Strains of Peanut Mottle Virus

O. R. Paguio and C. W. Kuhn

Department of Plant Pathology and Plant Genetics, University of Georgia, Athens 30602.
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ABSTRACT

Five virus isolates from peanut were identified as strains of peanut mottle virus. Relatedness was determined by similarities in particle morphology, serological reactions, ultraviolet absorbancy, host range, and properties in crude juice; mild strains cross-protected against the severe ones. Strains M1 and M2 induced mild mottle in peanut, whereas N, S, and CLP caused necrosis, severe mosaic, and chlorotic line pattern symptoms, respectively, Strains M1 and M2 were differentiated by three criteria: (i) symptoms on pea, (ii) incubation period in pea, and (iii) size of local lesions on bean. Other differences among the strains were local lesion size and virus production in peanut and pea.

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Mild mottle, necrosis, and chlorotic line pattern are symptoms of three virus diseases found in peanut fields in Georgia, and a severe mosaic disease has been found in North Carolina (14). Of these the mild mottle caused by peanut mottle virus (PMV) is the most prevalent (3, 7). Recent studies (10) revealed that this virus infects 75-90% of peanuts (*Arachis hypogaea* L.) in some areas of Georgia by midseason. The necrosis and chlorotic line pattern diseases were observed in only a few scattered plants and did not become widespread in commercial fields. These diseases, however, could be transmitted mechanically and they had a host range similar to PMV. Comparative tests were made to determine the relationship among these virus isolates obtained from peanuts.

MATERIALS AND METHODS.—Five virus isolates were used in this investigation. Four were obtained from peanut fields in Georgia and the fifth, a severe mosaic strain (PMV-S) (14), was furnished by T. T. Hebert. Each isolate was maintained in 'Argentine' peanut in a greenhouse with temperatures of 24 to 32 C. Mechanical inoculations were made by rubbing Carborundum-dusted leaves with a cheesecloth pad dipped in sap inoculum prepared in 0.01 M potassium phosphate buffer, pH 8.0, containing 0.01 M sodium diethyldithiocarbamate, 0.01 M sodium bisulfite, and 1% Celite.

Half-leaf local lesion assays were conducted on 'Topcrop' bean (*Phaseolus vulgaris* L.) (12). Bean was also used as a tester plant to determine the presence of virus in other hosts. Local lesion size for the various isolates was determined by measuring lesion diameters with a dial caliper.

For host range studies, five or more plants of each species or cultivar were inoculated. The plants were observed for about a month for symptom development, then they were back-inoculated to Argentine peanut to determine whether the virus was present, or if there was any change in symptomatology.

For longevity in vitro and dilution end point studies, both buffered and unbuffered sap preparations were made from 'Little Marvel' pea (*Pisum sativum* L.) infected 9 to 12 days. Virus preparations diluted 1/10 were kept in rubber-stoppered test tubes in the laboratory (22 to 25 C) until used.

For cross-protection tests, Argentine peanuts were segregated into two groups of 25 plants each. One group was inoculated with a mild mottle isolate (PMV-M2); the other group was not inoculated. When symptoms became apparent 12 to 15 days later, half of the PMV-M2-infected plants and half of the noninoculated plants were then inoculated with other isolates. Evaluation of cross-protection was determined by comparing symptoms which developed on challenged plants and the single inoculation to Argentine peanuts. Tests were conducted twice.

All virus isolates were cultured in Little Marvel pea for purification which was accomplished by chloroform clarification, polyethylene glycol precipitation, and density-gradient ultracentrifugation (12). Virus obtained from the single infectious zone in density-gradient columns was used for rabbit immunization, and serological and infectivity tests. Approximately 200 negatively stained (2% potassium phosphotungstate) particles of each isolate were measured by electron microscopy as described previously (12).

Antiserum for each isolate was prepared by giving alternate intramuscular and intravenous injections to rabbits at weekly intervals for six weeks. Antisera were stored in small volumes at -29 C. Serological relationships were studied by both ring interface, and microprecipitin tests (1). Three controls were used: (i) protein or nucleoprotein extracted from healthy peas (12), (ii) antiserum prepared against the protein from healthy pea, and (iii) normal rabbit antiserum.

RESULTS.—*Symptomatology on peanut.*—Four of the five virus isolates could be easily differentiated on the basis of symptoms expressed on several cultivars of peanut (Argentine, 'Starr', 'Florigiant', and 'Florunner'). Their characteristic symptoms are described below.

