# Some Properties of Citrus Variegation Virus

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Infectious variegation virus (CVV) has been extensively studied (*2, 3*), but much remains to be done on physiochemical and serological characterization of the virus as well as on possible interference or complementation phenomena between the two components of the virus. This paper reports the results of our continuing investigations in these areas.

## EXPERIMENTS AND RESULTS

**Purification.** The CVV isolate used in these studies is the same as that used in previous work (*9, 11*). The virus was propagated in French bean, *Phaseolus vulgaris* L. cv La Victoire. The purification procedure was similar to that previously outlined by Martelli *et al.* (*11*) and Corbett and Grant (*1*). Leaves were harvested 12 to 15 days after inoculation, chilled at 2°C, and homogenized in 1 vol of 0.3-M citrate buffer, pH 6.5, in the presence of 1 vol of cold chloroform and 0.1 per cent thioglycolic acid. Density-gradient centrifugation for 2.5 hours in 0.2- to 0.8-M sucrose columns yielded two opalescent bands. The bands had ultraviolet light-absorption spectra characteristic of nucleoproteins, and were infectious. These two nucleoprotein components were not completely separated, as shown by UV analysis of the sedimentation profiles with an ISCO density-gradient fractionator (fig. 1, summer). Two additional peaks were seen, indicating slow sedimenting material (fig. 1, winter). These zones were identified as host proteins and ribosomes (fig. 1, winter). Increasing centrifugation time in density gradient did not improve separation of the two virus components; thus, studies on interference or complementation were not possible. The purification procedure is consistent and repeatable. Virus yield was 5 to 6 $O.D._{260}$/100 gm of infected leaves, and the preparations, after density-gradient centrifugation, were free of detectable amounts of plant contaminants.

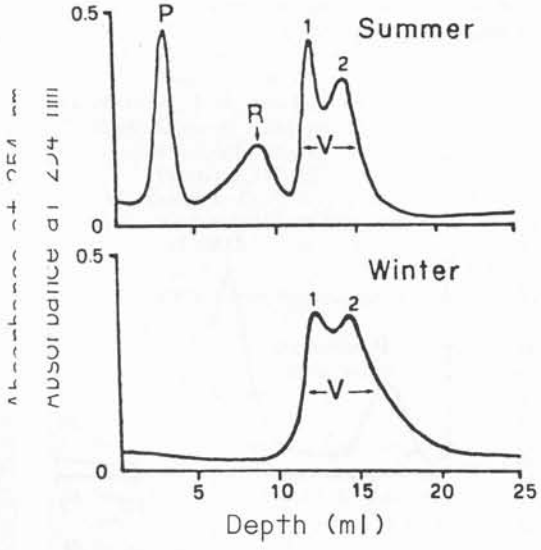


**Fig. 1. Sedimentation pattern of CVV in sucrose-density gradients after 2.5 hr centrifugation at 24,000 rpm in a Spinco SW 25.1 rotor. Profiles represent data obtained at different times of the year. P = plant protein; R = ribosomes; 1 and 2 = virus bands.**

**Sedimentation coefficient.** Sedimentation coefficient was determined by means of a Spinco Model E analytical ultracentrifuge equipped with Schlieren optics. Partially purified preparations yielded three to four peaks (fig. 2) similar to those seen in density-gradient sedimentation profiles. Purified preparations after density-gradient centrifugation yielded two faster sedimenting components. These were represented by two homogeneous peaks from which

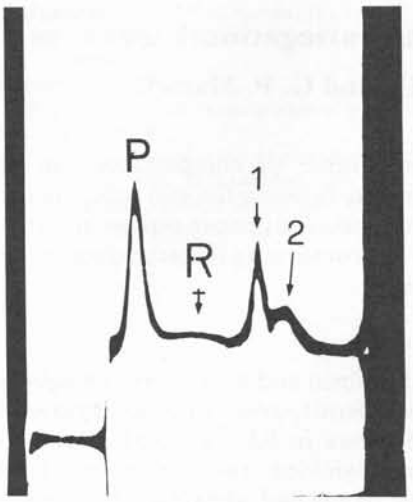


**Fig. 2. Sedimentation profile of partially purified CVV preparation. Photograph was taken 14.50 minutes after the speed reached 32,000 rpm. Schlieren angle is 30°, and temperature, 20°C. P = plant protein; R = ribosomes; 1 and 2 = virus.**

the sedimentation coefficients $S_{w20} = 86$ and 110 were calculated. These figures are in agreement with those of Corbett and Grant (*1*).

