ISOLATION, PURIFICATION, SEROLOGY AND NATURE OF ROSE MOSAIC VIRUS

by

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INTRODUCTION

The incidence of virus diseases of roses appears to be increasing in the United States, probably because of the methods used to propagate the great number of rose varieties affected. Numerous complaints regarding diseased stock, both foreign and domestic, attest to the seriousness of the problem. Growers in many areas are faced with the choice of investing in a certification program that would guarantee disease-free stock or having their plants quarantined.

Four different rose viruses have been reported in the United States. Common rose mosaic, rose yellow mosaic and rose streak are the most common, and rosette of rose or witches broom, which is more destructive than the mosaics, has been reported occasionally.

Surveys for rose mosaic in Oregon rose nurseries, and in test gardens containing roses from all parts of the United States, showed that most plants had symptoms suggestive of virus infection. Most infected varieties displayed symptoms on the late spring and early summer foliage, and some symptomless varieties were assayed and found to harbor a virus in a latent condition. Many rootstocks have become infected by the practice of cutting rootstock materials from plants in nursery rows which were previously budded with infected varieties, and many new varieties have been propagated on such stock.

The effect of rose mosaic and other viruses on the general vigor, longevity, hardiness, flower production, and color stability has not been reported. The high percentage of virus infection in roses, the

frequent latent characteristic of the virus in some varieties, and a lack of a reliable assay method, makes the selection of disease-free stock extremely difficult. Thermal inactivation of several viruses in living plants has now been demonstrated and may provide a method for the cure of virus infected rose plants. However, the results of heat inactivation studies would be difficult to evaluate if no virus assay methods are available. Before disease-free roses can be selected reliable assay methods must be developed and a certification or cleanup program initiated.

Viruses, suspected of being rose mosaic, have been transmitted from rose to herbaceous hosts, but the methods of transmission used were unreliable and time consuming. Identification of rose viruses has been difficult because of the variability obtained in results of transmission studies, and erroneous conclusions sometimes drawn. A reliable technique for the transmission of the rose viruses to herbaceous hosts would also facilitate a study of their physical and biological properties. A technique for the purification of rose mosaic virus has not been reported, consequently this virus has not been characterized by electron microscopy, by spectrophotometry or by serological methods.

This study was initiated to develop methods to recognize, isolate and study the viruses which infect rose and determine their effect on rose plants.

REVIEW OF LITERATURE

The first published records of a virus-like disease of rose in the United States was by Taubenhaus in 1923 (37) and 1925 (38). He described a mosaic symptom of rose in Texas, but no experiments were initiated to determine the true cause of the disease. Rose mosaic was first described as a virus disease by White in 1928 (45), and named "Infectious Chlorosis." Surveys for rose mosaic in commercial rose plantings throughout the United States, revealed a high percentage of affected plants (28, 46, 47, 48). When evidence of the widespread nature of rose mosaic was presented to the rose growers (33, 48, 49), resolutions were adopted to investigate the possibility of quarantine action. Furthermore, believing European rootstocks virus free, the growers resolved to lift import embargos (53). White (50, p. 50a) rejected these resolutions as an answer to the problem, and pointed out that "the source of the disease does not rest solely with the stock upon which forcing varieties are budded. The budwood itself is just as serious a carrier as the stock." He advocated inspection and certification of budwood as well as rootstocks.

Milbrath (32, 33) believed that many of the survey reports on the prevalence of rose mosaic could have been cases of mistaken identity. He maintained that insect damage, nutritional disorders and genetic disturbances were often mistaken for rose mosaic. He also questioned the authenticity of the disease, since infected buds grafted to healthy roses did not always produce symptoms on the host, or at least not the same type symptom. He postulated that if rose

mosaic were transmitted by grafting, it should become systemic, and all the leaves should subsequently show symptoms. He also noted that diseased field-grown roses did not show symptoms after growing for a period in a greenhouse.

In response to Milbrath's skepticism, White (51) in 1932, effectively segregated the rose mosaic syndrome from non-pathological disorders with similar symptoms. Chlorotic conditions of rose caused by common rose insects, genetic disturbances and nutritional disorders, were compared to those caused by rose mosaic virus. Rose mosaic was proven to be an authentic virus disease.

The occurrence of rose mosaic in American grown rootstocks was determined by Brierley and Smith (3, 4). Between the years 1929 and 1935, they indexed rootstocks on the rose variety Mme. Butterfly. No virus was detected in 103 plants of Texas Wax, or 27 of Multiflora, and only in one plant of 21 Odorata and 3 plants of 208 Ragged Robin rootstocks. Approximately 15 percent of 804 Manetti rootstocks were found to be infected. These figures were considerably lower than those reported from field surveys (28, 52).

Investigations of rose viruses by various American workers indicated that there were three basic rose viruses, 1) rose mosaic, 2) rose yellow mosaic and 3) rose streak. This compilation, recorded in the Review of Applied Mycology (7, p. 45-46), favors the classification proposed by Brierley et al. (3). Some of the numerous diseases originally described were concluded to be synonymous.

Infectious chlorosis, veinal chlorosis (51) and rose virus I (41) appeared to be synonymous with Brierley's rose mosaic. Thomas' (41) rose viruses 2 and 3 were considered to be rose yellow mosaic.

The rose virus disease, rose rosette, was reported in the United States by Thomas (42) in 1953. The description of this disfiguring disease resembles that of rose witches broom reported by Conners (8, 9) 12 years earlier in Canada.

Rose mosaic has been reported from Europe (22, 26) and New Zealand (14). Rose wilt, a lethal virus disease of rose, was also reported from Europe (18) and Australia (12, 20). This disease, which was characterized by a cellular necrosis in the cortex, medullary rays, and phloem, was shown by Grieve (20, 21) to be transmitted both mechanically and by insects. Neither Grieve nor Mushin (27) were able to produce an antiserum for the rose wilt virus.

The rose cowl-forming virus, reported from Europe, was demonstrated by Klastersky (24) to be sap transmissible. Lime and elm trees displaying similar cowl-like symptoms were suspected of harboring the same virus (25).

Fry and Hunter (14) reported that a rose line-pattern virus and rose vein-banding virus infected roses in New Zealand. The vein-banding and line-pattern virus manifestations were transmitted independently and retained their identity on different rose varieties. This phenomenon prevailed on the authors to report the diseases as separate entities.

Symptom manifestations of rose viruses have not been

dependable criteria of disease. Rose viruses can be latent in many varieties and show seasonal symptoms in others (3, p. 640-641; 41, p. 658). Baker et al. (1) demonstrated the effect of temperature on symptom expression of a rose mosaic virus. They showed that diseased plants, symptomless when grown out doors, developed symptoms when transplanted under glass which is contrary to the report by Milbrath (32, p. 542). They also noted that symptoms were somewhat depressed when the temperature in the greenhouse was lowered at night. A constant temperature of 17° C. appeared optimum for the expression of symptoms in the varieties Rome Glory and Peerless.

The rose viruses are not readily transmitted mechanically, so grafting methods are used predominantly. Fulton (15) reported an instance of mechanical transmission of rose mosaic to cucumber (Cucumis sativus L.) and to cowpea (Vigna sinensis (Torner) Savt.). Mechanical transmission of the rose virus from rose to herbaceous hosts was not accomplished after mid-summer. The rose virus isolate was transmitted from infected cowpea to 25 plant species in 7 families, including rose.

Numerous attempts to transmit the rose viruses with insects have failed. White (47, 48) and Nelson (34) were the first to attempt insect transfer, but reported negative results. Brierley et al. (3, p. 646-648) reported insect transmission trials with rose mosaic and rose streak. They tested 42 species in the families Cicadellidae, Cercopidae, Membracidae, Araepidae, Fulgoridae, Aphiidae and Coccidae in the order Homoptera and one species of thrips. None of these

insects transmitted the viruses. In addition, they demonstrated that viruliferous vectors of strawberry crinkle virus (Philaenum leucophthalmus L.), aster yellows virus (Macrosteles divisus Unler.) and red raspberry mosaic (Amphorophora rubi Kalt.) were unable to transmit any of these viruses to rose.

