adoption is the fact that so few trained zoologists know anything worth speaking of concerning nemas. It is suggested that trained zoologists can instruct themselves by a perusal of original nemic literature (not text-books—not encyclopedias) available in most large libraries, and by a few weeks study of living nemas with the aid of high-power immersion lenses. The nemas should be under sufficient pressure to prevent active motion, but not sufficient to altogether prevent them from moving.

THE CHALLENGE OF PLANT VIRUS DIFFERENTIATION AND CLASSIFICATION[1]

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FOR some time there has been no phase of phytopathology in greater need of cooperative thinking and action than that of plant virus differentiation and classification. Much uncertainty and confusion have existed in this field of investigation ever since the first recognition of a virus disease by Adolph Mayer in 1886. Soon after Mayer's work became known, it was claimed by some that his "Mosaikkrankheit" of tobacco included two distinct diseases, one the true infectious mosaic and the other a supposedly unrelated necrotic disease known elsewhere as "Pockenkrankheit." Although much attention was given to the subject, this disagreement has persisted almost to the present time, though it is now quite generally conceded that Mayer was correct in his interpretation that necrosis is one of the symptoms of the ordinary tobacco mosaic virus on tobacco as well as on certain other hosts.

In the meantime, the continued description of virus diseases on different hosts, on the basis of symptoms only, has led to serious confusion even in fundamental research concerning the nature of a virus. It was natural that a school of thought should develop which was inclined to the belief that only one, or at the most only a few, viruses existed in nature, or that a virus was a labile entity capable of adapting itself to various hosts and circumstances. This point of view has only recently been dispelled by those who maintain that many distinct and specific viruses exist in nature and that we have in the viruses a problem of differentiation and classification comparable in complexity if not in extent to that in mycology and bacteriology.

Unfortunately, however, the pendulum is apparently swinging too rapidly in this direction. The tendency to apply new names to a virus disease when only symptom expression is involved, either on an old or on a new host, is leading to new difficulties, the more serious because we are dealing with an unseen entity, the true nature of which may long remain a mystery.

The challenge is clearly before the workers on plant viruses, first, to check themselves and others as far as possible from adding to our present difficulties, and then to clear up as rapidly as may be done the confusion already existing in the literature.

While the reliable methods now available for the differentiation of plant viruses are not applicable in all cases nor entirely satisfactory in others, such methods are yet remarkably useful considering the early stage of development of this subject. New and better methods for the differentiation, determination and description of specific viruses are gradually becoming available, and important advances in this line of technique may be looked for in the future. Many of these methods are already familiar to most of the workers. In connection with a discussion of this kind, it may be well briefly to list the more obvious of these methods and to discuss their possibilities and their limitations.

Four chief types of differential or diagnostic features of plant viruses are recognized at the present time. These are: symptom expression, properties of the virus, modes of transmission and the cytological picture.

SYMPTOM EXPRESSION

Comparative symptoms on a single host species or variety have constituted the main diagnostic character relied on up to the present time in the recognition of specific viruses. The best example of the use of this type of differentiation lies of course in the potato virus group. The limitations of this method, useful as it has been in the past, are obvious to any one who has worked with this group of diseases. The symptoms produced may vary greatly with the variety of potato and its stage of development, and with the source and method of infection as well as with the environment. Consequently, descriptions of symptoms of the different viruses often overlap so extensively as to be quite unreliable even to authori-

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ties on the subject, who may unconsciously depend rather upon recognition through acquaintance than through description. However, all that is distinctly useful in this method of differentiation should be retained, but there is much in the way of detailed description which could advantageously be dispensed with.

The use of comparative symptoms on a range of different host species or varieties is a diagnostic character which has been less commonly used than the possibilities appear to justify. It is fundamental to the progress of virus investigation to know something of the host range of each virus, and where the host ranges are distinct, or even where the symptoms are distinct on some hosts although not on others, a good basis for differentiation or determination exists. It must be admitted that, in general, the use of the differential host method up to the present time has often served to confuse rather than to simplify our problem, but we believe that, with new facts which have been brought to light, the situation will soon be reversed.

A third group of differential factors which warrant consideration in the present connection is that of environmental influences, such as the effect of temperature on symptom expression. The remarkable influence of temperature on the symptom expression of certain potato viruses is now well known. Temperatures which may mask one virus disease may intensify the symptoms of another, as, for example, in the case of crinkle mosaic and rugose mosaic of the potato, using Schultz's terminology. This characteristic may consequently be of considerable value in the determination of these and other diseases where other more simple or reliable methods may not be at hand. In any case, factors of environment and other related circumstances, such as vigor and stage of development of the host, need to be taken into consideration in any attempted determination or description of a virus disease on the basis of symptom expression.

