



ORIGINAL ARTICLE OPEN ACCESS

Identification of Race-Specific, Clade-Specific and Broad-Spectrum Resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica* Diversity Fixed Foundation Sets

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Received: 23 March 2025 | **Revised:** 5 September 2025 | **Accepted:** 1 October 2025

Funding: This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC), the Natural Environment Research Council (NERC), the Department for Environment, Food and Rural Affairs (Defra), and the Scottish Government (BB/T010908/1, BB/T010916/1 and BB/T010924/1) and Defra (CH0110 and CH0112).

Keywords: black rot | *Brassica napus* | *Brassica oleracea* | breeding | disease resistance | vegetables

ABSTRACT

Black rot, caused by *Xanthomonas campestris* pv. *campestris* (Xcc), is the most damaging bacterial disease of vegetable brassicas (*Brassica oleracea*) worldwide. The prevalence of several genetically diverse Xcc races makes breeding for varietal resistance challenging. In this study, we have screened diversity fixed foundation sets (DFFSs) of *B. oleracea* and *Brassica napus* for resistance against isolates belonging to prevalent Xcc races 1, 4, 5 and 6. The DFFSs are designed to capture the genetic diversity available within the respective *Brassica* species gene-pools in smaller subsets of lines. Our findings revealed that resistances to race 1 and 4 were largely absent in *B. oleracea* but more prevalent in *B. napus*. Notably, resistance to race 4 was particularly common in *B. napus* (63% of lines showed resistance, 13% of lines showed partial resistance). Conversely, resistance to races 5 and 6 was more common in *B. oleracea* than in *B. napus*. In *B. oleracea*, there was no significant association between disease index and morphotype but, among the *B. napus* morphotypes, swede was the most susceptible to races 1, 5 and 6 but not to race 4. Ten *B. oleracea* and 67 *B. napus* lines showed resistance to more than one isolate. Further testing of a subset of these lines demonstrated that resistances were effective against additional diverse Xcc isolates in a race-specific, clade-specific or broad-spectrum manner. The resistant lines identified in this study offer a valuable resource for breeding programmes aimed to achieve durable and sustainable control of Xcc.

1 | Introduction

Xanthomonas campestris pv. *campestris* (Xcc) is a gram-negative bacterium that causes black rot disease of brassicas. It has been described as the most important pathogen of *Brassica oleracea* (Vicente and Holub 2013; Williams 1980) and is distributed worldwide wherever *Brassica* crops are grown (CABI 2022). The species *B. oleracea* is morphologically diverse and includes vegetable morphotypes like cabbage, broccoli, cauliflower, kale, kohlrabi and Brussels sprouts. Xcc can reduce quality and cause

complete yield loss of vegetable brassicas (AHDB 2023). In addition to *B. oleracea*, Xcc also infects other *Brassica* species, including *B. napus* (swede and oilseed rape), *B. rapa* (Chinese cabbage, turnip), *B. nigra*, *B. juncea* and *B. carinata* (mustards) as well as ornamental crucifers, some weeds and radish (Taylor et al. 2002).

Xcc is primarily transmitted via seeds (Williams 1980). A single Xcc-infected seed can lead to the infection of an entire crop, especially in vegetable brassicas, which are traditionally sown in

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modules in the glasshouse. Overhead watering systems in these settings facilitate the spread of Xcc through water splash, contaminating the crop before it is transplanted to the field (Roberts et al. 2007). While hot-water seed treatments are effective in reducing the severity and incidence of Xcc infection in brassicas (Roberts et al. 2022), there are limited curative treatments once infection is established. Traditional bactericides such as antibiotics and copper-containing compounds can be effective against Xcc, but their use is often strictly regulated or banned due to environmental concerns as well as their contribution to antimicrobial resistance (Haynes et al. 2020). Recent reports suggest that Xcc has acquired genes conferring resistance/tolerance to copper and antibiotics (Ramnarine et al. 2024). Alternative, more environmentally friendly methods of control include the use of resistant *Brassica* varieties. However, breeding for resistance to Xcc is challenging, due to the genetic diversity within this pathogen, whereby resistance to one Xcc genotype is not necessarily effective against other genotypes. There are at least nine races of Xcc, which are defined by their pathogenicity on a panel of *Brassica* accessions (Vicente et al. 2001; Vicente and Holub 2013) and isolates of the same race can be genetically diverse (Guy et al. 2013). A further challenge is that different races of Xcc are prevalent in different crop types and rotations. Races 1 and 4 are the most common in *B. oleracea* vegetables, whilst races 5 and 6 are found more frequently in other *Brassica* crops such as oilseed rape and turnips (Vicente and Holub 2013).

Several studies have identified resistance to Xcc in different *Brassica* species (Da Silva et al. 2020; Griesbach et al. 2003; Hong et al. 2021; Massomo et al. 2004; Sheng et al. 2020; Singh et al. 2018). However, some of these studies screened only a small number of *Brassica* lines and/or they are limited because they screened for resistance using just one or a small subset of Xcc isolates, in some cases mixtures of isolates, and often isolates of rare or unknown race. The latter makes it difficult to determine whether the identified resistances are broad-spectrum, race-, clade- or isolate-specific. Similarly, several commercial vegetable *B. oleracea* varieties claim tolerance or resistance to black rot, but the extent and specificity of these resistances are unknown (Greer et al. 2023). In this study, we have screened diversity fixed foundation sets (DFFSs) of *B. oleracea* and *B. napus* with Xcc isolates that represent the broad genetic diversity of the most prevalent races: 1, 4, 5 and 6. Each DFFS is a reference 'foundation' set of 100–200 *Brassica* lines that have been selected to represent the available genetic diversity in the species gene-pools that can be readily screened for traits. The *B. oleracea* DFFS primarily comprises lines selected from > 6000 lines held at the UK Vegetable Genebank (University of Warwick, UK) (Walley et al. 2012). Alleles within some of these *B. oleracea* lines have then been fixed by doubled-haploid production, whilst the remaining lines have undergone one to four rounds of single-seed descent (SSD) and therefore their levels of allele homozygosity can still vary (Table S1). The lines of the *B. napus* DFFS were sourced from genebanks and research collections from around the world; where alleles were not already fixed within a line, this was achieved through at least six generations of SSD or through doubled-haploid production (Table S2).

Resistances to Xcc races 1, 4, 5 and 6 have previously been identified in *Brassica* species and some have been characterised. For example, resistances to Xcc race 1 have been identified in *B. oleracea*

but they are typically partial and multigenic (Doullah et al. 2011; Iglesias-Bernabé et al. 2019; Kifuji et al. 2013). Strong single gene resistances to this race have been identified in other *Brassica* species (Dey et al. 2015; Sharma et al. 2016; Taylor et al. 2002; Vicente et al. 2002). Strong monogenic resistances to race 4 have also been commonly reported in *Brassica* species other than *B. oleracea* (Dey et al. 2015; Griffiths et al. 2009; Vicente et al. 2002). Resistance to Xcc race 5 has not been widely studied, perhaps because it is less prevalent than races 1 and 4 in vegetable brassicas, but nonetheless it has been isolated from vegetable *Brassica* fields (Jensen et al. 2010; Lema et al. 2012). Similarly, resistance to race 6 has not been well studied. In contrast to resistances to race 1 and 4, resistances to race 5 and 6 have been more commonly identified in *B. oleracea* than other *Brassica* species (Vicente et al. 2002). The gene-for-gene Xcc race model proposed by Vicente et al. (2001) defines race 6 as lacking race-specific avirulence (Avr) genes and therefore screening for resistance against race 6 isolates could help detect resistances that are not race-specific (Taylor et al. 2002).

The aim of this study was to identify and characterise sources of black rot resistance in *Brassica* species to better inform vegetable *Brassica* (*B. oleracea*) breeding programmes. Based on previous studies, we hypothesised that resistance to important Xcc races is rare in *B. oleracea* and occurs more frequently in other brassica species. To identify resistances, we first screened DFFSs representing the species *B. oleracea* and *B. napus* with UK isolates of Xcc representing the most prevalent races 1, 4, 5 and 6. *Brassica napus* is an allotetraploid (genome AACC, $2n = 38$) that arose from a cross between *B. oleracea* (genome CC, $2n = 18$) and *B. rapa* (AA, $2n = 20$) and contains two subgenomes, one from each parent (U 1935); the *B. napus* DFFS was screened for resistance as it shares part of the genome with *B. oleracea* and could harbour useful sources of resistance that could be deployed in *B. oleracea*. From the same screen, the disease severity scores of different morphotypes of *B. oleracea* and *B. napus* DFFS were compared to determine whether certain morphotypes were more resistant/susceptible to Xcc than others. Finally, the spectrum of identified resistances in the *Brassica* DFFS was determined by screening a subset of lines against an additional 10 Xcc isolates representing a wider diversity of the pathogen ascertained from phylogenomic analyses conducted in this study. This study identified several useful sources of race-specific, clade-specific and potentially broad-spectrum resistances in brassicas.

