

Techniques

Evaluation of Agarose Gel Electrophoresis for Resolving Nucleoprotein Components of Prunus Necrotic Ringspot Virus

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ABSTRACT

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The use of agarose gel electrophoresis for resolving the nucleoprotein components of three serologically indistinguishable isolates of Prunus necrotic ringspot virus (PNRSV) was evaluated. Although all nucleoproteins of the three PNRSV isolates used in this study had similar net surface charges, they resolved into three electrophoretic components when electrophoresed in 2% agarose gel at 100 V for 4-5 hr. Relationships among the electrophoretic components and components resolved in 10-40% sucrose density gradients were established. The top, middle, and bottom centrifugal components, in order of increasing sedimentation rate, were found to have electrophoretic mobility values that corresponded to those of the fast, middle, and slow electrophoretic components,

respectively. The specific reactions of the electrophoretic components in the gel with ethidium bromide, acridine orange, and Coomassie brilliant blue as well as with antibodies against PNRSV indicated that all components consisted of intact PNRSV nucleoproteins. Better resolution of the three classes of nucleoproteins was obtained with agarose gel electrophoresis than with sedimentation through sucrose gradients. The three isolates used could be distinguished by their electrophoretic mobility patterns although they were serologically indistinguishable. A procedure was described for rapid partial purification of PNRSV suitable for electrophoretic analysis.

Prunus necrotic ringspot virus (PNRSV) is one of 11 plant viruses included in the Ilarvirus group (12). Like other members of the group, PNRSV has a tripartite genome consisting of three

single-stranded, positive-sense RNA species of molecular weights about 1.3×10^6 , 0.89×10^6 , and 0.69×10^6 daltons (6,11). Although all three RNA species are essential for infection, they must be activated by either a subgenomic RNA 4 ($M_r 0.31 \times 10^6$) or a small amount of coat protein subunit ($M_r 25 \times 10^3$) (6). This activation phenomenon is one of the major characteristics of the Ilarvirus

group and readily distinguishes it from all other virus groups except the alfalfa mosaic virus (AMV) group whose genomic RNAs have similar activation requirements (2).

The four RNA species of members of the Iarvirus group are encapsidated into quasi-isometric particles of various sizes ranging from 20 to 38 nm. These nucleoproteins migrate as three or four components with sedimentation coefficients between 72 and 126 S (3). However, in the case of PNRSV, from one to three sedimenting nucleoprotein components have been detected after rate zonal or analytical centrifugation. Early reports (5,19) indicated only a single component. Tolin (17) demonstrated that a recurrent strain of the virus separated into two zones: 94–97 and 116–119 S. However, in a subsequent paper, Tolin (18) reported that her PNRSV isolate sedimented as three zones at 72, 95, and 117 S. Similarly, Loesch and Fulton (11) found that the nucleoproteins of PNRSV-G also were resolved into three components but with sedimentation coefficients of 72, 90, and 95 S. Working with the same isolate, Gonsalves and Fulton (7) confirmed the presence of three nucleoprotein components. More recently, we reported the presence of three nucleoprotein components in a rugose mosaic-inducing PNRSV isolate as well as in another isolate that remained symptomless in sweet cherry trees (16).

Rate zonal sucrose gradient centrifugation is the most common method used to resolve viral nucleoproteins of the Iarvirus group. However, it is frequently difficult to obtain complete separation of the closely sedimenting components. Only a few reports of separation of ilarviruses by electrophoresis have been published. Tobacco streak virus, the type member of the Iarvirus group, was reported to resolve into three components by polyacrylamide gel electrophoresis (10). Prune dwarf virus was separated into six or more components when electrophoresed for 16–24 hr in 2.8% polyacrylamide gels (8). Electrophoresis of AMV in polyacrylamide gels revealed the presence of at least 13 minor nucleoprotein species in addition to the four major species (1). Recently we showed that, when electrophoresed in 2% agarose gel, purified nucleoproteins of PNRSV resolved into three components (16).

In this report, we describe the nucleoprotein components associated with three serologically indistinguishable isolates of PNRSV as resolved by sucrose density gradients and in agarose gels. We also establish the relationships among the components resolved by the two systems.

In the course of developing this technique for diagnostic work, we found that partially purified viral nucleoproteins were suitable for electrophoretic analysis. They could be prepared from as little as 3 g of infected leaf tissues. The procedure to obtain such preparations is described in this paper.

