1971

Biophysical properties of tobacco ringspot virus associated with the line pattern mosaic disease of rose

Gary LaVerne McDaniel

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Agriculture Commons, and the Plant Pathology Commons

Recommended Citation

McDaniel, Gary LaVerne, "Biophysical properties of tobacco ringspot virus associated with the line pattern mosaic disease of rose" (1971). Retrospective Theses and Dissertations. 4485.

https://lib.dr.iastate.edu/rtd/4485

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
McDANIEL, Gary LaVerne, 1944-
BIOPHYSICAL PROPERTIES OF TOBACCO RINGSPOT
VIRUS ASSOCIATED WITH THE LINE PATTERN MOSAIC
DISEASE OF ROSE.

Iowa State University, Ph.D., 1971
Agriculture, plant pathology

University Microfilms, A XEROX Company, Ann Arbor, Michigan
Biophysical properties of tobacco ringspot virus associated with the line pattern mosaic disease of rose

by

Gary LaVerne McDaniel

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major Subject: Horticulture

Approved:

In Charge of Major Work

For the Major Department

For the Graduate College

Iowa State University Of Science and Technology
Ames, Iowa
1971
PLEASE NOTE:

Some Pages have indistinct print. Filmed as received.

UNIVERSITY MICROFILMS
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>15</td>
</tr>
<tr>
<td>Virus Identification</td>
<td>15</td>
</tr>
<tr>
<td>Source of line pattern mosaic virus</td>
<td>15</td>
</tr>
<tr>
<td>Rose inoculations</td>
<td>15</td>
</tr>
<tr>
<td>Transmission studies on roses</td>
<td>16</td>
</tr>
<tr>
<td>Transmission of LPMV from roses to herbaceous hosts</td>
<td>17</td>
</tr>
<tr>
<td>Host range studies</td>
<td>18</td>
</tr>
<tr>
<td>Physical properties of LPMV in plant sap</td>
<td>19</td>
</tr>
<tr>
<td>Intracellular inclusions</td>
<td>21</td>
</tr>
<tr>
<td>LPMV purification procedure</td>
<td>21</td>
</tr>
<tr>
<td>Preparation of antiserum</td>
<td>22</td>
</tr>
<tr>
<td>Serological methods</td>
<td>22</td>
</tr>
<tr>
<td>Biophysical and Serological Comparisons</td>
<td>23</td>
</tr>
<tr>
<td>Virus isolates compared</td>
<td>23</td>
</tr>
<tr>
<td>Virus purifications</td>
<td>24</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td>25</td>
</tr>
<tr>
<td>Density gradient analysis</td>
<td>25</td>
</tr>
<tr>
<td>Analytical ultracentrifugal analysis</td>
<td>26</td>
</tr>
<tr>
<td>Virus degradation and nucleic acid release</td>
<td>26</td>
</tr>
<tr>
<td>Serology</td>
<td>29</td>
</tr>
<tr>
<td>RESULTS</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>Virus Identification</td>
<td>31</td>
</tr>
<tr>
<td>Virus transmission and disease symptoms</td>
<td>31</td>
</tr>
<tr>
<td>Contact periods required for transmission of LPMV</td>
<td>34</td>
</tr>
<tr>
<td>Transmission of LPMV from diseased to healthy plants through root contact</td>
<td>35</td>
</tr>
<tr>
<td>Transmission of LPMV from roses to herbaceous hosts</td>
<td>36</td>
</tr>
<tr>
<td>Host range</td>
<td>42</td>
</tr>
<tr>
<td>Physical properties of LPMV</td>
<td>42</td>
</tr>
<tr>
<td>Intracellular inclusions</td>
<td>45</td>
</tr>
<tr>
<td>Purification</td>
<td>45</td>
</tr>
<tr>
<td>Serology</td>
<td>48</td>
</tr>
<tr>
<td>Biophysical and Serological Comparisons</td>
<td>48</td>
</tr>
<tr>
<td>Virus purifications</td>
<td>48</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td>52</td>
</tr>
<tr>
<td>Density gradient analysis</td>
<td>52</td>
</tr>
<tr>
<td>Analytical ultracentrifugal analysis</td>
<td>64</td>
</tr>
<tr>
<td>Virus degradation and nucleic acid release</td>
<td>69</td>
</tr>
<tr>
<td>Serology</td>
<td>72</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>89</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>102</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>106</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>115</td>
</tr>
<tr>
<td>APPENDIX: GRAFT TRANSMISSION AND HOST RANGE STUDIES</td>
<td>116</td>
</tr>
</tbody>
</table>
INTRODUCTION

The marketability of rose nursery stock is greatly influenced by the presence of diseases and cultural disorders. Wherever roses are grown, mildew, blackspot, and rust are recognized problems requiring regular applications of spray or dust for their control. Virus diseases of roses have been recognized for nearly fifty years, and are nearly as prevalent as the other rose diseases in some areas of the world. Virus infections not only affect the budding percentage of the plants in the nursery row, but may impair the grade, quality and yield of rose blooms.

Many rose cultivars have become infected with a latent or initially symptomless virus disease soon after introduction. Virus-like symptoms are common to many newly introduced cultivars and are present in some of the numbered selections undergoing pre-introductory trials. Virus diseases of rose have apparently spread from a few isolated areas to the extent that the crop may be diseased over a large portion of the world. The severity of some of these rose viruses has led to the ultimate embargo of rose stock entering the United States from fields in such areas as Australia, New Zealand, South Africa, and Italy.

Eight viruses are currently listed in the literature which normally infect roses. The most common, rose mosaic virus, has been divided into three categories according to
symptoms. A very common virus affecting nursery-grown roses is line pattern mosaic virus, that based on symptoms has been classed with the rose mosaic complex.

Research for this dissertation was initiated to isolate and characterize the virus associated with rose line pattern mosaic symptoms. Identification of this virus as tobacco ringspot virus, a virus previously not associated with roses, prompted further investigation of its relationship with the type strain of tobacco ringspot virus. Rose mosaic virus was also compared with tobacco ringspot virus and rose line pattern mosaic virus to determine whether they have common properties.
Evidence of the existence of a graft transmissible agent inducing a leaf chlorosis of roses was recorded in France as early as 1863 (67). However, viruses as causal agents of diseases of importance in rose culture did not attract attention until much later.

Rose mosaic, or infectious chlorosis, was first described by White from New Jersey and Massachusetts in 1928 (104). His description of rose mosaic listed three Hybrid Tea cultivars as being naturally infected, and successful experiments were reported in transferring the causal agent to healthy plants of a fourth Hybrid Tea cultivar by means of bud grafts. Other reports by White demonstrated that the mosaic virus of rose had already been widely distributed in the United States (105, 107, 108).

Symptoms described for the typical mosaic of rose relate to those appearing on selections of Hybrid Tea cultivars where the plants were dwarfed, but the degree of dwarfing depended on the cultivar, the severity of infection, and the environment. Buds were often imperfect on short stems and bleached. On 'Madam Butterfly' the petals were almost white instead of the normal light pink. Leaves were variously distorted with the midrib often bent and twisted. Leaflets showed chlorotic areas, especially along the midrib, which caused the leaflets to pucker and ruffle. Usually all leaflets
of a leaf displayed symptoms, but sometimes one leaflet was free from symptoms (106, 109).

Thomas and Massey differentiated between three types of mosaic according to symptom severity (95). Mosaic-1 was regarded as typical rose mosaic virus, with symptoms largely as described by White (106). Some cultivars in the field showed pale bands and lines on the leaves, but in general, the disease symptoms were more pronounced under glass.

Mosaic-2 showed more conspicuous chlorotic bands and blotches on leaves of cv 'Hollywood' than were caused by Mosaic-1, and occasionally leaf distortion occurred.

Mosaic-3 symptoms were more severe than those caused by Mosaic-2, but there was a tendency towards the formation of broad chlorotic blotches on the leaves with a decrease in the occurrence of lines and rings. Sometimes a conspicuous "oak-leaf" pattern was produced, often accompanied by a pronounced clearing of the veins.

The possibility that insects may transmit rose mosaic virus was studied by Brierly and Smith (9). They obtained no transmissions in 229 tests with 43 species of insects. Further, no natural spread of infection in the field was reported. They grouped Mosaics-2 and -3 together as rose yellow mosaic, characterized by brighter and lighter yellow patterns than are found in typical rose mosaic.

Fulton was the first to describe a host range for rose mosaic virus (36). He was successful in mechanically trans-
mitting virus from *Rosa setigera* Michx. to cucumber, where it caused chlorosis, necrosis, and death, and to cowpea, where it caused chlorotic ringspotting and eventual death. From infected cowpea, the virus was transmitted to 25 plant species in 7 families, including *Rosa*. He found that cowpea leaves macerated in buffer provided virulent inoculum.

Fulton also described the physical properties of rose mosaic virus (RMV). He found that RMV was inactivated by heating RMV-infected cowpea leaf discs in buffer for 10 min at 54°C, but not at 52°C. Extracts from macerated cowpea leaves in buffer became inactive within one hour unless reducing agents were present, which extended the longevity to six hours.

Fry and Hunter, in their account of rose mosaic in 1956, described two characteristic symptom forms (vein banding and line pattern) and concluded that they were caused by distinct entities (33). Since then a third symptom form, which was distinct from vein banding and line pattern, has been described and given the name "chlorotic mottle" (51). The symptoms of the three types of rose mosaic are (32, 50):

1. **Vein banding** is characterized by creamy-white or yellow bands bordering the leaf veins, both primary and secondary, or sometimes only on the fine veins near the leaf margins. Symptoms are only found on leaves formed in spring and autumn. No reduction in plant vigor was reported.

2. **Line pattern** with symptoms produced throughout the season. These appear on leaves as pale green, creamy-white or
yellowish wavy lines, broad bands, spots, ringspots or blotches. Reduction of plant vigor was associated with these symptoms. When symptoms of this type were recorded in England by Fletcher and Kingham (30), they reported stem necrosis occurring directly beneath the developing flower bud and causing death of the bud before the flower opened. Prolific development of the lateral buds accompanied bud death so that affected bushes could readily be picked out by this excessive growth.

3. Chlorotic mottle symptoms appear throughout the growing season. The mottle is formed by creamy-white areas varying in size from small spots to large blotches, with puckering of the center of the leaf blades and crinkling of the margins. The symptoms suggest that chlorotic mottle virus may correspond to Mosaic-1 of Thomas and Massey (95).

In addition to the various virus disease symptoms described as occurring naturally on rose, viruses associated with known diseases of Prunus or Malus have been transmitted to rose or have been found in rose. Thomas and Rawlins transmitted "winters peach mosaic" (peach yellow bud mosaic) virus to rose (96). Cochran transmitted peach ringspot virus from peach to apple and rose and found this virus occurring naturally in roses (20). Prunus ringspot (sour cherry necrotic ringspot virus) was also isolated from rose by Gilmer (41), Kirkpatrick et al. (55), and Harris and Milbrath (45). Thomas transmitted apple mosaic virus to rose (94). Mottling in apples was induced by budding from a mosaic infected rose, but
the symptoms were not characteristic of apple mosaic.

Traylor et al., however, found that Prunus ringspot virus cultures isolated from stone fruits and roses caused leaf symptoms on the mosaic indicator rose clone 'Condesa de Sastago' which could not be distinguished from typical rose mosaic (97). Reciprocal inoculations from rose virus cultures caused symptoms of Prunus ringspot in peach and Prunus tomentosa Thunb. seedlings, gave localized necrotic reactions on 'Shirofugen' flowering cherry, and reacted positively with Prunus ringspot virus (NRSV) antiserum in agar-gel diffusion tests.

Halliwell and Milbrath identified a virus from roses showing mosaic, which by serological methods was established to be tomato ringspot virus (43). They serologically differentiated four strains of RMV related to tomato ringspot virus. Electron microscopic examination showed that the particle sizes of RMV and tomato ringspot virus were identical.

Fulton presented evidence against this relationship between RMV and tomato ringspot virus (39). He was successful in isolating a virus from roses showing symptoms which Thomas and Massey would have classified as Mosaics-2 and -3. He clearly demonstrated that RMV was not serologically related to tomato ringspot virus, and had only a few antigens in common with NRSV.

He reported serological evidence suggesting that RMV, apple mosaic virus, NRSV, and Danish plum line pattern virus represent two serotypes, each composed of two strains (40).
He observed that NRSV and Danish plum line pattern virus cross-reacted strongly with each other's antiserum. RMV and apple mosaic virus cross-reacted strongly with each other's antiserum and weakly with NRSV and Danish plum line pattern antisera. In cross-absorption tests, RMV and apple mosaic virus were serologically identical.

Several spherical plant viruses have been shown to exhibit more than one schlieren peak when purified preparations are examined in an analytical ultracentrifuge. Mazzone et al. found that purified preparations of squash mosaic virus contained three particles with sedimentation coefficients of 57, 95, and 118s20,w (61). They concluded that the protein coats of the three particles were similar or identical and that the particles differed mainly in their content of RNA. This loss of the RNA in particles was demonstrated in turnip yellow mosaic virus by electron microscopy using the negative staining technique, when the term "ghost" particle was applied to the empty protein shell (8). Tobacco ringspot virus was shown to belong to this group of viruses that had more than one particle associated with purified preparations (89).

