Identification of Isolates that Cause a Leaf Spot Disease of Brassicas as *Xanthomonas campestris* pv. *raphani* and Pathogenic and Genetic Comparison with Related Pathovars

J. G. Vicente, B. Everett, and S. J. Roberts

First and second authors: Warwick HRI, The University of Warwick, Wellesbourne, Warwick CV35 9EF, UK; and current address of third author: Plant Health Solutions, 20 Beauchamp Road, Warwick CV34 5NU, UK.

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ABSTRACT

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Twenty-five *Xanthomonas* isolates, including some isolates received as either *X. campestris* pv. *armoraciae* or pv. *raphani*, caused discrete leaf spot symptoms when spray-inoculated onto at least one *Brassica oleracea* cultivar. Twelve of these isolates and four other *Xanthomonas* isolates were spray- and pin-inoculated onto 21 different plant species/cultivars including horseradish (*Armoracia rusticana*), radish (*Raphanus sativus*), and tomato (*Lycopersicon esculentum*). The remaining 13 leaf spot isolates were spray-inoculated onto a subset of 10 plant species/cultivars. The leaf spot isolates were very aggressive on several *Brassica* spp., radish, and tomato causing leaf spots and dark sunken lesions on the middle vein, petiole, and stem. Based on the differential reactions of

several *Brassica* spp. and radish cultivars, the leaf spot isolates were divided into three races, with races 1 and 3 predominating. A differential series was established to determine the race-type of isolates and a gene-for-gene model based on the interaction of two avirulence genes in the pathogen races and two matching resistance genes in the differential hosts is proposed. Repetitive-DNA polymerase chain reaction-based fingerprinting was used to assess the genetic diversity of the leaf spot isolates and isolates of closely related *Xanthomonas* pathovars. Although there was variability within each race, the leaf spot isolates were clustered separately from the *X. campestris* pv. *campestris* isolates. We propose that *X. campestris* isolates that cause a nonvascular leaf spot disease on *Brassica* spp. should be identified as pv. *raphani* and not pv. *armoraciae*. Race-type strains and a neopathotype strain for *X. campestris* pv. *raphani* are proposed.

Additional keywords: black rot, disease resistance, pathogen variants, races.

Leaf spot diseases caused by *Xanthomonas* spp. have been reported to cause severe damage in a number of crops belonging to the family *Brassicaceae* in different countries, including the United States (31), Japan (22), and possibly India and Turkey (3). These diseases have usually been attributed to either *X. campestris* pv. *armoraciae* (McCulloch) Dye or *X. campestris* pv. *raphani* (White) Dye. *X. campestris* pv. *armoraciae* (*Bacterium campestre* var. *armoraciae*) was first described by McCulloch (14) as being the causal agent of a nonvascular leaf spot disease of horseradish (*Armoracia rusticana*): this organism was reported to be only weakly pathogenic on cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*) (causing spots only on leaves, but no lesions on stems and petioles), stock (*Matthiola incana*), and lima beans (*Phaseolus* sp.), and was not pathogenic on kale (*B. oleracea* var. *acephala*), mustard (*Brassica* sp.), radish (*Raphanus sativus*), candytuft (*Iberis* sp.), wild cress (*Cardamine* sp.), *Nasturtium*, squash (*Cucurbita* sp.), poppy (*Papaver* sp.), cotton (*Gossypium hirsutum*), navy bean (*P. vulgaris*), and cowpea (*Vigna unguiculata*) (14). *X. campestris* pv. *raphani* (*Bacterium vesicatorium* var. *raphani*) was first described by White (28) as the cause of a bacterial spot disease of radish and turnip (*B. rapa*): this organism was reported to be pathogenic on cabbage, cauliflower, kale, Brussels sprouts (*B. oleracea* var.

gemmifera), mustard, tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum*), and tobacco (*Nicotiana tabacum*), and was not pathogenic on horseradish, garden cress (*Lepidium sativum*), *Nasturtium*, shepherd's purse (*Capsella bursa-pastoris*), bean (*P. vulgaris*), soybean (*Glycine max*), and garden huckleberry (*Physalis* sp.). All cultivars of radish, mustard and *B. oleracea* that were tested by White (28) were susceptible to this pathogen.

Serological, genetic and pathogenicity studies by Black and Machmud (2) and Alvarez et al. (1) did not support the separation of *X. campestris* pv. *armoraciae* from pv. *raphani*; these authors considered the names synonymous (with the designation *armoraciae* having priority over *raphani*). In contrast, White (28) and Tamura et al. (22) found differences between the two pathovars with *X. campestris* pv. *raphani* having a much larger host range that included radish and tomato, but not horseradish. Sahin and Miller (21) attributed a leaf spot disease of radish to a new pathotype of *X. campestris* pv. *armoraciae*; these isolates caused spots on several hosts including cabbage, kale, radish, and horseradish, but not on tomato and pepper.

According to the reclassification of the genus *Xanthomonas* based on DNA-DNA hybridization (24), the species *X. campestris* includes four other pathovars that can cause disease in cruciferous plants (pvs. *campestris*, *aberrans*, *barbareae*, and *incanae*). *X. campestris* pv. *campestris* (Pammel) Dowson causes black rot of brassicas, possibly the most important disease of crucifers worldwide (29). This pathovar and *X. campestris* pv. *aberrans* (Knösel) Dye have also been reported to cause leaf spots in brassicas (3,31), but the symptoms generally progress to include typical V-shape lesions and darkened veins caused by the vascular movement of the pathogen. For this reason, an isolate previously identified as *X. campestris* pv. *aberrans* has been included in

Corresponding author: J. G. Vicente; E-mail address: joana.vicente@warwick.ac.uk

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X. campestris pv. *campestris* (27). Recently, Vicente et al. (27) reported that isolates from ornamental crucifers, stock (*M. incanae*), wallflower (*Cheiranthus cheiri*), and candytuft (previously included in *X. campestris* pv. *incanae* (Kendrick & Baker) Dye, *X. campestris* pv. *campestris* and *X. campestris* pv. *armoraciae*, respectively), caused vascular diseases and showed pathovar-like specificity to their hosts of origin. In addition to distinctions based on host range (pathovars), several *Xanthomonas* species or pathovars can be further differentiated into races based on their interactions with differential cultivars. Thus, six races were proposed for *X. campestris* pv. *campestris* based on the response of certain *Brassica* species and cultivars (7,27). *X. campestris* pv. *vesicatoria* has been divided into two species, *X. axonopodis* pv. *vesicatoria* and *X. vesicatoria* (24), with different races (6).

Repetitive DNA-polymerase chain reaction (PCR)-based genomic fingerprinting (rep-PCR) with REP (targeting the repetitive extragenic palindromic sequence), ERIC (targeting the enterobacterial repetitive intergenic consensus), and BOX (targeting the DNA sequences of the BOXA subunit of the BOX element of *Streptococcus pneumoniae*) primers is a rapid and useful technique to assess the bacterial diversity at the species, pathovar, and isolate level (11). Rademaker et al. (16,18) have shown that rep-PCR can separate a number of *Xanthomonas* species and pathovars and that the results are highly correlated with results obtained in DNA-DNA homology studies and therefore seem to reflect their phylogenetic relationships.