2 | Materials and Methods

2.1 | Plant Lines and Cultivation

A total of 140 *B. oleracea* and 189 *B. napus* DFFS lines were screened for resistance to Xcc (Tables S1 and S2). These lines are kept at the UK Vegetable Genebank (University of Warwick, UK) (additional information available at <https://warwick.ac.uk/fac/sci/lifesci/wcc/genebank/collections/specialist>). Alongside the DFFS lines, as a control, we screened *Brassica* lines used for Xcc race determination: Wirosa (*B. oleracea*, Savoy cabbage, F_1 hybrid), Miracle (*B. oleracea*, cauliflower, F_1 hybrid), SxD1 (*B. oleracea*, doubled-haploid derived from cross between a cabbage and a rapid cycling line), FBLM1 (*B. juncea*, mustard, doubled-haploid), Just Right Turnip (*B. rapa*, turnip, F_1 hybrid) and COB60 (*B. napus*, oilseed rape, doubled-haploid)

(Vicente et al. 2001; Vicente and Holub 2013). For each of the DFFS and control lines, two or three seeds were sown into a single FP7 (7×7×8 cm) pot containing Levington M2 compost and cultivated in a glasshouse compartment at 20°C±2°C, supplemented with 16 h of light.

2.2 | Plant Inoculation Assays and Disease Scoring

Four- to five-week-old plants were inoculated following Taylor et al. (2002). Briefly, Xcc isolates of race 1 (WHRI 10018A, WHRI 6087, 6022A and 3843B), race 4 (WHRI 8978, WHRI 6367, 1279A and 3820A), race 5 (WHRI 8892A, WHRI 6382 and 3880) or race 6 (WHRI 8497, WHRI 6185 and 8806), were streaked onto King's B medium (King et al. 1954) and incubated at 28°C for 48 h prior to inoculation. Half a 10 µL inoculation loop of bacterial growth was scraped from the plate and mixed with 10 mL of sterile tap water to give a final suspension of OD₆₀₀=0.2 (corresponding to 2×10⁸ CFU/mL). Plants were inoculated by clipping secondary veins close to the leaf margin with mouse-toothed forceps wrapped in cotton wool and dipped in inoculum. The three youngest leaves were inoculated per plant and two or three plants were inoculated per DFFS line. Inoculated plants were kept in a glasshouse compartment at 20°C±2°C, supplemented with 16 h of light and watered every 2–3 days using a watering can to avoid splash.

Symptoms of infection were scored 2 and 3 weeks after inoculation. For each inoculated leaf, three metrics were scored: number of inoculation points, number of symptomatic inoculation points, and the relative size of the largest lesion (LS) on a scale of 0–9 (Table 1). Then for each individual plant the proportion of symptomatic inoculation points (PIP) was calculated and the average LS from the scores of up to three inoculated leaves per plant. A disease index (DI) score of 0–3 was then calculated for each plant from the scores obtained 2 weeks after inoculation, as after 3 weeks most of the leaves had dropped and were unsuitable for scoring. DI scores were calculated using the following formula:

TABLE 1 | Description of lesion size scores for *Brassica* leaves following clip inoculation with *Xanthomonas campestris* pv. *campestris*.

Lesion size score	Description
0	No lesion
1	Small lesion encircling the inoculation point
3	Lesion that reaches 25% of the way to the midvein from the inoculation point
5	Lesion that reaches 50% of the way to the midvein from the inoculation point
7	Lesion that reaches 75% of the way to the midvein from the inoculation point
9	Lesion stretching from the inoculation point to the midvein

Note: Adapted from Vicente et al. (2002).

$$\text{Disease index (DI)} = \text{PIP} \times \left(\frac{\text{average LS}}{3} \right).$$

DI scores for plants of the same DFFS line were then averaged to give a DI±standard deviation for each of the DFFS lines. These lines were then scored as resistant (R) if 0.00<DI≤0.10, partially resistant (PR) if 0.10<DI≤0.75 or susceptible (S) if 0.75<DI≤3.0 (Figure 1A) (Taylor et al. 2002). Where there was only one plant (due to poor germination) per DFFS line, the DI was not calculated, and resistance status was considered 'undetermined'.

2.3 | Experimental Design

In screen 1, 140 *B. oleracea* DFFS lines were inoculated in two batches (Batch 1: Bol1–Bol70 and Batch 2: Bol71–Bol140); 189 *B. napus* DFFS lines were inoculated in three batches (Batch 3: Bna1–Bna70; Batch 4: Bna71–Bna140; Batch 5: Bna141–Bna189) alongside controls with one of four Xcc isolates, race 1 (WHRI 10018A), race 4 (WHRI 8978), race 5 (WHRI 8892A) or race 6 (WHRI 8497). These isolates were obtained from the UK between years 2008 and 2019 and their race type was determined as part of a previous unpublished study (Table S3). Due to space limitations in the glasshouse, each batch of plants was then inoculated in two sequential sub-batches (Sub-batch 1: with race 1 or 4 in parallel and Sub-batch 2: with the race 5 or 6 in parallel). After inoculation plants were positioned on benches ~15 cm apart ordered from lowest to highest Bol/Bna number. Plants inoculated with different isolates were kept on separate glasshouse benches. The DI scores obtained from screen 1 were used to determine the resistance status of DFFS lines as well as to carry out morphotype analysis (see Section 2.4).

In screen 2, a subset of DFFS lines identified from screen 1 as having resistance to two or more of the UK isolates was retested against these isolates and a further 10 isolates representing the genetic diversity of race 1 (WHRI 6087, 6022A and 3843B), race 4 (WHRI 6367, 1279A and 3820A), race 5 (WHRI 6382 and 3880) and race 6 (WHRI 6185 and 8806) (Table S3).

2.4 | Morphotype Analysis

Using the DI scores obtained from screen 1 (see Section 2.3) a second analysis was carried out to determine if some morphotypes were more resistant or susceptible than others to each of four Xcc isolates, (race 1 WHRI 10018A; race 4 WHRI 8978; race 5 WHRI 8892A; race 6 WHRI 8497). For this, DFFS lines were grouped by morphotype (detailed in Tables S1 and S2) and statistical differences in DI scores (from screen 1) between morphotypes were determined for each of the DFFSs. Levene's and Shapiro–Wilk tests were first used to test for homogeneity of variance and normality of DI by morphotype, respectively (Levene 1960; Shapiro and Wilk 1965). A Kruskal–Wallis test followed by multiple pairwise comparisons with Bonferroni correction was then performed to determine statistical differences in the distribution of DI scores between morphotypes (Bonferroni 1936; Kruskal and Wallis 1952) for each of the four Xcc isolates tested. Statistical analyses were conducted in IBM SPSS Statistics v. 25.

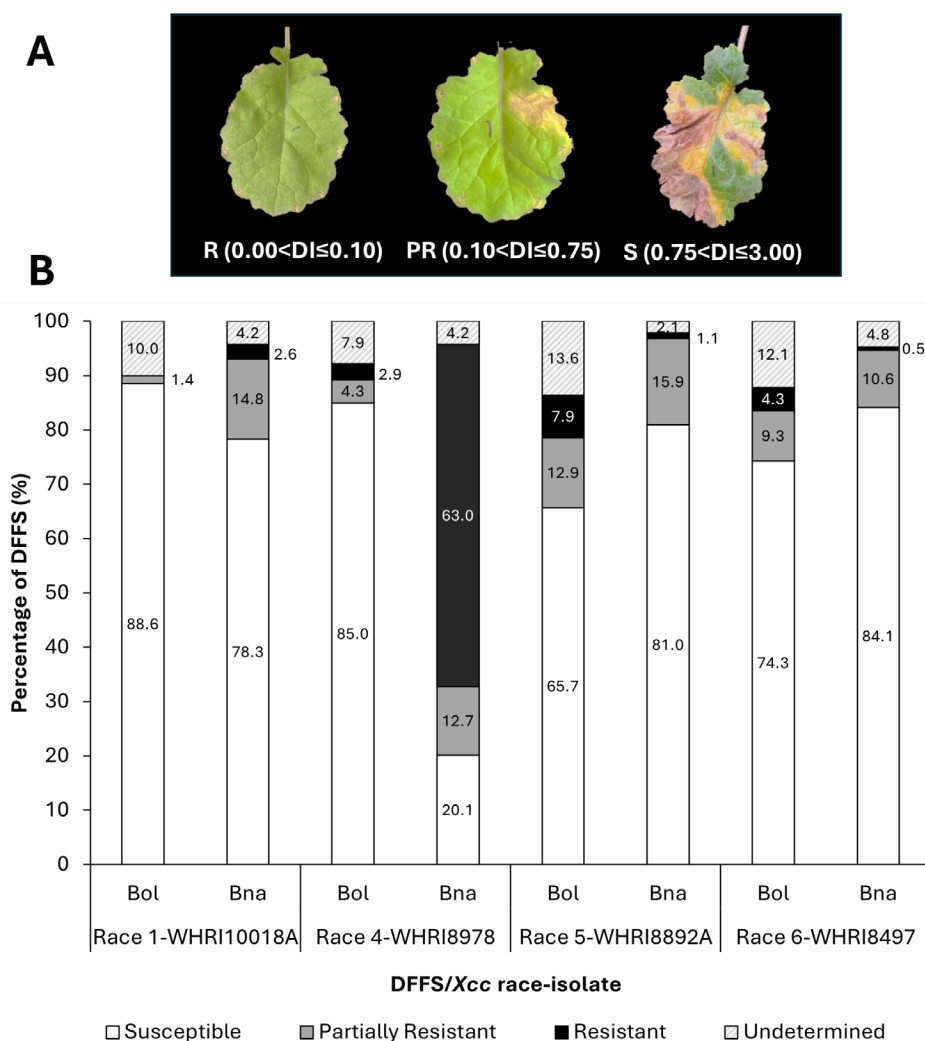


FIGURE 1 | (A) Example of phenotypes of resistant (R), partially resistant (PR) and susceptible (S) responses of *Brassica* diversity fixed foundation set (DFFS) lines 2 weeks after clip inoculation with isolates of *Xanthomonas campestris* pv. *campestris* (Xcc). (B) Percentage of lines from the *Brassica oleracea* (Bol, $n = 140$ lines) and *Brassica napus* (Bna, $n = 189$ lines) DFFSs that are susceptible (white), partially resistant (grey) or resistant (black) to single isolates of races 1, 4, 5 and 6; resistance status was not determined (grey crosshatch) when there was only one plant tested (due to poor germination).

