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Fabaceous and Cucurbitaceous Hosts Are Infected With Distinct Populations of the Powdery Mildew Species *Podosphaera xanthii*

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ABSTRACT

Two species of powdery mildew, *Podosphaera xanthii* and *Erysiphe vignae*, cause disease in mungbean (*Vigna radiata*) in Australia. *P. xanthii* is reported to have a wide host range, occurring worldwide on many different host families, including the Cucurbitaceae. It is unclear whether the *P. xanthii* populations that infect mungbean also infect other crops, such as cucurbits. In this study, we conducted cross-inoculation experiments to determine whether *P. xanthii* collected from mungbean infects a range of cucurbits; and conversely, whether *P. xanthii* from cucurbits infect mungbean and other *Vigna* species. *P. xanthii* collected from mungbean heavily infected black gram (*Vigna mungo*), native mungbean (*V. radiata* subsp. *sublobata*) and maloga bean (*Vigna lanceolata*); it did not infect zucchini (*Cucurbita pepo*), butternut pumpkin (*Cucurbita moschata*), cucumber (*Cucumis sativus*) or watermelon (*Citrullus lanatus*); and it produced small colonies on squash (*C. pepo*), marrow (*C. pepo*) and pumpkin (*Cucurbita maxima*). Conversely, *P. xanthii* from melon (*Cucumis melo*) heavily infected zucchini, pumpkin, butternut pumpkin and cucumber; it did not infect mungbean or black gram; it caused moderate infections on native mungbean and maloga bean. A search for genetic markers that differentiate the *P. xanthii* populations collected from mungbean and melon, used as inoculum sources in this investigation, revealed a 377 bp difference in the size of the promoter region of the *cyp51* gene. This was found to be a robust molecular marker in this work. This study revealed clear differences in the host range of the two *P. xanthii* populations infecting fabaceous and cucurbitaceous species.

1 | Introduction

Powdery mildew infections emerge in Australian mungbean (*Vigna radiata*) crops every season (Kelly et al. 2021). Mungbean is the most widely grown summer legume crop in Australia, produced predominantly in Queensland and northern New South Wales (Wells and Benjamin 2019). Black gram (*Vigna mungo*) is also grown in Australia as a minor crop (Chauhan and Willliams 2018). Large amounts of the Australian-grown mungbean and black gram are exported as food crops. Outside of Australia, mungbean and black gram are primarily grown throughout Asia, where they provide a vital source of inexpensive protein in cereal-based diets (Chauhan and Willliams 2018). Powdery mildew on Australian mungbean and black gram crops was recently confirmed to be caused by two pathogens, *Podosphaera xanthii* and *Erysiphe vignae* (Kelly et al. 2021).

The disease can reduce mungbean grain yields by up to 40% during conducive environmental conditions and without fungicide management (Kelly et al. 2017; Thompson 2016; Weir et al. 2017). All Australian mungbean and black gram commercial varieties are susceptible to powdery mildew to some extent, and disease management is largely based on fungicide applications (Kelly et al. 2024).

Podosphaera xanthii has been reported to cause powdery mildew on a wide range of hosts worldwide, including cucurbitaceous, fabaceous and asteraceous species, as well as on plants belonging to other families (Braun and Cook 2012; Hirata and Takamatsu 2001; Kelly et al. 2021; Kiss et al. 2008, 2020; Pérez-García et al. 2009; Polonio et al. 2021; Vela-Corcía et al. 2014). The pathogen has been most widely studied worldwide on cucurbits, including zucchini (*Cucurbita pepo*), squash (*Cucurbita pepo*), pumpkin (*Cucurbita maxima*), cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*) and other crops (Křístková et al. 2009; Lebeda et al. 2016, 2024; López-Ruiz et al. 2010; Pirondi, Pérez-García, et al. 2015; Pirondi, Vela-Corcía, et al. 2015). The disease caused by *P. xanthii* in glasshouse- and field-grown cucurbits can result in serious economic losses (Lebeda et al. 2016).