Viruses that commonly infect rosaceous hosts other than Rosa spp., have been graft-transmitted to rose. Thomas (39, p. 583; 40, p. 641; 41, p. 655-657) transmitted apple mosaic and peach mosaic to rose, and conversely, rose mosaic to apple but not to peach. Rose mosaic and apple mosaic were regarded as separate entities on the basis of symptom expression of the rose viruses in apple, and the extremely slow systemic movement of apple mosaic in rose (20 to 27 months to produce symptoms 6 inches from the graft). Peach mosaic was likewise separated from the rose viruses on the basis of results from cross-inoculation tests.

Plum line-pattern virus has been transmitted to roses (10) where it caused pronounced mottling, distortion and stunting of the leaves. A general reduction in vigor was also observed. Valleau (43, p. 101), in 1932, recognized the similarities of the symptoms of apple mosaic, plum mosaic (plum line-pattern) and rose mosaic on their respective hosts.

Cochran (6) placed buds from 11 standard rose varieties on peach and observed that virus symptoms developed on some of these trees. These symptoms were similar to those caused by the peach ring spot virus which he was studying from Prunus hosts. Gilmer (19) recovered

two viruses when he inoculated peach trees with buds from infected Multiflora rose. These viruses were isolated on cucumber and compared with 123 isolates from Prunus spp. The rose virus isolates were similar to Prunus ring spot virus with regard to thermal inactivation, aging in vitro, effects of ions on infectivity, and response to changes in pH. Electron micrographs prepared by Willison et al. (54, p. 100-101) of the rose viruses isolated by Gilmer, suggested that the two isolated were separate entities.

In Italy, Gualaccini (22) grafted rose buds infected with a yellows type of rose mosaic to Kwanzan flowering cherry; severe necrosis and death of Kwanzan resulted. He concluded that a severe strain of the ring spot of Prunus was the virus involved. However, Milbrath (30) showed that when this virus was present in pure culture it caused only mild chlorotic patterns on Kwanzan. Apparently the rose virus used by Gualaccini was not the ring spot virus of Prunus.

Fulton (16, 17) obtained clear infectious extracts containing rose mosaic virus by adsorbing the macerated tissue on hydrated calcium phosphate. Infected tissue macerates extracted in buffers of pH 6.5 to 8.7 produced infectious supernatants. Ionic strength of the extracting buffer was critical and infectious supernatants were only obtained within certain molar limits.

Willison (54) reported the partial purification of two rose viruses from cucumber by differential centrifugation. Electron micrographs showed that particles of one isolate were spheres about 40 mu in diameter while those of the other isolate were spheres with a

diameter of approximately 28 mμ. The particle sizes were determined on the basis of the differences in the distribution of the particles occurring in preparations from diseased tissue to those from healthy tissue.

Brierley et al. (2, 3, 4) submitted evidence that rose mosaic, yellow mosaic and streak were separate entities. Host reaction studies and cross-protection investigations showed that, in some cases, roses of the Talisman variety infected with both rose mosaic and yellows mosaic displayed characteristic symptoms of each virus. The rose mosaics, likewise, did not interfere with the development of the rose streak virus syndrome.

Exposure of virus-infected rose cuttings to high temperatures as a physio-therapeutic treatment was first investigated by Newton (35). He reported that immersion of infected rose cuttings for one hour in water at 45° C. or 15 minutes immersion in a one percent $KMnO_4$ solution was not effective. According to Thomas (41, p. 659-660), rose mosaic can survive in cuttings completely covered by sand held at 30° C. for 26 days, and in cuttings covered with moist sphagnum held at 36° C. for 14 days. Immersion of diseased cuttings in water at 45° C. for 30 minutes was also ineffective. He also found that rose yellow mosaic virus (rose virus 2 and 3 Thom.) survived similar treatments. In 1960 Holmes (23) presented evidence of successful heat inactivation of rose virus in vivo. Rose plants in which the soil temperature was held at 33.5 to 34° C. for one week and then for three weeks at 36° C. or longer, did not display a reoccurrence of of mosaic symptoms during a 40 week period of observation.

METHODS AND MATERIALS

I. Plant inoculation technique.

Woody plant seedlings were top-worked with the test plant using a whip-and-tongue graft, and inoculum was introduced by placing a chip bud below the graft. The inoculated seedlings were planted in number 10 cans and placed in the greenhouse.

Leaves of herbaceous hosts were inoculated mechanically. Infected leaves of herbaceous hosts were macerated in a mortar in 0.02 M Na_2HPO_4 at the approximate ratio of 1:5 (weight:volume). Carborundum powder (600 mesh) was dusted on the leaves and the extracted sap was rubbed on the healthy leaves with the forefinger, after which excess inoculum and carborundum were removed by washing with tap water.

II. Plant culture.

Herbaceous plants used in this study were grown from seeds sown on a loam-peat moss mixture in number 10 cans and covered with one half inch of sand. The plants were maintained in a greenhouse at an approximate temperature of 70° F. during the daylight hours and 65° F. at night. Sixteen hours of artificial light were provided during the winter months. All inoculations were made on young, vigorously growing seedlings.

RESULTS

I. Isolation of rose mosaic virus of rose.

A. Attempted mechanical transmission of rose mosaic virus from rose. Numerous techniques employed in attempts to mechanically transmit rose mosaic virus (RMV) from infected rose tissue were unsuccessful. Reagents were added to the extracting buffer in an attempt to inactivate the virus inhibitors present in rose tissue extracts. Chelating agents such as EDTA, cysteine HCl, and ascorbic acid were used to remove metallic ions; reducing agents, sodium sulfite and cysteine were added to protect the virus from oxidizing agents. KCN was used primarily to poison the proteinase enzyme systems, and a gelatin solution and nicotine sulfate were used to precipitate the tannins.

The reagents, separately or in combination, were added to the buffer in which the infected rose tissue was macerated. The solutions were also vacuum-infiltrated into the intercellular spaces of infected leaves prior to maceration. Mechanical transmission of RMV was not accomplished by any of these treatments.

B. Virus inhibitors in rose tissue extracts. Experiments were conducted to determine the effects of rose tissue extracts on the infectivity of viruses. One tenth of a gram of leaf tissue from black cowpea infected with alfalfa mosaic virus (AMV) was macerated in 25 ml of phosphate buffer and strained through four layers of cheesecloth. One ml of an extract from rose leaves of different

ages, or from roots, obtained in an identical manner, was added to one ml of the cowpea extract to give a dilution of each component of 1:500. Extract to be used on control plants was prepared by adding one ml of buffer to the virus preparation from cowpea in place of the rose tissue extract. Primary leaves of 20 black cowpeas were inoculated and observed for local lesion development. Similar tests were conducted, using only rose root extracts, on tobacco ring spot virus (TRS) and cucumber mosaic virus (CMV).

Extracts from mature and senescent leaves of rose had little inhibitory effect on AMV, but extracts from young succulent terminal leaves of rose reduced local lesion formation by 58 percent of that of the controls. Contrarily, rose root extracts stimulated AMV local lesion development on cowpea from 35 to 97 percent above that of the controls. The stimulatory effects of root extracts were less pronounced on the TRS and CMV isolates. Increases in local lesion count of 15 and 11 percent respectively were observed.

C. Isolation of rose mosaic virus by graft transmission to Vinca and Peach. Rose mosaic was transmitted to both peach and Vinca rosa L. when infected buds were chip- or T-budded on these hosts. Although no true organic union could be expected with these plants, the virus was able to move from the rose tissue and infect the host plants. Approximately 90 percent of the V. rosa plants became infected if at least 3 diseased buds were used on each plant.

When peaches were graft-inoculated with infected rose buds, RMV

could be recovered from the terminal leaves 6-8 weeks later by mechanical inoculation to cucumber, black cowpea, or Bountiful bean (Phaseolus vulgaris L.). Although the inoculated peaches were observed for 5 months, the only symptom that developed was a mild mosaic. Rose mosaic virus was mechanically transmitted from graft-inoculated V. rosa 3-4 weeks after inoculation to cowpea or cucumber, and since V. rosa did not require a dormant period, isolations could be conducted throughout the year. As symptoms of RMV infection in V. rosa were not always apparent, inoculated plants were assayed for infectivity on cucumber or cowpea. Mild mosaic symptoms on V. rosa were only observed on the terminal leaves during the spring and early summer months.

Rose mosaic virus was mechanically transmitted from graft-inoculated V. rosa to cowpea with greater ease and frequency than to cucumber. Symptoms on cowpea, indicative of infection, were generally not observed on the inoculated primary leaf or on the systemically infected first trifoliate leaves, but they did develop on subsequent trifoliate leaves. However, strong symptoms of infection developed on the inoculated primary and first trifoliate leaves of cowpea when the primary leaves were inoculated with a virus solution of high titer.

