Tomato Production under Mesh Reduces Crop Loss to Tomato Spotted Wilt Virus in Some Cultivars

M.J. Díez, S. Roselló, and F. Nuez

Departamento de Biotecnología, Universidad Politécnica de Valencia, Camino de Vera, 14, 46022 Valencia, Spain

J. Costa¹, A. Lacasa², and M.S. Catalá¹

Centro de Investigación y Desarrollo Agrario, La Alberca, Murcia, Spain

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Abstract. Seedlings of three tomato (*Lycopersicon esculentum* Mill.) cultivars ['RDD', carrier of the Sw5 gene, which confers resistance to tomato spotted wilt virus (TSWV); 'Pitihué', tolerant to the virus; and the susceptible cultivar Rutgers] were placed at the four- to five-leaf stage in cages containing a population of viruliferous thrips (*Frankliniella occidentalis* Perg.), and remained there for 0, 7, or 15 days. Plants were subsequently transplanted either into the open field or in tunnels protected with a mesh of 14 × 10 threads/cm. Systemic symptoms and number of dead plants were recorded and enzyme-linked immunosorbent assays (ELISA) were performed. 'Rutgers' exhibited severe systemic symptoms regardless of treatment and a high number of plants died. The level of infected plants remained low when protective measures were applied to seedlings of 'Pitihué' and acceptable yields were obtained. In open air cultivation, where seedling infection was severe, <20% of 'RDD' plants became infected and high yields were obtained; protected cultivation did not reduce yield. Although the percentage of infected plants was higher when cultivated under mesh, the yield of all three cultivars was greater than in the open field. The environment created under mesh stimulated growth, neutralizing the effect of the infection.

Because tomato spotted wilt virus (TSWV) causes severe economic losses in tomato, considerable effort has been focused on control methods (Roselló et al., 1996). Transmission of TSWV by thrips was first identified by Pittman (1927). Western flower thrips is currently considered to be the most important vector in Europe (Ananthakrishnan, 1980; Best, 1968). Attempts to control TSWV by preventive cultural practices, such as seedling protectors (antithrips mesh, chemical treatments) and control of vector populations, have often been ineffective. The use of virus-free seedlings, although difficult to obtain, would eliminate a source of primary inoculum. Chemical control has failed due to the vector's cryptic habitat and increasing resistance to insecticides (Robb, 1989) and biological control has not been satisfactory (Fischer et al., 1992). Physical barriers to control vector spread, such as protection with insect-proof screens (Antón et al., 1993; Berlinger et al., 1993; Lacasa and Contreras, 1993) or cultivation in tunnels with plastic mesh (Miguel and Serrano, 1991), can

help reduce the population of thrips. Even though resistant cultivars exist, response to TSWV infection is often variable depending on the specific conditions of culture.

One of the methods most often used to avoid host infection is protection with insect-proof screens. However, few studies have evaluated the efficacy of this practice. The value of using mesh depends on several factors. The objectives of this work were to determine the effect of 1) the level of inoculum, 2) protection of plants with tunnels made of antithrips mesh, 3) resistant or tolerant cultivars, and 4) the interactions between these three factors on disease development and on total yield.

Materials and Methods

Three tomato cultivars were chosen for this trial: 'RDD' (derived in the Polytechnic Univ. of Valencia from a line supplied by Dr. M.R. Stevens, Brigham Young Univ., Utah), carrier of the Sw5 gene for resistance to TSWV (Stevens et al., 1992); the Argentinian cultivar Pitihué (supplied by Dr. Calvar, Instituto Nacional de Tecnología Agropecuaria, Rio Negro, Argentina), which is tolerant to TSWV; and the susceptible cultivar Rutgers.

A population of viruliferous *F. occidentalis* was maintained in cages in a growth chamber programmed at 22 °C, relative humidity at 45% day/90% night, with a 14L : 10D photoperiod. This population has been maintained

for many generations on TSWV-infected plants. The thrips were reared on susceptible plants of the tomato cultivar Marmande and the pepper cultivar Sonar. Plants were replaced by younger ones when disease symptoms were severe to ensure continued feeding.

Plants were raised to the four to five true-leaf stage in 44 × 33 × 5.8-cm plastic trays containing a mixture of fertilized peat and perlite, which was autoclaved at 1 atm and 121 °C for 1 h. Twelve plants of each cultivar were placed in each tray. Three treatments were imposed: 1) plants not introduced to thrips in cages (T-0); 2) plants held in cages with viruliferous thrips for 7 d (T-7), and 3) plants held in cages with viruliferous thrips for 15 d (T-15). Four trays of each cultivar (48 plants) were randomized in the cages, but the presence of two trays of the same cultivar in the same cage was avoided. Because of limited space in the inoculation cages, plants of the T-7 treatment could be introduced into the cages only after the T-15 plants were removed. After treatment, all plants were sprayed with metiocarb (*N*-methylcarbamate of 3,5-dimethyl 4-methylthiophenyl) to kill adult thrips and larvae.

