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NEW DISEASE REPORT

First report of black rot caused by *Xanthomonas nasturtii* on watercress in Spain and Portugal

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bacterial plant disease, leaf spots, leaf edge senescence, Nasturtium officinale, wilt

Xanthomonas nasturtii was first identified as the cause of black rot of watercress (*Nasturtium officinale*) on plants grown in Florida, USA (Vicente *et al*., 2017). Similar symptoms had been reported earlier in Hawaii (McHugh & Constantinides, 2004) and an unidentified *Xanthomonas* sp. was isolated in Portugal in 2003 from wild watercress (Cruz *et al*., 2017). Since 2017, watercress crops in southern Spain have occasionally exhibited small yellow leaf lesions around the hydathodes, leaf spots, V-shaped leaf lesions, wilt, distortion and senescence (Fig. 1). Symptoms were more frequent during mild and humid periods from November to February, reducing yields by up to 60% and leading to rejection of some crops (Lascelles, 2019). In 2021, symptoms were seen on watercress produced in Portugal and sold in the UK.

Isolations were made from watercress leaves showing typical symptoms between 2017 and 2021 by excising and macerating small tissue fragments in sterile water and plating on King's medium B (KB). Seed produced in Spain was tested using a method based on the Interna-

FIGURE 1 Symptoms of black rot of watercress including leaf lesions around the hydathodes, spotting and wilting observed in a sample of watercress from southern Spain.

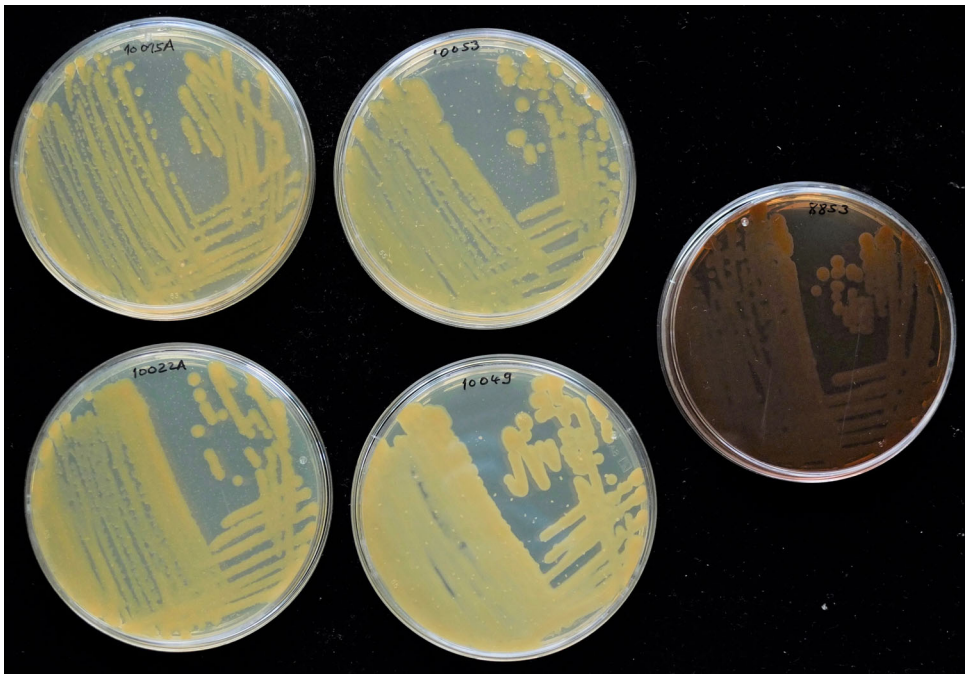

FIGURE 2 Two-week-old cultures of *Xanthomonas nasturtii* isolates from Spain (top, 10015A and 10053) and Portugal (bottom, WHRI 10022A and 10049) growing on King's medium B compared with the type strain from Florida (right, NCPPB 4600 = WHRI 8853).

