Collaboration for Plant Pathogen Strain Identification

Guidelines for the identification of *Pseudomonas syrinage* pv. *tomato* races using differential tomato varieties

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Host: *Solanum lycopersicum*
Pathogen: *Pseudomonas syrinage* pv. *tomato* (*Pst*)

Background:
Bacterial speck of tomato is caused by the gram-negative bacterium, *Pseudomonas syringae* pv. *tomato* (*Pst*) (Miller and Jones 2014). Bacterial speck was described originally in Taiwan and the US in 1933 and is an important disease of tomatoes worldwide (Bryan 1933). *Pst* is seedborne, likely contributing to its global distribution (Bashan and Assouline 1983; McCarter et al. 1983). Serious disease outbreaks are infrequent; however, problems can arise with cooler temperatures, high density planting, and high moisture environments resulting in economic loss (Pedley and Martin 2003; Preston 2000). Outbreaks on transplants in nursery production can also lead to losses or establishment problems in the field. The disease is characterized by small brown to black (5-10 mm) necrotic lesions (specks) surrounded by yellow halos on leaves and elongated lesions on stems of tomato plants (Miller and Jones 2014; Pernezny et al. 2012). Specks can coalesce as symptoms progress forming larger brown lesions (**Figure 1**). On fruit, smaller lesions (1-2 mm) occur and can reduce marketability of fresh-market varieties (Pedley and Martin 2003) (**Figure 2**).

Speck can be difficult to differentiate from bacterial spot, caused by Xanthomonas species (*X. euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri*) based on foliar symptoms alone (Pernezny et al. 2012). Environment-wise, both pathogens thrive in high moisture while speck favors cool temperatures (15-25°C), and spot favors warm temperatures. Additionally, fruit lesions characterizing bacterial speck are small and sunken, while for spot they are raised, often scabby, and larger (Miller and Jones 2014; Pernezny et al. 2012).

Figure 1. Lesions coalesced on tomato stems and leaves. *Photo: Cornell CALS.*

Figure 2. Specks on unripe tomato fruit. *Photo: Cornell CALS.*

Pst has remained a popular model system for studying host-pathogen interactions due to its application in a range of molecular biology techniques and its ability to infect *Arabidopsis thaliana* (Preston 2000). Additionally, the widely studied *Pst* strain, DC300, has a complete genome sequence available (Buell et al. 2003).

Management of the disease is primarily through the use of clean seed and disease-free transplants (Miller and Jones 2014; Pernezny et al. 2012). The bacteria spread via wind and water splash and enter the host plant through stomata or wounds, thus, minimizing handling of plants when wet and avoiding sprinkler irrigation can mitigate risk (Preston 2000). In the case of outbreaks, copper spays can reduce disease severity (Graves and Alexander 2002; Yunis et al. 1980; Zhang et al. 2021).

Races and resistance:
Resistance to *Pst* was initially identified in the wild species *Lycopersicon pimpinellifolium* (now *Solanum pimpinellifolium*) and was thought to be controlled by a single dominant gene (Pilowsky and Zutra 1982). Later studies identified the same gene for resistance in the tomato variety Ontario 7710 (which has *L. pimpinellifolium* in its pedigree) and found it to be semi-dominant (Carland and Staskawicz 1993; Kozik 2002; Pitblado and Kerr 1979, 1980; Pitblado and MacNeill 1983). This gene was named *Pto* and confers resistance to *Pst* race 0 (**Table 1**).

Pto was introgressed from wild tomato into cultivated tomato over 80 years ago and has been widely used in speck resistance globally since the 1980s (Pedley and Martin 2003). However, because the gene is semi-dominant, disease is occasionally observed on hybrids that carry only one copy. Pto is a cytoplasmically located serine-threonine protein kinase (Chandra et al. 1996). Direct physical interaction of Pto with either of the *Pst* race 0 effectors AvrPto or AvrPtoB constitutes the gene-for-gene recognition, upon recognition Pto acts with Prf (a nucleotide-binding leucine-rich repeat protein) to signal downstream pathways (Lin and Martin 2007). *Pst* race 1 is not recognized by Pto and can therefore attack tomato varieties with the *Pto* gene.

