

Organe bei Züchtung auf Invertzucker, wo die Concentration von dieser zur Atmung mehr geeigneten Zuckerart größer ist, wenn also in jedem Augenblicke mehr davon vorhanden ist. Die Annahme, daß die Ursache in irgend einem Entstehungszustande der einfachen Zuckerarten zu suchen wäre, scheint mir weniger wahrscheinlich.

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*Rhizostilbella rubra* (n. gen., n. spec.) a by-fruit form of *Ascobolus parasiticus* (nov. spec.); and its connection with the "Sclerotium disease" of certain tropical cultivated plants (*Sclerotium omnivorum* n. spec.).

By

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(With 1 col. plate.)

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Since several months I observed a large closed culture-pan in which plants and fruits of *Voandzeia subterranea* were actively decomposing. During the first few days since the introduction of the fresh plant-material to the culture-pan (as is here in the tropics so constantly seen) everything possible of the commoner and also of the less-commoner moulds made their appearance in turn on the decaying material. It was an alternate revelation and fading away of every variety of the shades of conidian colours; a keen struggle for existence, an ever-renewed pleasure to observing eyes, and a true source of enjoyment.

After this display had lasted for a month or so however, the "system" of lower moulds was gradually exhibited to the observer in broad traits. Then, in this decaying material the circumstances had evidently become untenable even for the most persistent saprophytes; the fruits, the stalks, the particles of earth had become as black as pitch, slippery and shining, with every sort of putrefactive bacterium, while an insupportable stink filled the culture-pan. Then after the first month had elapsed, the last mould visible to the naked eye utterly vanished, and the host of moulds seemed to have died out for good; a long mouldless period ensued. I continued to observe the culture-pan with its aspect of death, and finally my zeal flagged a bit, and the pan lay forgotten in a corner for two months or more.

What was my astonishment when one day glancing at the pan I was delighted by an unusually beautiful colour effect. Fruits and stalks were compactly covered with fiercely-green toadstools of the *Ascobolus*-type, while among this all, fruits, stalks and the pieces of earth were covered with fiery-red rhizomorph-like bands, while from these bands themselves there sprung a host of "conidium-bearers" (coremia), provided with little

yellow knobs, which at once betrayed themselves as representatives of the "Stilbella-type". It was evident that we had here to do with the complete fruitforming and its by-fruitforming of one of that always interesting group of „Conidien-Pilze“.

We will commence our description with this Conidium-form. The first, and most prominent habitus is presented in fig. 1. Here we see on a piece of soil the fiery-red rhizomorphic system spreading out in strong branchings, originally without the coremies (the left side of this figure) — later on provided with a more or less number of these coremies, on the top of which are little yellow, slimy knobs which, on microscopical examination are shown to be vastly multifold aggregations of thin Conidio-phores each of which with a very small conidium. The rhizomorphes average from 1½ to 2 mm breadth. They are markedly flattened and they narrow down towards the extremities.

Another illustration of habitus is represented in fig. 2 and fig. 3.

Fig. 2 is in fact a *Voandzeia* fruit thoroughly rotten, though remaining intact in form. We, in this figure now look at the fruit-wall and observe the great number of Coremies, which have penetrated the fruit-wall. Then let us now very carefully remove the fruit-wall then the illustration in fig. 3 presents itself before us, now looking at the seed-skin. We meet there with the above mentioned rhizomorphes and now it is evident that these rhizomorphes are present only between the seed-skin and the fruit-wall. This was the case with all the fruits.

A cream-coloured pulp, which was previously the cotyles, is found within this seed-skin. The rhizomorphes in the first instance originate from this pulp. This point however will be more fully considered in the second paragraph. For the sake of completeness I give fig. 4, where a rhizomorph swings spirally around a branch.

It is by this typical rhizomorphic character, manifesting itself everywhere through the flat bands which continually are pressed closely against the substratum that I have given to this fruit-form the specific name of *Rhizostilbella* and more fully *Rhizostilbella rubra*, owing to its deep red colour.

