Epidemiology and Seed Transmission of Two Tobacco Streak Virus Pathotypes Associated with Seed Increases of Legume Germ Plasm in Eastern Washington

WALTER J. KAISER, Research Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, Western Regional Plant Introduction Station, Washington State University, Pullman 99164-6402; STEPHEN D. WYATT, Associate Professor, Department of Plant Pathology, Washington State University, Pullman 99164-6430; and ROBERT E. KLEIN, Assistant Plant Pathologist, Irrigated Agriculture Research and Extension Center, Washington State University, Route 2, Box 2953-A, Prosser 99350-9687

ABSTRACT

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Two pathotypes of tobacco streak virus (TSV), designated I and II, were isolated from naturally infected bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*), adzuki bean (*Vigna angularis*), fenugreek (*Trigonella foenum-graecum*), alfalfa (*Medicago sativa*), and white sweet clover (*Melilotus alba*) at Central Ferry, WA. The two pathotypes could be distinguished by their host reactions, serology, and by SDS-polyacrylamide gel electrophoresis. Pathotype I infected cowpea (*Vigna unguiculata*), but pathotype II did not. Pathotype I isolates were similar, if not identical, to the red node strain of TSV in bean, whereas pathotype II isolates induced green to yellow mosaics in this host. Alfalfa and white sweet clover were overwintering hosts of both pathotypes. Pathotype I isolates were seed transmitted in naturally infected Black Turtle Soup (BTS) bean at a rate of 3.8%. Isolates of pathotypes I and II were seed transmitted in six of nine and five of nine mechanically inoculated bean cultivars, respectively, at rates of 0.9–15.1% and 0.5–2.4%. Inoculation of BTS bean in the greenhouse with pathotypes I and II reduced seed yields by 14–49%, and seed transmission ranged from 0.5 to 32.8% depending on the virus isolate and method of inoculation. In greenhouse trials, an isolate of pathotype I was seed transmitted in artifically infected adzuki bean (29%) but not in chickpea or fenugreek. Seed transmission of TSV was not detected in naturally infected alfalfa from Central Ferry. Inoculation of chickpea at Central Ferry with isolates of pathotypes I and II reduced seed yields at prebloom by 77–96% and at full bloom by 25–71%. Contrary to results from greenhouse tests, seed transmission of pathotypes I and II in prebloom-inoculated, field-grown chickpeas ranged from 1.1 to 11.1%. BTS bean trap plants placed in screened cages at biweekly intervals at Central Ferry became infected with both pathotypes of TSV. Transmission of TSV to bean plants in the cages differed greatly depending on the type of screen covering the cages. Maximum infection of 83% occurred on 9 July in cages with screens with larger openings (500 μm), compared with 11% of the trap plants in cages with screens with smaller openings (110 μm). Results suggest that an insect vector smaller than alate aphids is responsible for field spread of TSV to beans.

Germ plasm maintained by the Western Regional Plant Introduction Station at Pullman, WA, is periodically propagated in the field at Central Ferry, WA. Because TSV is reported to infect many plant species (3), we were concerned that germ plasm propagated at Central Ferry could become infected with seed-transmitted isolates of the virus. This could compromise our project's seed production efforts. We were particularly concerned with the bean germ plasm collection, which has more than 11,000 plant inventory (PI) accessions, because Thomas and Graham (16) reported that the red node strain of TSV was transmitted in an average of 1.47% of 21,424 seeds of several Pinto bean cultivars.

Isolates of tobacco streak virus (TSV) obtained from naturally infected white sweet clover (*Melilotus alba* Medik.) and Clay cowpea (*Vigna unguiculata* (L.) Walpers) at Central Ferry were grouped into two pathotypes (7). The pathotypes, designated I and II, belong to distinct serotypes. Both pathotypes were seed transmitted (<3%) in naturally infected white sweet clover. In greenhouse inoculation tests, a cowpea isolate of pathotype I was transmitted in 0.7–90.6% of the seed from mechanically inoculated plants of *Chenopodium quinoa* Willd., *Glycine max* (L.) Merr., *Gomphrena globosa* L., *Nicotiana clevelandii* A. Gray, and *V. unguiculata* (7). The isolate was also transmitted by thrips (7). In addition to being serologically distinct, the two pathotypes induced different symptoms in bean (*Phaseolus vulgaris* L.). Pathotype I isolates induced reddening of the nodes of infected bean plants, but pathotype II isolates did not. Based on serology and symptomatology, pathotype I isolates are similar, if not identical, to the bean red node strain of TSV (15).

The purposes of this study were to produce antisera to pathotypes I and II of TSV, observe the incidence and natural host range of isolates of these two pathotypes at Central Ferry, investigate seed transmission of both pathotypes in different bean cultivars and other legumes, determine the effects of virus infection on seed yield and quality of bean and chickpea (*Cicer arietinum* L.), and observe the time and amount of infection by placing bean trap plants in the field at biweekly intervals during the growing season.

