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.

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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 LEAVES[1]

M. W. Beijerinck

In 1885 Mr. Adolf Mayer[2] 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. Verhandelingen der Koninklyke akademie van Wetenschappen te Amsterdam. **65**: (2) 3-21, 1898. (With 2 plates).

[2] Landwirtschaftliche Versuchsstationen **32**: 450, 1886.

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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 appear[3] 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 "mikroaerophilie" in anaerobes[4] 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

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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 erythrodextrin and maltase from starch, while granulase produces only dextrin, aside from maltase, from starch as well as from erythrodextrin, 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 erythrodextrin 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 corpusculor 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.

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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

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 months[10] 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.

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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.

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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 blistery 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 monstrostities 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

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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.

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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 results[16] according to my several-times-repeated experiments with *Ulmus campestris*, *Acer negundo*, *Pelargonium zonale*, and *Utrica diocia*. 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 nonparasitic 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 intergrowth, 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 nonparasitic 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.

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

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. 1. 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 chlorophyl did not produce any distinct contrasts on the photographic plate.

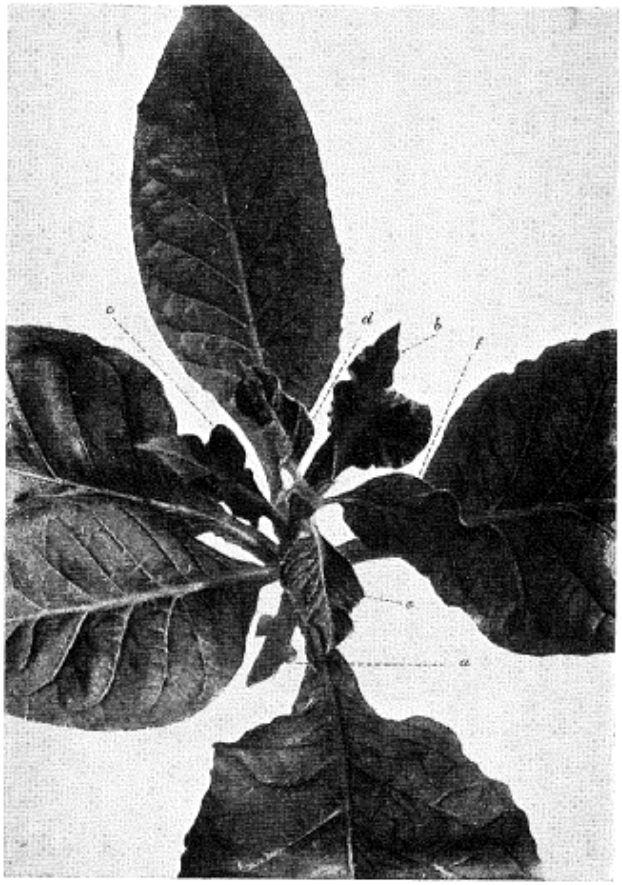
Fig. 2. A mildly diseased tobacco leaf in the second stage of the disease with a few brown spots that were produced through the premature necrosis of the tissues. The most important stage of the disease in which the dead, brown spots are increased by hundreds or thousands is not pictured.

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



Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.

Plate II

Beijerinck, M. W. 1898. Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopathological Classics, No. 7. American Phytopathological Society, St. Paul, MN.