In Australia, only a few studies have been undertaken on cucurbit powdery mildew (Pursley et al. 2010). The presence of *P. xanthii* was recently confirmed on watermelon and pumpkin in Australia (Kiss et al. 2020). Surprisingly, *P. xanthii* was also detected on other unrelated plant species, including a native woody species, *Trema tomentosa* (Kiss et al. 2020); rainforest spinach (*Elatostema reticulatum*) that is also native to Australia (Kiss and Vaghefi 2021); and an insectivorous plant, *Cephalotus follicularis*, that is native and endemic to Western Australia (Cunnington et al. 2008). Outside Australia, unusual records of *P. xanthii* include its identification as the causal agent of a serious disease of jellyfish tree (*Medusagyne oppositifolia*) that was imported from the Seychelles to the United Kingdom (Pettitt et al. 2010) and an infection of water poppy (*Hydrocleys nymphoides*), a monocot grown in pots in an urban area in Korea (Cho et al. 2018). These unexpected occurrences of the pathogen may indicate the existence of multiple *P. xanthii* lineages with broader or narrower host ranges, as suggested by several studies (Braun et al. 2001; Hirata and Takamatsu 2001; Lebeda et al. 2016, 2024; Miazzi et al. 2011).

It is unclear whether the *P. xanthii* populations infecting cucurbits also infect mungbean and black gram in Australia and elsewhere. The source of inoculum of *P. xanthii* on mungbean and black gram in Australia remains unknown. The sexual stage of *P. xanthii* has not been detected in Australia (Kiss et al. 2020); therefore, we expect that the pathogen survives on volunteer mungbean plants or other hosts outside of the cropping season. A recent study by Kelly et al. (2025) revealed *P. xanthii* on 18 different fabaceous hosts in Australia. It is possible that the pathogen survives on these 18 hosts, as well as cucurbits growing throughout Australia, which contribute to crop disease epidemics. The objectives of this study were to determine whether *P. xanthii* infecting mungbean is the same pathogen that infects cucurbits in Australia. Gaining a greater understanding of the host range of *P. xanthii* in Australia will aid in the development of management strategies.

2 | Materials and Methods

2.1 | Powdery Mildew Collection and Examination by Microscopy

In 2017, mungbean leaves infected with powdery mildew were collected from multiple plants in a crop located in Killarney, Queensland. Powdery mildew-infected melon (*C. melo*) leaves were collected from a home garden in Toowoomba, Queensland, in 2021. Several leaves of the original mungbean and melon samples were dried and pressed as herbarium specimens. The mungbean specimen was deposited in the Queensland Plant Pathology Herbarium under accession number BRIP 71599, and the melon specimen as BRIP 76745. The rest of the powdery mildew-infected mungbean leaves collected in 2017, and the melon leaves collected in 2021, were used on their days of collection to inoculate potted mungbean and cucumber plants, respectively, as described below. The living population of the powdery mildew obtained from mungbean leaves was designated as MUNG, and the population from melon leaves as CUC. These populations were maintained on mungbean and cucumber plants, respectively, for the duration of this study, as described below. The term 'population' here refers to a group of multiple powdery mildew sources obtained from multiple leaves collected in the field from the same host.

At the time of collection, both populations MUNG and CUC were identified as *P. xanthii* based on their morphological characteristics. Their morphological identification was confirmed by removing actively growing powdery mildew mycelia from leaves with 2–3 cm^2 pieces of clear sellotape, then mounting on microscope slides containing a droplet of lactic acid. Slides were viewed under an Eclipse Ni-U (Nikon) microscope with bright field and differential interference contrast (DIC) optics. The following characteristics were examined: shape and size of conidia; presence or absence of fibrosin bodies in fresh conidia; nature of conidiogenesis; morphology of the conidiophore; shape of hyphal appressoria; and, when observed, position of conidial germ tubes and shape of germ tube apices.

2.2 | Maintenance of Living Powdery Mildew Populations

On the day of collection in 2017, conidia of *P. xanthii* on several infected mungbean leaves were used to inoculate healthy potted mungbean plants cv. Jade-AU in an experimental glasshouse to maintain living populations of the pathogen. Inoculated plants were kept in isolation in BugDorm insect-rearing cages with very fine mesh in a glasshouse of the University of Southern Queensland (UniSQ). Powdery mildew colonies that developed on inoculated plants were propagated onto new, healthy potted mungbean plants produced from seeds in isolation in BugDorm cages. The procedure was performed every 3–5 weeks as described by Kelly et al. (2021).

The same procedure was performed when melon leaves infected with *P. xanthii* were collected in 2021. The pathogen readily infected potted cucumber cv. Long Green Supermarket plants grown from seeds in isolation and was then maintained on this cultivar for the duration of the study. The powdery mildew

populations originating from mungbean and melon and maintained in isolation on potted mungbean and cucumber plants, respectively, were used as inoculum sources in the molecular work and host range experiments described below.

