Discrepancies in the Intracellular Behavior of Three Strains of Tobacco Mosaic Virus, Two of which are Serologically Indistinguishable

A. L. Granett and T. A. Shalla

Department of Plant Pathology, University of California, Davis 95616. Present address of senior author: Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456.

Based on portions of a Ph.D. thesis submitted by the senior author.

This investigation was supported by USPHS Research Grant AI 07006 from the National Institute of Allergy and Infectious Diseases.

We wish to thank Arthur Allison and Jenifer Huang for their technical assistance during parts of this study.

Accepted for publication 1 October 1969.

ABSTRACT

Attempts were made to distinguish two common strains of tobacco mosaic virus, U-1(D) and U-1 (SB), and the U-5 strain by symptomatology, serology, and light and electron microscopy. In cells of Turkish tobacco (*Nicotiana tabacum*), the size, shape, and fine structure of the intracellular virus crystals formed by both U-1(D) and U-1(SB) were identical, but they were distinct from those formed by U-5. Also, the two common strains were indistinguishable from each other and different from U-5 on the basis of symptoms in various plants and in serological tests. The common strains appeared distinguishable only in differing tendencies to produce amorphous inclusions (X-bodies) and in the frequency with which the virus occurred in chloroplasts. The U-1(SB) strain was more effective than U-1(D) or U-5 in causing the formation of X-bodies. The chloroplasts frequently contained virions of either U-1(SB) or U-5, but only rarely U-1(D). Phytopathology 60:419-425.

The sites of synthesis of plant viruses in host cells have received considerable study in recent years (1, 5, 16, 18, 20). Earlier ideas that cellular organelles are involved in virus synthesis have lately received some support from electron microscope investigations (6, 7, 8, 23). A possible role in tobacco mosaic virus (TMV) synthesis has also been assigned to intracellular amorphous inclusions called X-bodies.

Esau & Cronshaw (7) presented some findings in startling discrepancy with earlier investigations of TMV by electron microscopy (9, 14, 15, 17, 22, 24). Despite research by others, theirs were the first convincing micrographs showing particles of common TMV in chloroplasts and nuclei. They also described more fully the fine structure of X-bodies, and implied that both intraorganelle virions and X-bodies were frequent in thin sections of diseased tissue, contrary to previous observations.

The discrepancies noted may possibly be explained as resulting from differences in virus strains and types of tissue studied. Shalla (23) found that virions of the U-5 strain were frequently found in chloroplasts, suggesting that Esau & Cronshaw (7) may have been working with a strain different from the U-1 (common) TMV. Also, the systemically infected leaves that Esau & Cronshaw (7) used were still developing when studied, whereas most previous work had been on manually inoculated leaves that were already mature. Differences in cells of developing and mature leaves could feasibly alter the relation of virus to various cellular components.

The present study compares the Davis strain of common TMV, U-1(D), the strain used by Esau & Cronshaw, U-1(SB), and the U-5 strain, with respect to the cytology of directly inoculated and systemically infected leaves. Particular attention was given to the comparative symptomatology and serology of the two common strains.

MATERIALS AND METHODS.—*Virus strains.*—One of the common TMV strains, here designated U-1(D), has been maintained in the Department of Plant Pathology at Davis since 1956 and has been used in previous studies in our laboratory (2, 21, 22, 23, 24). The other common strain was provided by Katherine Esau of the University of California, Santa Barbara, who originally obtained it from W. N. Takahashi. This strain, here designated U-1(SB), had been used in investigations of Esau & Cronshaw (6, 7). U-5 TMV, the *Nicotiana glauca* strain, was obtained from J. G. Bald of the Univ. of California, Riverside, and maintained in Turkish tobacco. We chose the U-designations because the three strains had characteristics similar to those described by Siegel & Wildman (25). The U-1 designation was subdivided for this study to emphasize that both those two strains had previously been considered as common TMV in studies conducted at Davis (D) and Santa Barbara (SB).

An earlier report (23) characterized the U-1(D) and U-5 strains by Siegel and Wildman's criteria (25). The U-1(D) strain systemically invaded leaves of *Nicotiana sylvestris* without producing local lesions, whereas U-5 produced only local lesions on directly inoculated leaves of this host. U-1(D) TMV was more resistant to ultraviolet irradiation than was U-5, and when U-1(D) was purified in the presence of 0.1 ionic strength cacodylic acid buffer, pH 6.9, the final high-speed pellet was brown, whereas that of U-5 was clear. Light microscopy revealed hexagonal virus crystals in cells infected with U-1(D), whereas bundles of needle-shaped crystalline inclusions predominated in cells invaded with U-5.

