Field Transmission of Tulip Breaking Virus and Serologically Related Potyviruses in Tulip

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ABSTRACT

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Tulip breaking virus (TBV) and related potyviruses were aphid-transmitted in tulips in Washington state throughout the growing seasons of 1985 and 1986. Aphids were trapped at sites where healthy tulips were exposed adjacent to TBV-infected tulips. Virus infection was detected serologically with monoclonal antibodies and polyclonal antisera using an antigen-coated form of indirect enzyme-linked immunosorbent assay (ELISA). Transmission was correlated with occurrence of *Macrosiphum euphorbiae* and *Dysaphis tulipae*, known vectors of TBV. Differential ELISA reactions with the monoclonal antibodies and polyclonal antisera indicated the occurrence in tulips of potyvirus isolates serologically related to, but distinct from, TBV. Infections detected by ELISA correlated well (87%; 27 of 31 affected plants from the 1986 exposure) with occurrence of flower break symptoms in the exposed tulips. In two cases, virus infection was detected by ELISA in the absence of flower break, and no virus was detected in two other plants with flower break.

The causal agent of classical or Rembrandt flower break of tulips (*Tulipa* spp.) is most commonly tulip breaking virus (TBV), a potyvirus. It is transmitted by aphids in the field during the growing season (17,18) and possibly during bulb storage (18). Typically, isolates of TBV infect only tulips and lilies (*Lilium* spp.), causing reduction in bulb yields and flower quality (26). A related potyvirus, tulip chlorotic blotch virus (TCBV), has been reported from Australia and also causes typical flower break in tulips (21). TCBV does not infect lily but does infect some other herbaceous host plants and is serologically related to, but distinguishable from, TBV (21). Several other viruses have also been reported to cause flower break in tulip, but both floral and foliar symptoms induced by these viruses differ from those typically induced by TBV. These viruses include arabis mosaic virus (2), cucumber mosaic virus (CMV) (25), lily symptomless virus (8), potato virus X (19), and tulip virus X (20). Asjes has also reported (in 16) another virus (possibly in the closterovirus group) associated with "broken" tulips. Of these viruses, CMV induces symptoms most similar to those associated with TBV (25,26).

Flower break symptoms associated with TBV infection occur in cultivars with dark-pigmented flowers, but not in most of those with yellow or white flowers (26). Foliar symptoms (a chlorotic mosaic) are typically produced in all cultivars, but may not be obvious. Asjes (3) has shown that cultivars differ in their apparent susceptibility to TBV, and that most do not become symptomatic until the season after infection. Spread of TBV can be reduced by isolating plantings, rogueing symptomatic plants, and using mineral oil sprays (1). Although plants showing flower break are commonly destroyed, asymptomatic cultivars may serve as significant reservoirs of virus for transmission to other tulips or lilies.

Several aphid species are natural vectors of TBV (5,6,14,17,18). Individual species may be of varying importance in different geographical areas because of climatic factors and occurrence of alternate host plants.

In western Washington there are approximately 160–200 ha of tulips grown each year. The estimated value at the farms of tulip bulbs and cut flowers produced during 1987 was in excess of $3 million. No information is available on the species of aphids vectoring TBV or the conditions favoring transmission of the virus in Washington. The purposes of this investigation were to identify the period in which aphid transmission of TBV is most likely to occur in the Puyallup Valley, one of the major tulip-growing areas in the state, and to correlate virus transmission with identified aphid species. We used monoclonal antibodies (MAbs) to detect TBV and to monitor aphid transmission of the virus. In the course of this investigation, some potyvirus isolates distinct from TBV were detected. These isolates could be serologically differentiated from TBV. This information should enable extension agents and growers to develop improved vector and disease control programs and minimize pesticide usage.

