Biophysical Differences Among Prunus Necrotic Ringspot Ilarviruses

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Accepted for publication 19 September 1991 (submitted for electronic processing).

ABSTRACT

Crosslin, J. M., and Mink, G. I. 1992. Biophysical differences among Prunus necrotic ringspot ilarviruses. Phytopathology 82:200-206.

Intact nucleoproteins of several isolates of Prunus necrotic ringspot virus (PNRSV) from cherry, peach, almond, and hop resolved as three components in rate-zonal sucrose density gradients and as two or three components on electrophoresis in agarose slab gels. Electrophoretic mobilities of the various nucleoprotein components differed among isolates. When electrophoresis was conducted on cellulose acetate sheets, PNRSV nucleoproteins resolved as single bands with marked differences in mobility noted among isolates. These results suggest that net surface charge is primarily responsible for differences in relative mobility among isolates, while differences in size of nucleoprotein components cause the different band patterns seen in agarose gels. Some differences in electrophoretic migration of the three genomic RNAs were noted among isolates under nondenaturing conditions. These differences were less pronounced among isolates than the differences in nucleoprotein migration. Relative molecular weights of coat protein subunits, determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), varied among isolates within a range of about 27–29 kDa but were constant for individual isolates.

Prunus necrotic ringspot virus (PNRSV) infects hops (*Humulus lupulus* L.), rose (*Rosa* spp.), and many species of *Prunus* (2,18). Sweet cherry trees infected with PNRSV exhibit a range of symptoms from none to severe rugose mosaic disease, depending on the virus strain or isolate (9). Mink (14) reported that enzyme-linked immunosorbent assay (ELISA) could be used for early detection of PNRSV-induced cherry rugose mosaic in sweet cherry orchards. Later, however, Mink and Aichele (15) found many apparently healthy trees that tested positive for PNRSV. These trees were infected with PNRSV isolates that were serologically related to rugose strains but that did not cause obvious disease symptoms. This severely limited ELISA as a tool with which to distinguish the different PNRSV isolates to reduce field spread of the disease.

Ong (19) and Ong and Mink (21) found that three biologically distinct but serologically indistinguishable isolates of PNRSV could be differentiated by their relative electrophoretic mobilities in agarose slab gels. There were also small differences in relative molecular weights of the coat protein subunits of two of these isolates.

This earlier work suggested that differences among PNRSV isolates in electrophoretic mobilities might be correlated with biological differences and consequently useful in distinguishing among isolates. Here we examined the electrophoretic migration pattern of nucleoprotein components, genomic RNAs, and coat proteins of several PNRSV isolates to determine which, if any, properties are isolate-specific and therefore useful for identification purposes.

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MATERIALS AND METHODS

Virus isolates. The virus isolates used in these studies are listed in Table 1. Isolates were maintained by weekly transfers from systemically infected *Chenopodium quinoa* Willd. or cucumber tissue. After inoculation, plants were maintained either in growth chambers at 22–25 C with a 16-h photoperiod or in a greenhouse equipped with supplemental lighting.

Virus purification. Viruses were purified using the low pH clarification procedure of Ong and Mink (21) with modifications. Systemically infected *C. quinoa* or cucumber tissue was harvested 7–14 days after inoculation and held at 4 C for 1 h. Five to 12 g of tissue were triturated in a Virtis homogenizer (The Virtis Co., Gardiner, NY) with 25 ml of ice-cold transfer buffer. The homogenate was centrifuged at 50,000 *g* for 15 min in a chilled type 30 rotor (Beckman Instruments, Palo Alto, CA). The supernatant was collected, pooled, adjusted to pH 4.5 by addition in drops of 5% (v/v) acetic acid, and again centrifuged at 50,000 *g* for 15 min. Pellets were discarded and viral nucleoproteins pelleted by centrifugation at 75,000 *g* for 4.5 h. The pellets were resuspended in 100 μl of TAE buffer (40 mM Tris-acetic acid, 1 mM disodium ethylenediaminetetraacetate [EDTA], pH 8) overnight at 4 C. The suspended material was placed into microcentrifuge tubes and centrifuged 5 min at 8,800 *g* in a microcentrifuge. The supernatant from the suspended high-speed pellet (SHSP) was used for infectivity assays, serology, and electrophoresis. In some experiments, the SHSPs were further purified by centrifugation in 10–40% rate zonal sucrose density gradients (21). Virus concentrations were estimated from the absorbance at 260 nm, assuming an extinction coefficient of 5 (12).

Serotyping. Isolates of PNRSV used in the current investigations were serotyped using previously described antisera (16,22), the anti-PNRSV antiserum PVAS 22 (ATCC, Rockville, MD), and the anti-apple mosaic virus (ApMV) antiserum PVAS 32 (ATCC). Agar double-diffusion gels were prepared according to Ong and Mink (20). Precipitation lines usually appeared after overnight incubation at room temperature. Gels were rinsed overnight in 0.85% (w/v) NaCl to remove unreacted proteins and stained with Crocein Scarlet-Coomassie Brilliant Blue (4). After destaining in 1% (v/v) acetic acid, gels were soaked for 1 h in 1% (v/v) glycerol and air-dried.

