The Structure and Physiological Significance of the Root-nodules of Myrica gale.

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The peculiar nodule formations on the roots of *Myrica gale* were first described and figured by Brunchorst* in 1886, who stated that they were caused by an inhabiting fungus with septate hyphæ and terminal spores. Möller† in 1890 placed this fungus in the group Frankia, naming it *Frankia Brunchorstii*, and considered it to be closely related to a similar fungus in Alder nodules. In 1902 Shibata‡ stated that the fungus is found exclusively in a peripheral sub-cork layer of tissue, one to three cells thick, and because of its peculiar ray-branching and club-shaped spores, it belongs to the group Actinomyces. Peklo§ in 1910, working on greenhouse-grown plants, supported Shibata's view.

Roots of *Myrica gale* were obtained for this investigation from plants growing wild in Wales, Ireland, and the North of England, and from cultivated plants growing in the Chelsea Physic Gardens. In all cases the roots were found to possess nodules of varying size. The young nodules are from 2—3 mm. long and 0·8—1 mm. broad, but these by branching form "clusters," sometimes as large as a nutmeg, and surrounded by peculiar rootlets which grow out through the end of each nodule or branch. The branching is associated with the outgrowth of lateral roots, and is not due to dichotomy of the apical meristem of the root as is the case in the nodules of Cycas, Alder, and Elæagnus.

A transverse section of the tubercle shows a central tetrarch vascular cylinder similar to that of a normal root, and indicates that the tubercle itself is a modified root. The stele is surrounded by an endodermis characterised by neither radial dot nor thickened walls, but by the cells being filled with oil drops. Outside the endodermis are several layers of cortical cells covered on the outside with a definite small-celled cork layer. In mature nodules the cortical tissue is characterised by the presence of (1) somewhat enlarged cells filled with bacteria; (2) cells filled with oil drops. By means of Kiskalt's amyl Gram stain the bacteria can be seen *in situ* in the enlarged cells as small rods. Towards the apex of the nodule

* J. Brunchorst, 'Unters. aus dem Bot. Inst., Tübingen,' 1886, vol. 2.
† H. Möller, 'Ber. d. Deutsch. bot. Ges.,' 1890, vol. 8.
‡ K. Shibata, 'Jahrb. f. Wiss. Bot.,' 1902, vol. 37.
§ J. Peklo, 'Cent. f. Bakt.,' 1910, (2), vol. 27.

zooglea threads of bacteria are seen passing from cell to cell, comparable with the "infection threads" first seen by Marshall Ward in leguminous nodules.

The nodules arise as modifications of normal lateral roots. The cortical cells of a young root, before its emergence from the main root, become infected by bacteria. The normal growth of the root is thereby checked, but by division and growth of the cells containing the bacteria, the characteristic nodule with its tetrarch stele is formed. When the nodule has reached its full size, the end of the stele, surrounded by a few cortical cells, grows out from the apex of the nodule and forms a thin rootlet. Around this three branches or nodules (occasionally only two) arise endogenously as outgrowths from the cells surrounding the stele, repeating exactly the growth and structure of the primary nodule. By repeated branching in this manner the peculiar "cluster" nodules are formed.

No fungal hyphæ were observed in any of the young nodules examined, but "infection threads" containing bacteria were numerous, and it was evident that the formation of the nodules is caused by the action of the infecting bacteria.

Pure cultures of the bacteria from the cortical cells of the nodule were made. These on examination were found to be identical in structure and growth with the organism *Pseudomonas radicicola* found in leguminous nodules. They gave the characteristic staining reaction with aniline gentian violet and amyl alcohol, and formed colonies of oval shape on maltose agar.

Cultures of the bacteria were made in flasks with a solution containing 1 grm. maltose, 0·5 grm. potassium phosphate, 0·02 grm. magnesium sulphate in 100 c.c. water. After incubation for seven days at 25° C. nitrogen determinations of the culture solution gave the following results :—

Control flask	0·53 mgrm. N	per 100 c.c.
Inoculated flask	2·58 „	„

showing a fixation of nitrogen of 2·05 mgrm.

Young Myrica plants were obtained from Heysham Moss, some having nodules on their roots, others having none. Both kinds were planted out in a greenhouse in pots containing soil deficient in nitrogen. The plants without nodules did not thrive, and soon died, whilst those possessing nodules flourished and made a good growth.

It is evident from these experiments that the root nodules of Myrica are concerned with nitrogen assimilation, and that to the four families of non-leguminous plants—Alder, Elæagnus, Cycas, and Podocarpus—known to possess the power of nitrogen fixation by means of root-nodules, a fifth—Myrica—must now be added.