REVIEW ARTICLE

Guidelines for the Identification and Characterization of Plant Viruses


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INTRODUCTION

Several hundred virus diseases of plants have been described and apparent new ones continue to be reported. Often the causal agents are described as new viruses on totally inadequate grounds. Within the last 10 years, virologists in the Plant Virus Subcommittee of the International Committee on Taxonomy of Viruses have established 23 virus groups and two families (Matthews, 1979) (Table 1). An additional 200 or more viruses in various stages of characterization remain ungrouped.

When an unknown virus disease is being investigated, it has to be determined if the virus (or viruses) concerned is identifiable with any that are already known. This 'diagnostic phase', in some instances may not need to go beyond determination of particle morphology to determine to which group the virus belongs, followed by an appropriate serological test to identify the virus. If the virus under investigation is not easily assigned to any known taxon, the diagnostic phase passes into the full 'descriptive phase' in which every property possible is investigated, to be sure that the virus is 'new', to discover its relationships and to publish an adequate description. Thus, what is required of diagnosis and of description may be quite different.

The literature contains too many examples of known viruses re-described under new names, or of viruses so inadequately described that it is doubtful whether they really are 'new'. The first purpose of this paper is to outline some of the basic procedures that can be used for virus diagnosis and description; many of these procedures are described in greater detail in Kurstak (1981). A second purpose is to offer guidelines to authors and editors for the standards of data acquisition and presentation considered desirable in publishing descriptions and identifications of new viruses. In connection with both purposes, 'Guidelines for Bacteriophage Characterization' (Ackermann et al., 1978) has useful information and advice to offer. The references we give for techniques are for guidance and are not intended to be a comprehensive list.

In the present text we emphasize the use of techniques available in well-equipped laboratories, and we would stress the importance of applying such techniques wherever possible. However, for our many less-advantaged colleagues who will find these proposals impossibly Utopian, a modified version is in preparation (Bock et al.) that will indicate what can and ought to be done using simpler facilities.
### Table 1. Basic characteristics of the groups of plant viruses*

<table>
<thead>
<tr>
<th>Group</th>
<th>Type member</th>
<th>Particle dimension (nm)†</th>
<th>Genome (mol. wt. × 10^-6)</th>
<th>Capsid proteins (mol. wt. × 10^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobravirus</td>
<td>Tobacco rattle virus</td>
<td>(E) 180–215 and 46–114 × 22</td>
<td>ssRNA (2) 2.4, 0.6–1.4</td>
<td>22</td>
</tr>
<tr>
<td>Tobamovirus</td>
<td>Tobacco mosaic virus</td>
<td>(E) 300 × 18</td>
<td>ssRNA 2.0</td>
<td>17–18</td>
</tr>
<tr>
<td>Hordeiviruses</td>
<td>Barley stripe mosaic virus</td>
<td>(E) 100–150 × 20</td>
<td>ssRNA (2–4) 1.1–1.5</td>
<td>21</td>
</tr>
<tr>
<td>Potexvirus</td>
<td>Potato virus X</td>
<td>(E) 470–580 × 13</td>
<td>ssRNA 2.1</td>
<td>18–27</td>
</tr>
<tr>
<td>Carlaviruses</td>
<td>Carnation latent virus</td>
<td>(E) 620–700 × 13</td>
<td>ssRNA 2.3</td>
<td>30</td>
</tr>
<tr>
<td>Potyvirus</td>
<td>Potato virus Y</td>
<td>(E) 680–900 × 11</td>
<td>ssRNA 3.0–3.5</td>
<td>32–36</td>
</tr>
<tr>
<td>Closteroviruses</td>
<td>Beet yellows virus</td>
<td>(E) 600–2000 × 10</td>
<td>ssRNA 2.3–4.3</td>
<td>23</td>
</tr>
<tr>
<td>Tobacco necrosis virus</td>
<td>Tobacco necrosis virus</td>
<td>(I) 28</td>
<td>ssRNA 1.3</td>
<td>23</td>
</tr>
<tr>
<td>Southern bean mosaic virus</td>
<td>Southern bean mosaic virus</td>
<td>(I) 28–30</td>
<td>ssRNA 1.4</td>
<td>30</td>
</tr>
<tr>
<td>Tomovirus</td>
<td>Tomato bushy stunt virus</td>
<td>(I) 30</td>
<td>ssRNA 1.5</td>
<td>41</td>
</tr>
<tr>
<td>Tymovirus</td>
<td>Turnip yellow mosaic virus</td>
<td>(I) 29</td>
<td>ssRNA 2.0</td>
<td>20</td>
</tr>
<tr>
<td>Luteovirus</td>
<td>Barley yellow dwarf virus</td>
<td>(I) 25</td>
<td>ssRNA 2.0</td>
<td>24</td>
</tr>
<tr>
<td>Pea enation mosaic virus</td>
<td>Pea enation mosaic virus</td>
<td>(I) 28</td>
<td>ssRNA (2) 1.7, 1.3</td>
<td>(2) 22, 28</td>
</tr>
<tr>
<td>Comovirus</td>
<td>Cowpea mosaic virus</td>
<td>(I) 28</td>
<td>ssRNA (2) 2.0, 1.4</td>
<td>(2) 25, 44</td>
</tr>
<tr>
<td>Nepovirus</td>
<td>Tobacco ringspot virus</td>
<td>(I) 28</td>
<td>ssRNA (2) 2.4, 1.4–2.2</td>
<td>55–60§</td>
</tr>
<tr>
<td>Bromovirus</td>
<td>Brome mosaic virus</td>
<td>(I) 26</td>
<td>ssRNA (3) 1.1, 1.0–0.7</td>
<td>20</td>
</tr>
<tr>
<td>Cucumovirus</td>
<td>Cucumber mosaic virus</td>
<td>(I) 28</td>
<td>ssRNA (3) 1.27, 1.13, 0.82</td>
<td>24</td>
</tr>
<tr>
<td>Ilarvirus</td>
<td>Tobacco streak virus</td>
<td>(I) 26–35</td>
<td>ssRNA (3) 1.1, 0.9, 0.7</td>
<td>25</td>
</tr>
<tr>
<td>Alfalfa mosaic virus†</td>
<td>Alfalfa mosaic virus</td>
<td>(B) 28–58 × 18</td>
<td>ssRNA (3) 1.1, 0.8, 0.7</td>
<td>24</td>
</tr>
<tr>
<td>Plant rhabdovirus‖</td>
<td>Lettuce necrotic yellows virus</td>
<td>(B) 160–380 × 50–95</td>
<td>ssRNA (1) 4–0</td>
<td>(5) 19–171</td>
</tr>
<tr>
<td>Tomato spotted wilt virus ‡</td>
<td>Tomato spotted wilt virus</td>
<td>(I) 85</td>
<td>ssRNA (4) 2.6, 1.9, 1.7, 1.3</td>
<td>(5) 27–90</td>
</tr>
<tr>
<td>Phytophlovirus‡</td>
<td>Wound tumour virus</td>
<td>(I) 70</td>
<td>dsRNA (12) 0.3–3.0</td>
<td>(7) 35–160</td>
</tr>
<tr>
<td>Fijiivirus‡</td>
<td>Fiji disease virus</td>
<td>(I) 71</td>
<td>dsRNA (10) 1.1–2.9</td>
<td>(7) 64–139</td>
</tr>
<tr>
<td>Caulimovirus</td>
<td>Cauliflower mosaic virus</td>
<td>(I) 50</td>
<td>dsDNA 4.8–5.0</td>
<td>37</td>
</tr>
<tr>
<td>Gemini virus</td>
<td>Maize streak virus</td>
<td>(I) 18 × 30</td>
<td>ssDNA (2) 0.7–0.8</td>
<td>28–34</td>
</tr>
</tbody>
</table>