MATERIALS AND METHODS

Source of virus. Most inoculation experiments were conducted with one isolate each of pathotypes I and II of TSV from Central Ferry. Isolate Mel 40 of pathotype I was from a *M. alba* plant infected from seed in 1984. Isolate Mel F of pathotype II was isolated from a naturally infected field plant of *M. alba* in 1981. In one chickpea inoculation study at Central Ferry, two additional isolates of pathotype II from Central Ferry were used. Isolates Mel 3 and BTS II-C2a were isolated from naturally infected plants of *M. alba* in 1984 and Black Turtle Soup (BTS) bean in 1986, respectively. All TSV isolates were maintained in *C. quinoa* or in infected leaves dried over Drierite (8-mesh anhydrous $CaSO_4$) and stored at 4 C. In inoculation studies, infected tissue of *C. quinoa* (dried or fresh) was triturated in 0.06 M K_2HPO_4 + 0.5% celite, to which a small amount of Carborundum (600 grit) was added. The triturate was rubbed on the leaves of test plants with the thumb and forefinger and immediately rinsed off with tap water. After 10–14 days, plants were assayed for systemic infection by mechanical inoculation to healthy *C. quinoa* indicator plants.

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Field studies. The incidence of infection of annual and perennial plant species by isolates of pathotypes I and II was determined at Central Ferry. Tissue samples were collected at periodic intervals and indexed for TSV infection on *C. quinoa* or tested by indirect enzyme-linked immunosorbent assay (ELISA). All hosts were annuals, except *M. alba* and alfalfa (*Medicago sativa* L.). Tissue of these two perennial hosts was collected from the same specific area at Central Ferry.

An experiment was initiated in 1986 to establish the time of infection of Black Turtle Soup 1 (BTS) (13) bean plants placed at three locations adjacent to an alfalfa field and volunteer plants of *M. alba* at Central Ferry. Healthy BTS bean seedlings were transplanted to pasteurized potting medium (55% peat moss, 35% pumice, 10% sand) in 15-cm-diameter plastic pots, with three plants per pot. When plants were 10–14 days old, three pots were placed in yellow plastic trays (28 cm wide × 34 cm long × 13 cm deep) to which approximately 2 L of tap water was added for irrigation purposes. At each site, trays of pots were placed in nylon screen cages (76 cm diameter × 90 cm high). The bottom edge of each cage was buried in soil, and the opening of the cage at the top was covered with a snug-fitting cover made of the same fine-mesh nylon screen. There were two cages per treatment per site. A treatment in which plants were exposed to the ambient environment (placed in yellow trays with no cage) was discontinued because plants were devoured by grasshoppers beginning in mid-June. Cages were covered with two types of fine-mesh nylon screen (Tetko, Inc., Elmsford, NY) to exclude larger insects, such as aphids (500-μm openings) (treatment 1) and to exclude or reduce ingress of smaller insects, such as thrips (110-μm openings) (treatment 2). Plants were placed in the field at 12- to 16-day intervals from 29 May to 3 September 1986. After exposure, plants were returned to Pullman, sprayed with diazinon, and incubated in an insect-protected greenhouse. After 4–5 wk, plants were indexed on *C. quinoa* or tested for TSV infection and other viruses by indirect ELISA. Seeds from TSV-infected plants were tested for seed transmission of TSV.

Virus-free seeds of chickpea PI 458870 were planted in single rows 5.3 m long with 1.5 m between rows in a randomized complete block design with four replicates of 50 seeds per row per replicate. Plots were manually inoculated with isolate Mel 40 of pathotype I and isolates BTS II-C2a and Mel 3 of pathotype II at prebloom (33 days after planting) and full bloom to early pod set (53 days after planting). Inoculum was increased in *C. quinoa*. Plants were inoculated following the procedure outlined earlier. Twenty-five to 30 days after inoculation, plants exhibiting virus symptoms were tagged and mortality counts were made after 70 days. Plants were harvested 96 days after planting, and seed yields were determined from 25 tagged plants in each plot. Seed size was determined by passing seeds through metal sieves with round holes. Seeds harvested from plants infected with the different TSV isolates in the prebloom and full-bloom plots were tested for seed transmission of the virus.

Greenhouse studies. Germinating seeds of healthy BTS bean were transplanted in pasteurized potting medium with one plant per 15-cm-diameter plastic pot. In two treatments, plants were inoculated in the primary leaf stage with isolates Mel 40 or Mel F. The third treatment consisted of seedlings that were indexed and found to be infected from seed with isolate Mel 40. A healthy control was included as the fourth treatment. There were 25 plants per treatment. Plants were incubated in the greenhouse at 20–25 C. On a per plant basis, information was collected on the number of pods, seeds per pod, total seeds, and weight of seeds. Seed from the different treatments was tested for seed transmission of TSV by planting seed in moist vermiculite, transplanting seedlings to sterile potting medium in metal trays (30 cm wide × 50 cm long × 7 cm deep) and indexing plants on *C. quinoa* after 2–3 wk.

