

# The white Root-Fungus of Cinchona

by

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In an enumeration of the diseases and fungi peculiar to the *Cinchona* tree <sup>1)</sup> I gave at the time a description of the two root-fungi, I had observed growing on the plantations in Java. These two fungi I distinguished as the grey or external and the white or internal fungus, as only the sterile mycelia were known hitherto. Both diseases frequently occur on the same plantation and it is only by examining the roots, that the separate causes become manifest. In both cases the disease first attacks the dead or decaying stems of a wood-tree, whence it gradually extends to the surrounding *Cinchona* trees.

By means of pure cultures and infections I was able to demonstrate, that the grey root-fungus, which possibly belongs to the genus *Rosellinia*, produces *Graphium* as lower fructification, both in nature and in pure cultures <sup>2)</sup>.

The white root-fungus, which mostly attacks trees of older growth, rather than does the grey, is usually found on *Cinchona Ledgeriana* Moens and *Cinchona robusta* Trimen. Between the bark and the wood of a diseased tree thin, tough, white layers of mycelium are found,

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<sup>1)</sup> A. Rant. De Ziekten en Schimmels der Kina. Mededeelingen van het Kina-proefstation. No. II, Buitenzorg, 1914.

<sup>2)</sup> A. Rant. Der graue Wurzelpilz von *Cinchona*. Bulletin du Jardin botanique de Buitenzorg. Deuxième Série, No. XXII, 1916.

which can change to a yellowish tinge under the influence of the sap of the tree; these layers frequently encircle the entire surface of smaller roots, killing the cambium layer, and consisting of extremely fine hyphae (diameter  $1\frac{1}{2}$ — $2\frac{1}{2}$   $\mu$ ) somewhat fatty in appearance; and when fresh material is taken from the roots and examined in water, alcohol or hydrate of chloral, it shows no septae at an enlargement of 740. On the external side of the bark rhizomorphs are found, but in no profusion; they develop lengthwise, branching out occasionally, are frequently flattened and black in colour with a white heart.

In this connection the fungus slightly resembles *Armillaria mellea* (Vahl) Quél. in Europe. In a former publication of Warburg discussing the so-called „*Cinchona* root-canker”<sup>1)</sup>, the writer mentions also having found rhizomorphs resembling at times those of *Armillaria*.

When inspecting the fungi generally, especially those growing on decaying or dead stumps of wood-trees on the *Cinchona* plantations, it is almost an impossibility to discover the true fruit form or forms of the white root-fungus, however it seemed to me important, not only as a matter of scientific interest, but also for the culture of the *Cinchona*, to trace the fruit form of this fungus, so that the toadstools could be destroyed, as is done with the *Armillaria mellea* in Europe. The safest way was therefore to start from pure cultures and to get to the fruit form in that way. For this purpose I selected diseased roots of *Cinchona* which were thoroughly cleaned and brushed under the flow of the water tap, then dipped into alcohol and set light to, so as to take away all

<sup>1)</sup> O. Warburg. Beitrag zur Kenntnis der Krebskrankheit der Chinabäume auf Java. Ber. d. Ges. f. Botanik zu Hamburg Heft III 1887.

O. Warburg. Bijdrage tot de kennis van den kanker der Kinaboomen. Tijdschrift voor Nijverheid en Landbouw in Nederl. Indië. Deel 35; 1887 blz. 195.

possible external infection<sup>1)</sup>. With a knife, sterilized in a flame, the bark was cut away and small pieces of the mycelium were placed in Petri dishes on an agar substrate of the following consistence:

water	
agar	$1\frac{1}{2}$ —2%
glucose	$\frac{1}{30}$ %
peptone	$\frac{1}{30}$ %
KH <sub>2</sub> PO <sub>4</sub>	$\frac{1}{20}$ %

acid. lacticum 1 % n. pro 100 cc agar.

On this substrate the growth of the fungus was very slow; from the white or yellowish bits of the mycelium, root-like rhizomorphs grew into the agar; these were white at first, then brown, finally black, and from older cultures in high Petri dishes they often grew out of the agar. In most cases only very scanty mycelium-growths were visible on the agar itself, except for a hard brown crust of mycelium. On more concentrated agars, damped rice, bread etc. the growth was somewhat accelerated, yet it maintained a slow process. In the substrate an abundant growth of brown or black rhizomorphs appeared, but on the substrate only very little mycelium was seen, it in most cases being brown in colour.

I tried several kinds of substrates, both liquid and solid kinds, but I will here not dwell upon these, because during my stay in Java, I was unsuccessful in obtaining fruit bodies. On my return to Holland in 1916 I brought some pure cultures over with me, and thanks to the kind assistance of Professor Dr. F. A. F. C. Went, I was permitted to cultivate the fungus in the Botanical Laboratory at Utrecht. On the advice of Miss Cath. Cool, of the Leyden Herbarium, I cultivated the fungus in large Erlenmeyer flasks filled with:

<sup>1)</sup> cf. A. Rant. Der graue Wurzelpilz von *Cinchona* l. c. p. 5.

white sand that had been first thoroughly washed and cleaned,

moss,

bits of living twigs of *Acer Pseudo-Platanus*  $\alpha$ , also thoroughly washed and cleaned,

water.

