Viruses in Plants

L. M. BLACK
The Rockefeller Institute for Medical Research, Princeton, N. J.

Introduction

Many people are unaware that plants have diseases,—that they are afflicted by the same pathogenic agents as attack animals and man. Fungi, bacteria, viruses, insects, and nematodes are important agents causing plant maladies. Plants also suffer from serious deficiency diseases. For example, a whole host of plant diseases recently have been shown to arise from an insufficiency of boron.

The study of viruses in plants is an intensely interesting one. In 1898, Beijerinck discovered that the infectious agent in juice from tobacco plants affected with mosaic disease could readily pass through filters that would hold back bacteria. As time passed, and more and more juices capable of initiating disease were tested, it became apparent that a considerable group of pathogens possessed this property. These agents also possessed in common the characteristics of being invisible under the microscope and of being unable to grow on any medium which did not contain living cells. This latter attribute made it necessary to develop a technique of handling viruses quite dissimilar in important respects from the bacteriological techniques developed earlier.

Symptoms

The symptoms produced in plants invaded by viruses are extremely variable. In the first place, the environment exerts a profound influence upon symptom expression. For example, the temperature at which the plants are growing may determine whether they will show any symptoms at all, even though they have been completely invaded by the virus. The most common first symptom of virus infection in plants consists of a clearing of the veins of the young leaves. The veins become more prominent, wider, lighter in color, or translucent. Leaves that are mature at the time the virus enters the plant seldom develop symptoms. The new leaves may be mottled by a mosaic of light and dark green areas, this condition persisting through the normal life span of the plant. Sometimes concentric light yellow rings appear on the leaf surface. Leaves may be blistered, puckered, curled, or otherwise distorted. In some diseases numerous shoots are produced. Potato plants may have so many miniature stalks that they look like moss on the ground. In other diseases the leaves, or even the whole plant, may be rapidly killed. In some cases the diseased plants may look stronger and more vigorous than the healthy plants, but they may yield poorly. In other plant virus diseases, after an initial phase in which symptoms are plainly evident, there regularly follows a stage in which practically no symptoms are discernible. However, virus can still be obtained from these recovered plants.

This is but a partial list of symptoms found on leaves. Flowers, fruits, and underground organs also show a great variety of symptoms. An interesting case is that of certain prized tulip varieties where the petals are streaked with red and white. These are nothing but red varieties infected with one of the tulip viruses. These special "varieties" of tulips are described in literature of the 16th century.

Economic Importance

Certain of the plant virus diseases are of tremendous economic importance. One of the outstanding cases in the United States is that of curly top of beets. The virus of this disease is transmitted by a leaf-hopper which in certain years spreads in great numbers from its breeding areas to the sugar beet fields. The beet crop in whole districts has sometimes been a complete failure and sugar beet factories were forced to dismantle and move elsewhere. Similarly, raspberry culture seems to have been abandoned in certain areas because of virus invasion. Spotted wilt virus makes the growing of tomatoes impossible in certain regions. The aster yellows virus made the culture of asters a practical impossibility in many sections. Numerous other instances could be cited.

The problem of potato degeneration can be taken as an example of the importance and mystery of certain diseases that attracted attention to the viruses. The degeneration of potatoes or their decline in vigor and yield in certain areas is not a new phenomenon. Since the earliest days of general potato culture by the western nations, this degeneration probably occurred. Early nineteenth century English descriptions of degenerated potatoes leave little doubt as to the virus nature of the trouble. One of the interesting features of the degeneration of the potato plant was its geographic localization. The more northerly countries or the regions of high elevation were not troubled seriously by potato degeneration. This knowledge led to a more or less continuous importation of seed potatoes from northern areas to southern areas; e.g., the importation of seed potatoes from Scotland into England. Thus, a partial control of the trouble was worked out with no knowledge as to its true nature. As the word "degeneration" implies, the commonly accepted understanding of the condition was that continuous asexual propagation had a devitalizing influence. Now we know that the degenerated potatoes were infected with one or more viruses. Degeneration proceeded more rapidly in warmer climates because there more plant lice infested the potatoes and spread the viruses from plant to plant. The asexual reproduction of the potato was involved in the degeneration process because the viruses were perpetuated in the seed tubers. If the potato plant began from the true seeds each year, there would be much less chance of degeneration because the transmission of plant viruses through true seed is the exception rather than the rule.

Natural Transmission

The study of the transmission of viruses in the field is an important practical problem. The method by which many plant viruses are transmitted in nature is still to be discovered. Tobacco mosaic virus seems to be disseminated principally by man himself. There is no insect known to transmit this virus efficiently. Workers may accidentally rub juice from chewing tobacco onto the leaves of young plants and thus inoculate them, for it has been shown that some of the best brands of chewing tobacco contain sufficient virus for the purpose. Later the virus is spread from plant to plant in the various handlings connected with tobacco culture.

