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

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Host: Tomato, *Solanum lycopersicum*

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 tomato (German, Ullman and Moyer 1992; Pappu 2009; Oliver and Whitfield 2016; Sherwood et all 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 tomato can vary with the host plant, time of year, as well as environmental conditions and include stunting, necrosis, bronzing, chlorosis, ringspots and ring patterns that affect leaves, stems and fruit (German et al 1992; Mumford et al 1996).

Fig. 1. Symptoms of early TSWV infection

Fig. 2. Symptoms of late TSWV infection

Fig. 3. TSWV fruit symptoms

Management of this 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 tomato cultivation: weed management, altered planting dates, thrips proof mesh tunnels where applicable, use of TSWV resistant tomato varieties, use of predatory mites and early pesticide applications to control thrips larvae (Adkins 2000; Cho et al 1989; Zitter and Momol 2014). In addition, the use of predatory mites has been useful to control thrips in greenhouse tomato production while populations are not exceedingly high (Messelink et al 2007).

Resistance and TSWV strains: The most commonly used resistance gene in tomato is the *Sw-5* cluster located on chromosome 9. The cluster originates from *Solanum peruvianum* (Stevens et al 1992). This source of resistance is also 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). Many commercial tomato 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 tomato production areas on a global scale. Mutations in the M RNA region that encode 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).

The resistance breaking strains of TSWV found in pepper are different from those found in tomato. In pepper, a different mutation in the S RNA region that encodes the non-structural protein (NSs) allows TSWV to break down the resistance provided by *Tsw* (De Ronde et al 2013). Strains still controlled by *Sw-5* or *Tsw* have been designated 0 and strains that overcome *Sw-5* or *Tsw* 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).

Many other tospoviruses including *Iris yellow spot virus* (IYSV) and INSV also have wide host ranges and are vectored by onion thrips (*Thrips tabaci*) and western flower thrips, respectively (Oliver and Whitfield 2016). Because so many tospoviruses affecting tomato 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 tomato leaves should be used to begin 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).

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

Preparation of host plants and inoculum: Susceptible tomato 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% Na_2SO_3 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. Cotyledons or first true leaves are gently rub inoculated Fig. 7 – 8). 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 tomato 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. Tomato seedlings, first true leaf emerging

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

Fig. 7. Dip a gloved finger into inoculum mixture

Fig. 8. Gently rub inoculum mixture onto cotyledons

Inoculation, incubation and evaluation for resistance and susceptibility: The cotyledons of test plants are rub-inoculated (Fig. 7 - 8) 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 tomato differentials to strains of TSWV

Differentiating host variety	TSWV 0	TSWV 1
Early Pak 7	S*	S
VFN8	S	S
Stevens	R	S

* S = Susceptible R = Resistant

In resistant plants, a hypersensitive reaction can be seen 4 – 5 days after inoculation (Fig. 9). Plants can be evaluated for their responses to TSWV 14 – 21 days after inoculation or when symptoms of disease such as stunting, chlorosis and bronzing develop in all tested susceptible controls (Fig. 9 - 10). Leaves of resistant plants remain symptomless (Fig. 10). There is currently no resistance to TSWV 1 in commercial varieties.

Fig. 9. Necrotic spots on cotyledons of resistant plants (L); Bronzing and chlorosis in susceptible plants

Fig. 10. Symptomless resistant (rear) and stunted susceptible (front) responses in tested seedlings.

A similar protocol for evaluating tomato varietal responses to TSWV can be found in the Guidelines for Distinctiveness, Uniformity and Stability (DUS) of new varieties published by the International Union for the Protection of New Varieties of Plants (UPOV). https://www.upov.int/edocs/tgdocs/en/tg044.pdf

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) Global database at: https://npgsweb.ars-grin.gov/gringlobal/search.aspx, then type in 'CPPSI*' in the search box for access to the CPPSI collections stored in GRIN.

Note: A limited supply of 50 seeds per differential can be ordered at no charge, as long as there is adequate seed in supply. The NPGS may not always have adequate seed of all the differentials listed above to provide a full set of differentials. Please contact Phyllis Himmel at cppsi@ucdavis.edu if this problem is encountered.

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.

Contacting CPPSI
Inquiries on how to participate and support CPPSI, provide feedback on new strains identified, views on the inoculation protocols, differential hosts, or any related matter are welcomed. Please contact:

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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 tomato cultivars.