2.3 | Identification of Molecular Markers Differentiating MUNG and CUC Populations of *P. xanthii*

Single-nucleotide polymorphisms (SNPs) have previously been detected in the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) of *P. xanthii* samples collected from diverse hosts (Hirata and Takamatsu 2001; Kelly et al. 2025; Kiss et al. 2020; Kiss and Vaghefi 2021). To identify SNPs that could serve as easily detectable molecular markers distinguishing the MUNG and the CUC populations, three conserved DNA regions were PCR-amplified and analysed. These were the nrDNA ITS region; an approximately 1000 bp long fragment of the β-tubulin (*tub2*) gene, previously proposed as a marker of intraspecific genetic diversity within *P. xanthii* populations (Vela-Corcía et al. 2014); and the lanosterol 14α-demethylase (*cyp51*) gene, which has been investigated in the context of fungicide resistance in *P. xanthii* (Vielba-Fernández et al. 2020). In addition, the promoter region of *cyp51* was also PCR-amplified because it was expected that it would be more variable than the other regions targeted in this work.

2.4 | Extraction of Total Genomic DNA, PCR Amplifications, Sequencing and Analysis

To perform DNA extractions, powdery mildew mycelia were removed from a symptomatic leaf of potted plants using either autoclaved fine artist's brushes or 1–1.5 cm^2 pieces of Sellotape. Powdery mildew materials, including the sellotape pieces when needed, were placed in a sterile 1.5 mL safe-lock Eppendorf tube and total genomic DNA was extracted using the Extraction and Dilution buffers from an Extract-N-Amp Plant PCR kit (Sigma-Aldrich) according to the manufacturer's instructions.

The nrDNA ITS region was amplified using the nested PCR method developed by Cunnington et al. (2003) and modified by Kiss et al. (2020). Primers PMITS1 and PMITS2 were used in the first PCR, and ITS1-F and ITS4 in the nested reaction (Kiss et al. 2020). To amplify an approximately 1000 bp long fragment of the *tub2* gene, primers TubRT6F (Vela-Corcía et al. 2014) and Bt2b (Glass and Donaldson 1995) were used as described by Kelly et al. (2021).

The *cyp51* gene and its promoter region were amplified with a total of four sets of primers developed in this study (Table 1). These primer sets have also been used in analyses of fungicide resistance markers in *P. xanthii* (authors' unpublished data). Primers were designed based on the *cyp51* gene of *P. xanthii* available in GenBank (accession number: GQ292524). To design primers for the upstream region, with the promoter included, the GQ292524 sequence was used to BLAST search against two *P. xanthii* genomes available in GenBank (accession numbers: GCA_028751805.1 and GCA_014884795.1). An approximately 800 bp long upstream fragment was extracted from both *P. xanthii* genomes. Sequences were aligned using Geneious Prime v. 2024.0.5 (Dotmatics) (https://www.geneious.com) and primers were designed manually based on the conserved regions. Primer Px-PF1 was developed in this process for the upstream region, and primer Px-PR4 for the downstream sequence. All primers were synthesised by Macrogen Inc. (Seoul, Korea). Table 1 includes the exact nucleotide positions of the amplicons within *cyp51* and in the upstream and downstream regions.

PCRs with the four *cyp51* primer sets were carried out in 25 μL final volumes, consisting of 2 μL total genomic DNA, 12.5 μL Hot Start Taq 2× Master Mix (New England Biolabs), 1 μL of each primer (10 μM) and 8.5 μL of DNase/RNase-free distilled water. PCR conditions were as follows: 94°C for 2 min; 30 cycles of 30 s at 95°C, 1 min at 55°C and 1.5 min at 68°C; and finally, 5 min at

TABLE 1 | Primers designed in this study and used to PCR-amplify the lanosterol 14α-demethylase (*cyp51*) gene and its promoter region in the populations of *Podosphaera xanthii* from mung bean (MUNG) and cucumber (CUC).

Primer set	Primer designation	Orientation	Sequence (5′–3′)	Nucleotide positions of amplicons within *cyp51* and in upstream and downstream regions[a]	Product size (bp)	
					MUNG	CUC
1	Px-PF1	Forward	AGTTCCGATGCCAGCTAAAG	(−725)–90	438	815
	Px-PR1	Reverse	TCGTCCTAGCATGTTCAGGT			
2	Px-PF2	Forward	TGGGTCTTTTGGCAACACTC	1–874	873	873
	Px-PR2	Reverse	GCAACTGTAGCCTGAGCACG			
3	Px-PF3	Forward	GCACCTCTCCCCCACAATAG	824–1684	860	860
	Px-PR3	Reverse	GACTTCACCCTTCTCTGCCA			
4	Px-PF4	Forward	TCCATGTGACAACCCCAGTA	1286–(+398)	800	800
	Px-PR4	Reverse	GTTATACCAAAGTCGCGCGT			

[a](−): before the start codon of *cyp51* of *P. xanthii* as available in NCBI GenBank under accession number GQ292524; (+): after stop codon.

