The Effect of Peanut Stripe Virus Infection on Peanut Composition

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ABSTRACT

Peanuts (*Arachis hypogaea* L.), cultivar Florunner, from plants inoculated with peanut stripe virus (PStV) were evaluated for chemical composition in comparison with peanuts from uninoculated plants. At harvest, seed were collected from plants which had been mechanically inoculated with PStV at emergence, or 20, 40, or 60 days after emergence and from uninoculated plants. The seed from PStV-infected plants had increases in manganese, selenium, zinc, iron, tartaric acid, raffinose, glucose, fructose, and total carbohydrate contents as compared to seed from uninoculated plants. Sucrose was increased in seed from plants inoculated with PStV at time of emergence. There was a decrease in the concentration of potassium, magnesium, protein, and total soluble phenolics of seed from plants inoculated with PStV. There were no changes in the concentration of stachyose, inositol, phosphorus, sulfur, calcium, copper, and oil.

Key Words: *Arachis hypogaea*, L. Florunner, carbohydrates, protein, minerals, oil.

In 1982, a new seed-transmitted peanut disease was reported in Georgia in plants raised from seed imported from the People's Republic of China (5). The leaves of the infected plants show striping and mosaic symptoms. The disease was named peanut stripe virus (PStV) (4). PStV, a member of the potyvirus group, is located in peanut cotyledons and embryos. It can be transferred from infected plants by sap innoculation or by aphids and it can infect other legumes (5). In greenhouse studies, peanut (*Arachis hypogaea* L.) plants, of the cultivar Florunner, infected with PStV produced fewer and smaller peanuts than uninfected plants (6).

Peanuts are characterized by high oil and protein concentrations. In the United States, over half the production of peanuts goes into domestic food use in products such as peanut butter, salted and roasted nuts and candies (1). Peanuts are a good source of calcium, potassium, phosphorus, iron, zinc, and magnesium (3,10). Carbohydrates provide the basic sweet taste of peanuts, and have also been shown to be precursors to compounds imparting the typical flavor and aroma characteristics of roasted peanuts and peanut butter (15,17). Phenolic compounds are also involved in the flavor of the peanut (14).

Since PStV could affect peanut quality, size, and yield, this study was designed to determine the effect of PStV infection on selected nutritional and flavor components of peanuts.

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Materials and Methods

Certified Florunner peanuts (*Arachis hypogaea* L.) were grown in 1985 at Tifton, GA, on loamy sand (fine-loamy, siliceous, thermic Plinthic Paleudults) to evaluate the effects of PStV on yield and on seed quality and chemical composition. The test was planted May 2 at ca. 120 kg seed/ha in twin rows, 81 cm between rows, on 1.83 m beds. The field was treated before planting with benefin (1.25 kg AI/ha) and vernolate (2.24 kg AI/ha). Alachlor (3.36 kg AI/ha) and naptalam + dinoseb (3.36 + 1.68 kg AI/ha, resp.) were applied at cracking for weed control as recommended by the Georgia Cooperative Extension Service (13). Beginning ca. 40 days after plant emergence, all plots were sprayed with chlorothalonil (2.48 l/ha) ca. every 10-14 days for control of leafspot diseases using an air-blast sprayer.

At emergence, two row plots were covered with Saran® screen cages (1.83 m wide x 1.83 m high x 3.66 m long) of 20 x 20 mesh screen supported by a conduit frame. This screen was placed over each plot to prevent aphid transmission of the virus between plots.

The experimental design was a randomized complete block with 9 replications. Treatments were: (a) uninoculated control, and mechanical inoculation (b-e) with PStV at (b) emergence (plants with at least one tetrafoliate completely expanded), (c) 20 days, (d) 40 days, or (e) 60 days after emergence. The plants in each inoculation treatment were dusted with the abrasive 600 grit Carborundum® powder prior to inoculation to increase the efficiency of inoculation. White lupine (*Lupinus alba* L.) infected with PStV was macerated in 0.25 M phosphate buffer (pH 7.2) with a mortar and pestle just prior to inoculation. Cheesecloth was then dipped into the buffer solution containing macerated tissue and rubbed on the peanut leaflets. Plants in uninoculated plots were not treated with Carborundum® or the buffer solution.

Optimum harvest date was determined using the hull scrape method (19). All plots were inverted on September 9-10 and allowed to remain in the field for 5-7 days for drying. Peanut pods from each plot were then harvested with a KMC Plot Thrasher®, placed in labeled bags, and dried at ca. 36 C to ca. 15% moisture. Samples for chemical analyses were then shelled with a peanut grade sheller.

Nitrogen and oil contents were measured by methods recommended by AOAC (2). Mineral contents were determined by X-ray analysis using an EG & G Ortec Energy Dispersive X-ray Analyzer System (Model Ortec 6110 TEFA) with a cryogenics Si/Li detector on 1 g defatted flour pressed into a 31 mm diameter disc at 137.9 MPa. The instrument was calibrated using N.B.S. wheat flour; therefore, mineral contents of these samples are relative and not absolute. Total soluble phenolics were determined on methanol extractions of defatted flour by the method of Forrest and Bendall (8) using ferulic acid as a standard.

