Seed Transmission of Peanut Mottle Virus in Peanuts

D. B. Adams and C. W. Kuhn

Department of Plant Pathology and Plant Genetics, University of Georgia, Athens, GA 30602. Present address of Adams: Cooperative Extension Service, Rural Development Center, Tifton, GA 31794.

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ABSTRACT

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Seed transmission of peanut mottle virus (PMV) in peanuts is a consequence of embryo infection. Virus was isolated from embryos but not from seed coats or cotyledons. Four isolates of PMV differed in frequency of seed transmission in Starr peanut: M1 = 0.3%, M2 = 0.0%, M3 = 8.5%, and N = 0.0%. Isolate M3 was transmitted at similar frequencies (average = 7.1%) in seed of four peanut cultivars. Isolate M2, however, was not seed-transmitted in large-seeded peanuts although it was transmitted at a low frequency (0.23%) in small-seeded ones. When seed were harvested from individual plants, seven of 30 peanut plants produced 777 seed free of PMV-M3 whereas the seed transmission varied from 0.5 - 8.3% of the remaining plants. Seed transmission of PMV was unrelated to the level of virus in leaves and flowers. When peanut plants were maintained at 21 or 35 C during flowering and pegging, seed transmission was reduced threefold when compared to greenhouse-grown peanuts.

Additional key words: epidemiology, primary source of inoculum.

Seed transmission of peanut mottle virus (PMV) occurs in peanuts (*Arachis hypogaea* L.) at relatively low frequencies (1, 3, 10, 13, 14). The importance of seed transmission has been established by epidemiological studies during the last five years; in Georgia, PMV-infected peanut seed provide the primary source of inoculum for diseases of two major crops, peanuts and soybeans, *Glycine max* (L.) Merr. (7, 8, 11, 14). No natural reservoir for PMV has been found, and the virus is not seed-transmitted in four hosts which have been found infected in nature: *Vigna unguiculata* (L.) Walp. subs. *unguiculata* (1, 6), soybeans (6, 7), *Pisum sativum* L. (1), and *Cassia obtusifolia* L. (1). Behncken and McCarthy (4) found PMV in navy beans (*Phaseolus vulgaris* L.) in Australia and reported a low frequency of seed transmission. However, no strains of PMV have been found which systemically infect beans in the United States. The objectives of this study were to determine the location of the virus in infected peanut seed and to determine factors which affect seed transmission.

MATERIALS AND METHODS

Three isolates of PMV, described previously (13), were used in this study: two (M1 and M2) caused mild mottle symptoms on peanut and the third (N) caused necrosis and stunting. A fourth isolate, designated M3, was obtained from a field-infected peanut plant in 1974. The new isolate caused mild mottle symptoms on several peanut cultivars and could not be distinguished from isolates M1 and M2 by symptoms on peanuts, host range, or serology. Isolates M1, M2, and N were maintained in *Pisum sativum* 'Little Marvel' and isolate M3 in peanut cultivar Starr.

For most of the seed transmission studies, 10- to 15-day-old peanut seedlings, grown in the greenhouse or field, were mechanically inoculated with PMV (13). In the study to determine seed transmission in individual plants, the test plants became naturally infected from a centrally-located row manually inoculated with virus. Mature pods on infected plants were handpicked 120-140 days after planting.

Frequency of seed transmission was determined by planting seed from PMV-infected plants in 10-cm diameter pots containing methyl bromide-treated soil (four seed/pot). Plants were maintained in a aphid-free greenhouse and observed for PMV symptoms for at least 30 days. Seed from the PMV-infected small-seeded peanut cultivars (Spanish type) were planted immediately after harvest or stored at 5 C. To obtain high percentage germination, seed of the large-seeded peanut cultivars (runner or Virginia type) were stored at 25-30 C for at least 60 days before planting (2) or the seed dormancy was broken by placing the seed in a sealed plastic container with slices of apples.

The presence of PMV in peanut plants with PMV symptoms was verified by grinding one leaflet in 1-2 ml of 0.01 M phosphate buffer, pH 7.5, containing 0.2% sodium bisulfite, 0.2% sodium diethyldithiocarbamate, and 1% Celite in a mortar and inoculating the PMV local lesion host, Topcrop bean. Furthermore, all abnormal-appearing plants and about 2,000 symptomless ones, grown from seed of PMV-infected plants, were tested similarly for PMV; none had the virus.

The relative infectivity of PMV in various plant parts was determined by conducting half-leaf assays on Topcrop bean (eight replications/treatment distributed

in a randomized block design). One leaflet, flower, pod, or immature seed was collected from ten randomly selected plants for each assay. Each gram of tissue was ground in a mortar containing 9 ml of buffer.

