

Association Between Tobacco Streak Iarvirus Seed Transmission and Anther Tissue Infection in Bean

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ABSTRACT

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Tobacco streak ilarvirus (TSV) seed transmission was investigated by reciprocal pollinations as well as by serological and infectivity assays of flower parts from beans (*Phaseolus vulgaris*) systemically infected with either TSV pathotype I (isolate Mel 40) or TSV pathotype II (isolate Mel F). Healthy and TSV-infected Black Turtle Soup (BTS) bean plants were reciprocally pollinated by using anthers from either healthy plants or plants systemically infected with Mel 40 or Mel F. When anthers from plants infected with Mel 40 were used to pollinate healthy plants, a high percentage of the resulting seedlings were infected with that isolate. Similar results were obtained from plants infected with Mel 40 that were allowed to self-pollinate. Seed populations from pollinations that used anthers from healthy plants and ovaries from infected plants produced

much lower seedling infection. The Mel F isolate was not seed-transmitted in cross-pollination experiments with BTS. Enzyme-linked immunosorbent assay results indicated that antigen levels of the two virus isolates were similar in flower petals and in ovaries of beans infected with either virus. However, TSV Mel 40 mean antigen levels in stamen tissues were much higher than those of TSV Mel F in similar tissues. The amount of infectious virus as measured by infectivity assays of flower parts on the local lesion host *Chenopodium quinoa* was also greater in stamens of plants infected with TSV Mel 40 than in stamens of plants infected with TSV Mel F. Seed transmission of TSV in beans may depend on early movement into and replication in pollen-associated tissues.

Most plant viruses described to date are not seed-transmitted, but the significance of seed transmission in the epidemiology of many important virus diseases is widely recognized. Seed transmission may occur by a variety of routes depending on early infection of floral meristematic tissues, enzymatic activity during seed maturation, virus strain, or host genotype (3-5,17,18). Bennett (1), Carroll (3), and others linked virus seed transmission to infection of micro- and megagametophytic tissues (8,21). Such virus-infected sporogenous cells can give rise to infected pollen and ovary-associated tissues. Most work indicates that in "true" seed transmission (the developing embryo is infected), early infection of the sporogenous tissues is essential (3).

Tobacco streak virus (TSV) pathotype I (isolate Mel 40) is efficiently seed-transmitted to progeny seedlings in bean (*Phaseolus vulgaris* L.) and is very similar to the bean red node strain of TSV (15). Infection levels as high as 32.8% have been observed in seedlings of the cultivar Black Turtle Soup 1 (BTS). TSV pathotype I, isolate Mel 40, and pathotype II, isolate Mel F, are distinct in terms of serology, seed transmission, and symptoms on *Chenopodium quinoa* Willd., BTS, and other hosts (15). Both Mel 40 and Mel F occur in naturally infected field hosts, including *Melilotus alba* Medik., *Medicago sativa* L., *Trigonella foenum-graecum* L., and *Vigna angularis* (Willd.) Ohwi & H. Ohashi. Previous work (15,16) demonstrated that beans could be infected with TSV in the field, presumably from infected field hosts. We used bean as an experimental host because of our concern about contamination of the Western Regional Plant Introduction Station's bean germ plasm collection with seed-transmitted viruses.

Many seed-transmitted ilarviruses are known to infect progeny seedlings through pollen (2,7,10,12,13,19,20). The purpose of our

study was to determine what floral tissues are critical for seed transmission of TSV in BTS. We compared the distribution of TSV isolates Mel 40 and Mel F in floral tissues from infected bean plants by means of serological and infectivity assays. We also determined the route of TSV Mel 40 seed transmission (via stamens or ovaries) by use of reciprocal pollinations between infected and noninfected plants.

MATERIALS AND METHODS

Virus and plant maintenance. Two previously described isolates of TSV were used in our study (15). The Mel 40 and Mel F isolates both originated from naturally infected *M. alba* at Central Ferry, WA (15). Both isolates were maintained in *C. quinoa* by mechanical transfer. Original isolates were maintained in infected leaf tissue dried over Drierite (8-mesh anhydrous CaSO₄) at 4 C or in frozen tissue at -80 C. Mel 40 was also maintained in seeds from infected *P. vulgaris*.

