Turnip Mosaic Virus-Induced Inclusions

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ABSTRACT

Cylindrical inclusions induced in the cytoplasm of *Brassica perviridis* by a Florida strain of turnip mosaic virus were studied with light and electron microscopy. In cross sections, the inclusions consisted of pinwheels with attached circles and laminated aggregates, and thus appear similar to inclusions induced by several members of the potato virus Y group. In extracts, the inclusions exhibited striations with a periodicity of 5 mμ. Phytopathology 60:85-88.

Cylindrical inclusions in the cytoplasm of plant cells are useful in diagnosing infection by viruses with particle lengths of 700-800 mμ (2). The present report describes cytological investigations of turnip mosaic virus (TuMV) in *Brassica perviridis* Bailey and demonstrates the presence of pinwheel inclusions in this host.

MATERIALS AND METHODS.—Leaves of mustard, *B. perviridis*, from healthy plants and from plants infected with the TuMV strain from Florida described by Purcifull (12), were used as source material for cytology. Epidermal strips were prepared for light microscopy by Christie's technique (1). Areas of leaves with abundant cytoplasmic inclusions were used for electron microscopy. Leaf extracts were prepared by modification of the leaf dip methods of Hitchborn & Hills (7). The extracts were negatively stained with either 1% potassium phosphotungstate or 1% ammonium molybdate (8) each at pH 6.7 and containing 0.025% bovine serum albumin. Leaf tissue for sectioning was fixed in 3% glutaraldehyde in 0.05 M phosphate buffer, pH 6.8, for 3 hr at room temperature, and postfixed in 1% OsO_4 in phosphate buffer for 1 hr at room temperature. After dehydration in an ethanol series, the leaf pieces, about 3 mm × 1 mm, were embedded in a Maraglas-Cardolite 70:30 modification of Freeman & Spurlock's mixture (5) and sectioned with a diamond knife. Sections were stained for 30 min in 1% uranyl acetate (9, 15), followed by a modification of Reynolds' (14) lead citrate for 10 min (4). Healthy leaf pieces treated by the same methods were used as controls. Measurements were obtained by comparing electron micrographs of leaf extracts with micrographs of a diffraction grating.

RESULTS AND DISCUSSION.—Light microscopy reveals a complex organization of small components within the large cytoplasmic inclusions in TuMV-infected cells (Fig. 1-A, B). Linear structures within the large inclusions consist of two types, (i) lines oriented in approximately the same direction (Fig. 1-A) or in different directions (Fig. 1-B) which resolve into plates; and (ii) lines which do not resolve into plates when the focus of the microscope is changed. Healthy cells did not contain inclusions. Terminology applied to cylindrical inclusions in this report was defined previously by us (2, 3).

Extracts from mustard leaves contained remnants of cellular organelles as well as flexuous rods, finely striated triangular bodies (Fig. 1-C), and rectangular bodies (Fig. 1-E). The triangular bodies are interpreted to be extracted laminated aggregates; the rectangular bodies are thought to be curved plates which form tubes or pinwheel arms. Triangular bodies were observed only in ammonium molybdate stain, indicating that the laminated aggregates were either not extracted in potassium phosphotungstate or were disrupted by this stain (11). Regularly spaced striations are parallel to one side of the laminated aggregates (Fig. 1-D). Measurements of distance between the striations averaged 5 mμ, the same striation spacing reported for tobacco etch virus and for watermelon-mosaic-induced inclusions (3, 13). Overlapping of striated surfaces in the scroll-like tubes results in various striation patterns (Fig. 1-E). None of the extracted or sectioned healthy tissues exhibited cylindrical inclusions or rods.

In sectioned tissue, TuMV particles and inclusions occurred abundantly in the cytoplasm of epidermal, mesophyll, and phloem cells. In Fig. 2-A, a portion of a mesophyll cell contains laminated aggregate and circular inclusions, but pinwheels are not present.

Four cylindrical inclusions are shown in a portion of a mesophyll cell in Fig. 2-B. The obliquely sectioned inclusion at the lower left of Fig. 2-B contains five pinwheel (pw) arms to which are attached two laminated aggregates (la), one attached to one arm, the other to three arms. The other cylindrical inclusions are cross-sectioned. The cylindrical inclusion in the lower center of Fig. 2-B consists of circular (c) inclusion attached to one arm and a laminated aggregate attached to three arms of the same pinwheel. The cylindrical inclusion in the center of Fig. 2-B has a circular inclusion attached to two arms of a five-armed pinwheel. A laminated aggregate attached to three arms of an eight-armed pinwheel is located in the upper portion of Fig. 2-B. Recently, Kamei et al. (10) reported that pinwheels, which indicate the presence of cylindrical inclusions in thin sections, were not present in tissues of *B. perviridis* infected with the "ordinary" strain of TuMV, although these authors described other inclusions known to form part of cylindrical inclusions. Furthermore, Hayashi et al. (6) showed pinwheels in tissue of *Petunia hybrida* infected with another isolate of TuMV (Fig. 6). Whether the virus isolate used in