Fig. 5 gives the longitudinal section of such a rhizomorph. In the central part there runs a skein of normal mycelium threads (just such as those which develop in the cotyles-pulp); these threads run loosely from each other, and run parallelly. This colourless mycelium-line is now indeed surrounded by a red-coloured skin of somewhat complicated formation. In the first place it appears that, where the central-mycelium runs lengthwise, the skin mycelium, on the other hand, runs crosswise, so that we perceive in our section a pseudo-parenchyma occurring. Now it is remarkable that this skin consists of four sharply defined layers. Proceeding from the periphery to the centrum we first come across a very thin layer of flat "pseudoparenchyma cells" as if we had to do with a "periderm". Following on, there comes as a second layer a "pseudoparenchyma", the "cells" of which are wider and isodiametric: these "cells" are somewhat wider than the width of the normal mycelium thread of the pulp. As a third layer there now occurs a row of enormously wide "pseudoparenchyma cells", while the fourth layer again corresponds to the

second. Then it is worthy of observation that the two external layers are of a dark red colour while the two inner layers are rose coloured.

The rhizomorphes as well as the coremies are very brittle. They break off in the hand like glass.

Fig. 6 is the longitudinal section of such a coremium. The deviating structure of the rhizomorph is immediately striking. The pith-mycelium threads do not run parallel to each other: they are more or less twined over each other. The "skin" is of simpler construction than that of the rhizomorphes. The pseudoparenchyma cells have here the width of normal mycelium threads.

The real conidiophores are threads, which are divided into irregular septa. Each of these threads bears at its extremity one very small oval conidium. The conidia thus do not become separated into chains. Each general "fruit-stalk" (coremium) constantly possesses but one knob; between the conidia and the conidia-bearing threads a yellow slimy substance is found, which give the typical slimy character to the knobs of the *Stilbella*-species in general.

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2. A single word now concerning the *Ascobolus*-fruit form of this mould. There is nothing very interesting about it to say here, since it corresponds to the usual type. Fig. 7 gives a section which sufficiently speaks for itself. The diameter of the "hat" is 3 mm; this is of a green colour, produced by a green pigment of slimy consistence which is present in the asci. Fig. 8 exhibits some of the branchless as well as of the branched paraphyses which, in very large number, are prominent far above the asci, just as we have already seen in fig. 7. The asco-spores are apparently brown. Their microscopical appearance is shown in fig. 9; they possess dark brown warty-processes, which run longitudinally over the spores.

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3. As already recorded in the first paragraph the *Ascobolus* and the rhizomorphes originate from the cotyles which have become a yellow pulp inside the seed-skin. It is remarkable that also already in this cotyle-pulp the mycelium threads have united in skeins and that these skeins then penetrate the seed-skin and there gives existence to the rhizomorphes or the *Ascobolus*. These skeins are by very careful preparation in some cases to be distinguished by a silken gloss by which they contrast with the yellow of the cream-coloured pulp. Fig. 10 gives a representation of such a preparation. The original mycelium-skein is divided into four. The two inmost skeins form the *Ascobolus* bodies, while the two outer skeins give life to the rhizomorphes of the *Rhizostilbella* fruit form.

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4. We have still to describe the third form of habitus of this remarkable mould.

In the first place the discovery of our mould is a result of exhaustive enquiry after a much dreaded "Sclerotium disease" of various culture plants. It is a disease which occurs in our Institute for Plantbreeding at Buitenzorg, most severely with *Voandzeia* and *Arachis* cultivations, and is then also very harmful.

It is important to know that therefore in our Institute the cultivation of *Voandzeia* finally is stopped. In course of years the soil has been so enormous infected with this mould, that, gradually, it has been impossible to cultivate crops which are somewhat sensible to this disease. Yet for the rest of the vegetable world also it is a very general malady, as all sorts of plants are liable to be more or less severely attacked by it in course of time. But with most of them the infection is not so dangerous from a practical agricultural point of view, although it may locally cause a great mortality among the growths.

