Etiology of Tomato Plant Decline in the California Desert

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ABSTRACT

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Tomato plant decline (TPD) has been a limiting factor in the production of fresh market tomatoes in the desert areas of California since 1977. Diseased plants are characterized by stunting, leaf rolling, and leaflet chlorosis. The disease occurs only in fields with a history of previous tomato crops and can reduce yields by as much as 80%. Although TPD is known to be soilborne, the cause of the disease has not been determined until now. Tomatoes grown at 14 C in soil collected from a field with a history of TPD became infected with a tombusvirus that was serologically indistinguishable from the BS-3 strain of tomato bushy stunt virus (TBSV). When tomato debris infected with TBSV was used to infest soil, seedlings planted in this soil developed symptoms of TPD and were positive for TBSV infection if grown at 16 C. In addition, TBSV was consistently found associated with field-grown, symptomatic plants collected during 1987 and 1988, as determined by ELISA. Eight tomato cultivars, which in field observations were considered to be susceptible or tolerant to TPD, were mechanically inoculated with TBSV and grown in the greenhouse. The symptoms were most severe on the TPD-susceptible plants, but were mild on TPD-tolerant plants. Symptom development was also found to be dependent on temperature in a manner consistent with temperature sensitive replication of the virus. These experiments implicate TBSV as the etiological agent of TPD.

Tomato plant decline (TPD) has affected tomato production in the desert of California since 1977 (8,20). The disease is soilborne and may affect entire fields of processing and fresh market varieties. Symptoms of TPD have been described previously (20); they include stunting and cessation of growth after the start of flowering. Leaves and leaflets are reduced in size and may be cupped and curled downward. Leaves become chlorotic as the disease progresses and may die prematurely. Yields of infected fields can be reduced as much as 80% by the disease (Van Maren, *unpublished*). Soil solarization during the summer and methyl bromide fumigation have been shown to reduce the incidence of disease, but do not eradicate it (Van Maren, *unpublished data*).

Researchers have been trying to determine the cause of TPD for several years (20). Studies attempting to implicate nematodes or root-infecting fungi as the cause of TPD have been unsuccessful (20; Van Maren *unpublished data*). Also, previous attempts to isolate, detect, or transmit viruses from field-collected symptomatic plants were negative (20; Van Maren, *unpublished data*). Other hypotheses as to the cause of TPD involve toxin accumulation in the soil and soil nutrient deficiencies; these hypotheses have also remained unproven (Van Maren, *unpublished data*).

An important aspect of TPD is its extensive occurrence. Growers in the Imperial Valley of California generally refrain from planting tomato crops for a third time within a span of 10 years in an effort to avoid expected disease losses in the third crop (Van Maren, *unpublished data*). Clearly the impact of the disease is having a dramatic effect on the choice to grow tomatoes in this area.

In 1986, we isolated a virus from tomato roots grown in soil at 14 C collected from a field with a history of TPD. Electron micrographs indicated that the virus isolate was composed of isometric particles, approximately 30 nm in diameter. Double-diffusion serological tests in agarose gels indicated the virus to be a member of the tombusvirus group. In this paper the virus is identified as tomato bushy stunt virus (TBSV), and its causal association with TPD is confirmed. In addition, some of the conditions conducive to disease development are described and possible controls identified.

MATERIALS AND METHODS

Isolate characterization. The virus was isolated from tomato roots collected from infested soil and designated T1. It was

