

JAMES

Phytopathological Classics

NUMBER 7

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TOBACCO PLANT

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AS A CAUSE OF THE SPOT-DISEASE OF
TOBACCO LEAVES

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4. ON THE ETIOLOGY OF INFECTIOUS VARIEGATION

By Erwin Baur

Translated from German

by

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With a preface and biographical sketches by the translator.

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*Jorge M. Hernandez
June 190*

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ACKNOWLEDGMENTS

The writer wishes to acknowledge the assistance of Miss Elizabeth von Oettingen, graduate assistant in German at the University of Wisconsin, for valuable aid in preparing the first rough drafts of the translations of three papers. Thanks are due to Dr. Benjamin M. Duggar for furnishing the originals of the photographs of Mayer, Ivanowski, and Beijerinck, used by Dr. Erwin F. Smith in his article on *Fifty Years of Pathology*, published in the Proceedings of The International Congress of Plant Sciences 1: 13-46, 1929. Many others, also, have been called upon for help and suggestions in preparing either the translations or in securing material for the biographies of the authors. Thanks are especially due to Dr. H. H. Whetzel, who generously offered to accept the translations for the *Phytopathological Classics*, and to Dr. H. B. Humphrey, upon whom the responsibility for final editing and publication has fallen.

TRANSLATOR'S PREFACE

Theory suggests that the viruses may be as old as life itself; written history indicates they existed as disease-inciting agents 500 years or more ago, but the science of the viruses is yet barely 50 years old. It is, perhaps, too early to evaluate even the first scientific contributions to this subject. In publishing the following translations as phytopathological classics, no exclusion of other meritorious papers by the same or other investigators is intended. If, however, only a limited number of papers are to be selected as representative of the historical background of the plant viruses, chronology and significance considered, it is believed that most plant pathologists would agree to including those translated here.¹ These papers individually and as a group have exercised a most profound and lasting influence on subsequent virus research and reasoning.

The more significant and interesting conclusions in these papers of Mayer, Ivanowski, Beijerinck, and Baur may be stated very briefly here in order that those readers who are not well acquainted with the plant viruses may sort out the more important conceptions from those that may be less significant or even erroneous.

The now commonly applied term for many virus diseases of plants, namely "mosaic," was first introduced by Adolph Mayer in his 1886 paper, which is translated in this series. Mayer is also generally conceded to have been the first to prove the infectious nature of the tobacco-mosaic disease, which later became the first known filterable virus. Although Mayer himself attempted to filter the infectious agent and could find no microorganisms accountable for the disease by modern bacteriological technique and therefore, suggested its similarity to enzymes, he reasoned that

¹ These translations were first made as contributions to the Ismé Aldyth Hoggan Memorial Collection on Plant Viruses at the University of Wisconsin. It is hoped that the publication of these translations will reciprocate in a small measure for the many valued separates on plant and animal viruses contributed to this collection by research workers throughout the world.

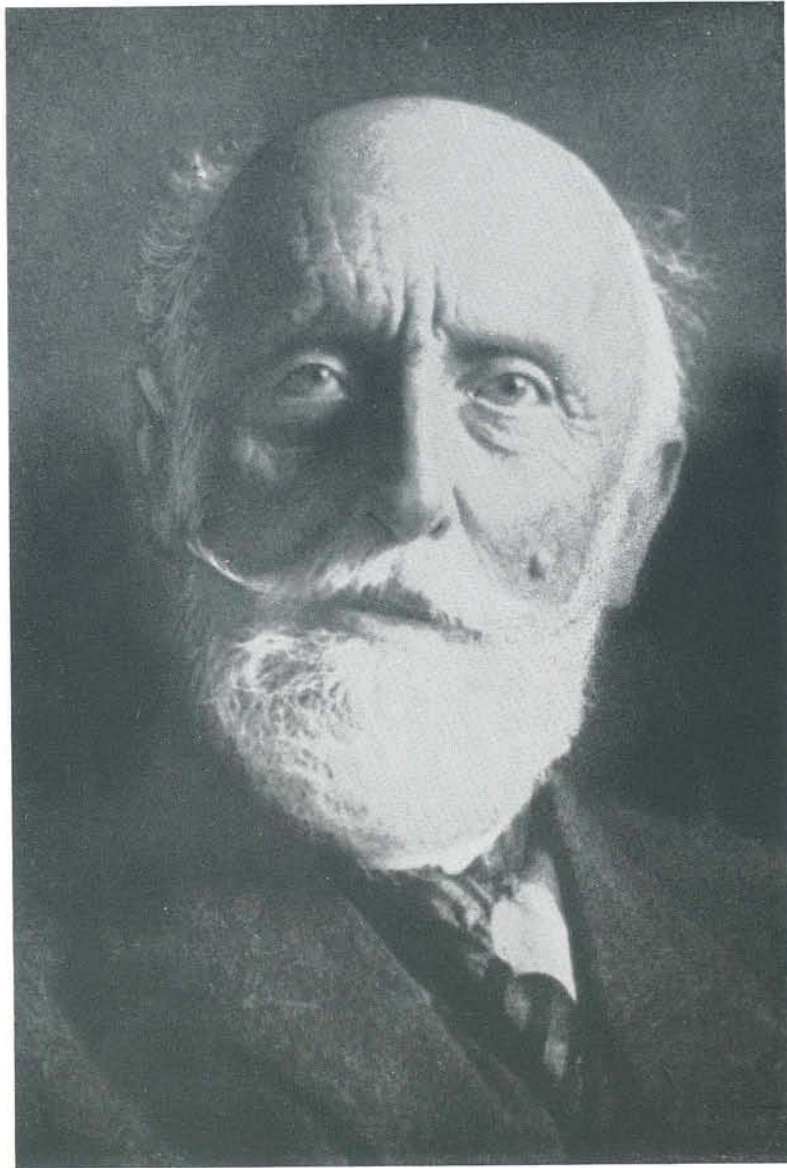
the disease must be of bacterial origin. The stimulating effect of this contribution on later research can well be imagined.

The short paper by Ivanowski, in 1892, is perhaps the most widely recognized article in both the plant-and animal-virus field. In a few brief sentences he essentially states that he filtered the juice of mosaic-diseased tobacco plants through a Chamberland filter, that the filtrates remained unchanged (sterile) and reproduced the typical disease. This is recognized as the first demonstration of a filterable infectious agent in either plant, animal, or human pathology. Curiously enough, this paper is devoted largely to a disagreement with Mayer over the identity of two types of symptoms of disease on tobacco with respect to cause, the contention upheld by Ivanowski now being generally believed to be erroneous. Ivanowski also is well known for a later paper, published in 1903, in which he first described abnormal intracellular inclusions in tobacco mosaic, including protozoan-like inclusion bodies as well as the crystal-like bodies now believed by some to be the virus itself.

Beijerinck's (1898) paper is perhaps most widely known because of his "contagious living fluid" concept. This conception, along with the first application of the term "virus" as contrasted to a poison or toxin or bacterial suspension, was not entirely new because Pasteur somewhat earlier was confronted with similar problems with rabies, and Loeffler and Frosh in the same year had filtered the causative agent of foot and mouth disease of cattle and were much concerned about what such a substance might be and how it could reproduce itself. Beijerinck's experimental material, namely tobacco mosaic (he used the names spot-disease, albinism or bunt) was better adapted for fundamental studies and he confronted himself with many questions, both practical and technical, which he attempted to answer by simple experimental methods. For example, he studied diffusion of the virus into agar, as a measure of its corpuscular or noncorpuscular nature. His *vivium fluidum contagium* appears to come very close to the now prevalent concept of a protein molecule as representing the physical structure of a virus.

Baur's 1904 paper was selected for translation not because of his interesting demonstrations on the transmissibility of infectious variegation by grafting but because of the

emphatic manner in which he used his knowledge of infectious variegation to reason against the generally accepted belief that all infectious diseases were necessarily caused by parasitic organisms. His idea of a virus and its reproduction, not unlike that of Beijerinck, serves to emphasize that progress in the understanding of the origin and nature of a virus has not advanced far since 1904.



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

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 TOBACCO¹

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 Versuchs-Stationen. 32: (451)-467, 1886. (With Plate III)

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 necessarily 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 once² 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.

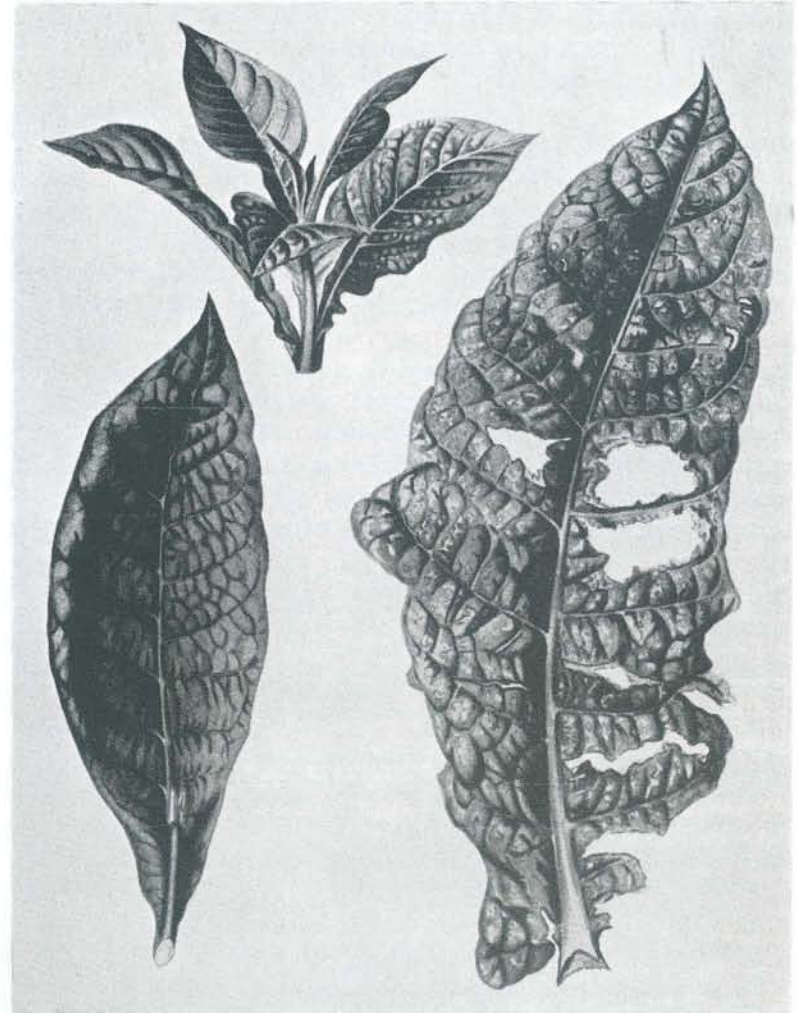


Plate III in color in the original, is here presented in black and white because of the cost of reproduction in color.

