Peanut Mottle Virus in Forage Legumes

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ABSTRACT

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Peanut mottle virus (PMV) was isolated from arrowleaf (*Trifolium vesiculosum*) and subterranean (*T. subterraneum*) clover, white (*Lupinus albus*) and blue (*L. angustifolius*) lupine, and the weed host *Desmodium canum*. The virus was identified by the use of indicator hosts, host range, and serology. This is the first report of natural infection of these forage legumes by PMV. The virus appears similar to the mild strain that commonly infects peanuts and soybeans in Georgia. Field observations in 1979 and 1980 indicated that PMV is quite prevalent and causes moderately severe symptoms in some forage legumes in southern Georgia.

During 1979 and 1980, typical virus symptoms such as vein chlorosis, mild mottle to severe mosaic, stunting, and rosette were observed on various forage legumes in southern Georgia. Routine isolation of viruses on indicator hosts confirmed the presence of viruses reported earlier (1). Some isolates, however, from arrowleaf (*Trifolium vesiculosum*) and subterranean (*T. subterraneum*) clover and blue (*Lupinus angustifolius*) and white (*L. albus*) lupine when inoculated on *Phaseolus vulgaris* 'Topcrop' produced lesions indicative of peanut mottle virus (PMV) (2).

PMV naturally infects and causes economic losses in peanuts (6) and soybeans (4). The virus is seed-transmitted in peanuts (6), and the infected plants derived from seed provide a source of virus for other peanuts and soybeans growing in proximity (3). In a previous study (8), a primary source of PMV could not be found except via peanut seed. Numerous members of the Leguminosae are susceptible to PMV by controlled inoculation, but the virus has a limited host range outside this family (2). Therefore, this study was done to determine the natural incidence of PMV in certain forage legumes.

MATERIALS AND METHODS

Plants with symptoms of PMV were collected primarily in Tift (in the peanut belt) and Spalding counties (70 miles north of the peanut belt), Georgia. Fresh leaf samples were removed from various forage legumes and possible weed hosts, placed in plastic bags, and returned to the laboratory.

Approximately 1 g of tissue was triturated with a mortar and pestle in 1 ml of 0.025 M sodium phosphate buffer (pH 7.2) containing 1% Celite. The buffered sap was used to mechanically inoculate the following isolation (recovery) hosts: *Chenopodium amaranticolor, Cucurbita pepo, Pisum sativum, Vigna sinensis,* and *P. vulgaris*. Presence of PMV was usually indicated by the development of large red local lesions on *P. vulgaris* 'Topcrop' and systemic mottle in *P. sativum* 'Alaska.' Single-lesion transfers in Topcrop beans and passage through *Arachis hypogaea* 'Florunner' or 'Argentine,' eliminated other viruses. The PMV cultures were maintained in *P. sativum* 'Little Marvel,' peanuts, or Bragg soybeans.

The diagnositic host range included *A. hypogaea* 'Florunner' and 'Argentine,' *Glycine max* 'Bragg' and 'Davis,' *Casia obtusifolia, C. occidentalis, Cyamopsis tetragonoloba, P. vulgaris* 'Bountiful' and 'Topcrop,' *P. sativum* 'Little Marvel,' and *Vicia faba*. The forage legumes tested for susceptibility in the greenhouse by mechanical inoculation were *T. vesiculosum* 'Yuchi,' *T. subterraneum* 'Mt. Barker,' *T. hybridum* 'common' (alsike clover), *T. pratense* 'Kenland' (red clover), *T. repens* 'Tillman' (white clover), *T. incarnatum* 'Dixie' (crimson clover), *Medicago sativa* 'Team' (alfalfa), *L. albus* 'Tifwhite-78,' and *L. angustifolius* 'Tifblue-78.'

Virus identification was confirmed serologically by a modified latex agglutination test (5) performed in microcapillary tubes.

We performed two separate tests in the greenhouse to determine the effect of PMV on fresh shoot and root weights of susceptible forage legumes. Seedlings were transplanted to a clay loam-vermiculite mixture in 15-cm clay pots and inoculated with PMV when the plants had approximately eight fully expanded leaves. Twelve inoculated and 12 healthy plants were alternately arranged on a greenhouse bench.