All plants were grown in sterile potting mixture in the greenhouse under natural light supplemented by incandescent lighting for a 16-h photoperiod at 20-25 C. Bean plants used in all experiments were grown in 15-cm plastic pots, whereas *C. quinoa* and bean seedling assay plants were grown in 7-cm pots.

Reciprocal pollination of infected and noninfected BTS beans. Tests to determine the frequency of pollen and ovule transmissions of TSV Mel 40 and Mel F were conducted with BTS bean plants. Infected parent plants were obtained by mechanical inoculation of BTS seedlings at the two-leaf stage by using triturates of fresh, infected *C. quinoa* (15). Flowers on mature, greenhouse-grown bean plants were hand-pollinated or allowed to self-pollinate to provide pods and seed for TSV transmission tests. Hand pollinations were made by using healthy and infected plants as both male and female parents. During morning hours, approximately 10 pollen donor flowers containing dehiscing anthers and pollen-covered stigmas were collected from healthy and virus-infected

BTS plants and stored on wet paper towels in covered petri dishes. Pollen receptor flowers were opened before dehiscence and emasculated with forceps. Anthers and/or pollen-covered stigmas from pollen donor flowers were gently rubbed over the stigmas of one or more emasculated flowers. The receptor flowers were then closed and labeled as to pollen source plant. Forceps used in pollinations were dipped in bleach and rinsed in distilled water between pollinations to minimize the possibility of transfer of pollen-bearing thrips from one plant to the next. All healthy or infected plants bearing hand-pollinated or self-pollinated flowers were indexed on *C. quinoa* and by enzyme-linked immunosorbent assay (ELISA). Fresh leaf tissue samples were used in assays at the primary leaf stage, after inoculation, and during maturation of the pods to confirm systemic infection or to detect possible infection during pollinations. Details of ELISA and local lesion assays are given below. Pods matured and dried on the plants. Mature seed was planted immediately after harvest or stored dry at 4 C until needed.

Seedling assay. To determine the incidence of seedborne Mel 40 or Mel F infection, approximately 2 cm² of unifoliate leaf tissue was taken from bean seedlings. The tissue was triturated in 1% K₂HPO₄ with 0.5% diatomaceous earth or Carborundum, and the triturate was rubbed on two leaves of *C. quinoa* indicator plants. Indicator plants were observed for symptoms for up to 2 wk post-inoculation.

ELISA of floral parts. Indirect ELISA was carried out according to previously published methods (15). To investigate relative levels of viral antigen in different flower parts, freshly opened, self-pollinated flowers were collected from healthy plants and plants infected with Mel F or Mel 40 and assayed immediately. Petals, stamens, and ovaries of single flowers were separated and individually triturated in 1.5 ml of standard ELISA buffer (6) with mortar and pestle. Aliquots of 200 µl each were loaded into duplicate wells of microtiter plates and incubated for 75 min at room temperature. Controls consisted of triturated flower parts from healthy bean plants. Polyclonal antisera to Mel F and Mel 40 isolates (15) were applied to wells at a dilution of 1:3,200 and incubated for 1 h. Goat anti-rabbit alkaline phosphatase conjugate and alkaline phosphatase substrate applications, as well

as plate readings, were also done by previously described methods (15).

Local lesion assay of BTS flower parts. To compare levels of viral antigen with levels of infectious virus, triturates of individual BTS flower parts were tested for infectivity by local lesion assay on *C. quinoa*. In the first experiment, self-pollinated flowers were selected at random from infected plants. Petals, stamens, and ovaries from equivalent numbers of healthy, Mel 40-, or Mel F-infected flowers were separately triturated in 1% K₂HPO₄ with 0.5% diatomaceous earth, and the triturate was rubbed on two leaves of *C. quinoa*. In the second experiment, triturates of filaments and anthers from randomly selected, self-pollinated flowers were used as inocula, and the mean number of local lesions was determined for each isolate.

RESULTS

We collected data on seedling infection derived from artificial pollinations of flowers on healthy, Mel 40-, or Mel F-infected plants. The data were compared with seedling infection derived from self-pollinations of healthy and infected flowers (Table 1). Mel F was not seed-transmitted. Pollination of flowers on healthy plants with pollen from plants infected with Mel 40 resulted in 26.4% infected seedlings, which was similar to the number produced by self-pollination of flowers on infected plants (21.2%). Pollination of flowers on plants infected with Mel 40 with pollen from healthy plants resulted in 3.4% infected seedlings.