Most plant viruses are transmitted by insects. At the beginning of the present century, Japanese workers showed that the virus of dwarf disease of rice was transmitted by a certain species of leafhopper. This was the first demonstrated case of the transmission of a plant virus by an insect. Since that time numerous plant viruses have been shown to be transmitted by insects. Insects that suck the juice from the phloem of plants are the chief offenders. Aphids carry most of the plant viruses known to be insect-borne, but leafhoppers, thrips, and other insects play an important part. In the majority of cases the process of transmission is purely mechanical,—the insect transmitting the virus only as long as its mouth parts are contaminated. This fact should not be forgotten when one is fascinated by the special biological relationships existing between certain viruses and the insects which carry them. In these more interesting cases, the virus may have quite a complex relationship with a specific insect. Thus, after the insect feeds on a diseased plant, an interval of hours or even days may elapse before it is capable of infecting a healthy plant. Some investigators have evidence that the virus multiplies in the insect during this period. Once the insect begins to transmit the virus, it may transmit it for the rest of its life without fresh access to a diseased plant. In one instance it has been clearly demonstrated that the virus passes through the insect egg, but not through the sperm, to the next generation. Sometimes the insect carries the virus through the winter in its body. It has also been demonstrated that within a single species of insect certain individuals are incapable of transmitting the virus, while others do so readily. In the leafhopper that transmits corn streak virus, a single sex-linked Mendelian factor controls this capacity. However, most plant viruses do not exhibit quite such special relationships with their insect carriers and some viruses are spread by numerous insects. It has also been shown that insects may carry a plant virus in their bodies for long periods, even though they are not capable of transmitting the virus. One of the most noteworthy facts about the virus-insect relationship is the absence of any detectable deleterious action by any plant viruses upon the insects carrying them.

The dispersal of viruses is also greatly facilitated by the large number of different kinds of plants which they are capable of invading. Some of the hosts may be biennials or perennials and so serve to carry them through the winter. Other hosts, cultivated or wild, while showing no evidence of disease, may be completely invaded by the virus in high concentration, and thus act as reservoirs from which insects transmit infinitesimal amounts to other plants that are severely damaged. An example of such a reservoir is the common American potato. All commercial varieties in America are 100% infected with a virus that does not produce any disease symptoms whatever in these potatoes.

Transmission in the Laboratory

The only method by which the presence of a virus can be demonstrated in any solution is by means of the infections it is capable of producing in plants. Some of the early methods by which viruses were introduced into plants were crude. To determine whether a plant disease is transmissible, one of the best tests is to graft a portion of the diseased plant on to a healthy plant of the same kind. Grafting is still indispensable in the study of certain plant viruses today, because this is the only known method of transmission. However, this method has serious handicaps for certain investigations on the virus itself. Presumably the virus in such transfers is always preserved in living protoplasm. One therefore cannot apply to the virus any test that will destroy the protoplasm of the host plant and all the information we gain about the virus is circumscribed by this environment. We cannot say that the virus would behave in the same manner if it were taken out of the host protoplasm. Some of the viruses that are difficult to transmit are also studied in the laboratory by means of the insects that carry them. This method of transmission has the same disadvantage that has been described for grafting.

Many viruses can be transmitted by rubbing the juice from diseased plants over the leaves of healthy plants with cheesecloth. Carborundum is often dusted over the leaf before rubbing to facilitate the transfer by wounding cells on the leaf surface. It is thought that transfer takes place only by the virus entering injured cells that recover or at least remain alive long enough for the virus to gain passage to neighboring uninjured cells. Most studies on viruses were originally based on the number of plants that were infected by a given inoculation. Some viruses make many lesions or "spots" on the inoculated leaves of certain of their hosts. It has been shown that each spot represents a point where the virus has entered the leaf and thus represents an infection. The usefulness of this reaction for quantitative work was demonstrated by Holmes in 1929. Thus, instead of using 200 plants in an experiment, the same number of infections might be obtained on a single leaf of a suitable species.

Classification

In the early days it was not known whether there was one virus infecting plants or many. Investigators trying to clarify this point discovered that viruses could be transmitted from species to species, genus to genus and finally from family to family. It became apparent that there were many plant viruses with widely overlapping host ranges. However, the host ranges of the viruses were not identical and this fact and the different disease symptoms produced by the viruses aided in their separation.

Johnson was one of the first to apply certain physical tests to the viruses for the purpose of differentiating them. For example, he found that the viruses varied in the extent to which they could withstand heating, dilution, or storage in the extracted juice and still be able to produce infection. For a time the study of plant viruses went through a period of intense activity in the describing and naming of new viruses. Great confusion was the result since it was always possible that two investigators were working with the same virus in different host plants. Means of identifying viruses, such as exchanging plant material, were not very satisfactory. However, more recently there has been a trend toward the grouping of some of the plant viruses in a more orderly relationship. Two lines of research have contributed especially to this result.

The identifying and natural grouping

of plant viruses has been facilitated by the development of serological tests similar to the agglutination tests that make blood identification possible. So far this method has been applied successfully to only a few plant viruses but if its use can be extended it promises much for an orderly classification.

