Natural Infection, Disease Reactions, and Epidemiological Implications of Peanut Mottle Virus in Cowpea

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ABSTRACT

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A mild strain of peanut mottle virus (PMV) was found in cowpea fields in Georgia in 1980 and 1981 but only when peanut fields were nearby. Infected cowpeas were either symptomless or showed a mild mottle, and plant growth and yield were not affected significantly by the virus in greenhouse studies. Ten of 24 *Vigna* entries were resistant to PMV. *Aphis craccivora* transmitted PMV to and from cowpeas and from cowpeas to peanuts. A low frequency of seed transmission was detected in one cowpea plant introduction. Field tests demonstrated natural transmission to and from cowpeas, from cowpeas to peanuts, and from peanuts to cowpeas. The necrosis strain of PMV caused a more severe disease in cowpea than the mild strain, and a mixed infection of PMV and cucumber mosaic virus caused a synergistic disease reaction in cowpea.

Peanut mottle virus (PMV) was first isolated from peanut (*Arachis hypogaea* L.) in 1965 (7). Since that time, other crops such as soybean (*Glycine max* (L.) Merr.) (8), garden pea (*Pisum sativum* L.), navy bean (*Phaseolus vulgaris* L.) (1), and some forage legumes (*Trifolium* and *Lupinus* spp.) (5) have been reported to be naturally infected with the virus. PMV may be restricted to the Leguminosae in nature although a few species outside this family are susceptible by mechanical inoculation (2).

The primary source of PMV appears to be peanut seedlings from infected seeds. Secondary spread occurs to other peanuts with subsequent transmission to other crops (1,3,7). The virus apparently is not seed transmitted in soybean (4), but Behncken and McCarthy (1) reported a low frequency of seed transmission in navy beans.

Naturally infected hosts are important because they may serve as an inoculum source of virus for other plants in the population or for other susceptible crops. In addition to yield losses that may result from infection, they may be involved in dual infections in which synergistic disease reactions could take place.

Although cowpea, *Vigna unguiculata* (L.) Walp. subsp. *unguiculata,* is recognized as an experimental host of PMV (7), natural infection has not been reported. Therefore, during virus surveys of cowpea fields in recent years, a diagnostic host for PMV was included in the greenhouse evaluation of diseased tissue from field plants. Studies were designed to detect and identify PMV in cowpea, to determine its effect on cowpea, and to determine the potential role of cowpeas in PMV epidemiology.

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MATERIALS AND METHODS

Virus identification. Leaf samples from diseased cowpea plants were collected during field surveys in Decatur, Macon, and Spalding counties, GA, in 1980 and 1981. Tissue was triturated in 0.25 M potassium phosphate buffer (pH 7.2) (1 g of tissue per milliliter) and rubbed onto several selected hosts dusted with 600-mesh silicon dioxide. When any sample caused necrotic local lesions on the PMV indicator host *P. vulgaris* 'Topcrop,' host range studies and serological tests were conducted to identify PMV. Antiserum to PMV (9) was used in sodium dodecyl sulfate immunodiffusion tests (12).

Disease reactions. Cowpea cultivars Clay, California Blackeye, Iron, and Dixiecream were grown in a soil-vermiculite mix (3:1) (two plants per 20-cm-diameter plastic pot) in the greenhouse to determine the effects of virus infection on yield. Eight plants of each cultivar were inoculated with PMV at the first true leaf stage, eight were inoculated near flowering, and eight were not inoculated. All plants were randomly distributed on greenhouse benches.

The reactions of 10 commonly grown cowpea cultivars (B. B. Brantley, Georgia Experiment Station) and 14 *Vigna* plant introduction (PI) lines (Southern Regional Plant Introduction Station, Experiment, GA) to PMV were determined by mechanical inoculation of 10-day-old seedlings grown in the greenhouse.

Assay procedure. In greenhouse and field experiments, indexing for PMV in cowpeas was accomplished by extracting plant sap as described previously and inoculating Topcrop bean.

Virus concentration in cowpeas was determined by local lesion assays on primary leaves of Topcrop bean (9). Infected tissue was triturated in 0.05 M potassium phosphate buffer (pH 7.5) containing 0.25 M $NaHSO_3$ and 1% celite. Treatment comparisons were made on a half-leaf basis in an incomplete block design with six replicates per treatment. Data were analyzed statistically on the basis of lesion numbers and transformations according to Kleczkowski (6). Differences at $P = 0.05$ were the same for both methods.

Transmission studies. For seed transmission tests, seeds from infected parent plants were sown in germination trays in the greenhouse. Seedlings were grown to the second true leaf stage before indexing to Topcrop bean. After indexing 100 individual seedlings from each entry with negative results, we pooled one leaflet each from five seedlings to index the remaining seedlings.

