Multiplication, Purification, and Properties of Ryegrass Mosaic Virus

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Cooperative investigation of the Chemistry and Biology Research Institute (Contribution No. 864) and the Research Station, Vancouver (Contribution No. 353).

Technical assistance of Rhoda Lau at Ottawa and of A. Valcic, S. Blevings and W. Ronald at Vancouver, is greatly appreciated. We are thankful to V. Helson and W. McCormick, Environmental Physiology Section, Ottawa Research Station for providing controlled environment facilities.

Accepted for publication 5 August 1975.

ABSTRACT

PALIWAL, Y. C., and J. H. TREMAINE. 1976. Multiplication, purification, and properties of ryegrass mosaic virus. Phytopathology 66: 406-414

Ryegrass mosaic virus, infected *Lolium multiflorum* 'S22' plants under 19,350 lx of light for 15 hours per day at 15, 20, and 25 C, but not at 30 or 34 C and the highest virus concentration in plants was reached at 15 C. In tests with different light intensities and photoperiods at 15 C, 21,400 lx of light and 15 hours photoperiod were optimum for virus multiplication and accumulation in plants. The virus, purified to apparent homogeneity, had a sedimentation coefficient of 166S, ultraviolet (259 nm) extinction coefficient of 2.50, $A_{259/280}$ ratio of 1.25, buoyant density (in CsCl) of 1.307 g/cm^3 at 15 C and 1.325 g/cm^3 at 25 C. The virus contains 5.3% ribonucleic acid (RNA) based on its phosphorus content of 0.48%. RNA of the virus prepared by treatment with sodium dodecyl sulfate (SDS), or SDS and phenol, was infectious and had a molecular weight of 2.7×10^6 daltons estimated from its mobility in polyacrylamide gels. The molar percentages of nucleotides in the viral RNA were 31% guanylic acid, 23% adenylic acid, 24% cytidylic acid, and 22% uridylic acid. Virus protein showed as a single component in SDS polyacrylamide gel electrophoresis and its molecular weight was estimated to be 29,200. The amino acid analysis indicated 263 amino acid residues in the protein.

Additional key words: light intensity, photoperiod.

Ryegrass mosaic (34) is caused by a sap-transmissible virus (RMV) widespread in the Gramineae in Britain (38). It occurs in British Columbia and Washington in ryegrass species (*Lolium perenne* L. and *L. multiflorum* Lam.) and orchard grass (3, 31). Isolates from U.K., Canada, and U.S.A. are related serologically (33). The virus particles are flexuous rods 703-nm long.

Problems encountered in preliminary work with RMV included low virus concentration in infected plants grown under usual greenhouse conditions, aggregation during purification, and instability of the virus. These problems are also common with potyviruses which are aphid-transmitted filamentous viruses 700-790 nm in length (13).

In early attempts to purify the virus, yields of RMV were highly variable. Differences in the concentration of the virus in infected plants were suspected to be one of the causes of this problem. The effects of environmental factors on virus multiplication were therefore investigated. We report in this paper: (i) the effects of temperature, light intensity, and photoperiod on multiplication of RMV in *L. multiflorum,* (ii) a method of purification that increased virus yields, and (iii) the physical and chemical properties of the virus and its components.

MATERIAL AND METHODS.—*The virus.*—RMV, isolated from Italian ryegrass (*Lolium multiflorum*) in British Columbia in 1960 (31), was propagated in *L. multiflorum* 'S22' or 'Gulf' for virus multiplication and purification experiments. Plants at the three- to four-leaf stage were sap-inoculated with the juice of infected plants mixed with 22-μm (600-mesh) Carborundum.

Plant growth condition.—The effects of temperature, different intensities and photoperiods of light (75% fluorescent and 25% incandescent) on virus multiplication, were tested in controlled environment growth rooms. Ryegrass plants grown in fibre pots in a separate growth room at 25 C and 17,100 lx (1,600 ft-c) illumination for 15 hours/day were transferred to the test conditions 2 days before inoculation. Five temperatures: 15, 20, 25, 30, and 34 C; three photoperiods: 8, 15, and 22 hours; and three light intensities (10,700, 21,400, and 26,750 lx) were tested. Plants thinned to eight per pot were inoculated 21 days after sowing. All plants were given a commercial 20-20-20 nutrient solution once every 2 weeks. Each nutrient application provided 200 mg each of N, P, and K and 450, 300, 25, 148, 70, and 400 μg of Mn, Fe, Cu, Zn, Mo, and B, respectively, per pot.

For virus purification work, plants were inoculated and maintained at 16 C with 20,400 to 21,400 lx of light for a 15-hour photoperiod per day, and received the nutrient supplement every week.

Virus assay.—A serological assay using precipitation end points as a measure of relative virus (antigen) concentration was used in all experiments. For determining the effect of temperature and light on virus

multiplication, 10 pots of virus-inoculated and 10 of uninoculated plants were kept in the test conditions. On each day of assay all the plants from one of the 10 pots and a corresponding control were harvested, mixed well and a 30-g sample was removed. Sap extracted from each sample was clarified by heating to 44 C for 15 minutes and centrifuging at 12,500 *g* for 15 minutes. Serial 1.5-fold dilutions of the sap of infected plants, and undiluted and a 2-fold dilution of sap from healthy plants were tested against a RMV antiserum (homologous titre, 1/1,280) at a dilution of 1/80 in a tube precipitin test. No reactions were observed with healthy plant sap and normal serum controls. The infectivity of clarified sap or purified preparations was tested by inoculating 2-fold dilutions to ryegrass plants and comparing the infectivity dilution end points.