Mild mottle isolates (PMV-M1 and PMV-M2).—PMV-M1 was obtained in 1961 and has been maintained in greenhouse-grown peanut plants. PMV-M2 was isolated in 1971 from a peanut field. These isolates induced mild mottling and upward curling of the leaf margins (Fig. 1-A and 1-E). Although upward cupping and depression of

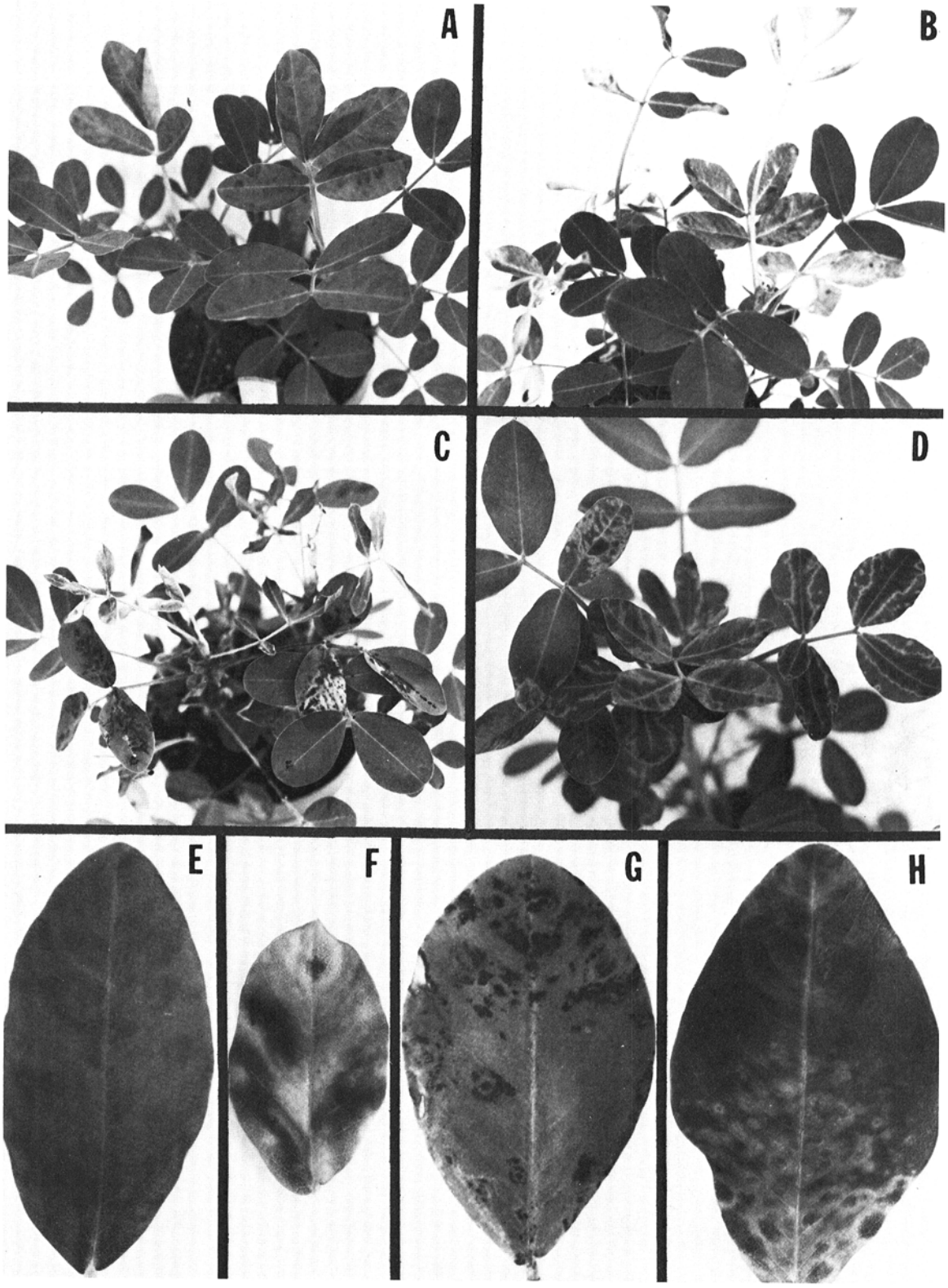

Fig. 1. Symptoms induced by different strains of peanut mottle virus (PMV) on 'Argentine' peanut. **A)** Mild mottle caused by PMV-M2 (M1 mottle is very similar), **B)** general chlorosis, leaf rolling, and stunting caused by PMV-S, **C)** necrosis, leaf rolling, and stunting caused by PMV-N, **D)** chlorotic line patterns caused by PMV-CLP, **E)** mild mottle caused by PMV-M2, **F)** chlorosis and reduced size caused by PMV-S, **G)** necrotic etching and rings caused by PMV-N, **H)** chlorotic ringspots caused by PMV-CLP.

interveinal tissue was more conspicuous with PMV-M1-than with PMV-M2 infected peanuts, their significance in diagnosis is questionable. Neither isolate caused reduction in vegetative growth of infected peanuts.

