AN ABSTRACT OF THE THESIS OF

John Elmore Elser for the M. S.
(Name of student) (Degree)

in Plant Pathology presented on
(Major) (Date)

Title: SOME CYTOLOGICAL ASPECTS OF STREAK MOTTLE VIRUS IN LILIUM SPECIOSUM THUNBERG

Abstract approved: T. C. Allen, Jr.

Cultivated lilies include many garden varieties in addition to the Easter lily, Lilium longiflorum Thunb. A popular member of the garden group is Lilium speciosum Thunb., whose leaves can be discolored by a virus symptom described as streak mottle. This disorder is characterized by dashes of chlorotic tissue interspersed with green.

In order to gain further insight about the nature of the virus inciting streak mottle on L. speciosum, leaf pieces of approximately 0.5 mm square were immersed in buffered glutaraldehyde fixative, treated with osmium tetroxide, dehydrated, and embedded in epoxy resin. After thin sectioning and staining, the specimens were examined with an electron microscope.

Configurations described as pinwheels and bundles, characteristic of 750 m" flexuous rod viruses, were seen in abundance within leaf mesophyll cells, epidermal cells, and to a lesser extent, in
xylem cells. In agreement with previous workers, the pinwheel and bundle inclusions were found to be different views of the same body. This intracellular body associated with streak mottle can be described as a cylindrical inclusion with curved plates emanating from a central hollow core. While some viruses appear to occur along the inner curvature of the plates, at least one has been reported as separate from the pinwheel-bundle figure. It appears that streak mottle virus is also one of the latter.

*L. speciosum* mesophyll cells were found to contain crystals, and these were observed in both diseased and healthy tissue. Diseased tissue displayed an additional crystalline body, which occurred in clusters and was often seen in close proximity to pinwheel-bundle figures. These bodies seem to be homologous to those found within nuclei of tobacco leaf mesophyll cells infected with tobacco etch virus, also a flexuous 750 μ rod.

The chloroplasts of both infected and healthy mesophyll cells showed large vacuoles of unknown significance. A smaller peripheral vesicle that occurs between the two layered plastid-limiting membrane seemed to occur more frequently in diseased cells than in healthy. Depletion of a chloroplast substance is suggested by the presence of these vesicles.

The remainder of the organelles, namely, dictyosomes, nuclei, and mitochondria, appeared to be similar in both healthy and
diseased cells. On the basis of preliminary findings further work may establish that streak mottle virus induces premature degeneration of host cells.
Some Cytological Aspects of Streak Mottle Virus in *Lilium speciosum* Thunberg

by

John Elmore Elser

A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Master of Science

June 1968
APPROVED:

Associate Professor of Plant Pathology in charge of major

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Date thesis is presented  

Typed by Opal Grossnicklaus for John Elmore Elser
ACKNOWLEDGMENT

I would like to offer gratitude to my major professor, Dr. T. C. Allen, Jr., for his advice and encouragement during this study. Sincere thanks also are extended to Dr. F. P. McWhorter for his helpful suggestions, Dr. Patricia Harris for her patience and cooperation and Mr. R. B. Addison for technical advice.

Grateful recognition is given to my parents, Mr. and Mrs. Elmore Elser, for their belief in the importance of educational advancement.
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SOME CYTOLOGICAL ASPECTS OF STREAK MOTTLE VIRUS IN LILIUM SPECIOSUM THUNBERG

INTRODUCTION

The lack of uniformity and appearance and disappearance of viral symptoms in liliaceous plants have made it necessary to use more than symptomatological studies to confirm or refute the association of a particular plant disorder with a given virus. It seems reasonable that elucidation of the different types of viruses capable of infecting lilies can serve to better categorize them. In addition, by arranging them in a fashion based upon what is known of structurally related viruses, it might be possible to effect appropriate control measures.

