

## ***Ampelocissus* plants harbor *Phakopsora* rust pathogens of grapevines and Boston Ivy**

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### **Abstract**

Susceptibility of Australian *Ampelocissus* plants to the grapevine leaf rust species complex was examined by urediniospore inoculation under laboratory conditions. Grapevine leaf rust fungus, *Phakopsora euvitidis*, and Boston Ivy rust fungus, *P. vitis*, infected and sporulated on the *Ampelocissus* plants, while *Ampelopsis* rust fungus, *P. ampelopsidis*, did not. The results confirmed the earlier report that *Ampelocissus* species support sporulation of *P. euvitidis* in northern Australia. Discovery of two *Ampelocissus* species to serve as the common hosts for two distinct *Phakopsora* species outside of their natural distribution area may throw light on the study on speciation process of the grapevine leaf rust species complex.

**Keywords** – *Ampelopsis* · *Parthenocissus* · Pucciniales · Vitaceae · *Vitis*

### **Introduction**

Leaf rust is among the most economically important fungal diseases of grapevines. Heavy rust infection results in early defoliation and reduction of fruits quality and yield. The causal pathogen, *Phakopsora euvitidis* Y. Ono, is known to be distributed widely in Asia (Ono 2000). Recently, this pathogen was inadvertently introduced to wine and table grape growing areas in northern Australia (Weinert et al. 2003) and in the states of Mato Grosso, Paraná and São Paulo in Brazil (de Souza 2004; Tessman et al. 2004). Since then, the rust fungus has been listed as the invasive rust species potentially hazardous to viticulture in the world (<http://www.cabi.org/isc/?compid=5&dsid=40016&loadmodule=datasheet&page=481&site=144>; [http://www.eppo.int/QUARANTINE/Alert\\_List/fungi/PHLLAM.htm?utm\\_source=www.eppo.org&utm\\_medium=int\\_redirect](http://www.eppo.int/QUARANTINE/Alert_List/fungi/PHLLAM.htm?utm_source=www.eppo.org&utm_medium=int_redirect); <http://nt.ars-grin.gov/taxadescrptions/factsheets/index.cfm?thisapp=Phakopsoraeuvitidis>).

In the native distribution areas, *P. euvitidis* alternates between *Meliosma myriantha* Siebold

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& Zucc. as spermogonial/aecial host and various *Vitis* species as uredinial/telial hosts (Ono 2000). Experimental inoculations did not prove additional vitaceous genera that harbor this rust pathogen in Japan (Ono 2000). However, these experimental results and continued field observations in Japan do not preclude the possibility that potential hosts for *P. euvitidis* exist in geographic regions where this pathogen has not been found and viticulture has been rapidly expanding. After the confirmation of its establishment in the Northern Territory, Australia, in 2001, *P. euvitidis* was proven to infect and sporulate on native *Ampelocissus acetosa* Planch. and *A. frutescens* B. R. Jackes under laboratory conditions (Daly et al. 2005) and, subsequently, one plant of unidentified *Ampelocissus* species was found naturally infected in the field by *P. euvitidis* (Daly and Hennessy 2006).

Discovery of the new host genus with two species for the grapevine leaf rust (GLR) fungus prompted the experimental study to elucidate whether *Ampelocissus* plants also support infection and sporulation of *P. vitis* and *P. ampelopsidis*, because knowing potential hosts for the pathogens is very basic in developing the rust control strategies and because the host specificity aids the study of evolutionary pathway of the *Phakopsora* pathogens on plants of the grape family.

### Materials and methods

Experimental inoculations were carried out in 2006–2010 at Ibaraki University campus in Mito, Ibaraki, Japan. Unidentified species of *Ampelocissus*, *Vitis* × *labruscana* L. H. Bailey cv. Kyoho, *Parthenocissus tricuspidata* Planch., and *Ampelopsis brevipedunculata* (Maxim.) Trautv. were planted in clay pots of 15 cm diameter or larger with loam soil and maintained in a greenhouse to avoid possible spontaneous rust infections.