Severe mosaic isolate (PMV-S).—Symptoms of PMV-S on all peanut cultivars were similar to those reported by Sun & Hebert (14). There was general yellowing of the leaves especially in the area near the veins, and as the disease progressed, the succeeding leaflets became distorted, narrow, and cupped upward (Fig. 1-B and 1-F). Infected peanuts were severely studied.

Necrosis isolate (PMV-N).—Initially, chlorotic spots were observed on young developing leaflets. These spots become necrotic 2 to 3 days later (Fig. 1-C and 1-G), sometimes forming well-defined concentric rings (Fig. 1-G). Necrosis also occurred on all new growth (11). In advanced stages, the whole plant became severely stunted. Leaves at this stage were distorted, narrow, and cupped upward similar to PMV-S symptoms except that the PMV-N leaves were greener and had irregular necrotic spots (Fig. 1-C). Necrosis symptoms were favored by high temperatures. More intense necrosis was observed in summer (25 to 32 C) than in winter months (22 to 28 C) in the greenhouse. The effect of temperature was noted also when inoculated peanuts kept at 16 C for 1 month did not develop necrosis. When these plants were transferred to the greenhouse (26 to 32 C), the new growth became necrotic.

Chlorotic line pattern isolate (PMV-CLP).—This isolate produced three types of symptoms. The initial symptom of vein-clearing on the newly developing leaflets, was followed by general chlorosis, with some dark green spots. The leaves turned pale as they matured with some dark green streaks along the secondary veins. Distinct chlorotic line patterns and chlorotic ringspots were observed on succeeding leaves (Fig. 1-D). Frequently, chlorotic spotting was concentrated at or near the basal end of leaflets (Fig. 1-H). The spots and line pattern became less intense as the plants grew older. The isolate caused little or no stunting of peanuts.

Symptomatology on other hosts.—The mild mottle isolates (PMV-M1) and PMV-M2) could be distinguished on Little Marvel pea by symptom severity and time of symptom appearance. PMV-M1 produced prominent vein-clearing 6 to 8 days after inoculation. Two to three days later, netlike vein chlorosis was observed. Subsequent leaves were mottled, slightly distorted, and reduced in size. PMV-M2 symptoms differed in two ways: (i) a general chlorosis rather than a netlike vein chlorosis followed the initial vein-clearing; and (ii) the initial symptoms occurred several days later, 12 to 14 days after inoculation.

The reaction of Little Marvel pea to the other isolates (PMV-N, S, and CLP) was similar to PMV-M2.

All isolates produced necrotic local lesions on Topcrop bean. Lesions usually appeared on the third day after inoculation for PMV-N and on the fourth day for the other isolates. Lesion size varied significantly between certain isolates; e.g., M1 was different from M2 and N, and N was different from M1, CLP, and S (Table 1). Isolates M1 and S produced the smallest lesions which could be distinguished easily from the larger ones caused by M2 and N (Table 1). Isolate CLP lesions were intermediate in size.

Other hosts produced similar reactions for all PMV isolates. Systemic mottle was observed on the following plants: *Phaseolus lunatus* L. 'Henderson', *Glycine max* (L.) Merr. 'Lee' and 'Bragg', *Vigna sinensis* (L.) Savi 'Early Ramshorn' and 'Clay', and *Pisum sativum* 'Early Alaska'. Diffuse chlorotic spots were produced on *Phaseolus vulgaris* 'Bountiful' and necrotic local lesions followed by systemic necrosis was observed on *P. vulgaris* 'Kentucky Wonder' and *Cassia tora* L.

Tests for a contaminating virus.—Similarities in host range and symptomatology suggested that the various isolates were either related to or contaminated with the original mild mottle isolate (M1). Therefore, attempts were made to separate a second virus from all isolates. With the exception of the CLP isolate, the virus obtained by serial dilution and single lesion isolation techniques always produced symptoms similar to the original source when they were inoculated back to Argentine peanuts. Furthermore, when each isolate was purified, a single infectious zone was found after density-gradient ultracentrifugation. Virus from the zone caused symptoms on peanuts similar to the source. The evidence is strong that a contaminating virus was not responsible for the different symptoms produced on peanut.

