expired, suddenly and peacefully, he was in the process of writing memoirs of greatmen he had met during his life time, a final essay on mango necrosis and a book on non-parasitic diseases of plants in India.

Prof. Dasgupta was honoured by various societies and was elected as the President of the Indian Phytopathological Society, Indian Botanical Society and Botany Section of the Indian Science Congress. He was an Honorary Fellow, Indian Phytopathological Society and Fellow, Indian National Science Academy, National Academy of Science and National Institute of Sciences, India and Mycological Society of USA. He was conferred D.Sc. degree (*Honoris causa*) in 1979, as the founder father, by the University of Kalyani, Kalyani. The famous Italian Naturalist, O. Campesse dedicated his monumental work on *Colture Tropicale*, Vol. VI, to Prof. Dasgupta as a token of esteem and appreciation.

Professor Dasgupta's life was so honest, truthful, sincere and disciplined that one would like to learn from it. He always was full of new ideas and generous in sharing them with his colleagues and students, often allowing them to take much of the credit. He was held in awe of his dynamic personality and simultaneously respected for his humanitarian attitude. He was Godfather to three children's institutions, that became known only after his death. He donated his large and magnificient personal library to the University of Kalyani. He lived in a shell, and only the few who could penetrate this hard shell knew his softness and love for the weak. He liked to help without being known as the helper, and the help was always in plenty, with modesty and with sympathy. His kindness and warmth of personality made him many friends.

Prof. Daspupta was a bachelor. But he has left behind a large family of mourners, which include his near relations, students, colleagues and admirers. May his soul rest in eternal peace.

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PLANT VIROLOGY IN DEVELOPING COUNTRIES*

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At the outset I would like to take this opportunity to thank the Indian Phytopathological Society for chosing me to receive this award.

I am sure the majority of you know the outstanding contributions made by Dr. M. S. Pavgi in Mycology and I am pleased that the Indian Phytopathological Society has constituted this award in his name. However, a virologist has been chosen to be the first recipient of this award and I hope that Dr. M. S. Pavgi will not mind if I devote my lecture to aspects related to research on plant virus diseases. Nevertheless it was interesting for me to note that he himself started his career with a position in Virology.

Prior to 1976 my research on plant viruses was mostly related to fundamental aspects. Since I joined ICRISAT in 1976 I have been devoting my entire time to applied research on plant viruses in developing countries. As a result I believe I know at least some of the problems faced by plant virologists in developing countries.

It is important to emphasize that applied research on plant virus diseases differs from that on fungal and bacterial diseases because of the special nature of viruses. I strongly believe that for formulating meaningful control measures, characterization of the causal virus/es and elucidation of its mode of transmission are essential. Several plant viruses produce very similar symptoms that can be described as ring spots, mosaic, mottle etc. Unrelated viruses can produce very similar symptoms, and strains of the same virus can induce different symptoms in the same species. Accurate symptom description is necessary for describing the *disease* (which results from interaction between host and viral genome). Nevertheless in the majority of cases it should not be used as proof for the identity of the causal virus/es. In order to achjieve precise virus identification, elaborate and expensive equipment are required. In addition to expertise required to operate and use the equipment appropriately, skilled electronic engineers are required to maintain them. Well trained scientific and technical staff are required to carry out the various techniques required for virus characterization (Tables 1 and 2).

CURRENT SITUATION OF PLANT VIRUS RESEARCH IN DEVELOPING COUNTRIES

In my opinion the situation that exists in India is in many respects similar to that found in many developing countries. My impressions are based on the publications, papers presented at meetings, and discussions held with virologists working in

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TABLE 1 : Steps in the diagnosis of plant virus diseases

1. Field observation of disease (incidence and distribution).
2. Maintenance of cultures by grafting.
3. Transmission tests: (a), Mechanical sap transmission; (b) Insect transmission.
4. Inoculation to a series of special test plants, back inoculation to a parallel range of test plants to check possible multiple infection and host range.
5. Identification of host/s which produces characteristic symptoms, especially local lesions (diagnostic hosts).
6. Identification of a systemically infected host which supports high virus concentration (for purification of viruses).
7. Determination of physico-chemical properties.
8. Examination under electron microscope of leaf dip preparations.
9. Development of methods to purify the virus.
10. Production of polyclonal antisera.
11. Fulfillment of Koch's postulates, especially using purified virus.
12. Analysis of infected tissue for virus-specific dsRNA (RNA viruses only).
13. Testing by ELISA or ISEM using appropriate polyclonal antisera.
14. Determination of interrelationships with similar viruses occurring elsewhere.

Modified from Bos (1976) and Reddy (1980).

