# 18. THE POSSIBILITIES OF THE USE OF THE ELECTRON MICROSCOPE IN HELPING THE DIAGNOSIS OF PLANT VIRUS DISEASES

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Virology was, probably, one of the scientific fields which benefited most from the introduction of the electron microscope (EM) as a research tool. Virus particles, being submicroscopic, are only visible in this instrument, and morphological investigations so far carried out, revealed that particles of each virus have usually a peculiar size and shape. Thus, it became easily acceptable that morphology, together with the chemical properties, is an intrinsic quality of the viruses, and both represent the basis for the modern virus taxonomy.

The knowledge of the virus particle morphology can be advantageously explored in helping the diagnosis of the diseases caused by a particular virus. It is well known that different virus diseases might show comparable pathological properties, although being caused by completely different viruses. In such a case, a single glance of the virus particle, in a quick, crude preparation for electron microscopy, made in a matter of few minutes, might result in a reasonably safe identification of the causal virus. In other cases, when virus etiology is suspected, a quick preparation examined in the EM, might confirm this hypothesis by revealing the presence of viruslike particles. Also, during routine inoculation tests, often some unexpected symptoms appear. EM examination can rapidly check for a possible contamination. Latent infections are also easily revealed by EM.

The possibility of using the EM in diagnosis, permits, in favorable cases, a significant economy in time, personal effort, and space in laboratory and greenhouse. But, by all means, EM cannot be considered as the only or the most efficient method for plant virus disease diagnosis. The results obtained are only reliable when analyzed together with pathological aspects of the disease, or further collaborated by other methods such as serology.

Concerning methodology, Johnson in 1951, was one of the first to suggest the possibility to use EM for diagnostical purposes by introducing the exudate method to collect samples for electron microscopy of plant viruses. Leaf exudates that appeared from cut veins, either spontaneously or induced by the application of water pressure at the leaf petiole, were collected in the Formvar-coated EM grids, and directly examined after shadow casting. Particles of some elongated plant viruses were easily identified among cell debris. However, the true potentiality of the method was only fully explored a few years later, at the Virus Institute at Braunschweig, Germany, initially with potato viruses, and later with a large number of other elongated viruses. Brandes, in 1957, introduced a more simplified technique to prepare the sample, known as leaf dip, in which a piece of infected leaf is dipped into a drop of distilled water. Materials from broken cells, including the virus particles, suspended in this drop of water were then

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examined in the EM. Brandes and co-workers studied a large number of elongated plant viruses, and noticed that each one had a typical modal length and a constant width. He coined this modal value as normal length (NL) and suggested that it represents the best estimate for the true particle length. The reasoning was that the leaf dip method was a relatively mild extraction procedure for the virions, and although some particles could suffer breakages and/or aggregations, most of them would retain their original size. Measurements of a large number of particles, at least 70–100, would then reveal this NL through the analysis of the particle length distribution curve. The NL is similar between strains of a given virus, and it is also independent of the host plant in which the virus is found.

Therefore, for elongated plant viruses, the general shape, which varies from short rigid rods to very long, flexuous threads, the NL can be used for diagnosis. Brandes also noticed that viruses with similar NL shared several biological and serological properties, and he came out with the first virus classification based truly in the intrinsic characteristic of the virus, in 1959.

A limitation of the leaf dip method to detect plant viruses was that it is relatively difficult to distinguish most of the isometric virus particles from particulated cell debris, after shadow casting. To solve this problem, we in Brazil, and Hitchborn in England, almost simultaneously in 1965, proposed the combination of the leaf dip method with the negative staining technique. This allowed the detection of some of the spheroidal viruses. Of course, to permit their detection, these viruses had to withstand the preparative steps and should occur in a relatively high concentration. Elongated viruses are also readily detected through this method, as well as membrane-bounded viruses such as rhabdoviruses and the tomato spotted wilt virus. The technique is basically the Brandes' leaf dip procedure where the water droplet is replaced by neutral phosphotungstic acid. Since no shadow casting is necessary, the time to prepare the sample is reduced to a few minutes.

In some cases, such as with plant diseases associated with mycoplasmalike (MLO) or rickettsialike (RLO) organisms, the leaf dip method is not usually suitable. In these cases, the best approach is to examine thin sections of the vascular region of affected plants to detect pleomorphic bodies in the sieve tubes or bacteroids in the xylem vessels. Also, examination of thin sections of virus-infected tissues offers frequently clues for diagnosis, because certain virus/host cell combinations produces typical cytopathic effects. Some examples of such cytological signatures of plant viruses are: intranuclear crystalline inclusions in bean yellow mosaic virus-infected cells; cytoplasmic pinwheels in cells infected by viruses of the potyvirus group; cell wall outgrowth associated with infection by some viruses of COMO- and NEPO-virus group; dense viroplasm containing virions, in cells infected by members of the CAULIMO virus group; association of virions and mitochondrion in cells invaded by the Brazilian tobacco rattle virus, etc.

Recently, another possibility to use EM for diagnostic purposes was developed, consisting in a combination of serology and leaf dip technique. A Formvar-coated grid is precoated with antisera against a given virus, and then floated onto a crude extract of the virus-infected material. If the virus and the antibody match, the particles of this virus will appear fuzzy after negative staining, revealing positive serological relationship.

Therefore, it seems clear that EM can be very useful in the diagnosis of the plant virus diseases. However, one of the most common problems is that plant virus laboratories in the developing countries do not usually have EM facilities, mainly due to the high cost to purchase the instrument and the accessories (up to US$100,000), as well as to maintain the equipment in working conditions. There are, on the other hand, possibili-

ties in doing some EM works without having the instrument in the laboratory, using the microscope from some other institution. If thin section works are involved, it is perfectly possible to organize a pre-electron microscopy laboratory, with an ultramicrotome and some other small items, spending less than US$10,000. If only leaf dip preparations are considered, all the recquired material would cost less than US$100/year. Then, an arrangement with an electron microscopy laboratory nearby in the same country, or even with laboratories from a friendly foreign country, would fullfill the purpose. Ultramicrotomy will need a somewhat specialized person, but leaf dip preparations can be prepared by people unfamilar with electron microscopy. Once prepared, leaf dip preparations can be dispatched by mail. If histological works are involved, either blocks or sections mounted on grids, can be sent by mail. When embedding facilities are nonexistent, it is possible to mail only the buffered tissues, after being pre-fixed by glutaraldehyde.

The last remark is to warn that EM is not suitable for mass diagnosis as is done by serology, for example in potato seed certification program. Although a large number of samples can be readily prepared, their examination is always time consuming, mainly when virus concentration is low. Thus, there must be a criterious preselection of the samples to be checked by EM to make this activity really profitable.

## Discussion

**D. A. Benigno,** Philippines: (1) Is your leaf dip method good for both broad and narrow leaf plants?

(2) How long do you dip the cut-leaf into the drop of PTA before you touch the drop with EM-grid?

(3) If you use leaf dip method for virus detection in the EM, how soon should you examine the sample after the leaf-dip?

**Answer:** (1) Apparently yes.

(2) Usually a few seconds. However, what I usually do, is to cut small pieces of the leaf under study, and put them floating onto the drop of the stain.

(3) I am using carbon-coated plastic (Formvar or Collodion) films, which withstand the preparation for very long period after it is made. Also, I prefer the use of 300 mesh grid, on which the film seems to be more stable.