The observation may here be inserted, that we speak of chance in nature, when small variations in the initial data occasion considerable variations in the final elements, because we cannot observe those small variations. Cyclic motions for instance will also always give rise to such cases.

For the special case considered here the result we have found may be formulated as follows: when in a purely mechanic, reversible process which occurs a great many times in the same way, events occur in which small variations in the initial data occasion considerable variations in the final state, then the total process gets the properties of an irreversible process.

Botany. — *"On a Sclerotinia hitherto unknown and injurious to the cultivation of tobacco."* (*Sclerotinia Nicotianae* OUD. et KONING). (By Prof. C. A. J. A. OUDEMANS and Mr. C. J. KONING).

The following communication contains five paragraphs.

Par. I gives an account of a visit to the tobaccofields in the Veluwe and Betuwe, in the autumn of 1902, about the time that the tobaccoleaves begin to be gathered.

Par. II contains an investigation of the disease which had attacked the plants, evidently a fungus, which had long been known as "Rot", but the nature of which had not yet been cleared up.

Par. III gives a summary of the experiments made with the Sclerotia of the fungus.

Par. IV deals with the anatomy of the Sclerotia and the *Sclerotinia* produced from them.

Par. V contains the result of some biochemical investigations.

Par. VI gives a few hints, the application of which may prevent or reduce the damage caused by *Sclerotinia Nicotianae.*

I. A VISIT TO THE TOBACCOFIELDS.

In order to study more closely the origin of the well-known patches and specks on dried tobaccoleaves, one of us repeatedly visited the tobaccofields in the Veluwe and Betuwe in September 1902. These visits repaid the trouble very well indeed, as they gave an opportunity of becoming acquainted with an evil which caused much damage, had not yet been clearly defined and so deserved a closer study.

In these visits one was first of all struck by the fact that the very extensive fields under cultivation were divided into smaller square plots by beanhedges and that these hedges consisted partly of scarlet runners (*Phaseolus coccineus* = *Ph. multiflorus*) and partly of "curved-beak" (a variety of French beans *Phaseolus vulgaris* SAVI [1]).

On account of their height these plants were considered effective as windscreens. Tobacco leaves namely, by their large surface as well as by their tender structure, cannot very well stand air-currents, which is proved by the fact that the scouring or rubbing of two leaves against each other by the wind, may cause discoloured spots, bruising of the tissues and even loss of substance.

Though the method of protecting the tobaccoplant against wind had evidently been well chosen, yet the growers themselves had noticed that it was wrong to use two different kinds of *Phaseolus*, because diseased tobaccoplants are much more frequent within hedges of scarlet-runners than of French beans. Experts are certainly right in their opinion that the reason of this is that scarlet-runners retain their leaves much longer than French beans. The latter begin to lose their leaves already in September and October, when the season can already be rather damp, whereas the scarlet-runners show no sign of it yet then. Hence the soaked soil as well as the damp plants can much better be dried by the wind within the hedges of French beans than of scarlet-runners. Accordingly the "rot" is in damp years always much stronger inside the leafed than inside the leafless hedges.

Another drawback of scarlet-runners is that their flower-clusters have not yet fallen off in September and October, so that, after having died, they not unfrequently drop down on the tabaccoplants and soaked through, remain hanging in the axils and in other places, where like wet sponges they foster the germination of conidia or spores.

In a visit to the tabaccofields of Mr. N. VAN Os at Amerongen on Sept. 27, 1902, many plants were found suffering from "rot". As such the growers considered specimens with limp, slippery leaves and with stems having discoloured stains. This was supported by the experience that such leaves and stems possess very infectious properties and that a single diseased leaf, carried to the drying-shed under a big heap on a wheelbarrow, can in one night easily infect some fifty others. Any precise idea of the agent here at work, was not found however among the experts, so that the only means of

[1]) The tobacco-growers themselves informed us that hedges of beans, especially of scarlet-runners and "curved-beaks" as windscreens, have been in use on tobacco fields as far back as can be remembered. In accordance with this they are mentioned by the late Prof. VAN HALL on page 60 and 61 of his "Landbuishoudkundige Flora" dating from 1855.

arriving at a scientific result was to take parts of sick plants to the laboratory and to study them there.

