Effect of Two Strains of Peanut Mottle Virus on Fatty Acids, Amino Acids, and Protein of Six Peanut Lines^{1,2} Allan R. Hovis,³ Clyde T. Young,^{*3} and Cedric W. Kuhn⁴

ABSTRACT

Peanut (Arachis hypogaea L.) cultivars (Starr and Florunner) and four peanut introductions (PI 261945, 261946, 261973, and 261980) were each separately inoculated with a mild strain (M2) and with the necrosis strain (N) of peanut mottle virus. The effects of these viral strains on the chemical composition of peanut seed were evaluated. The chemical characteristics varied with the type of viral infection. The greatest effect was on fatty acids and the least on the total amino acids. In general, peanuts infected with the necrosis strain showed: (1) a decrease in the percentages of stearic and oleic acids, while linoleic, arachidic, behenic, and lignoceric acids increased, (2) increases in the levels of the free amino acids glycine, alanine, isoleucine, histidine, lysine, and arginine, and (3) the total amino acids exhibited a slight decrease in aspartic acid and a slight increase in methionine. Peanuts infected with the mild strain generally showed: (1) a slight increase in linoleic acid, (2) little effect on the free amino acids, and (3) a small increase in tyrosine and a slight decrease in serine and aspartic acid for the total amino acids. No treatment effect was noted on protein content.

Key Words: Arachis hypogaea L., peanut, peanut mottle virus, fatty acids, free amino acids.

The major peanut (Arachis hypogaea L.) cultivars used in the United States are susceptible to peanut mottle virus (PMV) (6,8). The predominant strain causes very mild symptoms (12); yield losses, however, range from 17-30% (8). Although immunity from the disease has not been found (7), a recent study (9) demonstrated that a few peanut introductions are tolerant to PMV and yield losses are none or very slight.

Mondy and Koch (11) showed that enzymatic darkening was significantly greater and lipid content lower in potatoes (Solanum tuberasum L.) infected with Potato virus X. Demski and Jellum (3) reported that soybean (Glycine Max (L.) Merr.) infected by various viral strains, including PMV, had significant changes in fatty acid content. It was shown by Tu and Ford (17) that the free amino acid content of soybean leaves was affected by virus infection.

¹Paper no. 6002 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, N.C. 27650.

²Presented at the 8th Annual Meeting, APREA, Asheville, N.C. July 13-15, 1977.

³Research Analyst and Associate Professor, Dept. of Food Science, North Carolina State University, Raleigh, N. C. 27650.

⁴Professor, Dept. of Plant Pathology and Plant Genetics, University of Georgia, Athens, GA 30602. The purpose of this study was to determine the effects of PMV infection on the fatty acids, free amino acids, total amino acids, and protein content of the seed of six peanut lines.

Materials and Methods

Two peanut cultivars (Starr and Florunner) and four peanut introductions (PI 261945, 261946, 261973, 261980) were grown in 20.3 cm plastic pots in the greenhouse. Twelve plants of each of the six peanut lines were rubbed with inoculum buffer (0.01 M neutral potassium phosphate, 0.01 M NaHSO₃, 0.01 M sodium diethyldithiocarbamate, and 1% Celite), twelve with a mild strain (M2) of PMV, and twelve with the necrosis strain (N) (12). Seed from the Starr plants was harvested 120 days after planting and seed from all other lines at 140 days. After drying at 25 C, the seed was ground in a laboratory Wiley Mill equipped with a stainless steel head, blades, and 10 mesh screen. The full fat samples were frozen at -30°C until chemical analysis. The samples were analyzed for fatty acids, free amino acids, total amino acids, and protein. The resulting data were subjected to statistical analysis by the analysis of variance (15) and the Waller-Duncan (18) procedures.

Preparation and Analysis of Fatty Acid Esters

A 300 mg sample was weighed into a glass culture tube equipped with a Teflon lined screw cap. Petroleum ether (15 ml of boiling range 30-60°C) was added and the tube shaken for 30 min. The tubes were allowed to stand overnight and 5 ml of ether extract were pipeted into Teflon capped glass vials, and the ether was removed by evaporation under nitrogen gas. The fatty acids were esterified by the saponification-transesterification method described by Metalfe et al. (10). Methanolic NaOH solution (1.3 ml of a 0.5 N) was added to the vials containing the extracted oil (approximately 30 mg) and heated in a boiling water bath for 5 min. After cooling, 2 ml of 10% boron trifluoride in methanol were added to this mixture and boiled for 5 min. After cooling, 1 ml of petroleum ether and 2 ml of saturated NaCl solution were added and the vial thoroughly shaken. The vial was then centrifuged for 5 min at 1600 x g. The ether layer was removed with a Pasteur pipet and stored at -30°C until analysis.

A F&M model 810 Research Gas Chromatograph equipped with a 1.83 m x 2 mm I.D. glass column and F.I.D. was used to quantitate the methyl esters of the fatty acids. The column was packed with 100/120 mesh Gas-Chrome Q coated with 10% EGSS-X (Applied Science, State College, PA). The initial oven temperature was 180 C and was programmed to 200°C at 4 C/min. The injector temperature was 250°C and the detector temperature was 275°C. The carrier gas was helium at 60 psi inlet pressure. The percentage composition of a known mixture of methyl esters (Altech Assoc., Arlington Heights, IL) was confirmed with less than 1% error. Peak areas were measured by a computer data system. Peaks were identified by comparing their retention times to the standard mixture of fatty acids.

Extraction and Analysis of Free Amino Acids

The method of Young *et al.* (19) was used for extraction of the free amino acids. Two g of the full fat sample were thoroughly extracted with diethyl ether to remove the oil. The fat free residue was ground in 50 ml of methanol, chloroform, and water mixture (60:25:15; v:v:v) for 1 min using a Tekmar Tissumizer (Tekmar Instr. Co., Cincinnati, Ohio) at full speed. Ground samples were centrifuged ($1600 \times g$), and 30 ml portions of the supernatant were transferred to a 100 ml beaker and

allowed to evaporate to dryness under a hood. The dried extracts were resuspended in 2 ml of pH 2.2 citrate buffer, centrifuged and the supernatants frozen (-18°C) until analyzed.

