Resistant and Susceptible Reaction of Soybeans to Peanut Mottle Virus

J. W. Demski and C. W. Kuhn

Assistant Professor and Professor, Department of Plant Pathology, Georgia Agricultural Experiment Station, Experiment 30212, and College Station, Athens, Georgia 30602, respectively.

We thank E. E. Hartwig, Delta Branch Experiment Station, Stoneville, Mississippi, for providing soybean seed used in the screening test.

Accepted for publication 31 July 1974.

ABSTRACT

Seventy soybean cultivars and breeding lines were screened for reaction to peanut mottle virus (PMV). Fourteen cultivars were highly resistant to infection. Symptoms were not produced on resistant cultivars (with the exception of a few plants we considered derived from contaminated seed), and the virus could be isolated from only two of 674 plants. The resistance was effective under field conditions, and aphids could not transmit PMV to resistant cultivars. The incubation period and PMV concentration was similar in three susceptible cultivars; cultivar Laredo, however, had a longer incubation period and less virus in the lower leaves than the other susceptible cultivars. PMV significantly reduced plant height and yield of two susceptible cultivars in field tests.

Phytopathology 65:95-99

Additional key words: Glycine max, immunity, growth study.

Peanut mottle virus (PMV) was first found in soybean, *Glycine max* (L.) Merrill, in Georgia in 1971 (7). Surveys during the last three years have shown that PMV is one of the most prevalent soybean viruses in the state. The virus has been recovered from plants in numerous southern counties, and 50% of the plants in parts of some fields were infected. The results of field studies in 1972 and 1973 demonstrated that PMV had an adverse effect on plant height and yield. The high yield loss and widespread distribution of PMV, show the economic importance of this soybean disease. In 1973, Bock (2) reported that PMV caused very common and widespread diseases of both peanut (*Arachis hypogaea* L.) and soybean in East Africa, and recommended that improvement programs should include routine tests for reaction to PMV.

Bock (2) reported that all 21 cultivars and breeding lines of soybean tested were highly susceptible to PMV. However, it has been demonstrated that soybean germplasm does have resistance to other soybean viruses. Harris and Kuhn (3) found resistance to cowpea chlorotic mottle virus. Heinze (4), Koshimizu and Iizuka (5), and Ross (11) reported resistance to soybean mosaic virus. Therefore, these studies were initiated to find resistance in soybean to PMV, and to characterize the type of resistance.

MATERIALS AND METHODS.—The PMV isolate used was the mild strain (M-2) which is the most common strain found in Georgia peanut and soybean plantings (7, 8). Garden pea (*Pisum sativum* L. 'Little Marvel') was used for inoculum production. Mechanical inoculations were made with infected pea leaves ground in 0.05 M phosphate buffer (pH 7.5) containing 0.01 M $NaHSO_3$ and 1.0% Celite.

Screening for resistance was performed with 70 cultivars or breeding lines (hereafter called entries), using 20-40 plants per entry in greenhouse tests. Seed were planted in soil in 10-cm diameter pots, and unifoliolate leaves were inoculated 8-10 days after planting. They were reinoculated the next day to ensure a high percentage of infected plants.

To test for PMV infection in soybean, subinoculations were made to *Phaseolus vulgaris* L. 'Topcrop', on which reddish local lesions are produced (6). Infectivity assays were also conducted on Topcrop bean; a half-leaf experimental design, with at least six replications per treatment, was used.

The resistant reaction.—Resistant entries were grown in the greenhouse, inoculated with PMV, and tested for infectivity at different times after inoculation. Subinoculations were made after one week from the inoculated unifoliolate leaves, and again from the fifth trifoliolate leaves when they became fully expanded. Resistance in the field was tested by planting both resistant and susceptible entries between rows of susceptible entries which had been artificially inoculated with PMV. Natural field spread of virus to the test entries was monitored both by visual inspection and inoculation to Topcrop bean.

To determine the effect of high temperature on resistance, resistant and susceptible entries (20 plants per entry) were placed in a growth chamber at 36 C with a 12-hour photoperiod. After three days, the unifoliolate leaves were inoculated. The plants were maintained at 36 C for an additional eight days, then moved to the greenhouse. The unifoliolate and the fourth trifoliolate leaves were indexed to Topcrop bean 20 and 34 days after inoculation, respectively.