Another aid in classification has arisen from the study of virus strains or mutations. Viruses are very much like organisms in the frequency with which they mutate. If one examines 100 tobacco plants infected with tobacco mosaic one will almost certainly find, on some of the plants, leaves with bright yellow spots. If the virus is taken from this one spot and inoculated into healthy plants, the plants will develop tobacco mosaic but a more yellow form of the disease. By this technique and others, literally hundreds of different strains can be obtained. Some kill the plants, some cause severe distortion, some are symptomless. Yet when one goes into the field to obtain tobacco mosaic virus one always finds the same strain in the tobacco fields. Why? The strain in nature is fittest. It spreads from plant to plant more easily and invades each plant more quickly than any of the variants it has thus far produced. And once it has invaded the whole plant, it is difficult or impossible for the other strains to get in, or spread in the plant once they do. However, unrelated viruses gain entrance to the infected plant as readily as ever. Such reactions can, therefore, be used successfully to test and establish relationships and differences between viruses. However, once again it is necessary to add that the method has been applied to a limited number of viruses and several experimental difficulties will have to be overcome to make it of more general applicability.

Control

The control of many of the serious virus diseases has already been accomplished. Breeding plants for resistance to virus disease has probably been the most successful method employed. Workers in the United States Department of Agriculture have produced a sugar beet that gives a normal yield under conditions where the curly-top virus reduces the yield of the old varieties to almost zero. The production of sugar cane in certain Caribbean islands fell to very low levels after sugar cane mosaic virus invaded the islands and rose again with the introduction of disease resistant varieties. Progress is being made in breeding tobacco that for practical purposes will be immune to tobacco mosaic virus.

Methods other than plant breeding have also been employed. Losses from potato viruses have been held in check by a system of inspection and certification of fields in which seed potatoes are produced. Asters, free from aster yellows virus, are now grown commercially under large cages covered with coarse cloth netting which keeps out the leafhopper carrying the virus. Serious losses from cucumber mosaic can be prevented by eradicating weeds around the cucumber fields that provide a reservoir for the virus. Control by what would seem to be the simple and obvious measure of destroying the insects carrying the viruses is often the most difficult to accomplish. In fact, there are comparatively few cases where this method of control has been economically feasible. Each particular disease must be attacked at its most vulnerable point and this is usually revealed only after careful study.

Fundamental Nature

Investigations into the fundamental nature of viruses have been conducted ever since Beijerinck's demonstration in 1898 that the virus of the tobacco mosaic disease would pass through bacteria-proof filters. This experiment itself suggested that the viruses were unusually small pathogens—one of the fundamental facts known about viruses. The most significant recent discovery concerning viruses was Stanley's isolation of a crystalline protein material which seems to possess the properties of the tobacco mosaic virus. It is a remarkable chance that this latter finding should have been made with the same virus that first opened men's minds to the ultramicroscopic world of disease-producing agents. Between these two outstanding discoveries there were many less crucial but nevertheless important contributions. Many attempts were made to grow the viruses in lifeless media, but to the present day no one has succeeded in growing a virus in the absence of living cells. Abnormal bodies (inclusion bodies or X-bodies) were discovered in the cells of diseased plants and these were thought by some to represent a non-filterable stage of the virus, by others to be by-products of disease processes. Many of the inclusions resembled organisms. Now it seems evident that these bodies are products of disease processes. However, some of the abnormal bodies, less like organisms in appearance, are almost certainly aggregates of the protein crystals. Ultraviolet light microscopy failed to reveal any probable causal agent in diseased plant cells. Filtration experiments through special membranes and diffusion experiments were used to estimate the size of the plant viruses. It was shown that the virus could be sedimented by centrifugation, and measurements on the rate of settling also permitted estimation of the size of the virus particle. Experiments demonstrated that the juice from tobacco mosaic plants contained colloidal rod-like particles not present in non-infectious juice. The early estimates of virus size were remarkably close to the present figures. Now we know that the protein isolated from diseased plants consists of colloidal particles about 430 millimicrons long and 12.3 millimicrons wide. Convincing serological work was accomplished which indicated that the virus was closely associated with protein. All of these facts and probabilities had been established before 1935 when Stanley's crystallization of active protein in juice from tobacco mosaic plants provided a tangible focus for much of the future research.

Since 1935, many tests have been applied to the protein crystals in an effort to make certain that the protein is the virus and not simply a closely associated substance. Thus far no method has been found by which an active ingredient can be obtained separate from the characteristic protein, the colloidal particles of which are 42 million times as heavy as the hydrogen atom. Moreover, the application of the same methods of isolation to other plant viruses is yielding similar proteins. More recently, the English workers, Bawden and Pirie, have shown that the protein is a nucleoprotein. This is a point of great interest because nucleoproteins occur in the nuclei of cells.

It should be apparent from this brief account that the field of plant viruses is a young and active division of science and that the future probably holds many other interesting discoveries for the investigator.