GLOMERELLA MIYABEANA ON WILLOWS IN AUSTRALIA

James H. Cunnington[1,3], Rachel A. Powney[1], Robin J. Adair[2] and Kyla J. Finlay[2]

[1.] *Primary Industries Research Victoria (PIRVic), Department of Primary Industries, Knoxfield Centre, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia*
[2.] *Primary Industries Research Victoria (PIRVic), Department of Primary Industries, Frankston Centre, PO Box 48, Frankston, Victoria 3199, Australia*
[3.] *Corresponding author; email: james.cunnington@dpi.vic.gov.au*

Abstract

Glomerella miyabeana is recorded for the first time in mainland Australia. The fungus was isolated from leaf lesions on *Salix fragilis*, *S. alba* var. *vitellina* and *S. cinerea* in New South Wales, Victoria and the Australian Capital Territory. Ribosomal DNA ITS sequences from cultures isolated from *S. alba* var. *vitellina* were identical to *G. miyabeana* sequences on GenBank, but those from *S. fragilis* differed by one base. As *G. miyabeana* has been reported to cause occasional fruit rots, simple pathogenicity experiments were conducted using golden delicious apples and nashi pears. *Colletotrichum acutatum* was about twice as aggressive as *Glomerella miyabeana* on the apples, and three times as aggressive on the nashi pears.

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Introduction

During a survey for willow pests and pathogens in south-eastern Australia in 2005–2006, we found *Glomerella miyabeana* (Fukushi) Arx for the first time on mainland Australia. The fungus was isolated from leaf lesions on *Salix fragilis* (crack willow), *S. cinerea* (pussy willow) and *S. alba* var. *vitellina* (golden willow) in NSW, Vic. and the ACT. *Glomerella miyabeana* is recorded in Tasmania (Sampson and Walker 1982), although no voucher specimen is located in any Australian plant pathology herbaria. During these surveys, we found *G. miyabeana* on *S. fragilis* and *S. purpurea* (purple osier willow) in Tasmania. *Glomerella miyabeana* is an aggressive pathogen of willows, causing a black canker and leaf spot (Spiers and Hopcroft 1993). It occurs in England, North America, New Zealand and Japan on a wide range of *Salix* species. In England, it has been noted to be especially aggressive on *Salix fragilis* and *S. alba* var. *vitellina* (Rose 1989), while in New Zealand these species were not affected (Spiers and Hopcroft 1993). Phylogenetic studies have shown that *Glomerella miyabeana* is a distinct species in the *Colletotrichum acutatum* J.H. Simmonds complex (Guerber *et al.* 2003). A morphological, molecular phylogenetic and pathogenicity study was undertaken to confirm the identity of the fungus and to determine its potential to cause fruit rots.

Materials and Methods

The fungus was isolated directly onto potato dextrose agar (PDA). Cultures were grown under a combination of white and UV fluorescent lights for 2 weeks. Material was mounted in 100% lactic acid, warmed and examined using interference contrast. Cultures