MATERIALS AND METHODS

Virus source plants. Plants of *Tulipa* 'Lincolnshire' maintained at Puyallup, WA, since 1977 served as the source of virus infection. These plants had produced fewer flowering bulbs in each succeeding year and exhibited prominent flower breaking and foliar symptoms typical of TBV infection. Infection was confirmed by enzyme-linked immunosorbent assay (ELISA) with polyclonal antiserum and monoclonal antibodies (MAbs) to TBV, in tests made from 1984 through 1987. In the fall of 1984 and 1985, five groups of 20 infected bulbs were planted approximately 5 m apart in a row located at Washington State University's Farm Two near Puyallup to serve as the virus source for aphid transmission of TBV. Adjacent rows, on 1-m centers, were planted to other tulip cultivars that were planted for other experiments and were not tested for TBV incidence. These plants served to make the experimental plot more like a tulip field than a solitary row. In-furrow applications of quintozene (24 kg a.i./ha) were made to all bulbs before hilling to control soilborne diseases, and napropamide (4.5 kg a.i./ha) was applied after hilling to control weed growth.

Field exposure of virus-free test plants. During the 1984–1985 season, cultivar Apeldoorn tulips (8–9 cm circumference bulb size) served as virus-free test plants; during the 1985–1986 season, cultivar Paul Richter (8–9 cm) was used. Bulbs were obtained from grower stocks in which no flower break had been observed, but were not tested for TBV before exposure. Groups of nine bulbs were planted in each of 45 planting boxes (30 × 30 × 20 cm) at the same time the

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virus-infected Lincolnshire bulbs were planted in the field. The boxes of TBV-free bulbs were placed in an unheated, insect-proof screenhouse and covered with sawdust. Each year the boxes were transferred to the field and placed in an insect-proof screenhouse within 60 m of the exposure site during late February. Five boxes (one box = one replicate) of TBV-free bulbs were exposed for each of the five treatment periods (Table 1). During the exposure, boxes were placed in sunken 35 × 35 cm wooden frames located at either end of each group of Lincolnshire plants. One of the five boxes of TBV-free tulips that were exposed throughout the experimental period (treatment 5) was placed in one frame at the end of each group of Lincolnshire tulips. Single boxes from the other exposure periods (treatments 1–4) were placed in the frame at the other end of each group of Lincolnshire tulips during the specified exposure periods (Table 1). The five unexposed (treatment 6) control boxes remained in insect-proof cages.

After exposure, the boxes of tulips were removed from the field, sprayed with methamidophos (Monitor) to kill any aphids, placed in an insect-proof screenhouse, and observed for foliar and flower break symptoms. Boxes with dormant plants were stored in a lath house and covered with 5–10 cm of sawdust. On 3 February 1986 and 17 January 1987 the boxes of rooted bulbs were placed in an insect-free greenhouse and observed for foliar and flower break symptoms.

Tulips in boxes were watered as needed and fertilized weekly with calcium nitrate (2.4 g/L/box) during the period when they were placed in the field, and twice weekly while they were in the insect-proof greenhouse. Applications of iprodione and chlorothalonil were made as needed to control *Botrytis tulipae* (Lib.) Lind.

ELISA. TBV and/or related potyviruses were detected using an indirect ELISA with antigen-coated plates (15) and buffers as described (7), except as noted. Tulip leaf-tip samples (0.5–1.5 g) were extracted in 10 volumes of coating buffer containing 2% polyvinyl pyrrolidone (CB+PVP), filtered through cheesecloth, diluted to a final 1:100 with CB+PVP, and, after overnight storage at 4 C, were added to multiple wells of Immulon 1 MicroELISA plates (Dynatech, Chantilly, VA). After 2.5–3.5 hr of incubation, the plates were washed in PBS-Tween (PBS-T) and blocked for 0.5–1 hr with 1% bovine serum albumin in PBS before addition of antibodies. Monoclonal or rabbit polyclonal antiviral antibodies diluted in PBS-T were incubated for 2.5–3 hr. The plates were washed and alkaline phosphatase-labelled goat antimouse or goat anti-rabbit antibodies (Kirkegaard and Perry, Gaithersburg, MD) diluted in conjugate buffer were incubated overnight at 4 C. After washing, *p*-nitrophenyl phosphate (1 mg/ml in substrate buffer) was incubated 1–4 hr and read with a model MR700 Microplate reader (Dynatech), and sometimes read again after up to 8 hr. All incubations were at room temperature, except where noted. Control samples included CB+PVP, symptomless tulips (Apeldoorn or Paul Richter) protected in a screenhouse, healthy *Nicotiana benthamiana* Domin., *N. benthamiana* infected with potyvirus isolate "Wa tulip," healthy *N. edwardsonii* Christie and Hall, and *N. edwardsonii* infected with potyvirus isolate "Linc 2." Isolates "Wa tulip" and "Linc 2" were isolated from Lincolnshire tulip maintained at Puyallup. These isolates are serologically related to, but distinct from, typical TBV (J. Hammond, *unpublished*). Two wells per sample were used per antibody tested. Averaged values of absorbance at 405 nm were used for comparison, and values equal to, or in excess of, twice the healthy control values obtained in the same set of plates were considered positive.