MATERIALS AND METHODS

Virus isolates. Three PNRSV isolates were used in this study. Two of them, designated CH38 and CH39, had been transmitted earlier from sweet cherry trees (*Prunus avium* L.) to *Chenopodium quinoa* Willd. (13). The third isolate, designated FG, was transmitted from a mahaleb cherry tree (*P. mahaleb* L.), maintained by P. R. Fridlund at the Irrigated Agriculture Research and Extension Center, Prosser, WA, which had been previously inoculated with Fulton's isolate G (4). Isolate CH38 was transmitted from a cherry tree exhibiting rugose mosaic disease and induced primary lesions, tip necrosis, and dieback on *C. quinoa*. Isolate CH39 was transmitted from symptomless sweet cherry trees and induced a mild systemic chlorotic mottle in *C. quinoa*. Isolate FG induced only mild leaf spotting on sweet cherry trees. The systemic chlorotic mottle that it induced on *C. quinoa* was much milder than that induced by isolate CH39. All three isolates appeared to be serologically indistinguishable in agar gel immunodiffusion tests and enzyme-linked immunosorbent assays (16).

Viral nucleoprotein purification. Isolates CH38 and CH39 were increased for purification in *C. quinoa*. Isolate FG was increased in cucumber (*Cucumis sativus* L.) where it incited chlorotic lesions on the cotyledons followed by systemic mottle in the newly expanded leaves. *C. quinoa* plants were inoculated at the four- to six-leaf

stage, whereas cucumber plants were inoculated in the cotyledonary stage. Inoculated plants were kept in growth chambers at 24–27 C with 12-hr photoperiods. Tissues exhibiting symptoms were harvested 6–8 days for isolate CH38, 10–12 days for isolate CH39, and 7–10 days for isolate FG.

Infected tissues (15–50 g) were triturated in a Waring blender for 2 min with 50–75 ml of 20 mM potassium phosphate buffer, pH 8, containing 10 mM sodium diethyldithiocarbamate (DIECA) and 10 mM sodium thioglycollate. The extract was clarified by centrifugation in a Beckman model J2-21 centrifuge equipped with a JA-10 rotor (Beckman Instruments, Inc., Fullerton, CA) at 6,000 rpm for 30 min. The low-speed supernatant was filtered through two layers of Kimwipes paper tissues (Kimberly-Clark Corp., Roswell, GA), and the pH was adjusted to 4.5 with 36% glacial acetic acid. The slurry was allowed to stand for 30 min and clarified by centrifugation at 20,000 rpm for 15 min in a Beckman type 30 rotor, and the nucleoproteins were concentrated at 25,000 rpm for 4.5 hr in the same rotor. Pellets were resuspended overnight at 4 C in 20 mM potassium phosphate buffer, pH 8. After a second cycle of differential centrifugation, the viral nucleoproteins were resuspended in 1–2 ml of 20 mM potassium phosphate buffer, pH 8. The suspension was subjected to a low-speed centrifugation before use.

A "mini" purification was developed for electrophoresis studies that consisted of the following steps. Three to five grams of infected leaf tissues of *C. quinoa* was chilled at 4 C for about 0.5 hr and then triturated either in a homogenizer (The Virtis Co., Gardiner, NY) or in a mortar with 25 ml of cold 30 mM potassium phosphate buffer, pH 8, containing 10 mM DIECA and 10 mM sodium thioglycollate. Triturates were centrifuged at 15,000 rpm for 15 min in a Beckman type 30 rotor. (This step was found to be essential to obtain effective acid clarification later.) The supernatant was filtered through a layer of Kimwipes tissue paper and then clarified by adjusting the pH to near 5.0 with 36% glacial acetic acid. The final pH adjustment to 4.5 was accomplished using 10% glacial acetic acid. The resulting greenish white precipitate was removed by centrifugation at 20,000 rpm for 15 min in a Beckman type 30 rotor. Viral nucleoproteins then were pelleted at 25,000 rpm for 4.5 hr in the same rotor. Following centrifugation, the supernatant was discarded and the inside of the tube was carefully wiped dry with Kimwipes. The resulting pellet was resuspended overnight at 4 C in 20–60 μ l of 20 mM potassium phosphate buffer, pH 8. Subsequently the suspension was transferred, using a micropipet, to an Eppendorf tube and centrifuged at 11,500 rpm for 10 min in an Eppendorf microcentrifuge Model 5413 (Brinkmann Instruments, Inc., Westbury NY). Supernatant was collected and stored at 4 C for later use.

