Ultrastructural Comparison of Peanut Infected with Stripe and Blotch Variants of Peanut Stripe Virus

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ABSTRACT

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Two symptom variants of peanut stripe virus, stripe (PStV-S) and blotch (PStV-B), were compared ultrastructurally in the seventh quadrifoliolate leaf of systemically infected peanut (*Arachis hypogaea* 'Florigiant') sampled at five stages of expansion. The variants differed in the location of virus particles in cells and the severity of cytopathic effects, but not in particle length or type and location of cytoplasmic inclusions formed. Both variants of the virus are placed in potyvirus subdivision III. With either variant, cytoplasm in very young leaves was highly vesiculated, and pinwheel inclusions were located at the periphery of the cell, apparently attached to the plasmalemma near plasmodesmata. Scroll inclusions appeared in abundance in both PStV-B and -S infected cells at a later leaf expansion stage when symptoms were evident but indistinguishable as blotch or stripe. In more mature leaves expressing blotch or stripe symptoms, pinwheel and scroll inclusions were obvious in the cytoplasm and were often near mitochondria. Short, laminated aggregates were found infrequently. Virus particles were observed free in the cytoplasm, in linear arrays among membrane surfaces, and in monolayers sandwiched between membranes that extended as sheets through vacuoles. With PStV-B alone, particles were also observed in aggregates in the cytoplasm and along the arms of pinwheel inclusions. Membrane and organelle degradation was evident in cells infected with either variant, but was generally more severe with PStV-B. In fully developed leaves expressing distinctive symptom patterns, cells from light green areas contained numerous cytoplasmic inclusions and virus particles, whereas cells from dark green areas contained no observable cytoplasmic inclusions. Fewer virus particles were detected in extracts from dark green than light green areas and from PStV-S than from PStV-B tissue.

Additional keyword: groundnut.

In 1982, a new viral disease was described in peanuts at the Southern Regional Plant Introduction Station (SRPIS) in Experiment, Georgia (3). The causal virus, named peanut stripe (PStV), has since been found in several peanut-producing states (2), including Virginia (16). The virus was described by Demski and Lovell (2) as two symptom variants, stripe and blotch, caused by serologically indistinguishable potyviruses. Peanut plants infected with the stripe variant typically exhibit dark green stripes along the lateral leaf veins. As stripe-infected plants mature and the disease progresses, an oak leaf pattern develops on the leaves and becomes the predominant symptom expression. Plants infected with blotch variant develop dark green circular areas on the leaves.

This paper compares the progression of ultrastructural features corresponding with development of stripe and blotch symptoms in PStV-infected peanut and characterizes inclusion body types to allow placement of PStV into a potyvirus subdivision.

MATERIALS AND METHODS

Source and maintenance of viruses. PStV-stripe and PStV-blotch were isolated in 1983 from a single peanut (*Arachis hypogaea* L.) plant of cultivar NC-7 growing in research plots in Suffolk, VA having symptoms resembling peanut stripe. The virus was mechanically transmitted and maintained in the greenhouse in peanut cultivar Florigiant or NC-7. Plants were inoculated 14–16 days after seeding by rubbing neutral phosphate buffer-extracted sap or torn, infected leaf edges onto carborundum- (600 mesh) dusted leaflets of the first two quadrifoliolate leaves. The stripe- and blotch-symptom variation was observed upon inoculation of several peanut plants with the single isolate, and the separate variant cultures were derived by transfer from chlorotic local lesions on *Chenopodium quinoa* Willd. Both PStV-S and PStV-B reacted positively with antiserum to PStV provided by J. W. Demski (3) (Georgia Experiment Station, Experiment) in both immunodiffusion tests and enzyme-linked immunosorbent assay, confirming their identity. Furthermore, complete fusion of precipitin bands was observed between the Virginia isolates and a PStV-S isolate from Georgia in immunodiffusion tests (16) by using either Demski's stripe or blotch antisera or antiserum to zucchini yellow mosaic virus obtained from H. Scott (University of Arkansas, Fayetteville).