Analysis of Total Amino Acids

For amino acid analysis, the full fat samples were hydrolyzed by a modification of the method of Roach and Gehrke (14). In screw capped tubes, 100 mg of peanut meal in 20 ml of 6 N HCl were flushed with nitrogen and heated at 145°C for 2 hr. The pH was then adjusted to 2.2 with 12 N NaOH. Amino acids were quantitated by ion exchange chromatography as described by Spakman *et al.* (16) using a Durrum Model D-500 with a 1.75 mm x 48 cm column packed with Durrum highresolution cation exchanger (bead diameter, $8 \pm 1 \mu m$).

Protein Determination

The percent protein was determined by the macro-Kjeldahl method (2). The percentage nitrogen was converted to the percentage protein content by multiplying by 5.46 (5). Protein was also calculated using the total amino acid content as determined in the anlaysis of total amino acids.

Results

Previous studies (8,9) have established that PMV-M2 (predominant strain in nature) causes a seed yield loss of about 30% in susceptible peanut cultivars Starr and Florunner. With tolerant peanut lines PI 261945 and PI 261946, yield loss was none or negligible (9). In addition to these four peanut lines, two moderately tolerant (Yield loss

Table 1. Results of analysis of variance showing mean squares and level of significance on fatty acids in seed from virus infected peanuts.

Fatty Acid ^a	Variety	(V)	Sources of V Treatmen	VXT				
C16:0	4,166	**	0.093	 NS	0.073	NS		
C18:0	3.582	**	0.204	**	0.092	**		
C18:1	54.060	**	4,248	**	1.053	**		
C18:2	21.889	**	2,928	**	0.509	NS		
C20:0	0.502	**	0.004	NS	0.018	**		
C20:1	0.714	**	0.061	**	0.031	**		
C22:0	1.364	**	0.319	**	0,105	**		
C24:0	0.165	**	0.153	**	0.017	*		

^a Degrees of freedom for variety, treatment, and variety by treatment are 5, 2, and 10 respectively for each fatty acid.
** = significant at the 0.01 level.
* = significant at the 0.05 level.

NS = not significant.

of about 20%) lines (PI 261973 and PI 261980) were used in this study (Kuhn, unpublished).

Fatty Acids

The effect of viral infection (treatment) was significant for stearic (C18:0), oleic (C18:1), linoleic (C18:2), eicosenoic (C20:1), behenic (C22:0), and lignoceric (C24:0) acids (Table 1). The variety by treatment interaction was significant for stearic, oleic, arachidic (C20:0), eicosenoic, behenic, and lignoceric acids. Palmitic acid (C16:0) showed no

Table 2. Fatty acids (% of total) in seed of six peanut lines infected with two strains of PMV.

Treatment	C16:0	C18:0	C18:1	C18:2	C20.0	C20:1	C22:0	C24:0
			PI 261945 (H	ighlv Toler	ant)			
Control	11.43 a	2.22 a	37.39 a	40.59 a	1.29 a	1.53 ab	3.70 a	1.67 b
PMV-M2	11.28 ab	2.07 a	37.63 a	40.86 a	1.27 a	1.48 b	3.67 a	1.70 b
PMV-N	11.14 b	1.63 b	37.37 a	41.02 a	1.11 Ь	1.75 a	3.97 a	1.93 a
		I	PI 261 946 (H	ighly Toler	ant)			
Control	11.06 a	2.55 a	38.35 ab	40.21 a	1.39 a	1.28 b	3.61 a	1.53 b
PMV-M2	11.17 a	2.40 ab	39.13 a	39.67 a	1.27 a	1.35 b	3.39 a	1.58 b
PMV-N	11.00 a	2.27 b	37.49 b	41.03 a	1.33 a	1.46 a	3.68 a	1.71 a
		PI	261973 (Mod	erately Tol	erant)			
Control	11.64 a	1.95 a	36.62`a	41.03 a	1.19 Ь	1.63 a	4.07 ab	1.82 a
PMV-M2	11.39 a	1.99 a	36.16 a	41.34 a	1.35 a	1.62 a	4.29 a	1.81 a
PMV-N	11.48 a	2.05 a	36.80 a	41.05 a	1.28 ab	1.51 a	3.94 Ь	1.86 a
		PI	261980 (Mod	erately Tol	erant)			
Control	11.19 a	2.78 a	38.80 a	39.18 b	1.51 a	1.22 a	3.70 a	1.59 a
PMV-M2	11.21 a	2.58 b	38.52 a	39.69 a	1.46 a	1.28 a	3.68 a	1.55 a
PMV-N	11.29 a	2.85 a	37.78 b	39.78 a	1.56 a	1.23 a	3.89 a	1.59 a
			Florunne	r (Suscepti	ble)			
Control	10.61 a	1.31 a	43.43 a	37.25 b	0.95 a	1.78 a	3.07 b	1.82 a
PMV-M2	10.36 a	1.32 a	42.58 ab	38.06 a	1.00 a	1.91 b	3.09 b	1.63 b
PMV-N ²	10.12 a	1.19 b	42.08 b	38.26 a	0.97 a	2.03 c	3.33 a	1.91 a
			Starr (Susceptible)			
Control	12.07 a	2.76 a	39.32 a	37.62 b	́ 1.50 Ь	1.35 a	3.77 b	1.56 b
PMV-M2	12.22 a	2.76 a	38.51 ab	38.28 ab	1.57 a	1.25 b	3.90 b	1.48 b
PMV-N	12.23 a	2.48 b	37.52 b	38.94 a	1.52 b	1.37 a	4.35 a	1.68 a

Values with same letter within blocks are not significantly different at 0.05 level according to the Waller-Duncan multiple range test.

Table 3. Results of analysis of variance showing mean squares and level of significance on free amino acids in seed from virus infected peanuts.