But, though this disease is in practical sense not harmful for every crop, it is therefore so dangerous while in general, cultivators are extremely thoughtless, so that they do not pay attention in those cases in which a, a formerly harmful mould, suddenly becomes a dangerous enemy. This is, for instance, the case in our seedlings of rice, in which the mould is suddenly appeared in so a violent degree, that now, on the moment on with I write this, the seedling-beds are liable to a great mortality. In fig. 11 I give a representation of an attacked rice-seedling, on which the Sclerotia are very well to be seen. For further indication I name it here: "*Sclerotium omnivorum*". The infection occurs at the neck of the roots, the sickness being ultimately recognizable by the presence of a loose white covering of mycelium threads at the neck of the root, while gradually a great number of first white, later on light brown Sclerotia appear of the size of a pin's head, round or somewhat oval shaped. If the plant dies of it then the sclerotium layer often spreads over the whole of it, as frequently occurs with *Voandzeia* which constantly suffers most from this mould.

This sclerotium form has never been seen to fructify.

According to communications which have reached me there have already been many different generations of enquirers of the Agricultural Department at Buitenzorg who must have tried their hands on this mould with a view of acquiring a closer knowledge of its life, but always in vain; for the mould well know how to preserve with great stubbornness the veil of mystery that lay spread over its identification. My first attempts at causing this mould to fructify also failed, I cultivated it in every possible variety of artificial pabulum: indeed I obtained splendid pure-cultivations but always resulting in a splendid mycelium development followed by sclerotium formation. Although the sclerotia in their natural state only attained the size of a pin's head I got in my pure-cultures specimens of the size of a pea, brown with black spots; but no further results I attained.

I shall indeed enter no further into these interventions, as they are of but minor importance in this treatise. Now I have also followed from the beginning the method of keeping the sick plant materials in several closed culture-pans. I kept exact notes of the moulds that successively fructified in each of the culture-pans. At the beginning of the first paragraph of this treatise I have given a representation of the mould vegetations which successively manifested themselves on the decaying parts of the plants. I treated in this manner the sick plant materials as well as sound ones.

Now it occurred to me, in working out my notes, that the *Ascobolus* described, occurred only in the culture-pans in which were the plants

that had been directly infected by the "Sclerotium disease". This parallelism was so striking that I immediately began a systematic enquiry in the direction found. *Arachis* and *Voandzeia* plants and fruits were sterilized in boiling water and subsequently placed in sterilized culture-pans, the cover-edges of which were closed with cotton-wool. Some of these were now infected with the above mentioned pure-cultures of the Sclerotium-form, others remained uninfected as control pans. In these uninfected control pans there was never a mould vegetation to be perceived, only bacteria which I could not exclude, and these bacteria, indeed, were very welcome!

The infected culture pans exhibited nothing special during the first month; just as in the first paragraph, it appeared that the mould had need of an astonishingly long time in order to determine its real nature and to reveal it to man; for that the substratum must be in an advanced stage of decay, whereby it indeed does justice to its *Ascobolus* nature.

At last the *Ascobolus* fruit-bodies appeared only in the infected pans (infected with Sclerotia) and in some here and there also already the *Rhizostilbella* fruit forms.

They were the only moulds which occurred in the infected culture-pans and as I say above, in the uninfected pans never one mould appeared.

After that I brought the infection experiments to still further precision.

