

### Purification and Properties of a Severe Strain of Peanut Mottle Virus

M. K. C. Sun and T. T. Hebert

Former Graduate Research Assistant and Professor of Plant Pathology, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27607.

Journal Series No. 3591 of the North Carolina State University Agricultural Experiment Station.

Accepted for publication 31 January 1972.

#### ABSTRACT

A disease of peanut having chlorotic, sometimes necrotic, mosaic leaf symptoms is caused by a severe strain of peanut mottle virus (PMV-s). Of 30 mechanically inoculated species in eight plant families, only nine species of *Leguminosae* were infected by PMV-s. Peanut yield reduction in the field was 40 and 70% in cultivars Florigiant and NC2, respectively. Seed transmission was 0.001% in NC2. In sap from garden pea, properties were: thermal inactivation between 60 and 65 C; dilution end point between  $10^{-3}$  and  $10^{-4}$ ; and longevity at room temperature between 12 and 14 hr with a half-life of 83

min. Virus was purified from garden pea by chloroform-butanol clarification, polyethylene glycol precipitation, and resuspension of the virus in 0.025 M phosphate buffer, pH 7.4, containing 0.02 M sodium sulfite and either 0.2 to 0.6 M urea or 0.2 M guanidine-HCl. Particles of PMV-s are flexuous rods with a normal length of 740 nm. Virus inclusions were similar to those produced by members of the potato virus Y group. The virus was not serologically related to soybean mosaic, potato Y, or tobacco etch viruses.

Phytopathology 62:832-839.

*Additional key words:* virus yield, virus titer, temperature effect.

---

In 1950, Cooper (4) reported two virus diseases of peanut (*Arachis hypogaea* L.). One was designated "peanut severe mosaic"; the other, "peanut mild

mosaic". Both viruses were transmitted mechanically, by grafting and by aphids. Peanut severe mosaic usually occurred in scattered plants and showed an

intensive chlorotic, sometimes necrotic, mosaic pattern with severe stunting and distortion of the infected leaves. Peanut mild mosaic became widespread within fields, and showed faint chlorotic mottling or spot patterns in the infected leaves. It was not known whether the two diseases were caused by different strains of the same virus or by different viruses.

Kuhn reported peanut mottle in 1965 (15), and recognized the similarities between this disease and Cooper's peanut mild mosaic, but he considered Cooper's report too brief for meaningful comparison. Diseases similar to peanut mottle were later reported from Bulgaria (21), Venezuela (12), and Japan (13). Flexuous rod-shaped particles (12, 13, 21) and pinwheel inclusion bodies (12) have been observed in infected plants. No reports of purification or production of antiserum for peanut mottle virus (PMV) were found in the literature.

Since there are no reports, other than Cooper's, concerning peanut severe mosaic, this investigation was initiated to obtain information on the symptomatology, host range, purification, effect on peanut yield, and serological relationships of the severe mosaic virus. Because the evidence indicates that the virus studied here is a strain of peanut mottle virus, it is designated as the severe strain of peanut mottle virus (PMV-s).

**MATERIALS AND METHODS.**—An isolate of peanut severe mosaic virus (PMV-s) was obtained from peanut from Bertie County, N.C., and its identification was confirmed by the late W. E. Cooper. The isolate was screened through guar (*Cyamopsis tetragonoloba* [L.] Taub) to eliminate possible contamination with peanut stunt virus, and through two single lesion transfers on Topcrop bean (*Phaseolus vulgaris* L.). The virus was maintained in guar or peanut and increased in garden pea (*Pisum sativum* L. 'Early Alaska') for the studies of purification, host range, physical properties, and effect on yield of peanut.

Peanut mottle virus (PMV) was obtained from C. W. Kuhn and maintained in peanut. Peanut mild mosaic virus (PMMV) was supplied by W. E. Cooper; soybean mosaic virus (SMV) and antiserum, by J. P. Ross; potato virus Y (PVY) and tobacco etch virus (TEV) and antisera, by G. V. Gooding.

To study the effect of the virus on peanut yield, cultivars NC2 and Florigiant were grown in the field in soil treated with methyl bromide. Plants inoculated on the 10th day after germination were compared with noninoculated controls. There were three replications/treatment in a randomized block design with 10 plants/1.5 m row. Infection was determined by symptom observation or serological tests. The harvested seeds were weighed and graded for size. Seeds from infected field-grown plants were divided into three equal lots and sowed at different times in the greenhouse to determine seed transmission of the virus. Seeds from healthy plants were grown in the same greenhouse.

