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

D. E. Purcifull, J. R. Edwardson, and S. R. Christie

Associate Virologist, Plant Pathology Department; Agronomist, Agronomy Department; and Electron Microscope Technician, Plant Pathology Department, respectively; Institute of Food and Agricultural Sciences, University of Florida, Gainesville 32601.

Florida Agricultural Experiment Stations Journal Series Paper No. 3453.

Supported in part by Atomic Energy Commission Contract AT (40-1)-2583.

Accepted for publication 26 November 1969.

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.

Cylindrical inclusion bodies and related structures are characteristically found in thin sections of plant cells infected with rod-shaped viruses having particle lengths of 700-800 mμ (2). These inclusions have been studied in negatively stained extracts, and consist of finely striated structures of various shapes. Their function or precise composition is not known, but they are not aggregates of virus particles (4). Enzymatic digestion and immunocytochemical tests indicate that tobacco etch virus (TEV)-induced inclusions contain protein (10), but probably not viral protein (11).

In the present study, evidence is presented that differences in the shapes of striated inclusions can be useful for distinguishing tobacco etch virus from potato Y virus (PVY).

MATERIALS AND METHODS.—Most of the work was done with an isolate of TEV obtained from R. W. Fulton and an isolate of PVY provided by G. V. Gooding. A second isolate of TEV from G. V. Gooding, an isolate of PVY from A. A. Cook, and field isolates were also used. Extracts for electron microscopic examinations were prepared from the following species infected with TEV: *Capsicum annuum* L. 'Calwonder' (pepper); *Cassia tora* L.; *Datura stramonium* L.; *Lycopersicon esculentum* Mill. 'Homestead' (tomato); and *Nicotiana tabacum* L. (tobacco). Extracts from PVY-infected pepper, tomato, and tobacco plants were also studied. Controls consisted of extracts from healthy plants of all preceding species and tobacco infected with one of the following viruses: tobacco mosaic; potato X; cucumber mosaic; or tobacco ringspot.

Negative stains used were either 1% potassium phosphotungstate or 1% ammonium molybdate (6), each at pH 6.7 and containing 0.025% bovine serum albumin. Specimens were mounted on carbon-coated Formvar films mounted on 75 × 300 mesh copper grids. The extracts were prepared from symptomatic areas of systemically infected leaves. Cut edges of leaves were dipped in a drop of stain on a grid (1, 5), or leaf pieces were chopped in stain on a glass slide and a drop of the mixture was transferred to a grid. Excess fluid was removed with filter paper, and the specimen was allowed to air-dry prior to examination in a Philips 200 electron microscope.

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 nor 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