Purification procedure.—All procedures were carried out at 2-4 C. Infected plants harvested 20-21 days after inoculation were triturated in a motorized meat grinder and juice extracted through nylon cloth. The pulp was again ground in a mortar with acid-washed sand and soaked in 0.5 ml of 0.01 M sodium citrate, pH 8.0 (hereafter called "citrate") per g of leaves; the juice was extracted again. The pooled juice was heated to 44 C for 15 minutes, cooled in an ice bath, and centrifuged at 12,500 *g* for 15 minutes. Virus was precipitated from the supernatant by mixing 0.5 volume of saturated aqueous ammonium sulphate [$(NH_4)_2SO_4$] and centrifuging at 11,000 *g* for 15 minutes (low speed). The pellets were suspended in a volume of citrate equal to one-quarter that of the clarified juice, and centrifuged again at low speed. The supernatant was then centrifuged at 68,000 *g* for 90 minutes. The pellets, after soaking for 3 hours in citrate, were pooled, homogenized with a tissue grinder, and centrifuged at low speed. The pellet was suspended in citrate and centrifuged again to recover trapped virus particles. The pooled supernatant (50- to 60-fold concentrated, based on the volume of clarified juice) was subjected to rate zonal sucrose-density gradient (10-40% sucrose in citrate) centrifugation at 54,000 *g* for 2 hours in a Spinco SW25 rotor. A light-scattering zone at 29-32 mm depth had maximum infectivity and highest virus titre in the gradient column, and contained a high concentration of filamentous RMV particles. The virus zones removed from the tubes by piercing with a hypodermic needle were pooled, diluted 2.5-fold with citrate, and subjected to equilibrium zonal density-gradient (20-60% sucrose in citrate) centrifugation for 16 hours at 40,692 *g* in a SW25 rotor. A sharp virus zone at a depth of 43 to 46 mm contained the highest virus concentration in the gradient. A 2-ml quantity was removed from each tube, dialyzed overnight (or at least 5 hours) against citrate, then the virus was pelleted by centrifuging at 66,000 *g* for 2 hours. Suspension of the pellets in citrate, and sometimes an additional low-speed centrifugation, completed the purification. Virus preparations were assessed by serological assay, and by examination in a Philips EM 300 electron microscope after negative staining with 1% phosphotungstic acid.

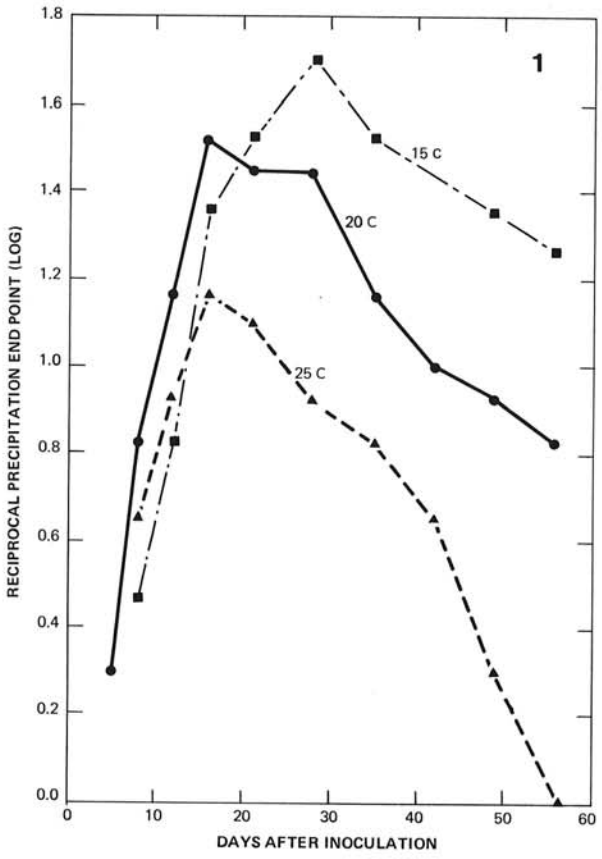

Fig. 1. Effect of temperature on relative ryegrass mosaic virus (antigen) concentration in ryegrass plants at different times after inoculation.

Ultraviolet extinction coefficient, sedimentation coefficient, and buoyant density determination.—The purified virus pellets were suspended in double-distilled water and the ultraviolet absorption spectrum recorded. Dry weight of the virus for calculating the extinction coefficient was determined by placing 0.2 ml of the virus suspension on a microscope coverglass and drying to constant weight at 90 C.

Sedimentation coefficients were determined in a Beckman Model E analytical ultracentrifuge using schlieren optics. Virus suspended in citrate with 0.1 M sodium chloride was centrifuged at 31,410 rpm, in an An-D rotor at 20 C. The graphical method of Markham (26) was used to calculate the sedimentation coefficient.

Buoyant density g/cm^3 of the virus was determined by equilibrium banding in cesium chloride (CsCl) in the analytical ultracentrifuge (7). Equilibrium centrifugation of the virus was also performed using the CsCl step gradient method (4) incorporating the modification of Sehgal et al. (29) and the buoyant density was determined from the refractive index of the sample containing the virus band. The CsCl step gradient consisted of 2 ml each of top and bottom solutions with densities of 1.252 and 1.500, respectively.