In this paper, we present the results of work done to clarify the taxonomy of *Xanthomonas* isolates that cause leaf spots on bras-

sicas and other hosts. We also present information on the occurrence of pathogenic variants (races), describe a differential series for the determination of races of *X. campestris* leaf spot isolates, and propose a gene-for-gene model to explain the interaction of races and differential cultivars. In addition, the genetic variability of *X. campestris* leaf spot isolates together with representatives of *X. campestris* pv. *campestris* and closely related *Xanthomonas* pathovars was studied using rep-PCR.

MATERIALS AND METHODS

Bacterial isolates. The isolates used in this study are listed in Table 1. Isolates originally identified as either *X. campestris* pvs. *armoraciae* or *raphani* were mainly obtained from researchers and seed companies from different countries. In addition, two isolates were obtained from commercial seed lots, as part of studies done at Warwick HRI, Wellesbourne (20). Isolates were initially considered to be *X. campestris* on the basis of colony characteristics on yeast dextrose chalk (YDC) agar (30) and/or King's medium B (KB) (8) and agglutination tests (12). The isolate HRI 6489 was similar to other *X. campestris* isolates except for lack of yellow pigmentation (15). For comparative purpose, representative isolates of the six races of *X. campestris* pv. *campestris* and of other closely related *X. campestris* pathovars from cruciferous hosts from the Warwick HRI culture collection (27) and representative *X. vesicatoria* and *X. axonopodis* pv. *vesicatoria* isolates (10) were also included (Table 1).

For long-term storage, bacterial growth was suspended in a liquid medium containing nutrient broth (8 g/liter) (Difco Labora-

TABLE 1. Source and origin of isolates used in this study

HRI isolate number (previous designation)	Source (reference) ^a	Host	Infected material	Geographical origin	Year of isolation
<i>Xanthomonas campestris</i> pv. <i>raphani</i> isolates (pathogenic on Savoy cabbage cv. Wirosa F ₁ and tomato cv. Moneymaker)					
RACE 1					
6489 (B-122)	M. Asma, ex. R. Morrison (15)			Arizona, U.S.	1984
6490 (P5034) ^b	M. Asma	<i>Brassica oleracea</i> var. <i>botrytis</i>	Seed	France	1995
6491 (P5043)	M. Asma	<i>B. oleracea</i> var. <i>botrytis</i>	Seed	France	1995
6493 (P5067)	M. Asma	<i>B. oleracea</i> var. <i>botrytis</i>	Seed	France	1995
6496 (A4641)	M. Asma, ex. A. Alvarez				
7974	Warwick HRI (20)	<i>B. oleracea</i> var. <i>capitata</i>	Seed	Southern Hemisphere	2000
7979	Warwick HRI (20)	<i>B. oleracea</i> var. <i>capitata</i>	Seed	Southern Hemisphere	2000
8079 (071)	H. Bouzar				
8297 (62-9)	D. Cuppels, ex. R. Stall (10)	<i>Raphanus sativus</i>			
8298 (0-71)	D. Cuppels, ex. W. Wiebe (10)	<i>Raphanus sativus</i>		Minnesota, U.S.	
RACE 2					
6497 (P5118)	M. Asma				1997
6518 (TMR61)	K. Tamura (22)	<i>B. rapa</i>	Leaves	Aichi, Japan	1986
6520 (TMR137)	K. Tamura (22)	<i>B. rapa</i> var. <i>pekinensis</i>	Leaves	Nagano, Japan	1986
8305 (BR-25) ^b	J. Damicone (31)	<i>B. rapa</i> var. <i>perviridis</i>	Leaves	Oklahoma, U.S.	1995
RACE 3					
6492 (P5056)	M. Asma	<i>B. oleracea</i> var. <i>capitata</i>	Seed	France	1995
6494 (P5082)	M. Asma	<i>B. oleracea</i> var. <i>capitata</i>	Seed	France	1995
6498 (P5155)	M. Asma		Plant		1998
6499 (P5159)	M. Asma		Plant		1998
6519 (TMR74) ^{b,c}	K. Tamura (22)	<i>Raphanus sativus</i>	Leaves	Shizuoka, Japan	1985
8299 (DC 91-2)	D. Cuppels (10)	<i>Lycopersicon esculentum</i>	Plant	Ontario, Canada	
8303 (BR-11)	J. Damicone (31)	<i>B. rapa</i> var. <i>utilis</i>	Leaves	Oklahoma, U.S.	1994
8304 (BR-16)	J. Damicone (31)	<i>B. rapa</i> var. <i>utilis</i>	Leaves	Oklahoma, U.S.	1995
8307 (XLS 6)	J. Damicone, ex. L. L. Black (31)	<i>B. oleracea</i> var. <i>capitata</i>		North Carolina, U.S.	
8308 (756)	J. Damicone, ex. D. Gabriel (31)	<i>B. oleracea</i> var. <i>capitata</i>	Seed	E. Asia	
8309 (3145)	J. Damicone, ex. Stall (31)			Florida, U.S.	

(Continued on next page)

^a Warwick HRI, Wellesbourne, U.K.; NCPPB, National Collection of Plant Pathogenic Bacteria, Sand Hutton, U.K.

^b Proposed race-type strains of *X. campestris* pv. *raphani*.

^c Proposed neopathotype strain of *X. campestris* pv. *raphani*.

^d Race-type strains of *X. campestris* pv. *campestris*.

^e NCPPB type strains of *Xanthomonas* species/pathovars.

tories, Detroit) and glycerol (150 ml/liter) and maintained on glass beads at -76°C (4).

Pathogenicity to *B. oleracea*. The cultivars and accessions used in this study are listed in Table 2. Plants of Savoy cabbage cv. Wirosa F₁ and cauliflower cv. Miracle F₁ were used for initial pathogenicity tests done by spray inoculation. Two plants of each cultivar were tested per isolate. Several re-isolations were performed to confirm the presence of bacteria in association with disease symptoms (leaf spots, vein and stem sunken lesions).

Host range. Twenty-one accessions/cultivars including hosts previously used in host range studies of *X. campestris* pathovars and races (27) (Table 2) were used to determine the host range of 12 isolates that caused leaf spots in the initial pathogenicity tests (HRI 6489, 6490, 6492, 6496, 6497, 6518, 7979, 8079, 8299, 8304, and 8305), two NCPPB isolates received as *X. campestris* pv. *armoraciae* (HRI 6375 and 6376), two *X. campestris* pv. *campestris* isolates (HRI 1279A and 3880), and an *X. vesicatoria* isolate (HRI 8300) by both spray inoculation and pin inoculation. Two to three plants of each accession were tested with each method except for horseradish (*A. rusticana*), cucumber (*Cucumis sativus*), pumpkin (*Cucurbita maxima*), and tobacco (*N. tabacum*) where only one plant was tested with each method.

