Effects of Time of Inoculation with Tobacco Ringspot Virus on the Chemical Composition and Agronomic Characteristics of Soybean

J. W. Demski, H. B. Harris, and M. D. Jellum

Assistant Professor of Plant Pathology, and Associate Professors of Agronomy, University of Georgia, College of Agriculture Experiment Stations, Georgia Station, Experiment 30212.

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ABSTRACT

Significant effects on the chemical composition and agronomic characteristics of Lee soybean were observed from infection with tobacco ringspot virus up to the end of the flowering period, which was 72 days after planting. Palmitic, linoleic, and linolenic acid proportions of the oil decreased and stearic and oleic acids increased as a result of infection during this period. The total seed oil decreased and total seed protein increased with early inoculation. Virus infection at or before flowering significantly reduced yield and plant height and delayed seed maturity. Early inoculation also affected seed quality and seed weight, but did not affect lodging. Phytopathology 61:308-311.

Additional key words: Glycine max, fatty acid composition.

A common disease of soybean (*Glycine max* [L.] Merr.) known as bud blight is caused by tobacco ringspot virus (TRSV) (1). With soybean production increasing in Georgia, there has been a subsequent increase in the observance of soybean plants suspected of being infected with TRSV. Isolation from these plants and identification by host range, physical properties, and serological tests, confirmed that TRSV is widespread in Georgia soybeans.

Exactly how TRSV is transmitted in nature is not known, although this mechanically transmitted virus has been shown to be transmitted by grasshoppers (5), nematodes (2), and possibly thrips (2). The most detrimental effects of TRSV infection, based on symptoms and yield, occurs when plants are infected during early stages of plant development (1, 9). Plant age at the time of infection has been shown to be of importance in determining seed set (1) and the frequency of seed transmission from infected plants (4, 10).

Tu & Ford (11) quantitatively measured the free amino acids in soybean leaves as affected by soybean mosaic virus and bean pod mottle virus; however, little data is available on the effect of TRSV infection on the chemical composition of soybean seed or certain agronomic characteristics based on the specific time of inoculation.

The purpose of this study was to evaluate the effects of TRSV when Lee soybeans were inoculated at various stages of development. Determination of (i) the fatty acid composition of oil from the seed; (ii) total seed protein and oil; and (iii) agronomic characters were made to ascertain when infection is the most detrimental in soybean production and to determine the critical time for control measures.

Materials and Methods.—In 1968, preliminary tests were conducted in the greenhouse and field to evaluate the agronomic characteristics of soybeans affected by TRSV. On 12 May 1969, Lee soybeans were field-planted in a randomized complete block with three replications. All inoculated rows were separated by noninoculated rows. Each row was 4.87 m in length with 97 cm between rows. The entire block was treated with 1.12 kg/hectare of 0,0-diethyl *S*[2-(ethylthio) ethyl] phosphorodithioate (disulfoton) in a band approx 9 cm from the rows. Disulfoton, a systemic insecticide, was used to control possible vector populations and reduce the natural spread of TRSV.

Plants were inoculated with a crude extract of TRSV by rubbing the youngest leaves. The extract was prepared by grinding infected leaves of *Phaseolus vulgaris* 'Topcrop' in 0.025 M phosphate buffer pH 7.2 containing 1% celite. Before inoculation, the soybeans were dusted with aluminum oxide.

Seed samples (25 g) for fatty acid analyses were ground in a small laboratory mill (10 mesh). Oil was obtained by overnight extraction of 0.3 g in a 1:1 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 (8). 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-11 HSB 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 analysed, and the mean was used for comparison. Total protein and oil analyses were performed on random seed samples of each treatment by R. L. Cooper, U.S. Regional Soybean Laboratory, Urbana, Ill.

Yield was determined by seed wt approx 30 days after harvest. Seed quality (visual ratings where 1 is excellent and 5 is very poor) and seed wt (g wt per 100 seed) were also determined at this time. Plant height, relative maturity, and lodging were recorded at the time of harvest.

Results.—The greatest changes in the percentage of individual fatty acids in the oil of Lee soybean seeds occurred in the earliest inoculated treatments. Figure 1 illustrates a typical chromatogram showing the resolution and retention time of the five major fatty acids. Early infection with TRSV significantly reduced the percentage of palmitic, linoleic, and linolenic acids, and increased the percentage of stearic and oleic acids (Fig. 2). The greatest total change was in linoleic and oleic

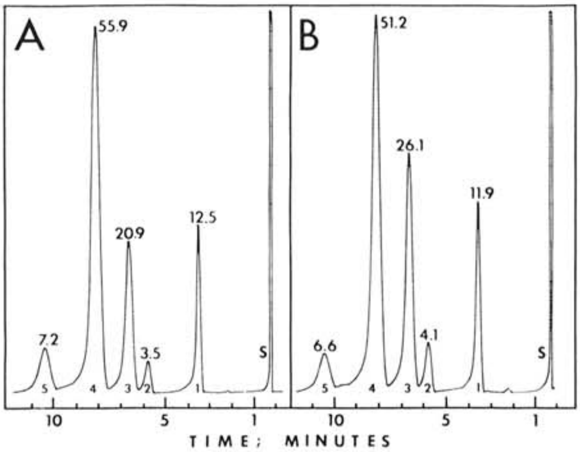

Fig. 1. Chromatogram illustrating the resolution and retention time of the five major fatty acids in Lee soybean (*Glycine max*) seed oil. **A)** Control (noninoculated); **B)** inoculated with tobacco ringspot virus 11 days after planting, 1 = palmitic, 2 = stearic, 3 = oleic, 4 = linoleic, and 5 = linolenic acid. The values reported are percentages of total fatty acids.

