On the Morphology and Development of *Phoma Richardiae* n. sp.

By

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(Schluß.)

Considerable difficulties lie in the way of direct proof of the theory above stated, in particular the power the fungus has of growing through ordinary porous membranes. By growing the fungus on an agar drop separated from another agar drop by a thin sheet of collodium, it was hoped that any substances formed in the one inoculated would find their way through the collodium into the second. But the fungus grew through the collodium and produced fruit on the agar on either side [1]). It grew through parchment paper, and goldbeaters' skin under similar conditions.

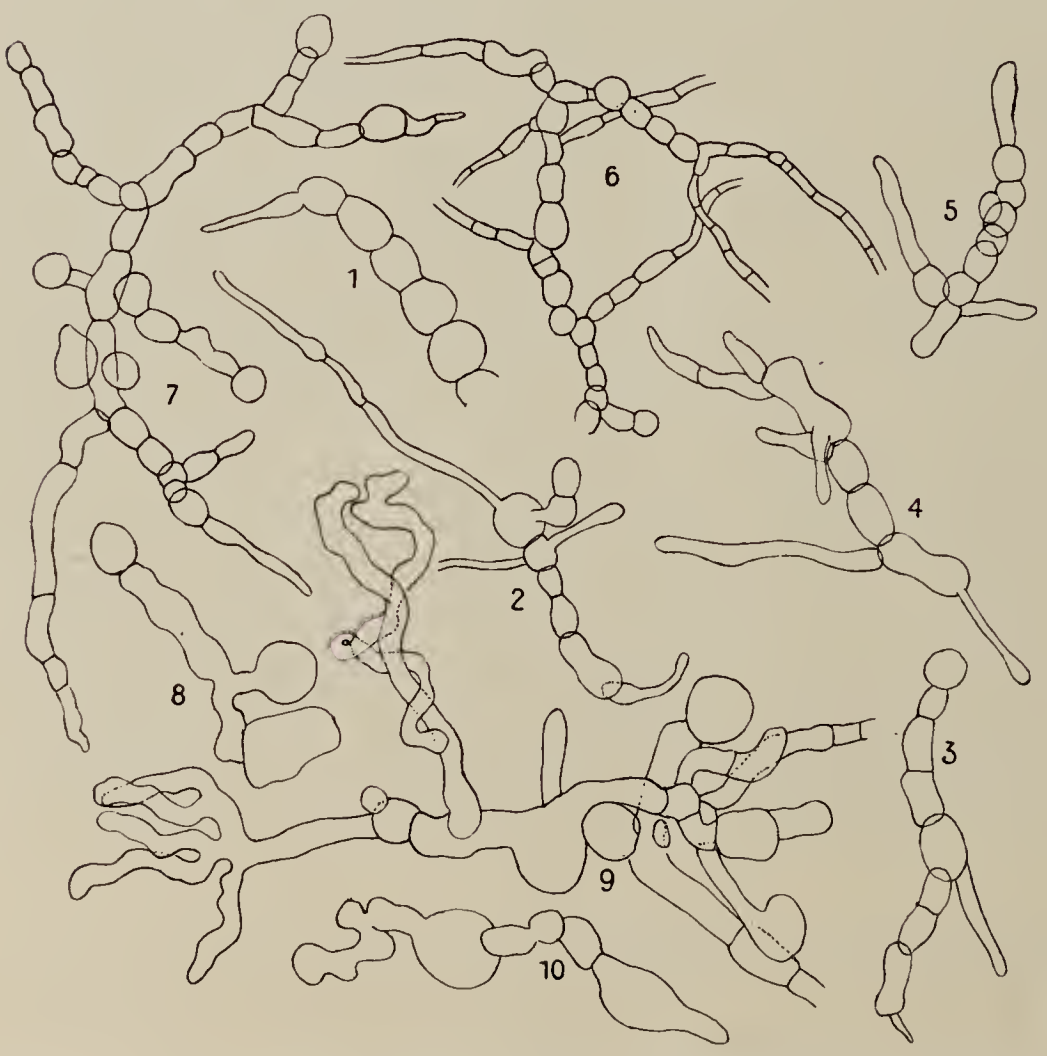

Fig. 6. *1—4:* Young plants from extruded spores on Casein gelatine. — *5—7:* Young plants on gelatine + 1% Oxalic acid. — *8—10:* Young plants on plum agar at 34° C. (Fig. *1—5* and *7—10* = $^{353}/_{1}$; Fig. 6 = $^{263}/_{1}$.)

It is unfortunately impossible to utilize the agar of old cultures for germination experiments. By the time it becomes dark coloured it is usually half dried-up, and in any case it is impossible to find fresh spores brought on to it, among the thousands of old.