Two aphid species, collected from peanut fields, were used in insect transmission studies. *Aphis craccivora* (Koch.) and *Myzus persicae* (Sulz.) were maintained on healthy cowpea, *Vigna sinensis* (Torner) Savi 'Early Ramshorn', and Chinese cabbage, *Brassica chinensis* L. 'Michihli', respectively. After being fasted 6-8 h, the aphids were allowed to make one probe on a PMV-diseased Starr peanut plant, and then transferred to a soybean or peanut plant (one aphid per plant) for 12 hours.

The susceptible reaction.—The incubation period of PMV in several susceptible soybean entries was determined by noting symptoms daily after seedlings had been inoculated. The relative amount of infectious PMV was compared in six susceptible entries. Unifoliolate leaves were inoculated, and plants were maintained until the seventh trifoliolate leaf was fully expanded.

PHYTOPATHOLOGY for January (65:1-94) was issued 16 January 1975.

TABLE 1. Aphid transmission studies of peanut mottle virus with susceptible and resistant soybean cultivars and Starr peanut

Test	Aphid	Soybean cultivar		Starr peanuts
		Ransom	Davis	
1	*Aphis craccivora*	3/30[a]	0/30	6/20
2	*Aphis craccivora*	1/30	0/30	5/20
3	*Myzus persicae*	5/30	0/30	11/20

[a]Number infected/total tested with one aphid allowed a single probe on a diseased peanut and then transferred to the indicator host for 12 hours.

Infectivity assays were made by macerating leaves (one leaflet from each of ten plants) in buffer (9 ml/g of leaf tissue) and inoculating Topcrop bean. Assays were made on a leaf-position basis at 5, 9, 16, 22, and 30 days after inoculation.

In a growth study, five soybean entries were grown in sterilized soil in 10-cm diameter pots (one plant per pot). The plants were rubbed with inoculum or buffer (controls) eleven days after planting. For each entry, there were 25 diseased and 25 healthy plants arranged randomly on two greenhouse benches. The test was conducted in August and September, and temperatures ranged from 21 to 32 C. Measurements of plant height, pods per plant, and dry weight (dried to unchanging weight at 80 C) of roots and shoots were made 33 days after inoculation.

Three field tests were conducted to determine the effect of PMV on plant height and yield. In each test, two susceptible entries, Jackson and Hampton 266A, were planted alternately in 4.9 m rows in a randomized block. There were four replications each of healthy and PMV mechanically inoculated treatments of each entry in each test.

The effect of plant age on susceptibility was tested in the greenhouse by making a weekly planting of three soybean entries (Bragg, Jackson, Ransom) and then inoculating all plants at one time. In the field, the susceptible entry Bragg was planted in May, and four 4.9 m rows were inoculated every ten days until mid-August.