Agarose gel electrophoresis. Electrophoretic separations (3,21) of nucleoprotein components of the various ilarviruses were conducted in 2% (w/v) low melting point (LMP) agarose (Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, MD) dissolved in TAE buffer. Sample wells were loaded with a mixture of 10–20 μl of SHSP or purified virus and 3 μl of electrophoresis sample buffer (TAE, 25% glycerol, 0.1% bromphenol blue). Electrophoresis of the samples was done at a constant 100 V (about 55 mA) at room temperature for 4 h. Gels were stained for 30 min with 2 μg/ml of ethidium bromide and photographed using a UV transilluminator. Most gels then were stained with 0.1% (w/v) Coomassie Brilliant Blue R in 5:5:1 methanol/water/acetic acid and destained 48 h in 40% methanol, 10% acetic acid (v/v). When destaining was complete, the gels were soaked in 1% glycerol for 1 h and air-dried.

RNA extraction. Ilarvirus RNAs were prepared for electrophoresis by combining 20 μl of SHSP, 3 μl of 10% aqueous sodium dodecyl sulfate (SDS), and 3 μl of electrophoresis sample buffer in autoclaved microcentrifuge tubes. The mixture was heated in a water bath to 55 C for 1 h, electrophoresed in 2.5% LMP agarose gels for 2–3 h at 100 V, stained with ethidium bromide, and photographed as described above. Some gels were subsequently stained with Coomassie Brilliant Blue in order to demonstrate the absence of intact nucleoproteins within the ethidium bromide-stained regions.

Phenol-SDS extraction and ethanol precipitation (13) also was used for isolating viral RNAs. RNA pellets were resuspended in 50–100 μl of TAE and 2 μl of 10% SDS. Resuspended RNAs were used for electrophoresis as described above.

Extraction of nucleoproteins from agarose gels. Nucleoprotein-containing regions were localized either directly by ethidium bromide staining or by staining the outside lanes of the gel and using them as markers for excision. The excised agarose was finely chopped with a sterile razor blade and placed into a sterile 0.5-ml microcentrifuge tube, the bottom of which had been punctured and plugged with cotton. One hundred to 150 μl of TAE or transfer buffer was added, and the samples were held at 4 C for 1–24 h to allow the nucleoproteins to diffuse out of the agarose. The 0.5-ml tube then was placed inside a 1.5-ml microcentrifuge tube and centrifuged 30 s at 8,800 *g*. The eluate was collected and an aliquot was electrophoresed as above to demonstrate recovery of the nucleoproteins. Some eluates were inoculated onto *C. quinoa* to determine infectivity.

Analysis of viral proteins. Suspensions of purified PNRSV (100–500 μg/ml) were mixed with an equal volume of 2× dissociation buffer (0.125 M Tris-Cl, pH 6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol) and boiled for 5 min. Electrophoresis was in SDS-polyacrylamide gels prepared according to the methods of Laemmli (11). Gels (4% stacking and 10 or 12% resolving) were cast in an SE400 vertical slab gel apparatus (Hoefer Scientific Instruments, San Francisco, CA). Electrophoresis was conducted at 100 V for 6 h. Gels were stained with Coomassie Brilliant Blue as described above.

Molecular weight markers (Sigma) included trypsin inhibitor (20,100 Da), trypsinogen (24,000 Da), carbonic anhydrase (29,000

TABLE 1. Origin and properties of Prunus necrotic ringspot and apple mosaic ilarviruses

Isolate	Original host[a]	Serotype[b]	Pathotype[c]		Reference or source[d]	State[e]
			C. quinoa	Sweet cherry		
CH3	Sweet cherry	CH3	D/M	R	16	WA
CH9	Sweet cherry	CH9	D	R	16	WA
CH19	Sour cherry	CH9	M	M	GIM	WI
CH30	Sweet cherry	CH30	M	R	16	WA
CH38	Sweet cherry	CH9	D	R	21	WA
CH39	Sweet cherry	CH9	M	M	21	WA
CH57	Sweet cherry	CH9	D	R	GIM	WA
CH61	Sweet cherry	CH9	M	M	16	WA
CH71	Sweet cherry	CH30	I	M	GIM	WA
CH133	Sweet cherry	CH9	D/M	R	JKU	CA
CH135	Sweet cherry	CH9	D/M	R	JKU	CA
CH136	Sweet cherry	CH9	D/M	R	JKU	CA
CH140	Sweet cherry	CH9	M	?	SWS	SC
CH141	Sweet cherry	CH9	M	?	JMC	OR
CH142	Sweet cherry	CH9	M	?	JMC	OR
AL14	Almond	CH9	D/M	NT	JKU	CA
PE4	Peach	CH9	M	NT	GNO	CA
PE5	Peach	CH9	M	NT	SWS	SC
PE8	Peach	CH9	M	NT	SWS	SC
RO2	Rose	CH9	M	NT	JMC	WA
G-ATCC	Sour cherry	CH9	M	NT	ATCC	WI
NRSV-Hop	Hop	Hop	M	NT	22	WA
ApMV-Hop	Hop	Apple	—	NT	22	WA

[a] Sweet cherry = *Prunus avium* L.; sour cherry = *P. cerasus* L.; almond = *P. dulcis* (Mill.) Webb; peach = *P. persica* (L.) Batch.; rose = *Rosa* spp.; hop = *Humulus lupulus* L.