Meanwhile a continued walk through the tobaccofields had revealed that this was a case not of a bacterial disease as had originally been supposed but of a sclerotial disease, since in various places in a greater or less degree spots were found on leaves and stems consisting of a white down and besides greater or smaller black grains, embedded in or lying on that down, so that on account of other observations made elsewhere, it seemed probable that these black organisms under favourable conditions might produce an ascigerous generation, from the morphological properties of which the place of the fungus in the system and its identity or difference with other known species might be inferred.

The richest crop of material for experiments was gathered in the dampest places, i. e. in the corners of hedges of scarlet-runners, while on the other hand in the vicinity of French beans often not a single grain was to be found. Where flowers or flower-clusters of scarlet-runners were held fast in the axils of tobaccoleaves, sclerotia were rarely sought in vain. It can be understood that the uninitiated — growers and working-men — imagined that the source of the evil had entirely to be sought in the blossoms of the scarlet-runners.

II. Investigation of the disease which had attacked the plants.

On various days of September 1902 sick parts of stems and leaves were taken home from the tobaccofields as well as from the drying sheds. In doing so each leaf and each stem were separately put into a sterilised tube and in the laboratory placed into a sterilised glass-box over wet filtering paper.

At a temperature of 22° C. a distinct change could already be observed in all the objects after 24 hours. They had developed a flimsy, transparent, much-branched mycelium. At a lower temperature the same phenomenon had occurred though less vigorously.

After 3×24 hours small bits of the obtained net of threads were with the necessary precautions placed on malt-gelatine and kept at 22°.. Already after 24 hours these bits had grown much and it was possible after another 24 hours to take away new bits from the margin of the circular cultures which had now grown to a diameter of 3,5 centimeters and to inoculate them on freshly prepared malt-gelatine. In this way a sufficient quantity of pure cultures were obtained in a relatively short time.

As healthy tobacco-plants were largely at our disposal, it was possible to carry the downy substance on them and to place the infected parts of leaves and stems in damp glass-boxes at 22° C. Again a beginning growth was noticeable after 24 hours.

The pure cultures on the malt-gelatine plates became more and more extensive, forming circles which after three days had diameters of 8, after four days of 13 centimeters.

By and by the malt-gelatine was peptonised and in a smaller or greater number of places, near the margin more than in the middle of the circles, small, white, glossy points arose, which secreted drops of a colourless, quite clear liquid, but which required no more than 12 hours to turn into black dots. These also continued the process of drop-formation for some time, when after some further increase in size they changed into shorter or longer, round or angular little bodies, which clearly belonged to the class of sclerotia. Having grown more and more independent of the hyphae which at first occluded them, these black bodies could now be removed without damaging them and they appeared to have reached a maximum length of 10 millimeters and a thickness of 5—6 millimeters.

The experiments on infection with parts of living tobaccoplants were all successful on condition that the place of inoculation was kept very wet, e.g. by wrapping it up in very wet cottonwool or some woodshavings steeped in water. The attacked tissues became discoloured also here.

From what precedes we may infer that the fungus cultivated on malt-gelatine does not differ from that of the tobaccofields, which was irrefutably proved later when from the sclerotia of both the same *Sclerotinia* was obtained.

It is worth mentioning that the myceliumcultures on the malt-gelatine which had produced the sclerotium, had besides given rise in several places to dull white, granulated spots, which microscopical examination revealed to consist of 1st. clusters of flask- or cone-shaped conidiophores, borne by erect or ascending hyphae and 2nd. a number of curious crystals pressed against the thread-shaped cells, partly loosely spread, partly assembled in clusters.