All tissue used in bioassays had comparable amounts of flower parts. Flowers from all plants appeared to be normal and had no obvious variations in size. Practical considerations prevented us from weighing individual flower parts. Previous experiments (data not shown) suggested that any delay between collection and immediate assay of fresh flower parts resulted in dramatically increased variation in results of ELISA and infectivity experiments.

The ELISA results, based on individual flower parts (Table 2), indicated that stamens of self-pollinated flowers from plants infected with Mel 40 had higher concentrations of viral antigen than stamens from flowers of plants infected with Mel F. Antigen levels in ovaries and petals were similar, despite infection by different virus isolates.

Local lesion assays were based on triturates of flower parts from equal numbers of healthy, Mel 40-, or Mel F-infected flowers. Triturates of stamens from plants infected with Mel 40 appeared

TABLE 1. Results of pollen and ovary transmission of tobacco streak virus (TSV) pathotype I (isolate Mel 40) and pathotype II (isolate Mel F) in bean (*Phaseolus vulgaris* 'Black Turtle Soup 1')^x

Virus-infected or control pollination type ^y	Number of pollinations	Number of seedlings assayed	Seedling transmission (%) ^z
Overy * pollen			
Isolate Mel 40			
H * H	27	149	0 a
I * H	32	149	3.4 b
H * I	48	163	26.4 c
I * I	48	203	21.2 c
Isolate Mel F			
H * H	31	156	0 a
I * H	29	116	0 a
H * I	51	227	0 a
I * I	61	312	0 a

^xResults based on harvest and virus-indexing of 296 pods resulting from 296 pollinations.

^yH = healthy; I = infected; H * H and I * I were self-pollinated; I * H and H * I were pollinated by hand.

^zMean percentage of seedlings infected per pollination (per pod, including noninfected pods). Transmission through pollen indicates a pollination using pollen from an infected plant to pollinate an emasculated flower on a healthy plant. Transmission through ovary indicates a pollination using pollen from a healthy plant to pollinate an emasculated flower on an infected plant. Numbers followed by the same letter are not statistically different from each other according to a two-sample analysis of variance (ANOVA) made between each of the two comparable distributions ($P = 0.05$).

TABLE 2. Indirect enzyme-linked immunosorbent assay (ELISA) absorbance of flower parts from bean plants (*Phaseolus vulgaris* 'Black Turtle Soup 1') systemically infected with tobacco streak virus (TSV) pathotype I, isolate Mel 40, or pathotype II, isolate Mel F

Flower parts	Absorbance values for TSV isolate or control infection type ^w			
	Mel F inoculated ^x (40)	Mel 40 inoculated (51)	Mel 40 seed ^y (29)	Healthy (43)
Petals	1.46 0.36-1.99	1.67 0.50-2.00	1.71 0.81-2.00	0.21 0.04-0.64
Stamens ^z	0.36 0.04-1.50	0.84 0.16-1.60	0.91 0.26-1.72	0.05 0.01-0.15
Ovaries	1.06 0.41-1.95	1.19 0.58-1.83	1.23 0.55-1.64	0.06 0.01-0.20

^wThe mean and range of ELISA absorbance values (A_{405nm}) of triturates of flower parts are listed. TSV antisera to Mel 40 or Mel F were prepared as previously described (15). Numbers in parentheses indicate number of tests.

^xPlants were infected with virus by inoculation at the seedling stage.

^ySeed = seedborne. Plants were infected with virus from seed of infected parent plant.

^zAccording to the Kolmogorov-Smirnov two-sample test, ELISA data from stamens of plants infected with Mel F came from a different distribution than data from stamens of plants infected with Mel 40 of either infection type. Data from petals and ovaries came from the same distribution, regardless of infection type.

to be about three times as infectious as triturates of stamens from plants infected with Mel F. Results of a second experiment also indicated higher infectivity of anthers and filaments from flowers of plants infected with Mel 40 than from plants infected with Mel F (Table 3, experiment 2). Infectivity of ovaries from plants infected with Mel F was somewhat lower than that of ovaries from plants infected with Mel 40. We did not consider this difference meaningful because of the high variation in numbers of local lesions induced by triturates from ovaries infected with Mel F. Although petals from flowers on plants infected with Mel F were more infectious than those of plants infected with Mel-40, this did not influence seed transmission and only served to confirm that the test plants were systemically infected.

