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## Identification Of Viruses Infecting Peanut In Alabama<sup>1</sup>

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### ABSTRACT

Leaf samples collected from 1,883 peanut plants in 158 fields in 14 counties during July-August, 1990 and 1991, were tested for peanut mottle (PMV), peanut stripe (PStV), peanut stunt (PSV), and tomato spotted wilt (TSWV) viruses by sap inoculations onto indicator plants and/or by enzyme-linked immunosorbent assay (ELISA). Of 889 plants showing virus-like symptoms, 58% were infected with TSWV alone or mixed with PMV or PSV, 36% with PMV alone or mixed with TSWV or PSV, and 5% with PSV alone or mixed with TSWV or PMV. Double infections of PMV with TSWV, PSV with TSWV, and PMV with PSV were detected in 24%, 3%, and 1% of the symptomatic plants. Of 994 apparent asymptomatic plants, 16% were infected with PMV alone or mixed with TSWV, 8% with TSWV alone or mixed with PMV or PSV, and 5% with PSV alone or mixed with TSWV. Double infections of PMV with TSWV and PSV with TSWV were detected in 2% and 1% of the asymptomatic plants. PMV and TSWV were found in at least one field in every county sometime during the two seasons; PSV was found in 13 counties. The most notable difference in virus incidence between the two years was that TSWV was found in 20% of the asymptomatic plants in 1990 as compared to 6% in 1991. PStV was not detected by ELISA in 1990 or 1991, and no reactions suggestive of PStV or any virus other than PMV, PSV, or TSWV were observed on indicator plants used to assay the 1990 collections.

Key Words: Virus diseases, peanut viruses, PMV, PSV, PStV, TSWV.

There is considerable information on virus diseases of peanut for many of the major production areas in the United States. However, little is known about viruses that infect peanut in Alabama. In annual surveys conducted since 1986, tomato spotted wilt virus (TSWV) was found to be widespread in peanut in the state, but at low incidence (10,12). Peanut stunt virus (PSV) was found in Alabama in 1971 (18), and peanut mottle virus (PMV) has occasionally been diagnosed over the past 20 years (9; R. T. Gudauskas, unpublished). During 1984-1985, leaf samples from peanut plants collected in 96 fields and from seedlings produced from 359 seed lots in Alabama were tested by enzyme-linked im-

munosorbent assay (ELISA) for peanut stripe virus (PStV), but the virus was never detected (R. T. Gudauskas and W. C. Johnson, unpublished). Except for the recent efforts with TSWV, there have been no systematic investigations of peanut viruses in Alabama. This study was undertaken to determine the identity and distribution of viruses that occur in peanut in the state. A preliminary report has been published (11).

## Materials and Methods

During the period from mid-July to mid-August in both 1990 and 1991, leaves were collected from symptomatic and asymptomatic peanut plants selected at random from fields throughout the 14-county peanut production area in Alabama. Symptoms included leaf chlorosis, mottling, necrosis, line patterns, distortion, or plant stunting. We did not determine the incidence of a particular virus or viruses within fields because our aim was just to identify the viruses infecting peanuts.

Samples from all leaves collected in 1990 were assayed by mechanical inoculation onto indicator plants and by ELISA. Leaves collected in 1991 were tested only by ELISA. For mechanical inoculation, a leaflet was ground in 0.1M potassium phosphate buffer containing 0.01M sodium sulfite, pH 7.0. The buffered sap was rubbed onto carborundum-dusted leaves of *Arachis hypogaea* L. cv Florunner, *Glycine max* (L.) Merrill cv Bragg, *Phaseolus vulgaris* L. cv Topcrop, *Nicotiana benthamiana* Domin., and *Nicotiana tabacum* L. cv Burley 21. Reactions considered to be indicative of PMV were mild mottle on Florunner peanut and Bragg soybean and necrotic local lesions on Topcrop bean; for PStV - striping or banding along veins and mild line patterns on Florunner peanut; for PSV - chlorosis and severe stunting on Florunner peanut and local and systemic chlorotic and/or necrotic rings and line patterns on Burley 21 tobacco; for TSWV - chlorosis, ringspotting and line patterns on Florunner peanut, large local and systemic chlorotic and/or necrotic rings and line patterns on Burley 21 tobacco, and local and systemic chlorotic and necrotic spotting, mottle, and plant collapse on *N. benthamiana*. For ELISA, a leaflet from each sample was ground in phosphate-buffered saline containing Tween-20 (8 g sodium chloride, 1.1 g dibasic sodium phosphate, 0.2 g monobasic potassium phosphate, 0.2 g potassium chloride, and 0.5 mL Tween /L; pH 7.4) and 2% polyvinylpyrrolidone (40,000 MW). The resultant sap was tested by the double antibody sandwich procedure (4) using antisera to PMV (provided by C. W. Kuhn, University of Georgia, Athens), PStV (provided by J. W. Demski, Georgia Experiment Station, Griffin), PSV (PSV-AS 47, obtained from American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852), and TSWV (provided by J. W. Demski). Some TSWV assays in 1991 were conducted with commercial kits obtained from AGDIA, Inc., 30380 County Road 6, Elkhart, IN 46514. Positive (virus-infected peanut) and negative (healthy peanut) controls were included in each ELISA plate. Absorbance at 405 and 490 nm for phosphatase- and peroxidase-based assays, respectively, was measured with an MR 700 Microplate Reader (Dynatech Laboratories, Inc., 14340