	Sources of Variation									
Amino Acid ^a	Variety (V)	Treatment (T)	VXT							
ASP	13089 **	14.28 NS	324.0 NS							
THR	1231 **	4.658 NS	17.53 NS							
SER	6909 **	2528 *	1830 *							
GLU	261733 **	13429 *	7357 *							
PRO	2185 *	1674 NS	2229 *							
GLY	140.5 **	91.59 **	35.29 **							
ALA	3606 **	1327 **	529.2 **							
VAL	25996 **	4856 **	1007 *							
ILE	184.5 **	40.47 **	5.893 NS							
LEU	119.9 **	17.74 **	3.404 NS							
TYR	127.9 **	26.08 *	11.03 NS							
PHE	32034 **	18816 **	4808 **							
HIS	32.61 **	19.02 NS	14.46 *							
LYS	48.68 **	14.23 NS	13.17 *							
NH4	1028 **	239.3 NS	369.0 NS							
ARĞ	4218 **	4082 **	1083 *							
PEP	891.1 **	47.25 NS	139.9 NS							

Degree of freedom for variety, treatment, and variety by treat-ment are 5, 2, and 10 respectively for each amino acid. = significant at the 0.01 level. = significant at the 0.05 level. a

NS = not significant.

Table 4. Free amino acids in seed of six peanut lines (µmoles per gram).