D. Comparison of rose mosaic virus isolates and tomato ring spot type culture on herbaceous hosts. Rose showing typical mosaic virus symptoms (Figure 1) were selected for isolation. Eight RMV isolates

were studied on herbaceous hosts and on the basis of host reactions, four different isolates were distinguished and selected for further study. Fulton's (15) isolate of RMV, designated F-1 in this sutdy, was treated as the type culture and Oregon RMV isolates, designated P-4, V-1B and P-2 were compared with this culture in herbaceous hosts. The symptoms and host range of the RMV isolates were similar to several isolates of tomato ring spot (TomRS) isolated from diseased gladiolus. These observations led to a comparative study of RMV and TomRS.

i. Chenopodium amaranticolor Coste and Reyn. Local lesions formed by the RMV isolates on the inoculated leaves of C. amaranticolor varied from small necrotic flecks, produced by isolates V-1B and P-2, to small chlorotic ring spots of isolate P-4. However, isolate P-4 would infect C. amaranticolor only during the spring months. American type culture 13 of tomato ring spot virus produced necrotic ring spots similar to those produced by RMV isolate F-1 (Figure 2). Systemic symptoms induced by the RMV isolates and TomRS virus were similar and the virus isolates could not be distinguished by the symptoms.

ii. Vigna sinensis, black cowpea. Local reactions on the inoculated primary leaves of black cowpea varied from chlorotic spots to large necrotic lesions. However, reaction on the primary leaves were unreliable because the occurrence of symptoms fluctuated more between individual plants, seasons of the year, and inoculum titers than between virus isolates.

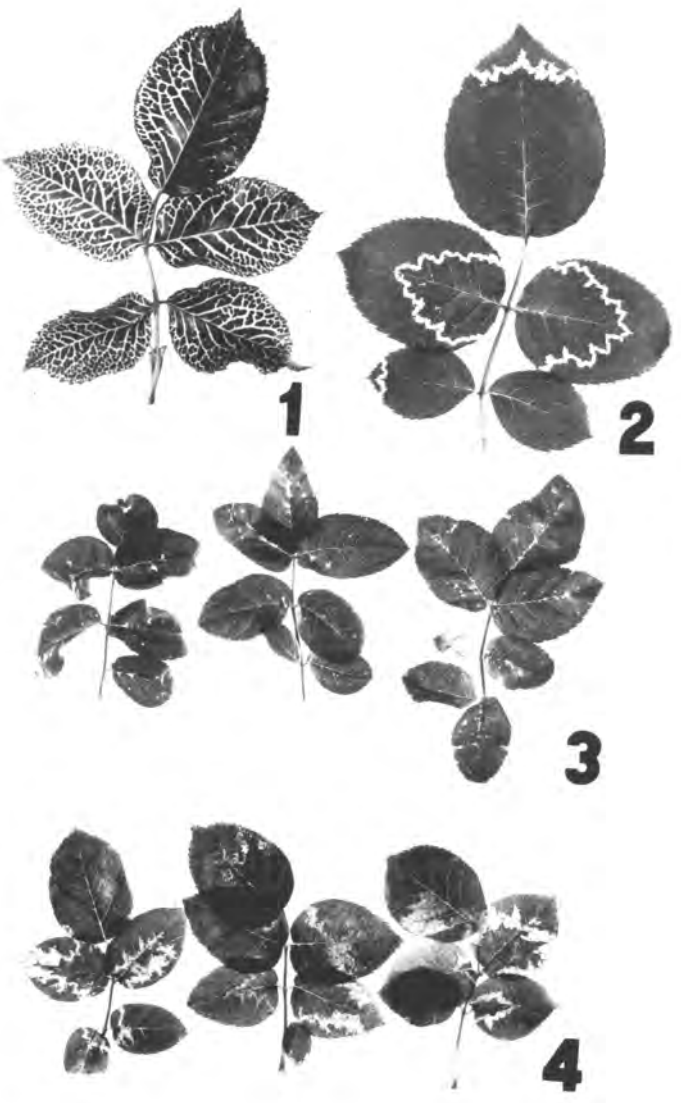

Figure 1. Typical rose mosaic in some hybrid tea roses. 1 and 2, rose yellow mosaic, 3 and 4, common rose mosaic.

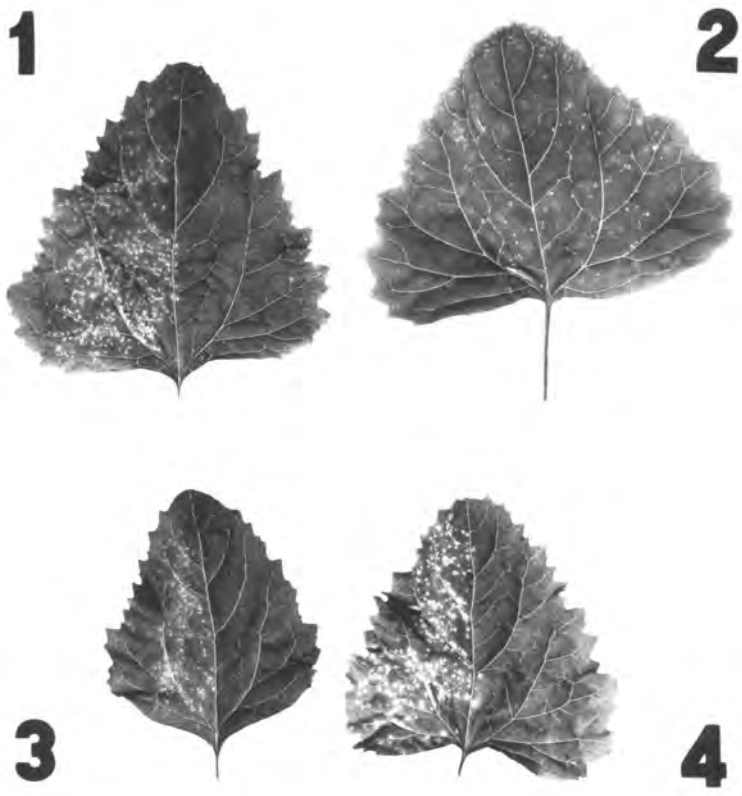

Figure 2. Comparison of local lesions caused by rose mosaic isolates and tomato ring spot virus-type culture on Chenopodium amaranticolor. 1) V-1B. 2) P-4. 3) F-1. 4) TomRS.

Systemic symptoms on the trifoliate leaves were consistently different among virus isolates and usually were not influenced by the factors which affected the occurrence of symptoms on the primary leaves. Isolate F-1 usually produced bright yellow chlorotic patterns in the form of vein banding, ring spots or line patterns, but necrotic lesions were seldom observed. Isolate P-4 generally remained latent except in the spring months or when inoculum of high titer was used, when vein clearing and necrotic flecking were observed. Isolate P-2 and V-1B both caused necrotic and chlorotic lesions on the trifoliate leaves. Tomato ring spot virus reaction was similar to that produced by P-4 in its symptom expression (Figure 3) and in its failure to produce symptoms except in the spring or with high titer. The severity and frequency of occurrence of systemic symptoms on cowpea, in the case of all five viruses tested, appeared to be influenced by the titer of the inoculum.

iii. Phaseolus vulgaris, varieties Bountiful and Sutter's Pink. Rose mosaic virus isolate F-1 and TomRS virus were tested on the following bean varieties: Bountiful, Sutter's Pink, Red Kidney, pole bean - Bluelake, Golden Cluster Wax, Dwarf Horticulture, Pinto and Great Northern. Bountiful bean and Sutter's Pink were the only two varieties in which symptoms occurred consistently. All four RMV isolates and TomRS virus caused chlorotic lesions on the inoculated primary leaves and necrotic lesions on the systemically infected trifoliate leaves. Differences among the five isolates were not distinguishable on the basis of host symptoms produced on either bean variety.