Old cultures were, however, boiled up with water and filtered. Small numbers of spores were then brought into hanging drops of the filtrate; they germinated normally. Evidently if a poisonous substance is formed, it is either not removed, or it is destroyed by boiling water.

1) Miyoshi (Jahrb. f. Wiss. Bot. 1895, **28**, p. 269) found that *Penicillium glaucum* and *Botrytis cinerea* both bored through layers of collodium 1,5 mm thick, the hyphae of the latter swelling and growing in a spiral form in the membrane. In the present case the hyphae were straight and slightly attenuated as they passed through the collodium.

It has already been noted that the production of mycelial gemmae, brown conidia, and "spore gemmae" was more marked on plum agar — which is acid — than on Salep, which is neutral. It seemed likely, therefore, that the use of media acidified with various substances might yield results of interest. Of the few substances tried up to the present, only one — Oxalic acid — has given any noteworthy result. Using 0,5 % acid in 5 % gelatine, germination and subsequent growth were slow, but otherwise normal. With 1 % acid the results were rather variable, due no doubt to the fact that the gelatine would not dissolve all the acid [1]). Sometimes growth was normal; more commonly it was very slow and the cells were greatly swollen and distorted. Young plants were produced exactly like those produced from spores extruded on old cultures, e. g. Casein gelatine (Fig. 6, *5—7*, comp. Fig. 6, *1—4*). The rate of growth gradually quickened, and the hyphae became more normal, possibly due to the deposition of crystals of acid, thus freeing the gelatine of a proportion hitherto dissolved. Occasionally the transition from slow distorted growth to normal was quite rapid (Fig. 6, *6*). Similar, but more regular results were obtained with plum agar acidified with Oxalic acid; in this case the slow distorted growth was maintained.

Though no development of the characteristic brown colouration has been produced on artificially poisoned media, it is nevertheless interesting to find that the form of the hyphae may be so similar to that on old cultures. The results of the experiments with Oxalic acid are sufficient to justify further trials, on a larger scale, and these I hope to carry out in the near future.

In the course of a few trials designed to test the effect of temperature on reproduction, the fungus was cultivated at 34° C. As might have been expected with a species whose form is so variable, the growth was greatly modified at this high temperature. Not only was development very slow, but the plants consisted of remarkably distorted hyphae (Fig. 6, *8—10*).

9. Infection Experiments.

Infection experiments on *Richardia africana* have yielded uniformly negative results. Healthy plants, plants which had been kept for a month in a state of drought, and in a water logged soil in a damp atmosphere respectively, and plants etiolated by a fortnight's darkness were all inoculated with no result. Following injury of leaves by a knife, by singeing and by treatment with steam, the fungus obtained a hold on the area actually killed, but did not penetrate into living tissue.

A considerable number of decaying *Calla* leaves from different districts have been examined in the course of this investigation, but only in the one case have *Phoma*-pycnidia been met with. There is little doubt therefore that the fungus is saprophytic only on *Calla*.

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1) The gelatine was made first, powdered acid to the required amount being then added. In hanging drops the amount of suspended acid appeared to vary slightly. When the acid is first dissolved in water and the gelatine then added, a part of the former is thrown out of solution. Had the medium been made in this way the results would probably have been more even, as the acid crystals in this case are finer than the particles obtained by grinding.

10. Systematic position of the fungus.

From the nature of the pycnidia and spores it is evident that the fungus is a species of either *Phyllosticta* or *Phoma*. These two genera are very similar in the form of their fruits.

According to Saccardo the distinguishing characters are:

Phyllosticta . . . maculicola, poro pertusa,
Phoma . . . . . non maculicola, papillata.

Allescher (in Rabenhorst's Cryptogamenflora) divides them according to the substratum:

Phyllosticta . nur Blätter bewohnend, seltener mit kleiner Papille,
Phoma . . . auf Stengelorganen etc., mit Papille.

In the case of fungi which are not proved parasites no great reliance can be placed on the substratum as a means of identification.

Even the neck is not a very certain means of distinction, for it varies a great deal with different species of *Phoma*. In *Phoma apiicola*[1]) it is straight, broad and tubular, set on the top of a spherical body, like the neck of a flask; in *Phoma suspecta*[2]) it is short, tapering, and continuous with the main part of the wall, resembling the truncated "neck" of a pear. On the other hand the apex of the pycnidium in some species of *Phyllosticta* — e. g. *P. tabifica*[3]) — is drawn out so that the fruit body approaches very closely in shape to that of some *Phomas*.

In the present case no neck is formed on most media though the apex may be slightly pushed out. In cultures on sterile *Calla*-leaf, however, a short pear-like neck is present. Of the culture media tried, *Calla*-leaf is certainly the most natural, and as the pycnidia are the most regular here, it is probable that the presence of a neck is a normal character. I regard the fungus therefore, as a species of *Phoma*.

