Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar
doi: http://dx.doi.org/10.5424/sjar/2012101-508-10

Spanish Journal of Agricultural Research 2012 10(1): 191-197
ISSN: 1695-971-X
eISSN: 2171-9292

Development of a *Tomato spotted wilt virus* (TSWV) risk evaluation methodology for a processing tomato region

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Abstract

A risk map for the *Tomato spotted wilt virus* (TSWV) was elaborated for the main Portuguese processing tomato producing region, the “Ribatejo e Península de Setúbal” region, where periodically this virus causes severe losses. Forty nine tomato fields were monitored. Risk factors for TSWV infection were identified and quantified according to their relative importance in TSWV incidence. The risk factors considered for each field were: (1) presence of TSWV in tomato plants; (2) presence of TSWV in weeds which are hosts of TSWV vectors; (3) presence of TSWV vector thrips; (4) presence of TSWV host crops previously (in the two years before), namely, tomato, potato and sweet pepper; and (5) presence of greenhouses, urban areas or TSWV host crops next to the field (up to about 100m from its borders). A risk estimator was calculated for each field. Among the thrips (Thysanoptera) identified, belonging to 11 genera, four vector thrips species were detected: *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* Lindman, the two most abundant ones, and *F. intonsa* (Trybom) and *F. schultzei* (Trybom). Blue sticky traps placed up to about 75 cm above the crop canopy caught *F. occidentalis* and *T. tabaci* more efficiently than the beating technique. The weeds *Datura stramonium* L., *Arctotheca calendula* (L.), and *Conyza bonariensis* (L.) were identified as TSWV winter repositories. This study proposes a methodology to be used by field technicians for the annual evaluation of TSWV risk at a regional level, for an improved planning of processing tomato crop in the following season.

Additional key words: GIS; monitoring; thrips; tomato for industry; tospovirus.

Resumen

Desarrollo de un método de evaluación de riesgos de incidencia de TSWV en una región productora de tomate para procesamiento

Se ha elaborado un mapa de riesgos de incidencia del *Tomato spotted wilt virus* (TSWV) para la región del Ribatejo y Península de Setúbal, la más importante productora de tomate de Portugal, y donde, periódicamente, el virus ocasiona importantes pérdidas. Se monitorizaron 49 parcelas de tomate al aire libre. Los factores de riesgo de infección por TSWV se identificaran y cuantificaran en base a su importancia relativa en la incidencia de la virosis: (1) presencia de TSWV en el cultivo de tomate; (2) presencia de TSWV en las hierbas adventicias hospedadores de los trips vectores; (3) presencia de trips vectores de TSWV; (4) presencia de cultivos hospedantes de TSWV previos (en los dos años anteriores), como tomate, patata y pimiento; y (5) presencia de invernaderos, áreas urbanas o cultivos hospedantes de TSWV en las proximidades (menos de 100 m de los bordes de las parcelas). Se estimó el riesgo de infección para cada parcela. Se detectaron cuatro especies de trips (Thysanoptera) vectoras del virus: *Frankliniella occidentalis* (Pergande) y *Thrips tabaci* Lindman, las más abundantes y *F. intonsa* (Trybom) y *F. shultzei* (Trybom), en menor proporción. *Datura stramonium* L., *Arctotheca calendula* (L.) y *Conyza bonariensis* (L.) actúan como reservorios invernales de TSWV. Se propone una metodología de evaluación del riesgo de infección del tomate por TSWV a escala regional, que ayude a los técnicos de campo a establecer las estrategias del cultivo de tomate en la siguiente campaña de cultivo.

Palabras clave adicionales: GIS; monitorización; trips; tomate de industria; tospovirus.

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Received: 21-12-10. Accepted: 07-02-12

Introduction

Tomato spotted wilt virus (TSWV) is responsible for numerous epidemics worldwide, causing heavy economic losses in several crops. Thrips (Insecta: Thysanoptera) are the only known TSWV vectors. The number of vector thrips species is continually being assessed and discussed, but at least eight species transmit the virus from infected to healthy plants (crops and weeds) (Ullman *et al.*, 2002; Whitfield *et al.*, 2005; Ohnishi *et al.*, 2006; Pappu *et al.*, 2009).