A number of viruses that infect roses have been classified as "nematode transmitted viruses with polyhedral particles", or simply NEPO viruses. These NEPO viruses have several properties in common in addition to those implied by the class designation. They all have particles approximately 25-30 nm in diameter, wide host ranges, and thermal inactivation points
in the range of 55-70°C. The viruses that are listed as NEPO viruses include: tobacco ringspot virus, tomato ringspot virus, raspberry ringspot virus, grape yellow vein virus, peach yellow bud mosaic virus, Arabis mosaic virus, and tomato black ring virus. Raspberry ringspot virus and Arabis mosaic virus are not found in roses in America, but cause severe losses to roses in Europe, South Africa, Australia, and New Zealand (15, 19, 48, 66). Tomato black ring virus has not been reported as pathogenic in roses.

Tobacco ringspot virus (TRSV) is considered as the classical NEPO virus (14). Steere reported that TRSV is an RNA virus consisting of spherical particles having an average diameter of 26 nm (88). Particle sizes from 19-28 nm have been reported by others (21, 22, 23, 80). Stanley reported the sedimentation constant of 115s\textsubscript{20,w} for TRSV (87). Chloroform-butanol extraction of TRSV yields bands consisting of 89s and 116s, in Svedberg units (89). Steere did not report a third component of 53s, which has been shown in the less stable strain used by Stace-Smith et al. (36). Salting-out of protein with ammonium sulfate in the purification procedure eliminates a schlieren peak that is present in both healthy and diseased sap and that sediments at a rate between 70 and 80 Svedberg units. Recent analysis of TRSV demonstrates that it consists of three components that separate as 53s, 94s, and 128s subunits (26, 86). Analysis of the three zones by density gradient centrifugation demonstrates that 97% of the virus
infectivity is located in the bottom component, while the remaining 3% is confined to the middle component (78).

The RNA isolated from the bottom component of TRSV by using phenol gave two major components on density gradient fractionation. The faster of these, representing 20% of the RNA and with a molecular weight of $2.2 \times 10^6$ daltons was infectious. The slower (MW of $1.2 \times 10^6$ daltons) was not infectious. RNA from the middle component was about the same size as the smaller RNA from the bottom component and was not infectious. TRSV nucleic acid is reported to be synthesized in the form of two pieces, which may or may not be identical and which later join to make an infectious unit (24).

The approximate composition of TRSV has been determined as 35% ribose nucleic acid and 65% protein, with a nucleoprotein particle weight of $5 \times 10^6$ daltons (89). The nucleotide composition of TRSV-RNA is estimated at 23.9 M adenylic acid, 24.7 M guanylic acid, 23.2 M cytidylic acid, and 28.2 M of uridylic acid per 100 moles of total nucleotide (54).

Biological agents implicated as natural vectors of TRSV are: the dagger nematode, *Xiphinema americanum* Cobb. (34, 35, 62, 77); the differential grasshopper, *Melanoplus differentialis* Thos. (27, 102); the tobacco flea beetle, *Epitrix hirtipennis* Melsheimer (79); thrips, *Thrips tabaci* Lindeman and *Frankliniella tritici* Fitch (6, 68); and red spider mites, *Tetranychus* sp (93). TRSV has also been shown to be trans-
mitted in the seed of some species (3, 4, 71, 99). TRSV is
denatured and inactivated when undiluted tobacco sap is heated
to 64 C for 10 min (89). The dilution end point of TRSV shows
slight infectivity at 1:1000, but only a trace of infectivity
at 1:10,000. TRSV remains infective in crude sap for 48 hr at
21 C, but not after 72 hr in crude sap (87). McKinney et al.
reported retention of 40-50% of infectivity in tobacco leaves
that were stored as desiccated tissue (64).

Tomato ringspot virus and its various strains constitute the
largest portion of the NEPO virus group. Tomato ringspot
(Tom RSV) has been much studied, first by Price (73, 74) and
later by Samson and Imle (76) and other workers. Like TRSV, it
has a wide natural host range and is transmitted through the
seeds of some of its hosts (52). No insect vectors have been shown to transmit TomRSV, but the dagger nematode (Xiphinema
americanum) can transmit the virus readily (35). Yet, TomRSV
is serologically unrelated to (91) and possesses physical
properties distinct from (53) TRSV. TomRSV is inactivated when
crude sap is heated for 10 min at 58 C, after standing for 27
hr at room temp, or when diluted 1:500 with water (76). The
average diameter of negatively stained particles is 27 nm (81,
103). Preparations of TomRSV showed only two particle types
on centrifugal fractionation. These were infectious bottom
component with $s_{20,w}$ of 126 and a noninfectious empty protein
shell ($s_{20,w}$ of 53), which was serologically indistinguishable
from the virus. The ultraviolet absorption spectra of the two
zones showed a protein top component and a bottom component of nucleoprotein (83).

The composition of TomRSV, based on analysis of recovered nucleotides and amino acids, was 40% RNA and 60% protein. Analysis indicated that the virus protein subunit was composed of approx. 217 amino acid residues. The molecular weight of the virus, based on sedimentation and diffusion rates, was $5.5 \times 10^6$ daltons (98). The nucleotide composition in mole percent was 25.7% guanine, 22.9% adenine, 21.7% cytosine, and 29.7% uracyl (84).

Literature reporting chemical properties of raspberry ringspot virus (RaspRVS) are sparse, possibly because it is identified as a strain of TomRSV (85). This virus is common in Europe and Great Britain (13) and is found in raspberry in North America (82). RaspRSV is a soil-borne virus (46) that is transmitted by a European nematode vector, *Longidorus elongatus* de Mann. (92). Preparations of RaspRSV lost infectivity when diluted to 1:10,000, upon standing for 21 days at 18°C, or when heated for 10 min at 70°C (12, 47). The particle size of RaspRSV was estimated at 30 nm and had three components identifiable by density gradient fractionation. These were: top = 50s, middle = 90s, and bottom = 128s, in Svedberg units. The bottom component was found to contain infectious intact virions (82).

Peach yellow bud mosaic (PYBMV), a soil-borne virus of peaches in California (101), is caused by a virus that is
mechanically transmissible between peach and herbaceous hosts (111) and has a nematode, *Xiphinema americanum*, as a vector (7). Physical properties of PYBMV showed that infectivity was lost when infective sap was heated for 10 min at 60 °C, but not at 58 °C, or when diluted more than 1:1000 with dist. water. Cross-protection and serological tests demonstrated that PYBMV and TomRSV are closely related (16). Particle sizes of PYBMV and TomRSV were found to be identical in negatively stained preparations (17).

Grape yellow vein is a disease of grapevines with which a mechanically transmissible virus has been associated (49). Gooding reported that grape yellow vein virus (GYVV) has a particle diameter of 28 ± 2 nm and is serologically related to PYBMV, but not to TRSV (42). Since PYBMV and TomRSV are serologically closely related, then the GYVV may be considered a strain of TomRSV also.

Although rose mosaic virus (RMV) has never been associated with a nematode vector, its physical properties and wide host range closely approximate those of the NEFC virus group. Sedimentation values were not determined for RMV, but preparations were electrophoretically homogeneous and separated into three zones during density gradient centrifugation. The uppermost zone was not infectious, while the heaviest component was infectious. The virus was spherical and about 25 nm in diameter (39).

Since sour cherry necrotic ringspot virus (NRSV, *PrunusRSV*)
shares a minor proportion of antigens with, and represents a distinct serotype of RMV (40), their properties would be expected to be similar. However, Fulton found that the virus had a spherical particle size of only 23 nm and displayed only a single zone in density gradient tubes (38). The preparation was not sufficiently concentrated for a minor component to have produced a visible zone.
MATERIALS AND METHODS

Virus Identification

Source of line pattern mosaic virus

The line pattern mosaic virus (LPMV) isolate was obtained from the Hybrid Tea rose cultivar 'Michelle Meilland'. LPMV-infected plants were selected on the basis of symptoms described by Allen (1) and Fry (32). LPMV-infected buds were grafted to 4 rose clones maintained at Iowa State University. These rose clones were propagated by cuttage from seedling stock plants that had never been bud grafted. 'Prairie Princess' (Iowa 60326-2) was selected as an ornamental rose with Hybrid Tea characteristics. The three other rose clones were obtained from the rose understock breeding program: 'Iowa 5710-2' (*Rosa multiflora* Thunb. *X* R. odorata Sweet). 'Iowa 60-5' (11), and 'Iowa 62-5' (56). The two latter selections were recently released to propagators.

Rose inoculations

Budwood was collected from field-grown 'Michelle Meilland' rose plants (systemically infected with LPMV) in August of 1968. All budwood was stored in moist paper towels at 15 C until required.

Six-inch stem pieces of 'Iowa 60-5', 'Iowa 62-5', 'Iowa 5710-2', and 'Prairie Princess' were trimmed and rooted under mist in August. Inoculations were made using the T-bud method (29). Bark pieces were also used as inoculum according to the
chip-bud method (69). This method involved removing a shield of bark from the stem and replacing it with a bark shield of the same size from an infected budstick. Both buds and shields were wrapped with a standard budding rubber to securely anchor the scion piece.

**Transmission studies on roses**

The experiments reported here involved determination of the length of time infected buds and bark must be in contact with healthy tissue before virus transfer occurs. Tissue containing LPMV was grafted by shield- or bud-grafts to the rose selections described earlier. These were maintained for varying lengths of time before the graft was removed with a knife and the wound covered with grafting tape. Inoculations were made in the spring, summer, fall, and winter seasons. The four rose cultivars were divided into four lots of five plants when inoculated for each grafting method. The grafted scions from the five plants in each lot were removed after a period of 7, 14, 18, 21, and 36 days. Final observations for LPMV symptom expression were made after 3 months.

A study was conducted to determine whether natural root grafts occurring between healthy and virus-infected plants might cause limited LPMV transmission in nursery rows. Conventional root grafting was not practical because the young cuttings produced only a thick fibrous root system. Therefore, a healthy, non-inoculated rose cutting was placed in a 4-inch
pot together with an inoculated cutting. These were allowed to become rootbound, assuming natural root grafts would result. Ten pairs of these healthy-inoculated combinations were prepared. These plants were left in the original pots for 6 months before observations were made.

Transmission of LPMV from roses to herbaceous hosts

Repeated attempts were made to transmit LPMV from roses to herbaceous hosts using rose leaves macerated in phosphate buffer, pH 7.5 (36). Tissue from both symptomless young leaves and older leaves displaying prominent LPMV symptoms were mechanically inoculated to a number of herbaceous plants previously found susceptible to RMV (36, 39).

A method used by Kirkpatrick et al. (55) for transmitting NRSV from rose was attempted. This consisted of rubbing the freshly cut surface of a stack of discs of LPMV-infected rose leaves over the surface of Carborundum-dusted and buffer sprayed leaves (110). This method of virus transmission minimizes phenolic compound oxidation, which is a major cause of virus inactivation.

Attempts were made to remove tannins and prevent oxidation of polyphenolic compounds in the rose leaves for sap transmission of LPMV. Ten grams of infected young rose leaves were washed in 20 ml of 0.01 M sodium diethyldithiocarbamic acid (Na-DIECA) and 20 ml of 0.02 M nicotine sulfate dissolved in 0.05 M phosphate buffer at 25°C under partial vacuum (39).
Leaves were then dialized against distilled water to remove most of the polyphenol oxidase. Leaf tissue was then macerated in 0.01 M phosphate buffer, pH 7.2, expressed through cheese-cloth pads and inoculated to Carborundum-dusted primary leaves of the following: *Cucumis sativus* L. 'Select National Pickling', *Phaseolus vulgaris* L. 'Bountiful' and 'Improved Tendergreen', *Cyamopsis tetragonoloba* (L) Taub., and expanded leaves of *Petunia hybrida* L., and *Nicotiana tabacum* type Turkish.

LPMV transmission to *Fragaria vesca* L. strawberry indicator plants was attempted by inserting an excised rose leaf into a strawberry leaf petiole (10). Approach grafts were also made between strawberry stolons and tender rose shoots (31). LPMV-infected strawberries were then runner grafted to healthy roses and other herbaceous species to attempt further virus transmission.

Petals were collected in the early spring from infected roses and macerated in 0.01 M phosphate buffer, pH 7.2, in a 1:1 (w/v) dilution. The expressed tissue was then rubbed on Carborundum-dusted leaves of Turkish tobacco, *Vincia rosea*, *Vigna sinensis*, *Phaseolus vulgaris* cultivars 'Bountiful' and 'Improved Tendergreen'.

**Host range studies**

The host range for LPMV was determined by inoculating 40 species in 13 plant families. Because the Turkish tobacco plants used to culture the isolate display prominent ringspot
symptoms, differential hosts known for the common ringspot viruses (TRSV and TomRSV) were included (74). Differential hosts for RMV were also inoculated (36, 39).