Currently there is no commercial *Pst* race 1 resistance. Researchers at Cornell University have identified *Ptr1* in wild tomato and are working with plant breeders to introduce it to tomato varieties that already have the *Pto* gene (BTI news article here). The *Ptr1* locus was found in the wild tomato relative, *Solanum lycopersicoides*, and confers resistance to several *Pst* race 1 strains by recognition of the effector AvrRpt2 (Mazo-Molina et al. 2019). Ptr1 encodes an intracellular immune receptor protein and is a pseudogene in cultivated tomato (Haefner et al. 2023; Mazo-Molina et al. 2020). Interestingly, *Ptr1* also confers resistance to some strains of *Ralstonia solanacearum*, the causal agent of tomato bacterial wilt.

Table 1. Expected responses of the *Solanum lycopersicum* differentials to *Pseudomonas syrinage* pv. *tomato* (*Pst*) races

		Race	
Tomato Differentials	**Gene**	**Pst: 0**	**Pst: 1**
VFN8	-	S	S
Ontario 7710	*Pto**	HR	S

*Ontario 7710 is *Pto* homozygous.
S = Susceptible, HR = Highly Resistant

Preparation of host plants and inoculum:

At least 20 plants per variety per isolate should be used when evaluating *Pst* resistance and performing race identification. Sow seed in potting mix in trays and grow plants in either a greenhouse or growth chamber with 12 hours of light and temperatures between 22-25°C during the day and 16-20°C at night. If seed is in excess, two seed per well can be sown and thinned as needed.

About 3 weeks post sowing, when plants have 3-4 expanded leaves, prepare fresh cultures of the *Pst* isolates. Streak the bacteria on King's B (KB) agar plates and incubate the plates at 28°C for 24 hours. Inoculum will be prepared by directly scraping the cells off the plates and resuspending cells in a saline buffer of neutral pH to an OD of 0.2 to 0.3. Note that plates can be stored but should be less than 2 days old. A small amount of Tween 20 or silwet can be added to the solution.

Inoculation, incubation and evaluation for resistance and susceptibility:

Tomato plants with 3 – 4 expanded leaves should be spray-inoculated ensuring full coverage of the leaf surface with the inoculum. Post inoculation plants are kept in the dark with high humidity for 48 hours at 20°C. After this period, plants are returned to the light and ambient humidity with temperatures 24-25°C. Too much humidity post the 48 hr incubation period can lead to secondary infections.

Symptoms can start to be evaluated 5 – 7 days post inoculation with a final observation at 2 weeks post inoculation (**Figures 3 and 4**). The resistant response should be no symptoms or pinpoint lesions. Susceptible plants will have larger lesions (specks) with chlorotic halos. No rating scale is used, individual plants are marked as either resistant or susceptible.

Figure 3. VFN8 tomato plants inoculated with *Pst* race 0 (left) or race 1 (right) showing bacterial speck symptoms including specks with chlorotic haloes on leaves. Plants are 2 weeks post inoculation. *Photo: Bayer.*

Figure 4. Ontario 7710 tomato plants inoculated with *Pst* race 0 (left) or race 1 (right). Ontario 7710 is resistant to Pst: 1 as illustrated by the healthy plants with no symptoms (left) and susceptible to Pst: 1 illustrated by the specks with chlorotic haloes on leaves (right). Plants are 2 weeks post inoculation. *Photo: Bayer.*

Ordering seed of the tomato differentials and *Pst* type isolates:
Type isolates of *Pst* and seed of the tomato differentials are available as CPPSI accessions via the USDA-ARS National Plant Germplasm System. Visit the USDA-GRIN website (https://www.ars-grin.gov/) and search "CPPSI*" to see a list of all accessions validated and deposited by CPPSI.

The CPPSI VFN8 seed is currently available via USDA-GRIN. For seed of Ontario 7710 please contact the CPPSI Director, Kelley Clark, at kjclark@ucdavis.edu for assistance.

Pst race 0 and race 1 type isolates were originally collected in California and are used as standards in the seed industry. ***Pst* is considered a native or naturalized plant pest by USDA APHIS and can be moved within the continental US without a permit.** See the APHIS Native and Naturalized Plant Pest List here.

For questions regarding ordering these reference races, contact:
Amy Gurza (amy.gurza@usda.gov) and/or **Andy Hagan** (andy.hagan@usda.gov)
National Lab for Genetic Resources Preservation Unit
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Liability waiver
CPPSI 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 races of Pst and resistance in the above tomato varieties.

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