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ORIGINAL ARTICLE

Glycine tabacina, native to Australia, is an alternate host of *Erysiphe diffusa* causing powdery mildew on soybean

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Abstract

Powdery mildew, caused by *Erysiphe diffusa*, is an emerging pathogen in Australian soybean crops. Since its initial detection in 2012, the disease has been observed every season in soybean paddocks throughout Australia. It is not known how *E. diffusa* survives between soybean cropping seasons in the Australian environment. This study identified the native *Glycine* species, *G. tabacina*, as an alternate host for *E. diffusa* in Australia. *G. tabacina* specimens naturally infected with powdery mildew were collected and the pathogen was identified based on morphological characters and nrDNA ITS and *MCM7* sequences. Cross-inoculation experiments demonstrated that the *E. diffusa* isolates infecting *G. tabacina* in the field were pathogenic to soybean. This study is the first to report *E. diffusa* on *G. tabacina* in Australia. As a perennial native often found in the vicinity of the annual soybean crops, *G. tabacina* can easily serve as an alternate host for *E. diffusa* and could be an example of a host range expansion in this powdery mildew species. Weed control in soybean crops, with special attention to the removal of the native *Glycine* species, may be an option for powdery mildew management for Australian soybean growers.

KEYWORDS
Glycine max, host range, *MCM7* sequences, phylogeny

1 | INTRODUCTION

On a global scale, soybean (*Glycine max*) is one of the major oilseed crops that are produced and consumed throughout the world (Hartman et al., 2016; Ratnaparkhe et al., 2011). Australia is one of the world's smaller soybean producers, although the crop has been commercially grown since the 1950s, largely for human food, animal feed and oil products (McGee, 2011). Additionally, soybean is considered an important part of the farming system, improving soil fertility and providing a disease break for crops, such as sugarcane, typically grown in rotation with soybean throughout Australia (McGee, 2011).

Powdery mildew infects soybeans worldwide and is reported to reduce yields and seed quality in susceptible varieties (Bui et al., 2023). Experimental data recorded in the 1970s and 1980s indicated that the disease may cause up to 35% yield losses in susceptible varieties if the environmental conditions are conducive and no management options are implemented (Dunleavy, 1978; Phillips, 1984). A global analysis of soybean yield losses caused by different diseases in 1998 revealed that powdery mildew was responsible for considerable losses in three out of the top 10 soybean-producing countries in that particular year (Wrather et al., 2001). Most recently, it was reported that powdery mildew caused up to

20% yield losses in a susceptible variety when used as an untreated control in field experiments conducted in Australia over four consecutive seasons (Dunn & Gaynor, 2020).

Soybean powdery mildew was first reported in Australia only in 2012 (McTaggart et al., 2012). The pathogen was identified as *Erysiphe diffusa* based on morphological characteristics of the asexual morph and analyses of the internal transcribed spacers (ITS) of the nuclear ribosomal DNA (nrDNA) region (McTaggart et al., 2012). *E. diffusa* is the most common powdery mildew species that infects soybean worldwide (Braun & Cook, 2012), although *Erysiphe glycines* has also been reported to cause powdery mildew on soybean outside of Australia (Takamatsu et al., 2002). Following the first report of *E. diffusa* on soybean in Australia (McTaggart et al., 2012), morphological studies and nrDNA ITS sequence analyses have confirmed this species in more recent soybean specimens collected in Queensland and New South Wales (Kelly et al., 2021; Kiss et al., 2020). The pathogen was also identified on *Glycine clandestina*, a species native to Australia (Kiss et al., 2020). The sexual morph of *E. diffusa* has not been reported in Australia (Kiss et al., 2020). *Erysiphe vignae*, a recently recognized powdery mildew species that infects mungbean (*Vigna radiata*) in Australia, is phylogenetically closely related to *E. diffusa* but cross-inoculation experiments have revealed that it does not infect soybean (Kelly et al., 2021).

Little is known of the host range and survival of *E. diffusa* in Australia. The identification of *E. diffusa* on *G. clandestina* based on nrDNA ITS sequences (Kiss et al., 2020) could be an indication that some *Glycine* species present in Australia may serve as alternate hosts of this pathogen between soybean cropping seasons. However, ITS sequences alone may not be sufficient to prove the identity of closely related powdery mildews that infect different host plant species (Jankovics et al., 2008; Kovacs et al., 2011). Secondary DNA barcodes (Bradshaw et al., 2022; Ellingham et al., 2019; Shirouzu et al., 2020) and, above all, cross-inoculation experiments are needed for this purpose. Amongst secondary DNA markers, the genes encoding mini-chromosome maintenance (MCM) proteins, and especially the *MCM7* sequences, were particularly useful in the identification of some powdery mildews that could not be distinguished based on ITS sequences (Ellingham et al., 2019; Shirouzu et al., 2020).