LPMV-infected leaf tissue was ground in 0.01 M phosphate buffer, pH 7.2, by mortar and pestle. Unless otherwise stated, all further inoculations of LPMV were made in this buffer. Inoculations were made by wiping Carborundum-dusted leaves or cotyledons with gauze pads dipped in LPMV-containing extracts and the leaves rinsed with distilled water (59). Plants were maintained in a greenhouse and observed daily for 3 weeks.

Plant species tested for susceptibility were grown from seed in most cases and inoculated when young and vigorously growing. Although a preinoculation dark period did not increase susceptibility of some species, most of the plants were darkened for 12 hr before inoculation (60). Since Turkish tobacco displays countable lesions and symptoms later become systemic, inoculations were made to this plant to determine whether infections were obtained. Several infected species showing only slight or no visible symptoms were also assayed by inoculation to cowpea, Vigna sinensis Endl.

**Physical properties of LPMV in plant sap**

Turkish tobacco plants were inoculated with LPMV to increase the virus for determination of physical properties. Systemically infected leaves were macerated with mortar and pestle. The undiluted sap was divided into 3 parts for
determining thermal inactivation point (TIP), dilution end point (DEP), and longevity of LPMV in vitro (LIV).

For TIP studies, 1.0 ml of undiluted sap was placed in thin-walled test tubes (15 X 130 mm) and each test sample was heated for 10 min according to the following schedule: 45, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 70, and 75 C. Heated sap was cooled immediately and inoculated manually on 5 plants of cowpea. Following inoculation, the plants were maintained in the greenhouse. Local lesions were observed after 4 days.

For DEP studies, 11 dilutions were made in buffer (undiluted, 1:1, 1:10, 1:50, 1:100, 1:500, 1:1,000, 1:2,500, 1:5,000, 1:10,000, 1:50,000, and 1:100,000) for LPMV. The same inoculation procedure previously described was used and local lesions read after 4 days.

For LIV, sap was diluted 1:2 (v/v) in buffer and held at both 25 C and -14 C. Samples held at 25 C were inoculated to cowpea each hr for the first 8 hr and each 8-hr period thereafter for 5 days. Samples from frozen tissue were inoculated every day for 15 days and 3, 6, and 8 weeks following freezing. Assays were made to cowpea and local lesions read after 4 days.

Tests for stability of LPMV in desiccated tissue were made, as described by McKinney (63). Systemically infected Turkish tobacco leaves were dried over CaCl crystals in a desiccator for 4 days. These dehydrated leaves were then cut into small pieces and placed in vials over 1 mg CaCl. Vials were corked, sealed in paraffin, and stored at -14 C. Tissue
was tested for infectivity after 10, 20, 30, 60, 90, and 180 days by inoculation to Turkish tobacco and cowpea.

**Intracellular inclusions**

Epidermal strips from systemically infected tobacco leaves were investigated for the presence of inclusion bodies. Strips were stained in 0.5% phloxine B and counterstained in 0.5% trypan blue without previous fixation (65). A stain combination of phloxine and methylene blue (1:20) in Methyl Cellosolve (ethylene glycol monomethyl ether) was also used (19). Strips were mounted in 0.8% NaCl and observed with a Nikon interference phase microscope.

**LPMV purification procedure**

LPMV was purified according to a modified chloroform-butanol procedure (42). Systemically infected cucumber (*Cucumis sativus* L. 'Ohio MR-17'), inoculated 8-10 days earlier, were harvested and frozen for 24 hr. Tissue was macerated in a Waring Blendor in cold 0.01 M phosphate buffer, pH 7.0-7.2 (w/v). Crude sap was expressed through gauze. Two volumes of a 1:1 mixture of cold n-butanol and chloroform were added slowly to 1 vol. of expressed juice, while stirring vigorously for 30 min at 4 C. This mixture was subjected to differential centrifugation at 16,300 g for 20 min, followed by 106,000 g for 180 min in a Spinco Model L-4 ultracentrifuge. Two more cycles of differential centrifugation were used to further purify the virus. The resulting pellet was resuspended in
phosphate buffer, pH 7.0, and stored at 4 C.

**Preparation of antiserum**

Antiserum was prepared by injecting a rabbit with purified LPMV (5-15 mg/ml). Normal serum was obtained by cardiac puncture prior to virus injection. Intravenous injections were made at 3-day intervals as follows: 3 injections of 0.5 ml each, 1 of 0.75 ml, and 1 of 1.00 ml of LPMV. Intravenous injections were followed by subcutaneous injections of 2 ml of purified LPMV emulsified with 2 ml Difco complete Freund's adjuvant 2 weeks after the last intravenous injection. Antiserum was collected by cardiac puncture 2 weeks following the subcutaneous injection. Serum samples were centrifuged at low speed, dispensed in sterile serum bottles, and stored at -14 C.

**Serological methods**

Antiserum titer was monitored by means of the microprecipitin test in plates (5). Two-fold dilutions of antigen and antiserum were applied, with controls consisting of saline, normal serum, and healthy clarified tobacco sap. Reactants were covered with mineral oil and stored at 25 C for 2 hr and then at 4 C for 24 hr. Plates were examined under a binocular microscope illuminated by unilateral light after 2 hr and 24 hr.

Serological identification of LPMV was accomplished by the Ouchterlony agar-double diffusion method (100). Plates containing 1% Ionagar #2 in 0.85% NaCl and 0.02% sodium azide (NaN3) were prepared. Antigen and antiserum wells, 4 mm in
diameter and spaced 6 mm from the center well, were cut with a 
#5 cork borer. Eight wells were prepared around a central well 
in each quadrant of the plates. Purified virus occupied the 
center well and either 2-fold serial dilutions of the various 
antisera or a series of antisera occupied the surrounding wells. 
A drop of 0.02% NaN₃ was added to the top of each well and 
plates incubated in a moist chamber at 21 C for 24 hr.

Biophysical and Serological Comparisons

Virus isolates compared

LPMV was compared with other ringspot viruses having 
similar particle morphology and size. These viruses, along 
with their sources, are listed in Table 1.

Table 1. Virus isolates used in comparisons with line pattern 
mosaic virus

<table>
<thead>
<tr>
<th>Virus isolate</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>tobacco ringspot virus (TRSV)</td>
<td>Cornell isolate--R. E. Ford, ISU</td>
</tr>
<tr>
<td>tomato ringspot virus (TomRSV)</td>
<td>Type strain--R.G. Grogan, U. of Calif., Davis, California</td>
</tr>
<tr>
<td>raspberry ringspot virus (RaspRSV)</td>
<td>R. Stace-Smith, Vancouver, British Columbia</td>
</tr>
<tr>
<td>(raspberry isolate of TomRSV)</td>
<td></td>
</tr>
<tr>
<td>rose mosaic virus (RMV)</td>
<td>Original isolate from rose. R.W. Fulton, Univ. of Wisconsin</td>
</tr>
<tr>
<td>cherry necrotic ringspot virus (NRSV)</td>
<td>R. W. Fulton, Univ. of Wisconsin</td>
</tr>
</tbody>
</table>
All isolates were cultured in cucumber (Cucumis sativus L. 'Ohio MR-17') utilizing the same inoculation procedures described earlier. However, RMV and NRSV required 0.02 M phosphate buffer, pH 8.0, with 0.02 M sodium diethyl dithiocarbamic acid (Na-DIECA) and 0.02 M 2-mercaptoethanol for stabilization. Differential local lesion hosts for the viruses included: TRSV—Vigna sinensis Endl. 'Early Ramshorn', Chenopodium quinoa Willd., or Cassia occidentalis L.; TomRSV—Vigna sinensis, Phaseolus vulgaris L. 'Bountiful', or Indigofera hirsuta L. (2); RMV—Cymopsis tetragonaloba (L.) Taub. or Momordica balsamina L. (36, 39); and NRSV—Momordica balsamina (38). TRSV, TomRSV, RaspRSV were selected as representative of NEPO viruses, while RMV and NRSV were selected as representative of rose viruses. Other NEPO viruses were obtained for comparison (peach yellow bud mosaic virus and grape yellow vein virus), but were discarded due to difficulties encountered in their purification.

Virus purifications

Clarification of tissue containing either RMV or NRSV was accomplished by homogenizing cucumber cotyledons in 0.02 M phosphate buffer, pH 8.0, and stirring with hydrated calcium phosphate (HCP) in a ratio of 1:1.5:0.2 (37, 38, 39). Stability of RMV was improved by addition of 0.02 M 2-mercaptoethanol, and NRSV by including 0.01 M Na-DIECA in extracts.

TomRSV and RaspRSV were purified by the method of Stace-Smith (33). Clarification was accomplished by homogenizing
tissue in 0.5 M boric acid buffer (pH 6.7). Ammonium sulfate (15 g/100ml) was added to extracts prior to centrifugation. TRSV was purified in the manner of LPMV, using the chloroform-butanol extraction method (89). All clarified extracts were subjected to differential centrifugation and final pellets suspended in original buffer for storage at 4°C, or in dist. water for analysis by analytical ultracentrifugation.

Electron microscopy

Purified virus preparations were photographed using either an RCA-EMU 3F or Hitachi HU-11C electron microscope. Negative staining of virus particles was accomplished by atomizing a mixture of purified virus (8 drops), 2% phosphotungstic acid (8 drops), and 1% Bovine serum albumin (1-2 drops) onto formvar-coated grids (90). Polystyrene bails (264 nm) in a 1:500 solution were added to this mixture occasionally to aid in determining particle sizes.

Density gradient analysis

Sucrose gradients were used to separate components of the viruses being compared. Gradients consisted of 1.0 ml layers each of 10, 20, 30 and 40% sucrose in 0.01 M phosphate buffer, pH 7.0. Gradients were allowed to equilibrate for 18-24 hr before 0.5 ml purified virus was layered on it. Sucrose gradient tubes were centrifuged in a Beckman SW50 rotor for 90-120 min at 204,000 g. Gradients were analyzed on an ISCO UV density gradient fractionator at 254 nm. Fractions from
each peak were assayed for infectivity after dialysis for 4-12 hr in the buffer system prescribed for the respective viruses.

Cesium chloride density gradients were prepared from 3.5 M CsCl (optical grade) and centrifuged for 18-24 hr at 204,000 g. Equilibrium gradient densities were standardized by micule markers (Microspheres, Inc.). Gradients were analyzed on an ISCO UV fractionator and the component fractions dialyzed against their respective buffers for 24 hr at 4 C. Virus components were analyzed in a Beckman DB-G spectrophotometer by scanning from 320 nm to 220 nm, using a quartz cell having a 1 cm optical path.

**Analytical ultracentrifugal analysis**

Sedimentation properties of the virus components were measured by centrifuging the purified virus (in dist. water) in a Spinco Model E analytical ultracentrifuge, using an An-D rotor with Schlieren optics at a 60 degree interference angle. Exposures were taken at 4 min intervals at a rotor speed of 31,410 rpm at 20 C. Sedimentation coefficients were determined using the graphic method described by Markham (57).

**Virus degradation and nucleic acid release**

**Protein determinations** Virus concentration and protein content analyses were determined by the Folin-Ciocalteau colorimetric test for proteins (75). Absorbancies at A_500 nm were read on a Spectronic-20 colorimeter and values compared with a standard curve prepared with Bovine serum albumin (BSA)
as a reference protein. Commercial Folin reagent (Fisher Scientific Co.) diluted 1:1 with dist. water was used in these determinations.

**Release of protein from intact virus** TRSV and LPMV were analyzed for protein and nucleic acid base ratios to determine their relationships with one another. All virus protein was derived from particles obtained from the bottom component of density-gradient tubes on sucrose gradients. Zones were collected, pooled, and dialyzed against 0.02 M phosphate buffer (pH 7.5) for 24 hr at 4 C. Virus was then lyophilized and sample weights recorded. Virus protein was isolated by employing 1N-HCl for 24 hr at 25 C to denature the protein and hydrolyze the nucleic acid (86). The denatured protein was recovered by low speed centrifugation, washed 3 times with anhydrous ether, and stored in 70% ETOH at 4 C for later analysis. Samples were analyzed on a DB-G spectrophotometer and were then again lyophilized and weighed.

**Nucleic acid release from intact virus** TRSV and LPMV samples from the bottom component of sucrose density gradient tubes were equilibrated to 5 mg/ml by reference with a standard tobacco mosaic virus (TMV) sample on a Beckman DB-G spectrophotometer. Nucleic acid release was achieved by the single-phase phenol system (25). One volume of virus (previously dialyzed against 0.2 M phosphate buffer, pH 7.0) was added to 1 volume of phenol reagent containing 3% sodium dodecyl sulfate (SDS) and 0.01 M disodium ethylene dianinetet-
raacetate (Na-EDTA). Each fraction was analyzed for activity and purity on a DB-G spectrophotometer. ETOH extraction and centrifugation was done until an absorption maxima reached $A_{260}$ nm. Nucleic acid preparations were assayed for infectivity by combining 1 drop sodium Bentonite (28) with a sample of purified nucleic acid and applying to half-leaves of cowpea.