After sterilization of the flasks and infection with pure cultures, the fungus was permitted to grow to a height of 18–20° C. for the space of one month, and then —

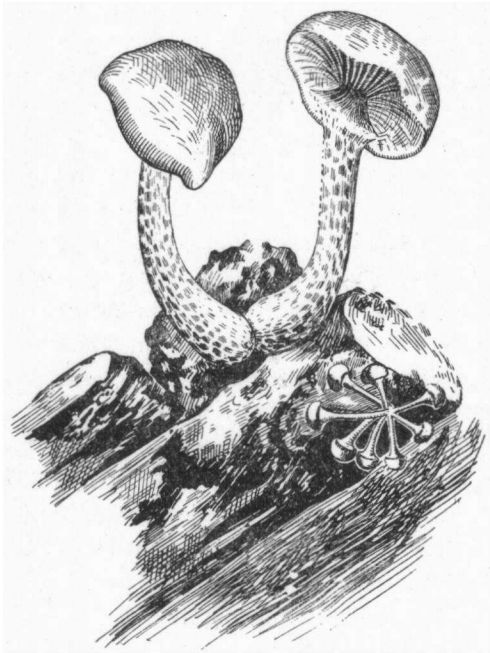


Fig. 1. Growths of the toadstools derived from pure culture.  
Mr. B. J. van Tongeren del.

in November 1916 — the flasks were placed in a room, where in winter, the temperature remained slightly over 0° C. In October 1917 two normal toadstools had developed in one of the flasks (Fig. XIV), but while the

flask was too full of material to allow of further toadstools maturing, there were several young fruit bodies showing (Fig. 1).

Through the courtesy of Prof. Dr. Johanna Westerdijk, then Lady Principal of the Willie Commelin Scholten Laboratory at Amsterdam, I learnt the name of the fungus. Mr. A. van Luyk, assistant of the above mentioned laboratory, was good enough to compare the fungus with *Armillaria mellea* found at Santpoort, near Haarlem, and he detected slight variations which are probably due to the abnormal growth in the Erlenmeyer flask. Of the cultivated fungus I can give the following diagnosis:

„Toadstools originating in clusters of two or more units; pileus 5—6 c.m. in diameter, honey-coloured, varying from yellowish brown to brown, peculiarly marked with small tufts of yellowish or brownish hairs, which disappear in the mature growths; stem: 5—6 c.m., diam 5—6 m.m., brownish colour; annulus: lacking in the mature specimens, but present in the young growths; gills: whitish, touching the stem and somewhat decurrent; spores: ovoid-elleptic or elliptic and slightly pointed;  $7-9 \times 5-6 \mu$  white and smooth; basidia  $27-38 \times 7-9 \mu$ ; cystidia at the margin of the gills:  $45-50 \times 10-12 \mu$ ; rhizomorphs resembling those of *Armillaria mellea* present in the culture and in free nature.”

According to Mr. van Luyk the fungus is *Armillaria mellea* (Vahl) Quél., the well known parasite of trees in Europe.

As far as I know only one description of the same fungus found in Java is quoted in the literature i. e. *Armillaria mellea* (Vahl) Quél. var. *javanica*. P. Henn. found by N. Fleischner at Tjibodas at a height of

1400 M. above the level of the sea <sup>1)</sup>, whereas my fungus was found on the Government *Cinchona* Plantation at a height of 1600 M. According to the description there are certain differences between this fungus and the one I cultivated. So far I have had no opportunity to compare the two fungi; in my opinion this would only be possible by cultivating them, the two examples having been grown under such different circumstances. And how important a part external conditions play in the growth, is a well known fact to every mycologist who has cultivated fungi on different substrates.

I very much regret to have no opportunity of making pure cultures of the *Armillaria* toadstools that were grown from my pure cultures of the white root *Cinchona* fungus to infect the *Cinchona* thereby, as it would be interesting to find out, whether this fungus is a true or wound parasite. Neither is it possible for me to investigate whether all of the sterile white internal mycelia, parasitic to the *Cinchona*, and which I called the white root fungus, will develop *Armillaria* fruit bodies in any case, or whether still other fungi form the peculiar mycelia I described above.

In concluding this article I desire to extend my warmest thanks to all those who have lent me their valuable assistance in making this investigation.

Amsterdam, October 1917.

<sup>1)</sup> P. Hennings, Fungi. Monsunia von O. Warburg; Band I. p. 20, Leipzig, 1900.

#### DESCRIPTION OF PLATE.

Plate XIV. The Erlenmeyer flask containing pure cultures of the white root-fungus and two toadstools photographed by Mr. Van der Gen, Cand. biol. Utrecht.