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

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 over-read 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

Reaction	Tobacco diseased		Only little diseased	
	1 weak %	2 acid %	3 weak %	4 acid %
Loss of ignition	2.9	7.3	3.0	5.6
Silicic acid	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	—

Soluble in dilute H Cl.

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.

doença nutricional

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
Loss on ignition	4.8	very similar
Water-holding capacity in per cent. vol.	36.4	to No. 3
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 hectare) 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 blight⁷ 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 fertilizer⁸ 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.

Nematoden

*doença
no processo
de produção
de mudas*

*Causa
nutricional
abnormal*

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 $\frac{1}{2}$ 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

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 unfaillingly 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.

transmission

teste dos portulacos de Koch fulvorum

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.

*doença
bacteriana*

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.

Ryksproefstation zu Wageningen, Fall of 1885.



DMITRII IVANOWSKI



DMITRII IVANOWSKI

DMITRII IVANOWSKI

-1924

Dm. Ivanowski (Iwanowsky) published his first paper on tobacco mosaic in 1890, which was apparently also his first scientific contribution. His age was, therefore, probably close to that of Baur, but his work on the mosaic disease was more nearly contemporaneous with that of Mayer and Beijerinck, who were considerably older, although they outlived him. Ivanowski's first paper was published in the Russian language and was based on observations in Crimea. The work was evidently supported or sponsored by the Botanical Laboratory of the Academy of Science of St.-Petersbourg, whence he also published on alcoholic fermentation as early as 1894.

It was on February 12, 1892, that Ivanowski read the paper before the Academy of Science, St.-Petersbourg, in which he reported the filterable nature of the tobacco-mosaic virus. This paper is now universally accepted as the first report of the filterability of either plant or animal viruses. The influence of this discovery on later biological thought and activity can hardly be overestimated. Ivanowski's claim to recognition does not, however, stop here. In 1903 he published what is probably his best and most detailed study on the mosaic disease. In this paper he was first to describe the inclusion bodies in the host cells of virus-diseased plants. This contribution is all the more significant when it is recalled that the Negri bodies of rabies were described in the same year. Although the Guarneri bodies of small-pox were described in 1894, it is doubtful that Ivanowski knew of them or suspected any similarity with his inclusion bodies. This paper was evidently his last on the mosaic disease, although he published on chlorophyll at least as late as 1914, his interests having evidently turned more directly to plant physiology.

In 1902-3, Dr. Ivanowski was listed as head of the Department of Botany of the Royal University of Warsaw. In

*DMV
Petersburg*

*Inclusion
bodies*

1907, he was Professor of Plant Physiology at the same institution, where he evidently continued his work for several years.

A notice only of Dr. Ivanowski's death is published in the 1924 volume of the *Berichte der Deutsche Botanische Gesellschaft*. It is unfortunate that more information is not now available about perhaps the most widely known man in virus research. An extensive search in the libraries in this country has failed to reveal any published biography. Correspondence with some European biologists in this country has failed to yield any clues on a published biography. Other efforts to secure information by correspondence with European biologists abroad have largely failed because of the war situation.

The great significance of Dm. Ivanowski's work was perhaps not fully recognized during his lifetime. His fame will no doubt grow with the passing of time, and it would be highly fitting and desirable for a biographer who knew him and his work to write a complete and satisfactory sketch of his life before it is lost to future generations of biologists.

CONCERNING THE MOSAIC DISEASE OF THE TOBACCO PLANT¹

Dmitrii Ivanowski

TWO YEARS AGO, in collaboration with Mr. Polowzow, I described a very wide-spread tobacco disease, which we called pock-disease (or pox-disease) and whose causes we set forth at that time.² On this occasion we also announced the supposition that the mosaic disease of tobacco,³ described by Ad. Mayer in Holland, really comprises two entirely different diseases, of which one (according to Mayer, the second phase of the mosaic disease) is the pock-disease studied by us. While investigating tobacco diseases in the Crimea in the summer of 1890, I was able to convince myself of the complete correctness of the supposition advanced by us at that time, for here I also met the form of disease described by Mayer as the first phase of the mosaic disease and could convince myself that this form is actually an entirely independent and in many respects a very interesting disease.⁴

Among the native tobacco planters (Tartars) it is known merely under the name of "bosuch" (i.e. disease); by some it also is called marble-disease. The outer appearance of the diseased plants, the course of development of the disease, and its distribution on the plantation correspond entirely to the description supplied by Mayer, so it will not be necessary to discuss this question. The difference in the statement begins only at the place where Mayer makes the assertion that "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 ex-

¹ Ivanowski, Dmitrii. Ueber die Mosaikkrankheit der Tabakspflanze. St. Petersburg. Acad. Imp. Sci. Bul. 35 (nouv. ser. i. e. ser. 4, v. 3): 67-70. Sept. 1892.

² Memoir of the Imperial Academy of Sciences of St. Petersburg, Vol. XXXVII, No. 7.

³ *Landwirtschaftliche Versuchstation* Vol. 32, pp. 451-67.

⁴ I shall designate it as mosaic disease even though it corresponds only to a stage of development in the disease described by Mayer under this name. *Melanges biologiques* Vol. 13, p. 237.

tensively than a similar spotting that often appears in fully ripe leaves without detriment to the value of the product." (p. 452). In the accompanying plate Mayer pictures a leaf densely covered with brown spots; these spots have become fused in places and some have dropped out of the leaf, as a result of which the leaf seems to have wide holes. In my opinion the leaf pictured has been attacked by two entirely different diseases: The mosaic disease (in the sense proposed by me) and the pock disease. Both diseases, although of an entirely different origin, may of course occur on the same plant.⁵ The brown spots are widely distributed and not always connected with the yellow parts of the leaf attacked by mosaic disease, which was the case according to Mayer; one can often find them in the middle of a dark green, healthy part. The independence of these two diseases may now no longer be doubted. For this I should like to adduce the following:

1. I observed the mosaic disease neither in Little Russia nor in Bessarabia, although the pock disease had reached a high degree of development there.

2. In the Crimea, where both diseases occur, one can, in inspecting the plantation, find examples [plants] that are suffering from the mosaic disease only; others, suffering only from the pock disease. These plants may most easily be differentiated through the very young leaves. In the plants attacked by mosaic disease, all new tissues (new leaves and shoots) show the changes peculiar to this disease (as Mayer has observed), that is, mosaic-like symptoms consisting of dark-green and yellow areas. When we, therefore, find examples of brown-spotted tobacco plants, in which, however, the very young leaves show no traces of the mosaic symptoms, one can say with certainty that these plants are suffering from the pock disease.

3. The mosaic disease is infectious, while the pock disease by no means has this characteristic.

4. The cause of the pock disease lies in the restriction of (water) transpiration through the leaves; the spots appear on entirely healthy leaves with a rapid and sudden increase in the transpiration of the plant; the causes of the mosaic

⁵ It may be possible even that the plant attacked by the mosaic disease is more susceptible to the pock disease than is the healthy one because diseased plants generally are more easily attacked by new diseases than are healthy ones.

disease are, on the other hand, of a very different sort, it is, as was mentioned above, infectious.

5. We found the pock disease in *Datura stramonium*, *Hyoscyamus niger* and many other plants; the mosaic disease, on the other hand, according to Mayer's observations, is not transmitted to other representatives of the family Solanaceae.

In support of the opinion that both diseases represent different stages of development of one and the same disease, Mayer only mentions the same geographical distribution and their succession in time. "It is true," writes Mayer, "that some growers 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 speaks against this (theory)." (l.c., p. 553).

My investigations on the mosaic disease are not yet completed, since I have met with great difficulties that must first be removed (as, for example, the inability of the tobacco microbes to develop on the usual artificial media). Nevertheless, I am already able to confirm at this time the following statements of Mayer:

1. *That the sap of plants suffering from the mosaic disease is infectious; inoculated into healthy plants it produces after a certain time the mosaic disease in the latter.*

2. *That in heating the sap of the diseased tobacco plant to a temperature near the boiling point, it loses its infectious qualities.*

3. *That, considering the absence of fungi and other parasites, infection through bacteria may be ascribed to the disease.*

On the other hand, I must contradict most emphatically the author's statement that the sap of leaves attacked by mosaic disease loses all its infectious qualities after filtration through double filter paper. According to my experiments the filtered extract introduced into healthy plants produces the symptoms of the disease just as surely as does the unfiltered sap. Moreover, this opinion of the author does not agree with my conviction that the mosaic disease is caused by bacteria, for a double layer (of filter paper) cannot, as is well-known, hold back bacteria. If this observation of

