

Cytoplasmic Cylindrical and Nucleolar Inclusions Induced by Potato Virus-A

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ABSTRACT

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Cytoplasmic cylindrical and lacunose nucleolar inclusions induced by potato virus-A (PVA) in cells of *Nicotiana clevelandii* were studied by light and electron microscopy. Differential staining with Azure A and Calcomine-Orange, Luxol Brilliant Green revealed that components of these inclusions were proteinaceous. Cylindrical inclusions appeared in thin

sections as pinwheels, scrolls, and short, curved laminated aggregates (subdivision-IV type inclusions). Neither cytoplasmic nor nucleolar inclusions were observed in healthy cells. The nucleolar inclusions induced by PVA are distinctive and can be used to separate infections caused by PVA from those caused by other potyviruses.

The name potato virus A was suggested (17) for the causal agent of the disease that Orton (18) had described as potato striate mosaic. Brandes and Wetter (5) assigned potato virus A (PVA) to group No. 10 in their classification scheme for elongated viruses. Matthews (16) classified PVA as a potyvirus.

PVA particles are flexuous and length measurements of 730 nm (4) and 730-750 nm (11,13) have been reported. Aphids transmit PVA in a nonpersistent manner (14). Additional properties of PVA have been summarized by Bartels (1,2).

Although light microscopy by Bawden and Sheffield (3) revealed no inclusions in PVA-infected potato tissues, deBokx and Wattereus (7) reported cylindrical inclusions in the cytoplasm of PVA-infected potato cells. Additional cytological studies of PVA-infected tissues are described in this report.

MATERIALS AND METHODS

Leaf tissue of healthy and of PVA- (Swiss isolate [11]) infected *Nicotiana clevelandii* were studied with light and electron microscopy.

Light microscopy. Epidermal strips were removed from tissue pieces (~3 × 5 mm) fixed in 1% glutaraldehyde, rinsed in phosphate buffer (pH 7.2), and differentially stained with Azure A, and with Calcomine-Orange, Luxol Brilliant Green (6) stains. Cells of healthy and infected tissues were compared under the oil-immersion objective. Micrographs were obtained on Polaroid 4 × 5 Land film, type 55.

Electron microscopy. Leaf tissues (~1 × 3 mm) were fixed in 1% glutaraldehyde, rinsed in phosphate buffer (pH 7.2), dehydrated in an ethanol series, stained with uranyl acetate, and embedded in Spurr's plastic. Tissues were sectioned with a diamond knife; stained with potassium permanganate, uranyl acetate, and Reynold's lead-citrate; and examined in a Hitachi EM600 electron microscope.

RESULTS

Nucleoli in many cells of epidermal strips from PVA-infected leaves stained in Azure A exhibited unstained inclusions (Fig. 1). Cytoplasmic cylindrical inclusions were also unstained indicating the absence of nucleic acids in the cytoplasmic and nucleolar

inclusions. In the Calcomine-Orange, Luxol Brilliant Green stain combination the nucleolar and the cytoplasmic cylindrical inclusions (Fig. 2) stained green, indicating the presence of protein.

In thin sections of PVA-infected tissues, cylindrical inclusions were observed in epidermal, mesophyll, and phloem cells. In most of the cells that contained inclusions, a preponderance of scrolls and tubes were observed (Fig. 3). In a few cells only the central portions of the cylindrical inclusions were observed (Fig. 4). Some cells contained curved plates that constituted the central portion of the inclusion (pinwheels in cross section and bundles in longitudinal section) to which were attached scrolls and/or short, curved, laminated aggregates (Figs. 5 and 6).

Nucleolar inclusions were detected in many epidermal, mesophyll, and palisade cells of PVA infected tissue (Figs. 7 and 8). These inclusions occurred much less frequently than did the cytoplasmic cylindrical inclusions. The lacunose nucleolar inclusions were more electron-opaque and exhibited a finer granularity than did the nucleolus or chromatin. The appearance of these inclusions in intact nuclei examined in the light microscope, and in sectioned nuclei in the electron microscope, indicate they are spherical.

No cytoplasmic or nucleolar inclusions were observed in healthy leaf tissues of *N. clevelandii*.