Since its first report in Australia in 2012, powdery mildew has been observed in Australian soybean crops every season (Dunn & Gaynor, 2020). The identification of alternate hosts of *E. diffusa* could contribute to the management of powdery mildew infections in soybean crops. Kiss et al. (2020) hypothesized that all powdery mildew species have been introduced to Australia from overseas only recently, most probably since the beginning of the European colonization of the continent, and some species have already broadened their host ranges in the new environment and have become pathogens of some Australian native plants, in addition to their introduced hosts. A conclusive identification of *E. diffusa* on native legume species in Australia that are not known as hosts of this fungus, or any of the other powdery mildew species overseas, would support this hypothesis. Therefore, the objectives of this research were to (a) identify native *Glycine* species as alternate hosts of *E. diffusa* in Australia based on morphological characters and nrDNA ITS and *MCM7* sequences; and (b) conduct cross-inoculation experiments to determine whether the *E. diffusa* isolates identified with DNA markers on native *Glycine* spp. are able to infect soybean in Australia.

2 | MATERIALS AND METHODS

2.1 | Plant and fungal materials

From 2021, naturally occurring *Glycine* spp. populations were monitored in southern Queensland, Australia, for symptoms of powdery mildew infections. Leaves naturally infected with powdery mildew were repeatedly collected from two sites in Toowoomba, Queensland in 2021. The identification of these *Glycine* spp. was made by Craig Marston (Department of Agriculture, Fisheries and Forestry, Brisbane). Soybean cv. PBA Dominator leaves infected with powdery mildew were collected for comparative studies from a commercial soybean crop in Kingsthorpe, southern Queensland, in April 2022. No other *Glycine* spp. naturally infected with powdery mildew were found during this study. After collection, all powdery mildew specimens were examined under a light microscope in the laboratory. Representative specimens were pressed and dried and deposited as herbarium materials at the Queensland Plant Pathology Herbarium (BRIP).

2.2 | Morphological characterization of the pathogens

An Eclipse Ni-U (Nikon) microscope with bright field and differential interference contrast (DIC) optics was used to examine each powdery mildew specimen. Actively growing hyphae, conidiophores and conidia were removed from infected leaf surfaces with cellotape, mounted on a microscope slide containing a droplet of lactic acid, and then viewed under the microscope and photographed with a DP23-CU 6.4MP (Olympus) microscope camera. The following characteristics were examined: shape and size of conidia ($n = 50$), presence or absence of fibrosin bodies in conidia, nature of conidiogenesis, morphology of the conidiophore, position of conidial germ tubes, shape of conidial germ tube apices and shape of hyphal appressoria.

2.3 | DNA extraction, amplification and sequencing

Powdery mildew hyphae, conidiophores and conidia were removed from infected leaves using 1–1.5 cm^2 pieces of cellotape. Total genomic DNA was then extracted from the cellotape pieces containing powdery mildew mycelia using an Extract-N-Amp Plant PCR Kit (Sigma-Aldrich) as per the manufacturer's instructions.

The ITS region from DNA samples was amplified using a modified version of a nested PCR method developed by Cunnington et al. (2003) using primers PMITS1 and PMITS2, and then ITS1-F and ITS4, as described by Kiss et al. (2020). PCR products were separated by gel electrophoresis on a 1% wt/vol agarose gel containing 0.01% GelRed (Gene Target Solutions) in TAE (containing a mixture of Tris base, acetic acid and EDTA) buffer and visualized under a UV source. PCR products of the nested reactions were purified and sequenced by Macrogen Inc. (Seoul, Korea) using primers ITS1-F and ITS4 (Kiss et al., 2020).