The colourless conidiophores were high 12—16 μ and broad 4—5 μ and consisted of a cylindrical body tapering a little towards the lower end, a thinner short neck and a spherical head, which latter just slightly exceeded the neck in breadth and produced spherical colourless conidia of 2.5 μ diameter, which were at first connected to short chains, but soon broke up and commenced an individual existence.

The crystals and other bodies, often striated, not occluded in cells, of varying shape and size, soluble in diluted hydrochloric acid in which they left a structureless residue, soon appeared to belong to the class of "calcospherites": organic compounds of calcium treated by the late Professor P. HARTING in 1872 in a quarto Treatise of the Royal Academy of Sciences, entitled: "Morphologie Synthétique sur la production artificielle de quelques formations calcaires organiques".

There could be no doubt that these calcospherites stood in no relation to the fungus, but had been produced by the gelatine, while on the other hand, the presence of conidia proved that the new *Sclerotinia,* like other species of the same genus, could multiply by conidia as well as by ascospores.

On the maltgelatine-plates which had been exposed to the air of the tobaccofields and in the drying-sheds, the same mouldy spots developed under the most favourable conditions of the laboratory, which had drawn our attention on the stems and leaves in the fields, and which had afterwards been artificially multiplied. More important still is that somewhat later the same sort of Sclerotia developed, the germination and further development of which gave origin to the formation of apothecia.

There cannot be the least doubt that the conidia floating in the air, by settling on the gelatine-plates, had produced the infection and the ensuing phenomena, so that these last experiments throw a clear light on the possibility of extensive tobaccofields being ruined in a very short time, as soon as by a prevailing uncommonly damp condition of the atmosphere a small patch of mould has anywhere found occasion to develop threads. At the same time they show that the opinion of VON TAVEL (Vergl. Morph. der Pilze, 1892, p. 105): "Es (die Arten von Sclerotinia) sind parasitische Pilze, deren Sclerotien im Innern der Pflanzentheile sich bilden ganz nach Art einer *Claviceps*" cannot be admitted for *Sclerotinia Nicotianae,* and that here an ectogenous formation of the Sclerotium has been substituted for an endogenous one.

III. CULTIVATION-EXPERIMENTS APPLIED TO SCLEROTIUM NICOTIANAE.

The sclerotia whose development it was desired to study were buried in sand, garden-soil, forest-soil and leaf-earth respectively, placed in suitable dishes partly in daylight, partly in dark, and after having been properly watered exposed to various temperatures among which that of 22° C. Not earlier than 6 weeks later the first sign of new life was observed in the shape of numerous black-brown little hills with a lighter-coloured top. The earliest appearance was in the dishes filled with forest-soil and placed in daylight at 15° C., whereas a temperature of 22° C. seemed to have hindered development. The culture in sand always remained backward. The hills gradually assumed the shape of little rods, but took 3—4 months to reach the appearance of thin little stems or threads, bent down over the surface. These latter moved in the direction of light.

The number of threads varied widely for the different grains (Fig. 2 and 5), but did not exceed 20. The progress of the growth was at first very small indeed (2 millimeters in 40 days) and was even insignificant between Nov. 1902 and Febr. 1903. But then the threads rapidly grew in length and in March measured as much as 6 centimeters.

After the thickness of the sprouts had very long remained unchanged, at last (in March) a distinct swelling appeared at their top, which at first club-shaped rounded and closed, soon divided into a somewhat inflated neck (apophysis) and a broader disc-shaped terminal piece, which latter could easily be recognised as an open shallow apothecium with the edge slightly bent inward (Fig. 8). The correctness of this view appeared when the miscroscopical examination had revealed the presence of spore-bearing asci and paraphyses in the disc (Fig. 9).

A single sclerotium appeared to be able to bear some six well-developed apothecia and besides some dwarfish rods.

Unburied Sclerotia do not develop, although they remain resting on the bed of mycelium-threads which produced them. Cultures in Petri-dishes were mostly spoiled by bacteria.

Bits of a fruit-stem, grown from a Sclerotium buried in humus, when placed on malt-gelatine gave origin to the development of white pads, which in their turn sometimes produced new Sclerotia in a week's time. Bits of white Sclerotial flesh behaved similarly.