PROPERTIES OF THE VIRUS

The properties of the virus itself seem to offer the most reliable and satisfactory characteristics for differentiation of certain viruses of the true mosaic type, or of those viruses which are quite readily artificially inoculable from plant extracts, as distinguished from those transmissible only by insects or by grafting, which are commonly of the "yellows" type of virus disease. Remarkable differences exist between these mosaic viruses with respect to the length of time they may survive in plant extract or apart from the living host. It is necessary only to call attention to the tobacco mosaic virus, which may apparently live outside the living host for as long as twenty-five years under certain conditions, in contrast to a certain potato mosaic virus which apparently becomes inactivated in two to four hours. Similarly, tolerances to dilution may vary from 1 to 10 to 1 to 10,000 or more with different viruses, and thermal death-points from 40° C. to 90° C. Corresponding variations in reaction to treatment with chemicals of various sorts are also known to exist, although this field as a means of virus differentiation is as yet relatively unexplored.

Not only are these properties of great value for diagnostic and descriptive purposes, but they may often serve, especially in combination with the use of differential hosts, as a ready means of separating combinations of viruses into their component parts. We have here a beginning of the technique for the isolation of what might be called "pure cultures" of viruses, which, followed up with some modification of Koch's postulates, may eventually place the determination of the mosaic type of virus disease on quite as sound a basis as that now available for diseases of bacterial origin.

MODES OF TRANSMISSION

The third means of differentiation of plant viruses, which merits more consideration than has been given to it from this point of view, is that of differential modes of transmission. We may pass over with bare mention the fact that it is possible in some instances to differentiate viruses by their behavior with respect to the source from which the inoculum is taken, and the method of inoculation used. Tobacco mosaic, for instance, is not recoverable from certain host plants although these may be readily infected with the disease; and Dr. Goss has shown, for example, that the spindle-tuber disease of the potato is transmissible by the cutting knife, whereas the common mosaic viruses of this host are not. Obviously, many viruses may be distinguished on the basis of their differential transmissibility through budding or grafting, insect vectors and virus extracts.

More interesting, however, and possessing greater possibilities for expansion within closely related groups is the isolation and differentiation of plant viruses through their specificity in insect transmission. The development of a method of differentiation on the basis of their insect relationships, particularly with respect to those viruses which are not readily transmissible by artificial means, may eventually serve to complete a satisfactory key for the determination of plant viruses in general. Our knowledge of this field is already sufficient to indicate that we are dealing here with at least three specific conditions affecting the transmissibility of a virus by an insect, namely: (1) The species of insect

involved; (2) the specific virus concerned; and (3) the species of host plant serving as a source of infection. It may also conceivably develop that the species of host plant serving as "suscept" may add to the possibilities of differentiation.

In some cases, at least, a highly specific relationship is known to exist between the virus and its insect vector. Certain diseases of the "yellows" type appear to be transmissible by a single species of leafhopper only, and, so far as is known at present, by no other type of insect; curly top of sugar beet by *Eutettix tenellus;* aster yellows by *Cicadula sexnotata;* and streak disease of maize by *Balclutha mbila.* Although the host ranges of the first two diseases are wide and may overlap to a certain extent, this specific relationship offers a ready means for the isolation and determination of the respective viruses concerned. Again, of the virus diseases affecting the raspberry, for example, the aphid *Aphis rubiphila* is said to spread curl only, and the aphid *Amphorophora rubi* the mosaic diseases only; and other examples may be quoted of different viruses which may affect the same host plant, each dependent upon a different specific insect carrier for its transmission.

It has furthermore been shown that cucumber mosaic is readily transmissible from tobacco by several different species of aphid, while ordinary tobacco mosaic is not so transmissible. Here again is a means of differentiation and a simple method for the separation of the two viruses involved should they occur in combination.

Only a single example may be cited at the present time of the influence of the host species serving as the source of infection on the transmissibility of a virus by an insect. The aphid *Myzus pseudosolani* is apparently unable to transmit the ordinary tobacco mosaic virus from tobacco and certain other solanaceous hosts, yet it will readily transmit this same virus from tomato. Although no adequate explanation of this peculiar host relationship can be offered at the present time, it is evident that here, at least, the species of mosaic host plant may exert a determining influence on the amount of insect transmission of a particular virus. Whether or not this is an entirely exceptional case, however, remains to be determined.

On the other hand, the relationship between insect and virus does not always appear to be so specific. Cucumber mosaic, for example, is said to be transmissible by at least five different species of aphid, as well as by two species of cucumber beetle. Further, the peach aphid has been reported as transmitting a number of different virus diseases, such as spinach blight, various potato mosaics, potato leaf-roll, lettuce mosaic, sugar-beet mosaic, celery mosaic, bean mosaic, and mosaic of Chinese cabbage, mustard and turnip. Although it has not yet been shown that the various diseases just named are actually due in all cases to different, specific viruses, our knowledge of some of them being practically confined to the symptomatology on a single host, yet several of these are definitely recognized as distinct; and it would consequently appear that the differentiation of viruses by means of certain insect vectors may be somewhat limited in its application.

