Estimation of the effect of *Tomato spotted wilt virus* (TSWV) infection on some yield components of tomato

Mehmet Ali Sevik · Miray Arli-Sokmen

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Abstract The effects of Tomato spotted wilt virus (TSWV) on yield and quality of tomato fruits were studied from May through November of 2004 at the Experimental Field of the Agricultural Faculty of Ondokuz Mayis University, in Samsun province, Turkey. TSWV caused 42.1% and 95.5% reduction in yield and marketable value of tomato, respectively. TSWV infection in tomato crop caused significant (P < 0.05) reductions in weight, total number, width and length of the fruits in infected plants. Reductions in yieldcontributing parameters were 26.61% in weight, 20.18% in number, 10.94% in width and 11.93% in length of fruits. It is difficult to estimate the actual yield loss and influence levels of TSWV in the field. Economic data are scarce, but in the present study it was estimated that the yield loss due to TSWV in tomatoes was approximately \$0.9 million in Samsun, Turkey.

Keywords Fruit · Tomato yield · Turkey · Yield reduction

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a crop of major importance in many countries (Engindeniz 2007).

M. A. Sevik (⊠) • M. Arli-Sokmen Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey e-mail: malis@omu.edu.tr

Turkey is the third largest tomato-producing country in the world after China and the USA (Anon. 2009), with almost 0.3 million ha under cultivation and a total annual production above 9 million tons. The tomato plant and fruit are susceptible to more than 40 viral diseases (Polston and Anderson 1997). Tomato spotted wilt virus (TSWV) is one of the most important viruses affecting tomatoes, occasionally leading to losses of up to 100% (Rosello et al. 1996). TSWV belongs to the genus Tospovirus within the Bunyaviridae (van Regenmortel et al. 2000). TSWV was first reported in tomato (Brittlebank 1919) and has a very broad host range estimated to be up to 1,090 plant species within 84 families (Parrella et al. 2003). The virus causes severe yield losses (producing less valuable or unmarketable plants, fruits or flowers) in many economically important crops (Ramkat et al. 2006). It is transmitted exclusively by at least nine species of thrips (Jones 2005) in a propagative-circulative manner (Wijkamp and Peters 1993). TSWV virions have spherical enveloped particles, about 80-110 nm in diameter (Adkins 2000).

Tomatoes infected by TSWV evince a wide variety of symptoms. Their appearance and severity depend on the genotype, the plant development stage at the time of infection, the virus isolate, and environmental conditions (Rosello *et al.* 1996). In tomato leaves, the virus causes characteristic bronzing or purpling, downward curling; leaf distortion; brown, reddish, bronze-colored, or yellowish concentric rings; irregular whitish or necrotic leaf spots and flecking; complete plant stunting and yellowing (Anon. 1991). Tomato fruit formed prior to infection appear normal whereas fruit that develop later may have pronounced symptoms. Green tomato fruit often develop pale green, white or yellowish blotches and spots (often with concentric zones of different colors), or small bumps on the surface. Severely affected plants may not form fruit or the fruit may be very small and unmarketable (Pataky 1991).

In recent years, various field surveys have been carried out on the incidence of TSWV in Samsun province of Turkey. From observations, it is known that the incidence and spread of TSWV have gradually reached high levels in tomato- and pepper-producing areas in Samsun. It is important to determine the economic effect of the virus to regulate virus management methods, and there is no information on the economic impact of TSWV in the tomato crop in Turkey. The purpose of the current study was to determine the effect of TSWV on tomato yield components under field conditions and to determine the yield loss in tomato due to TSWV infection for the Black Sea Region of Turkey. This article presents the results of research carried out in the Experimental Field of the Agricultural Faculty of Ondokuz Mayis University from May through November in 2004.

Materials and methods

Obtaining TSWV-infected source plants TSWV-tomato isolate (TWB01) was obtained from a fruit sample collected from a tomato field in Samsun province during surveys in 2002–2003 and maintained on *Nicotiana rustica*. Leaf extract from infected *N. rustica* plants was inoculated mechanically onto leaves of tomato seedlings at the 2–4 leaf stage after homogenization in 0.03 M phosphate buffer (pH: 7.0) including 0.1% Na₂SO₃ (sodium sulfite). Leaf-tobuffer ratio was 1:5 (1 g infected leaf to 5 ml buffer) (Gracia *et al.* 1999). After rinsing with tap water, plants were maintained in a growth cabinet at 20–23°C with a 16 hL:8 hD photoperiod for 2 weeks. Inoculated tomato plants were tested by ELISA to confirm TSWV infection.