Fresh weights of shoot-tops were determined by removing the tissue above the crown about every 3 wk with a total of four cuttings per test. The fresh weight of roots was obtained at the end of each test.

RESULTS

Virus isolation. Different viruses were isolated from forage legumes on the five isolation (recovery) hosts. Large red local lesions radiating along the veins in Topcrop bean indicated presence of PMV. A single virus culture was obtained by single local lesion passage through other Topcrop beans, then transferred through peanut systemically, and

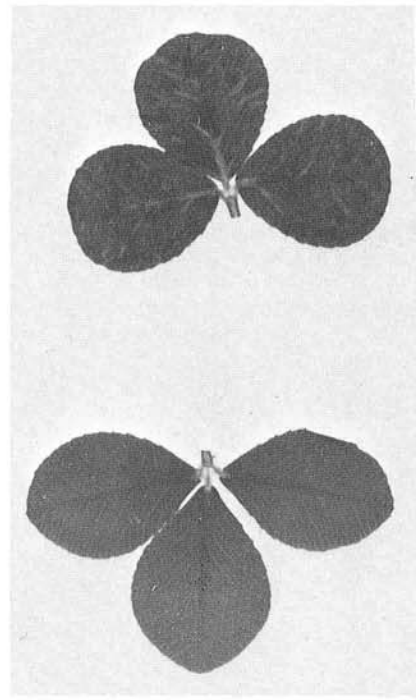

Fig. 1. Arrowleaf clover with peanut mottle virus: **(top)** infected, **(bottom)** healthy.

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returned to either Little Marvel pea or Bragg soybean.

Diagnostic host range. The isolates produced symptoms typical of the mild strain of PMV (2,7). Systemic mild mottle was observed on Florunner and Argentine peanuts, *Cyamopsis tetragonoloba, Cassia obtusifolia,* and *C. occidentalis.* Infected Bragg soybean and broad bean (*V. faba*) showed vein clearing followed by bright mottle, dark green islands, and mild distortion. Bright vein chlorosis followed by light green to severe mottle was observed on Little Marvel pea. Reddish local lesions spread dendritically along the veins and veinlets of the primary leaves of Topcrop bean, and Bountiful beans had chlorotic local lesions without systemic invasion.

No symptoms were observed on or virus recovered from Davis soybean. Other diagnostic hosts that did not have symptoms and from which virus could not be recovered were *Datura stramonium, C. amaranticolor, C. quinoa, Gomphrena globosa, Curcurbita pepo,* and *Nicotiana tabacum* 'Burley 21' and 'Samsun.'

Serology. Sap from naturally infected blue and white lupines flocculated latex beads sensitized with antiserum to PMV isolate NS (donated by C. W. Kuhn, University of Georgia). The antiserum (titer 1:256 in microprecipitin tests) at a dilution of 1:20 was optimum for good latex agglutination test reactions. Sap from healthy plants of the same species grown in the greenhouse served as controls.

Sap from infected subterranean clover, arrowleaf clover, and *Desmodium* did not react. When virus was transferred from these hosts to Little Marvel pea, however, pea sap produced a positive flocculation with PMV antiserum and confirmed the identity of virus from all hosts.

Natural infection. PMV was isolated from naturally infected arrowleaf and subterranean clover, white and blue lupine, and the weed host *Desmodium canum* in Tift County. In 1979, PMV was isolated from 13 of 25 samples of arrowleaf and nine of 20 samples of subterranean clover with viral symptoms. In the winter of 1979-1980, PMV reached epidemic proportions in a field of mixed blue and white lupines. The virus appeared to infect each species equally. In February 1980, almost 50% of the blue and white lupines were naturally infected. PMV has not been isolated from forage legumes in northern Georgia (Spalding County).

Forage legume susceptibility. We inoculated seven forage legume species (three separate tests) with a PMV isolate from white lupine to determine susceptibility. In addition to the species found naturally infected, crimson clover was susceptible to PMV by mechanical inoculation. Symptoms were not observed in and virus could not be recovered from alfalfa, alsike, or red or white clover. When a PMV isolate from *Desmodium* was used, we recovered the virus from one

Fig. 2. Blue lupine with peanut mottle virus. Entire plant has "bushy habit."