* Detailed properties of groups are described in Matthews, 1979; for description of group members, see Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses 1 to 215.
† E, Elongated particles; I, isometric particles (mean diameters); B, bacilliform particles.
‡ Grouping, but not group name, has been approved by the International Committee on Taxonomy of Viruses.
§ May be a stable tetramer; see Chu & Francki, 1979.
‖ Ungrouped member of the Rhabdoviridae.
¶ Genus of the Reoviridae.
** Particles appear to be constructed from two incomplete icosahedra; see Hatta & Francki, 1979.
Information useful in identifying a virus or deciding that it is 'new', and proceeding to a full description

The information to be sought during both the diagnostic and descriptive phases has been somewhat arbitrarily divided into two sections: firstly, what can be learned from virus in situ or from crude virus preparations and secondly, from purified virus preparations.

(A) Using crude virus preparations or virus in situ

1. Reproduction of the disease using the isolated virus

It often happens that plants are infected by one or more viruses that may or may not cause overt disease, and isolation of a virus from a diseased plant is no guarantee that the agent of the disease has been found. It is important, therefore, first to be sure that the causal virus has been isolated, i.e. that there is not a mixture of viruses present.

Two processes may be required: (i) testing for possible mixtures by inoculation of a series of specific indicator plants, and by serology, light or electron microscopy or a combination of these, and (ii) separation of mixtures by using differential vectors, test plants or single local lesion passages. Finally, the isolate should be re-inoculated to the original host which is then observed for typical symptoms of the disease. It is well to keep in mind that certain diseases are the consequence of mixed virus infection; failure to induce typical symptoms with one of several viruses isolated might suggest the possibility of two or more viruses as the causal agents. Inoculation of purified virus is desirable in order to 'work within the spirit' of Koch's postulates and great care should be exercised to prevent contamination of the preparation by other viruses being studied in the laboratory.

2. Host range

This is generally not a very precise or reliable guide to virus identification, and exhaustive host-range tests are usually no longer appropriate. However, the host plant in which the virus occurs may point to the literature (such as the Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses) on candidate viruses and in certain instances narrows the field considerably. In any case a preliminary host-range study is useful in discovering convenient laboratory hosts for subsequent multiplication, purification and tests of infectivity.

3. Symptom expression

As with host range, symptom expression is not usually a precise indication of virus identity and its interpretation should be treated with caution. Sometimes the type of symptom may be indicative of a distinct virus or virus group (e.g. pea enation mosaic virus). Environmental factors, especially light and temperature, can often have over-riding influences on the symptoms expressed, as can the strains or lines of test plants, such as Chenopodium amaranticolor or tobacco 'White Burley', that are used (van der Want et al., 1975). Minor strain differences between virus isolates may also be responsible for large symptom differences, while widely differing viruses may produce similar effects, e.g. the 'shoestring' symptom in tomato that may be caused by cucumber mosaic or tomato mosaic viruses and the 'yellows type' symptoms which may be caused by luteoviruses, closteroviruses, as well as by mycoplasma-like organisms. A short description of symptoms, therefore, may have its place but ought not to be treated as a substitute for more reliable and informative data.

4. Mode of transmission

Whether a virus is mechanically transmissible or not may be an important diagnostic feature although some viruses are only mechanically transmitted using special additives and
inoculation techniques. Almost invariably, the natural means of transmission is highly
diagnostic, as indicated in reviews by Costa (1976), Bird & Maramorosch (1978), Pirone &
Harris (1977), Harrison (1977) and Maramorosch & Harris (1979). There are also virus
groups without specific vectors, or for which the vectors are not known, or for which the main
route of transmission is by pollen, seed or vegetative propagation.

As the search for a vector may be long and painstaking, the results of other diagnostic
methods usually establish the identity of the virus and its relationship to others; such results
may also facilitate vector identification. Careful study of the mode of transmission is
nevertheless indispensable to the adequate description of a 'new' virus.

5. Type(s) of particle observed in negative stain by electron microscopy

Electron microscopy of particles in negative stain (Horne, 1967) is often very useful at the
'crude extract' stage, and sufficient diagnostic (though not fully descriptive) information can
often be obtained, especially with the aid of immunoelectron microscopy. Better particle detail
is usually seen in purified preparations; for convenience the electron microscopy of these and
crude preparations are discussed in section (B) 1.

