Effects of Cowpea Chlorotic Mottle Virus (Soybean Strain) on Chemical Composition of Davis Soybeans

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The effects of a strain of cowpea chlorotic mottle virus (designated CCMV-S) on total seed oil, protein, and fatty acid composition of oil from Davis soybeans grown at Experiment, Ga., in 1968 and 1969 was studied. Infection in inoculated plots was approximately 100% both years with no detectable spread of the disease to noninoculated plots. Early seedling infection with CCMV-S reduced total oil and increased protein percentage in 1968 but not in 1969. Of the five major fatty acids in the oil, infection with CCMV-S reduced the percentage of palmitic, linoleic, and linolenic acids and increased the percentage of stearic and oleic acids for the 2-yr period. On a yearly basis, the only significant change in oil composition due to CCMV-S infection was a decrease in linoleic acid in 1968, while palmitic and linolenic acids were decreased and stearic and oleic were increased in 1969. Although changes in chemical composition due to CCMV-S infection were significant, these changes were small and would not greatly affect the commercial value of infected soybeans.

A virus isolated from mottled soybean, *Glycine max* (L.) Merr., plants at Experiment, Ga., in 1967 was identified as a strain of cowpea chlorotic mottle virus by Kuhn (1968). Morphological changes in the soybean plant resulting from early infection with the virus (designated CCMV-S) include reduced vigor, leaf mottling with areas of light and dark green, a slight crinkling of the leaves, and leaves with a tendency to be more upright than those from noninfected plants.

The effects of soybean diseases on chemical composition of seed have not been extensively studied. Hartwig and Johnson (1953) reported that seed of resistant F_6 lines traceable to a single F_2 plant resistant to bacterial pustule, *Xanthomonas phaseoli* var. Sojensis, were higher in protein than comparable susceptible lines. Although total oil was not influenced by the disease, the iodine number of the oil was significantly increased at one of two locations in the diseased lines. This indicated a change in fatty acid composition of oil. In a similar study, Weber *et al.* (1966) found no difference in total protein or oil in the seed of resistant and diseased lines. Five virus diseases of hops, *Humulus lupulus*, were reported by Likens and Nickerson (1967) to have no influence on oil composition.

The purpose of this study was to determine the influence of seedling inoculation with CCMV-S on total protein, total oil, and fatty acid composition of oil from Davis soybean grown at Experiment, Ga., in 1968 and 1969.

MATERIALS AND METHODS

The seed for this study were grown in randomized complete blocks with five replications in 1968 and 10 replications in 1969 at Experiment, Ga. The entire test area was treated with 1.12 kg/ha of *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate (disulfoton) applied in a band beside recently emerged seedlings. Disulfoton (systemic insecticide) was used to control insect vectors and, thereby, prevent natural spread of CCMV-S within the plot area. The tests were planted on June 4, 1968, and May 27, 1969. Appropriate plots were inoculated on June 20, 1968, and June 13, 1969, with a crude extract from CCMV-S infected cowpea leaves containing 1% celite (Kuhn, 1968). Escapes were reinoculated as soon as they could be detected by lack of symptoms.

Random seed samples (60 g) from each of five replications in 1968 were taken from CCMV-S inoculated and control plots for total protein and oil analyses. Equal samples (12 g) from replications 1 through 5 and 6 through 10 were composited into two samples for total protein and oil analyses in 1969.

Random 25 g samples of seed from each treatment and replication (2-yr) were ground in a small laboratory mill (10-mesh). Three subsamples from the above were used for extraction of oil and subsequent fatty acid analyses by gas-liquid chromatography (glc). Oil was obtained by overnight extraction in an equal volume mixture of petroleum ether (Skellysolve F) and absolute methanol. The ground samples were left in 25-ml volumetric flasks during the methanol-sulfuric acid procedure for obtaining fatty acid methyl esters. Handling of oil samples after methylation in preparation for glc analyses has been previously described (Jellum and Worthington, 1966).

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-IIHSB digital integrator. Methyl esters were separated on a 2.4 m by 6.35 mm copper column packed with 10% (by wt) stabilized diethylene glycol succinate (DEGS) on Chromosorb W (80–100 mesh). Retention time for linolenic acid was 6 min and resolution of fatty acid peaks was similar to chromatograms shown in an earlier paper (Johnson and Jellum, 1969). Although soybean oil contains a number of fatty acids (Johnson and Jellum, 1969), only the five major components (palmitic,

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Table I. The Effect of CCMV-S Infection on Total Protein and Oil and on Oil Composition of Davis Soybean Seed Grown at Experiment, Georgia, in 1968 and 1969

