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](https://wileyonlinelibrary.com/journal/ndr2)  
**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).

National 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 F₁ (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 (1WHRI, culture collection of the School of Life Sciences, Wellesbourne, University of Warwick, UK; 2NCPPB, National Collection of Plant Pathogenic Bacteria, York, UK; CPBF, Coleção Portuguesa de Bactérias Fitopatogénicas, Oeiras, Portugal; 3Produced in Spain; 4From a bag of watercress obtained in a UK supermarket, Portugal stated as origin.)

<table>
<thead>
<tr>
<th>WHRI1 strain number</th>
<th>Other strain numbers2</th>
<th>Plant part</th>
<th>Year of isolation</th>
<th>gyrB result / GenBank Accession No. (length)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (type strain of Xanthomonas nasturtii) from USA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8853</td>
<td>NCPPB 4600</td>
<td>Leaves</td>
<td>2014</td>
<td>KX523291.1 (822 bp)</td>
</tr>
<tr>
<td>Isolates from Spain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10053</td>
<td>NCPPB 4622</td>
<td>Leaves</td>
<td>2017</td>
<td>As WHRI 8853</td>
</tr>
<tr>
<td>10015A</td>
<td>-</td>
<td>Leaves</td>
<td>2018</td>
<td>As WHRI 8853</td>
</tr>
<tr>
<td>10017B</td>
<td>-</td>
<td>Leaves</td>
<td>2018</td>
<td>OMB830432 (783 bp)</td>
</tr>
<tr>
<td>10054</td>
<td>Fera 21902792</td>
<td>Seeds3</td>
<td>2019</td>
<td>As WHRI 10017B</td>
</tr>
<tr>
<td>10055</td>
<td>Fera 2020028438</td>
<td>Leaves</td>
<td>2020</td>
<td>OMB830433 (616 bp)</td>
</tr>
<tr>
<td>10056A</td>
<td>-</td>
<td>Leaves inoculated with 10015A</td>
<td>2021</td>
<td>As WHRI 8853</td>
</tr>
<tr>
<td>Isolates from Portugal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10022A</td>
<td>CPBF 47</td>
<td>Leaves</td>
<td>2003</td>
<td>As WHRI 10055</td>
</tr>
<tr>
<td>10049</td>
<td>-</td>
<td>Leaves4</td>
<td>2021</td>
<td>As WHRI 8853</td>
</tr>
<tr>
<td>10058</td>
<td>-</td>
<td>Leaves inoculated with 10049</td>
<td>2021</td>
<td>As WHRI 8853</td>
</tr>
</tbody>
</table>

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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