Purification of PMV-s was done at 4 C by homogenizing systemically infected garden pea

plants, 8 to 14 days after inoculation, in 0.02 M sodium sulfite and 0.1 M phosphate buffer, pH 8.0 (2 ml/g tissue). The pulp was removed by passage through two layers of cheesecloth, and the extract vigorously shaken with 8:100 volume of chloroform-butanol (1:1), followed by centrifugation at 12,000 g for 10 min. The *n*-butanol was washed thoroughly with sodium bisulfite before mixing with chloroform. The water phase of chloroform-butanol-clarified juice was centrifuged at 12,000 g for 30 min after standing overnight. Sodium chloride and polyethylene glycol (PEG), MW=6000 (10, 11, 16), were added to the supernatant to a final concentration of 0.5 M and 4%, respectively. After 30 min or more, the virus was centrifuged at 12,000 g for 10 min. The pellet was suspended in a volume of buffer [0.025 M potassium phosphate, pH 7.4, containing 0.02 M sodium sulfite and 0.5 M urea (5) or 0.2 M guanidine-HCl equal to 20% of original extract volume]. Further purification was obtained by two additional PEG precipitations followed by one cycle of differential centrifugation.

To study the morphology of virus particles, virus preparations in 0.001% bovine serum albumin were sprayed on Formvar-coated copper grids and shadowed with palladium or mixed with potassium phosphotungstate at pH 7.3 and sprayed on Formvar-coated grids. The latter were reinforced before use with a light coat of carbon. Negatively stained virus particles from unpurified leaf homogenate were measured, and particle length was determined by the "arithmetic mean" method (3).

For the observation of virus inclusions, pieces of leaf tissue were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2; postfixed in unbuffered 1% osmic acid; dehydrated in alcohol; embedded in Epon; and sectioned with a diamond knife. Sections were stained with uranyl acetate (saturated in 50% ethanol) and lead citrate (0.05%) in 0.1 N sodium hydroxide (8, 14).

Antiserum to PMV-s was prepared by injecting rabbits intramuscularly four times at weekly intervals with 10 mg of purified virus emulsified with complete Freund's adjuvant per injection. The rabbits were bled periodically, starting 8 days after the last injection. The sera were pooled and freeze-dried.

PMV-s was compared serologically to other viruses of the potato virus Y (PVY) group (3) by a modified gel-diffusion method for flexuous rod-shaped viruses (9). The gel medium consisted of 0.8% Difco agar, 1% sodium azide, and 0.5% sodium dodecyl sulfate. Microprecipitin tests were also employed in the serological studies (1, 23).

**RESULTS.**—*Symptomatology and host range.*—Typical symptoms of PMV-s in peanut leaves are pale yellow or greenish-yellow mosaic patterns with variable green patches (Fig. 1-A). In plants inoculated at the two-leaf stage, the youngest leaves developed an intensive chlorotic, sometimes necrotic, mosaic pattern and were small and severely distorted. Inoculations at later growth stages initially caused faint yellow spots. In both cases, subsequent leaves showed the typical symptoms. No difference in