infection
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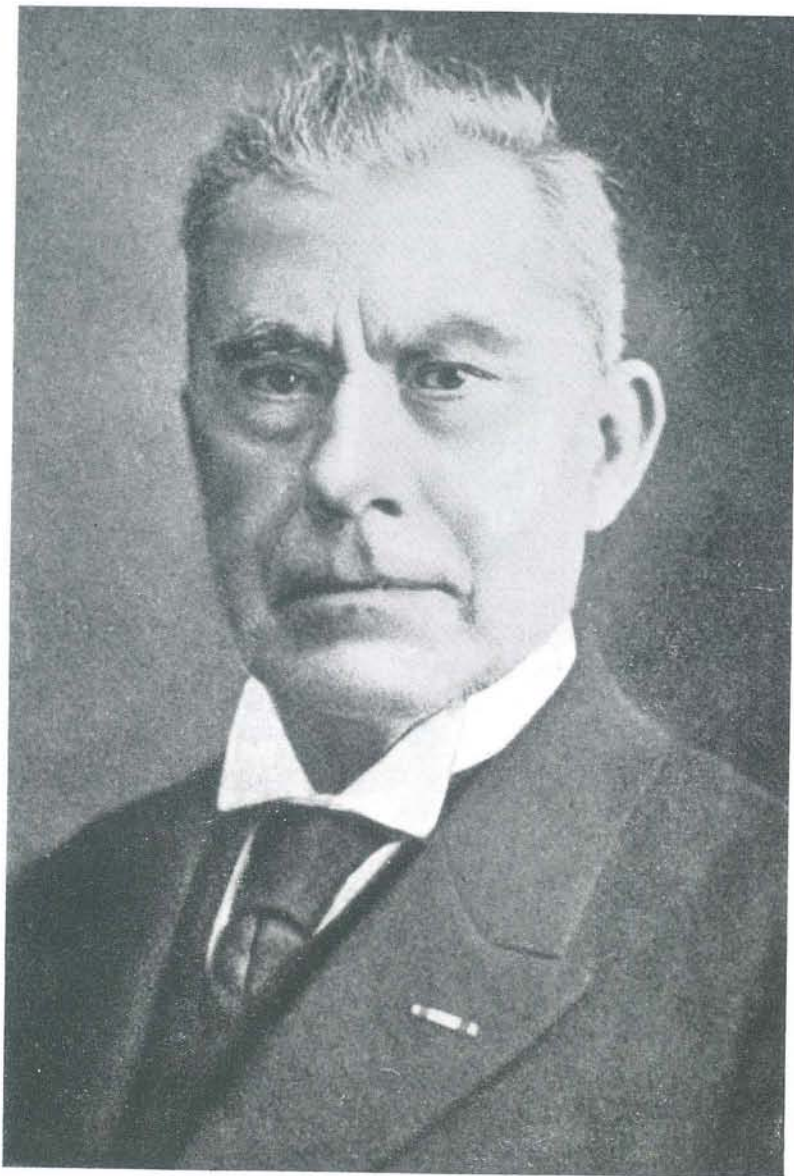
disease
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Mayer's were correct, one should rather come to the conclusion that the mosaic disease is not caused by bacteria, but rather by fungi whose spores cannot pass through the filter paper. Yet I have found *that the sap of leaves attacked by the mosaic disease retains its infectious qualities even after filtration through Chamberland filter-candles*. According to the opinions prevalent today, it seems to me that the latter is to be explained most simply by the assumption of a toxin secreted by the bacteria present, which is dissolved in the filtered sap. Besides this there is another equally acceptable explanation possible, namely, that the bacteria of the tobacco plant penetrated through the pores of the Chamberland filter-candles, even though before every experiment I checked the filter used in the usual manner and convinced myself of the absence of fine leaks and openings.⁶ I see further proof of the perfection of the filter-candles used by me in the fact that the liquids, most favorable to the development of bacteria, remained entirely unchanged for several months after filtration through this candle.

In any case, I hope that further investigations will clear up this question; the remarks under consideration have only the purpose to establish the independence of the two diseases, the mosaic and the pock disease, and to prove that they do not represent, as Ad. Mayer assumes, different stages of development of one disease.

⁶ It was impossible by means of a rubber bulb to press air through the filter-candles submerged in a cylinder of water.



MARTINUS WILLEM BEIJERINCK

MARTINUS WILLEM BEIJERINCK

1851-1931

Prof. M. W. Beijerinck is most famous as a soil microbiologist. If he had instead devoted his highly productive genius and energy to the study of his "contagious living fluid" it is highly probable that the science of the viruses would have been advanced 25 years beyond what it was in 1921 when he retired from active service. For a quarter of a century after his paper entitled "Ueber ein contagium vivium fluidum, als Ursache der Fleckenkrankheit des Tabaksblätter," the subject of plant viruses appeared to have reached a limit as far as research activity relating to the nature of the viruses was concerned. Beijerinck gave only a very small part of his brilliant career to the viruses, but many other important phases of agricultural and industrial science benefited by this distribution of his researches. Although Dr. Beijerinck was professionally a botanist and his first and last scientific interests were in this older field, the contributions for which he is most famous were adopted by bacteriology and soil microbiology. Commencing with studies on plant galls, his attentions were directed toward fermentation and he thus became a pioneer in microbiology, including, particularly, nutrition studies on algae, amoebae, yeasts, fungi, and bacteria. It was Beijerinck who, in 1888, isolated *Bacillus radicicola*, the nodule organism of leguminous plants. His studies, important to soil science, on the sulphur bacteria, azotobacter, and on denitrification came at about the same time as his work on the tobacco-mosaic virus. During all this busy period in research he was also a teacher with a highly stimulating influence on his pupils, thereby attracting numerous students of science to his desk and to his laboratories.

Martinus Willem Beijerinck was born in Amsterdam, Holland, March 16, 1851. He received the degree of "Chemical Engineer" from the Technical School of Delft in 1872 and obtained his Doctor of Science degree at Leyden in 1877.

He started his teaching career as a lecturer in 1873, including botany, physiology, physics, zoology, and geology, subjects taught before he had earned his doctor's degree. Such diversity of basic knowledge may account in part for his wide interests in later researches. As early as 1876 he lectured at the Agricultural School at Wageningen, Holland, and it was no doubt shortly after this that his interests in the tobacco-mosaic disease was fostered by Adolf Mayer who, about the same time, had come to Wageningen as Director of the Experiment Station. In 1887 Beijerinck became, at the invitation of Hugo de Vries, the microbiologist of the Netherland Yeast and Alcohol Factory at Delft. In 1893 he was made Professor of Bacteriology in the Technical School at Delft, which title was apparently changed to Professor of Microbiology in 1895. In 1897 Beijerinck founded the Microbiological Laboratory at the same institution where he performed his most important work on tobacco mosaic and labored on other problems until his retirement in 1921 at the age of 70 years. His numerous and brilliant papers were brought together on this anniversary in a collection of 5 volumes published by Gravenhage of Delft. Prof. M. W. Beijerinck died on Jan. 1, 1931 at a country home close to Gorssel in eastern Netherlands.

CONCERNING A CONTAGIUM VIVUM FLUIDUM AS CAUSE OF THE SPOT DISEASE OF TOBACCO LEAVES¹

M. W. Beijerinck

In 1885 Mr. Adolf Mayer² showed that the mosaic or leaf-spot^a disease of the tobacco plant is contagious. He pressed the sap from diseased plants, introduced it into capillary tubes, and pierced these into the leaves and stems of healthy plants growing out in the open. After a few weeks the latter were then attacked by the spot disease. He himself could not find any bacteria or other parasites in the diseased leaves through microscopic inspection. I was at that time Mr. Mayer's colleague at the Agricultural School at Wageningen, he showed me his experiments, and I, no more than he, could prove the presence of microbes in the diseased plants to which the disease could be ascribed. At that time, however, my bacteriological knowledge was so incomplete that I could not take my own direct observations as a conclusive proof.

Since that time I have been continually occupied with bacteriological experiments, and when I discovered the bacteria of the Papilionae nodules in 1887, I also took up the tobacco disease again. However, the result was also negative at that time. Since, however, in all my experiments at that time, the microscopic picture, on the one hand, had to be decisive, and on the other hand only cultural experiments pertaining to aerobes were carried out, the possibility still remained that anaerobes were vegetating in small numbers in the plant tissue, which defied direct observa-

^a The term Fleckenkrankheit (spot disease) as contrasted to Mosaikkrankheit (mosaic disease) and Pockenkrankheit (pock disease) as used in German by many writers, is often confusing. Flecken (spots) may apparently be either chlorotic or necrotic spots. As chlorotic spots, "fleckenkrankheit" would be synonymous with either mottling or variegation.

¹ Beijerinck, M. W. Ueber ein contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblätter. Verhandlungen der Koninklyke akademie van Wetenschappen te Amsterdam. 65: (2) 3-21, 1898. (With 2 plates).

² Landwirtschaftliche Versuchsstationen 32: 450, 1886.

tion, but were affecting the surrounding plant tissue with poisons, like the tetanus bacteria do with a poison, which is soluble, non-living, i. e., unable to reproduce itself.

It is well known that reduced pigments, which become colored when exposed to the air, often appear³ inside of cells of the organs of higher plants so that the possibility of the presence of anaerobes in the tobacco plant was from the very beginning not to be excluded. It is true that the presence of such microbes inside of the green organs of aerial plants, is highly improbable; yet, the discovery of "mikro-aerophilie" in anaerobes⁴ demands the greatest attention when facts of such far-reaching importance as those presented here are concerned, and these give special incentive to new experiments concerning the microbes appearing in the roots below the surface of the ground.

But after I had taken great pains to find anaerobes that could be causally connected with the disease, within or proximate to the diseased leaves and roots of the affected plants, but always with negative results, and I finally knew positively that these, too, were not present, the conclusion was no longer to be denied, that the spot disease is an infectious one that is not caused by microbes.

Then, in 1897, the resources of the newly erected bacteriological laboratory of the Polytechnical Institute at Delft were put at my disposal. This included a greenhouse with heating facilities, which I started using that same year for further experiments on the spot disease. I was, therefore, able to carry out a series of incontestable infection experiments the results of which I shall now briefly describe.

My experimental plants belonged mainly to the local variety from Amerongen and partly they came from seeds from Erfurt.⁵

1. *The infection is not caused by microbes, but by a contagium vivum fluidum.*

It soon became evident that the sap of diseased plants remains infectious when filtered through porcelain, through

³ I remind you for instance of the presence of *Indigo white* in the labellum of *Cattleya*.

⁴ On the relation of the obligatory anaerobes to free oxygen. Proceedings Royal Academy of Sciences. Amsterdam, May 28, 1898.

⁵ Diseased plants were sent to me from various sources, for which I here wish to express my thanks.

which process all aerobes are held back. However, I was not only concerned with the search for aerobes alone, but I also carried out careful experiments to determine the presence of anaerobes in the filtered juice, but with negative results, so that the sap used appeared entirely sterile.

The quantity of candle filtrate necessary for infection is extremely small. A small drop put into the right place in the plant with a Pravaz syringe can infect numerous leaves and branches. If these diseased parts are extracted, an infinite number of healthy plants may be inoculated and infected from this sap, from which we draw the conclusion that the contagium, although fluid, reproduces itself in the living plant.

Since, however, experiments using the candle filtrate are still open to criticism, especially when the possibility of the presence of anaerobes is not excluded, and, because of this, the corpuscular nature of the contagium has not been entirely disproved, I have carried out the following diffusion experiments, which, it seems to me, have produced entirely incontestable results from both points of view.

Drops of the extracted juice of diseased leaves, as well as ground-up diseased leaves, were put on the surface of thick (poured) agar plates, and left to diffuse with water for several days. I hoped in this way to separate the virus from the raw leaf substance, as well as from all bacteria, through diffusion, since the virus, if at all capable of diffusion, could penetrate into the agar downwards and sideways, thereby leaving as a residue all discrete parts, aerobic and anerobic bacteria and their spores. The experiment was, therefore, decisive in determining whether the contagium was actually capable of diffusion and, accordingly, had to be considered as soluble in water, or if not capable of diffusion, therefore, as extremely minutely distributed, yet as corpuscular, that is, as *contagium fixum*. It appeared that the substance causing the infection may penetrate into the agar plate to no small depth, as may be seen from the following circumstances.