Field experiment The field experiment was conducted at the Experimental Field of the Agricultural Faculty of Ondokuz Mayis University (41.3° N, 36.11° E) in Samsun, Turkey. Tomato seedlings of the cultivar "Y-65" were planted with 1 m between rows and 0.5 m between seedlings. Four thousand and eighty seedlings were planted in 60 rows, creating an experimental field of 2.100 m² (60×35 m). The field was equally divided into 16 plots, each of which had 15 rows and 255 plants. A total of 15 tomato seedlings were sapinoculated with TSWV-tomato isolate (TWB01) and placed at the center of the experimental field 4 weeks after tomato seedlings planted as inoculum source for natural thrips transmission. The plants were replaced by new inoculated ones every 2 weeks to maintain the virus infection pressure. One tomato plant from each row of the plots was randomly selected and marked with a white plastic label. Leaf samples from a total of 240 white-labeled plants (15 rows×16 plots) were collected and tested by ELISA each week for 10 weeks. Five each of uninfected (control) and TSWV-infected plants from white-labeled plants in each plot were selected and used for estimation of TSWV losses.

Yield and its components Fruits of infected and uninfected plants were harvested as they matured; fresh weight, width, length and marketability of fruits were recorded for each plot. Fruits with distorted shape, chlorotic ring spots, rough and/or discolored exterior and very small fruits were judged unmarketable, and those without symptoms and of normal fruit size were graded into marketable. Percent reductions of the yield-contributing characters including yield loss were calculated according to the following formula (Farooq *et al.* 2007)

$$P = \frac{A - A_1}{A} \times 100$$

where

- *P* Reduction percentage of yield-contributing character
- A Any parameter (yield-contributing character) of control (uninfected) plants
- A₁ Any parameter (yield-contributing character) of infected plants

Data analysis Experimental data were analyzed statistically using the *t*-test, and the differences between the uninfected and infected plants were measured for fruit weight, length and width. All statistical tests

Table 1 Average numbers and values of tomato fruits for uninfected tomato plants and those infected with Tomato spotted wilt virus

Plot no.	Uninfected fruit					Infected fruit				
	Number of fruits per plant	Unmarketable fruits	Weight (g)	Width (mm)	Length (mm)	Number of fruits per plant	Unmarketable fruits	Weight (g)	Width (mm)	Length (mm)
1	31	1	51.16	53.72	46.40	22	22	46.77	47.20	38.14
2	30	1	63.07	52.70	43.85	22	22	39.11	44.82	37.65
3	34	2	70.45	48.55	36.16	26	25	34.09	44.46	36.57
4	37	1	64.03	47.81	46.13	26	24	40.01	40.64	38.05
5	41	2	54.39	44.98	41.68	38	33	49.81	37.00	31.64
6	27	2	76.33	48.22	45.04	20	20	41.18	42.66	32.67
7	31	1	62.62	44.07	44.73	23	22	36.97	36.32	35.67
8	39	5	61.59	38.04	31.08	37	34	47.77	32.47	30.49
9	28	0	47.61	42.67	33.70	20	20	38.93	37.04	34.30
10	25	1	64.62	36.35	32.70	21	21	45.67	31.64	31.39
11	33	0	54.82	34.13	30.61	26	24	32.34	34.25	33.04
12	37	1	53.97	36.64	33.91	26	24	33.51	37.67	37.53
13	38	2	51.96	43.78	40.80	37	35	65.19	34.78	32.50
14	35	0	50.82	43.95	43.38	23	23	44.47	38.05	33.97
15	35	2	46.28	45.89	42.46	30	29	41.32	44.85	36.98
16	32	2	45.00	41.38	37.77	25	25	37.10	42.07	34.61
Average	33.31	1.43	57.42	43.93	39.40	26.34	25.18	42.14	39.12	34.70
% Reduction	0.0	4.3	0.0	0.0	0.0	20.18	95.5	26.61	10.94	11.93

were performed at the 0.05 level of significance using the statistical package SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

Serological testing DAS-ELISA (double antibody sandwich-enzyme linked immunosorbent assay) method was used to detect TSWV in tomato samples and applied according to Clark and Adams (1977) and instructions of the antisera's manufacturer (Loewe Biochemica, Sauerlach, Germany) for the polyclonal antisera of TSWV. In the DAS–ELISA method, samples were ground (1 g leaf in 5 ml buffer) in extraction buffer (PBS: 0.13 M NaCl, 0.014 M KH₂PO₄, 0.08 M Na₂HPO₄.12H₂O, 0.002 M KCl, pH 7.4) containing 0.05% Tween-20, 1% skimmed milk powder added to wells of microplate (TPP, Switzerland) after coating with TSWV-specific polyclonal antisera diluted in carbonate buffer (pH 9.6) and incubated at 4°C overnight. Plates were washed three times with PBS/Tween-20 buffer and coated with