To amplify a part of the *MCM7* gene, degenerate primers MCM7F2 (5'-TGTGATCGRTGYGGDTGTGA-3′) and MCM7R8 (5′-TCATYCCRTCRCCCATYTCYTTWG-3′) developed by Ellingham et al. (2019) were used. PCRs were carried out in 25 μL final volumes, consisting of 12.5 μL Hot Start Taq 2× Master Mix (New England BioLabs), 1.25 μL of each primer (10 μM), 0.25 μL of $MgCl_2$ (50 mM), 7.75 μL ultrapure water and 2 μL total genomic DNA at concentrations of 10–50 ng/μL. PCR conditions were as follows: 94°C for 2 min; 40 cycles of 10 s at 98°C, 30 s at 54°C and 1 min at 68°C; and finally, 5 min at 68°C. PCR products were purified and sequenced by Macrogen Inc. with primers MCM7F2 and MCM7R8.

2.4 | Phylogenetic analysis

The nucleotide sequences of the ITS and *MCM7* regions were edited using Geneious Prime v. 2024.0.3 (Biomatters Ltd). Initial sequences were compiled from chromatograms following visual inspections for potential sequencing errors. Forward and reverse sequences were trimmed and assembled to produce consensus sequences. The ITS and the *MCM7* consensus sequences produced in this study were deposited in NCBI GenBank (Table 1).

Multiple sequence alignments were constructed using MAFFT v. 7.388 (Katoh & Standley, 2013) with previously published *Erysiphe* sequences retrieved from GenBank (Table 1). Separate alignments were constructed for ITS and *MCM7* sequences before producing a concatenated alignment. The ITS + *MCM7* concatenated alignment dataset consisted of 35 sequences, including sequences from three specimens obtained in this study and 32 reference sequences of representative specimens obtained from GenBank (Table 1). Three ITS and eight *MCM7* sequences were determined in this study. *E. glycines* was used as the outgroup based on Shirouzu et al. (2020). This analysis resulted in an alignment with a total length of 1056 characters, including 706 identical and 350 variable sites.

Phylogenetic relationships were inferred using Bayesian inference (BI) and maximum-likelihood (ML) approaches. For BI, the Akaike information criterion estimated using MrModeltest v. 2.3 (Nylander, 2004) and PAUP v. 4.0 (Swofford, 2002) was used to determine the best-fit nucleotide substitution model for each individual alignment. MrBayes v. 3.2.4 (Ronquist & Huelsenbeck, 2003) was used to run two Markov chain Monte Carlo chains. One tree per 100 generations was saved, and the runs were ended when the standard deviation of split frequencies reached below 0.01. The 50% majority rule consensus tree was estimated after a 25% burn-in of the saved trees. For ML analysis, RAxML v. 8.2.11 (Stamatakis, 2014) was used with the GTR + GAMMA model of nucleotide substitution and 1000 bootstrap replicates.

2.5 | Cross-inoculation studies

To confirm whether the powdery mildew pathogen collected from the naturally infected *Glycine* plants is pathogenic to soybean, three cross-inoculation experiments were conducted. Mungbean, which is a host of *E. vignae*, a species that is closely related to the soybean pathogen *E. diffusa* (Kelly et al., 2021), was included in these experiments. Young plants of soybean cv. Bunya and mungbean cv. Jade-AU, six of each cultivar, were used as the test plants. These plants were grown from seeds sown into 10 cm diameter pots containing potting mix (Rocky Point Mulching). Two plants were grown in each pot; thus, three pots of soybean and three pots of mungbean were produced at the beginning of each experiment. Three BugDorm insect-rearing cages with 160 μm aperture mesh were used in these experiments to exclude unwanted infections with airborne powdery mildew inocula. A pot with soybeans and a pot with mungbeans were placed in each cage immediately after sowing. Our previous study had confirmed that the powdery mildew inocula, that is, conidia, can be kept in isolation in the cages if plants are watered without opening them (Kelly et al., 2021). Plants were grown in an experimental glasshouse, under natural daily illumination. The temperature was maintained at 18–26°C and the relative humidity at 70%–80% for the duration of the experiments. At the end of each cross-inoculation experiment, plants in the cages were abundantly sprayed with 70% (vol/vol) ethanol through the mesh to destroy all powdery mildew inoculum, then taken to another location where the cages were opened, and plants discarded.