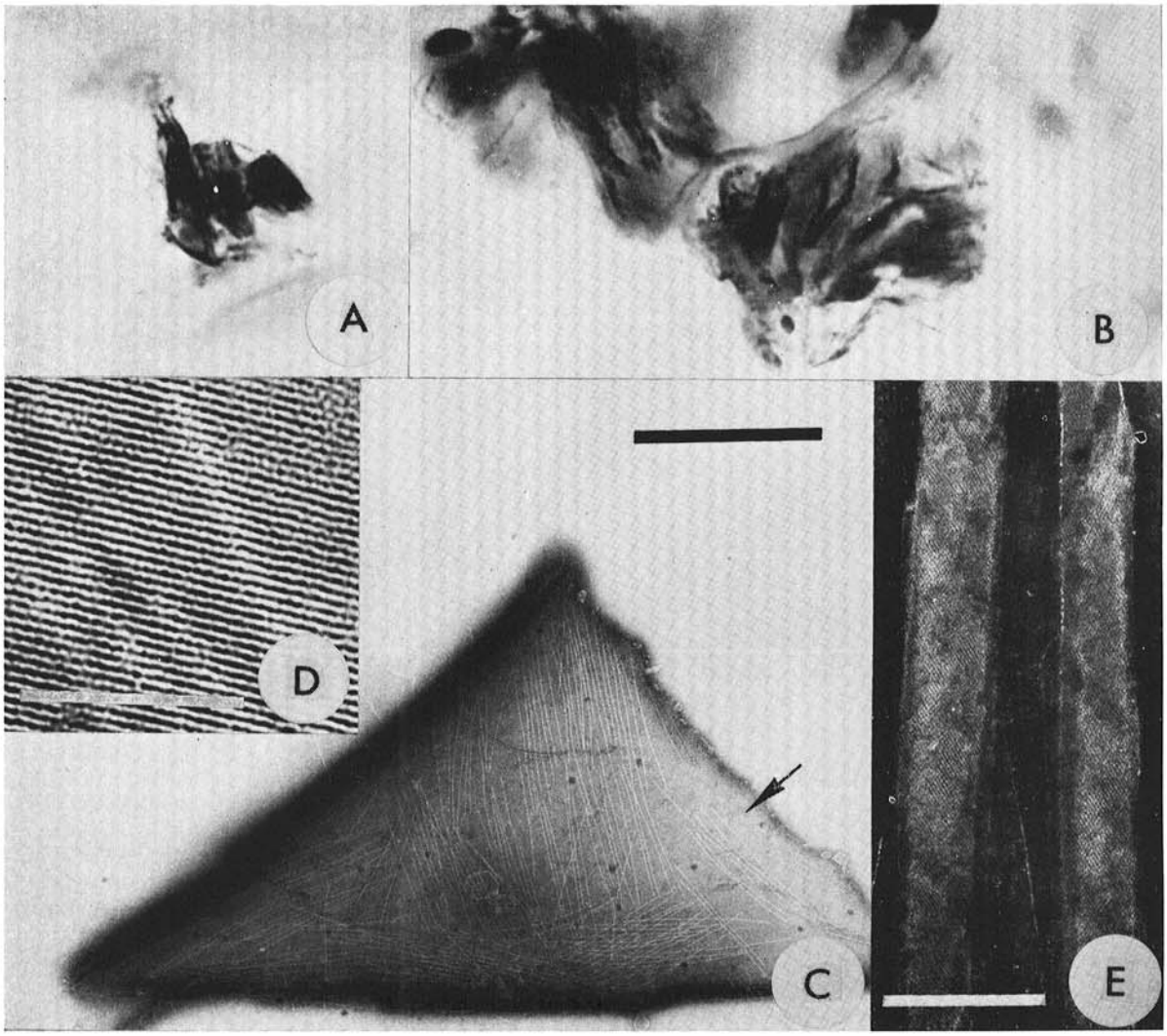

Fig. 1. Photomicrographs and electronmicrographs of turnip mosaic virus-induced inclusions in *Brassica perviridis*. **A)** Photomicrograph of inclusions showing parallel orientation of groups of plates in epidermal cell. ×1,700. **B)** Photomicrograph of adjacent epidermal cells; inclusions in left cell consist of linear structures not resolving into plates; inclusions in right cell consist of linear structures resolving into plates. ×1,700. **C)** Electron micrograph of negatively stained extracted triangular laminated aggregate in association with numerous virus particles. Bar = 0.5 μ. **D)** Inset, from region of inclusion in Fig. 1-C marked by arrow, showing striations, ammonium molybdate stain. Bar = 0.1 μ. **E)** Extracted rectangular inclusions consisting of flattened curved plates exhibiting overlapping striations and potassium phosphotungstate stain. Bar = 0.25 μ.

the present study is similar to those described previously (6, 10) is unknown; however, the Florida strain of TuMV does induce inclusions in *B. perviridis* which appear as pinwheels in cross sections.