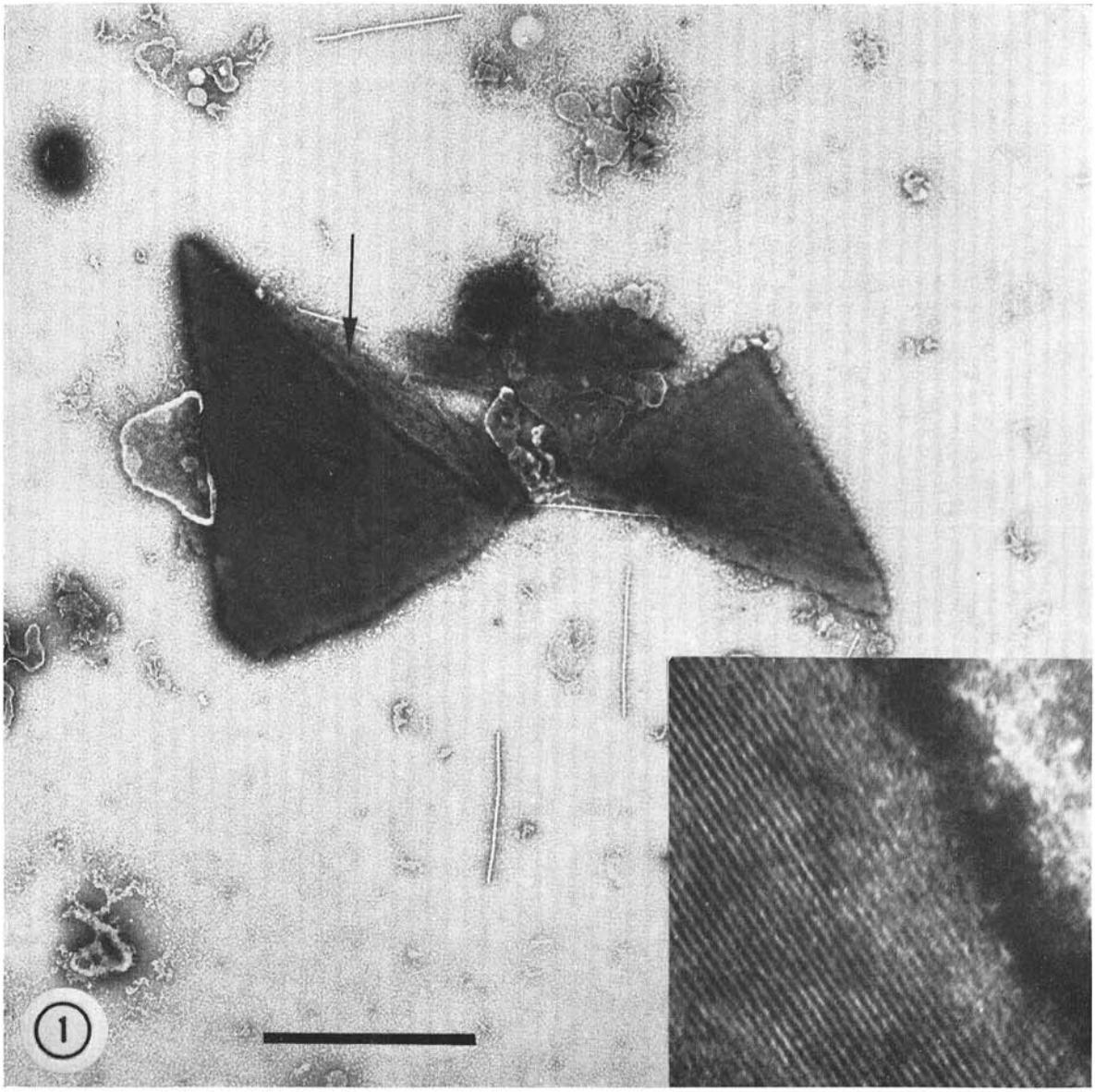

Fig. 1. Electron micrograph of leaf extract from tomato infected with tobacco etch virus, negatively stained with 1% potassium phosphotungstate, pH 6.7. Note triangular inclusions. Magnification mark = 1 μ. Striated nature of the inclusion is indicated in insert, which is a ×10 enlargement of the area indicated by the arrow.

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 mμ 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-