Seed transmission. Germinating seeds of different bean cultivars were transplanted in sterile potting medium in 15-cm-diameter plastic pots with one plant per pot. The plants were inoculated with isolates Mel 40 and Mel F in the primary leaf stage following the inoculation procedures previously outlined. Plants were indexed on *C. quinoa* 10–14 days after seed germination to identify those infected with TSV. Seeds from infected plants were planted in moist vermiculite, and germinating seeds were transplanted to pasteurized potting medium in metal trays. Two to 4 wk after being transplanted, plants were indexed on *C. quinoa* in groups of one to five plants. If test plants exhibited symptoms of TSV, each seedling in the group was indexed individually on *C. quinoa*. Seeds of naturally infected or artificially inoculated chickpea, fenugreek (*Trigonella foenum-graecum* L.), alfalfa, and adzuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi) were also tested for seed transmission of TSV.

Virus purification. Virus was purified from 50 to 100 g of infected tissue of *C. quinoa* 7–10 days after inoculation with a modification of the procedure of Uyeda (17) and Mink et al (11). Tissue was blended in 0.05 M acetate buffer, pH 6.5, containing 0.05 M EDTA, 0.01 M NaDIECA, and 0.01 M L-cysteine. Extracts were clarified by emulsification with chloroform (1 ml of chloroform per 4 ml of extract), followed by centrifugation for 10 min at 10,000 *g* and then a freeze- (−20 C) thaw treatment, followed by a second centrifugation at 10,000 *g* for 10 min. Two cycles of differential centrifugation were used to purify and concentrate the virus. The virus was pelleted by centrifugation at 50,000 rpm for 1 hr in a Beckman Ti 50.2 rotor, and the pellets were resuspended in 0.05 M acetate buffer, pH 6.5. For the second ultracentrifugation, the virus was sedimented through a cushion of 20% sucrose. For sucrose gradient centrifugation, 10–40% sucrose gradients were made in 0.05 M acetate buffer, pH 6.5, containing 0.002 M EDTA. Loaded gradients were centrifuged for 3 hr at 27,000 rpm in a Beckman SW 27 rotor. After centrifugation, the gradients were fractionated, and nucleoprotein was again sedimented by ultracentrifugation and injected into rabbits for the production of antiserum.

Virus protein samples were heated at 65 C for 5 min and electrophoresed on SDS-acrylamide gels. The gel was a 7–18% gradient and employed a buffer system as described by Laemmli (9). Virus (1 mg/ml) was treated with 0.06 M Tris buffer, pH 6.8, containing 2% SDS, 5% 2-mercaptoethanol, and 10% glycerol. Protein standards were from Sigma (VII-L).

Serology. New Zealand white rabbits were initially injected subcutaneously along the back at four places with a total of 4 ml of purified antigen (1 mg/ml) emulsified with Freund's complete adjuvant (1:1). Subcutaneous injections were repeated 2 wk after the first injections. Hyperimmune sera were produced by intravenous injections (1 mg/ml) every 2 wk starting 10 days after the second subcutaneous injection until usable antisera were produced. IgG was recovered from the serum by precipitation with 50% saturated ammonium sulfate, and the IgG preparations were cross-absorbed with acetone-precipitated healthy *C. quinoa*. The acetone powder was extracted with 80% ethanol at 50 C for 30 min and then washed with phosphate-buffered saline (PBS). The washed powder was incubated with 2 ml of purified IgG at 37 C for 1 hr and then removed by centrifugation at 10,000 *g* for 10 min.

For indirect ELISA, tissue samples were diluted 1:10 in pH 7.4 PBS-Tween 20 (0.05%), ovalbumin (0.2%), and polyvinylpyrrolidone (2%) and absorbed to Immulon 2 flat-bottomed plates (Dynatech Laboratories, Inc., Alexandria, VA) at room temperature for 1 hr. IgG was diluted to 1 μg/ml with the above buffer, applied to the plates, and incubated 1 hr. Anti-rabbit IgG:alkaline phosphatase conjugate (0.85 mg/ml) was diluted 1:3,200 in the above buffer, applied to the plates, and incubated for 3 hr at room

temperature. Microtiter plate wells were washed three times between each step with PBS-Tween 20. *p*-Nitrophenyl phosphate was used as alkaline phosphatase substrate, at 1 mg/ml, and plates were read at 410 nm on a Dynatech Minireader II.

Some pathotype II isolates of TSV induced yellow mosaic symptoms in bean that were similar to those induced by some systemic isolates of alfalfa mosaic virus (AMV). Indirect ELISA tests involving various polyclonal antisera were conducted with different TSV and AMV isolates in crude sap of *C. quinoa* to establish whether TSV isolates of pathotypes I and II and AMV were distinct and serologically distinguishable. Pathotypes I (from *T. foenum-graecum* [Tfg] provided by J. Larsen, WSU, Pullman) and II (Mel F from J. Larsen) and AMV (K. R. Bock, Lilongwe, Malawi) were compared. Pathotype I isolates of TSV included Mel 40, Tfg, and bean red node from G. I. Mink, IAREC, Prosser, WA (BRN-M). Pathotype II isolates included Mel F, Mel 3, BTS II-C2a, and BTS II-C2a seed transmitted in chickpea (BTS II-C2a-CST). Isolates of AMV were from alfalfa and the American Type Culture Collection (ATCC 105).

