ULTRASTRUCTURAL CYTOLOGY OF PEANUT INFECTED WITH PEANUT STRIPE VIRUS

by

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(ABSTRACT)

Two isolates of peanut stripe virus (PStV), stripe and blotch, were compared ultrastructurally in peanut (Arachis hypogaea L. 'Florigiant') at several stages of leaf expansion. Ultrathin sections of young leaves infected with either isolate of PStV revealed pinwheel inclusions attached to the cell wall near plasmodesmata. The cytoplasm of infected cells were highly vesiculated. Virus particles amassed in crystalline arrays were observed in blotch infected cells. Virus particles were observed along the arms of pinwheel inclusions. Scroll inclusions appeared in PStV infected cells at a later stage of leaf expansion. In more mature leaves, pinwheel and scroll inclusions occurred in the cytoplasm in association with mitochondria. Virus particles were observed free in the cytoplasm as well as concentrated in linear arrays along the inner surface of the tonoplast. Membrane and organelle degradation was evident in cells infected with

either isolate of the virus. Numerous cytoplasmic inclusions and virus particles were observed in cells from light green areas of the leaf. Cells from dark green areas did not contain cytoplasmic inclusions and contained few if any virus particles. Particle measurements show stripe and blotch isolates to have a mean length of 753 nm and 747 nm for leaf dip preparations and 746 nm and 745 nm for partially purified preparations, respectively. Both isolates had a modal length of 750 nm, regardless of the extraction procedure.

The relative virus titer of each isolate was determined in peanut leaves at five stages of leaf expansion and in dark green and light green areas of infected leaves. Virus titer increased significantly from the closed to the fully expanded stage, at which time the virus titer peaked and then decreased slightly. Virus titer was consistently higher in leaves infected with the blotch isolate at all expansion stages. Virus titer was also higher in cells from light green areas of the leaf than from dark green areas of the leaf, regardless of isolate.

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I. INTRODUCTION

Peanuts (Arachis hypogaea L.) continue to be a major economic commodity in the United States. Approximately 600,000 ha (1,482,600 acres) of peanuts are produced annually in the United States, having a farm value exceeding one billion dollars (Porter et al., 1984). Peanuts are a controlled commodity and are typically grown in states which have loose sandy soil and a long growing season. Peanut production is especially prevalent in the southern United States, with 98 percent of the production occurring in Virginia, Alabama, North Carolina, Georgia, Texas, Florida, and Oklahoma (Porter et al., 1984). As the intensity of peanut production increases, the potential development of new diseases increases as well. In 1982, a new viral disease was discovered on peanuts at the southern regional plant introduction station (SRPIS) in Experiment, Georgia. The virus, named peanut stripe (PStV) by Demski et al., (1984), was introduced into the United States via seed germplasm from the People's Republic of China. Recent investigations by Demski and Lovell (1985) show that two isolates of PStV exist. Peanut plants infected with the

stripe isolate typically exhibit dark green stripes along the lateral leaf veins. As stripe-infected plants mature and the infection progresses, an oakleaf pattern usually develops on the leaves and becomes the predominant symptom expressed. Plants infected with the blotch isolate develop dark green circular areas on the leaves. The blotch isolate could not be serologically distinguished from the stripe isolate and is considered to be a symptom variant of PStV (Demski and Lovell, 1985). Both stripe and blotch isolates are often found together in field grown peanuts. Peanut stripe has a seed transmission rate of 30 percent and is also aphid transmitted in a non-persistent manner (Demski et al., 1984). The virus is of economic importance since it has been shown to cause a 20 percent reduction in seed number and seed weight (Demski et al., 1984). Of the twelve groundnut cultivars most commonly grown in the U.S., all were found to be susceptible to mechanical inoculation with PStV (Demski et al., 1984).

During the 1983 growing season, PStV was found by P. M. Phipps at the Tidewater Research and Continuing Education Center (TRACEC) in test plots containing U.S and Chinese peanuts in Suffolk, Virginia. Isolations made from these peanuts revealed a mixed infection of the stripe and blotch isolates of PStV (personal communication with S.A. Tolin).

The potential for this disease to become epiphytotic, based on the frequency of seed transmission and the presence of the aphid vector, has encouraged further ecological studies concerning PStV. Other studies conducted to date have concentrated on the physical and serological properties of the virus. Little information exists on the basic effects of PStV on its host at the sub-cellular level. The specific objectives of my research are:

1) to examine the macroscopic symptomatology of PStV on peanut, and to select sub-isolates of virus that induce characteristic and reproducible symptom types and maintain the isolates by mechanical transmission.

2) to relate the progression of symptoms with plant development at both the morphological and cytological levels.

3) to determine the approximate and relative virus concentrations in leaves expressing different symptom types and at specific growth stages of the peanut plant infected with PStV.

4) to determine length of the virus particles inducing stripe and blotch symptoms.

II. REVIEW OF LITERATURE

A. PEANUT HISTORY

The peanut, Arachis hypogaea L., is a legume native to South America (Hammons, 1973). Peanut remnants dating back to 1500-1200 B.C., were recovered from ancient archeological sites on the northern coast of Peru. It was once believed that the peanut was first domesticated in the Gran Chaco region of southwestern Brazil; however, extensive investigations by Krapovickas (1968, cited by Hammons, 1982), and Cardenas (1969, cited by Hammons, 1982) support a Bolivian origin. Theories of an African or Asian origin were soundly disproved by de Candolle in 1882 (cited by Hammons, 1982). The earliest occurrences of peanut in China were dated after the discovery and conquest of America (Goodrich, 1937). Consequently, while the exact origin of the peanut remains unknown, South America is the unquestionable place of original cultivation (Hammons, 1982).

In the early 16th century, Spanish and Portuguese explorers found the native peoples cultivating the peanut in

several of the West Indian islands, Mexico, Argentina, Brazil, Paraguay, Bolivia and Peru (Hammons, 1973). The Portuguese explorers have been credited with the introduction of the peanut to African agriculture, and possibly to other countries, however, Hammons (1973) states that no record exists to document this supposition. The peanut was eventually brought to the southeastern coast of the United States. Again, however, the time and place of this introduction was not recorded (Hammons, 1973). The contention that the peanut was first introduced into the U.S. via slave ships from Africa rest primarily upon an interpretation made by Sloane on earlier writings of Clusius (Hammons, 1973).

Today, the peanut is recognized as an important commercial crop and is grown in over 50 countries on 6 continents. In the United States, peanuts are grown primarily for edible purposes, whereas in other countries peanuts are grown mainly for oil and meal (Woodroof, 1973). The most common variety grown in Virginia is Florigiant. This variety is a large seeded Virginia-type peanut used primarily for edible purposes such as candy and salted peanuts. Other varieties commonly grown in Virginia include: VA81B, NC7, NC6, NC8C and GK3. These varieties are also large seeded Virginia-type nuts.

Approximately two-thirds of the world's peanut crop is crushed for oil (Woodroof, 1973). Oil stock are those peanuts that are rejected or diverted from edible channels. Peanuts may be rejected because of low quality resulting from inadequate storage or because of an over supply of a particular variety. Peanut varieties used for oil extraction are usually mixed and considered collectively as "oil stock".

The importance of peanuts as a food crop has increased substantially in recent years. Peanut seeds are high in calories and contain 25% protein (McGill, 1973). Their use as an edible food crop is likely to increase due to an increased awareness of protein shortages existing in the world today.

B. PEANUT MORPHOLOGY

The cultivated peanut plant, Arachis hypogaea L., is an erect or prostrate, self pollinating, annual legume. The peanut is one of the few plants that produce flowers above ground and bear fruit, pods containing one to five edible seeds, below ground. Seeds of the cultivated peanut vary in size, shape and color (Gregory et al., 1973). Each seed is enclosed in a thin, papery seed coat and is composed of two cotyledons, an epicotyl with 6-8 differentiated leaves, a

hypocotyl and a primary root (Gregory et al. 1973). The dormant peanut embryo contains all of the leaves and above ground parts which the seedling will have for the first 2 to 3 weeks of growth (Gregory et al., 1973). Germination of the seed, characterized by the appearance of the radicle, occurs 24-36 hours after planting. The cotyledons and first leaves emerge 8-10 days after planting.

The root system of the peanut consists of a well developed tap root with many laterals. Adventitious roots that extend from the hypocotyl also serve to anchor the plant. The transition from the hypocotyl to the root is sharply marked by an abrupt constriction or collar. At the collar, the intact epidermis of the hypocotyl gives way to the non-epidermal outer layers of the primary root. It has been suggested that the uneven surface of the peanut root serves as an active absorptive surface (Richter, 1899, cited by Gregory et al., 1973).

Peanut leaves are pinnately compound with two opposite pairs of leaflets 2-5 cm long (Gregory et al., 1973). Leaflets are elliptical and range in color from dark dull green, dark blue green to yellow green depending on the cultivar (Gregory et al., 1973). The leaves are alternately arranged in a spiral (2/5 phyllotaxy on the n axis).

Flowers are born on inflorescences which are located in the axils of the leaves. However, flowers do not occur at the same nodes with vegetative branches. Each inflorescence bears three to several flowers. The hypanthium or calyx tube is filiform. There are two calyx lobes; an awl-like lobe opposite the keel and a wide, four notched lobe opposite the back of the standards. The five petals consist of a yellow-orange standard, two yellow-orange wings and two petals fused to form a keel. The staminal column is composed of ten filaments, two of which are sterile. The pistil has an ovary containing one to five ovules. A long filiform style extends along the hypanthium and results in a club-shaped stigma among the anthers.

After fertilization, a needle-like structure commonly called 'the peg' develops and elongates. The fertilized ovaries, located behind the tip of the peg, are carried down to and enter into the soil. The peg, positively geotropic, grows into the soil to a depth of two to seven cm, after which time the tip orients itself horizontally, the ovary enlarges and pod development begins.

C. ANATOMY OF THE PEANUT LEAF

The anatomy of the peanut leaf was described in detail by Brennan (1955). The peanut leaf is a normal angiospermous-type leaf comprised of an upper and lower epidermis, a palisade and spongy mesophyll layer, and a layer of large parenchymatous cells associated with water storage. The upper and lower epidermis is continuous around the lateral edges of the leaflets. Epidermal cells have thickened outer and inner walls with a thin cuticle present on the outer walls. Cells comprising the upper epidermis range from 35 microns in width by 35 microns in depth to 70 x 35 microns. Cells of the lower epidermis range from 50 x 15 microns to 90 x 20 microns in size. In the region of the midrib, cells of the lower epidermis are considerably smaller in size. Stomata were present on both the upper and lower epidermis. Brennan noted the presence of non-staining rhombohedral crystals in both upper and lower epidermal cells. He did not speculate as to their origin or function.

Below the upper epidermis, Brennan observed 3-4 layers of elongate parenchymatous cells also known as the palisade mesophyll layer. These cells contained nuclei and many chloroplasts. Brennan also noted that the first layer of palisade mesophyll cells were the longest, with each subsequent layer becoming progressively shorter. The

palisade mesophyll merges into the spongy mesophyll which is a region of loosely organized parenchyma cells of irregular shape and placement. Spongy mesophyll cells also contain nuclei and numerous chloroplasts, but are smaller and more rounded than cells in the palisade layer. Between the spongy mesophyll and lower epidermis is a layer of large parenchymatous cells 70-100 microns in diameter. These cells are devoid of chloroplasts and cell organelles, and are considered to be water storage tissue.

Brennan noted that each vascular bundle is surrounded by at least one layer of achlorophyllous parenchyma cells that extends from each vascular bundle to the upper epidermis by one or two rows of cells. These cells are also in contact with the water storage cells below the vascular bundles.

The midrib consists of several rows of large xylem tracheary elements arranged in a fan-like pattern. Four or five layers of immature sclerenchymatous cells were located to the adaxial side of the xylem. These sclerenchymatous cells were continuous with cells located in the petiole. Above this group of cells is three or four layers of parenchymatous cells, of which three or four large centrally located cells have developed into tannin sacs. There is no active cambium in the midrib. On the abaxial side of the

xylem are eight or nine layers of phloem cells. Of these, four or five are layers of small cells that consist of sieve elements, companion cells and phloem parenchyma, while the other four to five layers are of extraxylary fibers , similar to those found on the abaxial side of the vascular system in petiole.

D. DISEASE DESCRIPTION

1. History

During the 1982 growing season, virus-like symptoms different from those caused by the endemic peanut mottle virus (PMV) were observed on the foliage of peanuts by plant pathologist G. Sowell at the southern regional plant introduction station (SRPIS) in Experiment, Georgia. Host range tests were conducted by Demski et al. (1984) who detected mixed viral infections and subsequently derived pure cultures. Additional seed from lots used to make the original 1982 field planting were planted in the greenhouse and observed for symptoms. These lots consisted of seed from the People's Republic of China, Sudan, Senegal, Japan, India and Zimbabwe. Vein-banding and stripe symptoms similar to those observed in the field were only found in seedlings grown from Chinese seed. Serological tests conducted by Demski et al. (1984) showed that the isolate

failed to react with antiserum to peanut mottle virus (PMV). Single lesions were removed from Chenopodium quinoa plants previously inoculated with the virus and were used to inoculate peanut seedlings mechanically. The majority of these plants developed dark stripes along the lateral leaf veins for which Demski appropriately named the virus Peanut Stripe (PStV). Demski observed that several peanut plants inoculated from the single lesions exhibited dark green circular areas against a lighter green background that were not associated with the lateral veins. The isolate that induced such symptoms was called "peanut blotch". The blotch isolate could not be distinguished serologically from the stripe isolate and is considered a symptom variant of PStV (Demski and Lovell, 1985).

2. Symptomatology

Peanuts infected with PStV may exhibit one of several different symptom types. Demski et al. (1984) found that plants infected with the stripe isolate of the virus exhibit dark green stripes along the lateral leaf veins against a light green background. As these plants matured and the infection progressed, an oakleaf pattern developed on the leaves. Although the stripe and oakleaf pattern appeared on the same infected plant, they did not appear on the same

leaves and the oakleaf pattern became the predominant symptom as the plant matured. Peanut plants infected with the blotch isolate exhibit dark green circular areas or blotches on leaves that are light green in color. The dark green color that appeared on the stripe and blotch infected leaves appeared to be close in color to that of a healthy leaf. At no time did Demski observe the oakleaf pattern on blotch infected plants.

3. Host Range and Transmission

In preliminary host range tests, local lesions developed on inoculated Chenopodium quinoa and C. amaranticolor whereas a systemic mottle developed on Vigna unguiculata, Glycine max 'Yelredo', Lupinus albus, Trifolium incarnatum 'Dixie', T. subterraneum 'Mt. Barker', T. vesiculosum 'Yuchi' and Nicotina benthamiana. Tests were conducted by Demski et al., (1984) to determine if PStV was transmissible by aphids. Colonies of Aphis craccivora were starved for 1-12 hours and then allowed to make a single acquisition probe of 1 min on infected peanut leaves. Ten aphids were transferred to healthy Argentine peanut plants and allowed a 1 hr inoculation access period. Plants were tested for PStV by enzyme-linked immunosorbent assay (ELISA) and local lesion assay using C. amaranticolor as the assay

plant. PStV was found in 11 out of 14 peanut plants tested. Greenhouse tests were conducted by Demski et al., (1984) in order to determine the seed transmission rate of PStV. Seed collected from PStV-infected peanut plants were grown in the greenhouse and plants were tested individually for the virus using direct immunosorbent assay. Results of these tests indicate that PStV has a seed transmission rate of 30 percent.

4. Distribution

During the summer of 1983, PStV was detected by ELISA tests in peanut plants grown at two Georgia experiment stations. Infected peanuts growing at the Georgia Experiment Station and the Plant Materials Center originated from seed from the People's Republic of China (Demski and Lovell, 1985). Peanut stripe was also found in peanut plants located near the Chinese plants; these plants grew from seed originating from other countries. Peanut stripe was not detected in any of the 57 commercial fields seeded with Georgia certified seed (Demski and Lovell, 1985)

Surveys for PStV were conducted in other peanut growing states in the southeastern United States during the latter part of the 1983 growing season. Peanut stripe was identified on peanuts in North Carolina, Virginia, Florida

and Texas (Demski and Lovell, 1985). The virus was found in research and institutional test plots but not in commercial plantings.

5. Importance

Peanut stripe is a virus of legumes and infects several cultivars of soybeans, cowpeas, lupines and forage legumes such as arrowleaf, crimson and subterranean clovers. Twelve cultivars of peanuts commonly grown in the U.S. were all found to be susceptible to PStV. The effect of PStV on peanut yields is not known at this time. Preliminary greenhouse tests conducted by Demski et al. (1984) showed that seed collected from PStV infected plants showed a 20% reduction in seed number and seed weight.

There are several theories on how PStV could have entered and been disseminated in the United States (Demski and Lovell, 1985). One obvious means of entry and dissemination is from the Chinese seed entered into the U.S. plant introduction collection in 1979. Additional Chinese seed entered the U.S. in 1980 when a drought reduced the production of U.S. peanuts. In 1981, small samples of certain lines of peanuts were distributed by a delegation of visiting scientists from China who toured the United States. Contaminated seed may have also been exchanged unknowingly

by individuals representing personal business or research groups.

The exchange of germplasm within and between different countries is important since the improvement of peanut production depends to a large extent upon the incorporation of new germplasm. However, germplasm exchange should be balanced with adequate safeguards to prevent the spread of disease agents. Demski and Lovell (1985) stated that preventing further spread of PStV depends upon eliminating contaminated seed and permitting only virus-free seed to be distributed. Guidelines for controlling the spread of PStV have currently been instituted at at the University of Georgia. To date, no guidelines have been instituted in the state of Virginia.

E. DESCRIPTION OF THE VIRUS

1. Size and Structure

Recent investigations by Demski et al., (1984) demonstrated that PStV is a flexuous rod approximately 13 x 750 nm in size with a coat protein molecular weight of 34,000 daltons. Preliminary ultrastructural studies of leaf tissue infected with PStV revealed the presence of cylindrical inclusions, suggesting that this virus is a member of the potyvirus group.

2. Serological Relationships

Serological tests were conducted in order to assess the relationship between PStV and other potyviruses (Demski et al., 1984). In direct-ELISA, PStV cross reacted strongly with black eye cowpea mosaic virus (BlCMV), clover yellow vein virus (CYVV) and soybean mosaic virus (SMV) antisera, and a mild reaction was detectable with pepper veinal mottle virus (PVMV) antiserum. Antisera to bean yellow mosaic virus (BYMV), peanut green mosaic virus (PGMV), peanut mottle virus (PMV), potato virus Y (PVY) and tobacco etch virus (TEV) did not react with PStV. In indirect-ELISA using F(ab')2 fragments, PStV reacted strongly with antiserum to BlCMV, CYVV and to a lesser extent with PVMV and SMV.

3. Differentiation from other Potyviruses

There appears to be a biological distinction between PStV and other potyviruses infecting peanuts. These differences are based upon host range, symptomatology and the results of serological tests such as ELISA and Ouchterlony. Potyviruses that might be confused with PStV include peanut green mosaic virus (PGMV), groundnut eyespot virus (GEV), peanut mottle virus (PMV) and a virus causing a mild mottle on peanut (VPMM).

Peanut green mosaic virus, another recently described member of the potyvirus group, differs from PStV by not being seed transmitted (Sreenivasulu et al., 1981) and was not found to be serologically related to PStV by direct and indirect ELISA (Demski et al., 1984). Groundnut eyespot virus, characterized by dark green spots surrounded by a chlorotic halo on infected leaves, strongly resembles the blotch symptom type of PStV. However, host range tests conducted by Dubern and Dollet (1980) indicate that Chenopodium quinoa and C. amaranticolor are not hosts of GEV, whereas Demski et al., (1984) reported that local lesions resulted when Chenopodium sp. were inoculated with PStV. In addition, GEV did not react with antiserum to CYVV but did react strongly with antisera to pepper veinal mottle virus (PVMV) (Dubern and Dollet, 1980); whereas PStV reacted strongly with antiserum to CYVV and only reacted mildly to antisera of pepper veinal mottle virus.