TABLE 2 : Facilities for characterizing plant viruses*

1. *Maintenance of virus cultures and transmission*:
   —Glasshouses, small individual units
   —Growth chambers with constant temperature and humidity
   —Facilities for maintenance of insect cultures
   —pH meter, analytical balance, stirrers, refrigertor
   —Clinical centrifuge
2. *Virus characterization*:
   —Refrigrerated superspeed centrifuge
   —Refrigerated ultra centrifuge
   —Spectrophotometer with recording facilities (wave lengths between 200-400 nm)
   —ELISA reader
   —Gradient scanner with fraction collector
   —Apparatus for gel electrophoresis
   —Power packs
   —Transilluminator with a camera for instant photographs
   —Ultra low temperature freezer
   —Lyophilizer
   —Tube mixers
   —Micropipettes
   —Incubators
   —Water bath
   —Facilities for maintaining rabbits

*An electron microscope is deliberately eliminated because of its enormous cost to purchase and to maintain it. Nevertheless access to electron microscope is essential to know morphology of virus particles.

developing countries. The majority of the economically important virus diseases are yet to be fully characterized and their relationships with similar viruses occurring in other countries have still to be determined. New records of occurrence are usually based on symptoms and on biological properties such as mode of transmission, longevity *in vitro*, and thermal inactivation point (Nene, 1986). Serological techniques utilized are either gel diffusion, precipition tube or latex agglutinatgion tests, and only seldom are more sensitive techniques such as enzyme-linked immunosorbent assay (ELISA) used. Electron microscopy is seldom used for virus detection, though a large number of electron microscopes have been installed at various research centres in India. Additionally, physicochemical properties such as molecular weight of coat protein and nucleic acid, number of nucleic acid species encapsidated and their nature, and complementary DNA probes for determining interrelationships, are rarely used for precise virus characterization. I do not want to give you the impression that none of the plant viruses in developing countries have so far been characterized. There are reports, especially those published in recent times from India, where virus identification has been based on reliable and sensitive techniques. None-the-less they represent a small proportion of all the published reports on plant virus diseases.

If a new (virus) disease has indeed been observed and is suspected to be of viral origin on the basis of symptoms (similar symptoms *hitherto* have not been described from that country), transmission characteristics and biological properties such as thermal inactivation point and longevity *in vitro*, it is essential to publish this information especially in the journals which have wide circulation in the country where the disease has been observed (Table 3). However, author/s should use extreme caution in drawing conclusions on the identity of the causal virus. It is advisable not to give a new name to the causal virus. To give an example, "bud Necrosis" is a characteristic symptom in groundnut (*Arachis hypogaea* L.), caused by tomato spotted wilt virus. The disease can be described as "Bud Necrosis Disease". However, it should not be called "Bud Necrosis Disease". However, it should not be called "Bud Necrosis Virus". It is appropriate here to quote Hamilton et al. (1981), "It is far better to entitle a paper 'A disease of two leaved Solomon's seal caused by a strin of cucumber mosaic virus' than 'Solomon's seal mosaic virus, a new virus,".

TABLE 3 : Publication of results on occurrence of virus diseases

Information obtained	Suggestions for publication
1. Symptoms under field conditions and in laboratory tests. If the disease has not so far been described from the country perform steps 1-3 in table 1.	Name it as a Virus Disease. Publish results preferably as a short note. Do not give a name to the causal virus.
2. Depending on the facilities available follow steps given in table 1. It is possible to process samples till step '6' with minimum laboratory and green-house facilities; if processing could be done till step '6'.	Still do not give a name to the causal virus. Publish in a journal with wide circulation within the country of occurrence of disease.
3. Identification of causal virus was based on several criteria given in table 1.	Name the causal virus. Publish in a journal with international reputation for quality.

*If virus identification cannot be done because of lack of physical facilities, follow suggestions given on pages 7 and 8.

A PROPOSAL FOR IMPROVING FACILITIES FOR VIRUS RESEARCH IN DEVELOPING COUNTRIES

Before we look into ways to improve the current situation in developing countries it is essential to dwell upon the requirements for precise identification of plant viruses (Table 4). Factors which have contributed to lack of progress on virus identification in developing countries include:

- Lack of required facilities
- Lack of trained junior as well as senior staff
- Lack of motivation to utilize better techniques
- Frequent transfer of staff
- Lack of an atmosphere conducive for productive research