The electron microscope permits detailed anatomical observation of both viruses and the tissue invaded by them. While the total volume that can be examined by this method in a given time is small, it still is feasible to observe and record cytological alterations wrought by the virus.

The role of plant cell organelles in viral disease etiology is not fully resolved. The nucleus is known to assume a part in viral ribonucleic acid and viral protein synthesis, but viral assembly sites and areas of damage in the cell are not clearly identified. Uncertainty about the chloroplasts is especially acute with regard to virus
infection.

The present work deals with streak mottle virus of Lilium speciosum Thunb., a popular garden lily. The purpose of the study was to develop a method for processing lily tissue for electron microscopic examination and to study aspects of the infection as revealed by the techniques employed.
REVIEW OF LITERATURE

Lilies and Viruses

The genus Lilium represents a popular and diverse ornamental group whose best known members include the white, trumpet-shaped Easter lily, Lilium longiflorum Thunb. This plant is forced annually in commercial greenhouses and sold in sizable numbers due to its general acceptance as a symbol of Easter.

The garden-cultivated lilies are numerous; seven separate categories are listed in the 1963 Lily Yearbook (Slate, 1963). Even the group that includes the L. longiflorum species is comprised almost entirely of garden types. One of the more highly-regarded of these is Lilium speciosum Thunb., a plant capable of attaining four feet in height compared with the one to three foot range of the common Easter lily. Like L. longiflorum, L. speciosum is a Japanese native and originally was found in Shikoku, Kyushu and the islands of Koshiki-jima, part of the Japanese Archipelago. In 1794, Thunberg proposed both lilies as species in the Transactions of the Linnaean Society (Woodcock and Stearn, 1950). The first reported L. speciosum introduced to the United States came from Japan in 1876 (Rockwell, Grayson and de Graaf, 1950).

The importance of lily virus investigation is reflected by the
sponsored research that has been published (Guterman, 1930; McWhorter, 1963; McWhorter and Millsap, 1950) and the worthless plants that can result from virus infection. Closer scrutiny of the situation has enabled workers to argue that improper soil and climatic conditions, poor cultural methods, insufficient nutrition and other similar factors were not directly responsible for stunted plants, distorted flowers, discolored leaves, and poor bulb yields.

While it is not precisely known how early the growers of \( L. \) speciosum were confronted with the problems of virus, observations of its symptoms and effects were published in 1930 (Guterman, 1930). Concern for the possibly related Easter lily virus was raised much earlier on account of the rapid decline of the once-thriving Bermuda lily industry during the late nineteenth century. In addition to large numbers of Easter lilies, Guterman examined \( L. \) speciosum plants showing mottle symptoms. He noted that affected plants were stunted and the green areas of their leaves were interrupted by chlorotic areas occurring in streaks of varying width, sometimes proceeding along the whole leaf length.

\( L. \) speciosum was included with a large group of lily types in an attempt to compare the viruses affecting them (Brierley, 1940). Although viruses were not detected in western sources of \( L. \) speciosum, the eastern grown lilies gave positive indications of having both tulip and cucumber viruses. The tulip virus incited a strong
mottle in inoculated *L. longiflorum*.

The transmissibility of *L. speciosum* virus to tulips has been noted (McWhorter and Millsap, 1950). A later paper indicated that two distinct viruses may infect *L. speciosum* (McWhorter, 1963). The first virus was called fleck on account of the blocklike necrotic areas which it induced on its host. Fleck occurs naturally in rubrum lilies imported from Japan; it was found to be capable of infecting all *speciosum* varieties as well as corn (*Zea mays* L.). The second virus mentioned was transmissible to tulips; this was called mosaic to distinguish from the fleck disorder.

**Electron Microscopy**

In order to reinforce or supplant symptomatological data typified by the above accounts, plant virologists sought a different means of linking a particular virus with an infection. The potential means for this was made available with the advent of electron microscopy.