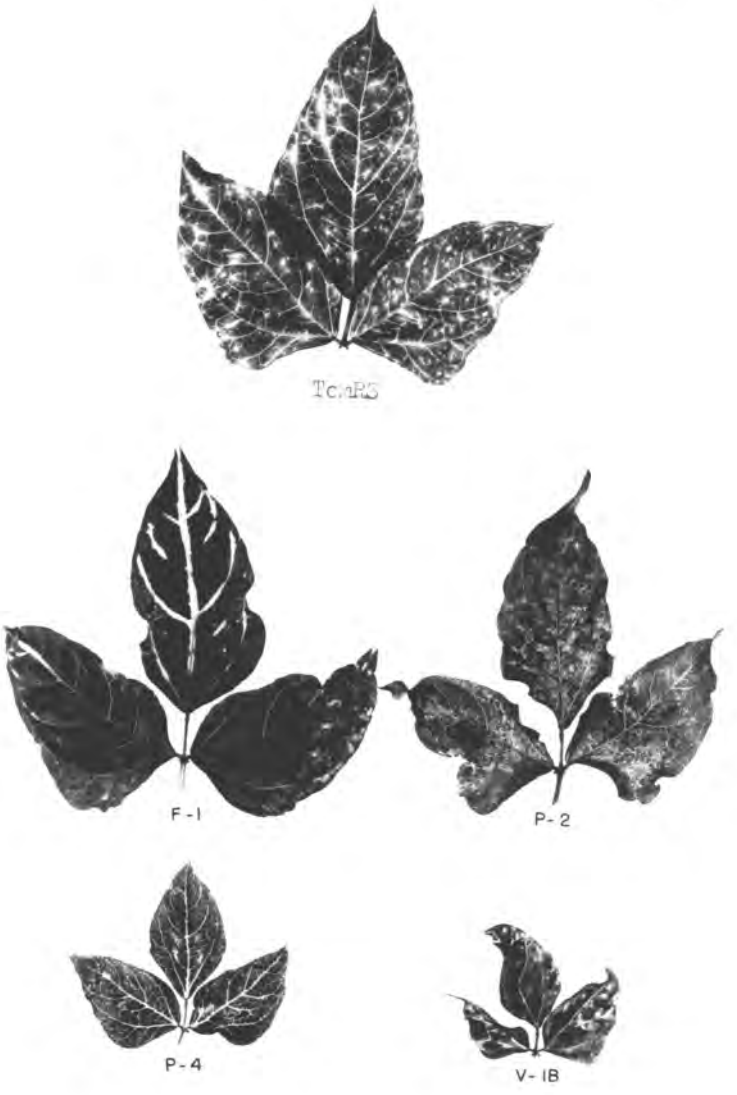

Figure 3. Comparison of systemic symptoms of some rose mosaic virus isolates and tomato ring spot virus on trifoliate leaves of black cowpea.

iv. Cucumis sativus, variety Chicago Pickling. The rose virus isolates and TomRS culture produced chlorotic lesions on the inoculated cotyledons, and necrotic lesions on the first true leaf of cucumber. Death of the infected plants generally followed within 1½ to 2 weeks after inoculation. Differences between the RMV and TomRS isolates were not readily distinguished by symptoms on cucumber.

II. Prunus ring spot virus in rose.

A. Prevalence of Prunus ring spot virus in rose. A survey for Prunus ring spot virus (PRSV) in rose rootstocks in commercial nurseries was conducted during the summers of 1959, 1960 and 1961. Budwood was selected from 71 Manetti, 181 Multiflora and 38 miscellaneous rootstocks. These were tested for PRSV on field grown Shirofugen (Prunus serrulata) (29). Fifty-five hybrid tea roses, grown on the Oregon State University plant pathology experimental farm, were also tested. Buds from a prune tree showing prune dwarf symptoms were used as a source of PRSV for a control.

None of the buds from the Manetti and Multiflora roses were infected. An Odorata rose tested positive for PRSV and 25 of the 55 hybrid tea roses proved to be infected with PRSV. Forty-four of the 55 tea roses had displayed symptoms of RMV, but there was no correlation between the PRSV infection and the plants showing RMV symptoms.

Evidence for the natural spread of PRSV among roses was

observed. Eleven of the 55 hybrid tea roses which had indexed negative for PRSV were re-indexed the following year. Seven of the eleven roses re-tested assayed positive for PRSV on Shiro-fugen.

B. Transmission of Prunus ring spot virus from rose to herbaceous hosts. A Prunus ring spot virus, of the non-yellows type (31), was mechanically and graft-transmitted from rose to cucumber. PRSV was recovered on cucumber from the terminal leaves of peach seedlings grafted with rose buds infected with PRSV. Mechanical transmission of PRSV directly from infected rose tissue to cucumber was accomplished on two different occasions. Rose leaves were macerated in phosphate buffer, and the extract was applied to the cotyledons of the cucumber. PRSV could not be transmitted to black cowpea or Bountiful bean, but V. rosa buds infected with PRSV caused a typical necrotic reaction when budded to Shiro-fugen. Symptoms caused by PRSV isolates from rose compared favorably with those caused by known isolated of PRSV of Cucurbita maxima Dcne. var. Buttercup and C. pepo L. var. White Scallop.

III. Survey for rose virus indicator hosts.

Five different host plants were tested during a search for an index host for the rose viruses. Each plant selected had either been observed or reported to be an indicator host for a specific virus. The indicator hosts included Jonathan apple, Hopa crab, Shiro plum, Prunus tomentosa, and Shiro-fugen, and the respective viruses included apple mosaic virus, stem pitting virus, plum

line-pattern virus and Prunus ring spot virus. These viruses were used as inoculum for the control plants.

No discreet virus symptoms were observed on the Hopa crab or Shiro plum seedlings inoculated with RMV, but Shiro-fugen and P. tomentosa reacted characteristically to the rose buds infected with PRSV (13, 29). Virus symptoms were not observed on Jonathan apple, but an increase in mildew (Podosphaera leucotricha (E & E) Salm.) susceptibility was observed on seedlings inoculated with the rose viruses.

The rose mosaic viruses were readily transmitted mechanically from the terminal leaves of infected Jonathan apple, but mechanical recovery of the virus was not accomplished from the other test plants.

IV. Purification of rose mosaic virus.

A. Selection of host and assay plants. Black cowpea, cucumber and Bountiful bean were infected with RMV and assayed for infectivity on C. amaranticolor and compared for virus titer. Juice extracted from cucumber cotyledons and trifoliate leaves of cowpea was more infectious than extracts from the primary and trifoliate leaves of Bountiful bean. Black cowpea was selected as a host over cucumber because of its ease of culture and maintenance.

B. Purification procedure. A modification of the technique, reported by Fulton (16, 17), for the clarification of tissue macerates infected with virus was adapted to the purification of RMV.

Trifoliate leaves of black cowpea infected with the F-1 isolate were briefly sprayed with Dow Corning anti-foam A and macerated in a morter in 1½ volumes (weight:volume) of 0.02 M Na_2HPO_4. The buffer was titrated to pH of 7.5 with phosphoric acid and 0.01 percent cysteine HCL added just prior to using. Buffer of the same formulation, less the cysteine complement, was used throughout the remainder of the procedure. The macerated tissue was expressed through four layers of cheesecloth with a hand press, and the extract centrifuged for 10 minutes at 4,200 rpm in a Servall angle centrifuge. Six-tenths gram of freshly prepared hydrated calcium phosphate (HCP) was added to the extract for each gram of original tissue. The mixture was homogenized in a beaker with a magnetic stirrir. Mixing was augmented by drawing and expelling the homogenate through a 30 ml syringe. The homogenate was centrifuged for 10 minutes at 2,500 rpm. The supernatant was again adsorbed with HCP as described above. The clear supernatant was then centrifuged for 15 minutes at 6,500 rpm to remove remaining traces of HCP. The extraction and clarification procedures were conducted in a cold room at 5° C., and the extract was at all times refrigerated or packed in ice.

Ultracentrifugation (Spinco No. 40 head) for 120 minutes at 38,000 rpm (127,640 x G), yielded an opalescent pellet and a free flowing non-opalescent, gelatinous pellet. Both pellets were resuspended in buffer and centrifuged for 15 minutes at 6,500 rpm. The virus was again pelleted by centrifugation for 120 minutes at 38,000 rpm, resuspended in buffer, and centrifuged for 15 minutes at 6,500 rpm.

Extraneous host materials were removed from the partially purified virus suspension by density gradient centrifugation. The virus-containing solution was layered on a 25 to 50 percent sucrose gradient and centrifuged for 240 minutes at 25,000 rpm (90,000 x G) in a Spinco SW 25 swinging bucket rotor. The virus zone was removed, diluted 1:5 with buffer and centrifuged at 38,000 for 120 minutes. The pellet was re-suspended in buffer and centrifuged for 15 minutes at 6,500 rpm. An outline of the purification procedure is illustrated in Figure 4.