When I thought a sufficient time had elapsed for the virus to have penetrated the agar plate to a considerable depth, if diffusion takes place at all, the plate was first cleansed with water, then washed off with a sublimate solution, and finally a layer of agar about half a millimeter thick was removed by means of a sharp platinum spatula from the outer

surface of the spot, where the raw material of the leaves or the extracted juice had lain. The mass lying immediately below was then removed in two successive layers and both parts used for the infection of healthy plants. The results left no room for doubt, in both cases the characteristic symptoms of infection were brought about, very intensively by the upper and more weakly by the lower layer of agar.⁶ After ten days the distance covered by the virus may have been at least two millimeters, perhaps considerably more. Although a diffusion distance of only a few millimeters was involved here, it seems nevertheless proved that the virus must really be regarded as liquid or soluble and not as corpuscular. This result might be of special interest in so far as it points to the fact that a similar forward movement of specific vital bodies inside of the plant meristem must be considered possible.⁷

The candle filtrate has a somewhat weaker effect on the plant than the extracted juice that has not yet been filtered. This may be seen from the following circumstance. Fresh extracted juice not only produced the peculiar spots of the leaves, which later become necrotic and are characteristic of the disease, but, if considerable quantities are used, it also causes an actual malformation of the leaves, which also remain small since the midrib does not reach its full size, and are less deeply lobate through disturbances in the growth of the edges and often show palmate-veining through which they become quite different from the normal tobacco leaves. Such malformations also may be produced by means of candle filtrates, if one wishes to do so, only much more material must be used for this. From this we must conclude that the virus is held back in the filter pores, at least at the beginning of the filtration process. How incorrect it would be to conclude from this that the virus is of corpuscular nature may be shown by the following experiment.

As is well known, a malt diastase consists mainly of a mixture of two enzymes, granulase and maltase, which may

⁶ Egg albumin and cooked potato starch also penetrate slowly into agar plates, which may be followed in the latter through the iodine reaction. Drops of soluble starch laid on gelatin plates diffuse much more easily than ordinary starch and also sideways to a relatively long distance.

⁷ I came to an identical conclusion earlier concerning the cecidiogene bodies causing gall formation: these bodies, too, must be soluble in water and capable of diffusion inside of the meristematic tissue.

be separated by diffusion.⁸ If, for example, a drop of malt extract is placed on a gelatin plate containing starch, the maltase soon precedes the granulase in diffusion. Maltase produces erythro-dextrin and maltase from starch, while granulase produces only dextrin, aside from maltase, from starch as well as from erythro-dextrin, which are not colored by iodine, so the action of iodine on the diffusion field of the diastase mixture will show the relative amount of maltase as compared with granulase through a red ring of erythro-dextrin on a blue background, which ring surrounds the colorless field of granulase. If the same malt-extract is passed through a porcelain candle, a considerable widening of the maltase rings is found in carrying out the diffusion experiment with the first parts of the filtrate, from which may be concluded that the filter pores more easily retain granulase, which diffuses slowly, than maltase, which diffuses more quickly. Later, when the wall of the filter is saturated with granulase, the original width of the maltase ring returns.

Therefore it was to be expected that a substance like the virus, which does not diffuse easily would flow through in a diluted form at the beginning of the filtration process yet without being composed of corpuscular parts because of this behavior.⁹

Although I had known for a long time that bacteria were not directly concerned with infection, I performed many inoculations on my experimental plants with the forms that happened to occur on the diseased tobacco leaves, as well as with those that developed in the extracted sap of diseased leaves, in order to make this fact absolutely sure. I always had negative results when the experiments were carried out correctly; never did a bacterial culture, free from the virus, produce symptoms of infection. In section 9, however, I shall show that, under these circumstances, it is not easy to entirely separate from the virus the bacteria isolated from the sap of diseased leaves, for these same bacteria, even after

⁸ The third enzyme of malt extract, glucase, is to be found only in small quantity in it.

⁹ I, therefore, cannot agree with the conclusion of Mr. Loeffler as regards the corpuscular nature of the virus of the foot and mouth disease (Centralblatt. für Bacteriologie. Part I. Vol. 24, p. 570, 1898). It would be interesting to know if the watery solutions of gold and platinum, produced by Mr. Bredig by means of the electrical luminous arc between metal electrodes in water, would pass the pores of the Bougie and could diffuse into gelatin or agar jelly.

Movimento
Na planta

Não é
bacteria

reinoculations, can contain enough virus to result in most marked chlorosis.

A proper experiment for the purpose of determining the fact that any microbe isolated from a diseased plant is not capable of causing the disease, therefore presupposes a carefully carried out colony culture, consisting of the isolated single germs that have been rinsed with much water and, under certain circumstances, even after repeated reinoculations, which are continued until the last traces of the virus, which has been absorbed or is clinging to the bacteria, have disappeared.

I believe that these remarks are not without importance. For I see in them an analogy to the experiences of the pathologists, according to which the organisms causing certain infectious diseases lose their virulence through culture outside of the organism and can increase it by repeated passage through susceptible animals. Although the analogy is not a very close one, it is certain that there is an analogy.

2. Only those organs of the plant that are growing and whose cells are dividing are capable of being infected; here only does the virus reproduce itself.

Only those tissues and organs of the tobacco plant are attacked by the virus that are not only in a state of active growth but in which the division of cells is still in full progress; all tissue that has reached its full growth is immune from it, but may under certain circumstances transport the virus. Leaves that are growing, but are beyond the expansion stage, can no longer be infected, although they are still suitable for the transmission of the virus to the stem.

If the stem is inoculated, only the young leaf-buds and the leaves that are newly developing from these growing points are infected. If young leaves are inoculated, the same thing takes place; the virus returns to the stem from the leaf and infects either the axillary buds or rises to infect the terminal bud. If fully matured organs are used for infection, be it stems or leaves, and very little virus is used, failure is certain;—obviously the virus then remains in the matured cells without having any effect. A larger quantity of the virus may, however, move out of the matured parts into the surrounding new tissues and affect them.

In any case, it is reasonably certain that the virus in the

plant is capable of reproduction and infection only when it occurs in cell tissues that are dividing, while not only the matured, but also the expanded tissues are unsuitable for this. Without being able to grow independently, it is drawn into the growth of the dividing cells and here increased to a great degree without losing in any way its own individuality in the process. In conformity with this, no ability of reproduction outside of the plant could be proved. It is true that a Bougie extract that was filtered clear and was entirely free from bacteria could be kept for over three months¹⁰ without losing its virulence or even seeming to decrease it. But an increase of infectivity was not to be observed, not even in the first period of the experiment, even though the extract had been prepared in such a way that not only diseased parts but also healthy buds and leaves were extracted, so that if nourishment in the usual sense had been able to bring about reproduction, this should have taken place. Also, in transferring the virus to appropriate gelatin-media, the color and index of refraction of the latter apparently remains unchanged throughout.

Yet, judging the original quantity of the virus, which is reproducing independently in the plant used for infection, is after all, difficult, and, since the question naturally is of special importance, new experiments in this direction are to be expected. For the time being I must, as I say, take for granted that propagation results only when the virus is connected with the living and growing protoplasm of the host-plant.

The behavior of the virus in connection with the growing tissues reminds one of similar relationships in gall formation, for the cecidiogenen substance also can affect only growing parts. As far as the movement is concerned, the latter bodies behave differently from the virus; they must, in order to be effective, be brought into meristematic tissues and they move only through these.

The method of reproduction of the virus reminds one in certain ways of that of the amyloplasts and chromoplasts, which also grow only with the growing cell protoplasm, but can also exist and function independently.

¹⁰ How long the extract may be kept I am not prepared to say as yet; in any case, longer than three months.

Virus is reproduced on second form

3. *The flow of the virus inside the plant. Various ways of infecting: Local and general infection.*

In artificial infection the virus may move with the flow of water through the xylem bundles. But I do not believe that the xylem is the normal path of the flow in a closed plant. For if young leaves are infected at the time they are in the seedling, or later, the mature stage, these leaves themselves remain entirely normal, as we have seen, but the virus from here returns to the stem in order to infect new tissues that are higher up. Now it seems to me, that such a movement can occur only when the so-called descending flow of sap is followed; but this is directed through the phloem. The existence of a flow of sap, which under normal conditions might be in a direction from the leaves toward the stem along the xylem or the parenchyma, is, however, improbable and, during strong transpiration, impossible, at least in the xylem.

If the virus really moves in the phloem bundles the direction of the movement should be both ascending and descending, and it should be subject to the laws followed by the usual food substances in the nutrition of new tissues or the depositing of reserve material. The flow necessary for this must, however, according to the circumstances, be directed either to the base or to the top of the organs. The symptoms of infection are in good agreement with this.

The slow flow of the virus along the phloem bundles is, in my opinion, shown in a strange manner in the order of the diseased leaves in one-sided local infection of the stem. Often (perhaps always) the leaf that first becomes diseased is situated directly above the wound left by the infecting needle. If the place of infection was closely circumscribed, for example to a single shallow puncture of the needle with the Pravaz syringe, the second diseased leaf, in a $\frac{3}{8}$ leaf position, may be exactly the ninth above the first one to become diseased. Then, or shortly before, an apparently fan-like spreading of the virus occurs through which at first the surrounding leaf series and finally everything around the stem appears to be infected. It is strange that the buds take up the virus less easily than its new tissues do, or perhaps, to speak more exactly, it is again able to rid itself of the virus, for it is a fact that under certain circumstances healthy organs may be again produced from it later.

I conclude that the virus may also move in the xylem and from there is able to infect the meristematic leaf-buds from the fact that many of my experimental plants produced leaves that were extremely malformed at their tips, even from the moment that they were first visible in the bud, while the general symptoms of the disease were then not yet noticeable on the lower part of the same leaflets. It seems as though in such cases a large quantity of virus is poured into the tip of the new tissues at once, which is probably only possible through the transpiration stream along the xylem bundles. Such leaves, however, later show the normal symptoms of the disease, also, in such a manner that they point in the usual way to the flow of the plastic nutritional substances as the carrier of the virus.

It was proved that the transportation of the virus for great distances through healthy and mature stems and roots areas is possible by attempts at infection with soil taken from the roots of potted tobacco plants. In order to approximate natural conditions, the experiments were arranged in a very simple manner, described in sections 6 and 7. It is sufficient to mention here that plants that were already two or more decimeters high and whose lower leaves had long since died off could be easily infected from the root by means of soil in which the dry virus was present and had therefore become infested. Just as in wound infection, all mature parts and even the leaves still in the process of expansion remain healthy while only the leaves newly formed from the terminal and axillary buds become diseased.

Under these circumstances, the movement takes place slowly and the symptoms may be expected only after a time of at least three weeks to a month. This period of time, however, depends on the phase of development of the plant, so that seedlings that are injured in the root show up the symptoms of the disease sooner after infection. My experiments with uninjured seedlings are not finished, so that I am yet unable to give the point of entrance of the virus into completely normal plants. For the tobacco grower, this question seems not to be an insignificant one, since the tobacco plants are transplanted after sowing whereby many open wounds on unavoidably severed fibrous roots at least make possible the entrance of the virus.