Plants and growing conditions.—Plants of *Nicotiana tabacum* L. 'Turkish' were used for all cytological work. Six-week-old seedlings were transplanted into 4-inch plastic pots containing sterilized soil and grown 3-6 weeks in a greenhouse kept at 25 C under 16 hr of light

maintained by white fluorescent tubes. All leaves more than 8 cm long were lightly dusted with 600-mesh corundum prior to applying inoculum with a cotton swab. In some experiments, the plants were transferred to a controlled-temperature chamber (Sherer, Model Cel 37-14) maintained at the same temperature and diurnal light regime as the greenhouse. Light, about 1,800 ft-c at plant height, was supplied with white fluorescent tubes and incandescent bulbs. Samples for light and electron microscopy were taken 1-4 weeks after inoculation. Newly emergent leaves, 5 cm or shorter, provided systemically invaded tissue samples. For virus purification, all leaves longer than 3 cm were removed from plants 6 to 12 weeks after inoculation.

Mature leaves of *Nicotiana tabacum* 'Xanthi n.c.' were used for infectivity assays. Leaves were lightly dusted, and inoculum was applied randomly to half-leaves with cotton swabs. Opposite halves were inoculated with purified homologous virus at a concentration producing 100-500 local lesions in 3 days.

Host-response tests were performed in the greenhouse with plants to be enumerated later.

Serology.—Antisera in rabbits were produced against the two U-1 strains after purifying them by the polyethylene glycol method of Gooding & Hebert (10). New Zealand white rabbits were immunized by injecting 1.5 mg virus/week for 3 weeks. Half of this injection was administered intravenously, while the other 0.75 mg was mixed with an equal volume (1 ml) of Freund's incomplete adjuvant (Difco, Detroit) and injected intramuscularly. Blood was obtained from each animal on the 4th week by cardiac puncture. Antisera were stored at −15 C until needed.

Using tube precipitin tests, both U-1(D) and U-1 (SB) antisera had titers of 1/1,280 against a final concentration of 1 mg/ml homologous virus.

Gel double-diffusion was used for most comparative serological tests (4). Agar gel consisted of 0.75% No. 2 Ionagar (Oxoid, Chicago), 0.85% sodium chloride, and 0.1% sodium azide, all dissolved in glass-distilled water. Twelve ml of this solution were poured into 100-mm-diam plastic petri dishes. A die produced eight 4-mm wells around a central well, also of 4 mm. These wells were filled with materials, and the dishes were then incubated in a moist chamber for 12 to 18 hr and observed by indirect light. With antisera in the center well, precipitin bands formed when homologous virus preparations were in peripheral wells, but no such bands developed with either macerated healthy tobacco leaf or clarified sap from such tissue in outside wells.

Intragel cross-absorption tests as described by van Regenmortel (28) and reciprocal cross-absorption in liquid suspension (19) were also performed to further clarify the serological relationship.

Light microscopy.—Epidermal strips were removed for light microscope studies from the abaxial surface of infected leaves. The strips were stained with a 1:4 mixture of calcomine orange-"luxol" brilliant green, each as a 1% solution in methyl Cellosolve, 95% ethyl alcohol, and distilled water (2:1:1) as described by Christie (3). Living hair cells were also studied by mounting unstained epidermal strips in distilled water. Observations were made with a Zeiss Model GFL microscope. Stained material was viewed with a ×100 objective lens, unstained specimens with either the ×40 or the ×100 phase-contrast Neofluar objective. Micrographs were made on Kodak Pantamic-X film with a Hasselblad camera attached to the microscope or with a Zeiss Ultraphot.

Electron microscopy.—Small pieces (about 5 × 15 mm) of infected leaf tissue were fixed for 2 hr under mild vacuum in cold 0.1 M sodium phosphate-buffered 5% glutaraldehyde (pH 7), and then rinsed in cold 0.1 M pH 7 phosphate buffer for 2 hr and postfixed in cold 1% buffered osmium tetroxide, pH 7. Dehydration was carried out in a cold ethanol series, after which the pieces were embedded in Araldite 6005 epoxy resin. Sections less than 100 mμ thick were cut on a Porter-Blum MT-2 microtome with a diamond knife, mounted on copper grids, and stained with uranyl acetate and lead citrate or with a 1% aqueous solution of potassium permanganate. The sections were examined on an RCA-EMU-3 electron microscope with a 50-μ objective aperture, operating at 50 kv.