2.5 | Genome Sequencing and Assembly

Genome sequences were generated for each of the 14 Xcc isolates used in this study, plus *X. campestris* pv. *raphani* NCPPB 1946 as an outgroup. Bacteria were cultured in King's B liquid medium at 28°C as described in protocols.io at <https://doi.org/10.17504/protocols.io.ewov1nr92gr2/v1> and genomic DNA was extracted from frozen bacterial pellets using the Qiagen MagAttract HMW DNA Kit (see <https://doi.org/10.17504/protocols.io.5jyl89428v2w/v1>). For all 15 isolates, paired, short reads were generated using the Illumina NovaSeq 6000 platform. For WHRI 10018A, WHRI 8978, WHRI 8892A and WHRI 8497, additional long-read sequencing was conducted using the Oxford Nanopore MinION platform. Short and long read sequences have been deposited in the Sequence Read Archive (SRA) (Leinonen et al. 2011).

SPAdes v. 3.15.1 was used for short-read genome assembly, as described in protocols.io at <https://doi.org/10.17504/protocols.io.kxygxzrqzv8j/v>.

The SPAdes command line was 'spades.py -careful -1 reads_file_1.fq.gz -2 reads_file_2.fq.gz -o output_directory'. The Unicycler v. 0.5.1 pipeline (Wick et al. 2017a, 2017b), a wrapper for SPAdes v. 4.0.0 (Bankevich et al. 2012), Flye 2.9.4-b1799 (Kolmogorov et al. 2019) and Racon v. 1.5.0 (Vaser et al. 2017) was used for hybrid assembly of combined short and long reads, following the 'short-read-first hybrid assembly' protocol described at <https://github.com/rrwick/Unicycler?tab=readme-ov-file#method-hybrid-assembly> (Wick et al. 2017a, 2017b).

2.6 | Phylogenomic Analysis

A phylogenomic tree was generated from genome assemblies using PhaME (Shakya et al. 2020), with FastTree 2 (Price et al. 2010) as its tree-building tool, which uses the generalised time-reversible model (Tavaré 1986). To assist in rooting the

tree, the genome of the pathotype strain of *X. campestris* pv. *raphani*, NCPB 1946 (Young et al. 2004), was included as an outgroup. For the FastTree 2 phylogenetic tree, 1000 bootstraps were performed. All command lines and configuration files are available via GitHub at <https://github.com/davidjstudholme/greer-et-al-xcc-phylogeny> and via Zenodo at <https://doi.org/10.5281/zenodo.15713394>. The resulting phylogenetic tree was rendered as graphics using the web-based Interactive Tree of Life (iTOL) v. 7.2 (Letunic and Bork 2021). For accession numbers of the genome sequences included in the tree see Table S3. These genome sequences were chosen because they had previously been assigned to one of the clades defined by Guy et al. (2013) and/or they had previously been assigned to a race (Vicente et al. 2001, unpublished).

3 | Results

3.1 | Identification of Resistance to Xcc in the *B. oleracea* and *B. napus* DFFSs

We first sought to identify resistance to Xcc isolates of prevalent races 1, 4, 5 and 6 in the *Brassica* DFFSs. Results for *B. oleracea* and *B. napus* are presented in Tables S1 and S2, respectively, and in Figure 1B for both species.

We identified only partial resistance ($0.10 < DI \leq 0.75$) to Xcc race 1 isolate WHRI 10018A in the *B. oleracea* DFFS (1.4% lines) in lines Bol52 (kale) and Bol120 (cauliflower). In the *B. napus* DFFS we identified both resistance ($DI \leq 0.10$) (2.6% lines) and partial resistance (14.8% lines) to this isolate. Resistance was identified in spring oilseed rape (SOSR; Bna10, Bna30 and Bna49), swede (Bna139) and winter oilseed rape (WOSR; Bna131). Partial resistance was found in these morphotypes as well as in forage rape (Bna118, Bna130 and Bna150), fodder rape (Bna106) and kale rape (Bna135 and Bna147).

Resistance to Xcc race 4 isolate WHRI 8978 was rare in the *B. oleracea* DFFS (2.9% lines), identified in four lines: Bol3 and Bol9 (broccoli), Bol14 (cabbage) and Bol18 (kale). Conversely, resistance to this isolate was exceptionally common in the *B. napus* DFFS (119 lines, 63.0%) and was identified in all *B. napus* morphotypes, for example, fodder rape (Bna88, Bna106 and Bna126), forage rape (Bna90, Bna118, Bna130, etc.), kale rape (Bna85, Bna121, Bna135, etc.), SOSR (Bna10, Bna16, Bna19, etc.), swede (Bna3, Bna32, Bna33, etc.) and WOSR (Bna6, Bna12, Bna15, etc.). Furthermore, partial resistance to WHRI 8978 was also less common in *B. oleracea* (six lines, 4.3%) than in *B. napus* (24 lines, 12.7%). Partial resistance was identified in cauliflower (Bol2 and Bol55), broccoli (Bol12), kale (Bol60 and Bol105) and ornamental cabbage ('other unknown' Bol91) morphotypes of *B. oleracea*. Partial resistance was identified in all *B. napus* morphotypes except fodder rape.

Unlike resistance to the Xcc race 1 and 4 isolates, we found that resistance to Xcc race 5 isolate WHRI 8892A was more common in *B. oleracea* DFFS (11 lines, 7.9%) than in *B. napus* DFFS (two lines, 1.1%). However, we found that partial resistance to this isolate was more common in the *B. napus* DFFS (30 lines, 15.9%) than the *B. oleracea* DFFS (18 lines, 12.9%). Resistance

to the race 5 isolate WHRI 8892A was identified in all *B. oleracea* morphotypes; it was identified in cabbage (Bol69), calabrese (Bol122), cauliflower (Bol33, Bol77 and Bol93), kale (Bol60, Bol61, Bol105 and Bol112), kohlrabi (Bol70) and tronchuda (Bol16) with partial resistance also identified in these morphotypes as well as in broccoli (Bol12, Bol13 and Bol126) and Brussels sprouts (Bol48 and Bol50). Resistance to WHRI 8892A was only identified in *B. napus* SOSR (Bna165) and WOSR (Bna138), but partial resistance was identified in all morphotypes except fodder rape.

Similar to the Xcc race 5 isolate, we found that resistance to the Xcc race 6 isolate WHRI 8497 was more common in the *B. oleracea* DFFS (six lines, 4.3%) than in the *B. napus* DFFS (one line, 0.5%) but that partial resistance was equally as common in the *B. napus* DFFS (20 lines, 10.6%) and the *B. oleracea* DFFS (13 lines, 9.3%). Resistance to the race 6 isolate WHRI 8497 was identified in all *B. oleracea* morphotypes except calabrese; resistance was identified in Brussels sprouts (Bol48), cabbage (Bol42), cauliflower (Bol44, Bol46, Bol47 and Bol130) and partial resistance was present in most morphotypes, except Brussels sprouts and cauliflower. The *B. napus* line with resistance to WHRI 8497 was a SOSR (Bna165) and partial resistance was identified in all *B. napus* morphotypes except fodder rape.

None of the *B. oleracea* and the *B. napus* DFFS lines segregated for resistance/susceptibility to any of the four Xcc races tested. This was expected for the *B. napus* DFFS as alleles within each line had been fixed by double-haploid production or several rounds of SSD (Table S2). However, as some of the *B. oleracea* DFFS lines had only undergone one to four rounds of SSD (Table S1), there was a possibility that some of these lines could segregate for resistance to Xcc, but that was not observed. Generally, there was a greater variability in DI scores between individuals of the same DFFS line when they had an intermediate response to Xcc ($0.1 < DI < 2$), compared to when they had a strong response, for example, resistant ($DI \leq 0.1$) or susceptible ($DI \geq 2$) (Tables S1 and S2).