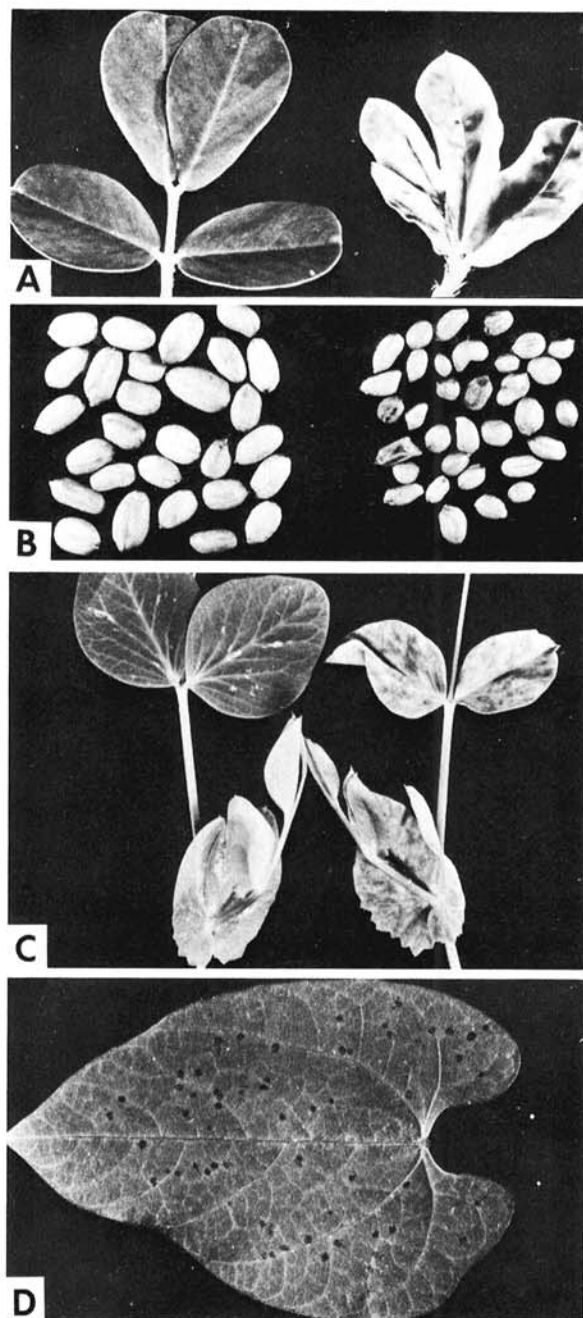


Fig. 1. Symptoms caused by a severe strain of peanut mottle virus. (Left) A, B, C) Healthy. (Right) A) Chlorosis and necrosis on NC2 peanut; B) seed size reduction on NC2 peanut; C) mottle and distortion on Early Alaska garden pea. D) Local lesions on Topcrop bean.

symptoms was observed among cultivars NC2, Florigiant, and Virginia Jumbo.

In Early Alaska pea, the virus caused vein clearing in the first leaves formed after inoculation; subsequent leaves were mottled, distorted, and reduced in size (Fig. 1-C). Five cultivars (Early

Alaska, Little Marvel, Laxton Progress, Thomas Laxton, Wando) were tested, and all were susceptible. In the Semnes, Dare, Lee, and Hill cultivars of soybean (*Glycine max* [L.] Merr), the leaves were mottled and malformed, the plants slightly stunted. Inoculated plants of the cultivars Ogden, York, and Hood showed no symptoms, and the virus could not be detected serologically in these plants. These three cultivars appear to be resistant to PMV-s.

Inoculated leaves of *Canavalia ensiformis* DC. developed large greenish-yellow areas, and plants wilted and died within 4 weeks after inoculation. *Trifolium incarnatum* L., *T. repens* L., and *Cyamopsis tetragonoloba* showed vein clearing followed by chlorosis with diffuse green blotches or green vein-banding. Systemically infected leaves of *Vigna sinensis* (Torner) Savi were mottled and malformed. Inoculated leaves of bean cultivar Tendergreen first showed faint chlorotic spots which later developed into necrotic local lesions and adjacent vein necrosis, whereas on the cultivar Topcrop, only necrotic local lesions appeared (Fig. 1-D). *Phaseolus coccineus* L. developed necrotic local lesions on inoculated primary leaves; systemically infected leaves were mottled and deformed.

*Cassia occidentalis* L., *C. tora* L., *Capsicum frutescens* L., *Cucumis sativus* L., *Chenopodium amaranticolor* L., *C. album* L., *Crotalaria spectabilis* Roth., *Datura stramonium* L., *Ipomea* sp., *Lycopersicon esculentum* Mill. 'Earliana', *Gomphrena globosa* L., *Medicago sativa* L., *Phaseolus aureus* Roxb., *P. mungo* L., *P. acutifolius* A. Gray, *Trifolium pratense* L., *Vinca rosea* L., *Zinnia elegans* L., *Nicotiana tabacum* L., and *N. glutinosa* L. were not infected by PMV-s. The virus could not be detected in inoculated or noninoculated leaves by local lesion assay on Topcrop bean and serological tests 2 to 8 weeks after inoculation.

*Effect on yield of peanuts and seed transmission.*—All plants inoculated 10 days after planting showed marked stunting 3 to 4 weeks after inoculation. Natural infection by the mild strain of peanut mottle virus occurred in many of the noninoculated plants. Individual uninfected plants selected from each block near the plot inoculated with PMV-s were used as controls. Yields were reduced by 72 and 41% in NC2 and Florigiant, respectively (Table 1). The reduction of weight was

TABLE 1. Effect of a severe strain of peanut mottle virus on growth and yield of peanuts inoculated 10 days after planting in the field

Cultivar	Treatment	Fresh wt of plants (g/10 plants)	Air dry wt of pods (g/10 plants)
NC2	Noninoculated	2,750.0**	995.3**
	Inoculated	810.2	279.9
Florigiant	Noninoculated	2,325.0**	684.3**
	Inoculated	1,230.0	404.4

\*\* = Significant difference (.01) between inoculated and noninoculated plants.

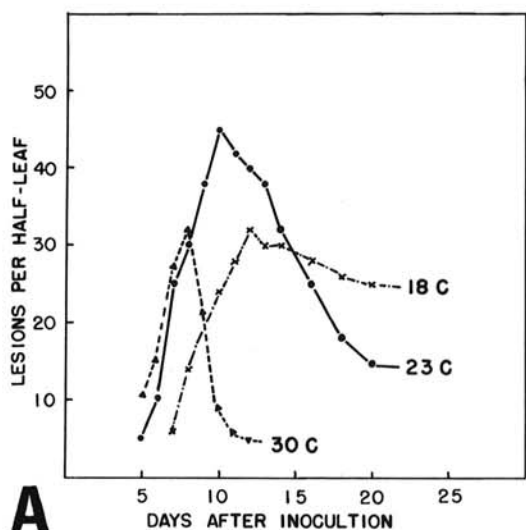
due primarily to reduction in seed size rather than in number of pods and kernels (Fig. 1-B).

