Incidence and Source of Inoculum of Peanut Mottle Virus and Its Effect on Peanut

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ABSTRACT

Peanut mottle virus (PMV) causes a major disease of peanuts (*Arachis hypogaea*) in Georgia. A mild mottle strain of PMV was predominant in commercial fields, but the necrosis and chlorotic line pattern strains were found infrequently. The source of primary inoculum appeared to be infected seed. PMV was transmitted through the seed of six peanut cultivars, obtained from a commercial source, at a rate of about 0.3%. We could not isolate PMV from weeds, trees, shrubs, or vines in or near peanut fields with PMV. Furthermore, the initial influence of an artificially inoculated plant did not extend more than 2 m, and the early-season spread within plots resulted in a greater number of adjacent (paired) infected plants than expected had the inoculum source been outside the plot. In test plots, 75-90% and 24-44% of the plants became naturally infected in 1971 and 1972, respectively. Yield losses of 20-25% for infected plants were similar and consistent in fields in 1971 and 1972. An early infection (June) caused a greater yield loss than a late one (August), but time of infection had no effect on the rate of seed transmission. PMV-infected plants produced more small seed than healthy plants, and the smallest seed had a PMV transmission rate of 3.7% as compared to 0 to 0.9% for larger seed.

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Peanut mottle virus (PMV) causes a disease (2, 5) of peanuts (*Arachis hypogaea* L.) that has been observed in the southeastern United States and in other peanut-growing regions of the world (1, 3, 4, 9). Despite its worldwide distribution, the disease has received little attention by peanut workers, probably because infected plants are difficult to recognize in the field. The primary symptom is a subtle mottling of the newest leaves; there is little or no reduction in the above-ground vegetative growth (5). There are several reasons why we think the importance of the disease has been overlooked: (i) five strains of PMV have been identified recently (8), (ii) greenhouse tests with a mild mottle strain of PMV showed significantly reduced yields (5), and (iii) a severe mosaic strain found in North Carolina reduced peanut production loss from 41 to 72% (11). Herein we report losses caused by the most prevalent strain of PMV in Georgia, the source of primary inoculum of PMV, and the incidence and spread of PMV within field plots.

MATERIALS AND METHODS.— This study was conducted in 1971 and 1972 on two sites in the peanut growing area (Coastal Plain region) of Georgia. Site 1 was on an experiment station farm, and site 2 was a commercial peanut field. The two sites were about 1500 m apart. Cultural practices were uniform for all plots. Peanuts were seeded at a distance of 7 to 10 cm in two-row beds, 1.0 m apart. Herbicide, fungicide, and insecticide applications were made in accordance with recommended commercial procedures.

The incidence of PMV was studied by observing plants in selected areas of relatively large plots. At site 1, the plots were 29 × 80 m and were planted with 'Starr' peanuts. There were two plots in 1971 and one plot in 1972. At site 2, the plots were 22 × 230 m; in 1971 two plots were planted with 'Florunner' and Starr peanuts, and the one plot in 1972 was Florunner. Counts of diseased and healthy plants were made by selecting five intraplots in each row at site 1 and six intraplots in every other row at site 2. Each intraplot was 7.6 m long and included about 100 plants. One intraplot was at the end of each row, and the others were scattered throughout the row. Counts were made at 2- to 3-wk intervals and 10,000 to 15,000 plants were observed per plot.

In 1972 four PMV infection levels were established by using Starr peanut seed obtained from site 1 of the previous year. The zero infection level was planted with seed from healthy plants. The second level, 0.3%, was obtained from naturally infected seed. Additional plants from the latter seed lot, selected at random, were mechanically inoculated with PMV two weeks after emergence to give the other two infection levels of 2.0 and 6.0%. Each infection level was planted in a 58-m^2 block with eight rows, had about 1,000 plants, and was replicated eight times in a completely randomized block design. Each of the plants was observed for peanut mottle at 1- to 2-wk intervals throughout the season.

Harvests to determine yields were made 19 to 20 wk after planting. Yields of individual diseased plants were compared with that from adjacent healthy plants by hand digging and picking. The plot with different infection levels was dug mechanically, the vines were air-dried for 3 days, and the pods were picked mechanically. Yields were based on 10% dry weight (100-g samples were dried 36 hr at 100 C).

