etc. In the other direction there was a further development of the apothecial type resulting in the typical *Pezizales*, *Helvellales* etc. By reduction, such forms as *Exoascus* arose from this type.

In some of these lines of development the formation of a separate antherid was gradually omitted, the sexual fusions taking place now between nuclei all of which originated within the carpogone.

I can not refrain at this point from urging the strict homology between the ascus, and the teliospore of the *Uredinales*. In both cases a union of two cells gives rise to one or several, ascogenous filaments, within which the cells are binucleate. At or near the end of such a filament cells arise within which the two nuclei unite, forming the young ascus or teliospore as the case may be. In this cell now begins the reduction division, leading to the production within the ascus of four or mostly eight ascospores, and in the teliospore (in some *Uredinales)* or in an outgrowth from it (promycelium), of four cells which may form germ tubes directly but usually form secondary spores, the so-called sporidia or basidiospores. By the same token the spores of the *Ustilaginales* and even the basidia of the *Basidiomycetes* are perhaps homologous to the ascus, and these groups, too, are probably derived from the *Ascomycetes*.

Protascus colorans, a new genus and a new species of the *Protoascineae*-group; the source of "Yellow-grains" in Rice.

By

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(With 1 col. plate.)

1. Introduction.

One of the most fearful calamities in the trade-rice of some countries, viz: — the rice which is exported from the Indies to the European market for consumption — is the appearance of the so-called "yellow grains". If a handful of rice be taken out of such an infected lot then "yellow grains" immediately come into view here and there. It is the endosperm that looks yellow. Now, it is no usual yellow colour that these same grains exhibit; the colour inclines a little to be brownish; so, one is more disposed to speak of brownish-yellow grains; sometimes there is an overlying deep orange glow. In all cases the grains are of a deep-yellow colour, a yellow fonce, so that one may without doubt speak of "yellow grains".

This phenomenon of "yellow grains" is not a matter of general spreading; but in the countries where it is endemic, it is in the highest degree dreaded by the rice-producers. Rice which has only just been harvested does not immediately exhibit yellow grains. They only appear in rice which has been garnered for a long time in large heaps. The yellow-colouring process progresses very slowly. It is not until after several months have elapsed that one has the chance of finding yellow

grains in these lots of heaped-up rice. If the yellow colouring happens during the voyage to Europe from the Indies then there is a very good chance of the whole of the infected portion being rejected on the market, or that in every instance its price would fall very seriously.

The European market is very markedly displeased at the presence of these "yellow grains". In my laboratory at the time of writing this treatise there was a lot of such rice with about 0,3% of yellow grains. This lot had been totaly rejected; about 0,3% of yellow grains was indeed considered to be an "amazing proportion". A very few yellow grains only are quite sufficient to depreciate the value of rice. If the yellow discoloration appears during storage in the barns in the Indies, where the rice must often remain for several months before shipping, then the producers must expent much time, money, and pains in sorting out and removing the yellow grains.

In short, these yellow grains are a veritable nightmare to the rice-dealer. It speaks for itself that this phenomenon has been for many years the subject of research; and yet one has never, up to the present, been able to trace out the quintessence of the matter, the actual cause. Formerly indeed the view was announced that yellow rice must be caused by the brooding of the rice, and it was reported once that one was indeed quite able to cause yellow rice to appear through artificial brooding by employing an extra high temperature, so that yellow grains were truly more regarded as products of some or other true chemical process. This opinion has in the course of time been upheld by many persons. It is however very remarkable that not one of them really seriously believed it to actually be due to brooding only. All of them are convinced that there is also still "something else" which must take part in it. This supposition is truly evident. Indeed those yellow grains certainly do not appear regularly in broodin grice parcels, and then on the other hand one sees them even in rice which one knows for certain has never brooded. So some persons begin to feel more conscious that the advent of yellow grains is much more to be attributed to dampness in particulare, so that the "brooding theory" begins to be discarded.

2.

I have been charged to once more make a systematic enquiry into the matter. For this I in the first place repeated the experiments with artificial brooding but without results; I have never been able to obtain yellow grains in this way. Very soon indeed dit I base the method of my experiments on the supposition that the yellow grains might originate by the infection of some or other organism. To a certain extent I had established reasons for such a supposition. I had e. g. cultivated more fungi in my laboratory for some time, which fungi were capable to a greater or lesser extent of colouring rice grains. Thus there are different species of *Aspergillus* which are able to impart a bright lemon-colour of the grains of rice. This lemon-colour of the endosperm is caused by the diffusion of a yellow pigment from the mycelium that permeates the endosperm. Further I have had a variety of *Penicillium* under examination which gives a dull lemon-colour to the rice grains, besides one variety of *Penicillium* which trows off a red pigment with which the grains are coloured. Sometime ago I found a *Bacterium* of a yellow colour.

