GUIDELINE FOR IDENTIFICATION OF PEPPER BACTERIAL LEAF SPOT RACES USING DIFFERENTIAL HOSTS

Authors: Chet Kurowski, Kevin Conn, Phyllis Himmel
Updated Nov 2015: Elisabetta Vivoda
Updated July 2019, June 2021: Phyllis Himmel

Host: *Capsicum annuum* L.

Pathogen: *Xanthomonas euvesicatoria, X. vesicatoria, X. perforans* and *X. gardneri*.

Background: Bacterial leaf spot symptoms include small, irregular, water-soaked, greasy-appearing lesions on leaf undersurfaces. Lesions develop rapidly in size, and become tan to reddish-brown. Often lesions are more numerous at leaf tips and margins where moisture accumulates. Symptoms are usually more severe and lesions reach a greater size following periods of prolonged leaf wetness. Defoliation occurs under heavy disease pressure. When conditions are dry, leaves become tattered as lesion centers and leaf margins dry and disintegrate. Stem lesions occur as narrow, light-brown, longitudinally raised cankers. Fruit spots begin as water-soaked areas that later become necrotic. These spots are rough in appearance and crack as they develop (7).

Until the early 1990's, bacterial leaf spot of pepper and tomato was thought to be caused by a single bacterial species, Xanthomonas campestris pv. vesicatoria. In the early 1990's, two distinct genetic groups were shown to exist within races of X. campestris pv. vesicatoria. In 1995, Vauterin et al. restructured the species within the genus Xanthomonas and proposed X. vesicatoria and X. axonopodis pv. vesicatoria. Subsequently, four taxonomically distinct xanthomonads were identified and placed into four groups, designated A, B, C, and D. Jones et al. showed these four groups to be distinct enough to deserve species status: X. euvesicatoria = X. campestris (axonopodis) pv. vesicatoria (group A), X. vesicatoria = X. vesicatoria (group B), X. perforans (group C), and X. gardneri (group D).

Pepper races found within X. euvesicatoria are the most widely distributed and cause the greatest economic loss in pepper. Xanthomonas vesicatoria and X. gardneri are also known to cause bacterial leaf spot on pepper and can have a significant impact in regions where they are found. Xanthomonas perforans races are occasionally found to cause disease on pepper. Pepper races found within *X. euvesicatoria* are the most widely distributed and cause the greatest economic loss in pepper. *Xanthomonas vesicatoria* and *X*. *gardneri* are also known to cause bacterial leaf spot on pepper and can have a significant impact in regions where they are found. *Xanthomonas perforans* races are occasionally found to cause disease on pepper. Races from all four species have been isolated from tomato. As resistance in tomato and pepper to bacterial spot is based on races that go across these species, use of the old and traditional name X. c. vesicatoria and its acronym Xcv continues.

Early work on bacterial leaf spot indicated that races recovered from tomato and pepper were pathogenic on both plant species, and for many years it was thought that cross infection could occur in the field. It was not until the 1970's that Cook (3) demonstrated host specificity was associated with a hypersensitive reaction (HR) (1, 2, 3). Currently, three groups of races are distinguished on the basis of virulence on tomato and pepper: tomato races are virulent on tomato only, pepper races are virulent on pepper only, and pepper-tomato races are virulent on both crops (11). Within the pepper and pepper-tomato groups, races of the pathogen can be distinguished by the reaction of various pepper lines each containing a resistance gene.

Development of resistance to bacterial leaf spot of pepper began when Sowell (14, 15) screened many plant introductions for resistance. Currently, five resistance genes, which induce a hypersensitive response, have been identified within pepper (1, 2, 3, 10, and 11). These genes were identified from the following plant introductions: PI 163192 (*Bs1* gene); PI 260435 (*Bs2* gene); PI 271322 (*Bs3* gene); PI 235047 (*Bs4* gene); *Capsicum baccatum* var. *pendulum* 1556

(Bs7). A hypersensitive response is observed as a confluent necrosis when leaves are infiltrated with a concentrated bacterial suspension. Growth of the bacterial population is arrested during the development of a hypersensitive response and disease symptoms are not observed (6, 17). The hypersensitive response is controlled according to the gene-for-gene model of resistance in that resistance is controlled by an avirulence gene in the pathogen and a resistance gene in the host (4, 5, 9).

A non hypersensitive response was identified in the breeding line Pep 13 and the accession PI 271322 and is controlled by bs5 and bs6, two recessive genes with additive action. Resistance is observed as yellowing and necrosis of the infiltrated area of the leaf. Growth of the bacteria is reduced during the development of the lesions and no symptoms are observed in resistant plants (10).

