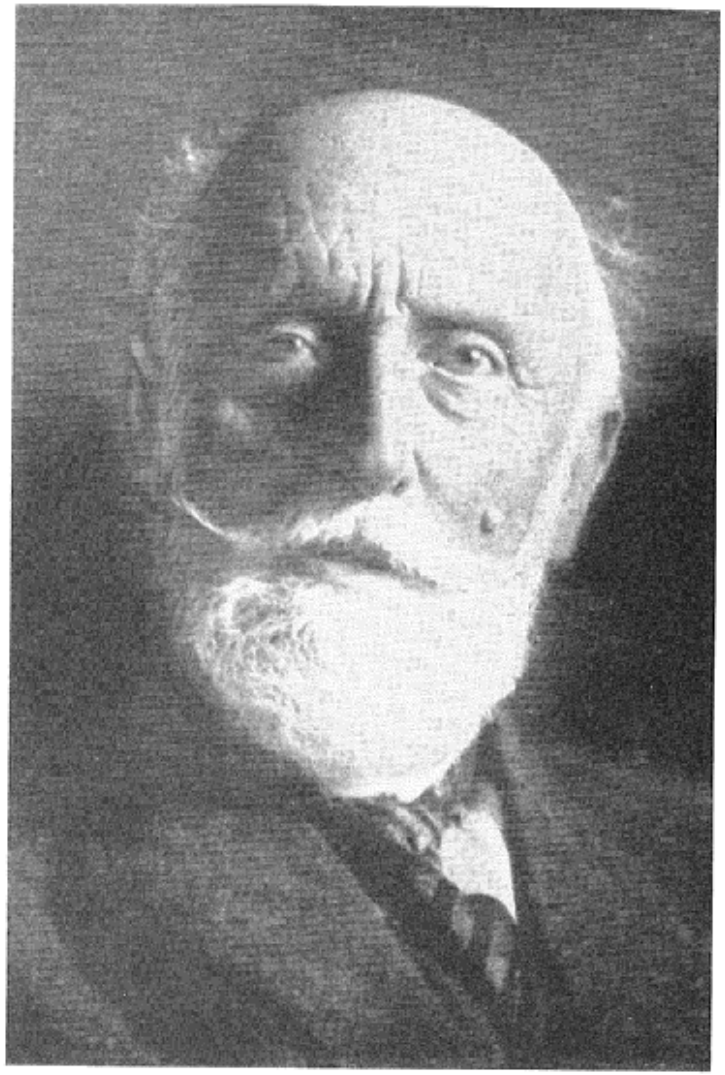

ADOLF MAYER

ADOLF MAYER
1843-

The period of scientific research on virus diseases is contemporaneous with the life of Adolf Mayer. His attention was first called to a peculiar disease of tobacco in Holland in 1879, when he was 36 years of age. Being already an experienced agricultural research worker and a professional chemist, Mayer at once analyzed the diseased tissues of the plants and the soil in which the plants were grown. Sixty years later (1939) he was alive to see many of the same problems confront the younger research workers of that decade. Mayer did not discover tobacco mosaic, although he was first to give it and similar diseases a permanent name. He himself refers to the opinions of growers as to the nature of the disease in 1857, and it was no doubt prevalent long before that, even in Europe where tobacco cultivation was not introduced until the 16th century. Dr. Mayer was, however, the first to do anything about the tobacco-mosaic disease in the modern sense of research, as is clearly evidenced in his highly significant 1886 paper entitled "Ueber die Mosaikkrankheit des Tabaks," (first published in Dutch in 1885). Mayer's research was naturally greatly influenced by the important contemporary bacteriological advances of that period; but, even considering this, his work was monumental in an entirely new field of thought and investigation. Mayer artificially transmitted for the first time a plant disease, the causal agent of which he demonstrated could not be seen or cultured. Pasteur was struggling at the same time with a similar problem in rabies, and advanced little further in the direction of the cause of these peculiar diseases than did Mayer.

