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Stagonospora Cassavae n. spec.

P. C. VAN DER WOLK, Arnhem (Holland). (With 11 Textfigures.)

1. In the early part of last year (1913), there appeared most unexpectedly in the Selection Garden at Buitenzorg a severe mould disease affecting the young slips of Cassava (Manihot utilissima). From the very beginning the disease was so virulent and progressed at once with such severity that the direst forebodings of an immediate and wide distribution of this sickness in the Cassava culture in Java appeared to be fully justified, seeing that vast quantities of selected Cassava material are distributed yearly from the Buitenzorg Selection Garden over the whole of the Indian-Archipelago, and that the disease appeared to be axceptionally infections. Where the disease came from has remained an enigma till now; so far as can be traced it was still unknown in the rest of Java and in the other islands. Since its appearance in the Selection Garden it has become quite habilitated, where it has become the greatest calamity which has yet befallen the Cassava plantations. Fortunately at the present time we have indeed completely succeeded in combatting the disease. The mould in question is a typical wound parasite; the seat of infection has till now exclusively been that cut surface of the slip which is situated above ground. The disease is absolutely combatted by the tarring of these over-ground cut surfaces when the slips are set in the ground immediately after they have been cut. Further tests should decide whether or not in the course of time infection can take place via the ground also, and what then the consequences would be of tarring the cut surface of the slip situated in the ground. If through certain circumstances the slips do not come directly into the ground immediately after they have been cut it is certainly of very great importance to very early well tar both cut surfaces of the slip. Independantly of this however the necessary care should be taken that the slips are treated with some caution lest they sustain an unnecessary injury. In any case we may be thankful that this very dangerous Cassava disease has been able to be so quickly combatted in such an efficient and extremely simple way.

2. The mould in question, which belongs to the Stagonospora-type and through its peculiar deviations has been brought under the heading of a new species, Stagonospora Cassavae, is pitch-black. It is a special destroyer of the wood elements in the body of the plant and spreads from the cut surface of the slip with incredible rapidity, by way of the woody elements in the bark and bast, over the scarcely opened buds and also over already old shoots, which acutely withered and go to ruin. Then the extension through the secondary woody part of the slip gradually takes place.

In the course of time the small pitch-black round pycnides are for-- med, which I shall return to presently. These pycnides are principally 15 Mycologisches Centralblatt, Bd. V.

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formed between the bast and the wood but do not break their way through the bast and the bark. Finally in the same way the pycnides arise also in little quantity from that part of the cut surface of the slip which is in open air. So long as the young expanded buds have not yet died off, the disease is directly recognizable by the black mycelial covering of the cut surface, yet it is quite too late to combat with it then. The mycelium appears, in the pure-cultures also, in the first place as a downy mass but towards the time of the forming of the pycnides this downy covering sinks together, and a humid loathsome pitch-black layer of moulds appears, as it were a covering of wet paint, where the forming of the pycnides, which lasts very long, continues to run its course.

3. I have been very successful in obtaining pure-cultures on several pabula, especially on boiled rice. For a finer investigation into the typical habitus of the mould this pabulum is but little adapted through its too great humidity, which conduces to the falling together of the mycelium which as it is this mould is too much addicted to. I have at length obtained the best results by nursing the mould on sterilized pieces of Cassava wood.

Something peculiar now makes its appearance in that mycelium.

In the mycelium threads more or less regularly arranged and for the most part in pretty large numbers there occur round bodies which at the first glance remind one of nuclei or oil drops or something similar.



Fig. 1. a Mycelium-thread of Stagonospora Cassavae with endospores. × 650.
b Germination of the endospores lying within the mycelium-thread. ×650. Such a thread is represented in figure 1a. I paid little attention to them in the beginning. I busied myself to show that these round bodies were no oil, nor glycogen, nor granuloselike substances, nor other reservematerial. What to think off of these obscure bodies! I, originally remained to view them as something rarely occuring pabulum, or, that my technics in indicating of this reserve-material was fault, or, that the used reagenti a were not pure, a matter which

already often had troubled my experimental works in the laboratoria of the Selection-and Seed Gardens of the Department of Agriculture at Buitenzorg.

In a such sceptical frame of mind I made acquaintance with the discovery of WEHMER¹) concerning the refractive globules in the spores of Merulius lacrymans. This rechearch opened new points of view and so I spared no trouble in trying to indicate that my obscure globules in *Stagonospora Cassavae* perhaps were also drops of aetherian oil: yet without succes.