TABLE 4. Basis for virus characterization

1. **Biological properties.**
   - 1.1 Transmission characteristics
     - 1.1.1 Mechanical sap transmission
     - 1.1.2 Transmission by biotic vectors (insects, mites, nematodes, fungi etc.)
     - 1.1.3 Transmission by seed or pollen
   - 1.2 Host range
     - 1.2.1 Symptoms on a set of special test or indicator hosts (local and systemic reactions)
     - 1.2.2 Reaction on a wide range of hosts
   - 1.3 *In vitro* properties
     - 1.3.1 Thermal inactivation point
     - 1.3.2 Longevity *in vitro*
       - at room temperatures
       - at refrigerated temperatures
     - 1.3.3 Dilution end-point
2. **Physico-chemical properties.**
   - 2.1 Molecular weight of coat protein/s
   - 2.2 Molecular weight of nucleic acid/s
   - 2.3 Nature of nucleic acid
   - 2.4 Single or multicomponent
   - 2.5 Particle density
3. **Morphology of Virus particles.**
   - 3.1 Size and shape
   - 3.2 Special features such as an outer membrane, core etc.
4. **Inclusion bodies.**
   - 4.1 In thin sections of plant tissues, observed under an electron microscope
5. **Interrelationships with similar viruses.**
   - 5.1 Serological relationships utilizing polyclonal antibodies.
   - 5.2 Serological relationships utilizing selected monoclonal antibodies.
   - 5.3 Serological relationship utilizing antibodies to specific regions of viral polypeptides.
   - 5.4 Relationships detected in western blots
   - 5.5 Percent nucleic acid homology utilizing complementary DNA probes (utilizing specific clones or random-primed probes).

Modified from Bos (1976).

- Lack of suitable curricula to include the most up-to-date information on virus identification
- Non-virologists teaching virology
- Lack of interaction with leading virologists.

I am sure you realize that not all these factors operate at each center where research is being done, none-the-less any single factor may impede progress.

In Table 1, I have listed the various steps required for the identification of plant viruses. I have slightly modified the schemes given earlier by Bos (1976) and Reddy (1980), especially in the light of an excellent report by Hamilton et al (1981) on the criteria for virus identification. Fortunately, the majority of plant virus diseases are caused by specific viruses. None-the-less it is becoming more apparent that mixed infections of unrelated viruses may be necessary for production of specific diseases. An excellent example is groundnut rosette disease where a luteovirus, a single-stranded RNA virus, and a satellite RNA are involved in etiology. Thus it is imperative, after purification of viruses through serial local lesion transfer, to determine that they can induce characteristic disease symptoms. In other words you should fulfil Koch's postulates. If indeed mixed infections of related non-sap transmitted viruses occur, they can often be separated by serial transmission through vectors (if they are insects) and through transmission to various hosts. By making isolations from local lesions and inoculation at nearly dilution end point, and by transmission to different hosts, it should be possible to separate mechanically transmitted viruses.

It is now regarded by some people as rather "old fashioned" to investigate the experimental host ranges of plant viruses. However, such studies can give vital information on occurrence of mixed infections and can be used to separate the viruses; they can provide local lesion hosts that are especially valuable for virus identification; and they can lead to identification of suitable hosts from which to extract virus for purification purposes.

Information on transmission is of course essential for formulating disease management practices.

Evidence has recently been presented to show that several RNA plant viruses can be identified by the analysis of doublestranded RNA (dsRNA) from infected tissues. The numbers and relative proportions of each dsRNA are characteristic for each virus and for each taxonomic group (Valverde *et al.*, 1986). dsRNA analysis is especially useful in cases where virus-like particles can not be detected. I should however mention that dsRNA, similar in size to those of some virus replicative RNA's, have been detected in healthy plant extracts (Wakarchuk and Hamilton, 1985; Sacks, W.A., I. Kirankumar, D.V.R. Reddy and Y. L. Nene (unpublished).

Serology is by far the most reliable method currently available for virus identification. When poor quality antisera are used extreme caution should be exercised in interpreting the results. Before publishing results it is essential to perform reciprocal tests using homologous antiserum and a range of heterologous antisera.

To elucidate the morphology of virus particles and their physico-chemical properties requires elaborate and expensive equipment. If such facilities and expertise are not

available, I would suggest that two alternative approaches be considered. These are to:

- Utilize facilities available in nearby institutes. Several institutes in India have excellent facilities for doing reserch in molecular biology (e.g., Center for Cellular and Molecular Biology in Hyderabad; Indian Institute of Science in bangalore; several universities supporting basic research). These facilities are adequate for virus characterization.
- Establish collabortive links with institutes where specialized virus research is being undertaken; e.g., International Agricultural Research Centers such as ICRISAT, if you are investigting viruses of their mandate crops; Virus laboratories located in IARI, New Delhi, the National botanical Research Institute in Lucknow, etc.). Laboratories located in Western Europe are ideal for handling problems on particularly difficult viruses occurring in countries with inadequate facilities for advanced virus research.

In the following section I suggest ways of improving the present situation in developing countries.

Improve physical facilities

They are listed in the Table 4.