Antibodies. Monoclonal antibodies were prepared at the American Type Culture Collection (ATCC), Rockville, MD, using TBV purified from tulip cultivar Texas Flame in The Netherlands (12). MAb TBV25D1D6 (25D1) ascitic stock fluid was diluted 1:4,000. Equal volumes of ascitic stocks of MAbs TBV17F3C4 (17F3) and TBV26A4F2 (26A4) were mixed and diluted 1:4,000 in PBS-T. Antibodies 25D1 and 26A4 react with both the Texas Flame (TBV-TF) and Jack Laan (TBV-JL) isolates of TBV, whereas 17F3 reacted with TBV-TF but not TBV-JL (12). MAb 25D1 also reacts with some isolates of bean yellow mosaic virus (10–12; J. Hammond, *unpublished*) and with potyvirus isolates "Wa tulip" (11) and "Linc 2" (J. Hammond, *unpublished*). Ascitic fluid stocks of antibodies TBV25A1B2 (25A1), TBV4C7D4 (4C7), TBV27C2H2 (27C2), TBV26B1G6 (26B1), TBV28D4H7 (28D4), and individual stocks of 26A4 and 17F3 were diluted 1:4,000, except for 4C7, which was diluted 1:1,000.

Polyclonal rabbit TBV antiserum (from the Bulb Research Center, Lisse, The Netherlands) was diluted 1:400; rabbit antiserum against TCBV (21) was diluted 1:2,000. A polyclonal rabbit antiserum against the "Wa tulip" isolate was prepared at ATCC by Nancy Elliott using virus purified at Beltsville (J. Hammond, *unpublished*); a sodium sulfate precipitated globulin fraction (13) was diluted 1:800.

Plant sampling. The distal portion of the oldest nonsenescent leaf of each plant was harvested and shipped to Beltsville for ELISA testing. Sampling of TBV-infected Lincolnshire tulip in 1985 showed that lower leaves yielded ELISA values equal to, or higher than, those from upper leaves (*data not shown*). Consequently, the oldest nonsenescent leaves were used in all subsequent testing. Apeldoorn tulip plants exposed in 1985 were sampled individually in the season of exposure as each treatment was removed from the field, except for several plants of treatment 4 that had senesced before removal. Control samples from the unexposed treatment 6 plants were included in all tests. The exposed Apeldoorn plants were retested in March of 1986. Three composite samples from three plants each were tested per replicate

Table 1. Number of tulip plants infected with tulip breaking virus (TBV) following exposure to TBV-infected tulips and aphids during 1985 and 1986 in the Puyallup Valley of Washington

	Cv. Apeldoorn (1985)			Cv. Paul Richter (1986)		
Treatment	Exposure dates	No. of days	TBV-positive[a]	Exposure dates	No. of days	TBV-positive
1	4 March–1 April	28	3	28 March–18 April	21	2
2	1 April–29 April	28	2	18 April–9 May	21	3
3	29 April–28 May	29	1	9 May–30 May	21	10
4	28 May–24 June	27	1	30 May–13 June	14	3
5	4 March–24 June	90	1	28 March–13 June	77	11
6	Not exposed	0	0	Not exposed	0	0

[a] Number of plants ELISA-positive for TBV out of 45 plants exposed during each treatment period.