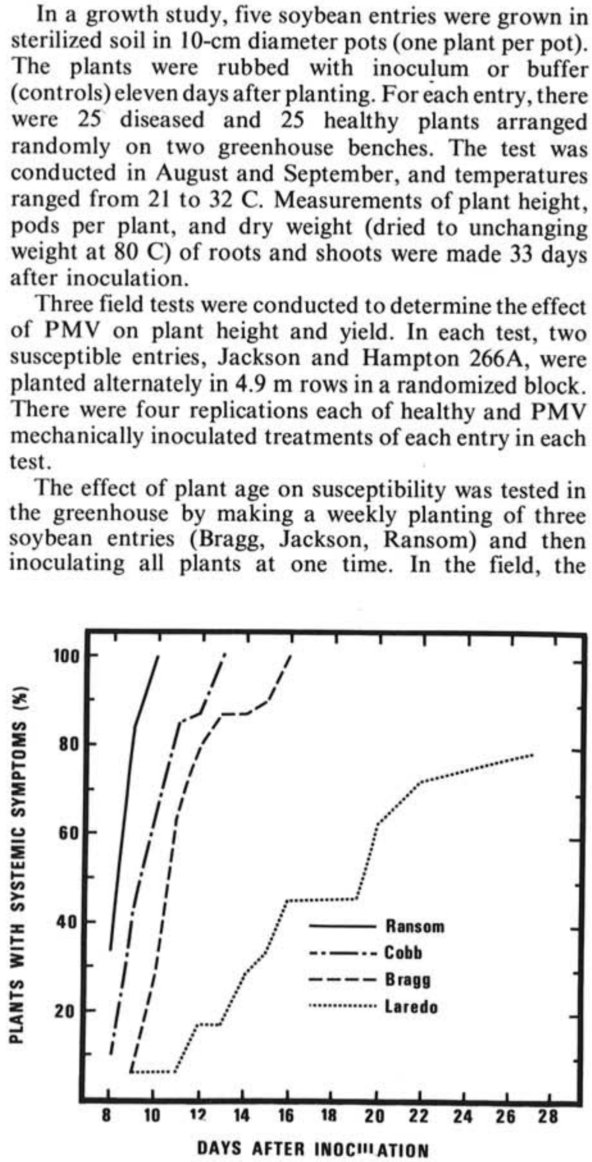

Fig. 1. Incubation period of peanut mottle virus in four soybean cultivars.

RESULTS.—In the screening test, 56 soybean entries were found to be susceptible to PMV because symptoms developed on 50-100% of the plants. Fourteen entries were judged to be resistant; no symptoms were observed on eleven of them, and three had symptoms on only 1-2% of their plants. PMV symtomatology was reported previously (7) for the soybean entries Bragg and Lee. Similar symptoms were noted on most of the susceptible entries; however, differences were observed in which entries had a more pronounced or a milder mottle than others. Furthermore, yellow-to-green ringspots occurred on systemically-infected leaves of a few entries, particularly when plants were grown in winter and spring months.

The resistant reaction.—The resistant entries were as follows: Arksoy, CNS, Curtis, Davis, Dorman, Ga 69-90, Haberlondt, Hale 3, Hale 7, Hardee, Peking, Pindell Perfection, Ralsoy, and Virginia.

The symptomless reaction was observed in three tests with approximately 30 plants per entry in each test. Subinoculations were made from the inoculated unifoliolate leaves of seven entries one week after inoculation and later, from the fifth trifoliolate leaf of all 14 entries. PMV was recovered from the unifoliolate leaf on one of 260 plants and from only one trifoliolate leaf of 414 plants.

Entries Davis, Hale, and Curtis consistently showed typical PMV symptoms in 1-2% of their plants. The virus was identified as PMV, and its origin was the mechanical inoculation since PMV is not seed-transmitted in soybean (Demski, *unpublished*). Seed harvested from three susceptible Davis plants were similar in seed coat color, size, and hilum color to seed from resistant Davis plants. However, over 90% of the progeny (208/230) of the susceptible plants were susceptible to PMV. Symptoms were similar on all plants, and there was no recognized segregation ratio for disease reaction.

Field resistance was correlated with resistance observed in the greenhouse. PMV spread from inoculated plants to six susceptible entries, and four resistant entries remained symptomless with the exception of one Davis plant. PMV was identified in the diseased plants by inoculating Topcrop bean, and the virus could not be isolated from the symptomless, resistant ones.

Heat treatment of resistant and susceptible entries showed that resistance was partially broken by a 36 C regime. A low concentration of PMV was detected in 18 of 20 plants of the inoculated unifoliolate leaves of the resistant entry Hardee, but no virus was found in any of the trifoliolate leaves. Virus could not be recovered from either the unifoliolate or trifoliolate leaves of two other resistant entries (Davis, Hale 7), but it was isolated from both unifoliolate and trifoliolate leaves of 19 of 20 plants of susceptible entry Ransom.

Both *Aphis craccivora* and *Myzus persicae* failed to transmit PMV to the resistant entry Davis (Table 1). The aphids transmitted the virus to the susceptible entry Ransom, but the transmission efficiency from peanut to soybean was only 3-17% as compared to 20-54% for peanut-to-peanut transmission (10).