A subset of 10 plant accessions (Table 2) including a selection of six *Brassica* cultivars, two radish cultivars, one wallflower cultivar, and one tomato cultivar was used to characterize a further 13 isolates that caused leaf spots, together with two isolates of *X. campestris* pv. *campestris* (HRI 3811 and 3883), two isolates of *X. vesicatoria* (HRI 5235 and 8302), and one isolate of *X. axonopodis* pv. *vesicatoria* (HRI 8301) by spray inoculation. One plant of each inbred line, F₁ hybrid, and self-fertilizing accession and two plants of each open pollinated accession were tested with each isolate.

Inoculations. Plants were raised from seed sown in 7 cm square plastic pots filled with Levington M2 compost (Scotts) and grown in a glasshouse with a minimum temperature of 20/15°C (day/night), venting at 22/17°C (day/night), and supplementary lighting from October to March to give 16 h days. Plants were inoculated approximately 4 weeks after sowing. Isolates were grown on KB at 30°C for 48 h before inoculation.

For spray inoculations, inoculum was prepared by suspending the bacteria in sterile tap water (10⁸ to 10⁹ CFU/ml). Bacterial suspensions were sprayed onto the plants including the upper and the under surface of leaves with a DeVilbiss Atomizer 15 (DeVilbiss Health Care, Inc., Somerset, PA) connected to an electrical pump. Plants were sprayed gently, until wet, by holding

TABLE 1. (Continued from preceding page)

HRI isolate number (previous designation)	Source (reference) ^a	Host	Infected material	Geographical origin	Year of isolation
<i>Xanthomonas campestris</i> pv. <i>campestris</i> isolates (black rot symptoms on Savoy cabbage cv. Wirosa F ₁ and one or more <i>Brassica</i> differentials) (27)					
RACE 1					
3811 (PHW1205) ^d	Warwick HRI (27)	<i>B. oleracea</i>		U.S.	
3818A	Warwick HRI (27)	<i>B. oleracea</i> var. <i>botrytis</i>	Leaf	Cornwall, U.K.	1994
6595	Warwick HRI (27)	<i>B. oleracea</i> var. <i>acephala</i>	Leaf	Lourinhã, Portugal	1997
RACE 2					
3849A ^d (2D520)	Warwick HRI (27)	<i>B. oleracea</i>	Seed	U.S.	
RACE 3					
3851A	Warwick HRI (27)	<i>B. oleracea</i> var. <i>botrytis</i>	Leaf	Greece	1989
5212 ^{d,e} (NCPPB 528)	Warwick HRI (27)	<i>B. oleracea</i> var. <i>gemma</i>		U.K.	1957
6412	Warwick HRI (27)	<i>B. oleracea</i> var. <i>botrytis</i>		France	1997
RACE 4					
1279A ^d	Warwick HRI (27)	<i>B. oleracea</i> var. <i>capitata</i>	Leaf	Cornwall, U.K.	1984
6189	Warwick HRI (27)	<i>B. oleracea</i> var. <i>italica</i>	Leaf	Muge, Portugal	1996
6312A	Warwick HRI (27)	<i>B. oleracea</i> var. <i>botrytis</i>	Leaf	California, U.S.	
RACE 5					
3880 (NCPPB 2986) ^{d,e}	Warwick HRI (27)	<i>B. oleracea</i> var. <i>capitata</i>		Australia	1975
3883 (NCPPB 1946) ^e	Warwick HRI (27)	<i>Raphanus sativus</i>		U.S.	1966
6382	Warwick HRI (27)	<i>B. rapa</i>		Canada	1953
RACE 6					
6181 ^d	Warwick HRI (27)	<i>B. rapa</i>	Leaf	Sardoal, Portugal	1996
6185	Warwick HRI (27)	<i>B. rapa</i>	Leaf	Castelo Branco, Portugal	1996
Other <i>Xanthomonas</i> isolates (not pathogenic on Savoy cabbage cv. Wirosa F ₁ nor on <i>Brassica</i> differentials) (27).					
Received as <i>X. campestris</i> pv. <i>campestris</i> (pathogenic on wallflower; new pathovar)					
3777A	Warwick HRI	<i>Cheiranthus cheiri</i>	Leaf	U.K.	1994
5219A (NCPPB 2517)	NCPPB, ex. L. A. E. Baker	<i>Cheiranthus cheiri</i>		U.K.	1970
Received as <i>X. campestris</i> pv. <i>armoraciae</i> (pathogenic on <i>Iberis</i> sp.; new pathovar)					
6375 (NCPPB 347) ^e	NCPPB, ex. G. B. Wallace	<i>Iberis</i> sp.		Tanzania	1954
Received as <i>X. campestris</i> pv. <i>armoraciae</i> (but not pathogenic or very weak on horseradish)					
6376 (NCPPB 1930)	NCPPB, ex. W. H. Burkholder	<i>Armoracia rusticana</i>			1939
<i>X. campestris</i> pv. <i>incanae</i> (pathogenic on stock)					
6377 (NCPPB 937) ^e	NCPPB, ex. C. E. Williamson	<i>Matthiola</i> sp.		U.S.	1950
6378 (NCPPB 1934)	NCPPB, ex. K. F. Baker	<i>Matthiola incana</i>		U.S.	1949
<i>X. vesicatoria</i>					
5235 (NCPPB 422) ^e	NCPPB, ex. D. W. Dye	<i>Lycopersicon esculentum</i>		New Zealand	1955
8300 (DC92-13)	D. Cuppels (10)	<i>Lycopersicon esculentum</i>		Ontario, Canada	
8302 (BA29-1)	D. Cuppels, ex. A. Alipi (10)	<i>Lycopersicon esculentum</i>		Argentina	
<i>X. axonopodis</i> pv. <i>vesicatoria</i>					
8301 (75-3)	D. Cuppels, ex. R. Stall (10)	<i>Lycopersicon esculentum</i>		Florida, U.S.	

the spray nozzle at a distance of 10 to 15 cm. In addition, the spray nozzle was held close to the under surface of a leaf in each plant to force the bacterial suspension into the tissues in two small areas (0.5 cm diameter). After inoculation, plants were kept at 20 ± 1°C, under conditions of high humidity, in cabinets with controlled mist spray equipment. Plants were kept in the mist cabinets for 48 h in the preliminary test and for 24 h in all subsequent experiments before returning to normal glasshouse conditions.

