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Final report:

Uredo rangelii life-cycle

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A report for Plant Health Australia Ltd

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Addendum

As part of this project, a paper based on the research presented in this report was prepared and submitted to a scientific journal in early 2013. At the request of reviewers, additional research was performed during 2013, with support from CSIRO. A revised paper with additional results was subsequently submitted and accepted for publication in the journal *Fungal Biology*. The citation for this paper is:

Morin L, Talbot MJ, Glen M. 2014. Quest to elucidate the life cycle of *Puccinia psidii* sensu lato. *Fungal Biology* 118:253–263.

Contents

Acknowledgments

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Summary

Elucidating the life cycle of rust fungi can improve our understanding of how variation may evolve in populations, which assists in the development of management strategies. There is controversy surrounding the life-cycle of the rust fungus *Uredo rangelii* (myrtle rust) that infects a wide range of plant species in the family Myrtaceae. The goal of this project was to elucidate the life-cycle of myrtle rust by performing a series of inoculations using basidiospores and investigating its nuclear behaviour at different stages of development. No spermagonia developed on leaves of the highly susceptible host *Agonis flexuosa* cv. Afterdark in any of the eight inoculation experiments performed using teliospore suspensions or basidiospores naturally discharged from telia. In half of the experiments however, sori morphologically indistinguishable from uredinia were observed. Presence of urediniospores among naturally discharged basidiospores in concurrent in vitro tests casts doubt that these sori were the result of basidiospore infections. Microscopic investigations further supported that basidiospore inoculation is most unlikely to lead to development of uredinia. The nuclear status of mycelia of uredinial sori, urediniospores and teliospores was typical of that of many rust fungi. The four-celled metabasidium exhibited a regular nuclear development, with uninucleate basidiospores becoming binucleate. Tri- or tetra-nucleate basidiospores were never observed. These initial results do not support the previous conjecture that myrtle rust is autoecious. It is possible that this rust fungus could be heteroecious, with an unknown alternate aecial host, but more research is required to obtain unequivocal proof.

1 Introduction

Uredo rangelii (myrtle rust) belongs to the guava/eucalyptus rust complex (*Puccinia psidii* sensu lato) that infects plant species of the Myrtaceae family, which include many Australian native species. It was first detected in April 2010 on the central coast of NSW (Carnegie et al. 2010) and despite considerable containment efforts it has now spread widely in NSW, Queensland and Victoria. CSIRO was involved in the response to this incursion by undertaking a series of trials to explore the extent of the host-range of the fungus within the Myrtaceae (Morin et al. 2012).

There is controversy surrounding the life-cycle of the guava/eucalyptus rust complex. The rust is considered to be autoecious by Figueiredo et al. (1984). These authors reported that basidiospore infections of *Syzygium jambos* led to the production of aecia and aeciospores that were morphologically indistinguishable from uredinia and urediniospores, but did not observe any spermogonia (pycnia). Simpson et al. (2006) however, questioned these findings and believe that the rusts in this complex are heteroecious, with an alternate aecial host that has not yet been identified.

Addressing knowledge gaps relating to the life-cycle of myrtle rust will improve our understanding of how variation may evolve in the population of this rust fungus. The source of genetic variability in this rust will influence its evolutionary potential, which in turn can have implications for the durability of genetic resistance bred into commercially important forestry species (McDonald and Linde 2002).

The goal of this project was to elucidate the life-cycle of myrtle rust by:

- performing a series of inoculations using basidiospores of myrtle rust, and
- investigating the nuclear behaviour of myrtle rust at different stages of development using microscopy techniques.

2 Material and Methods

2.1 Inoculation experiments

A series of successive inoculations were performed using teliospores of myrtle rust originating from three different host plants: *Agonis flexuosa* cv. Afterdark, *Lindsayomyrtus racemoides* and *Syzygium francissii*. The *A. flexuosa* cv. Afterdark plant had been inoculated with a bulk isolate of myrtle rust (ex. Somersby Plateau, New South Wales, Australia), as described in Morin et al. (2012), placed in a controlled-environment room at 24°C (12-h photoperiod) for 2 weeks and then transferred to another room at 20°C (12-h photoperiod) for c. 6 weeks until teliospores were found within uredinia. *Lindsayomyrtus racemoides* and *S. francissii* were inoculated with a single-uredinium isolate of myrtle rust as part of the host-range study reported in Morin et al. (2012) and had developed abundant telia within 3 weeks of inoculation.

Young *A. flexuosa* cv. Afterdark leaves were inoculated with a teliospore suspension or by basidiospores naturally discharged from telia (source of teliospores/telia for each inoculation listed in Table 1). Teliospores were removed from telia using a fine needle with the help of a stereomicroscope and suspended in liquid paraffin oil or in the inert speciality liquid hydrofluoroether HFE-7100 (3M) to prevent spore clumping. The suspension was then applied using a fine camel hair paint brush onto the adaxial surface of leaves still attached to plants (6–10 leaves per inoculation). Inoculated plants were incubated as described in Morin et al. (2012). Following each inoculation, an aliquot of the teliospore suspension was applied onto the surface of two 1 cm^2 blocks of 2% water agar and incubated under the same conditions as plants. After 24 h, agar blocks were placed on drops of blue-lacto-glycerol stain (0.02g aniline blue, 10 ml glycerol, 10 ml lactic acid, 10 ml deionised water) to stop the germination process. Teliospore germination and basidiospore production were assessed using a light microscope.