Adolf Mayer was born in Oldenburg, Germany, on Sept. 8, 1843. His scientific training was obtained at the Universities of Heidelberg, Ghent, and Halle. At 25 years of age he was a lecturer at Heidelberg, becoming a professor in the same institution in 1875. His field was chemical

Mayer, A. 1886. Concerning the mosaic disease of tobacco. Phytopathological Classics No. 7. American Phytopathological Society, St. Paul, MN.

technology, and as early as 1896 he published investigations on the fermentation of alcohol, followed by investigations on wood as building material, methods of street cleaning, artificial butter, burning quality of tobacco, plant nutrition, and similar industrial and agricultural problems.

In 1876 he was made Director of the Agricultural Experiment Station at Wageningen, Holland, which position he held until 1904. He returned to Heidelberg University as a Professor and continued a very active career, diverging from science sufficiently to adopt political economy in a serious way and to write drama in poetry and prose as a relaxation.

Professor Mayer's biography will probably not be written by a contemporary who knew him well in his most productive years, because he has evidently long outlived his generation, which included most of the true pioneers in modern research methods. His last address of which we know is 7, Moltke Street, Heidelberg, Germany.

CONCERNING THE MOSAIC DISEASE OF TOBACCO[1]

Adolf Mayer

IN THOSE regions of Netherlands where the cultivation of tobacco flourishes, that is in the provinces of Gelderland and Utrecht, there has been prevailing for many years a disease of this cultivated plant, to which it seems to be very important to draw the attention of the agricultural sciences; because the harm done by this disease is often very great and I myself know cases where it has caused the cultivation of tobacco to be given up entirely in a certain place. In spite of this, this disease has hardly been the subject of a thorough investigation as yet, for the simple reason that until recently the scientific treatment of technical agricultural questions had not yet taken a firm root in Holland.

The manifestations of this disease may be approximately described as follows. About 3-5 weeks after the young plant has been transplanted into the field, has taken root well, and has begun to grow vigorously, commonly around the middle of June, a map or mosaic-like coloring of light and dark green appears on the leaf surfaces, while otherwise the whole leaf still seems to be healthy. Soon afterwards one can, with the aid of a lens, and a little later also with the naked eye, discern that the leaf shows a more pronounced growth in thickness in the darker colored spots.

It soon appears that these thicker places of the leaf are growing more vigorously than the paler parts, which results in manifold and irregular distortions of the leaf surface. Finally, if the disease develops in the regular manner, some of the lighter and thinner parts of the leaf die prematurely, not entirely different from, only much more extensively than, the similar spotting which often appears in the fully ripe leaves without detriment to the value of the product. In the later stages of the disease, the darker parts

[1] Mayer, Adolf. Ueber die Mosaikkrankheit des Tabaks. Die Landwirtschaftlichen Versucha-Stationen. 32: (451)-467, 1886. (With Plate III)

Mayer, A. 1886. Concerning the mosaic disease of tobacco. Phytopathological Classics No. 7. American Phytopathological Society, St. Paul, MN.

of the leaf may take on the transparent and varnish-colored tint, generally peculiar only to leaves that have been injected, and in which the at first sharply delimited borders between light and dark gradually grow indistinct. (cf. Plate III, fig. 1, 2, 3). Finally, it is characteristic and a sure way of diagnosing older leaves that have already been disfigured by the disease, that, when a leaf has become diseased, all the younger leaves of the same plant also show the symptoms in corresponding earlier stages, so that the diagnosis to determine whether the *disease* is present must necessary always be made in the youngest leaves.

As far as concerns the distribution of the diseased plants in an affected field, one cannot set up a rule for this.

It is not unusual to find several diseased plants next to each other. Quite as often, one often finds healthy and diseased plants alternating in most arbitrary succession. It may be accepted for certain, that an obviously diseased plant is never a source of infection for its surroundings.

The disadvantages of the disease are obvious and may be listed under the following aspects:

1. Retardation of the growth and a consequent decrease in the yield.
2. Curling of the leaves, which renders them useless for the manufacture of cigars.
3. Brittleness of the leaves with the same result.
4. Insufficient ripening and, therefore, poor burn, also harmful to the aroma, as far as one can speak of such in European tobacco.

The disease that up to now is known only in Holland [Netherlands]—in spite of diligent search, I have found only once[2] in Southern Germany, near Karlsruhe, a similar phenomenon on a very small scale—has, up to now, received only regional names.