[b] Determined by agar gel double-diffusion using antisera to isolates CH3, CH9, and CH30 by the methods of Mink et al (16). Antisera to the hop isolates provided by C. B. Skotland (22).

[c] Symptoms observed on *Chenopodium quinoa* 10–14 days after rub inoculation with infected *C. quinoa* or cucumber tissue triturated in 30 mM potassium phosphate buffer, pH 8, containing 10 mM sodium diethyldithiocarbamate and 10 mM sodium thioglycolate. Symptoms were observed on sweet cherry trees two or more years after graft inoculation. M = Mild mottle (*C. quinoa*), mild mottle or none (sweet cherry); D = leaf necrosis and tip dieback; I = mottle with few necrotic spots or flecks; D/M = D-type when originally isolated, M-type in subsequent transfers; R = rugose mosaic disease (small twisted leaves, shot hole, enations); NT = not tested; ? = symptoms not recorded; — = not infected.

[d] GIM = G. I. Mink, WSU-IAREC; JKU = J. K. Uyemoto, UC Davis; GNO = G. N. Oldfield, UC Riverside; SWS = S. W. Scott, Clemson University; JMC = J. M. Crosslin, WSU-IAREC; ATCC = American Type Culture Collection.

[e] Abbreviation of state in which the original isolation was made.

Da), glyceraldehyde-3-phosphate dehydrogenase (36,000 Da), and egg albumin (45,000 Da). Distances to the protein bands were measured to the nearest millimeter and mobility was calculated as a percentage of the tracking dye migration. Relative molecular weights (Mr) of the coat proteins were determined by linear regression analysis.

Densitometry. Ethidium bromide-stained bands were localized graphically by scanning photographic negatives at 580 nm with a model 1312 gel scanner (Isco, Lincoln, NE). In some cases, dried Coomassie Brilliant Blue-stained nucleoprotein gels also were scanned. Distances to the peaks were measured and expressed as a percentage of the migration of the fastest nucleoprotein electrophoretic component of isolate CH39 (21).

Cellulose acetate electrophoresis. Electrophoresis of purified virus preparations (100–500 μg/ml) was on Optiphor-10 cellulose acetate sheets (Gelman Sciences, Ann Arbor, MI) in a Turner electrophoresis unit, model 310 (G. K. Turner Associates, Palo Alto, CA). About 2 μl of each sample was loaded onto TAE buffer-equilibrated sheets, and electrophoresis was in TAE, pH 8, at 10 mA for 15 min. Sheets then were stained in Ponceau S (Gelman) for 3 min and destained in several changes of 2% (v/v) acetic acid until the background was white. Regions containing protein were stained pink-red.

RESULTS

Virus purification. All PNRSV isolates tested separated into three centrifugal components in 10–40% rate-zonal sucrose density gradients (Fig. 1). Frequently, the middle component was less distinct than either the top or bottom components. With CH9, CH30, CH71, and AL14, the top component predominated. The sedimentation profile of isolate NRSV-Hop indicated a predominance of the bottom component and was similar to the sedimentation profile of an isolate of apple mosaic virus transmitted from hops (ApMV-Hop; data not shown).

The yields of purified nucleoproteins varied among isolates from about 1 to 12 μg/g of fresh tissue.

Nucleoprotein infectivity. High-speed pellets obtained from five PNRSV isolates and suspended in TAE buffer (SHSPs) were infectious when inoculated onto *C. quinoa* after storage at 4 C for 13–239 days. Other SHSPs of seven isolates were infectious after storage at −20 C for about 20 mo.

Nucleoprotein electrophoresis. Nucleoprotein components of PNRSV separated into two or three ethidium bromide-stained bands after electrophoresis in agarose gels at pH 8. Migration was towards the anode; however, migration rates for the individual components differed markedly among isolates (Fig. 2). No ethidium bromide-stained bands were evident in preparations from uninfected plants.

Isolate NRSV-Hop separated into two diffuse bands that migrated more slowly than any bands obtained with the PNRSV isolates transmitted from *Prunus* or rose (Fig. 2). AL14, the only almond isolate tested, exhibited the slowest migration of the viruses transmitted from rosaceous hosts.

Coomassie Brilliant Blue stained all electrophoretic bands that were detected by ethidium bromide (Fig. 2B), indicating that materials within the stained regions were nucleoproteins. Materials eluted from the combined bands within a given lane were infectious when inoculated onto *C. quinoa.* These results strongly suggest that the stained materials were intact PNRSV nucleoproteins.

