# Tobacco Streak Virus Isolated from Soybeans, Glycine max

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ABSTRACT

Tobacco streak virus (TSV) was isolated from naturally infected soybeans (*Glycine max*) for the first time in Iowa and in the USA. This TSV isolate had a thermal inactivation point of 58 C, dilution end point of 1:640, longevity in vitro of 24 hr, and a host range similar to a Brazilian strain of TSV isolated from soybean. It was inactivated more rapidly in tobacco sap than in cowpea or bean sap. At the end of 30 min, a 1:25 dilution of tobacco leaf sap infected with TSV was more infective than dilutions of 1:1 or 1:10. TSV infection in soybeans reduced seed and pod production and embryo vigor. Infective virus particles were 28 to 32 mμ in diam. The Iowa TSV isolate reacted only with TSV antiserum. *Cassia occidentalis* is a new host for TSV. Phytopathology 60:814-820.

Soybean plants with pods showing necrotic spots were noted in a late planting in Iowa in 1967. Infected plants were scattered throughout the field. The pod symptoms closely resembled those of the bud-blight disease caused by tobacco ringspot virus (TRSV) (1).

This paper describes studies that indicate that the cause of the problem noted in Iowa is the result of infection by tobacco streak virus (TSV) rather than TRSV.

MATERIALS AND METHODS.—The Iowa TSV isolate was maintained in cowpeas (*Vigna sinensis* Endl. 'Early Ramshorn') and tobacco (*Nicotiana tabacum* L. 'Samsun NN') by mechanical inoculation transfers at 5- to 10-day intervals. All plants were grown in a steamed loam soil:sand:peat mixture (2:1:1; v/v) in 4-inch clay pots in a 25-C greenhouse.

Inocula were prepared by grinding leaf tissue in a mortar with a pestle, expressing the sap through gauze, and diluting with 0.01 M potassium phosphate buffer, pH 7.0, containing 0.01 M sodium sulfite. All dilutions were by w/v unless stated otherwise, and all inocula were assayed immediately after preparation.

Assay plants (*Chenopodium quinoa* Willd.) were selected at the 10-leaf stage. The youngest six expanded leaves were inoculated by rubbing the entire leaf surface four times. One plant was used for each test sample, and each sample was duplicated. Plants were inoculated by rubbing inoculum with a pestle on leaves previously dusted with 600-mesh Carborundum. After inoculation, the plants were washed with water and maintained in a 25-C greenhouse for 3-6 days before local lesions were counted (Fig. 8).

TSV inoculum from cowpea primary leaves diluted 1:10 was used for the study of thermal inactivation point (TIP), dilution end point (DEP), and longevity in vitro (LIV) of the virus. Each physical property test was replicated and repeated four times. TIP studies were conducted by grinding each sample just before heating because of the rapid inactivation of the virus. Each 1-ml sample was heated for 10 min at either 54, 56, 58, 60, 62, or 64 C, cooled immediately in running tap water, and inoculated on the assay plants. Twofold dilutions were made from 1:10 to 1:1,280 to determine DEP. LIV of the virus was determined by grinding a sample of leaf tissue in buffer and holding the diluted sap in a test tube at 22 C. Samples were assayed at 0, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 hr.

Twenty-nine species of plants and five soybean cultivars were inoculated with TSV and observed for at least 3 weeks. If symptoms did not appear, a recovery inoculation was made to *C. quinoa* to test for masked TSV multiplication.

For inactivation studies, samples from 12-day inoculated NN tobacco leaves were diluted 1:1, 1:10, and 1:25 and assayed at 0, 5, 10, 20, and 30 min after grinding. Host effects were studied in cowpea, NN tobacco, and bean (*Phaseolus vulgaris* L. 'Bountiful'). The primary leaves of the cowpea and bean plants and the top six leaves of the tobacco plants were inoculated. Five days later, inoculated leaves were ground at a 1:10 dilution in buffer, allowed to set in the mortar at room temperature, and assayed at 1, 10, 20, and 30 min.

Several purification methods were used in attempts to purify the Iowa TSV isolate. Steere's method for TRSV (25) and Fulton's method for TSV (11) were unsuccessful. Two purification techniques were partly successful. One was an alternate low speed-high speed centrifugation of sap from inoculated leaves of NN tobacco, cowpea, and bean 5 days after inoculation. The leaves were ground in 0.01 M potassium phosphate buffer, pH 7.0 (0.5 ml buffer/*g* of leaf tissue) in a Waring Blendor, and the sap was expressed through cheesecloth and centrifuged at 10,500 *g* for 15 min. The supernatant was centrifuged at 105,000 *g* for 2 hr. The low speed-high speed centrifugation was repeated, and the resulting pellet was suspended in 3 ml of buffer and stored at 3 C.