The susceptible reaction.—The following entries were susceptible: Acadian, Avoyelles, Bienville, Biloxi, Bossier, Bragg, Cobb, Coker 68-33, Coker 68-38, Coker 68-41, Coker 69-85, Coker 69-86, Coker 69-87, Coker 102, Coker 208, Coker 318, Coker 4504, Dare, Dortchsoy, Essex, Forest, Ga69-132, Ga 69-136, Ga 69-178, Hampton 266A, Harrell, Hill, Hollybrook, Hood, Hutton, Improved Pelican, Jackson, Jackson 2, Laredo, Lee, Lee 68, Luthy, McNair 91-20, McNair 600, McNair 800, Mammoth Yellow, Missoy, Nansemond, N66-1783, Ogden, Old Dominion, Otootan, Palmetto, Pickett, Roanoke, Ransom, S-100, Tanner, Tokyo, Volstate, and Woods Yellow.

In the screening test, two observations suggested that soybean entries differed in their susceptibility to PMV: (i) the percent of disease plants varied from entry to entry and (ii) the time of symptom appearance (incubation period) also varied. Differences in the incubation period were verified in three tests in which symptoms were noted daily (Fig. 1). The first symptoms appeared on a few plants of entry Ransom about eight days after inoculation; all plants had symptoms by the tenth or eleventh day. Initial symptoms occurred on Cobb and Bragg about the same time as on Ransom, but it took a few days longer for all plants to show symptoms. Entry Laredo was distinctly different from the other entries. Symptoms developed on individual plants from 9-30 days after inoculation, and in only one of three tests did all plants show symptoms. These incubation period tests were conducted in June, July, and August in the greenhouse. The same general results were observed in winter months, but the time of initial symptom appearance was delayed, and a longer period was required for all plants to show symptoms.

To determine the relative amount of PMV infectivity in six susceptible entries (Bragg, Cobb, Laredo, Lee, Ransom, and S-100), eight bioassays were conducted at various times (5-30 days) after inoculation. The only consistent, significant difference among the entries was less infectivity in the lower leaves (unifoliolate and first, second, and third trifoliolate) of Laredo. Later-developing leaves of Laredo, however, had as much infectivity as the other entries. This infectivity observation seemed to be related to the delayed symptoms which occurred on Laredo (Fig. 1). Fig. 2 shows the average relative PMV infectivity in various leaves of susceptible entries 30 days after inoculation. At assay time, the eighth and ninth trifoliolate leaves were small and not expanded; they had no symptoms and very little virus could be isolated from them. In general, PMV infectivity was greatest in the leaves (second to seventh trifoliolate) with clearcut symptoms.

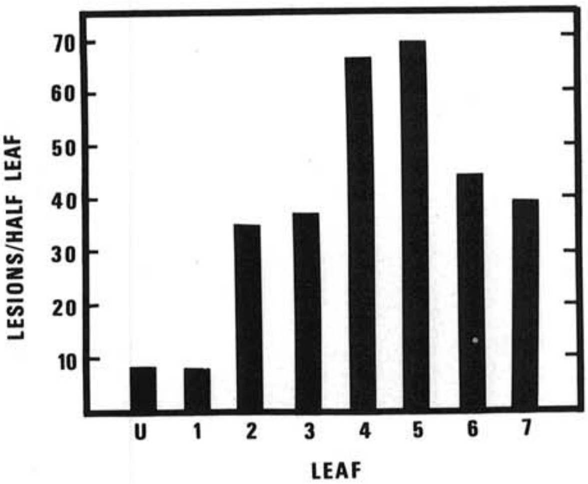

Fig. 2. Peanut mottle virus infectivity in the inoculated unifoliolate leaf (u) and the trifoliolate leaves (1 through 7) of six susceptible soybean cultivars. The data are the average number of local lesions of three assays conducted 30 days after inoculation.

In plant growth studies in the greenhouse, PMV significantly reduced plant height, root and shoot weight, and number of pods produced on entry Laredo (Table 2). Root and shoot weight of Ransom were significantly affected, but Lee 68 and the resistant controls were not significantly affected by PMV inoculations. In field studies, the plant height and yield of susceptible entries Jackson and Hampton 266A were significantly reduced about six and 20%, respectively (Table 3). Indexing from the field plants to Topcrop bean indicated that approximately 1 and 80% of the healthy and inoculated plants were infected with PMV, respectively.