Since 1997 severe infections by TSWV have been registered periodically in the processing tomato (*Solanum lycopersicon* L.) crop in the "Ribatejo e Oeste" region, Portugal. From 1998 to 2000, a TSWV eradication programme was undertaken in the region by the Ministry of Agriculture. Because it was concluded that the virus and its vectors were maintained in the fields mainly by the potato (*Solanum tuberosum* L.) — tomato rotation, it was decided that, in the high TSWV risk areas of that region, the cultivation of any Solanaceae in a field, namely potato and also sweet pepper (*Capsicum annuum* L.), was not allowed in the two years after a tomato crop in that field. After the adoption of this measure, TSWV incidence reduced, but periodically becomes a problem in the region, with significant economic losses. Tomato, potato and sweet pepper, intensively cultivated in the region, are known TSWV hosts (*e.g.* Cho *et al.*, 1986; Allen & Matteoni, 1991) and vector thrips hosts (EPPO, 1988; Chatzivassiliou *et al.*, 2001; Yarahmadi *et al.*, 2009). TSWV resistant cultivars are rarely used by growers there, except in the more critical fields and after having serious problems in the previous years.

This study proposes a methodology, to be used by field technicians, for the annual evaluation of TSWV risk at a regional level, for an improved planning of processing tomato crop in the following season.

Material and methods

Risk map

Field work took place from February to December 2004. Data were collected in a total of 49 tomato fields, selected at random: 47 tomato fields were located in the "Ribatejo e Península de Setúbal" region, included in the problematic region mentioned above, comprising 11 districts (Alcochete, Almeirim, Alpiarça, Azambuja, Benavente, Cartaxo, Montijo, Palmela, Salvaterra de Magos, Santarém, and Vila Franca de Xira); two extra fields in the neighbourhood Loures district were included owing to previous TSWV occurrence there. The central point of each field was registered by GPS. The tomato fields were part of a very patchy landscape, which included tomato, potato, sweet pepper, and several other vegetables, maize fields, vineyards and ornamental greenhouses. Crop fields were relatively small, the biggest ones with about 15 ha.

Five risk factors for TSWV incidence in a field were considered (four referred to each field, and a fifth related to its vicinity): (1) presence of TSWV in tomato plants; (2) presence of TSWV in weeds which are hosts of TSWV vectors; (3) presence of TSWV vector thrips; (4) presence of TSWV host crops previously (in the two years before), namely, tomato, potato and sweet pepper; and (5) presence of greenhouses, urban areas or TSWV host crops next to the field (up to about 100 m from its borders).

Risk factors were quantified according to their relative impact in TSWV incidence (Table 1), based on Bolkan & Reinert (1994), on the experience of the research team that conducted this work, and on the experience of the industry technicians associated to it. In each field, risk factors were assessed and the respective risk estimator was calculated by the sum of all risk factors values referred to that field.

In relation to risk factor 1, 100 tomato expanded leaves per field were collected (one leaf per plant) once during the crop period, in a zigzag sampling pattern, giving priority to plants showing TSWV symptoms. When sampled, tomato plants in the different fields were approximately in the same phenological stage, since sampling date was defined taking into account the plantation date. The large majority of the fields did not have TSWV resistant tomato cultivars (resistance by *Sw-5* gene). In each field, TSWV was also surveyed in weeds (risk factor 2) once in three sampling periods:

Table 1. Quantification of risk factors according to their relative impact in the *Tomato spotted wilt virus* (TSWV) incidence. Based on Bolkan & Reinert (1994), and on the field experience of the authors and the technicians who collaborated in this work

	TSWV in tomato	TSWV in weeds	Vectors	Previous host crops	Vicinity structures/ crops
Presence	4	4	2	2	2
Absence	2	2	0	0	0

before the establishment of the tomato crop (March/ April), during the crop period (June/ July), and in the post-harvest period (October/ November). In each sampling date the three most abundant weed species were selected, and 10 plants of each were collected randomly inside the field and in its margins, some showing TSWV symptoms and others not. Plant material collected (with and without symptoms) was analysed by ELISA test in laboratory (Bioreba antisera: IgG 190112, Conjugate 190122). Sample dilution was 1:20, and the test was considered positive if sample absorbance was at least three times greater than the negative control.

Vector thrips presence (risk factor 3) was monitored in each field using two sticky traps (24 × 20 cm, one blue and one yellow) located at the top of the crop canopy and replaced every two weeks. Vector thrips were identified using Mound & Kibby (1998) and Moritz *et al.* (2004) keys.

Previous crops in those fields (risk factor 4), as well as greenhouses, urban areas and crops in the vicinity (risk factor 5), were assessed by local observation and by a questionnaire directed to growers.

The map was obtained through geostatistical technique of kriging of categorical attributes, based on indicator kriging with a posterior classification (Soares, 1992; Goovaerts, 1997). GEOMS 1.0 software (CMRP, 1999) was used to estimate the spatial dispersion of risk classes, and ArcGIS 9.0 software (ESRI, 2004) was used to represent the output.