The molarity of the phosphate buffer used in the extraction process was found to be very critical. Infectivity of the clarified preparation decreased as the ionic strength of the buffer was either increased or decreased. Hydrogen ion concentration of the buffer was less critical. Virus activity was detected in clarified preparations extracted in buffers between the pH range of 7.0 to 8.0. Clarified virus preparations extracted in buffer without an oxidative protectant were low in infectivity. Agents with reducing and chelating potentials, such as cysteine HCl, added to the extracting buffer prevented loss of activity during clarification. Chelating agents incorporated in the buffer, with and without the cysteine complement, were not as effective as cysteine alone.

Longevity of the partially purified virus was greater when suspended in buffer than when suspended in distilled water. Virus suspensions in buffer were still infectious after 5 days storage at 5^{o} C., but suspensions in distilled water were no longer infectious after similar storage.

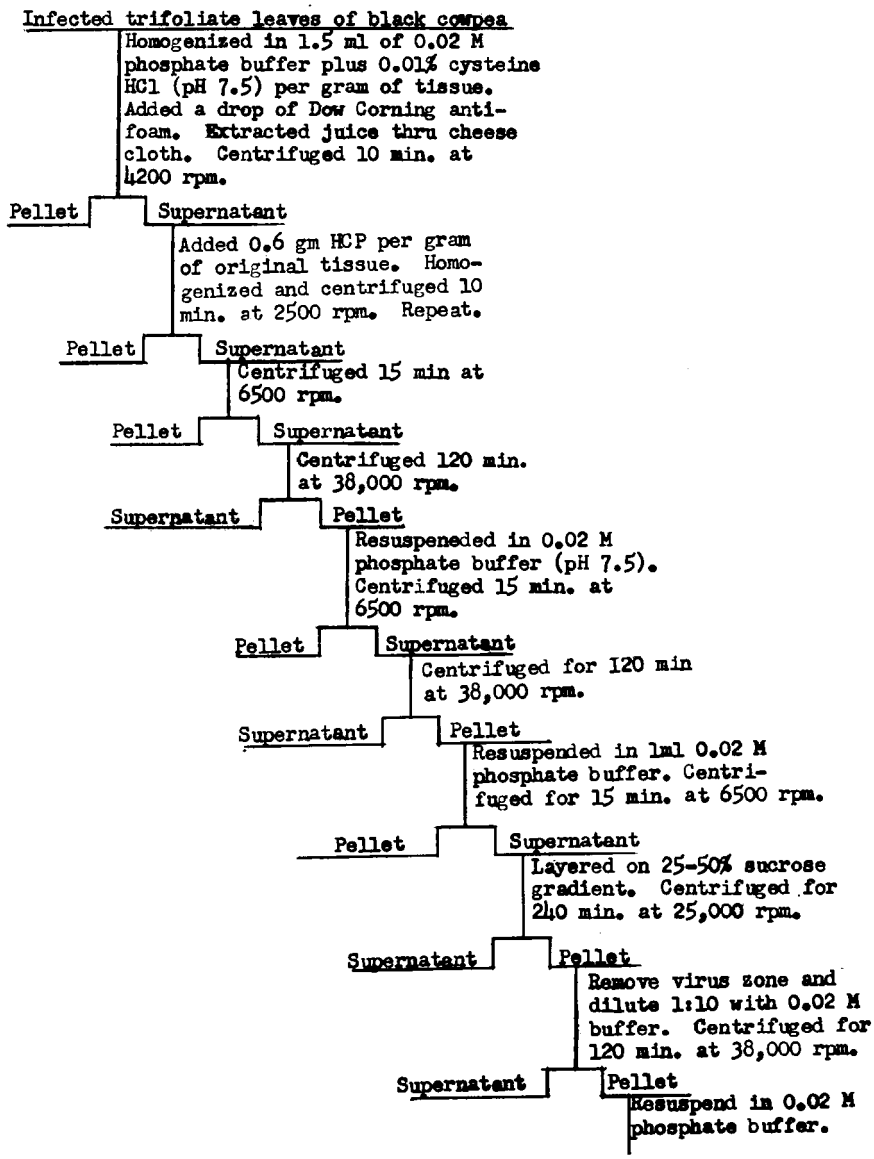

Figure 4. Outline of the procedure used in purification of rose mosaic virus isolate F-1.

Density gradient centrifugation of partially purified virus preparations effectively separated the mixture into four components, three opalescent zones and a pellet. The top zone and pellet were comparable to those observed in density gradient columns of similarly treated material from healthy plants. The particles from the bottom opalescent zone of the diseased preparations were shown to be infectious when assayed on C. amaranticolor, but the middle zone was not infectious. A low level of infectivity was usually present in the pellet. No additional zones were observed when the pellet from the 25-50 percent sucrose gradient was re-run on a 50 to 75 percent gradient.

Density gradient separations of preparations from healthy plants revealed an opalescent zone not observed in the same region of preparations from diseased plants (Figure 5).

Infectivity of the virus was very low when the density gradient sucrose was removed by dialysis with distilled water or buffer, but little infectivity was lost when the virus was removed from the sucrose by differential centrifugation.

V. Ultraviolet absorption of rose mosaic virus.

The ultraviolet adsorption spectrum of the particles occurring in the zones of a density gradient column of an RMV preparation was measured on a Carey recording spectrophotometer. The particles suspended in sucrose solution and in buffer were scanned for nucleoprotein with an absorption maximum and minimum near 260 and 240 mu.

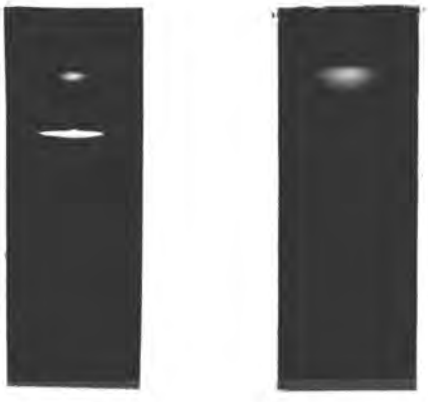

Figure 5. Twenty-five to fifty percent sucrose density gradient column of preparations from healthy cowpeas (left) and from cowpeas infected with rose mosaic isolate F-1.

Figure 6 illustrates the absorption spectrum of the various components separated from the partially purified preparations. The E 260/280 ratio of the infectious particles was 1.23 and the E max/min = 1.13. The ultraviolet absorption spectrum of this component was characteristic of a nucleoprotein. The top zone, which was comparable to that observed in healthy preparations, had an absorption spectrum characteristic of proteins. The spectrum of the material occurring in the second zone has not been characterized.

VI. Electron microscopy of rose mosaic virus.

Electron micrographs were taken of the RMV preparation components separated in a density gradient column with an RCA EMU-2D. The purified virus component, suspended in buffer, was sprayed on steel grids coated with nitrocellulose and shadowed with platinum at a grazing angle of 20°. A 1/8 dilution of the suspension showed good particle distribution with little aggregation. A standardized concentration of polystyrene latex of 1:1000 was used in each serial dilution. Examination of electron micrographs of air-dried preparations of purified RMV shows considerable flattening of the particles (Figure 7). An average of 50 measurements of the particles from the infectious preparation, calculated by comparison to polystyrene latex spheres of 264 mu, was 40 mu. The height of the particles, calculated from shadow measurements was 8.6 mu. If the virus particles were considered spherical, and compensations made for the flattening of the particles, a figure of 24 mu was derived.