Electrophorotypes. Isolates were divided into electrophorotypes (10) based on the relative distances of nucleoprotein migration in agarose gels (Fig. 3). Isolates having a fast nucleoprotein component (F^c) that migrated less than 60%, between 60 and 90%, or more than 90% that of the distance migrated by CH39

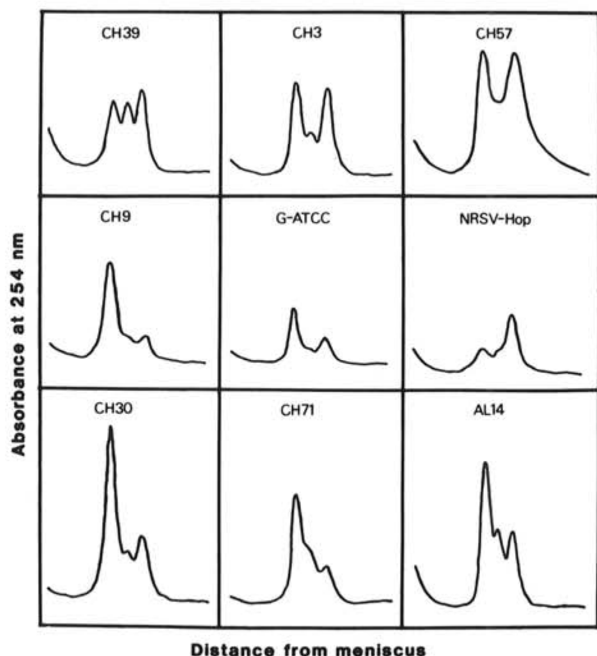

Fig. 1. Sedimentation profiles of nine Prunus necrotic ringspot ilarviruses after ultracentrifugation in 10–40% (w/v) sucrose density gradients for 4 h at 75,000 *g* in a Beckman SW25.1 rotor. Sedimentation is left to right.

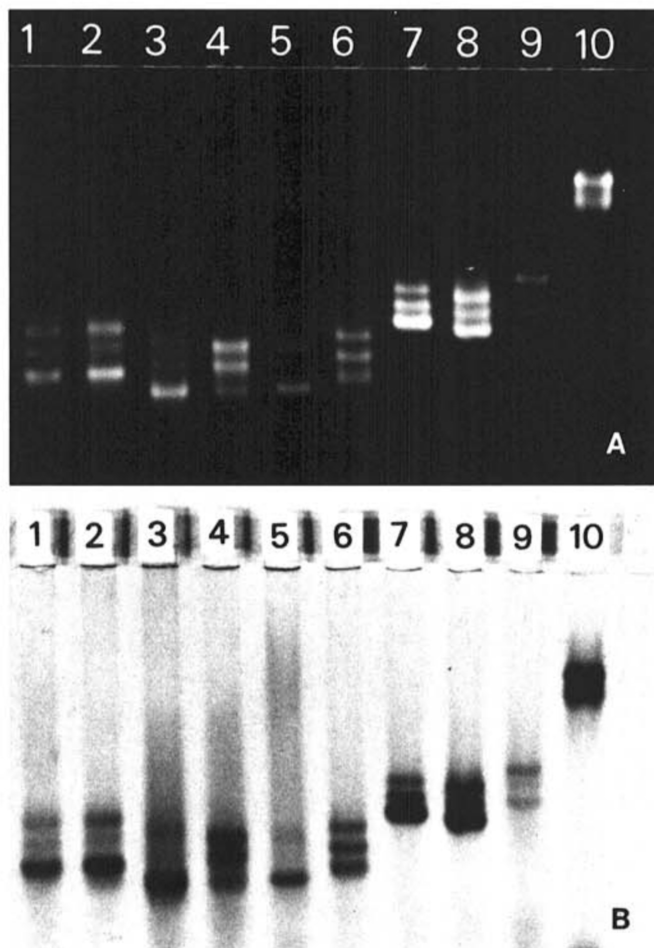

Fig. 2. Agarose gel electrophoresis of nucleoproteins of 10 Prunus necrotic ringspot virus isolates electrophoresed in 2% (w/v) low melting point agarose dissolved in TAE buffer (40 mM Tris-acetic acid, 1 mM EDTA, pH 8). Electrophoresis was conducted in TAE buffer for 4 h at 100 V. **A,** Gel stained with ethidium bromide. **B,** The same gel stained with Coomassie Brilliant Blue. Lane 1, CH71; lane 2, CH30; lane 3, CH38; lane 4, CH39; lane 5, G-ATCC; lane 6, CH135; lane 7, CH136; lane 8, CH133; lane 9, AL14; lane 10, NRSV-Hop.

F^e were classified as electrophorotypes I, II, and III, respectively. Only one isolate, NRSV-Hop, was assigned to electrophorotype I.

Of the isolates included in electrophorotype II, only one, RO2, originated from Washington State. The other members of electrophorotype II were from California, Oregon, or South Carolina. Conversely, isolates in electrophorotype III were from Washington, except for G-ATCC and CH19 (Wisconsin) and CH135 and PE4 (California).

The electrophoretic patterns of nucleoproteins of isolates CH38 and CH9 were unchanged after either three or 13 serial transfers in *C. quinoa*. For CH57, the band patterns were the same after one or 11 transfers. Nucleoprotein preparations obtained after numerous serial transfers of several other isolates showed that electrophoretic patterns were consistent between preparations. Nucleoprotein preparations of four isolates that had been stored at 4 C for 1–198 days showed no obvious change in electrophoretic patterns. These results indicate that electrophoretic patterns are a stable and reproducible characteristic of an isolate and that nucleoprotein preparations can be stored for extended periods without loss of these characteristics.