In the region of Rhenen and Amerongen it is called "bunt" (bont) referring to the first stage of spotting in the region of Wageningen and in the region mentioned above it is called "rust" (roest) referring to the later stage of the yellow colored spots that have died. "Smut" (vuil) is a name that is popular with the grower.

[2] The curling, or so-called "going crazy," of the Palatinate tobacco is an entirely different disease with much less far-reaching effects.

Plate III

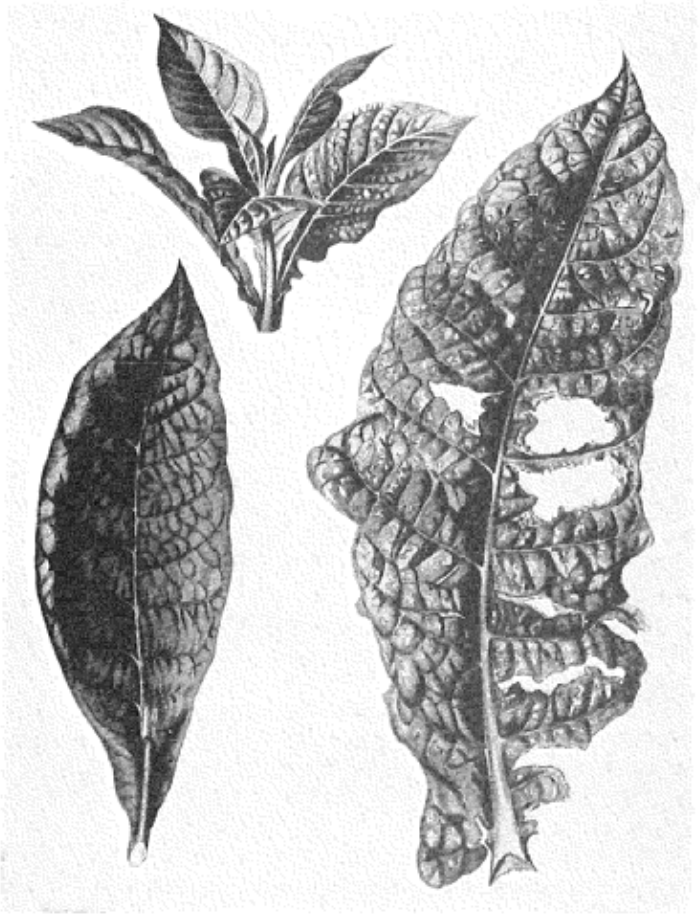

Plate III in color in the original, is here presented in black and white because of the cost of reproduction in color.

None of these expressions seems to me very desirable for general usage, least of all "rust," because this is used to designate a very characteristic fungus disease of all kinds of plants. In order to prevent a confusion that might easily take place, I should like to suggest for the time being as an international name "mosaic disease of tobacco." The term has not been used and gives a fair picture at least of the first apparent stage of the disease.[3]

What is the cause of this disease, and how may it be cured or avoided? These are two fundamental questions whose answers made a more thorough investigation necessary; therefore, the Experimental Station at Wageningen has devoted a continued study through several years to the subject,—with what measure of success, we shall see later in this article.

The earlier views of the growers about the causes of the mosaic disease had been widely divergent. A collection of them seems to us but a true chaos making one dizzy and at best useful to reaffirm the old experience, that man cannot exist without theories, and that the most practical of practitioners also usually has his specific favorite theories.[4]

[3] It is true, some growers claim to recognize two independent diseases in the two forms, or better, stages of the disease, but only because the first stage may not be apparent through superficial observation. The same geographical distribution and the succession in time of the two forms definitely speaks against this (theory).