Leaves of *G. tabacina* naturally infected with young colonies of the powdery mildew and collected from the field in Toowoomba, Queensland, Australia, on 14 June 2021, were used as the inoculum source in the first experiment. Powdery mildew-infected leaves of *G. tabacina* were brought to the laboratory in isolation. The powdery mildew fungus was identified microscopically and a small sample was retained for DNA extraction. The infected fresh leaves were then used to inoculate the first fully expanded, true leaves of four potted soybean and four potted mungbean plants kept in BugDorm cages in the glasshouse by gently touching the infected leaves against the healthy leaves. The inoculation process was the only time when the respective cages were opened during the experiment. All surfaces that might have been contaminated with the powdery mildew conidia during this procedure were cleaned with 70% ethanol. The two soybean and two mungbean plants kept in the remaining BugDorm cage served as uninoculated controls. All plants were kept in their cages in the glasshouse for another 2 weeks; then, the cages were opened, and powdery mildew samples were observed microscopically and used for ITS sequencing. The experiment was repeated

TABLE 1 Details of *Erysiphe* sequences used in the phylogenetic study.

Species	Host	Specimen number	GenBank accession number		Reference
			rDNA ITS[a]	*MCM7*[b]	
Erysiphe abeliicola	*Abelia spathulata*	MUMH4472	LC010069	LC517000	Shirouzu et al. (2020)
E. akebiae	*Akebia trifoliata*	MUMH4450	LC010068	LC517001	Shirouzu et al. (2020)
E. baeumleri	*Vicia hirsuta*	MUMH240	LC009933	LC517002	Shirouzu et al. (2020)
E. berberidicola	*Berberis amurensis*	MUMH201	LC009930	LC517003	Shirouzu et al. (2020)
E. berchemiae	*Berchemiella berchemiafolia*	MUMH259	LC009937	LC517004	Shirouzu et al. (2020)
E. betae	*Ambrina ambrosioides*	MUMH395	LC009946	LC516992	Shirouzu et al. (2020)
E. chloranthi	*Chloranthus serratus*	MUMH202	LC009931	LC516993	Shirouzu et al. (2020)
E. coriariae	*Coriaria japonica*	MUMH172	LC009927	LC517005	Shirouzu et al. (2020)
E. cruciferarum	*Raphanus sativus*	MUMH289	LC009943	LC516994	Shirouzu et al. (2020)
E. diffusa	*Glycine clandestina*	BRIP 68827	MT174188	–	Kiss et al. (2020)
E. diffusa	*Glycine max*	BRIP 71011	MW009056	PP027927	Kelly et al. (2021)
E. diffusa	*G. max*	**BRIP 76159**	PP023533	–	This study
E. diffusa	*G. max*	BRIP 71014	MW009059	–	Kelly et al. (2021)
E. diffusa	*Glycine tabacina*	**BRIP 76160**	PP023535	PP027928	This study
E. diffusa	*G. tabacina*	**BRIP 76624**	PP023534	PP027929	This study
E. epigena	*Quercus variabilis*	MUMH148	AB292718	LC517006	Shirouzu et al. (2020)
E. euphorbiae	*Chamaesyce nutans*	MUMH4646	LC010073	LC516995	Shirouzu et al. (2020)
E. glycines	*Desmodium podocarpum*	MUMH52	AB015927	LC516996	Shirouzu et al. (2020)
E. hommae	*Elsholtzia ciliata*	MUMH167	LC009926	LC516997	Shirouzu et al. (2020)
E. juglandis	*Juglans mandshurica*	MUMH278	LC009939	LC517007	Shirouzu et al. (2020)
E. lupini	*Lupinus* sp.	HAL 3378F	MZ265170	–	Bradshaw et al. (2021)
E. medicaginis	*Medicago polymorpha*	BRIP 70958	MT160214	PP027931	Crous et al. (2020)
E. medicaginis	*M. polymorpha*	BRIP 70957	MT160215	PP027930	Crous et al. (2020)
E. menispermi	*Menispermum dauricum*	MUMH282	LC009940	LC517008	Shirouzu et al. (2020)
E. paeoniae	*Paeonia lactiflora*	MUMH146	AB257438	LC516998	Shirouzu et al. (2020)
E. phyllanthi	*Phyllanthus flexuosus*	MUMH99	LC009921	LC517009	Shirouzu et al. (2020)
E. quercicola	*Quercus phillyraeoides*	MUMH885	AB193591	LC517010	Shirouzu et al. (2020)
E. quercicola	*Acacia sophorae*	BRIP 71600	MW293874	**PP027932**	Young and Kiss (2021)
E. trifoliorum	*Melilotus officinalis*	MUMH131	LC009924	LC517011	Shirouzu et al. (2020)
E. vignae	*Vigna mungo*	BRIP 68837	MT628284	–	Kelly et al. (2021)
E. vignae	*Vigna radiata*	BRIP 71005	MT628282	PP027933	Kelly et al. (2021)
E. vignae	*V. radiata*	BRIP 71006	MT628285	–	Kelly et al. (2021)
E. vignae	*V. radiata*	BRIP 71007	MW293894	–	Kelly et al. (2021)
E. vignae	*V. radiata*	BRIP 71598	MW293895	PP027934	Kelly et al. (2021)