3.2 | Xcc Resistance/Susceptibility Is Associated With *B. napus* Morphotype but Not *B. oleracea* Morphotype

We next investigated whether resistance ($DI \leq 0.75$)/susceptibility ($DI > 0.75$) to the four Xcc isolates representing races 1, 4, 5 and 6 was associated with crop morphotype. We found disease index (DI) was not significantly associated with morphotype in the *B. oleracea* DFFS when challenged with race 1 WHRI 10018A ($p = 0.673$), race 4 WHRI 8978 ($p = 0.205$), race 5 WHRI 8892A ($p = 0.349$) or race 6 WHRI 8497 ($p = 0.274$) (Figure S1 and Table S4) and therefore resistance/susceptibility was not associated with *B. oleracea* morphotype. Conversely, DI was significantly associated with morphotype in the *B. napus* DFFS when challenged with all isolates: race 1 WHRI 10018A ($p = 0.015$), race 4 WHRI 8978 ($p = 0.007$), race 5 WHRI 8892A ($p = 0.010$) or race 6 WHRI 8497 ($p = 0.002$) (Table S4 and Figure S2). For most isolates tested, swede was identified as more susceptible than other *B. napus* morphotypes. For race 1 WHRI 10018A, swede (mean rank = 114.50, $n = 32$) was

significantly more susceptible than winter oilseed rape (WOSR) (mean rank = 77.47, $n = 64$), $p = 0.023$. For race 5 WHRI 8892A, swede (mean rank = 116.32, $n = 33$) was significantly more susceptible than kale rape (mean rank = 50.78, $n = 9$), $p = 0.024$. Finally for race 6 WHRI 8497, swede (mean rank = 122.89, $n = 32$) was significantly more susceptible than WOSR (mean rank = 81.14, $n = 63$), $p = 0.005$ and the ‘Other/Unknown’ morphotype category (mean rank = 69.09, $n = 16$), $p = 0.016$. For race 4 WHRI 8978, swede was not a more susceptible morphotype, instead the ‘Other/Unknown’ morphotype category (mean rank = 123.56, $n = 17$) was significantly more susceptible than WOSR (mean rank = 78.80, $n = 64$), $p = 0.008$.

3.3 | DFFS Lines With Resistance to Multiple Xcc Races

Ten *B. oleracea* and 67 *B. napus* lines had resistance (resistant or partially resistant) to two or more of the Xcc isolates WHRI 10018A (race 1), WHRI 8978 (race 4), WHRI 8892A (race 5) and WHRI 8497 (race 6) (Table 2). The morphotypes of *B. oleracea* lines with resistance to multiple isolates were mainly kale (three lines) but also broccoli (two lines), kohlrabi (two lines), Brussels sprouts (one line), cabbage (one line) and tronchuda (one line). The 67 *B. napus* lines with resistance to multiple isolates represented all morphotypes, WOSR (24 lines), SOSR (18 lines),

TABLE 2 | Lines from the *Brassica oleracea* (Bol) and *Brassica napus* (Bna) diversity fixed foundation sets (DFFSs) that have resistance (R) or partial resistance (PR) to multiple isolates of *Xanthomonas campestris* pv. *campestris* belonging to four different races.

Race combination	Resistance to ^a				DFFS lines	No. of lines	
	R1	R4	R5	R6		Bol	Bna
R1 and R4	R	R	—	—	Bna: 10, 30, 49 and 131	0	25
	R	PR	—	—	Bna: 139		
	PR	R	—	—	Bna: 15, 21, 38, 57, 66, 74, 82, 97, 106, 107, 118, 122, 130, 135, 169 and 170		
	PR	PR	—	—	Bna: 27, 125, 136 and 147		
R1 and R5	—	—	—	—	—	0	0
R1 and R6	—	—	—	—	—	0	0
R4 and R5	—	R	PR	—	Bol: 14	4	20
					Bna: 12, 22, 32, 53, 58, 115, 121, 127, 129, 133, 134, 149, 151, 166, 167, 186 and 187		
	—	PR	R	—	Bol: 60 and 105		
	—	PR	PR	—	Bol: 12 Bna: 14, 173 and 180		
R4 and R6	—	R	—	PR	Bol: 3	1	13
					Bna: 64, 104, 108, 109, 111, 117, 141, 145, 160 and 185		
R5 and R6	—	PR	—	PR	Bna: 9, 71, and 156		
	—	—	R	PR	Bol: 70	5	1
	—	—	PR	R	Bol: 48		
	—	—	PR	PR	Bol: 15, 17 and 62 Bna: 52		
R1, R4 and R5	PR	R	R	—	Bna: 138	0	3
	PR	R	PR	—	Bna: 61 and 153		
R1, R4 and R6	PR	R	—	PR	Bna: 73	0	1
R1, R5 and R6	—	—	—	—	—	0	0
R4, R5 and R6	—	R	R	R	Bna: 165	0	3
	—	R	PR	PR	Bna: 148		
	—	PR	PR	PR	Bna: 155		
R1, R4, R5 and R6	PR	R	PR	PR	Bna: 150	0	1
Total						10	67

^aR1, race 1 WHRI 10018A; R4, race 4 WHRI 8978; R5, race 5 WHRI 8892A; R6, race 6 WHRI 8497 (Table S3).

swede (eight lines), kale rape (seven lines), other/unknown (five lines), forage rape (four lines) and fodder rape (one line).

We did not find any *B. oleracea* lines with resistance to both race 1 and 4 isolates, but five lines had resistance to both race 5 and 6 isolates (Table 2). Conversely, 24 *B. napus* lines had resistance to both race 1 and 4 isolates but only one *B. napus* line has resistance to the race 5 and 6 isolates. Resistance to both race 1 and 5 isolates or both race 1 and 6 isolates was not found in either *B. oleracea* or *B. napus*. Resistance to both race 4 and 5 isolates was found in four *B. oleracea* lines and in 17 *B. napus* lines. Finally, resistance to both race 4 and 6 isolates was found in one *B. oleracea* broccoli line and in 13 *B. napus* lines.

None of the *B. oleracea* lines had resistance to more than two of the four Xcc isolates. However, seven *B. napus* lines had resistance to three of the four Xcc isolates. Of these, three of these lines had resistance to the race 1, 4 and 5 isolates (SOSR: Bna61, WOSR: Bna138 and Bna153) and one of the lines to races 1, 4 and 6 (WOSR: Bna73) (Table 2). The remaining three *B. napus* lines had resistance to the race 4, 5 and 6 isolates (kale rape: Bna148, WOSR: Bna155 and SOSR: Bna165). No *Brassica* DFFS lines has resistance to race 1, 5 and 6 isolates. A single *B. napus* forage rape line (Bna150), was found to have resistance to all four isolates, with resistance to the race 4 isolate, and partial resistance to race 1, 5 and 6 isolates.

3.4 | Defining the Spectrum of Resistances Identified in the *Brassica* DFFSs

A subset of the *B. oleracea* and *B. napus* lines with resistance to multiple Xcc isolates was taken forward for further characterisation against additional genetically diverse isolates of the same four races 1, 4, 5 and 6 to determine the spectrum of resistance/susceptibility, for example, race-specific, clade-specific or broad spectrum (hereafter referred to as screen 2).

For the four Xcc isolates used in the initial screen of the *Brassica* DFFSs (hereafter referred to as screen 1) chromosome-level genome assemblies were generated. We generated contig-level genome assemblies for another 10 Xcc isolates that were used in screen 2 to test a subset of resistant DFFS lines. We also sequenced the genome of *X. campestris* pv. *raphani* NCPPB 1946, which was included as an outgroup for the phylogenomic tree. SRA accession numbers for the sequence reads and GenBank accession numbers for the assemblies are listed in Table S5.

Our phylogenetic analysis indicates that some of the most important Xcc races are paraphyletic (Figure 2), as previously shown by Guy et al. (2013) and Xcc isolates of the same race can belong to different clades. For example, Xcc race 6 isolates belonged to clades A, D and F. Furthermore, isolates that are genetically very similar can belong to different races. For example, WHRI 6022A (race 1), WHRI 3843B (race 1), 8004 (race 9), ATCC33913 (race 3) belong to different races but all belong to clade C (Figure 2). Our analysis showed that isolates from races 1, 4 and 6 can be found in at least three separate clades for each race, but isolates of race 5 (including WHRI 8892A) belonged to the single clade E. Among the isolates used in screen 1, three isolates belonged to the same clade A, despite belonging to different races: WHRI 10018A (race 1), WHRI 8978 (race 4) and WHRI 8497 (race 6).

In half of the *Brassica* DFFS lines (Bna12, Bna73, Bna131, Bna138 and Bna165) tested, resistance/susceptibility was consistent between screen 1 and screen 2 when challenged with WHRI 10018A, WHRI 8978, WHRI 8892A and WHRI 8497 (Table 3). For these lines resistance/susceptibility appeared to be race specific. For example, in screen 1, Bna131 was identified as being resistant to WHRI 10018A (race 1) and WHRI 8978 (race 4) and susceptible to WHRI 8892A (race 5) and WHRI 8497 (race 6) isolates and in screen 2, this line was resistant to all race 1 and 4 isolates and susceptible to all the races 5 and 6 isolates tested. For the other six *Brassica* DFFS lines tested resistance was not consistent for the four isolates used in both screens 1 and 2. This included all the *B. oleracea* lines tested (Bol60 and Bol62) and three *B. napus* lines (Bna52, Bna104 and Bna150). Where resistance was not consistent between the screens, lines were identified as having partial resistance ($0.10 < DI \leq 0.75$) in screen 1 and then susceptibility ($DI > 0.75$) in screen 2. The lines were then also susceptible to the additional Xcc isolates tested for that race. For example, Bol60 was identified as having partial resistance ($DI = 0.60$) to race 4 WHRI 8978 in screen 1 but was susceptible ($DI = 1.65$) to this same isolate in screen 2. Bol60 was then also susceptible to the other three race 4 isolates tested. However, resistance to race 5 WHRI 8892A in Bol60 was consistent between screen 1 ($DI = 0.00$) and screen 2 ($DI = 0.00$) and Bol 60 also had resistance to the two other race 5 isolates tested WHRI 6382 and WHRI 3880. In general, resistance was more stable than partial resistance across screens.