Table 2. Number of reported aphid vectors of tulip breaking virus trapped per exposure period during the 1986 treatments of cv. Paul Richter tulip in the Puyallup Valley of Washington

	Treatment period[a]				
Aphid vector	1	2	3	4	5
Dysaphis tulipae	···	5	79	15	99
Macrosiphum euphorbiae	4	13	16	21	54
Myzus persicae	···	···	1	···	1
Neomyzus circumflexus	···	···	2	···	2
All four species (total)	4	18	98	36	156

[a] Treatment exposure periods: 1 = 28 March–18 April, 2 = 18 April–9 May, 3 = 9–30 May, 4 = 30 May–13 June, and 5 = 28 March–13 June.

of nine bulbs. All of the individual plants within composite samples corresponding to positive or borderline ELISA reactions were retested separately. The individual samples were taken from the leaves previously used for the composite samples.

Paul Richter tulips exposed in 1986 were observed for flower break and foliar symptoms in the field and in the greenhouse. Tests of Paul Richter tulips from the greenhouse were begun in late February 1987. All tests were complete by the end of March 1987, well before the plants senesced. In initial sampling, all individual plants from treatment 3 (exposed 9–30 May 1986), and from one replicate of the unexposed treatment 6 (maintained in an insect-proof cage throughout the growing season), were tested. Three composite samples each of three individual plants were prepared per replicate by mixing equal volumes of the individual extracts to determine whether infection of a single plant in a composite sample could be detected.

All treatments were then assayed as three-plant composite samples with MAbs only. For these tests, leaf samples were pooled before extraction. Further samples were taken from each individual plant where discrepancies were observed between ELISA results and flower break symptoms. These samples were tested both with individual MAbs and the polyclonal sera. Samples of individual plants, for which initial ELISA results and visual observations concurred, served as internal controls. The samples that were assayed again by ELISA were separately tested for CMV by nucleic acid hybridization (27). Extracts of some samples were examined by electron microscopy for the presence of viruslike particles.

Aphid incidence. Insects were collected, at approximate weekly intervals during the experimental period, from three yellow pan traps (containing water and a small amount of Tween 20) set up at ground level within the row of infected Lincolnshire tulips. Collections were discontinued in June when most of the tulip foliage had senesced.

Aphids from the 1986 exposure were sorted, counted by date and pan location, and shipped to Beltsville for identification by the USDA-ARS Systematic Entomology Laboratory (to species level where possible, to genus where the state of the specimen precluded species identification).

RESULTS

Exposure of Apeldoorn tulip and aphid trapping (1985). Three plants in treatment 1 and two plants in treatment 2 were TBV-positive in the season of exposure. When retested in 1986, a total of eight plants among the five exposure treatments were TBV-positive (Table 1). None of the 45 unexposed plants (treatment 6) were found to be infected. Potyviruslike particles were observed by electron microscopy in four of five ELISA-positive samples, but not in one ELISA-negative sample. Only a single alate aphid was trapped during the entire 1985 exposure period.

Exposure of Paul Richter tulip and aphid trapping (1986). At least 26 aphid species were identified among the 1,146 aphids trapped. These included: *Acyrthosiphon dirhodum* Walker, *Acyrthosiphon pisum* Harris, *Acyrthosiphon solani* Kaltenbach, *Amphorophora* sp., *Aphis* spp., *Brachycaudus* sp., *Capitophorus* sp., *Cavariella aegopodii* Scopoli, *Cavariella hendersoni* Knowlton & Smith, *Chaitophorus* sp., *Dactynotus* sp., *Diuraphis holci* Hille Ris Lambers, *Dysaphis tulipae* Fonscolombe, *Fimbriaphis* sp., *Hyalopterus pruni* Geoffroy, *Macrosiphum avenae* F., *Macrosiphum euphorbiae* Thomas, *Myzocallis robiniae* Gillette, *Myzus ascalonicus* Doncaster, *Myzus persicae* Sulzer, *Nasonovia lactucae* L., *Neomyzus circumflexus* Buckton, *Pemphigus* sp., *Rhopalosiphum padi* L., *Schizaphis graminum* Rondani, and *Sipha* sp. Numbers of the four reported TBV vectors (5,6,14,17,18) trapped per treatment period are shown in Table 2.