Carbohydrate and tartaric acid contents were determined on methanol extractions of defatted peanut flour by high pressure liquid chromatography, HPLC. Two grams of hexane defatted peanut flours were extracted with methanol:water::85:15 (v/v). Methanol was removed from the extract on a rotary evaporator, and the aqueous portion was freeze-dried. The freeze-dried fraction was weighed, dissolved in aqueous methanol and suspended on a column of Dowex 50 in the acid form. The carbohydrates and organic acids were eluted with distilled water. The eluate was concentrated to 1 mL under nitrogen and filtered through a 0.45 μ Millex-HV filter. Ribitol was added as an internal standard. The samples were stored at -20 C until analyzed.

A Waters HPLC system consisting of a 6000A pump, a U6K injector, a R401 differential refractometer, and a 441 ultraviolet detector equipped with a 214nm filter was used. Two 300mm x 7.8 mm Bio-Rad columns packed with a strong cation exchange resin in the hydrogen form (Aminex HPX-87H) were preceeded by a guard column of similar material. The column eluates were monitored with a dual pen strip chart recorder (Kipp & Zonen) and data were acquired by a computer (Model 3357 Laboratory Automation System, Hewlett Packard). The mobile phase, 0.00175N sulfuric acid, was deoxygenated under reduced pressure in an atmosphere of nitrogen. The flow rate was 0.5

mL per min and the column temperature was 34 C. Samples and standards were injected with a 801w Hamilton syringe at a 10 μL volume. The run time was 60 min. Carbohydrates were detected by refractive index and tartaric acid by ultraviolet absorption. Sample analytes were identified by comparing their retention times to those of standards. All peaks were quantitated based on their heights in relation to the height of the internal standard.

Two samples of seeds from three replications of plots for each treatment were analyzed. Data were subjected to analysis of variance and means were separated by Duncan's multiple range test using SAS (18).

Results

In seed from plants inoculated with PStV the raffinose, glucose, fructose and total carbohydrate contents increased (Table 1). No significant differences were found in stachyose or inositol contents. There was a relationship between length of infection and increased content of sucrose, glucose, and fructose. A significiant increase in sucrose content was noted only in seeds from plants inoculated at emergence. Fructose and glucose contents, however, showed the largest increase in concentration in seeds from plants inoculated 60 days after emergence.

Table 1. Effect of Inoculation of peanut plants with PStV on carbohydrate content of peanut seeds.[1]

	Seeds from Plants Inoculated with PStV at Indicated Days After Emergence					
	Uninoculated Control	60	40	20	0	SE[2]
	g/100 g flour					
Stachyose	0.23	0.25	0.24	0.26	0.27	0.01
Raffinose	0.11a	0.20b	0.17b	0.21b	0.17b	0.01
Sucrose	3.05a	2.79a	3.30ab	3.17ab	3.59b	0.18
Glucose	0.28a	1.05c	0.82bc	0.90c	0.63b	0.10
Fructose	0.21a	1.07c	0.81bc	0.91c	0.60b	0.11
Inositol	0.11	0.10	0.11	0.11	0.11	0.01
Total Carbohydrates	4.04a	5.52b	5.47b	5.73b	5.38b	0.21

[1]Mean of three replicates (2 subsamples/replication). Values for each carbohydrate with like letters are not significantly different at p ≤ 0.05 by analysis of variance.

[2]Standard error of the mean

Seed from plants inoculated with PStV 60 days after emergence had significantly lower oil content than seed from uninoculated plants (Table 2). While oil content of seed from plants inoculated at emergence and 20 and 40 days after emergence differed from that of seed from uninoculated plants, the variations were not significant. Conversely, protein (% nitrogen x 5.46) content of seed was reduced, significantly, by inoculation with PStV at all times. Tartaric acid concentration of seed from plants inoculated with PStV was variable and unordered. Total soluble phenolics, on the other hand, were affected only by the earliest inoculation with PStV. The phenolic concentration of seed was reduced, significantly, when the plants were inoculated with the PStV at emergence (0 days) compared with that of seed from uninoculated plants.

Table 2. Effect of Inoculation of peanut plants with PStV on oil, protein, tartaric acid, and total soluble phenolic contents of peanut seeds.[1]

	Seeds from Plants Inoculated with PStV at Indicated Days after Emergence					
Measurement	Uninoculated Control	60	40	20	0	SE[3]
Oil (g/100 g seed)[1]	48.8b	46.2a	47.5ab	49.8b	47.3ab	0.83
Protein (g/100 g flour)[1]	50.2b	43.2a	45.8a	45.5a	43.6a	0.17
Tartaric Acid (g/100 g flour)[2]	0.033a	0.047b	0.045ab	0.052b	0.042ab	0.004
Total Soluble Phenolics (g/100 g flour)[2]	0.181b	0.169b	0.170b	0.153b	0.068a	0.020

[1]Mean of two replicates. Values with like letters are not significantly different at p ≤ 0.05 by analysis of variance.

