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

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(Fortsetzung.)

5. Germination of pycnospores.

The most vigorous germination takes place on plum agar. Here the spore swells to three or four times its original size, pushing out most commonly two, but sometimes one or three germ tubes 3,5—4 μ in diameter (Fig. 3, *1—12*). The germ tubes may be produced simultaneously (Fig. 3, *5, 7, 8*) or successively; the third germ tube, when formed, often arises after the others have attained considerable size (Fig. 3, *12*). During

germination the spore frequently elongates, and divides in two by a cross wall (Fig. 3, *4, 6, 8*); the elongated spore may become greatly pinched in where the cross wall is laid down, giving the impression of two distinct spores lying close together (Fig. 3, *3, 5, 10*). The bases of the germ tubes often swell irregularly (Fig. 3, *5, 7, 9*).

On Salep agar the spore swells very little and the germ tubes are narrower; in water the latter are only 2 μ broad (Fig. 3, *13—21*). On Lecithin gelatine they may commence as broad rounded buds, 7—8 μ in diameter (Fig. 3, *22—27*), though they develop later as normal tubes (Fig. 3, *28, 29*). On Cane Sugar gelatine the germ tubes are usually irregular, and the spore does not greatly enlarge (Fig. 3, *30—33*).

6. The brown conidia.

On the "Luft"-mycelium chains of conidia are formed in clusters or scattered singly (Fig. 3, *34—40*). The chains usually consist of a small number of spores, seldom more than five or six being attached together; they may be branched or simple. The spores do not readily fall apart. When produced in clusters, the chains are generally borne on a definite unbranched conidiophore 5—50 μ in length (Fig. 3, *34, 35*), but isolated chains often arise at the end of long branches which it is difficult to regard as conidiophores in the ordinary sense of the word (Fig. 3, *37—39*). They occasionally spring from the wall of the pycnidium (Fig. 3, *40*). Hyphae bearing chains of conidia are not infrequently thick walled and dark coloured in the region from which the conidia arise.

Fig. 3. *1—27*: Germinating pycnospores. — *1—12*: on plum agar; *13—21*: in water; *22—27*: on Lecithin gelatine. — *28, 29*: Later stages on Lecithin gelatine. — *30—33*: on Cane Sugar gelatine. — *34—39*: Brown conidia on aerial mycelium on plum agar. — *40*: The same on pycnidium wall. — *41—45*: Stages in development of brown conidia. — Fig. *1—33* = $^{353}/_{1}$; Fig. *34—45* = $^{153}/_{1}$.

The spores are roughly pear-shaped, being drawn out at the apex into a beak which may be up to 30 μ long. They vary in length between 20 and 40 μ and in breadth from 15 to 25 μ. Not infrequently they are oval, linear, or irregular in outline. They are smooth, thick-walled and divided by three or four cross walls; some of the cells

may be again divided in a longitudinal or oblique direction. They contain oil drops. During growth their colour changes from yellow-brown to brown-black; with age they may be impenetrable by light. The amount of constriction at the cross walls is very variable, but is usually well marked. These conidia are not produced in great abundance, especially on some media. On horse-dung agar, and on plum agar kept at 12° C — in both of which cases aerial mycelium is well developed — they are the most abundant. They never precede pycnidia formation; the majority are developed late in the life of the culture. Since they are thick-walled and dark-coloured, and arise on aerial mycelium, it is impossible to see their structure while still growing. By cutting out pieces of agar from PETRI-dish cultures and mounting in Lactophenol[1]), a sufficient number of examples in different stages of growth were obtained to enable their manner of evolution to be traced (Fig. 3, *41—45*). The conidia consist of special branches of the mycelium marked out in their earliest stages from vegetative branches, by their darker colour, and by the frequency of cross walls (Fig. 3, *41*). The base of the branch for a varying length remains unaltered, to form a conidiophore. Towards the apex the cells swell up and thicken their walls, to form the first conidium (Fig. 3, *42*, *43*). The apex meanwhile continues growth as a yellowish tube divided by cross walls into short cells (Fig. 3, *43*, *44*). A basal portion undergoes little alteration, forming the "Zwischenstück", while cells towards the apex again swell up and thicken there walls to form the second conidium (Fig. 3, *45*). The same process may be repeated several times. The first conidium may continue growth during the formation of the second; in some cases it remains smaller than the second (lowest chain Fig. 3, *34*).