Preparation of viral nucleic acid (NA) and nucleotide analysis.—A volume of purified virus was added to an equal volume of ribonucleic acid (RNA) extraction buffer, pH 9, containing 0.2 M ammonium carbonate, 0.002 M disodium ethylenediaminetetraacetate (EDTA), 0.2% sodium diethyldithiocarbamate, 2% sodium dodecyl sulphate (SDS), and about 200 μg/ml bentonite (2) and kept at 3-4 C for 1 hour. Two volumes of 80%

aqueous phenol with 0.1% 8-hydroxyquinoline were added to the virus-buffer mixture, stirred for 10 minutes at 2 C, and centrifuged at 3,200 *g* for 15 minutes. The top aqueous layer was removed, and bentonite was added to 100 μg/ml. Then the NA was precipitated by adding two volumes of 95% isopropanol and 0.1 volume of 1.0 M sodium acetate, pH 5.5. After storage at −20 C for 3 hours the mixture was centrifuged at 8,720 *g* for 15 minutes and the pellets were suspended in 0.015 M sodium citrate buffer, pH 7.0, containing 0.15 M sodium chloride (SSC). After reprecipitation with one volume of isopropanol, pellets obtained by centrifugation were washed twice with isopropanol to remove traces of phenol. The final pellets were suspended in SSC. In some experiments, the phenol step was omitted and the virus was incubated in SDS buffer for 3 hours or overnight. Sodium dodecyl sulfate was precipitated and removed by adding potassium chloride to 0.04 M, letting stand at 2 C for 4-5 hours and centrifuging. After addition of bentonite to the supernatant, the NA was precipitated with isopropanol and resuspended in SSC twice. For infectivity tests, the final pellet was suspended in 0.035 M dibasic potassium phosphate, and 0.05 M glycine (pH 9.2) a buffer useful in RNA infectivity studies with other viruses (Brakke, *personal communication*).

For nucleotide analysis the RNA preparations described above were hydrolysed in 1N HCl in a sealed glass tube in a boiling water bath for 1 hour. The purine and pyrimidine nucleotides were separated and determined as described by Markham (25).

Polyacrylamide gel electrophoresis of viral protein and nucleic acid.—Gel electrophoresis of proteins was performed in 2.5, 3.5, and 5.0% polyacrylamide gels containing SDS. Virus proteins and standards were boiled for 1 minute in 0.1 M sodium phosphate buffer, pH 7.2, containing 4 M urea, 1% SDS, and 1% mercaptoethanol. The protein markers were bovine serum albumin, ovalbumin, yeast alcohol dehydrogenase, carbonic anhydrase, and chymotrypsinogen A. The markers were applied separately and in various combinations with the virus protein at a concentration of approximately 10 μg per gel. Electrophoresis was at 7 mA/gel for 3 hours after which the gels were fixed and stained with coomassie blue in trichloroacetic acid by the method of Chrambach et al. (8).

The molecular weight of the nucleic acid was estimated by electrophoretic movement relative to standards in 2.4% polyacrylamide gels (23). Ryegrass mosaic virus and other viruses used as standards were dissociated before layering on the gels by adding 1 ml of a buffer pH 9, composed of 0.02 M Tris-HCl, 0.001 MEDTA, 1% SDS, and 4 M urea to 1-2 mg of the virus and heating for 10 minutes at 50 C.

Amino acid and phosphorus analyses.—The amino acid composition was determined with the nucleoprotein in seven virus preparations. In addition, two analyses were made on the virus protein which was isolated from the nucleic acid by dialysis of the virus against 1 N sodium hydroxide for 2 days at 4 C.

Cysteine and methionine determinations were made on separate samples of virus protein oxidized with performic acid for 24 hours. On completion of the oxidation, the excess performic acid was removed by dialysis. The cysteic acid and methionine sulfone contents were estimated by reference to the quantities of aspartic and glutamic acids determined in the same analysis. Aliquots of whole virus preparations were used for the analysis of tryptophan content (35).

Inorganic phosphorus was determined by the method

TABLE 1. Infectivity of sap from RMV inoculated plants maintained at three different temperatures

Days after inoculation	Dilution end points (reciprocal)[a]		
	15 C	20 C	25 C
5	...	32	8
8	48	128	64
21	512	512	192
28	1,024	512	128
56	256	128	16

[a]A portion of the sap clarified for serological assay was used to make a series of two-fold dilutions in distilled water. Data based on at least 30 plants (two experiments) inoculated with each dilution.