THE CYTOLOGICAL PICTURE

The fourth and last type of differentiation which we wish to mention is that of the cytological picture in the virus-affected tissues. This method is, of course, often used in the determination of certain animal viruses, although it has not yet been extensively developed for the plant viruses. It has been shown that the so-called "x-bodies," or vacuolate inclusions, are invariably associated with the tobacco mosaic virus regardless of the host on which it exists, provided that mottling or chlorotic symptoms are produced, but that they are not found in the case of the cucumber mosaic virus and certain other viruses on the same hosts even though host mottling may occur. The cytological picture of the potato viruses has hardly been studied sufficiently to warrant any definite conclusions, but we suspect that the details differ here also with certain different viruses. Characteristic cell inclusions are known to be constantly associated also with certain other plant virus diseases, and these may eventually prove to be a valuable diagnostic feature.

The usefulness of the cytological method will, of course, depend upon whether or not any simpler, quicker or more convenient means of differentiation exists where determination is required. The suggestion is merely put forward that cytological technique may eventually prove to be the best method of differentiating two or more specific viruses which are otherwise closely similar.

By the use of the various differential characteristics which we have now discussed, it has already been shown in certain instances that virus diseases of various hosts described in the literature are, or may be, due to one particular specific virus. The cucumber mosaic virus has, for instance, been shown to be the causal agent of mosaic diseases of a number of host species, where this relation was not suspected when the diseases themselves were originally described. There is room for considerably more reduction in synonymy than has so far been achieved. On the other hand, there has been, and there no doubt will be, a growing list of specific plant viruses

adequately described and accepted on both old and new hosts. The first challenge now before us is, however, whether there is any justification for a person to describe and name a virus disease on any host without adequately and thoroughly subjecting the virus concerned to a sufficient number of the differential tests available to ascertain whether or not the virus or the disease in question should be given a new name.

In America this problem of the promiscuous application of new names to virus diseases on the basis of symptom expression only has become so serious that it is generally felt that some concerted action should be taken for the protection of the virus workers themselves, as well as of those of the teachers and students of the future who may be obliged to cope with the subject. A strong feeling existed, therefore, at the last meeting of the American Phytopathological Society that it would be well to have a group of pathologists assigned to consider ways and means of reducing the difficulties before us. The initiative in this direction, to be most effective, should come rather from an international body of pathologists. A closely related phase of the subject of virus differentiation is the standardization of the requisite technique. Manifestly, a uniform procedure should be adopted in the determination of the properties of virus extracts. We are also obliged to recognize that the source of the inoculum itself with respect to the host species or variety as well as to other conditions may have a bearing on the results obtained. Finally, it must be recognized that the host plants to which the inoculum is applied may respond differently according to their age and vigor and to the surrounding environmental conditions. The subject of standardization of technique is one in which a good beginning could be made by the selection of some international group to help lead the way.

We are perhaps not yet sufficiently far advanced to go far into the field of strict classification of the plant viruses. Those of us who have attempted to comprehend the viruses as a group, however, are impressed by the fact that we appear to have several closely related classes or forms which may be compared to species of a single genus, while other groups of viruses are as distinct, certainly, as the most widely separated groups of bacteria. The development of a system of classification for the viruses seems to be almost inevitable in the near future, while this is at the same time a matter in which we can afford to move slowly.

The adoption of a uniform system of nomenclature for the viruses would prove to be highly desirable to the students of the subject. There appears to be no serious obstacle in the way of some satisfactory international agreement on this subject. Several proposals have already been made in the literature, but we wish to point out here that the effort should be fundamentally in the direction of naming the virus rather than the disease which it causes. In practice we may never overcome the synonymy and confusion of the common names of plant diseases, but there is no good reason why a single technical name should not be made to represent a specific disease-producing entity.

We have purposely taken this unusual opportunity to make such an appeal, rather than to present actual details of results and conclusions in this field of investigation. If the challenge of virus differentiation problems is to be met, we are convinced that nothing more helpful could come about than for some international body to come to some agreement on a system for plant virus differentiation, classification and nomenclature, and to use its best influence to secure the universal adoption of such a system or standard as will eventually place the subject of plant viruses in a position commensurate with their importance in the sciences.

SCIENTIFIC EVENTS

VIVISECTION IN ENGLAND

A BILL has been introduced in the House of Commons by Lieutenant-Commander Kenworthy to prevent the application of public moneys to vivisection experiments. The measure is a subsidiary bill promoted by the British Union for Abolition of Vivisection, and was previously before Parliament in 1922 and 1924, according to the London *Times*.

The British Medical Association is opposed to the bill and has addressed a letter to members of Parliament in which it is pointed out that the Act of 1876 lays down that no one but the holder of a license from the Secretary of State is permitted to use animals for experiments; that such work shall only be carried out at registered places; and that the experiments must be performed with a view to the advancement of physiological knowledge or of knowledge which will be useful for saving or prolonging life or alleviating suffering.

The letter of the association continues:

> This work is loosely termed vivisection, but no severe cutting operation is permitted under the Act without the use of an anesthetic of sufficient power to prevent the animal feeling pain. Very many of the so-called ex-