Identification might be assisted by a careful comparison with all the species in the two genera; but their great number, and their great similarity in morphological characters, renders the task an unenviable one, while the scanty descriptions given in many cases tends to nullify the advantages derivable from the labour.

There are many species in the two genera, occurring on Monocotylous plants, whose pycnidia and spores are comparable with those in the present case, but no species in either genus, with a similar secondary fruit form is mentioned in Rabenhorst. Only one species occurring on a member of the *Araceae* — *Phyllosticta nitida* Rob. (= Saccardo's *Phoma nitida*) — on dry leaves of *Calamagrostis arenaria* is there mentioned.

It is described thus — „Fruchtgehäuse auf der Blattoberseite zerstreut, klein, glänzend, halbkugelig, innen weiß, von der später der Länge nach gespaltenen Epidermis bedeckt, mit papillenförmiger Mündung; Sporen fast eiförmig, 5 μ lang".

The description is not very complete, particularly as regards the colour of the neck, and the presence or absence of sterigmata. As, how-

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1) vide Klebahn l. c.
2) vide Massee (Diseases of Cultivated Plants and Trees 1910, p. 406).
3) vide Rabenhorst l. c.

ever, the spore length is smaller than that of the *Calla*-fungus, it is probable that the two are distinct species.

HALSTED [1]) has cited a *Phyllosticta* occurring on *Calla* in America; but as no description has been published it is impossible to identify it.

Possibly the present fungus represents a new species. But comparative cultures with a great number of others would have to be carried out before this could be determined with certainty. It is desirable, however, that the fungus should have a distinctive title. I therefore propose that it should be named *Phoma Richardiae.*

In the identification of the second fruit form — the brown conidia — there is little doubt under what genus search must be made. In ENGLER and PRANTL only three genera in the *Dematiaceae-Dictyosporae* whose spores are formed in chains, are given, viz: *Sirodesmium*, *Fumago*, *Alternaria.*

The length of the conidiophore excludes the *Calla*-fungus from the first genus, while its vegetative habit is quite distinct from that of *Fumago.* LINDAU (in RABENHORST) describes three species of *Alternaria* which are comparable with it, as regards size and shape of spores, viz:

A. brevicolla PREUSS.

Ausgebreitet oder hier und da rasenartig, schwärzlich. Conidienträger aufrecht, cylindrisch, etwas büschelig, septiert, an der Spitze abgestutzt, rauchfarben, 45 bis 50 μ lang, 5 μ dick. Conidien umgekehrt eiförmig, an der Basis kurz zugespitzt, mit 3—4 Scheidewänden und mauerförmig, nicht eingeschnürt, grauschwarz, 20 μ lang, 12 μ dick. Auf Holzstückchen. (SACCARDO läßt es zweifelhaft, ob die Conidien kettenförmig stehen.)

A. humicola OUDEM.

Reife Rasen kreisförmig, schwarzgrün. Conidienträger articuliert, traubig verzweigt, hyalin, 3—5 μ dick. Conidien verschieden gestaltig, cylindrisch, umgekehrt keulig, länglich, flaschenförmig, zuerst hyalin, dann honigbraun, zuletzt braun und schwarzgrün bis rauchfarben, mit 3—7 Scheidewänden und mauerförmig, an den Wänden nicht oder kaum eingeschnürt, im Alter dicht und sehr fein an der Oberfläche punktiert und rauh, von sehr verschiedener Größe, bis 50 μ lang und 16 μ dick. Auf Gelatine, die mit humöser Erde aus dem Walde Spanderswoud inficiert wurde, im Laboratorium gewachsen.

A. chartarum PREUSS.

Weit ausgebreitet, unbestimmt in der Gestalt, zuerst braun, dann schwarz. Hyphen kriechend, aufsteigend oder aufrecht, verzweigt, septiert, mit unregelmäßigen, stielartigen Ästen. Conidien kugelig oder länglich, oben sich in einen Halsteil verlängernd und dann kettenförmig verbunden, braun oder schwarzgrün, mauerförmig geteilt. Auf Fliegenpapier und feucht liegendem Papier.

A. brevicolla thus differs materially from the fungus under consideration, in the small size of the spores, and the absence of constriction at the cross walls. The description of *A. humicola* agrees more closely; but the profuse branching of the conidiophore, and the finely "punktiert" surface of the spore are sufficient grounds for regarding it as distinct. The absence of spore measurements makes it impossible to judge whether *A. chartarum* is identical with the *Alternaria* form of the *Calla*-fungus; as no mention is made in the former case of marked irregularity in the form and size of the spores it seems extremely doubtful.

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1) I am indebted to Dr. B. D. HALSTED of New Jersey Exp. Stat. U. S. A. for this information.

It would seem, therefore, that the secondary fruit form of the *Phoma* represents a new species of *Alternaria*.

