Seed Transmission and Distribution of Tobacco Streak Virus in Six Cultivars of Soybeans

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Contribution No. 591, Department of Plant Pathology, Kansas Agricultural Experiment Station.
Supported in part by USDA, CSRS Research Agreement No. 216-15-48.
Accepted for publication 29 June 1973.

ABSTRACT

Six soybean cultivars at three stages of growth were inoculated with isolates of tobacco streak virus (TSV), one from soybean (A-TSV) and the other from tobacco (W-TSV). For either isolate, all plant parts except pollen, embryos of immature seeds, and non-inoculated leaves that had developed prior to inoculation, consistently assayed positive for virus. Depending on the cultivar, 0 to 20% of the seedlings from immature seeds from A-TSV-infected plants contained A-TSV; the percentage was higher in seedlings from mature seeds. Differences in seed transmission were noted among cultivars (2.6 to 30.6%). Of the cultivars tested, 'Wayne' had the highest percentage of transmission. W-TSV was not transmitted through either mature or immature seeds.

Phytopathology 64:112-114

The first authentic report of tobacco streak virus (TSV) in soybeans was in 1970 by Fagbenle and Ford (6). Prior to that, Melhus (12) and Johnson (9) in the United States and Costa et al. (5, reviewed in 6) and Costa and Carvalho (4) in Brazil reported a disease of soybeans with symptoms similar to those induced by TSV.

Attempts to detect seed transmission of TSV in tobacco, cotton, and pea have been unsuccessful (4, 13, 16, 18), although seed transmission has been reported for pinto beans (17), *Datura stramonium* (2), and *Chenopodium quinoa* (2). Fagbenle and Ford (6) recovered TSV from ground-up immature soybean seeds inoculated to *Chenopodium quinoa*. However, they could not demonstrate TSV transmission through mature soybean seeds.

This study was undertaken to determine the distribution of TSV in the soybean plant, and to reinvestigate virus transmission through soybean seeds.

MATERIALS AND METHODS.— Two isolates of TSV were used throughout this study. A-TSV was isolated from naturally infected 'Hark' soybeans at the Ashland Experiment Farm, Manhattan. W-TSV, from tobacco, was provided by R. W. Fulton. Each isolate was single-lesioned three times on cowpea and maintained on cowpea. A-TSV was identified by positive reaction to TSV antiserum by Ouchterlony agar double-diffusion serology and by symptoms on known indicator host plants. Symptoms of A-TSV on 'xanthi-nc' tobacco leaves correspond to Fulton's designation "entire" (7). Symptoms of W-TSV on 'xanthi-nc' tobacco leaves correspond to Fulton's designation "toothed" (7).

Two-, 4-, and 6-wk-old plants of six soybean cultivars (10 plants/treatment) (Table 1) were inoculated mechanically with each isolate of the virus. The 2-wk-old plants were inoculated on the unifoliate leaves and the others on the topmost expanded trifoliate leaves. Inoculum in all cases was fresh, heavily infected cowpea tissue homogenized in a Waring Blendor in three volumes of buffer (0.05 M Na_2HPO_4, pH 9.0 containing 0.1% Na_2SO_3). Inoculum was applied via pestle to Carborundum-dusted leaves, and the leaves were washed with tap water. Inoculum was kept in ice until applied. Greenhouse temperatures were about 25 C; most experimental work was done in the spring. Ten plants of each group and cultivar were kept as controls.

The average plant height per pot was measured before inoculation and at plant maturity or time of death.

All subsequent assays were done using 0.05 M Na_2HPO_4, pH 9.0 containing 0.02 M 2-mercaptoethanol. Cowpea with fully expanded unifoliate leaves served as assay host. For assay, 0.25 gm or less of tissue was ground in 2 ml of buffer and then inoculated via pestle to five Carborundum-dusted cowpea plants.