This Bacterium has anaerobic properties, and is under these circumstances able to call into existence a weak butylic acid ferment. I hope later to return in detail to these pigment forming moulds and bacteria, especially in connection with a peculiar appearance of polychromy. Regarding these moulds it is of the highest importance to particularly notice that they never occur spontaneously as rice colouring in rice garnering nor in the transport of rice. The reason for which is that they have need for their pigment forming of much more moisture than the rice heaps generally contain. It speaks for itself that the rice producers dry their rice as much as possible in order to safe guard it against the moulds. I have a special reason, for particularly emphasizing the above mentioned fact, especially as it is utterly in direct contrast to the conditions under which the moulds which cause the so-called "yellow grains" exert their dire influence. Therefore all the above mentioned different pigment forming moulds were of no importance in my investigation, yet they now formed none-the-less a hint that the so-called "yellow grains" would well be able to originate through a definite pigment forming microorganism.

There followed then a time of great disappointment and difficulty without my succeeding in discovering anything positive in this direction. At last, after long and unsuccessful attempts I was fortunate enough to actually succeed in isolating from the very "yellow grains" themselves a mould which in its turn, appeared to be able to transmit to healthy rice grains after artificial infection the same deep yellow colouring which is so characteristic of the true "yellow grains", that have been so much dreaded. Subsequently I was fortunately able on three occasions to isolate the same mould in the same manner; and in all these cases the mould was again able to originate these typical yellow grains. Consequently it is right to consider this mould as the cause of "yellow grains" in rice.

3.

This fungus appears even in the further investigations to have been unknown up to the present time. It belongs to the *Protoascineae* and certainly also to the sub-order of *Endomycetaceae*[1]). The deviations of form already described forms in this group have justified our bringing the mould so found under a new genus, for which purpose I have chosen the name *Protascus*, and with an eye to its rice colouring properties I shall name the mould *Protascus colorans*. In the plates appertaining to this treatise an illustration of this newly-found mould is presented in its different stages. This mould forms a strong mycelium and groows, especially at ordinary temperatures, quite luxuriantly on a pabulum of boiled rice or bread. In an immature state the mycelium is of a light pink colour; afterwards red bodies between the mycelium threads arise. These are globular enlargements, which arise at the end of the mycelium threads, and may be seen represented in fig. 1. These enlargements are the asci. They possess in this stage a more or less thick wall-standing protoplasm, which includes a large vacuole. When the culture grows older then the pink colour is altered to a blackish tint. The asci are then filled with dark coloured ascospores. This stage is represented

1) See J. Schröter, in Engler-Prantl, Natürliche Pflanzenfamilien 1^1.

in fig. 2. The number of the ascospores is not fixed; very many asci are only two-spored while on the other hand the greatest number of spores found per asci is fifteen. The fungus remains in this stage for a very long time. I therefore regard this stage as the normal condition of the full-grown mould. It is however noteworthy that in very old cultures there is a tendency for septa to form in the spores, so that we see here and there asci occuring with multicellular ascospores. Some of these are depicted in fig. 3. — I am inclined to regard the cell-multiplications as a degenerative phenomenon. We must however let this question rest aside.

In the remaining figures I have represented various transitional stages; so fig. 4 represents the primary beginning of asci-forming. We see here, just as is distinctly shown in fig. 1, that these asci principally occur at the top of the mould-threads; and begin as small rounded or pear-shaped enlargements. The asci are therefore not sexually formed. In some cases they exhibit outgrowths, wich outgrowths continuate like ordinary mycelium threads, so that in older cultures the impression arises that the asci are also originated intercalliarly. On their development it is seen that these are not really intercalliary formations, and that originally all the asci are terminally disposed. In fig. 5 asci are represented with similar out-growths and quasi intercallary habitus. Fig. 6 gives a representation of the transition stage that leads up to the spore-forming. We see herefrom that septa may arise in different ways, through which the relative positions of the spores in different asci also produce differently; this may easily be observed in fig. 2. I have not been able to discover conidia.

4.