Plants were checked from time to time to verify the presence of PMV, or to determine the presence of other viruses. Four newly-formed leaflets were ground in 2.0 ml of 0.01 M potassium phosphate buffer (pH 8) containing 0.01 M sodium diethyldithiocarbamate, 0.01 M sodium bisulfite, and 1% Celite and the extract was rubbed on 'Argentine' peanut and 'Topcrop' bean (*Phaseolus vulgaris* L.), a local lesion host.

Peanut seed from infected plants were planted in the greenhouse for seed transmission studies. The plants were observed for four wk, and the presence of PMV in suspected plants was verified by inoculation to Topcrop bean.

RESULTS.— *Virus identification*.— PMV was the only virus detected in our field plots. Identification was based on field and greenhouse symptoms on peanut, symptom reaction on Topcrop bean and 'Little Marvel' pea (*Pisum*

sativum L.), and serological tests (8). The predominant strain was PMV-M2, a mild mottle strain which caused no obvious reduction in shoot growth (5). Infection with the necrosis and chlorotic line pattern strains of PMV was observed but was negligible (less than 0.1%).

Disease incidence.— Disease development varied between years and between sites (Fig. 1-A). During a three-wk period in 1971 (6 to 9 wk after planting), the percentage of mottled plants increased from 3 to 18 at site 1 and from 5 to 90 at site 2. Although the disease incidence at site 2 remained similar thereafter, it increased to 75% at site 1 within 12 weeks after planting. There was a rapid disease buildup period at both sites, but it occurred at a later time at site 1.

The disease incidence was much less in 1972 than in 1971 (Fig. 1-B). Also, the incidence was less at site 2 than site 1, the reverse situation to that of 1971. Although specific counts were not made in other commercial peanut fields, observations indicated that the prevalence of PMV was much higher in 1971 than 1972, similar to our test plots.

Spread within plots.— The distribution of PMV-infected plants within plots was determined at different times during the growing season. At site 1, in both 1971 and 1972, infected plants were scattered throughout the plots during the first 6 weeks; there was no indication of increased numbers of diseased plants near the edges or in any part of the plots. At site 2, however, the disease incidence was greater near a wooded lot than in the rest of the large plot (22×230 m). At seven wk after planting, 40 and 50% of the diseased plants were concentrated in 3% of the total area of the plots in 1971 and 1972, respectively.

In 1971 it was difficult to follow virus movement because of rapid disease development (Fig. 1-A). In 1972, however, disease buildup was much slower and individual plants were observed at 2- to 3-wk intervals. The initial infection level within a plot influenced the number of diseased plants throughout the season (Fig. 1-C). Initial infection levels of 0, 0.3, 2.0, and 6.0% increased to 24, 27, 37, and 45%, respectively. Virus was apparently transmitted from the infected plots to the 0% infection plot; more than 50% of the diseased plants in the latter plot were in the two rows adjacent to infected plots, and the other infected plants were scattered in the remaining six rows.

Weekly field inspections of the plot with different infection levels in 1972, indicated that PMV spread was occurring from plant to plant (Fig. 2). As the season progressed, the number of adjacent infected plants in a row increased from 2-3 to 10-15. When van der Plank's test (13) was applied to our data, the observed number of doublets (paired adjacent plants) was much higher throughout the growing season than would be expected from random distribution (Table 1). At three weeks after mechanical inoculation, 20% of the inoculated plants had infected plants immediately adjacent to them.