Fig. 1. Reddening of the nodes (arrows) of Bountiful bean infected with isolate Mel 40, pathotype I, of tobacco streak virus.

Fig. 2. Stunting and necrosis of a Black Turtle Soup bean plant (arrow) infected with isolate Mel 40, pathotype I, of tobacco streak virus. Healthy plant, left.

RESULTS

Symptoms in bean. At Central Ferry, TSV pathotypes I and II were isolated from naturally infected bean cultivars Bountiful and BTS. In these two cultivars, pathotype I isolates, as exemplified by Mel 40, produce a reddening of the nodes (Fig. 1) and the pulvini of the leaves and leaflets. These symptoms are usually accompanied by a necrosis and reddening of the veins of primary and trifoliate leaves. Discoloration and necrosis of stem and shoot tips may follow. Sunken, reddish lesions often develop on young pods, and when necrosis is extensive, pods become shriveled and deformed. Seeds from symptomatic pods are often misshapen and discolored. Plants infected from seed or in the seedling or prebloom stage are frequently stunted (Fig. 2). Pathotype II isolates, typified by Mel F, induce a green to yellow mosaic (Fig. 3) in infected Bountiful and BTS bean plants. The yellow mosaic symptoms are reminiscent of the systemic symptoms induced in bean by some isolates of AMV (5).

Field studies. From 1981 to 1988, surveys were made of different plant species growing adjacent to our plots and naturally infected by pathotypes I and II of TSV at Central Ferry (Table 1). Both pathotypes of the virus were isolated from *C. arietinum*, *M. sativa*, *M. alba*, *T. foenum-graecum*, and *V. angularis*, whereas only pathotype I was isolated from *V. unguiculata*. A higher percentage of *M. alba*, *V. angularis*, and *V. unguiculata* were infected by isolates of pathotype I, whereas pathotype II isolates prevailed in *M. sativa*. In some years, infection of *M. alba* and *V. unguiculata* by pathotype I isolates exceeded 40%, whereas infection of *M. sativa* by pathotype II isolates ranged from 3 to 22%. TSV was symptomless in naturally infected *M. sativa*, *M. alba*, and *V. unguiculata*.

BTS bean trap plants were placed in the field at Central Ferry at 12- to 16-day intervals beginning on 19 May 1986. TSV was isolated from trap plants only during the period from 25 June to 4 August (Fig. 4). Maximum infection of trap plants by TSV in treatment 1 (plants in cages covered with nylon screen that excluded larger insects, such as aphids) occurred on 9 July when 83% of the bean plants were infected by the virus (50 and 33% of the plants were infected by isolates of pathotypes I and II, respectively). In treatment 2 (plants in cages covered with nylon screen that excluded or reduced ingress of smaller insects), 11% of the plants were infected with TSV (only isolates of pathotype I). On 4 August, only one plant in treatment 1 was infected with an isolate of pathotype I. Plants naturally infected with isolates of pathotype I frequently were stunted and exhibited reddening of the nodes and necrosis of the leaves, stem, and apical tissues. Plants were tested for other viruses, such as AMV, by ELISA and

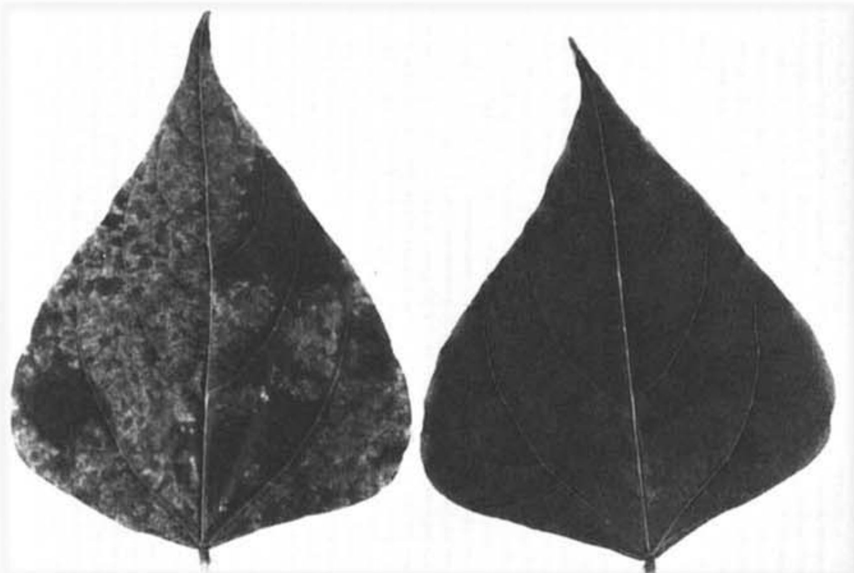

Fig. 3. Yellow mosaic symptoms in leaf of Black Turtle Soup bean (left) infected with isolate Mel F, pathotype II, of tobacco streak virus. Healthy leaf, right.