The CLP culture appeared to be a mixture of the CLP and M2 isolates. Initially, when peanuts were inoculated with CLP (sap from either peanut or pea), only 5 to 10% of the plants had CLP symptoms and

TABLE 1. Variation in local lesion size on 'Topcrop' bean and in virus production in 'Argentine' peanut and 'Little Marvel' pea among five isolates of peanut mottle virus

Isolate	Lesion area[a] (mm^2)	Virus production[b]	
		Peanut[c]	Pea[d]
M1	0.54[e]	22[f]	122[g]
M2	1.17	35	69
CLP	0.82	9	23
S	0.47	94	124
N	1.35	90	174

[a]Average of 320 lesions. Ten lesions, selected randomly, were measured on 32 plants, each plant representing a replication for statistical analysis.

[b]Sap extracts were used as inoculum for half-leaf local lesion bioassays (eight replications/treatment) on Topcrop bean. Assays were made 9 days after inoculations of peanut and pea.

[c]Peanut sap diluted 1/50.

[d]Pea sap diluted 1/100.

[e]LSD at 5% = 0.37; LSD at 1% = 0.50.

[f]LSD at 5% = 31; LSD at 1% = 43.

[g]LSD at 5% = 31; LSD at 1% = 42.

the remainder had mild mottle symptoms. An isolate which caused CLP symptoms on all plants was eventually obtained by serially selecting local lesions produced on Topcrop bean. CLP symptoms were obtained from purified virus samples (the zone from density-gradient centrifugation tubes) and by back-inoculation to peanut after inoculation of Kentucky Wonder bean, Little Marvel pea, and *Cassia tora*. Further evidence of heterogeneity of the particle population within an isolate occurred with PMV-M2; infrequently, a peanut plant with CLP symptoms occurred after inoculation with PMV-M2 which has showed no CLP symptoms for several months and for several serial inoculations.

Particle characteristics.—All virus isolates reacted similarly to the purification procedure adapted for PMV-M2 (12). They were inactivated by most conventional methods of clarification, and they severely aggregated when concentrated by differential ultracentrifugation and polyethylene glycol precipitation. However, incorporation of Cleland's reagent in the suspending buffer partially prevented and reversed aggregation. Thus, a single band was obtained following a second density-gradient centrifugation. The position of the absorption peak and the infectivity zone was similar (28 to 32 mm below the meniscus) for all isolates. The ultraviolet absorbancy showed further similarities. All isolates had maximum absorbance at 260 nm, minimum absorbance at 246 nm, and a 260:246 nm ratio of about 1.13.

Comparative electron microscopic examinations of purified preparations revealed that all isolates possessed flexuous rod particles with a most frequent length of 761, 725, 724, and 723 nm for M1, M2, N and CLP, respectively. The reported length for the S isolate is 738 nm (14). Whether these length differences are a characteristic feature of each isolate is not certain. Reports of particle length of PMV isolates of the mild form found outside the United States (2, 5, 6, 13) vary from 704 to 812 nm.

Serology.—Antisera prepared against each PMV isolate, reacted with all PMV isolates. The dilution end points of homologous and heterologous tests were similar. The titers of the microprecipitin tests varied between 1/64 and 1/128 and the ring-interface tests titers were between 1/256 and 1/512. Any differences were probably due to the inability to read the end point more sensitively since heterologous reactions were usually only one 2-fold dilution greater than homologous reactions. Attempts to use the modified Ouchterlony agar double diffusion for flexuous rod type viruses (4) were not very successful. Positive reactions (precipitin lines) were observed, but the virus antigen did not move sufficiently in the agar to allow definitive relationship interpretations.

All serological controls were negative and previous studies (12, 14) showed no serological relationship between PMV isolates (M2 and S) and other flexuous rod-type viruses of a similar length.

Virus production.—All isolates were purified from Little Marvel pea infected ten days. Based on the 260 nm absorbancy and the size of the density-gradient zone, the concentration of virus varied among the isolates. Local lesion assays on Topcrop bean confirmed the differences. In peanut, isolates N and S produced 3-4 times as much virus as isolates M1 and M2 and about 10 times as much as isolate CLP (Table 1). Differences in virus production were significant but not quite as striking when the isolates were cultured in pea (Table 1).

Cross-protection.—Since symptoms produced on peanuts by isolate M2 were mild and differed considerably from the more severe N, S, and CLP isolates, it was possible to conduct cross-protection tests. When 50 M2-infected peanut plants were challenged with the N, S, or CLP isolates, no necrosis, severe mosaic, or chlorotic line pattern symptoms were observed on these plants or on Argentine peanuts which were inoculated with sap from the challenged plants. Attempts to isolate N and CLP from single Topcrop local lesions obtained from challenged plants also failed, suggesting that these virus isolates did not multiply in the plants.

