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The proportion of viruliferous individuals in field populations of *Frankliniella occidentalis*: Implications for tomato spotted wilt virus epidemics in tomato

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Abstract

Tomato spotted wilt tospovirus (TSWV) infected plants and western flower thrips (*Frankliniella occidentalis* Perg., WFT) adult population densities were monitored during 1993 and 1994 in field tomatoes in Northeastern Spain. The proportion of viruliferous WFT adults in field populations was quantified. A significant association has been found between early population densities of WFT adult thrips and final TSWV incidence for early transplanted tomato crops. In contrast, for late transplanted tomato crops, whereas similar high final disease incidences of TSWV could be attained, very low WFT adult population densities were always detected. The significantly higher infectious potential of WFT populations collected during the early growth stages of late transplanted tomatoes could be relevant for the TSWV incidences attained in spite of the low thrips numbers detected.

Introduction

Tomato spotted wilt virus (TSWV) is a widely distributed plant virus that causes economically important diseases in many ornamental and vegetable crops throughout tropical and temperate climate zones (i.e., 1970; Reddy and Wightman, 1988). TSWV is the type species of the *Tospovirus* genus, the only plant-infecting viruses in the family *Bunyaviridae* (Milne and Francki, 1984; Francki et al., 1991). TSWV is transmitted by several species of thrips (*Thysanoptera: Thripidae*) (German et al., 1992; Goldbach and Peters, 1994), in a circulative and propagative persistent manner with a very specific relationship (Ullman et al., 1993; Wijkamp et al., 1993). The interactions between thrips and TSWV have been comprehensively studied (German et al., 1992; Ullman et al., 1992b; Wijkamp et al., 1995). The thrips can acquire the virus only during the larval stages (Sakimura, 1962) and transmit it as second instar larvae and as adults (Wijkamp and Peters, 1993). Adult thrips are thought to be most important in the dispersal of TSWV (Cho et al., 1989; German et al., 1992) because they move widely (Lewis, 1973) and remain capable of transmitting TSWV for long periods of time (Ullman et al., 1992b).

The epidemic outbreaks of TSWV disease in Spain have been related with the introduction and spread of the western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Lacasa, 1990). At present, TSWV is a limiting factor for production of many vegetable crops and severe economic losses have occurred in important crops like tomato, pepper, or lettuce.

Descriptions of TSWV epidemics have been based on data on the level of thrips populations (Cho et al., 1987; Puche et al., 1995). However, thrips numbers alone are not sufficient to indicate the risk of virus infection (Yudin et al., 1990; Ullman et al., 1992; Bandla et al., 1994). Usually, only part of the individuals of a population are able to transmit the virus, therefore, a measure of the proportion of inoculative

insects will be essential to analyse the epidemics (Irwin and Ruesink, 1986). A quash-blot technique has been recently described to screen viruliferous individuals in WFT populations (Aramburu et al., 1996) whose results strongly corresponded to transmission tests. This simple procedure reduces the time required by other methods to detect TSWV in individual thrips (Cho et al., 1988; Rice et al., 1990; Ullman et al., 1992a). Consequently, this procedure offers the simple tool required for assaying a sufficient number of thrips in order to provide a reliable measure of the proportion that transmit TSWV and might be used to forecast TSWV-induced diseases.

This paper reports the results of a study conducted during two growing season in commercial tomato plots transplanted in the open at different dates to determine the incidence of TSWV, the densities of WFT adult populations, the relative number of viruliferous WFT adults, and their possible relationships.

Materials and methods

Survey

A survey was conducted in 1993 and 1994 in commercial plots of field-grown pole tomatoes for fresh market on the Mediterranean coastal area, north of Barcelona in Spain, where vegetable crops are abundant and the incidence of TSWV is high in tomatoes (Laviña et al., 1996). In 1993 three plots transplanted in late April were sampled four times, in late May, and early June, July and August. In 1994 six plots, of which three were transplanted in early April and three in mid-June, were sampled weekly from transplanting to the end of the crop. Each plot was located in a different tomato field of the same growing region and consisted of approximately 5000 plants, 0.5 m apart, with 0.8 m between rows. Standard commercial production practices were used following the criteria of the growers. Data on TSWV incidence and thrips populations were taken and adult thrips samples were collected on every sampling date.

TSWV incidence

In a previous research done in the made area, a strong correlation was found between the TSWV incidence estimated by visual inspection and by ELISA analysis of systematically collected samples of the tomato cultivars usually grown (Royesta, Dario, Leopardo, Cobra). Good estimates of TSWV incidence were obtained by sampling 5 to 11% of the plants of a plot (Aramburu et al., 1994). Based on the results, in the present study, the TSWV incidence in a plot was expressed as the percentage of symptomatic plants among 500 inspected. Plant to be inspected during the growing season were selected at the first sampling date in five rows at a regularly spaced distance.