Electron photomicrographs of isolated tobacco mosaic virus particles taken from infected tobacco leaves were published (Kausche and Ruska, 1950). Soon other workers followed suit in a succession of papers describing the morphological properties of viruses *in vitro*, and their possible association to cellular components.

Electron microscopists believed they could derive more meaningful information from studies of viruses *in situ*. Micrographs of
tobacco mosaic virus in thin-sectioned plastic-embedded plant tissue were published in 1950 (Black, Morgan, and Wyckoff, 1950). Since that milestone, researchers have continually experimented with technique modifications for tissue preparation. Use of cross-linked plastics as opposed to the so-called monomers has resulted in more stable specimens for viewing; exposure of sectioned tissue to aqueous heavy metal salts (Reynolds, 1963) has improved the specimen image contrast; and, refined equipment--microtomes and electron microscopes--have enabled workers to produce more sharply defined micrographs. The results at present show an extensive collection of papers that concern ultrastructural studies of many different biological specimens, including plant tissue.

As revealing and detailed as electron images can be, the small amount of tissue that can be examined at one time is limited. The necessity of microtoming tissue to extremely thin slices (0.05 µ) makes studies of large volumes of cells formidable. Single cell studies are preferred to whole tissue studies; conclusions may be more safely drawn, and the exhaustive examination of so great a number of sections is avoided. Yet, until an acceptable plant tissue culture cell type is available to plant virology anatomists, use will continue to be made of selected portions of infected whole tissue for ultrastructural investigations.
Plant Viruses In Situ

At first intracellular plant viruses were described as rods, more or less rigid, and as spheres. A different manifestation of plant virus was observed (Yamaguchi, Kikumoto, and Matsui, 1963) in mosaic diseased tulips. Examination of infected flower petal tissue with an electron microscope revealed dense, fibrous masses or bundles scattered within the cytoplasm; never within mitochondria or plastids. Additionally, these workers observed masses they termed "looped profiles". Similar configurations were later reported in wheat streak mosaic-infected plant tissue (Lee, 1965).

A compilation of viruses, which were studied in situ, belonging to the so-called flexuous rod group (Brandes and Wetter, 1959) was made (Edwardson, 1966a); this list is as follows:

- Bean Common Mosaic Virus 750 mµ
- Sugarcane Mosaic Virus 750 mµ
- Lettuce Mosaic Virus 750 mµ
- Bean Yellow Mosaic Virus 750 mµ
- Watermelon Mosaic Virus 730 mµ
- Tobacco Etch Virus 730 mµ
- Potato Virus Y 730 mµ

It was found that each of these viruses in its respective host induced pinwheel and bundle inclusions. That these structures could serve as
diagnostic characters for viruses of the potato virus Y group was proposed. Subsequent work (Edwardson, 1966b) of assembling evidence through serial sectioning revealed that the pinwheels and bundles were different views of the same structure. This inclusion was described as a cylindrical body composed of curved plates whose inner edges converge around the central axis of the cylinder and whose outer edges diverge to form the outer boundary of the cylinder. The pinwheels were cross sections through this cylindrical structure, and the bundles represented longitudinal sections through differing numbers of curved plates.

There were some slight variations observed among the forementioned viruses pertaining to total structure. In some, such as bean yellow mosaic virus and lettuce mosaic virus, there was a reduced curvature of plates and the pinwheels had an open rigid appearance; in bean common mosaic and watermelon mosaic the pinwheels were more compact and the plates showed a greater degree of curvature. Tobacco etch and sugarcane mosaic viruses in situ displayed laminated structures attached to the arms of the pinwheels.

Investigators working with a virus of pepper (Capsicum annuum L.), considered the alternative concept, that pinwheels and bundles were separate manifestations of the virus (Rubio-Huertos and Lopez-Abella, 1966). They then reached the same conclusion as did the
previous workers concerning the three-dimensional structure of the cylinder. Following the structure in cross section through serial sections, they estimated the length of an intact figure to be in the range of 750 mµ.