Flower break symptoms and ELISA testing (1986 exposure of Paul Richter tulip). All of the control unexposed plants (treatment 6) remained free of foliar and floral symptoms of TBV throughout the experiments. None of these plants were found to be TBV-infected by ELISA (Tables 3 and 4). Ten individual plants of treatment 3 were TBV-positive by ELISA, and eight of these exhibited flower break symptoms (Table 3). Only one of these infected plants was not also detected as part of a composite sample. In this case, the individual plant had reacted weakly with the MAb 26A4/17F3 mix, and was not detected with MAb 25D1. The range of ELISA results from the comparison of individual and composite samples is

Table 3. Flower break symptoms and ELISA diagnosis of tulip breaking virus (TBV) per exposure period in cv. Paul Richter tulip exposed in 1986 in the Puyallup Valley of Washington

	Treatment period[b]					
Diagnosis[a]	1	2	3	4	5	6
FB−, ELISA−	42	42	35	42	33	45
FB+, ELISA+	2	3	8	3	11	...
FB−, ELISA+	...	...	2	...	...	...
FB+, ELISA−	1	...	...	...	1	...
TBV-infected[c]	2	3	10	3	11	...

[a] FB− = no flower break observed, FB+ = flower break observed, ELISA− = not infected as determined by ELISA, ELISA+ = infected with TBV as determined from ELISA absorbance at 405 nm in excess of twice that of healthy controls.
[b] Treatment exposure periods: 1 = 28 March–18 April, 2 = 18 April–9 May, 3 = 9–30 May, 4 = 30 May–13 June, 5 = 28 March–13 June, and 6 = unexposed throughout. Figures presented are numbers out of 45 plants exposed per treatment.
[c] ELISA-positive, with or without flower break.

Table 4. Comparison of optical density (A_{405}) values of individual and composite ELISA samples of cv. Paul Richter tulip exposed in 1986 to tulip breaking virus (TBV) in the Puyallup Valley of Washington, and tested in 1987

	Monoclonal antibody against TBV			
	25D1D6		26A4F2/17F3C4	
Sample	Individual	Composite	Individual	Composite
Coating buffer	0.01	0.01	0.01	0.01
Treatment 6[a]	0.05	0.06	0.04	0.06
Range	0.01–0.06	0.06	0.01–0.07	0.05–0.06
Treatment 3[b]				
Uninfected range	0.00–0.07	0.04–0.06	0.01–0.08	0.04–0.07
Treatment 3				
Infected range	0.09–2.0	0.26–1.19	0.09–2.0	0.09–2.0
Healthy				
Nicotiana benthamiana	0.05	...[c]	0.05	...
"Wa tulip"[d]				
N. benthamiana	0.40	...	0.07	...

[a] Treatment 6 plants were unexposed controls maintained free of TBV in aphid-proof cages in the field. The mean ELISA value, range obtained from nine single plants, and three composite samples of three plants each, are shown.
[b] Treatment 3 plants were exposed next to TBV-infected tulips from 9 to 30 May 1986. ELISA values are for individual and composite samples, separated into the range considered uninfected, and the range of values from TBV-infected plants.
[c] No composite samples of healthy or "Wa tulip"-infected *N. benthamiana* were tested.
[d] Virus isolate "Wa tulip" is serologically related to, but distinct from, TBV.

shown in Table 4.

Because most of the infected plants identified by testing plants singly were also detected in composite samples of three plants, all of the treatments were then tested as composite samples to reduce the number of tests needed. The results were compared with observed floral symptoms and, where appropriate, with previous test results. Where composite samples were TBV-positive by ELISA and at least one of the plants in that composite sample showed flower break, no further tests were made. In some instances, floral symptoms were observed in the absence of positive ELISA results from composite samples. In these cases, each of the plants in that sample was tested for CMV as described (27), and also by ELISA with each separate MAb and polyclonal antiserum. None of the samples was found to be infected with CMV by nucleic acid hybridization under conditions in which CMV-infected tomato plants are readily detected (*data not shown*). The additional ELISA testing confirmed that all but two of the plants with flower break were infected with TBV (Table 3). Despite twice assaying further samples of these two plants, no positive reactions were observed with any of the MAbs or antisera (samples T1-4-2 and T5-3-7) (Table 5). No potyvirus or other virus particles were detected by electron microscopy, nor was any virus detected by inoculation to a range of test plants. The cause of flower break in these two plants remains unknown.