A recently described potyvirus causing peanut mild mottle (VPMM) in China was found to react strongly with PStV antisera (Demski et al., 1984). However, major differences exist between the two potyviruses. Peanut mild mottle does not react with antisera to SMV and CYVV, does not infect Vigna unguiculata and has a seed transmission rate of 4.8 percent (Xu et al., 1983). In contrast, PStV reacts with

antisera to SMV and CYVV, infects Vigna unguiculata and has a seed transmission rage of 30 percent (Demski et al., 1984).

Peanut mottle virus (PMV) naturally infects both soybean (Glycine max (L.) Merrill) and peanut (Arachis hypogaea L.) in many parts of the world (Herold and Munz, 1968; Kuhn, 1965; Bock, 1973). Several investigators have reported that PMV was seed transmitted in peanut although at a lower rate than found for PStV (Kuhn, 1965; Bock, 1973). Peanut stripe differs from PMV by inducing local lesions on Chenopodium sp. and not infecting Phaseolus vulgaris 'Top Crop' or Pisum sativum 'Little Marvel'.

F. POTYVIRUSES

The potyvirus group, is presently recognized as the largest group of plant viruses. Potyviruses are typically long, flexuous rods ranging form 680 to 900 nm in length (Matthews, 1981). These viruses may be transmitted by sap, seed and commonly by aphids in a non-persistent manner (Matthews, 1981). The first system of virus classification based upon particle morphology and size was proposed by Brandes and Wetter in 1959. This system comprised many groups of rod-shaped viruses, and included a group with flexuous rods about 680-900 nm in length with potato virus Y

as a member. Viruses grouped in this manner also appeared to have certain biological properties in common, including the ability to be transmitted by certain vectors. The term potyvirus, a sigla for potato virus Y, was first used by Harrison et al., (1971), as a group name. Currently, the classification of viruses is based upon particle morphology and size, as well as nucleic acid type, the number of nucleic acid strands the particles possess, the number of virion types and genome fragments and whether the nucleocapsids are naked or enveloped (Bos,1983).

One of the distinctive characteristics of potyvirus infections is the presence of cylindrical inclusions in the cytoplasm of affected cells (Edwardson, 1966a; Edwardson and Christie, 1978). Studies conducted by Purcifull et al. (1973) showed that cytoplasmic inclusions are composed of viral encoded proteins that are serologically distinct from viral coat and host proteins. Recent investigations by Dougherty and Hiebert (1980) also support this premise. Analysis and identification of <u>in vitro</u> translation products of two potyviruses, tobacco etch (TEV) and pepper mottle virus (PeMV), by gel electrophoresis and immunoprecipitin tests provides direct evidence that the inclusions associated with potyviruses consist of viral encoded, nonstructural proteins (Dougherty and Hiebert, 1980).

Hiebert and Dougherty (1981), constructed a genetic map of the potyvirus genome based on the analysis of products of in vitro translations of five potyviral RNA's. Four of six distinct translation products detected were identified as virus specific proteins found in vivo. Hiebert and Dougherty presumed the translation products to be gene read throughs on the basis of serological reactions and size. The proposed genetic map of for the potyviral RNA is as follows:

5' end- 77-90 $x10^3$ Daltons (77-90 kd) protein gene - 49 kd protein gene - 41-50 kd protein gene - 68-70 kd cylindrical inclusion protein gene - 54-56 kd protein gene - 30-33 kd capsid protein gene - 3' poly A tail.

Variations in the viral genome can be expected to produce differences in inclusion morphology. According to Christie and Edwardson (1982), the inclusion type most commonly associated with potyvirus infections are the cylindrical inclusions. Cylindrical inclusion is a term used to describe the three dimensional aspects of proteinaceous inclusions induced by the potyvirus group. These inclusions may be observed as pinwheels, scrolls, loops and cylinders, depending upon the plane of section. A set of terms have been established by Edwardson to describe

the morphology of the various inclusion types. Pinwheels are cylindrical inclusions viewed in cross section and appear as a group of curved arms converging around a central core. The scroll inclusions result from the extension and rolling up of pinwheel arms. Bundle inclusions are longitudinal sections of pinwheel inclusions whereas tubules are longitudinal sections of scroll inclusions. Laminated aggregates have also been observed in cells infected with potyviruses. These aggregates appear as large, flat plates composed of many thinner plates in parallel orientation.

Edwardson et al., (1984) used morphological differences of cytoplasmic inclusions to group the potyviruses into four subdivisions. Viruses inducing scroll and pinwheel inclusions were assigned to subdivision-I; those inducing laminated aggregates and pinwheel inclusions were assigned to subdivision-II; those inducing pinwheels, scrolls and laminated aggregates were assigned to subdivision-III; and those inducing pinwheels, scrolls and short, curved laminated aggregates were assigned to subdivision IV. To date, PStV has not been assigned to a subdivision.

1. Methods For Studying Potyvirus Infections

Light Microscopy

One of the distinguishing features of potyviruses is the distinct intracellular inclusions which are associated with them. The light microscope, while generally thought to be incapable of resolving virus particles, has proven valuable for the study of viral inclusions. Inclusion bodies are usually abundant in leaf cells near areas showing distinct symptoms, and can be observed in epidermal strips or in fixed and mounted sections of leaf tissue. Stains have been employed to aid in the detection and identification of cellular inclusions at the light microscope level (Christie, 1967). Calcomine orange-"Luxol" brilliant green combination stain and Azure A are two stains commonly used for detecting inclusions. Paracrystalline inclusions induced by celery mosaic virus (CeMV) stain olive-green with O-G and red to violet with Azure A (Christie and Edwardson, 1982). Cylindrical inclusions stain olive-green to brown with O-G but do not stain with Azure A. Consequently, different inclusion types can be distinguished from one another be their staining reaction. Epidermal strips immersed in pyronin-methyl green stain nuclei blue and inclusions red (Kado, 1972). This staining method also gives some information about the chemical nature

of the material. The red color indicates the presence of ribonucleic acid (RNA), whereas the blue color indicates the presence of deoxyribonucleic acid (DNA). Antibodies labelled with fluorescent compounds have been used as a specific stain for locating viral antigens within cells. The fluorescent antibody method has been used to study the intracellular location and distribution of plant viruses within cells of host plants (Nagaraj, 1965) and insect vectors (Chiu and Black, 1969).

Electron Microscopy

The electron microscope has been a useful tool for studying the ultrastructure of virus infections. The high magnification and resolution capacity of the electron microscope has enabled virologists to visualize and study the morphology of virus particles which are too small to be seen with the light microscope. Depending on particle size and morphology, a virus can be tentatively assigned to a taxonomic group. Examination of virus induced inclusions with the electron microscope can also be useful for the identification and characterization of many plant viruses. Nine virus groups are known for inducing distinctive inclusions which are diagnostic for infections at the group level (Hamilton et al., 1981). These groups include:

Tobamovirus- cytoplasmic, crystalline plates;

Potyvirus- cytoplasmic, cylindrical inclusions (pinwheels, scrolls, bundles, tubes);

Closterovirus- banded bodies predominantly in the phloem;

Comovirus- cytoplasmic, crystalline inclusions;

Alfalfa Mosaic Virus- hexagonally, packed layers of particles;

Tomato Spotted Wilt Virus- particle aggregates enclosed in membranes;

Phytoreovirus- particles in tubules, particles in crystalline arrays, granular-fibrillar viroplasms;

Fijivirus- particles in tubules, particles in crystalline arrays, granular-fibrillar viroplasms;

Caulimovirus- viroplasms containing virus particles;

Edwardson et al., (1972) used light and electron microscopy to compare inclusions induced by cowpea virus (CV) and bean yellow mosaic virus (BYMV). Striking differences were found between inclusions of CV and BYMV, indicating that they are two distinct viruses belonging to the potyvirus group, and suggesting that inclusion morphology could be used as a basis for distinguishing between similar viruses.

Ultrathin and serial sectioning have been extremely useful for establishing the morphologic configuration of potyvirus inclusions. Using a series of ultrathin sections,

Edwardson (1966b) constructed a three dimensional diagram of cylindrical inclusions induced by tobacco etch virus (TEV). Ultrathin sections of vacuum dehydrated tissues and negatively stained leaf extracts showed that inclusion bodies induced by TEV and watermelon mosaic virus (WMV) had a substructure of fine striations with an average periodicity of 5 um (Edwardson et al., 1968). Differences in the morphology and substructure of inclusions and rod-shaped particles indicated that inclusions induced by TEV and WMV were not composed of aggregated virus particles. Later studies conducted by Purcifull et al., (1973) and Dougherty and Hiebert (1980) showed that cylindrical inclusions induced by potyviruses were protein products of the viral genome.

An electron image results from the interaction between the electron beam and the specimen. The quality of the image is improved by positively staining the specimen with heavy metal solutions such as uranyl acetate, lead citrate and phosphotungstic acid (Hayat, 1970). These stains have a selective specificity for cellular components which increases the electron density of the specimen and facilitates better contrast and resolution of the material being examined. Negative staining with electron-dense stains are used for revealing the detailed morphology of

virus particles or other particulate matter. In negative staining, the electron dense solution penetrates available spaces on the surface and around the virus particle, whereas positive stains bind and react chemically with the particle. Thin sectioning and use of heavy metal negative stains have been useful in resolving the substructure of inclusions and virus particles.

2. Detecting and Quantifying Virus Concentration

a. Infectivity Assays

The quantal assay was the first method used for measuring infectivity (Matthews, 1981). This method consists of inoculating groups of plants with different dilutions of the virus suspension (leaf extract) and recording the number of plants which become systemically infected. This assay was particularly useful for measuring the infectivity of viruses that did not have a suitable local lesion host or with viruses that had to be transmitted using insect vectors. The primary drawback of this assay is that it requires a considerable amount of time and plants to obtain a reliable answer.

In 1929, Holmes (cited by Matthews, 1981) introduced the local lesion assay for measuring the approximate

infective virus content of virus suspensions. The local lesion assay is based upon the theoretical principle that every infective virus particle is capable of inciting an infection/disease. The assay is conducted by inoculating a local lesion producing host with a specific amount of diluted virus suspension. The dilution of the virus suspension used in the assay is first determined by a dilution curve, which helps determine the dilution with which a change in virus concentration is accompanied by a relatively equivalent change in lesion number. This type of assay is limited to measuring virus particles that are biologically active, giving no indication of the number of virus particles present in the sample.

b. Density Gradients and Ultraviolet Absorption

Viruses, being nucleoproteins, have a characteristic absorption spectra. The maximum absorbancy of protein occurs at a wavelength of 280 nm, while the nucleic acid of a virus has an absorbance maximum around 260 nm. Virus concentration in purified preparations can be determined using an ultraviolet spectrophotometer or analyzer. The UV analyzer records the absorbance of flowing liquids and operates at wavelengths of 260 nm and 280 nm. The UV analyzer is often used with a density gradient fractionator

to determine the amount of virus in the sucrose density gradient after centrifugation (Brakke, 1963). The gradient containing the concentrated virus zone is passed through a flow cell where the continuous UV absorbance profile of the gradient is recorded. The area under the recorded peaks is proportional to the total mass of the absorbing material in the virus zone.

c. Enzyme-linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) is a serological test used to detect and quantitatively determine virus concentration in purified preparations and in crude tissue extracts (Clark and Adams, 1977). Double-sandwich or direct ELISA is conducted by adding a sample containing virus to a well of a microtiter plate which is first sensitized with a virus-specific antibody. If the antibody is specific for the virus being assayed the antigen will bind with the antibody and become stationary in the well of the plate. An enzyme-labelled antibody is added to the well and if the antibody is specific for the antigen, they will bind. However, if the antibody is not specific for the antigen the antibody will be removed by rinsing. An enzyme substrate is then added to the well. If the enzyme-labelled antibody is present, the two will interact and a yellow

color will result from the degradation of the substrate, indicating a positive reaction. If no color reaction occurs, then the antibody being used is not specific for the virus being assayed and a different specific antibody will have to be used. The virus concentration can be measured colorimetrically using a spectrophotometer at a wavelength of 405 nm.

d. Immunosorbent Electron Microscopy Assay

Several procedures have been employed to detect plant viruses from crude sap preparations. Brandes (1957) described a leaf-dip procedure for visualizing virus particles in sap exuded from a cut leaf. The procedure consisted of dipping a cut leaf into a drop of buffer on a grid. Ball (1971) expanded the leaf dip procedure with serology and developed what is now known as leaf-dip serology. This method consisted of placing a drop of diluted antiserum on a coated grid and dipping the edge of a freshly cut leaf into the drop for several seconds. The grid was then dried and negatively stained with a heavy metal. It was presumed that the antiserum would bind and decorate any specifically related virus during this procedure. Derrick (1973) described a quantitative assay for detecting plant viruses from crude sap. He called this method serologically

specific electron microscopy (SSEM); which is also known today as immunosorbent electron microscopy (Kerlan et al., 1981). This method involved coating a carbon-fronted filmed grid with specific viral antiserum. Virus particles become specifically attached and concentrated on the surface of the antiserum-coated grids when they are floated on drops of diluted extracts from virus infected tissue. The grids were rinsed with water to remove excess plant salts that might impair viewing and shadowed with platinum-palladium to enhance their viewing. This procedure proved to be considerably more quantitative than the leaf dip procedure described by Brandes (1957).

Dubochet and Kellenberger (1972) have shown that the adsorption of virions to grids is a highly selective process and not a random adherant of any particles present in the extract. Virus particles possess different specific affinities for the film surface which would have a significant effect on the number of particles that will adhere to the film. Yanagida and Ahmad Zadeh (1970) introduced the decoration modification into SSEM. This technique involves the addition of more virus-specific serum to the virions previously attached to the serum-coated grid. The term decoration implies the covering of antigenic sites by antibody molecules. This procedure creates a "halo"

antibodies around the virus. The stain accumulates more heavily around the added protein making observation and identification of the particle easier. After decoration, the grid is negatively stained with with a heavy metal stain such as uranyl acetate to enhance viewing. Milne and Luisoni (1977) modified Derrick's method which reduced the time requirement of 90 min per sample to 25 min per sample. This method involved coating the grids with diluted antisera, rinsing them in buffer and floating them on either purified virus preparations of crude sap extracted from infected leaves. Milne and Luisoni also added the decoration technique to this modified procedure in order to confirm the identity of the virus trapped on the grid.

G. COMPARISON OF ISEM & ELISA AS QUANTITATIVE VIRUS ASSAYS

Immunosorbent assays have been used to detect viruses in seed (Hamilton and Nichols, 1978; Roberts and Harrison, 1979); in plant tissue (Kerlan et al., 1981) and in various nematode vectors (Roberts and Brown, 1980). The most significant characteristic of immunosorbent assays is their ability to detect and quantify viruses in much lower concentrations than is possible by classical immunoprecipitation methods (Clark, 1981). This is achieved through 1) selectively trapping the virus with specific

antibodies previously adsorbed to the solid phase (polystyrene microtiter plate or electron microscope grid) and 2) using a sensitive detection system. In ISEM, detection is by direct visual observation of the virus particles, whereas in ELISA, detection and quantification is assayed colorimetrically.

Hamilton and Nichols (1978) compared ISEM with ELISA for the detection of pea seedborne mosaic virus (PSbMV), in seed lots containing various percentages of infected seed. The authors reported that ISEM detected PSbMV in seed lots containing 1-5 percent infected seed, while ELISA could not; indicating that ISEM was superior for detecting low levels of PSbMV infected seed. The low sensitivity of ELISA was thought to be due to the high background received from healthy seed which resulted in poor descrimination between healthy and diseased samples. Roberts and Harrison (1979) used ISEM for detecting particles of potato leaf roll virus (PLRV) and potato mop top virus (PMTV) in extracts of infected potato tubers, leaves and two aphid vectors. The authors found ISEM to be as sensitive as ELISA.

Kerlan et al., (1981) investigated the respective sensitivities of ELISA and ISEM with purified virus suspensions and crude leaf extracts from peach and plum trees infected with plum pox virus (PPV) and apple chlorotic

leaf spot virus (CLSV). Despite low virus concentrations in infected woody plants, PPV and CLSV could be detected by ISEM with the same sensitivity as ELISA. The authors reported that one advantage of using ISEM over ELISA with respect to these two viruses is that non-specific antibodies do not interfer with ISEM, whereas the sensitive and reliable detection of PPV and CLSV can not be obtained with ELISA without elimination of non-specific antibodies.

ISEM was also found to be very useful for detecting several nepoviruses in their nematode vector (Roberts and Brown, 1980). Viruses were detected much more reliably by ISEM that by "slash tests" in which nematodes are ground in buffer and rub-inoculated onto indicator plants to determine if they contain virus. ISEM can provide a rapid means of detecting viruses provided one has access to the necessary antisera and an electron microscope. Although ISEM can not replace ELISA in its convenience in diagnosing large numbers of samples, it is especially useful for routine diagnosis of small numbers of samples as well as for research purposes. In general, ISEM appears to be a sensitive, rapid and reliable serological alternative to ELISA.

H. CYTOLOGY OF POTYVIRUS INFECTIONS

The ultrastructural cytology of potyvirus infections within various plant hosts has been documented by several investigators. After conducting numerous cytological studies on the presence of cytoplasmic inclusions in cells infected with rod-shaped viruses, Edwardson (1966a) proposed that the presence of pinwheel and bundle type of cytoplasmic inclusions should be considered diagnostic for infection with viruses of the potato Y group. Further investigations on virus induced-inclusions by Edwardson and Christie (1978) led to the proposal of using virus induced-inclusions in classification and diagnosis of viral diseases. The structure of these inclusions, as elucidated by the reconstruction of serial sections for tobacco etch virus (TEV) by Edwardson (1966b) and for watermelon mosaic virus (WMV) by Purcifull and Edwardson (1967), was revealed as a cylindrical shape. In cross section this inclusion would be visible as a pinwheel with thin curved arms and thicker flat plates converging around a central core, whereas in longitudinal section it would appear as a bundle.

A study of pinwheel structures in wheat cells infected with wheat spindle streak mosaic virus was conducted by Langenberg and Schroeder (1973). They reported that membranous bodies consisting of convoluted plates and

tubules originated from the endoplasmic reticulum and appeared to give rise to pinwheel configurations. This is in agreement with earlier studies conducted by Hooper and Weise (1972). These investigators also visualized the formation of pinwheels from tubes or sacs which became ordered in membranous sheets interconnecting at regular points to form pinwheels. Endoplasmic reticulum has also been associated with bundle inclusions in cells infected with sweet potato russet crack virus (Lawson et al., 1971).

Several inclusion types have been reported within cells infected with potyviruses. Tubular structures were observed in the cytoplasm of mesophyll cells in plants infected with carnation vein mottle virus (CVMV) (Begtrup, 1976). Similar structures have been previously reported for datura shoestring virus (DSV) in Datura stramonium L. and D. metel L. (Weintraub and Ragetli, 1970) and for WSSMV in Triticum aestivum L. (Hooper and Weise, 1972). The tubular structures were seen in close contact with pinwheels and dense bands in the cytoplasm of stem and leaf cells of Dianthus barbatus L. and Silene armeria L. and are thought to be involved in the synthesis of virus particles (Begtrup, 1976). Investigations by Weintraub and Ragetli (1970) on Dianthus barbatus L. infected with carnation vein mottle virus (CVMV) found nuclei which were elongated and contained

electron dense chromatin. Cytoplasmic inclusions consisting of dense bands, loops and circles were observed throughout the cytoplasm.