68°C. Following all PCRs, products were visualised over a UV source following gel electrophoresis in 1% agarose gel.

All PCR products were purified and sequenced by Macrogen using the PCR primers. Chromatograms were visually inspected for potential sequencing errors, then the forward and reverse sequences were aligned, trimmed, and assembled with Geneious Prime v. 2024.0.5 to produce consensus sequences.

2.5 | Cross-Inoculation Experiments

To determine whether the *P. xanthii* population MUNG was pathogenic on cucurbits, five species belonging to the Cucurbitaceae were included in cross-inoculation studies as test plants. Conversely, the *P. xanthii* population CUC was used in cross-inoculation studies to determine its pathogenicity on mungbean and three other *Vigna* species. Cross-inoculation experiments were undertaken in a setup with 12 BugDorm cages with automated irrigation within a bay of an experimental glasshouse at UniSQ (Figure 1) under natural daily illumination with approximately 12h daylength at 18°C–26°C and 70%–80% relative humidity.

The following test plant species were included in each cross-inoculation experiment, performed twice with *P. xanthii* population MUNG and twice with population CUC: mungbean (*V. radiata*) cv. Jade-AU, black gram (*V. mungo*) cv. Onyx-AU, native mungbean (*V. radiata* subsp. s*ublobata*), maloga bean (*V. lanceolata*), zucchini (*C. pepo*) cv. Lebanese, squash (*C. pepo*) cv. Yellow Scallop, marrow (*C. pepo*) cv. Long Green Bush 2, pumpkin (*C. maxima*) cv. Golden Nugget, butternut pumpkin (*C. moschata*) cv. Butternut, and cucumber (*Cu. sativus*) cvs. Pickling Gherkin and Long Green Supermarket. Watermelon (*Ci. lanatus*) cv. Sugar Baby was also included in the experiments with *P. xanthii* population MUNG. For each experiment, two seeds of each test species were sown into 10-cm-diameter plastic pots containing pasteurised potting mix (Hortico). Three pots were prepared in this way for each test species and placed inside a BugDorm cage. A fourth pot with two seeds of the host of the *P. xanthii* population used in the respective experiment, that is, either mungbean cv. Jade-AU for population MUNG or cucumber cv. Long Green Supermarket for population CUC, was also included in each cage and was treated as the positive control. An additional two plants of the inoculum source host, that is, mungbean cv. Jade-AU or cucumber cv. Long Green Supermarket, were grown in a separate cage and served as uninoculated controls. After inoculation, all cages remained unopened on the glasshouse bench for the duration of the experiment.

FIGURE 1 | Powdery mildew host range experiments were conducted in 12 BugDorm very fine mesh insect-rearing cages with automated irrigation in a glasshouse bay with controlled temperature. [Colour figure can be viewed at wileyonlinelibrary.com]

In the first two experiments, the inoculum source was *P. xanthii* population MUNG. In the third and fourth experiments, inoculations were performed with *P. xanthii* population CUC. Leaves covered with fresh, 10- to 15-day-old colonies of the respective inoculum source were collected and brought to the glasshouse bay with 12 BugDorm cages (Figure 1) in isolation. These leaves were then gently touched against the first fully expanded leaves of all potted test plants grown from seeds in the cages. The inoculation process was the only time the respective cages were opened during the experiment. After 2weeks, all plants were examined for symptoms of powdery mildew infection without opening the cages.

Susceptibility was assessed visually on a 0–3 scale, where 0=no symptoms, 1=very limited sporulation with no spread of symptoms from the inoculation site, 2=moderate sporulation with some spread from the inoculation site and 3=heavy infection with extensive spread from the inoculation site, covering the entire leaf and spreading to other leaves. A hypersensitive response was noted when present. All plants remained in their cages in the glasshouse for an additional 2weeks to determine if any further symptoms developed. At the end of the 4-week period, cages were opened and powdery mildew-infected leaf samples were collected from each pot, in isolation, and observed microscopically to determine if colonies produced on test plants developed conidia. DNA was extracted from powdery mildew colonies developed on each test plant species in different cages. The *cyp51* promoter was PCR-amplified, and the size of the PCR product was determined following gel electrophoresis to confirm the identity of populations MUNG and CUC. At the end of each experiment, all plants were discarded, and cages were abundantly sprayed with 70% ethanol to destroy all powdery mildew inoculum.