Interestingly, the *B. napus* DFFS lines (Bna104, Bna150 and Bna165) displayed clade-specific resistance/susceptibility instead of, or as well as, race-specific resistance/susceptibility (Table 3). For example, Bna104 had resistance to all race 4 isolates tested (race-specific) and partial resistance to half of the race 1 isolates tested (clade-specific). Bna104 was resistant to race 1 isolates WHRI 6022A and WHRI 3843B, which both belonged to clade C of the study by Guy et al. (2013), but was susceptible to WHRI 10018A that belonged to clade A as well as WHRI 6087, which belonged to an unassigned clade.

From screen 1, Bna150 was identified as having potential broad-spectrum resistance to the four races. However, in screen 2 only the resistance to race 1 WHRI 10018A and race 4 WHRI 8978 was reproducible and susceptibility to race 5 WHRI 8892A and race 6 WHRI 8497 was observed (Table 3).

Bna73, Bna138 and Bna165 displayed potential broad-spectrum resistance that was effective against most of the 14 Xcc isolates they were challenged with (Table 3). Bna73 is a WOSR that showed resistance to all except race 5 isolates. Bna138 is a WOSR that showed resistance to all except race 6 isolates. Bna165 is a SOSR line that showed resistance to all isolates except race 1 WHRI 10018A, which belonged to Guy et al. (2013) clade A.

4 | Discussion

Overall, in our first screening we identified resistance (resistant and partial) in the *B. oleracea* and *B. napus* DFFSs to four Xcc isolates representing the most prevalent races (1, 4, 5 and 6).

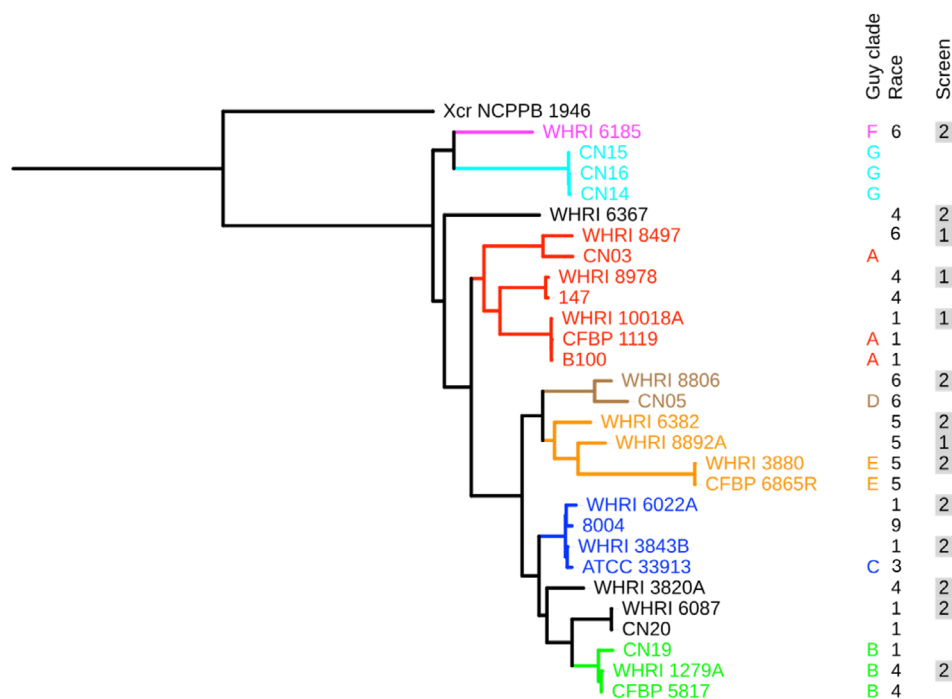


FIGURE 2 | Phylogenetic positions of the *Xanthomonas campestris* strains used for resistance screens 1 and 2 of the *Brassica* diversity fixed foundation sets (DFFS). The maximum-likelihood phylogenetic tree includes representatives of the colour-coded clades A–G previously described by Guy et al. (2013). Accession numbers for the genomes are provided in Table S3. Where known, races are indicated as described by Vicente et al. (2001). The tree is based on core genome sequences generated using PhaME (Shakya et al. 2020) and FastTree 2 (Price et al. 2010). The tree was graphically rendered using the Interactive Tree of Life (iTOL) v. 7.2 (Letunic and Bork 2021).

We found that race 1 and race 4 resistance is rare in *B. oleracea* (diploid genome CC) but more common in *B. napus* (allotetraploid genome AACC), particularly resistance to race 4. This finding suggests that resistance to these races originate from the *Brassica* A genome. Other studies have also proposed that resistances to races 1 and 4 probably originates from the *Brassica* A genome (and also the B genome) (Griffiths et al. 2009; Singh et al. 2018; Tonguç and Griffiths 2004; Vicente et al. 2002). It has also been reported that Xcc races 1 and 4 are more prevalent in *B. oleracea*, whereas races 5 and 6 are more common in *B. napus* (Guy et al. 2013; Vicente and Holub 2013). The rarity of resistance to Xcc races 1 and 4 in *B. oleracea* could explain why these races are more prevalent in this species. In contrast, the presence of resistance to races 1 and 4 in *B. napus* explains why they are not the prevalent races in *B. napus*. Furthermore, resistance to the race 5 and race 6 isolates was more commonly identified in *B. oleracea* than in *B. napus*. This is most likely to be as a result of resistance genes specific to the *B. oleracea* C genome that are absent from the *B. napus* C genome, either because they were not present in the ancestral lines that hybridised to form *B. napus* as a species (Chalhoub et al. 2014) or that they have been inadvertently lost as a result of extensive breeding programmes in *B. napus* aimed at improving oil quality. Xcc races are defined by *Brassica* resistance gene (*R*-genes) and Xcc avirulence (*Avr*) gene interactions (Vicente et al. 2001). As *R*-gene complements differ between *Brassica* species (Tirnaz et al. 2020), this most likely drives the evolution of Xcc races that are adapted to different host species.

Importantly, a total of 10 *B. oleracea* and 67 *B. napus* lines were identified as having resistance to more than one Xcc isolate/race

(Table 2). Resistance to both races 1 and 4 was not identified in *B. oleracea* but was identified in over 20 *B. napus* lines. Resistance to all four isolates was only found in one forage *B. napus* line Bna150, but this line's partial resistance to the races 5 and 6 isolates was not reproducible in the second screen. Similarly other partial resistances identified in screen 1, particularly in *B. oleracea* (Bol60 and Bol62), were also not reproducible in screen 2. The contrasting results between screens for some lines that probably have variable levels of partial resistance might be due to slight differences in conditions or level of plant development, as despite using standard glasshouse controls that include heating and venting and supplementary lights, the glasshouse temperature and light conditions showed moderate fluctuations depending on outside weather. Although partial resistances did not hold up between screens, they could still be useful in a field setting, as in this study inoculum pressure was very high, and inoculations were performed through marginal wounds; the level of inoculum in field conditions is generally much lower and the most common mode of entry will be through hydathodes (Cook et al. 1952; Hugouvieux et al. 1998) and therefore partial resistances might still be useful in field conditions. Conversely, resistance was reproducible between screens. Typically, resistance is mediated by more robust *R*-genes that are less influenced by environmental conditions than those conferred by partial resistance. These do, however, exert a stronger evolutionary pressure on pathogens, which can lead to resistance breaking down as well as tending to be race-specific (Gou et al. 2023).

We investigated whether Xcc resistance was linked to crop morphotype to help inform breeding programmes. To the best of our knowledge, this is the first example of such a study. In

TABLE 3 | Disease index (DI) scores and resistance status of lines from the *Brassica oleracea* (Bol) and *Brassica napus* (Bna) diversity fixed foundation sets (DFFSs) when clip inoculated with genetically diverse isolates of four races of *Xanthomonas campestris* pv. *campestris*.