The development of pinwheel inclusions induced by sweet potato russet crack virus in Ipomea setosa Ker. was documented by Lawson et al., (1971). Five days after inoculation, bundle inclusions were observed perpendicular to the cell wall and in close association with plasmodesmata. Pinwheel configurations appearing in the cytoplasm could be traced to the cell wall by serial sections, with the center of the pinwheel being observed directly over the plasmosdesmatal openings. Seven days after inoculation some bundle inclusions were found separated from the cell wall. Endoplasmic reticulum was observed at the base of the bundle inclusions. In addition, hoops and arcs were present in the cytoplasm. The sequence of pinwheel development and maturation in systemically infected tissue was found to be similar to the sequence of development in the inoculated leaf.

Pares and Bertus (1978) observed deep invaginations of the nuclear envelope in cells of Crinum sp. infected with a potyvirus. Although the authors did not specifically identify the virus, they did state that Hippeastrum mosaic virus is the only potyvirus recorded on Crinum sp.

Cytoplasmic inclusions were observed adjacent to the nuclear invaginations and on one occasion inclusions were observed in the nucleus. Wischnitzer (1974) suggested that convolutions of the nuclear envelope may be a morphological response that occurs to ensure adequate exchange of material between the nucleus and cytoplasm. Cells infected with WSSMV eventually resulted in the disruption of many organelles including the nucleus (Hooper and Weise, 1972). Investigations be Weintraub and Ragetli (1970) on Dianthus barbatus L. infected with CVMV found nuclei which were extremely elongated with the chromatin portions considerably more dense than in healthy cells. The presence of crystalline inclusions have been reported within the nucleus of cells infected with tobacco etch virus (TEV) (Matsui and Yamaguchi, 1964).

Changes in the number and appearance of mitochondria have been reported in cells infected with potyviruses. Pares and Bertus (1978) reported numerous mitochondria within cells infected with Hippeastrum mosaic virus. The appearance of large numbers of mitochondria was suggested to be a morphological expression of the increased respiration that occurs in virus infected tissue. Hooper and Weise (1972) reported the dissruption of mitochondria in wheat cells infected with WSSMV.

Membranous strands containing virus-like particles have been observed within cells infected with potyviruses (Weintraub and Ragetli, 1970; Lawson et al., 1971; Kitajima and Lovisolo, 1972; and Hunst and Tolin, 1983). Although the function of cytoplasmic strands has never been determined, some speculations have been made regarding their formation. Weintraub and Ragetli (1970) suggested that the formation of cytoplasmic strands within leaf cells infected with carnation vein mottle virus (CVMV) resulted from the tearing away of the outer layer of the cytoplasm due to the heavy mass of virus particles concentrated along this surface. It has been suggested that the formation of cytoplasmic strands in Datura sp. infected with henbane mosaic virus (HeMV) is a defense reaction of the infected cells (Kitajima and Lovisolo, 1972). Hunst and Tolin (1983) hypothesized that their formation was an intercellular virus localization mechanism which was responsible for the tolerant reaction of soybeans to several strains of soybean mosaic virus (SMV).

I. DARK-GREEN VS LIGHT-GREEN LEAF TISSUE

Viral infections often result in the visible mottling of leaf tissue. A detailed cytological study of dark green and yellow-green areas of leaves displaying mosaic symptoms was conducted by Goldstein (1926, cited by Atkinson and Matthews, 1970). She reported that the dark green areas appeared cytologically similar to healthy leaves whereas the yellow-green areas appeared abnormal. Pares and Bertus (1978) and Butterfield (1983) examined the ultrastructure of dark green and light green areas of leaf tissue of Crinum sp. infected with Hippeastrum mosaic virus and Cucurbita pepo L. infected with watermelon mosaic virus, respectively. Both investigators found that only cells from light green areas contained pinwheels and cytoplasmic inclusions. These results are in agreement with earlier findings that the cells in dark green areas of viral-infected leaves contain very little virus compared with cells in the yellow-green areas of the leaf (Reid and Matthews, 1966; Atkinson and Matthews, 1970; Loebenstein et al., 1977).

Atkinson and Matthews (1970) examined the virus content and distribution of plasmodesmata in cells near the junction between dark green and yellow-green tissue. They concluded that the dark green cells had normal plasmodesmatal connections with adjacent yellow-green cells and that TMV

rods that had been synthesized in yellow-green cells diffused or moved by cytoplasmic streaming into the dark green cells without multiplying in them.

III. MATERIALS AND METHODS

A. SOURCE, CULTURE AND MAINTENANCE OF VIRUS IN PLANTS

In October 1983, peanut plants growing at the TRACEC in Suffolk, Virginia suspected of having PStV were collected by P. M. Phipps. By mechanical transmission from these plants, S. A. Tolin isolated a virus with properties of PStV from one NC7 plant. By subsequent passage of this PStV-like isolate through cowpea (Vigna unguiculata) and Chenopodium quinoa, and then inoculation to Florigiant peanut, Tolin observed peanuts exhibiting the two symptom types typical of stripe and blotch variants described by Demski et al. (1984). These two isolates were maintained in the greenhouse in Florigiant or NC7 peanuts and were used throughout this study. Inoculum was prepared by grinding systemically infected leaves in a chilled mortar and pestle in 0.01M sodium phosphate buffer, pH 7.0. The sap was rubbed onto carborundum dusted leaves with a pestle.

For these studies, seeds of the peanut cultivar Florigiant were planted four to a pot in Spasoff's soilless mix and kept in the greenhouse. Cotyledons first emerged

9-10 days after planting before quadrifoliolate leaves were unfolded. Seedlings were transplanted individually into 4 " pots. Mechanical inoculation with either isolate was done when the first two quadrifoliolates were expanded and the fourth quadrifoliolate was just emerging from the stem (Fig. 1). This growth stage occurred approximately 14-16 days after planting. The first symptom was usually observed on the sixth quadrifoliolate, which appeared 2 1/2 weeks after inoculation. Samples for the following studies were taken from the seventh quadrifoliolate which emerged from the stem approximately 3 weeks after inoculation.

Peanut leaves go through several distinct stages of expansion and development. Peanut leaves first emerge from the stem in a closed state. The leaflets begin to expand and after two days become 1/3 expanded. Each day the leaves expand a little more becoming 2/3 and finally fully expanded. Seven to ten days after the leaflets have become fully expanded, the leaf is said to be in a mature form. Peanut plants are a particularly good system for studying the progression of sypmtom development since they have such distinct developmental stages.

Figure 1. Florigiant peanut seedling at inoculation stage.

B. DETERMINATION OF PARTICLE LENGTH

1. Preparation of Leaf Dips

Leaf dip preparations were made using leaflets taken from the 7th through the 10th quadrifoliolate of PStV-infected peanut. Preparations were made by dipping a freshly torn edge of a infected leaf in a drop of distilled water on a formvar-coated, carbon-backed electron microscope grid (200 mesh). The drop was removed after 5 min with filter paper. The virus particles were stained by placing the grids on drops of 1% aqueous uranyl acetate for 1 minute. The excess stain was removed from the grids with filter paper. The grids were examined for the presence of virus particles using a Zeiss 10CA transmission electron microscope at an acceleration voltage of 60kv.

2. Partial Purification

PStV-stripe and PStV-blotch were purified separately from Florigiant peanut using a modification of the PStV purification procedure described by Demski et al. (1984). Peanut stripe infected leaflets were harvested from the 7th through the 9th quadrifoliolates approximately 5 weeks after inoculation. The leaves (15 g) were homogenized in a Waring blender in 0.06M sodium phosphate buffer, pH 8.0, containing 0.02M $NaSO_3$ (1g tissue : 10ml buffer). The homogenate was

filtered through cheesecloth; 10% chloroform (v/v) was added for clarification and stirred for 3-5 min at 4° C. The emulsion was broken by centrifuging at 10,000 rpm for 10 min, and the aqueous layer was removed and placed in a cold room (4° C) for 1.5 h. The extract was then centrifuged at 10,000 rpm for 10 min to remove residual, insoluble plant proteins. After an additional low speed centrifugation, 20 ml of the virus suspension was layered on 15 ml of 30% sucrose prepared in 0.06M phosphate buffer, pH 8.0, containing 4% PEG (w/v) and 0.2M NaCl in a 1 x 3 1/2 " tube. After centrifugation at 22,500 rpm for 2 h in a Beckman SW 28 rotor, the pellets were resuspended overnight in 0.06M phosphate buffer, pH 8.0. After low speed centrifugation, a 2 ml aliquot of the virus suspension was layered on 10-40% linear sucrose density gradients prepared in 0.06 M phosphate buffer, pH 8.0, and then centrifuged for 2 h at 22,500 rpm. Virus zones were collected using an ISCO density gradient fractionator and UV analyzer and concentrated by high speed centrifugation (22,500 rpm) for 2 h. Purified virus was resuspended in 1 ml of 0.06M phosphate buffer, pH 8.0. Twenty microliter aliquots of the partially purified virus suspensions were placed on carbon-backed, formvar-coated 400 mesh electron microscope grids. These grids were stained with 1% uranyl acetate and used for obtaining particle lengths.

3. Particle Measurements

Virus particles were measured from both leaf dips and partially purified preparations. Prepared grids were viewed in the Zeiss 10CA transmission electron microscope and micrographs were taken at 20,000x. Virus particles were measured with the ZIDAS Image Analysis System from photographs enlarged to 50,000x. One hundred virus particles were measured from leaf dips and partially purified preparations of each isolate. A frequency distribution and mean particle length was determined for each isolate and extraction procedure separately.

C. TISSUE PREPARATION FOR TRANSMISSION ELECTRON MICROSCOPY

All tissue samples were taken from the 7th quadrifoliolate of Florigiant plants at selected times such that five stages of expansion were represented. The leaf samples were excised into 1 x 3 mm strips and fixed in a mixture of formaldehyde and glutaraldehyde (Karnovsky, 1965) for 12 h at 6° C. A solution of 0.1 M cacodylate buffer, pH 7.2, was used as a rinsing buffer between fixations. The material was post-fixed in 2% osmium tetroxide for 2 h at 4° C. The tissue was dehydrated in a graded ethanol series (15, 25, 30, 40, 50, 70, 80, 95, 100 percent) followed by an acetone rinse. The ethanol was changed every 20 min until

the 70% solution, in which the tissue was allowed to soak overnight. The tissue was then soaked in 80, 95 and 100 percent ethanol for 30, 45 and 60 min, respectively. To remove traces of alcohol, the tissue was rinsed in acetone three times for a total of 15 min. The leaf tissue was infiltrated with resin by soaking it in the following graded acetone-resin series: resin:acetone (1:3), 1/2 h; resin:acetone (1:1), 1 h; resin:acetone (3:1), 2h; pure resin (overnight). ERL epoxy resin (Spurr, 1969) was prepared according to the following protocol:

10.0g	ERL-4206	(vinyl cyclohexene dioxide)
6.0g	DER-736	(diglycidyl ether of propylene gylcol)
26.0g	NSA	(nonenyl succinic anhydride)
0.4g	DMAE	(dimethylaminoethanol)

The tissue was embedded in ERL epoxy resin and cured at 70° C overnight. Thin sections were cut with a Sorvall, Porter-Blum MT2-B ultramicrotome. All sections were picked up on copper, 400 and 300 mesh, electron microscope grids and stained with 2% uranyl acetate (Watson, 1958) and 0.4% lead citrate (Venable and Coggeshall, 1965). Grids were first placed on drops of 2% uranyl acetate for 30 min, then rinsed in distilled water and drained by removing excess water with filter paper. The grids were then placed on drops of 0.4% lead citrate for 10 min in a saturated NaOH

atmosphere in order to minimize the formation of lead precipitates. The grids were again rinsed with distilled water and excess water removed by absorption with filter paper. Sections were examined with a Zeiss 10CA transmission electron microscope at an acceleration voltage of 60kv.

D. QUANTITATIVE VIRUS ASSAY

1. Immunosorbent Electron Microscopy Assay

Virus titer was determined in leaves from the 7th quadrifoliolate when they were at a) five stages of expansion (closed, 1/3, 2/3, fully expanded and mature expanded) and b) in specific light-green and dark-green areas of leaf tissue at the mature expanded stage. The ISEM procedure used was the modified Derrick system of Milne and Luisoni (1977). Electron microscope grids (400 mesh) coated with a formvar-carbon film were sensitized by floating them on 20 ul drops of either stripe-specific or blotch-specific antiserum (1:1000 dilution) for 15 min. The antisera were kindly provided by J. W. Demski from the Georgia Experiment Station, Experiment, GA. The grids were washed by dipping into 0.06 M sodium phosphate buffer, pH 7.0. The sensitized grids were placed on 20 ul droplets of leaf extracts (1:10 dilution) for 20 min. For experiment A, sap from each

expansion stage was extracted from a pooled sample of leaf tissue (3.0 g) obtained from five Florigiant plants. This extract was also used to inoculate the Chenopodium amaranticolor plants used in the local lesion assay described in subsection 2. For experiment B, sap from light-green and dark-green leaf tissue was obtained from four leaflets taken from each of three plants. Sap from these respective areas, obtained from each peanut plant was assayed for virus concentration using ISEM. The grids were again washed in phosphate buffer, drained and placed on drops of antiserum diluted 1:1000 for 5 min in order to decorate the trapped virions. The grids were rinsed in 0.06 M phosphate buffer and distilled water and negatively stained with 1% uranyl acetate for 1 min. The grids were examined with a Zeiss 10CA transmission electron microscope at an acceleration voltage of 60kv. For experiment A, virus particles were counted from micrographs (1250x) taken of five randomly chosen fields on each of two grids. For experiment B, virus particles were counted from micrographs (1250x) taken of five randomly chosen fields on each of three grids.

The data from experiment A were analyzed as a two way factorial using the analysis of variance (ANOVA) procedure. The two factors examined were five expansion stages (closed,

1/3, 2/3, fully expanded and mature expanded) and two virus isolates (stripe vs blotch). Ten treatments, each consisting of a expansion stage-virus isolate combination, were replicated two times, with each replication represented as one grid. Five randomly selected grid squares were counted for virus particles for each replication. Differences among expansion stages were tested using the Duncan's multiple range procedure at the 0.05 level of significance. Single degree of freedom orthogonal comparisons were used to compare differences between isolates at each expansion stage.

The data from experiment B were also analyzed as a two way factorial using ANOVA. The two factors examined were tissue type (dark-green vs light-green) and virus isolate (stripe vs blotch). Four treatments, each consisting of a tissue type-virus isolate combination were replicated three times with each replication represented as one grid. Five grid squares randomly selected were counted for virus particles for each replication. Differences in virus titer between light and dark green tissue and stripe-infected vs blotch-infected tissue were tested using single degree of freedom orthogonal comparisons.

2. Local Lesion Assay

A local lesion assay was used to determine quantitatively the relative virus titer in peanut leaves from the 7th quadrifoliolate at five expansion stages. Forty eight plants of Chenopodium amaranticolor of similar size (8-10 leaves) were used as the assay plants in this study. Several leaves were removed from each plant so that only four leaves of uniform size remained. Approximately 0.3 g of leaf tissue collected at each growth stage was ground in 3 ml of 0.01 M sodium phosphate buffer, pH 7.0 in a chilled mortar and pestle. Each inoculum was then rubbed on the leaves of four plants previously dusted with carborundum. Four plants were inoculated with sap from a healthy Florigiant plant and with buffer as a control. Local lesions numbers were recorded 16 days after inoculation and expressed as the number of local lesions per leaf.

The data were analyzed as a two way factorial using ANOVA. The two factors examined were expansion stage and virus isolate. Ten treatments, each consisting of an expansion stage-virus isolate combination was replicated four times, with each plant representing one replication. Local lesions were recorded on four leaves (subsamples) per plant. Treatments were analyzed using the Duncan's multiple

range procedure at the 0.05 level of significance. Single degree of freedom orthogonal comparisons were used to compare differences between isolates at each expansion stage.

IV. RESULTS

A. MACROSCOPIC SYMPTOM DEVELOPMENT OF PSTV

The first symptom observed on peanut plants inoculated with either isolate of the PStV was a yellow-flecking of the leaves (Fig. 2). This symptom was usually observed on the sixth quadrifoliolate approximately 2 1/2 weeks after inoculation. Although this symptom was the first indication of virus infection, these leaves rarely developed full typical symptoms of stripe or blotch. The first leaves to exhibit full symptoms were consistently the seventh quadrifoliolate. These leaves were sampled for this study. Samples were taken from five expansion stages of this leaf in order to follow the progression of symptom development morphologically and ultrastructurally (Fig. 3). Typically, no distinct symptoms of stripe or blotch could be detected on the leaves until they were at the 2/3-expanded stage. Dark-green and light-green areas on the leaves became more pronounced as the leaves developed from the 2/3 to the mature-expanded stage.

Figure 2. Yellow flecking induced by peanut stripe virus on the 6th quadrifoliolate of Florigiant peanut.

Figure 3. Expansion stages of Florigiant peanut leaves.

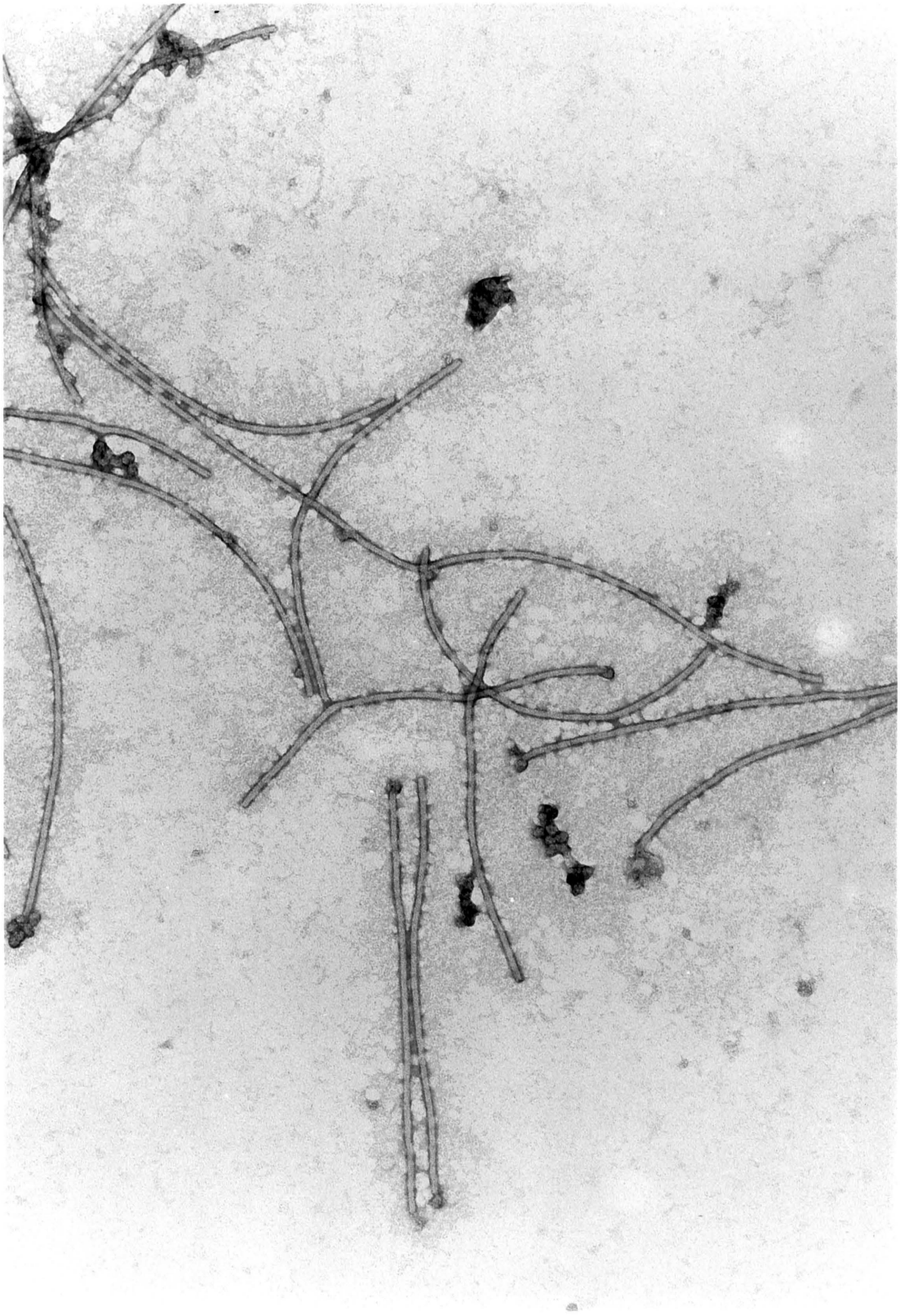
B. VIRUS PARTICLE LENGTH

One hundred virus particles were measured from leaf dips (Fig. 4) and from partially purified preparations of both stripe and blotch infected tissue. Particle lengths ranged from 670-820 nm for both isolates. The mean length of virus particles measured from stripe extracts of PStV were 753 nm and 746 nm for leaf dips and partially purified preparations, respectively. The mean length of virus particles from blotch extracts were 747 nm and 745 nm for leaf dips and partially purified preparations respectively. Both isolates had a modal length of 750 nm, regardless of the virus extraction procedure used (Figs. 5 and 6).