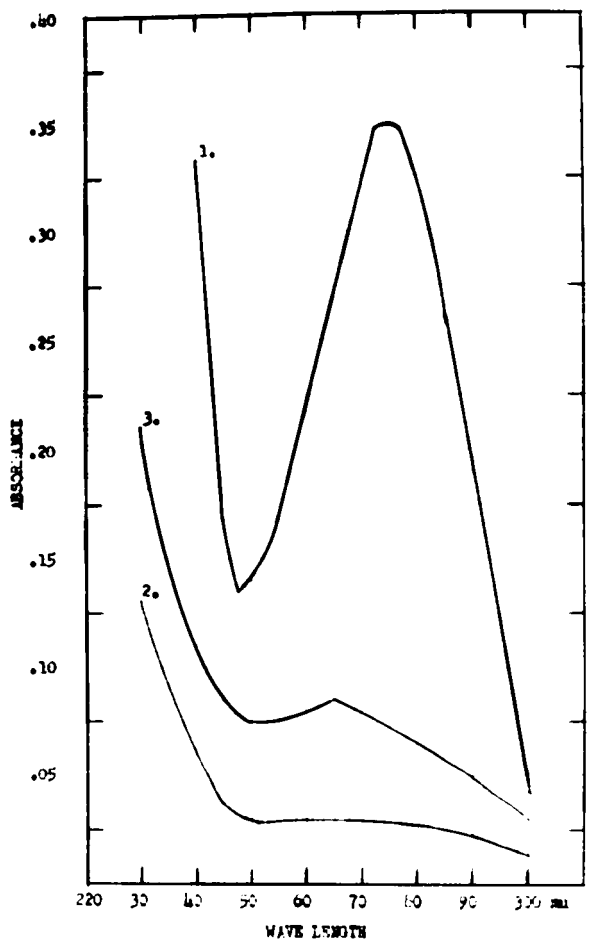

Figure 6. Ultraviolet absorption spectra of components separated from partially purified rose mosaic virus by density gradient centrifugation. 1) Spectrum of component from top zone, 2) middle zone and 3) the infectious bottom zone.

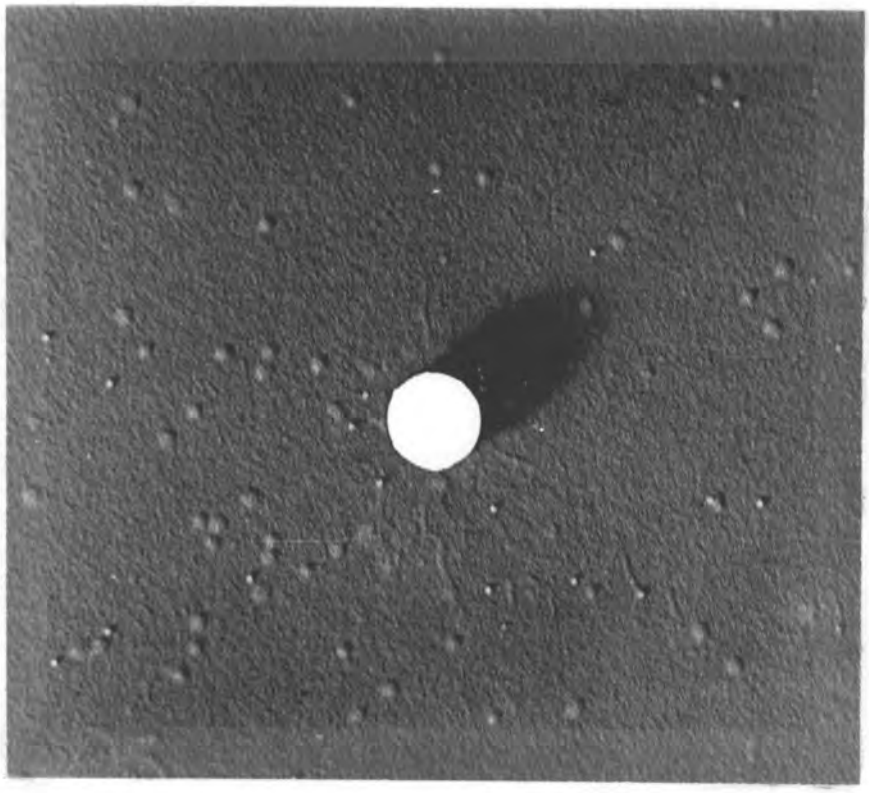

Figure 7. Electron micrograph of the particles occurring in the two zones of a density gradient column of a RMV preparation which were not observed in healthy plant preparations. The large particles were from the infectious bottom zone and the small particles from the middle zone. (x 50,000)

The particles from the middle zone of a density gradient column were approximately one-half the magnitude of the virus particles. These particles appeared to be particularly rigid and not as subject to flattening in air-dried preparations as was the RMV.

VII. Serological study of rose mosaic virus.

A. Preparation of antigen. Rose mosaic virus (F-1), extracted from black cowpea by two high-speed differential centrifugation cycles of the purification procedure, was prepared for injection into two rabbits. The RMV antigen suspended in buffer was used for intravenous injection, and for intramuscular injection the suspension was emulsified with Freunds incomplete adjuvant at a ratio of 1:1..

B. Immunization procedure. A combination of intramuscular and intravenous injections were used. Weekly intramuscular injections were generally followed every third or fourth day with an intravenous injection. The antigen prepared for each injection was assayed for activity on C. amaranticolor. Table I shows the immunization schedule.

C. Serological tests. The titer of the rose mosaic virus antiserum was determined by the reaction of adsorbed antiserum with partially purified virus preparations in micro-precipitin tests (44). The antiserum was adsorbed twice with partially purified preparations from healthy cowpeas. The titer of the adsorbed antiserum was 1/32.

Table I. Immunization schedule used in the preparation of RMV antiserum.

Type of injection	Date of injection	Date of test bleeding	Titer of antiserum
Intramuscular (IM)	1-8-62		
IM	1-16-62		
Intravenous (IV)	1-18-62		
IM	1-22-62		
IV	1-25-62		
IM	1-30-62		
IV	2-2-62		
IM	2-6-62	2-6-62	1/16
IV	2-9-62	2-12-62	1/64

Intramuscular injections were 3-4 ml of virus preparation emulsified with an equal volume of Freund adjuvant. Intravenous injections were 1-1.5 ml of virus preparation.

Crude sap preparations of RMV isolates P-4, P-2 and V-1B were reacted with the F-1 antiserum. Precipitation of the non-homologous antigens with the adsorbed F-1 antiserum had a dilution end point of 1/8. The low titer of the antiserum prohibited further tests to determine strain relationships. Normal serum was not observed to react with preparations from diseased or healthy cowpeas.

D. Serological relationship of rose mosaic virus to tomato ring spot virus.

i. Preparation of the antigen. Tomato ring spot virus was extracted from the inoculated primary leaves of Bountiful bean by the same technique previously described for RMV. The activity of TomRS from Bountiful bean was greater when the molarity of the extracting and suspending buffer was 0.01 M; otherwise the procedure was identical.

Infectious material used for injection received two high-speed differential centrifugation cycles of the purification procedure.

ii. Immunization procedure. Tomato ring spot virus was administered to two rabbits by a combination of intramuscular and intravenous injections, similar to the schedule described by Cadman et al. (5). The antigen for intramuscular use was emulsified at a 1:1 ratio with a 2:1 mixture of falba and mineral oil. Ivory soap was used as an emulsifying agent. Table II shows the immunization schedule.

Table II. Immunization schedule used in the preparation of TomRS antiserum.

Type of injection	Date of injection	Date of test bleeding	Titer of antiserum
Intramuscular (IM)	12-11-61		
IM	12-16-61		
IM	12-21-61		
IM	12-29-61		
IM	1-4-62		
Intravenous (IV)	1-11-62	1-15-62	1/64
IV	1-20-62	1-24-62	1/256

Intramuscular injections were 3-4 ml of virus preparation emulsified with an equal volume of 2:1 mixture of falba and mineral oil. Intravenous injections were 1.5 ml of virus preparation.

iii. Serological tests. The precipitin and micro-precipitin tests were used to determine antiserum titer. The micro-precipitin test, due to its relatively close check with the precipitin test, was used exclusively in later tests.

TomRS antiserum was absorbed twice with healthy plant antigen prepared by the same procedure as the virus antigen. Table III shows the titer of the adsorbed TomRS antiserum when reacted with the homologous antigen and non-homologous antigen (F-1).

The precipitation of the non-homologous antigen with the TomRS antiserum was clearly less dense than the reaction observed with the homologous antigen.

The dilution end point of the adsorbed antiserum when reacted with TomRS was 1/64, and 1/16 when reacted with RMV isolate F-1.

E. Cross precipitation tests. Tomato ring spot virus was reacted with the F-1 RMV antiserum. As previously stated, the titer of the RMV absorbed antiserum was 1/32 when reacted with the F-1 isolate. The dilution end point of the antiserum was 1/16 when reacted with partially purified tomato ring spot type culture virus. This again shows that TomRS virus and Fulton's RMV isolate are serologically related.

Serological reaction of tomato ring-spot antiserum with partially purified TomRS virus.