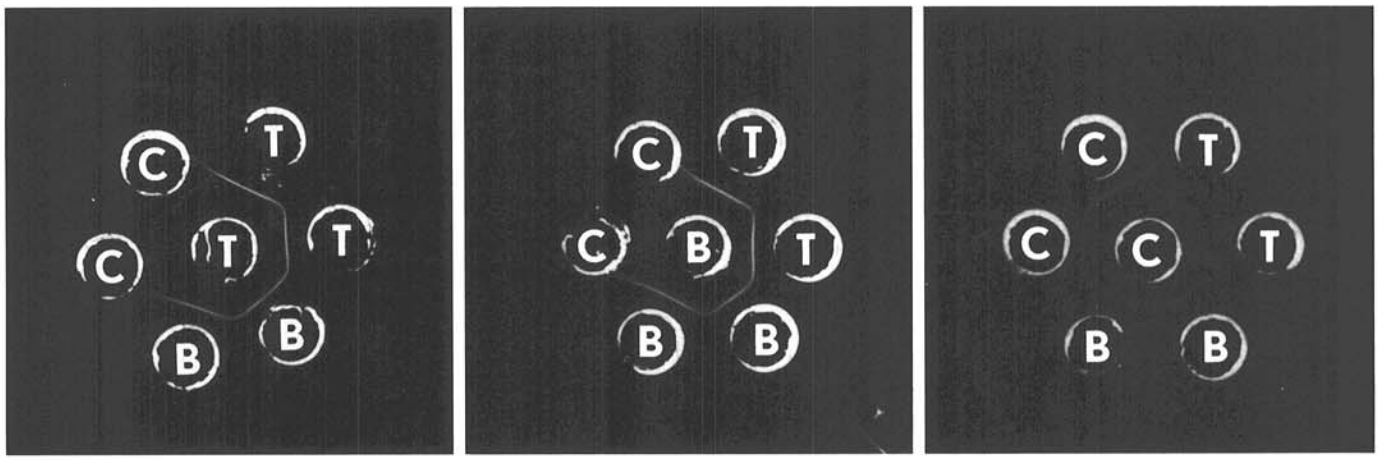

Fig. 1. Agar double diffusion of tomato bushy stunt virus isolate. Purified virus preparations were added to the outside wells, and antisera were added to the inside wells. Letters denote the isolate or its homologous antiserum; T = isolate T1, B = BS-3 strain, and C = cherry strain. When T1 or BS-3 antisera are used, solid precipitin lines formed between adjacent wells containing the two isolates, but spurs formed when these isolates were adjacent to the cherry isolate, indicating that T1 was closely related to BS-3.

propagated in the greenhouse in *Nicotiana clevelandii* Gray. Purified virus suspensions were prepared by extraction in 0.2 M sodium acetate, pH 5.0 followed by precipitation with 8% polyethylene glycol in 0.2 M NaCl as previously described (11). Purified virus (50 μg in 1 ml of phosphate buffered saline, pH 7.2 [PBS:0.14 M NaCl, 0.0025 M NaH_2PO_4, 0.0075 M Na_2HPO_4]) was injected intramuscularly into the thigh of a rabbit at weekly intervals for 6 wk, and the serum was collected 12 wk after the initial inoculation was used in these experiments. Purified virus suspensions of the BS-3 (28) and cherry (1,9,11) strains of tomato bushy stunt virus (TBSV) and antisera to these viruses were provided by B. I. Hillman and T. J. Morris (University of California, Berkeley). These isolates were propagated and maintained using the same procedures as for the T1 isolate.

Serological differentiation index (SDI) analyses of the three virus isolates based on serum titrations in agar-gel double diffusion tests were performed according to Koenig and Gibbs (17). This analysis is used to determine the degree of serological relatedness between virus isolates (30). The virus isolate suspensions were prepared in PBS at concentrations of 0.5 mg/ml; antisera dilutions started at 1:8, and twofold dilutions were tested to a concentration of 1:4096.

Electrophoretic comparison of the isolates was made on 1.0% agarose gels in 0.5 mM Tris, 30 mM glycine pH 8.2 (10). The gels were stained with ethidium bromide (1 μg/ml) and photographed with UV illumination.