Most observations were at an instrument magnification of approximately 6,000 times. Ten to 25 randomly selected micrographs were made of the groups of cells sectioned at a given layer in a tissue block. Care was taken to photograph only cells that were infected as evidenced by virions in the cytoplasm or vacuole. Observations were based on over 2,600 micrographs. The micrographs were graded according to whether virions were visible in plastids or nuclei, and whether X-body components were present.

RESULTS.—*Symptomatology.*—Host responses for the three strains were determined by inoculating different plant species and varieties. The 11 listed in Table 1 showed symptoms with one or more of the strains tested. The U-1 strains could be differentiated from U-5 on the basis of its effect on *Nicotiana sylvestris, N. glauca,* and *Phaseolus vulgaris.* U-1(D) could not be distinguished from U-1(SB), however, on the basis of symptomatology or host specificity. Also, the specific

TABLE 1. Host responses to three strains of tobacco mosaic virus

Host	Strain		
	U-1(D)	U-1(SB)	U-5
Chenopodium amaranticolor Coste & Reyn.	NL[a]	NL	NL
Datura stramonium L.	NL	NL	NL
Nicotiana clevelandii Gray	NL	NL	NL
N. glauca Graham	LI	LI	CS
N. glutinosa L.	NL	NL	NL
N. tabacum L. 'Glurk'	NL	NL	NL
'Havana 425'	NL	NL	NL
'Turkish'	CM	CM	RM
'Xanthi n.c.'	NL	NL	NL
N. sylvestris Speg. & Comes	CM	CM	NL
Phaseolus vulgaris L. 'Sutter Pink'	NL	NL	NI

[a] NL = Necrotic local lesions; CM = systemic chlorotic mottle; LI = symptomless infection; CS = bright, systemic chlorotic spotting; RM = rugose leaves and systemic chlorotic mottle; and NI = no infection.

infectivity of these two strains did not appear to differ on local-lesion hosts. When inoculum contained equivalent concentrations of purified virus (as estimated by spectrophotometry), the numbers of local lesions on hosts such as 'Xanthi n.c.' were about the same.

Serology.—The antisera produced against U-1(D) and U-1(SB) each had titers of 1/1,280 in tube-precipitin reactions with homologous or heterologous antigens.

Double-diffusion tests provided further evidence of serological identity between U-1(D) and U-1(SB). Figure 1 illustrates a double-diffusion test in which purified preparations of the three strains were placed in the peripheral wells. When the center well was filled with antisera against either U-1(D) or U-1(SB), continuous precipitin bands formed in the vicinity of wells containing U-1(D) and U-1(SB). Partial intersection of the bands occurred where either of the U-1 strains was adjacent to the wells containing U-5. This indicates that U-1(D) is serologically identical to U-1(SB), and that both are related to U-5 though not identical to it. Since, however, the lack of spur formation in double-diffusion tests cannot be taken as unequivocal proof of serological identity (27), intragel and reciprocal cross-absorption tests were also performed.

In the intragel tests, when U-1(D) or U-1(SB) antisera were first absorbed with homologous or heterologous virus, no precipitin bands formed in the vicinity of the antigen-containing wells. If either of these antisera were absorbed with U-5, however, a second band formed opposite the wells containing U-1(D) or U-1(SB) virus and no bands formed opposite the U-5 virus well. Similarly, when U-1(D) and U-1(SB) antisera were cross-absorbed with heterologous antigen, neither of the absorbed antisera reacted with homologous antigens in liquid suspension.

On the basis of these tests, we conclude that the U-1(D) and U-1(SB) strains of TMV are serologically identical and serologically related to U-5 TMV, though neither is identical to it.

Light microscopy.—Multilayered hexagonal crystals, as in Fig. 2 (above), predominated in living-hair and epidermal cells of leaves infected with either of the U-1 strains. Such crystals were found in 90% of the