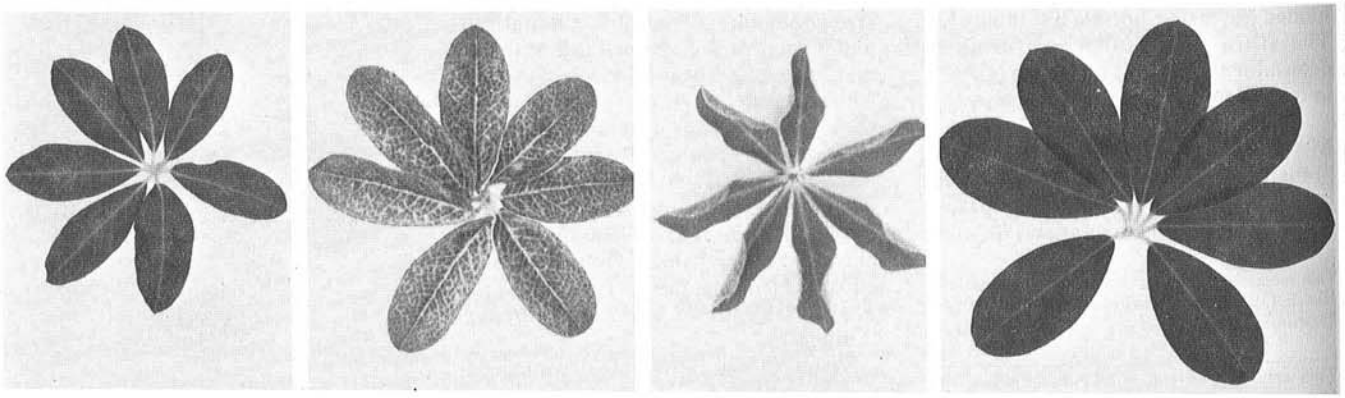

Fig. 3. Symptoms induced by peanut mottle virus in white lupine: **(left)** mild vein clearing, initial symptom in the field; **(left center)** bright vein clearing, field symptom; **(right center)** leaf curling, greenhouse symptom; **(right)** healthy.

Table 1. Effect of peanut mottle virus on fresh shoot and root weight of *Trifolium* sp. grown in the greenhouse

Clover	No. of plants (infected/inoculated)	Green shoot-top[a] weight per clipping per plant (g)		Green weight, roots[a] per plant (g)	
		Healthy	Inoculated	Healthy	Inoculated
Crimson *T. incarnatum* 'Dixie'	6/12	17.82	11.55*	3.67	2.48*
Subterranean *T. subterraneum* 'Mt. Barker'	4/12	13.19	9.46	3.23	2.45*
Arrowleaf *T. vesiculosum* 'Yuchi'	12/12	14.34	5.96	4.65	1.55*

[a] Each value is an average for the number of infected plants or paired healthy plants for each entry in one experiment. * = Significantly different ($P = 0.05$) from control according to paired T test.

of six alsike plants that had been mechanically inoculated.

Symptoms induced in some forage legumes by PMV were quite variable between greenhouse and fieldgrown plants suggesting that temperature, light, or both influence the symptoms. Generally, the mosaic or mottle was brighter in fieldgrown plants. Symptoms observed in the field were: chlorotic flecks and mild mottle on subterranean clover; vein clearing and chlorotic bands followed by mottle (Fig. 1) and reduction of leaf size or arrowleaf clover; general chlorosis, "bushy habit," and stunting, with mild mottle on leaflets of blue lupine (Fig. 2); and vein clearing and mottle of white lupine (Fig. 3).

In the greenhouse, subterranean clover and blue lupine showed no symptoms. Symptoms on arrowleaf clover in the greenhouse were similar to those in the field. Leaflet curling or cupping and occasional mild mottle developed in white lupine (Fig. 3), and crimson clover showed chlorotic flecks followed by chlorotic mottle (Fig. 4) that faded with age. Bright chlorotic mottle developed on *Desmodium* in the field and greenhouse when inoculated with a PMV isolate from *Desmodium*. *Desmodium* was not susceptible to an isolate from white lupine.

Leaflets of PMV-infected blue and white lupine were small, but visually this was not easily discernible. In greenhouse tests, average leaflet diameter was 10.3 mm for healthy and 8.4 mm for infected white lupine and was 3.0 and 2.2 mm for healthy and infected blue lupine, respectively. These differences were statistically significant at the 0.05 level, according to Duncan's multiple range test.