C. PARTIAL PURIFICATION

Partial purification of PStV isolates was conducted solely for the purpose of obtaining virus particles to be used for determining particle length. The modified procedure of Demski et al., (1984) was successful in obtaining virus particles from peanut leaves infected with either isolate of the virus. The results reported herein are from a single attempt to purify PStV from peanut.

Figure 4. Electron micrograph of a leaf dip from Florigiant peanut infected with the stripe isolate of peanut stripe virus (PStV), negatively stained with 1 % uranyl acetate.



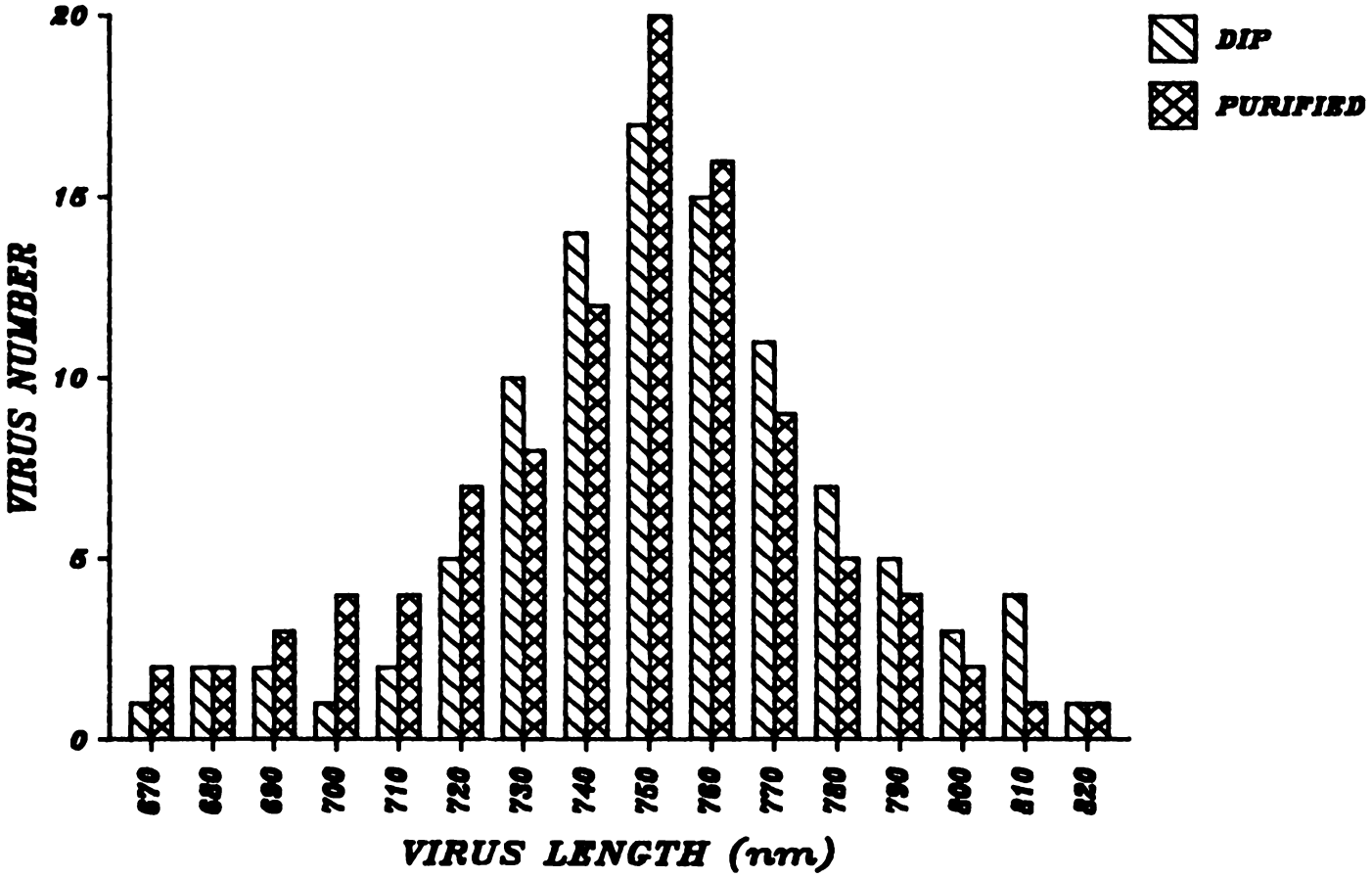


Figure 5. Frequency distribution of peanut stripe virus particles from partially purified and leaf dip preparations from Florigiant peanuts infected with the stripe isolate of peanut stripe virus.

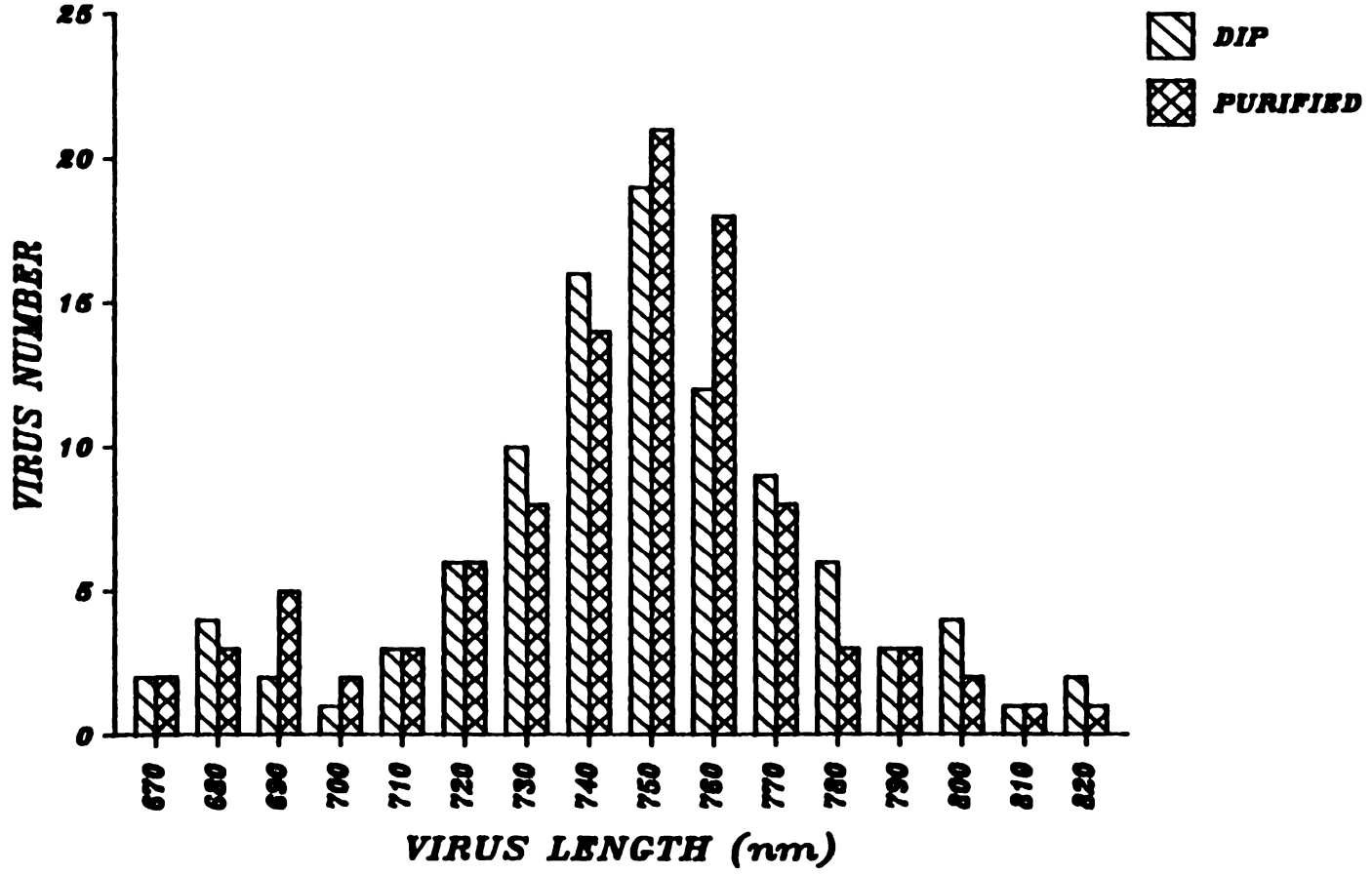


Figure 6. Frequency distribution of peanut stripe virus particles from partially purified and leaf dip preparations from Florigiant peanuts infected with the blotch isolate of peanut stripe virus.

Virus bands were observed in all tubes after rate zonal centrifugation. Absorbance profiles at 254 nm of sucrose density gradients were made as the virus bands were collected. Distinct peaks were obtained for both stripe and blotch isolates (Fig. 7). collected virus fractions and placed onto carbon-backed, formvar-coated 400 mesh grids. These grids were stained as previously described in Materials and Methods, and viewed in the TEM. Virus particles from both stripe and blotch preparations were observed on the grids.

Aliquots of stripe and blotch suspensions were taken from the The amount of virus estimated to be recovered from 15 g of leaf tissue was 0.20 mg/ml and 0.33 mg/ml for stripe and blotch respectively. The absorbance ratio (260/280) for the stripe and blotch suspensions was 1.15 and 1.24, respectively.

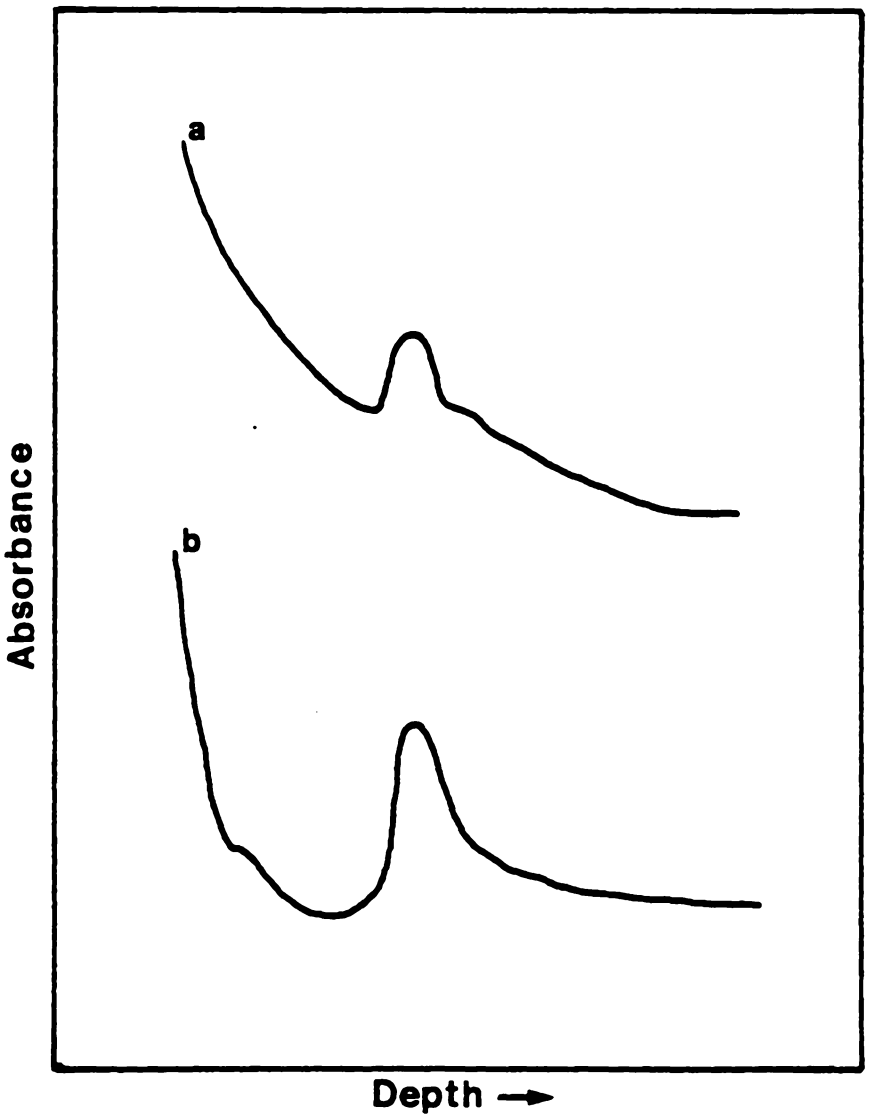
D. COMPARATIVE VIRUS TITER

1. Virus Titer at Different Growth Stages

a. Local Lesion Assay

Local lesions first appeared on Chenopodium amaranticolor approximately 11 days after inoculation with either isolate of PStV. Chenopodium plants inoculated with extracts from stripe infected leaf tissue showed an increase

Figure 7. Absorbance (254) nm profiles of partially purified flexuous rods from sucrose density gradients of a) stripe and b) blotch isolates of peanut stripe virus (PStV) from Florigiant peanut. Centrifugation was in linear, 10-40% sucrose gradients in 0.06M sodium phosphate buffer containing 0.02M Na_2SO_3, for 2 hours at 22,500 rpm. Absorbance scanned at 0-0.5 Optical Density (OD) units.


a
b
Absorbance
Depth

in the number of local lesions correlated with the increasing maturity of the source tissue (Table 1).

The most marked increase in local lesion number occurred from the closed to the 1/3-expanded stage, after which time, local lesion numbers continued to increase but at a lesser rate. Chenopodium plants inoculated with different stages of blotch-infected leaf tissue also showed an increase in the number of local lesions from the closed to the 2/3-expanded stage. However, at the fully expanded stage, the number of local lesion recorded per leaf decreased. There was an increase in the number of local lesions recorded on Chenopodium leaves inoculated with leaf tissue at the mature-expanded stage. Chenopodium leaves inoculated with leaf tissue at the mature-expanded stage had the greatest number of local lesions in both stripe and blotch-infected tissue.

The number of local lesions recorded from Chenopodium leaves inoculated with closed, 1/3-expanded and hardened leaf tissue was found to be significantly different. The number of local lesions recorded for leaf tissue at the fully expanded stage differed significantly between the stripe and blotch isolates.

Table 1. Infectivity assay of stripe and blotch isolates of peanut stripe virus (PStV) on Chenopodium amaranticolor for the 7th quadrifoliolate of Florigiant peanut at five stages of expansion.

Isolates	Mean number of local lesions per leaf on four plants				
	Expansion stages				
	Cl[1]	1/3	2/3	FE	ME
Stripe	5	28	50	67*	75
Blotch	7	33	41	30	72
	a[2]	b	c	c	d

*indicates significant difference between isolates at 0.05 level using single degree of freedom orthogonal comparisons.

[1]Cl= closed stage; 1/3= 1/3-expanded stage; 2/3= 2/3-expanded stage; FE= fully-expanded stage; ME= mature-expanded stage.

[2]Expansion stages not followed by the same letter are significantly different at 0.05 level according to the Duncan's multiple range test.

b. Immunosorbent Electron Microscopy Assay

Relative virus titer was determined in leaf tissue infected with either isolate of PStV, at five different stages of expansion. Leaves at the fully expanded stage had the highest virus titer in both stripe and blotch infected tissue (Table 2). There was also a significant difference in the number of virus particles counted at each growth stage, regardless of virus isolate. Leaves infected with the blotch isolate consistently had a higher virus titer than leaves infected with the stripe isolate at all growth stages.

There was a trend in the number of virus particles counted at the five different stages of development (Fig. 8). There was a reduction in virus titer at the 1/3-expanded stage as compared to the closed stage for both stripe and blotch isolates. An increase in titer was recorded at the 2/3 and fully expanded stage for both isolates. The titer also decreased in leaves when they were at the hardened stage.

C. Virus Titer in Dark-green vs Light-green Tissue

Immunosorbent electron microscopy was used to determine the relative virus titer in crude extracts of light-green and dark-green leaf tissue at the mature-expanded stage infected with either isolate of PStV. Light-green areas of

Table 2. Peanut stripe virus (PStV) particles observed in extracts of the 7th quadrifoliolate of Florigiant peanut at five expansion stages using immunosorbent electron microscopy (ISEM).

Growth Stage		No. of Virus Particles[3] PStV Isolates	
		Stripe	Blotch
Cl[1]	a[2]	52	65
1/3	b	31	41
2/3	c	77*	121*
FE	d	104*	293*
ME	e	97*	184*

*indicates significant difference between isolates at 0.05 level using single degree of freedom orthogonal comparison.

[1]Cl= Closed stage; 1/3= 1/3-expanded stage; 2/3= 2/3-expanded stage; FE= fully-expanded stage; ME= mature-expanded stage.

[2]Expansion stages not followed by the same letter are significantly different at 0.05 level according to Duncan's multiple range test.

[3]Mean number of particles counted in five fields from each of two electron microscope grids.