Table 1 Methodology and results of each of the inoculation performed.

Inoculation no.	Teliospore source (plant sp.)			Material inoculated[1]		Inoculation method			Sori observed
	Agonis flexuosa 'Afterdark'	*Lindsayomyrtus racemoides*	*Syzygium francissii*	Leaves attached to plants	Detached leaves	Teliospore suspension in oil[2, 3]	Teliospore suspension in HFE[2, 4]	Natural basidiospore discharge[5]	
1	✓			✓		✓			uredinial
2	✓			✓			✓		uredinial
3		✓		✓			✓		uredinial
4		✓		✓				✓	none
5		✓		✓				✓	none
6		✓			✓			✓	uredinial
7			✓		✓			✓	none
8			✓		✓			✓	none

[1] Only healthy *A. flexuosa* 'Afterdark' plants were used.
[2] The suspension was applied to leaves using a fine camel hair paint brush and also contained a few urediniospores.
[3] Liquid paraffin oil helps prevent clumping of spores.
[4] HFE-1700 (3M) is an inert speciality liquid used to clean electronic circuit, which helps prevent clumping of spores.
[5] Small pieces of plant tissue with one or two telia were excised and placed on water agar contained in plates (telia facing upwards). Plates were then inverted over leaves still attached to plants or over Petri dishes lined with moist paper towel into which detached leaves had been placed.

Alternatively, small pieces of plant tissue with one or two telia were excised and placed on 2% water agar contained in 9-cm Petri dishes. Plates, without lid, were then inverted over leaves (6–20 per inoculation) still attached to plants or detached and placed, adaxial surface facing upwards, on moist paper towel in 9-cm Petri dishes. Telia were directly located above leaves so that basidiospores produced were discharged onto their surface. Plates with telia were secured to dishes containing detached leaves with parafilm and

placed in a controlled-environment room at 20°C (12-h photoperiod) for 48 h. The telia plates were then removed and replaced with Petri dish lids. To prevent growth of saprophytic fungi, detached leaves were surface sterilised 5 days after inoculation by immersion in 1% NaOCl solution for 1.5 min followed with two 1 min rinses in sterile deionised water. Leaves were then blotted dry with paper towel and placed, adaxial surface facing upwards, on 2% water agar in 9-cm Petri dishes in a controlled-environment room (conditions as above).

For inoculation of attached leaves, one large plant was laid horizontally on a bench of a controlled-environment room (conditions as above) and plates with telia were inverted over selected leaves secured to the bench with tape. The whole set up was misted with water, covered with a plastic bag for 48 h and then dismantled. The plant was returned to its vertical position and left on the bench of the room.

Following each inoculation with naturally discharged basidiospores, telia were placed on a 2 cm^2 block of 2% water agar on the inside of the lid of a Petri dish, which contained a microscope glass slide placed on a moist filter paper. The plate was sealed with parafilm and incubated under the same conditions as inoculated leaves. After 48 h, drops of blue-lacto-glycerol stain were placed on the slide to stop the germination process. Presence of basidiospores and germination was assessed using a light microscope.

Leaves inoculated with the various methods were examined weekly for up to 4 weeks for fruiting bodies of the rust.

See Appendix A for a range of photographs relating to inoculations performed.

2.2 Microscopic examination of nuclear behaviour

Pieces of plant tissue with uredinia were excised from a *S. jambos* plant 3 weeks after inoculation with a single-uredinium isolate of myrtle rust (Morin et al. 2012). The pieces were fixed in a solution of 2% formaldehyde, 0.1% glutaraldehyde plus ~0.01% Tween-20 in 50 mM PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid) buffer for 3 h at room temperature. They were then dried off with paper towel and embedded in ~0.3% gel agarose (Type V, Sigma) contained in a mould. The agarose was removed from the mould once firmly set, trimmed to the required dimensions and glued on the block of a vibratome using superglue. Sections (~50 µm thickness) through the embedded tissue were then cut using a carbon steel blade. Each section was carefully collected with a small paintbrush and placed on microscope slides kept in moist conditions. Drops of a solution of the DNA-specific fluorochrome DAPI (4,6-diamidino-2-phenylindol) (5 µg/ml) and the fungal cell wall specific stain WGA (wheat germ agglutinin; Alexa Fluor® 488 conjugate; Invitrogen; 50 µg/ml) in deionised water were placed on the sections for 20 min in the dark at room temperature. The sections were gently rinsed with deionised water and examined immediately with a 40× objective or 63× water immersion objective on a Zeiss AxioImager fluorescence compound microscope.

Fresh urediniospores and teliospores were removed with a scalpel from *S. francissii* 5 weeks after inoculation with a single-uredinium myrtle rust isolate (Morin et al. 2012). Spores were placed on clean microscope glass slides and gently spread on the surface using a fine camel hair paint brush. The slides were then placed on moist paper towel contained in a 14-cm Petri dish in a controlled-environment room (conditions as above) for 24 h. Drops of a solution of DAPI (1 µg/ml) in deionised water were placed on the surface of the slides for 10 min in the dark at room temperature. The slide was then gently rinsed with deionised water and immediately examined as described above.

3 Results

3.1 Inoculation experiments

Fresh teliospores suspended in liquid paraffin oil or in HFE-7100 readily germinated when placed on water agar and produced basidiospores in all experiments that used teliospore suspensions for inoculation. Abundant basidiospores were also produced and naturally discharged from telia placed on water agar in all other experiments. A large proportion of basiodiospores discharged on a microscope glass slide beneath telia had germinated by the time of the assessment. For example in experiment no. 7 (Table 1), 45–49% of basidiospores discharged by four telia on the slide had germinated. Some urediniospores, germinated or not, were observed among teliospores on water agar and occasionally among basidiospores on glass slides.