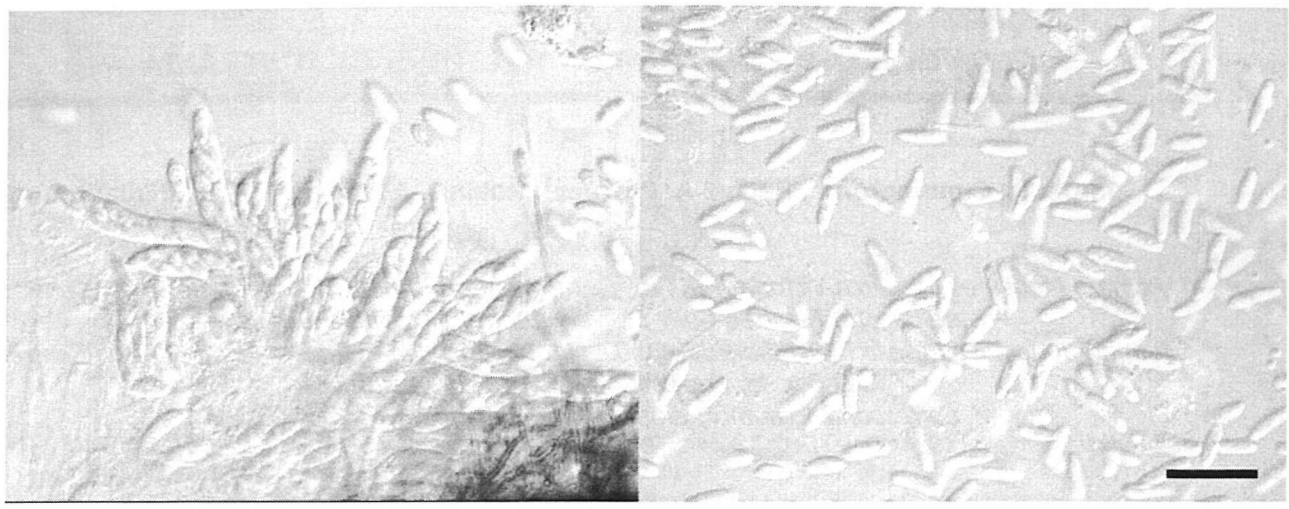

Figure 1. *Glomerella miyabeana* asci and ascospores (left), and conidia (right). Scale bar equals 30 μm.

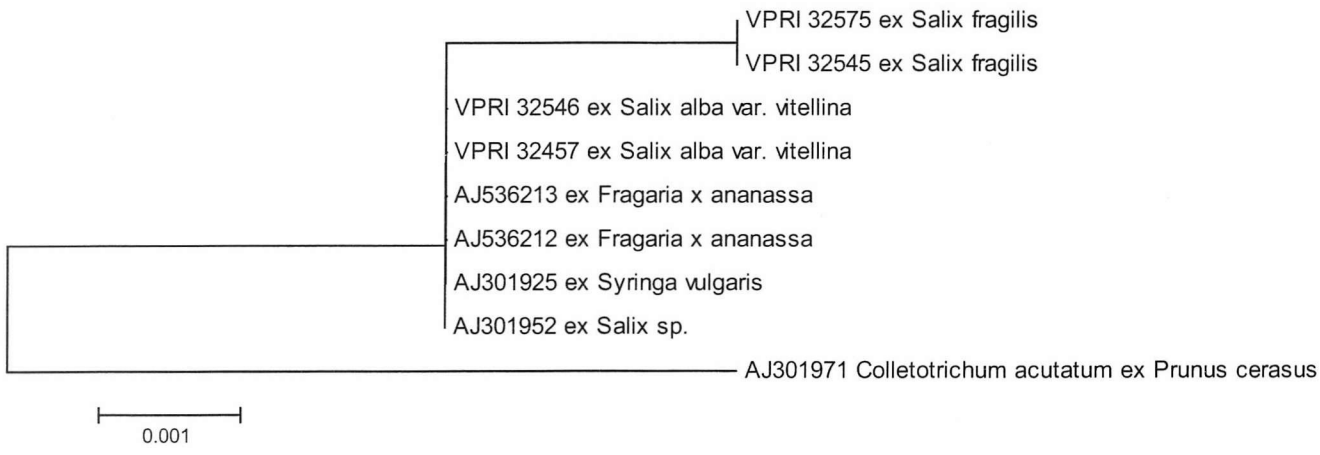

Figure 2. Minimum-evolution tree from rDNA ITS sequences showing the relationship between *Glomerella miyabeana* isolates from willow in Australia, and *G. miyabeana* sequences on GenBank. *Colletotrichum acutatum* was used as the outgroup and the scale bar equals one base change per 1000 bases.

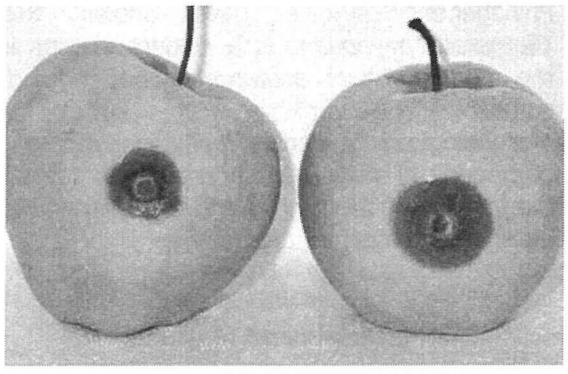

Figure 3. Comparison of *Glomerella miyabeana* (left) and *Colletotrichum acutatum* (right) as fruit rot pathogens of apple cv. Golden Delicious. Lesions one week after inoculation with mycelium plug.

and dried specimens have been deposited in herbaria VPRI and DAR (Table 1).