Thrips fauna and monitoring additional information

Thrips were collected in a tomato field (40 ha) in the same region from June to September 2006 using the beating technique and sticky traps.

Blocks of two traps were used, one trap placed at the top of the crop canopy and the other about 25 cm higher, facing the same direction. Every other week, two blocks of blue traps and two blocks of yellow traps were placed randomly in the field and stayed there during one week, except for the first two weeks, where just the lower traps were placed in the field, in a total of seven weeks and 48 traps. Weekly, the beating technique was used in the top 1/3 of the tomato plant, preferably directed to flowers and young leaves. Fifteen plants were selected randomly around the point where each block of traps was placed (in an 8 m diameter circle), in a total of 60 plants per week. The thrips species captured were identified (Mound & Kibby, 1998; Moritz *et al.*, 2004).

The capture of *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* Lindman by the two sampling methods was compared by the non-parametric Spearman's correlation ($p < 0.05$). The effect of trap colour and trap height in the capture of these two species was analysed by the Wilcoxon paired-sample test ($p < 0.05$) with SPSS for Windows 15.0 (SPSS Inc., Chicago, USA).

Results

Risk map

The TSWV risk map for the "Ribatejo e Península de Setúbal" region, in 2004, is presented on Figure 1. The information obtained in each field was valid along 14 km in the Northeast-Southwest direction, and along 6 km in the Northwest-Southeast direction. The risk

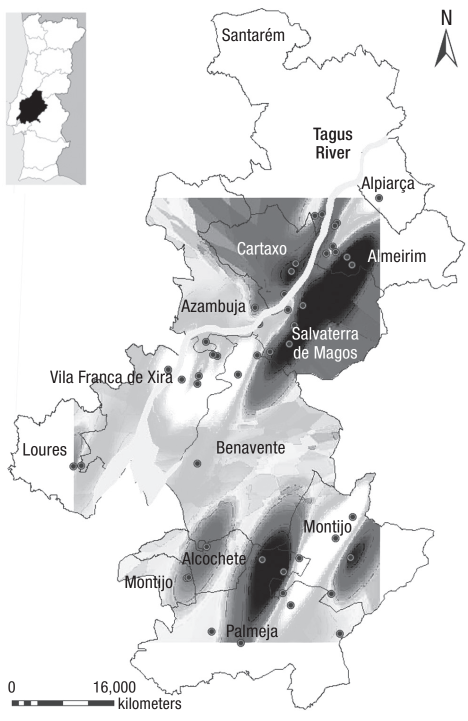

Figure 1. Risk map for *Tomato spotted wilt virus* (TSWV) infection in the "Ribatejo e Península de Setúbal" region, in 2004. The location of the region in Portugal is indicated; the district names are identified; points, some of them overlapped at the map scale, represent the fields surveyed; grey shades indicate TSWV risk areas intensity, being the higher risk areas represented in darker grey and the lower risk areas in lighter grey.

Table 2. Weeds infected with *Tomato spotted wilt virus* (TSWV) in processing tomato fields in "Ribatejo e Península de Setúbal" region tested by Elisa using TSWV- specific antiserum

Weed species	Before the crop (March/April)	During the crop (June/July)	After the crop (October/November)
Arctotheca calendula	×	×	×
Conyza bonariensis			×
Datura stramonium			×
Leontodon taraxacoides			×
Polygonum lapathifolium		×	
Other Asteraceae			×

" × " means TSWV infection detected in at least one plant sampled of that species.

was higher in Salvaterra de Magos, Almeirim and Palmela districts.

TSWV was detected in the crop and / or weeds in 29% of the fields, spread in the region surveyed. In weeds, it was present before the tomato crop was installed, during the crop period, and after harvest. The infected weeds were *Arctotheca calendula* (L.), which was present in the fields all year round; *Polygonum lapathifolium* L., during the crop period; and *Datura stramonium* L., *Conyza bonariensis* (L.), *Leontodon taraxacoides* (Vill.), and some other unidentified Asteraceae, after harvest, in the winter (Table 2).

TSWV vector and non-vector thrips were detected in the fields; when the crop was not installed thrips were found on weeds.

TSWV vector thrips were detected in all fields surveyed and in all sampling dates. Vector species were: *F. occidentalis*, *T. tabaci*, *Frankliniella schultzei* (Trybom) and *Frankliniella intonsa* (Trybom). The first two species were more abundant and frequent, especially *T. tabaci*. The proportion of vector thrips was low (inferior to 15%), revealing the presence of many non-vectors. Among them *Aeolothrips* sp., known predators, were very abundant.