Pathogenicity test. Tomato seed (the hybrid Jackpot, Ferry Morse Seed Co.) was planted in pasteurized soil in plastic pots (6″ in diameter) in an environmental control chamber at 16 C. Three weeks after planting, the leaves of seedlings were mechanically inoculated with isolate T1. Experimental controls remained uninoculated. Each plant was individually chopped after 6 wk, and the debris was incorporated into the soil in the pot in which the plant had grown. Tomato was once again seeded in the soil and incubated at 16 C. Water draining from each pot was collected and added back to the same pot. Ten weeks later the plants were evaluated for disease symptoms.

Disease survey. Plants were sampled in symptomatic and nonsymptomatic fields during the growing seasons of 1987 and 1988. Root and leaf tissue were indexed for infection by TBSV using double antibody sandwich ELISA (DAS-ELISA).

Cultivar reaction. During many years of field observations, one of us (Van Maren, *unpublished*) has observed significant differences in disease severity among different tomato cultivars. To determine if disease severity was related to concentration of virus antigen in host tissue, 3-wk-old seedlings of eight cultivars with differing field reactions to TPD were mechanically inoculated with isolate T1. The plants were grown in the greenhouse and were evaluated for local lesion and systemic symptom development. Relative virus antigen concentration of leaf tissue was measured with DAS-ELISA after 4 wk.

Fig. 2. Agarose gel electrophoresis of whole virions of tomato bushy stunt virus isolates. Lane 1 contains isolate T1, lane 2 contains BS-3 strain, and lane 3 contains cherry strain.

Temperature effects. Tomato cultivars were mechanically inoculated with isolate T1 and incubated at 16, 20, 24, and 28 C in environmental control chambers with a 12 hr light regime per 24 hr. The cultivars were evaluated for infection by observing local lesion development on inoculated leaves, systemic infection, and relative virus antigen content by DAS-ELISA.

RESULTS

Isolate characterization. The antiserum collected 12 wk after the initial injection had a titer of 1:1024 in agarose double diffusion. Serological comparisons by SDI analyses failed to reveal any significant differences between the T-1 and BS-3 isolates (i.e., SDI = 0). In contrast, the cherry strain could be distinguished from both tomato isolates with an SDI of 2.0. Both the T1 and BS-3 isolates formed precipitin lines at antisera dilutions of up to 1:1024, 1:256, and 1:125 with the T1, BS-3, and cherry antisera, respectively. The cherry strain formed precipitin lines at antisera

dilutions of up to 1:256, 1:64, and 1:512 with the same 3 antisera. When T1 antiserum was tested with its homologous virus and the BS-3 isolate in adjacent wells, complete fusion of precipitin lines was observed at all antiserum concentrations (Fig. 1). The reciprocal test produced the same reaction. However, when the cherry isolate was adjacent to either T1 or BS-3 isolates and challenged with one of their homologous antisera, strong spurs were observed on the precipitin lines.

During the course of this investigation, we collected 34 additional isolates of TBSV from symptomatic tomatoes from the field. These were paired next to isolate T1 in agar double diffusion test and challenged with T1 antiserum. All of these isolates reacted with T1 antiserum and failed to spur when in wells adjacent to isolate T1 (data not shown).

Fig. 3. Symptoms of tomato plant decline developing on tomato plants seeded into soil infested with tomato debris from plants inoculated with isolate T1 of tomato bushy stunt virus. The healthy plants on the right were grown in soil infested with healthy tomato debris.

Although the T1 and BS-3 isolates could not be distinguished in serological analyses, the virions could be readily differentiated by characteristic mobility differences in agarose gel electrophoresis performed at elevated pH (Fig. 2). Electrophoresis revealed that the T1 isolate was the least mobile of the three viruses tested. This approach provided the only available procedure for distinguishing among the isolates.

Pathogenicity test. Ten weeks after replanting, all the plants growing in soil infested with tomato debris infected with isolate T1 were showing severe symptoms of TPD (Fig. 3). Symptoms included severe stunting, reduced leaf and leaflet size, chlorosis, and epinasty. Plants growing in soil infested with the tomato debris not infected with isolate T1 appeared healthy. The symptomatic plants were positive for TBSV, as indicated by DAS-ELISA, and nonsymptomatic plants were negative.