In the seed transmission test, only one of 2,870 plants of NC2 showed symptoms of PMV-s, none in Florigiant (2,500 plants), and none in the check (3,000 plants of NC2). More than 90% of the seeds from infected plants germinated, and the plants grew normally.

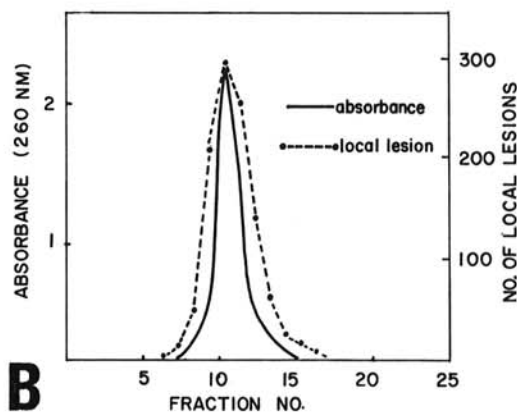
*Physical properties of PMV-s in sap.*—Tests of physical properties of PMV-s were done using sap from infected garden pea tissue ground in 0.1 M potassium phosphate, pH 8.0, containing 0.025 M sodium sulfite and assayed on Topcrop bean. The thermal inactivation point was between 60 and 65 C, and the dilution end point was between  $10^{-3}$  and  $10^{-4}$ . The inactivation by aging in sap (unbuffered) at 21-25 C was between 12 and 14 hr, with a half-life of 84 min. The method of Yarwood & Sylvester (24) as applied to plant viruses by Nitzany & Friedman (18) was used for the calculation of the half-life.

*Purification.*—Preliminary studies indicated that garden pea would be a suitable host for increasing PMV-s for purification because the virus titer is high in this host compared to other hosts, and the juice is easily extracted. A comparison was made of the virus titer in five cultivars of garden pea by inoculating Topcrop beans with juice from these cultivars (1 g tissue/5 ml phosphate buffer). Early Alaska, Little Marvel, and Laxton Progress produced about 300 local lesions/half-leaf, whereas Thomas Laxton and Wando produced less than 200 lesions/half-leaf. In a test to determine the effect of greenhouse temperature and age of infection on virus titer, it was found that the virus titer was slightly higher at 23 C than at 18 or 30 C, and that the maximum titer was reached after about 8 days at 30 C, 10 days at 23 C, and 12 days at 18 C (Fig. 2-A).

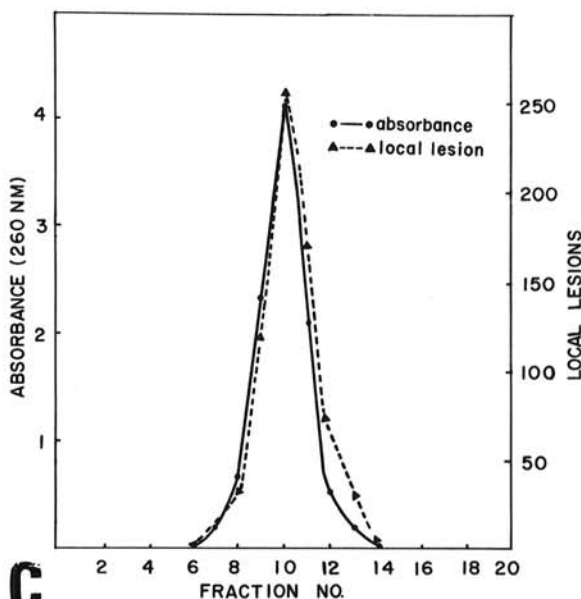
The procedure adopted for the purification of PMV-s is outlined in Fig. 3. Sodium bisulfite was added to butanol before mixing with chloroform (step 2) to remove any oxidized hydroxy groups (i.e., ketone and aldehyde) which could reduce infectivity of the virus. Polyethylene glycol was effective in precipitating the virus (10, 11, 16), and birefringence could always be observed at step 4 when the virus was resuspended in 2 ml buffer/100 ml of initial extract. On resuspension of the virus, urea or guanidine was chosen over ethanol, dioxane, and formamide to prevent and to reverse aggregation of the virus. They were tested by separately adding them at different concentrations to 0.025 M potassium phosphate, adjusting to pH 7.4, and resuspending PEG-treated virus. Effectiveness was assessed by observing



A



B



C

Fig. 2. Infectivity assays of severe strain of peanut mottle virus on Topcrop bean. A) Effect of greenhouse temperature and age of infection on virus titer in Early Alaska garden pea. B) Association of ultraviolet light absorbance and infectivity in successive fractions from a rate zonal sucrose density-gradient tube. C) Association of ultraviolet light absorbance and infectivity in successive fractions from a cesium chloride equilibrium density-gradient tube.