Inocula used were: *P. euvitidis* urediniospores formed on *V. × labruscana* cv. Kyoho, Ibaraki, Hitachiota, 21 Oct 2006, Y. Ono (YO) and S. Kodato (SK) (IBAR9779); Ibaraki, Suifu, 8 Sep 2009, YO (IBAR10112); Yamanashi, Koshu, 12 Sep 2009, YO & S. Pota (SP) (IBAR10123); *P. vitis* urediniospores formed on *P. tricuspidata*, Ibaraki, Suifu, 6 Sep 2006, YO & SK (IBAR9774); Ibaraki, Hitachiota, 21 Oct 2006, YO & SK (IBAR9780); Ibaraki, Mito, 8 Oct 2007, YO (IBAR9894); Fukushima, Iwaki, 5 Sep 2009, YO (IBAR10108); *P. ampelopsidis* urediniospores formed on *A. brevipedunculata*, Ibaraki, Hitachiota, 6 Sep 2006, YO & SK (IBAR9776); 8 Sep 2009, YO (IBAR10111); Ibaraki, Suifu, 8 Sep 2009, YO (IBAR10110); Ibaraki, Kasama, 12 Nov 2010, YO & H. Kamiki (IBAR10343); Fukushima, Iwaki, 12 Oct 2007, YO (IBAR 9895); Saitama, Chichibu, 12 Sep 2009, YO & SP (IBAR10116).

In each inoculation, five to ten apparently healthy leaves of each plant species (juvenile seedling leaves in unidentified *Ampelocissus* species) were inoculated by the method described by Ono (1994). The plant species from which the inoculum were taken were also inoculated with the same inoculum. The control plants were only sprayed with distilled water. The inoculations were carried out at room temperature between 15°C and 22°C; however, whenever possible, the inoculated plants were placed in a growth chamber at ca. 20°C with controlled artificial illumination for subsequent observations.

Vouchers from the successful inoculations are: *P. euvitis* on unidentified *Ampelocissus* species, Ibaraki, Mito, Ibaraki Univ. campus, 24 Nov 2006, YO & SK (IBAR9781); 2 Oct 2009, YO (IBAR10114); *P. vitis* on unidentified *Ampelocissus* species, Ibaraki, Mito, Ibaraki Univ. campus, 26 Sep 2006, YO & SK (IBAR9778); 24 Nov 2006, YO & SK (IBAR9782); 10 Nov 2007, YO (IBAR9941); 2 Oct 2009, YO (IBAR10113).

Uredinium structure and morphology of paraphyses and spores were examined under a differential interference contrast (DIC) microscope (Olympus BX51-DIC) equipped with an Olympus DP21 digital photography system. Spore size (20 spores/life cycle stage/specimen) was measured with an ocular micrometer.

### Results and discussion

All inoculations with urediniospores formed on *Vitis* and *Parthenocissus* plants resulted in successful infection and sporulation on *Ampelopsis* plants and either *Vitis* or *Parthenocissus* plants, from which the inoculum urediniospores were taken (Table 1). Latent periods in the inoculation on the *Ampelocissus* plants with *P. euvitis* urediniospores were 7-14 days and those with *P. vitis* urediniospores were 11-18 days. Urediniospores from *Ampelopsis* plants infected and sporulated only on plants of the same species; and no sign of infection was detected on the inoculated *Ampelopsis* plants. Control plants only sprayed with distilled water also showed no sign of infection.

Table 1 Results of urediniospore inoculations on unidentified *Ampelocissus* species<sup>a, b</sup>

Inoculum <sup>a</sup> from (Voucher <sup>c</sup> )	Origin of inoculum	Date of inoculation	Date of uredinium maturation	Voucher <sup>c</sup>
<i>Vitis</i> × <i>labruscana</i> cv. Kyoho (IBAR9779)	Ibaraki, Hitachiota	21 Oct 2006	4 Nov 2006	IBAR9781
<i>Vitis</i> × <i>labruscana</i> cv. Kyoho (IBAR10112)	Ibaraki, Suifu	8 Sep 2009	15 Sep 2009	IBAR10114
<i>Vitis</i> × <i>labruscana</i> cv. Kyoho (IBAR10123)	Yamanashi, Koshu	20 Sep 2009	30 Sep 2009	Not preserved
<i>Parthenocissus tricuspidata</i> (IBAR9774)	Ibaraki, Suifu	12 Sep 2006	23 Oct 2006	IBAR9778
<i>Parthenocissus tricuspidata</i> (IBAR9780)	Ibaraki, Hitachiota	21 Oct 2006	8 Nov 2006	IBAR9782
<i>Parthenocissus tricuspidata</i> (IBAR9894)	Ibaraki, Mito	8 Oct 2007	23 Oct 2007	IBAR9941
<i>Parthenocissus tricuspidata</i> (IBAR10108)	Fukushima, Iwaki	7 Sep 2009	18 Sep 2009	IBAR10113

<sup>a</sup> Urediniospores from *Ampelopsis brevipedunculata* were also inoculated with no successful infection leading to sporulation on *Ampelocissus* plants.