In susceptible entries, resistance to PMV increased

TABLE 2. Effect of peanut mottle virus (PMV) on plant growth of susceptible and resistant cultivars of soybean[a]

			Plant			
Cultivar	PMV reaction	Treatment	Height (cm)	Pods/plant	Root weight (g)	Shoot weight (g)
Laredo	Susceptible	Healthy	28.9x	11.9x	2.8x	24.2x
		Mottle	21.2y	8.7y	1.6y	16.7y
Ransom	Susceptible	Healthy	30.3	10.6	2.7x	21.5x
		Mottle	32.7	11.1	1.8y	18.3y
Lee 68	Susceptible	Healthy	33.9	18.0	3.0	29.2
		Mottle	33.0	17.5	2.4	26.8
Davis	Resistant	Healthy	28.4	11.0	3.0	25.8
		Mottle	30.7	10.9	3.1	23.0
CNS	Resistant	Healthy	29.6	14.2	2.6	25.5
		Mottle	27.0	15.5	2.8	27.5

[a]One plant per 10-cm diameter pot in soil. Plants rubbed with PMV inoculum or buffer eleven days after planting. Evaluations made 33 days after inoculations. All values are the average from 25 individual plants. Different letters denote significant difference ($P = 0.05$) for healthy and diseased pairs.

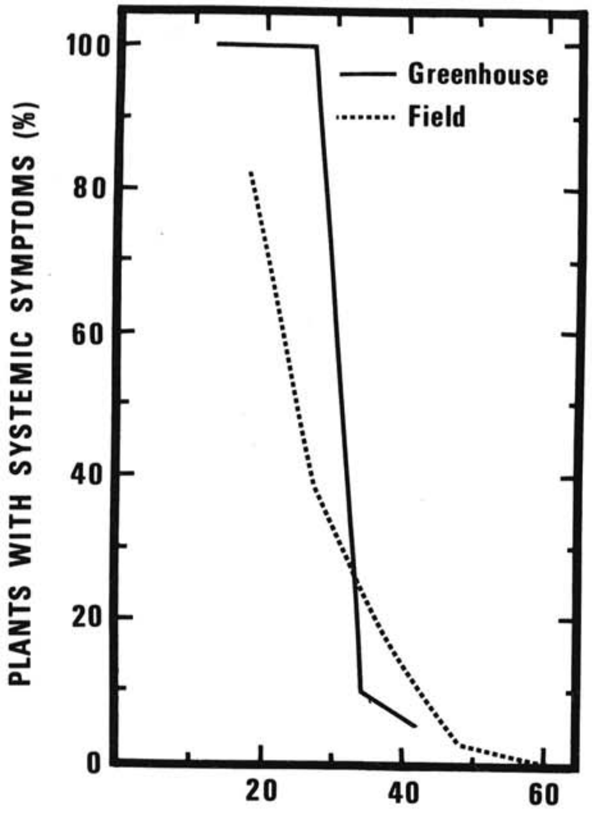

Fig. 3. The percentage of Bragg soybean plants systemically infected with peanut mottle virus, based on the age of plants when inoculated.

TABLE 3. Effect of peanut mottle virus on height and yield of two susceptible cultivars of soybean in a field test[a]

Cultivar	Treatment	Height (cm)	Yield (Kg/ha)
Hampton	Healthy	112[b]	1599
	Mottle	104	1263
Jackson	Healthy	114	1767
	Mottle	109	1452

[a]Plants were inoculated 19 days after planting.

[b]All values represent an average of three tests with four replications each. In each test, height and yield of each cultivar were significantly different, $P = 0.05$.

with plant age. The number of plants that could be infected by mechanical inoculation declined sharply after 20 and 30 days for plants in the field and the greenhouse, respectively (Fig. 3). Whereas 80-100% of the young plants could be systemically infected, only 1-2% became systemically infected when 40-60 day-old plants were inoculated. In the greenhouse, plants inoculated at 4 weeks developed both local and systemic symptoms. Subsequent inoculations at 5 and 6 weeks, however, caused chlorotic spots on the inoculated leaves and no systemic symptoms. Subinoculations to Topcrop bean showed that PMV could be recovered only from leaves with symptoms, and the virus concentration was low in leaves with chlorotic spots.