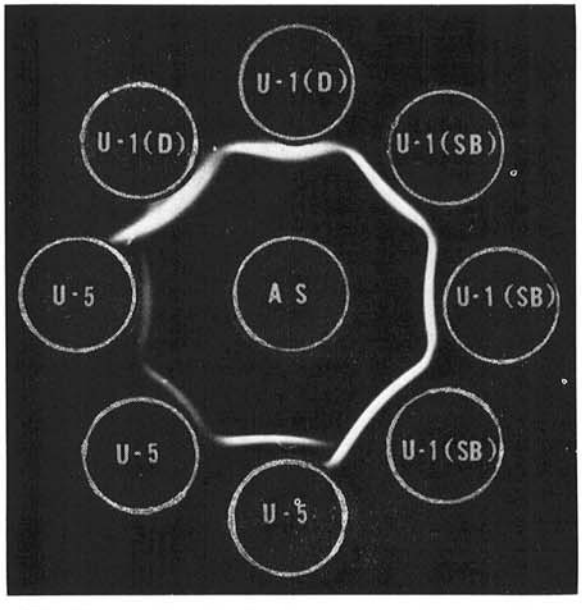

Fig. 1. Double-diffusion in agar of three strains of TMV. Outside wells are filled with purified preparations of the three strains diluted 1:1 with ethanolamine to a final virus concentration of about 1 mg/ml. Center well contains antiserum against U-1(SB).

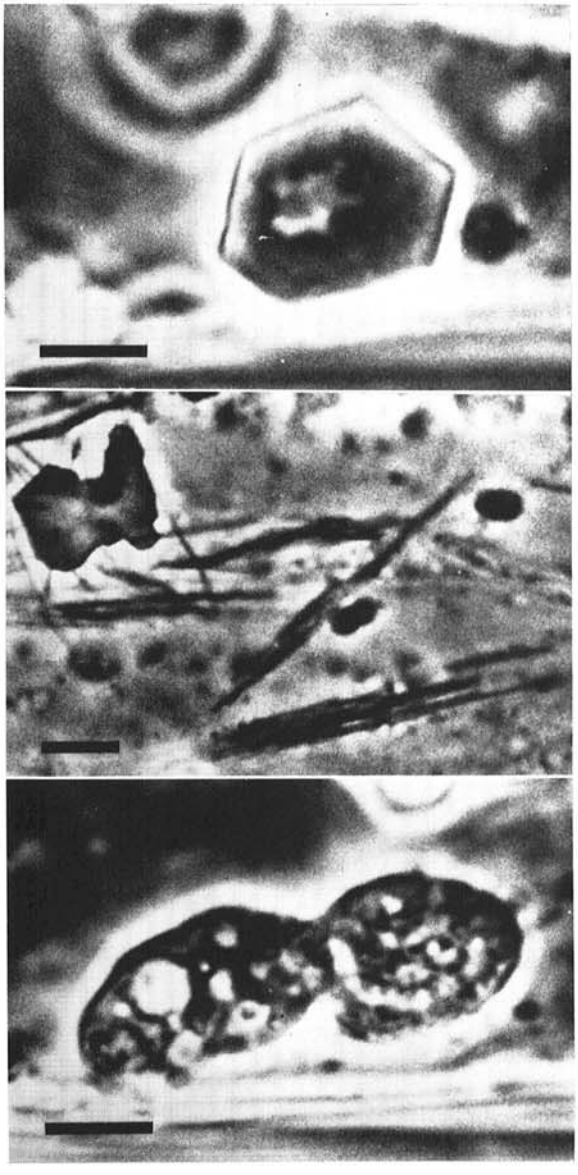

Fig. 2. Light microscopy of virus inclusions in tobacco hair and epidermal cells. Marker is 10 μ. Phase optics with the ×40 Neofluar objective lens were used. Above, hexagonal crystal typical of both common strains. Unstained. Center, combination of a small monolayer hexagonal crystal arrow and bundles of needlelike crystals characteristic of U-5. Unstained. Below, X-body inclusion found in tissue infected with U-1(SB). Stained with calcomine orange and "luxol" brilliant green.

cells of tissue infected with U-1(D), and in 99% of those with U-1(SB). Only 1% of cells in the U-5-infected tissue had hexagonal-type crystals, and these were usually small monolayer plates. On the other hand, 73% of the U-5-infected cells contained needlelike crystal bundles. Both needlelike crystals and small hexagonal plates may be in the same cell (Fig. 2, middle). Needlelike crystals were not observed in U-1-infected tissue.

The above data represent observations from both directly inoculated and systemically invaded tissues. With U-1(D) and U-1(SB), such combining results made little difference, since nearly all cells had hexagonal crystals, and needlelike crystals were absent. The U-5-infected tissue, in contrast, showed some differences when the mode of infection was considered. Small, monolayer, hexagonal plates in cells were more frequent in inoculated leaves (15%) than in systemically infected tissue (6%), although the needlelike crystals predominated in both cases.