The fungus-generation grows very rapidly on malt-gelatine as well as on bits of tobaccoplants at 22° C., though its temperature optimum is at about 24° C. At 37° C. the growth is arrested. Between 15° and 20° C. the development is still satisfactory.

IV. ANATOMICAL INVESTIGATION.

The mouldy threads which in the field develop on the surface of green parts of plants and which afterwards produce the Sclerotia, grow equally in all directions and so gradually form white discs, of increasing diameter, finally reaching an average breadth of 2 centimeters. These threads are colourless, 2 μ tick, much ramified, repeatedly septate, filled with a finely granulated protoplasm and occasionally accompanied by threads five times thicker, the significance of which could not be discovered.

From the thinner, creeping fibres others rise up on which either singly or in small clusters, flask- or cone-shaped organs develop, whose function is to split off conidia and which hence deserve the name of conidiophores. They are on an average 15 μ high and 3.5 μ broad and consist of a thick body, tapering a little at the bottom, a short, thick neck and a spherical head, only slightly thicker than the neck. From the spherical or knob-shaped head colourless, spherical conidia of 2.5 μ diameter come forth, which are very soon detached from each other, but the multiplication of which goes on for a very long time, as may be inferred from their extremely large number.

The Sclerotia, externally black, internally white, diverge little from the common type as far as their structure is concerned. They consist of a pseudoparenchym the cells of which are somewhat bigger in the middle of the grains, somewhat smaller near the surface, show various, mostly distorted shapes (fig. 7), have very thick walls and are not separated by intercellular spaces. The walls of the more superficial cells are black, of the more central ones colourless. If a sclerotium rests with part of its surface against the glass of a tube or box, the black colour does not develop there.

The spore-bearing generation (fig. 8) which under favourable conditions comes forth from not too old Sclerotia and consists of a long, thread-shaped stem and a miniature apothecium, shows, in the first-mentioned part short, cylindrical or column-shaped, closely packed cells, which at the surface bend dorsally, but in doing so assume the shape of clubs or retorts and turn their broadest part outside. They have a light-brown shade and impart to the stems and cups a peculiar appearance as if they were covered with downy scales.

The hymenium consists of asci and numerous loosely packed paraphyses, of which some protrude a little above the others (Fig. 10). The asci are tubular, with rounded tips, insensible to iodine, 160—180 $\times$ 6—7 μ and contain in their $^2/_3$ upper parts 8 inclined, colourless, oval spores in a single row. The paraphyses are only slightly swollen at the top and almost colourless. Germinating spores were not seen.

V. Biochemical investigation.

In order to study the conditions of life of *Sclerotinia Nicotianae*, the fungus was cultivated on and in different nutritive materials of known composition.

It appeared in the first place that the presence of free oxygen is absolutely necessary for its growth; with anaerobic methods of cultivation according to Buchner and Liborius no trace of development took place. It is not improbable that this is the reason why the mycelium only grows extremely slowly in nutrient liquids, where the quantity of oxygen below the surface is necessarily small.

On the other hand the fungus appeared to grow very rapidly when inoculated on malt-gelatine, malt-agar and also on parts of leaves and stems of the tobaccoplant, sterilised at a high temperature. Then a woolly mycelium developed, in some places rising above the surface. Below the surface of liquids or filtrates, obtained from parts of stems or leaves, after inoculation with the fungus, only a meagre cloudy mycelium appeared. As soon however as part of this had reached the surface of the liquid, its growth became much more vigorous. In some cases a floating sclerotium was even produced.