Rate zonal centrifugation. Rate zonal density gradient centrifugations of purified viral nucleoproteins were made in 10–40% sucrose gradients prepared by layering 7, 7, 7, and 4 ml of 40, 30, 20, and 10% (w/v) sucrose dissolved in 20 mM potassium phosphate buffer, pH 8. Density gradients (10–30%) were prepared by layering 5 ml each of 30, 25, 15, and 10% buffered sucrose solutions. Tubes containing 20–50% gradients were prepared by layering 7, 7, 7, and 4 ml of 50, 40, 30, and 20% buffered sucrose solutions. All gradients were stored at 4 C overnight before use. Two milliliters of partially purified preparations was applied to gradients and centrifuged in a Beckman SW 25.1 rotor at 24,000 rpm for up to 6 hr.

After centrifugation, gradients were scanned at 254 nm and fractionated. Absorbing zones were collected, pooled, and concentrated by centrifuging in a Beckman type 42.1 rotor at 40,000 rpm for 3 hr. The nucleoproteins were resuspended in 20 mM potassium phosphate buffer, pH 8, and stored at 4 C until needed.

Ultraviolet absorption spectra were determined using a Beckman DB-G spectrophotometer.

Cellulose acetate strip electrophoresis. Sephaphore X-supported cellulose membranes (Gelman Instrument Co., Ann Arbor, MI) were first immersed in TAE buffer consisting of 40 mM Tris-acetate and 1 mM disodium ethylenediaminetetraacetate, pH 8.0, and the excess buffer was removed with blotter paper. Purified viral nucleoproteins (10–15 μ l) then were applied with a Titan

serum applicator (Helena Laboratories, Inc., Allen Park, MI) 1.5 cm from the cathode end. Two runs were made to determine the electrophoretic mobilities of the nucleoproteins of each isolate. Electrophoresis was conducted at 25 amp for 30 min.

After electrophoresis, the cellulose membranes were stained with Ponceau S (Gelman Instrument Co., Ann Arbor, MI) for 3 min and then destained in several changes of 2% acetic acid until the background was white.

Agarose gel electrophoresis. A 2% agarose gel was prepared by melting 1 g of low-melting-point agarose, electrophoresis grade, in 50 ml of TAE buffer. Molten agarose was cast on an 11.5 × 14.5 cm Gelbond plastic support film (FMC Corp., Rockland, ME).

Viral nucleoproteins were mixed with TAE buffer containing 50% glycerol and bromophenol blue as a tracking dye at a nucleoprotein-to-buffer ratio of 5:1 before loading into sample wells. Electrophoresis was at 50 V for 30 min initially and then at 100 V for 4–5 hr.

Following electrophoresis, gels were stained with ethidium bromide at 2.5 μg/ml in 5 mM ammonium acetate or with acridine orange at 30 μg/ml in 10 mM potassium phosphate buffer, pH 7.0, for 15–30 min. Gels were destained in distilled water. Bands were visualized by placing the gels on an ultraviolet transilluminator (wavelength 302 nm). Photographs were taken with Polaroid Type 55 film, using a Polaroid Land Camera with an orange filter.

In some cases, gels that had been stained with ethidium bromide were subsequently stained overnight with 0.1% Coomassie Brilliant Blue R250 in water:methanol:glacial acetic acid (5:5:2) and destained for 48 hr in a solution of 30% methanol and 10% glacial acetic acid.

Immuno-electrophoresis. Viral nucleoproteins were resolved first by electrophoresis in 2% low-melting-point agarose as described above. Troughs then were cut between sample lanes parallel to the direction of electrophoresis. Antisera were added to the troughs, and the gels were incubated in a humid chamber for 24–48 hr. Rabbit polyclonal antibodies (against PNRSV isolates CH38, CH39, CH71, and CH61) prepared in this laboratory and mouse monoclonal antibodies (ATCC N46E10, NA49F10, and N63F10) prepared by Halk et al (9) were used. When immunoprecipitation lines became visible, each gel was stained with ethidium bromide. After it was photographed, the gel then was stained with Coomassie brilliant blue. The staining procedure using ethidium bromide was the same as described above. However, when gels were to be stained with Coomassie brilliant blue, they were washed with several changes of 140 mM saline and then with distilled water before staining.

RESULTS

To clarify presentation and discussion of our data, we designated the viral nucleoprotein components separated by rate zonal sucrose gradient centrifugation as top (T^c), middle (M^c), and bottom (B^c) in order of increasing sedimentation rate. Components resolved by agarose gel electrophoresis were designated as slow (S^c), middle (M^c), and fast (F^c) in order of increasing electrophoretic mobility.