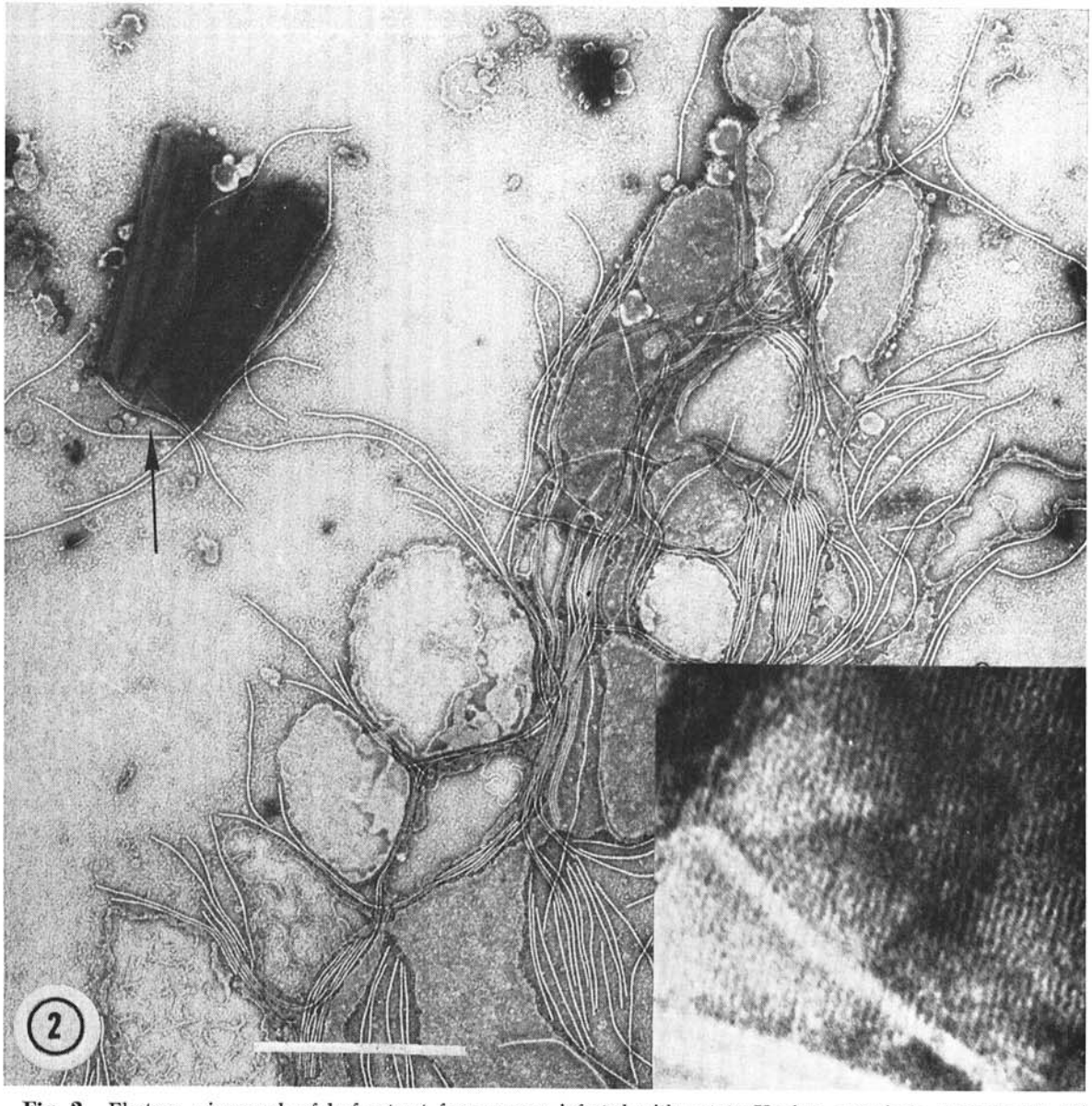

Fig. 2. Electron micrograph of leaf extract from pepper infected with potato Y virus, negatively stained with 1% potassium phosphotungstate, pH 6.7. Note quadrangular inclusions. Magnification mark = 1 μ. Striated nature of the inclusion is indicated in insert, which is a ×10 enlargement of the area indicated by the arrow.

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 mμ 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[a]

Specimens	Total samples[b]	Samples designated PVY	Samples designated TEV	Samples with no inclusions[c]
PVY	33	30	0	3
TEV	51	0	40	11
Other[d]	35	0	0	35

[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

1. BRANDES, J. 1964. Identifizierung von gestreckten pflanzenpathogenen Viren auf morphologischer Grundlage. Mitt. Biol. Bundesanstalt Land-Forstwirtsch. Berlin-Dahlem 110:130.
2. EDWARDSON, J. R. 1966. Electron microscopy of cytoplasmic inclusions in cells infected with rod-shaped viruses. Amer. J. Bot. 53:359-364.
3. EDWARDSON, J. R., & D. E. PURCIFULL. 1970. Turnip mosaic virus-induced inclusions. Phytopathology 60:85-88.
4. EDWARDSON, J. R., D. E. PURCIFULL, & R. G. CHRISTIE. 1968. Structure of cytoplasmic inclusions in plants infected with rod-shaped viruses. Virology 34:250-263.
5. HITCHBORN, J. H., & G. J. HILLS. 1965. The use of negative staining in the electron microscopic examination of plant viruses in crude extracts. Virology 27:528-540.
6. HORNE, R. W. 1967. Electron microscopy of isolated virus particles and their components, p. 521-574. *In* K. Maramorosch & H. Koprowski [ed.] Methods in Virology. Vol. III. Academic Press, N.Y.
7. KITAJIMA, E. W., I. J. B. CAMARGO, & A. S. COSTA. 1968. Intranuclear crystals and cytoplasmic membranous inclusions, associated with infection by two Brazilian strains of potato virus Y. J. Electron Microscopy 17:144-153.
8. MATSUI, C., & A. YAMAGUCHI. 1964. Electron microscopy of host cells infected with tobacco etch virus. I. Fine structures of leaf cells at later stages of infection. Virology 22:40-47.
9. RUBIO-HUERTOS, M., & F. GARCIA-HIDALGO. 1964. Ultrathin sections of intranuclear and intracytoplasmic inclusions induced by severe etch virus. Virology 24:84-90.
10. SHEPARD, J. F. 1968. Electron microscopy of subtilisin-treated tobacco etch virus nuclear and cytoplasmic inclusions. Virology 36:20-29.
11. SHEPARD, J. F., & T. A. SHALLA. 1969. Tobacco etch virus cylindrical inclusions: antigenically unrelated to the causal virus. Virology 38:185-188.