	P	EP		,	ASP			THR			SER		G	LU			P RO			GLY			A	LA		
- leal thy	52.	36	a	37	. 33	a		12 .89		45 14	(Hi 1.4	ghl 5 a	y To 544	ler .85	an t a		7.81	a	e	. 84	ь		52	.88	a	
PMV-M2	53.				. 79		19	.61	a				483			4	5.34	a		.88				. 29		
PMV-N	53.4	88	a	39	.63	a		.15					430				0.72	a	15	.26	a		57	. 70	a	
Healthy	50.	01	a	58	. 30	a		[2 .14					y To 464				6.34	a	14	.49	ь		77	.18	a	
PMV-M2	58.				.29			. 31					501			5	9.94	a		.97				. 35		
PMV-N ²	53.	08	a	49	.73	at	25	. 38	a	10	6.4	7 b	414	.40	Ь	6	4.90) a	17	.26	ia		62	.40	b	
Healthy	40.	74	a	31	. 75	a	PI 20	261 .92	973 a	(M	ode	rat 5 a	ely 436	To1	era a	nt)	1.65	at	, q	. 34	l h		52	.76	ь	
PMV-M ₂	39.				. 79			.51		10	3.3	2 a	386	.54	a		7.53			.74			50	.89	ь	
PMV-N ²	43.	16	a	40	.67	a	21	.40	a	11	9.2	2 a	461	.99	a	4	3.52	a	12	. 76	i a		62	. 86	a	
Healthy	60.	63		88	. 80	a		261 .31					ely 804			nt)	2.67	ь	15	.20	۱.		70	.10	ь	
PMV-M2	66.				.84			.00					886			5	1.86	Б		1.71				.54		
PMV-N	57.							.57		18	3.1	Ba	801	. 35	a	6	9.98	a		.16			94	.14	a	
Healthy	45.	••		117	01			F1	oru	nne	r (Sus	cept 506	ibl	e)		7.05		10	.68			70	.49		
PMV-M ₂	40.						43	05	ah	12	6.9 69	5 H	478	.04	a		4.23).19				. 49		
PMV-N ²	55.				. 26			. 06					493				4.02			.77				.85		
Healthy	66.4	••		07	.57			.07					ptib										~~	~		
PMV-M ₂	72.0				. 99			.0/ .97					605 590				1.72 9.00		16	.35	2 6			.21 .84		
PMV-N	53.				.47			.58					499				6.02			.54				.14		
Treatment	V	AL		I	LE			LEU					_	HE			HIS									
		_									TYR		P						LYS	; 	N	Η4			ARG	
	100								PI	261	945		ighl	ут		ran	t)					·				
Healthy PMV-Ma	123.	28	ab	12	. 80	a	3	.57	PI a	261 12	945	a	ighl 173	у Т .02	ab	ran 8	t) .36	a	2.99	a	44	.49	a	37	2.99	,
PMV-M ₂	123. 149. 79.	78	а	11	. 80 . 55 . 66	а	- 4		PI a a	261 12 11	945	a	ighl 173 268	ут	ab a	ran 8 13	t)	a i	2.99	a	44 42	.49	jа	- 3		2
PMV-M2 PMV-N	149. 79.	78 88	a b	11 11	.55 .66	a	4	.57 .10 .69	PI a a PI	261 12 11 8 261	945 .12 .85 .95 946	a a a (H	igh1 173 268 127 igh1	y T .02 .89 .97 y T	ab a b ole	ran 8 13 8 ran	t) .36 .06 .36 t)	a i a i	2.99 2.01 2.31	a a a	44 42 55	.49 .06 .16	5 a 5 a	3 4	2.99 0.35 1.26	5
PMV-M ₂ PMV-N Healthy	149. 79.	78 88 55	a b ab	11 11	.55 .66 .53	a a a	4 4 3	.57 .10 .69 .36	PI a a PI a	261 12 11 8 261 14	945 .12 .85 .95 946	a a (H a	igh1 173 268 127 igh1 115	y T .02 .89 .97 y T .89	ab a b ole a	ran 8 13 8 ran 10	t) .36 .36 .36 t) .66	a i a i	2.99 2.01 2.31 5.98	a a a a	44 42 55 50	.49 .06 .16	ja ja 2a	3 4 6	2.99	5
PMV-M2 PMV-N Healthy PMV-M2	149. 79.	78 88 55 19	a b ab a	11 11 11 10	.55 .66	a a a a	4 4 3 2	.57 .10 .69	PI a a PI a a	261 12 11 8 261 14 8	945 .12 .85 .95 946	a a (H a a	igh1 173 268 127 igh1 115 132	y T .02 .89 .97 y T	ab b ole a a	ran 13 13 ran 10 9	t) .36 .06 .36 t)	a i a i a i	2.99 2.01 2.31 5.98 2.42	a a a b	44 42 55 50 45	.49 .06 .16	5 a 5 a 2 a 3 a	3 4 6 3	2.99 0.35 1.26	955
PMV-M ₂ PMV-N Healthy PMV-M ₂ PMV-N	149. 79.1 107.1 134. 93.	78 88 55 19 21	a b ab a b	11 11 10 15	.55 .66 .53 .83 .15	a a a a	4 4 3 2 7	.57 .10 .69 .36 .71 .29 PI	PI a a PI a a 26	261 12 11 261 14 8 8 8	945 .12 .85 .95 .946 .66 .53 .71 3 (a a (H a a Mod	igh1 173 268 127 igh1 115 132 108 erat	y T .02 .89 .97 y T .89 .71 .89 ely	ab ole a a To	ran 13 13 13 10 10 10	t) .36 .36 t) .66 .19 .51 ant)	aiai	2.99 2.01 2.31 5.98 2.42 5.22	a a a b a	44 42 55 50 45 38	.49 .06 .16 .92 .18	5 a 5 a 2 a 3 a 4 a	3 4 6 3 5	2.99 0.35 1.26 3.15 1.56 7.95	
PMV-M2 PMV-N Healthy PMV-M2 PMV-N	149. 79.1 107.1 134. 93.	78 88 55 19 21 44	a ab ab b a	11 11 10 15	.55 .66 .53 .83 .15	a aaa a	4 4 3 2 7 4	.57 .10 .69 .36 .71 .29 PI .44	PI a a PI a a 26 a	261 12 11 261 14 8 8 197 8	945 .12 .85 .95 .946 .66 .53 .71 3.(.32	a a (H a a Mod	igh1 173 268 127 igh1 115 132 108 erat	y T .02 .89 .97 y T .89 .71 .89 ely .76	ab ole a a To	ran 13 13 10 10 10	t) .36 .36 t) .66 .19 .51 ant) .91	aiaiai	2.99 2.01 2.31 5.98 2.42 5.22 4.87	a a a b a a a a a a a a a a a a a a a a	44 55 50 45 38 70	.49 .06 .16 .92 .34	5 a 5 a 2 a 3 a 4 a 7 a	3 4 6 3 5 3	2.99 0.35 1.26 3.15 7.95 3.38	955 555 8
PMV-M2 PMV-N Healthy PMV-M2 PMV-N Healthy PMV-M2	149. 79.1 107.1 134. 93.	78 88 55 19 21 44 08	a b ab a b a a	11 11 10 15 13 12	.55 .66 .53 .83 .15	a a a a a a a	4 4 3 2 7 4 4	.57 .10 .69 .36 .71 .29 PI	PI a a PI a a 26 a a	261 12 11 8 261 14 8 8 8 197 8 7	945 .12 .85 .95 .946 .66 .53 .71 3 (a a (H a a Mod a	igh1 173 268 127 igh1 115 132 108 erat 109 118	y T .02 .89 .97 y T .89 .71 .89 ely	ab b ole a a To a a	ran 13 13 13 10 9 10 10 10 10 8 8	t) .36 .36 t) .66 .19 .51 ant)		2.99 2.01 2.31 5.98 2.42 5.22 4.87 3.50	a a a b a a a a a a a a a a a a a a a a	44 42 55 50 45 38 70 83	.49 .06 .16 .92 .18 .34	5 a 2 a 3 a 7 a 5 a	3 4 6 3 5 3 3	2.99 0.35 1.26 3.15 1.56 7.95	355 555 37