Race/Clade/Isolate ^d																
Race 1					Race 4					Race 5			Race 6			
A		-	C	C	A	-	B	-	E	E	E	A	F	D		
10018A	6087	6022A	3843B	8978	6367	1279A	3820A	8892A	6382	3880	8497	6185	8806			
Bna131 (SOSR)	R1 and R4	1	0.03	—	—	—	—	—	1.08	—	—	2.14	—	—		
		2	0.38	0.06	0.02	0.06	0.00	0.00	2.49	1.88	2.05	2.18	21.92	2.71		
Bna12 (WOSR)	R4 and R5	1	1.03	—	—	—	—	—	0.40	—	—	2.49	—	—		
		2	1.69	0.90	1.32	1.23	0.16	0.24	0.40	0.07	0.22	0.03	0.31	2.90	1.74	2.71
Bol60 (kale)		1	2.36	—	—	—	—	0.68	—	—	—	1.35	—	—		
		2	1.78	1.14	1.55	1.64	1.65	1.19	1.63	0.84	0.00	0.00	0.00	3.00	1.18	2.53
Bna104 (SOSR)	R4 and R6	1	1.45	—	—	—	—	0.00	—	—	—	0.30	—	—		
		2	1.22	0.89	0.44	0.27	0.00	0.05	0.05	0.00	2.03	2.43	2.89	2.73	0.95	2.66
Bna52 (WOSR)	R5 and R6	1	1.56	—	—	—	—	0.96	—	—	—	0.49	—	—		
		2	1.52	1.28	0.58	1.03	0.21	0.66	1.48	1.67	1.80	1.79	2.04	1.61	0.76	2.26
Bol62 (kale)		1	2.61	—	—	—	—	1.51	—	—	—	0.56	—	—		
		2	3.00	3.00	3.00	2.14	1.67	2.70	3.00	3.00	2.78	2.03	1.55	2.50	2.18	3.00
Bna138 (WOSR)	R1, R4 and R5	1	0.64	—	—	—	—	0.03	—	—	—	1.12	—	—		
		2	0.38	0.01	0.00	0.05	0.02	0.01	0.02	0.02	0.00	0.00	1.34	0.83	2.50	
Bna73 (WOSR)	R1, R4 and R6	1	0.15	—	—	—	—	0.00	—	—	—	0.32	—	—		
		2	0.43	0.22	0.26	0.03	0.00	0.00	0.00	0.00	2.03	1.16	1.67	0.25	0.11	0.15
Bna165 (SOSR)	R4, R5 and R6	1	1.43	—	—	—	—	0.00	—	—	—	0.04	—	—		
		2	1.71	0.00	0.03	0.43	0.00	0.01	0.09	0.00	0.07	0.02	0.00	0.13	0.03	0.36
Bna150 (forage)	R1, R4, R5 and R6	1	0.13	—	—	—	—	0.00	—	—	—	0.37	—	—		
		2	0.33	0.29	0.04	0.03	0.00	0.00	0.00	0.00	2.56	2.51	2.75	2.50	0.35	2.47

Note: Resistance status is highlighted according to DI scores where black = resistant 0.00 < DI ≤ 0.10, grey = partially resistant 0.10 < DI ≤ 0.75 and white = susceptible 0.75 < DI ≤ 3.00. White cells of the table containing “—” indicate isolates that were not tested in screen 1 and so DI scores are not available.

^aSOSR, spring oilseed rape; WOSR, winter oilseed rape.

^bR1, race 1 WHRI 10018A; R4, race 4 WHRI 8978; R5, race 5 WHRI 8892A; R6, race 6 WHRI 8497 based on screen 1 of the *B. napus* and *B. oleracea* DFFSs (Table S3).

^cScreen 1, initial screen of the *Brassica* DFFSs with four isolates representing races 1, 4, 5 and 6; Screen 2, screen with the same four isolates plus 10 additional isolates representing the diversity of the four races (Table S3).

^dClades described by Guy et al. (2013).

the *B. oleracea* DFFS, Xcc resistance was not linked to morphotype (broccoli, cabbage, calabrese, cauliflower, kale, kohlrabi, Brussels sprouts and troncchuda). However, in the *B. napus* DFFS swede was a significantly more susceptible morphotype than other morphotypes of the same species (fodder, forage, kale, spring and winter oilseed rape) when challenged with the initial race 1, 5 and 6 isolates. This suggests that the presence of susceptibility genes or the absence of resistance genes in this morphotype is responsible for the enhanced susceptibility to Xcc. Thus, swede represents a valuable resource to better understand Xcc–host interactions and engineer more resistant/tolerant *Brassica* crop types. Susceptibility genes to *Xanthomonas* pathogens of other crops have already been identified as targets for breeding, for example, *SWEET11-13*, *OsPIP3* and *OsImpα1a* in rice are utilised by the blast pathogen *Xanthomonas oryzae* pv. *oryzae* (Koseoglou et al. 2022). One of the limitations to our study was that in both DFFSs some of the morphotypes were not equally represented. For example, the *B. napus* fodder morphotype comprised only 2.6% (five lines) of the DFFS, whereas other morphotypes such as winter oilseed rape represented 34.9% (66 lines). However, by design the DFFS used in this study captured the genetic diversity of > 6000 *Brassica* accessions and are largely proportional to the popularity of crop morphotypes grown.

Our analysis has expanded the previous study by Guy et al. (2013) and has shown that Xcc isolates of the same race can be genetically diverse and belong to different phylogenetic clades. Guy et al. (2013) identified seven distinct clades (A–G) of Xcc and we have shown, using whole genome sequencing, that there is even more diversity in Xcc races as not all isolates fit in these clades. We have also shown that isolates of the same race can belong to more than one of these clades. For example, race 1 isolates can belong to clades A and C and race 6 isolates to clades A, D and F. This expands our knowledge on the known diversity of Xcc (Horta De Passo 2016; Jensen et al. 2010) and suggests that genetic determinants of race (Vicente et al. 2001) have been gained/lost multiple times so that races have convergently evolved. Understanding the genetic diversity of Xcc is necessary for breeding for broad-spectrum and durable resistance in *Brassica* as having resistance to just one race, or resistance to a single clade of a race will not give adequate black rot control.

By screening a subset of DFFS lines with isolates representing the diversity of four races of Xcc, we identified lines with race-specific (Bola60, Bna12 and Bna131), clade-specific (Bna104 and Bna150) and potential broad-spectrum (Bna73, Bna138 and Bna165) resistances. Race-specific resistance has been frequently reported, but this study has identified new sources of clade-specific and potentially broad-spectrum resistance to Xcc, both of which have been rarely identified in either *Brassica* (Soengas et al. 2007; Taylor et al. 2002) or the model plant *Arabidopsis thaliana* (Debieu et al. 2016). As a result of the diversity among a given race, DFFS lines with race-specific resistance(s) are likely to be recognising Avr factors in Xcc that underpin race (Vicente et al. 2001), whereas lines with clade-specific and broad-spectrum resistance could be recognising other more common Xcc factors not associated with race, for example, lipopolysaccharides or flagellin (Sun et al. 2006).

The *B. napus* line with the broadest resistance spectrum was Bna165, a spring oilseed rape line that had resistance to all

isolates tested except WHRI 10018A, which belonged to clade A (Guy et al. 2013). Given that resistance to race 4 was very common in the *B. napus* DFFS, we hypothesise that Bna165 possesses both this common race 4 resistance as well as a second resistance that recognises isolates of the other races, which may (or may not) also recognise race 4 isolates. Validation of this theory requires further inheritance and mapping studies. Nevertheless, this line provides a valuable genetic tool for further resistance characterisation alongside the four *B. napus* lines with race-specific resistance to the most prevalent and damaging races 1 and 4 (Bna73, Bna131, Bna138 and Bna150).

Our screening of the *B. oleracea* and *B. napus* DFFSs identified race-specific, clade-specific and potential broad-spectrum resistance to black rot, the most damaging bacterial disease of brassicas worldwide. These types of resistance can be used as a foundation for breeding programmes for environmentally friendly, sustainable and durable control of the disease.

Future work should focus on further characterisation of these types of resistance to determine modes of inheritance (e.g., dominant or recessive) and mechanisms of resistance. For this, genome-wide association studies (GWAS) or classical quantitative trait locus (QTL) mapping in populations segregating for the identified resistances could be conducted. These approaches would allow the identification of resistance-linked markers that could be used to accelerate introgression of identified resistance in commercial crop types by marker-assisted selection (MAS) (Ben-Ari and Lavi 2012). For the *B. napus* DFFS lines (genome AACC) it would also help identify the origins of the resistance, for example, from the *Brassica* A or C genome. Resistance originating from the *Brassica* C genome could be readily deployed in vegetable brassica crops where black rot is most damaging (*B. oleracea*, genome CC) through conventional breeding, but the types of resistance originating from the *Brassica* A genome would be more complex and may require the use of genetic modification/editing techniques. Resistant lines can also be crossed to stack different race-specific, clade-specific and broad-spectrum resistances. This would improve the spectrum of race-specific resistance, and if resistance mechanisms to the same race are different, could improve upon durability as it would make it more challenging for resistance-breaking isolates of Xcc to evolve.

Lastly, as resistance to the major Xcc races (1, 4, 5 and 6) is deployed in *Brassica* crops, other less prevalent races could become more prevalent. It will therefore be important to test identified types of resistance against less prevalent races of Xcc and to monitor for the emergence of new races that are not controlled by these resistances.

Author Contributions

Shannon F. Greer: conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, formal analysis, supervision. **Jamie Harrison:** investigation, writing – review and editing, visualization, validation, software, formal analysis. **Daisy Bown:** investigation, writing – review and editing. **Maria Serrano:** investigation, writing – review and editing. **Rana Muhammed Fraz Hussain:** investigation, writing – review and editing. **Srayan Ghosh:** investigation, writing – review and editing.