No spermagonia developed on leaves of *A. flexuosa* cv. Afterdark in any of the eight inoculation experiments performed using teliospore suspensions or basidiospores naturally discharged from telia (Table 1). Uredinial sori however, were observed on inoculated leaves in half of the experiments.

3.2 Microscopic examination of nuclear behaviour

Intercellular hyphae in the host mesophyll below a developing uredinium were binucleate (with presumably haploid nuclei) (Fig. 1 A–E). The rectangular, basal cells that give rise to urediniospores, and subsequently teliospores, were also binucleate (Fig. 1 F, G). Urediniospores were binucleate, whilst each of the two cells of teliospores had only one large nucleus (presumably diploid as a result of karyogamy during maturation process) (Fig. 2 A, B). When exposed to high relative humidity, each teliospore cell germinated by producing a metabasium (=promycelium) (Fig. 2 C). The nucleus migrated from the teliospore cell into the developing metabasidium and underwent a first division (presumably meiotic) (Fig. 2 D). A septum was laid down between the apical and basal cells of the metabasidium. A second nuclear division occurred in the apical metabasidium cell, presumably by meiosis, giving rise to four daughter nuclei (presumably haploid) (Fig. E, F). Septa were laid down between the daughter nuclei forming four uninucleate cells, from each of which a sterigma arose and a basidiospore was formed (Fig. 2 E–H). Soon after its entrance into the basidiospore, the nucleus typically divided, producing a binucleate (with presumably haploid nuclei), mature basidiospore (Fig. 2 H). Basidiospores examined possessed either one or two nuclei and never three or four. In some instances, it appeared that basidiospores germinated whilst still attached to the metabasium under the experimental conditions used (Fig. 2 F).