Differential ELISA reactions. Infections of "typical" TBV were characterized by reaction with each of the MAbs, and with TBV and "Wa tulip" virus (WaTV), but not TCBV polyclonal sera. Atypical reactions might indicate the presence of variants of TBV, or of related potyviruses such as WaTV and "Linc 2," which are serologically distinct from TBV and each other (Tables 4 and 5; *unpublished results*). Serial dilution of samples resulted in loss of detection by some antibodies earlier than with others (Tables 4 and 5; *data not shown*). Reactions were observed with the MAbs in several instances without corresponding reactions with one or another of the polyclonal sera. No infections were detected with the polyclonal TBV, TCBV, and "Wa tulip" sera that were not detected with the MAbs (Table 5). More infections in tulip were detected with the TBV polyclonal than with the "Wa tulip" polyclonal (*data not shown*). The only positive reactions of the TCBV antiserum were with the "Wa tulip" and "Linc 2" infected samples; the "Wa tulip" antiserum reacted with both of these controls as well as many of the individual tulip samples (Table 5). The strength of the polyclonal sera reactions was, in general, proportional to the reactions with the MAbs. Most notable were two tulip samples in which strong reactions were observed with all of the MAbs and with the "Wa tulip" antiserum, but no reaction was found with the Dutch TBV antiserum (samples T3-1-7A and T5-5-6A) (Table 5). These were the only indications in these assays of the presence in the test tulips of potyvirus isolates clearly differentiated from typical TBV. ELISA testing of further samples of T3-1-7A and T5-5-6A confirmed the earlier tests, but no virus was recovered by inoculation to a range of test plants (*data not shown*).

Electron microscopy. No viruslike particles were detected in unexposed (treatment 6) plants, nor were any potyvirus or any other viruslike particles detected in samples from the two ELISA-negative tulips with flower break that were described in a previous paragraph. In other samples, typical potyvirus particles of approximately 750 nm were observed and, in some instances, particles of about 650 nm (assumed to be of lily symptomless virus, the only carlavirus reported to infect tulips, and vectored by some of the same aphid species as TBV) (8) were detected in addition to potyvirus particles. No samples had only particles measuring approximately 650 nm. All samples in which particles of approximately 750 nm were observed had previously tested TBV-positive by ELISA. Electron microscopy, therefore, confirmed serological results but yielded no evidence for a viral cause of the flower break of two ELISA-negative plants (T1-4-2 and T5-3-7) (Table 5).

DISCUSSION

The lack of TBV detection in the unexposed plants (treatment 6) by either ELISA or occurrence of flower break

Table 5. ELISA (optical density at A_{405}) values of selected cv. Paul Richter tulip and control samples with monoclonal antibodies and polyclonal antisera against tulip breaking virus (TBV), tulip chlorotic blotch virus (TCBV), and "Wa tulip" virus (WaTV)

			Monoclonal antibody[d]				Polyclonal antiserum		
Sample[a]	FB[b]	ELISA[c]	25D1	26A4	17F3	28D4	WaTV	TCBV	TBV
T1-4-2[e]	+	−	0.02	0.02	0.01	0.01	0.06	0.11	0.03
T3-1-2	+	+	0.22*[f]	0.55*	0.01	0.02	0.15*	0.14	0.76*
T3-1-7A[e]	+	+	>2.0*	>2.0*	>2.0*	>2.0*	0.60*	0.18	0.08
T3-1-7B	−	−	0.03	0.02	0.01	0.02	0.06	0.11	0.04
T3-2-7	+	+	0.09*	0.17*	0.01	0.02	0.09	0.11	0.32*
T3-3-7	+	+	0.17*	0.43*	0.01	0.01	0.13*	0.10	0.63*
T3-5-1	+	+	0.61*	0.78*	0.01	0.02	0.25*	0.12	1.09*
T5-3-7[e]	+	−	0.03	0.03	0.02	0.03	0.07	0.11	0.04
T5-5-6A[e]	+	+	>2.0*	>2.0*	>2.0*	>2.0*	0.45*	0.15	0.09
T6-1-3	−	−	0.04	0.03	0.03	0.04	0.07	0.13	0.04
T6-4-4	−	−	0.03	0.03	0.02	0.04	0.05	0.12	0.05
Lincolnshire tulip[g]	+	+	0.44*	1.00*	0.10*	0.29*	0.26*	0.25*	1.35*
Healthy *Nicotiana edwardsonii*	NA	−	0.04	0.01	0.01	0.01	0.09	0.16	0.05
"Linc 2" *N. edwardsonii*	NA	+	0.28*	0.24*	0.26*	0.03	>2.0*	>2.0*	0.18*
Healthy *N. benthamiana*	NA	−	0.06	0.02	0.02	0.03	0.09	0.16	0.05
"Wa tulip" *N. benthamiana*	NA	+	0.47*	0.21*	0.18*	0.05	>2.0*	>2.0*	>2.0*

