

A *Contagium vivum fluidum** as the
cause of the mosaic disease
of tobacco leaves

1899 • M. W. Beijerinck

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THE LEAF SPOT DISEASE OF TOBACCO, also called the mosaic disease, is manifested first as a bleaching of the chlorophyll, occurring in spots over the leaf blade. This is followed later by the death of a part or all of the tissue of the spots. The discoloration first appears right next to the leaf veins and is manifested then by a strong increase in the amount of chlorophyll. Later the spaces between the spots become bleached usually to a yellow color, but in isolated cases they can

become completely albino. The dark green patches grow at the beginning more rapidly than the other parts of the leaf, leading to wart-like growths which arise from the upper surface of the leaf. However this phenomenon is observed more often in artificial infections than in tobacco fields, where the diseased leaves usually remain completely flat.

The third phase of the disease consists of localized death of the hundred or thousand small spots which are distributed randomly over the leaf. These then assume a brown color and become very fragile, so that holes are

* [*Contagium vivum fluidum* could be most likely translated: "living germ that is soluble."]

formed easily during the harvest of the leaves. These spots are the fear of the Dutch tobacco farmer, because they make the leaf worthless for cigar wrappers. . . .

Herr Adolf Mayer showed in 1887 that this disease is contagious. He expressed the sap from sick plants, placed it in capillary tubes and stuck these in healthy plants. He found that after 2-3 weeks, the latter plants became diseased.

In 1887 I attempted to discover if there was not a parasite which could be demonstrated to be the cause of the disease. Since microscopic studies were completely negative, the only type of bacteria that could be considered were those which could not be observed directly. But culture procedures showed that aerobic bacteria were completely absent, either from healthy or diseased plants. I later showed that anaerobic bacteria were also absent.

It seemed certain, therefore, that we were dealing here with a disease which was caused by a *contagium* which was not a *contagium fixum* in the usual sense of the words. This encouraged me to carry out new experimental infections in 1897 and 1898, in order to understand the properties of the *contagium* better. I would like to present here briefly the main results which were obtained in these studies.

It was first shown that the juice expressed from sick plants did not lose its virulence even after being filtered through a porcelain filter that was so fine that it rendered the juice completely sterile. This filtrate was tested for the presence of both aerobes and anaerobes, so that the experiment was completely unobjectionable. This filtrate was kept three months and remained completely bacterial free during this time but was repeatedly shown to induce the identical mosaic

disease when inoculated into plants. I do not know how long the virulence of this filtrate can be maintained.

The following experiments were designed to answer the question as to whether the virus should be considered particulate or soluble.

Pulverized tissue of diseased leaves was spread on thick agar plates and diffusion allowed to occur. A virus which was particulate would remain on the surface of the agar, since it could not diffuse into the molecular-sized pores of the agar plate. The deep layers of the agar would therefore not become virulent. But a water soluble virus ought to be able to penetrate to a certain depth in the agar plate. The experiment was discontinued after a diffusion time of about 10 days, which could be considered to be long enough, since I knew that diastase and trypsin would diffuse a considerable extent in this time. The upper surface of the plate was first washed with water and then with a strong solution of mercuric bichloride. After this, a sharp platinum needle was used to remove part of the agar, so that the inner layers could be reached, care being taken not to disturb the upper surface. Healthy plants were then infected with agar from these deep layers. The infection was just as extensive with this material as when the sterile filtrate was used. It can hardly be doubted, therefore, that the *contagium* must be considered to be fluid, or, more accurately, water soluble.

The experimental infections using plant juices were performed using the hypodermic needle of Pravaz. The most suitable place to infect is the youngest part of the stem which can be manipulated easily without causing extensive damage, since the closer the infection is to the meristem of the terminal bud, the earlier the results are seen. It has been shown that the

virus moves slowly through the plant, and further, that only the portions of young leaves that are undergoing cell division are sensitive to the infection. Both the mature leaves as well as the young leaves in which the cells have already stopped dividing are completely insensitive to the virus, even though they are able to transport it towards the meristematic regions. If stem internodes that are enlarging are infected, after 10–12 days the first symptoms of the disease can be observed in the young leaves which are coming out of the apical meristem. However, if an infection is carefully made as close as possible to the apical meristem, even after 3–4 days yellow spots and crisp distorted areas can be observed in the youngest little leaves that are still within the bud.

The amount of virus which is sufficient to infect a large number of leaves is quite small. It is then possible to obtain material from these diseased leaves which can be used to infect unlimited numbers of new plants. It is therefore quite clear that the virus is reproducing within the plant. From the above it is clear that this reproduction is not in the mature plant cells but in those tissues where cell division is occurring. . . .

Although the virus can exist outside the tobacco plant, it cannot reproduce under these conditions. I conclude this from the following facts: If a sterile filtrate of the virus is mixed with fresh plant sap of young tissues of healthy tobacco plants, it can be determined by experimental infections that no reproduction of the virus is obtained. Instead the virus is diluted in the same way as if pure water had been used instead of plant sap.