3 | Results

3.1 | Morphological and Molecular Identification of the Original Powdery Mildew Specimens and the MUNG and the CUC Isolates

Powdery mildews collected from mungbean in 2017 (specimen BRIP 71599) and melon in 2021 (specimen BRIP 76745) were both identified as *P. xanthii* based on their morphological characters and nrDNA ITS sequences as described in Kelly et al. (2021). The asexual morphs of populations MUNG and CUC could not be distinguished from each other based on their morphology. The ITS sequences obtained in this study from the two original specimens, BRIP 71599 and BRIP 76745, and also from the MUNG and CUC populations maintained on their host plants were identical to each other and to several other *P. xanthii* ITS sequences available in GenBank. These included MW293884, determined in a *P. xanthii* specimen collected from mungbean in Australia in 1974 (Kelly

et al. 2021); PQ509396, determined in *P. xanthii* from adzuki bean (*Vigna angularis*); and MK530412 sequenced in *P. xanthii* from melon. Similarly, the sequences of the *tub2* and the *cyp51* fragments determined in the MUNG and CUC populations were identical to each other and to some sequences retrieved from GenBank. These included the *tub2* sequence MW401671, determined earlier in a *P. xanthii* specimen collected from mungbean (Kelly et al. 2021); and the *cyp51* sequence GQ292524, determined in *P. xanthii* isolate SF48 that was collected from melon by López-Ruiz et al. (2010). Therefore, the ITS, *tub2* and *cyp51* sequences of populations MUNG and CUC were not useful to distinguish these two powdery mildew inoculum sources during host range studies.

The size of the promoter region of the *cyp51* gene amplified with the newly designed primers Px-PF1 and Px-PR1 (Table 1) was different in populations MUNG and CUC. The amplicons in population MUNG were approximately 450 bp, while a larger fragment of approximately 800 bp was obtained in population CUC (Figure 2). This clear difference in amplicon size was used as a robust marker to confirm the identity of populations MUNG and CUC during the host range experiments.

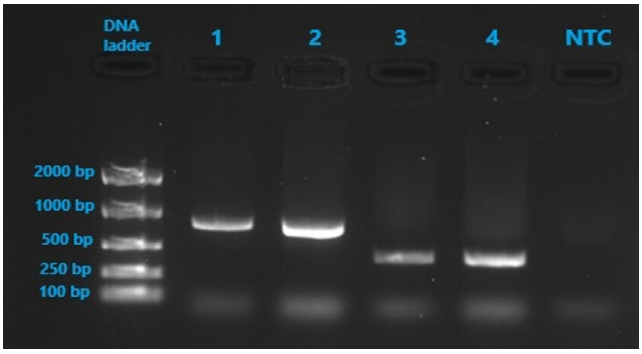

FIGURE 2 | Agarose gel to visualise the size difference in the promoter region of the *cyp51* gene in *Podosphaera xanthii* populations amplified with primers Px-PF1 and Px-PR1. Lanes 1 and 2: Population CUC from cucumber; lanes 3 and 4: Population MUNG from mung bean. NTC: Non-template (negative) control. [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Cross-Inoculation Experiments

The host range of populations MUNG and CUC was markedly different. In 2 weeks, population MUNG heavily infected all four *Vigna* spp. included in the tests, that is, mungbean, its original host, black gram (*V. mungo*), native mungbean (*V. radiata* subsp. *sublobata*) and maloga bean (*V. lanceolata*) (Figure 3a–c, Table 2). During that period, a few small and partly sporulating colonies had also developed on squash (*C. pepo*) and marrow (*C. pepo*) (Figure 3d,e, Table 2). Within the cucurbitaceous test plants inoculated with population MUNG, the number of sporulating colonies was highest on pumpkin (*C. maxima*) cv. Golden Nugget, especially on the older leaves (Figure 3f, Table 2). Population MUNG did not develop a single colony on any leaves of watermelon (*Ci. lanatus*), butternut pumpkin (*C. moschata*), zucchini (*C. pepo*) or cucumber (*Cu. sativus*) (Figure 3g–i, Table 2).