6. Cytopathology by light and electron microscopy

While cytopathology at the electron microscope level is generally more definitive, certain
advantages of the light microscope can make it a useful aid to diagnosis (Christie &
Edwardson, 1977; Edwardson & Christie, 1979; Fraser & Matthews, 1979). The light
microscope is easy to use, is readily available and has a large field of view permitting efficient
sampling of tissues. Many cytochemical and enzyme digestion tests, more difficult at the
electron microscope level, are available for the light microscope. Inclusions may be observed
in fresh, unfixed preparations, and the effects, sometimes destructive, of fixatives for use in
either type of microscopy can be observed as they occur. For diagnosis, the technique should
be used as an adjunct to electron microscopy and to other methods, rather than on its own.

Electron microscopic examination of inclusions can assist the rapid identification and
characterization of many plant viruses. Some individual viruses can be distinguished by the
inclusions they induce (Edwardson & Christie, 1978; Martelli & Russo, 1977; Maramorosh,
1977). Viruses within nine groups induce distinctive inclusions which are diagnostic for
infections at the group level (Table 2) and individual viruses within a group may sometimes
produce distinctive effects (Hull et al., 1970; Christie & Edwardson, 1977; Lesemann, 1977;
Gill & Chong, 1979; Shalla et al., 1980; Hatta & Francki, 1981a, b).

It should be noted that the classical use of 2 to 5% glutaraldehyde followed by 1% osmium
tetroxide as fixatives for electron microscopy can severely disrupt crystalline arrays of virus
particles such as those of tobacco mosaic virus (TMV) and the banded-body inclusions of the
potexviruses. However, fixation of tissues infected with these viruses, and perhaps others, in
unbuffered permanganate or in dilute (0.02 to 0.08%) osmium tetroxide (Warmke &
Christie, 1967) or in mixtures of glutaraldehyde and uranyl acetate (UA) (Hills & Plaskitt,
1968) causes less disruption of particle arrays although other cytological features may not be
so well preserved. Recommendations for description of cytopathology are as follows. (i) Host
species and cultivars, types of tissue, cells and cellular components should be specified, as
should the cultural conditions, age of infection, type of inoculation and whether the tissue was
directly inoculated or systemically invaded. (ii) The effects of the virus should be investigated
in several hosts if possible and at different times after inoculation. Inclusions, wall
modifications and so on should be visualized and described as three-dimensional, not
two-dimensional objects. Their appearance should be noted by light microscopy when
unfixed, and when fixed for both light and electron microscopy. For electron microscopy,
alternative fixation procedures should be used, with correlation of results from both types of
Table 2. *Cytopathic effects induced by viruses in different groups*†

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of members and possible members</th>
<th>Cytopathic effects</th>
<th>Group main characteristic</th>
<th>Inclusions† diagnostic for infection at the group level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobravirus</td>
<td>2</td>
<td>Insufficiently characterized</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tobamovirus</td>
<td>16</td>
<td>Cytoplasmic crystalline stacked plates</td>
<td>+ §</td>
<td>+</td>
</tr>
<tr>
<td>Hordeivirus</td>
<td>3</td>
<td>Particle aggregates predominantly cytoplasmic</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Potexvirus</td>
<td>24</td>
<td>Cytoplasmic banded bodies</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Carlavirus</td>
<td>21</td>
<td>Cytoplasmic banded bodies</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Potyvirus</td>
<td>73</td>
<td>Cytoplasmic cylindrical inclusion</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Closterovirus</td>
<td>12</td>
<td>Banded bodies predominantly in phloem</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tobacco necrosis virus</td>
<td>2</td>
<td>Cytoplasmic crystalline inclusions</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Maize chlorotic dwarf virus</td>
<td>2</td>
<td>Insufficiently characterized</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Southern bean mosaic virus</td>
<td>5</td>
<td>Particle aggregates occur in cytoplasm and nuclei</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tombusvirus</td>
<td>8</td>
<td>Cytoplasmic crystalline inclusions</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Tymovirus</td>
<td>16</td>
<td>Double membrane-bound vesicles in chloroplasts, clumped chloroplasts, empty protein shells in nuclei</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Luteovirus</td>
<td>28</td>
<td>Insufficiently characterized but particles confined to phloem tissues</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pea enation mosaic virus</td>
<td>1</td>
<td>Double membrane-bound vesicles extruded into cytoplasm from nuclear membranes</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Comovirus</td>
<td>12</td>
<td>Vesiculate-vacuolate and cytoplasmic crystalline inclusions</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Nepovirus</td>
<td>18</td>
<td>Vesiculate membranous bodies, particles aligned within tubules</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Bromovirus</td>
<td>3</td>
<td>Cytoplasmic crystalline inclusions</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Cucumovirus</td>
<td>4</td>
<td>Cytoplasmic crystalline aggregates predominantly in mesophyll; characteristic vesicles associated with tonoplast</td>
<td>+ ¶</td>
<td>—</td>
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<td>Ilarvirus</td>
<td>17</td>
<td>Insufficiently characterized</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Alfalfa mosaic virus</td>
<td>1</td>
<td>Hexagonally packed layers of particles</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Plant rhabdovirus</td>
<td>49</td>
<td>Particle aggregates in perinuclear space, or cytoplasmic vesicles</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Tomato spotted wilt virus</td>
<td>1</td>
<td>Particle aggregates enclosed in membranes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytooreovirus</td>
<td>2</td>
<td>Particles in tubules, granular-fibrillar viroplasms, particles in crystalline array</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Fijiivirus</td>
<td>9</td>
<td>Particles in tubules, granular-fibrillar viroplasms, particles in crystalline array</td>
<td>+</td>
<td>(+)**</td>
</tr>
<tr>
<td>Caulimovirus</td>
<td>7</td>
<td>Viroplasms containing particles</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Geminivirus</td>
<td>9</td>
<td>Insufficiently characterized</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

| Total                         | 345                                    |                                                                                   |                           |                                                        |

* Columns 1 to 4 adapted from Matthews (1979). † Column 5 adapted from Edwardson & Christie (1978).
‡ –, Inclusions are not main characteristics, or are not diagnostic. § +, Inclusions are main characteristics and are diagnostic.
¶ Hatta & Francki, 1981 b. ** (+), Inclusions cannot be used to distinguish phytoreoviruses from fijiviruses; they can be used to distinguish these viruses from viruses in other groups.
microscopy. (iii) Because of fixation-induced artefacts, thin-sectioning should not be used as the sole basis for diagnosis.