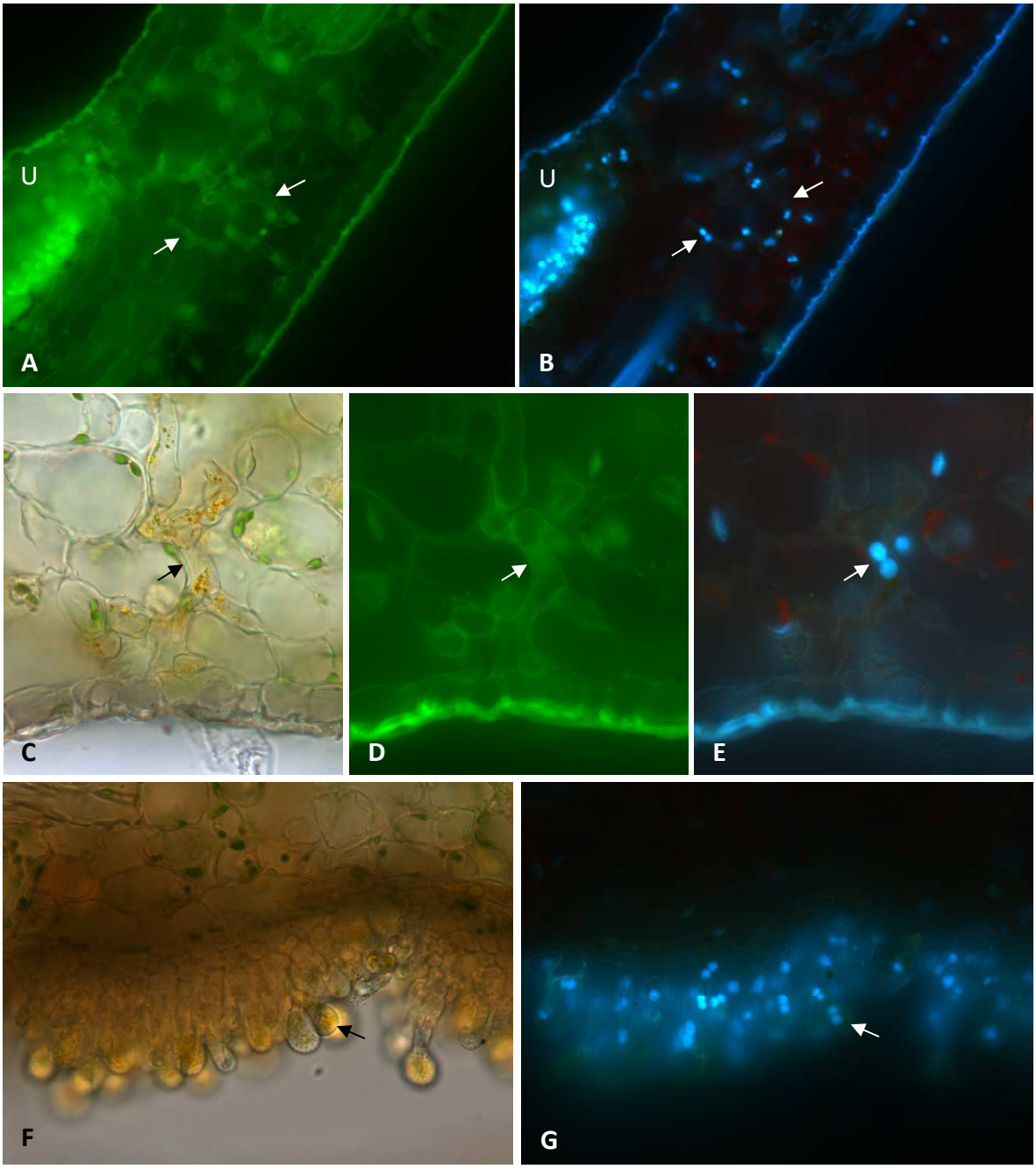


Figure 1. Cross-section of *Syzygium jambos* leaf infected by myrtle rust double-stained for intact fungal hyphae (WGA) and nuclei (DAPI). A & B. Network of intercellular hyphae (arrows) in the leaf mesophyll below a developing uredinium (U). Hyphae stained with WGA (A) and nuclei stained with DAPI (B). Note the binucleate status of hyphal cells. C–E. Close-up of an intercellular hypha (arrows). Bright-field image (C), hypha stained with WGA (D) and nuclei stained with DAPI (E). F & G. Developing uredinium. Bright-field image (F) and nuclei stained with DAPI (G). Arrows point to a binucleate urediniospore, which has developed from a basal cell that is also binucleate.

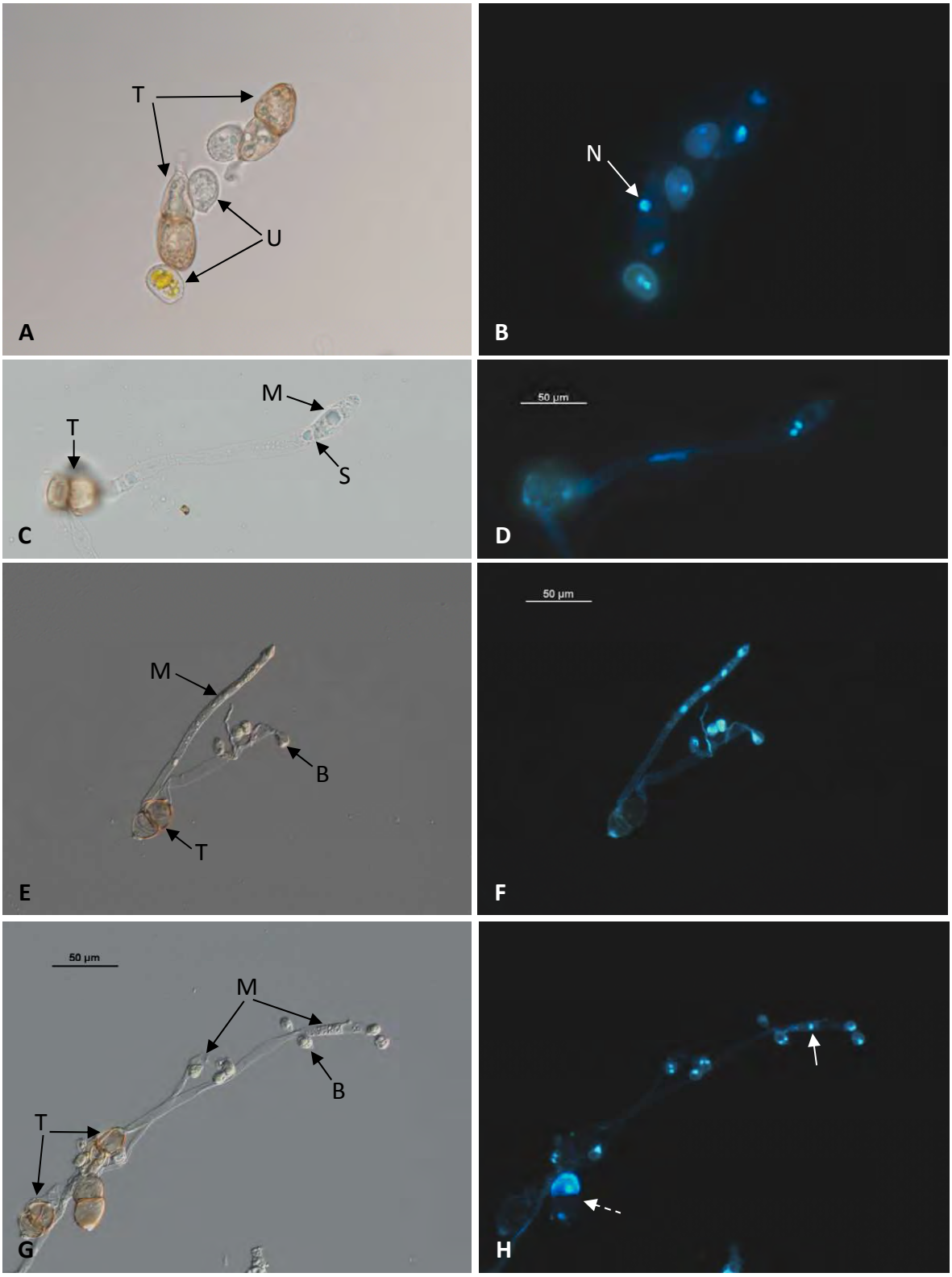


Figure 2. Nuclear status of known spore stages of myrtle rust. Bright-field mages on left and images of DAPI-stained nuclei on right. A & B. Binucleate urediniospores (U) and two-celled uninucleate teliospores (T). C & D. Germinated teliospore with a developing metabasidium (M) with two nuclei. Note the septum (S) laid down in the metabasidium. E & F. Teliospore with metabasidia produced from both cells. One metabasidium contains four nuclei but has not yet formed basidiospores (B). Basidiospores have formed on the other metabasidium and two of them have germinated in situ. G & H. Entangled metabasidia bearing binucleate and uninucleate basidiospores. Broken arrow indicates a teliospore that has not germinated and still contains its nuclei. Solid arrow indicates a metabasidium cell with a nucleus that has not yet formed a basidiospore.