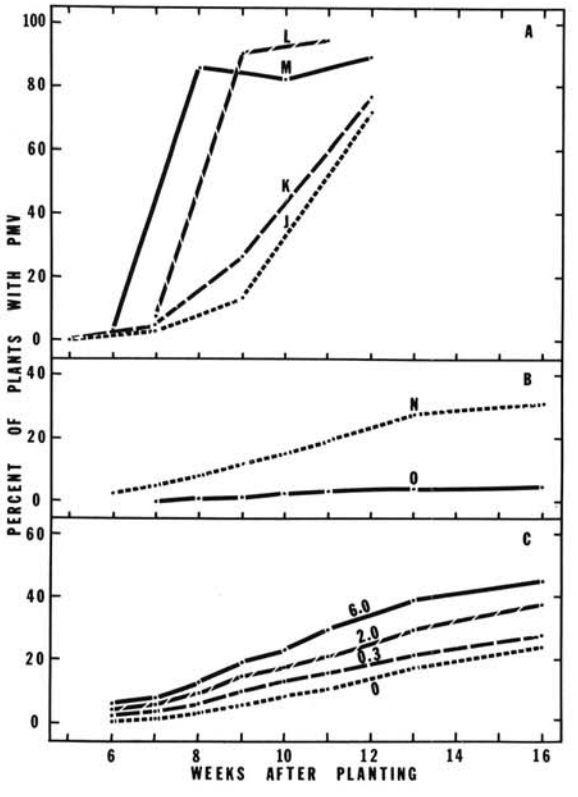

Fig. 1-A, B, C. Percentage of plants in field plots infected with peanut mottle virus. **A)** 1971 data — at site 1 plots J and K were planted with 'Starr' peanuts and at site 2 plots L and M were planted with Starr and 'Florunner' peanuts, respectively; **B)** 1972 data — sites 1 (plot N) and 2 (plot O) were planted with Starr and Florunner peanuts, respectively; and **C)** 1972 plots of Starr peanut with four initial levels of infection (0, 0.3, 2.0, 6.0%).

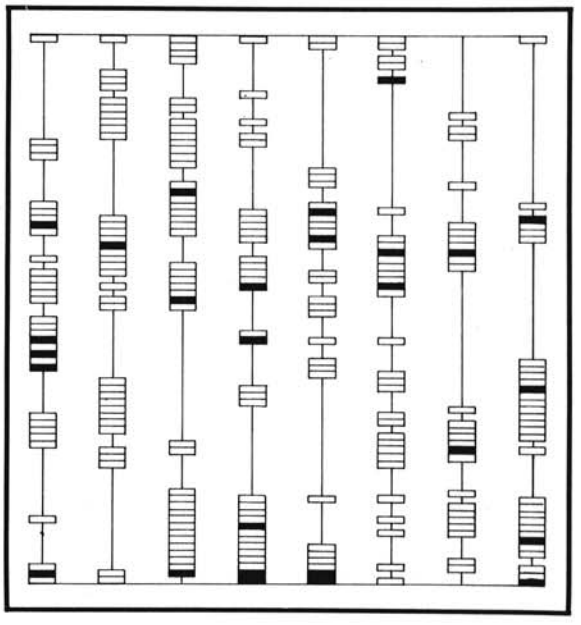

Fig. 2. Spread of peanut mottle virus in a field plot of 'Starr' peanuts. This is one representative block with an initial infection level of 2%. The block had eight rows and an area of 58 m². The black areas represent plants which were initially infected (both natural and artificial) with the virus, and the white areas represent plants which became infected during the growing season.

TABLE 1. Frequency of adjacent 'Starr' peanut plants infected with peanut mottle virus in a field plot[a]

Time of observation	Plants infected (No.)	Plants infected (%)	Doublets observed[b]	Doublets estimated[c]
June 6	783	5	222	49
June 22	1855	12	703	258
July 7	3013	19	1284	645

[a] Peanuts were planted on 19 April 1972; 324 of approximately 15,600 plants were mechanically inoculated on 3 May.

[b] A doublet is any two adjacent infected plants; a run of three infected plants is two doublets.

[c] This is the number of doublets expected if the distribution of infected plants is totally random. Estimate is based on the method developed by van der Plank (13); standard error = $\sqrt{\text{doublets estimated}}$.

TABLE 2. Yield of individual healthy and peanut mottle virus (PMV)-infected peanut plants and of peanut plots with four PMV infection levels[a]

Year of test	Treatment	No. of plants	Pod wt. per plant (g)	Percent reduction
1971	Healthy	160	58.3	—
	PMV	160	43.3	26
1972	Healthy	160	92.9[b]	—
	PMV	160	73.1	21
1972	PMV-24%[c]	3958[d]	42.1	0
	PMV-27%	3890	42.4	0
	PMV-37%	3869	39.1	7
	PMV-45%	3998	37.2[e]	12

[a] All tests with peanut cultivar 'Starr'.

[b] The difference between these two values is significant at the 1% level.

[c] The percentage of PMV-infected plants was determined two weeks prior to digging. Eight replications/treatment.

[d] Plot size was 58 m^2.

[e] This value is significantly different (5% level) from the 24 and 27% infection levels.

Source of inoculum.— Although PMV is known to be seed-transmitted (1, 5), we questioned if the low rate (less than 2%) was sufficient for the 75 to 90% disease incidence noted by midseason in 1971 (Fig. 1-A, B). Therefore, all types of plants, including peanut volunteers, in and near the two experimental sites plus two commercial fields, were checked for PMV. Sap from leaves of herbaceous plants, trees, shrubs, and vines was prepared in phosphate buffer containing 0.01 M sodium diethyldithiocarbamate and 0.01 M sodium bisulfite or 1% nicotine and rubbed on leaves of Argentine peanut and Topcrop bean. For woody-type plants, the first leaves to emerge in the spring were used for inoculum. No virus was found in 435 samples from 35 plant species.