Resolution of viral nucleoproteins in sucrose density gradients. The number and clarity of viral nucleoprotein components that were resolvable during rate zonal density gradient centrifugation was found to be dependent on both the type of gradient used and the length of centrifugation. When preparations were centrifuged on 10–40% sucrose density gradients at 24,000 rpm for 3 hr, nucleoproteins of all isolates separated into two distinct visible zones (Fig. 1A, B, and C). However, isolate CH39 often appeared as three closely spaced zones. When the centrifugation time was increased to a total of 6 hr, a middle component was resolved for isolates CH38 (Fig. 1D) and isolate CH39 (Fig. 1E). Occasionally, we did resolve the middle component of isolate FG; however, it was not apparent in the preparation illustrated in Figure 1F.

The relative sedimentation rates for the nucleoprotein components of all three isolates are compared in Table 1. It appears that nucleoprotein components of the different isolates sedimented at slightly different rates. Under the same

centrifugation conditions, all three components of CH39 sedimented more slowly than corresponding components of isolates CH38 and FG. They also were clearly resolved as separate light-scattering zones in gradient tubes (Fig. 1E) and as well-separated peaks when the gradients were scanned during fractionation (Fig. 2B). However, this was not the case with nucleoprotein components of isolates CH38 and FG. The M^c component of both isolates, when resolved at all, usually appeared as very faint light-scattering zones in the gradient tubes (Fig. 1D and F, and Fig. 3) and resolved poorly during fractionation (Fig. 2A and C).

To determine the sedimentation conditions for optimal separation of the viral nucleoprotein components, we compared three different sucrose density gradients: 10–30, 10–40, and 20–50%. The 10–30 and 10–40% tubes were centrifuged at 24,000

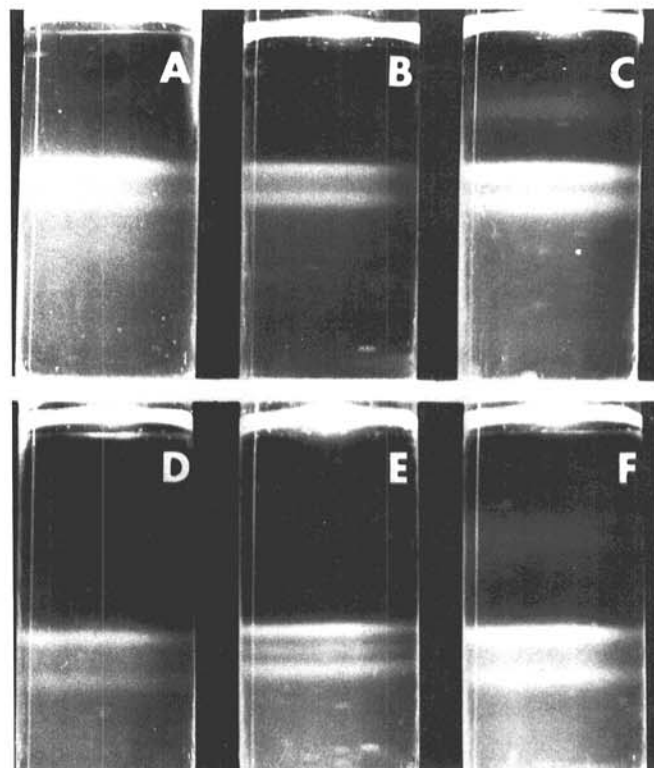


Fig. 1. Separation of nucleoprotein components of *Prunus necrotic ringspot virus* by rate zonal centrifugation. Upper row: gradient tubes with isolates A, CH38, B, CH39, and C, FG after 3 hr. Lower row: same gradient tubes with isolates D, CH38, E, CH39, and F, FG after 6 hr. Preparations were centrifuged in 10–40% sucrose gradients in a Beckman SW 25.1 rotor at 24,000 rpm. Sucrose was dissolved in 20 mM phosphate buffer, pH 8.0.

TABLE 1. Sedimentation of nucleoprotein components of *Prunus necrotic ringspot virus* isolates CH38, CH39, and FG

Centrifugation time ^a	Visible zone ^b	Isolate—distance from meniscus (mm)		
		CH38	CH39	FG
3 hr	T	17–20	16–18	17–20
	B	22–24.5	20.5–22.5	22–24.5
6 hr	T	28–30	26–28	27–29
	M	31–32	29–30	30–31 ^c
	B	34–36	31.5–33	33–35

^a Purified viral nucleoproteins were layered on a preformed 10–40% sucrose gradient and centrifuged in a Beckman SW 25.1 rotor at 24,000 rpm.