For pin inoculations, the middle vein and petiole of the three youngest expanded leaves were inoculated by stabbing in three to five places with a sterile entomological mounting pin charged with bacterial growth from a plate. Symptoms were recorded 7 and 14 days after inoculation.

rep-PCR. Isolates were grown on KB at 30°C for 24 h and the growth was used to prepare 3 ml of suspension containing 10⁸ to 10⁹ cells/ml. Cells from 1.5 ml of these suspensions were harvested by centrifugation for 10 min at 13,000 rpm (12,600 × g) in a microcentrifuge (MSE Microcentaur, Loughborough, UK). DNA was extracted using the Qiagen DNeasy tissue extraction kit (Qiagen Ltd., West Sussex, UK) according to the manufacturer's protocol for extraction of DNA from bacteria and animal tissues. DNA concentration was estimated after running samples on an agarose gel alongside known amounts of Lambda DNA. Primer sequences corresponding to REP1R (5'-IIICGICGICATCIGGC-3'), REP2I (5'-ICGICTTATCIGGCCTAC-3'), ERIC1R (5'-ATG-TAAGTCTCTGGGGATTCAC-3'), ERIC2 (5'-AAGTAAGTGA-CTGGGGTGAGCG-3'), and BOXA1R (5'-CTACGGCAAGGC-GACGCTGACG-3') (25,26) were synthesized by Operon Technologies, Inc. (Alameda, CA).

rep-PCR was carried out as described in Rademaker et al. (17) with minor modifications. PCR analyses were carried out in a total volume of 20 µl containing 1× Gitschier buffer (9), 3.5 mM MgCl₂ for REP and 1.75 mM for ERIC and BOX, 2 µl of dimethylsulfoxide (DMSO) (FULKA, Dorset, UK), 3.2 µg of bovine serum albumin (BSA) (Roche, Mannheim, Germany), 40 pmol of each primer (two primers for REP and ERIC and one

primer for BOX), 1.25 mM of each of four deoxynucleoside triphosphates, 1.6 unit of *Taq* DNA polymerase (Invitrogen), and approximately 40 ng of template genomic DNA. PCR amplification was performed in a Genius (Techne (Cambridge) Limited, Duxford, Cambridge, UK) thermocycler with the following program: one cycle at 95°C for 2 min; 30 cycles of denaturation at 94°C for 3 s and 92°C for 30 s, annealing at 40, 52, or 53°C for 1 min with REP, ERIC, and BOX primers, respectively, extension at 65°C for 8 min; single extension at 65°C for 8 min; and finally held at 4°C. Three PCR reactions were carried out with each set of primers and two control isolates (HRI 3811 and HRI 6519) were included in every run.

Aliquots of amplified REP- (7.5 µl), ERIC- (2.5 µl), and BOX-PCR (2.5 µl) products of each strain (and of blank controls) were analyzed by electrophoresis in a 1.2% agarose gel with ethidium bromide at 0.5 µg/ml in 0.5× Tris-borate-EDTA buffer for 8 h at 60V (approximately 1.8 V/cm) and photographed under UV light. Three 22-lane gels were run for each set of primers. A molecular mass marker (1-kb-plus DNA ladder, Invitrogen) was loaded into both edge and middle lanes of each gel.

The rep-PCR fingerprint profiles were used to measure the genetic similarity between strains. A digital image of each gel was analyzed using the Fingerprinting II software (Bio-Rad Laboratories, Hercules, CA). The disk background subtraction method was applied. Gels were normalized using the standards loaded into the edge and middle lanes of each gel. Cluster analysis of the pairwise similarity values was performed using the Dice similarity coefficient and unweighted pair-group method with arithmetic means (UPGMA) clustering technique. The results of REP-, ERIC-, and BOX-PCR genomic fingerprinting were combined using the Fingerprinting II software.

RESULTS

Pathogenicity of isolates on *B. oleracea*. In initial spray inoculations of Savoy cabbage cv. Wirosa F₁ and cauliflower cv. Miracle F₁, 25 isolates from brassicas, radish, and tomato, re-

TABLE 2. Origin of 21 plant accessions used for characterization of the host range and their reaction to the *Xanthomonas campestris* pv. *raphani* isolates^a tested in this study

Species/variety	Cultivar or accession	Type	Origin	Reaction
<i>Brassica</i> spp.				
Savoy cabbage (<i>B. oleracea</i> var. <i>sabauda</i>)	Wirosa F ₁ ^b	Hybrid	Bejo Zaden NV, Netherlands	Very susceptible (control)
Ethiopian mustard (<i>B. carinata</i>)	Selection of PI 199947 ^b	Inbred line	Warwick HRI; ex. Ethiopia	Very susceptible
Oilseed rape (<i>B. napus</i> var. <i>oleifera</i>)	Line 14R of Cobra ^b	Inbred line	Warwick HRI, U.K.	Resistant to race 1
Mustard (<i>B. juncea</i>)	Florida Broad Leaf Mustard ^b	Open-pollinated	Otis S. Twilley Seed Co., U.S.	Resistant to race 1
Turnip (<i>B. rapa</i> var. <i>rapifera</i>)	Just Right Hybrid Turnip ^b	Hybrid	Otis S. Twilley Seed Co., U.S.	Resistant to race 1
Turnip (<i>B. rapa</i> var. <i>rapifera</i>)	Seven Top Turnip	Open-pollinated	Otis S. Twilley Seed Co., U.S.	Variable; resistant to race 1
Cauliflower (<i>B. oleracea</i> var. <i>botrytis</i>)	Miracle F ₁ ^b	Hybrid	Bejo Zaden NV, Netherlands	Resistant to race 2
Radish and horseradish				
Radish (<i>Raphanus sativus</i>)	French Breakfast 3 ^b	Open-pollinated	Suttons Seeds, U.K.	Very susceptible
Radish (Mooli) (<i>R. sativus</i>)	Mino Early ^b	Open-pollinated	Suttons Seeds, U.K.	Resistant to race 2
Horseradish (<i>Armoracia rusticana</i>)	—	Vegetative propagation	Garden centre, U.K.	Weakly susceptible
Ornamental crucifers				
Hyacinth flowered candytuft (<i>Iberis</i> sp.)	White Empress	Open-pollinated	Unwins Seeds, U.K.	Variable
Perennial candytuft (<i>Iberis sempervivens</i>)	Snowflake	Open-pollinated	Unwins Seeds, U.K.	Variable
Stock (<i>Matthiola incana</i>)	Brompton Mixed colours	Open-pollinated	Suttons Seeds, U.K.	Resistant to all races
Stock (<i>M. incana</i>)	Heaven Scent	Open-pollinated	Suttons Seeds, U.K.	Resistant to all races
Wallflower (<i>Cheiranthus cheiri</i>)	Dwarf/Prince Primrose	Open-pollinated	Suttons Seeds, U.K.	Resistant to race 1
	Yellow			
Wallflower (<i>C. cheiri</i>)	Cloth of Gold ^b	Open-pollinated	Suttons Seeds, U.K.	Resistant to race 1
Others				
Tomato (<i>Lycopersicon esculentum</i>)	Moneymaker ^b	Self- or open-pollinated	Suttons Seeds, U.K.	Very susceptible
Pumpkin (<i>Cucurbita maxima</i>)	F ₁ Becky	Hybrid	Suttons Seeds, U.K.	Susceptible
Cucumber (<i>Cucumis sativus</i>)	Telegraph Improved	Open-pollinated	Suttons Seeds, U.K.	Susceptible
Tobacco (<i>Nicotiana tabacum</i>)	White Burley	Self-pollinated	Warwick HRI, U.K.	Susceptible
Pepper (<i>Capsicum annuum</i>)	Worldbeater	Self- or open-pollinated	Suttons Seeds, U.K.	Resistant to race 2

^a Twelve *X. campestris* pv. *raphani* isolates (HRI 6489, 6490, 6492, 6496, 6497, 6499, 6518, 7979, 8079, 8299, 8304, and 8305) were inoculated in all accessions. Other isolates were inoculated in a subset of 10 accessions.