[4] I have made a whole collection of examples and find in my notes among other things the following: (cf. also J. H. van Swieten: Tydschr. ter bevord. v. Nyverheid 1857, p. 157) One of the tobacco planters who fertilizes well and who, up to that time, had suffered little from the disease, maintains with an enviable sureness that only poor fertilizing, insufficient plant-food, is the cause of the disease. Many farmers blame the weather. With one it is the rays of the sun which are too strong, with others it is the cold nights or frosty fogs, with a third it is the cold together with the wet ground which causes the disease. There are very experienced farmers represented in this group. The same is true of the following group, in which the opinion has been formed that the condition of the seed (origin from plants that show the same disease) is of great influence. But here, too, serious comparative experiments to support the theory are not cited. Furthermore, the opinion that the procedure in planting is of great influence is very widespread. This goes so far, even, that the nickname Jan Bont was given to a farm-hand in Amerongen because he was known for his unlucky hand in planting and was therefore responsible for much "bunted" (spotted) tobacco. The opinions of this group as to what is the wrong treatment in planting, are also widely "colored" (divergent). Some have not formed a definite opinion about it. Others maintain that the evil lies in choosing a seedling with too strong a tap-root. Still others, that it is the plant with a long stem or also the somewhat etiolated plants that are the cause of it.

Still others maintain with great certainty that it is the planting in ground that has just been turned that is so disastrous, because in this way too much cold is brought into the subsoil. Some very intelligent farmers also put the blame on the hot beds in which the tobacco is sown. For example, they claim to have noticed that the disease has spread to a much more pronounced degree since the time that white paper, instead of gray paper, had been chosen for the transparent covering of the first place

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No matter how much one is justified in accepting, with a shrug of the shoulders, such attempts at an explanation, they are not always to be rejected as a point of departure for more exact questioning; and in every case we had to let ourselves be led by them for a while until we had finally come to the conclusion that it was necessary for us to commence again from the very beginning. No matter how much was undertaken by us in different directions, we believe that in this report we should briefly mention experiments that led only to negative results, even though in the cursory perusal granted to a single paper to which our so much overread age must limit itself, the reader is usually so tired out by the negative part that he has no more attention left for the positive results which are shown just before the gate closes.

Let me first mention in this short summary, that the first treatment of this question came to us from the outside. On the 23d of June, 1879, the Directors of the agricultural society (Afdeelingsbestuur van het Genootschap von Landbouw en Kruidkunde) of Wyk near Duurstede directed a communication to the board of directors of the Rykslandbouwschool, in which there was first of all a report of a discussion of the society on the following subject.[5] "What may be the reason why the tobacco plant has been suffering so much these last few years from the so-called rust?" Since no satisfying results were brought forth by this discussion, they had decided to turn to the Rykslandbouwschool. There were some healthy leaves and some leaves affected by the disease included in the letter.

As is usual in such cases, the board of directors of the Rykslandbouwschool immediately handed the letter and the samples over to the Experimental Station.

First of all a comparative chemical analysis of the healthy and the diseased tobacco leaves was undertaken. This could have possibly given some information as far as a difference in nutrition is concerned, although a difference in composition is not to be interpreted with certainty in this direction.

of planting. Others point to the excessive dryness of the hot beds, or to their being fertilized with pigeon-manure, etc.

And finally, there are many who hold the disease to be entirely unexplainable, a sort of magic, and several times the warning cry has reached my ear: You will never find it, never!

[5] Meeting of the 20th of May, 1879, in Rhenen, cf. the report about it in Wyksche Courant, 1879, No. 2, 31.

From these preliminary investigations, it was at least clear that it probably could not be a lack of nitrogen, nor of potassium, nor of lime, that had any connection with the leaves becoming diseased. Under usual circumstances there is no question anyway of a lack of phosphoric acid in the cultivation of tobacco, since the tobacco plant needs very little of this and, since, through the kind of cultivation here practiced, an excess of it is put into the soil.[6]

Furthermore, the following comparative analyses here may find their place.

Tobacco Soils from Rhenen

		Tobacco diseased		*Only little diseased*	
		1	2	3	4
Reaction		weak	acid	weak	acid
		%	%	%	%
Loss of ignition		2.9	7.3	3.0	5.6
Silicic acid	Soluble in dilute H Cl.	0.15	0.13	0.17	0.19
Aluminum and iron		1.34	1.11	1.55	0.91
Lime		0.14	0.08	0.21	0.08
Magnesium		0.09	0.01	0.11	0.01
Potassium		0.14	0.07	0.11	0.07
Sodium		0.05	0.22	0.05	0.21
Sulphuric acid		0.02	0.02	0.02	0.01
Phosphoric acid		0.23	0.12	0.25	0.10
Chlorine		0.03	0.01	0.02	—

From this collection of various experiences it became more and more plain that this malady could not possibly be interpreted as a mere nutritional disease. Aside from the analyses that have been made, one must add to these observations the fact of the sporadic appearance of the disease in soils that certainly have been uniformly fertilized, a phenomenon that never characterizes a mere deficiency in nutrition.