Sampling and electron microscopy. Tissue samples (1–3 mm^2) were taken from the seventh quadrifoliolate leaf of cultivar Florigiant peanuts at five stages of expansion and fixed in Karnovsky's (9) formaldehyde-glutaraldehyde fixative, postfixed in 1% osmium tetroxide, dehydrated in a graded ethanol series, and embedded in ERL epoxy resin (15). Sections were cut, stained with 2% uranyl acetate and 4% lead citrate, and examined in a Zeiss 10CA transmission electron microscope that was internally calibrated for astigmatism, resolution and, magnification by using perforated carbon film, graphitized carbon black, and potassium chloroplatinate standards. The five stages were: 1, leaflet less than one-third of its final size and not yet unfolded along the mid-rib; 2, leaflet open and expanded to approximately one-third of its final size; 3, leaflet open and expanded to approximately two-thirds of its final size; 4, leaflet expanded to final size but still tender and pale green; and 5, leaflet fully expanded and mature, darker green, and with a hardened surface.

Virus assays. Immunosorbent electron microscopy (ISEM) according to the modified Derrick system of Milne and Luisoni (12) was used to assay for the relative titer of virus particles in leaflet samples. Grids with a formvar-carbon membrane were sensitized by floating for 15 min on drops of a 1:1,000 dilution of antiserum provided by Demski. After washing with 0.06 M sodium phosphate buffer, pH 7.0, grids were floated for 20 min on 20 μl of leaf extract diluted 1:10 in phosphate buffer, washed as above, then decorated with 1:1,000 antiserum for 5 min and stained with 1%

uranyl acetate.

Relative titers of virus at the five expansion stages were determined from a total of 3 g of tissue of the seventh quadrifoliolate leaflets collected from five individual plants. These plants had been inoculated on the same date with crude extract (1 g/10 ml of 0.01 M neutral phosphate buffer) of leaf tissue from peanut plants inoculated with single *C. quinoa* lesions. Particles were counted from micrographs (1,250×) taken of five randomly chosen fields (one grid square each) on each of two grids. The extracts prepared for ISEM were also inoculated to *C. amaranticolor* Coste and Reyn., with each sample inoculated to four leaves on each of four plants.

Relative titers of virus in light green and dark green areas of fully expanded mature leaflets infected with either stripe or blotch variant were determined in four leaflets collected from each of three plants. Three grids were prepared from each 1–2 mm^2 piece of light green or dark green leaf. Particles were counted from micrographs (1,250×) taken of five randomly chosen fields on each of three grids. Data from both ISEM assays were tested for significance by analysis of variance, Duncan's multiple range, or orthogonal comparisons.

Length of PStV-S and PStV-B was determined from measurements of more than 100 particles from both leaf dips and partially purified (3) preparations. The measurements were made directly from the negatives by using a magnifying lens containing an internal metric scale and from micrographs enlarged to 50,000× with a ZIDAS Image Analysis System.

RESULTS

Initial symptoms in plants inoculated with either variant of PStV first appeared 16–18 days after inoculation as chlorotic flecking on the sixth quadrifoliolate leaflets. The seventh leaf consistently developed uniform symptoms in all four of the leaflets, and by the fourth or fifth sampling stage, symptoms characteristic of the two variants were well developed. Distinction between light green and dark green areas of the leaflet was sometimes evident at the third stage but not at earlier stages.

Tissue from plants inoculated with either variant appeared ultrastructurally similar at the closed stage. The cytoplasm of infected epidermal, palisade, and mesophyll cells was highly vesiculated and contained pinwheel inclusions (Fig. 1). Pinwheel inclusions were often observed appressed to cell walls near plasmodesmata. In sections at this and subsequent sampling stages, pinwheels were observed at various distances away from the cell wall (Figs. 2–4). Very few virus particles were found free in the cytoplasm in stage-one samples. However, aggregates of virus particles were found in stage-one samples of cells infected with the blotch variant (Fig. 5). Virus aggregates were never observed in leaf tissue infected with the stripe variant.