The other method of partial purification was a density-gradient centrifugation. Inoculated leaves were pestle-ground in a mortar at 1:10 dilutions by using the buffer just described. Sap was expressed through cheesecloth and centrifuged at 27,000 *g* for 5 min. The supernatant from each sample was layered on a sucrose density-gradient tube (10, 20, 30, and 40% sucrose) and centrifuged at 25,000 rpm in a Spinco SW

25.1 rotor. The contents of the tubes were scanned with an ISCO density gradient fractionator with a recording ultraviolet (254 mμ) densitometer.

Fractions from the peaks recorded from the density-gradient tubes were collected and dialyzed against a large volume of cold buffer for 3 hr. These fractions and samples from the low speed-high speed purification were mixed with 2% phosphotungstic acid (PTA) pH 7.4, atomized onto Formvar-coated grids, and examined with a Hitachi HU-11C electron microscope.

Ouchterlony agar double-diffusion tests (2) were made using partially purified virus preparations from NN tobacco, cowpea, and Bountiful bean. Antisera specific for TRSV, tomato ringspot virus (TomRSV), bean pod mottle virus (BPMV), cowpea chlorotic mottle virus (CCMV), cowpea mosaic virus (CPMV), cucumber mosaic virus (CMV), and tobacco streak virus (TSV) were used.

Seed transmission was determined on Bansei soybean plants inoculated 2, 4, and 6 weeks after planting. Five plants were inoculated at each time, and five healthy plants were maintained as controls. Seeds were harvested 10 weeks after planting. Pods per plant, seeds per pod, and seed maturity were recorded.

An immature seed from each group of plants was ground in 0.01 M potassium phosphate buffer, pH 7.0, and assayed on *C. quinoa*. Mature seeds were stored at 4 C for 3 months. Ten seeds/sample were then planted, and seedling heights were recorded 2 weeks after emergence.

RESULTS.—*Physical properties.*—The Iowa TSV isolate remained infective in plant sap from cowpea leaves for 24 hr, but not 36 hr. The TIP was 57 C. Infectivity was retained at dilutions of 1:640, but not at 1:1,280.

*Host range.*—Plants of 26 species were infected with TSV (Table 1). No infection occurred on *Datura stramonium* L., *Capsicum annuum* L., *Pisum sativum* L. 'Perfected Wales', or *Trifolium hybridum* L. Plants of *C. annuum* were stunted, and the leaves were slightly chlorotic with dark-green veins, but TSV could not be recovered.

Leaves of NN tobacco developed necrotic local lesions 2-3 days after inoculation. Local lesions and acute symptoms appeared as spots, concentric rings, ringspots, and (or) oak leaf patterns (Fig. 2). Leaves in the chronic stage of infection were generally symptomless, but flowers were sometimes split and had a filamentlike extension on each petal. Crenated leaves which sometimes lacked part of the lamina base appeared in the chronic stage of infection in Turkish tobacco (Fig. 1), and flower symptoms were like those of NN tobacco, but occurred more frequently (Fig. 3). Systemically infected *N. glutinosa* L. developed mosaic patterns in leaves that were often straplike, and pale, streaked flowers that were sometimes split (Fig. 4). An oak-leaf pattern appeared on previously symptomless inoculated leaves 45 days after inoculation. Systemically infected leaves of *N. acuminata* Hook. had a mosaic pattern, and the leaf edges were curved down. Floral tubes were split, and some sepals were petallike.

Inoculated primary leaves of cowpeas produced necrotic ringspots and necrotic veins in the winter, ringspot depressions on the leaf surface (Fig. 5), especially during April, but no symptoms in the summer. Systemic symptoms were terminal necrosis, thickened, blistered trifoliolates, and stunted plants. Inoculated leaves of *Vicia faba* L. *minor* also developed necrotic ringspots (Fig. 6).

Bountiful, Red Mexican, Great Northern, and Scotia bean cultivars developed irregular, ringlike local lesions and veinal necrosis on inoculated primary leaves (Fig. 9, 10). Reddish streaks appeared on the stems and petioles, and leaves often broke at the petiole base. Axillary buds often developed branches, and, in severe infections, terminal necrosis occurred.

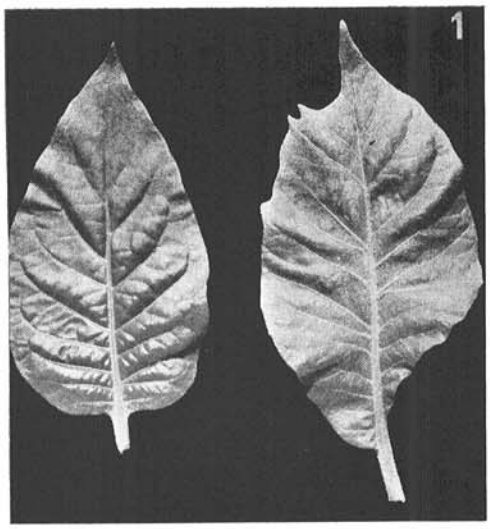
National Pickling, Imperial Long Green, and Ashley cucumbers developed chlorotic spots on inoculated cotyledons (Fig. 7). Systemically infected leaves were mottled, and smaller than those of the control.