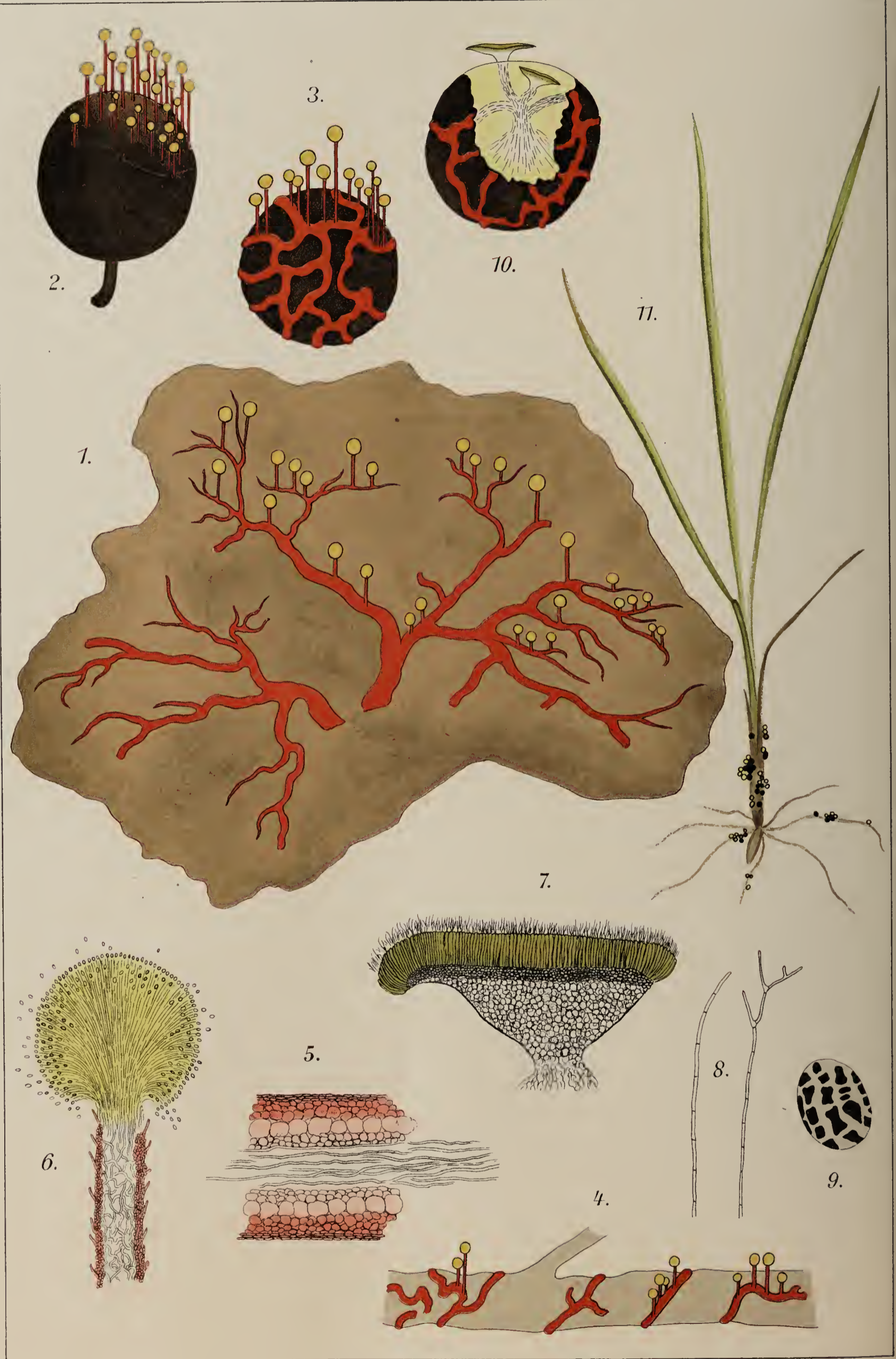
I laid in small PETRI-glasses only single small pieces of sterilized stalk; ten PETRI-glasses were infected with the pure-cultured *Sclerotium* material, and ten remained uninfected as control-glasses. The uninfected ones all remained absolutely free from any development of moulds; half a month after the beginning of the experiment, the evidently indispensable Bacteria decayed the stalks. Of the ten infected PETRI-glasses nine exhibited *Ascobolus* fruit bodies after 1½ months.

Indeed no further room for doubt was possible: the *Ascobolus* and the *Sclerotium* and the *Rhizostilbella* are all three forms of habitus of one and the same mould.