The influence of a known source of inoculum was studied by mechanically inoculating the first row of 12 or 16 rows of Argentine peanuts. When readings were taken eight wk after planting, 52% of the PMV infected plants were in the two rows (within 2 m) next to the inoculated row in test 1 (16 rows). In test 2 with 12 rows, 33% of the PMV-infected plants were within 3 m of the inoculated row. As the season progressed, the infection spread throughout the plots.

Yield loss.— Yield comparisons are difficult to make because control plots with PMV-free plants could not be maintained in the field. Therefore, we used two methods to compare yields. With the first, individual healthy and PMV-infected plants were selected in a one-acre peanut plot, and PMV caused yield reductions of 26 and 21% in 1971 and 1972, respectively (Table 2). Seed were bulked together in 1971, but the next year samples were collected for statistical analysis and the yield loss was highly significant. The second method involved peanut plots with different levels of PMV infection at the end of the growing season (plots are the same as those in Fig. 1-C). A 45% infection level caused a significantly greater loss (12%) than the 24 and 27% levels (Table 2).

Comparative studies of 1,000-g samples of pods revealed that yield losses were due mainly to the reduction in number and size of seed (Table 3). PMV had little effect on the number of pods produced, but fewer two-seeded pods were produced on the diseased plants. From PMV-infected plants, the weight per seed was less and there were more small seed and fewer large ones (Table 3).

The time of PMV infection affected the degree of yield reduction. Severe losses (48%) were noted if infection was observed within the first five wk of planting, and 26, 22, and 18% losses occurred when infection was observed at 7, 9, and 12 wk after planting, respectively (Fig. 3). The number and size of seed were progressively reduced by the longer infection periods.

Seed transmission.— The rate of seed transmission of PMV was similar regardless of time of infection (Table 4). Differences, however, occurred with different seed sizes. The transmission rate was highest for smaller seed. Plants from the smaller seed emerged later and were less vigorous than plants from larger seed; no particular difference in emergence and vigor was noted for plants from infected and healthy seed.

Relation to other peanut diseases.— A previous greenhouse study (5) demonstrated that PMV-infected plants produced more discolored pods than healthy ones. In this study, gray to brown patches were noted on some pods from both diseased and healthy plants, but the number

TABLE 3. Effect of peanut mottle virus (PMV) on pods and seeds from naturally infected 'Starr' peanut plants

Treatment	Pods/plant (No.)	Pods/plant (%)	Seed/plant (No.)	Seed/plant (%)	Percent of seed by size[a] 8.0 mm	6.5-7.9 mm	6.4 mm	Wt/seed (g)
Healthy	81	100	117	100	23	70	7	0.35
PMV-infected	77	95	100	85	13	71	16	0.32

[a] Based on 1,000-g samples which were screened for size.

was greater from diseased ones. The degree of discoloration seemed related to the duration of the PMV infection; the number of discolored pods from plants diseased at 5, 7, 9, and 12 wk after planting were 12, 8, 5, and 4% greater, respectively, than pods from healthy plants.

Cercospora leaf spot is common in most peanut fields, and we were concerned about a potential increase in leaf spot on PMV-infected plants. However, no significant differences in the number of dropped leaves or number of spots per leaflet were observed when 160 PMV-infected and 160 PMV-free plants were compared.

DISCUSSION.— These field studies confirm observations, made over a 10-yr period, that a mild mottle strain of PMV causes a major virus disease of peanuts in Georgia. Although peanut stunt virus and other strains of PMV have been noted in commercial fields, their distribution and frequency were limited. These other viruses should not be disregarded. In general, they have more drastic effects than the mild mottle strain on peanuts (7, 8, 11), and ecological factors may change and cause them to become more prevalent.

When individual PMV-infected peanut plants were compared to healthy ones, the yield loss caused by the virus was clearcut; it was in the range of 20 to 25%. Furthermore, significant yield differences in relatively large plots were detected when PMV infection levels, at the end of the season, varied from 27 to 45%. Despite these definitive data, the overall economic importance of PMV is difficult to ascertain because disease incidence varies from year-to-year and from field-to-field. There was a high disease incidence in 1971 in our field plots, and we estimated an overall PMV loss of 18%. In the same plots in 1972, the disease incidence was much lower and the estimated loss was only 4%.

