First report of powdery mildew of coastal wattle (*Acacia sophorae*) caused by *Erysiphe quercicola*

Key words

Powdery mildew, Australian flora

Abstract

A powdery mildew was found associated with the coastal wattle, *Acacia sophorae*, at Tullymorgan, in northern New South Wales, Australia. Morphological characterisation identified it as belonging to the genus *Erysiphe*, while internal transcribed spacer (ITS) sequencing and phylogenetic analysis confirmed it as *Erysiphe quercicola*, a pathogen with a worldwide distribution and a broad host range. This is the first record of *E. quericola* infecting *A. sophorae*, and the first powdery mildew infection recorded for this native Australian plant.

Coastal wattle (*Acacia sophorae*) is a shrub native to coastal and subcoastal south-eastern Australia. It is part of the vegetation of coastal heaths and forests, and also dunes as it tolerates sea sprays and sand blasts. In July 2020, heavy powdery mildew infections were observed on the leaves of *A. sophorae* in an open woodland forest at Tullymorgan, New South Wales, Australia. Whitish powdery mildew mycelium covered large parts of the abaxial leaf surfaces (Fig. 1a). Ten powdery mildew-infected leaves were collected from the spot, and pressed and dried for a week to produce specimens that were deposited at the Queensland Plant Pathology Herbarium under accession number BRIP 71600. A part of the herbarium material was taken to the laboratory for further investigations.

Morphological characteristics of hyphae, conidiophores and conidia were observed following rehydration of small samples of the powdery mildew mycelium using a method modified after Shin and La (1993). First, mycelial samples were removed from the dried leaves with 3-4 cm long pieces of cellotape. Each cellotape piece was immediately placed with mycelia downwards in a droplet of 100% v/v lactic acid pipetted onto a microscope slide. The slides were gently heated over an alcohol burner flame until boiling, then the cellotape pieces were lifted with a forceps, and the mycelial samples were scraped off with a scalpel into the lactic acid drops on the slides. The cellotape pieces were then removed, and a coverslip placed over the fungal samples. Slides were examined under a Nikon Eclipse Ni-U microscope (Nikon Co., Tokyo, Japan) with bright field and differential interference contrast (DIC) optics. Conidiophores produced conidia singly, and consisted of a foot-cell, straight or occasionally slightly curved-sinuous at the base, 22–41 × 7–12 µm, basal septum at the branching point, followed by one or two cells up to the same length as the foot-cell (Fig. 1b,c). Conidia were cylindrical or ellipsoid-cylindrical, and occasionally doliiform, 24–41 × 12–21 µm. Germinating conidia were also observed in the rehydrated materials. These produced germ tubes terminally or subterminally, ended in mostly multi-lobed appressoria, and were up to 3-4 times longer than conidia. Hyphal appressoria were also lobed or multi-lobed. All these morphological characteristics were diagnostic of the genus *Erysiphe*, but did not reveal the identity of the powdery mildew fungus at the species level.

To support the identification of the pathogen with molecular tools, total genomic DNA was extracted from powdery mildew mycelial samples removed from the host plant surfaces with 1–1.5 cm^2 pieces of cellotape. Cellotape pieces with mycelia were placed in 1.5 ml eppendorf tubes and DNA was extracted using the buffers of an Extract-N-Amp Plant PCR kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) of the powdery mildew fungus was amplified

from four DNA samples during a nested PCR as described by Kiss et al. (2020). The first PCRs used the powdery mildew-specific primers PMITS1 and PMITS2 developed by Cunnington et al. (2003). The nested reactions were done with the universal fungal primers ITS1-F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). PCR products of the nested reactions were purified and sequenced by Macrogen Inc. (Seoul, Korea) with primers ITS1-F and ITS4. Sequences were compiled from chromatograms following visual inspections for potential polymorphisms to identify any potential intra-sample variations in the nrDNA ITS sequences reported in some powdery mildews (Kovacs et al. 2011). Consensus sequences were trimmed and assembled with Geneious Prime 2019.1.3 (Biomatters Ltd.), and deposited in NCBI GenBank under accession number MW293874. These were identical to over 20 ITS sequences of *Erysiphe quercicola* available in GenBank, and Maximum Likelihood phylogenetic analysis conducted using MEGAX (Kumar et al. 2018) further confirmed the identity of the fungus (Fig 2).

