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Research Article

Incidence and possible sources of *Tomato spotted wilt virus* in tobacco grown in Denizli Province, Turkey

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Abstract

Tomato spotted wilt virus (TSWV) is economically prominent disease for its impact on tobacco (*Nicotiana tabacum* L.) production worldwide. An increase of the incidence of symptoms typical of TSWV has been observed in tobacco production areas in Denizli province of Turkey where tobacco is significantly grown. Surveys were conducted to determine the prevalence status of TSWV in tobacco cultivars and its possible sources of infections in four tobacco growing districts of Denizli province. A total of 501 plant samples from field-grown tobaccos, weeds, potential intermediate hosts, seedlings and seeds were collected during 2019 and tested by DAS-ELISA. Of these plants, 243 belong to 55 different weed species from 26 different families with intermediate host potential. Throughout the study, 40 crop plant samples which could be intermediate hosts and 39 tobacco seed samples were also taken for testing. Adult thrips specimens were picked up from the fields and brought to the laboratory for preparations. Four vector virus species were detected when adult thrips individuals were diagnosed: *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), *Aeolothrips intermedius* Bagnall (Thysanoptera: Aeolothripidae) and *Thrips major* Uzel (Thysanoptera: Thripidae). Of the 179 tobaccos sampled, 31.2% was positive; besides, of 243 weeds tested 10 were found to be infected. *Echinochloa crus-galli* and *Tordylium apulum* were determined to be new host recordings for TSWV infection. Only one tomato plant from the crop plants as intermediate hosts was infected. *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY) was also confirmed in tobacco fields.

Keywords: DAS-ELISA; tobacco; thrips; TSWV; viruses; weeds

Introduction

Tobacco (*Nicotiana tabacum*) is one of the most cultivated plants in many countries of the world. Turkey is a major tobacco producing country with a production of 70,000 tons per year (FAO, 2019). Denizli is located southwestern part of Turkey and tobacco is one of the most commonly grown field crops in Denizli.

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However, tobacco production is affected by several diseases and tobacco has a large number of virus diseases causing economic losses.

Tomato spotted wilt virus (TSWV), type member of the genus *Orthotospovirus*, is an economically significant pathogen that infects tobacco (Mila, 2011). It is the most serious problems in tobacco growing regions and transmitted persistently by at least eight species of thrips especially *Frankliniella occidentalis* (Whitfield *et al.*, 2005). It is noteworthy that in Japan (Tomaru *et al.*, 1982), USA (Martinez-Ochoa *et al.*, 2003), Zambia (Mayunga and Kapooria, 2003), Greece (Chatzivassiliou *et al.*, 2004), Serbia (Stanković *et al.*, 2011) and Bosnia and Herzegovina (Delić *et al.*, 2018) tobacco production was greatly affected due to TSWV infection. In recent years, virus diseases have become a problem causing high yield reductions in most tobacco-growing areas also, destructive effects of TSWV on tobacco was reported in Turkey (Azeri, 1981; Erdem, 2010; Günay, 2019). The wide range of host-plants including weed species and transmission by thrips makes difficult to control this virus (Groves *et al.*, 2002). As with the majority of plant viruses there is no effective curative treatment for TSWV. The aphid-transmitted *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Alfalfa mosaic virus* (AMV) and the mechanically transmitted *Tobacco mosaic virus* (TMV) are also thought to be the most prevalent ones (Chatzivassiliou *et al.*, 2004). Others such as *Tobacco ringspot virus* (TRSV) and *Potato virus X* (PVX) have been reported to have minor economic importance (Mayunga and Kapooria, 2003).

Tobacco cultivation is attacked by different pests and diseases thereby, cause severe damage to production. Among insects, the order Thysanoptera draws attention because they cause mechanical damage, such as feeding on areas close to leaf veins where they cause small silver spots and dotted with black spots (excrements) (McPherson *et al.*, 1999; Groves *et al.*, 2002; Arlı Sokmen *et al.*, 2005). In addition to that, the greatest damage of thrips is due to their ability to carry viruses and at least nine thrips species are known to transmit TSWV (Jones, 2005). TSWV is obtained by thrips from an infected plant in the first larval stage. But despite this, effective acquisition of the virus from an infected plant is less in the second larval stage. Followed by emerging adult thrips become infective and transmit the virus (Wijkamp *et al.*, 1995; Mason *et al.*, 2003). Although adult individuals can acquire the virus, but they cannot transmit it because of an age-dependent midgut barrier (Nagata *et al.*, 1999).