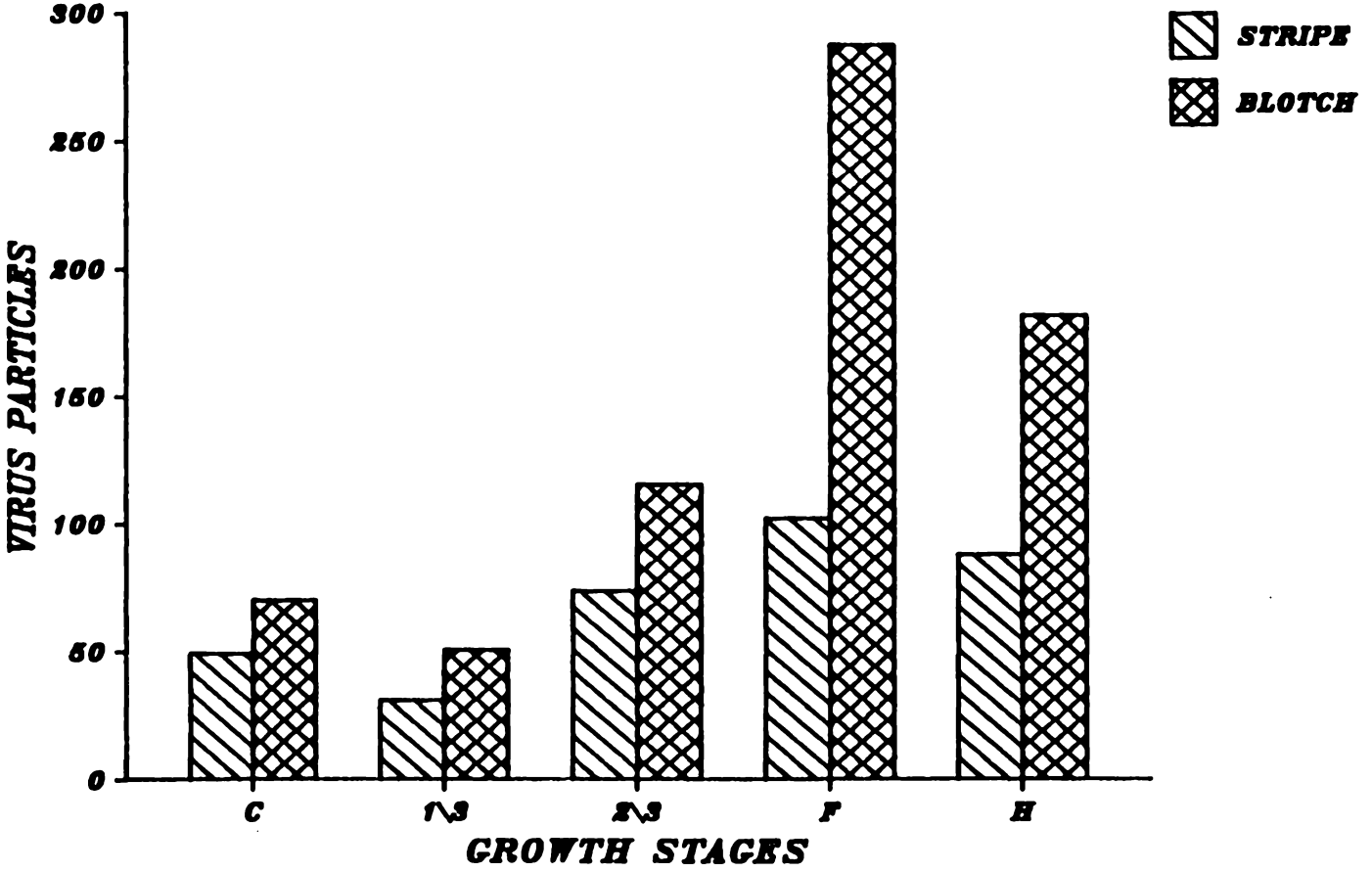


Figure 8. Comparison of peanut stripe virus titer in the 7th leaf at five expansion stages using immunosorbent electron microscopy (ISEM).

leaf tissue contained tremendously more virus than dark-green areas in the same leaf tissue (Table 3).

Virus content in light-green tissue infected with the blotch isolate was more than 2.5-fold higher than light-green tissue infected with the stripe isolate. Virus titer in dark-green tissue of stripe and blotch infected tissue was equally low.

E. CYTOLOGICAL EFFECTS OF PSTV

1. Closed Stage

Leaf tissue infected with either isolate appeared ultrastructurally similar at the closed stage. The cytoplasm of PStV-infected epidermal, palisade and mesophyll cells was highly vesiculated. Portions of pinwheel inclusions were observed in these vesiculated areas (Plate 1). Pinwheel inclusions were often observed attached to the cell wall near plasmodesmata (Plate 2). In subsequent sections, pinwheels were observed at various distances away from the cell wall (Plate 2). Very few virus particles were found free in the cytoplasm at this stage of leaf development. However, virus particles were found grouped together in a crystalline array in leaf tissue infected with the blotch isolate (Plate 3). This phenomenon was never observed in leaf tissue infected with the stripe isolate.

Table 3. Virus particles observed in extracts of light and dark green leaf tissue infected with peanut stripe virus (PStV) using immunosorbent electron microscopy (ISEM).

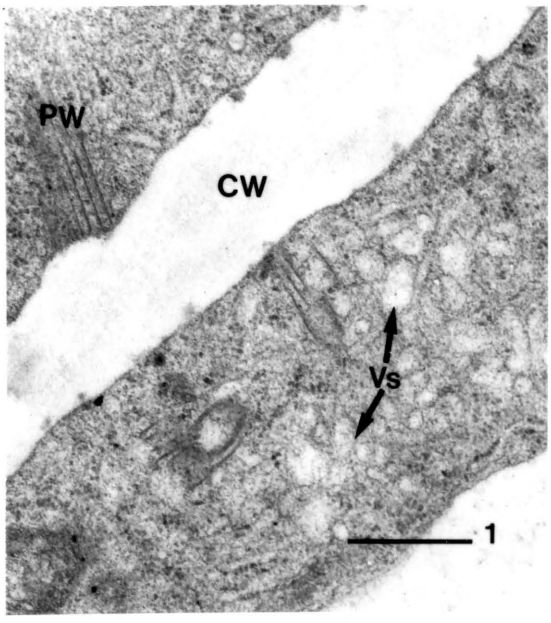
Tissue type	No. of Virus Particles[1]	
	PStV Isolates	
	Stripe	Blotch
Light-green	505*	1209*
Dark-green	36	34

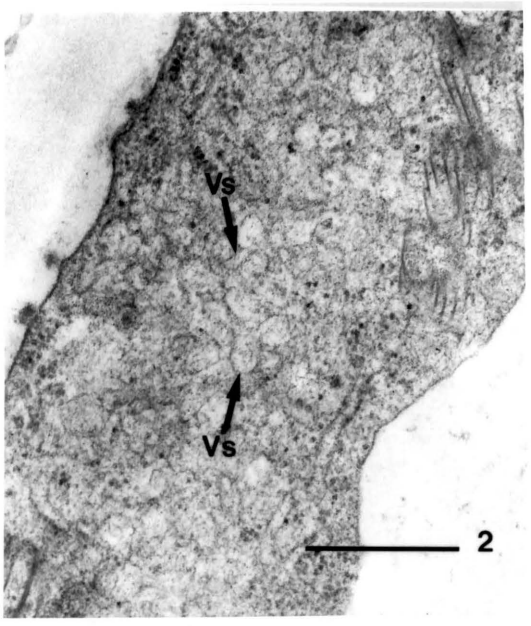
*indicates significant difference between isolates at 0.05 level using single degree of freedom orthogonal comparisons.

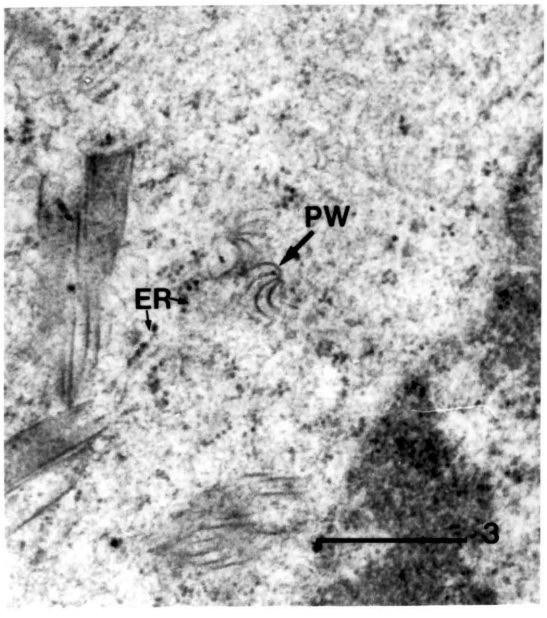
[1]Numbers represent the mean of five randomly chosen fields from each of three electron microscope grids.

Plates 1-11 depict the ultrastructure of Florigiant leaf tissue at different stages of expansion, infected with either the stripe or blotch isolate of peanut stripe virus (PStV).

Plate 1.1-1.4. Leaf tissue at the closed stage infected with PStV. Vesicles (Vs) are present in the cytoplasm of palisade cells infected with 1) stripe and 2) blotch isolates of PStV. Pinwheel (PW) inclusions perpendicularly attached to the cell wall and within vesiculated areas of the cytoplasm. 3) and 4) Portions of pinwheels (arrows) closely associated with endoplasmic reticulum (ER) within mesophyll cells. Bar Lengths: 1) .5 um; 2) .5 um; 3) .5 um; 4) .25 um.


PW
CW
Vs
1


Vs
Vs
2


PW
ER
3

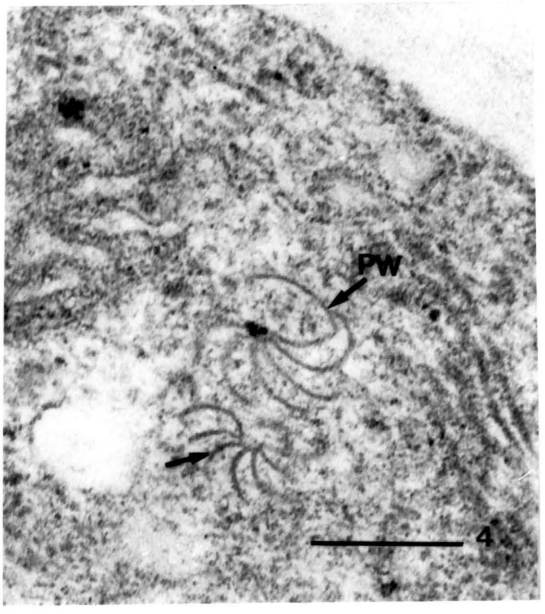
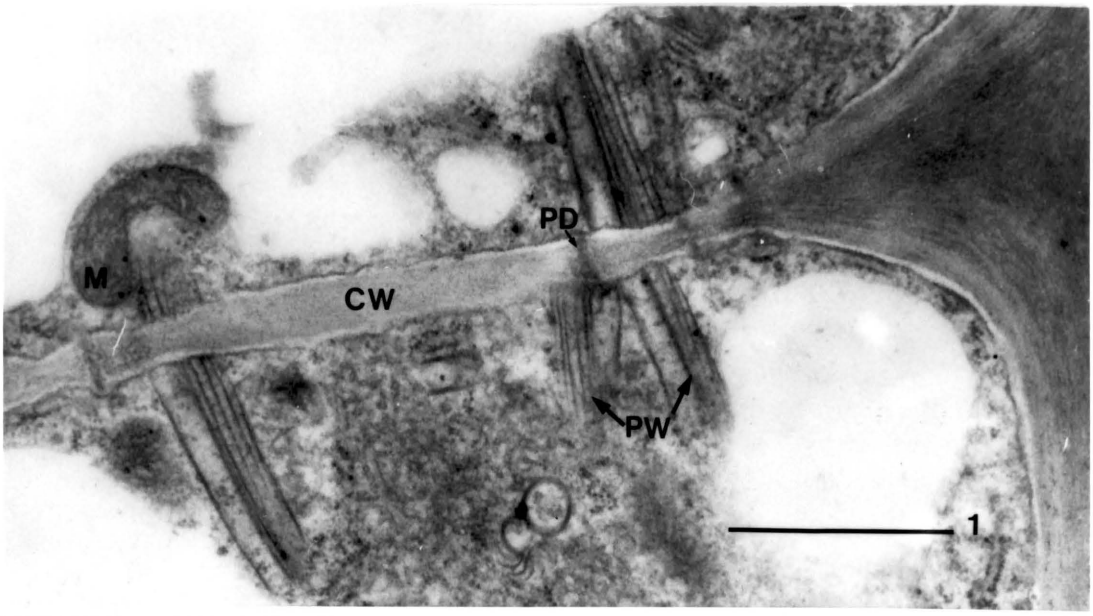

PW
4

Plate 2.1-2.2. Leaf tissue at the closed stage infected with the stripe isolate of PStV. 1) Pinwheels (PW) perpendicularly opposed to the cell wall (CW) near plasmodesmata (PD) in an epidermal cell. Mitochondria (M) closely associated with pinwheels. 2). Pinwheels (PW) opposed to the cell wall and at various distances away from the cell wall (arrows) in a mesophyll cell. Bar Lengths: 1) 1 um; 2) 1 um.


PD
M
CW
PW
1

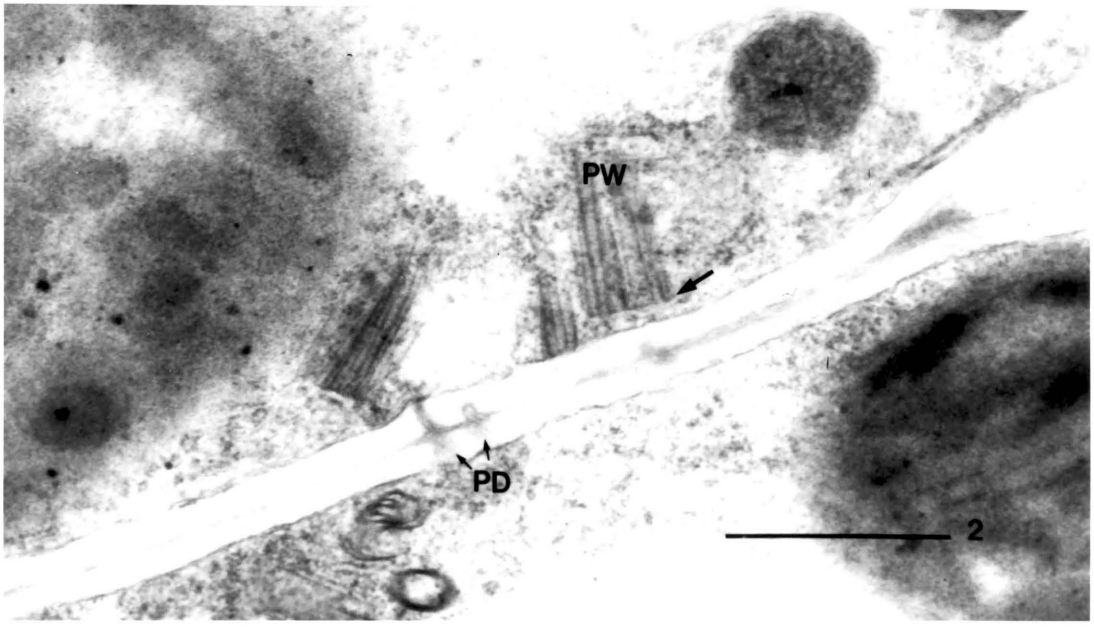
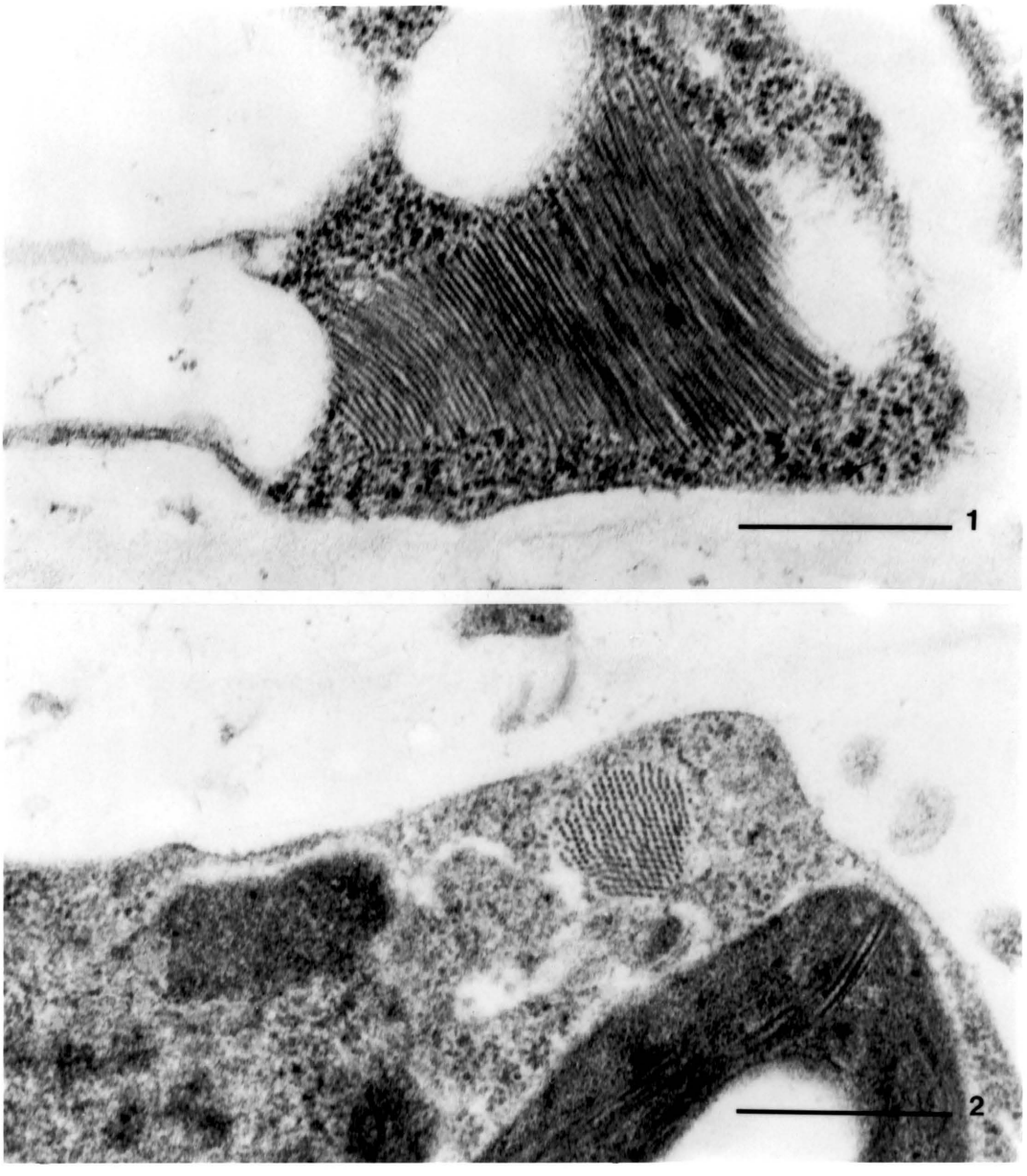

PW
PD
2

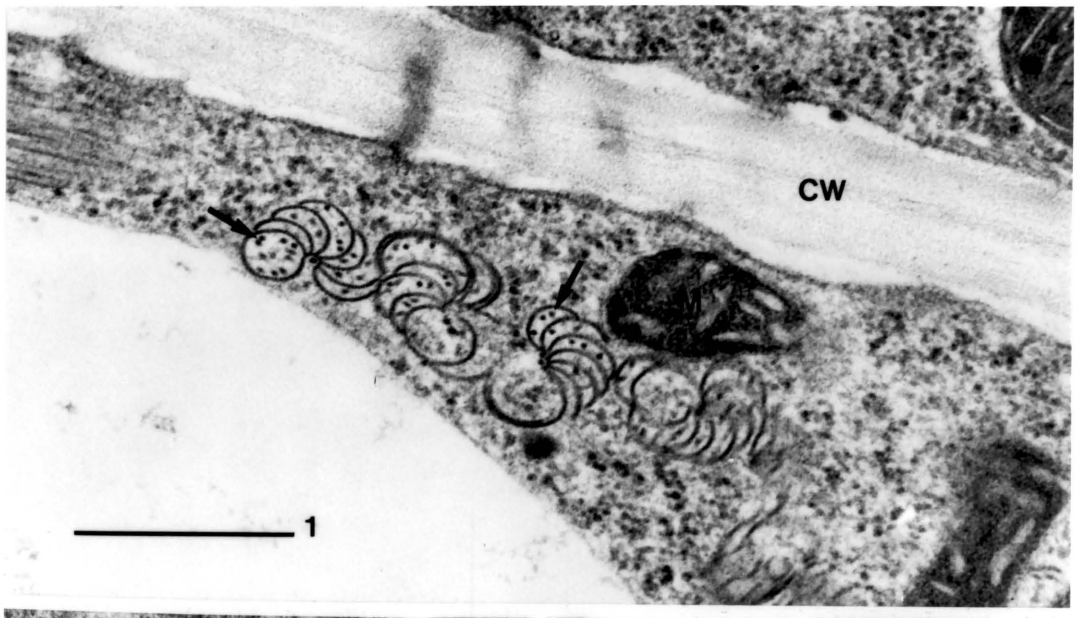
Plate 3.1-3.2. Leaf tissue at the closed stage infected with the blotch isolate of PStV. Virus particles grouped together in a crystalline arrays in 1) longitudinal section and 2) cross section within palisade cells. Bar Lengths: 1) .5 um; 2) .5 um.