Thrips populations

Thrips populations were monitored by sampling flowers (Salguero Navas et al., 1991). The number of WFT adults per flower was estimated in every plot from 50 fully opened flower clusters (one per plant) selected at random and sampled as described by Shipp and Zariffa (1991).

Proportion of viruliferous thrips

The proportion of viruliferous WFT in a plot was determined for samples of adult thrips collected from the maximum number of flowers that could be sampled by two people in twenty minutes. Adult thrips were also collected from adjacent crops (Tomatoes; lettuce, *Lactuca sativa* L; broad beans, *Vicia* faba L.; potatoes, *Solanum tuberosum* L.) in the four sampling dates of 1993 and in the first three sampling dates of May, in 1994. Thrips samples were observed with a stereomicroscope at 50 magnification and non-WFT adults were discarded. Then, thrips were fed during four to six days on healthy French bean pods (*Phaseolus vulgaris* L.) before testing for TSWV in order to avoid an overestimation of the infection with adult thrips that only fed on TSWV-infected plants but were not able to transmit the virus (Ullman et al., 1992a; Aramburu et al., 1996). After this period, thrips were assayed individually for TSWV infection by squash-blot on nitrocellulose membranes (Millipore Co., Bedford, USA) (Aramburu et al., 1996). Reactions were assessed by direct observation of the membranes or under stereomicroscope at a magnification of 5 to 50×. Virus-free and TSWV-infected adult thrips obtained from laboratory colonies were used as negative and positive controls, respectively. Based on preliminary studies (not shown), the proportion of viruliferous thrips was estimated only on samples of 50 or more individuals in order to avoid false null results due to a narrow sampling that could bias analyses and conclusions.

Table 1. Tomato spotted wilt virus (TSWV) incidence, *Frankliniella occidentalis* (WFT) adult population densities, and proportion of WFT viruliferous adults detected at different dates in three commercial tomato plots (A, B and C) monitored during 1993

Plot	Date	TSWV incidence (%)	Number of WFT adults/flower	n[a,b]	Viruliferous adults (%)[a]
A	May	0	0	. . . [c] (63)	. . . (0)
	June	3.3	0.15	88 (593)	0 (0)
	July	8.8	0.13	52 (410)	0 (0.2)
	August	17.1	0.10	132 (167)	1.5 (2.4)
B	May	1.1	0.02	51 (98)	0 (1.0)
	June	1.4	0.01	109 (280)	0 (0)
	July	1.4	0.08	94 (146)	0 (0)
	August	7.8	0.05	163 (152)	1.2 (0)
C	May	0	0.03	68 (228)	0 (0)
	June	4.1	0.59	66 (352)	0 (0)
	July	17.5	0.08	. . . (351)	. . . (0.3)
	August	43.4	0.04	. . .	. . .

[a] Values for samples collected from adjacent crops are indicated in brackets.
[b] Total number of WFT adults tested individually for TSWV by squash-blot.
[c] Not sampled or insufficient number of WFT adults collected.

Results

Three plots transplanted early in the growing season of 1993 were monitored for TSWV incidence and WFT adult densities (Table 1). A high TSWV incidence (43.4%) was attained in plot C, whereas plot A and B showed intermediate or low values (17.1% and 7.8%, respectively). Significant differences in thrips levels were found between plots (Student-Newman-Keuls' mean separation test, $P < 0.05$). The highest thrips density was detected in May in plot C when 0.59 adults were found per flower. A total of 3653 WFT adults were collected from the tomato plots and adjacent crops in 1993 and tested individually for TSWV by squash-blot (Table 1). Additional sampling efforts were required in plot B to collect enough thrips from tomatoes to accurately determine the proportion of adults infected. Viruliferous thrips were only detected in tomato plots in August. In plot A 1.5% of individuals were positive, and in B 1.2% (Table 1). During May, June, and July, viruliferous individuals were found in three samples collected in the adjacent tomato crops (July, plot A and C; May, plot B; Table 1).

As shown in Figure 1 and Table 2, comparable trends were found in the development of the TSWV incidence in the three tomato plots transplanted early in the season and monitored during 1994. The disease progress curves and the dynamics of WFT adult populations are presented in Figure 1. The values for TSWV incidences, WFT adult population densities, and proportion of viruliferous thrips are summarized in Table 2. The highest number of thrips found on tomato plants was detected between late May and late June. The possible relationships between thrips population levels and TSWV incidences in early transplanted plots was investigated. Also, the data sampled in 1993 can be considered, as weekly information for thrips populations was available (not shown). A significant correlation was obtained between cumulative numbers of WFT adults collected during the early growth stages of the crop (0 to 60 days after transplanting) and final disease incidences ($r = 0.78$, $P < 0.05$). Therefore, an important part of the variation in final disease incidence accounted for early thrips abundance. A total of 2571 thrips were collected and tested individually or TSWV throughout the season in these early transplanted plots. Low levels of viruliferous thrips were detected until early June, but increased thereafter towards the end of the crop. Only two viruliferous thrips were found among 501 WFT adults collected during May from adjacent lettuce and broad bean crops (Table 2).