Next the influence of the reaction of the nutrient liquid was studied. In a solution of 0.1°/₀ of potassium nitrate, 0.5°/₀ glucose, 0.050°/₀ magnesiumsulphate and 0.050°/₀ potassiummonophosphate, containing carbon and nitrogen assimilable by the fungus, *Sclerotinia Nicotianae* does not easily support free acid or alkali. The acid limit lies with this solution at about 1 cubic centimetre of $^1/_{10}$ normal sulphuric acid to 100 cubic centimetres of liquid, and the alkaline limit at 0.5 cM^3 of $^1/_{10}$ normal potassiumhydrate. Neither limit can be sharply drawn as the fungus only slowly produces acid in the solution mentioned. With 1.5 cM^3. of $^1/_{10}$ normal sulphuric acid no growth whatever takes place any longer; with the alkaline solution the limit could not be sharply defined.

Moreover an elaborate investigation was made as to which compounds were profitable to the fungus as carbonaceous and which as nitrogenous foods. As a carbonaceous food glycose, as a nitrogenous one saltpetre in the above-mentioned concentration, proved most satisfactory. Ammonium nitrate, a very good nitrogenous food, was not available of course in the presence of alkalies.

In the further experiments the saltpetre was replaced by a similar quantity (0.1 °/₀) of the nitrogen compound to be studied or the glycose by the carbon compound to be studied in the same concentration.

a. *Nitrogenous food.*

Nitrogen was offered to the fungus in the form of potassium nitrate, potassium nitrite, chloride, nitrate, phosphate, sulphate, carbonate of ammonia and ammonia. Ammonium nitrate gave the best results. The other compounds showed little difference. Of ammonia which was added in very small quantities, hardly anything was assimilated.

Of amido compounds, which are generally known as good sources of nitrogen for fungi, glycocoll, asparagine, aspartic acid, alanine, tyrosine and leucine gave good results in the present case also. The nitrogen of urea, creatine, parabanic acid and uric acid has little nutritive value. From the last mentioned substance also carbon can be assimilated.

Among aromatic compounds, only the nitrogen of ammoniumsalts has any nutritive value; among the derivatives of pyridine only the nitrogen of the residue, not the carbon. To develop the fungus glycose has consequently to be added to the nutritive material. Nicotine, being a free alcaloid can serve as a source neither of nitrogen nor of carbon.

If assimilable carbon is present, the nitrogen is used from the ammoniumsalts of oxalic, tartaric, citric and benzoic acids, least from ammonium succinate.

b. *Carbonaceous food.*

Of fatty acids only very dilute acetic acid (0.050 %) has a nutritive value for carbon.

The polyacid alcohols are bad sources of carbon, as was shown by an investigation with glycerine, erythrite, mannite, sorbite, adonite and dulcite. Least satisfactory was sorbite and also glycerine, a good carbon-food for many fungi, gave bad results here. Lactic acid in very small quantities, was available as a carbon-food.

Very differently behaved the sugars. As was already mentioned, glycose comes first in nutritive value. Besides were studied: arabinose, xylose, saccharose, fructose, maltose, lactose, raffinose and melibiose. Of all these only xylose and arabinose had any value as sources of carbon. In all other solutions only a trace of growth was observed. Though not without difficulty the fungus was able to derive carbon from cellulose. On filtering paper wetted with the above-mentioned nutrient solution, but without glycose, a snowwhite, woolly mycelium developed. Also from inuline carbon may be obtained.

c. *Nitrogenous and carbonaceous food.*

As mixed sources of carbon and nitrogen we must mention asparagine, aspartic acid and alanine. The addition of potassium nitrate improved the growth more with aspartic acid than with asparagine, which must probably be ascribed to the two carboxylgroups, active as sources of carbon.

Finally it must be mentioned that also peptone can furnish carbon as well as nitrogen, but that the nutritive value for nitrogen is increased here by adding glycose.

In accordance with the results of KLEBS, it was found that a high nutritive value of the liquid had influence on the formation of Sclerotia with alanine, leucine, aspartic acid and glycose. These bodies appeared under the mentioned favourable conditions at the surface of the liquid in about three weeks' time.