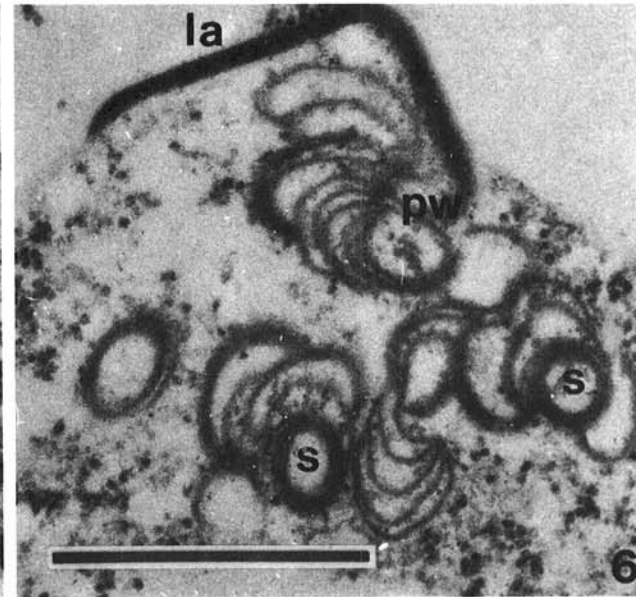
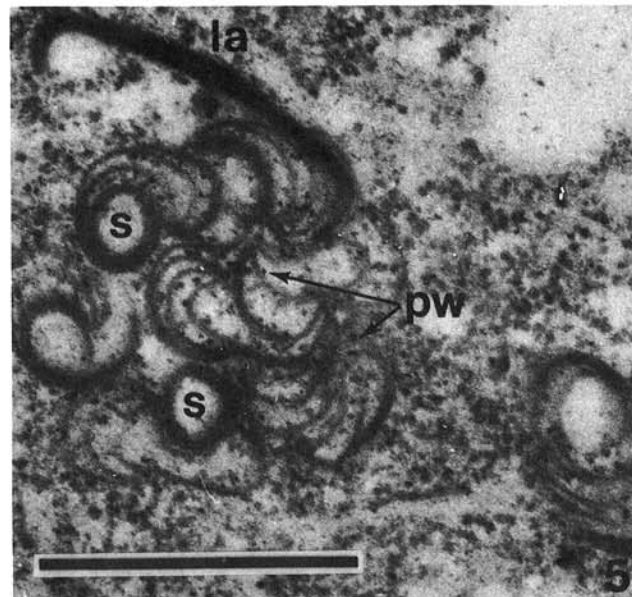
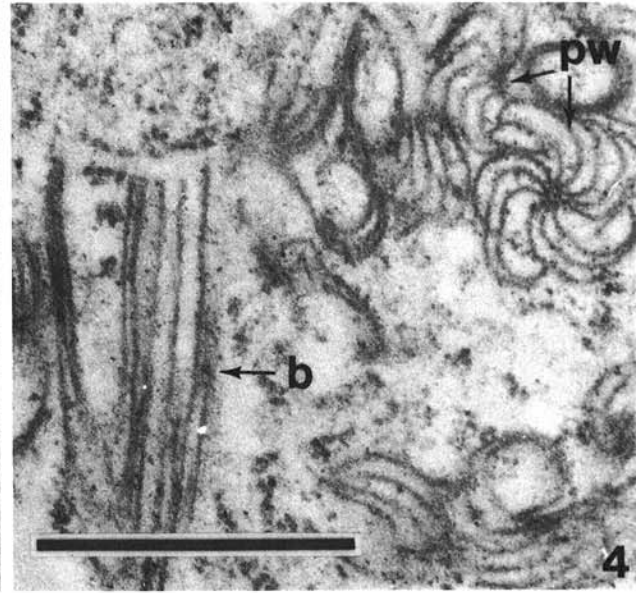
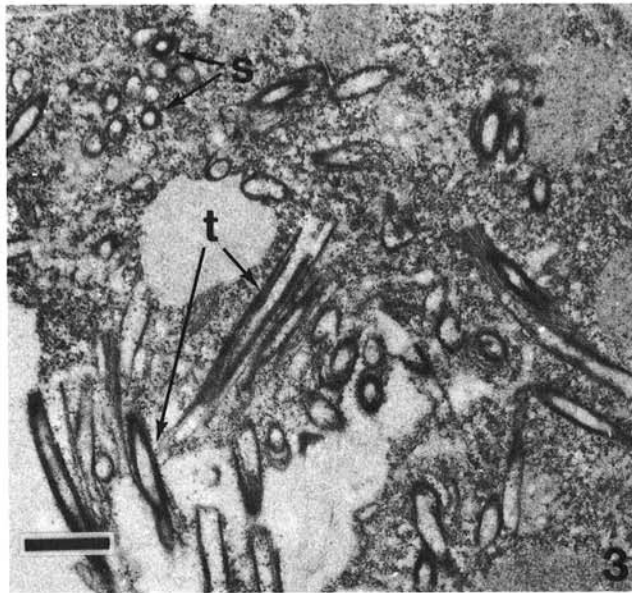
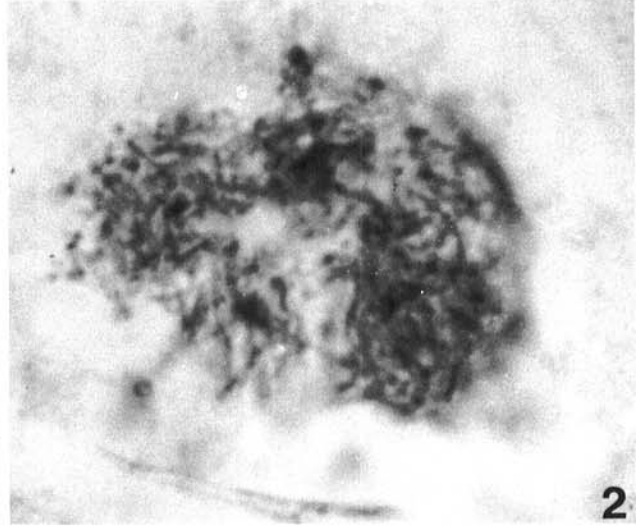
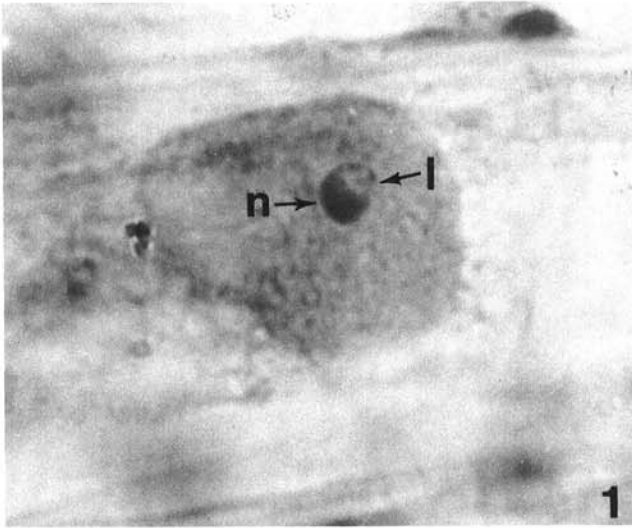
DISCUSSION