acids, which were basically mirror images of each other. Comparing the control (noninoculated) to the earliest inoculated plants, linoleic showed an 8% decrease in the virus inoculated treatment, oleic a 25% increase, palmitic a 5% decrease, stearic an 18% increase, and linolenic an 8% decrease. Although nonsignificant, linolenic was the only fatty acid in which the earliest inoculated treatment did not show the greatest percentage change.

Total seed protein significantly increased in early inoculated treatments, whereas total oil percentage decreased (Fig. 3). Inoculation later than 80 days after planting did not influence oil and protein percentages.

Yield reduction was dependent on inoculation time. Generally, the earlier the plants were inoculated, the greater was the yield loss (Fig. 4). Severe yield reduction was observed when plants were inoculated before or during the flowering period. Lesser effects were noted when inoculated after flowering. In some greenhouse studies, the earliest inoculations resulted in no seed production. Early inoculation also reduced plant height and seed quality, but had no effect on lodging. Plants which were inoculated at or before flowering matured later (Fig. 4). At maturity, plants inoculated early retained many leaves as compared with the control. Weight per 100 seed was higher in the earliest inoculated blocks where yield was the lowest.

Discussion.—The effect of TRSV on the fatty acid composition of oil in Lee soybean was in agreement with a study by Harris et al. (6) on the effects of the soybean strain of cowpea chlorotic mottle virus (CCMV-S) on soybeans. Tobacco ringspot virus caused an increase or decrease of the same fatty acids as did CCMV-S; however, the effect of TRSV was greater. Linoleic and linolenic acid proportions have been shown to be higher in soybean oil obtained from seed grown under cooler temp (7). Therefore, the delay in maturity due to early inoculation should have favored (cooler temp) an increased proportion of linoleic acid instead of the reductions as shown in Fig. 2.

Total oil and total protein of soybean seeds are generally inversely related. Although total oil decreased, the quality of the oil was not impaired, as the linolenic and linoleic acid content is still well within the normal range of edible seed oils.

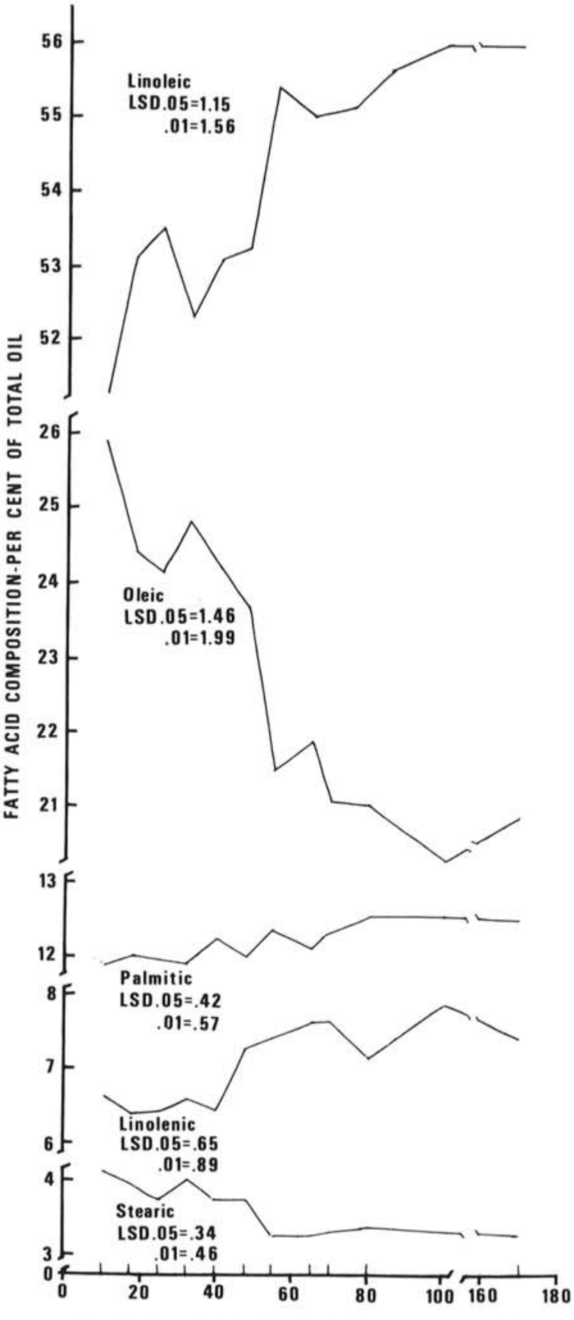

Fig. 2. Fatty acid composition of oil from Lee soybean (*Glycine max*) seed in relation to time of inoculation with tobacco ringspot virus. The last recorded plot for each fatty acid (170 days) is the control and also the time of harvest.