4 Discussion

Elucidating the life cycle and primary source of genetic variation in rust fungi is not always a straight forward venture. For example, the mystery surrounding the life cycle of *Puccinia striiformis*, which causes stripe rust of cereal crops and other grasses, lasted more than a century because the alternate host had never been identified despite considerable attempts (Jin et al. 2010). Other hurdles such as breaking innate dormancy of teliospores to enable experimentation can considerably delay investigations (e.g. Anderson et al. 2011). Atypical life cycles with vestigial and non-functional sexual spores, such as in *Hemileia vastatrix* (coffee rust) (Carvalho et al. 2011) and *Prospodium tuberculatum* (lantana rust) (Ellison et al. 2006), can be difficult to demonstrate as it involves proving the nonexistence or permanent loss of spore stages.

This project could not provide unequivocal proof that myrtle rust is autoecious. Leaves of *A. flexuosa* cv Afterdark, a highly susceptible host of the Australian accession of myrtle rust used in this project, never developed spermagonia following the many inoculations performed with either a teliospore suspension or naturally discharged basidiospores from telia. In half of the experiments however, sori morphologically indistinguishable from uredinia were observed on inoculated leaves. These results concur with those of previous experiments conducted with *P. psidii* s.l. in Brazil (Figueiredo et al. 1984; Glen et al. 2007). Microscopic examinations made in the concurrent in vitro tests performed with inoculum used in each of the experiments confirmed presence of basidiospores, with many of them germinated. Although extreme care was taken to only select telia without interspersed urediniospores in the experiments that involved naturally discharged basidiospores, a few, albeit rare, urediniospores, germinated or not, were also present in some of the in vitro tests. This observation inevitably casts doubt that the uredinial sori which developed on inoculated leaves, were the result of basidiospore infections. This contrasts with the interpretation of previous workers who considered these uredinial sori as aecia and on this basis concluded that *P. psidii* s.l. was autoecious (Figueiredo et al. 1984; Glen et al. 2007).

It is not possible to be totally certain that telia of *P. psidii* s.l. (including myrtle rust) are completely free of urediniospores prior to use in experiments. The presence of single urediniospores within a telium, as depicted in the SEM micrograph of a telium presented in Coutinho et al. (1998), could easily be overlooked during an examination of telia using a stereomicroscope. The use of a micromanipulator to isolate single basidiospores and transfer them onto young leaves could overcome problems with using for inoculations telia that potentially have some interspersed urediniospores (Ono 2002). Nonetheless, the microscopic investigations conducted in this project support the conjecture that basidiospore inoculation of Myrtaceae hosts does not lead to development of uredinia.

There are a number of rust fungi like *P. psidii* s.l., referred to as hemicyclic (Petersen 1974), for which only the uredinial and telial stages are known (for examples see Henderson 2000). Whilst these rusts may be heteroecious with undiscovered alternate hosts, it is also possible that their teliospores are no longer functional and that they solely survive through continued cycling via urediniospores or systemic mycelium in plants. Alternatively, teliospores may germinate into two-celled metabasium, instead of the usual four-celled, producing homothallic (self-fertile) basidiospores with two compatible nuclei, which give rise to uredinia upon host infection (Anikster et al. 2004).

The nuclear status of mycelium of uredinial sori, urediniospores and teliospores in myrtle rust was typical of that in many rust fungi (Saville 1939). There was no indication from the microscopic observations that teliospores were non-functional. They germinated normally without asynchronous or unstable nuclear division resulting in basidiospores of different size and shape as observed in other rusts (Ellison et al. 2006; Carvalho et al. 2011). The development of a four-celled metabasidium from each cell of *P. psidii* s.l. teliospores has been previously depicted (Pérez et al. 2010), and is similar to that commonly observed in many macrocyclic, demi-cyclic and microcyclic rust fungi (Ono 2002; Petersen 1974). The project demonstrated that the metabasidium of myrtle rust also exhibits a regular nuclear development, with the nucleus in the teliospore cell (presumed dikaryotic) dividing into four nuclei (presumably after meiosis),

which are subsequently separated into the four cells of the metabasidum. Basidiospores formed from each metabasidium cell were initially uninucleate and then became binucleate, as a result of what was assumed to be an additional mitotic division. Binucleate basidiospores have been commonly observed in rust fungi (Anikster 1983). Tri- or tetra-nucleate basidiospores were never observed. This suggests that the development of uredinia directly from basidiospore infections is most unlikely since nuclei of two different mating types are not present in single basidiospores.

A recent study of the population genetic structure of *P. psidii* s.l. in Brazil provided evidence of strong selection by hosts and a high rate of clonal reproduction with little or no gene flow among host-adapted pathogen genotypes (Graça 2011). These findings suggest that sexual recombination is rare or non-existent. The several, distinct microsatellite multilocus genotypes identified among rust isolates were uniquely associated with specific hosts. Previous cross-inoculation studies also demonstrated the existence of biotypes (races) of *P. psidii* s.l. with different host-ranges (e.g. MacLachlan 1938; Ferreira 1983; Aparecido et al. 2003). Rare mutation events within clonal lineages probably explained *P. psidii* s.l. adaptation to unique host species or clones bred for resistance (Graça 2011; Graça et al. 2011).