Treated plants were transplanted at Campo de Cartagena (Murcia, Spain) to either the open field or protected tunnels (10 × 5 m, covered with mesh of 14 × 10 threads/cm). Plants of treatments T-0 and T-15 were transplanted on 27 Apr. and plants of T-7 on 5 May. Four randomized blocks with six plants per block of each treatment were placed in both cultivation methods and blocks were separated by wide rows. Plant spacing was 0.5 m within the row and 1 m between rows. Plants were supported by vertical strings, and drip irrigation was used. No insecticide treatments against thrips were applied during the trials conducted in the open field in order to maximize feeding. In the screenhouse, insecticides were applied each 10 to 15 d to eliminate thrips. Fungicides prevented infection by the most common fungal diseases in the area, *Phytophthora infestans* Mont de Bary [controlled with Captan (cis-*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide), 40% + Zineb (zinc ethylene bis dithiocarbamate), 20%] and *Leveillula taurica*, Lev. [controlled with hexaconazole [(RS) 2-(2,4-dichlorophenyl) 1-(1*H*-1,2,4-triazol-1-yl)] hexanoic]. No viruses other than TSWV were detected by enzyme-linked immunosorbent assay (ELISA).

The experiment was a factorial with three variables. Three preinfestation treatments (T-0, T-7, and T-15) were applied to the three cultivars. All treated plants were transplanted both to the open field and to antithrips tunnels. The three effects studied were preinfestation treatment, cultivar resistance, and crop protection. Four replications per treatment and cultivation method were planted, using six plants per replication.

Symptoms and TSWV infection. Beginning on 2 May (for T-0 and T-15 treatments) and on 10 May (for the T-7 treatment), plants were observed weekly to detect characteristic virus-induced symptoms or death. Infection was

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¹Horticulture Dept.

²Plant Protection Dept.

verified by ELISA. Samples were tested with polyclonal antibodies against the TSWV BR-01 isolate (Loewe Biochemica GmbH, Sauerlach, Germany). All symptomless plants were analyzed every month. The numbers of infected and dead plants were recorded and the percentages calculated. The data were arcsin transformed prior to statistical analysis.

Vector population. Fifteen flowers and 10 leaflets were sampled from each treatment weekly beginning 5 June. Thrips were extracted with a homemade instrument, under incandescent light for 24 h. Thrips were kept in pots containing ethanol (10%) plus Tween 20® (polyoxyethylene-sorbitan monolurate; Sigma Chemical Co., St. Louis) as a wetting agent. The total number of adults and larvae per leaf/flower were recorded.

Harvests were carried out every 7 d beginning 14 July. The fruits harvested were classified as commercial, cull, and those with TSWV symptoms. The yield per plant at each harvest was calculated by dividing the yield of each block by the number of plants, including the plants killed by TSWV, after ELISA analysis, but excluding those that died as a consequence of transplanting. The total yield was calculated by adding the yields obtained in each harvest.

Results

Evolution of the vector population. Thrips populations in open air reached a maximum (mean of eight adults per flower) at the beginning of the growing period (Fig. 1D). A week later, the population decreased as a result of the presence of *Orius albidipennis* Reuter, a natural enemy common in this area. The population again peaked at the end of the growing period, with a mean of four adults per flower. Most of the thrips were adults, and were found mainly on the flowers.

Thrips were found on plants in the tunnels, in spite of chemical treatment, but their average number decreased to levels lower than one per flower (Fig. 1A and B). Only on T-15 plants in the tunnel (Fig. 1C) did the number reach four per flower in early June. However, chemical treatments had reduced the average number to less than one per plant by the end of the growing period. As in the open air, most of the thrips were adults and were located on flowers.

Effect of genetic resistance. The different levels of resistance inherent in each cultivar were the main effect detected by analysis of variance on the percentage of infected vs. dead plants and total yield, preinfestation being the main factor affecting early yield (Table 1).

After applying the treatments, the percentage of TSWV-infected and dead plants was low for 'RDD', medium for 'Pitihué', and high for the susceptible cultivar Rutgers, for both cultivation methods (Fig. 2).

Effect of preinfestation treatments on 'RDD'. No symptoms were evident on any plant of 'RDD' submitted to treatments T-0 or T-7 (Fig. 2 E and F), but the percentage of infected plants was ≈20% in treatment T-15. Some of these infected plants were eventually

Fig. 1. Effect of time of preinfestation with thrips and growing plants in tunnel vs. open air on the number of *Frankliniella occidentalis* adults and larvae on tomato plants from June to September. Means for three cultivars. Note that scales used for A and B differ from those used for C and D.

