

Collaboration for Plant Pathogen Strain Identification

Guidelines for the Identification of *Tomato Spotted Wilt Virus* races using Differential Pepper Hosts

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Host: Pepper, *Capsicum annuum*

Background: *Tomato spotted wilt virus* (TSWV) causes major economic losses globally due to an extremely wide host range involving more than 900 species of plants in 90 families that include important ornamental, fruit, and vegetable crops such as pepper and tomato (German, Ullman and Moyer 1992; Moury and Verdin 2012, Pappu 2009; Oliver and Whitfield 2016; Sherwood et al 2009). TSWV is the type species of the genus *Tospovirus* and is transmitted in a persistent manner mainly by western flower thrips, *Frankliniella occidentalis*. Western flower thrips acquire the virus as nymphs in order to transmit throughout their adult lives (Sakimura 1962).

Symptoms of the disease in pepper (Fig. 1 – 3) can vary with the pepper cultivar, age of the plant at the time of infection, time of year, as well as environmental conditions and include stunting, necrosis, chlorosis, ringspots and ring patterns that affect leaves, stems and fruit (German et al 1992; Mumford et al 1996).



Fig. 1. TSWV symptoms in susceptible pepper



Fig. 2 TSWV leaf rings and patterns



Fig. 3. TSWV pepper fruit symptoms

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Management of the disease and its thrips vectors is challenging at best due to the wide host range of both virus and vector, the large number of weed and perennial hosts that provide between-crop reservoirs, high thrips fecundity rates and the difficulty of controlling thrips with pesticides (Adkins, S. 2000; Cho et al 1989; Macharia et al 2015; Srinivasan et al 2014). A combination of approaches seems to be the best strategy to manage thrips and TSWV in pepper cultivation: weed management, altered planting dates, thrips proof mesh tunnels where applicable, use of TSWV resistant pepper varieties and early pesticide applications to control thrips larvae (Adkins 2000; Cho et al 1989; Pernezny et al 2003). In addition, the use of predatory mites has been useful to control thrips in greenhouse pepper production when populations are not exceedingly high (Messelink et al 2007).

Resistance and TSWV strains: The single dominant gene conferring resistance to TSWV in pepper is *Tsw*. It was identified in *Capsicum chinense* PI 152225 (Black et al 1991) and mapped to the distal portion of chromosome 10 (Jahn et al 2000). In plant tests, resistance is displayed as a local hypersensitive reaction in the host (Fig. 8) that prevents systemic spread of the virus. In time, the inoculated leaf often drops. Unlike *Sw5* in tomato, *Tsw* is not effective against *Tomato chlorotic spot virus* (TCSV), *Groundnut ringspot virus* (GRSV), *Alstromeria necrotic streak virus* (ANSV), *Chrysanthemum stem necrosis virus* (CSNV) and *Impatiens necrotic spot virus* (INSV) (Turina et al 2016). In addition, resistance can become unstable under continuous high temperatures, resulting in systemic infection (Moury 1998). Many commercial pepper varieties have been released containing this resistance gene. After years of the continuous use of these resistant varieties, resistance breaking strains of TSWV have been detected in pepper production areas on a global scale. Mutations in the S RNA region that encodes the non-structural protein (NSs) allow TSWV to break down the resistance provided by *Tsw* (De Ronde et al 2013).

The resistance breaking strains of TSWV found in tomato are different from those found in pepper. In tomato, mutations in the M RNA region that encodes the non-structural movement protein (NSm) allow TSWV to break down the resistance provided by *Sw-5* (Crescenzi et al 2015; Lopez et al 2011). Strains still controlled by *Tsw* or *Sw-5* have been designated 0 and strains that overcome *Tsw* or *Sw-5* have been designated 1 according to the ISF guidelines proposed by the International Seed Federation (Thomas et al 2019). In studies by Crescenzi et al (2015), pepper hybrids carrying the *Tsw* gene did not develop symptoms following inoculation with the TSWV 1 strain from tomato. In mechanical transmission experiments, Macedo et al (2019) demonstrated that TSWV 1 strains collected from pepper did not infect a *Sw-5* tomato cultivar, but did infect a tomato cultivar without the *Sw-1* gene.

Resistance-breaking strains of TSWV also vary by geographical region. Tomato varieties developed in Europe with resistance to TSWV 1 were found to be susceptible to TSWV 1 isolates found in California (2020 personal communication, R. L. Gilbertson).

Other tospoviruses such as INSV, TCSV and GRSV infect pepper and are vectored by several species of *Frankliniella* (Moury and Verdin 2012, Oliver and Whitfield 2016). Because all these tospoviruses cause similar symptoms, it is important to verify the species of tospovirus in question with additional serological and PCR based tools before determinations of the TSWV strain can be made.

TSWV maintenance: Thrips-inoculated symptomatic host leaves should be used to initiate a culture of TSWV. Thrips-inoculated leaves can be stored at -80°C for future use. After checking for purity, this material can be mechanically inoculated into a perennial susceptible host such as *Emilia sonchifolia* or *Gomphrenia globosum* and maintained in thrips proof cages in a greenhouse (Houle and Kennedy 2017).