It is not difficult to determine the accuracy of this statement, since the amount of virus used to infect plants has a great influence on the development of the symptoms of the mosaic

disease. With a small amount of virus, the usual results are obtained as described above. With large amounts of virus, highly deformed leaves of characteristic shape are obtained. In order to obtain these deformed leaves, it is necessary to inject much more of a diluted virus than of one not diluted. In this way it is easy to tell whether the virus has reproduced or stayed the same in any type of fluid. As mentioned above, I have not observed reproduction under artificial conditions, so that I believe the only mode of reproduction of the virus is in cells of the plant that are dividing. . . .

The ability of the virus to reproduce only when combined with the living protoplasm of the host plant may be related to its soluble or fluid nature. It is not easy to understand why a *contagium fixum*, even if so small that it could not be seen by direct microscopic examination, could not still reproduce away from the host, like ordinary parasitic bacteria. In addition, it also would seem probable that a microscopically invisible *contagium*, if particulate, could develop into macroscopically visible colonies on gelatin plates.

A soluble and diffusible virus, such as the mosaic virus, should bring about some coloration or change in refractive index of a gelatin or agar medium, if the chemical nature of the medium were altered when used as a nutrient by a reproducing virus. Such changes could not be seen when the virus was seeded onto malt extract gelatin or onto plates containing 10 per cent gelatin dissolved in a plant decoction containing 2 per cent cane sugar—both excellent media in my hands for the growth of parasitic and saprophytic plant bacteria. It also seems to me that reproduction or growth of a soluble body is not inconceivable, although difficult to imagine. It would not seem wise to assume a division process of

molecules which would lead to their reproduction, and the idea of molecules which feed themselves, which must be assumed to explain this, seems to me an unclear concept, if not actually contrary to nature.

A partial explanation would be the view that the *contagium* must be incorporated into the living protoplasm of the cell in order to reproduce, and its reproduction is so to speak passively brought about with the reproduction of the cell. But this would then leave us one mystery instead of two, since the incorporation of a virus into the living protoplasm, even if shown to be a fact, can in no way be viewed as an understandable process. . . .

If the soil in which a tobacco plant is growing is infected with the virus, after a time the disease is seen to appear in the apical bud. The length of time for its appearance is primarily dependent on the size of the plant. In young plants, I saw the first symptoms in two weeks, while in larger and older individuals, 4–6 weeks occurred before the first symptoms appeared in the newly formed leaves of the terminal meristem. Therefore, the roots and stem must be able to transmit the virus considerable distances. . . .

It is possible to infect the plants through the roots only when they are two or more decimeters high. It is uncertain whether wounds in the roots are necessary, or whether the uptake of the virus can occur through the intact surface of the root. Since the *contagium* can only attack the leaves that form after the infection begins, the number of healthy leaves below

the infected ones can be used to approximate the time of infection of plants growing naturally which have taken up the virus through their roots.

The virus can be dried without any change in its virulence. It could therefore overwinter in soil, where it perhaps would be partially destroyed, like so many bacteria and yeasts. .

An alcohol precipitate of virulent plant juice, dried at 40°C, retains its virulence.

The virulence is also maintained in dried leaves, so that two-year-old herbarium leaves are still suitable for experimental infections. Therefore the dried dust which forms easily during the harvest from the broken dead tissue of the leaf spots must undoubtedly be able to spread the disease.

As expected, in a moist environment the virus was inactivated by boiling water, as well as at 90°C. I have not determined the lowest temperature at which inactivation will occur but expect it would be between 70 and 80°C. . . .

It is possible that there are a whole series of plant diseases which are caused by a *contagium fluidum*, in a similar manner to the mosaic disease of tobacco plants. The diseases of peach trees described in America by Erwin Smith in 1894 under the names peach yellows and peach rosette seem, from his description, undoubtedly to belong here, although it is not yet certain if these diseases can be transmitted only through budding or grafting, as he describes, or, what is more likely, they can also be transmitted through the juice of the dead tissues.

Comment

The filterable nature of tobacco mosaic virus had been discovered in 1892 by Ivanowsky, but apparently Beijerinck was unaware of his work. Beijerinck

went much further in his investigations than Ivanowsky; therefore his paper is presented here.

Although Beijerinck was wrong about

the diffusibility of the tobacco mosaic virus, the rest of his observations are quite valid. He describes an agent which can pass through the smallest filters, can apparently reproduce only in the living plant, and seems to be quite stable. In attempting to explain these observations, he finds himself in a dilemma, since the physiological and biochemical facts of cell function were not yet available for him to use in explaining his observations. It is interesting that he comes as close to hitting the nail on the head as he does. His postulate that the virus becomes incorporated into the living protoplasm of the host plant is one which is about as close to current thinking on virus multiplication as it would be possible to

get in 1899. This hypothesis shows brilliant insight into the problem.

Beijerinck's descriptions of his experimental infections are detailed enough so that they could be readily reproduced. He showed that the amount of virus in a filtrate could be crudely quantitated. This is an important aspect of virology research, since it is necessary to have some idea of how much infectious material is present in a sample. Later methods of quantitation of tobacco mosaic virus were much more precise and ultimately made it possible to study the physics and chemistry of this virus, leading to its crystallization by Stanley (see page 160).