Necrotic pod blotching was the only symptom noted on field-grown soybean. In the greenhouse, however, TSV-inoculated primary leaves developed necrotic spots and dropped prematurely, new trifoliolate leaves showed necrotic spots, and some plants became stunted. Mo-

TABLE 1. Host range and symptoms of tobacco streak virus

| Plant species | Symptoms |
|---|---|
| *Antirrhinum majus* L. | S[a] |
| *Cassia occidentalis* L. | LLs[b] |
| *Chenopodium album* L. | LLs |
| *C. amaranticolor* Coste & Reyn. | LLc, SN[c] |
| *C. quinoa* Willd. | LLs, SN |
| *Cucumis sativus* L. 'National Pickling', 'Imperial Long Green', 'Ashley' | LLc, SM[d], St[e] |
| *Cucurbita maxima* L. 'Caserta' | LLs, SM |
| *Cyamopsis tetragonoloba* (L.) Taub. | LLs |
| *Dolicos lablab* L. | LLs |
| *Glycine max* (L.) Merr. 'Bansei' | LLs, SN, St |
| 'Lincoln' | LLs, SN, St, SM |
| 'Kanrich', 'Richland' | SN |
| *Gomphrena globosa* L. | LLs |
| *Helianthus annuus* L. | LLs, S |
| *Lycopersicon esculentum* Mill. 'Bonney Best' | LLs, S |
| *Nicandra physalodes* Gaertn. | LLs, S |
| *Nicotiana acuminata* Hook | SM, St |
| *N. bigelovii* S. Wats. | SM, St |
| *N. glutinosa* L. | SM, St |
| *N. tabacum* L. 'Samsun NN', 'Turkish' | LLsrt, SN, S |
| *Petunia hybridum* Vilm. | S |
| *Phaseolus vulgaris* L. 'Bountiful', 'Scotia', 'Great Northern', 'Red Mexican' | LLt, SN, St |
| *Pisum sativum* L. 'Wilt Resistant Perfection' | S |
| *Physalis floridana* Rydberg | LLs |
| *Trifolium incarnatum* L. | S |
| *Vicia faba* L. *minor* | LLr |
| *Vigna sinensis* Endl. | LLr, SN, St |
| *Vinca rosea* L. | S |