Selected References

Adkins, S. 2000. Tomato spotted wilt virus – positive steps towards negative success. Molecular Plant Pathology 1(3):151-157.

Cho, J. J., R. F. L. Mau, T. L. German, R. W. Hartmann, L. S. Yudin, D. Gonsalves, R. Provvidenti. 1989. A multidisciplinary approach to management of *Tomato spotted wilt virus* in Hawaii. Plant Disease 73(5):375-383.

Crescenzi, A., A. Fanigliulo, A. Viggiano. 2015. Resistance breaking *Tomato spotted wilt virus* isolates on resistant tomato cultivars in Italy. Pp.95-98 in Proc. IVthIS on Tomato Diseases. Eds. M. L. Paret et al. Acta Hort. 1069, ISHS.

De Rond, D., P. Butterbach, D. Lohuis, M. Hedil, Van Lent, J. W. M., Kormelink, R. 2013. *Tsw* gene-based resistance is triggered by a functional RNA silencing suppressor protein of the *Tomato spotted wilt virus*. Mol. Plant Pathol. 14(4):405-415.

German, T. L., D. E. Ullman, J. W. Moyer. 1992. Tospoviruses: Diagnosis, Molecular Biology, Phylogeny, and Vector Relationships. Annu. Rev. Phytopathol. 30:315-348.

Lopez, C., J. Aramburu, L. Galipienso, S. Soler, F. Nuez, L. Rubio. 2011. Evolutionary analysis of tomato *Sw-5* resistance-breaking isolates of *Tomato spotted wilt virus*. F. Gen. Virol. 92:210-215

Macedo, M. A., M. R. Rojas, R. L. Gilbertson. 2019. First report of a resistance-breaking strain of Tomato spotted wilt Orthotospovirus infecting sweet pepper with the Tsw gene in California, U. S. A. Plant dis. 103:5, 1048

Macharia, I., D. Backhouse, S. B. Wu, E. M. Ateka. 2015. Weed species in tomato production and their role as alternate hosts of *Tomato spotted wilt virus* and its vector *Frankliniella occidentalis*. Ann. Appl. Biol. 169:224-235.

Messelink, G., L., R. Van Maanen, S., E., F Van Steenpaal, A. Janssen. 2007. Biological control of thrips and whiteflies by a shared predator: Two pests are better than one. Biological Control 44:372-379.

Mumford, R. A., I. Barker, K. R. Wood. 1996. The biology of the tospoviruses. Ann. Appl. Biol. 128:159-183.

Oliver, J. E. and A. E. Whitfield. 2016. The Genus *Tospovirus*: Emerging Bunyaviruses that Threaten Food Security. Annu. Rev. Virol. 3:101 – 24.

Pappu, H. R., R. A. C. Jones, R. K. Jain. 2009. Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. Virus Research 141:219-236.

Sakimura, K. 1962. The present status of thrips-borne viruses. In Biological Transmission of Disease Agents, ed. K. Maramorosch, pp. 33-40. New York: Academic

Sherwood, J. L., T. L. German, J. W. Moyer, D. E. Ullman. 2009. Tomato spotted Wilt virus. *The Plant Health Instructor*. apsnet.org.

Stevens, M. L., S. J. Scott, R. C. Gergerich. 1992 Inheritance of a gene for resistance to *Tomato spotted wilt virus* (TSWV) from *Lycopersicon peruvianum* Mill. Euphytica 59:9-17.

Srinivasan, R., D. Riley, S. Diffie, A. Shrestha, A. Culbreath. 2014. Winter weeds as inoculum sources of *Tomato spotted wilt virus* and as reservoirs for its vector, *Frankliniella fusca* (Thysanoptera: Thripidae) in farmscapes of Georgia. Environ. Entomol. 43(2): 410-420.

Thomas, V., P. T. Himmel, P. R. Brown, S. Ruthner, V. Grimault. 2019 ISF Guidelines on the nomination of novel plant pathogen races and strains. Poster presented at the 2019 APS meetings.

Turina, M., R. Kormelink, R. O. Resende. 2016 Resistance to Tospoviruses in Vegetable Crops: Epidemiological and Molecular Aspects. Annu. Rev. Phytopathol. 54:347-371.

Zitter, T., T. M. Momol. 2014. Tomato spotted wilt virus. In Compendium of Tomato Diseases and Pests, eds. J. b. Jones, T. A. Zitter, T. M. Momol, S. A. Miller, pp 96-97. APS Press