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Suggested Procedures and Differential Hosts for Identifying Viruses

STANDARDIZED PROCEDURES, so important for scientific investigations, have not been fully employed in phytovirology. It is the purpose of this discussion (a) to indicate some areas of needed improvements in experimental methods, (b) to suggest procedures that may be helpful in diagnosing viral diseases and identifying or classifying viruses, and (c) to present a plea for working toward internationally standardized procedures.

Assumptions

The following basic assumptions are relative to this discussion:

1. Confirmation is necessary for full acceptance of new findings.
2. Species is the basic taxon for naming infectious agents.
3. Naming an infectious agent is necessary for intelligent discourse about the agent.
4. A useful and reliable classification of viruses must be based upon information obtained from procedures that are:
   a) applicable to all viruses,
   b) practical and available to a large majority of virologists, and
   c) standarized to the fullest feasible extent.

Characterization and Identification

The characterization or identification of the majority of plant viruses is largely limited to biological techniques based upon the range of sus-

ceptible hosts and the comparative responses of susceptible plant species, varieties, or clones to infection. Although a few viruses may be characterized and identified by such physicochemical techniques as serology, electron microscopy, sedimentation, and electrophoresis, many laboratories are not equipped for these techniques.

Standard Plants

Standard plants for determinative virology would provide a means of tentatively classifying all viruses since a virus must be transmissable by some method of inoculation. By inoculating a group of plant species, such as that in Table 1, with a virus and applying the information thus

TABLE 1. NUMBER OF VIRUSES KNOWN TO INFECT OR NOT INFECT SOME SPECIES OF PLANTS (INFORMATION FROM CODED-CARD FILE OF SUSCEPTIBLE AND INSUSCEPTIBLE PLANT SPECIES)

Species of plants	Number of viruses	
	Infecting species	Not infecting species
Beta vulgaris L.	33	62
Brassica oleracea L.	23	46
Chenopodium amaranticolor Coste & Reyn.	47	6
Cucumis sativus L.	67	77
Datura stramonium L.	70	67
Gomphrena globosa L.	26	16
Lycopersicum esculentum Mill.	60	76
Nicotiana glutinosa L.	78	67
Nicotiana tabacum L.	115	95
Phaseolus vulgaris L.	60	71
Pisum sativum L.	42	39
Solanum melongena L.	31	32
Solanum tuberosum L.	60	45
Vicia faba L.	35	40
Vigna sinensis Endl.	48	34
Zinnia elegans Jacq.	45	39

obtained to existing knowledge about susceptibility and insusceptibility, one should be able to determine whether the virus in question should be tentatively classified in a recognized group or as new. Final classification might require additional studies, but in many situations the tentative identification would satisfy the needs.

Standardized Procedures

Standarized procedures in this discussion relate to (a) genetic uniformity of test plants, (b) uniformity of the environment for the test plants prior to and after inoculation, insofar as possible, (c) inoculations to some or all of the standard test plants, and (d) comparison of results with information available on the susceptibility or insusceptibility of the test plants to plant viruses. The sequence of events and some details of the procedures are illustrated in Exhibit A.

Discussion and Plea

Standardization of some experimental methods in phytovirology is especially needed for ascertaining those properties that are used for viral classification. Without standardization, chaos in classification is inevitable. To achieve order it seems necessary to devise practical procedures and to obtain international agreement among virologists on what properties are to be criteria for classification. The core of the difficulty in obtaining these objectives seems a matter of mannerisms, attitudes, and customs rather than inherent difficulties in viral research.

Some suggested procedures are depicted in Exhibit A.

Agreement on criteria for classification seems obtainable if properties that are common to all viruses and within the scope of a majority of laboratories are selected for the primary stages of classification. The degree of subdivision of viruses into classes obviously would depend upon the information available and the amenability of any given virus to investigation. Infectivity is a basic property of all viruses, since transmission in series from an infected plant to noninfected plants by some method of inoculation is necessary to distinguish infectious agents from noninfectious agents as causes of diseases. By this process nonmicrobial infectious agents are classified in the category of viruses. By extending infectivity tests to a group of standard plants, one should be able to subdivide viruses into natural classes for which appropriate names could be given. Viruses that permit characterization by serology, electron microscopy, sedimentation, and electrophoresis could be classified into more specific groups as needed.

In addition to their usefulness in classification of viruses, these standard plants could be used as test plants in diagnosing the numerous specimens of diseases suspected to be viral infections. Some of the plants might even be used in assaying certain viruses.

A plea is made for international cooperation of phytovirologists to he end that a set of standard test plants (standard living culture media) be selected, adopted, maintained, and used for determining primary taxonomic properties of plant viruses. This cooperation seems attainable especially since the International Organization of Citrus Virologists has demonstrated such excellency in cooperation on viral problems in the citrus industry.

Exhibit A
Suggested Standardized Procedures for Determinative Phytovirology

1. Selection of a battery of about 15 standard test plants (species, varieties, selections, or hybrids) for the detection and basic classification of plant viruses.
2. Maintenance and increase of seed or clones of the test plants by methods that will assure reasonable genetic uniformity, freedom from virus, and a sufficient quantity for distribution internationally.
3. Production of healthy test plants in glasshouses or screenhouses that provide vector-proof conditions and reasonable control of factors influencing plant growth.
4. Inoculation of test plants by any feasible method (mechanical, dodder, tissue insertion, or living vectors).
5. Observations or records in the following order: First, plants insusceptible or susceptible for virus increase (– or +); Second, infection localized or systemic (viral invasiveness); Third, type of induced reactions (symptoms).
6. Comparison of results from inoculations with information in the literature relative to the selected test plants (Host index when available).
7. Exclusion of a number of viruses on the basis of insusceptibility of test plant (sequence of test-plant information is immaterial).
8. Exclusion of viruses on the basis of invasiveness in inoculated test plants.
9. Exclusion of viruses on the basis of comparative symptoms.
10. Tentative identification of virus as a known or new entity—whether similar or dissimilar to viruses not excluded by the procedure of elimination.