A STUDY OF THE ROOT-TUBERCLES OF PODOCARPUS CHINENSIS

BY

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Introduction.

Rayner (1927) in his review on Mycorrhiza has summarised and discussed all the work on root-infecting fungi and bacteria, published so far. A detailed review of the earlier literature on Mycorrhiza is given by Janse (1897), Gallaud (1905), Bernard (1909) and Rayner (1926). Reviewing the cases described, Rayner says, "It may be concluded that there is evidence that the formation of nodules by a number of plant species other than legumes, is directly related to invasion of the roots by strains of a bacterial organism closely related to Ps. radicicola. Whether this conclusion can be extended to include Podocarpae and other nodule forming conifers is at present doubtful, and requires confirmation by means of pure culture experiments on the species concerned." Waksman also mentions that the evidence that Podocarpus nodules are caused by Ps. radicicola is not conclusive.

In view of the conflicting nature of observations regarding nodule formation in Podocarpae made by Spratt (1912) as due to bacteria and Yeates as due to fungi respectively, the present investigation was undertaken. Not only has the morphology and physiology of the causal organism been studied but detailed anatomy of the roots as well as of the root-tubercles at different stages of their development been worked out.

Habit and External Morphology of the Root-tubercles.

The root-tubercles of Podocarpus chinensis are present on all plants that are growing in Lahore. They are found on the main roots as well as on the young rootlets. It is almost impossible to find a root which is free from tubercles. The tubercles are usually closely applied to the roots (Figs. III, VI. 4) and sometimes the roots are entirely covered over by such tubercles. In rare cases, the tubercles may not be closely applied to the roots (VI. 1). The colour of the root is brownish red and that of the tubercles, light tan. The tubercles are very brittle and measure 500 to 675μ in diameter.

Anatomy of the Root.

In transverse and longitudinal sections, the following tissue layers can be seen. In the centre is the stele (III, IV, V. St.) composed of xylem (III, X9) which consists of two bundles. Outside the xylem is the phloem (III pt.). Next are two layers of cells which contain large number of starch grains. The outer layer is the endodermis but the thickening of the cells on the radial walls is not clear (III, VII, 56 end). Breadth of the endodermal cells is about 38μ which is more than double the breadth of the adjacent cells. These cells are more or less rectangular. The outermost layer of the cortex contain dark brownish-red pigment, Bark (III, bk., IX. 12 bk.) is invariably found covering the roots. The bark consists of loosely arranged cells. At many places, the cells break down. In this layer are met with fungal hyphae (IX, 12 fh.) forming the so-called ectotrophic mycorrhiza. In the longitudinal section (VII, St.)—a side branch of the stele of the main root passes into the tubercle. The endodermis (VII, 5, 6 end) of the main root is also continuous into the tubercle and is very clear in the median longitudinal section.

Detailed Anatomy of the Tubercles.

The vascular cylinder does not go beyond the middle of the tubercle (VI, 5. St.) The end of the vascular cylinder is embedded in the spherical mass of parenchymatous cells, which constitute the tubercle proper. These cells contain a large number of fungal hyphae, which constitute the so-called endotrophic mycorrhiza. In the central cells of the tubercle the fungal hyphae are found in clusters filling the entire cavities of the cells (III fh.). When there is a single fungal hypha in a cell it usually coils itself (VIII, 8. fh), but when there are a number of hyphae in a cell, they do not limit their growth to that cell, but grow into the adjoining cells (IX. 10, fh.). The cell walls do not seem to resist in the least. The passage of the hyphae through the cell wall is very likely effected through secretions dissolving the cell wall (VIII, 9. d). The central cells of the tubercle are more or less rounded but the peripheral cells are elongated.

In most of the longitudinal and transverse sections, the tubercle is found to be surrounded either completely or incompletely by a tissue consisting of 3 to 4 layers of parenchymatous cells (IV, mt., VI, 2. mt, VII, 5 mt, VIII, 7. mt). This tissue belongs to the cortical portion of the root, which has been pushed out as the tubercle was being formed. The fungal hyphae have been found in this tissue. (VI, 8fh. IX, 11 fh.). Very young tubercles appear like small projections in the sections. (VII. yrb).

Isolation of Fungi.