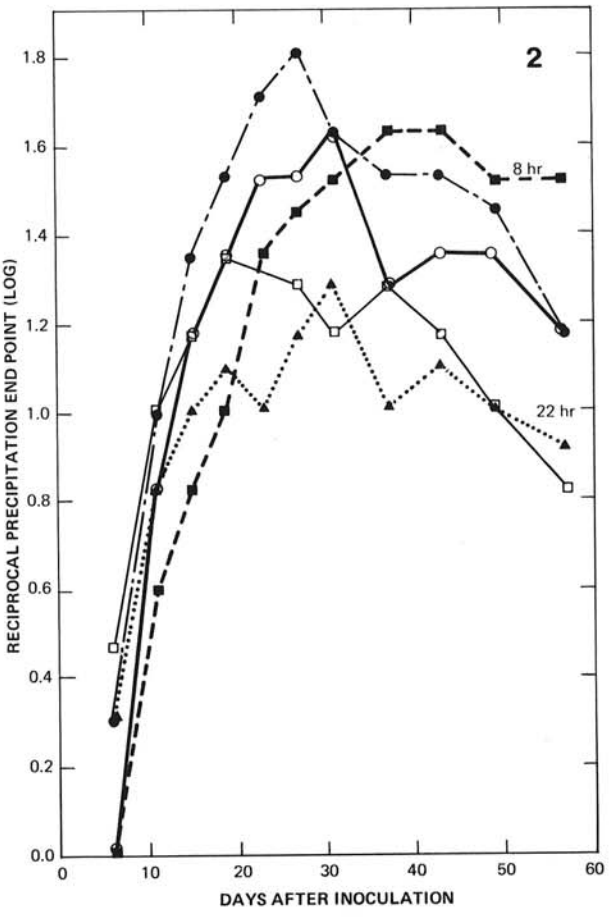

Fig. 2. Effect of light intensity and photoperiod on relative ryegrass mosaic virus concentration in ryegrass plants at different times after inoculation (temperature 15 C constant). o——o, 10,700 lx; ●- - - - -●, 21,400 lx; □——□ 26,500 lx of fluorescent and incandescent light for 15 hours per day, photoperiods tested ■- - -■ 8 hours and ▲....▲, 22 hours of 21,400 lx light per day.

of Chen et al. (6) from samples of virus in double distilled water after heating at 190-200 C for 15 minutes with 70% perchloric acid. The amount of virus protein in the samples was determined by amino acid analysis.

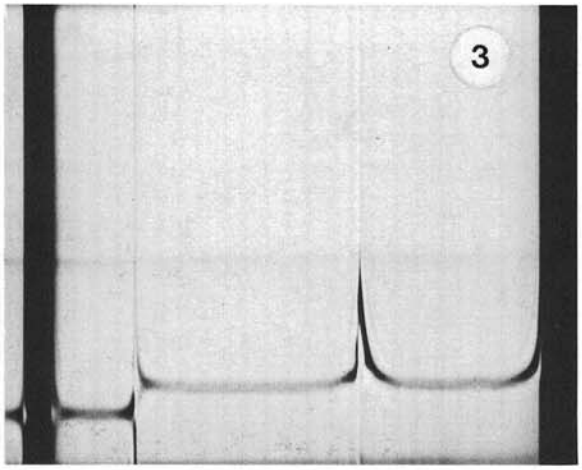

Fig. 3. Sedimentation pattern of purified ryegrass mosaic virus in 0.01 M sodium citrate with 0.1 M sodium chloride photographed through schlieren optics during an analytical ultracentrifuge run at 31,410 rpm. (20 C, 12 minutes after reaching speed).

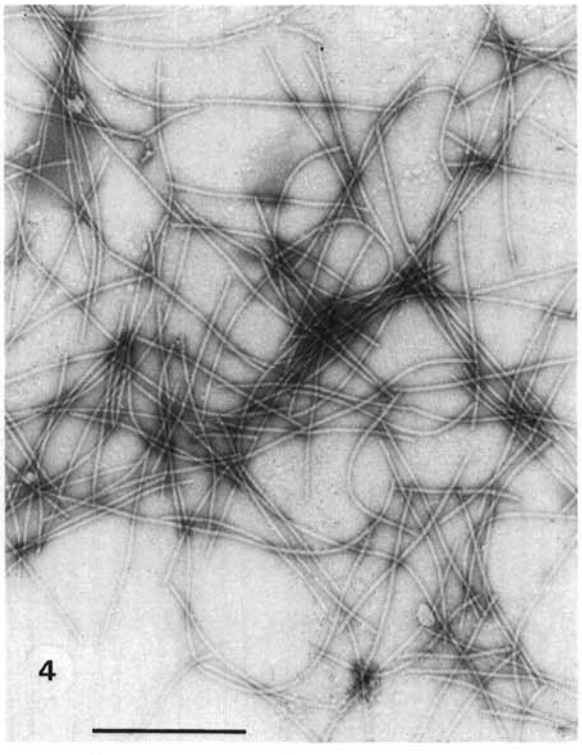

Fig. 4. Ryegrass mosaic virus particles in a diluted purified preparation in 0.01 M sodium citrate (pH 8.0). Small spherical spots are artifacts from formvar membrane and phosphotungstic acid negative stain. (Calibration bar = 0.5 μm).

Purified virus and RNA preparations, containing 0.2% sodium azide, were shipped from Ottawa to the Vancouver laboratory for determination of certain properties and analysis of protein and RNA.

RESULTS.—*Effect of temperature, light intensity and photoperiod on relative RMV concentration in plants.*—To study the effect of temperature, inoculated plants were grown under 19,350 lx with a 15-hour photoperiod per day. At 15, 20, and 25 C, symptoms of RMV appeared within 5-8 days after inoculation. At 30 and 34 C no symptoms appeared and the sap from these plants was not infective even at period up to 25 days after inoculation. Serological assays at different times after inoculation (Fig. 1) showed the highest virus concentration in plants kept at 15 C. At this temperature virus concentration was greatest at 28 days after inoculation and remained fairly high even after 56 days. At 20 and 25 C the highest virus concentrations were reached at day 16, but were only 75% and 33%, respectively, of the maximum at 15 C. After reaching peaks at higher temperatures the virus concentration decreased more rapidly than at 15 C and after 56 days at 25 C no virus was detectable.