In leaves sampled at the second stage, pinwheel inclusions were more abundant in the cytoplasm of both stripe and blotch-infected tissue. Pinwheel inclusions in blotch-infected tissue had virus-like particles measuring 13 nm in diameter situated between the arms of the inclusions (Fig. 3). Such particle arrangements were observed less often in stripe-infected tissue (Fig. 4). The formation of cytoplasmic sheets, which appear to be monolayers of virus particles sandwiched between two membranes to form sheet-like structures containing cytoplasm and virus, was first observed traversing vacuoles of cells at stage two of leaf expansion. These sheets were observed in cells infected with either variant of the virus and tended to become more abundant as the virus infection progressed (Fig. 6). All cellular organelles appeared normal at this stage. However, phytoferritin crystals were observed within some chloroplasts in PStV-infected palisade cells, but were not usually seen in comparable healthy tissue.

Scroll inclusions were regularly observed in abundance in infected leaves at the third (two-thirds expansion) stage with either variant of PStV (Figs. 7 and 9). Both scrolls and pinwheels were abundant in all cell types and often appeared to fill every available space in the cytoplasm. Short, laminated aggregates, some of which were curved, were observed at a low frequency (Figs. 7 and 9). Very few inclusions were found near the cell periphery. The cytoplasm was typically highly vesiculated, particularly in the areas that contained numerous inclusions (Fig. 9). Measurements of longitudinal sections of both scroll and pinwheels indicate these inclusions were 2–3 μm in length. Both pinwheel and scroll inclusions were near mitochondria (Figs. 7–8).

At the fourth stage (fully expanded), numerous virus particles were observed lining the edge of the tonoplast and other membranes (Fig. 10). Cytoplasmic sheets, observed in a variety of configurations, were commonly seen in all cell types and were larger and much more abundant within infected cells. The virus-like particles enclosed within the sheets had a mean diameter of 13 nm (Fig. 6).

Palisade and mesophyll cells at the fifth stage (mature, expanded) were filled with both scroll and pinwheel inclusions (Fig. 11). Numerous cytoplasmic sheets containing virus particles traversed these highly vacuolated mature cells. Virus particles were also observed lining the edge of the tonoplast around the entire vacuole. Mitochondria in cells infected with either isolate of PStV appeared much more degraded than they did in uninfected stage-five peanut leaves and were more degraded in PStV-B than in PStV-S infections. Accumulation of starch granules in the chloroplasts appeared to be in excess of that observed in uninfected cells.

Leaves from blotch-inoculated plants had more ISEM-detectable virus than did leaves from stripe-inoculated plants, but significance by orthagonal comparison was not noted until stage-three or later samples (Table 1). By Duncan's multiple range, there was also a significant difference (0.05 level) in the number of virus particles counted at each stage regardless of virus variant. Samples taken at stage four had the highest number of virus particles, and stage-two and -five samples had a decrease in titer. Results of infectivity assays were in agreement, except that differences between variants were not significant, and the decrease in titer at the two stages was not detected.

There was virtually no difference between particles associated with the stripe- or blotch-inducing variants of PStV; both had a modal length of 750 nm and similar frequency distribution plots (data not shown). Mean particle length for PStV-B and PStV-S was 753 nm and 747 nm, respectively, from leaf dips, and 746 nm and 745 nm from partially purified preparations in which buffer containing 0.02 M sodium sulfite as an antioxidant was used (3).

In fourth-stage samples from plants showing distinct symptoms of either variant, tissues from light green areas contained both scroll and pinwheel inclusions. Virus particles were observed throughout the cytoplasm and within cytoplasmic sheets. At the fifth stage, degeneration of mitochondria and of thylakoid membranes within chloroplasts was observed (Fig. 11). No characteristic virus-associated cytoplasmic inclusions were observed in tissues from dark green areas of the leaf (Fig. 12), and cells appeared essentially like those in healthy tissue. Few, if any, virus particles were observed in the cytoplasm of cells in dark green areas. A small number of structures resembling cytoplasmic sheets were observed in some cells. However, they were small and contained no obvious virus particles.