Table 1. F values from analysis of variance for the effects of cultivation method, preinfestation with TSWV virus, and cultivar of tomato on percentages of infected and dead plants and for early and total yield.

Source of variation	Infected plants ^a (%)		Dead plants ^a (%)		Early yield	Total yield
	12 June	16 Oct.	3 July	16 Oct.	7 Aug.	18 Sept.
Cultivation method (A)	42.54**	0.53	13.51**	0.17	16.60**	13.02**
Preinfestation (B)	14.97**	7.71**	8.62**	6.82**	33.33**	16.53**
Cultivar (C)	82.94**	87.02**	109.40**	133.70**	14.88**	35.84**
A × B	11.50**	0.16	0.65	2.16	3.39*	1.32
A × C	5.12**	1.30	11.26**	1.04	5.56**	8.94**
B × C	2.89**	1.96	10.74**	1.41	2.01	2.04
A × B × C	1.92	0.68	1.40	0.84	4.76**	2.82*

^aBased on analysis of arcsin transformed data.

*,**Significant at $P \leq 0.05$ or 0.01 , respectively.

able to localize the virus and grow quite normally, with no reduction in yield or quality of the fruits. No dead plants were recorded in treatment T-7, and in treatment T-15, the percentage of dead plants (data not shown) was lower than the percentage of infected plants.

Yield loss was lower in 'RDD' than in the other cultivars following treatment (Fig. 3 E and F). Tunnel cultivation did not reduce yield significantly ($F_{(2,9)} = 2.26$). Plants in treatments T-7 and T-15 yielded 70% to 80% of the yield of nontreated plants. In open air, both treatments T-7 and T-15 significantly reduced yield ($F_{(2,8)} = 14.11^{**}$), with higher losses in T-15 than in T-7. Yield of culls paralleled that of marketable fruits (data not shown). Preinfestation treatments delayed the first harvest.

Effect of cultivation method on 'RDD'. Plants grew more vigorously in protected cultivation than in the open air. Virus symptoms appeared earlier and the mortality rate was higher in tunnel cultivation in treatment T-15, but cultivation method did not significantly affect the percentage of dead plants.

Yield of 'RDD' was significantly higher under tunnel cultivation than in open air, regardless of treatment, but yield of culls was similar in both cultivation methods (data not shown). Symptoms appeared in some fruits, but did not cause economic losses. Plants grown in tunnel cultivation produced fruit earlier; at the beginning of August, the yield was twice that obtained in open air.

Effect of preinfestation treatment on 'Pitihué'. The infection level was greater in 'Pitihué' than in the resistant cultivar RDD, and preinfestation treatments increased the percentages of infected plants (Fig. 2 C and D). This effect increased with the number of preinfestation days. Nontreated plants also became infected because of the presence of thrips in the tunnel, but the percentage of infection was lower than in treated plants. Percentage of dead plants was higher in 'Pitihué' than in 'RDD' regardless of the treatments applied (data not shown). Yield losses were variable, depending on the treatment applied and on the cultivation method. Signifi-

Fig 2. Effects of time of preinfestation with thrips and growing plants in tunnel vs. open air on the course of infection of plants of three tomato cultivars. A and B—'Rutgers'; C and D—'Pitihué'; E and F—'RDD'. A, C, E—open air; B, D, F—tunnel.

cant differences were observed in the open field between treatments T-7 and T-15 vs. nontreated plants (T-0) ($F_{(2,7)} = 9.65^{**}$). However, no significant differences were detected when plants were grown in the tunnel. Few culls were produced in this cultivar, with a maximum of $\approx 11\%$ for T-15, and no disease symptoms appeared on fruits.

The treatments reduced earliness in the open field, but there were essentially no early yield losses in the tunnel.

Effect of cultivation method on 'Pitihué'. Tunnel cultivation increased the percentage of infected plants, and hastened infection on T-7 and T-15 plants, as determined by ELISA. The more rapid development of plants cultivated in the tunnel apparently favored the spread of the virus. Nevertheless, mortality was similar in both cultivation methods.

Protection of the crop did not increase yield significantly in 'Pitihué', in contrast with our results with 'RDD' (Fig. 3 C and D). However, the effect of preinfestation treatments was less pronounced on both yield and earliness. Fruits ripened earlier on noninoculated plants in the field, but not in protected cultivation.

Effect of preinfestation treatment on 'Rutgers'. 'Rutgers' had higher infection levels than other cultivars regardless of treatment. Even when seedlings were not pretreated (T-0 treatment) the percentage of infected plants was $\approx 70\%$ 1 month before the beginning of the

harvest period (mid-July). Preinfestation treatment increased the percentage of infection, which reached 100% in treatment T-15 (Fig. 2 A and B).