Note: Dashes indicate missing data; accession numbers of DNA sequences determined in this study are shown in bold.

[a]Internal transcribed spacer.

[b]Mini-chromosome maintenance protein.

twice, with the powdery mildew inoculum from *G. tabacina* collected a second time on 16 August 2021 and a third time on 25 November 2022, in different locations in southern Queensland. A part of the intact powdery mildew-infected *G. tabacina* leaves collected for the first cross-inoculation experiment was pressed and dried and deposited as a herbarium specimen at BRIP under accession number 76160.

3 | RESULTS

3.1 | Identification of the powdery mildews based on morphology

Three naturally occurring populations of *Glycine* plants infected with powdery mildew were collected in this study and identified

as *G. tabacina* based on morphological characteristics (Sheather & Sheather, 2020). White powdery mildew colonies covered the upper and lower leaf surfaces, and sometimes part of the pods of *G. tabacina* (Figure 1a,b). Heavy powdery mildew infections occurred in the field on soybean plants, with colonies covering more than 70% of the leaf area. Collected *G. tabacina* and soybean powdery mildew specimens were assigned herbarium accession numbers BRIP 76160 and BRIP 76159, respectively. The powdery mildews from both hosts were identified as *Erysiphe* species based on their morphological characteristics, as described by Braun and Cook (2012). The fungal hyphae were hyaline with lobed appressoria. Erect conidiophores, sometimes slightly flexuous at the base of foot cells, produced single conidia that were mostly ellipsoid or doliiform in shape, 27–38 μm in length and 13–15 μm wide, without fibrosin bodies, and germinated with short, lobed appressoria (Figure 1c–f) or sometimes with a *longitubus* pattern with a simple or swollen apex. No chasmothecia were found on naturally infected or inoculated *Glycine* species.

3.2 | Phylogenetic analysis

The ITS sequences determined in this study for all powdery mildews infecting the *G. tabacina* and *G. max* specimens were identical to each other and also to several *E. diffusa* ITS sequences originating from soybean and available in GenBank. These included MW009056, MW009057, MQ009059 and MT174188 published from Australia (Kelly et al., 2021; Kiss et al., 2020). All *MCM7* sequences produced in this study from *G. tabacina* and *G. max* specimens were identical to each other. No *MCM7* sequences available in GenBank on 2 April 2024 were identical to these newly determined sequences, and the closest match was LC517003, the *MCM7* sequence of *E. berberidicola* specimen MUMH201 from *Berberis amurensis* collected in Japan. LC517003 differed in 12 nucleotide positions from the *MCM7* sequences determined in this study in *E. diffusa* from both *G. tabacina* and *G. max*. All *MCM7* sequences produced in this study from *G. tabacina* and *G. max* specimens differed to those from *E. vignae* at one nucleotide position.

All concatenated ITS and *MCM7 E. diffusa* sequences obtained in this study grouped together into a single clade with a Bayesian posterior probability (PP) of 0.73 and an ML bootstrap probability (BS) of 91% (Figure 2). Five *E. vignae* sequences grouped together into the sister clade to *E. diffusa*, with a PP of 1 and a ML BS of 99% (Figure 2). The combined ITS+*MCM7* tree (Figure 2) was congruent with the single gene phylogenies.

3.3 | Cross-inoculation studies

Seven to ten days after inoculations, all soybean cv. Bunya plants inoculated with *E. diffusa* collected from naturally infected *G. tabacina* populations were heavily infected with powdery mildew in each

FIGURE 1 *Glycine tabacina* naturally infected with *Erysiphe diffusa* on a roadside in Toowoomba, Queensland, Australia in 2021. (a) Early symptoms on leaves. (b) Late symptoms on leaves and pods. (c) A conidiophore mounted in lactic acid. Bar = 10 μm. (d) A conidium in lactic acid. Bar = 10 μm. (e) Germinated conidium with lobed germ tube. Bar = 10 μm. (f) Lobed hyphal appressorium. Bar = 3 μm.