Adsorbed Antiserum	Antigen prepared from ..... Diseased plants 2	4	8	16	S	Healthy plants 2	4	8	16
2	3	3	2	1	0	0	0	0	0
4	3	3	2	1	0	0	0	0	0
8	3	3	2	1	0	0	0	0	0
16	2	2	1	1	0	0	0	0	0
32	1	1	t	t	0	0	0	0	0
64	1	t	0	0	0	0	0	0	0

Serological reaction of tomato ring-spot antiserum with partially purified rose mosaic virus (F-1).

Adsorbed Antiserum	Antigen prepared from ..... Diseased plants 2	4	8	16	S	Healthy plants 2	4	8	16
2	2	2	2	1	0	0	0	0	0
4	2	2	1	1	0	0	0	0	0
8	2	1	1	t	0	0	0	0	0
16	1	1	t	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0

Table III. Titer of tomato ring spot antiserum reaction with homologue (top) and rose mosaic virus isolate F-1 in micro-precipitin tests.
S = saline.

DISCUSSION AND CONCLUSIONS

Mechanical transmission of rose mosaic virus from rose was reported by Fulton (15) to be difficult and unreliable. He maintained that the infectivity of RMV was affected by rose tissue extracts, divalent ions and by the peculiarities of the virus. In the present study, virus inhibitors were found primarily in the extracts from young rose leaves. Mature and senescent leaves were shown to have little inhibitive effect on the viruses tested; however, RMV was not isolated from infected mature or senescent leaves. Numerous chemicals and different techniques used in the extraction process to counteract the effect of the virus inhibitors did not prevent inactivation of RMV. Rose root extracts had a pronounced stimulatory effect on the infectivity of alfalfa mosaic. This was most likely due to an interference by a substance present in the rose root with an inhibitor in the V. rosa or cowpea from which the AMV was extracted. The effect of rose root extracts on the infectivity of RMV was not investigated, but in view of the effect of this substance on the infectivity of AMV, it would appear that rose roots from infected plants would be the logical place from which to attempt isolations for RMV.

Several investigators have graft-transmitted rose viruses to peach, then have mechanically transmitted the viruses from peach to herbaceous hosts. In this study rose mosaic was readily transmitted by grafting to peach seedlings and also to V. rosa during any

period of the year. This virus could be mechanically tranmitted from both hosts to cowpea or cucumber with relative ease. RMV transmission to V. rosa was not 100 percent reliable, but provided an assay technique and a method of isolating RMV with a greater percent of success than by previously reported methods.

Reports (6, 19) of viruses isolated from rose which resemble Prunus ring spot virus are in all probability correct, but the contention that Prunus ring spot virus and RMV have a co-identity is unlikely. The present investigation shows that in tests for PRSV in 55 roses, 25 assayed positive for PRSV on Shiro-fugen. Forty-four of the 55 roses displayed symptoms of RMV. Evidence of spread of Prunus ring spot virus among roses was observed. Studies were not conducted to determine the mode of spread, but it was presumed to be by pollen transmission (11).

PRSV was transmitted by mechanical inoculation from rose to cucumber, but this was only accomplished twice and only in the spring of the year. This phenomenon was also observed by Fulton (15) for RMV. A combination of factors, including the titer of virus inhibitors, the virus titer and the balance between the two was probably responsible. The virus titer was probably at its peak during the rapid growth experienced during the spring months.

Jonathan apple, Hopa crab, Shiro plum, Prunus tomentosa and Shiro-fugen were inoculated with rose buds infected with RMV and PRSV. P. tomentosa and Shiro-fugen reacted characteristically to the PRSV (13, 29). None of these hosts proved to be indicators for RMV.

Oregon RMV isolates, Fulton's RMV isolate and tomato ring spot virus type culture reactions were compared on black cowpea, C. amaranticolor, Bountiful bean and cucumber. The host symptom reactions of the three Oregon RMV isolates and Fulton's isolate suggested strain relationships. The general spectrum of the host symptoms of all the RMV isolates tested was similar to that produced by TomRS virus. Serological reactions of Fulton's RMV with a TomRS type culture antiserum, verified this relationship, and furthermore, the TomRS culture reacted with a rose mosaic virus (F-1) antiserum in cross precipitin tests. The Oregon RMV isolates were not tested against the TomRS antiserum which reacted with Fulton's RMV, but did react with the antiserum produced from Fulton's RMV isolate. This indicates that the Oregon RMV isolates were also strains of tomato ring spot virus.

Fulton's isolate of rose mosaic virus (F-1) was purified. The clarification process, the most critical step in the procedure, relied on the adsorption of the host material to hydrated calcium phosphate (HCP). The ionic strength of 0.02 M of the extracting buffer prevented the virus from being adsorbed to the HCP. The addition of cysteine HCl to the extracting buffer effectively protected the virus from inactivation during extraction and clarification. Other chelating and reducing agents were not as effective. The virus was purified by density gradient centrifugation. Preparations from diseased plants contained two fractions not detected in similar preparations from healthy plants. The bottom zone of a

density gradient column contained infectious virus and had an ultraviolet absorption spectrum typical of a nucleo-protein. Sedimentation rates of the particles in the infectious zone were not determined. The middle zone from the diseased plant preparations did not have any of the characteristics of a virus and was not infectious. Investigations were not conducted to identify this component. The ultraviolet absorption spectrum, and the comparison of electron micrographs of this substance and the virus, did not indicate that these particles were virus precursors. Electron micrographs of TomRS published by Senseney et al. (36) contained small particles similar to those found in the middle zone of RMV preparations. This substance, occurring in RMV and perhaps TomRS infected plants, could be a normal plant component, the synthesis of which was increased, or a new component. In either case, the appearance of this product was induced by RMV infection. The pellet from the 25-50 percent columns contained some infectious material. This pellet, when re-suspended and re-run through a 50-75 percent density gradient column did not produce any new zones, and furthermore, the pellet at the bottom of this column still contained some infectious particles. This can probably be attributed to aggregated virus particles.

A density gradient separation of material prepared from healthy plants showed a zone in a region not apparent in the preparations from diseased plants. This zone had an absorption spectrum typical of a protein. The absence of this protein fraction in the preparations from the diseased plants was probably not due to the

clarification procedure or to an interference in the plant anabolic processes by the virus, but more likely due to a degradation action induced by the RMV infection.

Size of the RMV particles, determined by electron microscopy of purified material, are comparable to the dimensions of TomRS virus reported by Senseney et al. (35). He reports that TomRS was 43.0 x 13.5 mu. However, considerable flattening of the specimens was observed, and if the particles were considered spherical when in a normal state, they would measure approximately 27 mu. This figure closely agrees with the dimensions of 40.0 x 8.6 mu for RMV. However, if the RMV particles were considered to be spheres, a figure of 24 mu is derived.

SUMMARY

1. The effects of virus inhibitors present in rose tissue extracts have been studied. Data are presented to show that extracts from young rose leaves reduced the infectivity of alfalfa mosaic virus up to 58 percent, but those from mature and senescent leaves had little effect. Extracts from rose root increased the infectivity up to 97 percent.
2. A method for the transmission of rose mosaic virus by grafting infected rose buds to Vinca rosa and then mechanically to herbaceous hosts is discussed.
3. Rose mosaic virus isolates were compared on black cowpea, Bountiful bean, cucumber and Chenopodium amaranticolor. Four strains, including Fulton's isolate of RMV, were distinguished on the basis of host and serological reaction of RMV isolates to an antiserum prepared from Fulton's RMV.
4. Host reactions of the RMV isolates were compared with host reactions of tomato ring spot virus. Host and serological reactions of Fulton's RMV with TomRS antiserum were indicative of strain relationship between the two viruses. This relationship was further substantiated by cross-precipitin tests.
5. Fulton's RMV was purified by a method utilizing adsorption of the host materials to hydrated calcium phosphate, differential and density gradient centrifugation. Partially purified preparations from RMV diseased cowpea contained a non-virus component

not observed in similar preparations from healthy cowpea. Preparations from healthy cowpeas also contained a protein fraction not detected in preparations from RMV diseased cowpeas. The purity of the virus preparation was verified by electron microscopy and spectrophotometry. Electron micrographs of purified RMV revealed particles with dimensions similar to those reported for TomRS.