[a] T1-4-2 = treatment 1, replicate 4, plant 2, etc.
[b] FB = flower break symptoms: + = present, − = absent, NA = not applicable.
[c] ELISA diagnosis: + = positive result with at least one antibody, − = no positive result.
[d] Monoclonal antibodies: 25D1 = TBV25D1D6, 26A4 = TBV26A4F2, 17F3 = TBV17F3C4, and 28D4 = TBV28D4H7.
[e] Note that samples T1-4-2 and T5-3-7 had flower break but no significant ELISA reaction, and that T3-1-7A and T5-5-6A had high reactions with each monoclonal antibody and "Wa tulip" polyclonal, but no significant reaction with the TBV polyclonal serum.
[f] * = Positive reactions.
[g] Sample from the infected tulips used as TBV source.

symptoms indicates that the virus-free test plants of both cultivars were indeed uninfected.

The low incidence of TBV infection observed in the exposure of Apeldoorn in 1985 may have been due to low vector numbers; only a single aphid (tentatively identified as *Myzus persicae*) was collected from the pan traps during the period of exposure. Also, the cultivar Apeldoorn has been reported to have a degree of virus-suppressive resistance, leading to a slower spread of TBV within this stock (22,24). Apeldoorn and other Darwin hybrid tulips are reported to have low rates of TBV spread (3) and exhibit indistinct symptoms of infection (28). The few infected plants from the 1985 exposure of Apeldoorn were distributed among the exposed treatments, and no correlation could be made between exposure period and transmission. Fewer infected plants were detected among those exposed throughout the season (treatment 5) than those exposed during treatment period 1. Because no infection was detected among the unexposed treatment 6 plants, the TBV-infected plants in the exposed treatments were assumed to result from aphid transmission despite the low incidence of aphids observed.

Paul Richter tulip was substituted for Apeldoorn in the 1986 exposure and both incidence of aphids and of virus transmission were considerably greater than in 1985. Because tulips have been grown in the same field for several years, it is unlikely that the 1985 trial significantly influenced aphid incidence in 1986. Seasonal differences were probably the major determinant of vector populations. Clearly recognizable flower break was observed in many of the plants, and most of the plants with flower break symptoms were TBV-positive in ELISA (Table 3). The greatest amount of transmission was detected in treatment 3 (exposed 9–30 May 1986), and a similar number of plants were infected among those exposed for the whole season (treatment 5). Because not all plants from ELISA-positive composite samples were retested individually, it is possible that additional plants in these TBV-positive samples were infected in the absence of flower break and that the number of TBV infections was underestimated. The time periods during which virus transmission occurred was considered more significant than absolute numbers of plants infected.

At least 26 species of aphids were collected from the pan traps in 1986. Of these, *Aphis* spp. (*A. fabae* Scopoli and *A. gossypii* Glover), *Dysaphis tulipae*, *Macrosiphum euphorbiae*, *Myzus persicae*, and *Neomyzus circumflexus* have been reported as vectors of TBV (5,6,14,17,18). It is possible that some of the other aphid species trapped (especially those that are polyphagous) may also transmit TBV, and it would be desirable to test them for their ability to transmit this virus. However, such an undertaking was beyond the scope of the current study. Over 50% of all of the aphids trapped were *Pemphigus* sp., and most of these were trapped during treatment 4. *Pemphigus* sp. is probably not a vector of TBV. The Pemphiginae are not usually regarded as serious virus vectors (9) and *Pemphigus* spp. are not among the aphid species known to occur with any regularity on tulips, whereas all of the known vectors of TBV are often found on tulips (4).