Olpidium brassicae (Woronin) P. A. Dang. was frequently found infecting roots of tomatoes from both diseased and nondiseased fields. *Olpidium* species are known to vector tombusviruses (5,6,21,22,26,27). Studies were conducted to determine if TBSV could infect tomato roots without a soil fungal vector. When tomato seed was planted in pasteurized soil that was then infested with freeze-dried preparations of isolate T1 that was not infested with *O. brassicae*, the plants became infected with TBSV.

Disease survey. Results of the disease survey for 1987 and 1988 are listed in Table 1. Plants were considered to be positive by DAS-ELISA if the absorbance values were greater than the mean plus 3 × the standard deviation of the absorbance value of greenhouse grown control plants. A correlation of 68% was found

TABLE 1. Results of tomato plant decline surveys conducted in the California desert in 1987 and 1988 and detection of tomato bushy stunt virus (TBSV) in field samples by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)

1987[a]			1988		
Field number	Symptoms[b]	Samples tested for TBSV[c]	Field number	Symptoms	Samples tested for TBSV
1	+	12/12	1	+	25/30
2	+	6/7	2	+	5/5
3	+	1/7	3	+	5/6
4	+	14/14	4	+	20/28
5	+	0/2	5	−	4/14
6	+	1/1	6	−	0/6
7	−	3/20	7	−	5/12
8	−	0/6	8	−	1/4
9	−	0/9	9	−	2/5
			10	−	2/5

[a] 1987 samples were collected on 28 April in the Imperial Valley; 1988 samples were collected on 12 and 13 April in Blythe, CA and the Imperial Valley.
[b] Samples were collected in symptomatic (+) and nonsymptomatic (−) fields.
[c] DAS-ELISA was conducted with the samples using an antiserum made against isolate T1 of tomato bushy stunt virus. Samples were considered to be positive by DAS-ELISA if the absorbance value was greater than the mean plus 3 × the standard deviation of the absorbance value of greenhouse grown control plants. Numerators are the number of positive samples, denominators are the total number of plant samples collected from each field.

TABLE 2. Effect of isolate T1 of tomato bushy stunt virus on eight tomato cultivars

Cultivar	Source[a]	Field reaction[b]	Plants with LL[c]	Symptom severity[d]	Relative virus antigen content[e]
Celebrity	Peto	T	6	1(1)	0.28(.35)
H-100	Peto	T	5	3(2)	0.24(.40)
460	Peto	I	7	4(1)	0.89(.19)
204-9	Peto	I	7	4(1)	0.88(.32)
Valerie	Northrup King	S	9	3(2)	0.66(.35)
UC 82	Peto	S	9	5(1)	1.09(.40)
Jackpot	Ferry Morse	S	10	6(1)	0.84(.31)
6203	Peto	S	8	7(1)	1.11(.18)

[a] Name of seed company providing cultivar.
[b] T = Tolerant, I = Intermediate, S = Susceptible.
[c] Number of plants, out of 10, exhibiting local lesions 1 wk after inoculation.
[d] Plants rated on a scale of 0–9; 0 = no symptoms, 9 = dead; mean (and s.d.) of 10 replicates.
[e] Absorbance values (405 nm) of DAS-ELISA test; mean (and s.d.) of 10 reps. The average value for noninoculated plants was 0.03(1).

between symptoms and detection of TBSV by DAS-ELISA in 1987 and 80% in 1988. A correlation of 9% was found between nonsymptomatic plants and detection of TBSV in 1987 and 30% in 1988.

Cultivar reaction. Good agreement between field observation and reaction of cultivars in the greenhouse was obtained (Table 2). Local lesions were slower to develop on inoculated leaves, symptom expression was less severe, and virus titer was decreased on tolerant cultivars. A good correlation was observed between virus titer and symptom expression ($R = 0.73$).

