of nodule initiation (Tang et al., 1991).

Whether the failure of nodule initiation in lupins results from low internal iron supply or from external iron deficiency is unclear. Attempts to increase internal iron concentrations using different approaches such as foliar application of iron and split-root techniques have failed (Tang et al., 1990b; 1991). Nevertheless, in lupin plants pretreated with extremely high concentrations of iron (50 μM) before exposure to low iron concentrations (0.05 μM), nodule formation was not increased (Tang, 1991). If bradyrhizobial proliferation inside the root and the formation of nodule initials requires internal iron, questions arise about what form of iron in the host plant can be used and whether there is any barrier to

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**Figures 13, 14.** Light micrographs of cross section of the roots exposed to 0.05 or 0.25 μM iron (bar = 150 μm).

**Figure 13.** Section from a root exposed to 0.05 μM iron. On day 8 after inoculation, cell division has occurred in the outer cortex (arrows) but without formation of the meristem of nodule initials. **Figure 14.** At day 9 after inoculation, cell divisions in the cortex of roots exposed to 0.25 μM iron had ceased at the stage of day 4 after inoculation (Fig. 3). Some divided cells are darkly stained (arrow).

**Figures 15-18.** Electron transmission micrographs of cross section from selected areas of infected root tissues and nodules.

**Figure 15.** The centre of an infection site in the root 9 d after inoculation in 0.25 μM iron. An infection thread-like structure with a bacterium (arrow) is formed beneath a root hair (single asterisk), (bar = 1 μm). **Figure 16.** Centre of an infection site of a root receiving 0.05 μM iron. Many vesicles containing fibrous materials are present. Ribosomes appear to be disappearing and other organelles are hardly distinguishable. The arrow may indicate a bacterium released into the cytoplasm, and surrounded by peribacteroid membrane (double arrowhead). Electron opaque material with regular pattern (asterisk) may be chromatin separated from the nucleus, (bar = 0.5 μm). **Figure 17.** Thin section of a young nodule developed on a plant in 0.05 μM iron, taken 14 d after inoculation. Bacteroids surrounded by peribacteroid membranes occupy some part of the cytoplasm. Cells contain vacuoles and vesicles and peripherally-located amyloplasts. Many organelles, endoplasmic reticulum (arrows) and a nucleus with nucleolus are also present (bar = 2 μm). **Figure 18.** Electron micrograph, taken 14 d after inoculation, of a thin section of a nodule developed from a root exposed to 25 μM iron, shows numerous bacteroids occupying almost the entire cytoplasm. Vacuoles have become fewer. Most of the endoplasmic reticulum (arrows) and organelles are confined to the periphery of the cell (bar = 4 μm).
bradyrhizobia using the iron sources in root tissues. Total iron concentration in root tissues is much higher than the external concentration required for maximum nodulation, and the requirement of iron for bradyrhizobial growth in pure culture is low (O’Hara, Boonkerd & Dilworth, 1988).

In various *Rhizobium* species, common and host-specific nodulation genes determining infection and nodulation of specific legumes have been identified (Long, 1989). Cytological studies have suggested that nodule bacteria are able to elicit, at a distance, cortical cell differentiation and initiation of nodule organogenesis by means of extracellular signals (Bauer et al., 1985; Lerouge et al., 1990). The major alfalfa-specific signal, NodRm-1, has been purified from the culture supernatant of *R. meliloti* strains (Lerouge et al., 1990). Both common nodABC and host-specific nodH and nodQ genes of *R. meliloti* are involved in the production of extracellular symbiotic signals (Lerouge et al., 1990). Iron deficiency might therefore alter the expression of nodulation genes and inhibit the production of the bradyrhizobial signal required to establish nodule meristems in lupins. However, no work has yet been done on the effect of iron deficiency on nodulation gene expression and signal production in nodule bacteria.

The development of nodules in lupins is less sensitive than nodule initiation to iron deficiency. However, development was delayed under iron deficiency because the density of bacteroids in the invaded zone of iron-deficient nodules was lower than that of iron-sufficient nodules at early stages of nodulation development. Numbers of bacteroids in nodules of iron-deficient plants eventually reached similar levels to those in nodules of iron-sufficient plants. The ultrastructure of bacteroids was not affected by iron deficiency, unlike effects observed in nodules of iron-deficient soybean (Hecht-Buchholz et al., 1987). The low number of bacteroids in the developing iron-deficient lupin nodule mainly resulted from the delayed nodulation process. It follows that the multiplication of bacteroids in nodules is unlikely to have been limited by the low concentration of iron in the external solution.

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