Any cell of the conidium may give rise to a lateral branch developing in the same manner as the parent. In this way the chains may come to be branched (Fig. 3, *35*, *36*).

7. Mycelial gemmae.

Various gemma-like bodies are formed in old agar cultures. Since no two are exactly alike it is difficult to classify these growths; roughly, they may be divided into three groups, viz: —

a) The first variety consists of modified branches of the mycelium (Fig. 4, *1—5*). The evolution of this type of gemma, which follows closely that of the brown conidia, was followed by marking the position of a few in the earliest stages in a moist-chamber culture, and making drawings at intervals of a few days (Fig. 4, *1a* and *e*). Short branches arise and become divided by cross walls at close intervals (Fig. 4, *1a*); the cells thicken their walls and swell very irregularly, those in the middle as a rule becoming larger than the rest (Fig. 4, *1b* and *c*). The apex may continue growing, the cells thickening their walls as formed (Fig. 4, *1d* and *e*). From one or more of the cells short tubes may proceed, which divide and swell in the same manner as the parent. Simple growths may resemble distorted brown conidia (Fig. 4, *2*, *4*) while

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1) Lactophenol consists of approximately equal parts of Lactic acid, Phenol, Glycerine and Water.

complex forms are sometimes suggestive of chains of brown conidia (Fig. 4, *1*, *5*).

b) The second variety is represented by specialized stretches of the mycelium (Fig. 4, *9—16*). It has already been mentioned that in old mycelium the protoplasm becomes concentrated locally in bands of 2—10 cells' length. The cells divide, swell, thicken their walls, and assume a dark colour. Short branches with unthickened walls often arise from them (Fig. 4, *16*).

c) Gemmae may also take the form of grape-like masses of cells (Fig. 4, *17—21*). They may be terminal (Fig. 4, *17—19*) or in the middle of a hypha (Fig. 4, *20*, *21*). Their development was followed in the usual way, and takes place in the repeated division of a few adjoining cells, with concurrent swelling of the separate divisions. The walls gradually thicken and darken in colour; for the most part the masses have reached a considerable size before the thickening and darkening becomes apparent (Fig. 4, *22a—e*). This form of gemma is very characteristic of Salep agar cultures, and is comparatively rare on plum agar.

Combinations of the different forms of gemmae occur, e. g. a conidium-like growth arising from a gemma of type *b* or *c* (Fig. 4, *12*, *15*); there are many intermediate forms between types *b* and *c* (Fig. 4, *16*, *23*); while some are so irregular (Fig. 4, *6*, *7*, *8*) that it would be difficult to place them in any of the three classes.

All forms agree in being dark-coloured, the colour deepening with age, and in many cases becoming finally black. In old cultures gemmae are often impenetrable by light, even after boiling in Lactophenol, so that the structure cannot always be seen. They contain variable quantities of oil. With prolonged soaking in Lactophenol the colour is partially removed and they become transparent, many of the oil drops becoming at the same time invisible. In gemmae of all types it is frequently to be observed that the individual cells round themselves off, and in so doing partially separate (Fig. 4, *5—7*, *12*).

A halo of dark-coloured agar usually surrounds these bodies. In a moist-chamber culture it may be observed gradually increasing in density with the growth of the gemma; it is always darkest nearest the gemma, becoming gradually lighter in shade towards the periphery. On Salep agar the colouration is not so marked as on plum agar, and on gelatine media it is very faint or altogether absent.

The gemmae are all capable of germination. When the agar of an old culture is teased out and spread over fresh agar they germinate readily. Germination takes place in the pushing out of one or more germ tubes, whose bases are frequently swollen (Fig. 4, *24—27*). Usually the number of germ tubes produced is small. Germination also takes place, though more slowly, in hanging drops of water.

The simpler forms of type *a* are so similar to the brown conidia on the aerial mycelium, that they might well be regarded as conidia, distorted by reason of the fact that they are produced in the agar. But the gradation of forms from the simple to complex irregular types with no morphological resemblance to conidia is sufficiently complete to justify their inclusion together as gemmae.