Aphid transmission from cowpea to cowpea (five aphids per transfer) and from cowpeas to peanuts (one aphid per transfer) was determined using *Aphis craccivora* Koch. maintained on California Blackeye cowpeas. Aphids were starved overnight in glass vials and placed on PMV-infected leaves using a camel's hair brush. Aphids were permitted an acquisition access period of 1 min (observed under a binocular scope) and then transferred to recipient hosts for a 1-hr inoculation access period. After aphid removal, recipient hosts were maintained in the greenhouse for 2 wk before indexing for virus infection.

Natural spread of PMV was determined in field tests with cowpea and peanut cultivars planted in plots consisting of four rows 4 m long (1 m between rows) with about 50 plants per row. In two tests, the virus source was infected peanut seeds. In the third test, cowpea plants in the outer rows on each side of the plots were mechanically inoculated with PMV and the center two rows were either cowpeas or peanuts that were indexed for PMV. Indexing was done at flowering and again immediately before harvesting.

Mixed infection. In mixed infection studies, 9-day-old cowpea seedlings in 10-cm-diameter plastic pots were rubbed with inoculum containing a mixture of cucumber mosaic virus (CMV) and PMV. Control plants were rubbed with buffer and all plants were maintained in the greenhouse. Assays on Topcrop bean were conducted 19 days after inoculation, and all other evaluations were made at 26 days.

RESULTS

Virus identification. PMV was isolated from cowpeas in three widely separated areas of Georgia. In all cases, peanut fields were adjacent to the cowpeas (within 1–5 m). Field-grown cowpea plants with PMV were either symptomless or showed mottle to mosaic symptoms typical of CMV and/or blackeye cowpea mosaic virus (BlCMV). PMV was found in single infections and in mixed infections with either CMV or BlCMV; eg, of 10 diseased plants from one field, seven had CMV, six had BlCMV, two had PMV, and one had an unidentified virus.

No symptoms were observed on most field-grown cowpea plants infected with PMV alone. Under greenhouse conditions (21–30 C), however, the PMV isolate from cowpea caused veinclearing followed by mild mottle on California Blackeye and Clay cowpeas. Symptoms varied from extremely mild in summer months to clearly discernible in winter months. Recovery from symptoms occurred 2–3 wk after inoculation, after which plants remained symptomless.

Identification of PMV in field-grown cowpea plants was based on positive serological results (precipitin lines) and typical PMV disease reactions on Florunner peanuts (mottle), Bragg soybean (mottle), and Topcrop bean (necrotic local lesions). These reactions indicated that the virus was the same as or very similar to the mild strain (M) of PMV (10).

Disease reactions. Concentration of PMV in four cowpea cultivars was determined by local lesion assay. Sap diluted 1/10 from California Blackeye, Clay, Lady Peas, and Iron caused an average of 282, 153, 64, and 48 lesions per half leaf, respectively. (All differences were significant at $P = 0.05$ except the two lowest values.) The PMV infectivity level in California Blackeye and Clay was similar to that in peanut cultivars Argentine and Florunner but less than that in Little Marvel pea (9; C. W. Kuhn, *unpublished*).

In greenhouse studies, there were no significant differences in the number of pods, number of seeds, and seed weight of PMV-infected and control plants of Clay, California Blackeye, Iron, and Dixiecream cowpeas.

When 24 *Vigna* entries were evaluated for their reaction to PMV, symptoms were observed on very few plants; therefore, subinoculations were made from the inoculated plants to Topcrop bean. Virus infectivity was detected in Clay, Dixiecream, California Blackeye, Iron, and PIs 164316, 170857, 174965, 188701, 195416, 250161, 292907, 339588, 377366, and 406329. Virus infection was not found in Brown Sugar Crowder, Coronet, Early Pinkeye, Knuckle Purple Hull, Mississippi Silver, White Acre, or PIs 186454, 189230, 237669, or 328113.

PMV strains. In addition to the mild (M) strain, the necrosis (N) and chlorotic line pattern (CLP) strains of PMV have been isolated from peanuts in Georgia (10). The disease reaction caused by CLP in California Blackeye was very similar to the one caused by strain M; however, concentration of M was significantly ($P = 0.05$) greater than CLP (72 vs. 19 lesions per half leaf).

Strain N, on the other hand, caused a more severe disease in California Blackeye and Clay cowpeas than did strain M. Necrotic etching occurred on primary leaves inoculated with N and a clearly distinct mottle developed on trifoliolate leaves. Sap (1/10 dilution) from California Blackeye and Clay plants infected with strain N caused 328 and 401 lesions per half leaf, respectively, whereas strain M caused significantly ($P = 0.05$) fewer lesions, 72 for sap from California Blackeye and 175 for sap from Clay. Neither plant height nor dry weight of shoots measured at 4 wk after inoculation were significantly ($P = 0.05$) reduced by either strain.