Infectivity tests (Table 1) of a portion of the clarified juice used for six of the serological assays showed a similar pattern of virus multiplication, confirming the reliability of the serological assay of virus concentration in plants.

To test the effects of varying light intensity, inoculated plants were grown at 15 C under 10,700, 21,400 and 26,750 lx for 15-hour photoperiod per day. The highest virus concentration was reached under 21,400 lx, 27 days after inoculation and was maintained at a fairly high level until 48 days (Fig. 2). Under 26,750 lx, the virus multiplied almost as rapidly as at 21,400 lx for the first 12 days, but then the concentration decreased.

To test the effect of photoperiod, inoculated test plants were grown at 15 C under 21,400 lx. The 15-hour photoperiod was optimal for virus multiplication (Fig. 2). With a 22-hour photoperiod the pattern of virus multiplication was similar to that under high light intensity. The 8-hour photoperiod slowed virus multiplication; the virus concentration reached a maximum 10-12 days later than under a 15-hour photoperiod, but remained at fairly high levels even up to 57 days.

Virus purification.—As the inoculated plants were grown under conditions of temperature, light, and nutrition optimum for virus multiplication, virus yields from different batches of plants were reproducible, indicating that the virus concentration in plants was consistent.

Acidifying the juice to pH 5, or adding 0.01 M silver nitrate (11) did not give adequate clarification, and the virus aggregated. Shaking with 0.33 volume of chloroform gave good clarification, but after high-speed centrifugation of the clarified sap the pellets were difficult to suspend and a considerable amount of virus was lost in the low-speed centrifugation. Heating the sap to 44 C for 15 minutes provided the best clarification, but reduced the infectivity of the virus by 5 to 10%.

Side-to-side aggregation of virus particles was encountered during purification steps after juice

clarification. Virus yields were higher when $(NH_4)_2SO_4$ rather than polyethylene glycol 6000 was used for precipitation of virus in clarified sap. However, prolonged contact of the virus with $(NH_4)_2SO_4$ during precipitation caused irreversible aggregation of the particles. It is important, therefore, to centrifuge the sap within 10-15 minutes of mixing the $(NH_4)_2SO_4$ and to suspend the precipitated virus immediately in citrate. RMV tends to aggregate more with increasing degree of purification. Neutral 0.01 M potassium phosphate buffer, distilled water, and citrate were compared as solvents for the virus at different stages of purification and in sucrose density-gradient columns. Citrate minimized aggregation of virus particles, and in it the virus was relatively stable; only 15-20% of the particles showed some degradation after 8 days at 4-5 C. The virus aggregated in phosphate buffer and distilled water and some degradation of virus particles was observed in phosphate buffer preparations. Virus preparations purified in citrate were twice as infective as those purified in phosphate buffer, and virus yields (2.5 mg/kg of leaves) were about 40% greater than with phosphate buffer or distilled water. Phosphate buffer at pH 8 induced much less aggregation of the virus than at pH 7, but the virus preparations were not as infective as those prepared with citrate. Purified virus was denatured either by freezing or by dialysis against distilled water.

The purified virus in citrate showed a single peak in schlieren optics upon analytical ultracentrifugation (Fig. 3). Virus preparations were free of noticeable host contaminants and contained 703-nm-long filamentous particles typical of RMV (Fig. 4).

Ultraviolet absorption.—The purified virus, in citrate, had an absorption spectrum (Fig. 5) typical of a filamentous virus with a maximum at 259 nm and a minimum at 247 nm. A tryptophan shoulder at 288 nm was always present in the virus spectrum. The $A_{259/280}$ and $A_{259/247}$ ratios were 1.25 ± 0.05 and 1.08 ± 0.02, respectively. The $A_{280/260}$ ratio of different preparations varied from 0.81 to 0.83, but indicated a nucleic acid content of the virus near 6%. The extinction coefficient of the virus, at 259 nm determined by weighing the virus at different concentrations was 2.50 ± 0.04.

Sedimentation coefficients.—The sedimentation coefficient (S) of four suspensions (0.2 to 0.8 mg/ml) of the virus in citrate with 0.1 M sodium chloride increased with dilution of the virus, from 148S to 162S. The S_{20w} of RMV extrapolated to infinite dilution was 166S.

Buoyant density.—RMV at 50 μg/ml in a CsCl solution of 1.324 density was centrifuged 16 hours to equilibrium in the analytical ultracentrifuge at 25 C. The buoyant density (ρ) of the virus was calculated, using the formula of Ifft et al. (19), to be 1.325 ± 0.001 g/cm^3 in two experiments. The ρ of tobacco mosaic virus, centrifuged with RMV in the same cell and in another cell, also came to 1.325 g/cm^3 in two experiments. Equilibrium centrifugation of the virus in a Spinco SW40 rotor at 35,000 rpm and 15 C was performed using CsCl step gradients. In four experiments ρ of RMV was 1.3049, 1.3060, 1.3050, and 1.3103 g/cm^3 and of southern bean mosaic virus was 1.353, 1.359, 1.359, and 1.360 g/cm^3. Samples of RMV removed from the virus band were infectious and the virus retained its normal morphology. In one experiment, purified RMV was fixed by incubating with 5% glutaraldehyde in citrate (pH 7.2) for 6 hours. Buoyant density of the fixed virus, determined after dialysing against citrate to remove glutaraldehyde, was still 1.3049 g/cm^3.