Identification and understanding of the prevalence of viruses, their transmission and alternative hosts is very crucial in a crop production. In order to achieve this, a survey was conducted to investigate the incidence of TSWV infecting the tobacco crops and wild flora in order to better understand their epidemiology. The presence of PVY, CMV, AMV, TMV, TRSV and PVX among the collected samples was also analysed.

Materials and Methods

Collection of samples

The major tobacco producing districts in Denizli province were investigated for virus presence. Extensive surveys were performed in randomly selected tobacco growing nurseries and open fields in the districts of Acıpayam, Çameli, Kale and Tavas (Figure 1). Field work was carried out in two different periods. First carried out in tobacco seedbeds, and later stages of vegetative growth conducted in tobacco fields. Tobacco leaves showing virus-like symptoms were collected from seedlings and tobaccos during surveying nurseries and fields. Weeds and potential intermediate hosts were collected from the tobacco producing areas and their vicinity. They were taken irrespectively of the presence of symptoms, with most of them being symptomless. Seed samples of commonly used tobacco varieties in the region taken from nurseries and tobacco fields throughout the vegetation period. Also, seeds have been sent from relevant companies and picked up from farmers were used as material for virus tests. All of them were kept at 4 °C and immediately tested serologically.

Figure l. Districts of Denizli Province, Turkey

Identification of weed species

Weeds were prepared according to herbarium techniques and diagnosed according to the Flora of Turkey (Davis, 1965,1985).

Thrips detection and identification

During the observations in the region, adult thrips were collected from the fields and put into thrips storage liquid (9 parts 60% ethyl alcohol + 1-part glacial acetic acid + 1 part glycerine). The tubes containing thrips individuals were individually numbered and brought to the laboratory for preparations. For the preparations of the species, thrips were first taken from the thrips storage liquid and kept in petri dishes containing lactophenol for 30 minutes. Individuals were then placed ventrally on a Hoyer-dropped slide, and their wings, legs and antennae were straightened. Then, they were covered with a coverslip and kept in an oven set at 55 °C for 1 hour (Özsemerci, 2007). Samples prepared in this way were made ready for diagnosis. The species belonging to the order Thysanoptera were identified by Associate Professor Ozan DEMİRÖZER.

Virus detection and identification

All collected samples were analysed for the presence of TSWV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Serologic method was also performed for detection CMV, TMV, TRSV, AMV, PVY and PVX. Due to the low virus incidence in seeds, they were tested after germination (Figure 2). Specific antibodies for TSWV, CMV, TMV, AMV, PVY, PVX and TRSV were applied according to manufacturer's instruction. Commercial positive and negative controls were included in the assays. Samples with readings higher than the average of healthy control readings two times were considered to be positive.

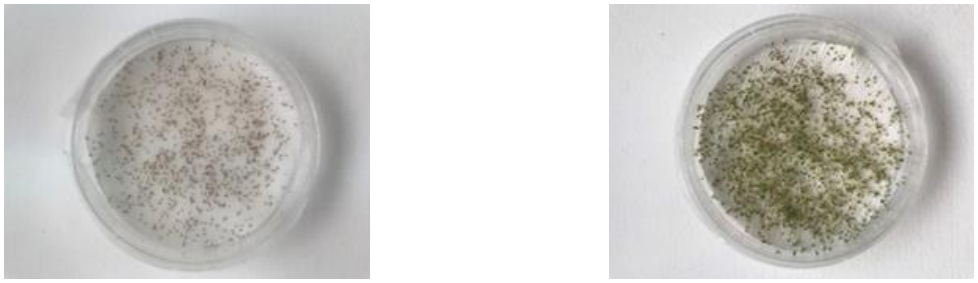

Figure 2. Germination processes of tobacco seeds before serological assays

Results

A total of 501 samples from field-grown tobaccos, weeds, intermediate potential hosts, seedlings and seeds were taken and analysed by serological test.

