Short communication

G143A Mutation Conferring Resistance to Quinone Outside Inhibitor Fungicides Detected in both *Podosphaera xanthii* and *Erysiphe vignae* Causing Powdery Mildew on Mung Bean (*Vigna radiata*) in Australia

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Abstract

Powdery mildew of mung bean (*Vigna radiata*) is caused by two species in Australia: *Podosphaera xanthii* and *Erysiphe vignae*. Currently, two fungicides are permitted for managing this disease in Australia: azoxystrobin and tebuconazole. The commercial fungicide products used for mung bean powdery mildew management contain either tebuconazole alone or a mixture of azoxystrobin and tebuconazole. This study detected the G143A mutation in the mitochondrial cytochrome b gene of both *P. xanthii* and *E. vignae*. The mutation was detected in two *P. xanthii* and one *E. vignae* populations out of a total of 15 populations sampled in south-east Queensland from 2017 to 2024. The G143A mutation is the major DNA marker of resistance to Quinone outside Inhibitor (QoI) fungicides, including azoxystrobin. This study confirmed that both powdery mildew species have developed QoI resistance in Australian mung bean fields.

Mung bean or green gram (*Vigna radiata* (L.) Wilczek) is the most widely grown summer legume crop in Australia, mostly produced in Queensland and northern New South Wales. During the past five years, its annual production was up to 80,000 to 100,000 tonnes, with almost 90% of the crop being exported (Chauhan and Williams 2018; Mahajan et al. 2023). Powdery mildew is one of the most common diseases of mung bean wherever this crop is grown (Pandey et al. 2018; Vaghefi et al. 2024). In Australia, the disease may reduce grain yields by up to 40% (Kelly et al. 2024a) and it is caused by two fungal species, *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff and *Erysiphe vignae* L. Kelly, L. Kiss & Vaghefi (Kelly et al. 2021). Both species infect hosts other than mung bean in Australia (Kelly et al. 2025). These two species can be distinguished based on their morphology and internal transcribed spacer (ITS) sequences of their nuclear ribosomal DNA (nrDNA) region (Kelly et al. 2021).

Currently, no mung bean varieties available in Australia offer adequate genetic resistance to suppress powdery mildew development under conducive conditions (Kelly et al. 2024a). Sowing early in the season is recommended as an opportunity to avoid infection (Grains Research & Development Corporation 2025). Two fungicides are registered and used for the management of mung bean powdery mildew in Australia: azoxystrobin, a Quinone outside Inhibitor (QoI) belonging to the Mode of Action (MoA) group 11 of the Fungicide Resistance Action Committee (FRAC) coding system; and tebuconazole, a Demethylation Inhibitor (DMI) that belongs to MoA group 3. Fungicide products permitted for the management of mung bean powdery mildew in Australia contain either tebuconazole alone (430 g/L) or a mixture of azoxystrobin (222 g/L) and tebuconazole (370 g/L) (Kelly et al. 2024a). Growers

typically apply one to three fungicide sprays per crop for powdery mildew, initiating at symptom onset or as part of a preventative program (Kelly et al. 2024a). A Decision Support Tool is freely available to explore the economics of fungicide use to control the disease in different situations (Sparks et al. 2020).

This study was initiated based on our observations of inconsistent efficacy in the field when applying a foliar product containing azoxystrobin (Fig. 1). The aim was to investigate whether inconsistent efficacy was associated with the development of azoxystrobin resistance in both *P. xanthii* and *E. vignae* populations infecting mung bean. Azoxystrobin inhibits mitochondrial respiration by blocking the electron transport chain at the cytochrome bc_1 complex (Bartlett et al. 2002). In all powdery mildew species that have exhibited resistance to QoI fungicides across diverse cropping systems, including *P. xanthii* infecting cucurbits, a mutation in the middle nucleotide position of codon number 143 of their mitochondrial cytochrome b gene (*cytb*) has always been detected (Ishii et al. 2001; Vielba-Fernández et al. 2020; Cowger et al. 2022; Zito et al. 2024). This point mutation, from G<u>G</u>T to G<u>C</u>T, known as G143A, leads to a change in the 143rd amino acid position of the cytochrome b (CYTB) protein, from glycine to alanine. As a result, the region targeted by QoI molecules within the CYTB protein is altered, preventing the fungicide from inhibiting CYTB and disrupting mitochondrial respiration (Fisher and Meunier 2005). In addition to powdery mildews, the G143A mutation also serves as the primary marker of QoI-resistance in many other crop pathogens (Yin et al. 2023; Horváth et al. 2024).