The level of mortality was high, even in plants from healthy seedlings. The percentage of dead plants was 90% in the field vs. 55% in the tunnel. Pretreatment increased the percentage of dead plants to 100% in the tunnel, and the mortality was also very high in the three field treatments.

Preinfestation treatments drastically reduced yield; no fruit was obtained in tunnel cultivation, and yield was reduced 50% in the field (Fig. 3 A and B). The percentage of culls was 49% of total yield in some cases. The effects of treatments on percentage of culls paralleled that on marketable yield.

Effect of cultivation method on 'Rutgers'. Protected cultivation improved the appearance and increased the number of infected plants on plants submitted to the three treatments (Fig. 2).

The effect of cultivation method on yield was evident only in treatment T-0, since treated plants died in protected cultivation. Healthy seedlings yielded three times as much in protected cultivation as in open air (Fig. 3 A and B).

Interactions between treatments. The interaction between cultivar and preinfestation treatment was reflected in higher infection and

mortality in treated plants of the susceptible cultivar (Table 1). At the beginning of the cultivation period, the percentage of infected and dead plants under mesh was much higher for 'Rutgers' than for 'Pitihué' or 'RDD'. An interaction between cultivation method and preinfestation treatments was evident in the higher percentage of infected plants under mesh than in the field as the time of preinfestation was prolonged (T-15 vs. T-7).

Protected cultivation increased yield in 'RDD' and 'Rutgers' (T-0 treatment) but not in 'Pitihué'. A triple interaction was also evident. The yield of 'RDD' was higher under mesh than in the field for all treatments, whereas the yield of 'Rutgers' was higher under mesh only in the absence of treatment. When seedlings were infected, the yield of 'Rutgers' was lower (no yield was obtained in T-15) in protected cultivation. The behavior of 'Pitihué' differed from that of the other cultivars. Whereas the yield obtained under mesh differed depending on the treatment, yields obtained in the field differed little, and were not affected by preinfestation treatment.

Discussion

In the field, without screen protection, the resistant cultivar 'RDD' remained free of disease even after exposure of seedlings to treatment T-7 (plants held in cages with viruliferous thrips for 7 d). Even when seedlings were submitted to treatment T-15 (plants held in cages with viruliferous thrips for 15 d), $<20\%$ of the plants became infected. Cultivation under mesh prevented yield reductions, even when contamination with viruliferous thrips during transplanting, occurred (T-15). Yield losses occurred in the field, but these were probably the result of causes other than TSWV. The anthrips mesh protected against the constant winds that hamper tomato cultivation in this area. In addition, early ripening under mesh could be due to earlier flowering and setting of fruits owing to the higher temperatures in the tunnel. This effect was very important in all treatments; in fact, in treatment T-7 there were no infected plants and in treatment T-15 the percentage of infection was lower than in protected cultivation. The use of resistant cultivars reduced the number of infected plants to very low levels, and high yields were obtained even under severe infection conditions.

The Argentinian cultivar Pitihué did not adapt well to our cultivation conditions. However, the level of infected plants remained low when protection measures were applied to ensure healthy seedlings. Early infection of seedlings increased the percentage of infected plants. Most plants with minimal symptoms regrew, reducing the level of mortality. The effect of preinfestation varied with the cultivation method. Yield was significantly reduced in the field, but not in protected cultivation; however, the percentage of infected plants was higher under mesh. One reason for this could be the regrowth of infected plants observed in tunnel cultivation; infected plants

Fig 3. Effects of time of preinfestation with thrips and growing plants in tunnel and open air on marketable yield of three tomato cultivars. A and B—'Rutgers'; C and D—'Pithu'; E and F—'RDD'. A, C, E—open air; B, D, F—tunnel.

grew more rapidly, outgrew the infection, and were able to produce marketable yields. This could explain the similar yield obtained in plants in treatment T-7 in both cultivation methods, although the percentage of infected plants was almost twice as great in protected cultivation. The greater vegetative development of plants grown in protected cultivation, and the mechanism of tolerance or resistance of this cultivar, could constitute advantages in controlling virus infection, giving rise to the observed regrowth. We conclude that tolerant cultivars grown in protected cultivation can

produce acceptable yields, even when seedling infection occurs and the thrips population cannot be totally controlled. Resistance of Argentinian varieties under field conditions has been reported in Argentina (Gallardo, 1990; Gallardo and Calvar, 1992) and Brazil (Boiteux et al., 1993).

The protection of seedlings and crop did not guarantee yield when susceptible cultivars were used, as mortality was almost total. Even when strict control measures were adopted, the yield was severely reduced due to the high plant mortality ($\approx 50\%$).

Clean transplants of TSWV-resistant cultivars yield best under protected cultivation. However, tunnel cultivation can enhance infection and hence reduce yields in susceptible and tolerant cultivars under high disease pressure.

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