[a] S = Systemic infection, no symptoms.
[b] LL = Local lesions; c = chlorotic spots; r = necrotic ringspots; s = necrotic spots; t = necrotic rings.
[c] SN = Systemic necrosis.
[d] SM = Systemic mosaic.
[e] St = Stunting of plant.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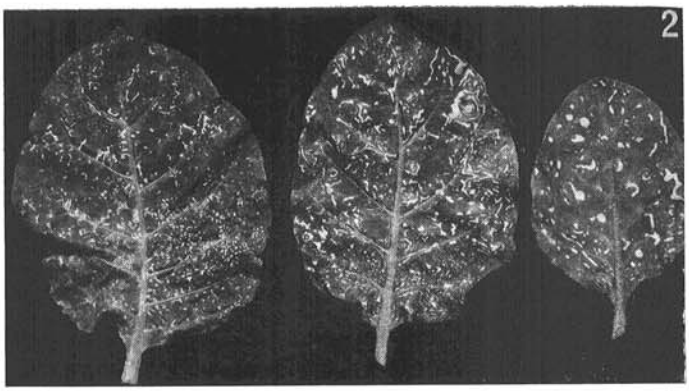
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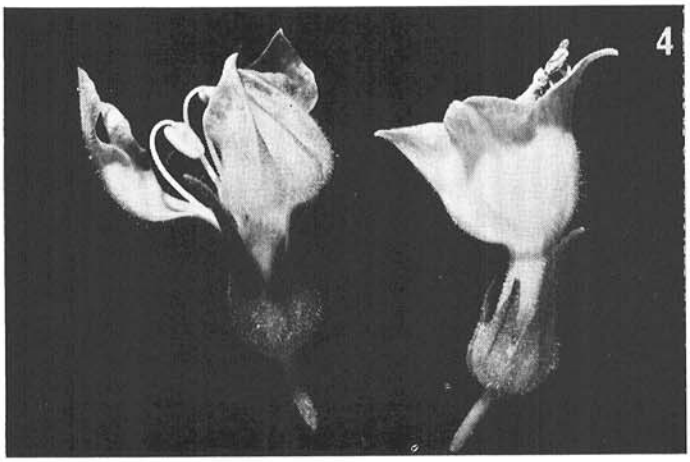
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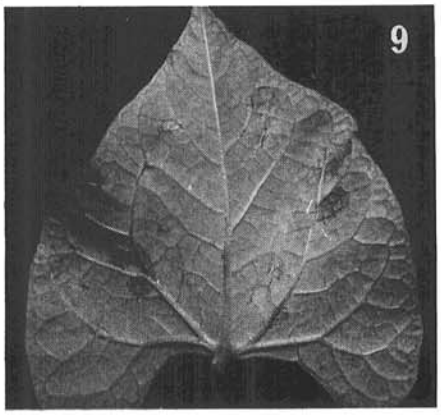
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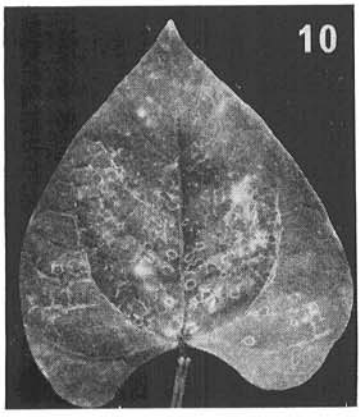
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**Fig. 1-10.** **1)** Leaves of Turkish tobacco, healthy (left) and chronically infected (right) by tobacco streak virus (TSV). **2)** Symptoms of TSV on NN tobacco. Acute stage systemic symptoms (left); local lesions (center and right). **3)** Flowers of a TSV-infected Turkish tobacco plant. The normal, healthy tobacco flower has a completely fused corolla tube without extensions on the petals. **4)** Flowers of *Nicotiana glutinosa,* TSV-infected (left) and healthy (right). **5)** TSV-inoculated cowpea primary leaf surface with indented, ringlike local lesions (×20). **6)** TSV-inoculated horse bean, *Vicia faba* L. *minor,* leaf with ringspot lesions. **7)** TSV-inoculated cucumber with chlorotic local lesions on the cotyledonary leaf. **8)** Typical assay of TSV on *Chenopodium quinoa* Willd. that develops chlorotic local lesions within 3-4 days. The lesions become necrotic within 6 days after inoculation. **9)** TSV-inoculated Bountiful bean primary leaf with ringlike local lesions and necrotic veinlets. **10)** TSV-inoculated Scotia bean primary leaf with chlorotic, ringlike local lesions and chlorotic vein symptoms.

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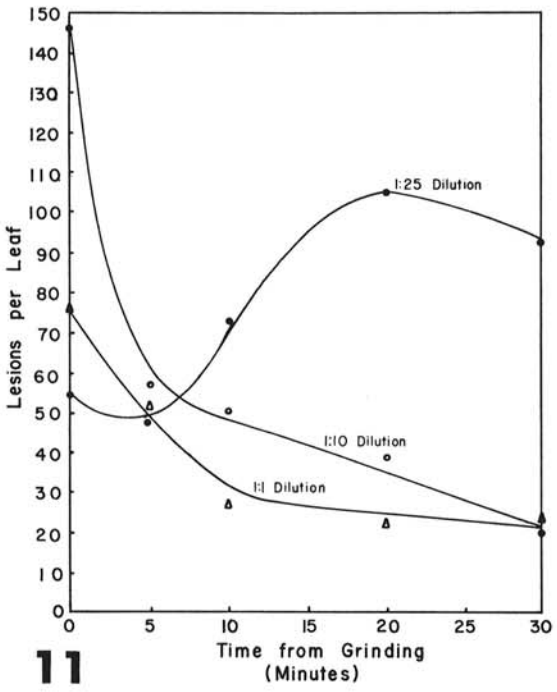


