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

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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 sectorised 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 16 hour lighting regime. Plants of wallflower cv. Cloth of Gold, cabbage cv. Wiroso 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 sectorised 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 tomato.

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 to the NCPPB with the number 4451.

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Figure 1: Gyrase B partial phylogeny of two *Xanthomonas campestris* strains (P763 and P764) from wallflower together with sequences from 18 other *Xanthomonas* spp. strains (sequences retrieved from GenBank). Sequences were trimmed to 530 bp and aligned using the ClustalW algorithm in MegAlign (DNASTar, USA). The phylogenetic tree was generated using a maximum likelihood model implemented by MegAlign with 1000 bootstraps.

Figure 2: Leaf spots and petiole and stem lesions on wallflower cv. Cloth of Gold caused by *Xanthomonas campestris* pv. *raphani* P764 following spray inoculation.

Figure 3: Symptoms on leaves of wallflower cv. Cloth of Gold caused by *Xanthomonas campestris* pv. *raphani* P764 (spray inoculated) and *X. campestris* P763 (pin inoculated).

Figure 4: Leaf spot symptoms on a leaf of turnip (*Brassica rapa*) cv. Just Right and absence of symptoms on a leaf of radish (*Raphanus sativus*) cv. Mino Early following spray inoculation of *Xanthomonas campestris* pv. *raphani* P764.

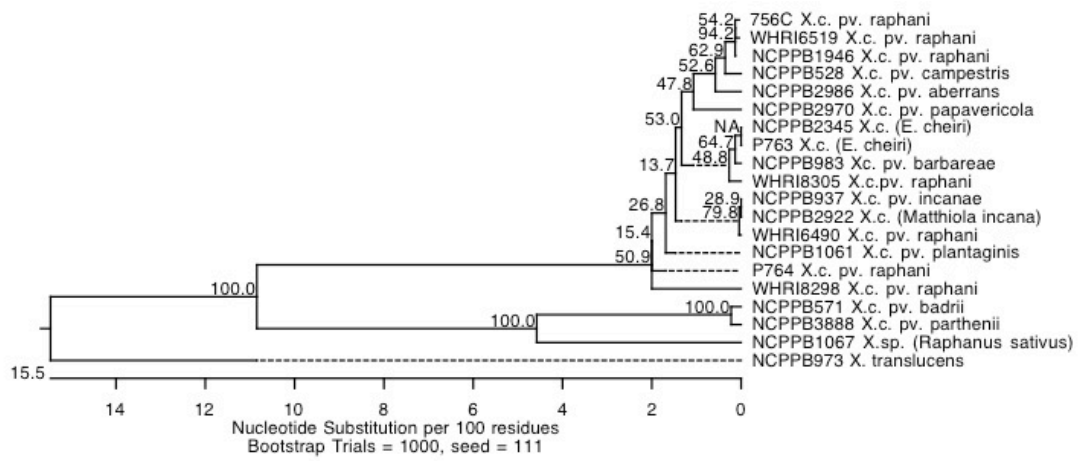


Figure 1.



Figure 2.



Figure 3.



Figure 4.