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

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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 strains of *X*. *campestris* pv. *vesicatoria*. In 1995, Vauterin et al. (18) 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 (8). 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 strains, and *X. gardneri* = group D strains. Pepper strains 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* strains are occasionally found to cause disease on pepper. Strains from all four species have been isolated from tomato.

Early work on bacterial leaf spot indicated that strains 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 strains are distinguished on the basis of virulence on tomato and pepper: tomato strains virulent on tomato only, pepper strains virulent on pepper only, and pepper-tomato strains virulent on both crop species (11). Within the pepper and pepper-tomato groups of strains, races of the pathogen can be distinguished by the reaction of various pepper lines.

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 an HR, 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 gene). An HR 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 an HR and disease symptoms are not observed (6, 17). The HR is controlled according to the gene-for-gene model of resistance: resistance is controlled by an avirulence gene in the pathogen and a resistance gene in the host (4, 5, 9).

Recessive resistance 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. Sometimes with *bs5* and often with *bs6* 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 *Capsicum pubescens* PI 235047. Identification of *Bs4* also allowed for differentiation of four additional races, 7 to 10.

Bs7 resistance gene allows the identification of recently described strains of X. gardneri with AvrBs7 gene and *X euvesicatoria* with the AvrBs1.1 gene (10). Further studies on these strain characterizations are underway.

A host differential table was developed to identify races from pepper 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 HR

Grow the pepper differential lines identified in **Table 1** for 3 to 4 weeks in a greenhouse or growth chamber at 28ºC 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 24 to 48 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 are visible. Reactions on ECW30R and 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.

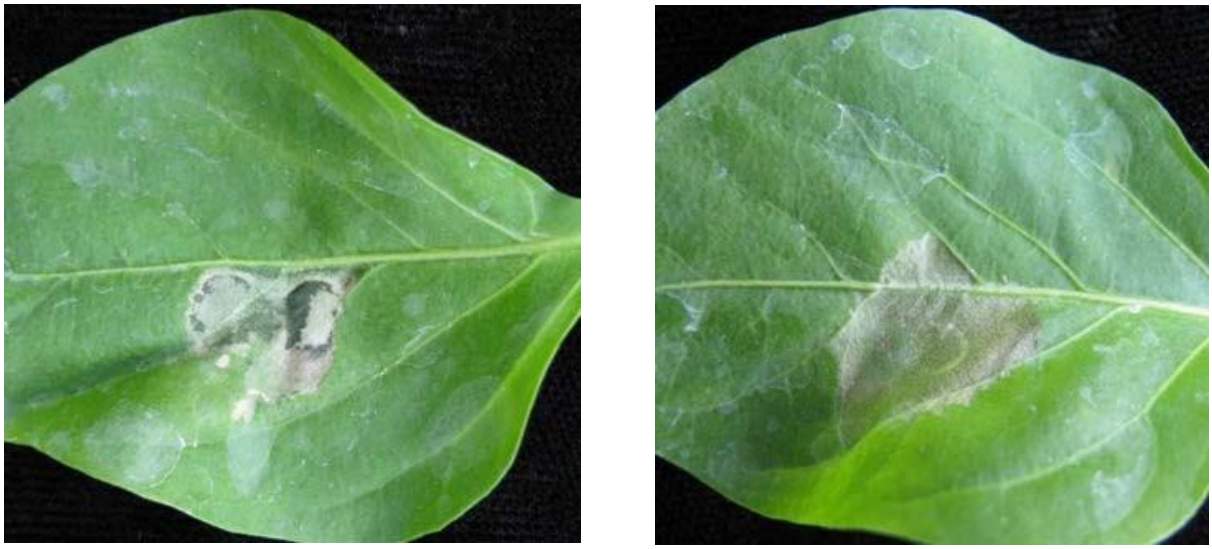

Figure 4. Susceptible reactions manifest 3-5 days after infiltration as chlorotic, water soaked tissue in the infiltrated area.

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

Grow the pepper differential lines 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 10^5 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. 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 (figure 8)

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
0	*avrBS1, avrBS2, avrBS3,*	S	HR	HR	HR	HR	HR
1	*avrBS2, avrBS3,*	S	S	HR	HR	HR	HR
2	*avrBS1, avrBS2*	S	HR	HR	S	S	HR
3	*avrBS2, avrBS4*	S	S	HR	S	HR	HR
4	*avrBS3, avrBS4*	S	S	S	HR	HR	HR
5	*avrBS1*	S	HR	S	S	S	HR
6	*avrBS4*	S	S	S	S	HR	R
7	*avrBS2, avrBS3*	S	S	HR	HR	S	HR
8	*avrBS2*	S	S	HR	S	S	HR
9	*avrBS3*	S	S	S	HR	S	HR
10	unknown	S	S	S	S	S	R

ECW = Early Cal Wonder

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

S = Susceptible reaction

HR = Hypersensitive-resistant reaction

R = resistant reaction non hypersensitive

PI 234057 (*Capsicum pubescens*): BS4 gene confers hypersensitive resistance to *Xcv*P6 and differentiates *Xcv*P1 from *Xcv*P7, *Xcv*P3 from *Xcv*P8, *Xcv*P4 from *Xcv*P9 and *Xcv*P6 from *Xcv*P10.

ECW70 R with AvrBs7 gene develop HR reaction when inoculated with X garderi with AvrBs7 gene and X euvesicatoria with Avr Bs1.1 gene.

Ordering seeds of pepper differential lines

Seeds of each of the differentials listed in Table 1 can be ordered from the USDA GRIN (Germplasm Resources Information Network) website of the USDA National Plant Germplasm System (NPGS) at:

Pepper Differential Set Accessions

Ordering strains of bacterial spot races

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

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