Fig. 1 Symptoms of *Tomato* spotted wilt virus appearing on tomato leaf (a) and fruit (b) in the field







alkaline phosphatase conjugated antibody diluted in extraction buffer and incubated for 2 h at 37°C. After washing, p-nitrophenyl phosphate in diethanolamine substrate buffer (0.5 mg ml⁻¹; pH 9.8) was added to wells and incubated at room temperature for 30– 180 min. Absorbance values were read at 405 nm using a microplate reader (Tecan Spectra II, Grodig/Salzburg, Austria). Virus-free tomato plants grown in an insect-proof growth chamber were used as negative controls. Samples were considered to be positive when the absorbance values at 405 nm values exceeded the mean of the negative controls by a factor of at least three (Arli-Sokmen *et al.* 2005).

Results

Marketable fruit and yield reductions due to TSWV infection Fruits were harvested and fresh weight,

width, length and marketability were recorded (Table 1). Due to infection with TSWV, young plants evinced inward cupping of leaves and turned bronze in color (Fig. 1a). Leaves developed numerous small dark spots, purple flecks or small necrotic rings. A yellowish concentric ring and/or mosaic were observed on the mature fruits (Fig. 1b).

Infected plants produced poor quality fruit (Fig. 2). The yield of marketable fruits of the plants infected with TSWV was significantly reduced. The yield of infected plants decreased significantly in comparison with uninfected plants due to less and small fruit production. Total weight of fruits harvested from each uninfected and infected plant selected from 16 plots was 61.21 kg and 35.41 kg, respectively. Yield loss was 25.8 kg and the percentage of yield loss calculated for 2004 in the experimental area was 42.1. TSWV also caused 95.5% reduction in the marketable value of tomato. The average weight per



Fig. 3 Average number of fruits of healthy plants and those infected with *Tomato* spotted wilt virus in each plot in the experimental area

fruit was 42.14 g for TSWV-infected plants and 57.42 g for uninfected ones. The average width per fruit was 39.12 mm and 43.93 mm for infected and uninfected fruits, respectively. The average length per fruit for infected plants was 34.7 mm and 39.4 mm for uninfected plants (Table 1).

This study indicated that the virus caused a reduction in fruit number (Fig. 3) by 20.18% as compared with the number produced by healthy plants (Table 1). Early plant infection with TSWV typically resulted in more severe symptoms or plant death.

The reduction in fruit length and width of infected plants was 11.93% and 10.94%, respectively. The average fruit weight reduction for the infected plant was determined to be 26.61%. TSWV infection caused significant reductions (P<0.05) in fruit weight, width and length. Also, the virus significantly (P<0.05) reduced the number of fruit on infected plants as compared with uninfected ones (Table 2).

Yield loss (YL) was estimated for the year 2004 in Samsun province by considering the following formula (Wallen and Jackson 1975) and using the yield loss factor (42.1%) in the experimental area as representative of field tomato production in Samsun and the percentage of area (36%) infected during surveys in Samsun province.

 $YL = AI \times ABP \times AvY \times YLF$

where

- AI Area infected in a specific year, expressed as a percentage;
- **ABP** Area (ha) in tomato production in a specific year [data of Turkish Statistical Institute (Anon. 2004)];
- **AvY** Average yield in kg ha⁻¹ in a specific year [data of Turkish Statistical Institute (Anon. 2004)];

 Table 2 Effects of Tomato spotted wilt virus on yield components of tomato fruits

Tomato fruit	Mean ± SEM	t
Weight (g)	49.312±0.202	24.397*
Width (mm)	43.872 ± 0.934	46.947*
Length (mm)	37.065 ± 0.904	40.994*
Number (per plant)	29.906 ± 0.113	26.362*

*Significant at 5% level

- YLF Yield loss factor, expressed as a percentage (yield loss in the current study)
- YL 0.36×6.920×5.351×0.421
- YL 5.612.103 kg (2004)

The Yield loss (YL)= $5.612.103 \times \$0.1613$ per kg (the price for 2004)=\$905,232

We estimated that a total annual crop loss due to TSWV in 2004 was nearly \$0.9 million for outdoor tomato production in Samsun.

