Single and Double Virus Infection of Soybean: Plant Characteristics and Chemical Composition

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Accepted for publication 15 May 1975.

ABSTRACT

Soybean *Glycine max* 'Hampton 266A' and 'Jackson' were inoculated singly and in double combinations with peanut mottle virus, cowpea chlorotic mottle virus, soybean mosaic virus, and tobacco ringspot virus. The effects of these viruses (singly and in combinations) on plant characteristics and chemical composition of soybean seed were evaluated. Yield and plant height were significantly reduced by all viruses and virus combinations, whereas other plant characteristics varied. In general, all single and double virus combinations caused an increase in seed protein content and a decrease in seed oil content of each cultivar. The percentage of the fatty acids linoleic and linolenic decreased while stearic and oleic increased in the oil of seed from virus infected plants. Virus infection had variable effect on palmitic acid levels. With double infections, all effects were additive or less than additive, and no synergism was observed.

Phytopathology 65:1154-1156

Additional key words: seed yield, plant height, protein content, oil content, fatty acid composition of oil.

Many viruses infect soybean [*Glycine max* (L.) Merrill] (1) and, depending on geographical location, the combination or virus 'mix' is quite variable. In Georgia, the four unrelated viruses naturally infecting soybean are peanut mottle virus (PMV) (9), cowpea chlorotic mottle virus (CCMV) (6), tobacco ringspot virus (TRSV) (3), and soybean mosaic virus (SMV) (2). This particular virus mix in soybean appears unique to the Southeast.

Unrelated viruses can infect and multiply within the same plant and even within the same cell (4, 10, 11). Soybean plants which were doubly infected with SMV and bean pod mottle virus were reported to give synergistic reactions for yield, seed mottling, seed size, and seed quality (10, 13) and for some free amino acids (15). In cowpea, double infection with CCMV and southern bean mosaic virus caused a synergistic reduction in weight, height, and yield (8).

The purpose of this study was to determine the effect of a single virus infection and the interaction between two viruses in soybean using the four viruses common in the southeastern United States.

MATERIALS AND METHODS.—Virus cultures were maintained in *Pisum sativum* L. 'Little Marvel', *Vigna sinensis* L. 'Early Ramshorn', *G. max* 'Bragg', and *Phaseolus vulgaris* L. 'Topcrop' for PMV, CCMV, SMV, and TRSV, respectively. The PMV isolate was the mild strain (M-2) which is common in Georgia peanut and soybean (12), but originally isolated from peanut. The CCMV, SMV, and TRSV isolates were obtained from soybean. Mechanical inoculations were made with infected leaves ground in 0.05 M phosphate buffer (pH 7.5) containing 0.01 M $NaHSO_3$ and 1% Celite for PMV, or 0.02 phosphate buffer (pH 7.2) containing 1% Celite for CCMV, SMV, and TRSV.

Six field tests were performed using the soybean cultivars Hampton 266A and Jackson. The design for each test was a split Latin square with four replications containing two single virus treatments, a double virus treatment, and a control. Each treatment consisted of a 4.9-m row with approximately 100 plants per row. This study had a total of 192 treatments which included all possible single and double virus combinations. All plants within a virus treatment were mechanically inoculated in the 1st true leaf stage after previously being dusted with 22-μm (600-grit) silicon carbide powder.

Plant characteristics.—The plant characters were rated as recommended by the U. S. Regional Soybean Laboratory at Urbana, Illinois. Height was measured to the nearest cm at the time of maturity. Maturity was rated in days earlier (or later) when 95% of the pods were ripe using the Jackson controls as the reference. Lodging was rated on a 1-5 scale in which 1 represents almost all plants erect and 5 almost all plants lodged. Leaf adherence was rated on a scale of 1-5 where 1 was all leaves abscissed and 5 all leaves attached. Seed quality (degree of wrinkling, defective seed coat, and moldy or rotten seed) was rated on a 1-5 scale where 1 was very good and 5 very poor. Seed weight was determined on 100 randomly selected seeds.

Plants of each 4.9-m treatment were hand harvested, placed in burlap bags in a dryer, and then passed through a hand thrasher. The seed were passed through a Clipper seed cleaner and dried to a uniform moisture content. Yields were recorded in kilograms per hectare (ha).

Chemical characteristics.—Oil and protein contents were determined on pooled seed samples of each treatment by R. L. Cooper, U. S. Regional Soybean Laboratory, Urbana, Illinois.