Nucleic acid to be used for purine base and pyrimidine nucleotide base analysis was treated separately. RNA in the aqueous phase from the phenol extraction was precipitated at 4 C with 2 volumes of 95% cold ETOH and 3 drops 1 M Na-acetate. Nucleic acid was collected by low speed centrifugation, washed with cold 70% ETOH with Na-acetate to remove phenol, and stored at -14 C in 70% ETOH.

Nucleic acid base ratio comparisons

Nucleic acid preparations from phenol-SDS extraction were treated with 2 volumes of 0.1 N-HCl to precipitate RNA. Following low speed centrifugation, RNA pellets were hydrolyzed for 1 hr at 100 C in sufficient N-HCl to give an approximate concentration of 20 mg/ml (58). Hydrolyzates containing purine bases and pyrimidine nucleotides were applied (10-20 ul) to Whatman #4 chromatogram paper strips that had previously been washed for 24 hr in 70% ETOH and buffer. Chromatograms were developed in 70% (v/v) tert. butanol-water (0.8 N with respect to HCl). Absorbing areas were located by UV light, cut out together with adjacent blank areas, and eluted in 5 ml of 0.1 N-HCl for 18-24 hr.
Pyrimidine nucleotides and purine bases of LPMV and TRSV were compared using a modified spectrophotometric method (58, 72). Differences in absorption maxima and minima of TRSV were determined for each UV-absorbing peak corresponding to adenine, guanine, cytidylic acid, and uridylic acid. These values were used to calibrate the DB-G spectrophotometer with respect to values found by Stace-Smith et al. (86). Molar ratio deviations were calculated for LPMV bases and values transposed to percent purine bases and pyrimidine nucleotides. All chromatographing and spectrophotometric estimations of nucleic acid were done in quadruplicate.

Serology

Preparation of antisera Antisera to the virus isolates were produced by intravenous injections to rabbits with 0.5 ml purified virus at 2-day intervals for 2 weeks. Intravenous boosters of 0.5 ml purified virus were administered periodically to maintain titer. A subcutaneous injection containing 1.0 ml purified virus in 1.0 ml Freund's complete adjuvant was given 2 weeks prior to collection of blood by cardiac puncture.

Immunodiffusion Agar-gel double diffusion tests were conducted using 1% Ionager #2 on thin glass slides (2.5 X 7.5 mm). Wells were cut using an LKB agar-gel punch, with either 4 or 8 wells surrounding a central well by a distance of 5 mm. Staining of slides was done after an 18-24 hr incubation period using 0.6% amido black 10B in methyl alcohol:acetic acid:water
(45;10:45) rinse solution (70).

**Immunoelectrophoresis**  Purified virus preparations were electrophoresed in neutral 0.02 M phosphate buffered 1% Ionagar #2 on LKB 6800-A electrophoresis equipment at 250 V-DC for 45 min. Following electrophoresis of viruses, a central trough was cut in the agar and antiserum added. When antisera were electrophoresed, purified virus was added to the central trough and antiserum-antigen allowed to react for 18-24 hr. Reaction slides were processed in the same manner as immunodiffusion slides.
RESULTS

Virus Identification

Virus transmission and disease symptoms

All rose clones inoculated by budding were systemically infected after 7 months. Inoculated plants were kept in an unheated greenhouse during January and February to provide an adequate dormant period. Following this, distinct symptoms appeared on new growth. Symptoms are pale green creamy white or yellow spots, usually associated with the midrib on newly emerging leaflets (Fig. 1A). During maximum growth in the spring, these symptoms completely disappear as the leaflets mature. Ordinarily, these symptoms are observed on only a few leaflets. All new leaflets emerging thereafter have symptoms. Leaflets expanding after mid-May display these early symptoms; however, the chlorotic areas eventually spread along the veins to form broad oak-leaf patterns, often giving the appearance of mottling (Fig. 1B). Leaves on infected plants are smaller than normal, but there is no apparent stunting of stem growth.

Symptoms on cv 'Prairie Princess' differ slightly, with symptoms expressed as narrow, wavy lines on the young leaflets, which form oak-leaf patterns as the leaflets mature (Fig. 1C). There is an apparent reduction in leaf size and stunting of growth in this cultivar. Unlike RMV (Fig. 1D) and vein banding mosaic virus, the symptoms of LPMV-infected roses persist throughout the growing season. However, the distinct oak-leaf
Fig. 1. Symptom expression of rose leaflets systemically infected with line pattern mosaic viruses

A. Rose leaflets displaying early chlorotic spot symptoms of LPMV compared with healthy rose leaflets

B. Systemic oak-leaf symptoms on rose leaflets infected with LPMV

C. Ring and line pattern symptoms on leaflets of the rose cultivar 'Prairie Princess' systemically infected with LPMV

D. Chlorotic ring symptoms on leaflets of the rose cultivar 'Queen Elizabeth' systemically infected with RMV.
symptoms are suppressed during the hot, dry period of July and early August. The stem necrosis observed by Fletcher and Kingham (30), which occurred directly beneath the developing flower bud, was not observed. No reduction or inhibition in flowering was apparent. When several understocks were cut back to the bud graft, all new growth from the bud showed the oak-leaf symptoms.

When an infected bark shield was used as inoculum, LPMV symptoms developed on 21 of the 30 plants. Failure of the remaining plants to become infected was associated with a blackening of the stem and prolific callus formation around the bark shield. This reaction was attributed to failure of the graft to heal and rejection of the shield before virus could pass between the tissues.

Contact periods required for transmission of LPMV

Contact periods of less than 15 days did not allow virus translocation, replication and ultimately development of symptoms in the bud-inoculated plants (Table A-1). Shield-inoculated plants required a minimum of 21 days for virus transfer (Table A-2). Removal of the grafted scion after these periods did not prevent virus transfer. Plants inoculated during summer or fall required longer contact periods. Shortest contact periods were obtained in early spring when plants were growing most rapidly. As the season advanced, the periods lengthened. Uniform results were obtained with plants
of the same age inoculated on the same date. Under the favorable growing conditions of early spring, LPMV passed from infected buds in from 14 to 21 days. At other seasons of the year, 21 to 36 days were required. Use of buds as inoculum source was superior to shield grafting for transferring virus from rose to rose; however, both grafting methods were successful.

Transmission of LPMV from diseased to healthy plants through root contact

None of the healthy rose cuttings expressed symptoms after a period of 6 months of root contact with LPMV-infected roses. Therefore, the plants were allowed to grow together in the same pots for an additional 3 months. Again, no symptoms were expressed by the healthy cuttings. Inspection of the roots with a binocular microscope revealed that natural root grafts had occurred between the plant pairs.

The plant pairs were divided and placed in separate pots. Inoculated roses were then approach-grafted to other healthy rose plants, while the original healthy plants were observed for an additional 3 months in isolation. In approximately 6 weeks the grafted healthy cuttings were displaying prominent LPMV symptoms. Healthy plants left in root contact with the infected cuttings displayed no symptoms after 12 months of observation.
Transmission of LPMV from roses to herbaceous hosts

All attempts to transmit LPMV from rose leaf tissue to any of the herbaceous plants tested were unsuccessful. Successful virus transmission was obtained from strawberry runner-grafts to infected roses after 3 months of contact. New leaves of LPMV-infected strawberry plants were chlorotic and mottled. These leaves were streaked with alternating green and white bands or contained mottling of dark-green islands surrounded by chlorotic bands as they matured (Fig. 2A). These symptoms were more comparable to mosaic symptoms than to those of typical ringspot. Eight of the ten strawberry plants originally grafted became infected. The two remaining plants that did not become infected also failed to unite at the graft union. The runners on the eight infected strawberry plants withered and were dead within one week before the first symptoms were expressed in the new leaves. No virus transmission resulted from excised rose leaves grafted to Fragaria vesca leaf petioles.

Runner-grafts from F. vesca to herbaceous plants failed to transmit LPMV. Grafted tissues failed to unite, and runners died within one week of contact. These infected strawberry plants were runner-grafted to healthy rose plants and to herbaceous plants used previously. Graft unions between rose and strawberry healed rapidly and runners remained alive throughout the period of the experiment. LPMV symptoms were observed on the grafted roses after 7½ months of contact. After 10 months,
five of the ten grafted roses showed systemic LPMV symptoms. No LPMV transmission was accomplished from *F. vesca* to any of the grafted herbaceous plants.

Inocula prepared from flower petals emerging during early spring caused ringspot symptoms in inoculated herbaceous hosts. Necrotic local lesions were produced on primary leaves of cowpea within 5 days after inoculation (Fig. 2B). Systemic infection of trifoliolate leaves resulted in death of the plants within 14 days. Both cultivars of *Phaseolus vulgaris* L. ('Bountiful' and 'Improved Tendergreen') reacted similarly, with a systemic chlorotic mottling of the trifoliolate leaves and eventual death of the plants. *Vinca rosea* is a satisfactory systemic host for maintaining LPMV for extended periods. Symptoms on inoculated leaves of Turkish tobacco are minute necrotic rings that are visible in 2-3 days. These rings usually become blanched or brown in 5 days. Secondary and tertiary rings are formed around the primary lesions within 10-14 days following inoculation (Figs. 3A and 3B). Later formed leaves are nearly devoid of symptoms, although these leaves often produce a faint greyish coloration. Inoculations from infected tobacco or cowpea demonstrate a marked reduction in virus infectivity from these recovered leaves, while systemically infected leaves displaying prominent symptoms produce high virus concentrations. Turkish tobacco plants were initially used for culturing the virus isolate.
Fig. 2. Symptom expression of line pattern mosaic virus in systemically infected *Fragaria vesca* leaves and inoculated primary leaves of *Vigna sinensis*

A. Symptoms of mottling and chlorosis on leaflets of *Fragaria vesca* following runner-grafting to LPMV-infected roses

B. Necrotic local lesion formation on primary leaves of *Vigna sinensis* following inoculation with LPMV
Fig. 3. Line pattern mosaic virus symptoms on inoculated and systemically infected leaves of *Nicotiana tabacum* type Turkish

A. Necrotic rings formed around primary lesions on tobacco leaves inoculated with LPMV

B. Ring and line patterns on tobacco leaves systemically infected with LPMV
Host range

*Cucumis sativus* L. (cucumber) consistently supported most relative infectivity of all other plant species tested. Typical virus symptoms for most susceptible hosts were a systemic ringspotting of newly formed primary and secondary leaflets. No symptoms were observed on *Momordica balsamina*, which is a common assay host for RMV.


Physical properties of LPMV

LPMV is inactivated after heating for 10 minutes at 64°C, but not after heating 10 minutes at 60°C. The DEP of LPMV is between 1:10,000 and 1:50,000. LPMV loses infectivity rapidly upon standing at room temperature (25°C) and is completely inactivated after 72 hours (Fig. 4).

Frozen LPMV extracts remained infective throughout the 8-week testing period (Table 2). Desiccated leaf tissue retained infectivity for 180 days and proved to be an adequate method for preserving LPMV.
Fig. 4. Physical properties of line pattern mosaic virus in tobacco sap

Each point represents average lesions per leaf of five cowpea plants (+ 5%)

A. Thermal inactivation point
B. Dilution end point
C. Longevity of LPMV in vitro
Table 2. Longevity of line pattern mosaic virus in frozen and desiccated tissue

<table>
<thead>
<tr>
<th>Frozen extract(^a)</th>
<th>Desiccated tissue(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>Lesions(^c)</td>
</tr>
<tr>
<td>1-14</td>
<td>200+</td>
</tr>
<tr>
<td>15</td>
<td>147.3</td>
</tr>
<tr>
<td>21</td>
<td>128.1</td>
</tr>
<tr>
<td>42</td>
<td>121.6</td>
</tr>
<tr>
<td>56</td>
<td>119.3</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Expressed sap of Turkish tobacco in phosphate buffer, pH 7.5, was stored at -14 °C.

\(^b\)Systemically infected Turkish tobacco leaves were dried over CaCl crystals, cut into small strips, and stored in sealed vials of the desiccant at -14 °C.

\(^c\)Average lesions/leaf of 10 cowpea leaves.

Intracellular inclusions

Isometric intracellular inclusion bodies were observed in both primary and systemic lesions of LPMV in Turkish tobacco. Inclusion bodies were confined to trichome cells within visibly lesioned areas that were surrounded by one or more necrotic rings (Fig. 5). Normal-appearing tissues surrounding the outermost rings and in the non-lesioned portion of the leaves were apparently free of the inclusion bodies.

Purification

The modified chloroform-butanol procedure proved satis-
Fig. 5. Intracellular inclusion bodies in tobacco leaf trichomes infected with line pattern mosaic virus (515X, Interference Phase)

A. Isometric intracellular inclusion in trichome cell in region of primary lesion of LPMV in Turkish tobacco

B. Pair of cuboidal inclusion bodies in trichome cell of Turkish tobacco leaf systemically infected with LPMV
factory for retaining most of the virus in the final pellet. Microprecipitin cross-reactions with homologous antiserum rarely demonstrated a purified LPMV titer over 1:16 and generally a titer of only 1:8 was obtained.