Establish banks for antisera and for seed of diagnostic hosts

I do not need to emphasize the importance of having ready access to specific antisera and seed of diagnostic hosts. Hosts commonly used in the diagnosis of virus diseases are listed in the descriptions of plant viruses published by the Commonwealth Mycological Institute (CMI)/Association of Applied Biology (AAB), currently AAB, and in the Virus Identification Data Exchange (VIDE) for "Viruses of Legumes" (Boswell *et al.* 1986) (An updated version of VIDE on "Tropical Viruses" will soon be published). In addition, a list of diagnostic hosts for the identification of mechanically transmitted legume viruses has been produced by Hampton *et al.* (1978).

Good quality antisera can be purchased from the American type culture collection in the USA, and from several commercial companies. When you are writing to institutes for antisera, it is essential that you approach only those where reliable methods are being used for virus characterization. For producing high quality antisera it is essential to use purified viruses with minimal host contamination. None-the-less for vriuses such as tomato spotted wilt virus, it is difficult to produce high quality antisera. Under these circumstances it is essential to use serological techniques such as ELISA with modifications to minimize reaction from healthy plant components.

Antisera are extremely expensive to produce, and because of this it may be that requests to highly reputed virus laboratories for quality antisera, are ignored. This is a particular problem when the requests come from scientists in developing countries from laboratories that have not established a reputation in the field. Thus I believe that high

Organization of training courses

Training in the detection of viruses should be organized at regular intervals. Emphasis should be on techniques such as ELISA and extraction and analysis of proteins and nucleic acids. Every effort should be made to give 'hands on' experience. It would be particularly helpful if kits containing antisera, chemicals and supplies required for ELISA and a micropipette with suitable tips could be provided to participants at the end of the course so that they could process a limited number of sampels when they return to their own laboratories.

I consider training to be potentially the most important channel through which to introduce virus detection methods to research workers in developing countries.

Access to literature and data bases on virus identification

Reputed international journals which publish articles on plant virus diseases are often expensive and many libraries in developing countries cannot afford to subscribe to them. Several abstracting journals such as "Virology Abstracts", and "Review of Plant Pathology" can provide information on the papers published and by writing to appropriate libraries it will be possible to obtain photostat copies of specific articles. Very few authors now provide reprints on request.

One of the most important sources of reliable information on the identification of virus diseases is the VIDE (Gibbs *et al.* 1989). A system called "DELTA", which is specifically designed to handle all forms of taxonomic information, has been used to store the information in computers. Very soon "VIDE for Tropical Viruses" will be published. Information on over 500 characters was included for each virus. Extensive information on host range, which I believe is vital for virologists working in developing countries, is provided. All test species recorded in "CMI/AAB Descriprions of Plant Viruses" are included.

CMI/AAB (currently AAB) descriptions of plant viruses provided reliable information on individual viruses as well as on each taxonomic group of plant viruses.

I should like to mention a worldwide information service called Semi-Arid Tropical Crops Information Service, (SATCRIS), which has been established at ICRISAT, and has the objectives of providing wide and efficient access to information on the five crops mandated to ICRISAT. SATCRIS offers several Services, and its Selective Dissemination of Information (SDI) service alerts scientists and others to current information in their specific areas of interest regularly each month. The SDI service draws its information from two global databases, viz., CAB International and AGRIS which is the International Information System for Agriculutral Sciences and Technology of the FAO.

The SDI service is particularly beneficial to developing country scientists and researchers since it provides access to a broad spectrum of information from two of the most comprehensive databases in agriculture. Furthermore it is backed up with ondemand access to photocopies of original papers that users found relevant.

Work environment

Last, but not least, it is important that senior staff and administrators should strive to create an atmosphere conducive for productive research. Inhibiting scientists for expressing their views; leaving obstacles to research by not providing adequate staff and funds; victimization of staff, especially those who do not agree with all the views of their superiors etc. are complaints I have often heard from various categories of staff. It is also imperative that research workers on their part should strive to improve their own capabilities and those of their subordinates, create a pleasant and cordial atmosphere for staff of all levels to function within, work hard and be totally committed to their research.

CONCLUSIONS

Rushing to publish research findings that are not based on reliable techniques should be avoided. Collaboration with reputed virus laboratories, cooperation among colleagues working on similar problems, access to data bases on virus identification, access to antisera and seed of diagnostic hosts could all play vital roles in improving research on plant viruses in developing countries. Training in techniques for virusdetection should be organized at regular intervals and wide publicity should be given to this activity. This would enable deserving candidates to apply to undergo this training.

Research results obtained on the basis of sound experimental methods could lead to their publication in journals with international reputation. This will contribute substantially to the career development of research worker/s involved. Additionally it can bring prestige to the concerned Institution as well as to host country and indeed can lead to attraction of much needed financial support for research on virus diseases.

In conclusion it is gratifying for me to note that many research workers in India and other countries are eager to use reliable and sensitive methods for virus identification and detection.

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