In a study to determine the location of PMV in mature peanut seed, seed were pretreated with a 2 min soak in trisodium phosphate (10%) followed by a 5 min rinse in running tap water. Seed then were separated into seed coats, cotyledons, and embryos; each part was ground in phosphate buffer and tested for virus by inoculating Topcrop bean.

RESULTS

Variation with virus isolates.—An initial seed transmission study was conducted in 1973 with isolate M2 in eight peanut cultivars. Despite previous results (10, 14) with M2 which showed about 1.7% seed transmission, only 27 of 16,888 (0.16%) plants were infected with PMV. Frequency of seed transmission was too low for productive research; therefore, a new PMV isolate (M3) was acquired and tested for seed transmission. When M3 was compared to three known PMV isolates, its frequency of seed transmission was much greater (Table 1), thus it was used for most studies reported herein.

Seed transmission in different cultivars.—Isolate M3 was transmitted at similar frequencies through the seed of four peanut lines with different types of plant growth and different seed coat colors: (i) Florunner, 4 of 78 (5.0%), (ii) Starr, 20 of 281 (7.1%), (iii) P.I. 261945 (light brown seed coat), 13 of 215 (6.0%), and (iv) P.I. 261946 (purple seed coat), 22 of 262 (8.4%). However, cultivar differences were observed with isolate M2. No seed transmission of M2 occurred in 5,158 plants of four cultivars of large-seeded peanuts (Early Runner, Florigiant, Florunner, and Virginia Bunch 67); it was transmitted through the seed of 27 of 11,730 (0.23%) plants of four small-seeded cultivars (Argentine, Spancross, Starr, and Tifspan).

TABLE 1. Seed transmission of four isolates of peanut mottle virus (PMV) in Starr peanut[a]

Virus isolate	Seed germinated (no.)	Plants with virus	
		Number	Percent
PMV-M1	381	1	0.3
PMV-M2	256	0	0.0
PMV-M3	177	15	8.5
PMV-N	213	0	0.0

[a]Seed, produced on greenhouse-grown plants, were planted in the greenhouse and observed for PMV symptoms for 30 days.

Virus in various plant parts.—Isolates M2 and M3 were found in all plant parts (leaves, flowers, pegs, pods, immature seed) of inoculated Starr and Florunner peanuts. The lowest incidence of infection occurred in immature seed, only 19% were infected when harvested at 12-14 wk after planting. Most of the virus apparently was inactivated during seed maturation since PMV-M2 could not be detected in any mature seed in this particular study, and PMV-M3 was found in only 5%, a level similar to the frequency of infected plants grown from the same lot of seed. When the seed parts were separated carefully, isolate M3 was found in the embryos of 15 of 269 (5.6%) seed of Starr peanut, but it could not be isolated from either the seed coats or cotyledons.

Transmission in individual plants.—Seed were harvested from ten individual plants of three peanut lines infected with PMV-M3. Seven of the 30 plants produced 777 virus-free seed (Table 2). For the other 23 plants, seed transmission varied from 0.5-8.3%. The average transmission was 2.0% which was similar for the three peanut lines.

Seed transmission and virus concentration.—The relative concentration of four isolates of PMV in various plant parts of Starr peanut was determined by bioassays. More virus was found in leaves with isolate N than with the three mild mottle isolates (Table 3). Relatively large quantities of virus could be isolated from the flowers, but no significant differences occurred among the isolates. All four isolates were found in the pods and immature seed, but the relative infectivity levels were low.

Effect of temperature on seed transmission.—When Starr peanut plants were maintained at different temperatures during flowering and pegging, 1 of 140 (0.7%) and 5 of 1,036 (0.5%) seed from plants kept at 21 and 35 C (16-hr photoperiod, 16,140 lx), respectively,

TABLE 2. Transmission of isolate M3 of peanut mottle virus (PMV) through the seed of ten individual plants of three peanut lines[a]

Plant no.	Starr			P.I. 261945			P.I. 261946		
	Seed germinated (no.)	Plants with virus		Seed germinated (no.)	Plants with virus		Seed germinated (no.)	Plants with virus	
		(no.)	(%)		(no.)	(%)		(no.)	(%)
1	135	3	2.2	63	1	1.6	183	0	0.0
2	174	4	2.3	60	0	0.0	144	8	5.6
3	213	11	5.2	154	3	1.9	95	2	2.1
4	86	0	0.0	32	1	3.1	34	2	5.9
5	114	0	0.0	96	0	0.0	83	0	0.0
6	179	1	0.6	84	7	8.3	74	1	1.4
7	143	1	0.7	122	2	1.6	120	6	5.0
8	146	3	2.1	103	5	4.9	127	2	1.6
9	194	1	0.5	116	3	1.9	118	1	0.8
10	154	2	1.3	155	0	0.0	112	3	2.7
Total	1,538	26	1.7	985	22	2.2	1,090	25	2.3

[a]Seed of the three peanut lines were produced in the field and tested for PMV infection in the greenhouse.

were infected. Both temperatures adversely affected seed transmission; seed transmission occurred in 38 of 2,123 (1.8%) seed produced in the greenhouse during the same time period. In another study, plants were moved to the same two controlled environments after pegging and maintained there until harvest; these temperature treatments did not alter frequency of seed transmission when compared to greenhouse-maintained plants.