Graham R. Teakle: resources, writing – review and editing. **Vardis Ntoukakis:** conceptualization, writing – original draft, funding acquisition, supervision, project administration, writing – review and editing, validation. **David J. Studholme:** conceptualization, investigation, funding acquisition, writing – original draft, methodology, validation, visualization, writing – review and editing, software, formal analysis, supervision, project administration. **Joana G. Vicente:** conceptualization, investigation, funding acquisition, writing – original draft, methodology, validation, visualization, writing – review and editing, formal analysis, project administration, supervision, resources. **Murray Grant:** conceptualization, funding acquisition, writing – original draft, methodology, validation, visualization, writing – review and editing, formal analysis, project administration, supervision.

Acknowledgements

The authors gratefully acknowledge the support of Horticultural Services at the University of Warwick for plant care. We also acknowledge the use of the University of Exeter's Isca high-performance computing facility. Parts of this work were carried out under a Plant Health Scientific Authorisation 100305/199069 from the Department for Environment, Food and Rural Affairs (Defra) of the UK. This work was funded by Biotechnology and Biological Sciences Research Council (BBSRC), Natural Environment Research Council (NERC), Defra, and Scottish Government grants BB/T010908/1, BB/T010916/1 and BB/T010924/1, and was part of the Strategic Priorities Fund Plant Bacterial Diseases programme under the project 'Xanthomonas plants diseases: mitigating existing, emerging and future threats to UK agriculture'. Part of this work was also funded by additional Defra grants CH0110 and CH0112.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

- AHDB. 2023. "Xanthomonas Black Rot of Vegetable Brassicas." <https://horticulture.ahdb.org.uk/knowledge-library/xanthomonas-black-rot-of-vegetable-brassicas>.
- Bankevich, A., S. Nurk, D. Antipov, et al. 2012. "SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing." *Journal of Computational Biology* 19: 455–477.
- Ben-Ari, G., and U. Lavi. 2012. "Marker-Assisted Selection in Plant Breeding." In *Plant Biotechnology and Agriculture*, edited by A. Altman and P. Hasegawa, 163–184. Academic Press.
- Bonferroni, C. 1936. "Teoria statistica delle classi e calcolo delle probabilità." *Pubblicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze* 8: 3–62.
- CABI. 2022. "Xanthomonas campestris pv. campestris (Black Rot). CABI PlantwisePlus Knowledge Bank." <https://doi.org/10.1079/pwkb.species.56919>.
- Chalhoub, B., F. Denoeud, S. Liu, et al. 2014. "Early Allopolyploid Evolution in the Post-Neolithic *Brassica napus* Oilseed Genome." *Science* 345: 950–953.
- Cook, A. A., J. C. Walker, and R. H. Larson. 1952. "Studies on the Disease Cycle of Black Rot of Crucifers." *Phytopathology* 42: 162–167.
- Da Silva, A. L. B. R., J. S. Candian, E. R. Do Rego, T. Coolong, and B. Dutta. 2020. "Screening Cabbage Cultivars for Resistance to Black Rot Under Field Conditions." *HortTechnology* 30: 448–455.
- Debieu, M., C. Huard-Chauveau, A. Genissel, F. Roux, and D. Roby. 2016. "Quantitative Disease Resistance to the Bacterial Pathogen *Xanthomonas campestris* Involves an *Arabidopsis* Immune Receptor Pair and a Gene of Unknown Function." *Molecular Plant Pathology* 17: 510–520.
- Dey, S. S., K. Sharma, R. Bhatia Dey, et al. 2015. "Inter Specific Hybridization (*Brassica carinata* × *Brassica oleracea*) for Introgression of Black Rot Resistance Genes Into Indian Cauliflower (*B. oleracea* var. *botrytis* L.)." *Euphytica* 204: 149–162.
- Doullah, M. A. U., G. M. Mohsin, K. Ishikawa, H. Hori, and K. Okazaki. 2011. "Construction of a Linkage Map and QTL Analysis for Black Rot Resistance in *Brassica oleracea* L." *International Journal of Natural Sciences* 1: 1–6.
- Gou, M., P. Balint-Kurti, M. Xu, and Q. Yang. 2023. "Quantitative Disease Resistance: Multifaceted Players in Plant Defense." *Journal of Integrative Plant Biology* 65: 594–610.
- Greer, S. F., A. Surendran, M. Grant, and R. Lillywhite. 2023. "The Current Status, Challenges, and Future Perspectives for Managing Diseases of Brassicas." *Frontiers in Microbiology* 14: 1209258.
- Griesbach, E., H. Löptien, and U. Miersch. 2003. "Resistance to *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson in Cabbage *Brassica oleracea* L." *Journal of Plant Diseases and Protection* 110: 461–475.
- Griffiths, P. D., L. F. Marek, and L. D. Robertson. 2009. "Identification of Crucifer Accessions From the NC-7 and NE-9 Plant Introduction Collections That Are Resistant to Black Rot (*Xanthomonas campestris* pv. *campestris*) Races 1 and 4." *HortScience* 44: 284–288.
- Guy, E., A. Genissel, A. Hajri, et al. 2013. "Natural Genetic Variation of *Xanthomonas campestris* pv. *campestris* Pathogenicity on *Arabidopsis* Revealed by Association and Reverse Genetics." *mBio* 4: e00538-12.
- Haynes, E., C. Ramwell, T. Griffiths, D. Walker, and J. Smith. 2020. "Review of Antibiotic Use in Crops, Associated Risk of Antimicrobial Resistance and Research Gaps. Report to Department for Environment, Food and Rural Affairs (Defra) and the Food Standards Agency (FSA) FS301082."
- Hong, J. E., K. S. Afrin, M. A. Rahim, H. J. Jung, and I. S. Nou. 2021. "Inheritance of Black Rot Resistance and Development of Molecular Marker Linked to Xcc Races 6 and 7 Resistance in Cabbage." *Plants* 10: 1940.
- Horta De Passo, V. I. 2016. "Molecular Genetics of Interactions Between *Xanthomonas campestris* pv. *raphani* and *Arabidopsis thaliana*. [PhD thesis]. University of Warwick."
- Hugouvieux, V., C. E. Barber, and M. J. Daniels. 1998. "Entry of *Xanthomonas campestris* pv. *campestris* Into Hydathodes of *Arabidopsis thaliana* Leaves: A System for Studying Early Infection Events in Bacterial Pathogenesis." *Molecular Plant-Microbe Interactions* 11: 537–543.
- Iglesias-Bernabé, L., P. Madloo, V. M. Rodríguez, M. Francisco, and P. Soengas. 2019. "Dissecting Quantitative Resistance to *Xanthomonas campestris* pv. *campestris* in Leaves of *Brassica oleracea* by QTL Analysis." *Scientific Reports* 9: 2015.
- Jensen, B., J. G. Vicente, H. Manandhar, and S. Roberts. 2010. "Occurrence and Diversity of *Xanthomonas campestris* pv. *campestris* in Vegetable Brassica Fields in Nepal." *Plant Disease* 94: 298–305.
- Kifuji, Y., H. Hanzawa, Y. Terasawa, Ashutosh, and T. Nishio. 2013. "QTL Analysis of Black Rot Resistance in Cabbage Using Newly Developed EST-SNP Markers." *Euphytica* 190: 289–295.
- King, E. D., M. K. Ward, and D. E. Raney. 1954. "Two Simple Media for the Demonstration of Pyocyanin and Fluorescin." *Journal of Laboratory and Clinical Medicine* 44: 301–307.
- Kolmogorov, M., J. Yuan, Y. Lin, and P. A. Pevzner. 2019. "Assembly of Long, Error-Prone Reads Using Repeat Graphs." *Nature Biotechnology* 37: 540–546.