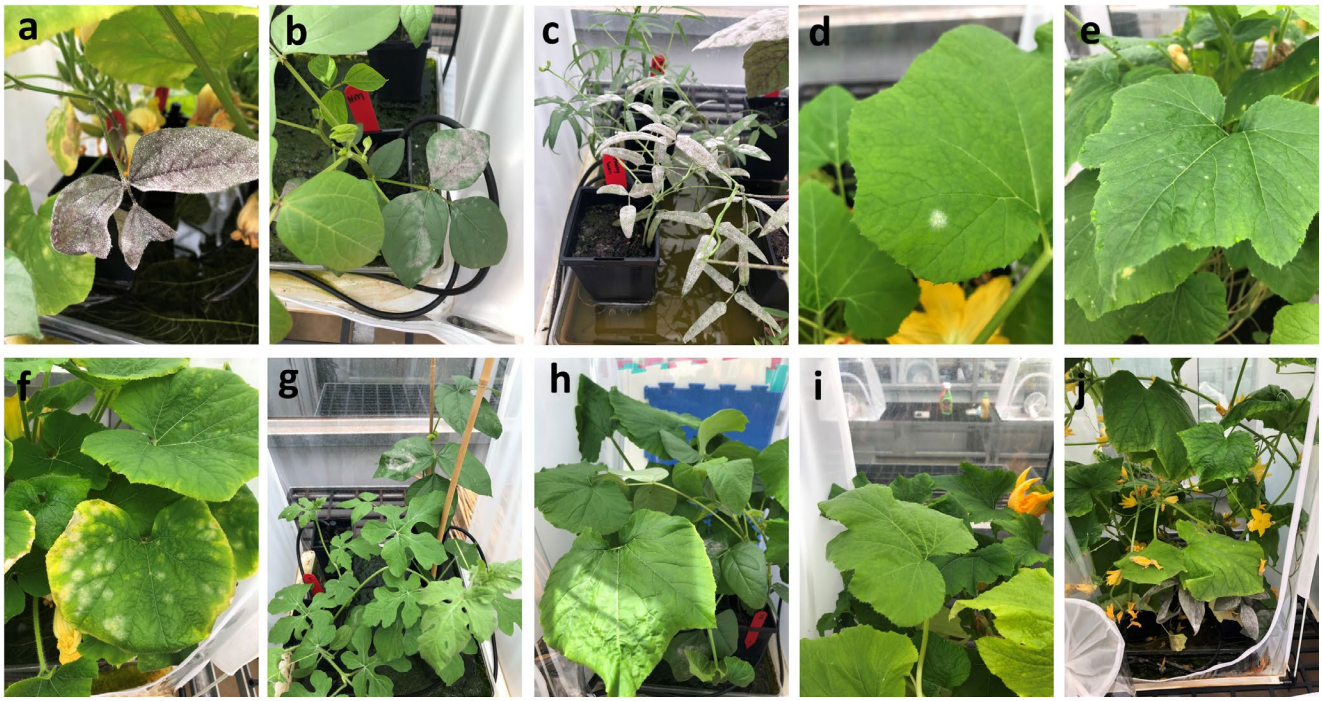

FIGURE 3 | Reaction of the test plants 4 weeks following inoculation with *Podosphaera xanthii* population MUNG from mung bean. Symptoms on (a) mungbean (*Vigna radiata*) cv. Jade-AU, (b) native mungbean (*V. radiata* subsp. *sublobata*), (c) maloga bean (*V. lanceolota*), (d) squash (*Cucurbita pepo*) cv. Yellow Scallop, (e) marrow (*C. pepo*) cv. Long Green Bush 2, (f) pumpkin (*C. maxima*) cv. Golden Nugget, (g) watermelon (*Citrullus lanatus*) cv. Sugar Baby, (h) butternut pumpkin (*C. moschata*) cv. Butternut, (i) zucchini (*C. pepo*) cv. Lebanese and (j) cucumber (*Cucumis sativus*) cv. Long Green Supermarket. [Colour figure can be viewed at wileyonlinelibrary.com]

Conversely, population CUC heavily infected both varieties of cucumber, zucchini and both varieties of pumpkin (Figure 4a–d, Table 2). Only a few, and mostly non-sporulating colonies were observed on squash and marrow leaves 4 weeks following inoculations (Figure 4e,f, Table 2). Interestingly, the population infected some of the older native mungbean and maloga bean leaves and produced large, sporulating colonies on these fabaceous hosts (Figure 4g,h, Table 2). When inoculated

TABLE 2 | Host reactions to inoculation with *Podosphaera xanthii* populations from mung bean (MUNG) and cucumber (CUC) at the end of the 4-week incubation period.