Seed samples (25 g) for fatty acid analyses were ground in a small laboratory mill with a 2.14-mm (10-mesh) screen. Oil was obtained by overnight extraction of 0.3 g in a 1:1 (v/v) mixture (2 ml each) of petroleum ether and absolute methanol. Handling of oil samples after methylation in preparation for gas-liquid chromatography analyses was as previously described (7). Analyses were made with a Varian Aerograph Model 1200-2 gas chromatograph (flame ionization detector) and peak areas were measured by an Infotronics Model CRS-208 digital integrator. The values reported are percentages of total fatty acids. Only the five major fatty acid components of oil (palmitic, stearic, oleic, linoleic, and linolenic) were measured. Three samples from each treatment were analyzed, and the mean was used for comparison.

RESULTS.—All virus inoculated treatments had between 82 and 100% of the plants infected. Although some natural virus spread occurred, no control treatment

had over 4% infection and all controls were treated as virus free.

Plant characteristics.—Plant height was significantly reduced by virus infection (except PMV) in both cultivars (Table 1). The average reduction by single infection was 9, 19, 25, and 35% for PMV, CCMV, SMV, and TRSV, respectively. Height reduction of doubly infected plants was 20 (PMV-CCMV), 24 (PMV-SMV), 36 (PMV-TRSV), 38 (CCMV-SMV), 38 (CCMV-TRSV), and 57% (SMV-TRSV).

The average maturity date was delayed by infection with SMV or TRSV. Maturity of Hampton 266A was significantly delayed by SMV or TRSV in three tests. In one of three tests, CCMV and TRSV significantly delayed maturity of Hampton 266A and Jackson, respectively. PMV did not affect the maturity of either cultivar.

In other plant characters, leaf adherence was significantly delayed in Hampton 266A when infected with SMV or TRSV but was not delayed in Jackson. PMV and CCMV had no effect on leaf adherence of either cultivar. Lodging was significant only for SMV infected plants in one of three tests. Seed quality of each cultivar was reduced by SMV or TRSV in two of three tests. Weight per 100 seed was altered by virus infection, but not always in the same direction (Table 1). In all three tests, the seed from plants infected with TRSV were larger while in all other tests, the seed from plants infected with PMV, CCMV, or SMV were smaller.

Yield of both cultivars was significantly reduced by single or double combination virus infection (Table 1) with the exception that the loss with PMV was not significant in one test. The average yield loss was 18, 31, 66, and 76% for single infection by PMV, CCMV, SMV, and TRSV, respectively. For doubly infected plants, the yield loss was 46, 78, 80, 82, 87, and 98% for PMV-CCMV, PMV-SMV, PMV-TRSV, CCMV-TRSV, CCMV-SMV, and SMV-TRSV, respectively.

Chemical characteristics.—Seed protein content was increased whereas oil content was decreased by virus infection (Fig. 1). Generally PMV and CCMV had the

TABLE 1. Influence of peanut mottle virus (PMV), cowpea chlorotic mottle virus (CCMV), soybean mosaic virus (SMV), and tobacco ringspot virus (TRSV), singly and in double combination on some characters of soybean

	Plant Characters			Fatty Acid Composition				
Treatment	Yield (kg/ha)	Plant Height (cm)	Weight 100 seed (g)	Linoleic (%)	Oleic (%)	Palmitic (%)	Linolenic (%)	Stearic (%)
Test A								
Control	1633 a[x,y]	117 a	14.9 a	56.0 a[z]	19.8 c	11.9 a	7.2 a	4.7 c
CCMV	1062 b	97 b	13.9 b	55.5 ab	20.6 c	11.7 ab	6.8 ab	5.0 bc
SMV	535 c	91 c	14.6 a	54.3 b	22.1 b	11.5 ab	6.4 bc	5.3 ab
CCMV-SMV	215 d	74 d	12.8 c	53.0 c	23.3 a	11.3 b	6.2 c	5.6 a
Test B								
Control	1603 a	115 a	14.2 b	55.6 a	20.0 b	11.9 ab	7.6 a	4.5 b
SMV	689 b	84 b	13.4 b	53.5 b	22.1 a	11.6 b	7.1 ab	5.2 a
TRSV	360 c	73 c	15.3 a	54.0 b	22.0 a	11.5 b	6.8 b	5.3 a
SMV-TRSV	33 d	49 d	13.6 b	53.0 b	22.8 a	12.1 a	7.0 b	5.1 a
Test C								
Control	1868 a	103 a	15.4 b	55.2 a	20.7 b	11.8 a	7.4 a	4.5 b
CCMV	1200 b	83 b	14.3 c	54.5 a	21.7 b	12.0 a	6.7 b	4.8 b
TRSV	397 c	71 c	16.9 a	52.3 b	23.9 a	11.8 a	6.2 c	5.3 a
CCMV-TRSV	289 c	64 c	16.3 ab	51.3 b	24.6 a	11.9 a	6.2 c	5.5 a
Test D								
Control	1454 a	115 a	14.5 a	55.6 a	19.9 b	12.1 a	7.2 a	4.7 b
PMV	1170 b	111 a	13.4 b	55.4 a	20.2 b	11.9 a	7.2 a	4.7 b
SMV	451 c	88 b	13.6 b	53.1 b	22.7 a	11.8 a	6.6 b	5.4 a
PMV-SMV	363 c	89 b	13.3 b	52.6 b	23.7 a	11.4 a	6.3 b	5.5 a
Test E								
Control	1918 a	117 a	14.6 b	55.5 a	20.3 c	12.1 a	7.2 ab	4.6 c
PMV	1465 b	110 a	13.9 b	55.0 a	20.4 c	12.0 a	7.4 a	4.7 c
TRSV	423 c	74 b	16.3 a	53.5 b	22.1 b	11.9 a	6.8 bc	5.1 b
PMV-TRSV	333 c	74 b	16.3 a	52.3 b	23.0 a	11.8 a	6.5 c	5.4 a
Test F								
Control	1679 a	108 a	15.4 a	55.1 a	21.1 a	11.8 a	7.4 a	4.3 c
PMV	1438 ab	99 a	15.2 a	54.7 a	21.5 a	11.9 a	7.1 a	4.4 c
CCMV	1153 bc	84 b	13.6 b	54.7 a	21.4 a	11.5 a	7.3 a	4.6 b
PMV-CCMV	897 c	84 b	12.7 c	54.2 a	21.9 a	11.5 a	7.0 a	4.8 a