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Sullyfield Circle, Chantilly, VA 22021). A sample was considered virus-infected if the average absorbance of duplicate wells was 3X that of the negative control.

## Results

A total of 1,883 peanut plants from 158 fields throughout the 14 county area were assayed for viruses during the 1990 and 1991 growing seasons (Table 1). Of the 889 plants showing virus-like symptoms, 58% were infected with TSWV alone or in combination with PMV or PSV, 36% with PMV alone or in combination with TSWV or PSV, and 5% with PSV alone or in combination with TSWV or PMV. Mixed infections of PMV with TSWV, PSV with TSWV, and PMV were detected in 24%, 3%, and 1% of the symptomatic plants, respectively. Of the 994 plants showing no apparent symptoms, 16% were infected with PMV alone or in combination with TSWV, 8% with TSWV alone or in combination with PMV or PSV, and 5% with PSV alone or in combination with TSWV. Mixed infections of PMV with TSWV and PSV with TSWV were detected in 2% and 1% of the asymptomatic plants, respectively. PMV and TSWV were found in at least one field in all 14 counties sometime during the two seasons; PSV was found in 13 of the counties. PSV was not detected by ELISA in any sample collected in 1990 or 1991, and no reactions suggestive of PSV or any virus other than PMV, PSV, or TSWV were observed on indicator plants used to assay the 1990 collections.

In 1990, TSWV and PMV were found in every county and over 90% of the fields surveyed; PSV was identified in 10 of the 14 counties and 27% of the fields (Table 2). A similar distribution of TSWV and PSV occurred in 1991, but PMV was detected in fewer fields and counties than in 1990. In 1990, TSWV and PMV were found in 61 to 68% of the

symptomatic plants and in about 20% of the asymptomatic plants. In 1991, TSWV was again identified in about 60% of the symptomatic plants, but in only about 6% of those showing no symptoms. PMV was detected in 12% and 15% of the symptomatic and asymptomatic plants, respectively. PSV was found in about 5% of both the symptomatic and asymptomatic plants.

**Table 2. Occurrence of peanut viruses in Alabama in 1990 and 1991.**

Sampling unit	No.	Incidence (%)					
		PMV <sup>a</sup>	PSV <sup>a</sup>	TSWV <sup>a</sup>	PMV+ TSWV	PMV+ PSV	PSV+ TSWV
1990							
Counties	14	100.0	71.4	100.0	100.0	28.6	42.9
Fields	70	91.4	27.1	94.3	81.4	10.0	17.1
Plants							
Symptomatic	388	68.0	5.7	61.1	45.6	2.8	2.8
Asymptomatic	154	18.8	0.6	20.8	12.3	0	0.6
1991							
Counties	14	92.9	64.3	100.0	64.3	0	50.0
Fields	88	62.5	26.1	80.7	29.5	0	14.8
Plants <sup>b</sup>							
Symptomatic	501	12.0*	4.8	55.7	7.2*	0*	3.4
Asymptomatic	840	15.0	5.2*	6.1*	0.5*	0	1.1

<sup>a</sup>Includes all single and mixed infections with this virus.

<sup>b</sup>Virus incidence compared between years using Student's T Test; \* = significantly different (p = 0.05) from corresponding value for 1990, e.g. 12% PMV incidence in symptomatic plants in 1991 compared to 68% in 1990.