7. Reactions with likely antisera

Rapid and accurate diagnosis is often possible directly on crude preparations using precipitin tests, immunosorbent assays (Voller et al., 1976, 1979) and various versions of immuno-electron microscopy (Milne & Luisoni, 1977). This is highly cost-effective and renders many of the other diagnostic tests redundant, providing of course that a clear positive answer can be obtained. For convenience, serological methods which can be applied to both crude and purified preparations are considered in detail in section (B) 2.

8. 'In vitro properties' and cross-protection tests

Although these methods are still used in some laboratories for diagnostic and taxonomic purposes, they have largely outlived their usefulness. None of the 'in vitro properties' including thermal inactivation point (TIP), longevity in vitro (LIV) and dilution endpoint (DEP) is a good taxonomic indicator, and this is especially true for viruses that are neither particularly stable nor unstable (Francki, 1980). For example, a virus whose TIP is between 65 and 70 °C could belong to any one of 14 different virus groups; one whose LIV is between 1 and 2 days, any one of 14; and whose DEP is between 10⁻⁴ and 10⁻³, any one of 18 groups. Although 'in vitro properties' are of very limited value in diagnosis, they may be useful in separating mixtures of viruses or for designing purification schemes.

Cross-protection tests have not been used as commonly as have the 'in vitro properties' in diagnostic and taxonomic work, probably because they are technically more difficult. Although there are examples of good correlation between degrees of cross-protection and serological relationships (Matthews, 1949), there are also many instances of incomplete, or no detectable cross-protection between virus strains shown to be antigenically related (Kassanis, 1963), as well as examples of cross-protection between obviously unrelated viruses (Kassanis, 1963; Hamilton, 1980).

(B) Using purified preparations

Purification, with its attendant aims of high quality and quantity, is often the major stumbling block in virus characterization. Precipitation of viruses from clarified extracts with polyethylene glycol (PEG) (Hebert, 1963; Gooding & Hebert, 1967) can often furnish virus of sufficient quantity and purity for limited characterization by electron microscopy, sedimentation and serology. However, highly purified preparations should be used for determination of the physicochemical properties of the virus and its protein and nucleic acid components and for the production of high quality antisera.

The choice of extraction buffer and additives can greatly influence the outcome of purification attempts. Extraction by a sap press or pestle and mortar rather than by a rotary homogenizer may reduce breakage of elongated viruses, especially closteroviruses (Bar-Joseph & Hull, 1974). Alkaline buffers (pH 8 to 9) of moderate ionic strength are generally preferred for extraction of elongated viruses which tend to aggregate or to be adsorbed to cellular debris (e.g. potyviruses) although alkaline buffers can break up some viruses with elongated particles, e.g. TMV (Francki, 1966). Acidic buffers of about pH 5 are useful for the extraction of some isometric viruses with the added advantage that most host proteins are precipitated from the extract. However, isometric viruses with isoelectric points above pH 5 are precipitated by this treatment, e.g. cucumber mosaic and tobacco ringspot viruses. Where pH-induced clarification may result in low virus yields, the use of chloroform:butanol (Steere, 1956) or detergents (Triton X-100, Nonidet P40) may be advantageous. Yields of some phloem-restricted luteoviruses can be enhanced by disruption of leaf tissue with a
mixture of cellulases and pectinases (Takanami & Kubo, 1979), whereas roots with their higher phloem content are a better source of some fijiviruses. Concentration of the virus by ultracentrifugation is commonly done but excessively high gravitational force may also cause breakage of flexuous rods (Huttinga, 1973); precipitation of viruses by PEG circumvents the problem but some viruses may not resuspend completely after precipitation. Final purification, especially of viruses contaminated by host materials, is often by rate-zonal centrifugation in sucrose or by isopycnic sedimentation in gradients of sucrose or caesium salts. A useful preservative for purified virus preparations is chlorbutanol (1,1,1-trichloro-2-methyl-2-propanol) which does not affect the biological properties of non-enveloped viruses nor is there interference in u.v. absorption spectrophotometry of preparations containing the compound. For detailed information on virus purification, see Francki (1972), Schumaker & Rees (1972), Venekamp (1972), Noordam (1973) and Gibbs & Harrison (1976). Specific information on purification methods for over 200 described viruses is contained in the Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses.

1. Fine structure of the particles in the electron microscope

One of the fastest ways to assign a particular virus to a taxonomic group is to examine preparations by electron microscopy using the negative stain procedure (Horne, 1967). Good photographs of viruses in negative stain are found in Maramorosch (1977) and Williams & Fisher (1974). Depending on particle morphology and size, a virus can be tentatively assigned to a taxonomic group; viruses with elongated particles are especially easy to assign in this manner because the size range of most groups of such viruses is sufficiently distinct from that of other groups (Brandes & Bercks, 1965) (Table 1).

The particle outline of isometric viruses is also useful in preliminary separation, e.g. round and smooth (cucumo, bromo); round and knobby (tombus, tymo); ovoid or imperfectly spherical (ilar); angular (tobacco necrosis, nepoviruses, comoviruses, broadbean wilt). Particle outline is best determined on solitary virus particles because those in close array will invariably exhibit an angular outline.