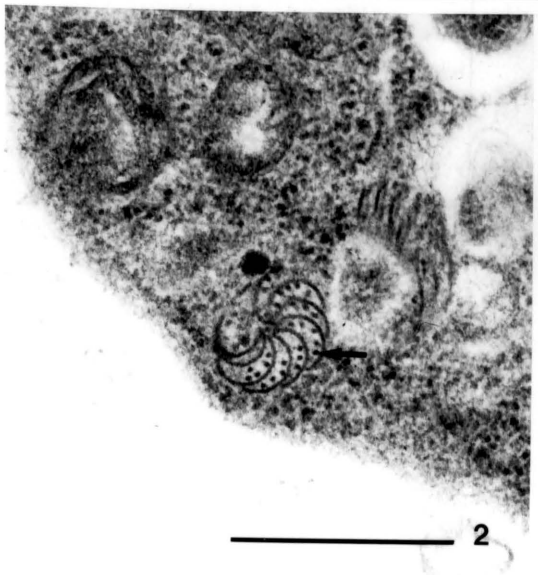

1
2

2. 1/3-expanded Stage

The ultrastructure of cells at the 1/3-expanded stage appeared similar to that of cells at the closed stage with the exception that pinwheel inclusions were more abundant in the cytoplasm of the more mature tissue. Pinwheel inclusions in blotch-infected tissue had what appeared to be virus-like particles situated between the arms of the inclusions (Plate 4). Such particle arrangements were observed less often in stripe infected tissue. Measurements of these particles show them to be 13 nm in diameter, placing them in the proper size range for flexuous virus particles. The formation of cytoplasmic strands was first observed in cells at the 1/3-expanded stage (Plate 5). These strands were observed in cells infected with either isolate of the virus, and tended to become more abundant as the virus infection progressed. All cellular organelles appeared normal during this stage. However, phytoferritin was observed within some chloroplasts in PStV infected palisade cells, and was not usually seen in comparable healthy tissue.

Plate 4.1-4.3. Leaf tissue at the 1/3-expanded stage infected with PStV. 1) and 2) pinwheels induced in the mesophyll cell by the blotch isolate of PStV. Virus particles (arrows) are spaced along the arms of the inclusion. 3) pinwheels induced in mesophyll cell by the stripe isolate of PStV. Note degenerative appearance of mitochondria (M). Bar Lengths: 1) .5 um; 2) .5 um; 3) .5 um.


CW
1


2

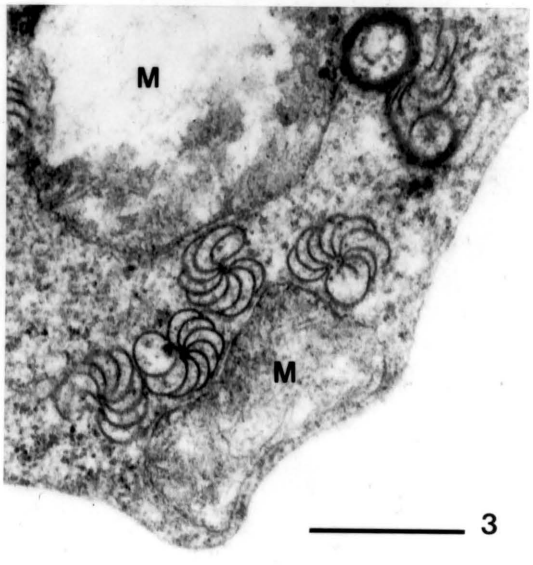
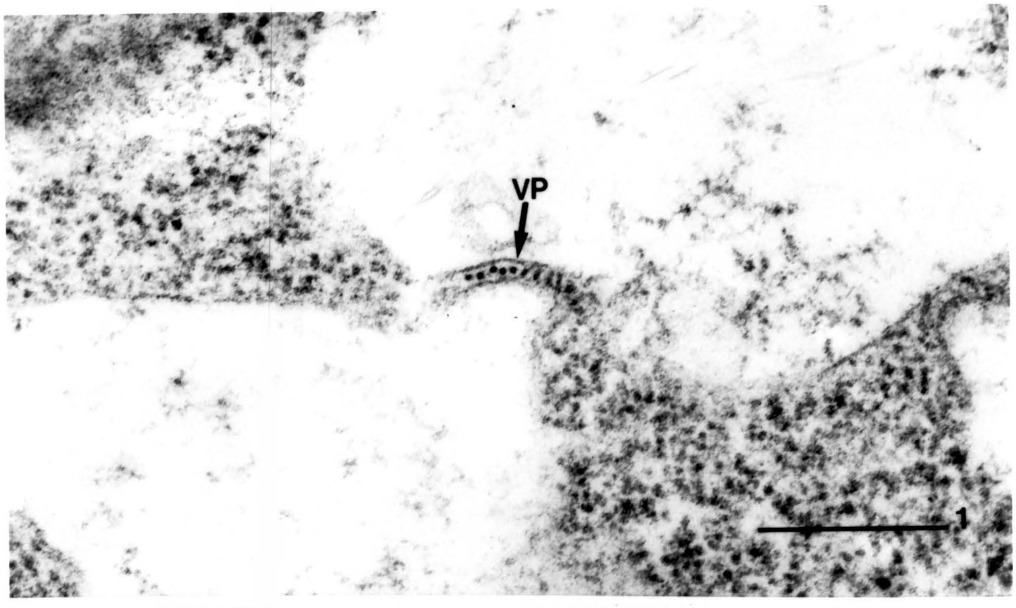
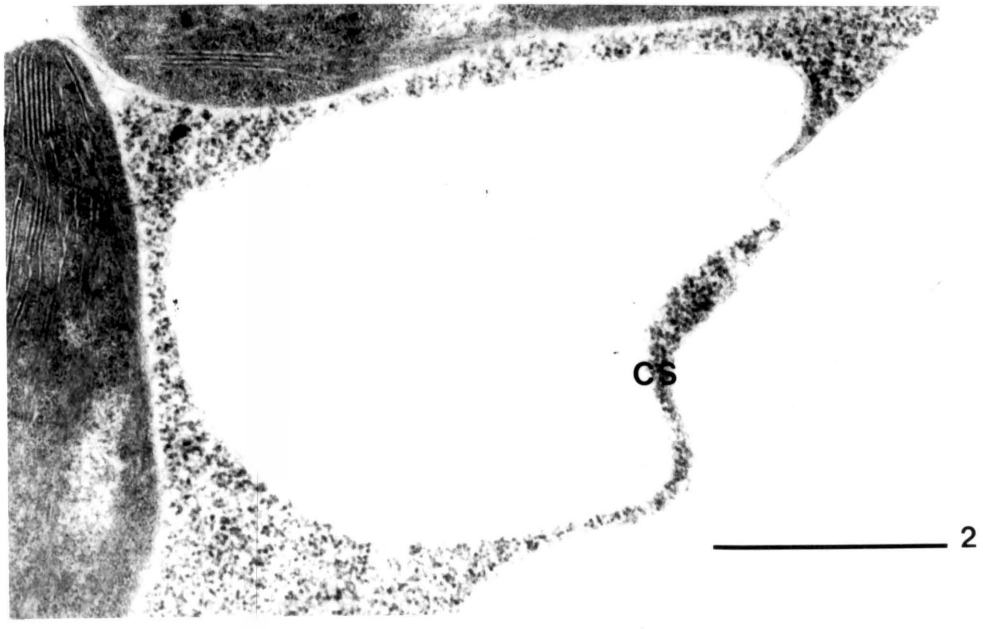

M
M
3

Plate 5.1-5.2. Development of cytoplasmic strands within palisade cells of 1) stripe-infected and 2) healthy leaf tissue. Note the presence of virus particles (VP) within the strand of the stripe-infected cell. Bar Lengths: 1) .5 um; 2) 1 um.


VP
1


CS
2

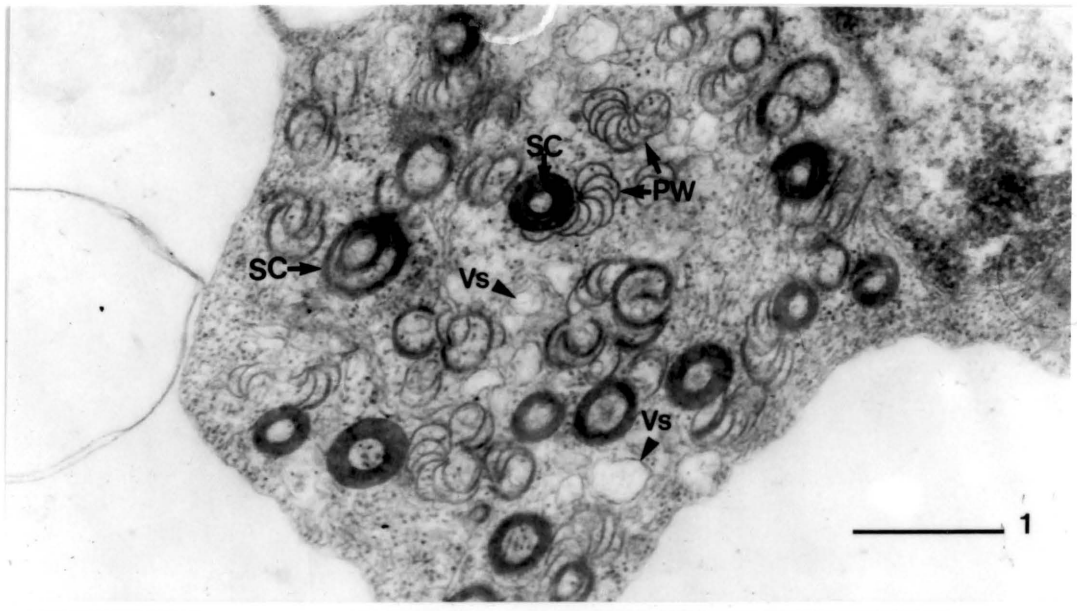
3. 2/3-expanded Stage

At the 2/3-expanded stage of leaf development, another type of inclusion body was observed in cells infected with either isolate of PStV. The additional inclusions, known as scrolls, are also typically associated with potyvirus infections (Plate 6). During this stage of the infection, both scrolls and pinwheels were abundant in all cell types and often appeared to fill every available space in the cytoplasm. Very few inclusions were found attached to the cell wall. The cytoplasm was typically highly vesiculated, particularly in the areas which contained numerous inclusions. Close examination of scroll inclusions indicate that they are composed of very thin filamentous sheets that are bound very tightly together. Both pinwheel and scroll inclusions were found closely associated with mitochondria (Plate 7). Measurements of longitudinal sections of both scroll and pinwheels indicate these inclusions range from 2-3 um in length (Plate 8). At this stage very few pinwheel inclusions were found attached to the cell wall.

4. Fully-expanded Stage

At the fully expanded leaf stage, numerous virus particles were observed lining the edge of the tonoplast and other membranes (Plate 9).

Plate 6.1-6.2. Palisade cells from leaves at the 2/3-expanded stage. 1) Closeup of pinwheel (PW) and scroll (SC) inclusions within a stripe infected cell. Numerous vesicles (Vs) are present in the cytoplasm among the inclusions. 2) Note the central location of the inclusions within the stripe infected cell. N= nucleus; M= mitochondria. Bar Lengths: 1) .5 um; 2) 2 um.


SC
PW
SC
Vs
Vs
1

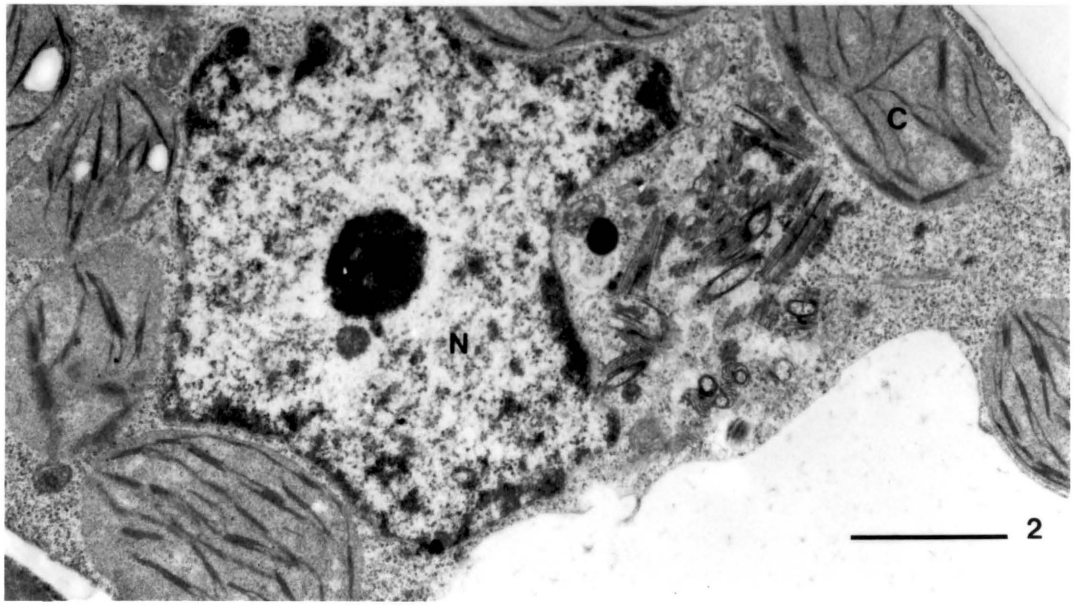

C
N
2

Plate 7.1-7.2. Leaf tissue at the 2/3-expanded stage. Pinwheels (arrows) and scrolls (arrowheads) were found closely associated with mitochondria (M) in 1) stripe and 2) blotch infected palisade cells. Bar Lengths: 1) .5 um; 2) .5 um.

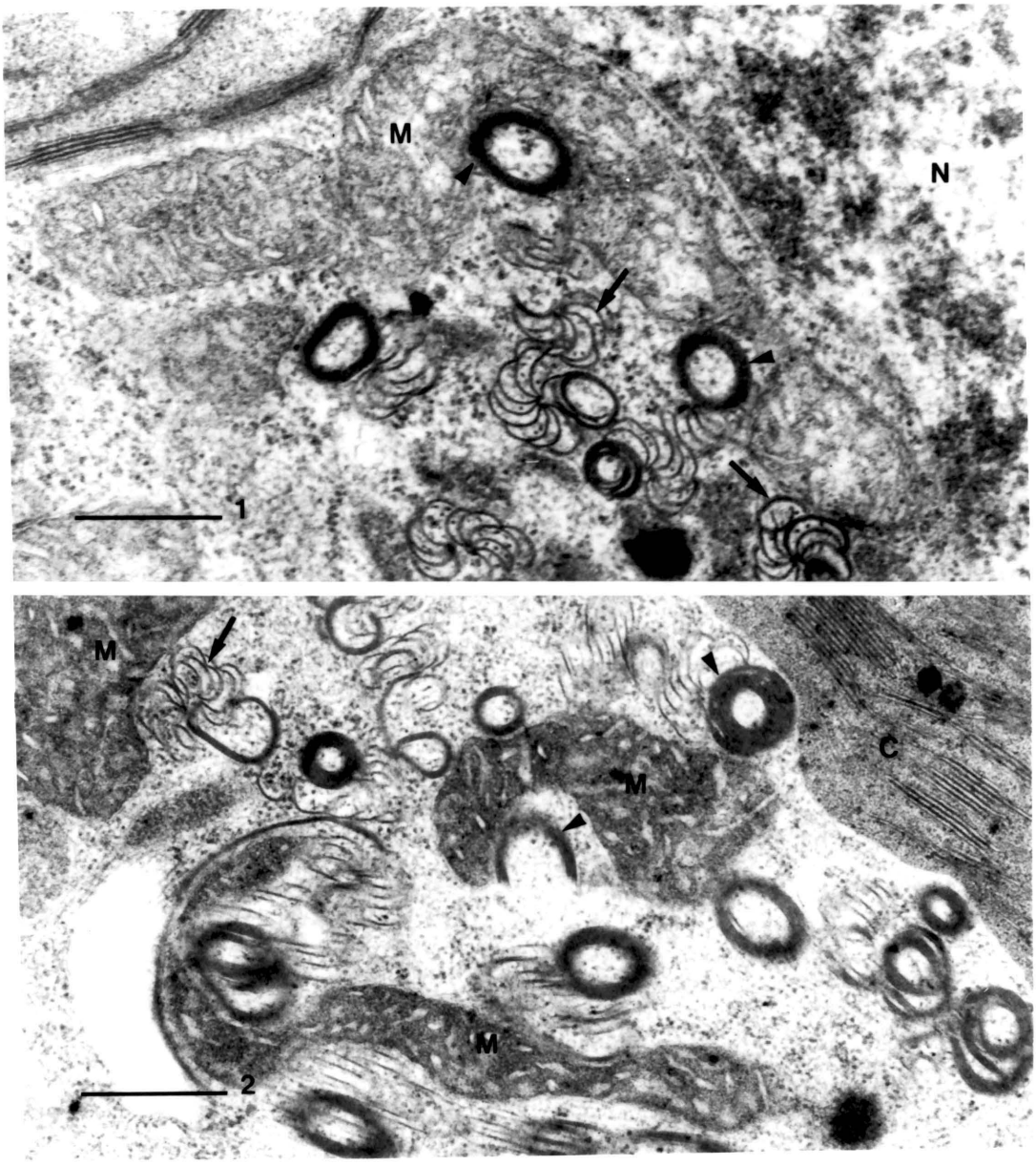

M
N
1
M
M
C
M
2

Plate 8.1-8.2. Micrograph of longitudinal sections of 1) scroll and 2) pinwheel inclusions within mesophyll cells infected with the stripe isolate of PStV. 1) scrolls appear to be composed of thin sheets which are held very tightly together. Mitochondria (M) are closely associated with both cytoplasmic inclusions. Bar Lengths: 1) .5 um; 2) .5 um.