The tobacco plants collected from the fields were showing stunting, ringspots, mosaic, chlorotic and necrotic spots. Symptoms were showed in Figure 3. TSWV was determined in 35 of the tobacco plants taken from Acıpayam district where 28 tobacco growing areas were visited (Figure 4). In addition, mixed TSWV and AMV infection was found in three of them, whereas single AMV infection was detected in only one (Figure 5). Single TSWV infection was in 40 tobacco samples and single CMV infection was only in one of them taken from Tavas district where 27 plots were examined.

Figure 3. Symptoms of collected tobacco plants: a) stunting, b) ringspots, c) mosaic, d) chlorotic spots and e) necrotic spots

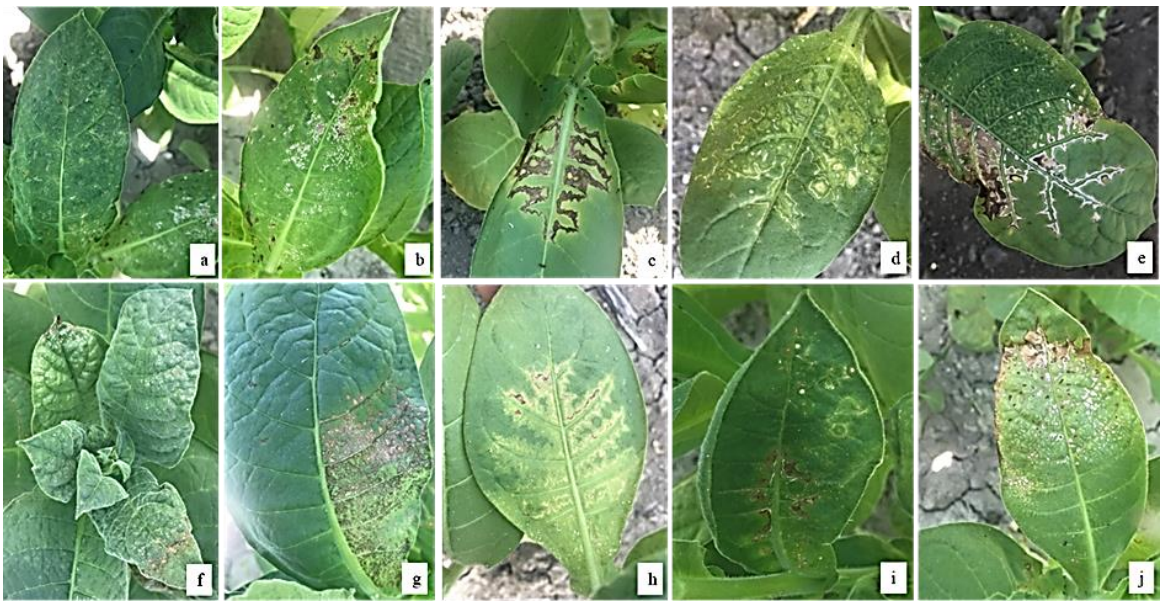

Figure 4. Symptoms of TSWV infected tobacco plants: a) chlorotic spots, b) chlorotic and necrotic spots, c) oak leaf shaped spot, d) chlorotic spots e) chlorotic and necrotic spots, f) chlorotic and necrotic spots, g) necrotic spots, h) oak leaf shaped spot, i) ringspots, j) chlorotic and necrotic spots