^b T, M, and B represent top, middle, and bottom viral nucleoprotein components, respectively, in order of increasing sedimentation rate.

^c Figures were extrapolated from another preparation with well-resolved middle component.

rpm for 3 and 6 hr, whereas the 20–50% tubes were centrifuged for 12 and 15 hr. Best resolution of the components was obtained after 6 hr in a 10–40% gradient (Fig. 4).

Ultraviolet absorbance spectra. Absorbance spectra of purified preparations of all three isolates after rate zonal sucrose density gradient centrifugation were typical of nucleoprotein preparations. All three isolates had absorbance maxima at 258 nm and minima at 244 nm. Considerable variation occurred in the 260:280 absorbance ratios among preparations. Calculated 260:280 ratios ranged from 1.45 to 1.57 (corrected for light scattering), suggesting a nucleic acid content between 11 and 14.5%.

Cellulose acetate strip electrophoresis. When purified virus preparations of all three isolates were electrophoresed on cellulose acetate membranes that had limited molecular sieving capability, the nucleoproteins of each isolate migrated as a single band. Furthermore, no differences in migration rates were observed among isolates, demonstrating a similar net intrinsic surface charge for each component of all three isolates.

Resolution of viral nucleoproteins by agarose gel electrophoresis. All three PNRSV isolates separated into three components with quite different electrophoretic mobilities during agarose gel electrophoresis. The best resolution of F^c, M^c, and S^c components

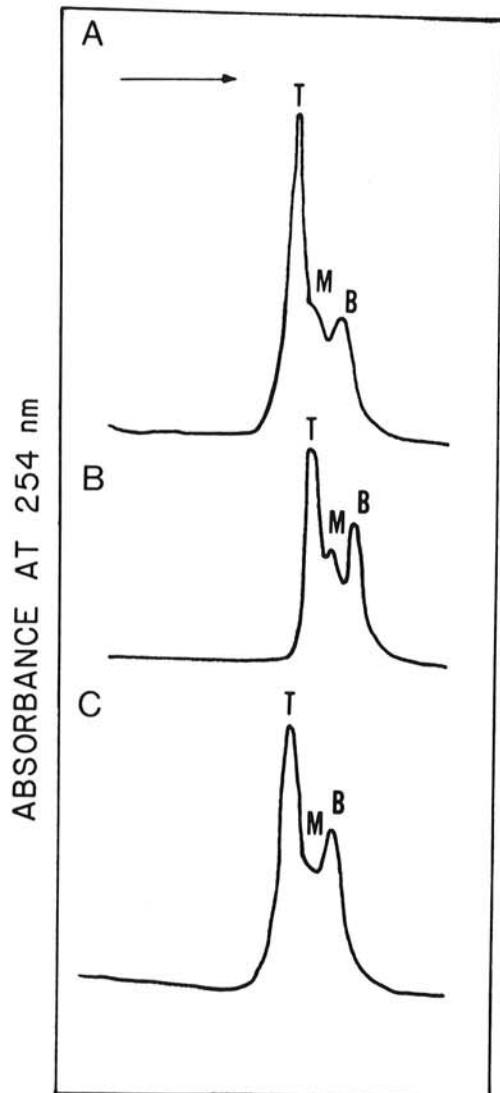


Fig. 2. Ultraviolet absorbance profiles of Prunus necrotic ringspot virus isolates **A**, CH38, **B**, CH39, and **C**, FG obtained during fractionation of gradient tubes in Figure 1. Preparations were centrifuged in 10–40% sucrose gradients in a Beckman SW 25.1 rotor at 24,000 rpm for 6 hr. Sucrose was dissolved in 20 mM phosphate buffer, pH 8.0. The top, middle, and bottom nucleoprotein components are designated as T, M, and B, respectively. Arrow indicates direction of sedimentation.



Fig. 3. Separation of nucleoprotein components of Prunus necrotic ringspot virus isolate FG by rate zonal centrifugation. Faint middle zone was visible. Preparation was centrifuged in 10–40% sucrose gradients in a Beckman SW 25.1 rotor at 24,000 rpm for 6 hr. Sucrose was dissolved in 20 mM phosphate buffer, pH 8.0.