^b Subset of 10 accessions used to study the host range of *X. campestris* pv. *raphani* isolates.

ceived from different researchers/seed companies as *X. campestris* pv. *armoraciae* or *X. campestris* pv. *raphani* or isolated at Warwick HRI, gave symptoms generally typical of the leaf spot disease described by White (28) as caused by *X. campestris* pv. *raphani*: circular dark spots were visible less than 1 week after inoculation; the spots later became light brown or gray, sometimes surrounded by a water-soaked halo (Fig. 1, left). In severely affected leaves, the spots coalesced and became irregular, but were not limited by the veins. Most isolates also produced black, sunken, elongated lesions on the middle vein, petiole, and/or stem in one or both cultivars (Fig. 1, left). In some cases, the petioles became bent and young leaves were distorted. Twenty-one of the isolates produced leaf spots on both cultivars, but four isolates (HRI 6497, 6518, 6520, and 8305) produced leaf spots only on cv. Wirosa F₁. The presence of *X. campestris* was confirmed after re-isolation from lesions; these isolates produced similar symptoms when re-inoculated into Wirosa F₁ and Miracle F₁ (data not shown).

Representative isolates of the six *X. campestris* pv. *campestris* races (race 1, HRI 3811; race 2, HRI 3849A; race 3, HRI 5212; race 4, HRI 1279A; race 5, HRI 3880 and 3883; and race 6, HRI 6181) were also spray-inoculated onto cvs. Wirosa F₁ and Miracle F₁. Some of these isolates initially produced small water-soaked dark spots and chlorosis, but the symptoms later progressed toward typical V-shape yellow lesions (Fig. 1, right). All *X. campestris* pv. *campestris* isolates produced black rot symptoms on cv. Wirosa F₁, except the race 2 isolate. The isolates of races 3, 5, and 6 did not produce any black rot symptoms on cv. Miracle F₁. Two isolates received from the National Collection of Plant Pathogenic Bacteria (NCPBB), York, UK, as *X. campestris* pv. *armoraciae* (HRI 6375 and 6376) and isolates of *X. vesicatoria*

(HRI 5275, 8300, and 8302) and *X. axonopodis* pv. *vesicatoria* (HRI 8301) were not pathogenic when spray-inoculated onto cvs. Wirosa F₁ and Miracle F₁.

Host range. In spray inoculations with the leaf spot isolates, hosts that were considered susceptible developed leaf spots and frequently showed dark sunken lesions on the middle vein, petiole, and/or stem (Fig. 2, left). In pin inoculations, these same hosts developed dark sunken lesions around the inoculation points (Fig. 2, center). The results are summarized in Table 2. Plants of *B. oleracea* cv. Wirosa F₁, a selection of *B. carinata* accession PI 199947, radish cv. French Breakfast 3 (Fig. 3), and tomato cv. Moneymaker (Fig. 4, left) were very susceptible to the 12 leaf spot isolates tested. Large numbers of spots and sunken lesions were observed in the leaves, petioles, and stems of spray-inoculated plants of these hosts. The *B. napus* cv. Cobra line 14R, *B. rapa* cv. Just Right Hybrid Turnip, *B. juncea* cv. Florida Broad Leaf Mustard, and the two wallflower cultivars were resistant to five leaf spot isolates (HRI 6489, 6490, 6496, 7979, and 8079), while *B. oleracea* cv. Miracle F₁ and radish cv. Mino Early were resistant to three leaf spot isolates (HRI 6497, 6518, and 8305) (Fig. 2, right). The *B. rapa* cv. Seven Top Turnip and the candy-tuft cultivars were mixtures and included both resistant and susceptible individuals when inoculated with the same isolate (Table 2). The two stock cultivars were resistant to all 12 leaf spot isolates tested. The pepper cv. Worldbeater was resistant to three isolates (HRI 6497, 6518, and 8305) and was susceptible to the other isolates (Fig. 4, center). The pumpkin cv. Becky F₁, cucumber cv. Early Telegraph, and tobacco cv. White Burley (Fig. 4, right) were susceptible to the 12 leaf spot isolates, although generally these isolates did not seem to be very aggressive on these hosts. The horseradish plants that were sprayed showed several

Fig. 1. Savoy cabbage cv. Wirosa F₁ leaves (abaxial side) spray-inoculated with *Xanthomonas campestris* pv. *raphani* isolate 8305 (left, 2 weeks after inoculation) and *X. campestris* pv. *campestris* isolate 3883 (right, 3 weeks after inoculation).

spots and the pin-inoculated plants had small lesions around the inoculation points, but the symptoms were generally weak.

The two *X. campestris* pv. *campestris* isolates (HRI 1279A and 3880) caused dark spots and chlorosis that progressed to typical black rot symptoms on *B. oleracea* cv. Wiroso F₁ and on radish cv. French Breakfast 3. The race 4 isolate (HRI 1279A) was not pathogenic on *B. rapa* cv. Just Right Turnip, *B. napus* line 14R of Cobra, *B. juncea* cv. Florida Broad Leaf Mustard, and *B. carinata* selection of PI 199947. The race 5 isolate (HRI 3880) was not pathogenic on *B. oleracea* cv. Miracle and radish cv. Mino Early and was just very weakly pathogenic on *B. juncea* cv. Florida Broad Leaf Mustard. *B. rapa* cv. Seven Top Turnip gave variable reactions with these two isolates. The two *X. campestris* pv. *campestris* isolates (HRI 1279A and 3880) were not pathogenic on other plants tested.

The *X. vesicatoria* isolate (HRI 8300) was only pathogenic on tomato and pepper. The symptoms on these hosts were distinct from the symptoms caused by the *X. campestris* leaf spot isolates: the spots caused by *X. vesicatoria* were generally smaller, darker and more irregular than spots caused by the *X. campestris* leaf spot isolates.

Host range on a subset of 10 hosts. The 13 leaf spot isolates tested were pathogenic on *B. oleracea* cv. Wiroso F₁, *B. carinata* selection of PI 199947, radish cv. French Breakfast 3, and tomato cv. Moneymaker. Five isolates (HRI 6491, 6493, 7974, 8297, and 8298) were not pathogenic on *B. napus* line 14R of Cobra, *B. rapa* cv. Just Right Hybrid Turnip, and *B. juncea* cv. Florida Broad Leaf Mustard; some of these isolates produced a small number of spots in wallflower. One isolate (HRI 6520) was not pathogenic on *B. oleracea* cv. Miracle F₁ and radish cv. Mino Early. The remaining seven leaf spot isolates were pathogenic on all of these hosts.