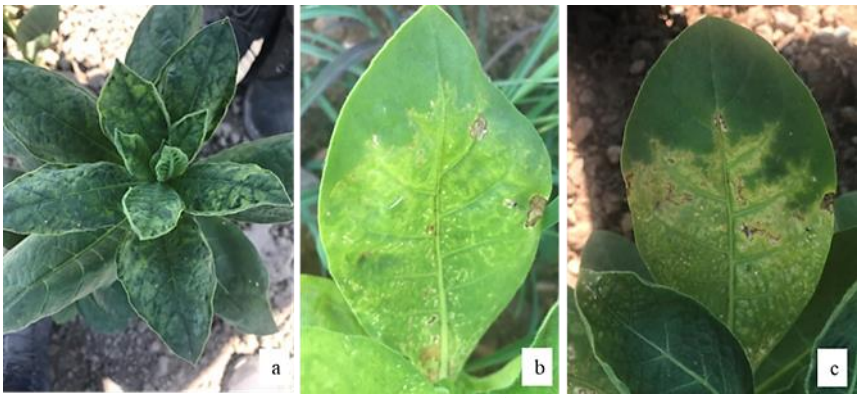

Figure 5. Symptoms of AMV infected tobacco plants: a) AMV infected, mosaic, b) AMV+TSWV infected, chlorotic and necrotic spots, c) AMV+TSWV infected, chlorotic and necrotic spots

In addition to this, mixed infections of CMV+PVY, TSWV+PVY and TSWV+CMV infections were recognized per one plant for each. The number of TSWV positive tobaccos was 22 from Kale district where 16 tobacco lands were visited. It was determined that 11 of the tobaccos taken from 2 tobacco farms in Çameli district were infected with TSWV. There were no other virus positive samples from Kale and Çameli districts. The ELISA results are mentioned in Table 1.

Table 1. The rate of tobacco infection according to the districts

Virus/Number of infected plants	District				
	Acıpayam	Tavas	Kale	Çameli	Total
TSWV	35	42	22	11	110
CMV	0	3	0	0	3
TMV	0	0	0	0	0
AMV	4	0	0	0	4
PVY	0	2	0	0	2
PVX	0	0	0	0	0
TRSV	0	0	0	0	0
Number of healthy plants	17	29	11	9	56
Number of tested plants	53	73	33	20	179

All the nurseries belonging to the producers of different companies and the tobacco varieties grown in the region was scanned. Ultimately, 26 tobacco seedling samples were taken from a total of 19 nurseries. No virus symptoms in the seedbeds were recognized and no infection with mentioned viruses was detected. Also, 39 tobacco seeds were tested and contamination with any viral agent was not found.

During the surveys weed samples belong to 55 different weed species from 26 different families (Table 2) and crop plant samples as intermediate hosts were collected. Of 243 weeds tested 10 were found to be infected with TSWV (Table 3). The infection was detected in only one tomato from the 40 crop plants taken as intermediate hosts (Table 4). The tomato plant was found TSWV+TMV positive.