For accurate description of a virus, the following recommendations are made regarding both crude and purified preparations: data presented should include details of host, extraction method, final resuspension buffer and preparation for electron microscopy. The minimum acceptable 'portrait' of a virus should be based on micrographs of good contrast, magnification not less than about $\times$ 200,000, essentially free of astigmatism, drift and focus errors, and not too burnt or contaminated. The results of using both UA and phosphotungstic acid (PTA) should be given, and use of additional stains (see below) or techniques would be helpful.

Data for elongated viruses should include the following. (i) A histogram of particle lengths measured in UA and in PTA. Each histogram should be derived from measuring not less than 100 particles preferably from crude preparations (e.g. leaf dips) where particle breakage or aggregation is much less likely than in purified preparations. Any differences due to host plants, extraction methods, final resuspension buffer or negative staining should be carefully noted. The magnification should be calibrated carefully and the method stated. Suitable methods would be: use of diffraction grating replicas, crystals such as catalase (Wrigley, 1968) or virus particles such as TMV [modal length 300 nm (Bos, 1975) and/or helix pitch 2.3 nm (Zaitlin & Israel, 1975)], with use of more than one method desirable. Particle lengths should be quoted as modal lengths. Where purified or fractionated material is used, evidence of infectivity (separately or in combination with other fractions) should be given where possible. (ii) Estimates of particle diameter. (iii) Information on flexuousness, e.g. rigid (tobamo, tobra, hordei), slightly flexuous (potex, carla), flexuous (poty), very flexuous...
Table 3. Fine structure characteristics of elongated viruses

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rigid rods, fine helix (pitch 2-3 nm) easily obscured by beam damage; clear axial canal</td>
<td>Tobamovirus</td>
</tr>
<tr>
<td>Rigid rods, robust helix (pitch 2.5 nm) easily seen; clear axial canal</td>
<td>Tobravirus, hordeivirus</td>
</tr>
<tr>
<td>Slightly flexuous rods with clear cross-banding (pitch 3.4 nm); longitudinal files of subunits often seen; axial canal sometimes visible</td>
<td>Potexvirus</td>
</tr>
<tr>
<td>Slightly flexuous rods (pitch 3-4 nm) showing about four clear longitudinal furrows between files of subunits; primary helix (cross-banding) usually not seen; axial canal usually not visible</td>
<td>Carlavirus</td>
</tr>
<tr>
<td>Flexuous rods (pitch 3.4 nm); usually no fine structure visible, but fine cross-banding and higher order helices visible in very good pictures; axial canal usually not visible</td>
<td>Putyvirus</td>
</tr>
<tr>
<td>Very flexuous rods; very open and obvious primary helix (pitch 3-7 nm); axial canal not visible</td>
<td>Closterovirus</td>
</tr>
<tr>
<td>Flexuous rods, but fine structures unlike any of the above. Viruses include those listed and possibly others. Not necessarily a homogeneous category</td>
<td>Potato virus T., apple stem grooving virus, lilac chlorotic leafspot virus</td>
</tr>
</tbody>
</table>

(clostro) (Table 3). (iv) Fine structure, e.g. presence/absence and type of cross-banding (i.e. morphology of primary helix) including visibility of higher order helices and Moiré effects due to contrasting of both sides of the particle; estimate of pitch, preferably with optical diffraction data; presence/absence of longitudinal files of subunits and of a visible axial canal; careful note of whether both ends of intact particles appear the same.

Data for ‘spherical’ viruses should include the following. (i) Evidence of the infectivity of the preparation examined, where possible and appropriate. (ii) The particle outline: round, ovoid, knobby, more or less angular, sharply angular. (iii) Particle diameter(s). With angular particles, dimensions should be quoted as measured side-to-side and corner-to-corner. Histograms are generally unnecessary, but care should be taken with magnification calibration. (iv) Surface detail: subunits, ‘eyes’ (artefactual areas of contrast due to superimposition of images of lower and upper faces of particles), granularity, central dimples; number and geometrical arrangement of subunits where discernible; views down proposed axes of symmetry, with supporting models, may be useful. (v) Presence or absence of particles penetrated by stain. (vi) Tolerance to UA and PTA.

Other kinds of virus, e.g. rhabdoviruses, alfalfa mosaic, tomato spotted wilt, carrot mottle, should be described at a similar level, as appropriate. Some problems in the interpretation of rhabdovirus structure are discussed by Francki & Randles (1975, 1980).

The particles of some viruses (cucumo-, ilar-, gemini-, alfalfa mosaic, tomato spotted wilt) are destroyed while others (como-, some reo- and closteroviruses) are damaged in PTA (2% phosphotungstic acid brought to pH 6.5 to 7 with NaOH or KOH) whereas their structural details are preserved in UA (2% aqueous unbuffered uranyl acetate pH ≤ 4.2). Such viruses are often salt-labile. Staining with PTA or UA may also damage some rhabdoviruses. A partial solution to this problem is to fix viruses on the grid using 1% glutaraldehyde in 0.01 to 0.1 M-phosphate pH 7 at room temperature for 15 min before negative staining. Useful alternative negative stains are PTA at pH 4 or 4.5, ammonium molybdate at pH 6-5, sodium tungstate adjusted to pH 6-5 with formic acid and unbuffered methylamine tungstate pH 6.4 (Fabergé & Oliver, 1974).

2. Serology

(a) Precipitin tests. Antigenic analysis of isometric viruses is perhaps best done by precipitin tests (e.g. Ouchterlony test) in agar gels (Bercks et al., 1972; Ball, 1974).
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elongated viruses, and those which have a strong tendency to aggregate or diffuse poorly into the agar, can be compared either by precipitin tests in liquid medium, e.g. the tube or preferably the slide precipitin tests (Bercks et al., 1972) or in the agar gel double-diffusion test after breakdown of particles with detergents (Purcifull & Batchelor, 1977), pyrrolidine (Shepard et al., 1974) or by sonication (Tomlinson et al., 1965). Antisera to the intact viruses do not always react with the degradation products; under these circumstances, it may be necessary to use degraded antigens for immunization (van Regenmortel, 1967; von Wechmar & van Regenmortel, 1968; Moghal & Francki, 1976; Chu & Francki, 1979).