As sources of bacterial leaf spot resistance have been identified, back-crossing these sources into the commercial, bacterial leaf spot-susceptible cultivar Early Cal Wonder was carried out for *Bs1*, *Bs2* and *Bs3, bs5, bs6 and Bs7*. Near isogenic lines were developed from Early Cal Wonder which became known as ECW10R, ECW20R, ECW30R, ECW12346R and ECW70R. These differential lines were used to identify races 0 to 5 of the pathogen. *Bs4*, which confers resistance to race 6, was identified in PI 235047. Identification of *Bs4* also allowed for differentiation of four additional races, 7 to 10.

The Bs7 resistance gene allows the identification of recently described races of *X. gardneri* with AvrBs7 gene and *X. euvesicatoria* with the AvrBs1.1 gene (10). The host differential table was developed to identify pepper races based on reactions on the ECW near isogenic lines, and PI 235047 (**Table 1.**).

Guidelines for differentiating races using the pepper differential lines:

Race identification based on the hypersensitive response: Grow the pepper differential lines identified in **Table 1** for 3 to 4 weeks in a greenhouse or growth chamber until the fourth true leaf is fully expanded. Make a 1 to 2 x 10^8 cfu/ml suspension of the appropriate bacterial strain(s) and pressure infiltrate each on the abaxial leaf surface near the midrib. A water-soaked area of leaf tissue 1 to 2 cm in diameter is sufficient. Evaluate reactions 48 to 72 hours after inoculation, depending on environmental conditions. Hypersensitive reactions are indicated by a rapid, necrotic collapse of the infiltrated area and generally are observed before susceptible reactions. Reactions on PI 235047 generally take longer to develop than on the other differentials.

Figure 1. Age or size of seedlings at inoculation

Figure 2. Infiltration of a leaf is accomplished by gently forcing the bacterial suspension into the underside of the leaf using a sterile syringe without a needle.

Figure 3. Resistant reactions can vary in appearance from bleached white with a dark border to uniformly dark brown throughout the infiltrated, collapsed area.

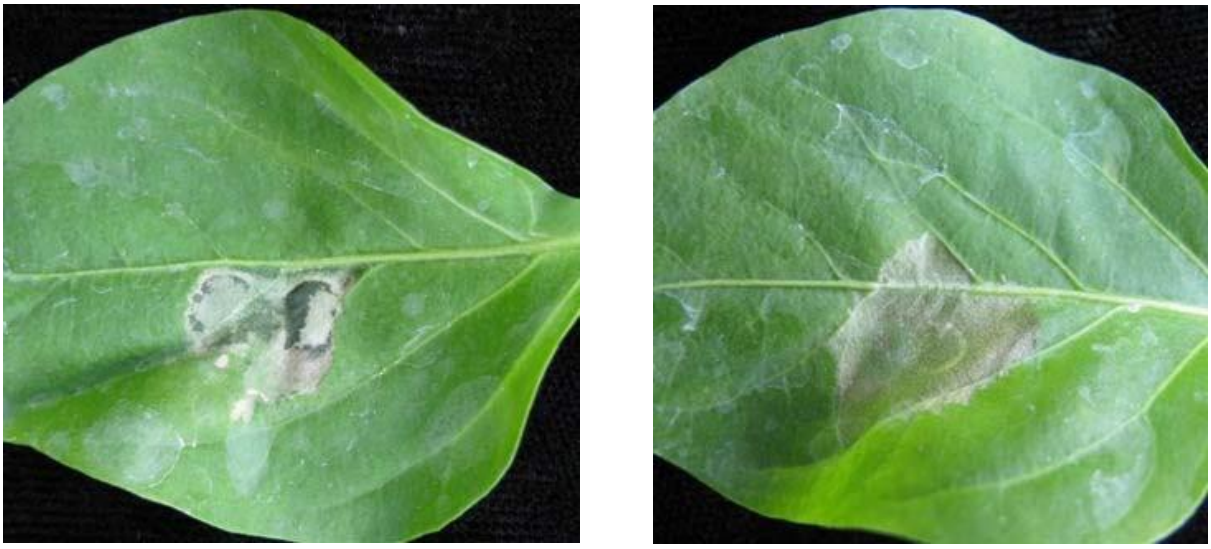

Figure 4. Susceptible reactions manifest 3-5 days after infiltration as chlorotic, water soaked tissue in the infiltrated area.