And, although under these circumstances I could find little motive for proceeding to more extensive chemical soil analyses, I still have some analytical figures from two tobacco-diseased soils that were determined by Dr. Pitsch,

[6] See my article on tobacco culture in Holland, Land. Wochenbl. für das Grossherzogthum Baden, 1879.

and had already been used for a different purpose once before.

These determinations are as follows:

Tobacco-diseased Soil from Amerongen

	No. 3 per cent	No. 4 per cent
Decantable part	19.0	not determined, but very similar to No. 3
Loss on ignition	4.8	
Water-holding capacity in per cent. vol.	36.4	
Apparent weight	1.31	1.31
Content of alkali-soluble organic matter	2.6	2.9
Content of phosphoric acid	0.37	0.40
Content of phosphoric acid in alkali-soluble organic matter	0.23	0.26

For us these figures are of value only insofar as they show us that these tobacco soils from Amerongen, even where the mosaic disease appears in them, are in an excellent state of fertility, and this, in spite of the fact that they were originally sandy soils that naturally did not have this wealth of organic matter and phosphoric acid. When one considers the manner in which the tobacco soils are fertilized every year and how old the culture of tobacco is in these regions, this result can hardly be surprising. However, it teaches us at the same time that the answer to the question submitted to us is not to be found in the field of nutrition.

In addition, I also further investigated comparatively a healthy and a diseased soil from Amerongen (Dr. Pitsch's No. 3) for lime, of which there usually is a dearth in the sandy soils in question and which tobacco needs in relatively large quantities (almost 200 kg. per hectar) and in both cases I found a little of this base, but no significant differences. In the same way, a lime-fertilization experiment on a tobacco-diseased field was without decided success.

These experiences, as has been said, compared with the results of manifold inquiries directed to experienced tobacco growers appeared to be sufficient to abandon the idea of a lack of nutrients as the cause for mosaic-disease and give the experiments another, presumably more useful, direction.

For a time, at first, we searched for nematodes in tobacco-diseased soils and in neighboring healthy soils, as well as in

the plants themselves, to which parasites my attention had been drawn by the studies on flax blight[7] that I was making at the same time.

Some nematodes were found, but they were of the type characteristic of humus inhabitants without proving to bear any relationship to the disease we were investigating.

Furthermore, as a result of communications from Herr Versteegh in Amerongen and other members of the "Genootschap voor Landbouw en Kruidkunde" our attention was directed to the growth conditions in the hot beds (small houses); and, in the spring of 1880, an experiment was undertaken in such a manner that the plants, in a hot bed constructed solely for this purpose, were kept colder or warmer, dryer or more moist, and more or less strongly fertilized with nitrogenous fertilizer[8] and their development carefully watched after they were transplanted in the open field.

They developed entirely normally without exception and grew very nicely towards the end of summer, although they were not so well developed as other seedlings planted earlier by us in the same soil and from ordinary hot beds. None of the modifications employed by us in the regulation of the hot beds has caused the mosaic disease.

According to this it is not to be accepted that the disease in question is determined by the differences represented in the seed beds themselves, i.e., stronger or weaker fertilization, greater or lesser warmth, more or less moisture in the first stages of growth.

Similar experiments were made with plants procured from growers whose hot beds were kept at different temperatures according to our records, and were transplanted by us; these, too, as has been indicated, gave negative results.

In order to comply with the opinion of some growers, other experiments were carried out simultaneously in connection with the above, in which the planting was purposely so done that the roots of the young plants were variously bent or injured in all kinds of ways. Even such abnormalities, as must often happen in careless transplant-

[7] Up to now only published in Dutch. cf. Tydschrift vor landbouwkunde 1881, p. 298.