Interestingly, in some crop pathogens, including rusts, *Pyrenophora teres*, and *Phyllosticta ampelicida*, a group I intron is located in the *cytb* gene after the 143rd codon (Grasso et al. 2006; Sierotzki et al. 2007; Miessner et al. 2011; Horváth et al.

2024). The G143A mutation has never been detected in these pathogens, with one notable exception (Hoffmeister et al. 2022). The lack of the G143A mutation in these fungi is explained by the 'intron hypothesis', which is based on the prediction that the G143A mutation coupled with this intron would lead to a splicing error in the maturation of the *cytb* mRNA that results in a nonfunctional CYTB protein (Horváth et al. 2024). So far, this intron has only been detected in a single powdery mildew species, *Podosphaera leucotricha* infecting apple (Koch et al. 2015; Strickland et al. 2023).

To determine whether the G143A mutation is present in populations of *P. xanthii* and/or *E. vignae* causing mung bean powdery mildew in south-east Queensland, Australia, a total of 20 samples were collected for molecular testing from leaves of mung bean cv. Jade-AU in commercial production fields and experimental plots from 2017 to 2024. Each sample consisted of one to three powdery mildew-infected leaves collected from a single plant. A single sample was collected at random in each field when the crop reached the green pod stage. Each year, except in 2020, one to four fields located 50 to 160 km apart were sampled in south-east Queensland. Each sample was collected in a paper bag in the field to avoid contamination with other powdery mildew samples. In the laboratory, powdery mildew tissues were removed from leaves with clear adhesive tape and examined under a light microscope in a droplet of water or lactic acid to identify the causal agents morphologically as described by Kelly et al. (2021).

DNA was extracted from powdery mildew tissues collected from leaf samples using one of the following methods: (a) approx. 1 to 1.5 cm^2 clear adhesive tape pieces as described by Kiss et al. (2020); (b) sterile artists' brushes; or (c) 6 mm diameter leaf discs cut out from leaves with a single-hole metal puncher. The extraction and the

dilution buffers of an Extract-N-Amp™ Plant PCR Kit (Merck/Sigma-Aldrich, Bayswater, VIC, Australia) were used to extract the DNA from each sample as per the manufacturer's instructions. To support the morphological identification of the pathogens, the nrDNA ITS region was amplified using a modified version of the nested PCR method developed by Cunnington et al. (2003). The first PCR step used primers PMITS1 and PMITS2, and the second ITS1-F and ITS4, as described by Kiss et al. (2020). PCR products were Sanger sequenced with primers ITS1-F and ITS4 (Macrogen Inc., Seoul, Korea) and chromatograms analysed as described by Kelly et al. (2024b).

A fragment of the powdery mildew *cytb* gene that included codon 143 was also amplified using a modified version of the PCR protocol using primers RSCBF1 and RSCBR1 developed by Ishii et al. (2001). The protocol was optimized for DNA samples extracted with the Extract-N-Amp™ Plant PCR Kit as follows: 2.5 min at 94˚C; 40 cycles consisting of 30 sec at 94˚C, 1 min at 58˚C, and 1.5 min at 68˚C; and a final extension of 8.5 min at 68˚C. Each 25 µL PCR reaction contained 7.75 µL of autoclaved Milli-Q water, 12.5 µL of Extract-N-Amp™ PCR ReadyMix™, 1.25 µL of 10 µM solutions of each of the RSCBF1 and RSCBR1 primers, 0.25 µL of 50 µM $MgCl_2$ and 2 µL of DNA template. PCR products were visualized after gel electrophoresis on a 1% (weight/volume) agarose gel containing 0.01% GelRed (Gene Target Solutions, Dural, NSW, Australia) in TAE buffer under ultraviolet light. A 100 bp DNA ladder was run with the PCR products in the gel. PCR products were then sent for Sanger sequencing with RSCBFI and RSCBR1 primers (Macrogen Inc.). Chromatograms were analysed using Geneious Prime v. 2024.0.3 (Dotmatics). PCR products that resulted in noisy chromatograms were repeated. When double peaks were detected at the mutation site, TA cloning of selected *cytb* PCR products