Of the known TBV vectors (Table 2), *Dysaphis tulipae* and *Macrosiphum euphorbiae* were trapped most often. However, the efficiency of TBV transmission by *D. tulipae* may be very low. McKenny Hughes (18) reported that *D. tulipae* (as *Anuraphis tulipae* B. de Fonsc.) transmitted TBV only during bulb storage, whereas Brierley and McKay (5) did not observe any transmission by *D. tulipae* either in the field or in storage (although it should be noted that in a concurrent series of tests they failed to obtain transmission with either *Myzus persicae* or *Neomyzus* [as *Myzus*] *circumflexus*, both of which transmitted TBV in other tests reported in the same citation). *Macrosiphum euphorbiae* was, therefore, probably responsible for most of the transmission, despite being trapped in lower numbers than several other aphid species. In comparison, *Myzus persicae* was the only reported vector of TBV trapped (in very low numbers) by Sutton and Garrett (23) during tests in which up to 44% of exposed tulips became infected. Two distinct periods of aphid activity and TBV transmission were observed in Australia (23).

The peak period for TBV transmission in 1986 was in treatment 3 (9—30 May 1986), which is similar to that reported in The Netherlands (3). Asjes (3) reported that the start of aphid activity in the Dutch tulip fields depends on the weather, and that peak virus spread occurred in May, although observed aphid numbers were lower in May than June (1). During the period of "big leaf growth" in May, the tulips may be more susceptible to TBV infection (1).

In Washington, some plants apparently became infected before the end of March. Aphid activity was observed during the early part of the growing season in 1986, even before the exposure treatments started. The results presented here suggest that transmission may occur earlier in the season in Washington than in The Netherlands, and that growers need to vary control measures accordingly.

Franssen and van der Hulst (10) compared the use of the same TBV MAbs to polyclonal antiserum and reported that the polyclonal antiserum was superior for detection of TBV in leaf samples. However, only double-antibody sandwich forms of ELISA were examined. It has been shown (11,12; J. Hammond, *unpublished*) that these MAbs react best using indirect ELISA with antigen-coated plates. In the present study, the MAbs were found superior to the polyclonal antisera. The antigen-coated ELISA has the advantage of simplicity and allows many antibodies (or antisera) to be compared simultaneously using a universal conjugate (or two conjugates if murine and rabbit antibodies are being compared in the same assay), provided that the epitope(s) recognized is not destroyed during antigen coating.

Differential activity of some of the separate MAbs used here has been demonstrated both with TBV isolates (12) and with other potyviruses (10–12; J. Hammond and R. L. Jordan, *unpublished*). However, mixed infections of TBV and "Wa tulip"-like isolates would not be easily distinguished serologically from TBV alone. Further differentiation was made possible through use of the polyclonal sera. Two samples produced strong positive results with all of the MAbs and the "Wa tulip" antiserum, but not with the Dutch TBV antiserum. Lack of reaction with TBV antiserum in this type of indirect ELISA implies a distinct relationship, because this assay allows broader cross-reactivity within virus groups than does antibody precoated ELISA (15). These two were the only samples among the test plants with potyvirus isolates distinct from TBV. However, the "Wa tulip" and "Linc 2" isolates also originated from Lincolnshire tulip, indicating that potyvirus isolates distinct from TBV do occur in the United States. Such isolates can be distinguished by differential ELISA reactions (Tables 4 and 5). Some of the isolates infect herbaceous hosts; TBV does not. Characterization of these isolates will be reported elsewhere.

In two instances, flower break was observed in plants that were consistently ELISA-negative for TBV and related potyviruses (Tables 3 and 5). The floral symptoms of CMV in tulip are the most likely of known viral diseases to be confused with TBV (25,26). Therefore, these plants were tested for CMV. CMV was not detected in these or any other tulip tested, nor was any other viral infection identified in these plants.

The work described here has shown that monoclonal antibodies can be successfully used to detect tulip breaking virus and related potyviruses in an indirect form of ELISA. It has been demonstrated that TBV transmission in Washington can occur throughout the growing season. In the 1986 season, the greatest number of plants were infected during May. These transmission data and ELISA detection should be useful in developing improved control strategies for TBV and related potyviruses in bulb

crops of commercial value in the northwestern region of the United States.

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