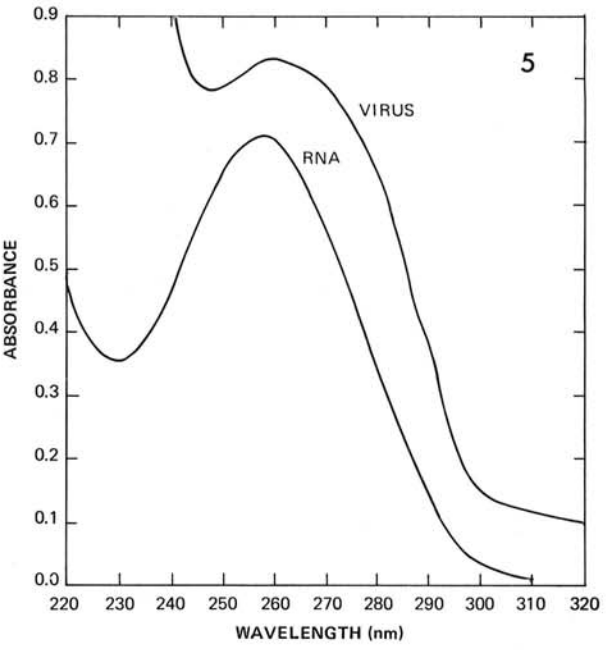

Fig. 5. Ultraviolet absorption spectra of purified ryegrass mosaic virus (RMV) in citrate and RMV-RNA in 0.15 M sodium chloride, 0.015 M sodium citrate (pH 7.0).

Properties of the viral nucleic acid.—Nucleic acid (NA) was prepared from virus either freshly purified or stored at 2 C. Nucleic acid was not separable from frozen virus which precipitated upon thawing. Yields of NA were 30-35 μg/mg of virus (estimated from $E^{0.1}_{259} = 24$). RMV-NA reacted positively with orcinol (30), but gave a negative diphenylamine reaction for deoxyribonucleic acid (5).

The ultraviolet absorption spectrum of RMV-RNA (Fig. 5), prepared by the SDS-phenol method and taken in SSC, had a maximum at 258 nm and a minimum at 232 nm. The $A_{258/232}$ values varied from 1.89 to 2.02 and $A_{258/280}$ values from 1.91 to 2.16 indicating minimal contamination with protein. These absorption ratio values were slightly lower for the RNA prepared without the phenol step. Preparations of RNA examined in the electron microscope, were devoid of virus particles.

When infectivity of each of four preparations containing about 60 to 90 μg/ml RMV-RNA and 100-200 μg/ml bentonite was tested on seven or eight ryegrass plants, 2,3, 0, and 1 plants became infected with RMV. Preparations of RNA made without the phenol step were always infectious and usually 50-75% of the inoculated plants became infected. Infectivity of RNA preparations was lost completely when treated with 0.1 μg of pancreatic ribonuclease per ml of RNA preparation for 30 minutes at room temperature.

A single component was observed when RNA from dissociated RMV was electrophoresed in polyacrylamide gels. Molecular weight of the RNA was estimated to be 2.7×10^6 (Fig. 6) by comparing its mobility with those of

RNA from tobacco mosaic virus having a molecular weight of 2.05×10^6 and with four components of brome mosaic virus having molecular weights of 1.06×10^6, 1.01×10^6, 0.74×10^6, and 0.31×10^6 (10). The molecular weight of white clover mosaic virus RNA determined in our tests was 2.3×10^6.

The base composition of the RNA based on the averages of five determinations with each of three preparations are shown in Table 2. Phosphorus analysis of the virus in triplicate on three preparations gave an average value of 0.48% indicating a nucleic acid content of 5.3%.

Properties of the protein.—The protein of RMV was examined by SDS polyacrylamide gel electrophoresis, and most preparations showed a single component with a mobility indicating a molecular weight of 29,200, but some preparations showed a minor component with an estimated molecular weight of 62,000, which was assumed to be a dimer. Estimated molecular weights of about 29,200 were obtained in gels with concentrations of 2.4 to 10%.

The molar percentages calculated from amino acid analyses of 24-hour hydrolyzates of virus preparations are presented in Table 3. The values were obtained by averaging analyses of seven virus preparations; each analysis was within 5% of the average value. Analyses of separated protein gave similar results, except that values for glycine were 4% lower. The content of the acidic amino acids, aspartic and glutamic, was twice that of the basic amino acids.

Since the molecular weight of the virus protein subunit was 29,200, the molar percentages were multiplied by 2.62 to yield relative molar ratios with integer values totalling 263. A protein subunit containing 263 amino acid residues would have a molecular weight of 29,100.