The development of the third type follows the same lines as that oft he meristogen pycnidia, and the gemmae are often fairly regular;

moreover their walls may thicken only when cell division has ceased. They might therefore be regarded as abortive pycnidia. But, again, numerous cases are met with where the resemblance to developing pycnidia is very slight.

Fig. 4. *1, a—e*: Development of mycelial gemmae; Type *a*. — *2—5*: Mycelial gemmae; Type *a*. — *6—8*: Mycelial gemmae; intermediate types. — *9—16*: Mycelial gemmae; Type *b*. — *17—21*: Mycelial gemmae; Type *c*. — *22*: Development of Type *c*. — *24—27*: Mycelial gemmae germinating. — (All = $^{263}/_{1}$.)

8. Changes in the pycnidial spores after extrusion.

The pycnospores undergo various changes after they are extruded. As, however, the sequence of events is difficult to follow in agar cultures owing to the massing of the spores, it will be convenient to describe first the changes undergone under other circumstances.

When a hanging drop of cane sugar solution is inoculated with a few spores, germination takes place readily and normal mycelium, bearing pycnidia, is formed. When a very large number of spores is used for inoculation their behaviour is very different.

a) The majority swell up to several times their original size, and assume a yellow-brown colour, gradually thickening their walls. Some remain as large round, oval, or kidney-shaped single cells (Fig. 5, *1—4*); a great many divide and grow further, forming small masses (Fig. 5, *5—12*) or bands of cells (Fig. 5, *13—16*). They may push out short hyaline buds or tubes (Fig. 5, *6—11*). Not infrequently the products of two separate spores become united by short hyaline bridging tubes (Fig. 5, *12*). It is convenient to designate these structures collectively as "spore gemmae" [1]).

b) Round the edge of the drop groups of spores swell, and, remaining hyaline, become joined by short bridging tubes; from one of them chains of pear-shaped yellow-brown conidia similar to those produced on the aerial mycelium may arise (Fig. 5, *17, 18*). This takes place especially where the spores happen to be very thickly massed around the edge. The chains are almost invariably directed outwards.

1) Spores of *Fumago vagans* behave somewhat similarly under the same conditions (vide Zopf, l. c.).

c) Some of the spores produce straight germ tubes which extend normally for a time without branching, and then their terminal cells divide, darken and thicken to form either gemma-like groups of cells, or pear-shaped conidia (Fig. 5, *19*).

d) Some divide to form a gemma-like group of cells from which a thin straggling, usually unbranched hypha proceeds (Fig. 5, *20—22*).

e) Sometimes a few adhering to the cover glass or remaining suspended in the body of the drop undergo no change beyond very slow swelling and darkening.

f) Finally a few spores lying on the edge of the drop germinate normally and give rise to mycelium on which pycnidia may be formed. The formation of short bridges between adjaient spores, and between the germ tubes of neighbouring spores is characteristic (Fig. 5, *23*, *24*). Occasionally the germ tubes unite at their tips (Fig. 5, *24*).

In all cases numerous oil drops are developed, whether in the gemmae or conidia — in which case they are both large and numerous — or in the mycelium.

While these changes are going on, the free surface of the hanging drop develops a skin which, at first pale straw colour and delicate, becomes eventually yellow-brown and tough. After about three weeks it is impossible to get the spores out separately with a needle; when this is attempted a piece of the skin with spores embedded in it is brought away. Around each developing spore, the skin is especially dark coloured, the colour being densest in the immediate proximity of the spore.

Similar results were obtained with Cane Sugar solutions varying in concentration from 40% to 0,5%, with varions strengths of Grape Sugar solution, with tap water, and with plum agar drops, provided a great mass of spores was used for inoculation in each case. The food medium does, however, exert some influence, for spores germinated normally on drops of very strong potato decoction gelatine; but when the medium was diluted with three or four times its volume of water many of the spores developed as in Cane Sugar solution.

The "spore gemmae" and "spore conidia" germinate readily when brought on to agar (Fig. 5, *25—30*).