DISCUSSION

Initial studies with PMV-M2 demonstrated the presence of virus in immature peanut seed but not in mature ones. This led to speculation (11) that seed transmission of the virus was caused by its association with the seed coat and that the virus might be sensitive to some type of seed treatment. The current studies, however, clearly establish that seed transmission of PMV-M3 is a consequence of embryo infection. Embryonic transmission (5) most likely eliminates seed treatment as a means to prevent seed transmission of PMV. The transmission difference between isolates M2 and M3 is probably due to the inability of M2 to infect the embryo of peanut seed. Isolate M2 may have lost its ability to be seed-transmitted because of maintenance in pea which may have caused selection of a variant of the original isolate.

The frequency of seed transmission of PMV seemingly varies from 0 to 20% (1, 3, 6, 10, 14). However, the highest frequency, observed in East Africa (6), was made with only 31 plants which were allowed to grow from subterranean pods left in situ in pots. Most of our studies (1, 10, 13, 14) with the mild mottle isolates have shown a transmission frequency of about 2%. Initially, the M3 isolate used in this study was transmitted at a frequency of over 8%; however, more recent tests (Table 2) showed a 2% level. Commercial peanut seed lots in Georgia have lower levels (0.1 - 1.0%) of infected seed (14) for at least two reasons: (i) not all plants are infected in fields where seed are produced, and (ii) the smallest seed which have the highest frequency of seed transmission (10, 14) are removed by screening. Since Georgia farmers plant 170,000 to 210,000 seed per hectare, even a 0.1% level of seed transmission (about two primary infection centers per 100 m^2) is significant in the epidemiology of the virus disease.

The relative concentration of PMV in various plant parts was unrelated to frequency of seed transmission. Isolate N had the most virus in leaves and was not seed transmitted. Isolate M3 had the least virus in leaves (not significantly different from isolates M1 and M2) and was the only isolate in this study to be seed-transmitted at a significant frequency. No significant differences in virus concentration of the different virus isolates were found in the flowers, and only small quantities of virus occurred in pods and immature seed. Therefore, embryonic infection by PMV apparently is controlled by some mechanism other than the ability of the virus to replicate in peanuts.

Immunity in peanut to PMV was not found in a screening test (12), and researchers in Africa, Australia, and Japan are unaware of any type of resistance in peanuts to the virus (K. R. Bock, G. M. Behncken, and T. Inouye, *personal communications*). Until some type of resistance has been found, the best control program for PMV will involve the use of virus-free peanut seed. A peanut seed production program which would yield virus-free seed or seed with extremely low levels of PMV has been proposed (11). The first step in implementation was successful; J. W. Demski (*personal communication*) produced virus-free seed in central Georgia in an area isolated from commercially-produced peanuts in 1975. Certain areas of Texas might be desirable also because PMV appears to spread very slowly there (9).

Future research in Georgia will consider two other methods to obtain PMV-free peanut seed. First, some individual peanut plants produce virus-free seed (Table 2), and it may be possible to select and culture specific lines. Second, a screening program will seek peanut cultivars and plant introductions in which PMV is not seed-transmitted. An inheritance study should allow this desirable characteristic to be incorporated into future peanut cultivars.

TABLE 3. Relative infectivity of four isolates of peanut mottle virus (PMV) extracted from various plant parts of Starr peanut[a]

Virus isolate	Lesions per half-leaf (no.)			
	Leaves[b]	Flowers[c]	Pods[d]	Immature seed
PMV-N	65 x[e]	33 x[e]	9	10
PMV-M1	31 y	35 x	7	1
PMV-M2	38 xy	53 x	3	1
PMV-M3	27 y	46 x	10	3

[a]Inoculum was prepared by extracting each gram of plant tissue in 9 ml of buffer.

[b]Average of four bioassays conducted on Topcrop bean at 1, 2, 6, and 10 wk after inoculation of 2-wk-old seedlings.

[c]Average of three bioassays conducted at 6, 8, and 10 wk after inoculation.

[d]Infectivity tested at 11 wk after inoculation.

[e]Data were statistically analyzed by using the average numbers of each assay as a replication. Treatment means with different letters in the same column are significantly different, $P = 0.05$, according to Duncan's multiple range test.

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