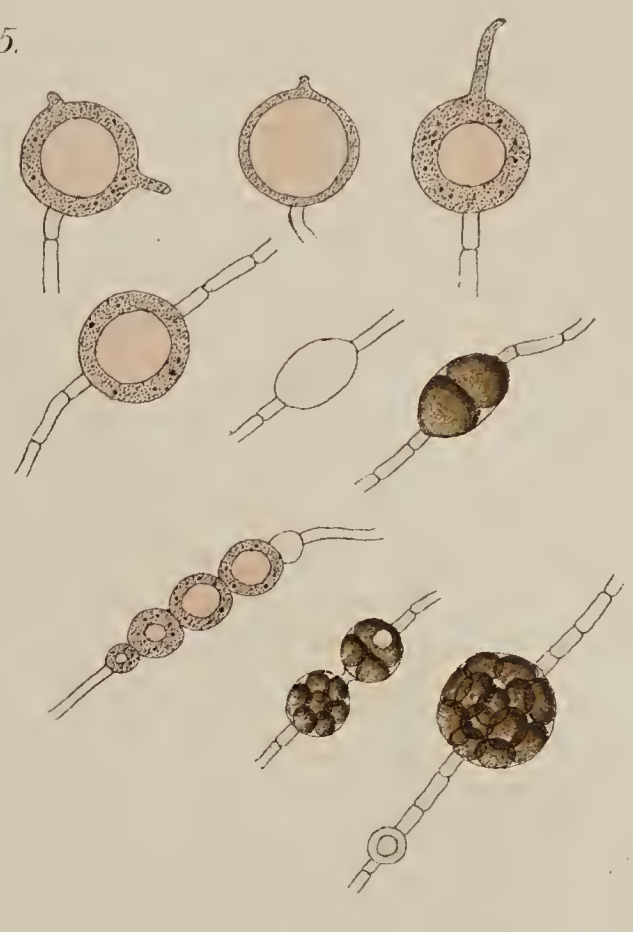
The mould was constantly cultivated at an ordinary in-door temperature, i. e. on the average 26° C. Under such conditions it flourished well. When it was submitted to higher temperatures its development declined; I constantly obtained the best results when no increase of temperature was introduced. This prevailed also with the artificial infections, as well as with the actual rearing of the mould from the "yellow grains" themselves.

Very beautifully succeeded experiments I obtained by drying up gradually, by little aud little the infected rice, without the least increasing of temperature. On this way I obtained the most characteristic "yellow grains". The experiments with higher temperatures never succeeded in so a striking degree. Gradually drying up of the infected rice is the real secret of the forming of "yellow grains".

Hereby the concept must fail that the yellow grains specially arise through brooding only. Yet this was indeed formerly assumed to be the case by practical observers. It is evident that "yellow grains" can occur in brooding rice, yet the crucial point is that it cannot be so through a high brooding temperature, but that in the first place the rice was infected by the mould, and in second place by the damp conditions favourable to the mould. The condition of humidity is in fact the cardinal point of the whole question. The absolute condition requisite for pigment forming is a very slight degree of humidity. Amid damp surroundings, wherein other moulds thrive luxuriantly, and produce an abundance of pigment, it is noteworthy that the pigment forming of *Protascus* is not at its optimum. Many of my experiments yielded no results owing to my

1.

5.



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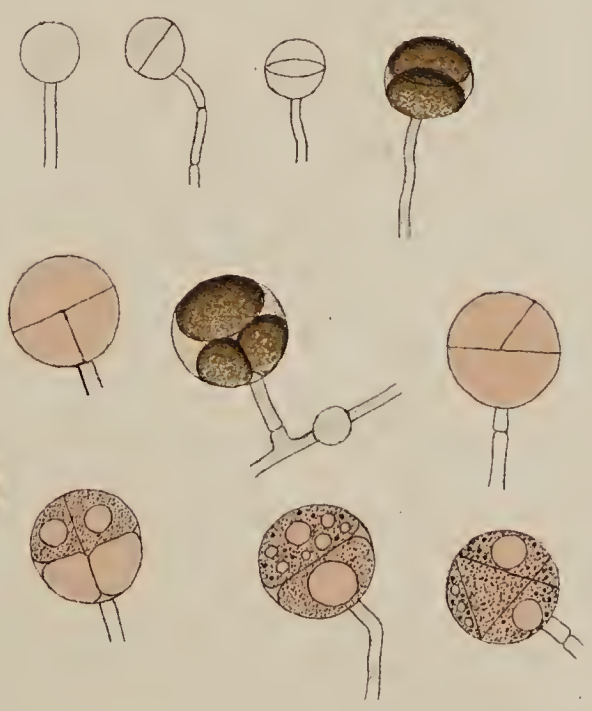