Proceeding now to the examination of spores extruded from pycnidia in normal agar cultures, precisely similar modifications are to be found. In this case, however, the changes take place much more slowly. Whereas in the sugar drop solution all the various growths detailed are to be met with in the course of a fortnight or three weeks, in normal cultures the first changes in the extruded spores are apparent only after this length of time.

The masses of spores then begin to assume a yellowish colour, and, passing through yellow-brown become finally black. At the same time numerous chains of dark pear-shaped conidia arise from them, radiating in all directions, giving the culture a remarkable appearance (Fig. 5, *31*). When the masses are sectioned it is found that only a comparatively small percentage of the spores are markedly altered. The majority have swollen to two or three times their original size, and assumed a smoky colour, while a few have behaved like the spores in sugar solution.

Spores remaining in the pycnidia behave similarly with age.

The number of spores in each mass is so great that it is rarely possible to see the exact connection between them and the chains of conidia. In one or two cases, however, where the mass had become somewhat disintegrated, after soaking for a month in Lactophenol it was possible to see that the conidia chains, like those in sugar solution, sprang from a group of spores hooked together by bridging tubes (Fig. 5, *32*).

Development may sometimes be watched in a moist-chamber culture where the extruded spores happen to spread out over the surface, instead of remaining in heaps. Fig. 5, *33—38* represents gemmae developed from spores extruded on the surface of a plum agar moist-chamber culture. The identity of these conidia with those produced on the "luft" mycelium is established by their agreement in form. They germinate readily on agar. In order to see whether the plants arising from them were in any way different from those produced from pycnospores a number were obtained from an old culture and spread out over sterile agar in a PETRI-dish. On germination (Fig. 5, *39—43*) isolated conidia were selected, examined under a high magnification to see that no pycnospores were adhering, and then, under a low power, picked out with two sterile needles, and brought on to hanging drops of agar in moist chambers. The resultant plants presented no features distinguishing them from those arising from pycnospores.

Fig. 5. *1—16* and *20—22:* "Spore-gemmae" in hanging drops of Cane Sugar. — *17—19:* Brown conidia in the same. — *23, 24:* Germinating spores in the same. — *25—30:* "Spore gemmae" germinating on plum agar. — *31:* Mass of extruded pycnospores, with chains of brown conidia arising therefrom (on old plum agar culture). — *32:* Single chain of brown conidia, showing origin (on old horse dung agar culture). — *33—38:* Spore gemmae from spores extruded on hanging drop of plum agar. — *39—43:* Brown conidia germinating on plum agar. — *44, 45:* Brown conidia from spores direct, and on mycelium respectively. — Fig. *1—16, 20—30, 33—37* = $^{608}/_{1}$; Fig. *17—19, 32, 38—45* = $^{263}/_{1}$; Fig. *31* = $^{153}/_{1}$.

The amount of modification which the extruded spores undergo varies with different media. On all the agar preparations used the changes were fairly uniform, but on gelatine media the alteration was confined to swelling of a limited number, with browning in a few instances.

On most other solid media e. g. Carrot, Radish etc., as also in liquid hanging drops the amount of change was similar to that on agar; on potato, however, and to a lesser degree on Asparagus a very large proportion of the spores swelled and divided forming dark masses, tissue-like in section.

On all media a few of the extruded spores germinated normally, but the growth of the resulting plants was usually limited and not infrequently abnormal; e. g. Fig. 6, *1—4* on Casein gelatine; frequently, too, gemmae were formed from the spore after germination.

The close resemblance between the alterations undergone in the normal course of events, when spores are extruded on to the food medium and those taking place in fresh spores in crowded hanging drops justifies the belief that the same factors are involved in each case, the changes being slower and less general in the former instance owing, probably, to lack of moisture. To make sure of this a piece of agar was cut out of an old Petri-dish culture, and covered with water in a sterile dish. A great number of spores floated to the surface, on which a skin quickly formed; there was soon a great development of the characteristic growths. Those remaining below the surface of the water, like those suspended in the hanging drops of sugar solution, underwent little change.