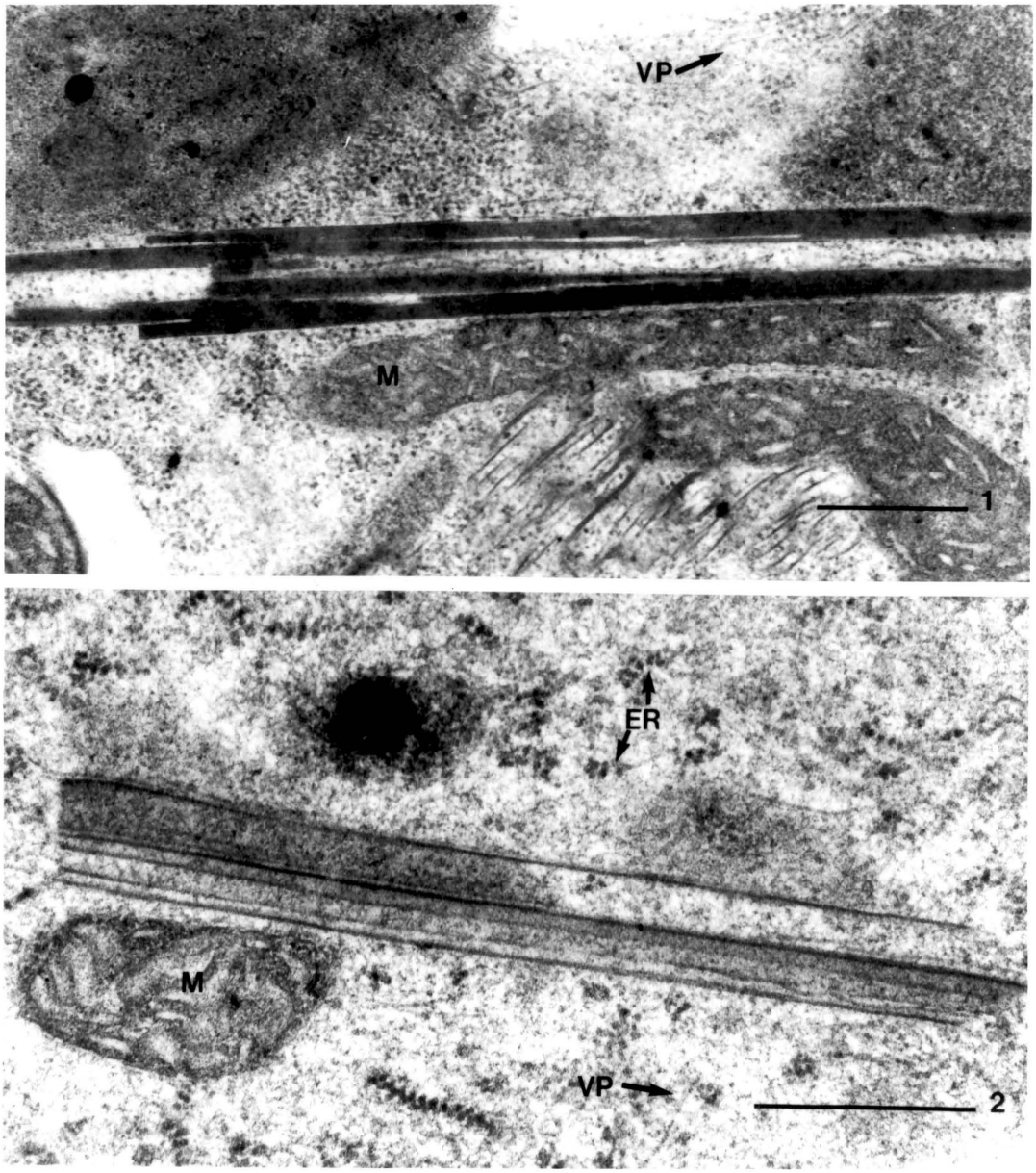
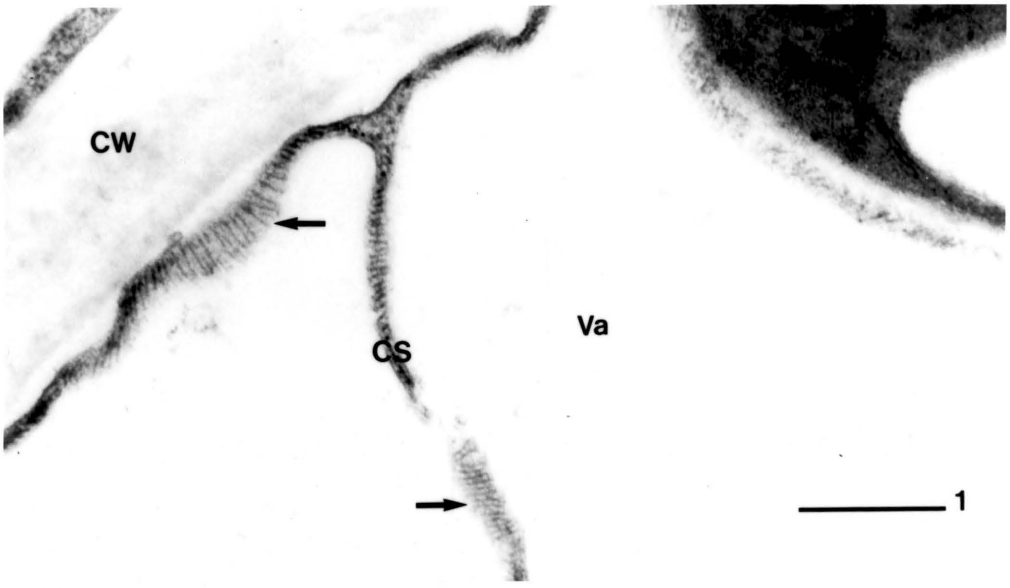
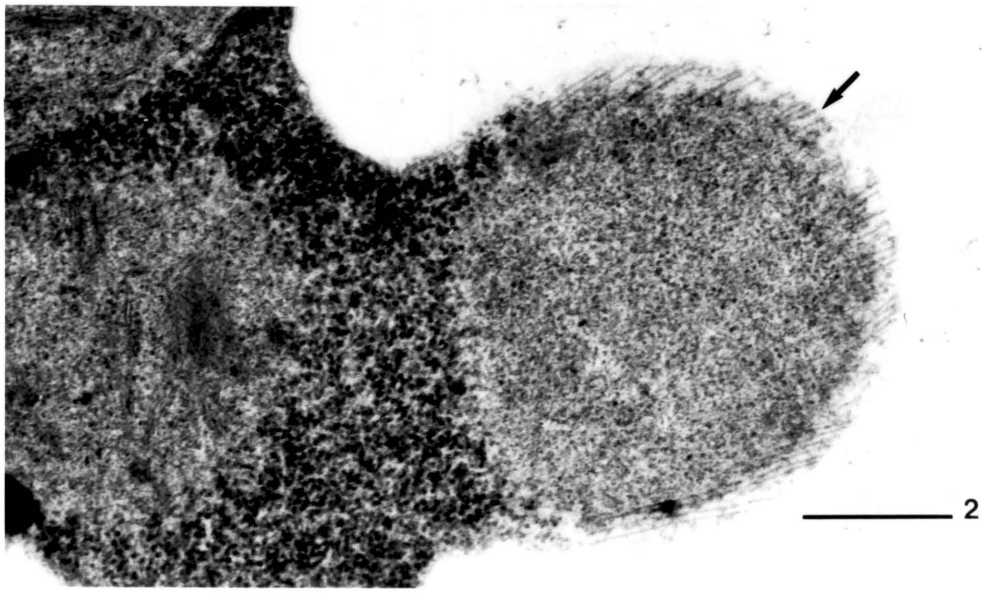

VP
M
1
ER
M
VP
2

Plate 9.1-9.2. Leaf tissue at the fully-expanded stage infected with PStV. Virus particles (arrow) line the edge of the tonoplast in 1)stripe and 2) blotch infected mesophyll cells. Virus particles can also be seen within the cytoplasmic strand (CS). Bar Lengths: 1) .5 um; 2) .5 um.


CW
CS
Va
1


2

Cytoplasmic strands were becoming larger and more abundant within infected cells. These strands appeared to contain virus-like particles. The particles enclosed within the strands had a mean diameter of 13 nm. Cytoplasmic strands were observed in a variety of configurations and were commonly seen in all cell types (Plate 10).

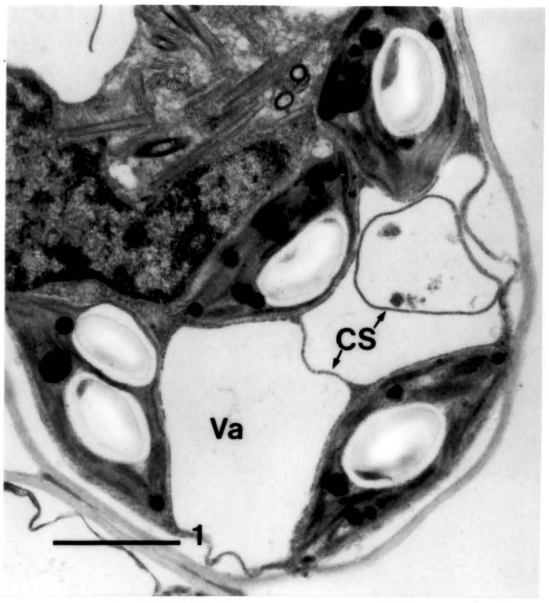
5. Mature-Expanded Stage

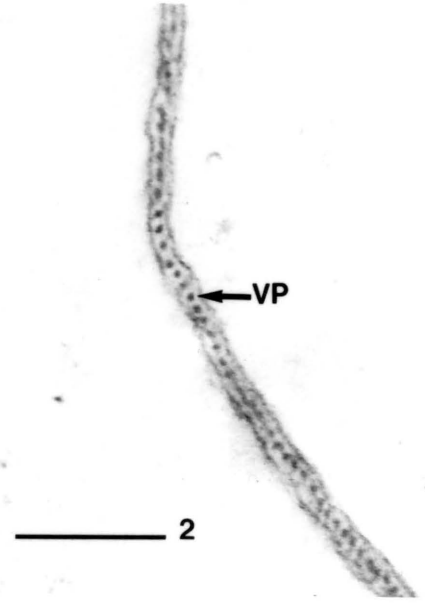
Cells at the mature-expanded stage of leaf development had an abnormal appearance. Palisade and mesophyll cells were plasmolyzed and filled with both scroll and pinwheel inclusions. Numerous cytoplasmic strands containing virus particles traversed highly vacuolated cells. Virus particles were also observed lining the edge of the tonoplast around the entire cell. Degradation of mitochondria was observed in cells infected with either isolate of PStV. Starch appeared to accumulate in the chloroplasts in excess of that observed in uninfected cells (Plate 11).

6. Dark-green vs Light-green Leaf Tissue

The ultrastructure of cells from light-green and dark-green areas of leaf tissue infected with either isolate was examined. Leaf tissue was sampled at the mature-expanded stage since true distinction between

Plate 10.1-10.3. Cytoplasmic strands within 1) blotch infected cell. 2) cross-section and 3) longitudinal/oblique section through cytoplasmic strands. Note the presence of virus particles (arrows) within each strand. Bar Lengths: 1) 2 um; 2) .25 um; 3) 1 um.


CS
Va
1


VP
2

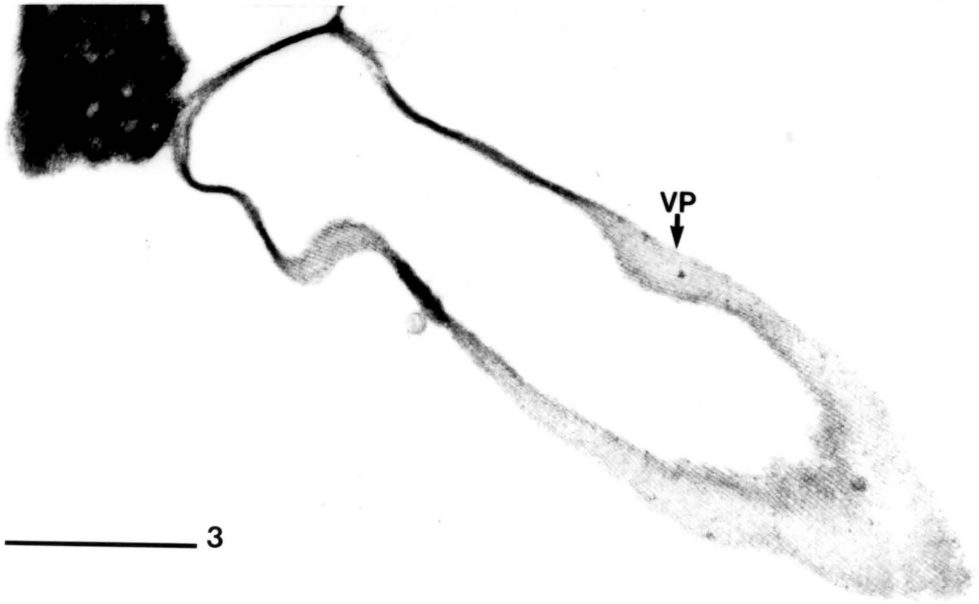
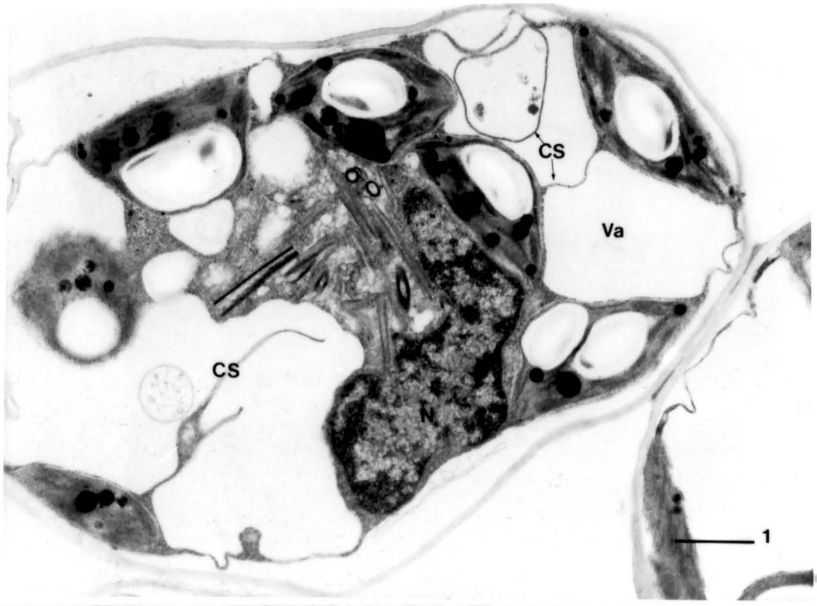
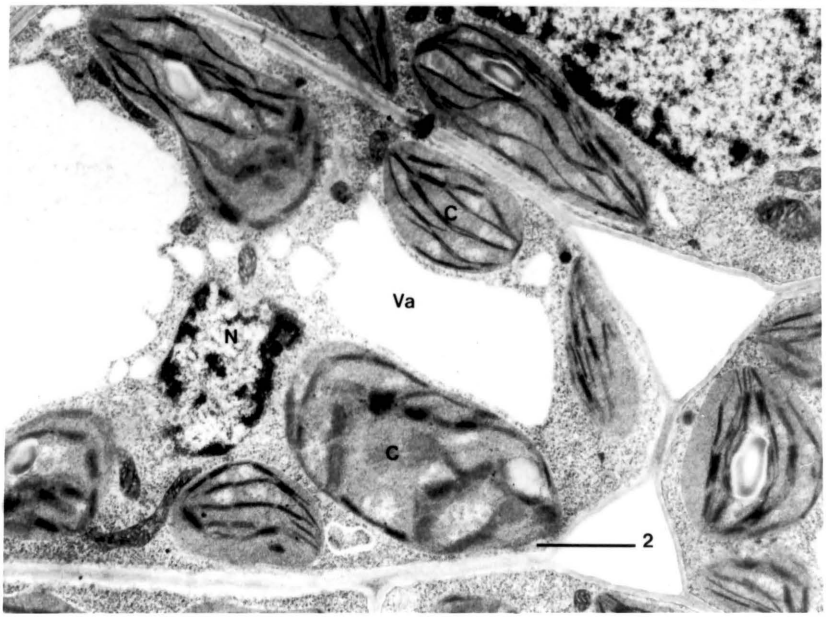

VP
3

Plate 11.1-11.2. Leaf tissue at the mature-expanded stage. 1) Mesophyll cell infected with the blotch isolate of PStV. Scrolls and pinwheels fill every available space in the cytoplasm. Cytoplasmic strands (CS) containing virus particles traverse the highly vacuolated and plasmolyzed cell. Note the presence of starch and lipophilic globules within the chloroplasts. 2) Healthy palisade cell. Bar Lengths: 1) 1 um; 2) 1 um.


CS
Va
CS
N
1


C
Va
N
C
2

light-green and dark-green areas was not even apparent until the 2/3-expanded stage. At the fully expanded stage, cells from light-green areas contained both scroll and pinwheel inclusions. Virus particles were observed throughout the cytoplasm and within cytoplasmic strands. At the mature-expanded stage, degeneration of mitochondria and of thylakoid membranes within chloroplasts was observed.

No characteristic cytoplasmic inclusions were observed in cells from dark-green areas of the leaf. Likewise, few if any virus particles were observed in the cytoplasm of these cells. A few cytoplasmic strands were observed in these cells, however, they were small and contained no obvious virus particles. In general, the ultrastructure of cells from dark areas appeared similar to that of cells from healthy tissue.

V DISCUSSION

A. VIRUS PARTICLE LENGTH

The mean particle lengths obtained for the stripe and blotch isolates of PStV were very similar for leaf dips and partially purified preparations. Separate measurements were made of particles obtained from leaf dips and those obtained by partial purification, in order to ascertain if the extraction procedures had any effect on particle length. The extraction procedures did not appear to have an effect on particle length since no appreciable difference in mean length was observed between procedures. Additionally, both isolates had a modal length of 750 nm regardless of the extraction procedure used. All particles were measured using a ZIDAS Image Analysis System. This system was found to be more accurate for measuring particle lengths than manually measuring the particles using an Ocular Scale Lupe (15x).

The protocol used to partially purify PStV was modified to minimize differences in the extracting conditions between the leaf dips, ISEM and the partially purified preparation.

The extracting buffer selected for both the partial purification and ISEM was 0.06 M sodium phosphate, whereas distilled water was used in preparing leaf dips. It was necessary to add 0.02 M $NaSO_3$ as an antioxidant to the buffer used in the purification procedure, however this was not a component of the buffer used for ISEM or for leaf dip preparations. The extraction buffer used by Demski et al., (1984) consisted of 0.1 M Tris-HCl buffer, pH 8.0, containing 0.02 M $NaSO_3$ and 0.05 M EDTA. The pH's of the extracting buffers used for purification and ISEM were pH 8.0 and pH 7.0, respectively. It was believed that this slight difference in pH would not have an effect on particle morphology.

Additives such as EDTA and urea were omitted from the purification procedure used in this study since previous studies have shown that these chemicals might interfere with particle morphology. Govier and Woods (1971) have shown that the composition of the extracting media can have an effect on the particle size and morphology of several potyviruses. These scientists tested the effect of adding magnesium during virus extraction and removing them from the extraction solutions by adding EDTA. They found that when particles of henbane mosaic virus (HMV) were exposed to buffers containing magnesium (0.05 M), the particles were

rigid and 900 nm in length. When these particles were exposed to buffers containing 0.05 M EDTA, the particles were flexuous and 800 nm in length. Similar morphological differences were found to occur with pepper veinal mottle virus (PVMV) and bean yellow mosaic virus (BYMV). Govier and Woods suggested that the Magnesium ions may form bridges between carboxyl groups of the virus protein and that these bridges may alter the conformation of the protein subunit, thus changing the length and rigidity of the virus particle. Huttinga (1974) tested the magnesium effect on six isolates of BYMV, pea seed-borne mosaic virus, lettuce mosaic virus and two isolates of potato virus Y. The addition and removal of magnesium was found to change the morphology of virus particles for all isolates and viruses tested, except for the two isolates of PVY.

B. COMPARATIVE VIRUS TITER

Significant differences in virus titer were observed between blotch and stripe infected leaves. This was more evident in ISEM assays rather than in local lesion assays. The ISEM assay was thought to be more accurate in assessing virus titer than the local lesion assay since it was able to detect more significant differences in titer between the two

isolates. Results of the ISEM assay showed that leaf tissue infected with the blotch isolate consistently had a higher virus titer than leaves infected with the stripe isolate. The ISEM assay also demonstrated that light-green areas of leaf tissue contained considerably more virus than dark-green areas; and that light-green areas from blotch-infected tissue contain more virus that light-green areas from stripe-infected tissue. The fact that dark-green areas from stripe or blotch infected leaves had approximately the same low virus concentration suggests that the dark-green areas represent essentially disease free tissue that does not support viral replication. The low amounts of virus in this tissue is thought to be due to passive movement of PStV particles from cells in light-green areas.