Table 1. Incidence of two pathotypes of tobacco streak virus (TSV) in seven plant species at Central Ferry, WA[a]

			Pathotype I[b]		Pathotype II	
Plant species	Year	Plants tested (no.)	No. infected	Percent infected	No. infected	Percent infected
Cicer arietinum	1986	40	1	2.5	1	2.5
Medicago sativa	1985	14	0	0	2	14.2
M. sativa	1986	168	2	1.2	3	1.8
M. sativa	1987	214	1	0.5	48	22.4
M. sativa	1988	170	2	1.2	33	19.4
Melilotus alba	1981	15	4	26.7	1	6.7
M. alba	1982	18	6	33.3	2	11.1
M. alba	1984	43	20	46.5	11	25.6
M. alba	1986	37	3	8.1	4	10.8
M. alba	1987	38	4	10.5	2	5.3
Phaseolus vulgaris	1986	288	38	13.2	22	7.6
Trigonella foenum-graecum	1984	10	2	20.0	2	20.0
Vigna angularis	1987	45	9	20.0	1	2.2
V. unguiculata	1981	30	3	10.0	0	0
V. unguiculata	1982	16	7	43.8	0	0

[a] TSV detected by serology (agar-gel diffusion tests and indirect ELISA) and by indexing on *Chenopodium quinoa*. Plants with mixed infections of pathotypes I and II were not detected.
[b] Pathotype I isolates of TSV induce red node symptoms in Black Turtle Soup bean, whereas pathotype II isolates induce mosaic symptoms in this host.

host indexing, but none were found. Seeds from infected BTS bean plants were tested for seed transmission of TSV. Isolates of pathotype I were transmitted in nine of 236 seeds (3.8%), but no transmission was detected in 106 seeds of plants infected with isolates of pathotype II.

Isolates of TSV pathotypes I and II induced similar symptoms in chickpea. Initial symptoms of infection were necrosis of shoot tips, chlorosis and mosaic, phloem discoloration, and stunting. Leaflets on virus-infected plants tended to be smaller and there was a proliferation of the axillary buds. Plants infected before flowering usually produced little or no seed, whereas pod and seed set were decreased with later infections. Inoculation of chickpea PI 458870 with isolates of pathotypes I and II reduced seed yields at prebloom by 77–96% and full bloom by 25–71% (Table 2). The largest yield reductions occurred with isolate Mel 40 of pathotype I regardless of plant growth stages, followed by isolate BTS II-C2a of pathotype II. Mortality was observed only in plants inoculated at prebloom and ranged from 20 to 22% with the two isolates of pathotype II to 89% with the pathotype I isolate. Seed transmission of isolate Mel 40 was detected in two of 18 seeds and in 2 of 189 seeds of isolate BTS II-C2a in prebloom-inoculated plants. No transmission of isolate Mel 3 was detected in 209 seeds from prebloom-inoculated plants. No transmission was observed in 538 seeds from plants infected at full bloom with isolates of both pathotypes.

Greenhouse studies. The effect of infection of BTS bean by isolates Mel 40 and Mel F of pathotypes I and II, respectively, on four yield parameters and seed transmission of TSV was studied under greenhouse conditions (Table 3). Pod number per plant was the only yield parameter that was not adversely affected by infection with either isolate. Isolate Mel 40 significantly reduced the mean number of seeds per pod and plant and weight of seeds per plant. When bean plants were infected from seed with isolate Mel 40 or inoculated in the seedling stage with isolates Mel 40 and Mel F, mean seed yields per plant were reduced by 49.5, 34.8, and 14.2%, respectively. Seed yield was inversely proportional to frequency of TSV seed transmission. Seed transmission of TSV ranged from 0.5 to 32.8%, depending on the virus isolate and method of infecting plants (Table 3). Isolate Mel F was transmitted in 0.5% of the seed from infected bean plants. Seed transmission of isolate Mel 40 was 15.1 and 32.8% in bean plants infected by mechanical inoculation or from seed, respectively.

Seed transmission. Seeds harvested from plants of *M. sativa* naturally infected with either TSV pathotype I or II at Central Ferry and of *C. arietinum*, *T. foenum-graecum*, and *V. angularis* mechanically infected with isolate Mel 40 of pathotype I were tested for seed transmission of the virus. TSV was seed transmitted only in *V. angularis*, in which 13 of the 44 seeds were infected with the virus. Plants of *V. angularis* infected from seed were symptomless, but the virus could be detected in leaf tissue by inoculation to *C. quinoa*.

In greenhouse inoculations of nine bean cultivars, TSV isolate Mel 40 of pathotype I was seedborne in six of nine cultivars, while isolate Mel F of pathotype II was seedborne in five of nine cultivars (Table 4). Seed transmission of TSV isolates Mel 40 and Mel F ranged from 0.9 to 15.1 and 0.5 to 2.4%, respectively. Seed transmission of isolate Mel 40 in bean cultivars Red Mexican UI 34 and BTS occurred at rates of 15.1 and 14.4%, respectively. Dubbele Witte and Stringless Green Refugee were the only bean cultivars in which TSV isolates Mel 40 and Mel F were not seed transmitted. Plants infected from seed with isolate Mel 40 often showed typical red node symptoms, whereas those infected with Mel F exhibited green to yellow mosaic symptoms.