In the tube and slide precipitin tests as well as in the agar gel double-diffusion test, viruses at low concentrations are compared on the basis of serum titres observed in homologous and heterologous reactions. Homologous serum titres are usually higher than heterologous ones and the number of twofold dilution steps separating the two titres is the serological differentiation index (SDI; van Regenmortel, 1975; Koenig, 1976). The SDI may serve as a measure of the degree of serological relatedness of two viruses. However, SDIs obtained with antisera from different rabbits or even different bleedings of the same rabbit may vary considerably; thus for semi-quantitative estimates a number of sera should be used. Whether two viruses are serologically very similar or very different can usually be determined with a single antiserum but if a low SDI is observed in tube or slide precipitin tests, it may be difficult to decide whether two viruses are serologically identical or only similar. In doubtful cases a serum may be adsorbed with the heterologous antigen until reactivity is no longer observed. A positive reaction between the adsorbed serum and the homologous antigen would indicate a serological difference between the two antigens.

In the agar gel double-diffusion test comparisons between closely related antigens are easier. Under balanced conditions (see e.g. Bercks et al., 1972; Hollings & Stone, 1975; Paul et al., 1980b) a fusion of precipitin lines usually indicates that the antigens are serologically identical, provided that the antiserum used is homologous for one of the antigens. Spur formation under balanced conditions indicates antigenic differences. The resolving power of the agar gel double-diffusion test can be further refined by intragel cross-absorption (van Regenmortel, 1967) which may reveal small differences between antigens not seen in the spur formation test. Immunoelectrophoresis may also reveal electrophoretic differences between antigens.

(b) Immunoelectron microscopy. Immunoelectron microscopical methods (e.g. Derrick & Briansky, 1976; Milne & Lesemann, 1978; Roberts et al., 1980; Conti et al., 1980; Nicolaieff & van Regenmortel, 1980; Lesemann & Paul, 1980) have the great advantage of being able to be applied to tissue homogenates and of requiring very low quantities of virus and antiserum. Relationships can be studied in different ways: (i) by the differential trapping of virus particles on electron microscope grids coated with antisera to different viruses; (ii) by endpoint dilution of an antiserum which effectively coats (decorates) the virus particles; (iii) by the observation of a clumping reaction at different serum concentrations. Pretreatment of grids with protein A of *Staphylococcus aureus* may enhance virus adsorption (Shukla & Gough, 1979) but meaningful results occur only under certain conditions (Lesemann & Paul, 1980; Milne, 1980). It may be useful to follow trapping of virus particles on serum-coated grids with decoration (Milne & Luisoni, 1977), particularly as trapping may be susceptible to bias due to non-specific adsorption of virus particles to the grids (Lesemann et al., 1980). Decoration of particles by antisera to distantly related viruses may be difficult to distinguish from non-specific decoration, however, especially in crude sap.

(c) Immunosorbent assays. The direct double-antibody sandwich form of ELISA (Clark & Adams, 1977) is apparently more specific than the usual precipitin tests for some viruses (Koenig, 1978; Rochow & Carmichael, 1979; Bar-Joseph & Salomon, 1980; Uyemoto, 1980) probably because of decreased avidity of enzyme-conjugated antibodies (Koenig,
This disadvantage can be overcome by the use of indirect ELISA methods (van Regenmortel & Burckard, 1980; Koenig, 1981) or of ¹²⁵I-labelled antibodies (Ghabrial & Shepherd, 1980). The application of monoclonal antibodies, already reported for animal viruses (Roehrig et al., 1980), may also be useful in antigenic comparisons of plant viruses.

3. Sedimentation properties

The sedimentation behaviour of viruses, whether determined by conventional analytical ultracentrifugation (Markham, 1967) or by gradient centrifugation in preparative centrifuges (Brakke, 1967) can yield information on important taxonomic characteristics, i.e. number of sedimenting components, their sedimentation coefficients and buoyant densities. Multi-component viruses such as the comoviruses can be distinguished from viruses with rather similar morphology, such as tobacco necrosis virus which sediments as a single species, and this is especially useful in categorizing spherical viruses. Rate-zonal centrifugation for determination of sedimentation coefficients or sedimentation equilibrium centrifugation for determination of buoyant densities, is more conveniently done in swinging bucket or angle rotors respectively in a preparative centrifuge primarily because of lower equipment costs and also because the results of infectivity assays can be correlated with a particular component, or mixture of components. For viruses that occur in high concentration in plant tissues, the analytical ultracentrifuge can be used to advantage with clarified crude sap (Markham, 1962) to give preliminary information about the number, sedimentation rate and concentration of sedimenting species.

(a) Rate-zonal centrifugation. Sucrose is the principal compound used to form the concentration gradients. Although glycerol gradients have a higher viscosity than those of sucrose gradients, they have the advantage that virus samples can be examined in the electron microscope without rinsing or dialysis as is necessary with other gradient media. Attention must be given to the composition of the buffer because of the possibility of affecting sedimentation properties [e.g. bromoviruses, Brakke, 1963; Lane, 1974; members of the southern bean mosaic virus (SBMV) group, Hull, 1977b; other viruses, Tremaine, 1977] although conditions leading to virion instability can be diagnostic. Sedimentation coefficients can be accurately and simply determined in conventional gradients (Brakke, 1958) or in ‘linear-log’ gradients (Brakke & van Pelt, 1970), using well-characterized markers such as TMV and SBMV. For details on constructing ‘linear-log’ gradients, see Jackson et al. (1977).

(b) Equilibrium centrifugation. Viruses can be sedimented to equilibrium in solutions of the salts of heavy metals, primarily CsCl and Cs₂SO₄. The buoyant densities of virions are calculated from the refractive indices of collected fractions using appropriate tables relating refractive index and density (International Critical Tables: Anderson & Anderson, 1973). Some viruses are unstable in CsCl and are better centrifuged in Cs₂SO₄ (Hull, 1976) although multiple bands may result from what should, by other criteria, be monodisperse components (Hull, 1977a).