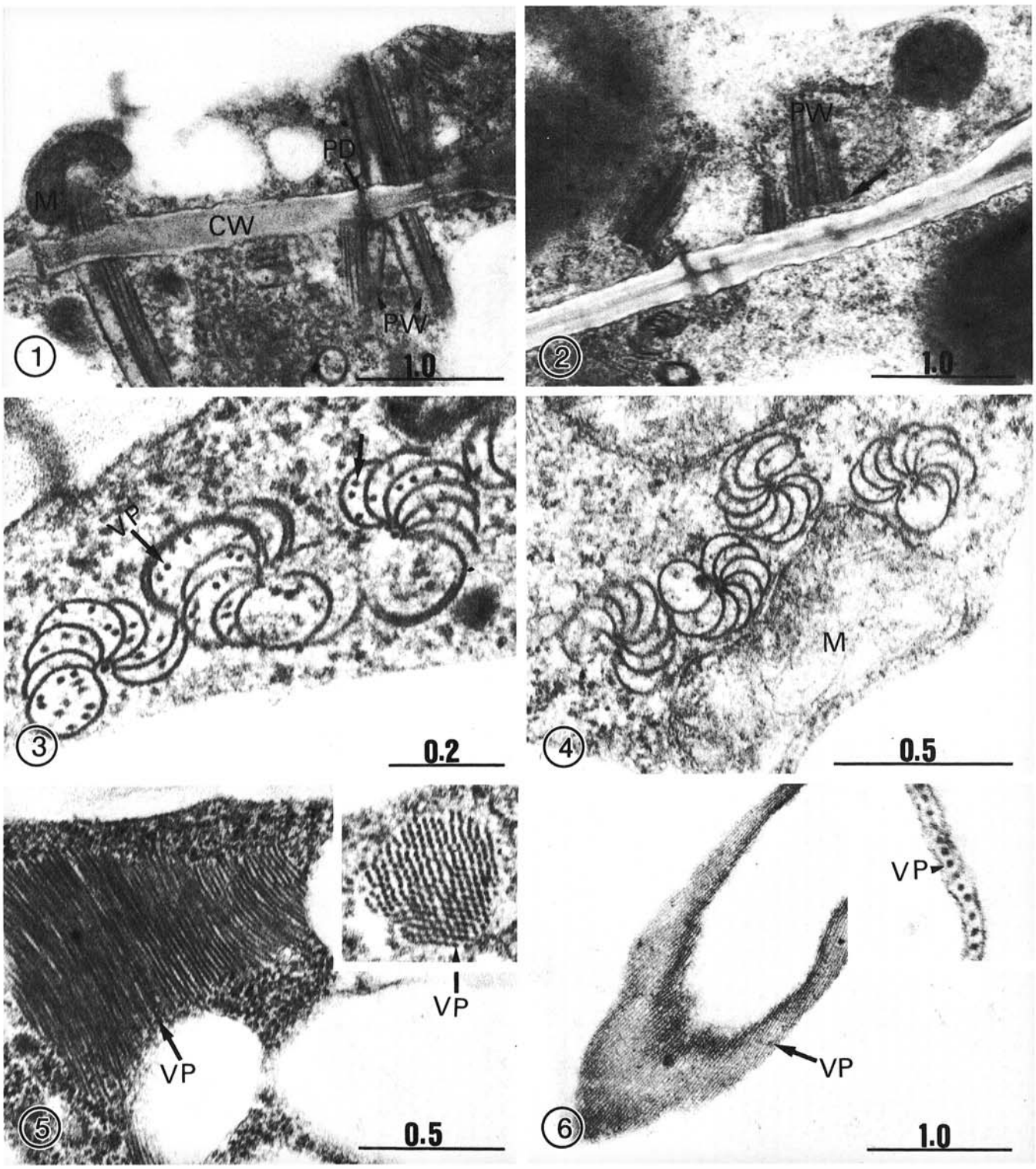
Assays by ISEM confirmed the intracellular observations, showing tremendously greater numbers of virus particles extractable from light green areas than from dark green areas in the same leaf infected with either variant (Table 2). Furthermore, virus content in light green tissue showing distinct blotches was more than 2.5 times higher than it was in comparable samples from light green tissues of stripe-infected leaves.