Then, I did not other effort to identify the substance of these obscure bodies. No one moment I thought that they should betray themselves as spores, for therefore, as is to be seen in the figures, the seizes are apparently too small for being spores of a higher mould as

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¹⁾ C. WEHMER, Die Natur der lichtbrechenden Tröpfchen in den Sporen des Hausschwammes (Merulius lacrymans). Ber. D. Bot. Ges., Bd. XXIX, 1911.

Stagonospora is, and, also, for a higher mould the number of the globules is apparently too great.

Afterwards, as I had absolutely no succes in identify the globules as pabulum or reserve material I regarded them as nuclei: but this point of view also did not satisfy tome, these nuclei-formings belonging to a very diverging type.

To my great surprise I discovered for the first time in an agarculture with an extract of Cassava wood that the round bodies were germinate! yes indeed, that this germination had even already occurred in the mother mycelium threads just as we may meet with among some of the lower *Phycomycetes*: see figure 1*b*. Figure 2 gives an enlargement of some of these round bodies. The mycelium threads are very

thin, the round bodies very small, a drawback in so far as concerns a closer carrying out of detail for which we have to thank the bad condition of the optical instruments and accessory appliances at the laboratory of the Selection Garden. Figure 3 gives a representation of the germination of one of the round bodies signified.

The round bodies in the mycelium thus appear on a closer investigation to be spores: endospores.

The germination of these appears indeed





Fig. 3. Germinating endospores. >10000.

only to catch on under very definite circumstances. In the very large number of pure cultures of this mould which I have had under my observation I have in only three cases perceived an abundant germination. What the conditions for germination are, I do not know; through an over pressure of work I have not been able to go farther into this enquiry Up to now I have considered this phenomenon so, that I have worked with different lines, with a greater number of varieties, of which only a single one possessed the fertilization of its spores to a marked degree, at least under the conditions in which I worked. In the course of my researches the fertilization of these peculiar spores has been a comparatively very rare occurence.

I repeat, no one would consider a priori those round bodies in my *Stagonospora Cassavae* as being spores. It is very well possible that they are degenerations of spores belonging to a former, "lower" genetic position of the mould. Then, the proposed degenerated caracter of the spores would explain why they cannot germinate; yet, among the greater number of varieties, there is one, in which the force of germinate is saved, is remained: but that it is a pure, great accident

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when one obtain this special variety in one's pure-cultures. One must this regard well.

We see, though the enigma of the round bodies in the mycelium of *Stagonospora Cassavae* being solved, there remains something very strange.

Still, after all it has been clearly demonstrated that we have to regard these puculiar round bodies in the mycelium of *Stagonospora Cassavae* as endospores.

Some divergences in form and relative grouping together of these endospores have been depicted in fig. 4: the drawings so speak for themselves that further comment is unnecessary.

The spores are not constantly spread over the whole mycelium thread in a regular manner; local accumulations may occur by which the spores attain but very small dimensions: and the whole were in conformity with the forming of swarmspores or the antheridia forming by *Phycomycetes*: see fig. 5. It is interesting that we are



obliged to view these cases in direct genetic relation with the somewhat rarely occuring accumulation forms such as those represented in fig. 6, the tendency to localise the spores in definite reservoirs.



Fig. 4. Mycelium threads with differently grouped endospores. >1000.

Fig. 5. Very marked local accumulation of endospores in a mycelium thread. $\times 1000$.

In this figure we are especially interested in 6d and 6e since they exhibit the tendency above indicated to an extreme degree, by bringing those reservoirs outside the normal mycelium. The acme of this tendency is mirrored in fig. 7, where we have obtained a habitus form which is very closely connected with those of some of the lower Ascomycetes, such as the Protoascineae, of which group a very typical representative I have already on a former occasion described in this periodical¹). Therefore I am inclined concerning this tendency, which aims at the localization of spores in definite morphological typically reservoirs, to regard it as a tendency to Asci-forming. I regard the reservoirs as Asci; the spores in question to therefore be true-Ascopores. It is well known that the genus Stagonospora and indeed the Sphaeropsidales in general have different joining-points to the Ascomycetes; but is interesting that the Ascomycetes type somewhat diverges from all the various species and that the Staganospora Cassavae clearly attaches itself to an Ascomycetes group which may not be freely ascribed to the true Ascomycetes. But it should be once more remarked that these pecu-

¹⁾ Protascus colorans, the cause of yellow grains in rice. Mycol. Centralbl. 1913, 3, 153.

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liar forms of habitat of the Stagonospora Cassavae are rare. They may not be used in the determination of the mould, for this pur-



Fig. 6. Different forms of local differentiated spore-reservoirs (proto-asci). ×650.