PMV-M2 PMV-N PMV-M2 PMV-M2 PMV-N Healthy PMV-M2 PMV-M2 PMV-N	149. 79. 107. 134. 93. 103. 93. 104.	78 88 55 19 21 44 08 77	a b a b a a a a	11 11 10 15 13 12 15	.55 .66 .53 .83 .15 .51 .24	a a a a a a a a a a	4 3 2 7 4 4 6	.57 .10 .69 .36 .71 .29 PI .44 .88 .54 PI	PI a a PI a a 26 a a 26	261 12 11 8 261 14 8 8 7 7 7 7 7	945 .12 .85 .95 946 .53 .71 3 (.32 .77 .55	a a (H a a a Mod a a Mod	ighl 173 268 127 132 132 108 erat 109 118 101 erat	y T .02 .89 .97 y T .89 .71 .89 .76 .76 .08 ely	ab ole a a a To a a a To a a To	ran 13 13 13 10 9 10 10 10 10 10 10 10	t) .36 .06 .36 t) .51 .91 .71 .90 ant)	a a a a a a	2.99 2.01 2.31 5.98 2.42 5.22 4.87 3.50 2.14	a a a b a a a a a a a a a a a a a a a a	44 42 55 45 38 70 83 58	.49 .06 .16 .92 .18 .34	5 a 5 a 2 a 3 a 3 a 7 a 5 a 0 a	34 63 5 33 3	2.99 0.35 1.26 3.15 7.95 3.38 5.37 3.63	000 000 073
PMV-M2 PMV-N PMV-M2 PMV-N Healthy PMV-M2 PMV-M2 PMV-M2 PMV-N Healthy	149. 79. 107. 134. 93. 103. 93. 104. 218.	78 88 55 19 21 44 08 77 50	a b ab a b a a a a a	11 11 10 15 13 12 15 18	.55 .66 .53 .83 .15 .15 .51 .24	a a a a a a a a a a	4 3 2 7 4 4 6 9	.57 .10 .69 .36 .71 .29 PI .44 .88 .54 PI .09	PI a a PI a a a 26 a a 26 a	261 12 11 8 261 14 8 8 7 7 7 7 7 7 198 14	945 .12 .85 .95 946 .53 .71 3 (.32 .77 .55 0 (.62	a a (H a a Mod a a Mod ab	ighl 173 268 127 132 108 erat 109 118 109 118 109	y T .02 .89 .97 y T .89 .71 .89 .76 .76 .76 .08 ely .11	ab ole a a a To a a b	ran 13 8 13 8 13 8 9 10 10 10 10 10 10 10 10 10 10 10 10 10	t) .36 .36 t) .51 .91 .71 .90 ant] .38	a a a a a a b	2.99 2.01 2.31 5.98 2.42 5.22 4.87 3.50 2.14	a a a a a a a a a a a a a a a a a a a	44 42 55 50 45 38 70 83 58 53	.49 .06 .16 .92 .18 .34 .77 .96 .60	5 a 5 a 2 a 3 a 7 a 5 a 7 a 3 a 7 a 3 a 9 a	34 635 333 3	2.99 0.35 1.26 3.15 7.95 3.38 5.37 3.63 6.17	955 555 373 7
PMV-M2 PMV-N PMV-M2 PMV-N PMV-N Healthy PMV-M2 PMV-M2 PMV-M2	149. 79. 107. 134. 93. 103. 93. 104.	78 88 55 19 21 44 08 77 50 66	a b ab a b a a a a a	11 11 10 15 13 12 15 18 20	.55 .66 .53 .83 .15 .51 .24	a a a a a a a a a a a	4 3 2 7 4 4 6 9 8	.57 .10 .69 .36 .71 .29 PI .44 .88 .54 PI	PI a a PI a a 26 a a a 26 a a	261 12 11 8 261 14 8 8 7 7 7 7 198 14 16	945 .12 .85 .95 946 .53 .71 3 (.32 .77 .55	a a (H a a Mod a a Mod a a	ighl 173 268 127 127 132 108 erat 109 118 109 118 101 101 165 238	y T .02 .89 .97 y T .89 .71 .89 .76 .76 .08 ely	ab ole a a a a a b a a b a	ran 13 8 13 8 13 8 9 10 10 10 10 10 10 10 10 10 10 10 10 10	t) .36 .06 .36 t) .51 .91 .71 .90 ant)	a a a a a a a a a a a a a a a a a a a	2.99 2.01 2.31 5.98 2.42 5.22 4.87 3.50 2.14 5.54 8.15	a a a b a a a a a a a a a a a a a a a a	44 55 50 45 38 70 83 58 53 63	.49 .06 .16 .92 .18 .34 .77 .96 .60	5 a 2 a 3 a 3 a 7 a a 3 a 3 a 3 a	3 4 6 3 5 3 3 3 2 5	2.99 0.35 1.26 3.15 7.95 3.38 5.37 3.63	000000000000000000000000000000000000000
PMV-M2 PMV-N PMV-N PMV-M2 PMV-N Healthy PMV-N Healthy PMV-N Healthy PMV-N	149. 79.1 107.1 134. 93. 103. 93. 104. 218. 237.1 203.	78 88 55 19 21 44 08 77 50 66 90	a b ab a a a a a a a	11 11 10 15 13 12 15 18 20 20	.55 .66 .53 .83 .15 .15 .51 .24 .96 .12 .35	a a a a a a a a a a a a	4 3 2 7 4 4 6 9 8 10	.57 .10 .69 .36 .71 .29 PI .44 .88 .54 PI .09 .89 .34	PI a a PI a a 26 a a a a	261 12 11 8 261 14 8 8 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	945 .12 .85 .95 946 .53 .71 3 (2 .53 .77 .55 0 (.62 .57 .78 oru	a a (H a a a Mod a a b nne	igh1 173 268 127 igh1 115 132 108 erat 109 118 119 118 119 118 119 118 119 118 119 118 119 118 119 118 119 118 119 118 119 118 119 118 119 118 119 119	y T .02 .89 .97 y T .89 ely .76 .76 .76 .08 ely .11 .95 .10 usc	ab ole a a To a b b ept	ran 13 8 13 8 13 8 13 10 9 10 9 10 9 10 9 10 9 10 9 10 9 1	t) .36 .06 .36 t) .51 .91 .71 .90 ant) .34 .64 e)	a a a a a a b a a	2.99 2.01 2.31 5.98 2.42 5.22 4.87 3.50 2.14 5.54 8.15 8.85	a a a a a a a a a a a a a a a a a a a	44 42 55 50 45 38 70 83 58 53 63 53	.49 .06 .16 .92 .18 .34 .77 .96 .60 .69 .93 .50	5 a 2 a 3	34 635 333 256	2.99 0.35 1.20 3.15 7.95 3.38 5.37 3.63 6.17 6.25 6.87	35555737757
PMV-M2 PMV-N Healthy PMV-M2 PMV-M2 PMV-N PMV-N PMV-N PMV-N PMV-N PMV-N Healthy Healthy	149. 79. 107. 134. 93. 103. 93. 104. 218. 203. 97.	78 88 55 19 21 44 08 77 50 66 90 26	a b ab aaa aaa aaa	11 11 10 15 13 12 15 18 20 20 7	.55 .66 .53 .83 .15 .15 .24 .96 .12 .35	aaaaaaaa b	4 3 22 7 4 4 6 9 8 8 10 6	.57 .10 .69 .36 .71 .29 PI .44 .88 .54 PI .09 .34 .34	PI a a PI a a 26 a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a 26 a a a a	261 12 11 8 261 14 8 8 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	945 .12 .85 .95 .95 .66 .53 .71 .32 .77 .55 .0 (.62 .57 .78 .78 .79	a a (H a a Mod a a Mod a b nne a	igh1 173 268 127 191 132 108 erat 109 118 101 erat 109 118 101 101 101 111 101 238 238 111 111 113 238 238 238 111 111 238 238 238 238 238 238 238 238 238 238	y T .02 .89 .97 y T .89 .71 .89 .76 .76 .08 ely .11 .95 .10 usc .31	ab ole a a a a b b a b a b a b a b a b a b a	ran 13 8 13 8 13 8 13 10 9 10 9 10 9 10 9 10 9 10 9 10 9 1	t) .36 .06 .36 .19 .51 .71 .90 .34 .64 e) .30	a a a a a a a a a a a a a a a a a a a	2.99 2.01 2.31 5.98 2.42 5.22 4.87 3.50 2.14 5.54 8.15 8.85 5.11	a a a a a a a a a a a a a a a a a a a	444255 504538 708358 536353 64	.49 .06 .16 .92 .18 .34 .77 .96 .60 .93 .50	5 a 2 a 3 a 7 a 3	34 635 333 256 6	2.99 0.35 1.20 3.15 7.95 3.38 5.37 3.63 6.17 6.25 5.87 6.25 5.87	955 555 373 757