**Serology**

Rabbit antiserum produced against LPMV had a titer between 1:1,024 and 1:2,048 as determined by the microprecipitin reactions with the virus. Immunodiffusion precipitation reactions occurred between purified LPMV and its homologous antiserum and with TRSV antisera obtained from R. G. Grogan and Microbiological Associates (Fig. 6). Extracts from a TRSV-infected Turkish tobacco leaf confirmed the identity of the virus isolate by giving a positive reaction with antiserum produced against LPMV and TRSV antisera.

Because *Fragaria vesca* L. is not a host for TRSV, antisera against TRSV and LPMV were tested with sap expressed from strawberry leaves infected with LPMV from runner-grafted roses. A very faint precipitation front occurred between the expressed sap and prepared antiserum at a dilution of 1:1, but no reaction was visible between the two sources of TRSV antisera.

**Biophysical and Serological Comparisons**

**Virus purifications**

The six virus isolates could be classified into three distinct groups, based on sensitivity to chemical treatment
Fig. 6. Ouchterlony agar double-diffusion plate showing antigen-antibody precipitation zones. Wells contained antisera for the following viruses: (A) rose mosaic virus, (B) tomato ringspot virus, (C) cherry necrotic ringspot virus, (D) tobacco ringspot virus (Grogan), (E) tobacco mosaic virus, (F) normal rabbit serum, (G) physiological saline, (H) tobacco ringspot virus (Microbiological Associates). The center well contained purified LPMV from rose
during initial tissue clarification. TRSV and LPMV were stable under 50% (v/v) chloroform-butanol levels of Steere’s purification method (89). TomRSV, RaspRSV, NRSV, and RMV were unstable at these high levels, resulting in particle dissociation and protein denaturation.

Hydrated calcium phosphate treatment during clarification resulted in very low yields of RMV or NRSV (2-5 mg/kg tissue). Acidification of initial tissue suspensions to pH 5.2, followed by addition of 4-5 ml chloroform/100 ml homogenate, resulted in 20-25 mg virus/100 g original tissue. Acidification of TomRSV and RaspRSV caused virus protein denaturation and precipitation, with loss of infectivity. TRSV and LPMV did not withstand acidification to pH 5.2; however, adequate clarification was obtained by reducing the amount of chloroform during extraction. Addition of 5 ml of chloroform/100 ml of homogenate in a Waring Blender resulting in relatively pure virus preparations without noticeably increasing preparation volume prior to ultracentrifugation.

Ammonium sulfate treatment of TomRSV and RaspRSV caused appreciable losses of virus yields. Large amounts of 18s ribosomal fractions were observed in Schlieren plates when Stace-Smith’s (83) purification methods were used. However, no better procedure was found for purifying these viruses. Virus yield was raised slightly by eliminating freezing of tissue prior to grinding in buffer, but more host protein and chlorophyllous material remained with the virus pellet.
Average yield of TomRSV or RaspRSV was never greater than 5 mg/100 g of tissue.

**Electron microscopy**

Polyhedral particles, about 25.5 ± 0.5 nm in diameter, were present in negative stained preparations of purified LPMV. Numerous particles were filled with PTA, suggesting partially empty virus particles (Fig. 7). Electron micrographs of the six viruses being compared demonstrated no significant variation in particle diameters from previously published values (Fig. 8). Measurements of both randomly spaced particles and particle aggregates of LPMV and TRSV revealed only a slight difference in particle diameter, with TRSV averaging 26 nm. RMV also measured approximately 25 nm in electron micrographs. NRSV particles varied between 22 and 24 nm, with an average diameter of 22.8 nm. No difference was observed between TomRSV and RaspRSV particle measurements, each having approximate particle diameters of 27.5 nm.

**Density gradient analysis**

Fractions collected from sucrose gradients demonstrated that the six viruses compared were maximally infective in their heavy component. Three light scattering bands were observed in sucrose gradients of all viruses except RaspRSV and TomRSV. NRSV also often displayed only 2 bands in sucrose gradients. Where 3 bands were recovered, the middle zone demonstrated slight infectivity also. Infectivity was never associated with
Fig. 7. Electron micrograph of purified LFMV in negative stained preparation (line scale represents 100 nm)
Fig. 8. Electron micrographs of the six icosahedral viruses studied (line scale represents 100 nm)

A. Line pattern mosaic virus
B. Tobacco ringspot virus
C. Tomato ringspot virus
D. Raspberry ringspot virus
E. Rose mosaic virus
F. Cherry necrotic ringspot virus
the top or lightest zone and was always found in the heaviest fraction for all viruses examined. Infectivity of the middle component of each virus analyzed was variable. Generally, these middle component fractions were less infectious than the heavier components.

Cesium chloride density gradients consistently demonstrated 3 narrow light scattering zones for all 6 viruses analyzed (Fig. 9). TRSV and LPMV showed comparable component densities on UV tracings, although the amount of top component varied with samples analyzed (Figs. 9A-9B). No differences could be observed in any UV fractionations of RaspRSV and TomRSV. In all cases, samples looked nearly identical when analyzed at the same time (Figs. 9C-9D). RMV and NRSV were similar in UV analysis, but differences in amount of top and middle components were generally observed (Figs. 9E-9F).

Measurement of component density of the viruses by Micule density markers proved inconsistent. The micules tended to rise to the top of the tubes before measurements could be made, thus invalidating previous measurements. Comparison of the viruses by their UV tracings shows that TomRSV and RaspRSV have heavier bottom components than the other viruses analyzed. The bottom components of RMV and NRSV are nearly indistinguishable on UV tracings and are markedly lighter than the bottom components of the other 4 viruses.

Spectrophotometric analysis of these viruses confirmed that they contain large proportions of nucleic acid. Analysis
Fig. 9. Recorder tracings of UV absorbance by viruses fractionated following sucrose density gradient ultracentrifugation (T = top, M = middle, and B = bottom components)

A. Tobacco ringspot virus

B. Line pattern mosaic virus
Fig. 9. (Continued)

C. Tomato ringspot virus
D. Raspberry ringspot virus
1.0 — TOMATO RINGSPOT VIRUS

C

1.0 — RASPBERRY RINGSPOT VIRUS

D

DEPTH, cm

ABSORBANCE AT 254 nm
Fig. 9. (Continued)

E. Rose mosaic virus

F. Cherry necrotic ringspot virus
of the bottom components gave a characteristic shift to an absorption maximum near 260 nm and a minimum near 240 nm. The top components, where analyzed, always gave a typical protein curve having a maximum absorption at 280 nm and a minimum absorption at 260 nm (Fig. 10). RaspRSV and NRSV absorption curves are not shown because they were identical to curves of TomRSV and RMV, respectively. Fractions collected from the middle components of RaspRSV or TomRSV were too dilute for spectrophotometric analysis (Fig. 10C), therefore they are not shown. Similarly, the top and middle components of RMV or NRSV could not be adequately separated in quantities required for spectrophotometric analysis following dialysis. Therefore, RMV bottom component was compared spectrophotometrically with the intact virus (Fig. 10D).

Analytical ultracentrifugal analysis

Three virus components of each virus were observed by Schlieren optics (Fig. 11). The sedimentation coefficients are as follows: LPMV = 54s, 88s, 128s (Fig. 11A); TRSV = 53s, 94s, 128s (Fig. 11B); TomRSV 52s, 92s, 126s (Fig. 11C); RaspRSV = 50s, 90s, 126s (Fig. 11D); RMV = 34s, 54s, 75s (Fig. 11E); and NRSV = 35s, 62s, 83s (Fig. 11F). The middle components of LPMV and TRSV demonstrated a slight difference in $s_{20, w}$, assuming an error of ± 5s. Considerable quantities of 18s ribosomal fractions were observed in samples of TomRSV and RaspRSV when ammonium sulfate was used in their purification.
Fig. 10. Spectrophotometric absorbance curves of virus components obtained from sucrose density gradient tubes

A. Tobacco ringspot virus
B. Line pattern mosaic virus
C. Tomato ringspot virus
D. Rose mosaic virus
Fig. 11. Comparison of sedimentation patterns of viruses from Schlieren optics. Sedimentation is from left to right. Symbols are: $T =$ top, $M =$ middle, $B =$ bottom components, and $R =$ 18s ribosomal fraction

A. Tobacco ringspot virus
B. Line pattern mosaic virus
C. Tomato ringspot virus
D. Raspberry ringspot virus
E. Cherry necrotic ringspot virus
F. Rose mosaic virus
RMV and NRSV have markedly lighter particles than the NEPO viruses used in this comparison, with bottom components less than 100s and top components substantially lower than either TRSV or TomRSV.

**Virus degradation and nucleic acid release**

**Protein and nucleic acid contents**  No significant differences were observed between the protein contents of the bottom components of TRSV and LPMV samples. Absorption curves for the protein stripped from the bottom components of TRSV and LPMV show the characteristic absorption spectra for virus protein. Determination of percent protein in the bottom components of TRSV and LPMV are shown in Table 3.

Table 3. Protein and nucleic acid content of LPMV and TRSV bottom components

<table>
<thead>
<tr>
<th>Virus</th>
<th>Bottom component sample weight (mg)</th>
<th>Protein wt (mg)</th>
<th>Calculated % protein</th>
<th>Percent nucleic acida</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPMV</td>
<td>24.5</td>
<td>14.2</td>
<td>57.96%</td>
<td>42.04%</td>
</tr>
<tr>
<td>TRSV</td>
<td>57.8</td>
<td>33.9</td>
<td>58.65%</td>
<td>41.35%</td>
</tr>
</tbody>
</table>

aNucleic acid content determined from protein content.

**Nucleic acid release from intact virus**  Spectrophotometric analysis of nucleic acid samples indicated that purity of samples was difficult to detect using the shift to A260 nm as the only criterion. Both TRSV and LPMV have absorption
Fig. 12. Spectrophotometric absorbance curves of virus bottom component protein and nucleic acid

A. Tobacco ringspot virus

B. Line pattern mosaic virus
TOBACCO RINGSPOT VIRUS

LINE PATTERN MOSAIC VIRUS (Rose-TRSV)

A

B
spectra that correspond very closely with the nucleic acid alone (Fig. 12). However, precipitation and centrifugation of samples until no additional pellet could be obtained were more helpful in determining purity. When the supernatant was analyzed spectrophotometrically following each centrifugation, the absorption spectrum of the supernatant could be observed to shift from a maximum of 262 nm to 280-285 nm. This criterion was therefore used in determinations of nucleic acid purity in samples for base ratio comparisons.

**Nucleic acid base ratio comparisons**  Purine bases and pyrimidine nucleotides hydrolyzed from LPMV nucleic acid did not deviate significantly from values obtained for TRSV. Purine bases for LPMV gave values slightly higher than TRSV, while pyrimidine nucleotides gave molar ratio deviations just under those obtained for TRSV. Purine bases and pyrimidine nucleotides of LPMV were compared with values obtained by Stace-Smith et al. (86) for TRSV (Table 4).

**Serology**

**Immunodiffusion**  TRSV reacts strongly with antisera produced against LPMV and TRSV (Figs. 13A, 13B). No reactions occurred between TRSV and antisera for TomRSV, RaspRSV, RMV, NRSV, or normal rabbit serum.

LPMV shares antigens with TRSV, RaspRSV, RMV, and NRSV (Figs. 13C, 13D). Two light precipitation bands are observed in reactions with LPMV and its antiserum, while only a single
Table 4. Percent of purine bases and pyrimidine nucleotides in LPMV compared with those for TRSV

<table>
<thead>
<tr>
<th>Bases(^a)</th>
<th>Maximum wavelength(^b)</th>
<th>LPMV</th>
<th>TRSV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average max-min difference(^c)</td>
<td>Molar ratio deviations(^d)</td>
</tr>
<tr>
<td><strong>Purines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanine</td>
<td>A250</td>
<td>+0.021</td>
<td>+0.028</td>
</tr>
<tr>
<td>Adenine</td>
<td>A260</td>
<td>+0.013</td>
<td>+0.017</td>
</tr>
<tr>
<td><strong>Pyrimidine nucleotides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>A280</td>
<td>-0.0135</td>
<td>-0.018</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>A260</td>
<td>-0.022</td>
<td>-0.030</td>
</tr>
<tr>
<td>Unaccounted</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Bases obtained in order of appearance on ascending paper strips in acid developing medium.

\(^b\) Wavelength of maximum absorption for each base and wavelength used for standardization of DB-G spectrophotometer.

\(^c\) Differences obtained at full-strength and half-strength hydrolyzates.

\(^d\) Deviations of LPMV from values obtained with TRSV maximum minus minimum values.

\(^e\) Percent base of LPMV hydrolyzate with respect to TRSV values.