X-bodies (Fig. 2, below) were observed in stained epidermal strips from diseased plants. Figure 3 represents data accumulated from 20,000 cells. Cells from plants infected with U-1(SB) had significantly more X-bodies (94% of all cells) than did cells of plants infected with the other two strains. U-1(D)-infected cells had some X-bodies (17%), whereas U-5 cells were nearly devoid of such structures (1%). Inoculated tissue did not differ noticeably from systemically infected tissue in the prevalence of these amorphous inclusions.

These observations are consistent with previous reports for common TMV and the U-5 strain (1). Although U-1(D) and U-1(SB) were indistinguishable by the form of the intracellular virus crystals, the pattern of similarities is broken when X-bodies are considered. More are found in U-1(SB) tissue than in tissues infected with either of the other two strains.

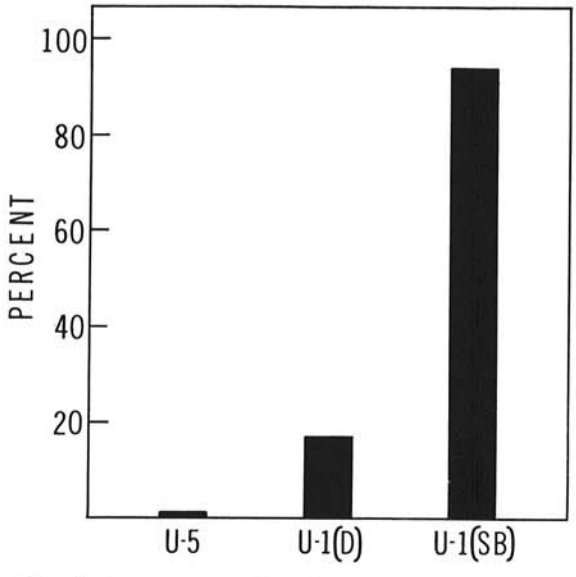

Fig. 3. Frequency of X-body occurrence in epidermal strips of tobacco infected with three strains of TMV. Frequency is based on per cent of the total number of cells with evidence of virus infection (crystals) that contain X-bodies.

Electron microscopy.—Figures 4 and 5 are representative electron micrographs of virus crystals found in TMV-infected tissue. The herringbonelike crystal (Fig. 4) is typically found in cells infected with either U-1(D) or U-1(SB), and represents a cross-sectional view of the hexagonal crystals seen with the light microscope (6, 26, 30). The cross-hatch pattern of viral aggregation (Fig. 5) is similar to those found with the aucuba strain of TMV (29) and with U-5 (23), and represents a cross-section through the needlelike crystals observed with the light microscope. In the survey of U-5-infected cells, a few (17 of 300) micrographs contained packets of loose parallel-packed virions in addition to the cross-hatch form.

These results are consistent with the light microscope data; that is, the U-1(D) and U-1(SB) virus crystals are essentially indistinguishable and about equally prevalent. Both strains differ markedly in this respect from U-5.

The appearance of X-bodies under the electron microscope is well established (6). Their most conspicuous feature is dense filaments about 70 mμ in diam (Fig. 6) which, at high magnification, have been shown to be composed of microtubules each about 24 mμ in diam (7, 14).

In systemically invaded leaves, the X-body tubules were observed more frequently in cells infected with U-1(SB) than in cells infected with U-1(D) (Table 2). This is consistent with the frequency with which X-bodies were observed with the light microscope (Fig. 3), although the differences between U-1(D) and U-1(SB) were less pronounced. Also, in directly inoculated leaves there was no significant difference in the frequency with which X-bodies were observed with the electron microscope.

Over 6,000 chloroplasts were observed with the electron microscope in cells of diseased tissue. Figure 7 is a portion of a chloroplast containing virus particles. Virions were observed in chloroplasts much more frequently with U-5 inoculation than with either U-1(D) or U-1(SB) (Table 3). Furthermore, virions of U-1 (SB) were found in plastids more frequently than were virions of U-1(D). In systemically invaded tissue, however, there were no significant differences among the three strains. Virions of all three were found in about 15% of the respective plastids.

TABLE 2. Frequency with which X-bodies were observed in thin sections of Turkish tobacco cells infected with three strains of tobacco mosaic virus

Mode of infection	No. of micrographs	Strain		
		U-1(D)	U-1(SB)	U-5
Directly inoculated	1,430	1.1[a]	0.5	0
Systemically invaded	1,153	3.0	10.0	0

[a] Per cent electron micrographs containing sectioned X-bodies.