PMV-M2 PMV-N Healthy PMV-M2 PMV-M2 PMV-N Healthy PMV-M2 PMV-M2 PMV-M2 PMV-M2 PMV-M2 PMV-M2	149. 79.1 107.1 134. 93. 103. 93. 104. 218. 237.1 203.	78 88 55 19 21 44 08 77 50 66 90 26 60	a b a b a a a a a a a a a a	11 11 10 15 13 12 15 18 20 20 7 8	.55 .66 .53 .83 .15 .15 .51 .24 .96 .12 .35	a a a a a a a a a a b i	4 3 2 7 4 4 6 9 8 10 5 7	.57 .10 .69 .36 .71 .29 PI .44 .88 .54 PI .09 .89 .34	PI a a PI a a 26 a a 26 a a a 26 a a a a a	261 12 11 8 261 14 8 8 7 7 7 198 14 16 12 12 5 5	945 .12 .85 .95 946 .53 .71 3 (2 .53 .77 .55 0 (.62 .57 .78 oru	a a (H a a Mod a a Mod a b n n a a	ighl 173 268 268 127 127 132 108 erat 109 118 101 101 101 101 101 101 101 101 101	y T .02 .89 .97 y T .89 ely .76 .76 .76 .08 ely .11 .95 .10 usc	ab b cole a a a b b a b a b a b a b a b a b a b	ran 13 8 13 13 13 13 10 9 10 9 10 10 11 8 8 13 11 11 6 7 7	t) .36 .06 .36 t) .51 .91 .71 .90 ant) .34 .64 e)	a a a a a a a b a a b b b b b b b b b b	2.99 2.01 2.31 5.98 2.42 5.22 4.87 5.54 4.87 5.54 8.85 8.85 8.85 5.11	aaa abba aaa baa baa baa	44255 504538 7083858 533633 536441	.49 .06 .16 .92 .18 .34 .77 .96 .69 .93 .50 .29 .19	5 a 23 a 34 a 35 a 36 a 37 a 38 a 39 a 39 a	34 635 333 256 68	2.99 0.35 1.26 3.15 5.37 3.38 5.37 3.63 6.17 6.25 6.87 2.59	
PMV-M2 PMV-N Healthy PMV-M2 PMV-M2 PMV-M2 PMV-M2 PMV-M2 PMV-M2 PMV-M2 PMV-M2 PMV-M2 PMV-M2 PMV-N	149. 79.3 107.1 134. 93. 103. 93. 104. 218. 203. 97. 93. 93. 92.	78 88 55 19 21 44 08 77 50 66 90 26 60 29	a b a b a a a a a a a a a a a a	11 11 10 15 13 12 15 18 20 20 7 8 10	.55 .66 .53 .83 .15 .15 .24 .96 .12 .35 .29 .35 .12	aaaaaaa bata	4 3 2 7 4 4 6 9 8 8 10 5 7 8	.57 .10 .69 .36 .71 .29 PI .44 .88 .54 .09 .89 .34 .78 .55 .79	PI a a PI a a a 26 a a a a a a a a a a a a a a a	261 12 11 14 8 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	945 .12 .85 .95 946 .53 .71 3 (1 .53 .73 .55 0 (2 .57 .78 0 (1 .62 .57 .78 0 (1 .62 .57 .78 0 (1 .62 .57 .57 .57 .57 .57 .57 .57 .57 .57 .57	a a (H a a a Mod a a b n n a a a rr	ighl 173 268 268 127 127 132 108 erat 109 118 101 101 101 101 101 101 101 101 101	y T .02 .89 .97 .71 .89 ely .76 .76 .08 ely .11 .95 .10 usc .31 .00	ab ole a a To a a To b b b ept	ran 13 8 13 8 13 13 10 10 10 10 10 10 10 10 10 10 10 10 10	t) .36 .06 .19 .51 .71 .90 .38 .34 .64 e) .58 8 .88	a a a a a b a a b b a b b a b b a b b a b b b a b	2.99 2.01 2.31 5.98 2.42 5.22 4.87 3.50 2.14 5.54 8.15 8.85 5.11 7.16 8.38	a a a a a a a a a a a a a a a a a a a	44255 50458 708358 53353 6441 58	.49 .06 .16 .92 .18 .92 .92 .93 .50 .29 .19 .31	5 a 23 a 34 a 35 a 36 a 37 5 a 38 a 39 a 39 a 30 a 30 a 30 a 30 a 30 a 30 a 31 a 32 a 31 a 32 a 31 a 31 a 31 a 31 a 31 a 31 a 31 a 31	34 635 333 256 68	2.99 0.35 1.26 3.15 5.37 3.38 5.37 3.63 6.17 6.25 6.87 2.59	
PMV-M2 PMV-N Healthy PMV-M2 PMV-N Healthy PMV-N Healthy PMV-N Healthy PMV-N Healthy PMV-N Healthy Healthy	149. 79.3 107.3 134. 93. 103. 93. 104. 218.3 203. 97. 93.	78 88 55 19 21 44 08 77 50 66 90 26 60 29 52	a b a a a a a a a a a a a a a a a a a a	11 11 10 15 13 12 15 18 20 20 7 8 10 14	.55 .66 .53 .83 .15 .15 .24 .96 .12 .35	aa aaa aaa bata b	4 3 2 7 4 4 6 9 8 8 10 10 5 7 8 9 9 8 9 9 9 9 9 9	.57 .10 .69 .36 .71 .29 PI .44 .88 .54 PI .09 .89 .34 .78 .55	PI a a PI a a 26 a a 26 a a a a a b	261 12 11 8 261 14 8 7 7 7 5 198 14 16 12 5 5 5 6 8	945 .12 .85 .95 .66 .53 .71 3 ((.32 .77 .55 0 (.62 .57 .78 0 .02 .02	a a (H a a a Mod a a Mod a b n n a a a rr a	ighl 173 268 127 127 132 108 erat 109 118 101 erat 165 238 101 r (\$ 37 45 53 (\$ 139	y T .02 .89 .97 .71 .89 ely .76 .76 .08 ely .11 .95 .10 usc .31 .00	ab ole a a a To b a b b c a a c b a b c a a a c b c a a a c b c a a a c b c a a a c b c a a a c b c a a a c b c b	ran 8 13 8 13 13 13 10 10 10 10 10 10 10 11 11 11 10 10 10	t) .36 .06 .19 .51 .71 .91 .38 .34 .64 e) .30 .58 .88	a a a a a a a a a a a a a a a a a a a	2.99 2.01 2.31 5.98 2.42 5.22 4.87 3.50 2.14 5.54 8.15 8.85 5.11 7.16 8.38	a a a b a b a a b a b a a b a	44255 504538 708358 5336353 644158 57	.49 .06 .16 .92 .18 .34 .77 .96 .60 .69 .93 .50 .50 .29 .19 .31	5 a 23 a 34 a 35 a 36 a 37 5 38 a 39 a 30 a 30 a 30 a 30 a 30 a 30 a 30 a 30	34 635 333 256 68 811	2.99 0.35 1.26 3.15 5.37 3.38 5.37 3.63 6.17 6.25 6.87 2.59	955 555 373 757 917 3