DISCUSSION

There was essentially no difference between the types of inclusion bodies induced by two symptom variants of PStV and their appearance relative to the development of a systemic infection in the seventh quadrifoliolate leaf of peanut. Pinwheel inclusions were observed in cells of leaflets infected with either PStV-B or PStV-S that had not yet unfolded and were less than one-third of their ultimate size. The PStV-induced pinwheels form apposed to the plasmalemma and eventually became detached and

drifted into the cytoplasm. Similar findings were reported for soybean mosaic virus-infected soybeans (8), for sweet potato russet crack-infected morning glory (11), and for many other potyviruses.

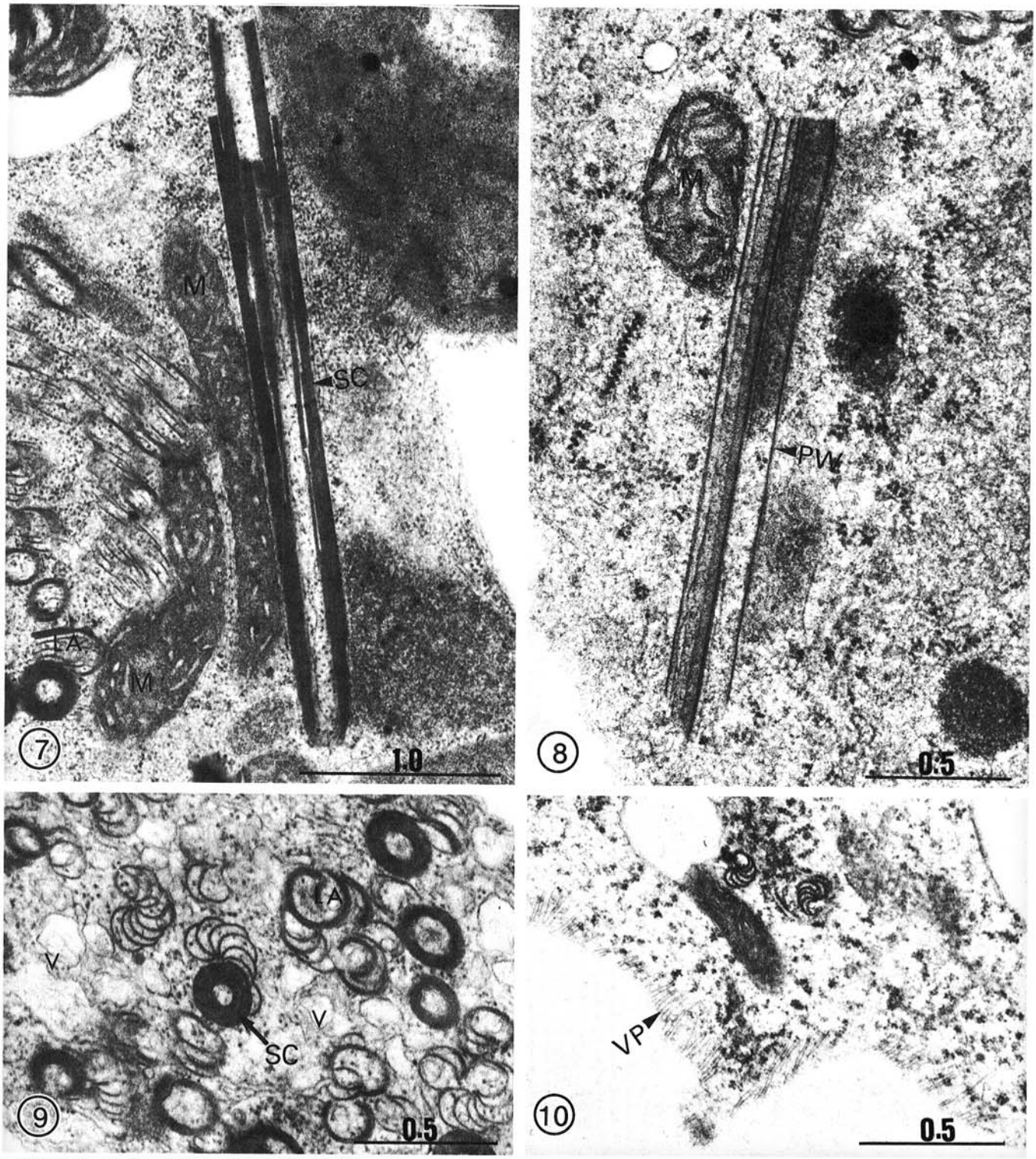
Numerous vesicles were observed in the cytoplasm of PStV-infected cells at all stages of leaf development. In leaf tissue at stages one and two, pinwheels were found within these vesiculated areas. Most of the pinwheel inclusions observed at this stage were closely associated with, if not apposed to, the cell wall. At stages three and four, the number of inclusions per cell had increased substantially, and most of the inclusions observed were located in