\(^f\) Values obtained by Stace-Smith et al. (86) for TRSV.

zone is observed with LPMV and antisera of TRSV or RMV. Secondary reaction bands are observed between NRSV and antisera for LPMV and NRSV, but only a single band is produced with RMV.
antiserum (Fig. 13C). No reaction is observed between NRSV and antisera for TRSV, RaspRSV, or TomRSV.

A strong precipitation band was formed between NRSV and its homologous antiserum. A secondary band was formed behind this primary reaction band and also between NRSV and antisera of RMV and LPMV (Fig. 13E). No reactions were observed between NRSV and normal serum and antisera for TRSV, RaspRSV, elm mosaic virus (EMV), or TomRSV. RMV reacted strongly with antisera for RMV and NRSV (Fig. 13F). Secondary precipitation bands formed around the RMV well are not distinct enough to make further analysis of antigen-antiserum relationships. Separation of wells and use of freshly purified RMV (Fig. 13G) shows a reaction with only homologous antiserum. Allowing this RMV preparation to age 4 days at 4°C shows that RMV no longer produces a distinct reaction zone with its own antiserum (Fig. 13H). However, no relationships are observed between these preparations and antisera of LPMV, NRSV, TomRSV, RaspRSV; or TRSV.

TomRSV reacts strongly with antisera prepared against both RaspRSV and TomRSV (Figs. 13I, 13J). No reactions are observed between TomRSV and antisera for RMV or LPMV. RaspRSV reacts strongly with antisera prepared against both RaspRSV and TomRSV (Figs. 13K, 13L). No reactions are produced between RaspRSV and antisera for TRSV, LPMV, RMV, NRSV, or normal rabbit serum.

A strong precipitation band formed between TRSV antiserum and TRSV, near the center well. Secondary, diffuse bands formed around all antigen wells were not normal reactions and indicate
antibodies were reacting with denatured virus protein subunits, rather than intact virus particles (Fig. 13M). A precipitation band corresponding to the band formed near the TRSV well was not present near the LPMV well. No reactions were observed between TRSV antiserum and RaspRSV or TomRSV.

A strong reaction band formed between wells of LPMV antiserum and TRSV (Fig. 13N). A clear reaction spur is observed in bands formed near wells of LPMV and TRSV. However, the band that crosses in front of the RMV well is not sharp and cannot be considered a true precipitation band. Secondary bands are observed also between LPMV antiserum and wells of LPMV and TRSV. LPMV antiserum also reacts strongly with both TomRSV and NRSV, but not with NRSV, EMV, or healthy cucumber sap.

Comparison of freshly purified virus with older preparations demonstrated that age of virus can influence precipitation reactions. Preparations of TRSV and LPMV (Fig. 13N) were compared with freshly purified cultures (Fig. 13O). Strong bands (bottom and right) are formed with fresh antigen, while more diffuse bands and secondary bands from antibody reactions with protein subunits are formed with older antigen (top and left). This is demonstrated further with LFMV antiserum and a 7-day old culture of RMV (Fig. 13P). Again, older (10-15 days) preparations of TRSV and LPMV were added to wells. A strong precipitation band is observed between LPMV antiserum and LPMV. No reaction is observed between LPMV antiserum and healthy cucumber sap.
Fig. 13. Ouchterlony agar immunodiffusion plates of antigen-antiserum reactions

Numerical designations are as follows:

1. Line pattern mosaic virus
2. Tobacco ringspot virus
3. Tomato ringspot virus
4. Raspberry ringspot virus
5. Rose mosaic virus
6. Cherry necrotic ringspot virus
7. Line pattern mosaic virus antiserum
8. Tobacco ringspot virus antiserum
9. Tomato ringspot virus antiserum
10. Raspberry ringspot virus antiserum
11. Rose mosaic virus antiserum
12. Cherry necrotic ringspot virus antiserum
13. Elm mosaic virus antiserum
14. Elm mosaic virus

NS Normal rabbit serum
HY Healthy cucumber sap
Fig. 13. (Continued)

Numerical designations are as follows:

1. Line pattern mosaic virus
2. Tobacco ringspot virus
3. Tomato ringspot virus
4. Raspberry ringspot virus
5. Rose mosaic virus
6. Cherry necrotic ringspot virus
7. Line pattern mosaic virus antiserum
8. Tobacco ringspot virus antiserum
9. Tomato ringspot virus antiserum
10. Raspberry ringspot virus antiserum
11. Rose mosaic virus antiserum
12. Cherry necrotic ringspot virus antiserum
13. Elm mosaic virus antiserum
14. Elm mosaic virus
NS Normal rabbit serum
HY Healthy cucumber sap
**Immunoelectrophoresis** Electrophoresis of LPMV followed by reaction against LPMV antiserum produced a light spur that was not evident between electrophoresed TRSV and LPMV antisera (Fig. 14A). This reaction is graphically presented to make this reaction spur clearly evident (Fig. 15A). No difference was observed in precipitation reactions following electrophoresis of TRSV and LPMV followed by reaction with TRSV antiserum (Fig. 14B). Electrophoresis of LPMV and TRSV antisera shows clear separation of the two antisera when LPMV is used (Fig. 14C). The isoelectric point of the TRSV antiserum component that reacts with LPMV is near neutral, while LPMV antiserum is positively charged and migrates toward the cathode. The TRSV antiserum component that reacts with TRSV is positively charged at pH 7.0 and is not distinguishable from LPMV with TRSV antiserum (Fig. 14D).

LPMV and RaspRSV are clearly distinct viruses and share no common antigens (Figs. 14E, 14F). They do not react with each other's antiserum following separation of components by electrophoresis. Electrophoresed RaspRSV produces a strong precipitation band and 2 lighter reaction spurs, when reacted with its homologous antiserum (Fig. 15B). Numerous reaction spurs are observed behind the primary precipitation band produced when electrophoresed RMV is reacted with its homologous antiserum (Fig. 14G). Electrophoresed LPMV produces secondary reaction spurs when reacted with RMV antiserum (Fig. 15C), but does not react with the primary component of RMV antiserum.
RMV components migrate toward both the anode and cathode when electrophoresed at pH 7.0. One LPMV component also migrates towards the cathode when reacted with RMV antiserum, but this component is not identifiable in reactions with LPMV antiserum (Fig. 14H). There is no serological relationship between TRSV and RMV using separation by electrophoresis. No reactions are observed between RMV and TRSV antiserum (Fig. 14I) or between TRSV and RMV antiserum (Fig. 14R).

Electrophoresed TomRSV and RaspRSV are identified with difficulty by reactions with heterologous antisera (Fig. 14J). RaspRSV produces 2 very diffuse spurs directly above the primary precipitation band with RaspRSV antiserum that do not occur in TomRSV precipitation zones (Fig. 15D). No differences occur in reactions of TomRSV or RaspRSV against TomRSV antiserum (Fig. 14K). TomRSV antiserum produces a secondary reaction band directly ahead of the major precipitation band when reacted against TomRSV that is not present in reactions with RaspRSV antiserum (Figs. 14L, 15E). No differences are detectable between these two antisera in reactions with RaspRSV (Fig. 14M).

RMV and NRSV demonstrate similar immunoelectrophoretic migration properties, with antigen components moving toward both anode and cathode at pH 7.0 (Fig. 14N). Two bands and reaction spurs are observed with RMV antiserum when RMV is electrophoresed (Fig. 15F), but are not present in electrophoresed NRSV (Fig. 14N). Both electrophoresed RMV and NRSV gave
similar reactions with NRSV antiserum (Fig. 14O). When NRSV antiserum is electrophoresed, it produces 2 light reaction bands with RMV and only one with NRSV (Figs. 14P, 14Q).

Electrophoresed RMV antiserum reacts strongly with RMV, but also produces 2 light spurs and a light secondary reaction band (Fig. 15G). When electrophoresed RMV antiserum is reacted with NRSV, only a single spur is observed (Fig. 15H).
Fig. 14. Immunoelectrophoresis slides of antigen-antibody reactions

Anode (+) is at the left side of plates. Numerical designations are as follows:

1. Line pattern mosaic virus
2. Tobacco ringspot virus
3. Tomato ringspot virus
4. Raspberry ringspot virus
5. Rose mosaic virus
6. Cherry necrotic ringspot virus
7. Line pattern mosaic virus antiserum
8. Tobacco ringspot virus antiserum
9. Tomato ringspot virus antiserum
10. Raspberry ringspot virus antiserum
11. Rose mosaic virus antiserum
12. Cherry necrotic ringspot virus antiserum
Anode (+) is at the left side of plates. Numerical designations are as follows:

1. Line pattern mosaic virus
2. Tobacco ringspot virus
3. Tomato ringspot virus
4. Raspberry ringspot virus
5. Rose mosaic virus
6. Cherry necrotic ringspot virus
7. Line pattern mosaic virus antiserum
8. Tobacco ringspot virus antiserum
9. Tomato ringspot virus antiserum
10. Raspberry ringspot virus antiserum
11. Rose mosaic virus antiserum
12. Cherry necrotic ringspot virus antiserum
Fig. 15. Graphical representation of immunoelectrophoresis slides showing weak spur production and minor precipitation bands

Numerical designations are as follows:

1. Line pattern mosaic virus
2. Tobacco ringspot virus
3. Tomato ringspot virus
4. Raspberry ringspot virus
5. Rose mosaic virus
6. Cherry necrotic ringspot virus
7. Line pattern mosaic virus antiserum
8. Tobacco ringspot virus antiserum
9. Tomato ringspot virus antiserum
10. Raspberry ringspot virus antiserum
11. Rose mosaic virus antiserum
12. Cherry necrotic ringspot virus antiserum
DISCUSSION

Line pattern mosaic virus of rose is as prevalent as rose mosaic virus in Continental United States. However, this virus is more a problem for nurserymen than it is for the consumer, because propagation is the only known means for disseminating LPMV in commercial roses. LPMV is readily transmitted to susceptible roses by various methods of budding and grafting. Inarch grafts, patch grafts, shield grafts, and T-buds have all proved effective when union is established. T-budding has been used most extensively for propagating roses commercially because of its convenience.

The interval between budding and the appearance of the first recognizable symptoms of LPMV is highly variable. During this period union must be accomplished, also the virus must replicate and be translocated to a growing point where young leaves are expanding. Canes are of approximately the same stage of growth when suitable for budding, but the growth of the inserted bud and changes in shoot dominance after budding are subject to wide variations. The best circumstances for prompt production of LPMV symptoms includes rapid development of a young lateral shoot directly below the transferred bud.

LPMV is carried in cuttings or scions taken from infected plants. Field budding is the primary means of spreading LPMV and is the best place for virus eradication. Two general practices for maintaining a supply of rootstock wood for cuttings
are followed in California. In the first, a 'mother block' is maintained as a source of cuttings, and hundreds of cuttings are rooted from each established plant each year it is used. Therefore, scions are never worked on the plants in the 'mother block' that provides the cuttings.

In the second, and most common procedure, the 'mother block' is eliminated and cuttings are collected at random from the tops of field-budded plants when the latter are cut back to force the inserted buds. Such cuttings from budded plants are subject to contact with LPMV-infected buds. In each practice the understock cuttings are lined-out in the field for rooting and then budded the following summer. Should a 'mother block' plant become infected, all vegetative progeny from it would carry virus and transmit it to any scion wood grafted to them. However, if the 'mother block' is free from LPMV, symptoms will appear only in plants grown from LPMV-infected bud-wood. When cuttings are taken from budded plants, any wood from an infected plant may transmit LPMV to healthy buds inserted in the next season. Therefore, LPMV-infected bud stock infects the wood to be used for buds the following season. Such infection is avoided by the 'mother block' system.

The practice of taking cuttings for understocks from the tops of budded plants is an economically commercial practice, but it provides an efficient means of preservation and dissemination of virus diseases, such as LPMV and RMV, in roses.
Indexing of 'mother block' plants or bud stock plants by virus indicator plants is an effective means for identifying virus infections for eradication programs. Indicator plants to be used for LPMV-infected roses have not been investigated, but effective control will depend on such measures. Use of Fragaria vesca indicator plants may be adequate until better indicator hosts are found, although runner-grafting is not the most desirable commercial indexing method and may not be completely reliable if virus-free strawberry plants are not readily available.

Some rose clones do not display recognizable LPMV symptoms at all seasons and it is difficult to determine the presence of LPMV in infected understocks propagated from these budded plants. Thus, high percentages of infection can occur without symptom expression. When a clone capable of expressing strong LPMV symptoms is budded on such understocks, a sudden appearance of LPMV symptoms may result, and are usually attributed to natural spread. The practice of taking cuttings from budded stocks is so well suited to preservation of LPMV and its dissemination to new cultivars that it appears adequate to account for all observed spread of LPMV in commercial nurseries.

The rose understock clone recently released by Iowa State University, now designated as 'Iowa 62-5', has shown promise as a source of tolerance for LPMV. Experimental evidence provides a basis for further examination of its capability for adaptation to the current propagation practices. This clone displays
easily recognizable LPMV symptoms, yet very low percentages of infected cuttings are observed. LPMV-infected bud survival is up to 50% lower on 'Iowa 62-5' than 'Iowa 60-5', indicating that this clone may act to "clean up" infected cultivars in the field. This understock clone could prove a decided advantage over the highly susceptible, symptom suppressing 'Manetti' understocks used frequently in nursery practices.