[2]Mean of three replicates (2 subsamples/replicate). Values for each measurement with like letters are not significantly different at p ≤ 0.05 by analysis of variance.

[3]Standard error of the mean

In comparison with seeds from uninoculated plants, the concentration of potassium showed a significant decrease in seeds from plants at all times of inoculation with PStV (Table 3). Similarly, significant reductions in magnesium content were noted in seeds from plants inoculated 0, 20, and 60 days after emergence. Calcium concentration was higher in seed from inoculated plants

Table 3. Effect of Inoculation of peanut plants with PStV on mineral contents of peanut seeds.[1]

	Seeds from Plants Inoculated with PStV at Indicated Days After Emergence					
	Uninoculated Control	60	40	20	0	SE[2]
Macroelements						
g/100 g flour						
P	0.25	0.25	0.25	0.25	0.25	0.007
S	0.28	0.28	0.29	0.28	0.28	0.006
K	1.50b	1.39a	1.35a	1.35a	1.37a	0.02
Ca	0.36a	0.41a	0.44b	0.40a	0.39a	0.02
Mg	0.83c	0.78b	0.79bc	0.77b	0.73a	0.01
Microelements						
ppm						
Mn	15.15a	20.39b	19.35b	20.57b	20.13b	0.65
Fe	18.67a	22.08b	22.05b	22.04b	21.47ab	0.94
Cu	5.15a	5.96b	5.46a	5.34a	5.63ab	0.26
Zn	39.21a	51.01b	43.13c	47.05d	46.48d	0.93
Se	0.79a	0.90bc	0.83a	0.89b	0.94c	0.01

[1]Mean of two replicates. Values with like letters are not significantly different at p ≤ 0.05 by analysis of variance.

[2]Standard error of the mean

as compared with seed from uninoculated plants, but the increase was significant only in seed from plants inoculated 40 days after emergence. No significant differences were noted among treatments in the concentration of phosphorus or sulfur.

The microelements manganese, zinc, and selenium were significantly increased in concentration in seed from plants at all times of inoculation as compared with seed from uninoculated plants. Iron content was also increased after inoculation PStV, but increases were significant only after inoculation at 20, 40, and 60 days after emergence. Concentration of copper was increased significantly over the untreated control only in seed from plants inoculated 60 days after emergence.

Discussion

Glucose and fructose are known flavor and aroma precursors in roasted peanut products (15,17). The increased concentration of glucose and fructose was highest in seeds from plants inoculated later in the growing season, but this increase was not at the expense of sucrose. The PStV may influence the translocation of these sugars from the plant to the seed. A reduction in carbohydrates in potatoes infected with potato leaf roll virus can be accompanied by an accumulation of carbohydrates in the leaves (16). An increase in glucose and fructose content in seeds from plants infected with PStV could lead to changes in the flavor quality of roasted peanuts and other peanut products. The oligosaccharide, raffinose, is responsible for flatulence caused by legumes(11). The level of raffinose was increased substantially in seed from PStV infected plants.

High contents of phenolic compounds are associated with a bitter taste in oilseeds (15). The concentration of soluble phenolics was decreased, significantly, in the seeds from plants inoculated at time of emergence. Since slight, although not significant, decreases were noted in seeds from plants inoculated at all other times, PStV infection should not impose a bitter flavor to roasted peanuts or peanut products.

The quantity of oil found in the peanut seed from inoculated plants was reduced compared with the oil content of seed from uninoculated plants. The reduction was only significant in seed from plants inoculated 60 days after emergence. The nutritive value of peanuts is dependent in part on their protein content. PStV caused a significant decrease in the protein concentration in seeds from inoculated plants as compared to that in seeds from uninoculated plants. The virus may be inhibiting translocation of amino acids to the peanut seed or could be using the amino acids in their metabolic processes.

The calcium, magnesium, and potassium contents reported here are higher than those reported in other studies (7,9,10). However, the other studies were done on whole seed rather than defatted flour and atomic absorption spectrophotometry was used rather than x-ray analysis. This could account for the differences. Also, in this study, it is more pertinent to compare mineral contents in seed from inoculated plants with those in seed from uninoculated plants than it is to have absolute values. Peanuts are considered a good source of potassium and magnesium (3,10). The decrease in magnesium and potassium content in peanuts from inoculated plants may indicate an adverse effect of PStV on the nutritive value of the peanut.

In field studies in 1985 and 1986, PStV infection initiated by mechanical inoculation at emergence and 20, 40, and 60 days after plant emergence did not significantly influence yield or grade of Florunner peanuts (13). However, evaluation of seeds from that study, as reported here, suggest that PStV infection could decrease the nutritive value of peanuts and could change the flavor of peanuts and peanut products.

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