Figs. 1–6. Transmission electron micrographs of thin sections of peanut leaves infected with peanut stripe virus, blotch (PStV-B) or stripe (PStV-S) variants. (Bars = micrometers.) **1**, Epidermal cell of a closed, stage-one leaf infected with PStV-S, showing pinwheels (PW) on cell walls (CW) near plasmodesmata (PD) and near a mitochondrion (M) (25,000×). **2**, Parenchyma cell of a closed-stage leaf infected with PStV-S, showing pinwheels (PW) at various positions (arrow) in relation to the cell wall (25,000×). **3**, Parenchyma cell of a second-stage leaf (one-third expanded) infected with PStV-B, showing pinwheel inclusions in cytoplasm with virus particles (VP) in a matrix between the pinwheel arms (100,000×). **4**, Parenchyma cell from a second-stage leaf infected with PStV-S, showing pinwheel inclusions associated with mitochondria (M) in cytoplasm. Note that few virus particles are associated with these pinwheels (62,500×). **5**, Aggregates of virus particles (VP) in longitudinal (50,000×) and cross (inset: 75,000×) sections of a palisade cell from a closed-stage leaf infected with PStV-B. **6**, Longitudinal oblique (25,000×) and cross (inset: 100,000×) sections of virus particles (VP) encased in sheets of cytoplasm traversing the vacuole of a cell in a fully expanded leaf infected with PStV-B.

the vesiculated areas. Similar vesicles have been previously reported in virus-infected tissues (6,8,11,14). Hoefert et al (6) reported vesicles of unknown origin containing networks of fibrils in phloem parenchyma cells in older leaves infected with beet yellow stunt virus and associated their presence with early signs of cell degeneration. Powell et al (14) suggested that the vesicles are a part of an intercellular transport system for cellular macromolecules and may also carry viral RNA intercellularly.

