

First occurrence of *Xanthomonas campestris* pv. raphani on wallflower (*Erysimum cheiri*) in the United Kingdom

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In a study of diseases caused by *Xanthomonas campestris* on UK nursery-grown wallflowers (*Erysimum cheiri*), one isolate (P764) obtained in 1990 from Sussex differed from the others. The affected plants (cv. Bedder Mixed), showed distorted growth with chlorotic and necrotic spots, but no sectored V-shaped wilting, the most common symptom associated with xanthomonads in wallflowers. Other *X. campestris* isolates (including P763), were obtained from wallflowers with typical wilting. Infected plant material was comminuted in sterile 0.1% peptone solution and loopfuls streaked onto yeast dextrose chalk agar and nutrient dextrose agar (Lelliott & Stead, 1987). Plates were incubated for up to 72 hours at 28°C. *Xanthomonas*-like colonies were purified by re-streaking and isolates were maintained at -80°C (Protect System, UK).

To study the relationship between the wallflower isolates and other *X. campestris* isolates, fatty acid profiles (Stead *et al.*, 1992), rep-PCR (using REP, ERIC and BOX primers) fingerprints (Vicente *et al.*, 2006) and partial gyrase B (*gyrB*) sequences (Parkinson *et al.*, 2009) were analysed. The partial gyrB sequences of P763 and P764 were deposited in GenBank (Accession Nos. KX289616 and KX289617). All analyses clearly placed both P763 and P764 in *X. campestris*. In the gyrase B phylogeny, P764 had up to 99.1% similarity with sequences of *X. campestris* (Fig. 1).

Host test classification of pathovars within X. campestris has been previously established (Vicente et al., 2001; Vicente et al., 2006; Fargier & Manceau, 2007). Unlike other X. campestris pathovars, X. campestris pv. raphani (Xcr) causes local necrotic leaf spots in tomato and cruciferous hosts. Host tests appropriate to pathovar determination were done in glasshouses maintained at 20-30°C, c. 70% relative humidity and a 16 hour lighting regime. Plants of wallflower cv. Cloth of Gold, cabbage cv. Wirosa F1 and tomato cv. Moneymaker were inoculated with a range of strains representing X. campestris pvs. campestris (Xcc) and raphani, and wallflower strains P763 and P764. Cell densities were adjusted to c. 10⁸ cfu/ml. Stab and spray inoculations were done on three replicate plants (Vicente et al., 2006). P764 produced pale tan leaf spots in wallflower (Fig. 2) whereas P763 produced V-shaped sectored wilt symptoms (Fig. 3). P764 also produced leaf and petiole spots in cabbage and tomato with symptoms identical to strains of all three races of Xcr (WHRI 6490, WHRI 8298(R1), WHRI 8305(R2) and WHRI 6519(R3)). Typical V-shape vascular lesions, as caused by Xcc on susceptible cabbage, were not seen with these Xcr strains. The Xcc reference strains (including WHRI 5212 and WHRI 1279A) produced vascular symptoms on cabbage, but not on wallflower or

In a second experiment, P764 was used to inoculate the *Xcr* susceptible *Brassica* lines/cultivars Wirosa F1, PIC1 (PI 199947), tomato cv. Moneymaker, the *Brassica* differentials COB60 (Cobra), Just Right Turnip, FBLM2 (Florida Broad Leaf Mustard), Miracle F1, SxD1, radish cvs. Mino

Early and French Breakfast, and ornamental crucifers, wallflower cvs. Cloth of Gold, Blood Red and Primrose Yellow, candytuft cv. White Empress, and garden stock cvs. Heaven Scent and Mixed Colours. Spray and pin methods were used (Vicente *et al.*, 2006). All cultivars were susceptible, showing typical *Xcr* leaf spots and sunken lesions, except Miracle F1, SxD1, radish Mino Early (Fig. 4) and garden stock lines indicating that P764 is race 2 *Xcr*.

These results confirmed P764 as *Xcr*. The pathovar status of P763 has not been determined, other than it does not belong to *Xcr*. This is the first documented record of *Xcr* in the UK and is the first record of its occurrence worldwide in *Erysimum cheiri*. *Xcr* P764 has been accessioned in the NCPPB with the number 4451.

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References

Fargier E, Manceau C, 2007. Pathogenicity assays restrict the species *Xanthomonas campestris* into three pathovars and reveal nine races within *X. campestris* pv. *campestris*. *Plant Pathology* **56**, 805-818. http://dx.doi.org/10.1111/j.1365-3059.2007.01648.x

Lelliot RA, Stead DE, 1987. Methods for the diagnosis of bacterial diseases of plants. In: Preece, TF, ed. *Methods in Plant Pathology*, Volume 2. Oxford, UK: Blackwell Scientific Publications, pp. 216.

Parkinson N, Cowie C, Heeney J, Stead DE, 2009. Phylogenetic structure of *Xanthomonas* determined by comparison of *gyrB* sequences. *International Journal of Systematic and Evolutionary Microbiology* **59**, 264-274. http://dx.doi.org/10.1099/ijs.0.65825-0

Stead DE, Sellwood JE, Wilson J, Viney I, 1992. Evaluation of a commercial microbial identification system based on fatty acid profiles for rapid, accurate identification of plant pathogenic bacteria. *Journal of Applied Microbiology* **72**, 315-321.

http://dx.doi.org/10.1111/j.1365-2672.1992.tb01841.x

Vicente JG, Conway J, Roberts SJ, Taylor JD, 2001. Identification and origin of *Xanthomonas campestris* races and related pathovars. *Phytopathology* **91**, 492-499.

http://dx.doi.org/10.1094/PHYTO.2001.91.5.492

Vicente JG, Everett B, Roberts SJ, 2006. Identification of isolates that cause a leaf spot disease of brassicas as *Xanthomonas campestris* pv. *raphani* and pathogenic and genetic comparison with related pathovars. *Phytopathology* **96**, 735-745. http://dx.doi.org/10.1094/PHYTO-96-0735





Figure 4



Figure 2 Figure 3

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