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EARLY NODULE DEVELOPMENT IN MYRICA GALE[1]

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Seedlings of *Myrica gale* were grown aeroponically and inoculated with suspensions prepared from mature nodules of *M. gale* plants or with a homogenized preparation of a pure culture of the actinomycete isolated from *Comptonia* nodules. Morphological, anatomical, and cytological studies were made of early nodule development in these plants, and comparisons were made with similar stages in *Comptonia* and *Casuarina*. Root hair infection was followed by prenodule formation in a manner similar to that in *Comptonia*. In *M. gale*, most nodules originated with one or two primary nodule lobes. Thereafter, secondary nodule lobes formed sparsely and in a precisely ordered sequence. In 3-mo-old seedlings, nodules with up to five nodule lobes were observed, but the majority of nodules at this age were still one-to-three lobed. In *Comptonia* the number of primary nodule lobes is much higher than in *M. gale*. In *M. gale* nodule roots developed from many but not all nodule lobes, usually in an ordered sequence.

Introduction

There have been relatively few developmental studies of root nodules initiated by soil actinomycetes which invade the root, causing the formation of organized structures capable of symbiotic fixation of atmospheric nitrogen. Only in recent years has a clearer understanding of initiation and development of these nodules begun to be available. Of the two general types of nonleguminous nodules formed on roots of woody dicot species, the *Alnus* type which lacks nodule roots has been studied (TAUBERT 1956; BECKING 1968; ANGULO 1974).

Nodules of the *Myrica* type, characterized by the development of upward-growing determinate nodule roots, were studied by BOND (1952) and by FLETCHER (1955) in *Myrica gale* L., by TORREY (1976) in *Casuarina cunninghamiana* Miq., and by BOWES, CALLAHAM, and TORREY (1977) and CALLAHAM and TORREY (1977) in *Comptonia peregrina* (L.) Coult. Nodule root development was analyzed by TORREY and CALLAHAM (1978) in *M. gale* as part of a comprehensive study of nodulation in this species. Details of root hair infection in *Casuarina*, *Comptonia*, and *Myrica* are presented by CALLAHAM, NEWCOMB et al. (1979).

Material and methods

Seeds locally collected from plants of *Myrica gale* were germinated in sand after cold treatment or following treatment with 500 ppm gibberellic acid for 24 h. Young seedlings were transferred either to water culture or to aeroponic culture and inoculated with nodule suspensions prepared from mature nodules of *M. gale* or a suspension of the cultured endophyte from *Comptonia peregrina* (CALLAHAM, DEL TREDICI, and TORREY 1978). Observations on nodule root development in these plants have been reported (TORREY and CALLAHAM 1978). Nodules were fixed, embedded in Araldite resin, and sectioned for microscopic observation by the methods of CALLAHAM and TORREY (1977) and NEWCOMB et al. (1978).

Observations

ROOT HAIR DEFORMATION AND INFECTION.—As in *Casuarina*, *Comptonia*, *Alnus*, and other genera (ANGULO 1974; TORREY 1976; CALLAHAM and TORREY 1977; CALLAHAM, NEWCOMB et al. 1979), root hair deformation is the prelude to the invasion of the root hair by the soil actinomycete causing nodulation. Details of this process are discussed by CALLAHAM, NEWCOMB et al. (1979). In *Myrica gale* one root hair infection may suffice for each nodule formed, but occasionally more than one root hair may be infected on the same lateral root.

PRENODULE DEVELOPMENT.—The endophyte filaments pass from the root hair cell base into adjacent cortical cells of the lateral root, penetrating cell walls and passing through cell cytoplasm. Cortical cells are stimulated to divide and to hypertrophy so that, soon after infection, a swollen region of the infected root is evident either in cross section (fig. 1) or in longitudinal view (figs. 2, 3). This swollen region with its infected cells is termed the prenodule (CALLAHAM and TORREY 1977). Epidermal cells appear not to subdivide under the stimulus of the endophyte, but midcortical cells are especially responsive. The course of the filamentous endophyte can be traced radially from the root hair cell base to midcortical cells (fig. 4) and thence to inner cortical cells, but the endophyte never penetrates the endodermis of the lateral root (fig. 5), which is characteristically composed of tannin-filled cells forming a continuous cylinder around the vascular tissues. In the early prenodule state the stimulus to cortical cell response is local (figs. 2, 3).