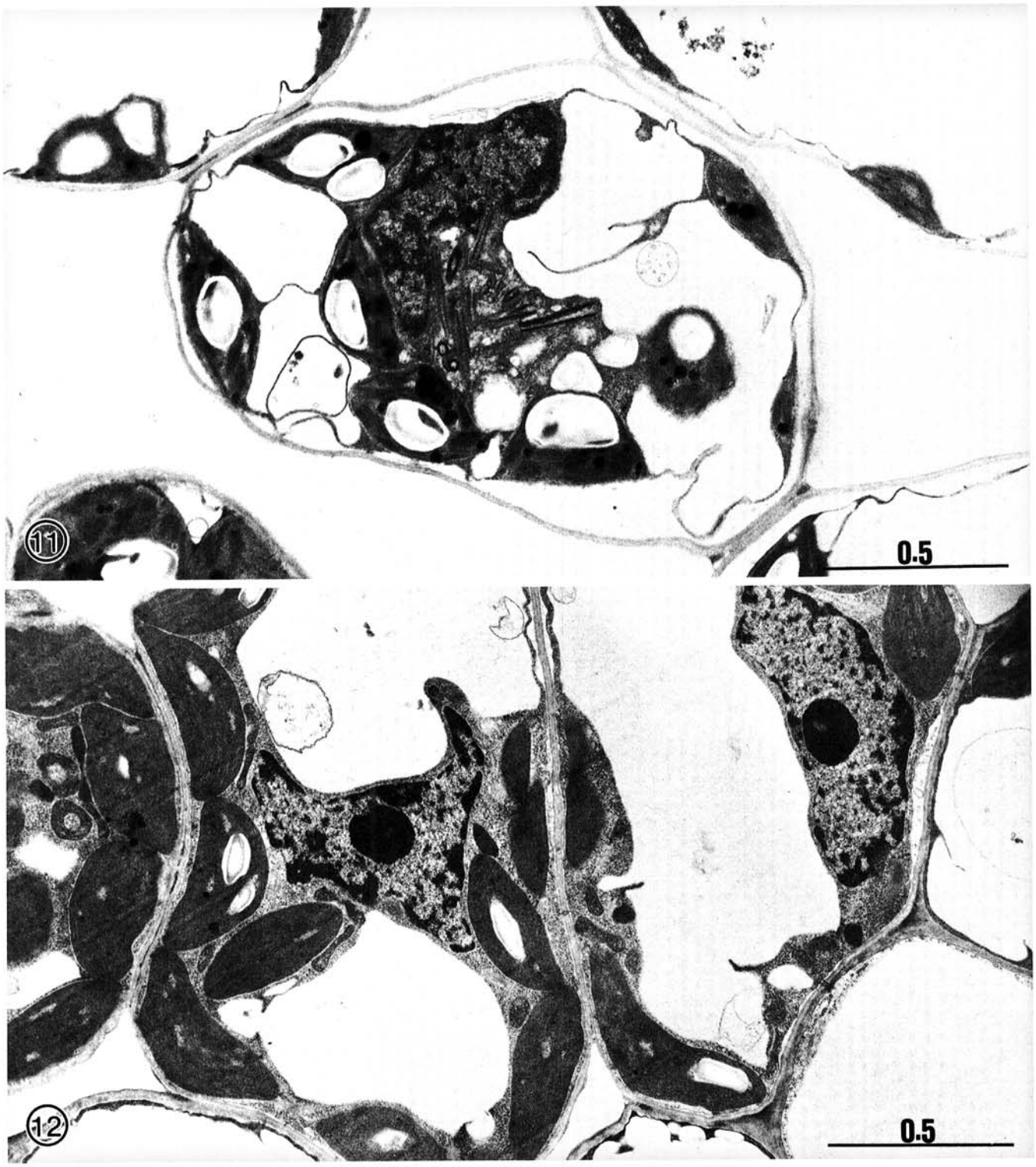
Monolayers of virus particles in cytoplasmic sheets were observed in both stripe- and blotch-infected tissue as early as the second stage. As the leaf tissue matured and the disease progressed, these structures became more abundant. Leaves at the mature, expanded stage had reduced cytoplasmic content, but contained numerous sheets filled with virus particles. Although such sheets



Figs. 7–10. Transmission electron micrographs of thin sections of peanut leaves infected with peanut stripe virus, blotch (PStV-B) or stripe (PStV-S) variants. (Bars = micrometers.) **7**, Parenchyma cell in a third-stage (two-thirds expanded) leaf infected with PStV-S showing mitochondria (M) along the length of a scroll inclusion (SC) and with pinwheel inclusions and a laminated aggregate (LA) (35,000×). **8**, Parenchyma cell in a third-stage (two-thirds expanded) leaf infected with PStV-S showing a pinwheel inclusion (PW) in longitudinal section associated with a mitochondrion (M) (50,000×). **9**, Palisade cell in a third-stage (two-thirds expanded) leaf infected with PStV-S showing scrolls (SC) in cross section, short, curved laminated aggregates (LA) and vesicles (V) (50,000×). **10**, Palisade cell in a fourth-stage (fully expanded) leaf infected with PStV-B showing virus particles (VP) lining tonoplast membrane (50,000×).

and cytoplasmic strands have been reported for several potyviruses, their function has never been determined (1,8,10,17). Suggestions have included a defense reaction of the infected cells (10) and an intercellular virus localization mechanism responsible for a tolerant symptom response (8). With PStV, this may also be a virus localization mechanism and not simply accidental trapping, because the prevalence of the structures increased with time of infection, regardless of PStV variant and symptomology.

Although some scroll inclusions were observed in early stages, they were not seen in abundance in PStV-infected cells until the third stage, when symptoms were also evident. Short, laminated aggregates were not observed until this stage and did not increase in abundance in later stages. The appearance of scrolls in the later stages of infection could be the result of a change in expression or posttranslational modification of viral-encoded genes, or a change in the assembly of the viral-encoded proteins to form scrolls and laminated aggregates instead of, or in addition to, pinwheels. Both pinwheel and scroll inclusions were often found near mitochondria, but it can only be speculated that a functional relationship might exist between inclusions and mitochondria.