INFECTED TISSUE<sup>a</sup>

1 -homogenize in 0.1 M phosphate, pH 8.0 containing 0.02 M Na<sub>2</sub>SO<sub>3</sub> (2 ml/g tissue)

## HOMOGENATE

-remove pulp with cheesecloth; add 8% (v/v) chloroform-butanol (1:1)

2 -stir 10 to 15 min; centrifuge at 12,000 g 10 min

SUPERNATANT ..... pellet<sup>b</sup>

3 -stand overnight; centrifuge at 12,000 g 30 min

SUPERNATANT ..... pellet<sup>b</sup>

-add 4% (w/v) polyethylene glycol 6,000 and 0.5 M NaCl

4 -incubate 30 min or more; centrifuge at 12,000 g 10 min

PELLET ..... supernatant<sup>b</sup>

5 -resuspend in phosphate buffer (one-fifth original volume)<sup>c</sup>

-centrifuge at 12,000 g 10 min

SUPERNATANT ..... pellet<sup>b</sup>

6 -repeat step 4; repeat step 5 (in one-tenth original volume)

SUPERNATANT ..... pellet<sup>b</sup>

7 -repeat step 4; repeat step 5 (in one-fiftieth original volume)

## VIRUS PREPARATION

<sup>a</sup> The purification was performed at 4 C.

<sup>b</sup> Discard.

<sup>c</sup> Buffer = 0.025 M potassium phosphate, pH 7.4, containing 0.02 M Na<sub>2</sub>SO<sub>3</sub> and 0.5 M urea or 0.2 M guanidine HCl.

Fig. 3. Flow diagram for purification of a severe strain of peanut mottle virus from *Pisum sativum* 'Early Alaska'.

birefringence, absorbance 260 nm at different dilutions, and width of the band in sucrose density-gradient centrifugation. The effectiveness of different reducing agents in the buffer used to extract and resuspend the virus was compared using similar procedures. Sodium sulfite was found to be superior to 2-mercaptoethanol, thioglycolate, ascorbate, or cysteine. The enzyme inhibitor, diethylthiocarbamate, was of little value in the purification process.

The virus preparation purified through this procedure contained no detectable amounts of host antigens. The antihost serum used was produced by injecting partly purified host protein into rabbits. The

host protein was purified through the same procedure used for the virus purification except that 4% chloroform-butanol (1:1) was used in clarification and 15% PEG in the precipitation of host protein.

When the virus preparation was centrifuged on a sucrose density-gradient column (2), an absorption peak was located in the 10th and 11th 1-ml fraction from the top (2.8 to 3.0 mm beneath the meniscus) (Fig. 4). The material in this peak was found to be associated with infectivity (Fig. 2-B). The density gradient was prepared by layering 5, 5, 7, and 7 ml of 0.025 M phosphate buffer, pH 7.4, containing 20, 30, 40, and 50 g of sucrose, respectively, per 100 ml solution. Centrifugation was made in the Spinco SW25.1 rotor 3 hr at 60,000 g. The solution was fractionated by a density-gradient fractionator.

In equilibrated cesium chloride density gradients (22), a single prominent light-scattering band 2.0 to 2.2 mm beneath the meniscus was present. The material in this band was found to be associated with infectivity in tests on Topcrop bean (Fig. 2-C) and on peanut. The density gradients were prepared by mixing 1.8 ml of saturated cesium chloride (in 0.025 M phosphate buffer containing 0.02 M sodium sulfite) with 3.2 ml of virus solution in a 5-ml tube. The virus reached its buoyant density zone in the gradients formed during 26 to 30 hr of centrifugation at 100,000 g in the SW39 rotor.

Final preparations, after rate density-gradient centrifugation and/or equilibrium density-gradient centrifugation, had absorption spectra typical of tubular plant viruses with maxima at 260 nm and minima at 247 nm. The ratio of absorption at 260/280 was 1.24, suggesting that the virus had a low percentage of nucleic acid (about 5.5%). The purified preparation lost its infectivity and birefringence when frozen in phosphate buffer plus sodium sulfite.