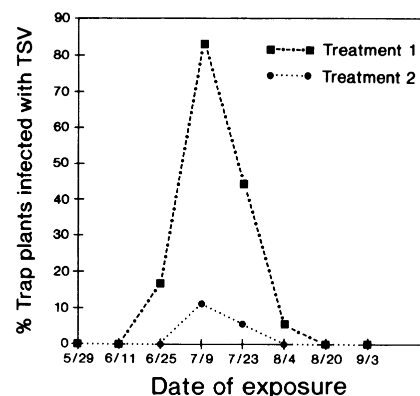

Fig. 4. Incidence of tobacco streak virus (TSV) in bean (*Phaseolus vulgaris*) cv. Black Turtle Soup trap plants placed in the field at Central Ferry, WA, for 12- to 16-day intervals beginning on 29 May 1986. Plants were placed in cages with fine-mesh nylon screen, the openings of which varied in size (treatment 1 = 500 × 500 μm; treatment 2 = 110 × 110 μm). Infection by TSV was determined by inoculation of *Chenopodium quinoa* virus indicator plants and by indirect ELISA.

Virus purification and strain comparison. TSV isolates representative of pathotype I (Tfg) and pathotype II (Mel F) were purified from *C. quinoa* tissues. Upon centrifugation into sucrose density gradients, three nucleoprotein zones (106S, 84S, and 76S) typical of TSV were observed. There was no difference between isolates Tfg and Mel F with respect to sedimentation rates. Because gradient profiles suggested that our virus preparations contained some slow-sedimenting host material, virus preparations were routinely purified by sucrose grad-

Table 2. Effects of infection by three isolates of tobacco streak virus (TSV) on seed yield, seed size, mortality, and seed transmission in chickpea PI 458870 inoculated at two stages of growth at Central Ferry, WA

TSV isolate[v]	Growth stage[w]	Mean seed yield per plant (g)[x]	Mortality (%)	Seed size[y] (mm) Yield retained by metal sieve (%)			Seed transmission		
				9.6	8.0	6.4	Seeds (no.)	No. infected	Percent infected
Healthy control		23.6 a[z]	0	34.3 a	59.1 a	5.2 c	100	0	0
BTS II-C2a	Prebloom	1.9 d	22.0	7.5 c	49.4 b	53.0 a	189	2	1.1
	Full bloom	14.7 b	0	24.3 b	65.6 a	8.6 c	140	0	0
Mel 3	Prebloom	5.5 c	20.2	11.5 c	57.3 a	24.6 b	209	0	0
	Full bloom	17.7 b	0	32.1 a	61.5 a	5.4 c	148	0	0
Mel 40	Prebloom	1.0 d	89.2	0 d	12.2 c	7.4 c	18	2	11.1
	Full bloom	6.9 c	0	11.6 c	55.3 a	24.8 b	250	0	0

[v] TSV pathotypes I (Mel 40 = bean red node) and II (BTS II-C2a and Mel 3).
[w] Prebloom and full-bloom plots were inoculated 33 and 53 days after planting, respectively, and plots were harvested after 96 days.
[x] Mean yield per plant is based on seed harvested from 25 plants in each of four 5.3-m single-row plots for all treatments except the Mel 40 prebloom plots, where yield was based on four plants per plot because of high mortality.
[y] Seed was graded into different sizes by sieving.
[z] Numbers in the same column followed by the same letter do not differ significantly ($P = 0.05$) from each other according to Duncan's new multiple range test.

Table 3. Effects of infection by two pathotypes of tobacco streak virus (TSV) on four seed yield parameters and seed transmission in Black Turtle Soup bean in greenhouse inoculation trials[x]

Treatment[y]	Mean pods per plant (no.)	Mean seeds per pod (no.)	Mean seeds per plant (no.)	Mean seeds per plant (wt.)	Seed transmission		
					Seeds (no.)	No. infected	Percent infected
Healthy	16.79 a[z]	5.63 a	94.05 a	20.81 a	354	0	0
Inoculated pathotype II	15.58 a	5.33 a	82.86 ab	17.85 b	366	2	0.5
Inoculated pathotype I	19.80 a	3.54 b	69.64 bc	13.57 c	378	57	15.1
Seedborne pathotype I	15.58 a	3.72 b	58.14 c	10.51 d	402	132	32.8

[x] Results based on harvest of pods and seeds from 25 plants grown singly in 15-cm-diameter plastic pots.
[y] TSV pathotypes I (Mel 40 = bean red node) and II (Mel F) inoculated in the seedling stage. Seedborne pathotype I was infected from seed.
[z] Numbers in the same column followed by the same letter do not differ significantly ($P = 0.05$) from each other according to Duncan's new multiple range test.