Figs. 11–12. Transmission electron micrographs of thin sections of parenchyma cells in mesophyll tissue of peanut leaves infected with peanut stripe virus blotch (PStV-B) sampled at the fifth stage (mature, expanded) from an area showing light green (Fig. 11: 6,400×) or dark green (Fig. 12: 6,400×) symptoms. (Bars = micrometers.)

TABLE 1. Peanut stripe virus (PStV) particles observed in extracts of the seventh quadrifoliolate leaf of Florigiant peanut at five expansion stages by using immunosorbent electron microscopy

Expansion stage[a]	No. of virus particles[b]	
	Stripe	Blotch
1	52	65
2	31	41
3	77*	121*
4	104*	293*
5	97*	184*

[a] 1 = closed stage; 2 = one-third expanded stage; 3 = two-thirds expanded stage; 4 = fully expanded stage; 5 = mature, expanded stage.
[b] Mean number of particles counted in five fields from each of two electron microscope grids. An asterisk indicates a significant difference between variants at 0.05 level with single degree of freedom orthogonal comparison.

TABLE 2. Virus particles observed in extracts of light and dark green leaf tissue infected with peanut stripe virus (PStV) by using immunosorbent electron microscopy

Tissue type	No. of virus particles[a]	
	Stripe	Blotch
Light green	505*	1,209*
Dark green	36	34

[a] Numbers represent the mean of five randomly chosen fields from each of three electron microscopy grids. An asterisk indicates a significant difference between variants at 0.05 level with single degree of freedom orthogonal comparison.

In the dark green areas of leaflets infected with either variant, no virus-related cyloplasmic inclusions were observed, suggesting that this tissue did not support replication of the virus, because these inclusions consist of viral-encoded proteins that are expressed during coat protein synthesis (5). The few virus particles found by ISEM in extracts of dark green tissue could be the result of passive movement of virus from cells of light green tissue or from incomplete tissue separation during sampling. These observations with PStV are in agreement with studies conducted by Pares and Bertus (13), who found that only cells from light green areas of potyvirus-infected leaves of *Crinum* sp. contained pinwheel and tubular inclusions, as well as virus particles scattered throughout the cytoplasm and in densely aggregated masses.

Two main ultrastructural differences were observed between the stripe- and blotch-infected tissue with regard to location of virus particles within cells. First, the assembly of virus-like particles measuring 13 nm in diameter into aggregates was observed only in cells infected with the blotch variant. Similar aggregations of virus-like particles have been reported in wheat infected with wheat spindle streak mosaic virus (7), in morning glory infected with sweet potato russet crack virus (11), and with other potyviruses. The second difference in blotch-infected tissue was that pinwheel inclusions have virus-like particles situated between the arms of the pinwheels. Such particle-pinwheel associations were rarely observed in stripe-infected tissue, even though it is thought that pinwheels function in the assembly of potyvirus particles (4,5). Possible explanations for the differences are different virus concentration in cells and differential stability of the particles.

Particle assays by ISEM demonstrated that PStV-B attains a higher titer than does PStV-S, but infectivity and relative amount of virus recovered by partial purification were approximately equal for the two variants. Comparison of negatively stained particles of the two variants, either in leaf dips or from partially purified preparations in the presence of antioxidant, revealed no observable differences in virion stability.

Differences in the morphology of cytoplasmic inclusions have been used to separate potyviruses into four subdivisions (4), but the published micrographs of PStV (2) do not allow its placement into a subdivision. In numerous ultrathin sections of PStV-infected leaf tissue, pinwheels and scrolls characteristic of subdivision I are the primary cytoplasmic inclusions induced by both PStV-B and PStV-S. But on close examination of micrographs, it was observed that both variants also induce a low number of short, laminated aggregates. We thus conclude that PStV should be placed in subdivision III, but concur with Francki et al (5) on the difficulty of distinguishing between subdivisions I and III.

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