*Virus recovery in various purification steps and yield of purified virus.*—Infectivity of samples

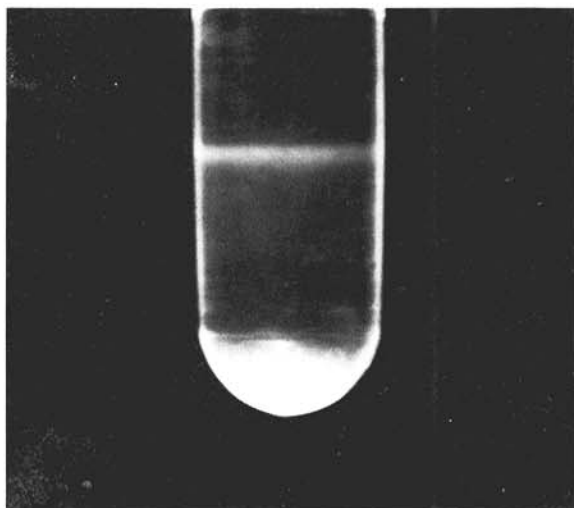


Fig. 4. Zone of a severe strain of peanut mottle virus in rate zonal sucrose density-gradient tube. The virus was purified by three cycles of polyethylene glycol precipitation followed by one cycle of differential centrifugation.

TABLE 2. Infectivity on Topcrop bean of samples taken at various steps in the purification of a severe strain of peanut mottle virus

Step	Total vol (ml)	Dilution for inoculation	Lesions <sup>a</sup> /half-leaf
Initial extract <sup>b</sup>	2,000	1	80
Water phase of chloroform-butanol clarification	1,500	8/10	29
First PEG <sup>c</sup> precipitation	400	2/10	26
Second PEG precipitation	200	1/10	23
Third PEG precipitation	200	1/10	22
High-low centrifugation	20	1/100	21

<sup>a</sup> Average no. lesions per eight half-leaves.

<sup>b</sup> 1,000 g of infected garden pea were used in this purification.

<sup>c</sup> PEG = polyethylene glycol 6,000 MW.

collected at various steps in the purification procedure was used to estimate recovery of the virus. Samples were diluted to the concentration of the initial extract, placed in separate dialysis tubes, and dialyzed together against the same buffer solution (0.025 M potassium phosphate, pH 7.4, containing 0.02 M sodium sulfite) to eliminate possible inhibitors existing in the virus solution of any particular step of purification. The main loss of infectivity was in chloroform-butanol clarification (Table 2). The loss of infectivity at this step varied with samples used, ranging from 30 to 75%. The loss of infectivity by PEG precipitation was negligible.

To estimate yield of purified virus, the virus preparation obtained after 3 cycles of PEG precipitation was centrifuged in cesium chloride equilibrium density-gradient columns, and dialyzed against deionized distilled water for 48 hr (4 changes of water). The virus solution was transferred to a tared serum bottle, freeze-dried for 48 hr, then dried over calcium chloride to a constant weight. In two tests, 28 mg and 32 mg of virus/1,000 g of infected garden pea were obtained.

*Virus particles and virus inclusions.*—After three precipitations with PEG, the virus preparation consisted primarily of flexuous rod-shaped particles (Fig. 5-A). No host antigens could be detected serologically. After sucrose density-gradient centrifugation, the virus particles were more uniform (Fig. 5-B), with a mean length of 738 nm (Fig. 6-B). This corresponded very closely to the mean length of 740 nm obtained by measurement of particles in unpurified leaf homogenate (Fig. 6-A). In both preparations, the small peaks observed at ca. 1,480 nm probably represented end-to-end aggregates of virus particles.

Thin sections from infected leaves of garden pea revealed the presence of inclusion bodies having the shapes of pinwheels (Fig. 5-C), bundles, or circles similar to those observed by Herold & Munz (12) and to other members of PVY group (6, 7, 17).

*Serological relationship to some other viruses.*—In

gel diffusion tests, antiserum to PMV-s reacted with Cooper's peanut mild mosaic virus (PMMV) and Kuhn's PMV, and produced a distinct curved band near the antigen well. The reaction lines formed continuous bands without spur formation. No attempts were made to detect the degree of relationship, nor were the reciprocal serological tests done because no antisera against PMV and PMMV were available. Anti-PMV-s serum did not react with potato virus Y, soybean mosaic virus, or tobacco etch virus in either gel diffusion or microprecipitin tests.