In following the morphology of this fungus interesting physiological questions are raised, in particular that of poisonous excretions. It is known that many bacteria excrete substances inhibitory to their own growth, e. g. *Pseudomonas destructans*[1]). Wehmer[2]) has shown that *Penicillium italicum* and *Penicillium olivaceum* similarly poison their own food medium by excreted waste products. The behaviour of the present fungus is at times very suggestive of the results of a self-poisoning action. The concentration of the protoplasm of the mycelium in short stretches, the formation of thick-walled brown conidia and gemmae are apparent indications of an adaptation to unfavourable circumstances; while the fact that these growths only occur late in life[3]) is suggestive of a disturbance of physiological, not purely physical, balance.

The darkening of the agar in a culture can be due to only one of two causes; either to some substance excreted from the fungus, or to purely chemical changes going on in the agar as a result of the absorption of some of its ingredients. In either case the effect would be cumulative. So, too, the formation of an especially dark halo around the gemmae could be explained on either ground, for as the dark patches are formed around parts of the plant in which protoplasm is massed (as opposed to the greater part of the mycelium, which loses its contents after a time) it is natural that the changes in the agar should be most marked in these regions. The variation in the amount of discolouration in different media

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1) Potter, Journ. Agric. Sc. 1908, Vol. III, Part I, p. 103.

2) Wehmer, Beiträge zur Kenntnis einheimischer Pilze II, 1895.

3) A Petri-dish is overspread by the mycelium in about a week, but the brown conidia and gemmae appear chiefly after the culture is a month old.

may be explained, also, on either ground. But the gemmae begin to assume a dark colour before the halo appears; the latter spreads from the gemmae, gradually extending its edge further afield, and always being densest in the centre; further, the brown conidia on the "luft" mycelium — which do not come in contact with the agar at all — also develop a dark brown colour. These facts taken together indicate that the cause of the colouring, both of fungus and agar must be sought for in some vital process, rather than in a purely chemical change in the food medium; though the food medium obviously exerts an influence, as witness the variation on different media.

The theory of self-poisoning receives support from the behaviour of the pycnospores. It is to be noted that the changes observed, occurred only where a great number of spores were present in a limited space, under conditions where germination was possible. It the plant does excrete a poisonous substance, the most marked effects would be looked for where a great number of spores germinated, or attempted to germinate in a confined space Whatever the cause, it is evident that the spores soon find themselves in the same position as the mycelium in an old culture; they either grow out to gemmae direct, or pass over at once to the formation of brown conidia. Those at the edge of the drop which can send out mycelium into the air, retaining only sufficient contact with the liquid to permit of food absorption, and thus having comparatively little surface exposed to poisonous effects, are alone capable of normal growth. Were it purely a matter of competition it would be expected that the outermost spores in the drop would fare the worst; and that the rest would tend to form thin straggling hyphae instead of large conidia and gemmae rich in oil. It is difficult to account for the spore changes on the assumption that they are caused by the purely chemical alteration in the food medium, consequent on the absorption of some of their ingredients, since they take place in a solution of Cane Sugar in distilled water, and in ordinary tap water. (Schluß folgt.)

Referate.

JAVILLIER, M., Influence du zinc sur la consommation par l'*Aspergillus niger* de ses aliments hydrocarbonés, azotés et minéraux (Compt. Rend. Acad. Sc. 1912, **155**, Nr. 2, 190—193).

Bei Anwesenheit von Zink ist *Aspergillus niger* imstande, alle für sein Wachstum notwendigen Nährstoffe besser auszunutzen; der Pilz haushaltet sparsamer, indem er, zugunsten seines Wachstums, wenig für seine Erhaltung verbraucht. So verbrauchte z. B. der Pilz für die Bildung von 1 g Trockensubstanz bei Anwesenheit des Stickstoffs in Form von Ammoniumtartrat nach zweitägiger Cultur ohne Zink 11,45 g, mit Zink 3,70 g Zucker. Der Verbrauch von Stickstoff betrug bei Abwesenheit von Zink 0,091, bei Anwesenheit desselben 0,054 pro Gramm Trockensubstanz, wenn der Stickstoff als Ammoniumtartrat in der Lösung enthalten war.

Die Zusammensetzung der Asche von *Aspergillus niger* wird ebenfalls durch die Anwesenheit von Zink beeinflußt. Der Gehalt an Si und