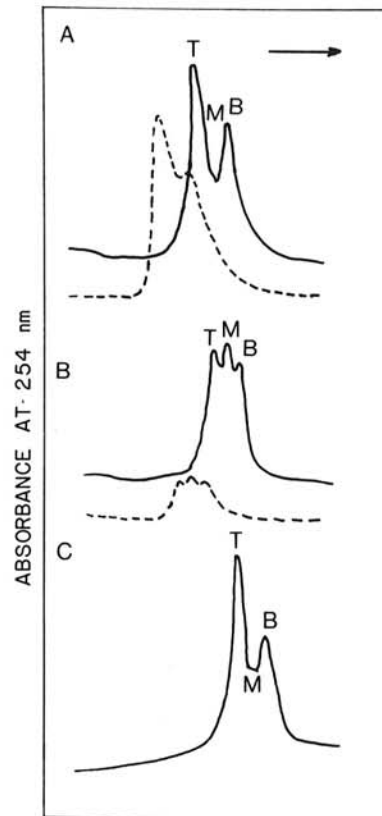


Fig. 4. Ultraviolet absorbance profiles of nucleoprotein preparations of Prunus necrotic ringspot virus in 10–40% sucrose density gradients for 6 hr (solid line) and 10–30% sucrose density gradients for 3 hr (broken line). **A**, CH38; and **B**, CH39. **C**, CH38 in 20–50% sucrose density gradients for 12 hr. Preparations were centrifuged in a Beckman SW 25.1 rotor at 24,000 rpm. Sucrose was dissolved in 20 mM potassium phosphate buffer, pH 8.0. The top, middle, and bottom nucleoprotein components are designated as T, M, and B, respectively. Arrow indicates direction of sedimentation.

of isolates CH38 and CH39 was obtained in 2.0 to 2.5% agarose gel (Fig. 5). However, the M^c band of isolate FG was not clearly resolved under these conditions. Components of isolate CH39 migrated faster than the corresponding components of isolates CH38 and FG (Table 2).

We inferred that all the components resolved were nucleoproteins because of their specific reactions to nucleic acid and protein stains. When stained with ethidium bromide, the electrophoretic bands fluoresced orange-red, indicating the presence of nucleic acids. They fluoresced red when stained with acridine orange, suggesting that the nucleic acids were single stranded. These bands also were readily stained with Coomassie brilliant blue, indicating the presence of proteins.

Relationships between centrifugal and electrophoretic components. Highly purified preparations of T^c, M^c, and B^c from each of the three isolates were prepared by two or three successive rate zonal sucrose density gradient centrifugations. During electrophoresis, these components migrated as F^c, M^c, and S^c, respectively (Fig. 6).

Immunoelectrophoresis. The serology of F^c, M^c, and S^c components was tested using seven PNRSV antisera including four rabbit polyclonal and three mouse monoclonal antibodies. Immunoprecipitation lines, distinctly outlining the diffused nucleoprotein bands, were apparent in the ethidium bromide-stained gels (Fig. 7). However, only a weak reaction line was obtained between monoclonal antibody N46E10 and components

of CH38.

Detection of viral nucleoproteins in partially purified preparations. We were able to detect all three viral nucleoprotein components in samples prepared from 3 and 5 g of leaves using the "mini" purification procedure described above. No apparent bands were obtained in preparations from 1 g of leaves (Fig. 8).

As expected, nucleoprotein preparations obtained with the "mini" procedure had more proteinaceous contaminants than preparations obtained by the normal purification procedure (Fig. 9). However, these contaminants did not interfere with the separation and resolution of the viral nucleoprotein components.

A band that migrated faster than component F^c was detected in some preparations of isolate CH38. This band was stained by Coomassie brilliant blue, but not by ethidium bromide. It reacted with all three monoclonal antibodies tested.

DISCUSSION

Separation of nucleoprotein components of small isometric multicomponent viruses has been done primarily by rate zonal centrifugation. To obtain highly purified preparations of individual components, fractions need to be pooled from successive density gradient centrifugations. Such procedures are not only time consuming but also result in substantial loss of viral nucleoproteins. Hence, it is often difficult to obtain sufficient materials for critical studies. This problem has been especially acute with PNRSV because of its inherent instability and relatively low concentration in infected tissues. Our study shows that the nucleoprotein components of PNRSV are detected easily by electrophoresis in agarose gels even with partially purified preparations. The demonstration that the electrophoretic components are the same (but in reverse order) as those resolved by density gradient centrifugation will greatly accelerate studies on the nature of these components.