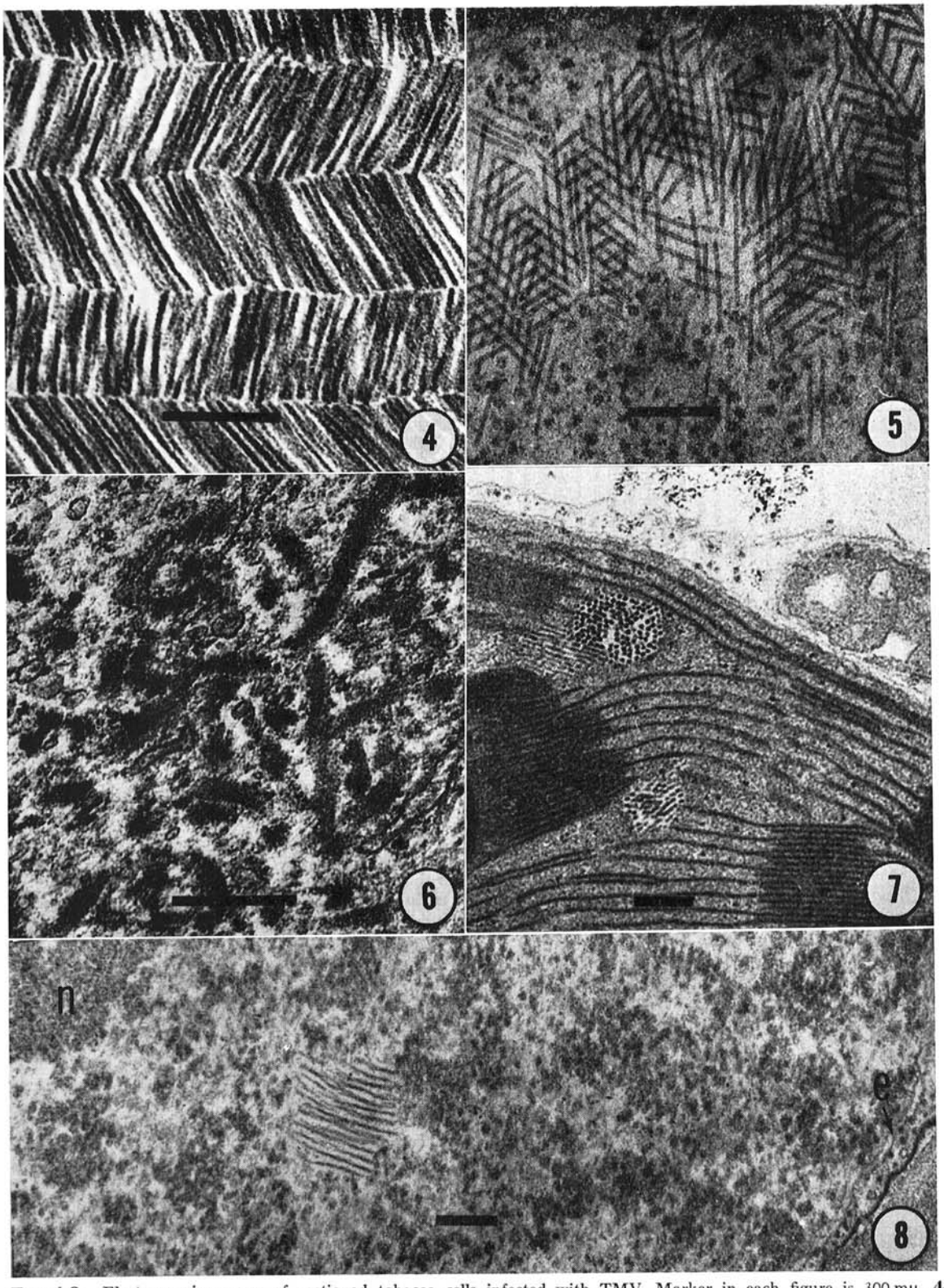

Fig. 4-8. Electron microscopy of sectioned tobacco cells infected with TMV. Marker in each figure is 300 mμ. **4)** Multilayered herringbonelike crystal typical of those found in U-1(D) and U-1(SB) infected cells. **5)** Cross-hatched crystal in cytoplasm of a tobacco cell infected with U-5. **6)** X-body components in cytoplasm of U-1(SB)-infected tissue. **7)** Portion of a chloroplast from a tobacco leaf inoculated with U-5. Virions can be seen both longitudinally and in cross-section. **8)** Portion of a nucleus from a tobacco leaf inoculated with U-1(D). Packet of virions is in the nucleoplasm at center between the nucleolus (n) and nuclear envelope (e).