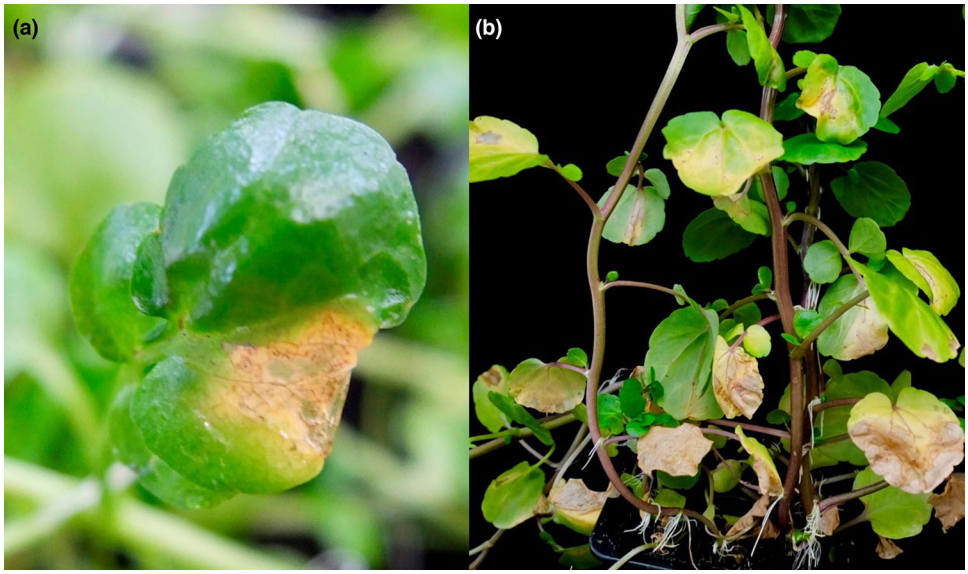

FIGURE 3 Symptoms observed in watercress inoculated with *Xanthomonas nasturtii* isolates: (a) WHRI 10053 from Spain (15 days after inoculation), (b) WHRI 10022A from Portugal (14 days after inoculation).

tional Seed Testing Association method for detection of *Xanthomonas campestris* pv. *campestris*. Mixtures of bacterial colonies were observed on the plates after incubation for 48–72 hours at 28°C and colonies resembling *Xanthomonas* (yellow, mucoid) were sub-cultured. Isolates were purified and stored at -76°C in nutrient broth with 150 g/l glycerol (Vicente *et al*., 2017) and/or at -80°C in Protect® (Protect System, UK). In contrast to the type strain (NCPPB 4600) from Florida, isolates from Spain and Portugal did not cause browning of the KB medium (Fig. 2).

Pathogenicity was tested on plants of three watercress accessions and Savoy cabbage cv. Wirosa F_1 (Bejo Zaden B.V., The Netherlands) by stabbing with an insect pin charged with bacteria (Vicente *et al*., 2017). The *Xanthomonas*-like isolates were pathogenic to all three watercress accessions (Fig. 3) whereas no symptoms were observed

TABLE 1 Origin of *Xanthomonas nasturtii* isolates obtained from watercress and their partial *gyrB* sequences ([1]WHRI, culture collection of the School of Life Sciences, Wellesbourne, University of Warwick, UK; [2]NCPPB, National Collection of Plant Pathogenic Bacteria, York, UK; CPBF, Coleção Portuguesa de Bactérias Fitopatogénicas, Oeiras, Portugal; [3]Produced in Spain; [4]From a bag of watercress obtained in a UK supermarket, Portugal stated as origin.)