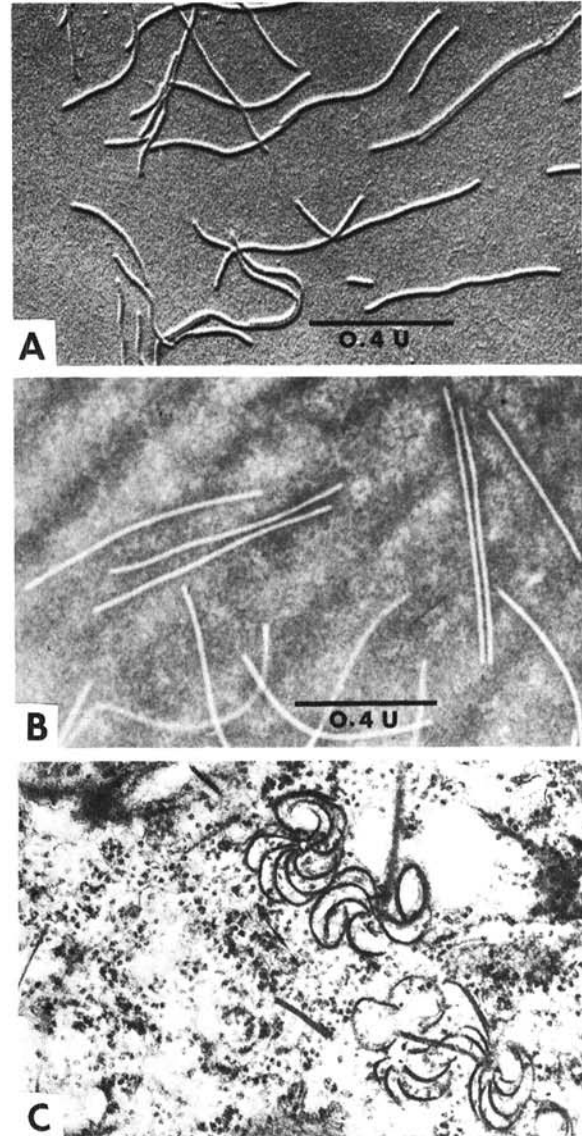


Fig. 5. Electron micrographs of particles and inclusion bodies of severe strain of peanut mottle virus. A) Palladium-coated virus particles after three polyethylene glycol precipitations. B) Negatively stained virus particles after further purification by sucrose density-gradient centrifugation. C) Pinwheel inclusion bodies observed in thin sections.

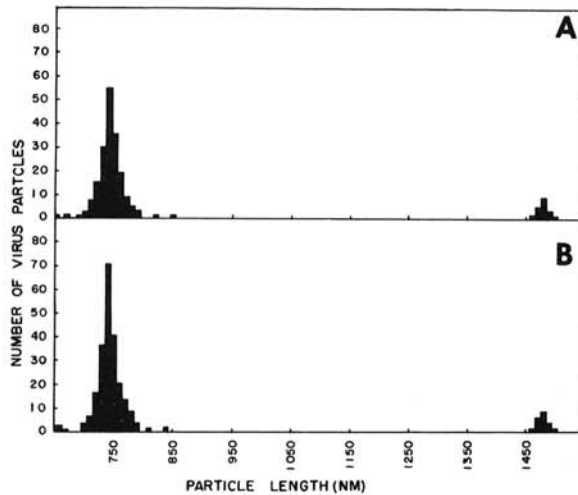


Fig. 6. Histograms showing the number of particles of severe strain of peanut mottle virus in each 10-nm length group from electron micrographs. A) Garden pea leaf homogenate. B) Purified virus from garden pea.

In similar experiments, PMV-s did not react with antisera to those three viruses.

**DISCUSSION.**—On the basis of serological reaction, host range, and physical properties, it is concluded that the severe mosaic of peanut reported by Cooper (4) is caused by a strain of the peanut mottle virus described by Kuhn (15), and is designated as the severe strain of peanut mottle virus (PMV-s). The principal differences between PMV and PMV-s are that PMV-s causes more severe symptoms on peanuts and certain other hosts than does PMV; PMV-s causes symptoms in guar and in *Phaseolus coccineus*, whereas these are symptomless hosts of PMV; *Cassia tora* and *C. occidentalis* are infected by PMV (15) but not by PMV-s; and the rate of seed transmission is lower in PMV-s (0.001%) than in PMV (2%). The peanut mild mosaic reported by Cooper (4) and the peanut mottle reported by Kuhn (15) appear to be the same disease. The virus isolates causing these two diseases both reacted serologically with antiserum to PMV-s and produce similar symptoms in peanuts and other hosts.

Sodium sulfite played an important role in the purification of PMV-s. All tests showed that birefringence could be observed after the first PEG precipitation only when sodium sulfite was present during homogenation. Resuspension of the virus was more complete with sodium sulfite than with other reducing agents in the buffer. Sodium sulfite probably acted not only as a reducing agent by inhibiting phenoloxidase and by combining with quinones (19), but also by inhibiting virus aggregation.