If one wishes to convince oneself in the shortest possible

time of the virulence of the contagium it is best to deeply wound with a knife the youngest part of the stem below the terminal bud, which still may be easily treated without injury, and to place into the wound a piece of fresh, diseased tissue. The newly formed leaves will then plainly show the first traces of the disease after ten to twelve days; after three weeks the disease symptom is clearly distinguishable, even to the layman.¹¹

The difference between the plants infected from the soil and those infected through wounds in the stem is notable. While the former show a general infection from the moment that the disease makes its appearance, which means that the diseased leaves are all around the stem, in the latter the infection, as has been discussed before, is at first confined to a stem segment as a local infection, which later develops into a general infection.

4. *The virus may be dried without loss in strength of infection.*

Pieces of dried diseased leaves put into healthy plants showed themselves capable of inducing infection even after they had been kept in my herbarium for two years. The same was true of the pieces of filter paper that had been moistened with the expressed sap of diseased leaves and carefully dried at 40°C. Extremely small pieces of leaves may be used for infection, which shows infinitely small quantities of the contagium are sufficient for infection. The virulence of the dried materials is, however, always less than that of the fresh materials, and for the present, I shall ascribe this difference to the partial destruction of the virus in drying and not to a change into a modified, weaker form, for, with a small amount of fresh virus, I obtained symptoms apparently identical to those resulting from use of a much greater amount of the dried virus. It, therefore, still seems doubtful whether the word virulence is really applicable here. I placed the dried virus into parts of the stem, as well as into the central veins of young leaves, and obtained the usual results.

I wish to mention at this time that the alcohol precipitate of virulent extracted sap retains its virulence after drying

¹¹ Recently, I was able to reduce to three days the time interval necessary to appearance of the disease by treating parts of buds that were yet much younger than those used before.

at 40° C. However, strong alcohol is also not injurious to many bacterial spores.

5. *The virus may winter in its dry state outside of the plant in the soil.*

In the fall of 1897 I let a diseased plant in a large flower pot in the shed die through lack of water. The plant was pulled up, the soil shaken from the root system into the pot, and the latter was kept in a dry place. In the following spring I divided the soil between four pots, partly filled with fresh soil. One pot was larger and was set with three plants; the three smaller pots received a plant apiece, all of which were bearing several leaves of which the lower ones had already dried off. All these plants were without a doubt entirely healthy. After about four weeks the conditions were as follows: Of the three plants in the large pot, one had become diseased; the two others were healthy and remained so until the end of the experiments. The plants in the three small pots all became diseased. One of them developed poorly from the very beginning, became strongly diseased, and soon showed the peculiar malformed leaves so characteristic of the more pronounced, artificial-wound infection. The plant also produced several chlorotic leaves. Since the other typical symptoms of the disease were also very pronounced in this plant, it is certain that the virus can retain its full virulence after wintering in air-dried soil. The other plants showed the normal development of the disease. Since I had subsequently stirred the soil of some of these plants in the pots with a piece of wood, I presume that the malformed plant had received large root injuries in the process, through which many entrances may have been opened to the virus.

6. *Other attempts at infection through the roots.*

On the 6th of June, 1898, a series of healthy plants, growing in pots and several decimeters high, were infected in the following manner. A severely diseased plant was taken out of the ground with a lump of soil, and the soil shaken out of the root system and strewn in small quantities on the soil close to the main stem of potted plants. The plants were then sprinkled with tap water and the soil partly dug under, avoiding injury to the roots. After almost four weeks all the

infectious
em. fructo
1000 spores
2 años

Phenomenon
por fenomeno
na raíz

experimental plants showed general infection in the newly formed leaves. Then, to my astonishment, came a period of recovery, so that at the end of August I considered the plants to be healthy. Later, however, their leaves again became diseased, although not in very virulent form.

I feel that I must conclude from these experiments that normal roots are capable of taking up the virus from the soil through their closed outer epidermis. I admit, however, that this conclusion may not be reliable, for animals living underground may have made possible or facilitated the entrance of the virus by means of root injuries. Only experiments with plants grown in nutrient solutions would, in my opinion, yield absolute certainty on this question.

7. *The virus becomes ineffective in boiling temperatures. The effect of Formalin.*

For a long time I considered it possible that some anaerobe might have something to do with the infection. Especially when I learned of the characteristics of a group of these organisms occurring in manure and faeces, to which I will give the name of "skatolbacteria," did I think I had reason for this conception. The "microaerophilie" is of such a nature in these forms that one is forced to infer a relatively large consumption of oxygen. More than that, some varieties have such extremely small, either spherical or oblong spores that the possibility of their passing through the Bougie pores is not entirely unthinkable. For this reason, I carried out several experiments with freshly extracted heated juices, and juices after filtration through the candles.

These experiments produced a result that was definitely negative so far as concerned the presence of bacterial spores. Boiling completely destroys the virus. It cannot even stand 90° C.; the shortest period of heating is sufficient to destroy the virus. I have not as yet ascertained the minimum temperature, but do not doubt that it is only a matter of a pasteurization temperature. Through these experiments the phantom of the anaerobes and their spores was forever banished.

This is perhaps the place to say a word about the sterilization of the utensils used in the experiments, especially of the Pravaz syringe. The modification by Koch, it is true, is better suited for sterilization, but its rubber ball does not

allow the injection of the fluid with as great a pressure as does the original form. I therefore tried to sterilize the latter with Formalin, since it does not stand the heat very well. The success of this, however, is only partial and only with greater quantities of Formalin. Weak solutions of Formalin mixed with the virus do not destroy it, but they do so lengthen the period of incubation that the disease cannot become apparent until six or more weeks after infection. In any case, one must be sure that the last traces of Formalin have completely evaporated from the syringe before using it again, for it has become apparent that Formalin is very poisonous for the tissues of the tobacco plant, much more so than to the virus itself. In section 9 I shall come back to this matter. Here I wish to emphasize that Formalin, once it has entered the vessels, is carried with noticeable rapidity through the leaf veins, whose living cells soon die off. Through careful injection of Formalin into the midribs, in such a way that the xylem bundles are not mechanically injured, it is possible to destroy all living tissues, without disturbing the flow of water in the least, so that the leaf remains fresh and can continue growing.

8. *Different symptoms of the disease. Development of malformed leaf tissues through large quantities of virus.*

In my opinion the spot disease (mosaic) of the tobacco leaves in its milder form is a disease of the chlorophyll particles and, in its more intensive forms, a general disease of the living protoplasm.¹² The course of the milder form is as follows: With artificial infection of the virus in stem wounds below the terminal bud, those leaves that unfold within ten days remain healthy. The leaves that develop later present a yellow-spotted appearance, which in itself is not particularly characteristic and often occurs in healthy leaves. After two to three weeks a characteristic symptom of the disease appears (Table II, Fig. 1). In the neighborhood of the lateral veins of the 2nd or 3d division, the color becomes very dark-green in places, namely in rectangular areas that are cut in half by the veins; in the rest of the leaf the coloring process is somewhat slower than under normal conditions and sometimes even recedes to complete albi-

¹² For the time being I must skip the anatomical relationships because they have not as yet become entirely clear to me.

*Pravaz
syringe
virus*

bolton

nism. In any case, dark-green spots develop on a light-green background. The border between the two colors is either sharply defined and sudden or it is diffused as if diluted. Since the darker parts grow more rapidly than the lighter ones, they soon stand out over the even surface of the leaf more or less convexly, whereby, in the acute cases, pronounced bullate blisters develop on the upper surface of the leaf. Later (Plate II, fig. 2) one can observe a necrosis of the leaf cells developing at the edge or even in the middle of the dark spots, which soon leads to the small, tan, dead, and dried spots feared so much by tobacco growers because the leaf thereby becomes unsuitable for use as a wrapper for cigars. Although most of the dead tissue spots develop in the manner described near or in the dark-green fields near the veins, the origin of some of them remains uncertain; apparently, they also may develop in the yellow spots. The symptoms in the tobacco fields are usually not of as great an intensity as in artificial infection, especially the blistering outgrowth of the dark-green parts on the leaf blade is entirely lacking. In contrast to this, the necrosis and drying of leaf spots were not observed in some of the greenhouse plants.

With artificial injection of fresh extracted sap, or with inoculation with diseased tissue the disease may reach a higher stage of intensity than I have as yet observed under natural conditions.¹³ I mean the abnormal tissues of the newly formed leaves (Plate I b, c, d, Plate II, fig. 4 and 5). This is no doubt connected with the quantity of infectious material used for the experiment. Therefore, it is much easier to produce leaf monstrosities with fresh extracted juice than with the Bougie filtrate, since, as has been remarked earlier, more of the latter must be injected in order to obtain the same effect, which certainly is remarkable for a contagium that increases through growth.

The first noticeable symptoms of the striking appearance of leaves becoming malformed is the retardation of the growth in the direction of the midrib and of the principal lateral veins. Oval or circular leaf surfaces develop because of this. Later the intensively green spots are to be observed, which rise blister-like and are peculiarly contrasted to the

¹³ Probably because severely diseased plants are soon noticed and taken out.

rest of the leaf-surface, which remains a much lighter color and has a tendency towards albinism, particularly at the veins. Once, I got a small beautifully formed "ascidium" instead of an abnormality of the kind described. Such entirely unrecognizable leaves always remain much smaller than the leaves that develop later and are, by the way, healthy and fresh, as, on the whole, the symptoms of the disease never become particularly detrimental to the plant. Even specimens that have been severely attacked produce stalks of normal height and thickness and, finally, at the end of the vegetation period often entirely healthy leaves, bloom and fructify normally, and, as far as is known, their seeds are always healthy. I do not yet know whether it will be possible to artificially infect the blossoms and seeds, because I began the experiments in connection with this too late.

The symptoms of the disease in plants growing in the open are so variable in intensity that one involuntarily thinks of individual predisposition. If this impression is correct, and if it is a matter of something other than the uneven quantity of the virus originally introduced, it probably will be easy to produce an immune race in which, of course, artificial infection would have to be used as a criterion. The facility of such infection experiments seems to place success within reach.

9. *Albinism or "bunt" as an incidental result of artificial infection.*

In many of my experimental plants, spots appeared on the leaves in which chlorophyll was entirely lacking. In a few cases the spots were spread over the surface by the hundreds and in such elegant order that really decorative colored plant-leaves resulted (Plate II, fig. 3). Up to now I do not have the thing in hand; it is questionable whether it will be possible to make any experiment out of it with constant results. I wish to mention a few cases here, where it appears that there is a causal connection.