Various aspects of the central portions of PVA-induced cylindrical inclusions, pinwheels, and bundles appeared in the report of deBokx and Wattereus (7). Scrolls, short curved laminated aggregates, pinwheels, and bundles were observed in this study (Figs. 5 and 6). We attribute the differences in cylindrical inclusion morphology, which appear in these reports to differences in sampling. While all of the components of cylindrical inclusions may occur within a cell, all the components will not appear in every thin section of that cell. Sections may have neither scrolls or laminated aggregates (Fig. 4), only scrolls (Fig. 3), only laminated aggregates, or both scrolls and laminated aggregates (Figs. 5 and 6). PVA induced many more scrolls than laminated aggregates.

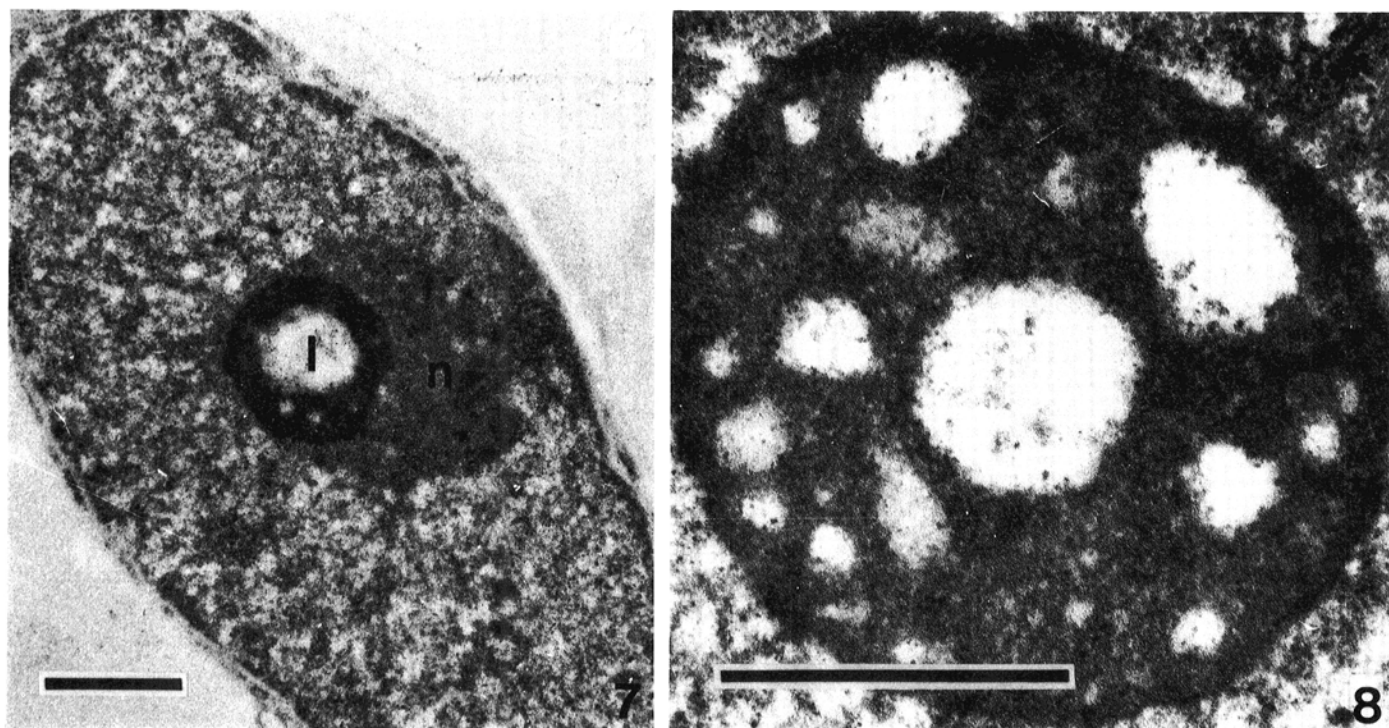
Fifteen potyviruses exhibiting similar configurations in the proteinaceous plates constituting their cylindrical inclusions have recently been separated from other members of the group into subdivision-IV (9). On the basis of its cytoplasmic inclusions, PVA is a member of subdivision-IV.