[8] This is also in accord with the experiences in the culture of tea, communicated to me by the former inspector of the East Indian cultures Herr K. W. von Gorkum, that one must not use a better soil for the tea plant beds than that which the tea plantation offers to the older plants if one does not want to experience a considerable reverse after transplanting.

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ing, proved fairly harmless as far as obtaining good plants is concerned, and were in any case without influence on the production of the disease.

In order to answer the question, which also presented itself to us, as to whether the mosaic disease of tobacco could possibly be connected with the sudden transition from conditions of great warmth with restricted evaporation in the hot bed to the opposite conditions, which the open field presents, further experiments were instituted in the spring of 1881. These were carried out in the following manner: tobacco was grown in a room of unusual and continually high temperature, and later transplanted into the open field. If the disease occurred in samples thus treated, the question was to be answered positively; if it did not occur, or at least not more frequently than usual, the question was to be answered in the negative.

Since the latter was the case, I can be brief here in the description of the experiments undertaken. On the 24th of March the tobacco seed (the same that had been used for the experiments in the preceding year) of the type "Onde Groene," which is almost exclusively cultivated in this region, and is supposed to be a Maryland tobacco that has become acclimated, was sown in large prepared flower pots.[9]

The pots were covered with large glass beakers, set up near a sunny window, and kept warm day and night by means of a large water-bath whose temperature corresponded approximately to that of the growers' warmest hot beds, as they generally are in May. As far as concerns the restriction of evaporation, this was achieved to a degree not usually reached; and our problem was the study of the influence of this factor in its sudden change.

The results of these experiments also were purely negative. It is true that in some cases the sudden transition proved detrimental to the growth of the plants, but the disease was not caused by this.

The opinion that plants standing too close together

[9] In this, large and small seeds were kept separate. Under "small" is to be understood in this case smaller than ½ mm. in diameter; "large" equals larger than this border line. The latter at first produced larger plants. I did not follow up these observations any further, but should like to recommend them to the growers, remarking at the same time that the selection of seeds in the cultivation of tobacco seems to be an entirely unexplored field.

(etiolated) in the hot bed later acquired the disease was also investigated and disproved.

In the meantime my attention was turned in another direction in the summer of 1881, mainly through corresponding with the well-known horticulturist Witte in Leyden and through a study of the disease in several experimental fields of the Rykslandbouwschool, where foreign tobacco varieties were planted for a different purpose. In the year 1881 there was an excellent opportunity to observe the mosaic disease. It was generally prevalent in the surroundings of Wageningen and still more in those of Rhenen. Now it was striking that all the foreign varieties of tobacco, even though it was just these that had undergone a direct change of climate of considerable extent, were entirely spared by the disease, while in the rest it never was entirely absent.

Experiments of this kind were carried out in the year 1882, on field plots that had been under observation before, because of the frequent appearance of the mosaic disease, as well as on plots on which tobacco had never before been cultivated. These experiments were also combined with others in which tobacco seed was used that had been produced by us in 1881 under precautionary measures that prevented self-fertilization (cutting out the unripe stamens and transferring foreign pollen to the stigma) and with others in which seed from diseased plants was used.

All these differences in the origin of seed proved to be without influence on the incidence of the disease, which developed on the land already known to be disposed to the disease, but on the other hand, did not appear on land devoted for the first time to tobacco. Through this and other observations the conjecture that we were dealing with a disease caused by parasites was naturally strengthened.

Finding the Cause of the Disease

Simultaneously with the experiments indicated above, other experiments had been undertaken, which were concerned with the discovery of a plant parasite. I have already mentioned incidentally that we also searched for Anguillulen (nematodes) in the tobacco-diseased soil and in others. But much earlier, immediately after the disease had first been observed on living plants, their tissues were diligently searched for fungi, animal parasites, etc., not only by me

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but also several times by research workers of my acquaintance. This investigation was at first without successful results. Only one authority in the field of plant diseases claimed to find fungus hyphae in the diseased parts of the leaves, "which might develop into Leptoria (Septoria?) or Phoma." However, a fully developed fungus never has been found on the living plant; these unidentified threads, therefore, must have been a secondary infection on the wilted leaf.