Table 2. Weed species detected in tobacco nurseries and fields

No	Scientific Name	Common name	Family	Life span /Features
1	*Aegilops triuncialis*	Barb goatgrass	Poacae	Annual/Monocotyledon
2	*Althaea officinalis*	Common marsh-mallow	Malvaceae	Annual/Dicotyledon
3	*Amaranthus albus*	Tumbleweed	Amaranthaceae	Annual/Dicotyledon
4	*Amaranthus retrofelexus*	Redroot pigweed	Amaranthaceae	Annual/Dicotyledon
5	*Anagallis arvensis*	Scarlet pimpernel	Primulaceae	Annual/Dicotyledon
6	*Anthemis arvensis*	Field chamomile	Asteraceae	Annual/Dicotyledon
7	*Asphodelus albus*	White asphodel	Asphodelaceae	Annual/Dicotyledon
8	*Avena sterilis*	Winter wild oat	Poacae	Annual/ Monocotyledon
9	*Bromus tectorum*	Downy brome	Poacae	Annual/ Monocotyledon
10	*Capsella bursa-pastoris*	Shepherd's purse	Brassicaceae	Annual/Dicotyledon
11	*Centaurea solstitialis*	Yellow starthistle	Asteraceae	Annual/Dicotyledon
12	*Chenopodium album*	White goosefoot	Chenopodiaceae	Annual/Dicotyledon
13	*Cichorium intybus*	Chicory	Cichoriaceae	Annual/Dicotyledon
14	*Cirsium arvense*	Canada thistle	Asteraceae	Perennial/Dicotyledon
15	*Convolvulus arvensis*	Bindweed	Convolvulaceae	Perennial/Dicotyledon
16	*Cuscuta campestris*	Field dodder	Cuscutaceae	Parasite
17	*Cynodon dactylon*	Bermuda grass	Poaceae	Perennial/Monocotyledon
18	*Daucus carota*	Wild carrot	Apiaceae	Annual/Dicotyledon
19	*Echinochloa crus-galli*	Barnyard grass	Poacae	Annual/ Monocotyledon
20	*Equisetum arvense*	Field horsetail	Equisetaceae	Fern
21	*Fumaria officinalis*	Common fumitory	Fumariaceae	Annual/Dicotyledon
22	*Galium aparine*	Stickywilly	Rubiaceae	Annual/Dicotyledon
23	*Helichrysum italicum*	Curry plant	Asteraceae	Annual/Dicotyledon
24	*Heliotropium europaeum*	Common heliotrope	Boraginaceae	Annual/Dicotyledon
25	*Hordeum murinum*	Wall barley	Poacae	Annual/ Monocotyledon
26	*Hypericum perforatum*	St John's wort	Hypericaceae	Annual/Dicotyledon
27	*Lactuca serriola*	Prickly lettuce	Asteraceae	Annual/Dicotyledon
28	*Lamium amplexicaule*	Henbit deadnettle	Lamiaceae	Annual/Dicotyledon
29	*Lolium perenne*	Perennial ryegrass	Poacae	Annual/ Monocotyledon
30	*Malva sylvestris*	Common mallow	Malvaceae	Annual/Dicotyledon
31	*Matricaria chamomilla*	Wild chamomile	Asteraceae	Annual/Dicotyledon
32	*Orobanche ramosa*	Branched broomrape	Orobanchaceae	Parasite
33	*Papaver rhoeas*	Common poppy	Papaveraceae	Annual/Dicotyledon
34	*Plantago lanceolata*	Ribwort plantain	Plantaginaceae	Perennial/Dicotyledon
35	*Poa annua*	Annual bluegrass	Poacae	Annual/ Monocotyledon
36	*Polygonum aviculare*	Prostrate knotweed	Plygonaceae	Annual/Dicotyledon
37	*Portulaca oleracea*	Purslane	Portulacaceae	Annual/Dicotyledon
38	*Ranunculus arvensis*	Corn buttercup	Ranunculaceae	Annual/Dicotyledon
39	*Raphanus raphanistrum*	Wild radish	Brassicaceae	Annual/Dicotyledon
40	*Rubus fruticosus*	Blackberry	Rocaceae	Perennial/in bush form
41	*Salvia officinalis*	Sage	Lamiaceae	Annual/Dicotyledon
42	*Secale vulgare*	None	Poacae	Annual/ Monocotyledon
43	*Sinapis alba*	White mustard	Brassicaceae	Annual/Dicotyledon
44	*Sinapis arvensis*	Field mustard	Brassicaceae	Annual/Dicotyledon
45	*Sisymbrium officinale*	Hedge mustard	Brassicaceae	Annual/Dicotyledon

46	*Sorghum halepense*	Johnson grass	Poacae	Perennial/Monocotyledon
47	*Tordylium apulum*	Mediterranean hartwort	Apiaceae	Annual/Dicotyledon
48	*Tragopogon dubius*	Yellow salsify	Asteraceae	Annual/Dicotyledon
49	*Tribulus terrestris*	Puncture vine	Zygophyllaceae	Annual/Dicotyledon
50	*Urtica urens*	Burning nettle	Urticaceae	Annual/Dicotyledon
51	*Veronica hederifolia*	Ivy-leaved speedwell	Plantaginaceae	Annual/Dicotyledon
52	*Vicia cracca*	Bird vetch	Fabaceae	Annual/Dicotyledon
53	*Viscum album*	Mistletoe	Loranthaceae	Half parasite
54	*Xanthium spinosum*	Sping cocklebur	Asteraceae	Annual/Dicotyledon
55	*Xanthium strumarium*	Common cocklebur	Asteraceae	Annual/Dicotyledon