Treatment	Percentage		Fatty Acid Composition of Oil, %				
	Protein	Oil	Palmitic	Stearic	Oleic	Linoleic	Linolenic
			1968				
Control	37.0	24.7[b]	12.1	3.9	25.8	52.3[a]	6.0
Inoculated	38.9[a]	23.7	11.9	4.1	27.2	51.0	5.7
			1969				
Control	39.2	23.9	12.3[b]	3.5	21.7	55.6	6.9[b]
Inoculated	39.5	23.9	12.0	3.6[b]	22.4[b]	55.4	6.7
			2-yr average				
Control	38.1	24.3[a]	12.2[b]	3.6	23.1	54.5[b]	6.7[b]
Inoculated	39.2[b]	23.8	11.9	3.8[b]	24.0[b]	53.9	6.3

[a,b] Differences significant at 5 and 1% levels, respectively, between control and inoculated treatments.

stearic, oleic, linoleic, and linolenic acids) were measured. Paired comparison analyses were used to evaluate observed differences (Snedecor, 1946).

RESULTS AND DISCUSSION

Seedling infection with CCMV-S was essentially 100% in the inoculated plots both years, with no apparent spread of the disease to control plots. Grain yield was reduced 20% in 1968 and 31% in 1969 by seedling infection with CCMV-S.

Total Protein and Oil. Total seed protein was significantly ($P \leq .05$) increased in diseased plots in 1968, but not in 1969 (Table I). When the data for both years were combined into a single analysis, the increase in protein in the seed from the diseased plots was highly significant ($P \leq 0.01$). Total oil was inversely related to protein. Oil percentage was decreased by infection with CCMV-S in 1968, but not in 1969. In the 2-yr combined analysis, the oil content of seed from CCMV-S diseased plants was significantly lower than oil content of the control seed.

A number of environmental factors influence the chemical composition of soybean seed (Howell, 1963). Parker and Harris (1962) reported that seed protein was increased and oil was decreased by application of lime or molybdenum. They concluded that one of the apparent functions of lime and molybdenum fertilization was to increase microbial *N*-fixation that resulted in darker green foliage color and increased protein. Diseased plots in this study had a darker green foliage color than control plots, which is similar to that obtained from heavier *N*-fertilization. Therefore, the increased seed protein observed in the diseased plots in 1968 could have been the result of stimulation of *Rhizobia* to greater activity or greater utilization of *N* by the host plant. On the other hand, more energy is required to synthesize oil than protein (Howell, 1970). Since the energy available for oil production in CCMV-S diseased plants was probably lower than for noninfected plants, it seems logical that a lower available energy level would have resulted in a decreased oil production. Oil and protein percentages in soybean seed are generally inversely related (Howell, 1963).

Fatty Acid Composition of Oil. The only significant change in oil composition in 1968 was a decrease in linoleic acid (Table I). However, observed *t* values for differences in stearic and oleic acids (2.743 and 2.735, respectively) were near the required level (2.776) for significance. In 1969, infection with CCMV-S caused highly significant decreases in palmitic and linolenic acids and increases in stearic and oleic acids when compared with the control. The decrease in linoleic was not significant in 1969. Although differences in palmitic, stearic, oleic, and linolenic acids were nonsignificant in 1968 between the control and diseased plots, each fatty acid was affected in the same way as in 1969 (Table I). This is substantiated by the highly significant differences between control and diseased plots when the data for both years were combined into one analysis.

Howell and Collins (1957) showed that linolenic and linoleic acid content of soybean oil was inversely related to temperature during seed maturation. Several other environmental factors, including photoperiod, light intensity and quality, and soybean nutrition, had little or no effect on these oil components. Since the seed from the control and diseased plants were from adjacent plots and only a slight change in maturity (1–2 days) was evident, it seems unlikely that a temperature variable caused the change in oil composition we observed.

The commercial quality of the oil was not adversely affected by the presence of the disease since linolenic, which is undesirable in edible oils, was decreased by CCMV-S infection. Although significant, the changes in oil composition were small and would probably have little or no effect on its commercial utilization.

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LITERATURE CITED

Hartwig, E. E., Johnson, H. W., *Agron. J.* **45,** 22 (1953).
Howell, R. W., "The Soybean. The Physiology of the Soybean," pp. 75–124, Academic Press, New York (1963).
Howell, R. W., *Soybean News* **21,** 1 (1970).
Howell, R. W., Collins, F. I., *Agron. J.* **49,** 593 (1957).
Jellum, M. D., Worthington, R. E., *Crop Sci.* **6,** 41 (1966).
Johnson, B. J., Jellum, M. D., *Agron. J.* **61,** 379 (1969).
Kuhn, C. W., *Phythopathology* **58,** 1441 (1968).
Likens, S. T., Nickerson, G. B., J. AGR. FOOD CHEM. **15,** 525 (1967).
Parker, M. B., Harris, H. B., *Agron. J.* **54,** 480 (1962).
Snedecor, G. W., "Statistical Methods," pp. 75–88, Iowa State College Press, Ames (1946).
Weber, C. R., Dunleavy, J. M., Fehr, W. R., *Agron. J.* **58,** 544 (1966).

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