Erysiphe quercicola was recognised by Takamatsu et al. (2007) as a distinct taxon within the species complex *E. alphitoides s. lat.* infecting diverse oak species (*Quercus* spp.). Soon after its description, *E. quercicola* was identified on several unrelated woody hosts, such as mango (*Mangifera indica*), rubber tree (*Hevea brasiliensis*), *Citrus* spp., and a number of other tropical and subtropical tree species (Kirschner and Liu 2014; Takamatsu et al. 2015; Desprez-Loustau et al. 2017, 2018; Meeboon and Takamatsu 2020). In Australia, *E. quercicola* was recorded on oak, mango, and also on the native *Acacia holosericea*, *A. mangium*, and *Eucalyptus camaldulensis* based on ITS sequence analyses (Limkaisang et al. 2006; Takamatsu et al. 2007, 2015). This is its first report on *A. sophorae* worldwide.

A recent comprehensive list of powdery mildew species identified in Australia indicated that all the species of this large group of plant pathogens, the Erysiphales, were introduced to Australia since the beginning of European colonisation of the continent (Kiss et al. 2020). Most

Australian native plants have never been reported as hosts of any powdery mildews. Those that are known to be infected with these pathogens were always attacked by species introduced from overseas. Kiss et al. (2020) hypothesised that all the powdery mildew infections of plants native to Australia recorded so far have been the results of quick host range expansion events of introduced species that are known to have wide host ranges overseas. This is supported by molecular clock evidence that suggests powdery mildews emerged in the late Cretaceous (Takamatsu 2013), after the separation of Australia from Gondwana. It is likely that the powdery mildew infection of *A. sophorae*, reported here for the first time globally, is a new example of a host range expansion of *E. quercicola*.

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Figure Captions

Fig. 1 (a) *Acacia sophorae* leaves infected with powdery mildew in New South Wales, Australia. (b) An immature conidiophore of *Erysiphe quercicola* removed from an *A. sophorae* leaf following rehydration in hot lactic acid. (c) A mature conidiophore of *Erysiphe quercicola* removed from an *A. sophorae* leaf following rehydration in hot lactic acid. Bars = 10 µm. Images were edited using Adobe Photoshop.

Fig. 2 Maximum-Likelihood Tree of an ITS sequence alignment using MEGAX. The scale bar shows 0.005 changes per site, and bootstrap support values from 1,000 replicates are shown at the nodes. Specimen from this study indicated in **bold**. Hosts and regions are indicated. Genbank accession numbers are provided in parentheses.