Table 3. Virus contamination status of weeds collected in surveys

Sampling date	Number of plants tested / TSWV infected plants	TSWV infected weed species
06.05.2019	46/0	-
20.05.2019	30/0	-
10.06.2019	90/4	*Cynodon dactylon* *Sinapis arvensis* (2 samples) *Cuscuta campestris*
24.06.2019	63/5	*Sinapis arvensis* *Chenepodium album* *Sorghum halapanse* *Tordylium apilum* *Echinochloa crusgalli*
08.07.2019	0/0	-
25.07.2019	8/0	-
06.08.2019	0/0	-
22.08.2019	6/1	*Orobanche romosa*
Total	**243/10**	

Table 4. Crop plants in tobacco nurseries and fields

Name	Scientific Name	Family	Number of tested plants
Tomato	*Solanum lycopersicum*	Solanaceae	11
Sweet pepper	*Capsicum annuum*	Solanaceae	3
Eggplant	*Solanum melongena* L.	Solanaceae	1
Cucumber	*Cucumis sativus*	Cucurbitaceae	1
Zucchini	*Cucurbita pepo*	Cucurbitaceae	1
Watermelon	*Citrullus lanatus*	Cucurbitaceae	1
Melon	*Cucumis melo*	Cucurbitaceae	3
Wheat	*Triticum aestivum* L.	Poaceae	6
Barley	*Hordeum vulgare* L.	Poaceae	3
Rye	*Secale cereale* L.	Poaceae	1
Corn	*Zea mays* L.	Poaceae	1
Lettuce	*Lactuca sativa* L.	Asteraceae	4
Sunflower	*Helianthus annuus*	Asteraceae	1
Onion	*Allium cepa* L.	Amaryllidaceae	2
Beetroot	*Beta vulgaris*	Amaranthaceae	1

As a result of the diagnosis, it was revealed that *Thrips tabaci*, *Frankliniella occidentalis*, *Aeolothrips intermedius* and *Thrips major* species were found in the region.

Discussion

Surveying the main tobacco producing areas in Denizli Province, Turkey we found that TSWV was present in all tobacco sampling areas. Of the samples tested, 61.45% were found to be infected with the virus. Therefore, it can be considered as a prominent viral threat for Turkey tobacco industry. Its incidence highly depends on the presence of alternative virus sources and active vector populations (Grooves *et al.*, 2002). The first infections are predicted to occur in the field since it was noted that after planting the tobacco seedlings the growers did not apply any pesticides for pest management until the first hoeing. This suggests that the contamination most likely occurred in this period. Out of 196 tobacco plants collected from Samsun, is a city on the north coast of Turkey, TSWV infection rate was found 5.61% by DAS-ELISA (Erdem, 2010). In Zambia, 72 tobacco plants were analysed and the virus was not present (Mayunga and Kapooria, 2003). In Greece, incidence of it in tobacco fields has been searched and the infection rates was found between 0.4% and 100% by ELISA in different counties (Chatzivassiliou *et al.*, 2004). In Serbia, 380 tobacco plants analysed serologically and it was the most frequently found virus with 37.9% infection rate (Stanković *et al.*, 2011). Out of 26 tested tobacco plants from open field in district of Bosnia and Herzegovina serological and molecular results revealed that 25 were positive (Delić *et al.*, 2018). Similarly with our results, it was also noted that necrotic spots, necrosis and stunting were observed on tobacco plants.

Although virus identification showed that TSWV was the most commonly found virus, we have encountered several viruses. The infection by CMV, AMV and PVY was also confirmed in tobacco fields in the Turkey western region. It was found that the aphid-borne viruses AMV, CMV and PVY spread was notably limited. In our study, they were considered of minor importance, due to a really limited spread within tobacco fields. Our data showed that 6.70% of tobacco plants were double-infected. However, TMV, PVX and TRSV were not found in the examined provinces.

TSWV is not transmitted through seeds which indicates that it is not a source of inoculum. None of the investigated viruses especially TSWV were present in seedbeds. It can be concluded that the necessary care has been shown in the thrips control in the seedbeds. This suggests that seedlings are not virus sources for further spread in the field. Incidence of TSWV in tobacco seedbeds were investigated in different areas in Greece. The infection rates varied between 2% and 10.7% in seedbeds (Chatzivassiliou *et al.*, 2004). No seed-borne viral agents have been determined in tobacco fields. These findings support the idea that sufficient attention is paid in seed production and distribution.