was performed by Macrogen Inc. Twenty fragments of directly amplified fragments were cloned and sequenced per PCR sample, and their chromatograms were analysed. Those 20 clones per PCR sample were selected at random during the TA cloning procedure. Cloning and sequencing multiple fragments selected at random from direct PCRs has been useful in confirming sequence variations in multi-copy DNA regions (Kovács et al. 2011; Tanner et al. 2015).

Light microscopy (Fig. 2A-C) and ITS sequences confirmed that four samples collected in three locations in 2018 and 2019 contained *E. vignae* only; 11 samples from five locations collected from 2017 to 2024 were *P. xanthii* only; and five other samples contained both *E. vignae* and *P. xanthii*. The latter five samples were not included in the *cytb* PCRs because the RSCBF1 and RSCBR1 primers may have amplified *cytb* fragments from both powdery mildew species. The ITS sequences determined in this study were identical to those of *E. vignae* and *P. xanthii* reported by Kelly et al. (2021).

Following repeated *cytb* PCRs and sequencing, chromatograms with clear sequences for the G143A codon were obtained from all the15 samples. In one of the four *E. vignae* samples, collected in 2019, and one of the 11 *P. xanthii* samples, collected in another location in 2023, chromatograms contained double peaks for bases G and C in the point mutation site (Fig. 2D, E). These double peaks indicated the presence of both the G143A mutation and the wild-type codon (G143) in the sampled powdery mildew populations. In a *P. xanthii* sample collected in 2021, there was only one clear peak for a C base at the mutation site, indicating that all the *cytb* genes carried the G143A mutation in that sample. The other three *E. vignae* and nine *P. xanthii* samples exhibited a peak for only a G base at the mutation site, suggesting only the presence of the wild-type codon.

After cloning and sequencing 20 directly amplified fragments from each of the two PCR samples with double peaks at the mutation site, five and nine clones out of 20 exhibited the G143A mutation in the *E. vignae* and *P. xanthii* samples, respectively. The rest of the clone sequences contained the wild-type codon (Fig. 2F, G). These cloned sequences confirmed the results of the direct PCRs. Representatives of the consensus sequences with the QoI resistance codon G143A are available in GenBank under accession numbers PV189423 and PV189424.

These results confirmed the presence of the G143A QoI resistance marker in both *P. xanthii* and *E. vignae* populations causing mung bean powdery mildew in south-east Queensland. The work was based on a limited number of samples; therefore, no conclusions could be drawn in terms of the frequency of the mutation in the sampled populations. Allele-specific quantitative PCR methods are currently being developed to address this question. The detection of the G143A mutation in both powdery mildew species confirmed that these can develop QoI resistance, unlike apple powdery mildew that has an intron after the 143rd codon of its *cytb* gene (Koch et al. 2015; Strickland et al. 2023). The results were communicated to the industry to highlight the importance of fungicide resistance management measures that have been developed for Australian broadacre cropping systems (Ireland et al. 2021). With little genetic resistance to powdery mildew in the currently available varieties, fungicides that belong to other FRAC MoA groups could also be considered by the mung bean industry. Alternative plant protection products, such as biofungicides, should also be tested for their potential in mung bean powdery mildew management.

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The authors declare no conflict of interest.

Figure captions:

FIGURE 1 A mung bean cv. Jade-AU plot with powdery mildew symptoms following two applications of a fungicide product that contained azoxystrobin.