Values with same letter within blocks are not significantly different at the 0.05 level according to the Waller-Duncan multiple range test.

Table 5. Results of anlaysis of variance showing mean squares and level of significance on total amino acids in seed from virus infected peanuts.

6	Sources of Variation									
Amino Acid ^a	Variety (V)	Treatment (T)	VXT							
ASP	84.413 **	9.342 **	3.850 *							
THR	2.626 **	0.171 NS	0.101 NS							
SER	12.535 **	1.545 *	0.630 NS							
GLU	181.548 **	13.579 *	7.534 *							
PRO	50.896 **	17.843 *	8.116 *							
GLY	4.159 **	1.576 *	0.726 NS							
ALA	6.660 **	0.928 *	0.256 NS							
CYS	2.357 **	0.059 NS	0.097 NS							
VAL	11.170 **	0.280 NS	0.368 NS							
MET	3.604 **	0.065 NS	0.268 *							
ILE	6.388 **	0.193 NS	0.378 NS							
LEU	28.580 **	0.750 NS	1.433 NS							
TYR	9.843 **	2.530 NS	0.933 NS							
PHE	23.208 **	3.663 NS	0.926 NS							
HIS	5.717 **	1.579 *	0.596 NS							
LYS	1.058 **	1.168 NS	0,391 NS							
	1.453 NS	1.265 NS	1.155 NS							
ARG	81.790 **	6.021 NS	6.233 NS							

a Degrees of freedom for variety, treatment, and variety by treatment are 5, 2, and 10 respectively for each amino acid. = significant at the 0.01 level.

= significant at the 0.05 level.

NS = not significant

significant differences for either treatment or variety by treatment interaction. The change in fatty acid content for each peanut line infected with two strains of PMV were variable (Table 2). The necrosis strain showed more significant changes than the mild strain for all six lines. There were almost twice as many significant changes in the fatty acids of the susceptible lines than in the tolerant or moderately tolerant lines. In general, peanuts infected with the necrosis strain showed a decrease in the percentages of stearic and oleic acids, while linoleic, eicosenoic, behenic, and lignoceric acids increased. In peanuts infected with the mild strain there was a slight increase in linoleic acid.

Free Amino Acids

The effect of the virus infection (treatment) was significant for serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, and arginine (Table 3). Variety by treatment interactions were significant for serine, glutamic acid, proline, glycine, alanine, valine, phenylalanine, histidine, lysine, and arginine.

Changes in the free amino acid content for each line infected with the two strains of PVM were observed (Table 4). Results were variable with the necrosis strain again showing the most effect. Peanuts infected with the necrosis strain tended to show increases in the levels of glycine, alanine, isoleucine, histidine, lysine, and arginine. For the mild strain there were no general trends evident. There was little correlation between degree of susceptibility to virus infection and changes in free amino acids.

Total Amino Acids

Significant differences were found for virus infection (treatment) for aspartic acid, serine, glutamic

PEANUT SCIENCE

Table 6. Total amino acids (mg per g) in seed of six peanut lines infected with two strains of PMV.

Treatment	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	NH4	ARG
lealthy	38 50 =	7 01 =	16 50	. 52 0	10 00	. 17 90	12 46	PI	261945 (Highly	Tolerant) a 13.84 a		12.04	10.70				
MV-M2 MV-N	3/.5/ a	\/.68 a	1 16.08	a 51.52 a	3 17.6/	a 17.44	a 12.17	a 5.73	a 13.34	a 5.43	a 14.33 a a 14.10 a	23 30 a	11 27	a 10 ∩ 2 a	0 30 :	. 0.5/ .	5 07 ·	27 10
≘althy MV-M2 MV-N	JO.JD 8	1.95 a	1 10.38	a 52.81 a	1 20.54	a 18.08 .	a 12.50	a 6.31 a 5.84	a 13.61 b 13.81a	b 6.10	Tolerant) a 14.67 a b 14.14 a ab 14.77 a	23.15 a	12 57 :	18 20 -	10 17 :	. 10 17 .	6 00 -	07 70
ealthy MV-Mo	41.75 a	8.17 a	17.45	a 57.22 a	23.48	a 18.84al	13.45	PI 26	1973 (Mo a 14 71	deratel	y Toleran	t) 24 70ab	12 60	10 66 5	10 51 -	10 56 -	6 74	42.00
MV-N2	41.41au	0.20 0	1 1/.220	D 30./2 2	1 22.17	a 18.91 i	a (.449).	ab 6.27 b 6.03	a 14.44a a 14.03	b 6.24 b 5.74	a 15.40 a a 15.29 a	25.62 a 24.55 b	12 0/	10 06 -	10 20 4	10 AC -	6 65 .	40 00
ealthy MV-M2 MV-N	JO.05 D	/.00 a		0 20.02 0	20.35	a 17.50 a	1 1 2 118	a 5.53	a 13.62	a 5.30	y Toleran b 14.88 a b 14.61 a a 15.01 a	24.00 a	11 66 1	16 00 -	10 07 .	. 10 61 6	C 07	
ealthy MV-M2 MV-N								a 5.25		a 4.42	tible) b 13.25 a b 13.16 a a 13.29 a							
ealthy MV-M ₂ MV-N	00.10 0	/.U/ a	17.22 6	J 43.04 D	10.00	0 10./1 /		a 5.10	a 12 57	a 5.02	ble) a 14.33 a a 13.40 b a 13.55 b	20 26 5	10 70 1	15 00 6	0 05 -	. 10	E 01	04 OF