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5. The *Ascobolus* is the highest fruit form, the chief-fruit form, of this mould; and I have given it the name that is most characteristic for it, I have called it *Ascobolus parasiticus*, so comprehending in it the highest fruit form together with the most notorious by-form, viz: the parasitic Sclerotium. The *Ascobolus* fruit-bodies as such, are generally recognized as typical saprophytes. Also in my cultures *Ascobolus* in narrow sense appears only as a saprophyte; it is even such a pronounced saprophyte that I have never met with it in free-nature: nor on the old dead *Voandzeia* nor on the *Arachis* plants, nor on the manure which is used here on so large a scale in the Experimental Gardens. It thrives evidently only under the conditions found in a culture-pan, that is to say on a substratum that is utterly decayed and very wet. It is therefore quite peculiar that it should have an allied form of habitus which is pure parasitic, viz: — "the Sclerotium state." It is consequently not to be wondered at that one has never been able to completely discover this mould, since the *Ascobolus* and *Rhizostilbella* forms are





removed so astonishingly far from the Sclerotium state, viz: — by at least a month, so that, as through chance, one has no suspicion of it, one would never come to the conclusion that there was any connection.

I have also found out the cycle of development of these forms only through persevering in the above mentioned systematic noting of the development of successive moulds in culture pans.

May it not be possible that, following on the lines of this method, one also might be capable of elucidation some of the legion of Sclerotium-forms which are to be found in the mould world, of which next to nothing is known.

To resume I have then in this treatise arrived at the result that *Ascobolus parasiticus* has three forms of habitus; they are:

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|--|--------------------------------|
| 1 <sup>st</sup> the Ascobolus fruit form . . . . . | <i>Ascobolus parasiticus</i> , |
| 2 <sup>nd</sup> the Conidia fruit form . . . . .   | <i>Rhizostilbella rubra</i> ,  |
| 3 <sup>rd</sup> the Sclerotium form . . . . .      | <i>Sclerotium omnivorum</i> .  |

### Explantation of the plate.

**Fig. 1.** Rhizomorphes of *Rhizostilbella rubra* creeping over a piece of earth, and upward directed "conidian heads". Magnified about 2 ×.

**Fig. 2.** Decayed fruit of *Voandzeia subterranea* with "conidian heads" of *Rhizostilbella rubra* which have broken through the fruit-wall. Magnified about 2 ×.

**Fig. 3.** The same as fig. 2, but with the fruit-wall removed, in order to demonstrate the rhizomorphes between fruit-wall and seedskin.

**Fig. 4.** Branchlet of *Voandzeia subterranea* with rhizomorphes spirally creeping out over it. Magnified about 2 ×.

**Fig. 5.** Longitudinal section of a piece of the rhizomorph of *Rhizostilbella rubra*. Magnified about 50 ×.

**Fig. 6.** Longitudinal section of a conidian-head of *Rhizostilbella rubra*. About 50 ×.

**Fig. 7.** Section of the fruit-body of *Ascobolus parasiticus*. Magnified about 20 ×.

**Fig. 8.** Branched and unbranched paraphyses of *Ascobolus parasiticus*. About 80 ×.

**Fig. 9.** Ascospore of *Ascobolus parasiticus*. Magnified about 500 ×.

**Fig. 10.** Seed of *Voandzeia* with *Rhizostilbella rubra* and *Ascobolus parasiticus* in order to show the mutual relation of these two.

**Fig. 11.** Young riceplant attacked by *Sclerotium omnivorum*. Natural size.

## Versuche über die Bedingungen der Holz-Ansteckung und -Zersetzung durch *Merulius* [Hausschwammstudien V].

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Mit 1 Textfigur und 2 Tafeln Photographien.

### 2. Die Ursache des Mißerfolges der Mycelimpfungen<sup>1)</sup>.

Die früher geschilderten Ergebnisse<sup>1)</sup> waren mir — wie ich offen gestehe — unerwartet. Lebende Hyphen des *Merulius*-Mycels vermochten

1) Schluß von p. 321, 3, 1913/14, H. 7.



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