[x]Data are an average of cultivars Hampton 266A and Jackson (four replications of each).
[y]Values with a common letter within blocks are not significantly different, $P = 0.05$, according to Duncan's multiple range test.
[z]Data on fatty acids are reported as percentages of total fatty acids.

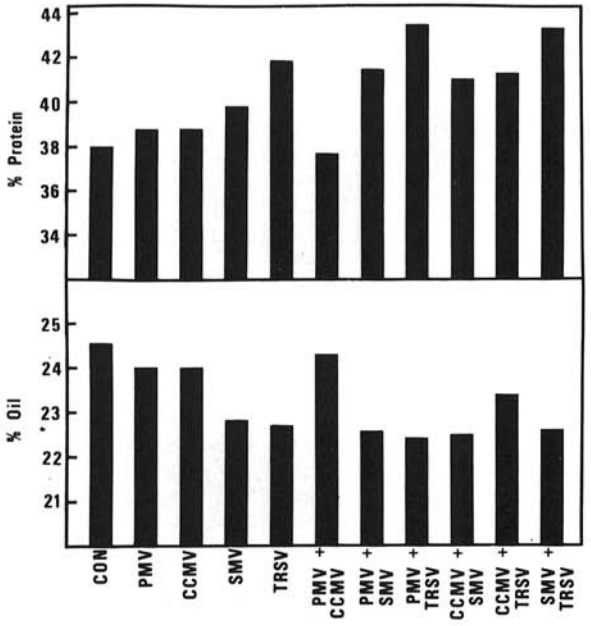

Fig. 1. Changes in oil and protein content of soybean seed as influenced by single and double virus infection. Data represents the mean of cultivars Hampton 266A and Jackson.

least effect and SMV and TRSV had the greatest effect on seed protein and oil.

The percentage composition of individual fatty acids of seed oil from each cultivar was influenced by single and double virus infection (Table 1). Linoleic and linolenic acids were reduced, whereas oleic and stearic acids were increased. Changes in palmitic acid were variable, but palmitic was generally reduced in Hampton 266A by virus infection with little or no change in Jackson. Greater changes in all fatty acids occurred in Hampton 266A than in Jackson, but the direction of change was the same for both cultivars. The order of greatest to least percentage changes was stearic, oleic, linolenic, linoleic, and palmitic.

DISCUSSION.—Generally, the viruses or virus combinations causing the most severe symptoms caused the greatest changes in the various plant and chemical characteristics measured in this study. PMV caused the least change in growth habit and likewise had the least effect on yield, seed quality, oil content, protein content, and fatty acid composition of oil. SMV and TRSV caused more leaf distortion and stunting which resulted in greater yield loss, lower seed quality, and significant chemical composition change.

All doubly infected plants showed effects that were additive or less than additive and no synergism was observed.

Directional changes in the chemical composition were the same for the four viruses tested and is in agreement with previous work with TRSV and CCMV (3, 5). Also, it is interesting to note that southern green stink bug *Nezara viridula* (L.) damage of soybean seed (14) affects the fatty acid composition of oil similar to that of virus infection as reported herein.

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