Our results showed occurrence of a wide range of weeds in leading tobacco producing districts in Denizli province. Most of the weed species that are identified as alternate hosts of TSWV were distributed in all the production areas. TSWV was detected in the weeds represented different plant families. Despite this, no symptom expression was observed during surveys on these weeds, indicating a latent infection. In our study, the infection by TSWV was recorded on perennial monoctyledon plants which are *Cynodon dactylon* and *Sorghum halepense*. It has been reported that the occurrence of TSWV in these species by DAS-ELISA (Jordá *et al.*, 1995, Jordá *et al.*, 2000). However, it has not been confirmed in these weed species in vegetable producing areas of the eastern Mediterranean region of Turkey during the years 2004-2006 (Atakan *et al.*, 2013). TSWV was also determined in different weed species such as *Chenopodium album*. Plants belong to this weed species near tobacco and vegetable growing areas have been identified in Hawaii (Cho *et al.*, 1986), Italy (Grieco *et al.*, 2000) and Greece (Chatzivassiliou *et al.*, 2001) whereas have not in Turkey (Atakan *et al.*, 2013).

Assays showed that *Sinapis arvensis* harbor TSWV, similarly as occurred in Greece (Chatzivassiliou *et al.*, 2001). Whereas species such as *Sinapis alba* and *Raphanus raphanistrum* belong to same family, did not.

Similar results have been obtained when plants of the *Raphanus raphanistrum* were collected (Atakan *et al.*, 2013). *Cuscuta campestris* is a commonly found parasitic plant in the study area and found to be infected. Field dodder is not only parasite also a vector of virus diseases which affect crop growth. TSWV was transmitted to healthy plants by means of *Cuscuta campestris* (Hosford, 1967). It was also found on seeds of *Orobanche romosa* which is parasitic plant only germinates when a host root is nearby its roots. Further investigations will be required to determine whether or not it serves as vector in transmitting the virus when infests new plants.

Field surveys also indicated that *Echinochloa crus-galli* and *Tordylium apulum* were infected by TSWV. To the best of our knowledge, these species are newly reported hosts of TSWV. Virus could not be found out by DAS-ELISA in plants of *Aegilops triuncialis, Avena sterilis, Bromus tectorum, Hordeum murinum, Lolium perenne, Poa annua, Secale vulgare* and *Daucus carota* from same families as these weed species. It has been reported that no TSWV could be detected in *Poa annua* (Atakan *et al.*, 2013). Although some species such as *Convolvulus arvensis, Malva sylvestris* (Lavina *et al.*, 1996), *Capsella bursa-pastoris* (Wilson, 1998), *Xanthium spinosum, Xanthium strumarium, Tribulus terrestris, Heliotropium europaeum, Papaver rhoeas, Lactuca serriola, Fumaria officinalis, Polygonum aviculare* (Chatzivassiliou *et al.*, 2001), *Plantago lanceolata* (Groves *et al.*, 2002) and *Cirsium arvense* (Dikova, 2013) have been reported to be TSWV hosts previously, were observed not to be infected in our study. Similarly, any *Heliotropium europaeum, Capsella bursa-pastoris, Fumaria officinalis, Polygonum aviculare, Bromus tectorum, Anagallis arvensis, Ranunculus arvensis* and *Urtica urens* contaminated with TSWV has not been found (Atakan *et al.*, 2013). The findings also have been showed infection in some weed species belong to different families such as Amaranthaceae (Groves *et al.*, 2002), Portulacaceae (Grieco *et al.*, 2000), Rubiaceae and Lamiaceae (Chatzivassiliou *et al.*, 2001). In contrast, *Amaranthus albus, Amaranthus retrofelexus, Galium aparine, Lamium amplexicaule, Salvia officinalis* and *Portulaca oleracea* belong to these families were not infected. Plants of *Lamium amplexicaule* and *Galium aparine* have been found negative in ELISA (Atakan *et al.*, 2013). Other species, such as *Althaea officinalis,* Anagallis arvensis, *Anthemis arvensis, Asphodelus albus, Centaurea solstitialis, Cichorium intybus, Equisetum arvense, Helichrysum italicum, Hypericum perforatum, Matricaria chamomilla, Rubus fruticosus, Urtica urens, Vicia cracca* and *Viscum album* appeared to be not infected. Our data showed that PVY, CMV, AMV, PVX, TRSV or TMV were not identified in weeds. Studies were conducted in Greece to assess the potential reservoir hosts of PVY, CMV and AMV among weeds and different species from various families were found infected (Chatzivassiliou et al., 2004).