ALBINISM IN A MIXTURE INFECTION OF A BACTERIUM WITH THE VIRUS

Extract of diseased leaves after having stood for a day at room temperature was plated out on a culture medium for

the isolation of the bacteria that had developed in it. The following mixture was used: Decoction of 20 grams of clover leaves in 100 grams of water and 2 grams of cane sugar solidified after filtration and boiling with 10 per cent of gelatin.

Mainly two types of bacteria developed of which the one, a non or weakly-liquefying and non-fermenting bacterium, which I have called *B. anglomerans*,¹⁴ and which occurs very commonly on plants in general, occurred in millions per cc. In the first plating, the extracted juice was just poured over the gelatine plate, so that it had to be taken for granted that each bacterial colony was infected with the virus. Without further isolation, parts of these colonies were transferred into test tubes on the culture medium mentioned above, so that a trace of the virus might, however, have been transmitted, even if it had not reproduced itself in the bacterial colonies. Since the bacteria grow quickly, much material was soon formed with which a suspension was prepared in tap water and a plant was amply infected on the 30th of September. At first I thought that the plant would remain entirely healthy; but, on the 15th of October, I noticed a beginning of the disease, which, however, did not continue to develop in the regular manner but produced a beautiful albino plant.

The second variety of bacteria treated exactly as the first remained without effect in the infection experiment.

Since I have saved the bacterial cultures, I shall be able to repeat the experiment in the future. At present I am mainly interested in the question of the virus occurring only as a contamination in the colonies or in its increase either between the bacteria or in the bacteria proper. In the latter case a variation in the characteristics of the virus is not impossible.

ALBINISM THROUGH INFECTION WITH VIRUS COMBINED WITH FORMALIN

The observation to be discussed here was an entirely incidental one. When I cleaned my Pravaz syringe with Formalin before using it, a trace of Formalin remained in the hollow needle in one case and entered the experimental

¹⁴ Bot. Zeit. 1888 p. 749.

plant along with the virus, which I immediately noticed because of the necrosis of cells adjacent to the wound. Later the plant showed the injury only indistinctly, but, afterwards, several leaves became mottled.¹⁵

ALBINISM THROUGH INFECTION FROM THE SOIL

Some of the plants, which had become diseased very late in the season in the greenhouse through infection from the pot-infested soil are to be described more as variegated than as spot-diseased. The dark-green spots near the veins had become barely visible, while the discoloration in the rest of the leaf parenchyma had appeared particularly early and intensively. However, only part of the areas of the colored leaves became white, the majority of them remained yellowish. In one of these plants the lower leaves remained very small and became malformed in the manner described before.

Of the three cases of bunt (mosaic) that I have mentioned in this section the first two may agree in that the virus entered the plant in a greatly diluted state; but I do not believe that dilution is here an essential factor, because the third case gave the impression that especially much virus had been active. However, I consider it, if not proved, at least highly probable that there is some connection between the virus of the spot disease and the bunt (mosaic), and the old question whether the bunt (mosaic) is always of the same origin has come back into discussion with these experiments.

10. Other infectious plant diseases caused by a contagium fluidum and not by parasites.

Even if the symptoms of the spot disease coincide so closely with certain forms of albinism or bunt (mosaic) that both may unhesitatingly be put under the category of infectious diseases of the chlorophyll bodies, there yet remains, according to known observations, a difference in the principle of the mode of transmission of the contagium, a difference that leads one to consider both of them as separate kinds of diseases, each with its particular virus. The form of albinism (variegation) suitable for transmission is

¹⁵ I repeated this experiment, but found only the usual symptoms of the disease, although very late. If the virus remains long in contact with even strongly diluted Formalin, it is completely destroyed.

Distrip
gave, why
albinism
or bunt

↑
virus #3

namely transferred only when the living albino tissues grow together with the living-tissues of the green plant by means of grafting or budding, while simple inoculation of green plants with the tissues or the extracted juice of variegated varieties of the same kind, remains entirely without results¹⁶ according to my several-times-repeated experiments with *Ulmus campestris*, *Acer negundo*, *Pelargonium zonale*, and *Urtica dioica*. It appears, therefore, that the contagium of variegation is transmissible but that it stands in a much closer relationship to the protoplasm of the plant than the contagium of the spot disease, in that it cannot exist, like the latter, outside of the plant and dies when the plant cells that carry or continue it themselves die. My preceding observations, however, sufficiently show that the last word has not yet been spoken on this subject. Since the question of the contagiousness of variegation is important, for the evolution theory as well as for the theory of variability, further experiments on the subject would be very desirable.

Another disease, which surely belongs here, is that known in America as "peach yellows."¹⁷ The symptoms of this disease consist mainly in immaturity of the fruits, growth of the latent buds at unusual places into thin brooms, which are often colorless, and yellow discoloration of the leaves, which is followed in a few years by the death of the tree. According to Mr. Smith, bacteria and parasites are definitely not the cause. Nevertheless, it was easy to transmit the disease to healthy trees simply through grafting or budding with a bud of a diseased tree. This experiment showed that it is necessary for the bud to unite if the disease was to be transmitted, for the virus is not capable of infecting healthy trees without the connection of the living tissues, according

¹⁶ It is true that some researchers have doubts as to the transmissibility of variegation as such and have expressed the opinion that those green plants that become variegated themselves through grafting with variegated ones would have become so without any grafting whatsoever, i.e., through spontaneous bud variation. They remark that the stocks used (*Abutilon*, *Jasminum*, *Pelargonium*) are garden plants whose green specimens have a strong tendency towards variegation anyway. Such objections, however, are not sufficiently grounded (see Lindemuth, *Vegetative Bastardenerzeugung durch Impfung*, *Landwirtschaftliche Jahrbücher* 1878, no. 6 and Vochting, *Transplantation*, p. 13, 22, 92, and 112, Tübingen 1892.)

¹⁷ Erwin F. Smith, *Peach Yellows and Peach Rosette*, U. S. Department of Agriculture. *Farmers' Bulletin* No. 17, Washington, 1894. I know this short but interesting discussion only through the separate which the author kindly sent. Much to my astonishment, I was not able to find a word about it in the scientific literature available to me.

to Mr. Smith. He neglects to point to the agreement of this observation with the mode of transmission of variegation in *Abutilon* and *Jasminum*.

"Peach rosette" is, according to Mr. Smith, another non-parasitic disease, closely related to "peach yellows," which is easily transmissible through budding and root grafting. The disease manifests itself in that all buds, dormant as well as active ones, grow into small rosettes that consist of single large leaves and several hundred small leaves. The color of the leaves is yellow. The fruit does not ripen but dries and falls to the ground prematurely. Here, too, we find the peculiarity I have described in the spot disease, that the virus moves laterally with difficulty but upwards with ease, so that a tree may become diseased on the side on which the rosette bud was grafted, while the opposite side remains healthy for years.¹⁸

Smith says that the epidemic character of yellows, as well as rosette, leads one to the conclusion that there must exist another mode of transmission than that of tissue inter-growth, but he does not believe that the virus can come from the soil; however, he notes that, particularly in rosette, a whole tree may become diseased in almost all its parts at the same time, which, as we saw earlier, is not compatible with local infection but rather points to a general infection similar to the spot disease when the tobacco plant is infected from the soil.

Since Smith did not carry out any experiments with artificially transferred sap, the possibility remains, even the probability, that these, too, could give a positive result. If this should really be the case, the virus would probably also be capable of existence outside of the plant, and infection from the soil through the roots would be possible, and yellows and rosette would then approximate much more closely the spot disease than is apparent from the descriptions given.

I consider it highly probable that many other non-parasitic diseases of unknown cause may be ascribed to a *contagium fluidum*. It seems useful to me in further research on this matter to distinguish sharply between the two forms

¹⁸ The latter observation seems to exclude entirely the possibility that in peach rosette we are concerned with a "phytoptus" invasion, although the other symptoms of the disease seem to point to it.

7
in which, according to the available knowledge, such a *contagium* may appear, namely, firstly as an independent *contagium*, which is capable of existence outside of the plant, even if only for a time, as in the leaf-spot disease of the tobacco plant, and secondly as a *contagium* that exists only in living tissues as in the form of variegation, which is transmissible through graftage only.

EXPLANATION OF THE ILLUSTRATIONS

Plate I

A young tobacco plant that has become diseased through artificial infection with much virus. The virus was introduced through a wound at *a*, which penetrated through the whole stem. The diseased leaves, *b*, *c*, *d*, which first developed, are malformed; those following, *e*, *f*, are diseased, though not malformed.

Plate II

Fig. I. A young tobacco leaf, in the first stage of the disease, with a moderate amount of virus. The dark-green spots are visible next to the vein, the local changes, by the way, in the color of the chlorophyll did not produce any distinct contrasts on the photographic plate.

Fig. 2. A mildly diseased tobacco leaf in the second stage of the disease with a few brown spots that were produced through the premature necrosis of the tissues. The most important stage of the disease in which the dead, brown spots are increased by hundreds or thousands is not pictured.

Fig. 3. A vari-colored tobacco leaf of a plant that had become vari-colored through the mixed infection of the virus with *Bacillus anglomerans*.