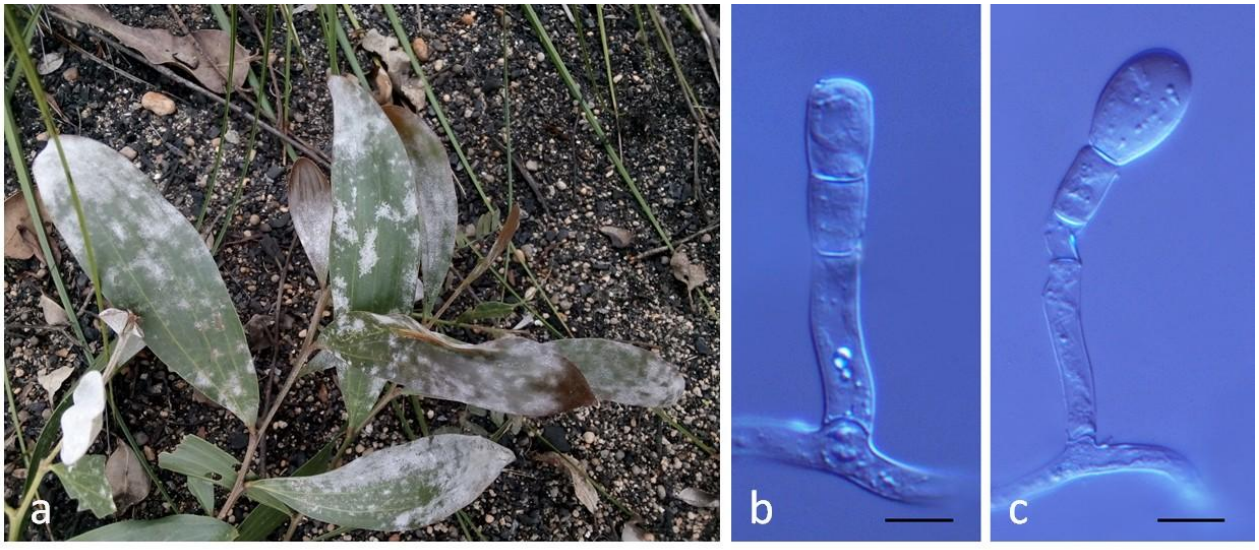
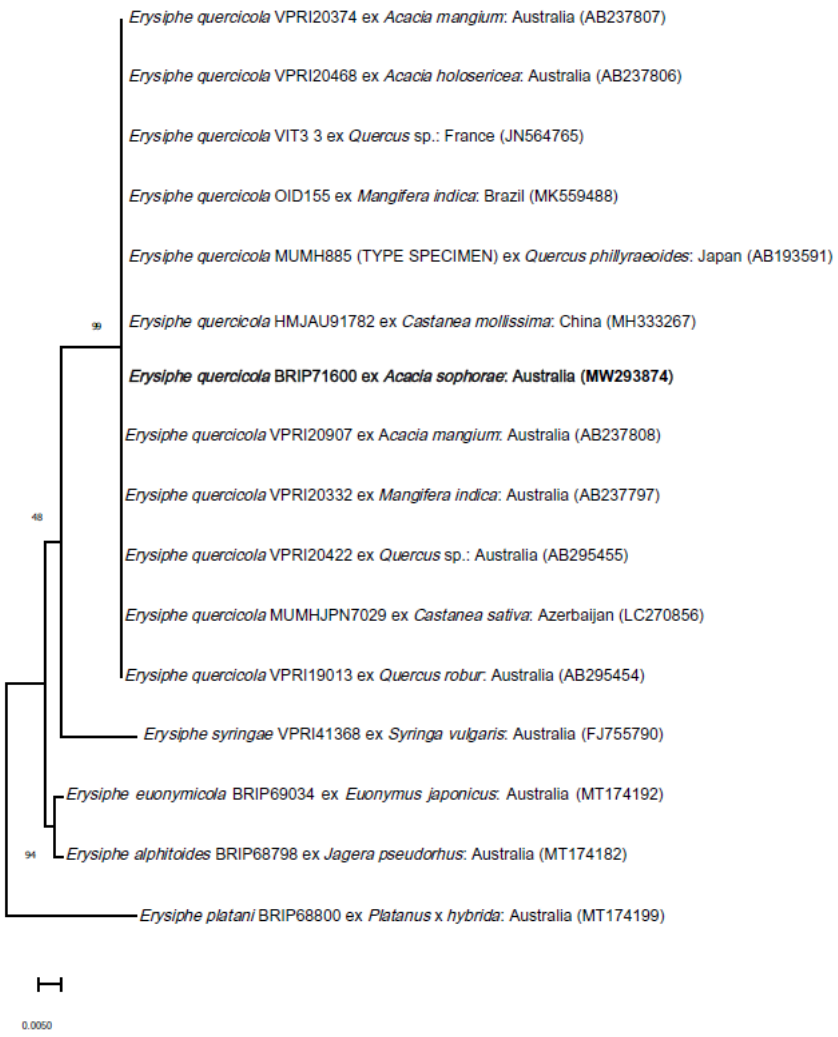


Fig. 1


Erysiphe quercicola VPRI20374 ex *Acacia mangium*: Australia (AB237807)
Erysiphe quercicola VPRI20468 ex *Acacia holosericea*: Australia (AB237806)
Erysiphe quercicola VIT3 3 ex *Quercus* sp.: France (JN564765)
Erysiphe quercicola OID155 ex *Mangifera indica*: Brazil (MK559488)
Erysiphe quercicola MUMH885 (TYPE SPECIMEN) ex *Quercus phillyraeoides*: Japan (AB193591)
99
Erysiphe quercicola HMJAU91782 ex *Castanea mollissima*: China (MH333267)
***Erysiphe quercicola* BRIP71600 ex *Acacia sophorae*: Australia (MW293874)**
Erysiphe quercicola VPRI20907 ex *Acacia mangium*: Australia (AB237808)
Erysiphe quercicola VPRI20332 ex *Mangifera indica*: Australia (AB237797)
48
Erysiphe quercicola VPRI20422 ex *Quercus* sp.: Australia (AB295455)
Erysiphe quercicola MUMHJPN7029 ex *Castanea sativa*: Azerbaijan (LC270856)
Erysiphe quercicola VPRI19013 ex *Quercus robur*: Australia (AB295454)
Erysiphe syringae VPRI41368 ex *Syringa vulgaris*: Australia (FJ755790)
Erysiphe euonymicola BRIP69034 ex *Euonymus japonicus*: Australia (MT174192)
94
Erysiphe alphitoides BRIP68798 ex *Jagera pseudorhus*: Australia (MT174182)
Erysiphe platani BRIP68800 ex *Platanus* x *hybrida*: Australia (MT174199)
0.0050

155

156 **Fig. 2.**