In 47% of the fields, TSWV host crops had been cultivated there in the two previous years, especially potato and sweet pepper, and 57% of the fields had greenhouses, urban areas or TSWV host crops next to them.

Thrips fauna and monitoring additional information

No significant differences between the two trap height levels tested were found regarding *T. tabaci* and *F. occidentalis* captures (Table 3). However, blue traps performed better than yellow ones in catching both species (Table 3).

F. occidentalis and/or *T. tabaci* were always detected by the traps, whereas in five of the 12 sampling dates vectors were not detected by the beating technique. The correlation analysis between the two sampling techniques indicated weak and non significant correlation for *F. occidentalis* (r_s = 0.104, p = 0.74) and for *T. tabaci* a statistically but not biologically significant correlation (r_s = – 0.701, p = 0.02).

Considering the overall captures obtained by both monitoring methods, eleven thrips genera were detected. The genus *Thrips* was the most abundant (49%), followed by *Aeolothrips* sp. (19%) and *Frankliniella* sp. (18%). *Ceratothrips* sp. constituted 5% of the total thrips abundance, and the other genera were less representative: *Anaphothrips*, *Chirothrips*, *Limothrips*,

Table 3. Comparison between thrips captures in high and low traps, and in yellow and blue traps by the Wilcoxon paired-sample test. p = significance level, Z = Wilcoxon test statistic, N = number of trap pairs compared

Comparison	Trap	*Frankliniella occidentalis*			*Thrips tabaci*		
		p	Z	N	*p*	Z	N
Height (low *vs* high)	Blue	0.33	– 0.980	10	0.45	– 0.764	10
	Yellow	0.07	– 1.826	10	0.24	– 1.186	10
Colour (blue *vs* yellow)	Low	0.02	– 2.429	13	0.02	– 2.411	13
	High	0.03	– 2.197	10	0.01	– 2.803	10

Melanthrips, *Odontothrips* and *Scolothrips*. Tubulifera were also detected (5%), and among them *Haplothrips* sp. was present. Some specimens were identified up to species level, namely, *Aeolothrips tenuicornis Bagnall*, *Anaphothrips obscurus* (Müller)*, Ceratothrips discolour* (Haliday)*, F. intonsa*, *F. occidentalis*, *F. schultzei*, *F. tenuicornis* (Uzel)*, Melanthrips fuscus Sulzer, Thrips australis* (Bagnall), *Thrips angusticeps* Uzel and *T. tabaci*.

Discussion

Different factors combine to influence the risk of TSWV incidence and losses in the tomato crop. Some factors are more important than others. A higher score (4) was attributed to the presence of inoculum in a field (factors 1 and 2). Its non-detection was scored with the value of 2, as a conservative attitude, in a region where TSWV is spread. The presence of other factors that raise the risk of TSWV infection in a field (factors 3 to 5) was scored with the value of 2 because their presence does not necessarily mean an infection. For example, vector thrips must be viruliferous and conditions must be favourable for virus transmission. The combination of several factors involved allows risk estimation.

Surveys conducted in 1998 and 1999, during the implementation of the TSWV eradication programme mentioned above (DGPC, 1998, 1999), identified Palmela and Montijo districts as the highest risk areas in the region. The risk map presented here (Fig. 1), obtained with 2004 data, which was not a severe year of TSWV in processing tomato in that region, confirmed Palmela as a high risk district, but not Montijo which had medium risk, and added Salvaterra de Magos and Almeirim as high TSWV risk districts. This dynamic panorama underscores the need for a regular evaluation of the risk.

Field observations on TSWV incidence in the following year (2005) in the region confirmed the risk areas indicated in the map and so the associated methodology here proposed for its elaboration, but a complete validation must still be undertaken through the collection of data in the region during some more years. Field technicians' routine work is essential in this process. The change in the agricultural practices in the problematic areas from one year to the next, as for example the use of TSWV resistant/ tolerant cultivars or the change on the cultivated crops in order to escape the virus, is a challenge to the validation process. On the other hand, factors as tomato cultivars, planting date, planting spatial design and weather conditions may be integrated in the future, if necessary, however keeping in mind that overall methodology must be simple to be used periodically by field technicians, which are the end users.