The contact experiments reported here were designed to examine the possible sources of transfer of the virus under nursery conditions. These tests indicate that natural root grafts between healthy and virus-infected plants are not sufficient for allowing translocation of virus under the conditions provided by this experiment. Further tests of these root contact studies must be conducted, under both greenhouse and field conditions, and more exhaustive microscopic studies made before the possibility of root graft transmission can be eliminated. The budding and grafting techniques are highly effective means of transferring virus to healthy nursery stock. The period of contact necessary to induce virus symptoms suggests that the graft union must be fully developed and plasmodesmata present between the host and implanted tissue before virus may pass freely from one to the other.

Most rose cultivars bud-inoculated with LPMV did not show symptoms obviously different from roses infected with RMV, NRSV, or TomRSV (97). Typical RMV produces patterns of chlorotic lines and rings on rose leaves (36), which are also present on
LPMV-infected roses. However, LPMV-infected rose leaves display more prominent line patterns or 'oak-leaf' symptoms than rings in the more advanced stages of infection.

LPMV symptoms observed on rose leaves are apparently caused by extensive ring formation at the acute stage of infection in late spring. Failure of symptoms to develop in newly formed leaves indicates a slightly different host-virus association, the chronic stage, observed in LPMV-infected tobacco. Observations of LPMV symptoms on field-grown roses in late August may be attributed to climatic factors again favorable for renewed virus replication.

Use of petals as a virus source offers several advantages when virus is transmitted from woody ornamentals. Formation of quinones from naturally occurring phenolic substances by the action of the polyphenol oxidase enzyme system usually are not encountered in petal extracts (98). Therefore, the high tannin content (polyphenol substances) of rose leaf extracts may be avoided. However, it is possible that more than one virus may be present in rose leaf tissue, yet only one virus is present in petals. This virus mixture would be undetected using only petal extracts for virus transmission to susceptible herbaceous hosts.

Transmission of LPMV to susceptible herbaceous hosts provided a starting point for examinations of physical properties, host range, and its serological properties. Initial serological examinations showed that LPMV was related to TRSV, the type
virus of the NEPO virus group. This provided a basis for further comparisons of LPMV with viruses of the NEPO virus group and two viruses already found to infect roses.

All six of the viruses compared are composed of three components in qualitative examinations. These viruses demonstrated comparable components consisting of: 1) a top component, which is noninfectious protein region in gradient tubes; 2) a middle component consisting of nucleoprotein particles that are only partially infectious; and 3) a heavy bottom component that is completely infectious and represents the major virus fraction. There are numerous theories concerning the nature and origin of these virus components of the NEPO virus group. The current theory is that all components are present in variable quantities in the infected cells, the quantity of each component present depending on host differences and time after infection of the cell. The bottom or heavy component is comparable to the single virus band observed in gradient tubes containing TMV. These bottom component particles consist of infectious virus RNA surrounded by a protein coat and is capable of replication by the nucleic acid. The middle component particle is lighter because less nucleic acid is present, perhaps lacking nucleotides that render this RNA noninfectious (24). The top component of protein is virus related protein that has autoaggregated without the inclusion of virus nucleic acid. The amount of these top component protein particles decreases with age of infection, as more
virus nucleic acid is replicated. This accounts for the variability observed in amounts of top component protein observed in the various analytical analyses.

The six viruses may be separated into pairs, according to their analytical properties. Cesium chloride density gradient centrifugation of these viruses showed that the bottom components of RaspRSV and TomRSV were slightly heavier than TRSV or LFMV. However, Schlieren examinations showed that TRSV or LFMV bottom components have $s_{20,w}$ rates of 128s, while RaspRSV or TomRSV have $s_{20,w}$ rates of 126s. RMV and NRSV, while also showing 3 virus components, consistently demonstrated the lightest virus fractions. RMV contains the lightest bottom component particles (75s) of the six viruses examined. No virus of the NEPO virus group has yet been observed having a bottom component particle $s_{20,w}$ rate less than 100s. If RMV or NRSV can be classified with the NEPO viruses, then they will represent the lightest viruses in this classification.

In addition, NRSV apparently has a particle diameter slightly smaller than RMV or the NEPO viruses. Any correlation to particle measurements and $s_{20,w}$ rates would probably not be relevant, however, because of the numerous conflicting particle measurement reports. One criterion established for NEPO viruses is that they have particle diameters near 30 nm, yet the type virus for this classification (TRSV) has been reported to have a diameter of 19-28 nm.

Determinations of protein and nucleic acid contents of
TRSV and LPMV were made on bottom component fractions alone. This was done to standardize comparisons, thus eliminating influence of variations in top component protein or nucleic acid differences in middle component particles. Previous data for percent protein and nucleic acid content of TRSV have been variable, because all three virus components have been included in determinations. Stanley reported a TRSV protein and nucleic acid content of 68.2% and 31.8%, respectively (87). Steere reported a TRSV nucleic acid content of 34.4% from preparations showing only two electrophoretic components (89). Stace-Smith et al. report a TRSV nucleic acid content of 41.9%, which was determined from bottom component particles from sucrose gradients (86). This value for TRSV nucleic acid content correlates very closely with the calculated figure of 41.35% bottom component nucleic acid for the Cornell strain of TRSV analyzed. The value determined for LPMV (42.04%) bottom component nucleic acid falls inside the 5% confidence limits of 41.9 ± 0.7%, as does the value for TRSV. These values indicate that there is no significant difference between the protein or nucleic acid contents of the two viruses.

The immunodiffusion examinations made during LPMV identification have slightly differing results than the comparative tests conducted later. In the original tests, LPMV reacted only with its homologous antiserum and two sources of TRSV antiserum, but no spurs were observed occurring between LPMV and antisera of RMV, NRSV, or TomRSV. The original tests were
conducted in petri plates with wells spaced farther apart than later tests on agar slides. In addition, antigen was of lower titer because virus was purified from tobacco rather than from cucumber, as in later tests. Serological tests on slides are also apparently more sensitive to minor component reactions, partly because these precipitate zones were stained.

Length of time virus is stored following purification greatly affects the quality of precipitation bands formed in immunodiffusion tests. Precipitation bands forming rings around the center well and very diffuse bands that do not form sharp leading edges are an indication of antigen breakdown to smaller protein subunits. When this occurs, antibodies may react with protein subunits or specific amino acid fragments from degraded virus protein coats. Thus serological cross-reactions may be observed as faint precipitation bands or diffuse zones between antibodies and denatured protein subunits from a virus that may not actually be serologically related to it. Positive relationships, however, are observed between TRSV and LPMV, NRSV and RMV, and TomRSV and RaspRSV in immunodiffusion tests. Additional serological relationships between these viruses cannot be adequately correlated from these immunodiffusion data. Therefore, more qualitative serological examinations were conducted using immunoelectrophoresis tests on agar slides.

Immunoelectrophoretic properties of viruses and antisera are not commonly reported in literature. The basis for most
antigen-antiserum relationships are most often correlated with immunodiffusion data alone. However, when antigen-antiserum relationships are distant and reactions are weak, then immunoelectrophoretic separation allows specific examination of these weakly reacting components.

Minor components and reaction spurs are observed more easily when components are separated by their electrophoretic mobilities. Much of the background denatured protein or subunits migrated rapidly across the slides and was not present after 45 min of electrophoresis at pH 7.0. Electrophoretic separation allows identification of secondary reactions that are not evident in immunodiffusion tests and indicates the charges of components relative to antiserum. Such examination shows that LPMV differs in its antiserum isoelectric point from that of TRSV antiserum. These antiserum isoelectric point differences are detected only when reacted with LPMV.

The secondary reactions observed on immunodiffusion slides are shown as reaction spurs or secondary bands located in back of, or trailing behind, the primary precipitation band in most electrophoresis tests. These secondary bands show the degree to which antigen and antisera are related. Identification of each component would then determine to what degree two viruses share common antigens.

Serological comparisons of the six virus isolates and antisera demonstrates direct relationships between the following virus pairs: RMV and NRSV; TomRSV and RaspRSV; and TRSV
and LPMV. RMV and NRSV share common minor antigens, but are serologically distinct viruses. RaspRSV and TomRSV are more closely related serologically than RMV and NRSV, but immunoelectrophoresis of antigen or antiserum allows detection of minor component differences. TRSV and LPMV serological differences are observed by immunoelectrophoresis. This indicates that LPMV should be considered a strain of TRSV, rather than as being identical to the type strain of TRSV.

RMV and LPMV appear to share antigenic components, based on these serological examinations. Although NRSV and LPMV show no common antigenic properties, NRSV and RMV are closely related and may be grouped together in serological properties. The same may be said for TomRSV and RaspRSV. No serological evidence was shown for relationships between LPMV and RaspRSV, but LPMV apparently shares antigens with TomRSV. These results indicate that LPMV shares antigens with viruses of the three classes of viruses examined: 1) TRSV group, 2) TomRSV group, and 3) RMV group. This information correlates with results observed in analytical examinations of RMV and NRSV. Results from these serological and analytical studies clearly indicate a basis for classifying RMV and NRSV in the NEPO virus group. However, this can take place only when a nematode vector has transmitted RMV or NRSV.

Analytical analyses and serological data clearly indicate that LPMV is related to TRSV, yet is different enough to be regarded as a TRSV strain. The middle component of LPMV is 6s
units lighter than the same component of the type strain of TRSV. This difference is greater than the 5% error involved in calculations. Also, the spurs observed in immunodiffusion and immunoelectrophoresis demonstrate at least a one-way antigen-antiserum difference between these strains. This difference in serological reactions resides in the amino acid sequences of the two viruses, which are dictated by the respective virus nucleic acids. However, no significant quantitative differences were detected in their nucleic acid bases or nucleotides. The strain differences can, therefore, only be detected by rigorous amino acid analyses or by determinations of their nucleic acid base sequences.

The symptomatology, physical properties, and analytical analyses of LPMV suggest its close relationship to TRSV. This was confirmed by serological data. Therefore, it is concluded that LPMV symptoms of rose are caused by TRSV infection and will be designated as rose-TRSV. This is the first report of TRSV in woody species, with the possible exception of florist's hydrangea (2).

Tobacco ringspot virus has a wide natural host range and has been transmitted experimentally to many species in a large number of families of flowering plants. Line pattern symptoms are common on field-grown roses and methods for eradicating rose-TRSV from infected plants will have to be developed. Therefore, rose-TRSV will have to be included in any nursery improvement or quarantine program. Although no nematode
vector has yet been implicated with natural spread of rose-TRSV, the evidence brought forth by this investigation shows that nematode control measures in rose fields is a sound practice. *Rosa multiflora* is a common bait plant for *Xiphinema americanum*, the native nematode implicated in spread of viruses in the NEPO virus group. Until further investigations prove contrary, it may be assumed that rose-TRSV, rose-TomRSV, and possibly RMV are also carried by a nematode vector. Further analysis of properties of these viruses will be necessary to elucidate their relationships with one another and provide a further basis for placing these rose viruses in a single classification.
SUMMARY

Line pattern mosaic virus was isolated from field grown 'Michelle Meilland' roses. Symptoms on rose leaflets consist of pale green, yellow or white spots along the midrib. Leaflets emerging after late spring display ring and line symptoms, which form broad oak-leaf patterns on most rose clones.

LPMV passed from infected buds in a minimum of 14 days, when roses were grafted in early spring. Longer contact periods were required at other seasons. When bark shields were used as inoculum, a minimum of 18-21 days were required for LPMV transmission to healthy roses. Although both grafting methods were capable of virus transfer, T-buds were most efficient. No virus was transferred from inoculated roses after 12 months of root contact with healthy plants, even though natural root grafts occurred.

No virus was recovered by various methods of isolation from rose leaf tissue. LPMV was transmitted from infected roses to healthy plants by Fragaria vesca. Runner grafts with F. vesca were successful in virus transmissions from rose to rose, but not from rose to herbaceous plant species. LPMV symptoms on grafted strawberry leaves consist of alternating green and white bands or patterns of dark green islands surrounded by chlorotic bands, often resembling mottling. Inocula prepared from flower petals formed in early spring caused distinct ringspot symptoms on inoculated herbaceous hosts.
Necrotic local lesions are produced on inoculated cowpea and infection becomes systemic within 5-7 days, causing death of plants. Symptoms on inoculated Turkish tobacco are minute necrotic rings formed within 3 days. Within 15 days secondary and tertiary rings are formed around primary lesions. Oak-leaf symptoms are formed on leaves systemically infected with LPMV within 15 days following inoculation. Later formed leaves are nearly devoid of symptoms.

Host range studies show that LPMV has a wide host range and symptoms on inoculated herbaceous hosts resemble TRSV or TomRSV infections. LPMV is inactivated after heating 10 min at 64°C, but not after heating 10 min at 60°C. LPMD fails to produce lesions on inoculated cowpea leaves, when diluted more than 1:50,000 in buffer. LPMV loses infectivity rapidly upon standing at 25°C and is no longer infective after 72 hr. LPMV inocula may be stored for at least 8 weeks as frozen clarified sap or for at least 180 days as desiccated leaf tissue preparations.