Values with same letter within blocks are not significantly different at the 0.05 level according to the Waller-Duncan multiple range test.

acid, proline, glycine, alanine, and histidine (Table 5). Variety by treatment interactions were significant for aspartic acid, glutamic acid, proline, and methionine. Changes in total amino acid content for each line for both types of virus infection were found (Table 6). The results were quite variable with no correlation between degree of susceptibility and changes in total amino acids.

Protein Content

The pooled data of protein content revealed no significant differences for viral infection (treatment) or variety by treatment interactions.

Discussion

The chemical composition of peanut seed was altered by infection with PMV. The infection caused significant changes in 75% of the fatty acids, 59% of the free amino acids, and 39% of the total amino acids. There was no significant effect on the protein content.

Chemical changes in seed were correlated with disease severity which was evaluated two ways: (i) different virus strains and (ii) peanut lines with different degrees of susceptibility. With susceptible cultivars, PMV strain N causes a much more severe disease than strain M2 (9, 12); plant growth is stunted 35% as compared to 12% for M2, and seed yield loss is about doubled, 64% as compared to 30% (9). When compared to seed from uninoculated plants, strain N caused changes in 63% of the fatty acids as compared to 31% for M2; 38% of the free amino acids were altered by N and only 6% by M2. Although chemical changes (fatty acids, free amino acids, and total amino acids) were noted for seed from infected tolerant plants, the number of significant changes was only two-thirds as great as those observed for seed from susceptible plants.

The chemical alterations noted in this study apparently are not correlated with seed transmission of PMV which occurs at a low frequency (1, 6, 8). Significant chemical differences were observed between susceptible and tolerant peanut lines; however, the frequency of seed transmission of a mild strain of PMV was nearly the same for the two types of lines (1).

Since virus infection changes the chemical composition of peanut seed, there is need for caution when chemical analyses are used to evaluate various seed factors such as oil quality and genetic characteristics. PMV is worldwide in distribution (4, 8), and its incidence in some fields is very high (13); therefore, the virus effect could be significant on a variety of standard tests.

Literature Cited

- 1. Adams, D. B., and C. W. Kuhn. 1977. Seed transmission of peanut mottle virus in peanuts Phytopathology 67: 1126-1129.
- 2. Amer. Oil Chemists' Society. 1951. Official and Tentative Methods.
- Demski, J. W. and M. D. Jellum 1975. Single and double virus infection of soybean: Plant characteristics and chemical composition. Phytopathology 65:1154-1156.
- 4. Demski, J. W., D. H. Smith, and C. W. Kuhn. 1975. Incidence and distribution of peanut mottle virus in peanuts in the United States. Peanut Science 2:91-93.
- 5. Jones, D. Breese. 1931. Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. U. S. Dept. of Agric. Circular No. 183, 16 pp.

- Kuhn, C. W. 1965. Symptomatology, host range, and effect on yield of a seed-transmitted peanut virus. Phytopathology 55:880-884.
- Kuhn, C. W. Grover Sowell, Jr., J. H. Chalkley, and H. F. Stubbs. 1968. Screening for immunity to peanut mottle virus. Plant Dis. Reptr. 52:467-468.
- 8. Kuhn, C. W. and J. W. Demski. 1975. The relationship of peanut mottle virus to peanut production. Ga. Agric. Exp. Sta. Res. Report 213, 19 p.
- Kuhn, C. W., O. R. Paguio and D. B. Adams. 1978. Tolerance in peanuts to peanut mottle virus. Plant Dis. Reptr. 62(4):365-368.
- 10. Metcalfe, L. D., A. A. Schmitz, and J. R. Pelka. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38:514.
- Mondy, N. I. and R. L. Koch. 1978. Effect of potato virus X on enzymatic darkening and lipid content of potatoes. J. Food Sci. 43:703-705.
- 12. Paguio, O. R. and C. W. Kuhn. 1973. Strains of peanut mottle virus. Phytopathology 63:976-980.
- 13. Paguio, O. R. and C. W. Kuhn. 1974. Survey for peanut mottle virus in peanut in Georgia. Plant Dis. Rep. 58: 107-110.

- 14. Roach, D. and C. W. Gehrke. 1970. The hydrolysis of proteins. J. Chromatog. 52:393-404.
- Snedecor, George W. and William G. Cochran. 1967. Statistical Methods, Sixth Edition. Ames, Iowa: The Iowa State University Press.
- Spakman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in chromatography of amino acids. Anal. Chem. 30:1190.
- 17. Tu, J. C. and R. E. Ford. 1970. Free amino acids in soybeans infected with soybean mosaic virus, bean pod mottle virus, or both. Phytopathylogy 60:660-664.
- Waller, R. A. and D. B. Duncan. 1969, 1972. A bayes rule for the symmetric multiple comparison problem. Journal of the American Stat. Assoc. 64:1484-1499. Corrigenda 67: 253-255.
- Young, Clyde T., Timothy G. Young and John P. Cherry. 1974. The effect of roasting methods on the flavor and composition of peanut butter. Proc. of Amer. Peanut Res. and Ed. Assoc. 6(1):8-16.

Accepted July 31, 1979