DISCUSSION.—Natural infection of soybean with PMV has been observed in several areas of the world: United States (7), Africa (2), and Australia (1). The virus causes economic loss, and sources of resistance are needed to control the disease. Bock (2) tested a wide range of soybean germplasm, and found all lines susceptible to PMV. In our studies, however, 14 of 70 entries tested were resistant to the virus. Bock (2) used two breeding lines which had Acadian as one parent. This was the only entry used in common in both studies and it was always found susceptible. Therefore, different strains of PMV and/or different soybean genetic material could account for the different results.

The resistant reaction in soybean to PMV may be characterized as a very high degree of resistance. Immunity may be applicable to describe the reaction, but this term implies an absolute quality that is very difficult to measure. PMV could rarely (two of 674 plants) be isolated from any portion of the inoculated plants which suggests a high degree of resistance to the establishment of infection. Plants held at a continuous high temperature of 36 C caused one of three resistant entries to become infected only in the inoculated unifoliolate leaves. Since PMV could not be recovered from the trifoliolate leaves, it is suggestive of soybeans having a high degree of resistance to virus translocation. Furthermore, studies with a susceptible entry showed that plants inoculated 5-6 weeks after seeding had only localized virus development, indicating resistance to virus translocation. Therefore, based on these studies, it appears that soybeans have a high degree of resistance to both virus establishment and virus translocation. For resistant entries, no symptoms (except from plants of a contaminated seed lot) were observed under field or greenhouse conditions, and plant growth was unaffected by PMV inoculations. Resistant entries could not be infected by aphids in greenhouse tests, and they did not become infected under field conditions. The nature of the resistance to PMV was both stable and practical, and it should be quite useful to plant breeders.

The general effect of PMV on susceptible soybean entries was similar to its effect on peanut (6, 9). For both soybean and peanut, there was a moderate effect on above-ground shoot growth, whereas yield and root weight were affected more severely.

It is doubtful if all 56 susceptible soybean entries react exactly the same way to PMV. However, our research, which included a wide variety of genetic material, did not lead to a general classification involving various degrees of susceptibility. Laredo was the only entry which reacted differently from the other entries which were classed as susceptible. The long incubation period, and the low virus concentration in the lower leaves, led us to conclude that Laredo was more resistant than entries such as Cobb, Bragg, and Ransom. However, the growth study suggested that PMV would have a greater effect on the yield of Laredo than the other entries.

LITERATURE CITED

1. BEHNCKEN, G. M., and G. J. P. MC CARTHY. 1973. Peanut mottle virus in peanuts, navy beans and soybeans. Queensl. Agric. J. 99:635-637.
2. BOCK, K. R. 1973. Peanut mottle virus in East Africa. Ann. Appl. Biol. 74:171-179.
3. HARRIS, H. B., and C. W. KUHN. 1971. Influence of cowpea chlorotic mottle virus (soybean strain) on agronomic performance of soybeans. Crop Sci. 11:71-73.
4. HEINZE, K. 1941. Feldinfektionsversuch mit dem Sojabohnevirus. Mitt. Biol. Reichsanst. Landforstwirtsch. 65:23.
5. KOSHIMIZU, Y., and N. IIZUKA. 1963. Studies on soybean virus diseases in Japan. Bull. Inst. Agric., Tohoku Univ. 27:1-103.
6. KUHN, C. W. 1965. Symptomatology, host range, and effect on yield of a seed-transmitted peanut virus. Phytopathology 55:880-884.
7. KUHN, C. W., J. W. DEMSKI, and H. B. HARRIS. 1972. Peanut mottle virus in soybeans. Plant Dis. Rep. 56:146-147.
8. PAGUIO, O. R., and C. W. KUHN. 1973. Strains of peanut mottle virus. Phytopathology 63:976-980.
9. PAGUIO, O. R., and C. W. KUHN. 1974. Incidence and source of inoculum of peanut mottle virus and its effect on peanut. Phytopathology 64:60-64.
10. PAGUIO, O. R., and C. W. KUHN. 1975. Transmission of peanut mottle virus by aphids. Annu. Proc. Am. Phytopathol. Soc. for 1974. 1: (In press).
11. ROSS, J. P. 1969. Pathogenic variation among isolates of soybean mosaic virus. Phytopathology 59:829-832.