FIGURE 2 Morphology of the asexual morphs of *Podosphaera xanthii* and *Erysiphe vignae* infecting mung bean in Australia, and representative chromatograms obtained following direct amplifications of a fragment of their *cytb* gene; and following TA cloning of 20 directly amplified fragments selected at random. **A.** Conidiophore of *P. xanthii*. Bar = 25 µm. **B.** Conidia of *P. xanthii* with fibrosin bodies (white arrows). Bar = 10 µm. **C.** A conidiophore, a hyphal appressorium (black arrow) and plasmolysed conidia of *E. vignae*. Bar = 10 µm. **D** and **E**. Representative, noisy chromatograms of directly amplified *cytb* fragments in *P. xanthii* and *E. vignae*, respectively, with double or multiple peaks in codon 143. **F** and **G**. Representative chromatograms obtained following cloning and sequencing of 20 directly amplified *cytb* fragments per PCR product with double or multiple peaks in codon 143. Those 20 clones per sample were selected at random during the cloning procedure. Chromatograms of cloned fragments exhibit either the G143 (GGT) or the A143 (GCT) codon. In each chromatogram shown in figures D-G, the middle nucleotide position of codon 143 is indicated by a yellow box.

FIGURE 1 A mung bean cv. Jade-AU plot with powdery mildew symptoms following two applications of a fungicide product that contained azoxystrobin.

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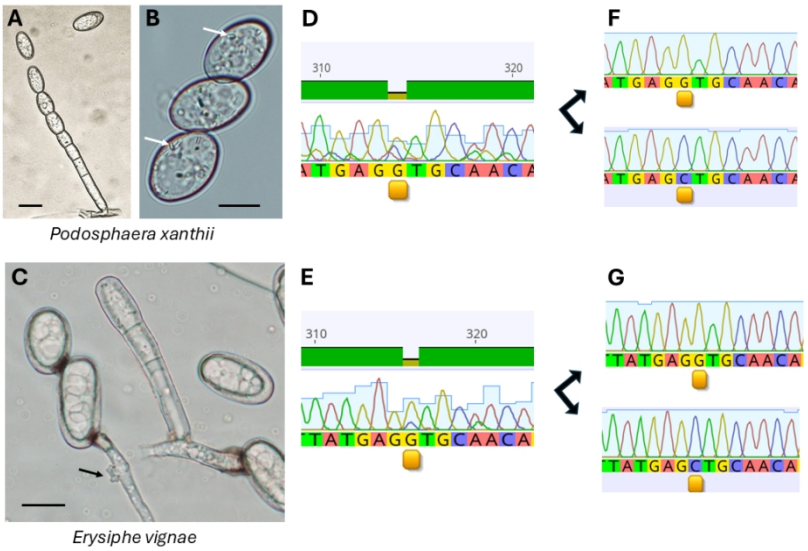

FIGURE 2 Morphology of the asexual morphs of Podosphaera xanthii and Erysiphe vignae infecting mung bean in Australia, and representative chromatograms obtained following direct amplifications of a fragment of their cytb gene; and following TA cloning of 20 directly amplified fragments selected at random. A. Conidiophore of P. xanthii. Bar = 25 µm. B. Conidia of P. xanthii with fibrosin bodies (white arrows). Bar = 10 µm. C. A conidiophore, a hyphal appressorium (black arrow) and plasmolysed conidia of E. vignae. Bar = 10 µm. D and E. Representative, noisy chromatograms of directly amplified cytb fragments in P. xanthii and E. vignae, respectively, with double or multiple peaks in codon 143. F and G. Representative chromatograms obtained following cloning and sequencing of 20 directly amplified cytb fragments per PCR product with double or multiple peaks in codon 143. Those 20 clones per sample were selected at random during the cloning procedure. Chromatograms of cloned fragments exhibit either the G143 (GGT) or the A143 (GCT) codon. In each chromatogram shown in figures D-G, the middle nucleotide position of codon 143 is indicated by a yellow box.

338x190mm (96 x 96 DPI)