Vector populations levels and their inoculative potential varied for the tomato plots transplanted later in the season of 1994, while TSWV incidences were comparable to those detected in early transplanted plots (Figure 1, Table 3). Significantly lower levels of thrips

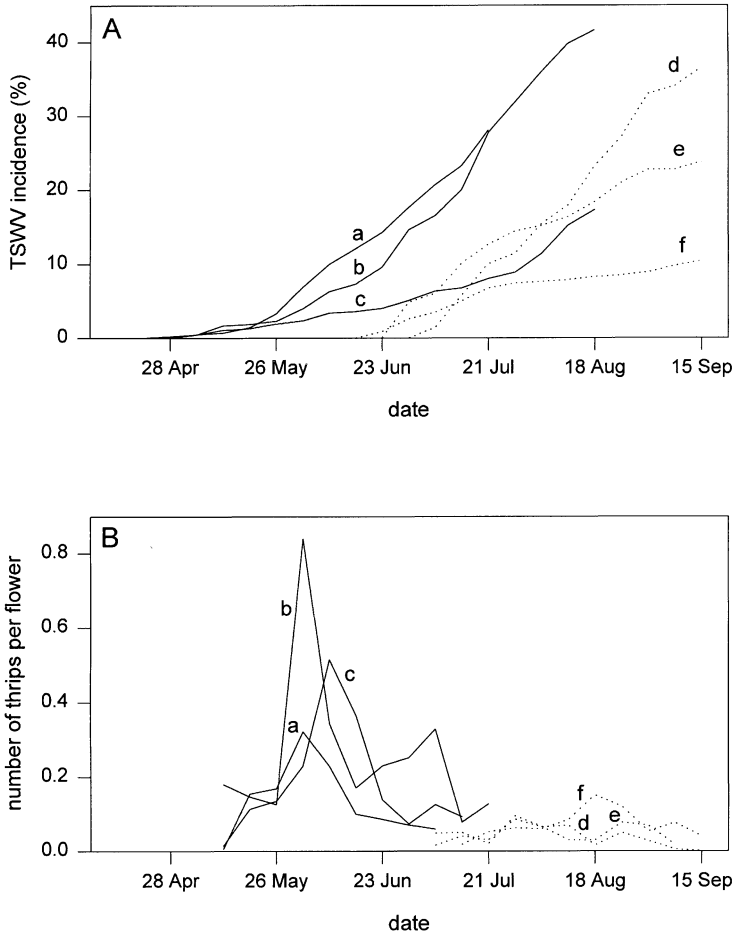

Figure 1. Disease progress curves (A) and dynamics of WFT adult populations (B) for three tomato plots transplanted in early April (a, b, c) and three transplanted in mid-June (d, e, f) monitored in 1994.

were detected in late transplanted plots throughout the season (Hierarchical ANOVA Plot × Date with an orthogonal contrast of early transplanted vs. late transplanted, $P < 0.05$) and additional efforts were required to collect enough thrips for a reliable estimation of the proportion of viruliferous individuals. The analysis of 1090 WFT adults collected from these plots showed that significantly higher levels of viruliferous adults were detected during the early growth stages of this crop (0 to 60 days after planting) when compared to early transplanted one (one way ANOVA, $P < 0.05$). In addition, cropping practices result in that late transplanted tomato crops were surrounded by early transplanted ones, and during early growth stages of the former (mid-June to mid-August) high numbers of infective thrips were present in the latter, as suggested by data in Table 2. No significant differences were detected between early and late transplanted plots in the proportion of viruliferous thrips in samples collected in later phases of the crop.

Attempts were done to find any statistical association between TSWV incidences and data for viruliferous thrips. No significant relationships were found indicating that other factors are also involved in the process and/or that the limited number of data available difficult to show up possible dependences.