PRIMARY NODULE LOBE FORMATION.—In a manner analogous to nodule lobe initiation in *Comptonia*

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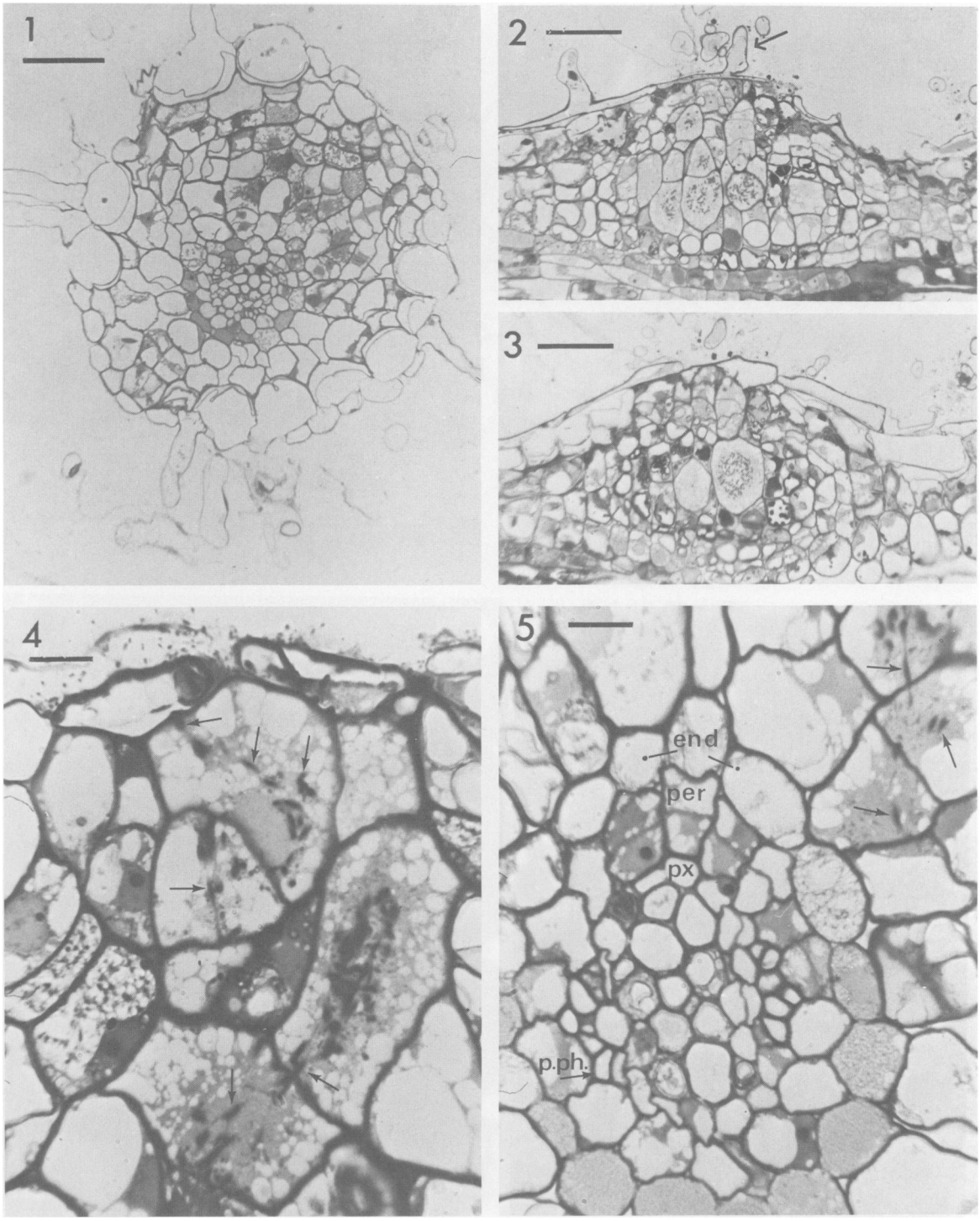
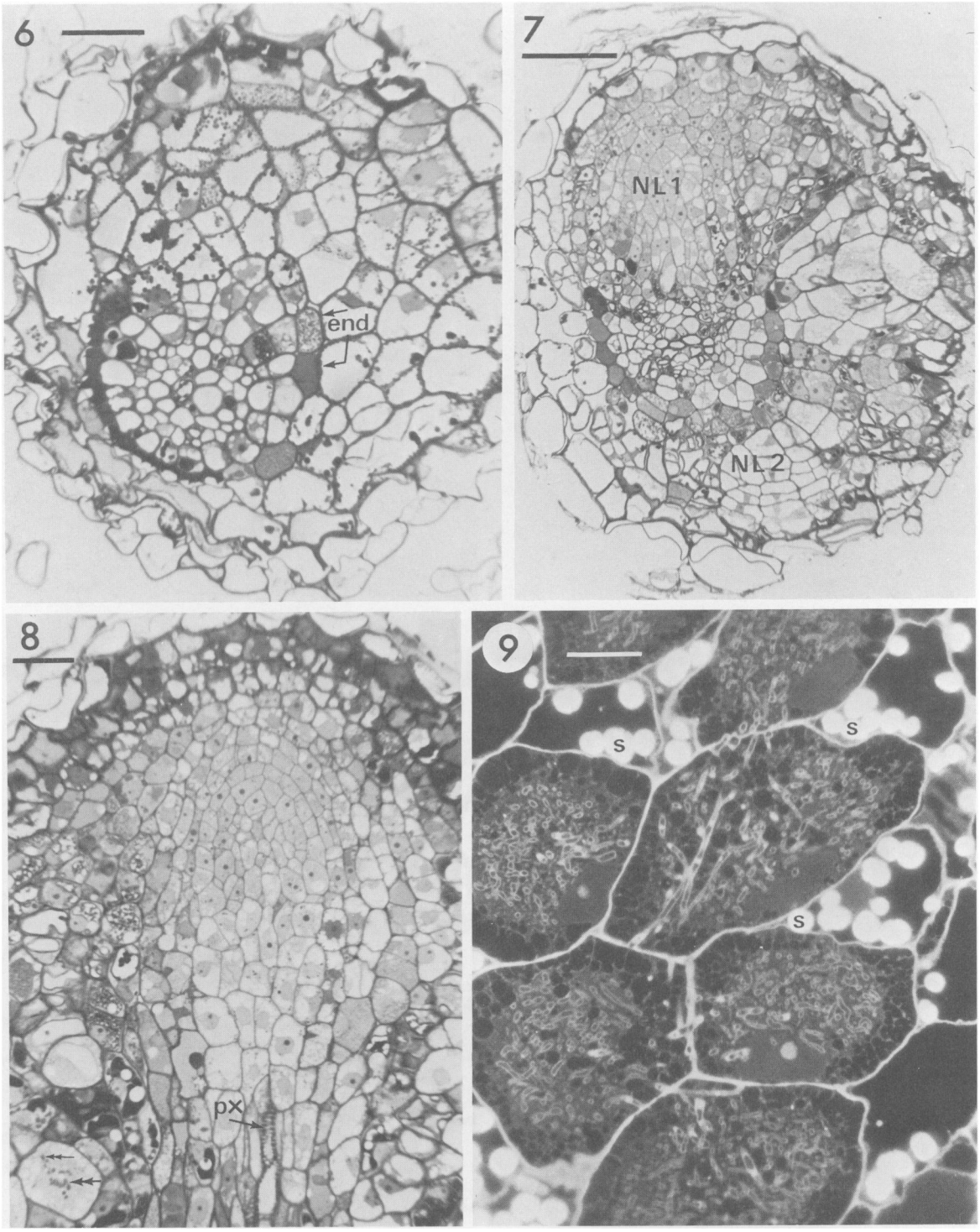