Differences in virus titer between the two isolates may be due to differences in the viral genome which directs viral replication. It is also possible that differences in titer may be due to the proportion of light-green vs dark-green areas of the affected leaves; since my studies show that dark-green areas of the leaf contain considerably less virus than light-green areas of the leaf. This observation has also been documented by other researchers (Reid and Matthews, 1966; Atkinson and Matthews, 1970).

Consequently, it is feasible that different proportions of light and dark green areas in affected leaves may have a direct effect on the virus titer on a per leaf basis. In order to determine if differences in titer are due to proportions of light and dark green leaf areas, it should be possible to measure these respective areas using the ZIDAS Image Analysis System and to compare them to the virus titer measured in these leaves.

Several researchers have reported on the unequal distribution of virus particles in dark-green vs light-green areas of the leaf (Reid and Matthews, 1970; Loebenstein et al., 1977). Several theories have been put forth to explain why these differences in virus titer exist. Goldstein (1926) suggested that the dark-green areas represent tissue which had escaped infection by the virus during the early stages of leaf development. Reid and Matthews (1966) speculated that some process, like lysogeny, might be occurring within cells, thus giving dark-green tissue its resistance to infection. Studies conducted by Atkinson and Matthews (1970) indicate that cells from dark-green areas were unable to support replication by tobacco mosaic virus (TMV). The authors speculated that "resistance" to replication was due to the presence of a diffusible agent that was as of yet still unidentified. However, there is no

convincing evidence which supports the presence of virus specific inhibitors in these dark-green areas. Studies conducted by Atkinson and Matthews negated the theory that dark-green areas can arise from the progeny of a single cell. Studies conducted by Loebenstein et al., (1977) found that dark-green areas of tobacco leaves infected with cucumber mosaic virus (CMV) did not contain infectious RNA and were resistant to reinfection with three strains of CMV.

The dark-green areas of tissue may not always persist in an essentially virus-free state. Several studies have shown that a breakdown of "resistance" leading to viral replication can take place after the plants had been placed in an environment with an elevated temperature (Loebenstein et al., 1977). Leaf dip and ISEM preparations made throughout the coarse of the year have shown that dark-green areas from PStV infected peanuts remained essentially virus-free, regardless of temperature ranges of 15-29 C.

The age and/or stage of leaf development at the time of virus infection is thought to be related to the proportion of leaf tissue that develops into dark-green areas (Atkinson and Matthews, 1967). This is in agreement with what I observed on peanut plants infected with isolates of PStV. The first indication of virus infection was observed on the fourth leaf above (6th quadrifoliolate) the inoculated

leaves. This leaf exhibited yellow flecks up through the fully expanded stage but did not exhibit full symptoms of stripe or blotch at the hardened stage. Very few dark-green areas were observed on these leaves. The 7th quadrifoliolate exhibited full symptoms of stripe and blotch. However, these leaves had a lower proportion of dark-green areas than the 8th quadrifoliolate. In general it appeared that the ratio of dark-green to light-green areas in affected leaves increased up until three weeks after inoculation, after which time it leveled off.

Since my assays indicated that both isolates of PStV could be found in measurable quantities in leaf tissue at the closed stage, I thought that it should be possible to detect and quantify the virus at even earlier stages of leaf development. Consequently, a preliminary experiment was conducted in order to ascertain if virus could be detected in the 7th, 8th and 9th quadrifoliolates at much younger stages of development. Plants were dissected when the 7th quadrifoliolate was at the closed stage and the younger, developing leaves were removed from the stem. The 8th quadrifoliolate consisted of very small (0.05 cm in length) but fully developed leaflets while the 9th quadrifoliolate consisted primarily of meristematic tissue. The 7th, 8th and 9th quadrifoliolates were removed from stripe and blotch

infected plants and prepared for ISEM as described previously. The 7th quadrifoliolate was also tested for virus and used as a check to compare with previous results at the stage. Virus particles were only found in the 7th quadrifoliolate of stripe infected plants and were not found in any of the quadrifoliolates excised from blotch infected plants.

The explanation for not obtaining virus in any of the other quadrifoliolates sampled may be due either to actual absence of the virus or to flaws in the procedure used. It was difficult to thoroughly grind up such small pieces of tissue in the small aliquot of grinding buffer used to give the 1:10 dilution. A spot plate consisting of small wells made out of ground glass would probably increase the accuracy of this test immensely. It is also possible that some plants sampled for this test were not infected with the virus. However, this is highly unlikely since the 6th quadrifoliolate was first checked for yellow-flecking and only plants positive for the trait were used. In addition, plants used for sampling were kept and the laterals were checked for symptoms. All plants used in sampling were found positive for either of the two isolates of PStV. Although this preliminary test did not demonstrate the presence of virus in very immature tissues, the limited

nature of the experiment suggests it would be appropriate to repeat the experiment on a larger scale before assuming that virus does not occur in these tissues.

It has been said that the apical meristem of systemically infected plants may contain little or no virus (Gibbs & Harrison, 1976). The apical meristem is known to be a dynamic region where cell division is constantly and rapidly occurring. Consequently, viruses may have a difficult time invading and becoming established in this area. Apical meristem culture is now widely used to produce virus-free stocks of vegetatively propagated plants such as potatoes and chrysanthemums. However, there is evidence that some viruses may invade meristematic tissue. Virus particles of tobacco rattle virus (TRV) have been observed in meristematic cells of both root and shoot apices (Kitajima and Costa, 1969). Walkey and Webb (1968) examined squashes of dissected apical meristem tissue by electron microscopy and were able to show the presence of several viruses in the apices of various hosts.

More tests of this nature are needed in order to better understand the progression of virus infections. Fixation and examination of tissue sections in the electron microscope would also supplement the understanding of virus-host relationships.

C. COMPARISON OF QUANTITATIVE VIRUS ASSAYS

Immunosorbent electron microscopy and local lesion assays were used to determine virus titer in peanut leaves infected with isolates of PStV. Based on the results of these studies, ISEM was found to be a more accurate method for assessing virus titer than the local lesion assay. The results from ISEM were more reproducible than the results obtained from the local lesion assay. In addition, more significant differences among the two isolates were detected by ISEM than by the local lesion assay, indicating that ISEM is a more sensitive assay for quantifying virus titer. Local lesion or infectivity assays are limited to measuring only virus particles that are biologically active, giving no indication of the total number of virus particles present in the preparation. The ISEM assay is especially advantageous since it requires smaller amounts of leaf tissue and less preparation time than a local lesion assay. Results of an ISEM assay can be obtained the same day, provided one had ready access to an electron microscope; whereas results of a local lesion assay can be obtained 1-2 weeks after inoculation.

D. ULTRASTRUCTURAL CYTOLOGY OF PSTV-INFECTED PEANUTS

Differences in the morphology of cytoplasmic inclusions have been used to separate potyviruses into four sub-divisions (Edwardson et al., 1984). Examination of numerous ultrathin sections of PStV-infected leaf tissue indicate that pinwheel and scrolls are the only two cytoplasmic inclusions induced by isolates of PStV. Based upon these findings, PStV should be assigned to sub-division I.

Pinwheel inclusions were observed in cells as early as the closed leaf stage in peanut leaves systemically infected with either isolate of PStV. The results of this study suggest that pinwheels form opposed to the plasmalemma and eventually become detached and drift into the cytoplasm. Longitudinal sections of pinwheels drifting away from the cell wall reveal the presence of a membrane closing the one end of the inclusion. This membrane is always located on the end of the pinwheel which has been in contact with the plasmalemma. I could not determine if this membrane was part of the plasmalemma which had torn off with the pinwheel or if it was also a product of the viral genome, as is the inclusion itself. The plasmalemma along the cell wall where the pinwheels were attached did not appear to be torn or disrupted, which supports the theory of the membrane being

of viral origin. Similar findings were reported for SMV-infected soybeans (Hunst, 1983) and for sweet potato russet crack (RCV) infected morning glory (Lawson et al., 1971). Lawson et al., (1971) suggested that pinwheels are formed at the plasmalemma and then migrate into the cytoplasm. Hunst (1983) reported that pinwheel inclusions appeared to form initially at the plasmalemma, however as the infection progressed, pinwheels were observed forming from one side of the double-membrane sac of endoplasmic reticulum (ER) in the cytoplasm.

Numerous vesicles were observed in the cytoplasm of PStV-infected cells at all stages of leaf development. In leaf tissue at the closed and 1/3-expanded stage, portions of pinwheels and some completely formed pinwheels were found within these vesiculated areas. However, most of the pinwheel inclusions observed at this stage were closely associated to if not opposed to the cell wall. At the 2/3 and fully expanded leaf stage, the number of cytoplasmic inclusions per cell had increased substantially. Most of the inclusions observed were present in the vesiculated areas. These observations suggest that the vesicles may be involved in the formation of inclusions, particularly during later stages of infection. Lawson et al., (1971) also observed vesiculated areas in the cytoplasm of RCV-infected

morning glory cells. Pinwheels, loops and circle configurations were found associated with these areas. Consequently, the authors interpreted the appearance of the vesiculated areas in the cytoplasm as sites of pinwheel degradation rather than sites of the pinwheel formation.

The function of these vesicles could not be determined in this study. Similar vesicles have been previously reported in other virus-infected tissues (Hoefert, et al., 1970; Lawson et al., 1971; Powell et al., 1977; Hunst 1983). Hoefert et al., (1970) reported the appearance of vesicles containing networks of fibrils in phloem parenchyma cells in older leaves infected with beet yellow stunt virus. Their source of origin was not determined however, their presence was associated with early signs of cell degeneration. Physiological studies conducted by Powell et al., (1977) suggest that the vesicles are apart of an intercellular transport system for cellular macromolecules and may also carry viral RNA intercellularly. The presence of the vesicles in pinwheel forming areas suggests that these vesicles may play a similar role in PStV-infected cells.

Areas composed of proliferated ER have also been associated with the formation of pinwheels (Hooper and Weise, 1972; Hunst, 1983). Pinwheels induced by SMV and WSSMV were observed to form abutted to double-membrane sacs

in these areas. Pares and Bertus (1978) also observed partial inclusions in areas of endoplasmic reticulum and polysomes, and occasionally in areas of electron opaque deposits.

The presence of cytoplasmic strands was observed in both stripe and blotch infected tissue at the 1/3-expanded leaf stage. As the leaf tissue matured and the virus infection progressed, these strands became more abundant. Leaves at the mature expanded stage had reduced cytoplasmic content, but did contain numerous cytoplasmic strands filled with virus particles. Although cytoplasmic strands have been reported for several potyviruses, their function, has never been determined (Begtrup, 1976; Kitajima and Lovisolo, 1972; Weintraub and Ragletti, 1970; Hunst and Tolin, 1983). It has been suggested that their formation within cells of Datura infected with henbane mosaic virus is a defense reaction of the infected cells. Hunst and Tolin (1983) hypothesized that the formation of cytoplasmic strands is an intercellular virus localization mechanism. They suggested that this mechanism was responsible for the tolerant response of Essex soybean plants to three mild SMV strains. Weintraub and Ragetli (1970) proposed that the low virus concentration obtained from the purification of carnation vein mottle virus was due to their failure to

disrupt the strands containing virus particles during the purification process.

Scroll inclusions were observed in PStV-infected cells at the 2/3-expanded leaf stage. These inclusions were located among the pinwheel inclusions in the vesiculated areas of the cytoplasm. In some cases pinwheels and scrolls appeared to share a common arm. Close examination of scroll inclusions indicate that they are composed of thin filaments that are held together very tightly by some means. The appearance of scrolls in the late stages of infection indicates that either a new part of the viral genome is being read at this point, or the packaging of the virus-coded protein is in some way altered to form scrolls instead of, or in addition to pinwheels.

Both pinwheel and scroll inclusions were often found in close association with mitochondria. It is possible that this is a forced association due to overcrowding of the cytoplasm with inclusions. However, the frequency in which this was observed suggests that a more intricate relationship between inclusions and mitochondria exist.

1. Light and Dark Green Tissue

Several differences were observed between the ultrastructure of dark-green and light-green leaf tissue. Cells from dark-green areas did not contain any cytoplasmic inclusions and contained few, if any virus particles. In contrast, cells from light-green areas were filled with both scroll and pinwheel inclusions. Virus particles were observed scattered through the cytoplasm, aggregated in crystal arrays and parallel along the tonoplast.

Based upon ultrastructural observations of both dark-green and light-green leaf tissue infected with either isolate of PStV, I believe that the dark-green areas on the leaves represent healthy tissue which does not support replication of the virus. The virus particles that were found in cells of dark-green tissue are believed to be the result of passive movement from cells of light-green tissue. This is supported by the fact that no cytoplasmic inclusions were observed within cells of dark-green tissue, and if viral replication were taking place, one would expect to find corresponding inclusions in these cells. In addition, the virus titer in dark-green tissue of stripe and blotch infected leaves was nearly identical, which suggests that this low number is an accurate assessment of titer in dark-green tissue.

These observations are in agreement with studies conducted by Pares and Bertus (1978) who found that only cells from light-green areas of Crinum sp. infected leaves contained pinwheel and tubular inclusions. Virus particles in Crinum infected cells were observed scattered throughout the cytoplasm and in densely aggregated masses only in light-green areas. A similar report was made by Butterfield (1983) with respect to watermelon mosaic virus-infected pumpkin leaves.

2. Differences in Stripe and Blotch infected Tissue

The stripe and blotch isolates of PStV differed in both symptom expression and in some cytopathic effects on Florigiant peanut plants. Although studies conducted by Demski et al., (1984) found the two isolates to be virtually identical serologically, they clearly induce different symptom patterns on infected leaves. Symptoms induced by the blotch isolate appear to be slightly more severe than those induced by the stripe isolate. This becomes especially apparent two months after inoculation when the patterns induced on the leaves become less pronounced on the blotch infected leaves and very faint on stripe-infected leaves. Although symptoms at this stage of the infection appear less severe, ultrastructural studies have not been

conducted on these leaves to ascertain the effect of the virus at this stage of leaf maturity.

Two main ultrastructural differences were observed between the stripe and blotch infected tissue. Virus particles grouped together in crystalline arrays were only observed in cells infected with the blotch isolate. Ultra-thin sections of leaf tissue containing longitudinal and cross-sections of these virus crystals were examined and measured. The particles measured 13 nm in diameter. Similar aggregations of virus-like particles have been reported in wheat infected with WSSMV (Hooper and Weise, 1972) and in morning glory infected with RCV (Lawson et al., 1971).

Pinwheel inclusions in blotch-infected tissue appeared to have virus-like particles situated between the arms of the inclusion. Such particle-pinwheel associations were rarely observed in stripe-infected tissue. The function of virus particles between the arms of the inclusion could not be determined from this study. The presence or absence of virus particles between the arms of the pinwheel is thought to be correlated with the concentration of virus in the cell. The results of the virus titer assay of blotch-infected light-green tissue had a 2.5 fold higher concentration of virus than stripe-infected tissue. This

may explain why this phenomenom is typically seen in blotch infected tissue. Pinwheels of this nature have also been reported by Hooper and Weise (1972) in WSSMV infected wheat.

E. VIRUS TITER AND ULTRASTRUCTURAL OBSERVATIONS

There is a relationship between virus titer as determined by ISEM and the cytopathic effect induced by isolates of PStV. During the early stages of systemic viral infection, virus titer was found to be very low, but detectable in leaves at the closed and 1/3-expanded leaf stage. Ultra-thin sections of leaf tissue at these stages showed that pinwheel inclusions were present but in very low numbers. In addition, very few virus particles were observed in cells at these stages. As the virus infection progressed, virus titer measured in leaves at the 2/3 and fully expanded stage were found to increase substantially with the highest titer occurring at the fully expanded stage. Thin sections of these respective leaf tissues showed that the number of cylindrical inclusions in affected cells had increased to the point that inclusions filled all available spaces in the cytoplasm. Many virus particles were also observed scattered throughout the cytoplasm and aligned along the tonoplast. Assuming that the proteins which make up the cylindrical inclusions are products of the

viral genome (Purcifull et al., 1973; Dougherty and Hiebert, 1980) one would expect to find more inclusions in cells as the infection progressed and as virus titer increased.

Cytoplasmic strands were also more abundant in cells in which the virus titer was high. This supports the virus localization theory of Hunst and Tolin (1983). The reduction in virus titer that was seen in leaves at the hardened stage may be due to failure to disrupt the strands containing virus particles as proposed by Weintraub and Ragetli (1970) or due to the actual degeneration of virus particles during late stages of disease.

SUMMARY

Two Virginia isolates of peanut stripe virus (PStV), 'stripe' and 'blotch' were compared ultrastructurally in peanut (Arachis hypogaea L. 'Florigiant') at five stages of leaf expansion. Ultrathin sections of leaf tissue at the closed and 1/3-expanded stage revealed pinwheel inclusions attached to the cell wall near plasmodesmata and in vesiculated areas of the cytoplasm. Two main ultrastructural differences were observed between stripe and blotch infected tissue. Virus particles grouped together in crystalline arrays were observed only in cells infected with the blotch isolate. Pinwheel inclusions in blotch infected tissue had virus-like particles situated along the arms of the inclusion. This association was rarely observed in stripe infected tissue. The presence or absence of virus particles between the arms of the pinwheel is believed to be correlated with the concentration of virus in the cell. The results of the virus titer assay indicated that leaf tissue infected with the blotch isolate contained substantially more virus than leaf tissue infected with the stripe isolate. Cytoplasmic strands containing virus particles

were observed forming at the 1/3-expanded stage. All organelles within young, PStV infected cells appeared normal with the exception of chloroplasts which contained phytoferritin. Scroll inclusions appeared in infected cells from leaves at the 2/3-expanded stage. At the fully expanded stage, numerous pinwheel and scroll inclusions were present in the cytoplasm and closely associated with mitochondria. Virus particles were observed free in the cytoplasm as well as concentrated in linear arrays along the inner surface of the tonoplast. The cells from mature leaves infected with either isolate of the PStV were highly vacuolated and filled with inclusions and cytoplasmic strands. Degradation of mitochondria was also observed within these cells.

The ultrastructure of cells from light-green and dark-green areas of PStV infected leaves were examined. Cytoplasmic inclusions were only observed in cells from light-green areas of the leaf. Numerous virus particles were observed free throughout the cytoplasm and within cytoplasmic strands. Cells from dark-green areas of the leaf contained few if any virus particles and appeared normal. Cytoplasmic strands were present but did not contain any virus particles. Dark-green areas of the leaf are believed to represent healthy tissue that does not that does not support virus replication.

Particle measurements obtained from leaf dips and from partially purified virus preparations of stripe and blotch isolates indicated that essentially no difference in particle length existed between the two isolates. Mean particle lengths for stripe and blotch isolates were 751 nm and 741 nm from leaf dips and 746 nm and 745 nm from purified preparations, respectively. Both isolates had a modal length of 750 nm.

The relative virus titer of each isolate was determined in peanut leaves at five expansion stages and in dark-green and light-green areas of the leaf. A significant increase in virus titer was noted for each expansion stage up until the fully expanded stage. Fully expanded leaves had the highest virus titer in both stripe and blotch infected tissue. A decrease in titer was observed in the mature-expanded leaves. Leaves infected with the blotch isolate consistently had a higher virus titer than leaves infected with the stripe isolate at all growth stages. Virus titer was also substantially higher in cells from light-green areas rather than dark-green areas of the leaf regardless of isolate. Differences in virus titer between the two isolates is believed to be due to differences in the viral genome which enhances viral replication and also to the proportion of light-green vs dark-green areas of affected leaves.

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