6. Prunus ring spot virus was detected in 45 percent of a plot of hybrid tea roses, but most of the rose rootstocks tested were free from this virus. Evidence of natural spread of PRSV among the hybrid tea roses is presented.
7. Jonathan apple, Hopa crab, Shiro plum, Prunus tomentosa and Shiro-fugen were surveyed as indicator hosts for RMV and PRSV. PRSV infected rose buds caused a characteristic reaction on P. tomentosa and Shiro-fugen. No reactions were observed on the hosts inoculated with RMV.

BIBLIOGRAPHY

1. Baker, Kenneth F. and Earl H. Thomas. The effect of temperature on symptom expression of rose mosaic. Phytopathology 32:321-326. 1942.

2. Brierley, Philip and Floyd G. Smith. Streak, a virus disease of roses. (Abstract) Phytopathology 24:7. 1935.

3. _____. Mosaic and streak diseases of roses. Journal of Agricultural Research 61:625-660. 1940.

4. _____. Spread of rose virus diseases. American Nurseryman July 1, 1940. pp. 5-8.

5. Cadman, C. H. and R. M. Lister. Relationship between tomato ring spot and peach yellow bud mosaic viruses. Phytopathology 51:29-31. 1961.

6. Cochran, L. C. Infection of apple and rose with ring spot virus. (Abstract) Phytopathology 40:964. 1950.

7. Common names of virus diseases used in the Review of Applied Mycology. Vol. 35:Supplement. 1957.

8. Conners, I. L. Twentieth Annual Report of Canada. Plant Disease Survey. 1940. p. 98.

9. _____. Twenty-first Annual Report of Canada. Plant Disease Survey. 1940. p. 98.

10. Current research and investigation. Orchard. N. Z. 31:307. 1958. (Abstracted in Review of Applied Mycology. 38:239. 1959.)

11. Das, C. R. and J. A. Milbrath. Plant-to-plant transfer of stone fruit ring spot virus in squash by pollination. Phytopathology 51:489-490. 1961.

12. Diester, E. A. Plant Diseases. Rose wilt virus. Control of black spot of grape. Agric. Gaz. N. S. W. 64:487-489. 1958. (Abstracted in Review of Applied Mycology 38:283. 1959.)

13. Fink, H. C. Prunus tomentosa as an index plant for sour cherry virus. Phytopathology 45:320-323. 1959.)

14. Fry, P. R. and J. R. Hunter. Rose mosaic virus in New Zealand. New Zealand Journal of Science and Technology Sec. A. 37:478-482. 1956.

15. Fulton, Robert W. Mechanical transmission and properties of rose mosaic virus. Phytopathology 42:413-416. 1952.

16. _____. A rapid method for partial purification of some unstable plant viruses. (Abstract) Phytopathology 47:521. 1957.

17. _____. Purification of sour cherry necrotic ring spot and prune dwarf viruses. Virology 9:522-535. 1959.

18. Gigante, R. Una nuova virosi della rosa in Italia. Boll. Staz. Pot. Veg. Roma. N. S. 16:76-94. 1936. (Abstracted in Review of Applied Mycology 16:179-180. 1937.)

19. Gilmer, R. M. Behavior of juice extracts of certain virus isolates from Prunus and from rose in cucumber plants. (Abstract) Phytopathology 46:241. 1956.

20. Grieve, B. J. Rose wilt and dieback, and virus diseases of rose occurring in Australia. The Australian Journal of Experimental Biology and Medical Science 8:107-121. 1931.

21. _____. Further observations on rose wilt virus. Proc. Roy. Soc. Victoria 54:229-238. 1942. (Abstracted in Review of Applied Mycology 23:18. 1942.)

22. Gualaccini, Franco. Una virosi della Rosa nuova per l'Italia. Suoi rapporti con le virosi dei frutteferi. Bollettino della stazione de Patologia Vegetale 15:79-88. 1957.

23. Holmes, Francis O. Rose mosaic cured by heat treatments. Plant Disease Reporter 44:46-47. 1960.

24. Klastersky, I. A new morphogenic virosis on roses. Stud. bot. Cechosl. 10:1-13. 1949. (Abstracted in Review of Applied Mycology 29:28. 1950.)

25. _____. A cowl-forming virosis in roses, lime trees and elm trees. Stud. bot. Cechosl. 12:73-171. 1951. (Abstracted in Review of Applied Mycology 31:38. 1952.)

26. Kristensen, H. R. and A. Thomsen. Visussygdomme hos roser I. Tidsskr planteavl. 63:369-393. 1959. (Abstracted in Biological Abstracts 35: no. 22349. 1959.)

27. Mushin, Rose. Serological studies on plant viruses. Australian Journal of Experimental Biology and Medical Science 20:59-63. 1942.

28. McWhorter, F. P. Further report on rose mosaic in Oregon. Plant Disease Reporter 15:1-3. 1931.

29. Milbrath, J. A. Selecting stone fruit trees free from virus diseases. Oregon State University. Agricultural Experiment Station. Technicial Bulletin 522. p. 1-27. 1952.

30. _____. The epinasty virus reaction of Kwanzan and Shiro-fugen flowering cherry. Phytopathology 50:495-497. 1960.

31. _____. The relationship of stone fruit ring spot virus to sour cherry yellows, prune dwarf and peach stunt. In: Proceedings of the Fourth Symposium on Virus Diseases of Fruit Trees in Europe, Lyngby, 1960. Tidsskrift for Planteavl 125-133. 1961.

32. Milbrath, D. G. A discussion of the reported infectious chlorosis of the rose. California State Department of Agriculture Monthly Bulletin 19:535-544. 1930.

33. _____. A discussion of the reported infectious chlorosis of rose. Florist Exchange and Horticultural Trade World July 26, 1930. pp. 11 and 33.

34. Nelson, Ray. Infectious chlorosis of the rose. (Abstract) Phytopathology 20:130. 1930.

35. Newton, W. Chlorosis of rose. In: Report of Dominion Botanist for year 1930. Division of Botany. Canada Department of Agriculture. p. 23. 1931. (Abstracted in Review of Applied Mycology 11:245. 1932.)

36. Senseney, C. A., Robert P. Kahn and Paul R. Desjardins. Particle size and shape of purified tomato ring spot virus. Science 120:456-457. 1954.

37. Taubenhaus, J. J. Diseases of ornamentals. Plant Disease Reporter. Supplement 29:447. 1923.

38. _____. Diseases of ornamentals. Plant Disease Reporter. Supplement 42:361. 1925.

39. Thomas, Earl H. Apple mosaic. Hilgardia 10:581-87. 1937.

40. _____ and T. E. Rawlins. Some mosaic diseases of Prunus species. Hilgardia 12:623-644. 1939.

41. _____ and L. M. Massey. Mosaic diseases of the rose in California. Hilgardia 12:647-663. 1939.

42. _____ and C. E. Scott. Rosette of rose. Phytopathology 43:218-219. 1953.

43. Valleau, W. D. A virus disease of plum and peach. Kentucky Agricultural Experiment Station. Bulletin 327:89-103. 1932.

44. Van Slogteren, D. H. M. Serological micro-reactions with plant viruses under paraffin oil. In: Proceedings of the Second Conference on Potato Virus Diseases, Wageningen, Netherlands, 1945. Wageningen, H. Veenman & Zonin, 1955. pp. 51-54.

45. White, R. P. An infectious chlorosis of rose. Plant Disease Reporter 12:33-34. 1928.

46. _____. An infectious chlorosis of rose. New Jersey Agricultural Experiment Station. Annual Report. Bulletin 42:42-43. 1929.

47. _____. An infectious chlorosis of rose. (Abstract) Phytopathology 20:130. 1930.

48. _____. An infectious chlorosis of rose. The American Rose Annual 15:87-90. 1930.

49. _____. Infectious chlorosis of rose. Florists Exchange and Horticultural Trade World January 25, 1930. p. 46.

50. _____. Quarantines and rose chlorosis. Florists Exchange and Horticultural Trade World March 15, 1930. pp. 50a and 54.

51. _____. Chlorosis of the rose. Phytopathology 22:53-69. 1932.

52. Weiss, F. and F. P. McWhorter. Pacific coast survey for rose mosaic. Plant Disease Reporter 14:203-205. 1930.

53. Wildon, C. E. Michigan rose men discuss infectious chlorosis. Florists Exchange and Horticultural Trade World March 8, 1930. p. 58.

54. Willison, R. S., M. Weintraub and J. D. Ferguson. Purification and electron microscopy of viruses causing cherry yellows and related diseases. Canadian Journal of Botany 34:86-103. 1956.