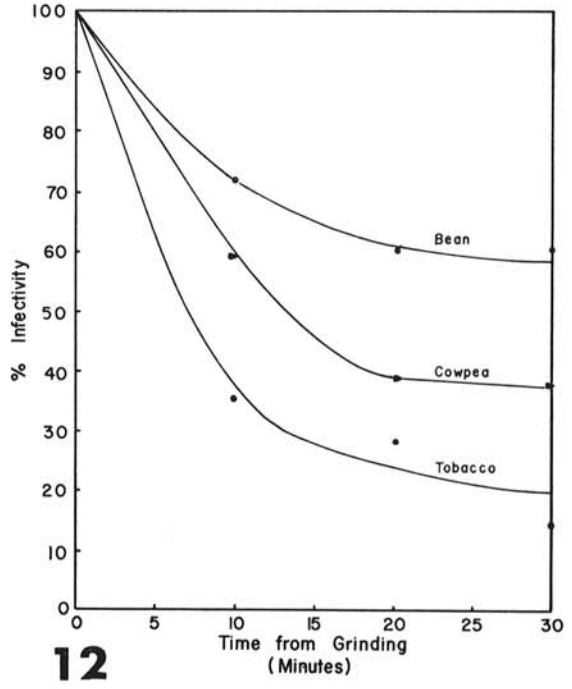


**Fig. 11-12.** **11)** Effect of dilution of inoculum from tobacco on the rate of inactivation of tobacco streak virus (TSV). Diluent = 0.01 M potassium phosphate buffer, pH 7.0, with 0.01 M sodium sulfite. **12)** Rate of inactivation of TSV in infective sap from bean, cowpea, and tobacco, each diluted 1:10 with 0.01 M potassium phosphate buffer, pH 7.0, with 0.01 M sodium sulfite.

saic symptoms and necrotic streaks at primary leaf nodes sometimes occurred.

*Inactivation.*—A dilution of 1:10 produced the greatest number of lesions at zero time, but both the 1:1 and the 1:10 dilutions were rapidly inactivated. The 1:25 dilution produced fewest lesions at zero time, but the number produced after 30 min was four times greater than those produced by the other two dilutions (Fig. 11). The infectivity of the 1:25 dilution increased for 20 min, then decreased between 20 and 30 min.

TSV was more rapidly inactivated at a 1:10 dilution in tobacco sap than at the same dilution in bean or cowpea sap. Of the three hosts tested, the rate of inactivation was lowest in bean (Fig. 12).

*Purification.*—Preparations from alternate low speed-high speed centrifugations and from sucrose density-gradient purifications produced lesions on test plants.

When scanned on the ultraviolet density-gradient fractionator, the contents of the tube with infected bean sap produced ultraviolet absorption peaks 6, 10, 12, and 18 mm from the meniscus. Samples from the top, second, third, and bottom peaks produced 0, 0.5, 17, and approximately 200 lesions/leaf, respectively. The top peak had the highest absorbancy, and each zone below absorbed less than the previous one. Infected cowpea leaves produced peaks 6, 10, and 13 mm from the meniscus. Samples from the lowest peak produced an average of one lesion/leaf; the other two produced no lesions. Leaves from infected NN tobacco produced peaks 5, 8, and 10 mm from the meniscus. None of the samples from the zones from tobacco was infective.

*Electron microscopy.*—Spherical virus particles with 28-32 mμ diam (Fig. 13) were observed in negatively stained preparations from the alternate low speed-high speed purification. Slightly larger particles were observed from the two middle peaks in the density-gradient tubes containing infected bean sap; no particles were observed from the top peak.

*Serology.*—A positive reaction occurred between TSV antiserum obtained from R. W. Fulton, Madison,