VI. HINTS ABOUT THE PREVENTION OF THE SCLEROTINIA-DISEASE ("ROT") IN TOBACCOFIELDS.

As a damp soil and a damp atmosphere are both absolutely necessary for the development of the "rot" or *Sclerotinia*-disease and as this disease in wet years appears about the time when the tobacco-leaves begin to be gathered, it is absolutely necessary, for the reasons given above, to stop the cultivation of scarlet-runners (*Phaseolus coccineus*, also named *Phas. multiflorus*) on the tabaccofields and only to admit and to continue the cultivation of French beans (*Phaseolus vulgaris* SAVI).

Besides limp leaves or stems or such as are covered with the least quantity of a white down must immediately be removed and burned.

The leaves that have been carried into the drying-sheds must at once be laid asunder and hung up to be dried. Suspected leaves must be sorted out and destroyed.

DIAGNOSIS LATINA.

Sclerotinia Nicotianae *Oud.* et *Koning*. — *Sclerotiis* ad superficiem caulium et foliorum primo in compagine densissimo filorum mycelii niveorum absconditis, celeriter mole augentibus, mox itaque expositis, tandemque a substratu decidentibus, extus nigris, intus albis, nunc subglobosis, tunc iterum oblongis, 10 maxime mill. longis, 5—6 mill. maxime crassis, teretibus vel subangulosis. — *Ascomatibus* plurimis (usque ad 20) ab uno eodumque sclerotio protrusis, longe stipitatis, tenerrimis; stipite filiformi, tereti, flexuoso, 4—6 centim. longo, $^1/_2$ mill. crasso, deorsum scabro, sursum laevi, summo obesiore, sic ut ascoma satis longe apophysatum videatur, una cum ascomate pallide fuscescente, floccoso-squamuloso. *Ascomate* proprio minimo, primo coniformi, clauso; dein p. m. expanso, perforato; tandem patelliformi, late aperto, 0.8 mill. in diam., 0.2 mill. alto, margine incurvato. — *Ascis* cylindricis, apice rotundatis, iodo haud caerulescentibus, deorsum breve stipitatis, 160—180×6—7 μ, paraphysibus obvallatis, octosporis. — *Sporidiis* ellipticis, 5—7×3—4μ, in partibus ascorum $^2/_3$ superioribus oblique monostichis, levibus, hyalinis.

Paraphysibus filiformibus, summo subclavatis, numerosissimis, dense congestis, ascos paullo superantibus, $2^1/_3$ μ crassis, protoplasmate dilute-fuscescente farctis.

Ex mycelii hyphis repentibus hyalinis, septatis, ramosis, numerossimae assurgunt hyphae basidiiferae; basidiis sive conidiophoris lageniformibus utplurimum conglobatis, summo conidia sphaerica, hyalina, diam. 2.5 μ, in catenas breves coadunata procreantibus.

Conidia ex aëre in patellam gelatina praeparata repletam delapsa, mox germinare incipiunt, myceliumque proferunt, cujus hyphae, quum plurimis locis arctius inter se coalescant, sclerotiorum novorum exordia edunt.

EXPLANATION OF THE PLATES.

Fig. 1. Four mature sclerotia (4—8×3—4 mill.), magnified.

Fig. 2. Two Sclerotia with a certain number of sprouts (juvenile ascomata) magnified.

Fig. 3. Microscopical representation of erect branches of the mycelium, against the top of which free calcospherites (from the gelatine) and also clusters of them have fastened.

Fig. 4. Microscopical representation of lying and ascending mycelium-threads, with the conidiophores produced by them and the apical conidia and chains of conidia originated therefrom.

Fig. 5. A Sclerotium with partly immature, partly full-grown long-stemmed Sclerotia, magnified.

Fig. 6. Section of a Sclerotium, magnified.

Fig. 7. Microscopical picture of part of a section of a Sclerotium.

Fig. 8. Nearly full-grown and full-grown ascomata, of which one cut longitudinally, magnified.

Fig. 9. Microscopical representation of part of a longitudinal section of a mature ascoma, with spore-bearing asci and paraphyses.

Fig. 10. Part of Fig. 9. enlarged.