			Host reaction to *P. xanthii* populations[a]	
Host species	**Common name**	**Cultivar**	**MUNG**	**CUC**
Vigna radiata	Mungbean	Jade-AU	3	0, HR
Vigna mungo	Black gram	Onyx-AU	3	0, HR
V. radiata subsp. *sublobata*	Native mungbean	—	3	2, HR
Vigna lanceolata	Maloga bean	—	3	2, HR
Cucurbita pepo	Squash	Yellow Scallop	1	1
C. pepo	Marrow	Long Green Bush 2	1	1
Cucurbita maxima	Pumpkin	Golden Nugget	1	3
Cucurbita moschata	Pumpkin	Butternut	0	3
C. pepo	Zucchini	Lebanese	0	3
Cucumus sativus	Cucumber	Pickling Gherkin	0	3
C. sativus	Cucumber	Long Green Supermarket	0	3
Citrullus lanatus	Watermelon	Sugar Baby	0	Nt

Abbreviations: HR, hypersensitive response in host; Nt, not tested.
[a]0, no powdery mildew symptoms; 1, limited sporulation; 2, moderate sporulation; 3, heavy sporulation.

FIGURE 4 | Reaction of the test plants 4 weeks following inoculation with *Podosphaera xanthii* population CUC from cucumber. Symptoms on (a) cucumber (*Cucumis sativus*) cv. Pickling Gherkin, (b) zucchini (*Cucurbita pepo*) cv. Lebanese, (c) pumpkin (*C. maxima*) cv. Golden Nugget, (d) cucumber (*Cu. sativus*) cv. Long Green Supermarket, (e) squash (*C. pepo*) cv. Yellow Scallop, (f) marrow (*C. pepo*) cv. Long Green Bush 2, (g) native mungbean (*Vigna radiata* subsp. *sublobata*), (h) maloga bean (*V. lanceolota*), (i) black gram (*V. mungo*) cv. Onyx-AU and (j) mungbean (*V. radiata*) cv. Jade-AU. [Colour figure can be viewed at wileyonlinelibrary.com]

with population CUC, a hypersensitive response occurred on the inoculated leaves of mungbean and black gram (Figure 4i,j, Table 2).

The *cyp51* promoter size marker confirmed the identity of the *P. xanthii* populations at the end of the cross-inoculation tests. No infection developed in the non-inoculated control plants, indicating that the cages effectively prevented contamination between *P. xanthii* populations.

4 | Discussion

The cross-inoculation experiments performed in this study demonstrated that the *P. xanthii* population MUNG, collected from mungbean in 2017, does not infect butternut pumpkin, cucumber, zucchini or watermelon. Conversely, the *P. xanthii* population CUC, collected from melon in 2021 and maintained on cucumber, did not cause disease in mungbean or black gram. These results indicate that the host ranges of *P. xanthii* populations collected from mungbean and melon are markedly different but also partly overlapping (Figure 5). Both populations heavily infected the test plants representing the plant families of their original hosts, the Fabaceae and the Cucurbitaceae, respectively. However, a few sporulating powdery mildew colonies were still produced on some of the test plants that represented the other family during cross-inoculation tests, sometimes followed by a hypersensitive response of the infected leaf tissues. Nevertheless, most of those infections resulted in only a few colonies that mostly appeared on the older leaves, 3–4 weeks following inoculations. Yeh et al. (2021) have also observed that *P. xanthii* colony development was confined to older leaves in some host species. These observations may indicate that the respective test plant species or cultivars are not true hosts of the population used for inoculations, and those infections are unlikely to occur under natural field conditions. Alternatively, those plant species may harbour low levels of inoculum to maintain populations in the environment. Earlier, an accidental observation in a glasshouse followed up by a small-scale experiment led to a similar conclusion. In that case study, *P. xanthii* infecting cucumber was able to produce a few colonies with sparse sporulation on two bean varieties, but the infections had always triggered a hypersensitive reaction in the infected bean leaves (Kiss and Szentiványi 2001).