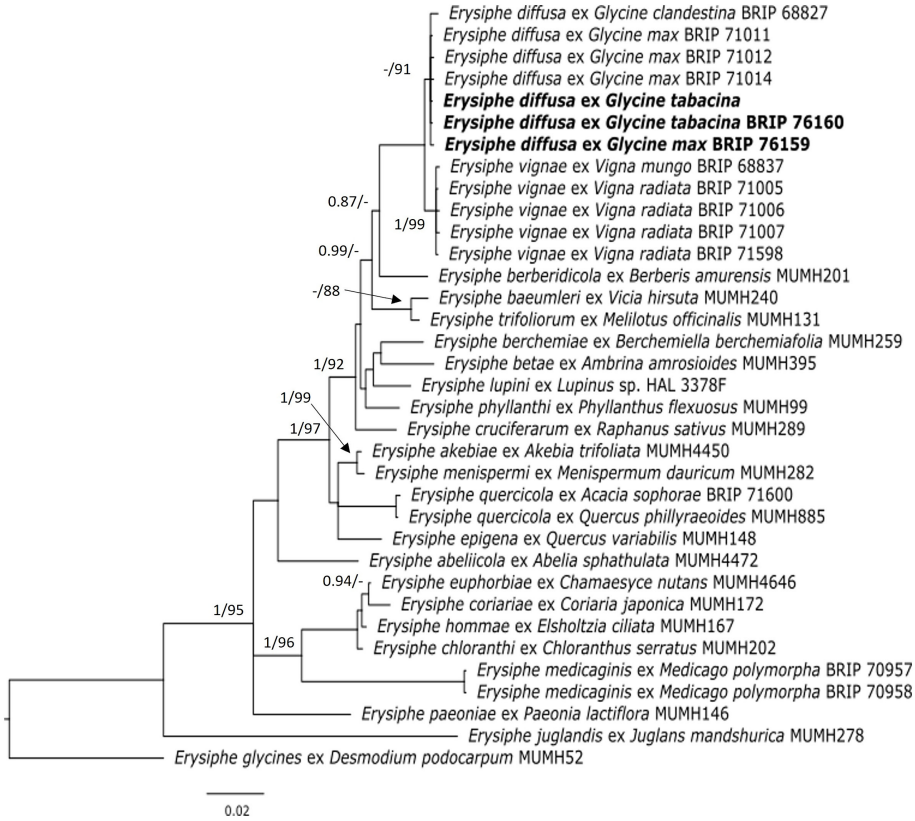

FIGURE 2 Maximum-likelihood phylogram was inferred from a concatenated alignment of rDNA internal transcribed spacer (ITS) and mini-chromosome maintenance protein (*MCM7*) sequences of powdery mildew specimens belonging to the genus *Erysiphe*. The specimens collected in this study (Table 1) are in bold. All other specimens were obtained from GenBank (Table 1). Taxon labels include species names followed by the host and specimen number, where available. The tree is rooted to *Erysiphe glycines* ex *Desmodium podocarpum* MUMH52 (Shirouzu et al., 2020). Maximum-likelihood bootstrap values >80% and Bayesian posterior probability values >0.80 are shown beside the branches. Thickened branches represent maximum-likelihood bootstrap values of 100% and Bayesian posterior probability of 1. The scale bar represents nucleotide substitutions per site.

replicated experiment (Figure 3a). A hypersensitive response was always induced in mungbean cv. Jade-AU by the *E. diffusa* inocula, which were eventually overcome by the fungus to establish small, sparse and weakly sporulating colonies (Figure 3b). Microscopy and ITS sequencing confirmed the identity of the *E. diffusa* isolates on potted soybean and mungbean plants at the end of each cross-inoculation test.

4 | DISCUSSION

The morphological and phylogenetic studies in this work revealed that *E. diffusa* causes powdery mildew on both soybean and *G. tabacina*. The results of the cross-inoculation studies demonstrated that *G. tabacina* can serve as an alternate host of *E. diffusa* and should be considered an inoculum source for powdery mildew when established near a soybean crop.