The particle morphology and inclusion bodies in infected plants indicate that PMV-s is a member of the potato virus Y group (3, 6, 7). It is not closely related serologically to three other members of this group, soybean mosaic virus (SMV), potato virus Y,

and tobacco etch virus. Of the seven soybean cultivars tested, three were resistant and four were susceptible to PMV-s. These seven cultivars show the same response when inoculated with several SMV isolates (20). Genes for resistance to SMV in soybeans may also confer resistance to PMV-s. If so, this would indicate a relationship between PMV-s and SMV.

Peanut severe mosaic is usually found in only a few scattered plants in the field, whereas the peanut mottle virus usually infects a high percentage of the plants in many fields (4). The low percentage of seed transmission of the severe strain may be partly responsible for the low incidence of this strain in the field. Also, since such a large percentage of the plants in the field are usually infected by the mild strain, this strain may be protecting the plants from infection by the severe strain. Although seed transmission is thought to be the primary source of inoculum for PMV and PMV-s, the importance of other hosts as sources of inoculum has not been adequately investigated.

#### LITERATURE CITED

1. BALL, E. M. 1964. Serology: techniques used in plant virus research, p. 235-252. In M. K. Corbett & H. D. Sisler [ed.]. Plant virology. Florida Press, Gainesville.
2. BRAKKE, M. K. 1960. Density gradient centrifugation and its application to plant viruses. *Advances Virus Res.* 7:193-224.
3. BRANDES, J., & C. WETTER. 1959. Classification of elongated plant viruses on the basis of particle morphology. *Virology* 8:99-115.
4. COOPER, W. E. 1950. Two virus diseases of peanuts. *Phytopathology* 40:6.
5. DAMIRDAGH, I. S., & R. J. SHEPHERD. 1970. Purification of the tobacco etch and other viruses of the potato virus Y group. *Phytopathology* 60:132-142.
6. EDWARDSON, J. R. 1966. Electron microscopy of cytoplasmic inclusions in cells infected with rod-shaped viruses. *Amer. J. Bot.* 53:359-364.
7. EDWARDSON, J. R., D. E. PURCIFULL, & R. G. CHRISTIE. 1968. Structure of cytoplasmic inclusions in plants infected with rod-shaped viruses. *Virology* 34:250-263.
8. FAHMY, A. 1967. An extemporaneous lead citrate stain for electron microscopy. 25th Annu. Meeting Electron Microscop. Soc. Amer. Proc. 148 p.
9. GOODING, G. V., & W. BING. 1970. Serological identification of potato virus Y and tobacco etch virus using immunodiffusion plates containing sodium dodecyl sulfate. *Phytopathology* 60:1293.
10. GOODING, G. V., & T. T. HEBERT. 1967. A simple technique for purification of tobacco mosaic virus in large quantities. *Phytopathology* 57:1285.
11. HEBERT, T. T. 1963. Precipitation of plant viruses by polyethylene glycol. *Phytopathology* 53:362.
12. HEROLD, F., & K. MUNZ. 1969. Peanut mottle virus. *Phytopathology* 59:663-666.
13. INOUE, T. 1969. Peanut mottle virus from peanut and pea. *Nogaku Kenkyu* 52:159-164.
14. KAY, D. H. 1965. Techniques for electron microscopy. F. A. Davis Co., Philadelphia. 560 p.

15. KUHN, C. W. 1965. Symptomatology, host range, and effect on yield of a seed-transmitted peanut virus. *Phytopathology* 55:880-884.
16. LEBERMAN, R. 1966. The isolation of plant viruses by means of "simple" coacervates. *Virology* 30:341-347.
17. MATTHEWS, R. E. F. 1970. *Plant virology*. Academic Press, New York & London. 778 p.
18. NITZANY, E. F., & S. FRIEDMAN. 1963. Application of the half life concept to some plant viruses. *Phytopathology* 53:548-551.
19. PIERPOINT, W. S. 1966. The enzymic oxidation of chlorogenic acid and some reactions of the quinone produced. *Biochem. J.* 98:567-580.
20. ROSS, J. P. 1969. Pathogenic variation among isolates of soybean mosaic virus. *Phytopathology* 59:829-832.
21. SCHMIDT, H. B., & K. SCHMEIZER. 1966. Elektronenmikroskopische Darstellung und Vermessung eines saftübertragbaren Virus der Erdnuss (*Arachis hypogaea* L.). *Phytopathol. Z.* 55:92-96.
22. SIEGEL, A., & W. HUDSON. 1959. Equilibrium centrifugation of two strains of tobacco mosaic virus in density gradients. *Biochim. Biophys. Acta* 34:254.
23. SLOGTERON, D. M. H. VAN. 1955. Serological micro-reactions with plant viruses under paraffin oil, p. 51-54. 2nd Conf. Potato Virus Dis. Proc. Lisse-Wageningen.
24. YARWOOD, C. E., & E. S. SYLVESTER. 1959. The half life concept of longevity of plant pathogens. *Plant Dis. Repr.* 43:125-128.