**Table 1. Viruses identified in symptomatic and asymptomatic peanut plants in 14 Alabama counties in 1990 and 1991.**

County	No. fields	No. plants <sup>a</sup>	No. plants infected					
			PMV <sup>b</sup>	PSV <sup>b</sup>	TSWV <sup>b</sup>	PMV+ TSWV	PMV+ PSV	PSV+ TSWV
Barbour	12	52 S	21	0	24	13	0	0
		84 NS	19	1	23	4	0	1
Bullock	8	65 S	31	2	29	23	0	0
		49 NS	19	2	5	4	0	1
Butler	6	36 S	11	0	7	2	0	0
		43 NS	0	0	0	0	0	0
Coffee	17	40 S	18	1	19	10	0	0
		71 NS	7	0	4	0	0	0
Conecuh	7	51 S	22	6	29	9	0	5
		57 NS	5	8	10	3	0	0
Covington	10	50 S	25	1	16	6	0	0
		71 NS	11	0	4	0	0	0
Crenshaw	7	35 S	15	4	16	14	4	3
		38 NS	11	0	11	7	0	0
Dale	12	71 S	15	3	53	14	0	2
		78 NS	9	0	2	2	0	0
Escambia	7	41 S	17	5	26	12	1	3
		39 NS	8	7	17	2	0	7
Geneva	16	109 S	44	9	89	37	0	9
		105 NS	12	5	2	0	0	0
Henry	19	139 S	50	6	93	34	5	2
		100 NS	20	0	3	0	0	0
Houston	18	64 S	21	1	44	20	1	1
		119 NS	27	3	0	0	0	0
Pike	12	81 S	26	7	45	18	0	2
		86 NS	5	12	1	0	0	0
Russell	7	55 S	8	1	26	1	0	1
		54 NS	2	7	1	1	0	0
Totals	158	889 S	324	46	516	213	11	28
		994 NS	155	45	83	23	0	9

<sup>a</sup>S = symptomatic plants; NS = asymptomatic plants.

<sup>b</sup>Total of all single and mixed infections with this virus.

## Discussion

These and other recent findings show that TSWV is established throughout the peanut production area in Alabama and many other states (3, 7, 10, 12, 13, 20). Our results indicated that PMV also is prevalent in Alabama and that PSV occurs at lesser but significant levels as well. The widespread occurrence of PMV was not unexpected in view of its general occurrence in neighboring Georgia (15) and other peanut producing states (9). Finding PSV in 13 of the 14 counties surveyed, and at rather high incidence in some, was surprising and it indicates a need for continued awareness of possible outbreaks of the virus. PSV has not been associated with any particular disease problem in peanuts since its discovery in the state in 1971 (18), even though it had also been detected in white clover (1, 17) which occurs in the peanut production area. To our knowledge, PSV has not been an economic problem in peanut in other states in recent years (15, 22).

The detection of PMV, PSV, and TSWV in asymptomatic plants established higher incidence levels of each than would have otherwise been apparent and underscored the importance of including some assay procedure in this type of study. Possibly, symptoms were very mild or simply overlooked at the time of collection. This could have been particularly true in 1990 when many fields were under drought stress, which could have confounded or obscured symptoms of virus infection. Symptoms of PMV can easily be overlooked because they often are rather mild and tend to fade or become masked as plants mature. Symptoms of TSWV usually are much more discernible; however, they too might be mitigated in plants under water stress (2, 5). In 1991, when moisture levels in general were adequate throughout the growing season, TSWV was detected in only

6% of the asymptomatic plants as compared to 20% in 1990. The numbers of asymptomatic plants in which PMV and PSV were detected in 1991 were about the same or higher than in 1990. Culbreath *et al.* (6) detected TSWV in roots but not leaves of 23% of the asymptomatic peanut plants they tested in 1990, but the water status of the plants was not mentioned.

As have others (20), we often detected mixed infections of PMV and TSWV. More mixed infections were found in 1990 when incidence of PMV was generally higher than in 1991. Mixed infections of PMV and PSV (14) and PMV and PStV (8, 15, 19) have also been reported in peanut in the United States. We found a few plants that were doubly infected with PMV and PSV or with PSV and TSWV, but we did not identify PStV in any sample tested in this or a previous survey of peanuts in Alabama. Although most of our samples came from commercial fields, both surveys included collections from research plantings on the Wiregrass Substation at Headland, AL. Findings of PStV in other states have been restricted primarily to research plantings of peanut (8, 15, 16, 21).

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