Preliminary serological examinations determined that LPMV is related to TRSV. This rose-TRSV isolate was compared with viruses of the NEPO virus group (TRSV, TomRSV, RaspRSV) and rose viruses (RMV, NRSV) to determine common analytical and serological properties among the virus groups. These viruses are grouped into 3 virus classes according to their ability to withstand chemical treatment during tissue clarification. TRSV and LPMV withstand chloroform-butanol concentrations equal to
initial weight of tissue (w/v), but TomRSV, RaspRSV, RMV, and NRSV are denatured by this treatment. Calcium phosphate in clarification of RMV and NRSV does not yield as much virus in final pellets as acidification to pH 5.2 prior to centrifugation. TomRSV and RaspRSV were purified using ammonium sulfate in clarification procedure, although virus yields were low. Electron micrographs of LPMV contained spherical particles approximately 25-26 nm in diameter. Particle diameters of other viruses examined are as follows: TRSV = 26 nm, TomRSV and RaspRSV = 27.5 nm, RMV = 25 nm, and NRSV = 22.8 nm.

All six viruses demonstrated 3 light scattering bands in sucrose and/or cesium chloride density gradient fractionations. UV spectra of all viruses examined show high nucleic acid content of their bottom component fractions or unfractionated virus preparations. Schlieren optics examination of viruses gave the following sedimentation coefficients: LPMV = 54s, 88s, 128s; TRSV = 53s, 94s, 128s; TomRSV = 52s, 92s, 126s; RaspRSV = 50s, 90s, 126s; RMV = 34s, 54s, 75s; and NRSV = 35s, 62s, 83s. A middle component was observed in TomRSV preparations (92s), which corresponds to that of RaspRSV and was not previously described. New sedimentation rates are given for RMV and NRSV.

Protein and nucleic acid contents of LPMV bottom component particles are 58.0% and 42.0%, respectively. TRSV nucleic acid content of bottom component particles was estimated to be 41.4%. Nucleic acid values for both viruses are not significantly
different than previously published figures for TRSV (41.9%). Comparison of TRSV and LPMV nucleic acid purine bases and pyrimidine nucleotides shows that no significant differences in nucleic acid occur. Apparently, differences are either in nucleotide sequence and/or amino acid sequences in protein coats; however, these determinations were not made.

Immunodiffusion tests on agar slides gave variable results with the six viruses and antisera. Virus protein denaturation, caused by storing virus preparations for extended periods following purification, resulted in unexplainable secondary cross-reactions with antisera. Therefore, serological analyses were conducted using immunoelectrophoresis procedures. Numerous secondary reaction bands and spurs were observed on reaction slides of electrophoresed antigens reacted with various antisera. Distinct similarities in serological properties exist between TRSV and LPMV, RMV and NRSV, and TomRSV and RaspRSV; thus, showing strain relationships between them. Immunoelectrophoretic cross-reactions also are observed between LPMV and antisera of RMV, TRSV, and TomRSV. This indicates that LPMV shares antigens with viruses of TRSV, TomRSV, and RMV groups.
LITERATURE CITED


associated nucleoproteins and nucleic acids. In Biochemical Regulation in Diseased Plants or Injury. Symposium. Phytopathological Society of Japan, pp. 21-34.


78. Schneider, I. R. and T. O. Diener. 1966. The correlation between the proportions of virus-related products and the


91. Tall, M. G., W. C. Price, and K. Wertman. 1949. Differentiation of tobacco and tomato ring spot viruses by cross
immunization and complement fixation. Phytopathology 39: 288-299.


ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. Griffith J. Buck for his assistance and useful instructions during the entire course of this study. The financial support provided by the American Rose Foundation made this study possible, and their assistance is gratefully acknowledged. Thanks also are given to Dr. Ervin L. Denisen for his assistance and encouragement throughout the period of graduate training.

Special gratitude is given to Dr. Richard E. Ford for his technical assistance, use of equipment and laboratory space, and for his sincere interest in this research program.
APPENDIX: GRAFT TRANSMISSION AND HOST RANGE STUDIES
Table A-1. Contact periods required for line pattern mosaic virus transmission by bud grafts

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact periods(^a)</td>
<td>Contact periods(^a)</td>
<td>Contact periods(^a)</td>
<td>Contact periods(^a)</td>
</tr>
<tr>
<td>Rose</td>
<td>7 14 18 21 36</td>
<td>7 14 18 21 36</td>
<td>7 14 18 21 36</td>
<td>7 14 18 21 36</td>
</tr>
<tr>
<td>Iowa 5710-2</td>
<td>0(^b) 0 0 2 3 0 0 0 2 4 0 1 3 5 5 0 0 0 1 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iowa 62-5</td>
<td>0 0 0 0 2 0 0 0 0 3 0 0 0 1 3 0 0 0 1 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iowa 60-5</td>
<td>0 0 0 4 5 0 0 0 2 5 0 1 2 3 5 0 0 1 3 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prairie</td>
<td>0 0 0 3 4 0 0 1 3 5 0 2 3 5 5 0 0 0 2 4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Period (days) in which buds were in contact with stock before buds were removed.

\(^b\)Each datum indicates the number of plants in 5 which expressed symptoms in 3 months.
Table A-2. Contact periods required for transmission of line pattern mosaic virus by shield grafts

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rose cultivars</td>
<td>Contact periods&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Contact periods&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Contact periods&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Contact periods&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iowa 5710-2</td>
<td>0&lt;sup&gt;b&lt;/sup&gt; 0 0 0 0 3</td>
<td>0 0 0 1 3</td>
<td>0 0 0 2 5</td>
<td>0 0 0 1 3</td>
</tr>
<tr>
<td>Iowa 62-5</td>
<td>0 0 0 0 2</td>
<td>0 0 0 1 1</td>
<td>0 0 0 1 3</td>
<td>0 0 0 0 2</td>
</tr>
<tr>
<td>Iowa 60-5</td>
<td>0 0 0 1 3</td>
<td>0 0 0 2 3</td>
<td>0 0 0 2 4</td>
<td>0 0 0 0 3</td>
</tr>
<tr>
<td>Prairie Princess</td>
<td>0 0 0 1 4</td>
<td>0 0 0 2 2</td>
<td>0 0 0 2 5</td>
<td>0 0 0 1 4</td>
</tr>
</tbody>
</table>

<sup>a</sup>Period (days) in which shields were in contact with stock before being removed.

<sup>b</sup>Each datum indicates the number of plants in 5 which expressed symptoms in 3 months.
Table A-3. Partial host range of line pattern mosaic virus

<table>
<thead>
<tr>
<th>Species tested</th>
<th>Inoculated</th>
<th>Systemic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species tested</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apocynaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vinca rosea</em> L. 'Pinkie'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Begoniaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Begonia semperflorens</em> Link.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chenopodiaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Beta vulgaris</em> L. 'Baby Canning'</td>
<td>C1R</td>
<td></td>
</tr>
<tr>
<td><em>Chenopodium album</em> L.</td>
<td>NLL</td>
<td></td>
</tr>
<tr>
<td><em>C. amaranticolor</em> Coste &amp; Reyn.</td>
<td>NLL</td>
<td></td>
</tr>
<tr>
<td><em>C. quinoa</em> Willd.</td>
<td>NLL, Cl</td>
<td>Cl</td>
</tr>
<tr>
<td><em>Spinacia oleracea</em> L. 'Bloomsdale'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compositae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chrysanthemum morifolium</em> L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Prairie Sun'</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Zinnia elegans</em> Jacq.</td>
<td>C1R</td>
<td></td>
</tr>
<tr>
<td>'Fire Flame'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cruciferae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brassica oleracea</em> L. 'Early Spartan'</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Plants were inoculated with expressed tobacco sap in phosphate buffer. Abbreviations are: Cl = general chlorosis, C1R = chlorotic rings, D = eventual death, E = epinasty, M = mottled, NLL = necrotic local lesions, NR = necrotic rings, NS = necrotic spots, OL = oak-leaf pattern, Rec = recovery from symptoms, St = stunted, VCl = veinal chlorosis, + = symptomless carrier, = noninfected. The presence of virus was confirmed by reindexing on Turkish tobacco and Vigna sinensis.
Table A-3. (Continued)

<table>
<thead>
<tr>
<th>Species tested</th>
<th>Inoculated</th>
<th>Systemic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cucurbitaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Citrullus vulgaris</em> Schrad. 'Dixie Queen'</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Cucumis melo</em> L. 'Hale's Best'</td>
<td>Cl</td>
<td>ClR, Cl, M</td>
</tr>
<tr>
<td><em>C. sativus</em> L. 'National Pickling'</td>
<td>Cl</td>
<td>ClR, Cl, M</td>
</tr>
<tr>
<td><em>Cucurbita maxima</em> Duchesne 'Butternut'</td>
<td>N</td>
<td>NR, M</td>
</tr>
<tr>
<td><em>Momordica balsamina</em> L.</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td><strong>Geraniaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pelargonium hortorum</em> Bailey 'Cardinal'</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td><strong>Labiatae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Coleus blumei</em> Benth. 'Candidum'</td>
<td>ClR</td>
<td>ClR, OL</td>
</tr>
<tr>
<td><strong>Leguminosae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cassia occidentalis</em> L.</td>
<td>NLL</td>
<td>N</td>
</tr>
<tr>
<td><em>C. tora</em> L.</td>
<td>NLL</td>
<td>N</td>
</tr>
<tr>
<td><em>Cyamopsis tetragonoloba</em> (L.) Taub.</td>
<td>NLL</td>
<td>E, M, D</td>
</tr>
<tr>
<td><em>Indigofera endecaphylla</em> L.</td>
<td>NLL</td>
<td>_</td>
</tr>
<tr>
<td><em>I. hirsuta</em> L.</td>
<td>NLL</td>
<td>_</td>
</tr>
<tr>
<td><em>I. subulata</em> L.</td>
<td>NLL</td>
<td>_</td>
</tr>
<tr>
<td><em>I. tinctoria</em> L.</td>
<td>NLL</td>
<td>_</td>
</tr>
<tr>
<td><em>Phaseolus aureus</em> Roxbg.</td>
<td>ClR</td>
<td>ClR, St, M</td>
</tr>
<tr>
<td><em>P. vulgaris</em> L. 'Bountiful', 'Red Kidney'</td>
<td>NS</td>
<td>ClR, N, D</td>
</tr>
<tr>
<td>Species tested</td>
<td>Inoculated</td>
<td>Systemic</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td><strong>Leguminosae (Continued)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. vulgaris</em> L. 'Wade',</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Improved Tendergreen',</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'White Marrowfat', 'Pea Bean',</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Resistant Cherokee', and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Valentine'</td>
<td>NS</td>
<td>ClR, N</td>
</tr>
<tr>
<td><em>Pisum sativum</em> L. 'Progress N 9'</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Vicia faba</em> L. 'Long Pod Fava'</td>
<td>NLL</td>
<td>N</td>
</tr>
<tr>
<td><em>Vigna sinensis</em> Endl.</td>
<td>NLL</td>
<td>N, D</td>
</tr>
<tr>
<td>'Early Ramshorn'</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Malvaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Abutilon theophrasti</em> Medic.</td>
<td>+</td>
<td>E, D</td>
</tr>
<tr>
<td><strong>Rosaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fragaria vesca</em> L.</td>
<td></td>
<td>Cl, M</td>
</tr>
<tr>
<td><em>Rosa multiflora</em> Thunb.</td>
<td></td>
<td>NS, VCl, OL</td>
</tr>
<tr>
<td><strong>Scrophulariaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Antirrhinum majus</em> L. 'Glacier'</td>
<td>NR</td>
<td>NR, N, D</td>
</tr>
<tr>
<td><strong>Solanaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Capsicum annuum</em> L. 'Yolo Wonder'</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lycopersicon esculentum</em> Mill.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Diamond State'</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Nicotiana glutinosa</em> L.</td>
<td>NR</td>
<td>Cl, St, Rec</td>
</tr>
<tr>
<td><em>N. tabacum</em> L. 'Samsun NN'</td>
<td>NR</td>
<td>Cl, St, Rec</td>
</tr>
</tbody>
</table>
Table A-3. (Continued)

<table>
<thead>
<tr>
<th>Species tested</th>
<th>Inoculated</th>
<th>Systemic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solanaceae (Continued)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>N. tabacum</em> L. type Turkish</td>
<td>NR</td>
<td>N, OL, St, Rec</td>
</tr>
<tr>
<td><em>Petunia hybrida</em> Vilm.</td>
<td>ClR</td>
<td>M, St, Rec</td>
</tr>
<tr>
<td>'Red and White Delight'</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Solanum melongena</em> L.</td>
<td>+</td>
<td>M, NS, Cl</td>
</tr>
<tr>
<td>'Black Beauty'</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> L. 'Norland'</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>