Species belonging to the order Thysanoptera generally spend the winter as pupae or adults in the soil, weeds or plant residues. The females, which come out with the warming of the weather in the spring months, begin to lay eggs in the plant tissues or on the plant. The wintering places of individuals are important (Anonymous, 2008). For this reason, in this study, it was tried to determine the weeds that thrips species can overwinter. For this purpose, individuals were taken from winter weeds in or around the tobacco field.

T. tabaci and *F. occidentalis* are pests that have been reported as vectors of TSWV. *T. tabaci* is a cosmopolitan and polyphagous species which has wide range of hosts including tobacco, cotton, clover, onions, garlic, tomatoes, eggplant, potatoes, peas, zucchini, cucumbers and beets. They usually spend the winter on various plants in adult form. *F. occidentalis* is a polyphagous species and its hosts are fruit trees, ornamental plants, field crops, vegetables as well as weeds. They usually spend the winter in the soil, under weeds or plant debris (Kirk and Terry, 2003). These two species were found in most of the observed regions. Especially since the beginning of August, it has been observed that the density has increased considerably. As of mid-September, no two individuals were found due to the fact that tobacco harvesting was almost completed.

Aeolothrips intermedius feeds on the flowers of various plants belonging to the families Asteraceae, Cruciferae and Fabaceae. Although rare, there may be cases where it displays a predatory behaviour. It is recorded that it fed on *T. tabaci* and *F. occidentalis* (Alavi and Minaei, 2018). In the observations made in the region, this individual was encountered in Acıpayam, Kale and Tavas and only 3 individuals were seen. *Thrips*

major is a pest that causes fruit losses by feeding directly on nectarines, peaches and strawberries and also by laying eggs in the tissue (Atakan *et al.*, 2014). Only 1 individual of this species was observed in Çameli. *A. intermedius* and *T. major* species are not species reported as TSWV vectors.

Conclusions

The main purpose of this investigation was to determine the incidence of TSWV in tobacco fields. Weeds may serve as reservoirs for TSWV and its vectors. It was also aimed at revealing some commonly found weed species which may be potential hosts for the virus. TSWV was mainly found in surveyed districts where some tobacco fields were totally infected. It is recognized every year in almost all examined fields and high infection rates of TSWV causing high crop losses (results not shown). This virus consistently prevailed in Western Turkey. Virus incidence, as estimated by the number of symptomatic tobacco plants present in the field, depending on the tobacco variety and sampling area. Also, number of vector species present and their ability to transmit greatly affect virus spread in the field. Our results on the incidence of TSWV in tobacco crops and the associated weeds suggest that the thrips transmitted TSWV consist the main threat for the tobacco production. The wide host range of TSWV increases the difficulty of managing the virus. It is crucial to prevent destructive virus spread and subsequent crop losses. Management of weeds that are hosts of the virus and thrips is essential for disease control. The elimination of weeds that consists the main alternative virus and vectors' reservoirs, is generally suggested as an effective procedure for managing virus epidemics. More extensive researches should be carried on the relationships between weeds as thrips hosts and virus source to spread of TSWV in tobacco growing areas.

Authors' Contributions

Funding acquisition: MG and DB; Investigation: NG, SGT, PO, MG and DB; Methodology: NG, SGT and PO; Project administration: MG; Resources: MG; Supervision: MG; Writing - original draft: NG, SGT and PO; Writing - review and editing: MG and NG. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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