4. Analysis of the nucleic acid

For reviews on isolation and properties of plant virus nucleic acids, see Hull (1979), Zaitlin (1979) and Lane (1979). Extraction of nucleic acid by phenol–SDS procedures (Brakke & van Pelt, 1976; Peden & Symons, 1973) or by SDS–perchlorate (Wilcockson & Hull, 1974) is simple and efficient.

(a) Type of nucleic acid. Viral DNA can be distinguished from RNA by its lower buoyant density in gradients of caesium salts (Birnie & Rickwood, 1978), its differential sensitivity to DNase and pancreatic RNase, and by its base composition. A simple procedure is to treat the
nucleic acid preparation with nucleases and then electrophorese the products. Alternatively, gels containing native viral nucleic acid can be soaked in the enzyme solutions (Morris & Dodds, 1979). The major problems with enzymic methods are insufficient deproteinization of the nucleic acid and impure nucleases. DNase contaminated by RNase is difficult to purify, but DNase can be inactivated in RNase preparations by heating at 100 °C for 10 to 15 min.

(b) Strandedness. Whether the nucleic acid is single-stranded (ss) or double-stranded (ds) can be determined by its melting behaviour (Shepherd et al., 1970) or by using nucleases and gel electrophoresis (Morris & Dodds, 1979; Luisoni et al., 1979). Double-stranded RNA can be distinguished from ssRNA by its stability to RNase as high ionic strength (e.g. in 0-3 M-NaCl) and its lability to the enzyme at low ionic strength. As a rule, dsDNA can be distinguished from ssDNA by its sensitivity to dsDNA-specific restriction endonucleases such as EcoRI and HindIII (Smith & Nathans, 1973); however, certain restriction endonucleases can cut ssDNA (Godson & Roberts, 1976). Single-stranded DNA such as that occurring in the geminiviruses can be hydrolysed by S1 nuclease under conditions where calf thymus DNA is unaffected (Goodman, 1977). Another method of determining strandedness depends on the effect of temperature on the hyperchromicity or a shift in the wavelength of maximum absorption of ultraviolet light following reaction of single-stranded nucleic acids with formaldehyde (Fraenkel-Conrat, 1954; Sinsheimer, 1959).

(c) Number and size of polynucleotides. These values are conveniently estimated by gel electrophoresis using the tris–phosphate–EDTA buffer system of Loening (1969) or the tris–borate–EDTA buffer of Peacock & Dingman (1967). Extensive secondary structure of ssRNAs may be a problem in accurate estimation of mol. wt. but such base-pairing can be reduced by the use of glyoxal (McMaster & Carmichael, 1977), methyl mercuric hydroxide (Bailey & Davidson, 1976), or 8 M-urea (Murant & Taylor, 1978); extreme care is recommended in the use of methyl mercuric hydroxide because of its high mammalian toxicity. Electrophoresis of glyoxylated RNA is recommended over other electrophoretic methods for determining the mol. wt. of RNAs between $1.7 \times 10^6$ and $3.5 \times 10^6$ (Murant et al., 1981). Marker RNAs should be well characterized; ribosomal RNAs of Escherichia coli (Stanley & Bock, 1965) are probably the best for the purpose. Mol. wt. of dsRNAs can only be accurately estimated using dsRNA markers (Bozarth & Harley, 1976) e.g. the RNAs of reovirus (Shatkin et al., 1968).

(d) Base composition and sequence. The limited value of base ratio data in virus classification has prompted the use of other methods (e.g. nucleic acid hybridization) to determine relationships. Comparisons of the degree of homology between complementary negative-strand RNAs or complementary DNAs of reference strains and test strains of TMV (Vandewalle & Siegel, 1976; Zaitlin et al., 1977) and some cucumoviruses (Habili & Francki, 1974; Gonda & Symons, 1978) showed either a high degree of homology or no homology at all. In some instances, relationships established by hybridization studies were similar to those established by amino acid sequence (Vandewalle & Siegel, 1976) and by serology (Gonda & Symons, 1978; Piazzolla et al., 1979), but no homologies were detected (Kummert et al., 1978) between the RNAs of two tymoviruses that are closely related serologically (Koenig, 1976). Studies on base sequence homology are still in their early stages, however, and their usefulness in virus taxonomy can only be assessed when more data become available.

Two factors should be considered when interpreting data from molecular hybridization. Firstly, there may be similar nucleotide sequences at different loci, representing, for example, RNA with various control functions and therefore of little significance in relating two viruses. Secondly, the capsid protein, on which many of the factors used in relating viruses are based (e.g. serology, vector specificity, susceptibility to denaturing agents) constitutes only about 10 to 20% of the total coding capacity of the typical viral genome. Furthermore, those amino acid sequences positioned on the 'surface' of the virus particle are more directly involved in
some of these properties than are those in the rest of the polypeptide. Hence, it is not unexpected that serologically related viruses may show considerably less than complete homology or even none, within the limits of detectability.

5. Analysis of the coat protein

The number of polypeptides and their mol. wt. are very useful in assigning a virus to a taxonomic group. Many methods are available for the isolation of viral proteins (Ralph & Bergquist, 1967; Tsugita & Hirashima, 1972) although direct dissociation of virus followed by electrophoresis (Hull & Lane, 1973) is more convenient for most types of analysis and gives comparable results. The principal technique used to determine both numbers of polypeptides and their mol. wts. is SDS–polyacrylamide gel electrophoresis (Laemmli, 1970; Maizel, 1971).

(a) Number of polypeptides. The problem of protein degradation during virus purification and storage (Koenig et al., 1970; Tremaine & Agrawal, 1972; Hiebert & McDonald, 1973; Al Ani et al., 1979) is especially significant for its effect on estimates of polypeptide number by SDS–gel electrophoresis. Such degradation by plant proteases can often be minimized or prevented by rapid purification of virus from freshly harvested material, by avoiding prolonged incubation of sap extracts and by the use of chelating agents. Occasionally, bonds between the polypeptides are not broken by the denaturant and additional migrating species are observed (Sehgal & Hsu, 1977). Another source of error may be impure virus preparations.