For molecular comparison with other *Glomerella* and *Colletotrichum* species, the ribosomal DNA ITS region was sequenced from two isolates from each *Salix* species (Table 1). DNA was extracted using a DNeasy™ Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Each 25 μL PCR reaction contained 5ng of DNA, 200 μM of each dNTP, 1.5 mM $MgCl_2$, 2.5 μL 10x buffer, 4 ng each of primers ITS1F (Gardes and Bruns 1993) and ITS4 (White *et al.* 1990), and 0.5 units of Taq polymerase. Reaction cycles were 35 cycles of 30 sec. at 94°C, 30 sec. at 50°C, 1 min. at 72°C. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen) and sequenced directly using primers

ITS1F and ITS4, with an ABI PRISM® BIGDYE™ Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions. Sequences have been deposited in GenBank (Table 1). A minimum evolution tree was constructed with MEGA2 (Kumar *et al.* 2001) to show the phylogenetic relationships of these sequences. *Colletotrichum acutatum* was used as the outgroup.

To compare the fruit rotting pathogenicity of *G. miyabeana* with *Colletotrichum acutatum*, apple (cv Golden Delicious) fruit and nashi pear fruit were inoculated with 6 mm agar plugs of each fungus according to the methods described in Pitt *et al.* (1991). The culture of *C. acutatum* (VPRI 16247) originated from apple fruit. Twelve replicates of each fungus on each fruit were prepared. Fruits were placed in a plastic bag and left at room temperature for 1 week.

Results and Discussion

Leaves had large blackened lesions that appeared to be extending quickly along the blade. Leaves ultimately shrivelled and died. The morphology of the fungus agreed well with recent descriptions of *G. miyabeana* (Ellis and Ellis 1985, Spiers and Hopcroft 1993). Ascocarps were about 2 mm in diameter, initially formed singly, but later in clumps. Asci were clavate, about 50–70 × 10 µm, and ascospores were hyaline, aseptate, oblong, about 13–6 × 5 µm (Figure 1). Most isolates produced a pink-red pigment. The conidial state (*Colletotrichum* sp.) was not formed in culture, but was seen on moist incubated leaves. Conidia were hyaline, aseptate, straight to slightly curved, approximately 20 × 5 µm (Figure 1).

The two isolates from *S. fragilis* had identical ITS sequences. These differed from the two *S. alba* var. *vitellina* isolates by one nucleotide. These latter sequences were identical to four sequences on GenBank, none of which were labelled *G. miyabeana*. These include two isolates from strawberry in New Zealand collected by Johnston and Jones (1997), and identified by them as *G. miyabeana*, that were later sequenced by Martinez-Culebras *et al.* (2003) who called them *Colletotrichum acutatum*. The ITS sequences were also identical to two sequences designated *G.* cf. *cingulata* that were isolated from *Salix* sp. and *Syringa vulgaris* (Nirenberg *et al.* 2002). It seems likely that these two collections are *G. miyabeana* given the identical ITS sequence and host relationship (at least for the isolate from *Salix*). The next most similar sequences in GenBank belonged to the *C. acutatum* complex. These differed by at least five bases. The phylogenetic relationship between these sequences is shown in Figure 2.

Although *G. miyabeana* is primarily a willow pathogen, it has been recorded in New Zealand from ripe fruit rots on strawberry, apple, nashi pear and tomato (Johnston and

Table 1. Collection details, herbarium accession numbers and GenBank accession numbers of *Glomerella miyabeana* specimens examined.

Specimen	Host	Collection details	ITS GenBank accession
VPRI 32546	*Salix alba* var. *vitellina*	Brindibella, ACT, Nov. 2005	EF452724
VPRI 32547	*Salix alba* var. *vitellina*	Brindibella, ACT, Nov. 2005	EF452725
VPRI 32735	*Salix cinerea*	Cann River, Vic., Mar. 2006	-
VPRI 32545	*Salix fragilis*	Tumut River, NSW, Nov. 2005	EF452726
VPRI 32575	*Salix fragilis*	Tumut River, NSW, Nov. 2005	EF452727
VPRI 32737	*Salix fragilis*	Queenstown, Tas., Feb. 2006	-
VPRI 32736	*Salix purpurea*	Blackfish Creek, Tas., Feb. 2006	-

Jones 1997). We compared the pathogenicity of *G. miyabeana* with *C. acutatum*. After one week, *C. acutatum* lesions had an average radial growth of 11 mm on the apples and 9.5 mm on the nashi pears. *Glomerella miyabeana* lesions had an average radial growth on 6.5 mm on the apples and 3.5 mm on the nashi pears. It is unlikely that *G. miyabeana* will be a fruit rot pathogen of significance in Australia as willows, the primary inoculum source, are generally not associated with fruit orchards in Australia. This contrasts with orchards in New Zealand where willows are commonly used as shelter belts (Johnston and Jones 1997).

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