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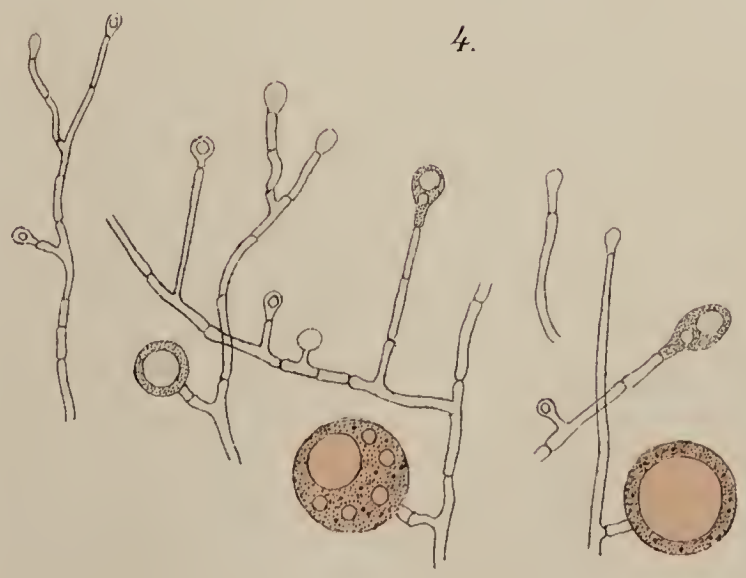

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having kept the grains and the mould in much too damp surroundings. It appears that the infection progressed most favourably when I let the grains dry slowly. Only under these circumstances did pigment-forming occur, and coupled with it, the diffusion within the endosperm

Pigment forming is a phenomenon of the withering away of the *Protascus*. The explanation therefore must be in this way only, why it is so exceptionally difficult to rear the mould from the "yellow grains" themselves. I have so tediously experimented on a vast number of yellow grains, yet only in four cases have I been able to isolate the *Protascus*, although the mycelium is to be found throughout the whole endosperm. It is therefore highly probable that she mould continues to die off, after to have formed the pigment as a phenomenon of decay. It is truly in any case a pathological phenomenon.

This fact gives the explanation of the appearence df "yellow grains" in brooding rice, and how the possibility could arise of a brooding-theory. By the high temperatures of the brooding rice, the *Protascus* deceases gradually, in which stage, as we have mentioned above, the forming of a yellow pigment takes place. The yellow pigment is the sweats of death of the *Protascus colorans*.

5.

The simplest way of preventing the occurrence of yellow grains is to keep the heaped up rice absolutely dry. In this way, of course, infection is not to prevent, yet in every case there is no advance and yellow grains are not found. It is of course safest to sterilize or disinfect the rice, and then keep it absolutely dry. Yet the difficulty lies not so much in making it dry as indeed in keeping it dry. The mould can develop in the very least degree of dampness. If then we do not take care that the rice remains absolutely dry, then we have in the end always a chance that is may be infectet by *Protascus*, which can manifests itself in the forming of "yellow grains". But to keep the rice absolutely dry is then very difficult, because the rice at once becomes a place of refuge to harbour all sorts of insects, the water-vapour of which set free by their respiration is sufficient to bring the rice into so damp a condition that infection by *Protascus* colorans may manifest itself by produciny "yellow grains". Thus the production of yellow grains in rice can only be combatted by first desinfect the rice, and afterwards storing it in an absolutely dry condition, and moreover in such a way that no insects may have access to it. I take it as a matter of course that all precautions are taken to prevent the rain gatting at it. In very damp regions repeated drying is absolutely necessary.

Buitenzorg, July 1913.

Explanation of plate.

Protascus colorans nov. gen. et spec. — Asci and ascospores.

Fig. 1. Mycelium with young asci at the top of the mould-threads.
Fig. 2. Asci with dark coloured ascospores.
Fig. 3. Spores with septa (asci with multicellular ascospores).
Fig. 4. Primary beginning of asci-forming.
Fig. 5. Asci with out-growths, intercallary habitus (see explanation p. 156).
Fig. 6. Asci, stages leading up to the spore-forming.