Fig. 8. Germinating endospore with ascus and ascospores. >>10 000.

pycnides are leathery, pitch-black and round with a very faint notching of the upper surface, without subiculum and without an opening papilla: see fig. 9. These pycnides may become a source of errors in the determination. It is indeed an astonishingly long time before they are quite full grown. They are round only when in this full grown

condition. Before that time only pycnidia-like bodies are met with, already provided with Conidies which may however have all kinds of fantastic forms: a black kernel surrounded by a loose some what brownish tinted envelope. But this envelope also is more and more compressed in a centripetal direction, from which compression

Fig. 10. Pcynidoconidies of Stagonospora Cassavae. × 300.

process after the lapse of time the full-grown pycnidiae result, so indeed as has been previously said conidia are already present in the pycnides

of the mould, for this purpose the Pycnides exclusively serve.

A remarkable-Ascus forming is represented in fig. 8, also already in fig. 3 (the lowest figure). We must positively reconize two-different forms in the germ-mycelium: the normal mycelium and the sacculate (Ascus) forming one. This is clearly seen in fig. 3 and 8. The very small



Fig. 7. Proto-asci forming of Stagonospora Cassavae. ×650.



Fig. 9. Pycnide of Stagonospora Cassavae. $\times 50$.

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which are not yet full-grown. One must well regard this; it may be a source of error in the determination.

The conidia are hyaline, crescent-shaped and as such curved to a greater or lesser degree. Fig. 10 gives a representation. The conidia are 4 to 6 celled; yet through the great accumulation of reserve materials and the fineness of the septa, these septa can only be perceived when one treats the preparation with chloral-hydrate. In ordinary water for example there is nothing to be seen of the septa and the conidia appear to be completely unicellular, which can give rise to many errors.

Buitenzorg April 1914.

Studien über einige Rhizopus-Arten.

Von J. HANZAWA aus Sapporo.

(Mit 12 Textbildern und 14 Tabellen.)

[Aus dem Techn.-Bacter. Laboratorium des Techn.-Chem. Instituts der Kgl. Techn. Hochschule Hannover.]

Nachdem EHRENBERG (1820) Mucor stolonifer als Rhizopus nigricans von der Gattung Mucor abgetrennt und beschrieben hatte, wurden allmählich über 20 verschiedene Rhizopus-Arten aufgestellt. Neben wirtschaftlich wertvollen (Stärkeverzuckerungspilze) finden sich darunter bekanntlich mehrere pathogene Arten. Die sichere Artbestimmung dieser Pilze ist leider äußerst schwierig, nicht selten kaum möglich.

A. FISCHER¹) hat die Rhizopus-Arten nach der Gestalt der Sporen in zwei Gruppen eingeteilt und dadurch die morphologischen Merkmale einzelner damals bekannter schärfer betont. Später hat VUILLEMIN²) darauf hingewiesen, daß bei der vergleichenden Untersuchung von Rh. iaponicus, Rh. tonkinensis, Rh. Oryzae und Rh. nigricans außer den morphologischen auch physiologische Kennzeichen - Temperatur- und Culturbedingungen — zur Artbestimmung wünschenswert seien. Neuerdings hat LENDNER³), von diesem Grundsatz ausgehend, die beschriebenen 22 Rhizopus-Arten zu ordnen versucht. Auf die innerhalb dieser Gruppe bestehenden besonderen Schwierigkeiten infolge des sehr gleichförmigen Aufbaues und der ähnlichen physiologischen Merkmale ist von WEHMER⁴) anläßlich seiner Zusammenstellung der practisch wichtigeren Arten bereits hingewiesen, auch die Notwendigkeit eines directen Vergleiches der bislang beschriebenen Species betont worden. In diesem Sinne ist auf Anregung desselben nachfolgende Arbeit, über die ich Einzelnes bereits früher mitteilte⁵), durchgeführt.

Das Bacteriologische Laboratorium des Technisch-Chemischen Instituts zu Hannover besitzt mehrere botanisch noch nicht näher bestimmte *Rhi*zopus-Arten, ich habe diese einer vergleichenden Untersuchung an der

1) A. FISCHER, *Phycomycetes* (RABENHORSTS Cryptogamenflora Deutschlands, 2. Aufl., 1, 4. Abt., p. 228).

2) VUILLEMIN, P., Revue Mycologique 1902, 24, Nr. 94, p. 45.

3) LENDNER, Les *Mucorinées* de la Suisse (T. III, fasc. 1 des «Materiaux pour la flore Cryptogamique Suisse», Berne 1908, p. 111).

4) WEHMER, C., Mucoraceengärungen (LAFARs Handbuch der Technischen Mycologie 1907, 4, p. 490).

5) HANZAWA, J., Mycologisches Centralblatt 1912. 1, p. 408.

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