This has been done by dilution method, as well as by allowing the fungus to grow out under aseptic conditions in a moist Roux tube. For dilution cultures root-tubercles were steeped in formalin water (5 per cent) for 10 minutes and then washed in four successive tubes of sterilised distilled water. The tubercles were then crushed aseptically and a suspension in sterilised distilled water made. From this suspension, successive dilutions were made in series of 4 melted culture tubes (at 40° C) of potato-glucose, bean pod agar, malt extract agar and purple lactose agar and poured into sterilised petri dishes. These were incubated at 25° C. In the Roux tubes, the roots with the tubercles before being put in were steeped in formalin and washed in distilled water as before. The fungi grew out of the tubercles in 3 to 4 days at 25° C, when sub-cultures in different media were made. In dilution culture, the fungus made its appearance in 2 to 3 days' time. Three different fungi, viz., the following, were isolated from dilution cultures (1) a sterile mycelium producing one, (2) Fusarium sp. and (3) Aspergillus sp. The first one was in abundance and in the Roux tubes cultivations, only the first one came out. It had the same kind of hyphae as that found within the tubercles.

Mycelial Character.

The hyphae are non-septate and they measure 3.7 to 5.5μ in diameter. In different culture media the thickness differs. Inside the host tissue, the ends of hyphae swell up in the form of vesicles. No reproductive bodies or spores of the fungus have been noticed in the host tissue or in cultures excepting abundant chlamydospore formation in old cultures.

Modes of Infection.

Fungal hyphae have been noticed (1) in the bark of the root forming the so-called ectotrophic mycorrhiza, (2) in the cortical portion of the root, which has been pushed out by the growing tubercle and (3) in the tubercles proper filling up the whole tissue. By inoculation experiments, the progress of the fungus inside the tissue of different host has also been followed.

From the observations made it seems that the infection of the bark takes place first from the surrounding humus. The mycelium then penetrate the outermost layer of cortex which is broken at places. As a result of infection and the irritating presence of the fungus, hypertrophy occurs resulting in tubercle formation. It has not been possible to inoculate Podocarpus roots with the isolated fungus as no tubercle free root has been found But inoculation experiments have been carried out on other roots, viz., on Cycas and Casuarina, with the sterile mycelium forming fungus. Big glass vessels with metal covers were filled up to 1 3rd with socked sands and sterilised, on which the roots after being treated with formalin and washed in distilled water, were rolled in the culture plates and then planted in the sand beds. The fungus grew readily forming a mantle and by cutting sections, it has been found that they penetrate the epidermal layers of the roots. The fungus penetrated with more facility the epidermis of the Casuarina roots. The presence of the fungus in Casuarina or in Cycas, produced no excessive growth leading to tubercle formation as in Podocarpus. It is hoped to grow Podocarpus from seeds aseptically and then carry on the inoculation experiment on Podocarpus.

Nitrogen Fixation by the Fungus and Relationship to Its Host

That the plants of Podocarpus are not suffering from parasitism, becomes obvious on a casual examination of the roots. Though not one root is free from the tubercles and that whole surfaces of the roots are simply covered by thousands of these tubercles, yet the plants do not appear to be affected in the least. It is a case of some sort of give and take. As Nobbe and Hiltner (1897) had experimentally showed that these tubercles were actively concerned in the fixation of atmospheric nitrogen, hence all the three fungi isolated from the root tubercles were tested for their power of fixing atmospheric nitrogen, if any. For this 100 cc. of Beijerinck's solution (Eyre) in hard glass flasks was used. A set of 6 flasks were inoculated with each of the three fungi. The flasks were fitted with cotton plugs through which plugged glass tubes passed dipping into the liquids. Through these tubes, the liquid media in the flasks were aerated on alternate days for 2 minutes by means of an electric blow pump (120 to 150 bubbles given per minute). At the end of eight weeks, these flasks were tested for nitrogen. Only in the first case, i.e., with the sterile mycelium positive result was obtained. It has not been possible to do any quantitative estimation.

Discussion.

The histological feature and cultural characters that have been described show complete justification for considering that the fungus with the sterile mycelium which has the power of nitrogen fixation, causes tubercle formation in Podocarpus. Many hypotheses have been put forward to explain the relationship of the fungi and the roots. It was Pfeffer who first called attention to the possib'e symbiotic action between the roots of plants and fungi.

Rayner in his papers on Orchid Mycorrhiza gives a historical account from this point of view. Frank considered the ectotrophic mycorrhiza behaving as root hairs for the roots of higher plants and suggested that in endotrophic mycorrhiza the host plant procured nitrogenous food for itself by digesting the fungus, which fills the more internal cells.