It is well documented that virus-infected seed can be the source of primary inoculum for several virus diseases [review by Shepherd (10)]. When seed from PMV-infected plants were tested, the PMV transmission rate was about 2% (1, 5). However, the transmission rate was much lower, about 0.3%, in commercially produced peanuts (C. W. Kuhn, *unpublished*), probably because small seed were eliminated before planting and not all plants that produced seed were infected with PMV.

Since we suspected that the PMV transmission level may be too low to be the sole source of primary inoculum, a thorough search was made to find another host for PMV in areas adjacent to peanut fields. None was found, not even near site 2 where a high disease incidence was observed close to a wooded lot. The high incidence was probably related to the initial movement of insect vectors from the wooded area to the edge of the field. Two other types of evidence suggest that the inoculum source was within the peanut field. First, according to van der Plank's test (13), the pattern of spread was from plant to plant, even during the earliest stages of disease development. Second, when field plants were artificially inoculated, immediately adjacent plants were the first to become naturally infected, and the initial influence of the artificially inoculated source was most evident within 2 to 3 m. The evidence is strong that PMV-infected seed are the source of primary inoculum.

Undoubtedly, the rate of seed transmission that is important in the epidemiology of a virus disease is dependent on the vector-virus-host combination. With favorable environmental conditions, a very low seed transmission rate is sufficient to cause important economic losses. PMV in peanut may be similar to lettuce mosaic virus in lettuce. The incidence of lettuce mosaic at harvest varied directly with the amount of seed-borne virus (12, 14), and if seed transmission of lettuce mosaic virus exceeded 0.1%, control was unlikely to be satisfactory (14).

Although insect transmission was not included in this study, the mode of dissemination, studied by other investigators (1, 3, 4), and our preliminary tests indicate that PMV is transmitted in the field by aphids. The abundance and relative activity of these vectors probably explain the differences in PMV incidence and spread in 1971 and 1972.

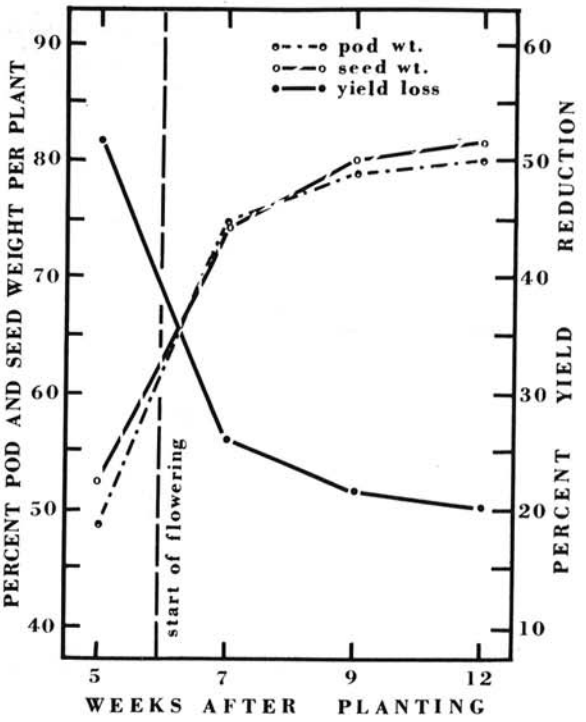

Fig. 3. Effect of the time of infection of peanut mottle virus on the yield of 'Starr' peanuts. The total pod wt, seed wt, and yield of PMV-free plants equals 100%.

TABLE 4. Transmission of peanut mottle virus through different sized seed of 'Starr' peanut plants infected for different lengths of time

Seed size (mm)	5 weeks[a]		12 weeks[a]	
	Seeds germinated (no.)	Infected plants (%)	Seeds germinated (no.)	Infected plants (%)
> 8.5	39	0	59	0
7.9-8.5	59	0	221	0.9
6.5-7.9	378	1.6	367	2.0
6.0-6.5	167	3.6	114	2.6
< 6.0	195	2.0	54	3.7
Total	838	1.9	815	1.7

[a] Seed from plants diseased at 5 and 12 weeks after planting.

Peanut mottle virus occurs naturally in at least three plant species other than peanut: *Cassia tora* L. (5), *Glycine max* (L.) Merr. (6), and pea (4). The relationship of these plants to the epidemiology of peanut mottle is unknown, but they must be considered when control measures are being developed.

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