There is no evidence that infection by any virus 700-800 mμ in length induces only tubes and/or laminated aggregates in the host cytoplasm. Since electron microscopy of virus-induced inclusions is in its infancy, there is a lack of information about the effects of environment, genotype, and developmental stage of host, and virus strain variations on the formation of inclusions. Interactions between these factors and viruses may influence the rates of formation and dissolution, and thereby influence the abundance of the different components of cylindrical inclusions.

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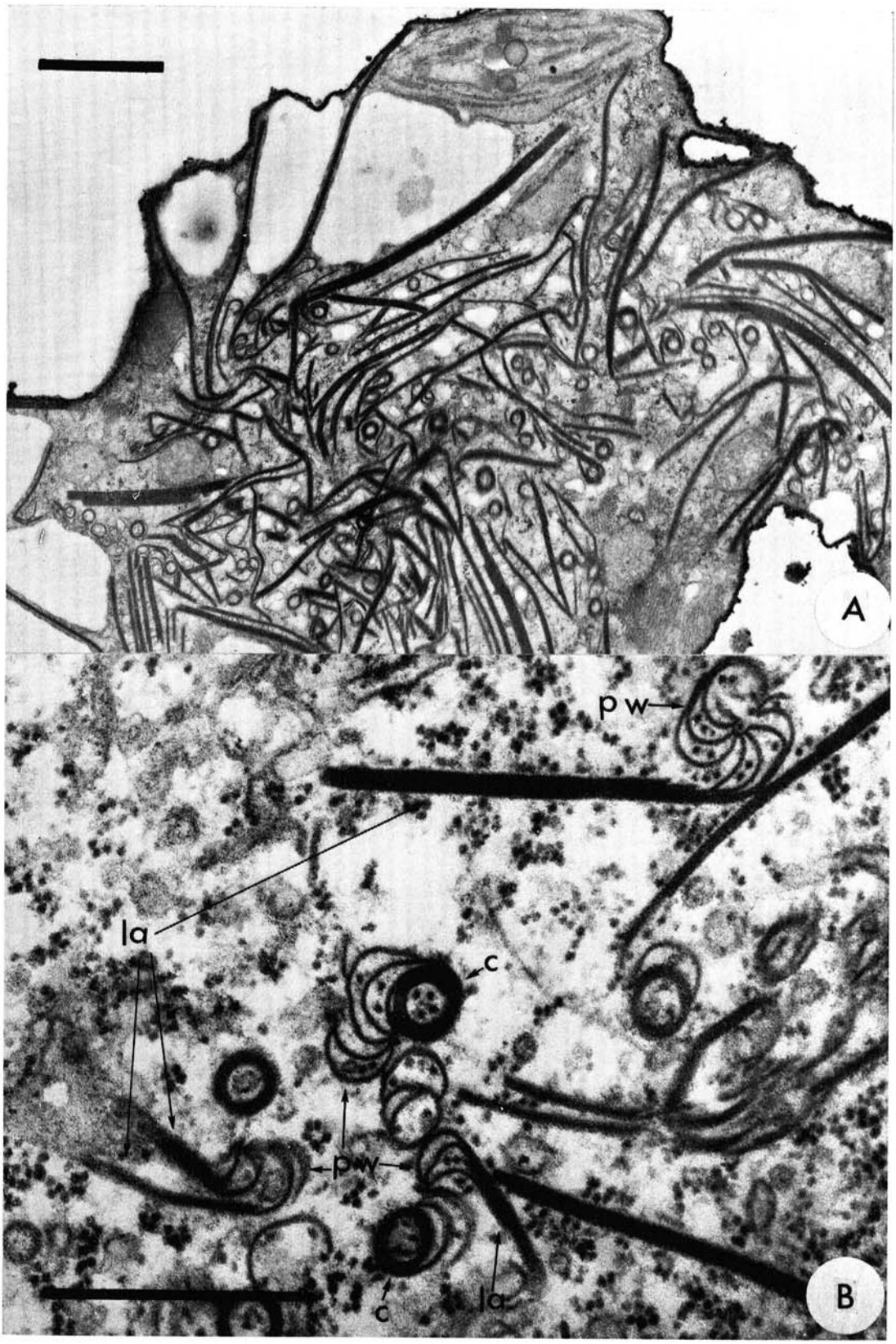

Fig. 2. Electron micrographs of portions of mesophyll cells from turnip mosaic virus-infected *B. perviridis* leaf tissue. **A)** Numerous laminated aggregate and circular inclusions. Bar = 1 μ. **B)** Cylindrical inclusions sectioned in various planes. Oblique and cross sections of cylindrical inclusions showing pinwheels (pw) with attached circular (c) and laminated aggregate (la) inclusions. Bar = 0.5 μ.

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