Temperature effects. The effect of temperature on symptom development and virus titer was substantial (Fig. 4). The number of plants that became infected greatly decreased as the temperature increased. At 16 C the infection rate was 100%, but it decreased to 50% at 20 C, 12.5% at 24 C, and 0% at 28 C. The virus titer in the infected tissue also decreased dramatically as the temperature increased.

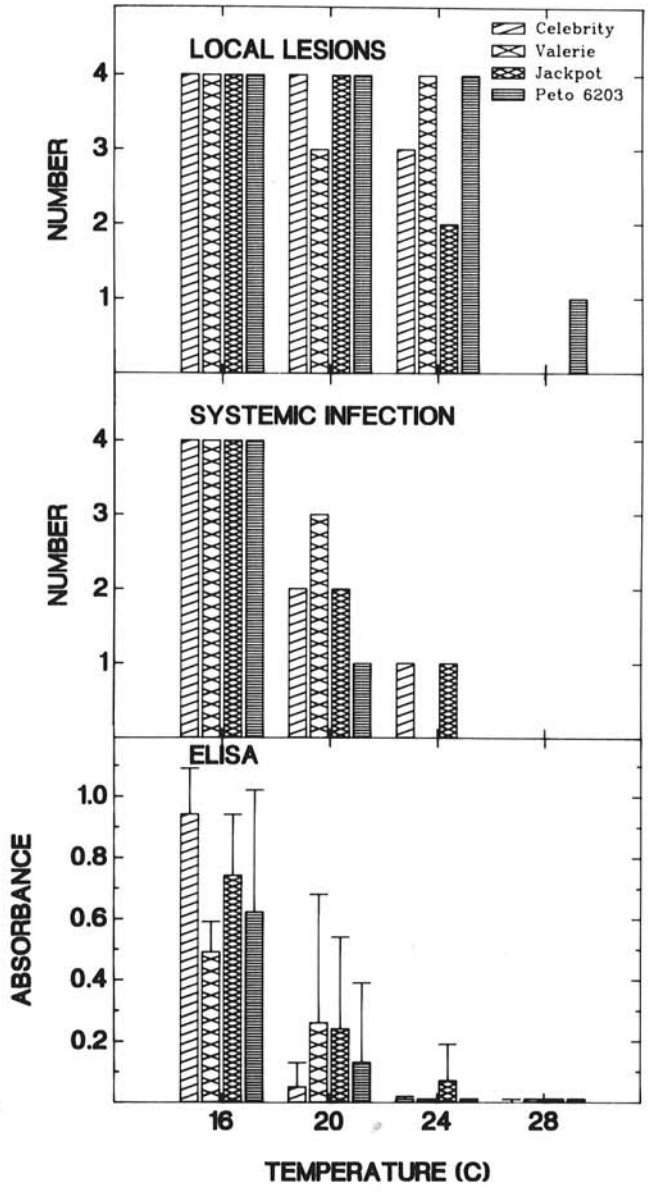

Fig. 4. Affect of temperature on infection of four tomato cultivars by tomato bushy stunt virus (TBSV). Tomato cultivars were mechanically inoculated with isolate T1 and incubated at 16, 20, 24, and 28 C in environmental control chambers. Four plants of each variety were tested at each temperature. Values for local lesions are the number of plants which exhibited local lesions on inoculated leaves 6 days after inoculation. Values for systemic infection are the number of plants which were systemically infected with TBSV as determined by double antibody-sandwich-ELISA 15 days after inoculation. Values for ELISA are the means of the absorbance values of the four plants tested; bars are one standard deviation of the mean. The average value of the noninoculated plants was 0.01.