The *X. campestris* pv. *campestris* race 1 isolate (HRI 3811) caused black rot symptoms in three *Brassica* cultivars (Wiroso F₁, Miracle F₁, and Just Right Turnip) and both cultivars of radish;

the race 5 isolate (HRI 3883) caused black rot symptoms on three *Brassica* cultivars (Wiroso F₁, selection of PI 199947, and Just Right Hybrid Turnip) and radish cv. French Breakfast, and was weakly pathogenic on *B. juncea* cv. Florida Broad Leaf Mustard and radish cv. Mino Early.

The *X. vesicatoria* (HRI 5235 and 8302) and *X. axonopodis* pv. *vesicatoria* (HRI 8301) isolates produced small dark irregular spots on tomato and no symptoms on other hosts.

Occurrence of races. The differential reactions of *Brassica*, radish, and wallflower cultivars were used to separate the *X. campestris* leaf spot isolates into three races (Fig. 5). Race 1 isolates were not pathogenic on *B. napus* line 14R of Cobra, *B. rapa* cv. Just Right Hybrid Turnip, and *B. juncea* cv. Florida Broad Leaf Mustard; these isolates were also not pathogenic or very weakly pathogenic on wallflower cv. Cloth of Gold. Race 2 isolates were not pathogenic on *B. oleracea* cv. Miracle F₁ and radish cv. Mino Early. Race 3 isolates were pathogenic on all these cultivars. Ten *X. campestris* leaf spot isolates were included in race 1, four in race 2, and 11 in race 3 (Tables 1 and 3).

rep-PCR. The patterns of the fingerprints of 50 *Xanthomonas* isolates were generated by REP-, ERIC-, and BOX-PCR. These patterns were complex with a number of polymorphic bands. Twenty-four bands were identified in REP, 32 in ERIC, and 38 in BOX-PCR (ranging from approximately 200 bp up to 4.0 kb). The cophenetic correlation (that measures the correlation between distance values calculated during dendrogram building and the observed distance) was 0.79, 0.68, and 0.74 for REP, ERIC, and BOX dendrograms, respectively. The data obtained with the three sets of primers were combined and used to generate a dendrogram (Fig. 6). The cophenetic correlation for this "combined" dendrogram was 0.83, showing that the clustering was more accurate after combining the results. The profiles of the two isolates that were repeated in every PCR and every gel were 91 to 99% similar (data not shown); therefore, differentiation of isolates that appear more than 90% similar might be unreliable.

Fig. 2. Leaves (abaxial side) of radish cv. Mino Early spray-inoculated with *Xanthomonas campestris* pv. *raphani* isolate 6496 (left, susceptible reaction, 1 week after inoculation) and pin-inoculated with *X. campestris* pv. *raphani* isolates 6490 (center, susceptible reaction, 2 weeks after inoculation) and 8305 (right, resistant reaction, 3 weeks after inoculation).

At 60% similarity, the *X. vesicatoria* and *X. axonopodis* pv. *vesicatoria* isolates were separated from all the *X. campestris* isolates included in this study; at approximately 68% similarity, the *X. axonopodis* pv. *vesicatoria* isolate separated from the three

Fig. 3. Radish cv. French Breakfast (swollen hypocotyl) spray-inoculated with *Xanthomonas campestris* pv. *raphani* isolate 8298 (2 weeks after inoculation).

X. vesicatoria isolates. At 73% similarity, the isolates obtained from wallflower (HRI 3777A and 5219A) separated from other *Xanthomonas* isolates from crucifers. At 75% similarity, two groups of isolates from crucifers were differentiated: one cluster included all leaf spot isolates, an isolate from horseradish (HRI 6376), and the two isolates from stock (HRI 6377 and 6378); the other cluster included all *X. campestris* pv. *campestris* isolates plus an isolate from candytuft (HRI 6375). Within each race of the leaf spot isolates and each race of *X. campestris* pv. *campestris*, there were isolates that were very similar or identical, but there were also isolates that were quite distinct.

DISCUSSION

The nomenclature used in previous papers for isolates of *X. campestris* that cause a nonvascular leaf spot disease on *Brassica* spp. is confusing, and isolates have been identified as either *X. campestris* pv. *armoraciae* or *X. campestris* pv. *raphani*. Such isolates are sometimes obtained during tests on commercial brassica seed (20) and the confusion in their nomenclature presents a problem when reporting results, particularly if the seed is crossing international boundaries (e.g., both *X. campestris* pvs. *armoraciae* and *raphani* are officially classified as nonindigenous in the UK). This study aimed to clarify the nomenclature of *X. campestris* leaf spot isolates.

The symptoms observed after spray and pin inoculation of the *Xanthomonas* leaf spot isolates were similar to the symptoms described by White (28) (Figs. 1, 2, and 3). The isolates were only weakly pathogenic on horseradish, but were very pathogenic in some *Brassica* spp., radish, and tomato cultivars (Fig. 4). In contrast with the disease described by White (28), the horseradish disease described 1 year earlier by McCulloch (14) had a narrower host range and was not reported to produce dark sunken lesions on stems and petioles. The only isolate studied here that originated from horseradish (HRI 6376) was nonpathogenic and failed to produce leaf spots on either horseradish or the other hosts tested. Although other cultivars of horseradish might be more susceptible to the disease, it is unlikely that we have seen the organism described by McCulloch (14). Because the diseases described by McCulloch (14) and White (28) seem to have different host ranges and possibly produce different symptoms, both their pathovar names should stand and one name should not have priority over the other as previously considered by Black and Machmud (2) and Alvarez et al. (1). Previously, Tamura et al. (22) identified his leaf spot isolates from Japan as *X. campestris* pv.

Fig. 4. Leaves (adaxial side) of tomato cv. Money Maker (left), pepper cv. Worldbeater (center), and tobacco cv. White Burley (right) spray-inoculated with *Xanthomonas campestris* pv. *raphani* isolate 6490 (2 weeks after inoculation).

raphani because they had a large host range that included radish and tomato, but not horseradish. The leaf spot disease of radish described by Sahin and Miller (21) as caused by a different pathotype of *X. campestris* pv. *armoraciae* also seems to be distinct from the disease studied here as the pathogen attacked horseradish, but not tomato. Therefore, we consider that all the leaf spot isolates that we have studied should be considered as *X. campestris* pv. *raphani* and not *X. campestris* pv. *armoraciae*.