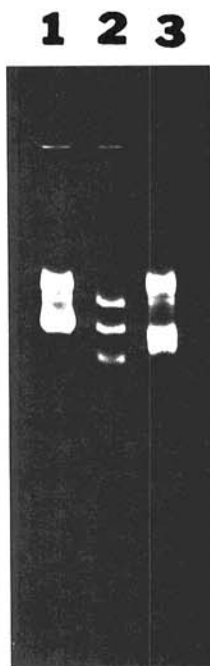


Fig. 5. Electrophoresis of nucleoproteins of Prunus necrotic ringspot virus in 2.5% low-melting-point agarose gel. Lane 1, FG; lane 2, CH39; and lane 3, CH38. Nucleoproteins were electrophoresed in low-melting-point agarose gel dissolved in 40 mM Tris-acetate and 1 mM ethylenediamine-tetraacetate, pH 8, at 100 V for 5 hr. Gel was stained with ethidium bromide.

TABLE 2. Electrophoretic mobilities of Prunus necrotic ringspot virus isolates FG, CH38, and CH39 in agarose gel electrophoresis^a

Isolate	Component—distance from sample well (mm)		
	Slow	Middle	Fast
FG	31	32	36
CH38	33	37	41
CH39	35.5	39.5	43.5

^a Purified viral nucleoproteins were electrophoresed in 2% low-melting-point agarose gel at 100 V for 4.5 hr.

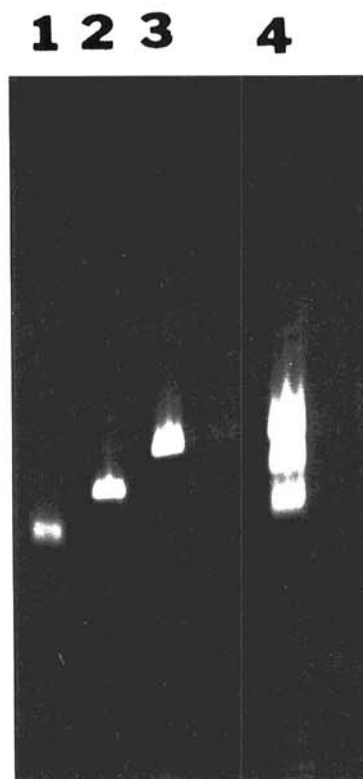


Fig. 6. Electrophoretic analysis of isolated nucleoprotein components separated by successive cycles of sucrose density gradient centrifugation of isolate CH39. Lane 1: top, lane 2: middle, and lane 3: bottom centrifugal components, and lane 4: total viral nucleoproteins before rate zonal centrifugation. Nucleoproteins were electrophoresed in 2% low-melting-point agarose gel in 40 mM Tris-acetate and 1 mM ethylenediamine-tetraacetate, pH 8, at 100 V for 5 hr and stained with ethidium bromide.

In addition to improved resolution, some other advantages of using electrophoretic separation are that more treatments can be compared under identical conditions, components can be selectively stained with various nucleic acid or protein stains, and loss of nucleoproteins during successive rate zonal centrifugation is avoided. We found that as little as 3 g of infected leaf tissues can be used for electrophoretic analysis (17).

Nucleoprotein components of the three PNRSV isolates used here were found to have different rates of migration during agarose gel electrophoresis. These differences appear to result primarily from the sieving effect of the gel matrix due to differences in size and shape of the nucleoprotein components because all components appear to have the same net surface charge when electrophoresed in cellulose acetate strips.

We used isolates CH38 and CH39 in our studies because they represented two major groups of biological variants of PNRSV commonly found in sweet cherry orchards in Washington (16). Isolate CH38 typifies the group of isolates that induces rugose mosaic disease. Isolate CH39 represents isolates that remain symptomless in infected trees and thus cause little or no economic damage (14). Although these isolates differ in their biological properties, they are indistinguishable serologically. Results obtained here demonstrate that they can be readily differentiated by their electrophoretic mobility patterns. Perhaps more significantly, preliminary studies have shown that at least three

other rugose-inducing and three other symptomless isolates also exhibited mobility patterns similar to those of isolates CH38 and CH39, respectively (C.-A. Ong and G.I. Mink, *unpublished*).