TABLE 3. Frequency with which tobacco mosaic virus (TMV) virions were observed in chloroplasts of plants infected with three strains of TMV

Virus strain	Tissue sampled	
	Directly inoculated	Systemically invaded
U-1(D)	1[a] (616)[b]	12 (1,429)
U-1(SB)	7 (1,058)	17 (1,322)
U-5	92 (1,040)	14 (608)

[a] Per cent of chloroplasts containing virus particles.
[b] Total no. plastids observed in infected cells (as evidenced by the presence of virions in the cytoplasm or vacuole).

Sections of almost 800 nuclei were observed in micrographs of infected cells, and only 9 of these contained virus particles (Fig. 8). In inoculated leaf tissue, none of the 10 nuclei of U-1(D)-infected cells contained observable virus; 6 of 12 nuclei of U-1(SB)-infected cells contained virus, and 2 of 13 nuclei of U-5-infected cells contained virus. Many more nuclei were seen in the smaller cells of the systemically invaded tissue, but only one of the 305 nuclei in U-1 (SB)-infected tissue had virus that was detected. In material systemically infected with U-1(D) and U-5, the 335 and 113 nuclei, respectively, observed contained no virus.

Since few nuclei were observed, the apparent greater nuclear invasiveness of U-1(SB) is open to question. Some intranuclear virus may not have been detected because the organelles were not completely sectioned. In some instances serial sections were examined, but complete serial sections were not obtained of all nuclei.

DISCUSSION.—It is now clear that various investigators gave different locations for TMV within the host cells because they studied different strains. Closely related virus strains can differ in intracellular behavior. The two common TMV strains, U-1(D) and U-1(SB), were identical in serology and symptomatology, but were effectively separated by quantitative cytological differences. The U-1(SB) strain was characterized by more intraplastidial virions and more frequent X-bodies.

Esau & Cronshaw (7) observed clusters of TMV particles in nuclei of infected cells, and similar observations were made in the present study using their isolate. Failures in this laboratory to find intranuclear virions in tissue infected with the U-1(D) strain suggest that the U-1(D) and U-1(SB) strains may also differ with respect to the frequency with which virions may be found in nuclei.

The literature gives other examples of serologically identical virus strains with detectable differences. Knight (12, 13) showed that the M-TMV strain has the same coat-protein and is serologically identical to the common strain, but produces masked or only very mild symptoms in tobacco plants. This phenomenon might be explained by a hypothesis involving the virus cistrons. Even though the cistron which controls coat-protein synthesis may be the same for the two strains, one or more other cistrons that are somehow involved in symptom expression could vary (11). Similarly, specific cytologic responses may also be controlled by viral cistrons other than that which presumably directs coat-protein synthesis.

Some differences were noted when directly inoculated leaves were compared with those invaded systemically. Although intraplastidial virions were more prevalent in leaves inoculated directly with U-1(SB) than in those inoculated with U-1(D), no difference was detected in systemically invaded leaves. Also, at the electron microscope level, X-bodies were encountered more frequently in leaves systemically invaded by U-1(SB) than U-1(D), but no significant difference was detected in directly inoculated leaves. Using the light microscope, however, U-1(SB) was found to induce many more X-bodies than U-1(D) in both types of infection. It may be that, for quantitative measurements to be meaningful at the electron microscope level, still more sections need to be examined.

Esau (6) has published micrographs suggesting that virions in the cytoplasm may become incorporated into newly formed nuclei during mitosis. In the present study, TMV clusters were seen in nuclei in leaves mature at the time of inoculation. One would expect little, if any, cell division in leaves at this stage of development. It seems clear that TMV virions can arrive within the nucleus by some mechanism other than entrapment during mitosis.