Although the *X. campestris* pv. *raphani* isolates can cause leaf spots when inoculated in pumpkin, cucumber, and tobacco (Fig. 4), they were generally not very aggressive on these hosts and

therefore it seems unlikely that they would cause disease in these crops in commercial practice. However, it is likely that the isolates studied here would cause disease on members of the family *Brassicaceae* including different *Brassica* spp., radish, and some ornamental crops (e.g., wallflower) and, outside this family, on tomato. In very susceptible hosts, a water-soaked halo was frequently observed around the leaf spots. In weakly susceptible hosts, this was less frequent or was not observed. In these cases, to confirm that the lesions are a sign of pathogenicity and not just a hypersensitive response, the bacterial population in the lesions could be studied to check if the bacteria are actively multiplying.

X. vesicatoria and *X. axonopodis* pv. *vesicatoria* also cause spots on tomato and pepper, but not on *Brassica* spp. and radish, and the symptoms are distinct from those produced by the *X. campestris* leaf spot isolates.

We have confirmed that *X. campestris* pv. *campestris* can also cause spots on leaves of *Brassica* spp. (Fig. 1) and possibly on other crucifers, but not on other hosts like tomato. The symptoms are distinct from those caused by pv. *raphani* and generally progress toward typical vascular chlorotic black rot lesions. The reactions of the different *X. campestris* pv. *campestris* races when sprayed on the differential *Brassica* cultivars agreed with previous results obtained in wound inoculations (27), but the *B. napus* cv. Cobra line 14R seem to be more resistant in spray inoculations than in wound inoculations as it did not show symptoms when sprayed with two of the isolates (HRI 3811 [race 1] and HRI 3883 [race 5]).

Most leaf spot isolates studied here originated from *B. oleracea*, *B. rapa*, or radish, but one isolate from tomato was also identified as *X. campestris* pv. *raphani* (Table 3). Kuflu and Cuppels (10) also noticed that this isolate was distinct from *X. vesicatoria* and *X. axonopodis* pv. *vesicatoria* as it produced large spots on tomato and pepper and it was also highly pathogenic on radish and cabbage seedlings and they hypothesized that it was related to *X. campestris* pv. *raphani*. Jones et al. (5) also reported that an *Xanthomonas* isolate from tomato produced leaf spots and stem lesions on cabbage, and previously, Robbs (19) found that some isolates obtained in Brazil from tomato could infect pepper and cabbage and were probably *X. vesicatoria* (syn. *campestris*) pv. *raphani*.

Two isolates were obtained from *B. oleracea* seed tests and were originally considered nonpathogenic because, when inoculated on the secondary veins around the leaves using a pin or mouse-tooth forceps, they only produced small necrotic areas around the inoculation points (data not shown). Therefore, we suggest that routine pathogenicity tests of potential *X. campestris* isolates should be done on a brassica cultivar susceptible to both *X. campestris* pv. *campestris* and pv. *raphani* (e.g., Wirosa F₁) and that some inoculation points should be done in the middle vein. Isolates that do not cause typical black rot symptoms, but cause dark sunken lesions can then be spray-inoculated to confirm their identification as *X. campestris* pv. *raphani*.

The inoculation results indicate that *X. campestris* pv. *raphani* can be divided into at least three races (1, 2, and 3) based on the differential reaction of *Brassica*, radish, and wallflower cultivars (Fig. 5). Races 1 and 3 appear to be the most common races in *B. oleracea* crops. Although the number of isolates studied is not

Fig. 5. Leaves (adaxial side) of Savoy cabbage cv. Wirosa F₁ (left), *Brassica napus* line Cobra 14R (center), and cauliflower cv. Miracle F₁ (right) spray-inoculated with *Xanthomonas campestris* pv. *raphani* isolates HRI 8297 (bottom; 2 weeks after inoculation), 6520 (middle; 2 weeks after inoculation), and 8303 (top; 1 week after inoculation).

TABLE 3. Frequency of occurrence, host of origin, and geographical distribution of *Xanthomonas campestris* pv. *raphani* races among 25 isolates

Race type	Host of origin (number of isolates)	Geographical origin (number of isolates)	Total number of isolates
1	<i>Brassica oleracea</i> (5), <i>Raphanus sativus</i> (2), unknown (3)	France (3), Southern Hemisphere (2), United States (2), unknown (3)	10
2	<i>Brassica rapa</i> (3), unknown (1)	Japan (2), United States (1), unknown (1)	4
3	<i>Brassica oleracea</i> (4), <i>Brassica rapa</i> (2), <i>Raphanus sativus</i> (1), <i>Lycopersicon esculentum</i> (1), unknown (3)	France (2), Japan (1), Canada (1), United States (4), E. Asia (1), unknown (2)	11

large enough to reach a conclusion, there appeared to be no relationship between race and geographical origin of the isolates, but most race 2 isolates seem to have originated from the same host species, *B. rapa* (Table 3). A differential series is shown in Table 4 together with a postulated gene-for-gene model to explain the relationship between races and cultivars. The differential series only includes accessions that are F₁ hybrids or uniform selections, and therefore should always give uniform reactions. It is only necessary to inoculate three *Brassica* cultivars to determine the pathogenicity and the race-type of potential *X. campestris* pv. *raphani*; we recommend the use of *B. oleracea* cvs. Wirosa F₁ and Miracle F₁ and a selection of *B. napus* cv. Cobra (Fig. 5), as all these accessions have also been previously included in the *X. campestris* pv. *campestris* differential series (27). The model presented is the simplest hypothesis involving the smallest number of genes (two matching gene pairs) necessary to explain the observed interactions. In this hypothesis, although not tested, gene homology was assumed for cultivars with the same pattern

of reaction. The genes that confer resistance to races 1 and 2 were designated R1 and R2, respectively. The gene R1, present in *B. napus*, *B. rapa*, and *B. juncea* lines, confers resistance to race 1 isolates (with A1). This gene could have originated in the A genome of *B. rapa*. R2, present in *B. oleracea* cv. Miracle F₁ and radish cv. Mino Early, confers resistance to race 2 isolates that have A2. This model provides a basis for understanding the interactions of races and cultivars and can be expanded if new races and differentials are identified.

rep-PCR-based genomic fingerprinting was used to assess the genetic diversity of the *Xanthomonas* leaf spot isolates and to compare them with representative isolates of *X. campestris* pv. *campestris* and related pathovars (Fig. 6). The results obtained from REP-, ERIC-, and BOX-PCR fingerprints were combined, as recommended by Rademaker et al. (16), to obtain more significant and consistent results. The results indicated that *X. campestris* pv. *raphani* is genetically distinct from *X. campestris* pv. *campestris*. In contrast, previous results of carbon source oxida-

Fig. 6. Dendrogram of genetic similarity of 25 *Xanthomonas campestris* pv. *raphani* isolates together with 25 other *Xanthomonas* isolates. Similarities were calculated from the combined data of repetitive extragenomic palindromic (REP), enterobacterial repetitive intergenic consensus (ERIC), and BOX primer sets using Dice's coefficient. The dendrogram was obtained using the unweighted pair-group method with arithmetic average method.