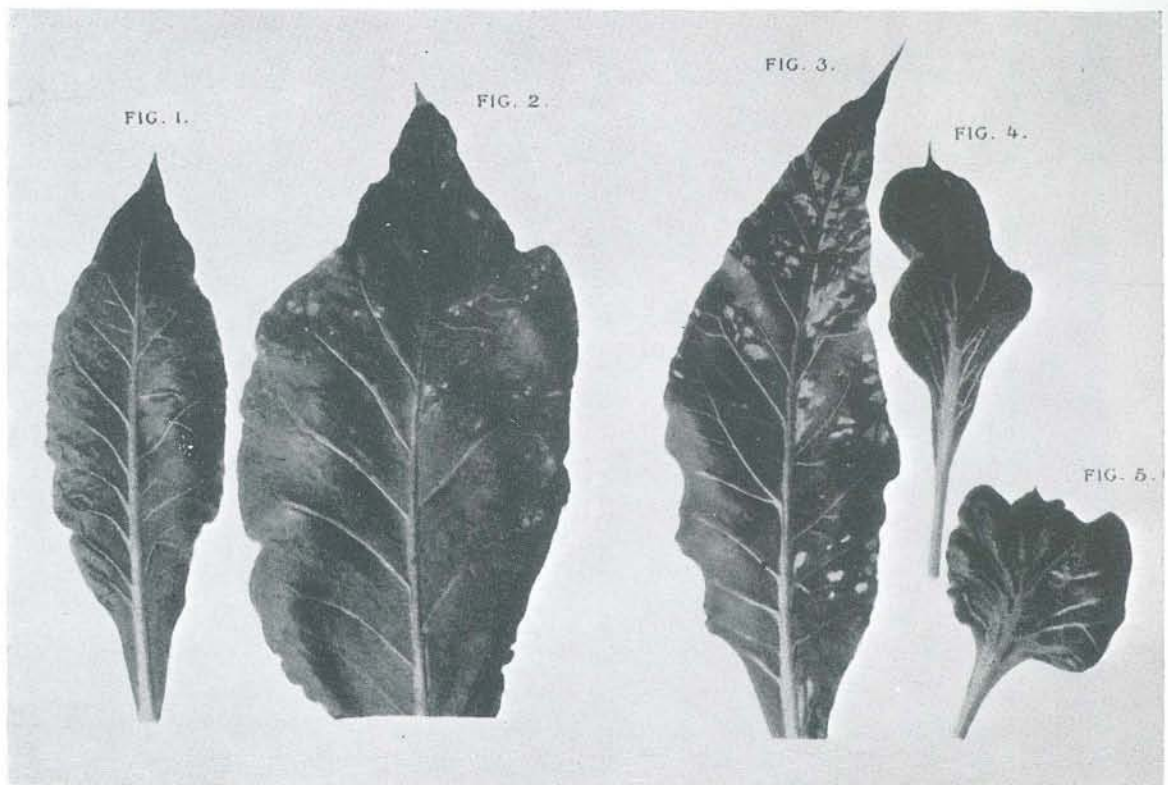
Fig. 4 and 5. Small malformed tobacco leaves, produced by the introduction of large amounts of virus into the stem.

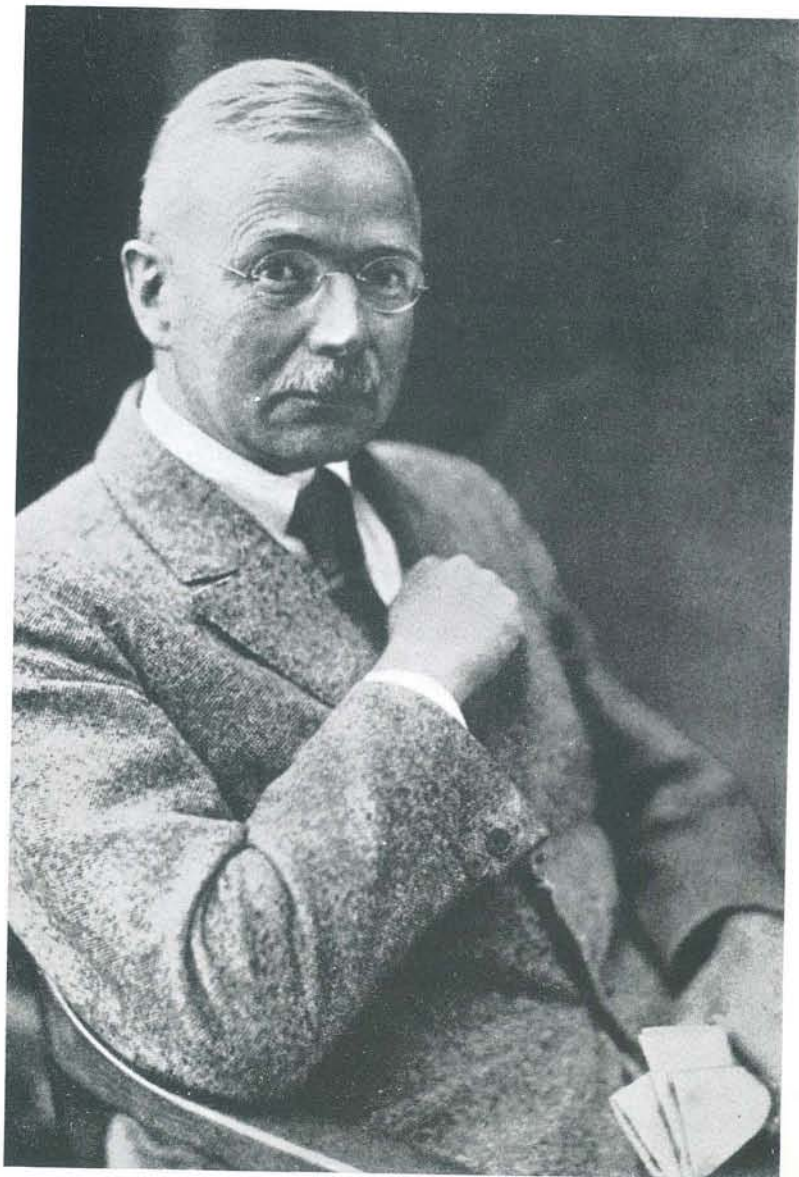
[These plates, in color in the original, are here presented in black and white because of the high cost for reproduction in color.]

Plate I



Plate II





ERWIN BAUR

ERWIN BAUR

1876-1933

Dr. Erwin Baur published several papers on infectious variegation or chlorosis in plants between the years 1904 and 1909. The first of these, published in 1904, entitled "Zur Aetiologie der infektiösen Panachierung" was evidently Baur's first botanical paper. It is perhaps a credit to his versatility that his interesting work on infectious variegation is not even mentioned by some of his biographers. After his early work on variegation Baur became one of Germany's first and best known geneticists, and a strong promoter and administrator in the development of studies on heredity in his country. In plant genetics he is best known for his work on the snapdragon, *Antirrhinum majus*. His work in applied genetics as related not only to plants, but to animals and man, also represents important pioneer efforts in these subjects.

Erwin Baur was born on April 16, 1875, at Ichenheim in Baden, South Germany. Following his elementary schooling he studied medicine at the Universities of Heidelberg, Freiburg, Strassburg, and Kiel, receiving his Doctor's degree in Medicine in 1900. His medical career lasted but a short time, for, in 1903, he returned to study botany at Freiburg, where he obtained his degree of Doctor of Philosophy under Dr. Oltmanns in the same year, and became an assistant in the Botanical Institute at the University of Berlin, where he also became full professor in 1910. It was during this period that he did his work on infectious variegation as contrasted to non-infectious variegation, which later led him into the field of heredity. He founded a professorship in genetics at the Botanical Institute, the first and perhaps still the only institute in Germany teaching pure and applied genetics. Baur was a magnetic and impressive teacher, and attracted many students into his field. He also founded and in 1929 became director of the Kaiser Wilhelm

Institute for Genetic Research at Berlin-Dahlem, one of the largest and most productive research centers of its kind.

Professor Baur possessed a strong personality and faith in himself and in his own judgment, a characteristic that is well illustrated in his early papers on infectious chlorosis. He received high honors from many scientific societies in other countries and became, as well, a prominent figure in social and political circles in Berlin. Nevertheless, one of his biographers, Hans Stubbe, writes, "He needed but very little for himself. With the simple mind of a farmer, living close to nature we find combined in him the great intellectual powers of his countrymen which made him a skillful diplomat and a powerful opponent."

Professor Baur died Dec. 3, 1933, at the early age of 58 years, when at the height of his career and of the many great labors he had undertaken to perform.

ON THE ETIOLOGY OF INFECTIOUS VARIEGATION¹

Erwin Baur

Presented on Oct. 11, 1904

THAT a certain type of leaf-variegation, panachure, as the horticulturists call it, can be transferred to healthy plants that have been green-leaved up to that time, is a phenomenon² known to gardeners almost 200 years. In the course of time a mass of literature pertinent to this subject has been collected. This literature, however, is not very satisfactory, mainly because, under the term panachure (albinism), most authors include two completely heterogeneous phenomena, which have only a very superficial resemblance to each other. There is, namely, a very frequent panachure (variegation) which is *not infectious*, but, on the contrary, more or less perpetuated by seed, and secondly a much rarer, though very similar, type of variegation frequently appearing, which is definitely infectious and is not perpetuated by the seed. The former type belongs to the realm of leaf variations or mutations,³ the latter is an infectious disease.

Only a few authors make this distinction, among them, e.g., Vöchting.⁴ Lindemuth also, who has perhaps undertaken most experiments in regard to the infectiousness of variegation, distinguishes the two types very carefully, according to verbal accounts. For the remainder, as mentioned, most authors confuse these two variegations.

As far as we are concerned only the latter infectious type of variegation interests us here, for which I shall in the following discussion use the term infectious chlorosis (*Chlorosis infectiosa*), thereby eliminating these constant confusions.

¹ Baur, Erwin. Zur Aetiologie der infectiösen Panachierung. Berichte der Deutschen Botanischen Gesellschaft. 22: 453-460. 1904.

² Historical references concerning this especially in: Meyen, *Plant Pathology*, Berlin, 1841. Lindemuth, *Vegetable Bastardization through Grafting*, Berlin, 1878.

³ These designations used in the De Vries sense.

⁴ On transplantations (graftings) on the Plant Body. Tübingen 1892, p. 92.

Variegation
gene form
vs
infectious

Such an infectious chlorosis is known especially in the Malvaceae family, although quite a similar one has been observed several times in Jasmine. Reliable, more detailed researches are extant only for the Malvaceae.⁵

The course of the infections has already been described so frequently that I need only recapitulate it briefly here. In order to infect a green-leaved Malvacea, normal up to the moment, e.g. *Abutilon sellowianum* Regel, it is best to proceed in the following manner: graft a twig—frequently only a leaf⁶ suffices—of a fleck-diseased (variegated) specimen of the same or even another species on a healthy plant. In this case, e.g., a twig of the variegated *Abutilon striatum* Dicks.⁷ Soon after a callus union has resulted between stock and scion there occurs in the newly-forming leaves of *Abutilon sellowianum* Reg. the symptoms of the disease, the intensely yellow flecks. Now one can again cut off the grafted, diseased twigs of *striatum*, the *Abutilon sellowianum* has become permanently variegated, all leaves growing out later are variegated. One circumstance I should especially like to emphasize here is that only very young, still embryonic leaves can be infected; all leaves that, at the time of inoculation, are already fully developed, or are already in the phase of expansion, do not become infected.

From the once-infected plants twigs can be cut off, in order to use them as cuttings, and thus many similarly infected offshoots can be secured at will. Likewise, other plants may be again infected by grafting of the twigs of these thus infected specimens, etc.

On the other hand, the infectious chlorosis is not transferred to the seedlings through seeds.

The various Malvaceae-species are varyingly susceptible to the infectious chlorosis. On this subject Lindemuth⁸ especially has undertaken a series of experiments, whose re-

⁵ Morren, Bulletins de l'Academie Royal de Belgique, 2^{me} ser., vol. XXVIII, 1869, No. 11. Lindemuth, l.c. Further publications by the same author in: Gartenflora, Vol. 50, 1901, Vol. 51, 1902, Vol. 53, 1904, p. 421.