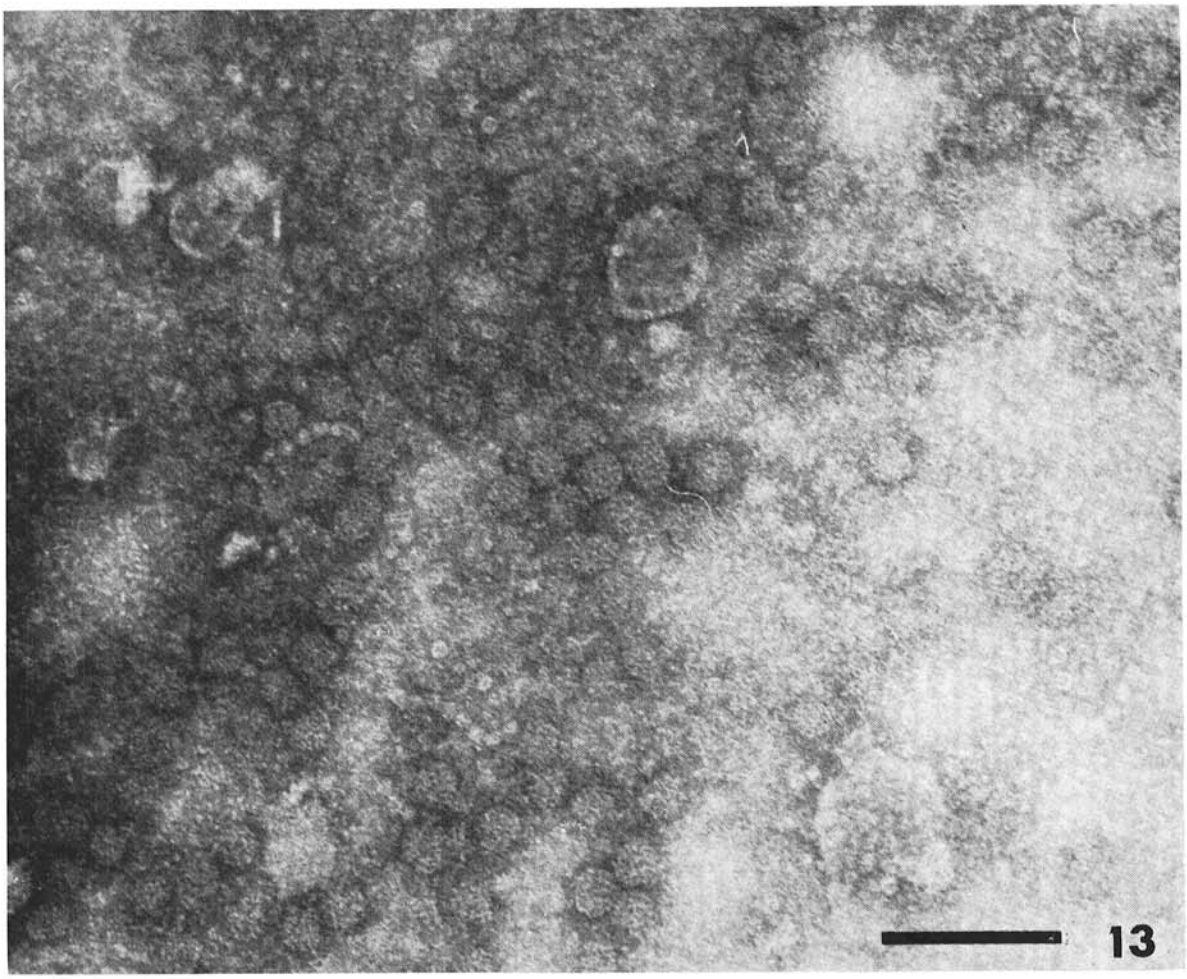


**Fig. 13.** Tobacco streak virus, from high speed-low speed purification, negatively stained with neutral phosphotungstic acid. Bar represents 100 mμ.

Wisconsin, and the Iowa TSV isolate. The strongest reaction was with infected bean extract; infected tobacco sap seldom produced a reaction zone. No reactions were observed in at least three tests with antisera from TRSV, TomRSV, PMV, CCMV, CPMV, or CMV.

*Effect of TSV on soybean seeds.*—Early inoculation of soybean plants with TSV reduced the number of pods per plant, and inoculation at any age delayed seed maturation (Table 2). TSV could be recovered from immature seeds from infected plants, especially from plants inoculated early. Mature seeds from plants inoculated early produced seedlings that were stunted, with conspicuously shortened petioles (Table 3).

Discussion.—TSV can cause symptoms closely resembling TRSV in soybean (7). Symptoms similar to bud blight reported in Brazil (7) in soybean were (i) stunting of the plants; (ii) curving and death of the stem tips; (iii) necrosis in the pith of the stems, especially near the nodes; (iv) development of axillary buds; (v) dwarfing of leaves; and (vi) prevention of seed production if infection occurred early. The virus was identified as tobacco streak virus (TSV), with physical properties similar to TRSV (1, 14, 23).

Symptoms reportedly induced by TRSV in fields of soybeans (13, 16, 20) could have been caused by TSV, as viruses can cause stunting, curling of stem tip, systemic necrotic spots on leaves, discoloration of stem

Table 2. Seed and pod production of Bansei soybeans inoculated with tobacco streak virus (TSV) in an approximately 25-C greenhouse

| Time of TSV inoculation after seeding | Yield[a] | | |
|---|---|---|---|
| | Pods/ plant | Seeds/ pod | % Mature seeds |
| *weeks* | | | |
| 2 | 5.8[b] | 1.38 | 32.4[c] |
| 4 | 11.0 | 1.54 | 49.4[c] |
| 6 | 9.8 | 1.69 | 46.2[c] |
| Control | 9.8 | 1.63 | 61.7 |

[a] Each value is the mean of five replicates.
[b] Significant at the 5% level by Students' t-test.
[c] Significant at the 10% level by Students' t-test.

TABLE 3. Height of seedlings grown at approximately 25 C in a greenhouse from seed harvested from infected Bansei soybeans

| Time of TSV inoculation after seeding | Length[a] (mm) | | |
|---|---|---|---|
| | Cotyledon to primary leaf | Primary leaf to 1st trifoliolate | Petiole length |
| *weeks* | | | |
| 2 | 31.8[b] | 10.1[c] | 19.6[c] |
| 4 | 39.3[d] | 13.7[c] | 16.8[c] |
| 6 | 38.4[d] | 18.6 | 39.7 |
| Control | 42.1 | 18.4 | 34.7 |

[a] Each value is the mean of six replicates.
[b] Significant at the 5% level by Students' t-test.
[c] Significant at the 3% level by Students' t-test.
[d] Significant at the 10% level by Students' t-test.

pith, stem streaking, pod blotching, and reduction in number of pods per plant in soybeans (1, 7). The Iowa isolate of TSV also produced similar symptoms in the greenhouse. Symptoms caused by both TSV and TRSV occur at the same time of year (28), and symptom similarities make field identification, diagnosis, and differentiation between the two viruses impossible at present. It is interesting that Costa et al. (7) suggested that in addition to TRSV, TSV may cause bud blight "da soja" in the USA, but no one has reported it to date.