<sup>b</sup> *Vitis* × *labruscana* cv. Kyoho and *Parthenocissus tricuspidata* plants were also inoculated, with successful sporulation only in the combinations of the same inoculum and inoculated host species.

<sup>c</sup> Vouchers are deposited in the Herbarium of Systematic Mycology, Ibaraki University (IBAR)

Uredinia formed both by *P. euvitis* and *P. vitis* were minute, surrounded by necrotic host tissues and never expanded (Figs. 1a, b). The sori were sometimes covered with resinous fluid exuded from the infected host tissues and the sporulation did not last long. Uredinial paraphyses from the *P. euvitis*-inoculated plants were cylindrical, weakly to moderately incurved, 29–52µm high and 7–15µm wide.

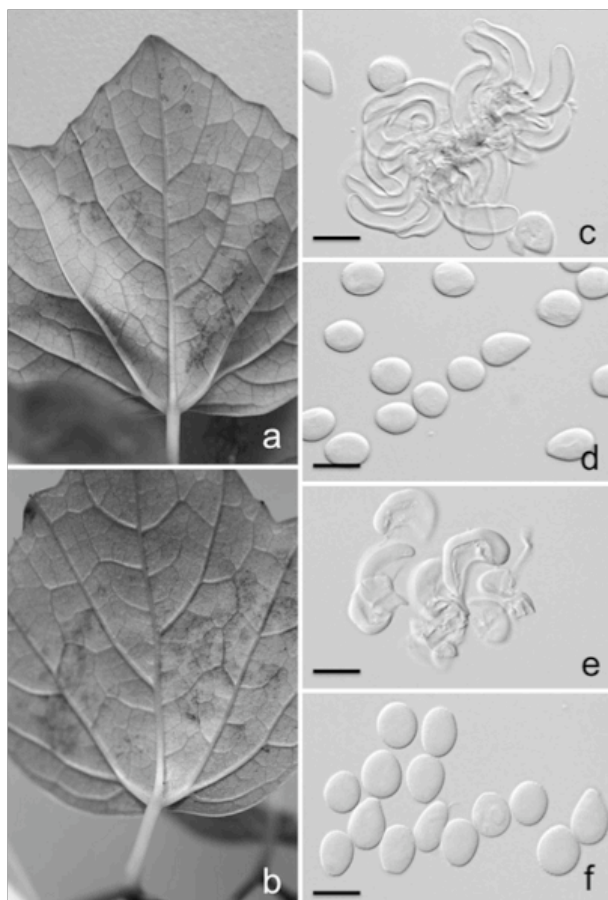


Figure 1. Uredinia and urediniospores formed on unidentified *Ampelocissus* species by experimental urediniospore inoculations. **a.** Uredinia formed on abaxial leaf surface by inoculation of urediniospores from *Vitis x labruscana* cv. Kyoho. **b.** Uredinia formed on abaxial leaf surface by inoculation of urediniospores from *Parthenocissus tricuspidata*. **c & d.** Uredinial paraphysis and urediniospores formed by inoculation of urediniospores from *V. labruscana* cv. Kyoho. **e & f.** Uredinial paraphysis and urediniospores formed by inoculation of urediniospores from *P. tricuspidata*. Bar: 20μm.