The actual emplacement of the virus particles with reference to the three dimensional rosette figure is not definite for all viruses in the flexuous rod group. Serial sections of tobacco etch virus (Zettler, Christie, and Edwardson, 1967) and watermelon mosaic virus (Edwardson, Purcifull, and Christie, 1968) suggested that virus particles lie along the inner curvature of each plate. The notion that the virus actually is present along the plates is strengthened by autoradiographic demonstration of tritiated uridine and leucine uptake by tobacco etch virus-infected tissue (Hayashi and Matsui, 1967). Masses of inclusions showing the pinwheel-bundle pattern showed uptake of both the nucleic acid base label and the amino acid label. This suggested that virus is present within the figures.

Intranuclear crystalline inclusions, originally observed in tobacco etch virus-infected cells (Rubio-Huertos and Garcia-Hidalgo, 1964), also were studied by autoradiography (Hayashi and Matsui, 1967). Only radioactive leucine appeared to be concentrated by these inclusions.

Recent studies of wheat streak mosaic virus (Shepard and Carroll, 1967) noted that virus-like particles directly associated
with the pinwheel figures could not be found. Instead, rods, morphologically similar to those seen in particulate preparations of the virus, were found in clusters separate from the pinwheel-bundle cylinders.

**Viral Cytopathology**

The interest that ultrastructural anatomy of virus infections in plants has raised is evidenced by two recent review papers on the subject. Anatomical data since the commencement of viral cytopathological studies (Esau, 1967; Matsui and Yamaguchi, 1966) is examined. The present knowledge of viral cytopathology is discussed in considerable detail in both papers, so unless an aspect applies to the work herein, it will best be consulted in one or both of these forementioned works.

Aside from the visualization of viruses and their development in plant cells, the chief area of interest about them has been the achievement of pictorial data on cell alterations wrought by the viral invaders. The organelles, especially the chloroplasts, are the objects of detailed scrutiny on account of their relative abundance and the presumed alteration of their pigments which accompanies many plant virus infections.

Micrographs suggesting a destruction of chlorophyll and a suppression of plastid development in systematically infected embryonic leaves of *Nicotiana tabacum* L. have been published (Shalla, 1959).
Specifically, chloroplasts in tissue fixed during active virus synthesis showed disorganization, clumping or dissolution of stromatic material, and a lack of normal lamellar structures. While chloroplasts from more mature leaves appeared to have assumed a more spherical shape in response to infection, they showed no evidence of disorganization.

*Datura stramonium* L. infected with tobacco mosaic virus and examined with the electron microscope showed the release of chloroplasts into the cell vacuole and fewer numbers of these organelles.

In a study systematically following the different stages of virus synthesis, large vacuoles were encountered within the chloroplasts (Shalla, 1964). Infected leaf tissue chloroplasts sometimes contained virus-like particles in these vacuoles; healthy tissue chloroplasts also showed similar vacuoles containing cytoplasmic components such as mitochondria or ribosomes. A vacuolation of chloroplasts 4 to 48 hours after inoculation of tobacco leaves with tobacco mosaic virus has also been noted (Milne, 1966).

Plants locally infected with turnip yellow mosaic virus showed globular and enlarged chloroplasts with large amounts of starch, fewer osmiophilic bodies, and many small vesicles around the periphery of the plastid (Chalcroft and Matthews, 1966).

Progressive destruction of chloroplasts has been reported in the case of a corn virus (Herold, Bergold, and Weibel, 1960); in
contrast, it has been suggested by others that there is no abnormality within the chloroplast of a mature infected cell (Matsui and Yamaguchi, 1966). In young leaves, developed after a virus infection, grana lamellae have been found reduced or entirely lacking.