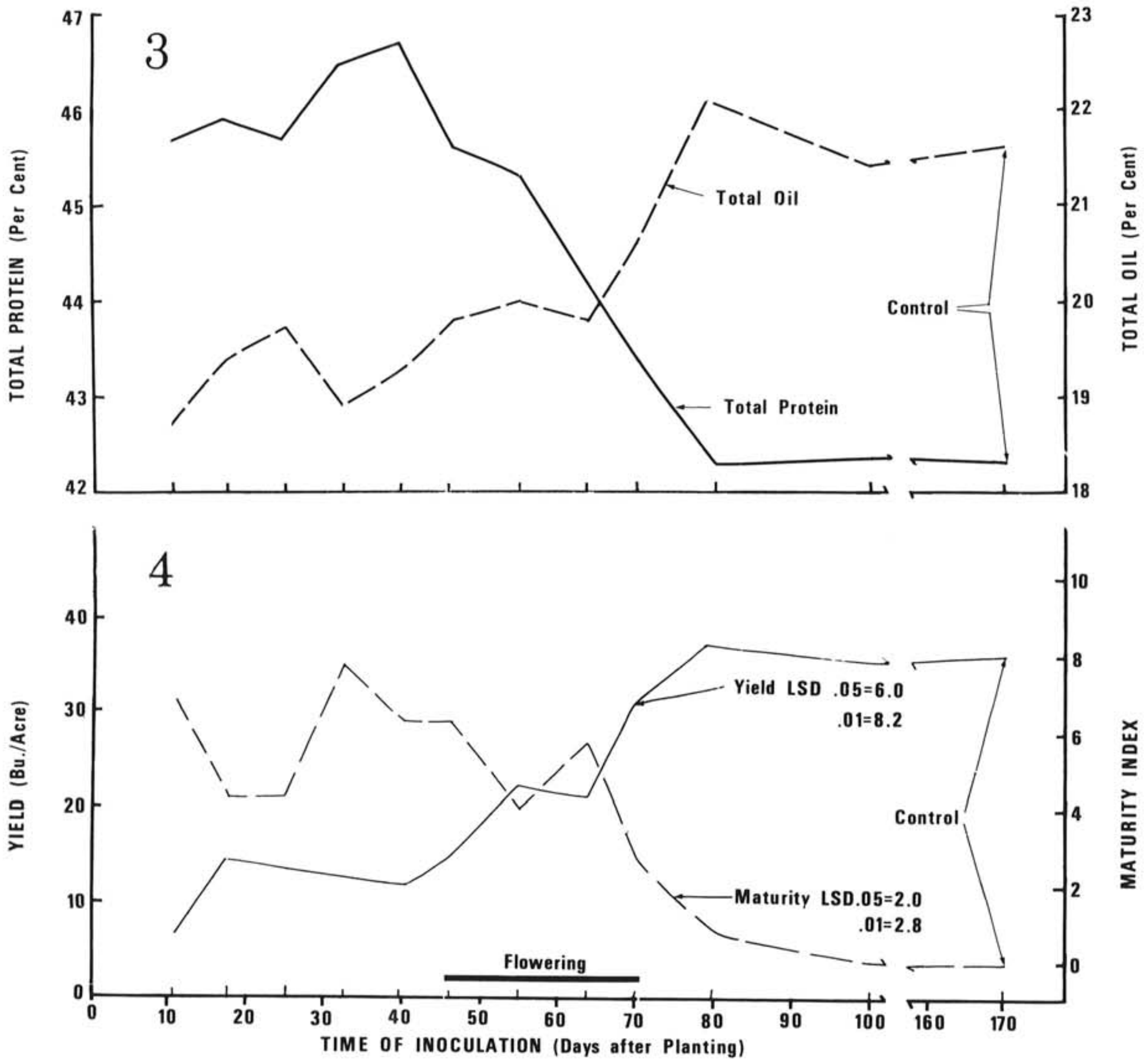

Fig. 3-4. **3)** Total protein and oil percentage; and **4)** yield loss and maturity index of Lee soybeans (*Glycine max*) inoculated at different times with tobacco ringspot virus. Harvest was at 170 days.

Crittenden et al. (3) reported yield losses of over 50% in naturally infected plantings. In this study, yield was reduced 79% by early inoculation. Determination of yield loss due to virus diseases is quite difficult where virus spread cannot be fully controlled and the mode of natural transmission is unknown. Use of the insecticide disulfoton did not prevent infection of noninoculated plants. The indexing of 50 plants/treatment at the end of the vegetative growing season revealed an average of 12 infected plants/100. The time at which these plants became infected is unknown, but the fact that some infected plants occurred in the controls should tend to emphasize the large yield reduction of 79% between the earliest inoculated and control treatments. Yield losses resulted from reduced seed numbers, since the three earliest inoculated plots (lowest yielding) had the largest seed. Yield losses and other agronomic characteristics were in agreement with the data obtained in the 1968 greenhouse and field studies.

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