**Nucleic acid.** The percentage of nucleic acid of a mixture of the two components, calculated spectrophotometrically from UV-absorbance values, was about 12 per cent ($E_{max}/E_{min} = 1.27$; $E_{280}/E_{260} = 0.71$).

Nucleic acid was extracted from purified virus preparations by the single-phase phenol system of Diener and Schneider (*4*). The recovered nucleic acid had a low level of infectivity (5 per cent of the intact virus used as control). Infectivity was calculated on the basis of $O.D._{260}$ units of virus inoculated on the local-lesion host *Phaseolus aureus* Roxb.

Nucleic acid after extraction was centrifuged in 0.2- to 0.8-M sucrose density-gradient columns containing 1 ml of Mg-activated bentonite (*5*). The top component of chicory yellow mottle virus ($S_{w20} = 50$) was used as an internal standard (*15*). The nucleic acid appeared to be composed of two species sedimenting at 21 S and 24 S (fig. 3). These values correspond to molecular weights of about $0.8 \times 10^6$ and $1.1 \times 10^6$ daltons, respectively (*8*).

It is tempting to suggest that both sedimenting classes represent native RNA that originates directly from the two virus components, rather than from the breakage of a larger RNA molecule. If true, the two virus components would contain different nucleic acids, a common occurrence in many multicomponent plant viruses.

Intact virus was lyophilized and digested with 1 N HCl for 1 hour at 100°C. Bases were separated chromatographically on Whatman No. 1 paper, by the method of Markham and Smith (*10*). The molar base ratio was: A 24.8; G 26.6; C 20.7; U 27.9. Treatment of whole virus with 1.8 per cent formaldehyde at 27°C for 24 hours produced a 25 per cent increase in optical density and a shift of about 8 nm in wavelength maximum (from 258 to 260 to 267 to 268 nm), indicating single strandedness of the nucleic acid (*12, 16*).

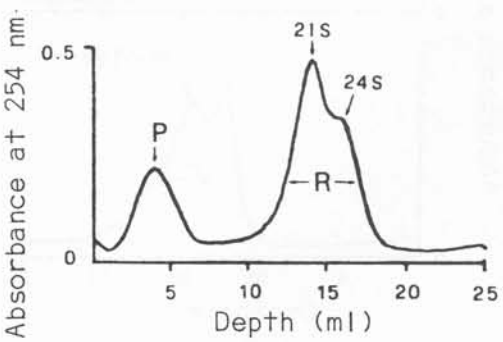


**Fig. 3. Extinction profile in sucrose-density gradients of CVV-RNA released by the single-phase phenol system. Gradients were centrifuged 11 hours at 24,000 rpm in a Spinco SW 25.1 rotor. P = virus protein; R = RNA with 21 and 24 S components.**

**Serology.** Immune sera were obtained by injecting rabbits with purified virus preparations fixed, or not, with glutaraldehyde. Antisera were prepared with titer varying from 1:64 to 1:256. Some of the antisera showed a weak reaction (1:2 to 1:4) against healthy bean plant sap.

## CONCLUSIONS

The purification method adopted yielded virus preparations sufficiently concentrated and "clean" to allow some physio-chemical studies of the virus complex. From these studies, CVV was determined to be a single-stranded RNA virus. The cryptogram: R/1 : */*: S/S: S/* is proposed. Symptoms of CVV in the herbaceous hosts are similar to those characterizing ILAR viruses (*6*) and the almond strains of prunus necrotic ringspot virus (*13, 14*). Similarity is seen in morphology, number of sedimentation classes, sedimentation coefficients (*7*) and molar percentage of nucleotides (*7*).

## ACKNOWLEDGMENTS

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