Aggregated particles presumed to be tobacco mosaic virus within chloroplasts have been reported (Esau and Cronshaw, 1967). These clumps, lacking a bounding membrane, evidently displaced the chloroplast grana. Invaginations of the inner membrane of the chloroplast envelope, termed peripheral vesicles, also could be seen.

In sieve element plastids of older sugar beet leaves infected with curly top virus, the following features have been noted (Hoefert and Esau, 1967); 1) presence of rodlike material; 2) tightly compacted internal membranes; 3) proteinaceous material in smaller proportion and often displaced; 4) an accumulation of osmiophilic material, distinct from that seen in normal and degenerating plastids, and 5) breakdown of plastid membranes and general dissolution of the contents of the plastids. In healthy plants degeneration did not take place until the sieve element ceased to function.
MATERIALS AND METHODS

For ultrastructural studies, small pieces of healthy or diseased leaves were placed in chilled glutaraldehyde that had been diluted to 6.5% with 0.07 M phosphate buffer (Na$_2$HPO$_4$-KH$_2$PO$_4$), pH 7.0 (Kolehmainen, Zech, and von Wettstein, 1965). Rapidity of fixative penetration was enhanced by mild aspiration. A four- to six-hour fixation was followed by an overnight wash in cold buffer. Post-fixation was carried out in 2.0% osmium tetroxide-buffer (1:1), succeeded by dehydration in graded ethyl alcohols. Two 15 minute washes of propylene oxide preceded the introduction of the tissue to the first Araldite 6005-Epon 812 mixture which consisted of one part of the epoxy resin mixed with twice the amount of prescribed accelerator (DMP-30, or 2,4,6, -dimethyl aminophenol) to two parts of propylene oxide; the next step consisted of a fresh mixture of the forementioned ingredients with the resin and propylene oxide proportions reversed. Final embedding material consisted of pure resin with three drops of DMP-30 from a Pasteur pipette per 4 ml of resin. This mixture was vigorously mixed and evacuated of bubbles by placing it in a vacuum oven at 60°C. The plant tissue then was added to the resin and allowed to remain outside the oven for several hours to overnight to ensure maximal resin penetration. Final curing at 60°C was accomplished in 24-36 hours.
Sections were cut from trimmed tissue blocks on a Porter-Blum MT-I ultramicrotome using a diamond knife. Sections of silver-grey interference colors were picked up on Formvar-coated grids, stained with uranyl acetate and lead citrate (Reynolds, 1963). The sections then were examined with a Philips EM-300 electronmicroscope operated at 60 Kv with a 20μ objective aperture.

Negatives were printed on Kodabromide F paper of varying contrast depending on the quality of the negative.
RESULTS

Initial work to obtain satisfactory plant tissue preservation and embedding was attempted using a chromic acid-osmium tetroxide fixative and maraglas plastic embedment. Failure to attain adequate infiltration of the epoxy resin prompted the use of differently proportioned mixtures and other ingredients. The hardness of Epon offset its rapid infiltration advantage, so an Epon-Araldite mixture which seems to combine the easier cutting quality of Araldite and the more rapid infiltration of Epon was used.

On account of the success obtained with glutaraldehyde fixation (Kolehmainen, Zech, and von Wettstein, 1965), this writer followed a similar procedure. The use of a diamond knife for sectioning proved advantageous. Aqueous uranium and lead salts (Reynolds, 1963) were the consistently used stains.

The cells studied were primarily mesophyll-parenchymatous types. These were characterized by their relatively thin walls, living photosynthetic state, and large central vacuoles. With the bulk of the cell contents being vacuolar, only a small portion of the total cell volume in a given section shows discernible cytoplasm and organelles, and these appear closely abutted to the wall (Figures 2, 3). More often than not, the chloroplasts and a thin film of plasma-lemma -bounded cytoplasm would be the only objects of interest in
a given field.

Examination of a mesophyll tissue region revealed cells of diverse ages; healthy and diseased tissue alike showed chloroplasts ranging from those with uniform lamellae and packed grana to those with scattered lamellae and swollen grana.