According to Stahl the fungus in the root gave to the higher plants the products of assimilation of mineral salts which it procured in humus soil. Magnus and Shibota have shown the digestion of the fungus in the host cells. Gallaud considers the fungus to be internal saprophyte deriving all its food material from the host plant. Bernard also looks upon the fungus as a parasite. McDugal though considering the fungus as a parasite considers that the roots also receive benefit from the digestion of the fungus hyphae. Melin and also Burgeff failed to show nitrogen fixation by the fungi isolated, but Rayner showed that in Ericaceæ the plants would utilize atmospheric nitrogen with the help of Mycorrhiza. McLennan on Lolium found definite symbiosis, though the exchange was not of a nitrogenous character. Chaudhuri and Raja Ram have shown experimentally that the Mycorrhiza in Marchantia nepalensis lead a symbiotic life and that the host cannot properly develop without the fungal infection.

Duffrenoy has suggested that symbiosis is a form of parasitism in which equilibrium exists between the invading powers of the fungus and the resisting power of the host. As long as this equilibrium exists, it is profitable to both, but ultimately one of them gains advantage over the other symbiont, causing a great disadvantage or death to the other. Rayner found similar condition in *Calluna vulgaris*. The present authors have found that at least the host does not at all suffer from the fungal infection, and as found by Frank, Magnus, Shibata and Bernard, they have also found degeneration and digestion of fungal hyphae in the older nodules. The relationship may not be a case of real symbiosis but may be a sort of equilibrium of Duffrenoy which ultimately breaks down in favour of the host.

Summary and Conclusion.

The anatomical study of the tubercles, supplemented by cultural experiments, show complete justification for considering that the root tubercles in Podocarpus chinensis are caused by fungal infection. The presence of the fungus has been shown even in the youngest tubercle and the fungus is present in all stages of the development of the tubercles. Bacteria, viz., Ps radicicola which have been described by many authors as causing nodule formation, have been found to be absent in most of the sections studied. The fungus which inhabits the nodules and which has been isolated, has been shown to possess the power of fixing atmospheric nitrogen. Though inoculation experiment on tubercle free Podocarpus roots has not been possible for want of material, the fungus has been shown to have the power of penetrating the epidermis of roots of other plants, viz., Casuarina and Cycas but no tubercles are formed in these cases.

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Explanation of Plates.

Fig. I. Photograph of roots of Podocarpus chinensis with tubercles (tb). × 1/2.

Fig. II. Photograph of roots, more magnified.

Fig. III. Microphotograph of a transverse section of Podocarpus root with a tubercle. Bk.—bark; cx.—cortex; end.—endodermis; fh.—fungal cluster; ph.—phloem; pcl.—parenchymatous cells of the tubercle; rt—root; st.—stele; tb.—tubercle; xy.—xylem. × 95.