Table 4. Transmission of two pathotypes[a] of tobacco streak virus (TSV) in seed of different bean cultivars[b]

Bean cultivar	Pathotype I Tested (no.)	Pathotype I Infected[c] (no.)	Pathotype II Tested (no.)	Pathotype II Infected (no.)
Black Turtle Soup	146	21	158	1
Bountiful	235	2	210	1
Dubbele Witte	122	0	148	0
Great Northern UI 123	136	0	130	3
Monroe	77	1	130	0
Pinto UI 111	213	18	214	1
Pinto UI 114	105	8	151	2
Red Mexican UI 34	106	16	116	0
Stringless Green Refugee	84	0	120	0

[a] TSV pathotypes I (Mel 40 = bean red node) and II (Mel F).
[b] Seeds were harvested from plants that were mechanically inoculated with each isolate of TSV in the seedling stage.
[c] Bean seedlings were indexed on *Chenopodium quinoa* to detect infected seedlings.

ient centrifugation as a final step. Virus yields were typically 0.1 mg of virus per gram of tissue at 5–7 days postinoculation.

Although both Tfg and Mel F were purified with the same technique, they were shown to differ in one physical test. Tfg extracts could be clarified by shifting the pH from 6.5 to 4.8 briefly before centrifugation at 10,000 *g*, but Mel F could not. Such a pH shift or attempted purification of isolate Mel F in pH 5.0 and 6.0 (0.05 M) sodium acetate buffer resulted in a considerable loss of virus during purification.

To determine purity of our virus preparations and coat protein molecular weights, preparations of Tfg and Mel F were treated with SDS and electrophoresed on SDS-polyacrylamide gels. In four electrophoretic experiments, Tfg migrated more slowly than Mel F, indicating molecular weights of approximately 25,500 Da, but the differences were qualitative rather than quantitative (Fig. 5). There was no indication of protease reduction of Mel F protein size. Virus preparations were slightly contaminated with traces of other proteins. Mel F and Tfg dimers were also detected by staining. Dimerization was confirmed by electrophoresis of V8 protease digested excised gel bands (1).

Serology. Rabbit antisera were prepared against Tfg and Mel F isolates. The antisera were routinely cross-absorbed with protein isolated from healthy *C. quinoa*. IgG fractions were efficient at specifically detecting virus protein when used in indirect ELISA (Table 5). TSV isolates can be identified to serotype with a combination of homologous and heterologous tests. Although Mel F antiserum was specific for pathotype II isolates, the specificity of Tfg antiserum for pathotype I isolates required adsorption by dilution of the antiserum in Mel F-infected *C. quinoa* extract before placement in the ELISA plate. Otherwise, Tfg antiserum cross-reacted with Mel F at about a 2–3:1 ratio. Neither Tfg nor Mel F antisera reacted with two isolates of AMV. The AMV antiserum reacted only with the two AMV isolates and not with any of the TSV pathotype I and II isolates.

DISCUSSION

TSV pathotypes I and II have been isolated from six hosts at Central Ferry,

including white sweet clover (7), adzuki bean, alfalfa, bean, chickpea, and fenugreek. To our knowledge, this is the first report of natural infection of adzuki bean, chickpea, and fenugreek. TSV seed transmission was detected in field studies with chickpea and in greenhouse studies with adzuki bean but not in greenhouse studies with chickpea and fenugreek. Chickpea and adzuki bean appear to be new hosts of TSV in which the virus is seed transmitted.

About 4% seed transmission of pathotype I isolates was observed in BTS beans naturally infected with TSV at Central Ferry. In greenhouse inoculation tests with TSV pathotypes I and II, isolates Mel 40 and Mel F were seed transmitted in seven of nine bean cultivars, with transmission rates of up to 15%. Both pathotypes were seed transmitted in two Pinto bean cultivars, one of which (Pinto UI 111) was also tested by Thomas and Graham (16) in the only report of TSV seed transmission in bean. Various factors can affect seed transmission, such as host genotype, virus isolate, time of infection, and environmental conditions (10). It is possible that one or more factors critical for TSV transmission in bean seeds is lacking because, although TSV infects beans in different growing areas in the western United States, there is only one report of TSV seed transmission in bean.

Natural infection of BTS bean trap plants by TSV at Central Ferry occurred over a 5-wk period beginning 25 June 1986, with a peak occurring 9 July. More than 80% of the trap plants in treatment 1 were infected by TSV on 9 July, compared with only 11% in treatment 2. An aerial vector appears to have been responsible for TSV spread to the trap plants contained in the screened cages, particularly in treatment 1. (The screen excluded larger insects, such as alate aphids but allowed passage of smaller insects, including thrips.) Although the screen in treatment 2 excluded or reduced ingress of nearly all insects including thrips, it was not possible to completely protect the trap plants from potential TSV vectors during irrigation and transportation into and out of the field. The relatively low level of TSV infection in treatment 2 is likely a result of this. TSV pathotype I is transmitted by the western flower thrips (*Frankliniella occidentalis* (Pergande)) and/or the onion thrips (*Thrips tabaci* Lind.) (7), and both species readily colonize white sweet clover and alfalfa at Central Ferry (W. J. Kaiser, *unpublished data*), the most important reservoir hosts of both TSV pathotypes. The period of maximum trap plant infection by TSV (9 July and 23 July) also coincided with the period of profuse flowering of white sweet clover. Converse (2) showed that infection of *Rubus* species by TSV in Oregon was flower-related and that viruliferous pollen may be the primary means by which TSV spreads into the crop. Sdoodee and Teakle (14) found that thrips were transporting TSV-infected pollen from tomato to *C. amaranticolor* Coste & Reyn. test plants and that virus released from the pollen probably infected the test plants via thrips feeding wounds. This method of thrips-mediated pollen transmission could explain how TSV was spread to BTS bean trap plants at Central Ferry and to flowering and nonflowering *Rubus* spp. in Converse's field trials (2).