Besides the widely recognized ground substance, the stroma, green plastids had a grana and a lamella network. In addition to these there seemed to be at least two distinct types of vacuolar areas within chloroplasts; both were seen in healthy and diseased leaf tissue.

The first type, found in some chloroplasts, can be described as larger vacuoles with an interior membrane amoebalike in appearance (Figures 2, 3, 5). This space was frequently occupied by cytoplasmic components such as mitochondria and ribosomes; as far as is now known, this type of phenomenon is normal. In the case of tobacco mosaic virus infected plant cell chloroplasts, particles resembling rods of the virus may be found within this vacuole (Milne, 1966; Shalla, 1964).

The second type, found in numerous chloroplasts is somewhat smaller than the first type and occurs close to the surface of the plastid in most cases (Figures 3, 4, 5, 6, 8). The invaginated portion always occurred between the first and second layer of the bilayered plastid limiting membrane despite the fact that the vesicle
sometimes protruded deeply into the interior of the plastid. Occasionally openings were found leading directly to the cytoplasm, or the vesicle was extended proximally to the grana situated within the chloroplast.

Comparison of diseased and healthy tissue at relatively young age reveals that the chloroplasts in diseased tissue seem to have more of the second type of vacuolar region, namely the peripheral vesicles (Figure 5).

Advanced infection (Figure 10) conveys the impression of incipient cell breakdown. Chloroplast grana integrity appears to be disrupted, a condition noted with reference to wheat striate mosaic virus (Lee, 1964).

The presence of a large crystalline body within cells of mesophyll tissue (Figure 7), while illustrated here in diseased tissue, also has been noted in healthy Lilium leaf mesophyll cells.

Initial work with \textit{L. speciosum} streak mottle virus infected leaves gave the impression that virus structures occurred only in parenchyma cells. This later was found to be untenable. Epidermal cells (Figure 11) and what appear to be portions of the vascular regions of the leaf (Figure 12) contain the typical pinwheel and bundle configurations. Serial sections (Figure 6) depict both how clustered these viral structures occur and the apparent continuity of the figures from section to section. Like many others, these cylindrical
inclusions occurred in the midst of a field of chloroplasts. Further
evidence for the continuity of the pinwheels is seen where the "spokes"
were cut obliquely. Here the curvature of the plate is thickened
(Figure 10).

The small clusters of crystalline bodies, which do not appear
to be like the previously noted ones, seem to be associated with the
streak mottle infection (Figures 8, 9, 10). No comparable struc-
tures were seen in healthy tissue of L. speciosum. When seen in
diseased tissue, these were often associated with the pinwheel fig-
ures.
DISCUSSION

Plant viruses intimately associate with their hosts. The insidiousness of their action is attested to by the fact that damage assessments are difficult to make; the inability of one to judge cell ages in tissue creates obstacles for distinguishing cells that are showing natural degeneration and those that are affected by the invading virus.

Fixation and staining for electron microscopy are relatively non-specific, so absence of structures, macromolecular alterations, and chemistry of components and their situations within cells are not always detectable. Some exceptions to be noted are specific localization studies with reference to plant virus. One method involves the use of antibody conjugated to ferritin (Shalla, 1967). Here, otherwise undetectable tobacco mosaic virus protein can be detected in host cell nuclei. Autoradiography (Hayashi and Matsui, 1967), though limited to certain compounds such as tritiated uridine, has been useful for certain applications, such as nucleic acid detection.

The focal points for damage assessment in this study are primarily the chloroplasts. The mitochondria, dictyosomes, and other organelles do not seem to be noticeably affected by the streak mottle virus. Judging from external chlorotic symptoms on affected plants, one might expect to see some discernible changes in the
photosynthetic organelles.