At 3-5 wk after inoculation, two plants of each cultivar of the 4- and 6-wk series were assayed for virus in root, unifoliolate leaf, and topmost expanded trifoliolate leaf. Two plants of each cultivar of the 2-wk series were assayed for virus in root and in unifoliolate (inoculated) leaf, because few trifoliolates developed. If the unifoliolate leaf had dropped, the next trifoliolate up the stem was assayed. New trifoliolates arising from the axil of unifoliolate leaves also were assayed from all three series when present.

Flower petals, anthers, and pistils were assayed from plants in the 6-wk series inoculated with A-TSV. Plants inoculated with W-TSV did not produce enough flowers for such assays.

Two immature pods per cultivar per virus isolate were assayed from the 6-wk series. The pod coat was assayed separately, and each of the seeds in the pod was separated manually into seed coat and embryo and were assayed separately. In initial experiments, we decontaminated the seed coat and embryo by soaking them in running tap water for about 20 min prior to inoculation (11). Mature seeds were assayed similarly, except that seeds were soaked in distilled water for 18 to 24 h before being separated manually into seed coat and embryo.

A few immature seeds per cultivar for either isolate were grown in steam-sterilized soil. Some were sown intact, others with the seed coat removed. The topmost trifoliolates of the resulting seedlings were assayed for virus. Mature seeds from infected plants also were grown in steam-sterilized soil, and the topmost trifoliolates of the resulting seedlings were assayed for virus.

RESULTS.— Plants of all cultivars in each series developed obvious virus symptoms within 10 days after inoculation. Most plants in the 2-wk series displayed terminal necrosis and bending of the stem tips within 10 days of inoculation, and many were dead within 4-6 wk. Average maximum height ranged from 18 to 38 cm, compared with

TABLE 1. Seed-transmission rate in seedlings from mature soybean seeds of cultivars inoculated at three growth stages with two isolates of tobacco streak virus

Soybean cultivar	Growth stage when inoculated						Total % transmission (A-TSV)
	2-week		4-week		6-week		
	A-TSV	W-TSV	A-TSV	W-TSV	A-TSV	W-TSV	
'Columbus'	10/23[a]	–[b]	2/36	–[b]	4/35	0/31	17.0
'Wayne'	9/20	–	17/33	–	0/32	0/35	30.6
'Cutler'	–[b]	–	9/37	–	0/35	0/24	12.5
'Kent'	1/6	–	1/32	–	0/31	0/12	2.9
'Dare'	1/5	–	0/36	–	1/35	–[b]	2.6
'Calland'	5/32	–	10/35	–	0/24	0/24	16.4

[a]Ratio of infected seedlings/number of seedlings tested.
[b]Dash indicates that no seed was produced.

55 to 57 cm for controls. Surviving plants in this series generally produced vestigial pods or normal-sized pods with aborted seeds, though we collected a few normal-sized pods containing seeds that appeared normal.

Infected plants of the 4- and 6-wk series developed typical leaf puckering and darkening along petioles and veins. Darkening of nodes, crooked growing points, dwarfed trifoliolate leaves, and supernumerary production of vestigial pods were evident at later stages of growth.

Roots, inoculated unifoliolate leaves, and topmost trifoliolate leaves that developed after inoculation, gave assayable quantities of TSV from all plants tested. Except for those directly inoculated, leaves that developed prior to inoculation had no assayable virus. However, trifoliolates emerging from the axil of any of the leaves below the point of inoculation always gave a high virus titer when tested.

Flowers from infected plants appeared to be normal but contained varying amounts of assayable virus. Virus was detected in petals but not in anthers of all cultivars of the 6-wk series inoculated with A-TSV.

W-TSV-infected plants were stunted more severely than were A-TSV-infected plants. For either isolate, pod blotching was not consistently present, although virus always could be recovered from pods of infected plants, either below or above the point of inoculation.