DISCUSSION.—Since the highest concentration of the virus in ryegrass developed at 15 C with 21,400 lx of light, and infection did not occur at 30 and 34 C, RMV can be designated as a low-temperature virus with a relatively high light requirement. Although multiplication of the virus was rapid at 20-25 C, 15 C seems to be optimum for virus accumulation in plants. The rate of degradation of the virus in vivo, increased with temperature and was apparently higher than the rate of multiplication at 20 and 25 C, especially 20 days or more after inoculation. The filamentous viruses of the Gramineae differ considerably in their temperature and light requirements. Maize mosaic virus (a strain of sugarcane mosaic virus) favours relatively high temperature (27-43) and long days for its multiplication (27). The other extreme is represented by wheat spindle streak mosaic virus with an optimum development in wheat at 8-10 C and about 10,000 lx, 12 hours per day (32). An intermediate temperature, 20 C, was found to be optimal for multiplication of wheat streak mosaic virus in wheat plants (1). Environmental conditions optimal for multiplication appear to be specific properties of a virus, and they may vary considerably within a single group of viruses having many common attributes.

Ryegrass mosaic virus, like most other 700-to790-nm-long filamentous viruses, occurs in low concentrations in the sap of infected plants. Yields of purified virus were increased by growing plants under optimum conditions for virus multiplication and by minimizing aggregation of virus particles during purification. Side-to-side aggregation of virus particles, a serious problem in RMV purification, was minimized by maintaining the virus at pH 8 and at low temperature throughout the purification procedure. Phosphate ions seem to be detrimental to the virus; phosphate buffer, even at pH 8, reduced infectivity, and the virus was still partially aggregated. Phosphate buffer has been reported to reduce infectivity and/or serological activity of several rod-shaped viruses (37), and causes degradation of apple chlorotic leaf spot virus (22).

The chemical and physical properties of four viruses with morphology and vectors common to members of the aphid-transmitted potyvirus group have been partly established. Ryegrass mosaic virus is the first virus of Gramineae with similar morphology but with a mite vector to be purified and fully characterized by its physical and chemical properties. Hiebert and McDonald (14) found two protein components, termed slow and fast, in SDS polyacrylamide gel electrophoresis of tobacco etch virus, turnip mosaic virus, and potato virus Y. The slow form was found in preparations purified quickly and it moved anomalously in gels of different concentrations. Similar protein components were reported for maize dwarf mosaic virus B (15). RMV preparations showed a single protein component with an estimated molecular weight of 29,200 which is similar to values of 26,500, 26,840, 28,600, and 28,500 obtained for the fast components of tobacco etch, turnip mosaic, potato virus Y, and maize dwarf mosaic virus, respectively. The

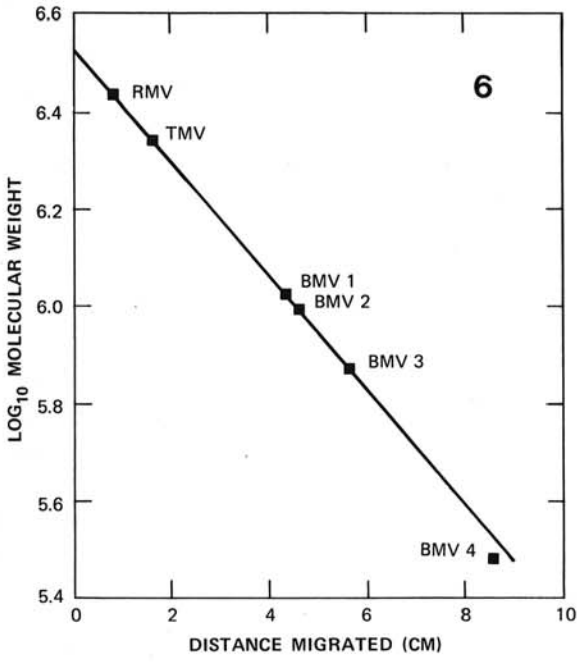

Fig. 6. Determination of molecular weight of ryegrass mosaic virus RNA by electrophoresis on 2.4% polyacrylamide gels. The line is obtained by plotting the log of molecular weight of marker RNA's against their migration distances. Abbreviations are: TMV, tobacco mosaic virus RNA (2.05×10^6); BMV-1, BMV-2, BMV-3, and BMV-4 are components of brome mosaic virus with molecular weights of 1.06×10^6, 1.01×10^6, 0.74×10^6, and 0.31×10^6 daltons, respectively.

TABLE 2. Molar percentages of recovered nucleotides of ryegrass mosaic virus RNA compared with those for other members of the potyvirus group (except potato virus Y)

Virus	Guanylic acid	Adenylic acid	Cytidylic acid	Uridylic acid
Ryegrass mosaic[a]	31	23	24	22
Tobacco etch[b]	22.8	30.0	20.1	27.1
Turnip mosaic[c]	22.4	34.5	22.3	20.8
Maize dwarf mosaic[d]	20.2	33.5	16.2	30.1
Pea seed-borne mosaic[e]	22.8	44.0	17.6	15.6

[a]Based on averages of five determinations with each of three preparations.
[b]Damirdagh and Shepherd (9).
[c]Hill and Shepherd (16).
[d]Hill et al. (15).
[e]Knesek et al. (20).