The vacuoles found in chloroplasts may be virus-induced. The larger type, present in both healthy and diseased cells, can only be speculated upon as to its role in plastid function or ontogeny. The smaller type is noticeably more common in virus-diseased tissue than in healthy. The suggestion that these represent a depletion of a chloroplast-based substance cannot be ignored at this time (Matsui and Yamaguchi, 1966).

Regions proximal to the plastid grana that appear vacant are conspicuous in certain cell chloroplasts; these may very well be extensions of the peripheral vesicles discussed previously; they bear a resemblance to an overall disrupted effect seen in chloroplasts of degenerating cells. Perhaps this is an accelerated effect brought about by the viral infection.

The frequently disorderly arrangement and clumping of chloroplasts in diseased mesophyll cells is a discernible feature of streak mottle infection. Compared with the orderly lining of the cell wall in healthy cells, these areas crowded with green plastids suggest a considerably disruptive effect by the virus.

It must be emphasized that while the photographic evidence is presented herewith, it is still somewhat subjective. Safer assumptions will be possible when the parameters of a normally functioning, healthy chloroplast are firmly established; then the natural
differences in chloroplast structure will be taken into consideration when assessing disease damage.

In view of the fact that chloroplasts of older tissue in early stages of degeneration had numerous invaginations, it is possible that the virus infection accelerated the breakdown of the plastid. If the virus is responsible for the removal or depletion of a substance from the plastid, it would be helpful to identify this compound. Since chloroplasts as well as mitochondria have their own genetic systems, in addition to their structural materials and metabolic chain constituents (Gibor and Granick, 1964), the role of the plastid in viral disorders could shed some light upon viral infection from both the standpoint of metabolic disturbances and nucleic acid changes occurring in cell organelles.

Pinwheels and bundles in streak mottle virus of L. speciosum are abundant in infected cells. Based upon serial sectioning of the infected tissue, there seem to be views of similar cylindrical inclusions, agreeing with the findings of others (Edwardson, 1966b; Rubio-Huertos and Lopez-Abella, 1966). Besides concurring with the previous statement, the latter mentioned authors in the previously cited works found these structures to measure approximately 750 m\(\mu\) in depth. This plus the demonstration that the virus particles occur lengthwise along the inner curvature of the "spokes" (Zettler, Christie, and Edwardson, 1967), would disclose the basic structure
of the cylinder. Yet, the proposed structure might require qualification, it may apply to the virus found in *Capsicum annuum* L. (pepper) (Rubio-Huertos and Lopez-Abella, 1966), watermelon mosaic (Zetter, Christie, and Edwardson, 1967), but the virus location may be different for other flexuous rod viruses. For instance, in the case of wheat streak mosaic virus (Shepard and Carroll, 1967), there were no signs of the virus along the inner curvature of the plates; rather, particles morphologically similar to particulate preparations of wheat streak mosaic virus (*in vitro*) were seen removed from the pinwheel figures. The exact function of the seemingly protective cylinder is not known. Vacuum-fixed tissue (Edwardson, Purcifull, and Christie, 1968) containing these showed them to have a crystalline structure.

The presence of this inclusion with its sheaves emanating from a common cylindrical core seems to be characteristic of all 750 mµ flexuous rod virus infections. Its role in viral infectivity could be revealing in view of the difficulty researchers have experienced in purifying viruses of this type and keeping them infective (Brakke and Staples, 1958). One might surmise that the conditions necessary to release the viruses from the protective body are deleterious to their integrity; thus, they would be rendered noninfective during purification attempts.

When considering the variations that seem to occur within this
virus group, streak mottle virus is more similar to bean common mosaic or watermelon mosaic (Edwardson, 1966a). Like these, its inclusions have tight, compact coils and no lamellar ends attached to the tips of the pinwheel "spokes".

Taken by itself, L. speciosum streak mottle virus in situ produces an abundance of particles with intensive clustering within its host often presenting views where individual pinwheel boundaries are difficult to discern. These clusters create uncertainty about the three dimensional structure.