Then I suddenly made the discovery that the juice from diseased plants obtained by grinding was a certain infectious substance for healthy plants. For instance, if one grinds up finely a leaf that is clearly diseased with the addition of a few drops of water and sucks the thick green emulsion thus obtained into fine capillary glass tubes and then sticks these into the thick leaf veins of an older plant in such a manner that they remain without penetrating to the back of the leaf, in nine cases out of ten one will be successful in making the healthy plant, of which the leaf thus treated is a part, heavily diseased.

The time between inoculation and the first unquestionable signs of the disease is regularly 10-11 days. At the end of this period the disease puts in its appearance, not in the leaf that has been inoculated, but in the very youngest leaves, particularly those not yet developed at the time of inoculation; and once a leaf shows this mosaic-like coloring, it also appears unfailingly on all the younger leaves and on all the shoots that develop in the axils of the diseased leaves. The plant is diseased in all its younger parts, with perhaps the exception of the blossom, if one does not break it off,—in all its older parts it is healthy.

It is self-evident from this that the disease will be the more violent the younger the plant is when inoculated. It seems to depend to a much lesser degree on the quantity of the inoculum. One only has to be careful that the inoculum is really sucked up, which succeeds most easily with watery inocula and when the leaves of the plant to be infected are slightly wilted.

It follows that after this striking discovery the investigation of protoplasmic bodies of the extract from diseased tobacco was again taken up with special zeal. It is true that the frequently repeated microscopic scrutinization of this

extract at first did not show any decisive results, for reasons which are easily comprehensible to anyone who is familiar with anything about bacteriological investigations and such. Firstly, the juice pressed out of healthy and out of diseased tobacco is rich in almost colorless particles in the protoplasm,[10] which have a shape not unlike that of the red blood corpuscles, only a little more sickle-like (half-moon-like) and often cover up other, principally smaller, things. Besides this, the extract in both cases (although apparently predominantly in diseased leaves) is rich in smaller tetrahedric particles, which slowly disappear in hydrochloric acid and probably must be interpreted as being calcium oxalate. Whatever other smaller particles one may see in the sap they are so indefinite, even when strongly magnified, that one may not with certainty designate them as anything organized.[11]

Later I tried to isolate these questionable organisms according to Koch's method and other methods; in many cases I proved the presence of bacteriological vegetation. However, none of these, used as inoculum, were infectious to healthy tobacco. Likewise I inoculated the latter with a great number of well-known bacteria and fluids containing bacteria, which were in many cases isolated according to the method given by Zopf, without resultant disease in a single case. In order to spare other experimental workers in this field fruitless labor, I mention here as such inocula:

1. *Bacterium tumescens*
2. Lactic acid bacteria
3. *Bacterium subtilis*
4. Glycerin bacteria
5. Acetic bacteria
6. Pigeon manure (a manure frequently used for tobacco hot beds)
7. Sheep manure (the usual tobacco manure in Holland)
8. Chicken excrements
9. Cattle manure
10. Outhouse manure (used several times in "practice" in cases where the disease appeared)

[10] These, however, do not show the albumen reaction, nor do they react to methyl violet, but they *are* somewhat colored by iodine.

[11] Sap from healthy plants does not produce the disease, as I have proved experimentally—although to some it may seem superfluous to have tried this.

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11. Grated old cheese
12. Horse manure
13. Extract from tobacco diseased soil
14. Putrefied legumes

However, there is another means of answering the question, with what kind of disease one is dealing in this case, than that at present generally used by mycologists. One should realize that a definite capacity to infect, as has been proved in our case, may be determined either by an unorganized or an organized ferment. It is true that the former would be rather unusual as a cause for a disease, and also that an enzyme should reproduce itself is unheard of. Yet this situation has been taken under consideration in the following.

An organized ferment also may be: a fungus or a bacterium, and these two form-groups can be distinguished with the aid of a microscope and also by a mechanical method. May I remind you, that a mixed alcoholic and lactic acid fermentation becomes purely the latter after filtration through ordinary filterpaper, because the lactic acid bacteria go through the pores of the paper by the thousand, while the *Saccharomyces* cells do not.

The following experiments were carried out in order to weigh these three possibilities.

First the inoculum, which swarmed with cell-contents, was filtered through ordinary filter paper and the filtrate used for a great number of further inoculations.