Transmission studies. *A. craccivora* transmitted PMV from cowpeas to one of five peanut plants and from cowpeas to two of seven cowpea plants. Seed transmission occurred with two of 240 seeds of cowpea PI 292907 but not with 2,374 seeds of six other susceptible cowpea entries or in 699 seeds of two other susceptible *Vigna* spp.

Natural transmission of PMV from peanuts to cowpeas and vice versa was evaluated in three field experiments. In test 1, California Blackeye (susceptible) and Coronet (resistant) cowpeas were planted in alternate rows with Florunner peanuts, which had a seed transmission frequency of 0.9%. After pods had set on the cowpeas, individual plants were indexed to Topcrop bean; infection percentages were 24, 8, and 0 for Florunner, California Blackeye, and Coronet, respectively. Seed from the same lot of California Blackeye showed no PMV infection when planted in two plots 470 and 690 m from the peanuts. In test 2, Iron cowpeas were planted at random in a field of naturally infected peanuts; 11 of 18 cowpea plants indexed at flowering were infected with PMV. In the third field test, PMV spread from mechanically inoculated cowpeas to adjacent cowpeas in each of two trials and from cowpeas to peanuts in each of two trials; PMV incidence in recipient plants was more than 50% in all four trials. PMV was not isolated from plants in two cowpea and two peanut plots where a source of virus was not provided.

Mixed infection. A mixed infection of CMV and PMV strain M caused a synergistic reaction in California Blackeye cowpeas. New symptom types, necrosis and stunt, were observed with the mixed infection, and plant height and shoot and root growth were reduced more than the additive effects of single infections (Table 1). PMV infectivity level was decreased in the mixed infection (Table 1). Similar synergistic effects were found with strains N and CMV in California Blackeye, but a mixed infection of strains CLP and CMV had very little effect on symptoms or plant growth. No synergism occurred in Clay cowpeas with mixed infections of CMV and any of the PMV strains.

Table 1. Effects of single and double infections of cucumber mosaic virus (CMV) and mild strain of peanut mottle virus (PMV) on symptoms, plant growth, and infectivity in California Blackeye cowpeas[x,y]

Treatment	Symptoms	Plant height (cm)	Dry wt (g)		Lesions[z]
			Shoots	Roots	
Control	None	38.9 a	8.33 a	2.97 a	...
CMV	Mottle	38.2 a	7.82 a	4.17 a	...
PMV	Mild mottle	38.4 a	7.92 a	2.81 a	169
CMV + PMV	Mottle, stem necrosis, stunt	28.7 b	3.54 b	1.13 b	77

[x] Plants were inoculated 9 days after seeding, 12 plants per treatment; all values are average per plant.

[y] Treatment means in the same column followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] Average number of local lesions per half leaf in three assays, significantly different at $P = 0.05$.

DISCUSSION

These studies establish that cowpea is a natural host for PMV. Infected plants were found in widely separated areas in Georgia during surveys in 1980 and 1981. The cowpea disease appears to be of little economic importance at this time. Symptoms are very mild and probably indiscernible in field plants, and greenhouse studies did not demonstrate any yield loss caused by PMV.

Despite the mild disease reaction, PMV in cowpea should be considered important for at least three reasons: 1) epidemiological implications, 2) potential

of naturally occurring severe strains of PMV, and 3) potential synergistic interactions with unrelated viruses. Cowpea can serve as a source of PMV inoculum, and this becomes even more important with the demonstration of potential seed transmission in cowpea. Cowpeas are frequently grown in areas immediately adjacent to other legumes susceptible to PMV, eg, peanut, soybean, and forage legumes. When multiple and continuous cropping is practiced, spread from one crop to another is likely, which would reduce the importance of seed transmission in peanut as providing the primary source of inoculum (1,3,7).

Four naturally occurring strains of PMV have been reported in the United States (10), and it is probable that other variants exist in nature. For example, an isolate that systemically infects *P. vulgaris* has been found in Australia (1). In the current studies, strain N caused a significantly more severe disease than strain M.

The most prevalent cowpea virus in Georgia is CMV (J. W. Demski and C. W. Kuhn, *unpublished*); it is seed transmitted in all commercial cowpea cultivars and occurs in all plantings. In a mixed infection with the potyvirus BlCMV, CMV causes a synergistic disease reaction (11). PMV also is a potyvirus and it also caused a synergistic reaction with CMV in one cowpea cultivar although not as strong a reaction as the combination of CMV and BlCMV.

Several cowpea lines were identified as resistant to PMV. Virus could not be isolated from inoculated plants, and no natural infection occurred in one of the lines planted in a field test.

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