The wall was colorless and dorsally 1.2–2.6μm thick (Fig. 1c). Urediniospores were subglobose, broadly ellipsoid or obovoid, and 19–22 × 12–17μm in size (Fig. 1d). The wall was colorless, uniformly ca. 1.5μm thick and evenly echinulate. These paraphysis and spore morphologies were the same as those of the inoculum formed on the *Vitis* plants. Uredinial paraphyses from the *P. vitis*-inoculated plants were clavate to short-cylindrical, strongly incurved, 24–42μm high and 9–17μm wide (Fig. 1e). The wall was colorless and dorsally 2.4–6.7μm thick. Urediniospores were subglobose, broadly ellipsoid or obovoid, and 17–25 × 13–17μm in size (Fig. 1f). The wall was colorless, uniformly ca. 1.5μm thick and evenly echinulate. These paraphysis and spore morphologies were the same as those of the inoculum formed on the *Parthenocissus* plants.

Successful inoculations of unidentified *Ampelocissus* species with *P. euvitidis* urediniospores confirmed the earlier report of experimental inoculations with *P. euvitidis* urediniospores onto *A. acetosa* and *A. frutescens* in Darwin, Australia (Daly et al. 2005). The experimental inoculation study was prompted by the inadvertent introduction of *P. euvitidis* to Darwin, Northern Territory, Australia, in 2001 (Weinert et al. 2003). *Cayratia maritima* B. R. Jackes and *Cissus adnata* Roxb. were also inoculated to find out if they also serve as potential hosts for *P. euvitidis*; however, the result was negative. Although moderately susceptible reactions were observed on the *Ampelopsis* plants in the experiments carried out in Australia, less susceptible reactions were observed in this study. These different host reactions might have arisen from genetic differences in the pathogen's virulence and the host plant susceptibility, and environmental conditions under which the inoculations were carried out. It is also possible that the rust populations in East Asia and Australia are different in species level (Chatasiri and Ono 2008).

The inoculation experiments proved that *Ampelocissus* species native of Northern Territory, Australia, can be the host of *P. euvitidis*. A single unidentified *Ampelocissus* plant was found to have a low level of sporulation in Darwin in 2006 (Daly and Hennessy 2006). Nevertheless, deciduous nature of the plants suggests little contribution of native *Ampelocissus* plants as the persistent source of inoculum for GLR outbreaks in this geographic region (Daly et al. 2005; Daly and Hennessy 2006).

In this study, *Ampelocissus* plants were found, for the first time, to harbor *P. vitis*. *Phakopsora vitis* is distributed in China, Korea, Japan, Taiwan and the Philippines (Ono 2000); and it could be inadvertently introduced to Northern Territory, Australia, by rust infected Boston Ivy plants. However, as in *P. euvitidis*, it is unlikely that the Boston Ivy rust pathogen persists on *Ampelocissus* plants in this tropical region.

Discovery of the common *Ampelocissus* hosts for phylogenetically different *P. euvitidis* and *P. vitis*, and of the inability of the *Ampelocissus* plants to support infection of *P. ampelopsidis* may stimulate the study of evolutionary diversification of the GLR species complex (Chatasiri and Ono 2008). Recent phylogenetic analyses of vitaceous genera with three chloroplast markers (Soejima and Wen 2006), complete chloroplast genome sequence (Jansen et al. 2006), nuclear GAI1 gene sequences (Wen et al. 2007) and noncoding plastid *trnC-petN*, *trnH-psbA*, and *trnL-F* sequences (Ren et al. 2011) showed the consistent phylogenetic pattern with various detailed resolutions. The *Ampelopsis* clade is sister to the *Rhoicissus/Cissus striata* clade. This inclusive clade is sister to another inclusive clade, in which the *Parthenocissus* clade is sister to *Yua* clade. This further inclusive clade and the *Vitis/Ampelocissus/Pterisanthes* clade are in sister relationship. On the other hand, in phylograms generated from ITS2 and D1/D2 sequences (Chatasiri and Ono 2008), the *P. euvitidis* clade is sister to the *P. ampelopsidis* clade, and this inclusive clade and the *P. vitis* clade are in sister relationship. These reconstructed phylograms for the vitaceous genera and their pathogens do not exactly match each other. Apparent incongruence between the phylogenetic patterns of the vitaceous genera and the GLR species complex suggests the speciation had taken place by the jump rather than the host-tracking evolution (co-evolution) (Roy 2001; Chatasiri and Ono 2008).

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### Disclosure

Inoculation experiments carried out with plant pathogenic fungi onto cultivated plants did not violate current Japanese laws and regulations. No part of the paper has been published previously.

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