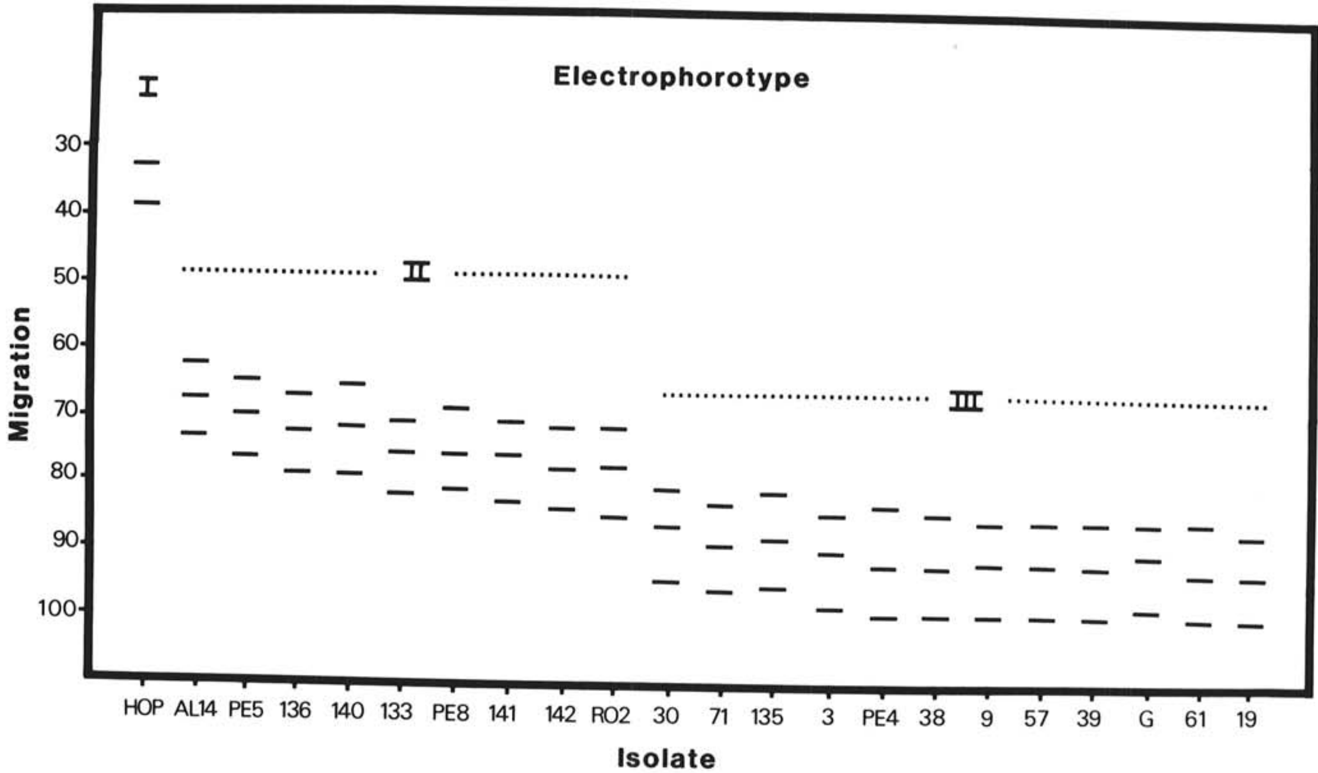

Fig. 3. Electrophorotypes of Prunus necrotic ringspot virus. Migration of individual components of a given isolate is expressed as a percentage of the migration of the fast electrophoretic component of isolate CH39. AL, almond; G, G-ATCC; HOP, NRSV-Hop; PE, peach; RO, rose; other isolates with numbers only are from cherry. Isolate sources are shown in Table 1. Data are the means of 1–10 values. Electrophoretic conditions as in Figure 2.

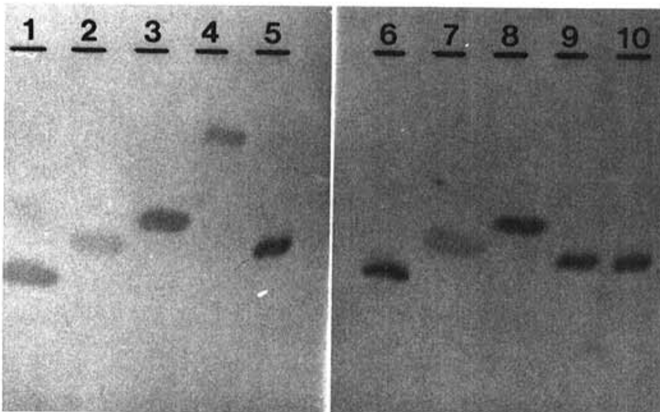

Fig. 4. Electrophoretic migration of Prunus necrotic ringspot virus nucleoproteins on cellulose acetate sheets. Electrophoresis of purified virus (2–4 μl at 100–500 μg/ml) was conducted in TAE buffer (40 mM Tris-acetic acid, 1 mM EDTA, pH 8) for 15 min at 10 mA and stained with Ponceau S. Lane 1, CH9; lane 2, RO2; lane 3, AL14; lane 4, NRSV-Hop; lane 5, CH30; lane 6, CH57; lane 7, RO2; lane 8, AL14; lane 9, CH71; lane 10, CH61.