The crystals associated with this virus appear to be homologous to those seen within the nucleus in tobacco etch virus infections (Rubio-Huertos and Garcia-Hidalgo, 1964). Proof of this would be attainable through autoradiography; the crystals would have to show leucine uptake, not uridine.

Inability to clearly see virus particles within the inclusions raises some doubt as to particles being present within the cylindrical bodies. This will have to be resolved by further work.

The name streak mottle virus was given this particular entity on account of the symptomatology and the need to distinguish it from other virus disorders of L. speciosum. Mottle, for instance, does not manifest pinwheel and bundle inclusions. Mosaic, besides appearing in mottle infections, does not accurately describe the symptoms seen on plants sampled for this study.
Due to the difficulty that this virus has presented in isolation attempts, it may be necessary to adapt the findings of other morphologically similar viruses that more readily lend themselves to isolation studies in order to clearly define transmission properties, chemical and physical properties.
SUMMARY

1. A virus belonging to the 750 mµ flexuous rod group is believed responsible for a streak mottle disease in *Lilium speciosum* Thunb.

2. Streak mottle virus seems to be responsible for excessive numbers of peripheral vesicles in chloroplasts and the premature breakdown of infected cells.

3. Crystalline structures associated with the infection are suggested to be homologous to intranuclear crystals seen in tobacco etch virus infections.

4. Streak mottle is not tissue specific, although it is predominantly seen in leaf mesophyll cells of infected *Lilium speciosum* Thunb.

5. Occurrence of actual virus particles is not established; it cannot be definitely stated that they exist within the curved plates of the cylindrical inclusion associated with the disease.

6. Disruption of chloroplasts along the walls of mesophyll cells infected with streak mottle is frequently seen.


Figure 1. *Lilium speciosum* showing symptoms of streak mottle virus.
Figure 2. Portion of a healthy mesophyll cell from leaf of *L. speciosum*. × 6990

N = nucleus
C = chloroplast
V = vacuole
CW = cell wall
Figure 3. Portions of four adjacent healthy mesophyll cells of *L. speciosum* leaf. × 14,227

V = vacuoles of chloroplasts

arrows = peripheral vesicles
Figure 4. Streak mottle-infected leaf mesophyll cell of *L. speciosum.* × 5,360

AGG = aggregates of pinwheels and bundles

N = nucleus

C = chloroplast
Figure 5. Streak mottle infected leaf mesophyll cell of *L. speciosum.* $\times 22,000$

- $B =$ bundles
- $P =$ pinwheels
- $C =$ chloroplast
- $CW =$ cell wall
- $PV =$ peripheral vesicles
- $V =$ vacuole
Figure 6. A-G. Serial sections through pinwheel-bundle configurations of streak mottle virus in *L. speciosum*. Each plate $\times 32,000$
Figure 7. Crystalline structure and pinwheels in parenchyma cell of streak mottle-infected L. speciosum leaf. × 24,282

CRYS = crystalline structure
P = pinwheel of virus
CW = cell wall
C = chloroplast
Figure 8. Portion of diseased mesophyll cell showing pinwheels and bundles associated with steak mottle virus infection. The cluster of crystalline substance (arrow) has been seen only in infected tissue. × 30,784
Figure 9. Higher magnification of the crystalline substance associated with streak mottle infection in mesophyll cell. × 81,000

arrow = crystalline substance
N = nucleus
NM = nuclear membrane
Figure 10. Mesophyll cell of infected *L. speciosum* leaf showing signs of degeneration. 
$\times 27,360$.

C = chloroplast

arrows = crystalline substance associated with streak mottle infection.
Figure 11. Portion of an epidermal cell of infected L. speciosum. X 24, 200

B = bundle
P = pinwheel
CW = cell wall
Figure 12. Portion of xylem cell showing pinwheel and bundle figures in bordered pit region. 
× 30,848

PB = pit border
B = bundles