11. Principal Results.

1. A fungus which cannot be identified with any species previously described, and to which it is proposed the name of *Phoma Richardiae* should be given, was found on decaying leaves of *Richardia africana*. It is not parasitic on the latter plant.
2. The fungus has two conidial forms, and produces mycelial gemmae. It may be described as follows:

   Mycelium at first hyaline and colourless, with numerous oil drops; yellow-brown with age.

   Pycnidia round or oval 120—150 μ in diameter, at first yellow, darkening with age to brown-black; with a short, usually tapering neck 20—30 μ in diameter, darker than the body in early life. Cavity very densely packed with spores. Wall of two or three layers of irregular, undifferentiated cells. An extremely variable organ; on artificial media often very irregular in shape, with no neck, and varying between 20 μ and over 200 μ in diameter.

   Pycnospores unicellular, hyaline, oval or egg shaped, with two or three oil drops towards either end, 6—7 μ long and 4 μ broad; borne directly on the innermost wall cells, with no sterigmata; extruded in masses.

   Free conidia borne in simple or branched chains on unbranched conidiophores, 5—50 μ long, or at the end of long branches. Spores pear-shaped, oval, or irregular, brown-black, thick walled, smooth, with three or four cross walls, and sometimes one or two longitudinal or oblique, 20—40 μ long and 15 to 25 μ broad. „Zwischenstück“ up to 30 μ long.

   A species of *Alternaria*, not identifiable with any previously desbribed.

   Gemmae developed on mycelium, in old cultures, dark coloured, thick walled, very variable in shape; of three types:

   a) Special branches resembling distorted brown conidia or chains of conidia,
   b) Local stretches of hyphae, 2—10 cells in length,
   c) Grape-like masses of cells, intercalary or terminal.

3. The pycnidia are evolved in two distinct ways:

   a) Symphyogen. Hyphae weave together and branch profusely, forming a dense cellular mass or primordium. A cavity, in which the spores are formed, arises in the centre, while the mass is still growing in bulk, and expands with the mass.

   b) Meristogen. The primordium is formed by the repeated division of a few adjoining cells of a hypha, usually aided by the fusion of short branches.

   Under certain circumstances the pycnospores may divide in this manner to form pycnidia direct.

   Many intermediate methods of formation occur, a more or less regular sequence from one extreme to the other being observed in some cultures.

4. The pycnospores undergo complex changes with age:
   a) They may grow out directly into chains of brown conidia,
   b) They may form gemmae.
5. Young spores behave similarly when crowded in hanging drops of Cane Sugar or other media.
6. It is suggested on the following grounds that the fungus excretes a substance poisonous to itself:
   a) The behaviour of the spores with age, and in crowded hanging drops.
   b) The production of gemmae, late in life,
   c) The discolouration of agar in cultures, especially around the gemmae.
7. Partial success has attended the attempts up to the present made at experimental proof of the self-poisoning theory advanced.

Hausschwammstudien III[1]).

3. Ansteckungsversuche mit verschiedenen Holzarten durch *Merulius*-Mycel.

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(Mit 3 Textfiguren.)

Infectionsversuche mit Hausschwamm müssen, wenn ihre Resultate auf practische Verhältnisse übertragbar sein sollen, selbstverständlich unter möglichst natürlichen Bedingungen angestellt werden. Von diesem Gesichtspunkte ausgehend, begann ich im Mai vorigen Jahres eine Reihe solcher Experimente mit verschiedenen Hölzern in meinem Versuchskeller direct auf dessen porösen Backsteinfußboden. Zu der constanten Feuchtigkeit der Kellerluft kommt hier als mitwirkender Factor also die Bodenfeuchtigkeit, welche aufgelegte Holzproben allmählich näßt, Glasschalen beschlagen unterseits mit feinen Wassertropfen. Der Pilz wuchs auf diesem Fußboden in üppigen weißen Mycelrasen, ausgehend von einem größeren inficierten Fichtenholzstück.

Der Ausfall dieser Versuche widerlegt zunächst die Meinung, daß nur wasserdampfgesättigte Luft für den Befall unsterilisierten Holzes optimale Bedingungen schafft, höherer Feuchtigkeitsgehalt des Holzes unter natürlichen Verhältnissen also von Nachteil sei. Im Gegenteil wächst und wirkt unser Pilz da am besten auf direct feuchtem Substrat, also auch auf den wassergetränkten Teilen sonst lufttrockener Hölzer[2]). Hier vermag er bei längerer Einwirkung selbst Eichenholz in wenn auch beschei-

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1) Dieses Centralblatt 1912, **1**, 2, 133, 166.

2) Zu den übrigens nicht näher belegten Angaben bei R. Falck (*Merulius*-fäule, p. 305) steht das in directem Gegensatz.