In the introduced range, a unique, single microsatellite multilocus genotype was observed among *P. psidii* s.l. isolates collected from different hosts in Hawai'i (Zhong et al. 2011; Graça 2011) and among those collected in Australia (M Glen pers. comm.). This supports the hypothesis that a single rust genotype was introduced in each of these regions and that only clonal reproduction has occurred since the incursion despite the presence of viable teliospores (Loope 2010; Carnegie & Lidbetter 2012). In contrast, three different microsatellite multilocus genotypes were identified among four isolates originating from Florida (Zhong et al. 2011). More than one rust genotype may have been introduced from South America to Florida or alternatively it is possible that in the 33 years since the first detection of *P. psidii* s.l. in Florida (Marlatt & Kimbrough 1979), new variant forms of the rust have evolved as has been reported in the native range (Zhong et al. 2011; Graça 2011).

To obtain the ultimate proof that *P. psidii* s.l. is a heteroecious rust, its alternate aecial host will have to be found. As suggested by Simpson et al. (2006), it is possible that the 68 *Aecidium* species listed from Brazil (Hennen et al. 2005) may represent the aecial state of *P. psidii* s.l. Investigations combining DNA sequence analysis with inoculations of susceptible Myrtaceae species with fresh spores of these rusts are warranted. Nonetheless, the clonal population structure of *P. psidii* s.l. in Brazil, revealed by Graça's (2011) genetic study, is a strong indication that its putative alternate host is extremely rare or even extinct in the native range. It is conceivable that contemporary populations of *P. psidii* s.l. are solely maintained via continued asexual reproduction (urediniospores) on its primarily non-deciduous Myrtaceae hosts and that mutation is the key process for the emergence of new genotypes. Strict asexual reproduction would considerably reduce its evolutionary potential, although other factors such as effective population size and mutation rate are likely to still play a role and contribute to the emergence of distinct genotypes with different virulence profile over time (McDonald & Linde 2002).

Appendix A Photographs relating to inoculations performed

Source of teliospores for inoculations

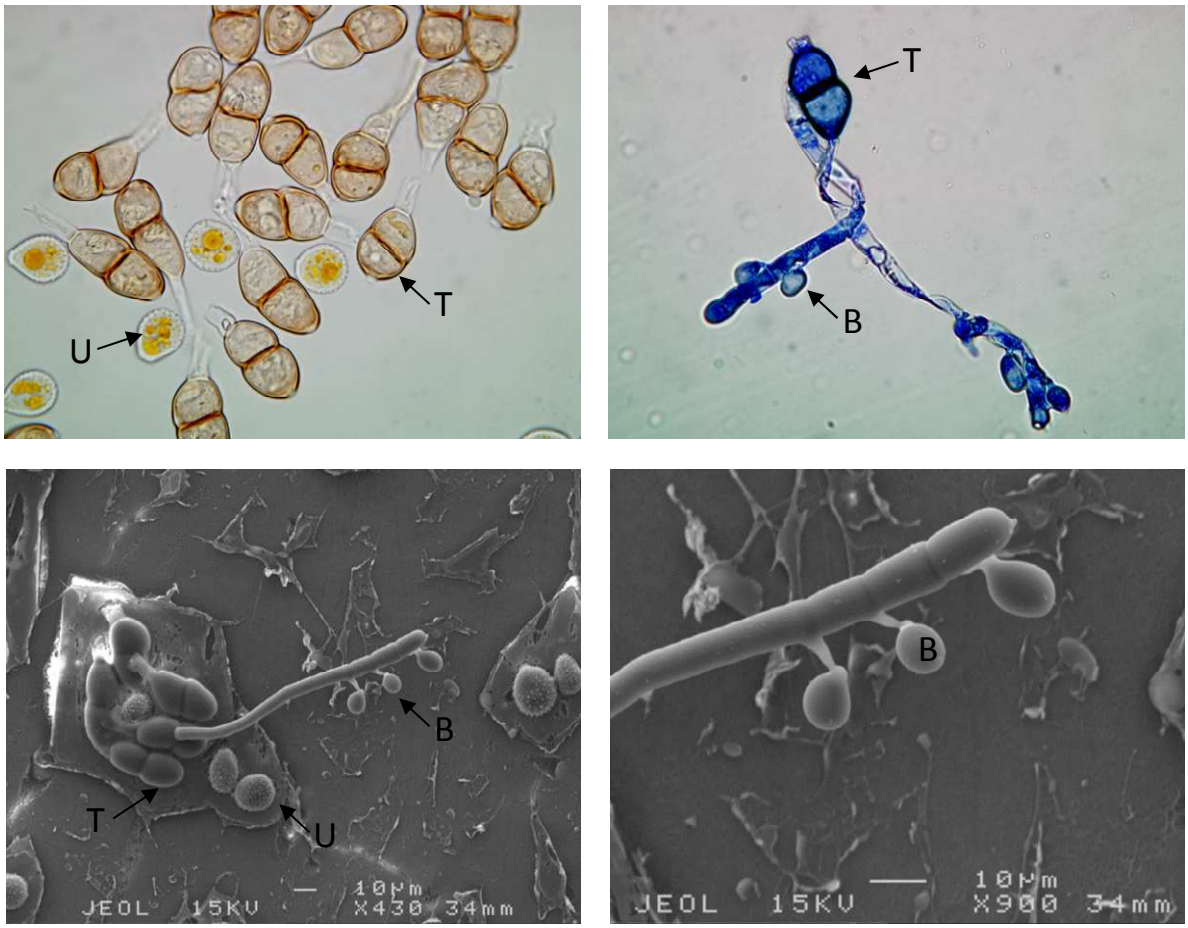
Agonis flexuosa 'Afterdark'