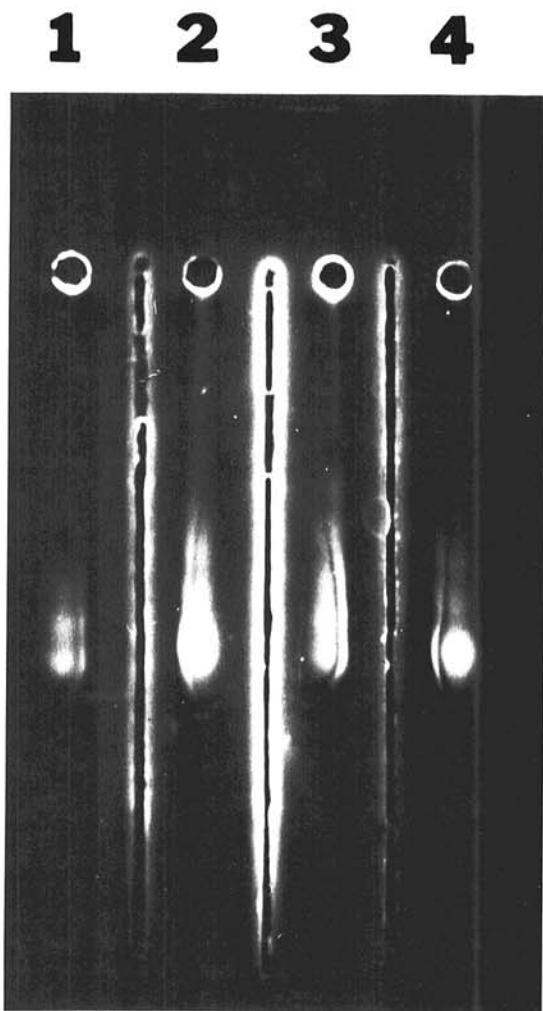


Fig. 7. Immunoelectrophoresis of nucleoprotein components of Prunus necrotic ringspot virus using mouse monoclonal antisera. Wells 1 and 3 were charged with purified nucleoproteins of isolate CH39; wells 2 and 4 with those of isolate CH38. Nucleoproteins then were electrophoresed in 2% low-melting-point agarose gel in 40 mM Tris-acetate and 1 mM ethylenediaminetetraacetate, pH 8, at 100 V for 5 hr. After electrophoresis, monoclonal antibodies ATCC NA49F10, N46E10, and N63F10 (left to right) were applied to the troughs.



Fig. 8. Electrophoretic analysis of partially purified preparations of Prunus necrotic ringspot virus from 1 g (lane 3), 3 g (lane 2), and 5 g (lane 1) of leaves of *Chenopodium quinoa* infected with isolate CH38. Conditions of electrophoresis: 2% agarose gel in 40 mM Tris-acetate with 1 mM ethylenediaminetetraacetate, pH 8.0; 100 V for 5 hr; stained with ethidium bromide after electrophoresis.

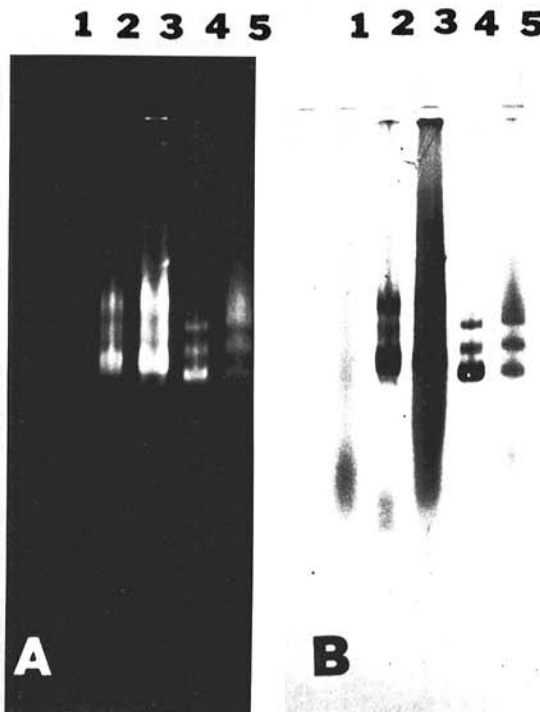


Fig. 9. Electrophoretic analysis of nucleoproteins of Prunus necrotic ringspot virus. Viral nucleoprotein bands stained with **A**, ethidium bromide, and **B**, Coomassie Brilliant Blue R250. Healthy control (lane 1), purified (lane 2), and partially purified (lane 3) preparations of isolate CH38; purified (lane 4) and partially purified (lane 5) preparations of isolate CH39. Conditions of electrophoresis: 2% agarose gel in 40 mM Tris-acetate with 1 mM ethylenediaminetetraacetate, pH 8.0; 100 V for 5 hr; stained with ethidium bromide after electrophoresis.

These results suggest that electrophoretic analysis may be a useful diagnostic tool for distinguishing PNRSV variants in commercial orchards. So far as we are aware, this is the first report of isolates of the same virus, isolated from plants of the same crop showing different symptoms, that are serologically indistinguishable and yet have different electrophoretic mobility patterns.

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