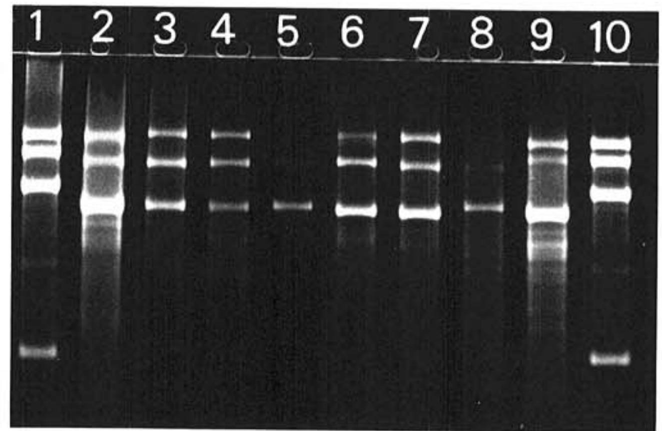

Fig. 5. Electrophoretic separation of Prunus necrotic ringspot and cowpea chlorotic mottle virus (CCMV) RNAs. Nucleoproteins were dissociated in 1% sodium dodecyl sulfate at 55 C for 1 h. Electrophoresis was in 2.5% low melting point agarose gels for 3 h at 100 V in TAE buffer (40 mM Tris-acetic acid, 1 mM EDTA, pH 8). Gel was stained with ethidium bromide. Purified CCMV RNA was a gift from S. D. Wyatt. Lanes 1 and 10, CCMV; lane 2, CH3; lane 3, CH19; lane 4, CH39; lane 5, CH38; lane 6, G-ATCC; lane 7, CH61; lane 8, CH71; lane 9, CH30.

Cellulose acetate electrophoresis. Purified preparations of PNRSV migrated as single bands during electrophoresis on cellulose acetate sheets in TAE buffer. The migration distances, however, varied among isolates (Fig. 4). The relative distances migrated by the various isolates on cellulose acetate corresponded to the relative mobility of the isolates in agarose gels. For example, nucleoproteins of isolate NRSV-Hop had the slowest mobility of the isolates tested on either medium. Similarly, AL14 and RO2 showed reduced mobility in relation to the other isolates.

RNA electrophoresis. After SDS dissociation of nucleoproteins, the RNAs of all isolates listed in Table 1, except for NRSV-Hop and ApMV-Hop, separated into three or four major bands (Fig. 5) (RNA 1, slowest; RNA 4, fastest) (12). RNA 2 of CH30 (lane 9) migrated distinctly more slowly than the RNA 2 of other isolates. Several minor bands are present in some lanes, particularly in preparations of CH3 (lane 2) and CH30 (lane 9). However, the significance of these bands is not known. The RNA 4 band was weak or absent in most of these PNRSV preparations. Similar results were found with RNAs prepared by phenol-SDS extraction. The RNAs of NRSV-Hop and ApMV-Hop were not well resolved by either method (data not shown).

PNRSV coat protein. The coat protein of each PNRSV isolate resolved as one major band by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 6). Estimates of Mr varied among isolates within a range of approximately 27–29 kDa (Table 2). Protein of isolate CH9 also showed a minor band of approximately 20 kDa. In most preparations, minor bands of approximately 55–60 kDa were observed and may consist of coat protein dimers.

Although the calculated Mr of the coat proteins varied somewhat among experiments (Table 2), the differences observed among isolates were reproducible and consistent among experiments. Differences in coat protein Mr among isolates were verified by electrophoresis of isolate mixtures (Fig. 7).

Serology. When reacted with antiserum PVAS 22 (Fig. 8; additional results not shown), nucleoproteins of all isolates transmitted from *Prunus* and rose, except CH71 and CH30, produced fused precipitin lines with isolates classified as CH9 serotypes (16,21; Table 1). Isolates CH30 and CH71 produced strong spur reactions with isolates of the CH9 serotype and fused precipitin lines with each other. Thus, CH71 is considered to be CH30 serotype and was the only other isolate of the CH30 serotype detected. Isolate CH3, which was classified as a third serotype (CH3) of PNRSV (16), reacted similarly to CH9 serotypes in these tests. Additionally, CH3 could not be differentiated from CH9 serotypes using antiserum that had previously been used for this purpose (16). The reason for this difference is not known.

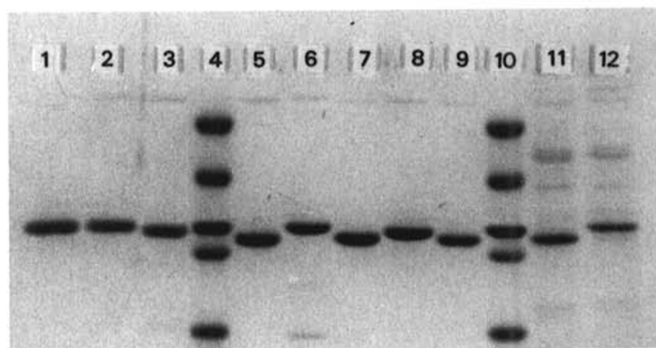

Fig. 6. Differences in coat protein subunit apparent molecular weight among isolates of Prunus necrotic ringspot virus determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to Laemmli (11) using 4% stacking and 10% resolving gels. Electrophoresis was conducted for 6 h at 100 V. Gel was stained with Coomassie Brilliant Blue. Lanes 4 and 10, molecular weight standards of 45, 36, 29, 24, and 20.1 kDa (top to bottom); lane 1, G-ATCC; lane 2, CH38; lane 3, CH39; lane 5, CH3; lane 6, CH9; lane 7, CH30; lane 8, CH61; lane 9, CH71; lanes 11 and 12, partially purified CH71 and CH9, respectively.