Result: filtered extract has about the same effect (the percentage of diseased plants is somewhat smaller) as the original. If this frequently corroborated result seems to establish the fact that the solid (organized) cell-contents are not responsible for the transmission of the infection, one may add immediately, that the particles described in more detail above, are all small enough to go through the pores in the filter paper, even though in a somewhat different relationship. It is not until one has repeated the filtration through double filters that one finally succeeds in getting a clear filtrate. This also was used for many infection experiments.[12]

Result: Filtrates that are clarified (purified) in any way do not have the capacity for infection.

[12] Since for all these experiments whole rows of plants were used.

With this (result) already, the possibility of an infection through an enzyme-like body would be excluded; because it definitely contradicts all the known characteristics of these peculiar substances to be removed from a fluid in which they have been dissolved by means of simple filtration. This conclusion is supported by the fact that an attempted isolation, or better, concentration of an enzyme from the unfiltered extract by precipitation with weak alcohol, and redissolving in water, a method that brings one nearer the goal with all enzymes, led to no preparation capable of producing infection, in which experiments it was, however, necessary to be careful to use finally a clear solution in which no bacteria could be found.

At the same time experiments were undertaken with extracts kept heated at certain degrees of temperatures for hours.

Result: Continual heating at 60° does not alter demonstrably the capacity for infection, at 65-75° it becomes weaker. Heating the sap at 80° for several hours kills the infectious substance.

These experiments,[13] therefore, confirm the fact that the infectious substance in question is subject to the living conditions of organic ferments. But, according to the preceding experiments the object sought could be found only in the organized particles. The question is more and more narrowed down to bacteria and fungi, and, even to this, the experiments described above give a quite unmistakable answer. Fungi universally have too great a dimension to go through the filterpaper. One might perhaps think of a gonidial stage with particularly small spore-like reproductive organs, but it would then be incomprehensible, how such a passing stage in the life cycle of a fungus could again produce the same disease of which it was a product. Also, it is impossible to assume that a fungous disease in any of its stages should not have been recognized as such by us or by experienced observers who took the trouble to inspect the diseased plants microscopically.

In short, I conclude, not basing my conclusions entirely on new experimental facts, but also deducing in part from

[13] Of further experiments, whose point of departure are not connected in any way with the presentation here chosen, I attempted: Infection of diseased plants with the sap of healthy plants and infection of other Solanaceae with the sap of diseased tobacco—both without success.

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facts already known, that we are concerned with a bacterial disease. A closer knowledge of the form and mode of life of the responsible bacteria cannot, of course, be obtained in this way and must be reserved for future research.

On the whole, I feel justified from my preliminary studies, which at least have reached somewhat of a termination, in drawing the following conclusions:

1. The mosaic disease of tobacco is a bacterial disease, of which, however, the infectious forms are not isolated nor are their form and mode of life known.

2. The capacity for infection of the disease from plant to plant under the artificial conditions of extract mixture is proved with certainty. Under natural conditions no significant infection takes place from plant to plant. The seed from diseased plants can produce healthy plants.

3. The spreading of the disease substance must be looked for in the soil of the tobacco plantations and in the hot beds; because certain and particularly fields repeatedly grown to tobacco are especially likely to be diseased. A case of transmission of the disease with the soil has not been verified.

Of course, for the time being only uncertain precautionary measures can be mentioned, whose introduction by way of trial is nevertheless commendable.

Wherever the disease appears in the hot beds, one should in any case change the soil and on the tobacco plantations themselves a rotation of crops should be instituted. The diseased plants standing in the fields and the stalks remaining in the fields after the harvest should be removed in such a way that no part of them is returned to a tobacco field.

If possible, one should fertilize with materials that have no lower organisms in them, as, for instance, pulverized peat and artificial fertilizers (among which a mixture of saltpeter and potassium chloride is commendable); and, if this is not possible, one should use only one kind of natural fertilizer and, in any case, carefully record the experiences that are derived from this.

Rykspoefstation zu Wageningen, Fall of 1885.

Mayer, A. 1886. Concerning the mosaic disease of tobacco. Phytopathological Classics No. 7. American Phytopathological Society, St. Paul, MN.