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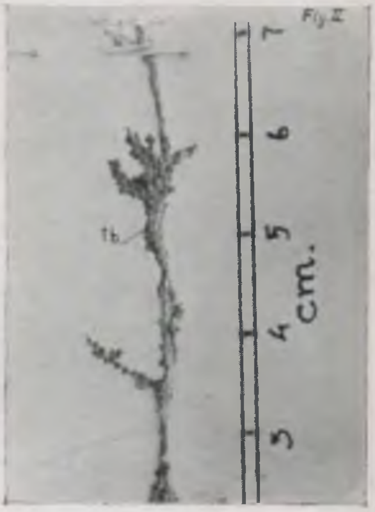
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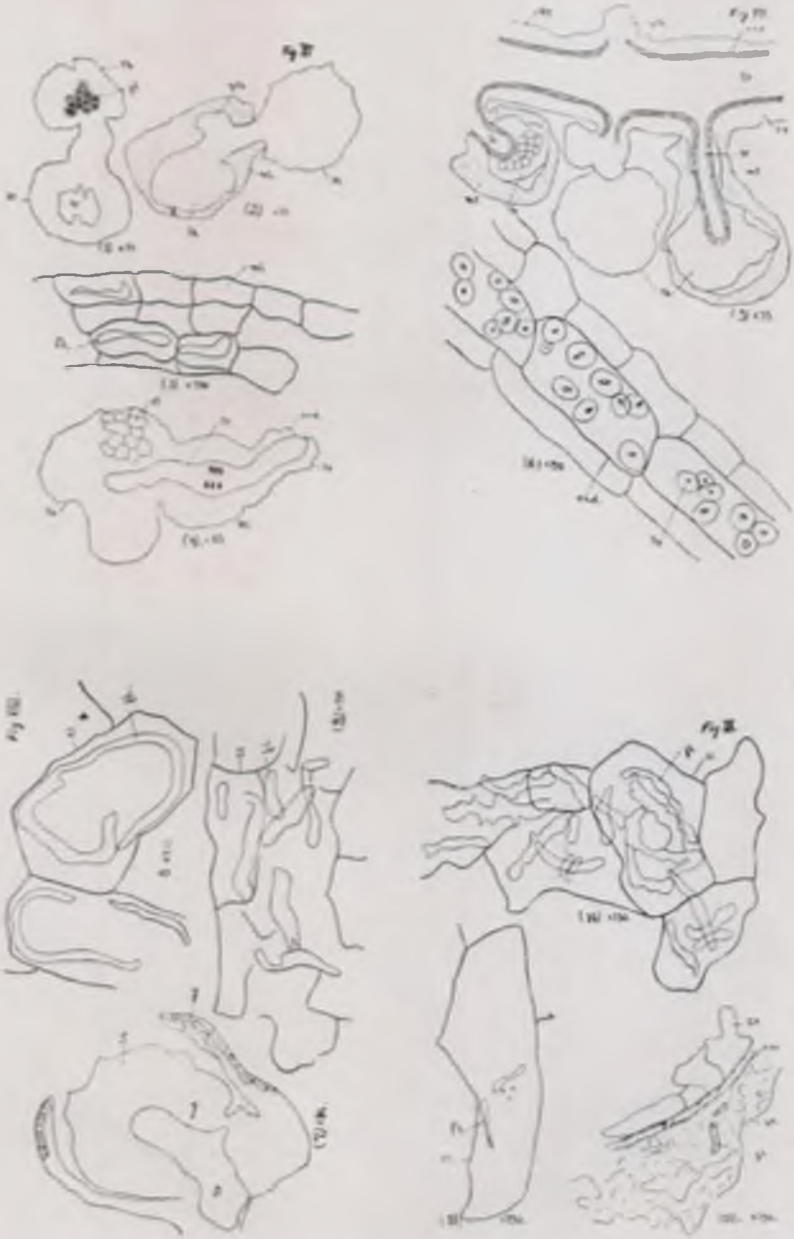

Fig. IV. Podocarpus root with tubercles in longitudinal section. Bk.—bark; fh.—fungal cluster; mt.—mother tissue; st.—stele; tub.—tubercle; ytb.—young tubercle. × 94.

Fig. V. Another longitudinal section of Podocarpus root with tubercles. Bk.—bark; cx.—cortex; mt.—mother tissue; st.—stele; tb.—tubercle. × 45.

Fig. VI. 1. T. S. with a tubercle. × 55.

2. Do. × 55.

3. A portion of the mother tissue of No. 2 magnified (the portion marked ×). × 750.

4. T. S. a root with 2 tubercles. × 55.

Cl.—cells of the tubercle; end.—endodermis; fh—fungal cluster; mt.—mother tissue; rt.—root; st.—stele; tb.—tubercle; t'b.—young tubercle; ytb.—very young tubercle.

Fig. VII. 5. L. S. root with tubercles. × 55.

6. Endodermis of the above. × 750.

Cx.—cortex; end.—endodermis; mt.—mother tissue; rt.—root; st.—stele; sg.—starch grains; tb.—tubercle; ytb.—young tubercle.

Fig. VIII. 7. T. S. Tubercle with mother tissue. × 94.

8. 2 cells of the tubercle showing hyphae. × 750.

9. Some cells of the tubercle showing hyphae. × 750.

Cl.—cell; d.—cells which have broken down; end.—endodermis; fh.—fungal hyphae; st—stele; tb.—tubercle.

Fig. IX. 10. Some cells of the tubercle. × 750.

11. A cell from the mother tissue showing a fungal hyphae with vesicle. × 750.

12. A portion of the bark showing fungal hyphae. × 750.

Bk.—bark; cx.—cortex; fh.—fungal hyphae; ocx.—outermost layer of cortex.

N. B.-Magnifications given are of original drawings. Plate I has been reduced to half and Plate II to one-third in printing.