DISCUSSION

As previously described by Kaiser et al (15) and in agreement with Ghanekar and Schwenk (11), seed transmission of TSV appeared to vary with virus isolate or strain. In contrast to work by Ghanekar and Schwenk, isolate Mel 40 was present at high levels in pollen-associated tissues and presumably infected seed and seedlings via those tissues. There appeared to be 3–10 times more infectious virus per flower part in flower stamens from plants infected with Mel 40 than in stamens from plants infected with Mel F (Table 3). Seed transmission levels from self-pollinated plants infected with Mel 40 were similar to those obtained by pollinating healthy plants with pollen from infected plants. Although low levels of seed transmission resulted from pollinations of ovaries on infected plants with pollen from healthy plants, this may have resulted from incomplete emasculatation of flowers or to a low natural rate of Mel 40 transmission through ovules. A pollen-associated route of seed transmission is consistent with the finding that Mel 40 was present at higher levels in anther tissues than Mel F.

The differing levels of virus antigen and infectivity appeared to be tissue-specific but also were dependent on virus isolate. Overall, ELISA results suggested some tissue-specific influence on antigenicity in flower parts from plants infected with either isolate. In contrast, infectivity of Mel 40 appeared to be less affected by tissue type than Mel F. The approximately 10-fold difference in infectivity between Mel F- and Mel 40-infected filaments and anthers contrasted with the difference in levels of antigenicity and infectivity between stamens of similar flowers (Tables 2,3). This disparity might have resulted from our having used slightly different tissue samples or filaments and anthers from more flowers in local lesion experiment 2 than were represented by triturates of stamens in experiment 1 or in ELISA. The comparable differences in antigenicity and infectivity between stamens from plants infected with either isolate suggested that Mel 40 is present in greater numbers of virus particles than Mel F. However, the ELISA data may still reflect greater amounts of Mel F coat protein than the numbers of competent, assembled

Mel F virus particles in stamen tissue. There could be several reasons for this, including virus degradation or faulty encapsidation. Mel F is more pH-sensitive than Mel 40 (15; M. H. Walter, S. D. Wyatt, and W. J. Kaiser, *unpublished*), which might have affected the infectivity of Mel F in stamen tissues. If isolate Mel F had infected the stamen tissues early enough and replicated normally, then degradation could have reduced infectivity but might not have decreased viral antigen levels as detected by ELISA. We also could not rule out the possibility that Mel F was subject to physical barriers, because the distribution of virus within tissues was not determined cytologically.

The ability of Mel 40 to infect developing microgametophytic tissues, thus infecting pollen, could have been due to increased replication in the pollen-associated tissues, greater ability of virus to move from cell to cell, lack of physical barriers to Mel 40, or a reduction of in situ degradation of virus. In addition, virus-coded movement factors play a role in cell-to-cell movement of many viruses (14) and may have accounted for differences in amounts of virus in flower tissues. The effect of virus-coded movement factors on seed transmission of viruses in otherwise systemically infected hosts is unknown. Work describing any such effect would have to distinguish virus movement from replication, as has been done with TMV (9).

Virus maintenance or increase could occur via seed transmission under field conditions, depending on host and/or isolate. Both Mel 40 and Mel F occur in naturally infected field hosts, yet Mel F appears to be seed-transmitted at a much lower rate than Mel 40 in beans. This may suggest that Mel F can be maintained in the field by some other means, possibly by transmission via thrips (15,16). Investigations of the low seed transmission of Mel F are underway.

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TABLE 3. Local lesion assay of flower parts from bean plants (*Phaseolus vulgaris* 'Black Turtle Soup 1') systemically infected with the Mel 40 or Mel F isolates of tobacco streak virus

Flower parts ^c	Mean number of local lesions ^b	
	Mel 40	Mel F
Experiment 1		
Petal	143 (57)	231 (102)
Ovary	145 (147)	104 (92)
Stamen	146 (58)	52 (37)
Experiment 2		
Filament	262 (111)	30 (51)
Anthers	161 (103)	16 (31)

^aIndividual plant parts were triturated in 1% K₂HPO₄ + 0.5% celite, and the triturate was rubbed on two leaves of *Chenopodium quinoa*. Standard deviations are in parentheses.

^cThe different parts from six and 6–12 flowers in Experiments 1 and 2, respectively, taken from four to eight plants were assayed for virus on *C. quinoa*.

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