Soybean is grown annually over a wide area of eastern Australia, ranging from inland growing regions of southern Queensland and New South Wales to the coastal regions of northern Queensland and New South Wales (McGee, 2011; Soy Australia, 2023). *G. tabacina* is a native, perennial legume, commonly found throughout eastern Australia in grassy woodlands and forests (Sheather & Sheather, 2020). Based on the results of this study, it is possible that *G. tabacina* is an alternate host for *E. diffusa* infecting soybean, allowing a means of survival of the pathogen between soybean cropping seasons. This presumption is supported by the distribution of *G. tabacina* throughout eastern Australia (Atlas of Living Australia; https://ala.org.au). Removal of this alternate host from nearby soybean crops may offer Australian soybean growers an effective disease management tool that reduces an inoculum source that contributes to infections and limits the survival of the pathogen between cropping seasons. Currently, powdery mildew infections in Australian soybean crops are managed through host resistance and the application of fungicides. Dunn and Gaynor (2020) reported that powdery mildew reduced soybean yields by up to 20% in a susceptible variety when no fungicides were applied in field experiments conducted during the 2014/2015 season in New South Wales, Australia. Tebuconazole fungicides are currently registered in Australia to control powdery mildew infections in soybean. Overreliance on these demethylation inhibitor (DMI) fungicides may lead to the development of fungicide resistance in *E. diffusa* populations. Weed management focusing on

