A Morphological Comparison of Inclusions Induced by Tobacco Etch and Potato Y Viruses

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ABSTRACT

Striated inclusions were found in negatively stained leaf extracts from plants infected with either potato Y virus (PVY) or tobacco etch virus (TEV). As viewed in an electron microscope, the PVY-induced inclusions were generally quadrangular, but triangles were never observed. By contrast, the TEV-induced inclusions were characteristically triangular, although quadrangles were also observed. Characteristic shape of the inclusions was tested as a basis for distinguishing TEV from PVY by identifying coded samples. Phytopathology 60:779-782.

RESULTS.—Virus particles, striated inclusions, and cellular organelles were found in negatively stained leaf extracts from all plant species infected with either TEV or PVY. As previously described (4), the inclusions associated with TEV infection were usually triangular in outline (Fig. 1), although quadrangles were also observed. By contrast, PVY-induced inclusions were characteristically quadrangular (Fig. 2), but pentagonal structures were also seen. The triangular inclusions characteristic of TEV were never observed in extracts from PVY-infected plants. Striations were clearly discernible on both TEV and PVY-induced inclusions (inserts, Fig. 1, 2).

Experiments were performed to determine if inclusion morphology was a reliable basis for distinguishing between infections caused by PVY and TEV. One of us made leaf dips from virus-infected and control plants of the species listed previously. The grids were coded and presented to another worker as unknowns. Each grid was examined for a maximum of 10 min, while looking initially for the presence of rod-shaped particles. When these were found, the remaining time was devoted to a search for inclusions, using a magnification of ×3,600. Inclusions were checked for striations at ×230,000. If characteristic triangular inclusions were found, the sample was designated “TEV”. Samples containing several groups of the quadrangular inclusions and no triangular inclusions were designated “PVY”.

In all cases where sufficient inclusions were found, the samples were designated either TEV or PVY, and all these designations were correct (Table 1). No inclusions were found in some individual samples in the arbitrary 10 min allowed, although flexuous rods were found in these same preparations. All of the PVY negatives were from tomato, and the TEV negatives were either from tomato or tobacco. In one sample of PVY, the extract from an old leaf contained abundant inclusions, but no particles were observed. Striated inclusions were not observed in extracts from noninoculated controls or from plants infected with control viruses.

To test the applicability of characteristic inclusion shape for distinguishing TEV from PVY in field samples, leaves showing mottling and vein-banding...
symptoms were collected from several naturally infected tobacco plants by W. C. Smith, Jr., near Mayo, Fla. Extracts for electron microscopy were prepared from each of 15 leaves and examined for the presence of rod-shaped particles and inclusions. Based on inclusion morphology, nine of the samples were designated TEV and six were designated PVY. Serological tests confirmed the accuracy of these identifications. Pepper and tomato plants naturally infected with TEV or PVY also have been examined. Extracts from pepper contained abundant inclusions, and TEV was readily distinguished from PVY, but tomato samples sometimes yielded too few inclusions to provide a basis for judgment.

Discussion.—This study has shown that inclusion morphology can be used to distinguish TEV from PVY infections. These viruses would be difficult to distinguish by electron microscopy on the basis of particle morphology, since they are both flexuous rods about 730 m\textmu{} long. The negative staining technique provides a simple and rapid means of evaluating the shape of flattened inclusions in extracts. Differences in the shape of inclusions induced by TEV and PVY also have been demonstrated by in situ methods. The cylindrical in-
clusions and laminated aggregates induced by TEV (2, 4, 8, 9, 10) differ from the cylinders and scrolls induced by PVY (2, 7).

All of the 15 filamentous viruses having particles 700-800 m long that we have examined induce striated inclusions in their hosts and, in some cases, the inclusions resemble those shown for PVY and TEV. Turnip mosaic virus, for example, induces both triangular and quadrangular inclusions in mustard (3). Not enough information is available at the present time to know whether inclusion morphology alone can be used to distinguish TEV and PVY from the other viruses that induce striated inclusions in their hosts.

The method of sample preparation had some effect on the ease of finding striated inclusions, and it was advantageous to make leaf extracts in both phosphotungstate and ammonium molybdate. Although the virus particles were seen in greater contrast in phosphotungstate, this stain had some disruptive effect on the triangular inclusions associated with TEV infection. Better preservation of these inclusions was achieved with molybdate. Usually more inclusions were observed when the leaf pieces were chopped, rather than dipped, in stain.

Although no inclusions were found in some samples in the experiments reported herein, this was apparently...
Table 1. Use of inclusion morphology to distinguish tobacco etch virus (TEV) from potato virus Y (PVY) in extracts from leaf samples

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Total samples</th>
<th>PVY samples designated</th>
<th>TEV samples designated</th>
<th>Samples with no inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVY</td>
<td>33</td>
<td>30</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>TEV</td>
<td>51</td>
<td>0</td>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>Other</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>35</td>
</tr>
</tbody>
</table>

a Single grid from each sample was scanned for a maximum of 10 min in an electron microscope. The coded samples were judged infected with TEV based on presence of triangular striated inclusions, or infected with PVY if quadrangular but not triangular inclusions were found in negatively stained extracts.

b Flexuous rods were observed in all TEV samples and in all but one of the PVY samples.

c No characteristic inclusions found in these preparations.

d Extracts made from healthy plants or plants infected with control viruses.

due in part to the short scanning time (10 min) and the fact that only one grid was checked. If a sample is to be evaluated for presence and type of inclusions, it may be necessary to check several grids and to extend the examination time. We were often able, however, to locate and characterize striated inclusions in extracts after 1 to 2-min examination.

LITERATURE CITED