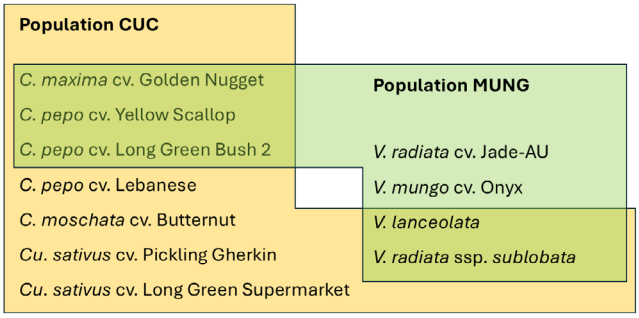

FIGURE 5 | Distinct, but partially overlapping host ranges of *Podosphaera xanthii* populations MUNG from mungbean and CUC from cucumber. [Colour figure can be viewed at wileyonlinelibrary.com]

Due to the race structure of some *P. xanthii* populations infecting cucurbits (Lebeda et al. 2016, 2024; Miazzi et al. 2011), we cannot exclude the possibility that there are other *P. xanthii* populations infecting fabaceous or cucurbitaceous hosts in Australia with host ranges that differ from populations MUNG and CUC. To our knowledge, no studies have investigated the race structure of *P. xanthii* populations infecting cucurbits or any other host in Australia.

The existence of multiple races of *P. xanthii* infecting cucurbits has been known for almost 100 years (Lebeda et al. 2024). Different races vary in their ability to infect different genotypes, cultivars or breeding lines of a single host (Lebeda et al. 2024). Since their detection, researchers have established a uniform system for the characterisation and determination of cucurbit powdery mildew races based on (1) a standard set of race differentials, (2) a uniform method of screening, (3) a uniform code for host–pathogen interactions and (4) a system that allows the addition of new accessions (Lebeda et al. 2024). No such approach has been considered for powdery mildew in many other cropping systems, including mungbeans.

Despite early reports that *P. xanthii* from cucumber and cosmos (*Cosmos bipinnatus*) (Asteraceae) were co-infectious (Abiko 1978), a more recent study found that *P. xanthii* from cosmos was not pathogenic on cucumber, although isolates from cucumber were able to produce small colonies on cosmos (Hirata and Takamatsu 2001). That study found six nucleotide substitutions in the nrDNA ITS region between *P. xanthii* isolates from cosmos and cucumber (Hirata and Takamatsu 2001). Although the ITS sequences of powdery mildews from the same species that infect different host plants may differ in one or more nucleotide positions (Jankovics et al. 2008; Kovács et al. 2011), this is not a general rule within the Erysiphaceae. The current study found that the ITS sequences were identical in *P. xanthii* populations collected from mungbean and melon. Recent phylogenetic analyses of *P. xanthii* isolates collected from different fabaceous and other hosts in Australia have also revealed that their ITS sequences were identical or highly similar to each other and sequences from hosts belonging to other families (Kelly et al. 2021, 2025; Kiss et al. 2020; Kiss and Vaghefi 2021). A genome-wide diversity analysis of a large number of *P. xanthii* samples collected from diverse cucurbits across Europe and North America indicated very low levels of genetic diversity (Pirondi, Vela-Corcía, et al. 2015). That comprehensive study did not reveal any variations in eight genomic regions, including a fragment of the *cyp51* gene and the nrDNA ITS sequences; and an amplified fragment length polymorphism (AFLP) analysis revealed a high similarity between the sampled populations (Pirondi, Vela-Corcía, et al. 2015). The present study focused, for the first time, on the promoter region of the *cyp51* gene and identified a reliable genetic marker that distinguished the two *P. xanthii* populations included in this work.

Developing varieties with host resistance to powdery mildew has been one of the aims of the National Mungbean Improvement Program in Australia. Currently, host resistance is assessed under field conditions relying on natural powdery mildew infections. The species composition of the inoculum, that is, the presence and the ratio of *P. xanthii* and/or *E. vignae* that infect

mungbean (Kelly et al. 2021), the inoculum load, and the timing of infections cannot be controlled in those field trials. The possibility that at least *P. xanthii* infecting mungbean is composed of different races or pathotypes would further complicate mungbean breeding efforts. Despite extensive research in cucurbits, the mechanisms of host resistance to *P. xanthii* are not well understood (Lebeda et al. 2024).

Recently, it was suggested that *P. xanthii* may be split into several distinct species (Yeh et al. 2021). New, phylogenetically informative loci may be identified based on the already existing high-quality genome assemblies available for *P. xanthii* infecting cucurbits (Kim et al. 2021; Polonio et al. 2021) and other powdery mildews (Bindschedler et al. 2016; Vaghefi et al. 2022). A whole-genome sequencing project is currently underway to produce an assembly of a *P. xanthii* isolate that infects mungbean and to compare the candidate effector repertoire of that isolate with those infecting cucurbits. Further host-range studies, similar to those reported in this study, are also needed to disentangle the complexity of the interactions between *P. xanthii* and its many hosts across diverse plant families and support future studies on candidate effectors of *P. xanthii*.

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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.

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