^a The isolates were identified according to the results of this study and according to the results of the pathogenicity tests done previously by Vicente et al. (27). The isolate HRI 6375 from candytuft (*Iberis* sp.) and the isolates HRI 3777A and HRI 5219A from wallflower (*Cheiranthus cheiri*) should be included in new *X. campestris* pathovars.

tion (Biolog) profiles, serological tests using monoclonal antibodies, restriction fragment length polymorphism (1), BOX-PCR alone (31), and even REP-, ERIC-, and BOX-PCR combined (18) failed to distinguish the two pathovars. Although *X. campestris* pv. *raphani* and *X. campestris* pv. *campestris* isolates are highly variable even within each race, some groups were formed. Alvarez et al. (1) also reported that isolates that cause black rot and isolates that cause leaf spots were both genetically heterogeneous. The clusters formed seemed to be more related to the races than to the host or geographical origin of the isolates. These findings contrast with results obtained by Massomo et al. (13) and Tsygankova et al. (23) that indicated that geographical origin was a major factor for *X. campestris* pv. *campestris* genetic diversity.

The results of our genetic diversity study showed that the *X. campestris* isolates from wallflower are quite distinct from *X. campestris* pv. *campestris* and *X. campestris* pv. *raphani*. We have showed previously that they cause a vascular disease of wallflower (27). These isolates should not be included in *X. campestris* pv. *campestris*, and should be considered a distinct (new) pathovar. Isolate HRI 6375 (NCPPB 347) from candytuft was not included in the *X. campestris* pv. *raphani* cluster (that also included the horseradish isolate), but was closer to some *X. campestris* pv. *campestris* isolates. Rademaker et al. (18) also noticed

that the isolate from candytuft and the isolate from horseradish had distinct fingerprint profiles. This isolate causes a vascular disease in candytuft and was also pathogenic in some radish plants (27), but is not pathogenic on *Brassica* spp. or horseradish. Therefore, this isolate should not be included in and should not be designated as the pathotype strain of *X. campestris* pv. *armoraciae*, and should be considered a distinct (new) pathovar. The isolates from stock were included in the *X. campestris* pv. *raphani* cluster. These isolates cause a vascular disease of stock (27) and are included in a separate pathovar (*X. campestris* pv. *incanae*). We were unable to confirm the existence of *X. campestris* pv. *armoraciae*; the single isolate from horseradish was included in the *X. campestris* pv. *raphani* cluster, but does not appear to be pathogenic. The current information on the host range of the *X. campestris* pathovars is summarized in Table 5.

A major problem of many previous studies on the diversity of *X. campestris* isolates was the lack of reliable, pathogenic type-strains that represent isolates that cause vascular disease in *Brassica* spp. and other crucifers, and type-strains of isolates that only cause leaf spots and petiole and stem sunken lesions. Our work has shown that the type-strain of *X. campestris* pv. *raphani* (HRI 3883, NCPPB 1946) does not cause the leaf spot disease described by White (28) (Fig. 1); this isolate was previously shown to correspond to race 5 of *X. campestris* pv. *campestris* (27). We propose that isolates HRI 6490 (race 1), HRI 8305 (race 2), and HRI 6519 (race 3) should be designated as new race-type strains of *X. campestris* pv. *raphani*. As race 3 seems to be the most common race, we propose that isolate HRI 6519 from radish should be designated as the neopathotype strain. These strains together with the proposed gene-for-gene model should assist in genetic and pathogenic studies of *Xanthomonas* spp. and could be used for the selection of resistant material for breeding programs. Our model needs to be further supported by detailed genetic and molecular data from both the host and the pathogen to be fully validated.

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TABLE 4. Postulated gene-for-gene model to explain the relationship between different hosts and races of *Xanthomonas campestris* pv. *raphani*

Differential cultivars or accessions ^a	Resistance (R) genes	Races/avirulence (A) genes ^b		
		1	2	3
		A1	A2	
Wirosa F₁ , selection of PI 199947, radish cv. French Breakfast 3, tomato cv. Moneymaker		+	+	+
Cobra 14R , Just Right Hybrid Turnip, Florida Broad Leaf Mustard	R1	–	+	+
Miracle F₁ , radish cv. Mino Early	R2	+	–	+

^a Recommended accessions for race-typing *X. campestris* pv. *raphani* isolates are bold.

^b +, compatible interaction (susceptibility); –, incompatible interaction (resistance).

TABLE 5. Host range of *Xanthomonas campestris* pathovars from cruciferous hosts based on studies by McCulloch (14), White (28), Vicente et al. (27), and the present study

Pathovar	Host of origin	Pathogenicity to ^a						
		Cabbage cv. Wirosa (<i>Brassica oleracea</i>)	Radish (<i>Raphanus sativus</i>)	Stock (<i>Matthiola incana</i>)	Wallflower (<i>Cheiranthus cheiri</i>)	Candytuft (<i>Iberis</i> sp.)	Horseradish (<i>Armoracia rusticana</i>)	Tomato cv. Moneymaker (<i>Lycopersicon esculentum</i>)
<i>X. campestris</i> pv. <i>campestris</i> ^b	Various (<i>Brassica</i> sp., <i>Raphanus sativus</i> , <i>Sinapis arvensis</i> , and other cruciferous weeds)	v +	v +/-	–	–	–	–	–
<i>X. campestris</i> pv. <i>incanae</i>	<i>Matthiola</i> sp.	–	–	v +	–	–	nt	–
<i>X. campestris</i> ^c	<i>Cheiranthus cheiri</i>	–	–	–	v +	–	nt	–
<i>X. campestris</i> ^d	<i>Iberis</i> sp.	–	v +/-	–	–	v +	–	–
<i>X. campestris</i> pv. <i>raphani</i> ^e	Various (<i>Brassica</i> spp., <i>Raphanus sativus</i> , <i>Lycopersicon esculentum</i>)	ls +	ls +/-	–	ls +/-	ls +/-	ls (+)	ls +
<i>X. campestris</i> pv. <i>armoraciae</i> ^f	<i>Armoracia rusticana</i>	ls (+)	–	–	–	–	ls +	–

^a v, vascular infection; ls, leaf spot symptoms; +, compatible interaction (susceptibility); (+), weakly pathogenic; –, incompatible interaction (resistance); +/-, variable reaction between and/or within cultivars; and nt, not tested

^b Some of these isolates were previously included in *X. campestris* pv. *aberrans* (HRI 3880) and *X. campestris* pv. *raphani* (HRI 3883).

^c Isolates HRI 3777A and HRI 5219A previously included in *X. campestris* pv. *campestris*.

^d Isolate HRI 6375 previously included in *X. campestris* pv. *armoraciae*

^e Postulated based on the description of White (28) and results of the present study.

^f Postulated based on the description of McCulloch (14). The existence of this pathovar has not been confirmed (27); the only isolate from horseradish (HRI 6376) studied here was nonpathogenic and failed to produce leaf spots on either horseradish or the other hosts tested.

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