Discussion

Good correlations between thrips abundance and TSWV incidences have been reported in certain cases (Cho et al., 1987) but such relationships might not exist in other areas with a different epidemiological situa-

Table 2. Tomato spotted wilt virus (TSWV) incidence, *Frankliniella occidentalis* (WFT) adult population densities, and proportion of WFT viruliferous adults detected at different dates in three tomato plots transplanted in early April monitored during 1994

Date	TSWV incidence (%)[a]	Number of WFT adults/flower[a]	n[b]	Viruliferous adults (%)[a]
7 April	0	–[c]	–	–
14 April	0	–	–	–
21 April	0	–	–	–
28 April	0	–	–	–
5 May	0.4	. . .[d]	. . .	. . .
12 May	1.1	0.07	. . .	. . .
19 May	1.5	0.14	. . .	. . .
26 May	2.5	0.14	322	0.1
2 June	4.4	0.46	825	0.2
9 June	6.5	0.37	386	1.4
16 June	7.7	0.21	492	1.6
23 June	9.3	0.15	342	2.8
30 June	12.4	0.13	129	1.8
7 July	14.5	0.17	75	5.3
14 July	16.7	0.07	. . .	. . .
21 July	21.3	–		–
28 July	20.4	–		–
4 August	23.7	–		–
11 August	25.7	–		–
19 August	29.5	–		–

[a] Values are means for the plots sampled.
[b] Total number of WFT adults tested individually for TSWV by squash-blot.
[c] No flowers present on the plants.
[d] Not sampled or insufficient number of WFT adults collected.

Table 3. Tomato spotted wilt virus (TSWV) incidence, *Frankliniella occidentalis* (WFT) adult population densities, and proportion of WFT viruliferous adults detected at different dates in three tomato plots transplanted in mid-June monitored during 1994

Date	TSWV incidence (%)[a]	Number of WFT adults/flower[a]	n[b]	Viruliferous adults (%)[a]
16 June	0	–[c]	–	–
23 June	0.3	–	–	–
30 June	2.5	–	–	–
7 July	3.7	0.03	. . .[d]	. . .
14 July	7.0	0.04	. . .	. . .
21 July	9.8	0.03	. . .	. . .
28 July	11.1	0.08	156	1.2
4 August	12.7	0.13	445	0.8
11 August	14.0	0.06	53	1.9
19 August	16.6	0.06	110	3.6
25 August	18.9	0.08	. . .	. . .
1 September	21.6	0.05	193	3.4
8 September	22.2	0.03	79	0
15 September	23.6	0.02	54	7.4

[a] Values are means for the plots sampled.
[b] Total number of WFT adults tested individually for TSWV by squash-blot.
[c] No flowers present on the plants.
[d] Not sampled or insufficient number of WFT adults collected.

tion. Little information is available in the literature about inoculative thrips in TSWV epidemics of field-grown tomato crops. The present report provides data that could be useful for prediction and management of TSWV epidemics in the Mediterranean coastal area north of Barcelona, Northeastern Spain.

Evidences are found to affirm that early thrips abundance could be related with final disease incidence for early transplanted tomato crops. Similarly to that reported by Webb et al. (1970) and Salguero Navas et al. (1991) for flower thrips in tomato, our data show that thrips populations usually reached the highest number in that period. A low proportion of thrips appeared to be viruliferous is those populations. It would be interesting to investigate whether a decrease of TSWV incidence could be obtained by an appropriate management of thrips populations during that critical period.

The relationship found in early transplanted tomatoes between TSWV incidence and early thrips abundance is similar to that reported for lettuce crops by Cho et al. (1987) in Hawaii, and for peanut crops by Reddy et al. (1983) in India. In contrast, a totally different picture is obtained for late transplanted tomatoes. In this case, low WFT adult numbers were detected throughout the season whereas TSWV incidences were comparable to those found in early transplanted crops. WFT adult population densities were so low that the proportion of inoculative individuals were difficult to estimate. The analyses of data showed that significantly more infective thrips are present in the initial growth phases of late transplanted crops when compared with early transplanted ones. Therefore, late transplanted crops are more probable to be infected in that period than early transplanted crops. This observations may explain why high TSWV incidences occur in late transplanted plots in spite of the low WFT population numbers present. The sources for those highly infective thrips populations are, probably, nearby crops like early transplanted tomatoes, where an increase in the infectious potential of thrips is detected in that period (Table 2). If adult thrips move over large distance, as suggested by Lewis (1973), they can easily reach and infect the newly transplanted tomatoes coming from infected crops present in their neighborhood. In these conditions, strategies different to continuous spraying of chemicals for thrips control should be

explored for management of spotted wilt disease in tomato. Inhibition or avoidance of virus transmission by the thrips in relevant moments, as suggested by Allen et al. (1993) or the use of tomato cultivars tolerant or resistant to TSWV are good candidates. Conclusions drawn by this work strongly suggest that initial phases of the crop (0–60 days after transplanting) are critical for the development of TSWV disease. Therefore, more attention should be paid to this significant period when developing control strategies for management of TSWV in field tomatoes. The results presented contribute to the understanding of the epidemiology of TSWV in field tomatoes and offer valuable information for disease management.

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