Greenhouse studies indicate that only early infections cause a reduction in number of pods per plant. Infection at any age caused a delay in seed maturation that could result in losses at harvest. Attempts to transmit TSV by seed of tobacco, pea, and bean were unsuccessful (6, 19, 26, 29). Transmission by soybean seed prior to our study had not been attempted. We were able to show that TSV enters immature soybean seeds, causing a reduction in embryo and seedling vigor. Although the virus could be recovered from immature seed, we could not demonstrate TSV transmission by mature seeds.

The physical properties of the TSV isolate from Iowa resembled those of TSV more than those of TRSV (1, 6, 14, 23), but differences were so light that they could be mistakenly attributed to isolate variations.

The host ranges of TSV and TRSV are extensive (9, 12). TSV occurs worldwide [3, 4, as reported by Devergne in 1961, cited by Brunt (4), and by Gigante in 1961 and Jochems in 1930, cited by Costa & Carvalho (6)]. TSV naturally infects tobacco (3, 8, 14, 28), field bean (18, 22, 26, 29), alfalfa (30), garden bean (29), sweet clover (27), dahlia (4, 6), cotton (7), pea (19), and dodder on a desert shrub (15). Lack of infection in some plant species may be due to low concentration of inoculum, time of year, or age of plants. A distinct yellow mottle in pepper was observed in this study and by Zaumeyer (30), but virus was not recovered in either case.

Various isolates of TSV have been described (18, 19, 26, 29, 30). Costa (5) found the Brazilian isolate of TSV to be similar to the United States type strain (10). He differentiated the Brazilian isolate by (i) the incomplete symptom recovery of tobacco plants, which resulted in stiffness and narrowing of leaves that we observed on *N. glutinosa* and *N. tabacum*; (ii) petiolated leaves in sessile varieties; and (iii) more severe systemic symptoms.

Symptoms caused by the Iowa TSV isolate in tobacco more closely resemble those of the Brazilian isolate (6) than any reported for American isolates from or near field crops. Although there have been reports of flower and leaf abnormalities (5, 15, 26) in Turkish tobacco plants infected with the American isolates of TSV, Costa (5) differentiated the Brazilian isolate from American isolates by the severe flower modifications, split corollas, and filamentlike appendages on petals in many species and cultivars of *Nicotiana*. The Iowa TSV isolate caused similar flower modifications in three *Nicotiana* species, and other species would probably show similar symptoms. Crenated leaves in Turkish tobacco occurred here as was previously reported (26). *Cassia occidentalis* is a new host for TSV.

The effects of dilution on inactivation of TSV by oxidation are similar to those reported earlier (10). There may be other causes of inactivation, however (11).

TSV has been purified by Steere's method for TRSV (25), by charcoal-freezing (17), and by a method using 2-mercaptoethanol and hydrated calcium phosphate (11), which did not work well for us. Purification was difficult, probably because of the rapid inactivation of the virus. The density-gradient purification was the fastest method and the most successful, producing preparations of high infectivity.

Spherical particles with diam of 28-32 mμ were associated with infective preparations, particles with larger diam were associated with some of the noninfective fractions, and no particles were observed from the top component from the density-gradient tubes. The size of the infective virus particles is within the range of sizes reported for TSV (25-30 mμ) (4, 11, 17, 21) and TRSV (24). Fulton (10) observed particle disruption in preparations of TSV in electron-microscope studies, and associated the disruption with the possible fragility of the protein coat of the virus. The larger particles associated with the middle zones in density-gradient tubes in this work may have been due to expansion of virus particles, which decreased their density, resulting in complete disruption leaving individual protein subunits. Loss of infectivity may have been due to the action of RNase, as the protein subunits of the virus particles separated and exposed the viral RNA. Four zones from the red node strain of TSV (17) also may have been a result of virus inactivation, but the number of zones is thought to be related to the time of the host infection. Present results showed four zones with preparations from tissue infected for 5 days. Fulton (11) suggested that the upper components may have been lost in his purification procedure, and the homogeneous size of particles found in the final pellet from low speed-high speed purification in this study suggests that he was correct.

The serological tests provided conclusive evidence

that this Iowa soybean virus was TSV because a positive reaction occurred only with TSV antiserum. Serology is the only effective method of distinguishing between soybeans infected by TSV and TRSV until quick and clear-cut host differentials are demonstrated.