WHRI[1] strain number	Other strain numbers[2]	Plant part	Year of isolation	*gyrB* result / GenBank Accession No. (length)
Control (type strain of *Xanthomonas nasturtii*) from USA				
8853	NCPPB 4600	Leaves	2014	KX523291.1 (822 bp)
Isolates from Spain				
10053	NCPPB 4622	Leaves	2017	As WHRI 8853
10015A	-	Leaves	2018	As WHRI 8853
10017B	-	Leaves	2018	OM830432 (783 bp)
10054	Fera 21902792	Seeds[3]	2019	As WHRI 10017B
10055	Fera 2020028438	Leaves	2020	OM830433 (616 bp)
10056A	-	Leaves inoculated with 10015A	2021	As WHRI 8853
Isolates from Portugal				
10022A	CPBF 47	Leaves	2003	As WHRI 10055
10049	-	Leaves[4]	2021	As WHRI 8853
10058	-	Leaves inoculated with 10049	2021	As WHRI 8853

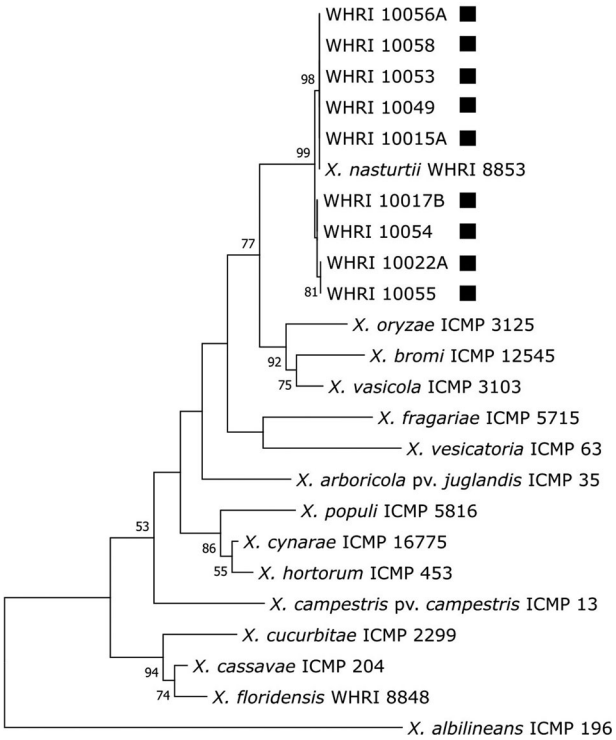

FIGURE 4 Phylogeny of partial *gyrB* sequences of seven *Xanthomonas nasturtii* isolates obtained from watercress from Spain and Portugal and two re-isolates (marked with black square), together with the type strain of *X. nasturtii* WHRI 8853 from Florida and sequences from 14 other *Xanthomonas* spp. strains (including *X. floridensis* WHRI 8848, a non-pathogenic isolate from watercress) retrieved from GenBank. Sequences were trimmed to 600 bp. Bootstraps are shown next to the nodes with bootstraps < 50 omitted.

on cabbage. To complete Koch's postulates, re-isolations were made from symptomatic watercress leaves inoculated with isolates WHRI 10015A (from Spain) and 10049A (from Portugal). Isolates with the same morphological characteristics were obtained.

Fatty acid profiling (Weller *et al.*, 2000) was performed on isolates grown on trypticase soy agar plates at 28°C for 48 hours; comparisons with libraries that did not include *X. nasturtii*, indicated the highest matches of 0.38 to 0.68 with *X. axonopodis* and *X. campestris*. DNA was extracted as outlined in Vicente *et al.* (2017). The partial 16S rRNA gene was amplified and sequenced for isolates from Spain and confirmed their identity as a *Xanthomonas* species. The partial *gyrB* gene was amplified and sequenced for selected isolates according to Parkinson *et al.* (2007) (Table 1). The phylogeny (Fig. 4) confirmed that isolates from Spain and Portugal belong to *X. nasturtii* and shows that there is some genetic diversity amongst them.

This is the first report of *X. nasturtii* on watercress crops in Europe. We suspect that black rot of watercress may be widespread in production areas of the Iberian Peninsula and, as the crop is grown in water beds, the pathogen is likely to spread relatively easily. Based on detection in a watercress seed lot produced in Spain, the pathogen may be seed borne and therefore it is important to test watercress seed to avoid introducing the disease into clean beds and new areas/countries.

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