Discussion

The objective of this study was to reveal the effect of TSWV on yield parameters of tomato under field conditions at the Experimental Area of the Agricultural Faculty of Ondokuz Mayis University, from May to November 2004. Characteristic symptoms noticed on tomato fruits were distinct concentric rings on fruits, which later turned into brown, uneven ripening. The crop loss caused by TSWV on tomato in this study was 42.1% and there was a 95.5% reduction in marketable tomatoes. Similarly, the severe symptoms of TSWV were observed on tomato fruits by Wangai et al. (2001) and a 90% reduction in marketable yield was observed in variety Cal J. by Ramkat et al. (2006). Moriones et al. (1998) also stated that the quality (marketable) of tomato yield is dramatically reduced by TSWV infection in infected plants. TSWV is now one of the ten most economically destructive plant viruses, with worldwide losses exceeding one billion dollars annually (Saidi and Warade 2008). The economic impact of TSWV is great, due to its wide geographical distribution and to its broad host range. Crops in which important losses due to TSWV have been reported are tomato, pepper, lettuce, eggplant, papaya, French beans, celery and ornamental plants (Gragera et al. 2003; Rosello et al. 1996). Infection rates of 30-100% lead to major losses in commercial vegetable crops. These losses include reduction of yield, quality, and occasional crop failures where virus infections have been excessive (Cho et al. 1986; German et al. 1992).

Percentage of marketable fruits is perhaps a more useful criterion than yield reduction in evaluating economic data (Salama and Sill 1968). If the nonmarketable fruits were excluded, the losses would have been much greater, since more than 95% of the fruits harvested from the infected plants were nonmarketable (Al-Shahwan *et al.* 1995). Similarly, 95.5% of the fruits were found to be nonmarketable in the present study.

In the current study, infection of tomatoes with TSWV significantly reduced the average fruit weight per plant in all infected plants. In tomato, the average yield per fruit was 42.14 g for TSWV-infected and 57.42 g for uninfected plants.

TSWV-infected plants may be reduced greatly in fruit size (width and length, see Table 1). Successful tomato production is usually associated with healthy vegetative top growth throughout the growing season (Francki and Hatta 1981; Gitaitis *et al.* 1998; Moriones *et al.* 1998). Díaz-Pérez *et al.* (2003) found that there is an increasing reduction of vegetative top fresh weight, fruit number and fruit yield (total and marketable) with increasingly earlier expression of TSWV symptoms during tomato plant development. Compared with symptomless plants, total fruit yield of symptomatic tomato plants was reduced by 2.3% for each day before harvest that plants first exhibited symptoms of TSWV.

Kim *et al.* (2004) reported that the virus caused yield and quality losses of paprika fruit in Korea. Tomato crops in several districts in Kenya were affected by a disease suggestive of TSWV infection during the November 1999 to March 2000 tomato-growing season. Farmers reported up to 80% losses of their potential yields (Wangai *et al.* 2001).

It is difficult to estimate the actual levels of TSWV that have an effect in the field. Economic data are scarce, but in the present study, we estimated yield losses due to TSWV about \$0.9 million for tomato crop in Samsun, Turkey. Similarly, estimates in the Netherlands indicated annual losses from direct damage by Western flower thrips of \$30 million, and by TSWV of a further \$19 million (Roosjen et al. 1998). Australian vegetable growers estimated total annual crop losses due to vegetable pathogens of up to \$150,000 and \$54,000 for greenhouse and outdoor vegetable crops, respectively. Of the viruses, TSWV was the most predominant (Porter et al. 2007). TSWV is a serious problem for tomato production in Georgia, USA, causing as much as \$8.8 million in losses in a single year (Riley 2000). TSWV and thrips vectors have had a tremendous negative impact on the yield of various crops including peanut, tobacco, tomato, and pepper, causing an estimated \$100 million in losses annually across all of these crops in Georgia. In Phytoparasitica (2012) 40:87-93

tomato, TSWV can often reduce marketable yields by 50% besides greatly increasing the incidence of irregular-ripened tomatoes (Riley 2004). In Georgia, flue-cured tobacco is also seriously affected by TSWV, causing stand loss in excess of 30% with an estimated loss of more than \$17 million in 2004 (Pearce 2005). Also, TSWV causes millions of dollars in losses to the peanut industry in Georgia, USA (Chu *et al.* 2004).

To the best of our knowledge, the current study is one of the few that present quantitative data on the effect of viruses on crops in Turkey and the first report of quantitative data on the effect of TSWV on tomato plants in Turkey.

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