LITERATURE CITED

1. BALD, J. G. 1966. Cytology of plant virus infections. Advances Virus Res. 12:103-125.
2. CARROLL, T. W. 1966. Lesion development and distribution of tobacco mosaic virus in *Datura stramonium*. Phytopathology 56:1348-1353.
3. CHRISTIE, R. G. 1967. Rapid staining procedures for differentiating plant virus inclusions in epidermal strips. Virology 31:268-271.
4. DUDMAN, W. F. 1965. Differentiation of strains of tobacco mosaic virus by immune diffusion in agar plates. Phytopathology 55:635-639.
5. ESAU, K. 1967. Anatomy of plant virus infections. Annu. Rev. Phytopathol. 5:45-76.
6. ESAU, K. 1968. Viruses in plant hosts. Univ. Wis. Press, Madison. 225 p.
7. ESAU, K., & J. CRONSHAW. 1967. Relation of tobacco mosaic virus to the host cells. J. Cell Biol. 33:665-678.
8. GARDNER, W. S. 1967. Electron microscopy of barley stripe mosaic virus: comparative cytology of tissues infected during different stages of maturity. Phytopathology 57:1315-1326.
9. GEROLA, F. M., F. CHRISTOFORI, & G. DASSU. 1960. Richerche sulle infrastrutture delle cellule del mesofillo fogliare di piante sane e virosate di tabacco (*Nicotiana tabacum*). B: Piante virosate. Caryologia 13:367-378.
10. GOODING, G. V., JR., & T. T. HEBERT. 1967. A simple technique for purification of tobacco mosaic virus in large quantities. Phytopathology 57:1285.
11. KADO, C. I., M. H. V. VAN REGENMORTEL, & C. A. KNIGHT. 1968. Studies on some strains of tobacco mosaic virus in orchids. I. Biological, chemical, and serological studies. Virology 34:17-24.
12. KNIGHT, C. A. 1947. The nature of some of the chemical differences among strains of tobacco mosaic virus. J. Biol. Chem. 171:297-398.
13. KNIGHT, C. A. 1963. Chemistry of viruses. Protoplasmatologia 4:1-177.
14. KOLEHMAINEN, L., H. ZECH, & D. VON WETTSTEIN. 1965. The structure of cells during tobacco mosaic

virus reproduction. Mesophyll cells containing virus crystals. J. Cell Biol. 25 (3, pt. 2):77-97.
15. MATSUI, C. 1958. Pathological cytology of tobacco leaf infected with tobacco mosaic virus III. J. Biophys. Biochem. Cytol. 4:831-832.
16. MATSUI, C., & A. YAMAGUCHI. 1966. Some aspects of plant viruses in situ. Advances Virus Res. 12:127-174.
17. MILNE, R. G. 1966. Multiplication of tobacco mosaic virus in tobacco leaf palisade cells. Virology 28:79-89.
18. MILNE, R. G. 1967. Plant viruses inside cells. Sci. Progress, Oxford 55:203-222.
19. MILNE, K. S., & R. G. GROGAN. 1969. Characterization of watermelon mosaic virus strains by serology and other properties. Phytopathology 59:809-818.
20. SCHLEGEL, D. E., S. H. SMITH, & G. A. DE ZOETEN. 1967. Sites of virus synthesis within cells. Annu. Rev. Phytopathol. 5:223-246.
21. SHALLA, T. A. 1959. Relations of tobacco mosaic virus and barley stripe mosaic virus to their host cells as revealed by ultrathin tissue-sectioning for the electron microscope. Virology 7:193-219.
22. SHALLA, T. A. 1964. Assembly and aggregation of tobacco mosaic virus in tomato leaflets. J. Cell Biol. 21:253-264.
23. SHALLA, T. A. 1968. Virus particles in chloroplasts of plants infected with the U-5 strain of tobacco mosaic virus. Virology 35:194-203.
24. SHALLA, T. A., & A. AMICI. 1967. The distribution of viral antigen in cells infected with tobacco mosaic virus as revealed by electron microscopy. Virology 31:78-91.
25. SIEGEL, A., & S. G. WILDMAN. 1954. Some natural relationships among strains of tobacco mosaic virus. Phytopathology 44:277-282.
26. STEERE, R. L. 1957. Electron microscopy of structural detail in frozen biological specimens. J. Biophysic. Biochem. Cytol. 3:45-60.
27. VAN REGENMORTEL, M. H. V. 1966. Plant Virus Serology Advan. Virus Res. 12:207-271.
28. VAN REGENMORTEL, M. H. V. 1967. Serological studies on naturally occurring strains and chemically induced mutants of tobacco mosaic virus. Virology 31:467-480.
29. WARMKE, H. E. 1968. Fine structure of inclusions formed by the aucuba strain of tobacco mosaic virus. Virology 34:149-159.
30. WARMKE, H. E., & R. G. CHRISTIE. 1967. Use of dilute osmium tetroxide for preservation of three-dimensional crystals of tobacco mosaic virus. Virology 32:534-537.