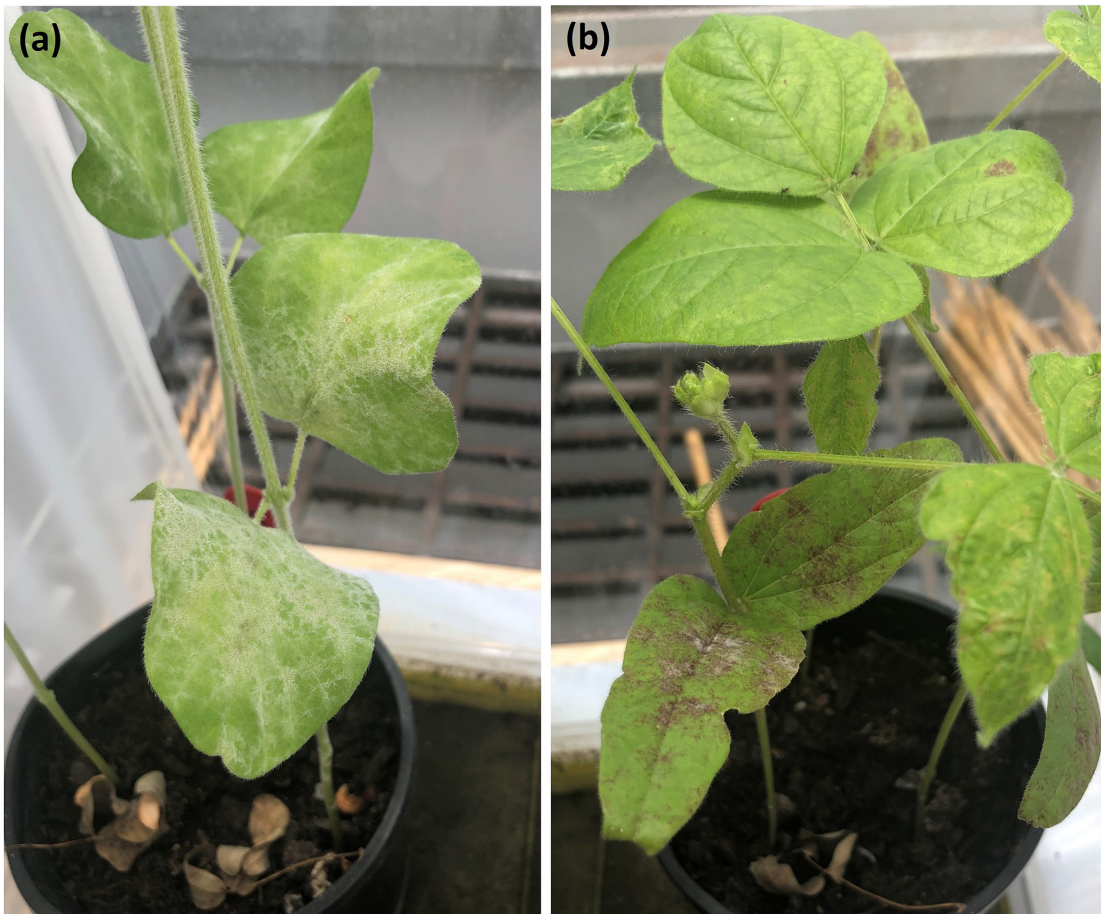

FIGURE 3 Cross-inoculation experiment in a BugDorm insect-rearing cage. The source of inoculum was *Erysiphe diffusa* isolate BRIP 76160 from *Glycine tabacina*. Soybean cv. Bunya and mungbean cv. Jade-AU plants were tested for their susceptibility to the *E. diffusa* isolate. (a) Powdery mildew colonies developed on inoculated soybean plants. (b) Mungbean plants developed a hypersensitive response with sparse sporulation.

the control of *G. tabacina* populations in the vicinity of soybean paddocks is proposed as a new method that may contribute to the reduction in disease pressure without fungicide applications.

Kiss et al. (2020) hypothesized that all powdery mildew species have only recently been introduced to Australia from overseas, since the beginning of the European colonization of the continent. Their hypothesis has implied that some powdery mildew species may have broadened their host ranges since their introduction to Australia and have become pathogenic on a few Australian native plant species, in addition to their original hosts. The results of this study support this hypothesis. *E. diffusa* was only reported in Australia in 2012, as a soybean pathogen (McTaggart et al., 2012), and is now also recognized as a pathogen of an Australian native plant. It remains unclear whether soybean or *G. tabacina* was the original host of *E. diffusa* in Australia. The perennial nature of *G. tabacina* makes this weed an ideal alternate host for the soybean pathogen *E. diffusa*, allowing a means of survival between soybean cropping seasons for longer periods on a single host.

Cross-inoculations, microscopy and phylogenetic analyses of ITS and *MCM7* sequences done in this study reaffirmed that *E. diffusa* is a different species to *E. vignae* that infects mungbean. Results of cross-inoculation experiments reported in this work confirmed those of a previous study (Kelly et al., 2021), whereby *E. diffusa* induced a hypersensitive response in mungbean leaves, which eventually are overcome by the pathogen to produce weakly sporulating colonies. ITS and *MCM7* sequences of *E. diffusa* differed to *E. vignae* by four and one conserved nucleotide positions, respectively. These results highlight the value of these two DNA regions in the identification of the powdery mildew species (Bradshaw et al., 2022; Ellingham et al., 2019; Shirouzu et al., 2020) and provide a case study where cross-inoculation experiments and ITS and *MCM7* sequence analyses mutually support the differentiation of two closely related taxa, *E. diffusa* and *E. vignae*.

This study is the first to report *G. tabacina* as a host of *E. diffusa* in Australia. Other legumes may also harbour *E. diffusa* between soybean cropping seasons in Australia. During glasshouse inoculations, Mignucci and Chamberlain (1977) reported that at least 15 different legume species, including *G. tabacina*, other species of *Glycine*, *Phaseolus* and *Lespedeza*, as well as cowpea (*Vigna unguiculata*), pigeonpea (*Cajanus cajan*) and pea (*Pisum sativum*), were all susceptible to *Microsphaera diffusa* (syn. *E. diffusa*) to some degree in the United States. This study should be repeated using Australian isolates of

E. diffusa that infect soybean to identify other alternative hosts of the soybean powdery mildew pathogen in Australia. When studying the powdery mildew taxa on fabaceous hosts in Australia, Cunnington et al. (2004) identified *E. pisi* as a pathogen of pea, and *Podosphaera xanthii* as a pathogen of *Phaseolus* and *Vigna* species. More recent Australian studies confirmed that amongst the legumes, *E. diffusa* infects soybean and *G. clandestina; P. xanthii* infects mungbean, black gram (*Vigna mungo*) and cowpea; and *E.* c.f. *trifoliorum* infects pea (Kelly et al., 2021; Kiss et al., 2020). There are currently no records of powdery mildew on *Lespedeza* species in the Queensland Plant Pathology Herbarium (BRIP), and only one record of powdery mildew on pigeonpea, reported as an *Oidium* species. *G. tabacina* is one of six wild *Glycine* species that are native to Australia (Ratnaparkhe et al., 2011). Further studies should investigate whether the other native *Glycine* species, or the various other fabaceous hosts that readily grow in close proximity to Australian soybean crops, are hosts of *E. diffusa* and contribute to powdery mildew infections in soybean crops.

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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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