Weed reservoirs in the fields constitute sources of inoculum and those present before the crop is installed allow an early infection of tomato crop, with higher losses. A reservoir host must not only be susceptible to TSWV but also serve as food source for larvae development of thrips vector species (Cho *et al.*, 1986). This Tospovirus is acquired by thrips larvae and transmitted mainly by the remaining adults. In the present study, TSWV was detected in some weed species, before the installation of the crop, during the crop and after its harvest, which are recognized as TSWV hosts in literature (Parrella *et al.*, 2003), except *Leontodon taraxacoides*, for which no reference was found. However, in a conservative attitude, while this is not clarified, this species was also considered TSWV host. According to Chatzivassiliou *et al.* (2001), many plant TSWV hosts, if not all, are also hosts of thrips vectoring tospoviruses. It is already known that *Datura stramonium* is a breeding host of *F. occidentalis* (Bautista & Mau, 1994; Chatzivassiliou *et al.*, 2001) and of *T. tabaci* (Chatzivassiliou *et al.*, 2007), which also breeds in *Conyza bonariensis* (Mo, 2006). Vector thrips were detected in *Polygonum lapathifolium* (Raspudic *et al.,* 2009), and *Polygonum* sp. was identified as ovipository hosts of *F. occidentalis* (Stobbs *et al.*, 1992). *Arctotheca calendula* is recognized as a feeding host of vector thrips, with no information being given about larvae development (Jelinek, 2008); no information is available for *Leontodon taraxacoides*, however taking into account the large number of Asteraceae which are hosts of vector thrips, this species was taken into consideration in this TSWV risk analysis. The methodology proposed here would be improved if the percentage of infected plants (tomato and weeds) in each field was taken into consideration in the scores attributed to risk factors 1 and 2, instead of just presence/ absence information.

Other surveys (DGPC, 1999; Serra *et al.*, 2003) also identified other weed species as important TSWV winter repositories: *Sonchus oleraceus* L., *Chamaemelum fuscatum* Vasc., *Anthemis arvensis* L., *Raphanus raphanistrum* L. and *Armoracia rusticana* Gaertn., Mey et Scherb., besides *Arctotheca calendula* (L.), the most frequently infested one.

Four thrips vector species were identified in the Portuguese region "Ribatejo e Península de Setúbal", namely *Thrips tabaci*, *Frankliniella occidentalis,* the most abundant ones, and *F. schultzei* and *F. intonsa*. Transmission tests revealed that *F. occidentalis* local populations are very efficient vectors while *T. tabaci* ones are weak vectors (Cortez *et al.*, 2003). Vector efficiency of *F. schultzei* and *F. intonsa* local populations has not been tested yet. It would be an improvement to the methodology here suggested, if vector efficiency was taken into consideration in the score attributed to risk factor 3: instead of just considering vector thrips presence/ absence in each field, quantification of the species involved should also be included. Thrips fauna in the crop presented in this study adds information about this taxonomic group presence in the region.

Greenhouse crops are frequently TSWV and vector thrips hosts. Urban areas are usually associated with ornamentals, which have the same problem. In fact, according to the national agricultural authority, ornamentals constitute TSWV infection foci during the winter in the region (DGPC 1998, 1999). So the presence of greenhouses and/or urban areas in the vicinity of tomato fields was considered a risk factor.

Additional information given in our study indicates that vectors should be detected using sticky traps since they were more efficient in detecting thrips than the beating technique. Efficiency of the latter method would be intensified by increasing the number of plants surveyed, but such an increase would be time consuming in a monitoring programme. Traps should be placed up to about 75 cm above the crop canopy. Blue traps performed better than yellow ones in capturing the two most abundant vector thrips (*F. occidentalis* and *T. tabaci*). Traps with different colours were not tested side by side because the distance between them in this type of studies has not been determined yet. There must be enough distance to avoid any interference between colours; however, this distance must be short enough so traps can operate in the same ecological conditions. In this study colours were tested separately; the location changed weekly, at random. Blue and yellow sticky traps have been widely used for monitoring vector thrips as a result of several studies which indicate their atractiveness (*e.g.* Kirk, 1984; Brodsgaard, 1989; Vernon & Gillespie, 1990; Teulon & Penman, 1992; Brodsgaard, 1993; Mateus & Mexia, 1995; Mateus *et al.*, 2003; Chen *et al.*, 2004).

With this study, field technicians are provided the tools and the incentive to update the risk map annually by evaluating risk factors in their fields, allowing for better regional planning of the processing tomato crop, that is, where to plant tomato, which varieties should be used, and the TSWV and vector monitoring effort to be dedicated. After the crop is installed in the field there are no known control measures against TSWV. Prevention is the only way to control the disease.

Acknowledgements

This work was financed by the national project PO Agro 189. The authors thank: all field technicians who helped in the data collection; Ângela Silva for the ELISA tests; Amílcar Soares and Júlia Carvalho (Instituto Superior Técnico, Lisboa) for geostatistical advice; Tiago Teixeira for help in making the map; and Genevieve Gwynne for English revision of the manuscript.

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