Wild-sourced *Chamelaucium uncinatum* have no resistance to *Puccinia psidii* (myrtle rust)

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Abstract *Chamelaucium uncinatum* is a West Australian endemic shrub that is grown as a garden ornamental and for the cut flower market. Commercial cultivars are highly susceptible to infection by *Puccinia psidii*, which causes leaf, stem, bud and flower rust. Pathogenicity tests showed that wild-sourced *C. uncinatum* plants have no apparent natural resistance to this pathogen.

Keywords *Puccinia psidii* · Myrtle rust · Geraldton waxflower · *Chamelaucium uncinatum*

Since *Puccinia psidii* was introduced into Australia in 2010 (Carnegie et al. 2010) the rust fungus has established on a wide range of species within the Australian Myrtaceae, including 346 taxa in 57 genera (Giblin and Carnegie 2014). *Puccinia psidii* has already spread geographically with records from north of Cairns (16°91'S 145°77'E) in Queensland (Pegg et al. 2013) to west of Melbourne (38°07'S 144°31'E) in Victoria (Kriticos et al. 2013). The pathogen was recently reported in Burnie, Tasmania (41°05'S, 145°90'E) (<http://biosecurityadvisory.dpipwe.tas.gov.au/> accessed 5

March 2015). There are concerns that *P. psidii* will devastate natural ecosystems, dominated by plants in the Myrtaceae, as well as commercial cut flower, native food, essential oil and forestry industries (Glen et al. 2007; Carnegie and Lidbetter 2012).

The natural distribution of *C. uncinatum* (Geraldton waxflower) is the coastal region north of Perth to Kalbarri, Western Australia (WA) (Considine and Webb 1999). Accounting for around 40 % of all Australian wildflower production, the farmgate value of Geraldton waxflower is estimated to be more than \$20 million per year (Michael 2011). Internationally Geraldton waxflower is an important commercial crop with large areas of cultivation in Israel and California (Considine and Webb 1999).

Controlled inoculations of commercial Geraldton waxflower cultivars have shown them to be highly susceptible to infection by *P. psidii* (Morin et al. 2011, 2012). Seed set is low in this species, and the cultivation of new plants is from cuttings. Though most Australian myrtaceous species have been shown to be vulnerable to infection by *P. psidii*, a small proportion of resistant individuals have usually been found in wild populations (Morin et al. 2012; Pegg et al. 2014). Such individuals may be useful for breeding programs to improve resistance to *P. psidii*.

To determine the potential for variability in susceptibility within the Geraldton waxflower population across its native range, cuttings were taken from 56 wild plants at 11 locations north of Perth, WA during early April 2014. Population locations were identified from previous field work conducted by the University of Western Australia with the aim of capturing as much of the natural genetic diversity as possible (Considine and Webb 1999). Location

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Fig. 1 *Chamelaucium uncinatum* (Geraldton waxflower) specimens were collected from locations north of Perth, WA as indicated by black diamonds, using co-ordinates in GoogleMaps

co-ordinates (latitude and longitude) for each accession were recorded and mapped (Fig. 1).

Plant material was transported to the Plant Breeding Institute (PBI), University of Sydney. Cuttings (around 10 cm in length) were made from the collected samples, dipped in Yates Clonex Purple[®] rooting gel (6 mg/L indole-3-butyric acid) and propagated in sterile vermiculite/peat mix (50:50) in 100 cell plug trays. Trays were kept on a mist bench at ~22 °C constant temperature. Once roots were established (approximately 2 months), plants were potted into 100 mm pots and hardened off. The 11 populations represented a range of phenotypes with the most northerly Hutt River (28°07'35"S, 114°29'01"E) plants having larger leaves (~35 mm in length) and flowers (~18 mm in diameter)

compared to the Guilderton (31°20'21"S, 115°29'27"E) and Seabird populations (~15 mm leaves and ~14 mm flowers).

Successfully propagated plant accessions were inoculated under controlled greenhouse conditions. Urediniospores from a single isolate (Sandhu and Park 2013), maintained in liquid nitrogen at the Plant Breeding Institute, were placed in 10 mL of Isopar[®] and the mixture agitated. Spore concentration was measured at 8.1×10^5 spores per mL. Aerosol spraying of this inoculum was conducted in a chamber before moving the plants to a dark, humid room where they were incubated at 20–22 °C overnight (12 h). Uninfected *Syzygium jambos* plants were inoculated as positive controls for rust infection (Morin et al. 2012).

Fig. 2 *Chamelaucium uncinatum* (Geraldton waxflower) plants showed no sign of resistance to controlled inoculation with *Puccinia psidii*. Pustules were present on all specimens at 14 days post inoculation

Following inoculation, plants were moved into poly-glasshouse conditions and watered with drip irrigation. Temperature was set to 23 °C with a fluctuating range from 17 to 24.5 °C.

Within 14 days, under these controlled inoculation conditions, all 56 accessions developed lesions with abundant sporulation on new growth leaves of all plants, as well as stems, buds and flowers of some specimens (Fig. 2), and were classified as highly susceptible. No evidence of hypersensitive response or resistance was detected in any of the 56 accessions tested. *Syzygium jambos* plants also developed pustules within 14 days.

Results from the testing of wild-sourced Geraldton waxflower plants collected across a wide geographic range (28°07'35"S, 114°29'01"E to 31°20'21"S, 115°29'27"E) indicate that this species is highly susceptible to *P. psidii*. The plants tested all displayed similarly high levels of susceptibility with regard to timing and infection phenotype. These results indicate that *P. psidii* poses a serious threat to Geraldton waxflower in natural populations as well as in commercial cultivation. The susceptibility of flowers may reduce seed set and threaten the longer term survival of natural populations of this important species.

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Conflict of interest The authors declare no conflict of interest in the reporting of these results.

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