DISCUSSION

The serological results from SDI analyses and immunodiffusion demonstrate that the T1 tomato isolate from California is indistinguishable from previously reported tomato isolates such as the BS-3 strain. This result is consistent with other recent outbreaks of TBSV-caused diseases in solanaceous crop plants (2,7,16). The ability to distinguish very minor differences in electrophoretic mobility between closely related isolates of TBSV (13,16–18) was demonstrated in our study as well and proved useful in distinguishing the novel field isolates from the TBSV-BS-3 and cherry strain standards.

The pathogenicity test conducted in the environmental control chambers, together with the pathogen association data collected in the disease surveys, provides bona fide evidence of the etiological significance of TBSV in TPD. The field survey data from 1988 did not correlate with symptom development as well as the 1987 data, perhaps due to the warmer growing season in 1988, in which infected plants could have remained nonsymptomatic. Previous workers have found that temperature dramatically affects symptom expression in plants infected with tombusviruses (11,12,23,25). In our experiments we have shown that temperatures greater than 20 C will attenuate both symptom development and virus titer. These results correlate with field observations. Late in the season, when temperatures are higher, previously symptomatic plants will tend to produce normal, asymptomatic foliage (Van Maren, *unpublished*); also, late in the season, virus titers in infected plants are reduced to nondetectable levels (Gerik, *unpublished*). Our field observations on the temperature sensitivity of the disease are consistent with recent studies that have demonstrated that TBSV replication is temperature sensitive in both plants (11) and protoplasts (14). The interrelation of temperature and virus titer may also explain the difficulty encountered by previous researchers in detecting a pathogenic virus (20). Attempted isolations from mature symptomatic plants collected from the field in May or June, when temperatures have increased, may be unsuccessful due to the very low virus titer in plants growing at higher temperatures.

The reaction of different tomato cultivars indicates that varietal resistance may be an applicable control measure for this disease, but the data from the growth chamber studies imply that temperature could be an important factor in the expression of resistance. The cultivar Celebrity, observed in the field and greenhouse to be tolerant, had reduced virus titer at 20 C and higher but not at 16 C.

We have shown that infection by isolate T1 can occur without a fungal vector. This phenomenon has been previously documented for tombusviruses (3,15), but this observation does not preclude the possibility that vectors may be involved in this disease. Cucumber necrosis virus, another tombusvirus, does have a fungal vector (5,6,26,27). A fungal vector would account for efficacy of solarization and fumigation as controls of the disease.

The common occurrence of TPD throughout the Imperial Valley and the Blythe area of California is an interesting and unusual phenomenon. Several previous studies have identified tombusviruses in association with river water throughout the world, and there is strong suggestive evidence that these viruses can enter a field in irrigation water (19,22,29). This seems a reasonable explanation for the widespread incidence of the disease because the entire agriculture area involved in the epidemic shares the Colorado river as a common irrigation water source. Hence, we have begun studies to detect the virus directly in the water from the river and irrigation canals in the area. The observation that the disease is seen only in the third and later crops indicates that the virus is not only increasing during the previous tomato crops but also persists in the soil in the absence of tomatoes.

Although TPD has been observed since 1977 until now, the causal agent has remained unknown. Tomato bushy stunt virus

has been widely studied, but has been of little etiological importance (13,22). Previously, most reported epidemics of TBSV have been in greenhouse-grown solanaceous crops (2,13,16,31). Three previous epidemics of field-grown tomatoes caused by TBSV have been reported (4,7,24). The disease in California differs from the previous reports in that all fields known to have had three crops of tomatoes since 1977 exhibit symptoms of the disease and the incidence in infested fields nears 100%. Also, fruit symptoms such as chlorotic blotching, rings, and line patterns previously reported to be the major cause of loss due to TBSV have not occurred in California. The manifestation of symptoms of TPD in California has been unique and devastating.

In conclusion, our data have shown that the cause of TPD is TBSV. This is the first step in the development of control measures for this disease. Many approaches exist for control, including varietal resistance and soil solarization and fumigation. Cultural approaches, such as the delaying of planting until the soil warms above temperatures optimal for the virus, may be effective. Research is continuing to find a control for this disease.

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