- Koseoglou, E., J. M. Van Der Wolf, R. G. F. Visser, and Y. Bai. 2022. "Susceptibility Reversed: Modified Plant Susceptibility Genes for Resistance to Bacteria." *Trends in Plant Science* 27: 69–79.
- Kruskal, W. H., and W. A. Wallis. 1952. "Use of Ranks in One-Criterion Variance Analysis." *Journal of the American Statistical Association* 47: 583–621.
- Leinonen, R., H. Sugawara, and M. Shumway. 2011. "The Sequence Read Archive." *Nucleic Acids Research* 39: D19–D21.
- Lema, M., M. E. Carrea, T. Sotelo, P. Velasco, and P. Soengas. 2012. "Discrimination of *Xanthomonas campestris* pv. *campestris* Races Among Strains From Northwestern Spain by *Brassica* spp. Genotypes and Rep-PCR." *European Journal of Plant Pathology* 133: 159–169.
- Letunic, I., and P. Bork. 2021. "Interactive Tree of Life (iTOL) v5: An Online Tool for Phylogenetic Tree Display and Annotation." *Nucleic Acids Research* 49: W293–W296.
- Levene, H. 1960. "Robust Tests for Equality of Variances." In *Contributions to Probability and Statistics*, edited by I. Olkin, 278–292. Stanford University Press.
- Massomo, S. M. S., R. B. Mabagala, I. S. Swai, J. Hockenhull, and C. N. Mortensen. 2004. "Evaluation of Varietal Resistance in Cabbage Against the Black Rot Pathogen, *Xanthomonas campestris* pv. *campestris* in Tanzania." *Crop Protection* 23: 315–325.
- Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. "FastTree 2—Approximately Maximum-Likelihood Trees for Large Alignments." *PLoS One* 5: e9490.
- Ramnarine, S. D. B. J., J. Jayaraman, and A. Ramsuhag. 2024. "Crucifer Lesion-Associated *Xanthomonas* Strains Show Multi-Resistance to Heavy Metals and Antibiotics." *Current Microbiology* 81: 136.
- Roberts, S. J., L. Branfield, and S. R. Payne. 2022. "Improved Management of Bacterial Diseases of Horticultural Crops. Agriculture and Horticulture Development Board CP191 Final Report."
- Roberts, S. J., J. Brough, and P. J. Hunter. 2007. "Modelling the Spread of *Xanthomonas campestris* pv. *campestris* in Module-Raised Brassica Transplants." *Plant Pathology* 56: 391–401.
- Shakya, M., S. A. Ahmed, K. W. Davenport, M. C. Flynn, C.-C. Lo, and P. S. G. Chain. 2020. "Standardized Phylogenetic and Molecular Evolutionary Analysis Applied to Species Across the Microbial Tree of Life." *Scientific Reports* 10: 1723.
- Shapiro, S. S., and M. B. Wilk. 1965. "An Analysis of Variance Test for Normality (Complete Samples)." *Biometrika* 52: 591–611.
- Sharma, B. B., P. Kalia, D. K. Yadava, D. Singh, and T. R. Sharma. 2016. "Genetics and Molecular Mapping of Black Rot Resistance Locus *Xcalbc* on Chromosome B-7 in Ethiopian Mustard (*Brassica carinata* A. Braun)." *PLoS One* 11: e0152290.
- Sheng, X. G., F. Branca, Z. Q. Zhao, et al. 2020. "Identification of Black Rot Resistance in a Wild *Brassica* Species and Its Potential Transferability to Cauliflower." *Agronomy* 10: 1400.
- Singh, S., S. S. Dey, R. Bhatia, J. Batley, and R. Kumar. 2018. "Molecular Breeding for Resistance to Black Rot [*Xanthomonas campestris* pv. *campestris* (Pammel) Dowson] in Brassicas: Recent Advances." *Euphytica* 214: 196.
- Soengas, P., P. Hand, J. G. Vicente, J. M. Pole, and D. A. C. Pink. 2007. "Identification of Quantitative Trait Loci for Resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica rapa*." *Theoretical and Applied Genetics* 114: 637–645.
- Sun, W., F. M. Dunning, C. Pfund, R. Weingarten, and A. F. Bent. 2006. "Within-Species Flagellin Polymorphism in *Xanthomonas campestris* pv. *campestris* and Its Impact on Elicitation of Arabidopsis FLAGELLIN SENSING2-Dependent Defenses." *Plant Cell* 18: 764–779.
- Tavaré, S. 1986. "Some Probabilistic and Statistical Problems in the Analysis of DNA Sequences." *Lectures on Mathematics in the Life Sciences* 17: 57–86.
- Taylor, J. D., J. Conway, S. J. Roberts, D. Astley, and J. G. Vicente. 2002. "Sources and Origin of Resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica* Genomes." *Phytopathology* 92: 105–111.
- Tirnaz, S., P. E. Bayer, F. Inturrisi, et al. 2020. "Resistance Gene Analogs in the *Brassicaceae*: Identification, Characterization, Distribution, and Evolution." *Plant Physiology* 184: 909–922.
- Tonguç, M., and P. D. Griffiths. 2004. "Development of Black Rot Resistant Interspecific Hybrids Between *Brassica oleracea* L. Cultivars and *Brassica* Accession A 19182, Using Embryo Rescue." *Euphytica* 136: 313–318.
- U, N. 1935. "Genome Analysis in *Brassica* With Special Reference to the Experimental Formation of *B. napus* and Peculiar Mode of Fertilization." *Japanese Journal of Botany* 7: 389–452.
- Vaser, R., I. Sović, N. Nagarajan, and M. Šikić. 2017. "Fast and Accurate de Novo Genome Assembly From Long Uncorrected Reads." *Genome Research* 5: 737–746.
- Vicente, J. G., J. Conway, S. J. Roberts, and J. D. Taylor. 2001. "Identification and Origin of *Xanthomonas campestris* pv. *campestris* Races and Related Pathovars." *Phytopathology* 91: 492–499.
- Vicente, J. G., and E. B. Holub. 2013. "*Xanthomonas campestris* pv. *campestris* (Cause of Black Rot of Crucifers) in the Genomic Era Is Still a Worldwide Threat to Brassica Crops." *Molecular Plant Pathology* 14: 2–18.
- Vicente, J. G., J. D. Taylor, A. G. Sharpe, I. A. P. Parkin, D. J. Lydiate, and G. J. King. 2002. "Inheritance of Race-Specific Resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica* Genomes." *Phytopathology* 92: 1134–1141.
- Walley, P. G., G. R. Teakle, J. D. Moore, et al. 2012. "Developing Genetic Resources for Pre-Breeding in *Brassica oleracea* L.: An Overview of the UK Perspective." *Journal of Plant Biotechnology* 39: 62–68.
- Wick, R. R., L. M. Judd, C. L. Gorrie, and K. E. Holt. 2017a. "Unicycler: Resolving Bacterial Genome Assemblies From Short and Long Sequencing Reads." *PLoS Computational Biology* 13: e1005595.
- Wick, R. R., L. M. Judd, C. L. Gorrie, and K. E. Holt. 2017b. "Completing Bacterial Genome Assemblies With Multiplex MinION Sequencing." *Microbial Genomics* 3: 132.
- Williams, P. H. 1980. "Black Rot: A Continuing Threat to World Crucifers." *Plant Disease* 64: 736–742.
- Young, J. M., C. T. Bull, S. H. De Boer, G. Firrao, G. E. Saddler, and D. E. Stead. 2004. "Names of Plant Pathogenic Bacteria, 1864–2004." *Review of Plant Pathology* 75: 721–763.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Disease index (DI) scores of *Brassica oleracea* diversity fixed foundation set lines from screen 1 grouped by morphotype after clip inoculation with *Xanthomonas campestris* pv. *campestris* isolates (A) WHRI 10018A race 1, (B) WHRI 8978 race 4, (C) WHRI 8892A race 5 and (D) WHRI 8497 race 6. Resistant $0.00 < DI \leq 0.10$, partially resistant $0.10 < DI \leq 0.75$ and susceptible $0.75 < DI \leq 3.00$. Boxes represent the interquartile range (IQR), horizontal lines indicate the median, whiskers extend to $1.5 \times$ IQR, and dots indicate data points. Significant differences between crop types are indicated by $*p < 0.05$ and $**p < 0.01$. **Figure S2:** Disease index (DI) scores of *Brassica napus* diversity fixed foundation set lines from screen 1 grouped by morphotype after clip inoculation with *Xanthomonas campestris* pv. *campestris* isolates (A) WHRI 10018A race 1, (B) WHRI 8978 race 4, (C) WHRI 8892A race 5 and (D) WHRI 8497 race 6. SOSR, spring oilseed rape; WOSR, winter oilseed rape. Resistant $0.00 < DI \leq 0.10$, partially resistant $0.10 < DI \leq 0.75$ and susceptible $0.75 < DI \leq 3.00$. Boxes represent the interquartile range (IQR), horizontal lines indicate the median, whiskers extend to $1.5 \times$ IQR, and dots indicate data points. Significant differences between crop types are indicated by $*p < 0.05$

and $**p < 0.01$. **Table S1:** Details of *Brassica oleracea* diversity fixed foundation lines (Bol) used in this study including crop type, genetic status and their disease symptoms: the proportion of positive inoculation points (PIP, scale: 0 to 1), average lesion size (LS, scale: no lesion = 0 to largest lesion = 9), average disease index (DI, scale: resistant = 0 to susceptible = 3), standard deviation of average DI scores (SD) and overall resistance status (RS, highlighted as black = resistant $0.00 < DI \leq 0.10$, grey = partially resistant $0.10 < DI \leq 0.75$ and white = susceptible $0.75 < DI \leq 3.00$) when clip inoculated with four different races of *Xanthomonas campestris* pv. *campestris*. Averages were determined by inoculating 2 to 3 plants per line and 3 leaves per plant. **Table S2:** Details of *Brassica napus* diversity fixed foundation lines (Bna) used in this study including crop type, genetic status and their disease symptoms: the proportion of positive inoculation points (PIP, scale: 0 to 1), average lesion size (LS, scale: no lesion = 0 to largest lesion = 9), average disease index (DI, scale: resistant = 0 to susceptible = 3), standard deviation of average DI scores (SD) and overall resistance status (RS, highlighted as black = resistant $0.00 < DI \leq 0.10$, grey = partially resistant $0.10 < DI \leq 0.75$ and white = susceptible $0.75 < DI \leq 3.00$) when clip inoculated with four different races of *Xanthomonas campestris* pv. *campestris*. Averages were determined by inoculating 2 to 3 plants per line and 3 leaves per plant. **Table S3:** Details of *Xanthomonas campestris* pv. *campestris* isolates used for plant inoculations and phylogenomic analysis. **Table S4:** Kruskal–Wallis test statistics for the association of disease index (DI) with morphotype in the *Brassica oleracea* (Bol) and *Brassica napus* (Bna) diversity fixed foundation sets (DFFSs) after clip inoculation with *Xanthomonas campestris* pv. *campestris* isolates of different races. **Table S5:** Accession numbers for genome sequence data generated in this study and summary statistics for genome assemblies.