Until 1987, 300–400 bean germ plasm accessions in the Pullman collection were propagated annually at Central Ferry. This practice ceased in 1987 because many bean lines were becoming infected with bean common mosaic virus, which is seed transmitted in bean (8). Bean germ plasm may have become contaminated with TSV during these field propagations. TSV seed transmission has not been detected in the germ plasm collection (W. J. Kaiser and R. E. Klein, *unpublished data*), but this requires further investigation.

Alfalfa is an important reservoir of several viruses infectious to various food legumes in the Pullman germ plasm collection, including bean, broadbean (*Vicia faba* L.), chickpea, and lentil (*Lens culinaris* Medik.). Alfalfa was not cultivated in the vicinity of the research station at Central Ferry until 1983 when the Army Corps of Engineers planted a strip of alfalfa cv. Ladak between the station and the Snake River. A survey of the alfalfa in 1985 detected alfalfa mosaic and pea streak viruses as well as TSV pathotype II. More than 30% of the plants were infected with AMV and some were infected with two or more viruses. In subsequent surveys, bean leafroll virus and TSV pathotype I were also detected. Only AMV was found to be transmitted in seed (<5%) of plants from this alfalfa planting. TSV has been reported in alfalfa in Ontario, Canada (12), and in central Washington (4) and it is possible that alfalfa serves as an important reservoir of different TSV pathotypes in other alfalfa growing areas of the Pacific Northwest.

The major diseases affecting chickpeas at Central Ferry are caused by viruses (W. J. Kaiser, *unpublished data*). At this location, chickpeas are infected by several viruses (6), including TSV. Both pathotypes of TSV were isolated from naturally infected chickpeas, although the incidence was low and sporadic. In inoculation trials at Central Ferry, yields of chickpea inoculated at prebloom with one isolate of pathotype I and two of

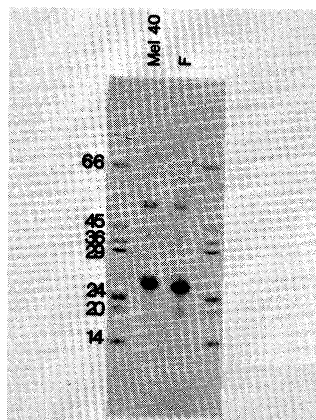

Fig. 5. SDS-acrylamide gel electrophoresis of SDS-coat proteins of isolates Mel 40 and Mel F of tobacco streak virus. The numbers on the vertical scale indicate kilodalton sizes of known protein standards.

Table 5. Indirect ELISA absorbances of various tobacco streak (TSV) and alfalfa mosaic (AMV) virus isolates in *Chenopodium quinoa* against three different TSV and AMV antisera

	Antiserum[b]			
	TSV			
Viral isolate[a]	Tfg	Tfg[c]	Mel F	AMV
TSV pathotype I				
BRN-M	0.66	...[d]	0.09	0.06
Tfg	1.00	0.81	0.04	0.06
Mel 40	0.29	...	0.04	0.05
TSV pathotype II				
Mel F	0.42	0.06	1.16	0.05
BTS II-C2a	0.33	...	0.89	0.05
BTS II-C2a-CST	0.43	0.08	1.03	0.05
Mel 3	0.61	...	1.30	0.06
AMV				
ATCC 105	0.09	0.06	0.03	1.11
Alfalfa	0.09	...	0.03	1.00
Healthy	0.07	0.05	0.03	0.06

[a] Pathotype I isolates of TSV: BRN-M (bean), Tfg (*Trigonella foenum-graecum*), and Mel 40 (*Melilotus alba*). Pathotype II isolates of TSV: Mel F (*M. alba*), BTS II-C2a (bean), BTS II-C2a-CST (*Cicer arietinum*), and Mel 3 (*M. alba*). AMV isolates: alfalfa (*Medicago sativa*) and ATCC 105 (ATCC).

[b] TSV antisera of pathotypes I (Tfg) and II (Mel F) prepared by J. Larsen and AMV antiserum from K. R. Bock.

[c] Antiserum diluted in Mel F-infected *Chenopodium quinoa* extract.

[d] Not tested.

pathotype II were reduced by more than 75%. Isolate Mel 40 and BTS II-C2a of pathotypes I and II, respectively, were transmitted in seeds from prebloom-infected plants. The plants infected from seed were stunted and the leaflets were reduced in size. Apparently, this is the first report of a virus being seed transmitted in chickpea. Only one chickpea line was used in the inoculation study. Additional tests are needed with both pathotypes of TSV to establish the effects of infection on yield, quality, and seed transmission in other chickpea lines.

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