Isolate NRSV-Hop produced distinct spurs with CH9 and CH3 serotypes and a faint spur with isolate CH30 that is not evident in the photograph. However, antiserum prepared against NRSV-Hop (22) reacted strongly only with NRSV-Hop and produced very faint precipitin lines with isolate G-ATCC (data not shown). NRSV-Hop antiserum did not produce a visible reaction with CH9 and CH30. Isolate ApMV-Hop reacted strongly with the NRSV-Hop antiserum and produced a distinct spur with isolate NRSV-Hop (data not shown), confirming earlier results (22). Apple mosaic virus antiserum obtained from ATCC (PVAS 32) reacted similarly to the NRSV-Hop antiserum, except that there was no visible reaction with G-ATCC (data not shown).

These results indicate that isolates NRSV-Hop and ApMV-Hop are serologically distinct from the PNRSV isolates transmitted from rosaceous hosts and are serologically related to each other. With the exception of CH30 and CH71, the PNRSV isolates from rosaceous hosts were not differentiated serologically in these tests and appear to be of the CH9 serotype.

DISCUSSION

Sedimentation profiles in sucrose density gradients of the PNRSV isolates used were similar to earlier results (7,12,21). These authors noted that the top centrifugal component was frequently the most prominent and that the middle component was often poorly resolved. The PNRSV isolate studied by Loesch and Fulton (12) was reported to sediment into three zones of approximately 72, 90, and 95 S. Although sedimentation coefficients of the centrifugal components were not determined in this study, nucleoproteins of all isolates used here sedimented to approximately the same position, suggesting that sedimentation coefficients do not vary greatly among isolates. Our work demonstrates that isolates of PNRSV display considerable electrophoretic diversity. A total of 22 virus isolates were tested and assigned to three electrophorotypes based on their migration rates. However, it seems probable that a continuum of electrophoretic mobilities exists among PNRSV isolates that will become more evident as more isolates are examined.

Because of the nonsizing nature of cellulose acetate sheets, electrophoretic mobility on this medium is determined primarily by net surface charge. Little or no separation occurs due to variation in particle size as evidenced by the fact that all PNRSV isolates migrated as single bands. Agarose gel electrophoresis, on the other hand, separated the nucleoprotein components on the basis of both size and net charge (14), the overall distance migrated being a function of the net surface charge with the resolution of specific components due to differences in particle size.

TABLE 2. Mean relative molecular weight (Mr) of PNRSV capsid proteins[a]

Isolate	Mr[b]	N[c]	SD[d]	Isolate	Mr	N	SD
NRSV-Hop	27.3	3	174	CH39	28.7	5	354
CH140	27.6	1		PE4	28.7	1	
CH3	27.8	6	677	PE6	28.7	1	
ApMV-Hop	27.8	1		PE8	28.7	1	
CH71	27.9	5	480	RO1	28.8	1	
CH30	27.9	2	97	RO3	28.8	1	
PE5	28.0	1		CH61	28.8	5	269
CH133	28.2	1		G-ATCC	28.9	6	379
CH141	28.2	1		RO2	29.0	2	327
CH142	28.2	1		CH57	29.2	5	81
CH143	28.2	1		CH9	29.3	4	448
AL14	28.6	2	290	CH38	29.3	6	348

[a] Determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using the methods of Laemmli (11).
[b] Values (in kilodaltons) are based on linear regression analysis of migrations of molecular weight standards (45.0, 36.0, 29.0, 24.0, and 20.1 kDa).
[c] Number of determinations included in the mean.
[d] Standard deviation (Daltons).

Isolates CH133, CH135, CH136, and AL14 all produced severe necrosis and tip dieback in *C. quinoa* when initially isolated from *Prunus* trees. These isolates, however, produced relatively mild systemic mottles in later transfers. Others (24) have observed that cultures of PNRSV maintained in cucumber frequently produced less pronounced symptoms after several serial transfers. These so-called run-out cultures also yielded relatively less bottom centrifugal component as compared with recently established cultures (12). In this study, isolates CH57 or CH9 purified from *C. quinoa* plants that exhibited either tip dieback or mild systemic symptoms produced similar, if not identical, electrophoretic migration patterns in agarose gels. These results suggest that migration patterns are unaffected by factors that induce changes in isolate symptomatology.