FIGS. 1–5.—Stages of prenodule formation in lateral roots of *Myrica gale*. Fig. 1, Cross section of small lateral root after root hair infection showing asymmetry caused by proliferation and hypertrophy of cortical cells invaded by actinomycete; bar = 50 μm. Fig. 2, Longitudinal section of early prenodule showing root hair invasion site (arrow) and endophyte within divided cortical cells of the lateral root; bar = 50 μm. Fig. 3, Longitudinal section as in fig. 2, showing hypertrophied cortical cells invaded by endophyte; bar = 50 μm. Fig. 4, Enlarged view of cross section of outer tissues of lateral root adjacent to root hair invasion site, showing course of endophyte (arrows) through subdivided cortical cells; bar = 10 μm. Fig. 5, Cross section of central cylinder of lateral root with tannin-filled endodermal layer and cortical cells containing endophyte (arrows). Note periclinal division in pericycle (*per*); *px* = primary xylem; *p.ph.* = primary phloem; *end* = endodermis; bar = 10 μm.



FIGS. 6–9.—Early stages in nodule lobe formation in *Myrica gale*. Fig. 6, Cross section of lateral root tangential to prenodule, showing pericyclic proliferation initiating primary nodule lobe; the tannin-filled endodermis (*end*) is interrupted by pericyclic divisions; bar = 25 μm. Fig. 7, Cross section of lateral root, showing later stages in primary nodule lobe formation; one lobe cut longitudinally (*NL1*) shows early organization while a second lobe (*NL2*) cut tangentially shows early predominantly periclinal divisions; bar = 50 μm. Fig. 8, A primary nodule lobe cut longitudinally showing its rootlike appearance; primary xylem connections to the lateral root are cut longitudinally (*px*); the endophyte (double arrows) has begun to invade what will be the cortical cells of the lobe; bar = 25 μm. Fig. 9, Section of cortical cells of a well developed nodule lobe, prepared with a polysaccharide-specific dye and photographed in ultraviolet light; large starch grains (*s*) occupy uninfected cells; the endophyte nearly fills some cells and shows clearly the polysaccharide encapsulation material around every filament; bar = 10 μm.

(CALLAHAM and TORREY 1977), pericyclic proliferation is initiated near the site of root hair infection but typically not in the same transverse plane as the infected hair. Periclinal divisions of the pericycle followed by anticlinal divisions of the derivatives (fig. 6) serve to disrupt the continuity of the endodermal layer. Subdivisions of cortical cells external to the pericycle contribute to the newly forming primordium which shows rootlike characteristics. The primordium arises endogenously and soon shows a predominantly radial and longitudinal orientation of the cellular derivatives (fig. 7). The primordium may arise opposite a primary xylem pole (fig. 6) or at an angle to it (fig. 7), as was observed also in *Comptonia*. Since we did not observe crushing of endodermal cells (fig. 6), we conclude that endodermal cells divide and the derivatives are incorporated into the primary nodule lobe primordium. Similarly, cortical cells are stimulated to divide and become a part of the forming primordium (fig. 7).

At a later stage, when the primordium has not yet broken through the epidermis of the lateral root and is still less than 0.2 mm long, it already shows root-meristem-like organization when observed in longitudinal section (fig. 8). A terminal series of cells, the outermost filled with tannins, form a root-cap-like structure enclosing the densely cytoplasmic cells of the nodule lobe meristem itself. Once formed, the nodule lobe appears to be in an arrested state, judging from the dormant-appearing root cap zone, the lack of mitoses, and the nearness of fully mature primary xylem in the central cylinder which attaches at right angles to the vascular tissue of the main axis root. Already at this stage, one can observe filaments of the endophyte which have invaded the cortical region of the newly formed primordial lobe (fig. 8). The state of arrest of this primordium is variable (TORREY and CALLAHAM 1978). At an unknown signal, the primordium lobe resumes cell division, forming new cells whose elongation gives rise to the nodule root. In *M. gale*, nodule roots grow vertically upward to 3–4 cm and then cease further growth. The endophyte does not invade cells of the nodule root itself.

Cortical cells of the nodule lobe are of two types, those invaded by the endophyte, which are enlarged and ultimately gorged by the filamentous actinomycete (fig. 9), and smaller, uninfected cortical cells in which starch grains are prominent structures. Staining for total polysaccharide (fig. 9), emphasizes these differences. The encapsulation of the endophyte is pectic (LALONDE and KNOWLES 1975), and its formation by the host utilizes a substantial part of the energy and substrates in the infected cells. Barriers between infected and uninfected cells which must exist are not understood.