LITERATURE CITED


1. ALLINGTON, W. B. 1946. Bud blight of soybean caused by the tobacco ring-spot virus. Phytopathology 36: 319-322.
2. BALL, E. M. 1961. Serological tests for the identification of plant viruses. Brochure. Amer. Phytopathol. Soc., St. Paul, Minn. 16 p.
3. BERKELEY, G. H., & J. H. H. PHILLIPS. 1943. Tobacco streak. Can. J. Res. (C) 21:181-190.
4. BRUNT, A. A. 1968. Tobacco streak virus in dahlias. Plant Pathol. 17:119-122.
5. COSTA, A. S. 1945. The relationship between American tobacco streak and Brazilian "necrose branca" or "couve". Phytopathology 35:1029-1030.
6. COSTA, A. S., & A. M. B. CARVALHO. 1961. Studies on Brazilian tobacco streak. Phytopathol. Z. 42:113-138.
7. COSTA, A. S., S. MIYASAKA, & A. J. D'ANDREA PINTO. 1955. Queima dos brotos da soja, uma moléstia causada pelo vírus da necrose branca ou couve. Bragantia 14:vii-x.
8. DIACHUN, S., & W. D. VALLEAU. 1954. Reaction of some species of *Nicotiana* to tobacco mosaic virus, tobacco streak virus, *Pseudomonas tabaci,* and *Phytophthora parasitica* var. *nicotianae.* Ky. Agr. Exp. Sta. Bull. 618. 12 p.
9. FULTON, R. W. 1967. Purification and some properties of tobacco streak and tulare apple mosaic viruses. Virology 32:153-162.
10. FULTON, R. W. 1948. Hosts of the tobacco streak virus. Phytopathology 38:421-428.
11. FULTON, R. W. 1949. Virus concentration in plants acquiring tolerance to tobacco streak. Phytopathology 39:231-243.
12. HENDERSON, R. G., & S. A. WINGARD. 1931. Further studies on tobacco ringspot in Virginia. J. Agr. Res. 43:191-207.
13. JOHNSON, F. 1943. Soybean streak in Ohio. Plant Dis. Reptr. 27:86-87.
14. JOHNSON, J. 1936. Tobacco streak, a virus disease. Phytopathology 26:285-292.
15. LACKEY, C. F. 1966. Recovery of a virus resembling tobacco streak from dodder growing on a desert shrub in southern California. Plant Dis. Reptr. 50: 479-482.
16. MELHUS, I. E. 1942. Soybean diseases in Iowa in 1942. Plant Dis. Reptr. 26:431-432.
17. MINK, G. I., K. N. SAKSENA, & M. J. SILBERNAGEL. 1966. Purification of bean red node virus. Phytopathology 56:645-649.
18. PATINO, G. 1958. A new strain of tobacco streak virus. Phytopathology 48:463 (Abstr.).
19. PATINO, G., & W. J. ZAUMEYER. 1959. A new strain of tobacco-streak virus from peas. Phytopathology 49: 43-48.
20. SAMSON, R. W. 1942. Tobacco ringspot on edible soybeans in Indiana in 1941. Plant Dis. Reptr. 26:382.
21. SCOTT, H. A., M. VINCENT, & W. J. ZAUMEYER. 1961. Serological studies of red node and pod mottle viruses. Phytopathology 51:755-758.
22. SKILES, R. L., & W. D. THOMAS, JR. 1948. Red node and shiny pod viruses of field beans. Colo.-Wyo. Acad. Sci. Jour. 3:44 (Abstr.).
23. SMITH, K. M. 1957. A textbook of plant virus diseases. Little, Brown and Co., Boston, Mass. [2nd ed.]. 652 p.
24. STEERE, R. L. 1953. Tobacco ringspot virus, a polyhedron purified with the use of butanol. Phytopathology 43:484 (Abstr.).
25. STEERE, R. L. 1956. Purification and properties of tobacco ringspot virus. Phytopathology 46:60-69.
26. THOMAS, H. R., & W. J. ZAUMEYER. 1950. Red node, a virus disease of beans. Phytopathology 40:832-846.
27. VALLEAU, W. D. 1940. Sweet clover, a probable host of tobacco streak virus. Phytopathology 30:438-440.
28. VALLEAU, W. D., E. M. JOHNSON, & S. DIACHUN. 1952. Tobacco diseases. Ky. Agr. Exp. Sta. Bull. 581. 62 p.
29. VIRGIN, W. J. 1943. An unusual bean disease. Phytopathology 33:743-745.
30. ZAUMEYER, W. J. 1959. New tobacco streak virus from symptomless alfalfa infectious to beans. Phytopathology 49:555-556 (Abstr.).