Syzygium francissii

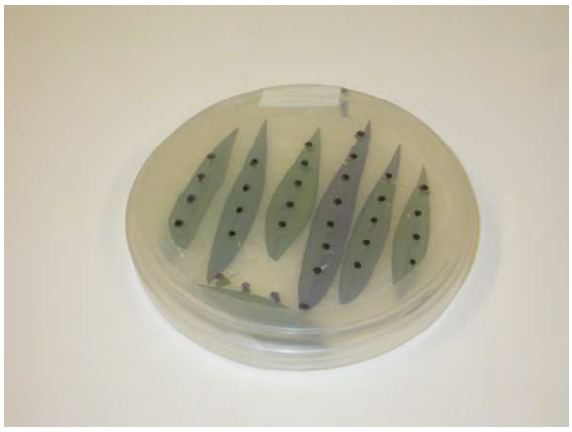
Lindsayomyrtus racemoides

The different types of spores of myrtle rust:

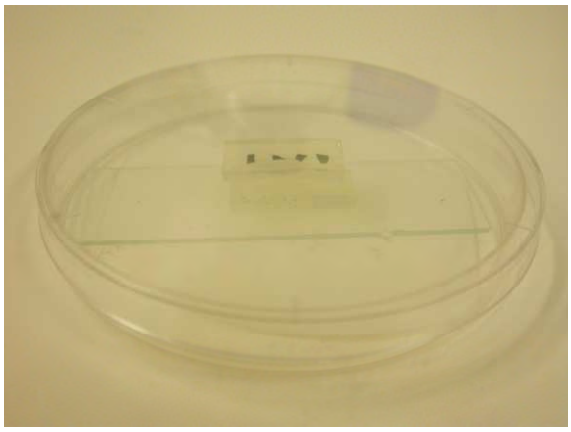
T= teliospores, U = urediniospores, B = basidiospores



Inoculation of detached leaves by natural discharge of basidiospores



Pieces of telia placed on agar plate inverted over detached leaves



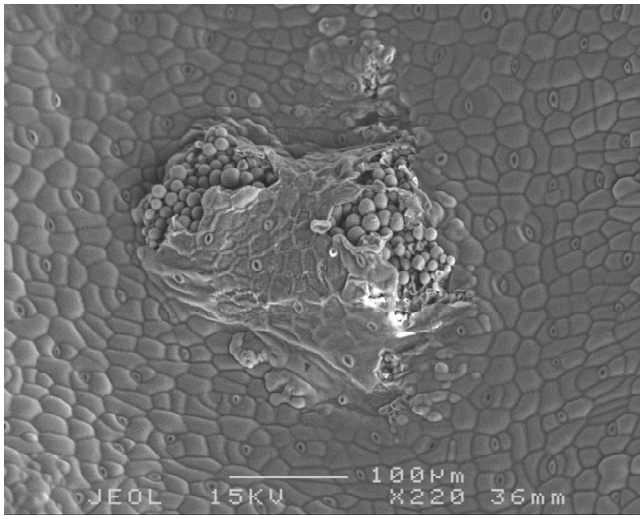
Pieces of telia placed on agar block inverted over glass slide to check basidiospore production and germination

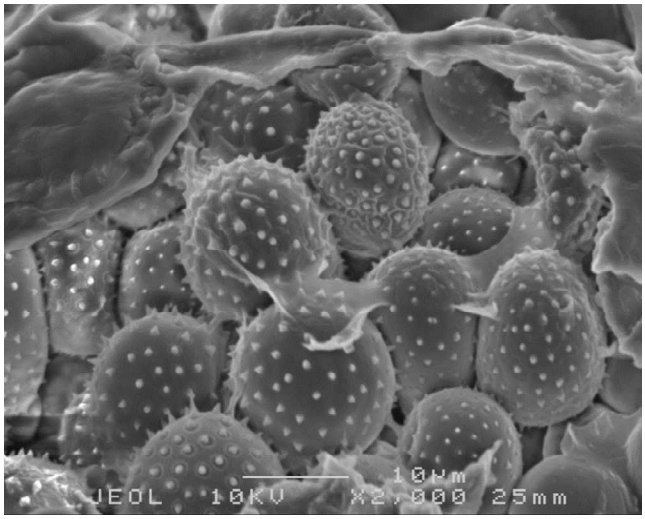
Fruiting bodies that developed on attached leaves following inoculation with a suspension of teliospores in oil (Inoculation no 1)

Upper surface

Under surface

SEM photographs of a fruiting body that developed on attached leaves following inoculation with a suspension of teliospores in HFE (Inoculation no 2)





Close-up of spores

Fruiting bodies that developed on detached leaves following inoculation by natural discharge of basidiospores (Inoculation no 6)

References

Anderson FE, Díaz ML, Barton J, Flemmer AC, Hansen PV, McLaren DA, 2011. Exploring the life cycles of three South American rusts that have potential as biocontrol agents of the stipoid grass *Nassella neesiana* in Australasia. *Fungal Biology* 115:370–380.