COMPARISONS BETWEEN M. GALE AND COMPTONIA PEREGRINA.—In aeroponics, *M. gale* seedlings, inoculated either with a ground nodule suspension from *M. gale* plants or with a suspension of the *Comptonia* isolate, readily form numerous small nodules along the length of the root. Each nodule is initiated in the deformed root hair region proximal to the lateral root apices of the seedling root system. In *Comptonia* we have observed that ground nodule suspensions form nodules sparsely, perhaps one to three per seedling, but this difference disappears when seedlings are inoculated with a suspension of the *Comptonia* isolate, whereupon large numbers of nodules form seedling roots (CALLAHAM et al. 1978).

The most striking difference between nodules of *M. gale* and *Comptonia* centers on the number of primary nodule lobe primordia formed at each infection site. In *Comptonia* the number of primary nodule lobe primordia per infection site is very variable (BOWES et al. 1977). This variation is shown in a sample of seedlings with as few as one lobe or as many as 14 lobes being initiated within very close proximity of the root hair infection (fig. 10*B*). In *M. gale*, in contrast, only one or two primary nodule lobe primordia are initiated at most infection sites (fig. 10*A*), although infrequently as many as three, four, and five primordia arise at a common site.

Discussion

Early nodule development in *Myrica gale* is similar to that in *Comptonia peregrina* (CALLAHAM and TORREY 1977), which may not be surprising since these two species are closely related. The two differ largely with respect to the number of primary nodule lobe primordia initiated at the outset. In *M. gale* the average number, usually one or two, is consistent and much smaller than observed in *Comptonia*.

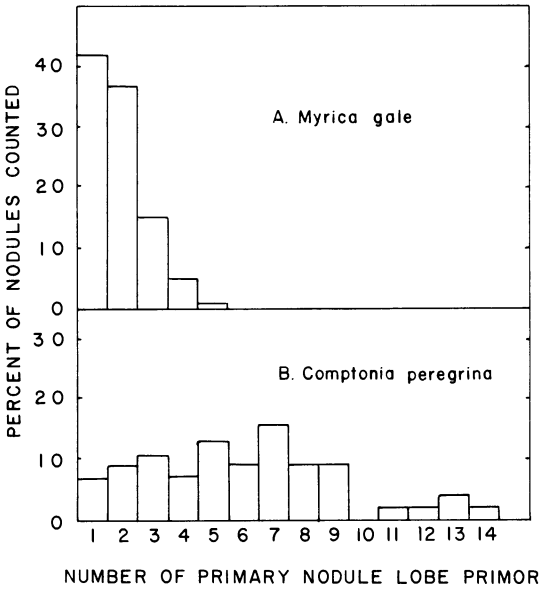


FIG. 10.—Percentage of nodules formed with different numbers of primary nodule lobe primordia in inoculated seedlings of *Myrica gale* (*A*) and *Comptonia peregrina* (*B*). *Myrica* seedlings sampled (100 nodules) at 12 wk; *Comptonia* seedlings sampled (45 nodules) at 4 wk.

Details of root hair infection, prenodule formation, and primary nodule lobe development are quite similar in the two species. Although prenodule formation was not as carefully studied in *Casuarina* (TORREY 1976) as in these two species, there seem to be great similarities in structural detail. In *Casuarina cunninghamiana*, the number of primary nodule lobe primordia is typically low, more comparable with the situation in *M. gale* than in *Comptonia*.

The early stages of nodule development in *Alnus*, insofar as they have been studied, tend to resemble the situation described here for *M. gale* and earlier for *Comptonia*. We know infection in *Alnus* is by root hair invasion (TAUBERT 1956; ANGULO 1974) followed by cortical cell invasion. According to ANGULO (1974) and ANGULO, VAN DIJK, and QUISPEL (1975), a prenodule stage precedes primary nodule lobe initiation and is followed more or less simultaneously by primary nodule lobe formation typically involving one, two, or three lobes from one infection site. In this respect, *Alnus glutinosa* resembles *M. gale*. Unlike *M. gale*, however, elongate nodule roots do not develop, and the *Alnus* nodule retains its coralloid nature, later showing secondary or higher lobings on the primary nodule lobes which, although arising endogenously, give the morphological appearance of dichotomous branching. Whether this pattern of development occurs in most actinomycete-induced nodules of the *Alnus* type remains to be shown.

Acknowledgments

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