⁶ Morren, l.c. p. 437.

⁷ The variegated specimens of some species have a horticultural value as favorite decorative plants because of their frequently very beautiful green and yellow marbled leaves, and are, accordingly, cultivated and propagated by nurserymen. Usually they pass for "varieties" under their own name, e.g., the diseased *Abutilon striatum* under the name *Abutilon thompsoni*, the variegated *Kitaibelia vitifolia* Willd. under the name *Kitaibelia lindemuthi*.

⁸ Lindemuth, l.c. For much further oral information I am greatly indebted to Inspector Lindemuth.

sults have actually been published only in part. Among them, the observation is especially interesting that there are certain species very highly susceptible—two, especially,—which are grown in the local university nursery under the names of "*Abutilon indicum*" and "*Sida abutilon*." In these cases some of the leaves are more or less extensively flecked; others do not become green at all, and remain small and wrinkled. The infected plants soon starve, because of the lack of CO₂ assimilation, which, after all, has been completely prevented. The other species, as is generally known, are not much injured by chlorosis, since, in their case, only parts of the leaves are, after all, prevented from carrying on assimilation.

Briefly, in anatomical terms, in infectious chlorosis it is in general a question of a change in the chlorophyll bodies. These are smaller than normal in the yellow leaf areas and they are more or less free from chlorophyll. Perhaps worthy of mention also is the statement of Woods⁹ that, in the yellow leaf areas, oxidases occur in much larger amount than in the green areas.

There is nothing to be seen in the infected plants of any kind of parasitic foreign organisms. Parasitic organisms as a cause of infectious chlorosis are also out of the question for quite different reasons, as I shall point out later.

However, before I discuss this proof, which is the purpose of this entire report, I should like to discuss one other point.

In the course of years the conclusion has been advanced for the great majority of infectious diseases of animals and plants that the causes of the diseases are parasitic microorganisms, and for those infectious diseases, for which up to now it has been impossible to find an organic causative agent of that kind, a similar etiology has been suspected. Though by far most pathologists who have considered this question from a theoretical point of view go a step farther.¹⁰ They say that only living organisms are at all conceivable as causative agents of infectious diseases. The assumption, according to them, that non-living matter could be the cause of any infectious disease is illogical and invalid. It is the following simple line of reasoning that seems to lead to that

⁹ Centralblatt für Bakteriologie- und Parasitenkunde. II. sec. Vol. V. 1899.

¹⁰ Cf. on this point, e.g. Joest, Unknown Infection Matter. Centralblatt für Bakteriologie I, Vol. 31, 1902.

Not transmissible per se

conclusion. In all infectious diseases, which, after all, is just their characteristic feature, a healthy individual can be infected with a minimal amount of infectious matter. From the thus infected individual can be derived many hundred-fold the amount of infectious matter used for the infection, with which other individuals can be infected, etc. *ad infinitum*. One person, sick with the pox-disease, can infect a whole population in a short time. From this it is said to follow with certainty that the virus of an infectious disease must multiply in the body of the diseased animal or diseased plant. Such a growth and increase, so the reasoning goes, can be attributed only to organisms, only to "living substances," according to our experiences up to the moment. A non-organized, let us say a pure chemical substance, which would be able to assimilate foreign substances, in order to rebuild itself from them, such a substance is yet unknown to us. Furthermore, even the existence of such a substance has not been made probable by any observations. Therefore, if we come in contact anywhere with a "something" that has this capacity of growth and multiplication, then we must conclude, on the basis of our present knowledge, that this "something" is a living organism. Infectious diseases, i.e., diseases in which the virus increases within the infected individuals, can, therefore, be produced only by living organisms.

This whole line of reasoning indeed seems to be logical; at any rate, this is the *dominant* theory today in pathology, of plants, animals and humans. Now, however, infectious chlorosis is quite a typical infectious disease. By the grafting of a single diseased leaf we can infect another plant, it in turn produces an unlimited number of infected leaves, and with each one of these leaves we can infect yet another plant, etc., *ad infinitum*, the virus of the infectious chlorosis must, therefore, without a doubt reproduce within the infected plant. In spite of this *the virus certainly cannot be a living organism*, for the following reasons: We have already shown that infectious chlorosis can be transferred by the grafting of infected twigs, or even leaves. Prerequisite for an infection is only the condition that there exist between the tissue of the grafted infected twig or leaf and the tissue of the plant to be infected a firm callus union and that it endure a certain short time. *A different mode of infection is not known.*

Although, for as long as 35 years in many florists' nurseries and in most botanical gardens, variegated Malvaceae have now been grown, together with healthy specimens in direct contact with them, and frequently together in the same plant beds or in one and the same flower pot, in spite of this, not one single case is known where a variegated plant has "spontaneously" induced infection in another. It can indeed be said with certainty that all variegated Malvaceae existing in gardens today derive their disease from the *Abutilon thompsoni*, introduced commercially in 1868, and, to be sure, all of them by means of infection by grafting.¹¹

During the course of the past summer I carried on further infection experiments in greater number, and I owe Mr. Engler a debt of gratitude for allowing me the use of an experimental bed in the Royal Botanical Garden at Dahlem. It was my purpose to determine actually whether an infection could not be produced in another way than by that of grafting. All experiments had the same completely negative result. I shall briefly describe the most important of the attempted methods of infection. By cutting and mashing infected variegated leaves I produced a pulp and then inflicted rather extensive wounds on healthy specimens of several strongly susceptible species of Malvaceae, and into the wounds I smeared the leaf-pulp. Furthermore, from such a leaf-pulp I expressed a juice and then injected this juice, filtered and unfiltered, into healthy twigs, which I cut off for this purpose, and, after injection, again either grafted onto the old mother-plant or cultivated as cuttings. In these injection experiments I naturally assured myself that a really considerable amount of the expressed juice penetrated the twigs. I cut off the tips of the twigs for that purpose and injected into the basal cut surface until the juice dripped out from the upper end of the twig, I thus treated a considerable number of these twigs with the expressed juice for a number of days, the juice to be sure being considerably diluted with water. Of these latter twigs, indeed, only a fraction, about 30%, remained alive.

Furthermore, I took twigs that had been dipped into vessels completely filled with the extracted juice of infected leaves and evacuated them up to 20 mm. Hg under the

¹¹ Cf. Morren, l.c. p. 436.

pneumatic air-pump receiver and then under normal atmospheric pressure allowed the juice to be forced into the intercellular spaces. Many of the twigs, about 50%, outlived this manipulation, took root as cuttings and continued to grow, *but all remained completely healthy, just as did the injected twigs.* In the case of some of the specimens under reduced air pressure, there appeared on the first newly-formed leaves a spotty discoloration bearing some similarity to variegation, which for a long time I considered as a symptom of infection; but the later developing leaves were always quite normal and green. I was then also able to determine that such indistinct spotty colorations occurred also occasionally on the ordinary cuttings of healthy plants that had not been injected nor evacuated and filled with expressed juice. I then kept unpotted healthy plants for hours and days with their roots in the unfiltered juice of infected leaves, all with the same negative result.

Following these experiments, though especially in consideration of the fact that, in the 35 years in which we have known the infectious chlorosis of Malvaceae, we have become acquainted with no single case of a different type of infection than by grafting, we can conclude, I think, that *prerequisite to an infection is the callus union of a healthy with a diseased plant.*

With that it is, in my opinion, also proved that the infectious substance unknown to us *cannot be a living organism.* I arrive at this conclusion by the following line of reasoning: If the virus of infectious chlorosis were an organism, then its ability to exist would have been dependent upon the occasional graftings carried out by gardeners. Such an organism would not have had any possibility of existing before 1868, for cases where two specimens of the Malvaceae under discussion standing close together would form a natural graft are too rare to be considered. All the plants attacked by infectious chlorosis reproduce in nature only by seed and not by runners or the like. How then would the hypothetical causative agent move from one plant to the other if the first one dies? A parasitic organism that has such a limited capacity to move from one host-plant to another is not at all capable of existence. Therefore, *the virus of the infectious chlorosis cannot be an organism.*

Against this conclusion only one objection can be raised,

namely that although *in our country* this variegation is transferable only artificially by grafting, but that after all it spreads spontaneously in the tropical native habitat of the plants attacked by it in other ways, perhaps through an intermediary host not existing here (analagous, e.g., to malaria or yellow fever). But this objection, too, has no basis. There is no information in regard to the assumption that in the countries (Central and South America), which are to be considered as the native habitat of the *Abutilon* species under discussion, infectious chlorosis occurs at all more frequently or spreads spontaneously; and this is important, for the variegated plants are, as has been said, favorite decorative plants, and much attention is paid to the occurrence of such leaves everywhere, not only through scientific considerations, but, also, what is more important, observed through practical and economic interests. Therefore, once more be it said, infectious chlorosis *cannot* be produced by an organism.

Consequently, the dogma that an infectious disease without a living organic (organized) cause is inconceivable, which dogma dominates the whole field of the pathology of infectious diseases, must be wrong. There must, therefore, be a mistake in the line of reasoning that has led to such a conclusion. In fact, there are several mistakes therein. First of all one must not by any means conclude that a virus must grow "actively," as an organism grows, from the mere fact that a virus in the diseased plant multiplies and increases in volume. There are also other possibilities. The following would be conceivable, e.g., the virus might function as a product of metabolism of the diseased plant, and this would then indeed have to be a product of metabolism with the following quite conceivable characteristics. It would have to exert on the embryonic leaf processes a formative stimulus of such a type that these develop into variegated abnormalities, into pathologic forms, instead of into normal green leaves, which pathologic forms, then, again reproduce this same pathologic product of metabolism, which, in turn, exerts the formative stimulus on the young leaf processes, etc. Thus, it by no means follows that a virus grows "actively" from the fact that it increases in volume in the diseased plant. There are, as the one example described here shows, still *other* possibilities.

Not to
transmit
infection

Furthermore, it is not yet proved, that a series of phenomena like that of an infectious disease must under all circumstances be produced by a *material virus*. Quite *different influences* would be conceivable. But I do not intend to go more deeply into such purely theoretical questions. For the time being it is merely important to show *that there is a typically infectious disease for which living organisms cannot be considered as causative agents*. This proof seems to be of considerable importance. After all, a whole series of infectious diseases¹² is known where all our knowledge to date contradicts organisms as a cause. For a further insight into the etiology of these diseases the old dogma of the unconditionally parasitic nature of all infectious diseases seems to me to be only an obstruction.

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¹² Of the plant diseases, I consider under this point above all, the mosaic disease of tobacco.