The relative molecular weights (Mr) of PNRSV coat proteins varied among isolates from about 27 to 29 kDa. Among other members of the ilarvirus group, coat protein Mr varies from about 19 to 30 kDa and most members have coat proteins of approximately 25–29 kDa (7). Barnett and Fulton (1) reported a Mr of 25 kDa for PNRSV isolate H based on amino acid analysis. Using 7.5% polyacrylamide gels, Gonsalves and Fulton (7) reported a Mr of 25 kDa for isolate G. More recently, Ong (19) noted variation in Mr among three PNRSV isolates: 26 kDa for FG and CH39, and 26.5 kDa for CH38. Our results also indicate a difference in coat protein Mr between CH38 and CH39. However, our estimates of Mr are more than 2 kDa larger for these isolates. The reasons for this discrepancy are not known. The determinations of Ong (19) were made using 10% polyacrylamide gels and, although the apparent Mr of a protein may vary depending on the polyacrylamide concentration (8), there were no major differences among our Mr determinations made in 10 or 12% gels (data not shown). Although isolates CH38 and CH39 were transmitted from rugose mosaic-diseased and nondiseased sweet cherry trees, respectively, our results with several rugose and nonrugose isolates suggest there is no obvious relationship between symptom severity of isolates on sweet cherry or *C. quinoa* and the coat protein Mr. Interestingly, coat protein Mr varied among isolates that cannot be differentiated serologically, such as CH38 and CH39. It is not known whether the observed variability in Mr among ilarvirus coat proteins results from differences in size, amino acid composition, or structural configuration. Noel et al (17) reported that single amino acid substitutions could alter the migration of a protein in polyacrylamide gels.

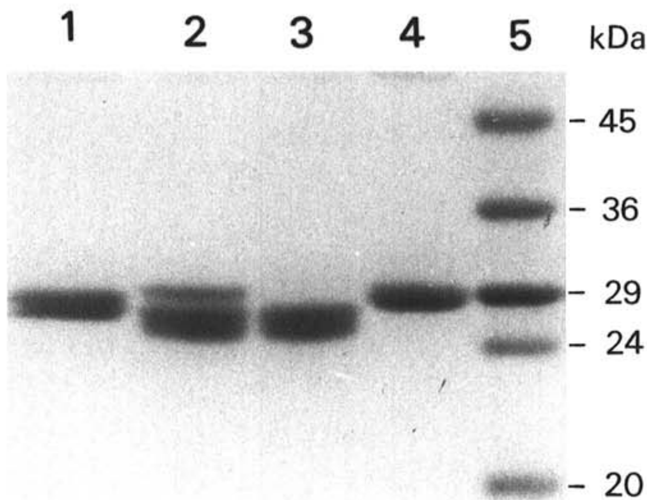

Fig. 7. Resolution of mixtures of Prunus necrotic ringspot virus coat proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, using 4% stacking and 12% resolving gels and methods described in Figure 6. Lane 1, isolates CH61 and CH71; lane 2, isolates CH57, CH3, and NRSV-Hop; lane 3, isolates CH3 and NRSV-Hop; lane 4, isolates CH38 and CH39; lane 5, molecular weight standards. The isolates within a given mixture are listed in order of decreasing molecular weight.

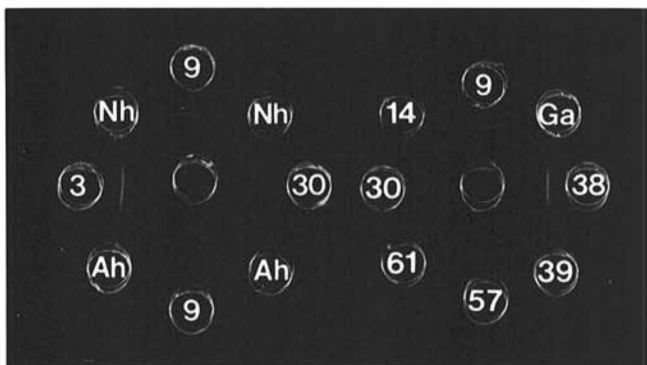

Fig. 8. Agar gel double-diffusion tests with several Prunus necrotic ringspot virus isolates and antiserum PVAS 22. Center wells were filled with antiserum diluted 1:4 in phosphate-buffered saline, pH 7.4. Antigens consisted of purified or partially purified nucleoproteins. Nh, NRSV-Hop; Ah, ApMV-Hop; Ga, GTCC; 14, AL14; 3, 9, 30, 38, 39, 57, and 61, CH isolate numbers.

Although the RNAs of most isolates tested exhibited similar electrophoretic mobilities under nondenaturing conditions, some differences were observed. For example, the relatively slow migration of RNA 2 of isolate CH30 allowed differentiation of this isolate from the serologically and electrophoretically indistinguishable isolate, CH71. Whether the differences in migration of the RNAs resulted from differences in molecular weight or secondary structure was not determined (13). However, differences in RNA electrophoretic mobilities can be useful markers for isolate identification.

Although there was diversity in nucleoprotein and RNA electrophoretic patterns among the PNRSV isolates tested, not all isolates could be specifically identified using only electrophoretic techniques. However, comparison of nucleoprotein and RNA electrophoretic patterns, coat protein molecular weight, and symptomatology allowed specific identification of virtually all isolates. The ability to identify individual virus isolates will be useful in identifying isolate mixtures, evaluating pseudorecombinants, differentiating serologically similar isolates, and studying cross protection (9) among PNRSV biotypes.

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