Race identification based on resistance to the recessive genes bs5 and bs6:

Grow the pepper differential lines listed in table 1 for 3 to 4 weeks in a greenhouse or growth chamber until the fourth true leaf is fully expanded. Make a 1 to 2 x 10^5 cfu/ml suspension of the appropriate bacterial race(s) and pressure infiltrate each on the abaxial leaf surface near the midrib. A water-soaked area of leaf tissue 1 to 2 cm in diameter is sufficient. Incubate for 3 weeks in greenhouse conditions before evaluation. Evaluate reactions 3 weeks after inoculation, depending on environmental conditions, according to the following reading scale:

1 = no disease symptoms (figure5)

2 = slight to moderate yellowing and slight necrosis (figure 6)

3 = extensive yellowing and moderate necrosis (figure 7)

4 = complete necrosis (figure 8)

Figure 5 Severity 1 No disease symptoms

Figure 6 Severity 2 = slight to moderate yellowing and slight necrosis

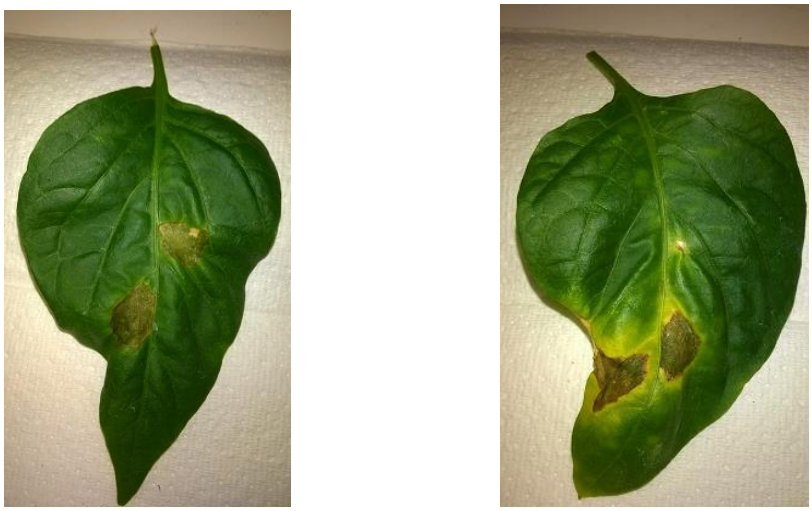

Figure 7. Severity 3 = extensive yellowing and moderate necrosis

Figure 8. Severity 4 = complete necrosis

Table 1. Differentiation of bacterial spot races using known resistance genes in pepper

Race	Functional avirulence gene	ECW No R gene	ECW 10R BS_1 gene	ECW 20R BS_2 gene	ECW 30R BS_3 gene	PI 235047 BS_4 gene	ECW 12346R Bs1, Bs2, Bs3, bs5,bs6 genes
Xcv: 0	*avrBS$_1$, avrBS$_2$, avrBS$_3$,*	S	HR	HR	HR	HR	HR
Xcv: 1	*avrBS$_2$, avrBS$_3$,*	S	S	HR	HR	HR	HR
Xcv: 2	*avrBS$_1$, avrBS$_2$*	S	HR	HR	S	S	HR
Xcv: 3	*avrBS$_2$, avrBS$_4$*	S	S	HR	S	HR	HR
Xcv: 4	*avrBS$_3$, avrBS$_4$*	S	S	S	HR	HR	HR
Xcv: 5	*avrBS$_1$*	S	HR	S	S	S	HR
Xcv: 6	*avrBS$_4$*	S	S	S	S	HR	HR*
Xcv: 7	*avrBS$_2$, avrBS$_3$*	S	S	HR	HR	S	HR
Xcv: 8	*avrBS$_2$*	S	S	HR	S	S	HR
Xcv: 9	*avrBS$_3$*	S	S	S	HR	S	HR
Xcv: 10	unknown	S	S	S	S	S	HR*

ECW = Early Cal Wonder

ECW 10R, ECW 20R, ECW 30R and ECW12346R are near isogenic. ECW 10R, ECW 20R and ECW 30R differ by the presence of the BS1, BS2 and BS3 genes, respectively.

S = Susceptible reaction

HR = High resistance with hypersensitive response

HR* = High resistance without a hypersensitive response

PI 234057 (*Capsicum pubescens*): BS4 gene confers hypersensitive resistance to *Xcv:* 6 and differentiates *Xcv*: 1 from *Xcv*: 7, *Xcv*: 3 from *Xcv*: 8, *Xcv*: 4 from *Xcv*: 9, and *Xcv*: 6 from *Xcv*: 10.

Ordering seeds of differential lines

Seeds of each of the differential lines listed in Table 1 can be ordered from the USDA GRIN (Germplasm Resources Information Network). 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.

To set up an account, go to https://npgsweb.ars-grin.gov/gringlobal/search and select 'New user' at the top of the opening page and follow instructions to create a profile and establish an account.

To order seeds, go to https://npgsweb.ars-grin.gov/gringlobal/search and log in to your USDA GRIN account. Type in 'pepper Bacterial spot differentials' 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.

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.