Fifteen potyviruses have been reported to induce inclusions in nuclei or nucleoli of infected cells; these inclusions are fibrous-

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Figs. 1-6. Cells of *Nicotiana clevelandii* containing inclusions induced by potato virus A. **1,** Epidermal cell containing nucleolar inclusion, stained with Azure A ($\times 1,940$). n = nucleolus, l = nucleolar inclusion. **2,** Epidermal cell containing cytoplasmic cylindrical inclusions, stained with Calcomine-Orange, Luxol Brilliant Green ($\times 1,940$). **3,** Portions of cylindrical inclusions exhibiting scrolls (s) and tubes (t). **4,** Central portions of cylindrical inclusions, pinwheels (pw) and bundle (b). **5,** Cylindrical inclusions exhibiting pinwheels (pw), scrolls (s) and a short, curved laminated aggregate (la). **6,** Cylindrical inclusions exhibiting pinwheels (pw), scrolls (s), and an angled laminated aggregate (la). Bars = 1 μ m.



Figs. 7 and 8. Nuclei of cells of *Nicotiana clelandii* infected with potato virus A. 7, Lacunose nucleolar inclusion. n = nucleolus, l = nucleolar inclusion. 8, Nucleolar inclusion containing many lacunae. Bars = 1 μ m.

paracrystalline, crystalline, or granular-spherical (8). The inclusions induced by celery mosaic (8) and hippeastrum mosaic (12) viruses are granular-spherical, but have not been reported to be lacunose. Beet mosaic virus (BMV) induces granular-spherical lacunose inclusions in nucleoli of infected cells (10,15,19). Numerous fibers in paracrystalline arrays develop in some of the lacunae of the inclusions in advanced stages of infection (15). The granular-spherical lacunose inclusions associated with nucleoli of PVA infected cells (Figs. 7 and 8) have not been observed to contain fibers; however, different stages of infection were not addressed in the present study. PVA infections could be distinguished cytologically from beet mosaic virus infection if it were shown that fibers are not associated with PVA-induced inclusions. The nucleolar inclusions induced by PVA distinguish this virus from the other members of the potyvirus group.

PVA and BMV can be distinguished cytologically on the basis of differences in the morphologies of the cylindrical inclusions they induce. The slightly curved pinwheel arms and long laminated aggregates of BMV and the markedly curved pinwheel arms, scrolls and short usually curved laminated aggregates of PVA are quite dissimilar.

PVA, PVY, and tobacco etch virus (TEV) have been reported to infect potatoes. PVA can be distinguished cytologically with light or electron microscopy from PVY and TEV on the basis of its spherical granular lacunose nucleolar inclusions. Two strains of PVY have been reported to induce crystalline nuclear inclusions; other PVY strains have not been reported to induce nuclear inclusions. TEV strains induce crystalline nuclear inclusions.

Leaf crinkle symptoms are exhibited by potatoes infected with PVA in combination with potato virus X, and top-necrosis symptoms are induced by infections with PVA and PVY. Mixed infections of PVA and PVY could not be distinguished cytologically from single infections with PVA. Mixed infections of PVA and PVX can be identified cytologically with light or electron microscopy, by the presence of PVA induced nucleolar inclusions and PVX-induced laminar cytoplasmic inclusion components.

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