TABLE 3. Amino acid compositions of ryegrass mosaic virus and members of the potyvirus group

Amino acid	Ryegrass mosaic virus		Molar percentage				
	Molar percentages[a]	Relative molar ratio	PVY[b]	TEV[c]	TUMV[d]	MDMV[e]	PSBMV[f]
Lysine	3.78	10	6.50	5.02	5.63	4.50	3.82
Histidine	1.84	5	2.32	3.05	3.46	1.96	2.54
Arginine	6.75	18	5.90	6.67	7.36	5.39	6.11
Aspartic acid	9.80	26	11.37	12.70	12.55	10.12	12.72
Threonine	9.21	24	6.90	6.58	6.93	9.55	5.60
Serine	8.72	23	5.10	4.60	4.33	7.50	7.63
Glutamic acid	11.91	31	11.91	11.94	9.96	11.01	12.98
Proline	5.99	16	5.67	4.31	3.90	3.74	4.07
Glycine	6.10	16	6.78	6.97	6.49	12.74	7.12
Alanine	10.47	27	8.40	9.59	7.36	8.63	9.16
Cysteine	1.86	5	0.36	0.51	0.43	0.38	...
Valine	4.01	10	6.50	6.31	5.19	4.67	6.36
Methionine	2.11	6	4.25	5.34	4.33	4.17	5.34
Isoleucine	2.12	6	6.00	2.43	4.76	2.91	4.58
Leucine	7.71	20	5.02	6.91	8.66	5.08	5.85
Tyrosine	2.26	6	3.35	3.43	3.46	3.39	3.31
Phenylalanine	3.05	8	2.47	2.62	3.90	2.73	2.80
Tryptophan	2.29	6	1.19	1.03	1.30	1.51	...

[a]Determined from amino acid analysis of seven virus preparations, by performic acid oxidized protein of two preparations and tryptophan determinations on two preparations.
[b]Calculated for potato virus Y (PVY) from Stace-Smith and Tremaine (36).
[c]Calculated for tobacco etch virus (TEV) from Damirdagh and Shepherd (9).
[d]Calculated for turnip mosaic virus (TUMV) from Hill and Shepherd (16).
[e]Calculated for maize dwarf mosaic virus (MDMV) from Hill et al. (15).
[f]Calculated for pea seed-borne mosaic virus (PSBMV) from Knesek et al. (20).

absence of a slow component in RMV is not too significant because Hill and Shepherd (17) observed only the fast component in their studies of tobacco etch and turnip mosaic viruses.

The amino acid compositions of four members of the potyvirus group and RMV are compared in Table 3. All have high contents of aspartic and glutamic acids and other features in common. However, conclusions drawn from a comparison of amino acid compositions must await determination of the chemical difference between the fast and slow protein forms in the potyvirus group.

The RNA content determined by phosphorus analysis was 5.3% and is similar to values of 5.4%, 5.1%, and 5.0% determined by the same method for potyviruses potato virus Y, tobacco etch, and turnip mosaic viruses, respectively (9, 16, 36). Hill et al. (15) reported a higher value, 6%, for the RNA content of maize dwarf mosaic virus.

The nucleotide composition of RMV nucleic acid is different from the reported compositions of potyviruses (Table 2). RMV has a higher content of guanylic acid and a lower content of adenylic acid than the four potyviruses. Hill et al. (15) proposed that a high adenylic acid content may be a characteristic of potyviruses, but Table 2 shows that contents of uridylic acid vary considerably with these viruses.

The molecular weights of the RNA's of 700-to790-nm filamentous viruses should be similar if they have identical helical structures. The molecular weight of the RNA of RMV determined by polyacrylamide gel electrophoresis is 2.7×10^6 (Fig. 6). Similar values of 2.8×10^6 for wheat streak mosaic virus (2), 2.7×10^6 for maize dwarf mosaic virus (28), and 3.1×10^6 for potato virus Y (24) were determined with formaldehyde-treated RNA's using sedimentation rates in linear log sucrose gradients. A higher value of 3.5×10^6 for the molecular weight of

turnip mosaic virus RNA was reported by Hill and Shepherd (16) using the polyacrylamide gel electrophoresis method. In the same study, Hill and Shepherd (16) also reported values of 3.1×10^6 and 2.8×10^6 for the molecular weights of the RNA's of two strains of white clover mosaic, a 480-nm filamentous virus. These values are higher than the 2.3×10^6 value we obtained, and also higher than the 2.4×10^6 value Koening (21) obtained for the RNA molecular weight of white clover mosaic virus using the same method.

The buoyant density of RMV (1.325 g/cm^3) determined at 25 C in a CsCl density gradient in the analytical ultracentrifuge agreed with values of 1.318, 1.321, 1.330, and 1.326 g/cm^3 determined for four members of the potyvirus group; i.e., bean yellow mosaic virus, pea mosaic virus, lettuce mosaic virus, and potato virus Y, respectively, at 20 C by Huttinga and Mosch (18). The variation in density between members of the potyvirus group is difficult to interpret since these authors reported variable quantities of high and low molecular weight viral proteins in their preparations. A change in the size of the protein relative to the RNA would affect the buoyant density greatly. The average value for buoyant density of RMV determined at 15 C in a swinging bucket rotor (1.307 g/cm^3) is lower than that determined for this virus in the analytical ultracentrifuge. On the other hand, the buoyant density of maize dwarf mosaic virus was reported to be 1.300 g/cm^3 at 25 C in a SW 50 rotor by Gordon and Gingery (12). The range in values of potyviruses reported by Huttinga and Mosch (18) may be dependent on the degree of limited proteolysis of the virus occurring in plant sap described by these authors, or it may be a valid difference based on differences in amino acid and nucleotide composition and structure. The reason(s) for difference in buoyant density of RMV at 15 and 25 C are not clearly understood at present.

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