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Fig. 4. TSWV culture in *G. globosum*

Preparation of host plants and inoculum: Susceptible pepper seedlings for a fresh culture, inoculum production or for strain testing are grown until the first-true leaves are emerging (Fig. 5). Ten seedlings per replication and two to three replications per variety are recommended for resistance and susceptibility testing. Inoculum is collected from the cultured host and prepared by flash freezing fresh symptomatic plant tissue with liquid nitrogen (Fig. 6), then homogenized in chilled 0.01M phosphate buffer, pH 7.0 to which 0.1% Na₂SO₃ is added (1:10 tissue to buffer weight by volume). If liquid nitrogen is not available, symptomatic tissue can be macerated in chilled phosphate buffer then diluted as described above. The buffered inoculum should remain chilled throughout the inoculation process. Abrasive agents (eg., carborundum or celite) may be used to enhance inoculation efficacy. The first or second pair of true leaves are gently rub inoculated (Fig. 7). After 2 – 3 weeks, the inoculum is ready for use. Depending on the time of year, seedlings for strain testing should be planted so test seedlings and inoculum are ready at about the same time. One to two inoculations may be needed to ensure 100% of the susceptible seedlings develop symptoms.

Our experience in screening pepper lines for resistance to TSWV, has demonstrated that symptom development is best facilitated by minimizing the number of sequential mechanical inoculations between thrips inoculated material and the tested plant lines. Three sequential mechanical inoculations are used in this protocol.



Fig. 5. Pepper seedlings, first true leaves emerging



Fig. 6. Frozen fresh symptomatic tissue ready for homogenizing with a mortar and pestle in cold buffer

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Fig. 7. Dip a gloved finger into inoculum mixture

Inoculation, incubation and evaluation for resistance and susceptibility: The cotyledons of test plants gently are rub-inoculated (Fig. 7) with a gloved finger using freshly prepared inoculum as described above. Inoculated plants can be incubated in the greenhouse at 25°C with 12 hours of light.

Table 1. Expected reactions of pepper differentials to strains of TSWV

Differentiating host variety	TSWV: 0	TSWV: 1
Yolo Wonder B	S	S
CHI-7	HR	S
PI 152225	HR	S

S = Susceptible HR = Highly Resistant

In resistant plants, a hypersensitive reaction can be seen on the inoculated leaves 4 – 5 days after inoculation (Fig. 8). Frequently, the inoculated leaves drop off leaving behind seemingly asymptomatic resistant plants. Seedlings can be evaluated for their responses to TSWV 14 – 21 days after inoculation or when symptoms of disease such as stunting, necrosis, chlorosis, ringspots and ring patterns develop in all tested susceptible controls (Fig. 9R). Leaves of resistant plants often remain symptomless (Fig. 9L). There is currently no resistance to TSWV P1 in commercial varieties.



Fig. 8. Necrotic spots on leaves of PI 152225 – chlorotic, a resistant response



Fig. 9. Asymptomatic resistant (L) and stunted susceptible plants (R)

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Ordering seeds of host differentials:

Seeds of each of the differential lines listed in Table 1 can be ordered from the USDA GRIN (Germplasm Resources Information Network: <https://www.ars-grin.gov/>). You may search the USDA GRIN database without logging in, but cannot order seeds until you create an account and log in to the database.

Type in 'CPPSI*' in the search window. Select the differential hosts to order. Select the cart button at the top of the page to generate an order form. Select 'submit' to place your order.

A limited supply of seeds per differential can be ordered at no charge, as long as there is adequate seed in supply. The USDA National Plant Germplasm System in which the GRIN database is housed may not always have adequate seed of all the differentials listed to provide a full set of differentials.

If you have difficulties ordering seeds, contact Kelley Clark at kjclark@ucdavis.edu for assistance.

Ordering strains of the pathogen:

Reference strains of TSWV 0 can be obtained by contacting:

Dr. Raquel Salati

Scientist, United Genetics Seeds
8000 Fairview Rd
Hollister, CA 95023

Phone: (831) 636-4882

E-mail: raquel.salati@unitedgenetics-usa.com

You must send a valid APHIS permit to the supplier in order to receive reference strains. There will be a nominal fee charged that covers the cost of culturing, purity checks, validation of pathogenicity and shipping by the supplier.

Feedback

Inquiries on how to participate and support CPPSI, provide feedback on new races identified, views on the inoculation protocols, differential hosts, or any related matter is welcomed. Please contact: Kelley Clark at kjclark@ucdavis.edu.

Liability waiver

The CPPSI Collaboration for Plant Pathogen Strain Identification, USDA NPGS/GRIN, APS, ASTA, and all other associated members and participating organizations or companies have done their best to provide information that is up-to-date and published in refereed journals and, therefore, no liability for the use of this information is accepted. The inoculation protocol described in this document has been demonstrated to be effective at identifying strains of TSWV and resistance in the above pepper cultivars.

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