Effects on growth. Fresh shoot-top and root weights were significantly reduced in inoculated plants (Table 1). In addition, four of six crimson clover and two of six arrowleaf clover plants died about 120 days after inoculation. Observations of naturally infected plants in the field, however, did not indicate that plant death was common.

DISCUSSION

This is the first report of forage legumes naturally infected by PMV. The increase of forage legumes for winter pasture in the Southeast maintains a susceptible species in the field during most of the year. Seeding dates cause an overlap of the vegetative stages of the forage legumes and peanuts and soybeans; a vegetative source of PMV may thus always be present.

Although PMV has a narrow host range, naturally infection of some winter annual forage legumes poses new problems for developing control programs. Winter annual forage legumes may provide a source of virus during most periods of the year. *Desmodium canum* is killed by frost from central Georgia north but it grows naturally as a perennial throughout southern Georgia and Florida. In this southern clime, *D. canum* can also serve as a source of PMV for cultivated crops. Previous to this report, the only known overwintering source of PMV was peanut seed (3,8). We have now isolated PMV from forage legumes in southern Georgia (in the peanut belt) and from blue lupine in Florida but not in northern Georgia.

The most common strains of PMV in peanuts and soybeans are the mild strains (7). Two years' data on the PMV isolates from forage legumes indicate that the mild strains are also the most common in these species. Further studies are in progress to determine to what degree the PMV-infected forage legumes contribute to the spread of virus within this group and also to peanuts and soybeans. If the source of PMV for forage legumes is peanuts or soybeans, planting of forage legumes in the peanut belt could be delayed until the fall frost.

Numerous viruses have been isolated from the forage legumes. The use of Topcrop bean as an indicator host to determine the presence of PMV appears unique. Two to 4 days after inoculation, small dark local lesions appear, enlarge (often to 2 mm), become reddish, and spread dendritically along the veins. This reaction on Topcrop has been illustrated previously (2,6), but its usefulnes in the isolation and identification of PMV justifies reemphasis. The appearance of the previously described symptoms on Topcrop beans suggested the presence of PMV, which was confirmed by host range and serology in numerous isolations from various plants.

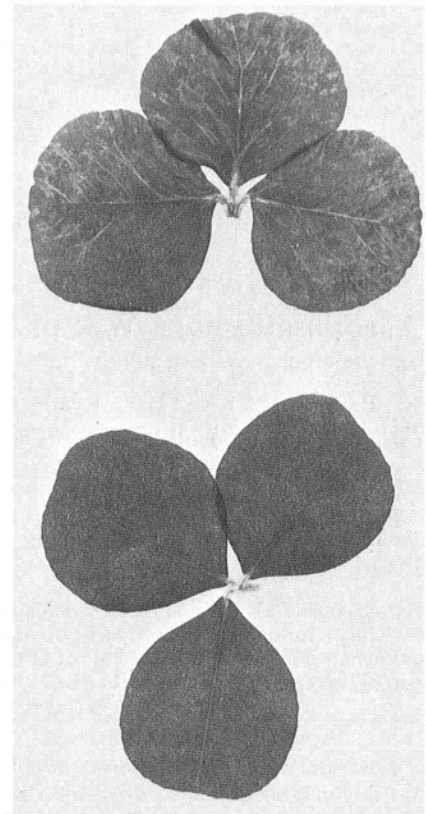

Fig. 4. Crimson clover with peanut mottle virus: **(top)** infected, **(bottom)** healthy.

Separation of PMV from bean yellow mosaic, peanut stunt, white clover mosaic, and cucumber mosaic viruses was accomplished by passage through Topcrop bean and Florunner peanut. The rapid development of local lesions in Topcrop and restricted susceptibility of Florunner to other viruses permitted the isolation of PMV free of other viruses.

Additional importance of PMV in the forage legumes was indicated by greenhouse studies which showed that PMV causes economic loss and death in arrowleaf and crimson clover and yield loss in subterranean clover. Observations on the natural infection of blue and white lupines indicate that PMV may be a serious problem in lupine production in the peanut belt in Georgia.

ACKNOWLEDGMENT

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