(b) Mol. wt. of polypeptide(s). The mol. wt. of viral proteins are usually estimated by SDS–gel electrophoresis. Most proteins bind $\approx 1.4 \text{ g SDS/g protein}$ with the result that migration depends on size rather than on charge. The mol. wt. of an unknown protein is estimated by comparing its mobility with those of other proteins of known mol. wt. (Shapiro et al., 1967; Weber et al., 1972). The relationship is essentially linear, depending on gel concentration, in the mol. wt. range of 15,000 to 100,000 but deviations are significant at very low and very high mol. wt. Commonly used marker proteins and their mol. wt. include $\beta$-galactosidase (116,000), phosphorylase a (94,000), bovine serum albumin (67,000), ovalbumin (43,000), chymotrypsinogen A (25,700), myoglobin (17,200) and cytochrome c (11,700).

There are at least three possible sources of error in the estimation of mol. wt. Firstly, the proteins (the markers and unknowns) may not bind the same amount of SDS per unit weight. Anomalous binding of SDS to viral coat proteins has been reported (Koenig, 1972; Koenig et al., 1973); atypical binding of SDS can be tested by using the methods of Hedrick & Smith (1968). Secondly, proteins which may have been reduced, may re-form S–S bonds by re-oxidation; this problem can be prevented by alkylation of reduced proteins (Lane, 1978). Thirdly, the protein(s) may have been degraded during virus extraction; see (a) above.

(c) Amino acid composition and sequence. The amino acid composition of the coat proteins of a number of viruses is known but is of little use in distinguishing between similar viruses, because similar compositions may arise from quite different sequences. There is more agreement between relationships suggested by amino acid composition and serology in the tobamovirus group than in the tymovirus group (Paul et al., 1980a). Better comparisons may be made using ‘peptide maps’ obtained by limited proteolysis of purified protein followed by gel electrophoresis (Cleveland et al., 1977).

CONCLUSIONS

Once the relevant data have been accumulated, and the comparisons made with the literature, it may emerge that a newly studied virus is indeed different from those already described. Depending on the degree of relationship the virus bears to established viruses, it
Table 4. Guidelines for establishing the taxonomic status of new virus isolates

No serological cross-reactivities with similar established viruses

I

New isolate

Serological cross-reactivities with similar established viruses: serological differentiation indices* with several antisera are usually

greater than 3

II

New virus

The new isolate differs from similar viruses in natural and artificial host ranges and symptomatology and usually also in other properties†

III

New virus

The host range of the new isolate is similar to that of an established virus

IV

Natural and artificial host ranges of the new isolate and established viruses are very dissimilar; few or no common hosts found. Pronounced differences in other properties†

V

Host range and possibly also other properties† of the new isolate differ from those of established viruses. The differences are not as great as in IV

New virus

New strain of established virus

(e.g. strains of caetus virus X)

New virus

(e.g. turnip yellow and Kennedia yellow mottle viruses)

* Tests with a broad specificity such as the slide precipitin test or the agar gel double-diffusion test should be used for determining serological differentiation indices [see section (B) 2 (a) in text for definition].

† Examples of 'other properties' are: morphology, cytopathic effects, vector species, base composition of nucleic acid, electrophoretic properties.
may be legitimately considered to be a 'new' virus, and be given a new name, or it may be classified as a strain or isolate of an 'old' virus. In order that the wisdom of this decision may be judged (and justified), we suggest that all the closely relevant literature on the virus be briefly reviewed in the published description.

Viruses do not always fit neatly into taxonomic schemes, and due to the clonal rather than sexual nature of virus reproduction, the distinction between viruses and strains will always be arbitrary. The problem is especially common in the potyvirus and tymovirus groups (Bos, 1970; Beczner et al., 1976; Koenig, 1976; Lesemann et al., 1979). As an aid in deciding whether a 'new' entity shall be considered as a new virus or a strain of an already established virus, we would like to endorse the scheme outlined in Table 4. Here, decreasing requirements on serological differentiation are counterbalanced by increasing requirements on other differentiation properties such as host range and vector species. The scheme should serve as a guide rather than a set of firm rules.

Reports of apparently new viruses or previously unrecognized virus diseases continue to appear. A survey among several journals [Phytopathology, Phytopathologische Zeitschrift and Plant Disease Reporter (now Plant Disease)] from 1977 to 1979 inclusive shows that many of the apparently new viruses, especially rod-shaped ones, have been assigned at least to a virus group, but that about 30%, mostly isometric viruses whose taxonomy is not well developed, could not be identified. It is also interesting to note that within the last three years the ssDNA-containing geminiviruses have come to light (Goodman, 1977; Harrison et al., 1977). The recent description of a dsDNA-containing elongated virus (Sela et al., 1980) must await confirmation but no doubt new viruses and perhaps new kinds of virus will continue to emerge.

Unfortunately, the expertise in characterization is not always where the new virus diseases are discovered, and workers without adequate facilities or experience have to seek assistance from colleagues who are better equipped. Cooperation between individuals in well-equipped institutions and their fellow virologists wherever they are located is an ideal shared by all the contributors to this paper and a goal we are prepared to seek. In this way, and also, we hope, with the help of this paper, newly discovered viruses may be better characterized at the time of publication, thus avoiding the unnecessary cluttering of the literature with inappropriate and often redundant virus names. It is far better to entitle a paper 'A disease of two-leaved Solomon's seal caused by a strain of cucumber mosaic virus' than 'Solomon's seal mosaic virus, a new virus'.

This paper was prepared by a study group in response to a suggestion by the Plant Virus Subcommittee of the International Committee on Taxonomy of Viruses that guidelines were needed for the characterization of plant viruses and the subsequent publication of descriptions.

REFERENCES