If you have difficulties ordering seeds, contact us at cppsi@ucdavis.edu for assistance.

Ordering races of bacterial spot races

Reference races of races of the bacterial spot pathogen can be obtained for a service fee by contacting

Dr. David F. Ritchie
Professor
North Carolina State University
Plant Pathology Dept, Thomas Hall
100 Derieux Place, Campus Box 7616
Raleigh, NC 27695-7616 USA

Phone: (919) 515-6809
Fax: (919) 515-7716
E-mail: david_ritchie@ncsu.edu

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: Dr. Phyllis Himmel at pthimmel@ucdavis.edu

Liability waiver

The 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 races of the pepper bacterial spot pathogen and resistance traits of pepper cultivars.

References

1. Burkholder, W. C., and Li, C. C. 1941. Variation in *Phytomonas vesicatoria*. Phytopathology 31:753-755.
2. Canteros, B. I., Minsavage, G. V., Bonas, U., Pring, D., and Stall, R. E. 1991. A gene from *Xanthomonas campestris* pv. *vesicatoria* that determines avirulence in tomato is related to

avrBs3. Mol. Plant-Microbe Interact. 4:628-632.

3. Cook, A. A. 1973. Characterization of hypersensitivity in *Capsicum annuum* induced by the tomato strain of *Xanthomonas vesicatoria*. Phytopathology 63:915-918.
4. Ellingboe, A. H. 1984. Genetics of host-parasite relations: An essay. Pages 131-151 in: Advances in Plant Pathology, Vol. 2, D. D. Ingram and P. H. Williams, eds. Academic Press, New York.
5. Flor, H. H. 1955. Host-parasite interactions in flax rust – Its genetics and other implications. Phytopathology 45:680-685.
6. Hibberd, A. M., Basset, M. J. and Stall, R. E. 1987. Allelism tests of three dominant genes for hypersensitive resistance to bacterial spot of pepper. Phytopathology 77:1304-1307.
7. Jones, C., Conn, K., and Himmel, P., eds. 2006. Bacterial spot. Pages 2-3 in: Pepper Eggplant Disease Guide, Seminis Vegetable Seeds, Oxnard, California.
8. Jones, J. B., Lacey, G. H., Bouzar, H., Stall, R. E., and Schaad, N. W. 2004. Reclassification of the Xanthomonads associated with bacterial spot disease of tomato and pepper. Syst. Appl. Microbiol. 27:755-762.
9. Minsavage, G. V., Dahlbeck, D., Whalen, M. C., Kearney, B., Bonas, U., Staskawicz, B. J., and Stall, R. E. 1990. Gene-for-gene relationships specifying disease resistance in *Xanthomonas campestris* pv. *vesicatoria*–pepper interactions. Mol. Plant-Microbe Interact. 3:41-47.
10. Potnis, N., G. Minsavage, J. K. Smith, J. C. Hurlbert, D. Norman, R. Rodrigues, R. E. Stall, and J. B. Jones. 2012. Avirulence proteins AvrBs7 from *Xanthomonas gardneri* and AvrBs1.1 from *Xanthomonas euvesicatoria* contribute to a novel gene-for-gene Interaction in Pepper. Mol. Plant-Microbe Interact. 25:307-320.
11. Reifschneider, G. J. B., Bongiolo, N. A., and Takatsu, A. 1985. Reappraisal of *Xanthomonas campestris* pv. *vesicatoria* strains – Their terminology and distribution. Fitopatologia Bras. 10:201-204.
12. Ritchie, D. F., and Dittapongpitch, V. 1991. Copper- and streptomycin–resistant strains and host differentiated races of *Xanthomonas campestris* pv. *vesicatoria* in North Carolina. Plant Dis. 75:733-736.
13. Sahin, F., and Miller, S. A. 1998. Resistance in *Capsicum pubescens* to *Xanthomonas campestris* pv. *vesicatoria* pepper race 6. Plant Dis. 82: 794-799.
14. Sowell, G., Jr. 1960. Bacterial spot resistance of introduced peppers. Plant Dis. Rep. 44:587-590.
15. Sowell, G., Jr., and Dempsey, A. H. 1977. Additional sources of resistance to bacterial spot of pepper. Plant Dis. Rep. 61:684-686.
16. Stall, R. E., Jones, J. B., and Minsavage, G. V. 2009. Durability of Resistance in Tomato and Pepper to Xanthomonads Causing Bacterial Spot. Annu. Rev. Phytopathology 47:265-284.
17. Stall, R. E., and Cook, A. A. 1966. Multiplication of *Xanthomonas vesicatoria* and lesion development in resistant and susceptible pepper. Phytopathology 56:1152-1154.
18. Vauterin, L., Hoste, B., Kersters, K., and Swing, J. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45:472-489.