Assayable quantities of virus always could be obtained from the seed coats but inconsistently from the embryos of immature seeds. Embryo assays were conducted only for 'Cutler' and 'Calland'; 36% and 30% of the embryos of the two cultivars tested, respectively, contained A-TSV, but none from those infected with W-TSV contained W-TSV. Decontaminating the embryo and seed coat in running tap water had no effect on results. Mature seeds tested similarly gave negative results for both seed coat and embryo.

Seedlings from immature soybean seeds gave negative assay for all cultivars except Calland, which gave 20% transmission of the A-TSV isolate. No seed transmission was obtained for the W-TSV isolate in immature seeds of any cultivar.

In the 6-wk series, more than 89% of the mature seeds from virus-infected plants germinated; seeds from 'Kent' (78% for A-TSV, 80% for W-TSV) and Calland (67% for A-TSV, 69% for W-TSV) were exceptions. Seed transmission varied with cultivar for seedlings assayed from mature seeds from plants infected with A-TSV (Table 1). Only 2.6% total transmission was obtained from 'Dare'; 30.6% was obtained from 'Wayne'. The W-TSV isolate was not seed-transmitted in mature seeds of any cultivar. Seeds from the 4-wk series, notably from the cultivar Wayne , produced the most infected seedlings. Tissue from several infected seedlings was tested serologically with TSV antiserum; results were positive.

A-TSV infected seedlings were allowed to develop into mature plants. Three cultivars, viz. Wayne, Calland, and Dare, produced 60, 21, and 1 seeds per 10 plants, respectively; the others produced no seeds. Eight of these Wayne seeds were planted; six of the eight seedlings which developed gave a positive assay for the virus.

Virus was never detected in assays of healthy controls or their seed parts.

DISCUSSION.—Symptoms of leaf puckering and dark streaks in petioles and veins conform with an earlier report (3). The supernumerary vestigial pod formation we observed has not been reported previously. Our results show that regardless of time of infection (2, 4, or 6 wk), the virus moved throughout an infected soybean plant and reached assayable quantities in all plant parts tested, except for pollen and for those leaves that had developed prior to inoculation.

A recent report (11) on southern bean mosaic virus in *Phaseolus vulgaris* Logan indicated that immature embryos not decontaminated gave positive assays and that immature embryos decontaminated in running tap water for about 0.5 h gave negative assays. We obtained a positive assay for seed coats consistently, and for embryos occasionally, despite decontamination steps. In addition, whenever a positive assay was obtained for both seed coat and embryo, local lesions counts for each were high, suggesting that the embryo indeed was infected rather than contaminated.

The consistent presence of virus in immature seed coats might be related to the virus' origin in the plant and to the conducting tissue between the seed coats and the rest of the plant (1, 15). Absence of virus in seed coats of mature seeds corresponds with inactivation of virus in mature seed coats, as reported by Inouye (8, reviewed in 10) and Schippers (14, reviewed in 10). Because our primary interest was to study seed transmission, most of the limited seed supply was planted; only a few seeds were

assayed directly. We might have obtained positive assays from mature seed embryos, had more been assayed.

Seedlings from mature seeds of plants infected with A-TSV showed seed transmission, whereas seedlings from mature seeds of plants infected with W-TSV consistently assayed negatively. This indicates that seed transmission of TSV may vary with the virus strain.

The mature-seed-transmission data also indicate that plant age at time of inoculation affected seed transmission of TSV. Plants inoculated at 4 wk were more prone to seed transmission than were plants inoculated at 2 or 6 wk. The fact that seedlings produced from infected seeds can develop into plants that in turn can produce infected seeds, indicates one way the virus may be maintained and/or increased slowly under field conditions from year to year.

We found no virus in the pollen. Thus, the virus probably enters the developing seed directly from the mother plant.

Based on differences in symptom severity and seed transmissibility, A-TSV and W-TSV are distinctly different. Only the A-TSV strain was transmissible through mature soybean seeds, perhaps because A-TSV was isolated from soybean and W-TSV from tobacco.

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