Anikster Y, 1983. Binucleate basidiospores – a general rule in rust fungi. *Transactions of the British Mycological Society* 81:624–626.

Anikster Y, Szabo LJ, Eilam T, Manisterski J, Koike ST, Bushnell WR, 2004. Morphology, life cycle biology, and DNA sequence analysis of rust fungi on garlic and chives from California. *Phytopathology* 94:569–577.

Aparecido CC, Figueiredo MB, Furtado EL, 2003. Effect of temperature on infection, teliospore formation and basidiospore production for *Puccinia psidii* (Uredinales). *Summa Phytopathologica* 29:239–243.

Carnegie AJ, Lidbetter JR, 2012. Rapidly expanding host range of *Puccinia psidii* sensu lato in Australia. *Australasian Plant Pathology* 41:13–29.

Carnegie AJ, Lidbetter JR, Walker J, Horwood MA, Tesoriero L, Glen M, Priest MJ, 2010. *Uredo rangelii*, a taxon in the guava rust complex, newly recorded on Myrtaceae in Australia. *Australasian Plant Pathology* 39:463–466.

Carvalho CR, Fernandes RC, Carvalho GMA, Barreto RW, Evans HC, 2011. Cryptosexuality and the genetic diversity paradox in coffee rust, *Hemileia vastatrix*. *PLoS ONE* 6: e26387.

Coutinho TA, Wingfield MJ, Alfenas AC, Crous PW, 1998. Eucalyptus rust: a disease with the potential for serious international implications. *Plant Disease* 82:819–825.

Ellison CA, Pereira JM, Thomas SE, Barreto RW, Evans HC, 2006. Studies on the rust *Prospodium tuberculatum*, a new classical biological control agent released against the invasive alien weed *Lantana camara* in Australia. 1. Life-cycle and infection parameters. *Australasian Plant Pathology* 35: 309–319.

Ferreira FA, 1983. Eucalyptus rust. *Revista Árvore* 7:91–109.

Figueiredo MB, Coutinho LN, Hennen JF, 1984. Estudos para determinacao do ciclo vital de *Puccinia psidii*. *Summa Phytopathologica* 10, 53–54.

Glen M, Alfenas AC, Zauza EAV, Wingfield MJ, Mohammed C, 2007. *Puccinia psidii*: a threat to the Australian environment and economy - a review. *Australasian Plant Pathology* 36:1–16.

Graça RN, 2011. Genetic diversity of *Puccinia psidii* populations. PhD Thesis, University of Viçosa, Brazil.

Graça RN, Aun CP, Guimarães, Rodrigues VA, Zauza EAV, Alfenas AC, 2011. A new race of *Puccinia psidii* defeats rust resistance in eucalypt. *Australasian Plant Pathology* 40:442–447.

Henderson DM, 2000. A checklist of the rust fungi of the British Isles. British Mycological Society, Surrey, UK.

Hennen JF, Figueiredo MB, de Carvalho AA Jr, Hennen PG, 2005. Catalogue of the species of plant rust fungi (Uredinales) of Brazil. Instituto de Pesquisas, Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil. Available: http://www.jbrj.gov.br/publica/uredinales/. Accessed 27 Nov. 2012.

Jin Y, Szabo LJ, Carson M, 2010. Century-old mystery of *Puccinia striiformis* life history solved with the identification of Berberis as an alternate host. *Phytopathology* 100:434–435.

Loope L, 2010. A summary of information on the rust *Puccinia psidii* Winter (guava rust) with emphasis on means to prevent introduction of additional strains to Hawaii. U.S. Geological Survey Open-File Report 2010-1082. U.S. Geological Survey, Reston, Virginia. Available: http://pubs.usgs.gov/of/2010/1082/. Accessed 27 Nov. 2012.

MacLachlan JD, 1938. A rust of the pimento tree in Jamaica, BWI. *Phytopathology* 28:157–170.

Marlatt RB, Kimbrough JW, 1979. *Puccinia psidii* on *Pimenta dioica* in south Florida. *Plant Disease Reporter* 63:510–512.

McDonald BA, Linde C, 2002. Pathogen population genetics, evolutionary potential and durable resistance. *Annual Review of Phytopathology* 40, 349–379.

Morin L, Aveyard R, Lidbetter JR, Wilson PG, 2012. Investigating the host-range of the rust fungus *puccinia psidii* sensu lato across tribes of the family myrtaceae present in Australia. *PLoS ONE* 7, e35434 doi:10.1371/journal.pone.0035434.

Ono Y, 2002. Life cycle and nuclear behaviour in three rust fungi (Uredinales). *Mycoscience* 43:37–45.

Pérez CA, Wingfield MJ, Altier NA, Simeto S, Blanchette RA, 2010. *Puccinia psidii* infecting cultivated Eucalyptus and native myrtaceae in Uruguay. *Mycological Progress* 10:273–282.

Petersen RH, 1974. The rust fungus life cycle. *The Botanical Review* 40:453–513.

Saville DBO, 1939. Nuclear structure and behaviour in species of the Uredinales. *American Journal of Botany* 26:585–609.

Simpson JA, Thomas K, Grgurinovic CA, 2006. Uredinales species pathogenic on species of Myrtaceae. *Australasian Plant Pathology* 35, 549–562.

Zhong S, Yang B, Puri KD, 2011. Characterization of *Puccinia psidii* isolates in Hawaii using microsatellite DNA markers. *Journal of General Plant Pathology* 77:178–181.

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