Fig. 11. Top of an ascus and a couple of spores, still more enlarged.

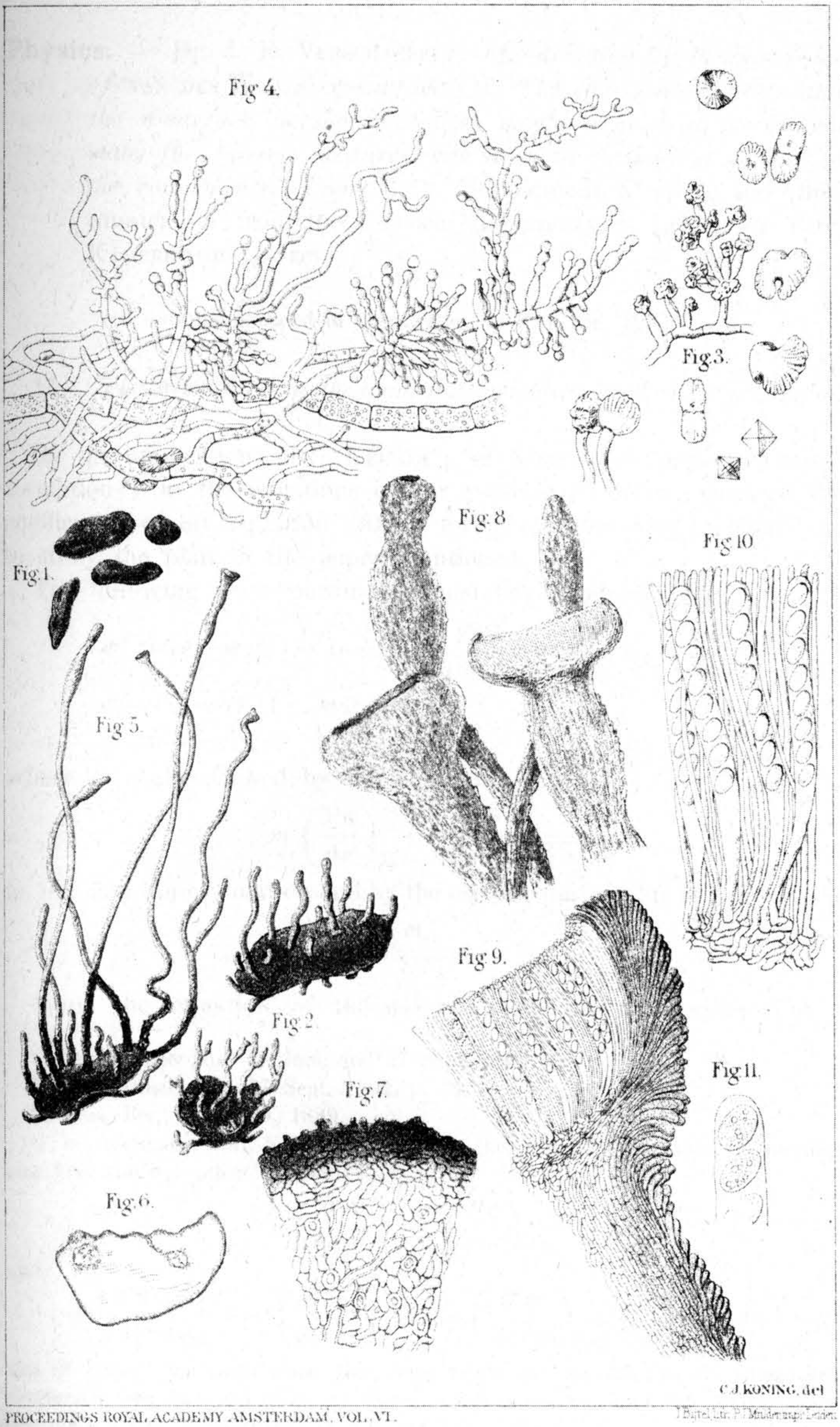
Fig 4.
Fig.3.
Fig.1.
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C. J. KONING. del