Properties in crude juice.—Longevity in vitro and dilution end point values for all PMV isolates from unbuffered preparations were substantially the same as those reported for the mild isolates (2, 5, 7, 14). Since low numbers of lesions were obtained with unbuffered sap preparations, tests were made with 0.05 M potassium phosphate buffer, pH 8.0, containing 0.01 M Na_2SO_3. All isolates remained active up to 120 but not 144 hr at 22 to 25 C. Isolates M2, CLP, and S had a dilution end point between 10^{-3} and 10^{-4} and isolates M1 and N between 10^{-4} and 10^{-5}.

Seed transmission.—The rate of seed transmission in peanut may vary between the mild (M1, M2), and severe (N, S), isolates. For PMV-M2, 1.6% (14 of 838) of the seed produced diseased plants, a value similar to the 2% reported for PMV-M1 (7). The PMV-N transmission rate was 0.6% (5 of 836), and Sun & Hebert (14) found only one of 5,370 (0.02%) peanut plants with PMV-S.

Comparison of PMV-N-induced necrosis and a ringspot disease.—In 1964, Kuhn et al. (9) reported a ringspot disease of several peanut introductions. Since the symptoms of the disease appeared to be similar to the necrosis and ringspot symptoms induced by PMV-N (Fig. 1-G), it was desirable to investigate their possible relationship. Three methods were employed: (i) mechanical inoculation of ringspot-infected leaves ground in various buffers (acetate, phosphate, borate) including the buffer-antioxidant system used for routine inoculation of PMV-N, (ii) phenol extraction of ringspot tissue to determine if the causal agent exists as a free nucleic acid, and (iii) cross-protection tests. The source of ringspot disease was seeds of two peanut introductions (P.I. 261986 and P.I. 262009) in which the disease was originally observed. All attempts, including the phenol extraction, to mechanically transmit the ringspot disease were unsuccessful. No symptoms occurred on Argentine peanut or Topcrop bean, two common hosts of PMV-N. The ringspot disease did not cross-protect

against PMV-N since necrotic symptoms developed on new growth of the peanut introductions with ringspot when they were inoculated with PMV-N, and PMV-N was isolated from the challenged plants 15 days after inoculation. From these results, it was concluded that ringspot disease is not related to the peanut necrosis disease induced by PMV-N. Whatever causes the ringspot on peanut is not known. The disease seemed to be localized rather than systemic as observed with PMV-N. Ringspot occurred only on mature leaves which developed 3 to 4 weeks after planting and none developed on later growth.

DISCUSSION.—The five peanut virus isolates studied herein are clearly related, and should be regarded as variants of PMV. A close relationship among all isolates was established based on particle morphology, serological reactions, cross-protection tests, and host range studies. We believe the five isolates are distinct strains of PMV. Although strains M1 and M2 produced similar mild mottle symptoms on peanut, they could be clearly differentiated by other criteria: (i) symptoms on pea, (ii) incubation period in pea, (iii) virus production in pea, and (iv) size of local lesions on bean. The other three strains (N, S, CLP) caused distinctly different symptoms on peanut. Furthermore, lesion size and virus production established differences among the PMV isolates (Table 1). Four of the strains (M1, M2, N, S) have reacted similarly in the greenhouse and laboratory for at least two years; they appear to be stable, predictable entities. Strain CLP varied somewhat until single lesion isolations produced a stable isolate. Originally, the CLP isolate was a mixture of CLP and M2.

All five PMV strains were obtained from commercial peanut fields. Field surveys (*unpublished*) and isolation tests in 1971 and 1972 (10) have established that M2 is the most prevalent strain in Georgia. It is not clear whether the original mild mottle strain (M1), isolated in 1961, has changed in the greenhouse environment or if the prevailing strain has changed in the field. Strains N and CLP were found in only a few plants in a few fields (10). PMV-S has not been observed in Georgia, but it occurs in scattered peanut plants in North Carolina (14). The limited distribution of the N, CLP, and S strains in the field may be explained by a very low rate of seed transmission, absence of a major source of primary inoculum, more than one insect vector which may differ in their ability to transmit different strains, cross-protection by a prevalent mild mottle strain, or a combination of these factors.

The presence of different PMV strains poses a problem in developing control measures. Although a mild strain may offer protection against the more severe strains, its existence should not be accepted because it causes yield losses between 18 and 26% (10). Furthermore, PMV-M2 has been found in commercial soybeans (8) and pea (6), and a shift in the predominating strain is possible.

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