Elucidating the life cycle of myrtle rust: *not that easy...*

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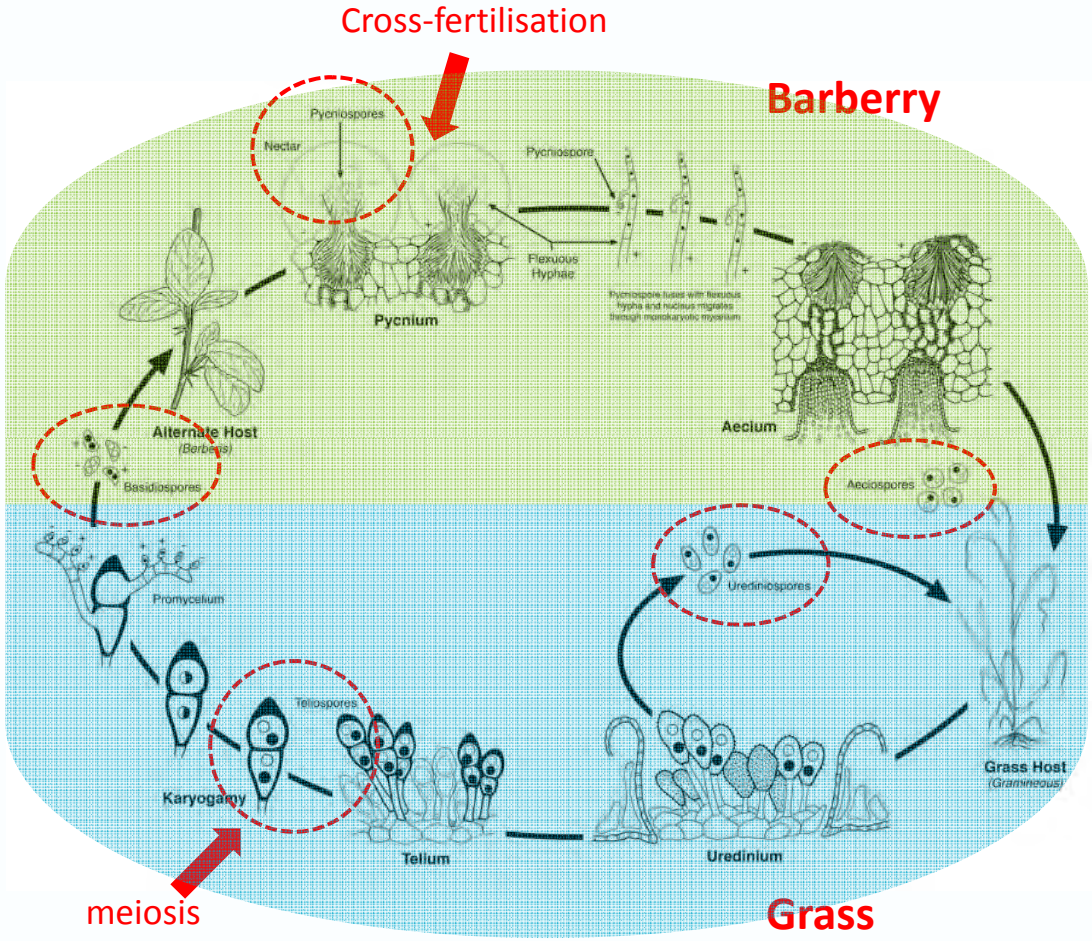
Introduction

- Rust fungus life cycle – why do we need to know?
  - Evolutionary potential
  - Appropriate management strategies

- Many different life cycles:
  - Up to five spore stages
  - Clonal reproduction (+ / – genetic recombination)
  - Autoecious vs heteroecious

Introduction

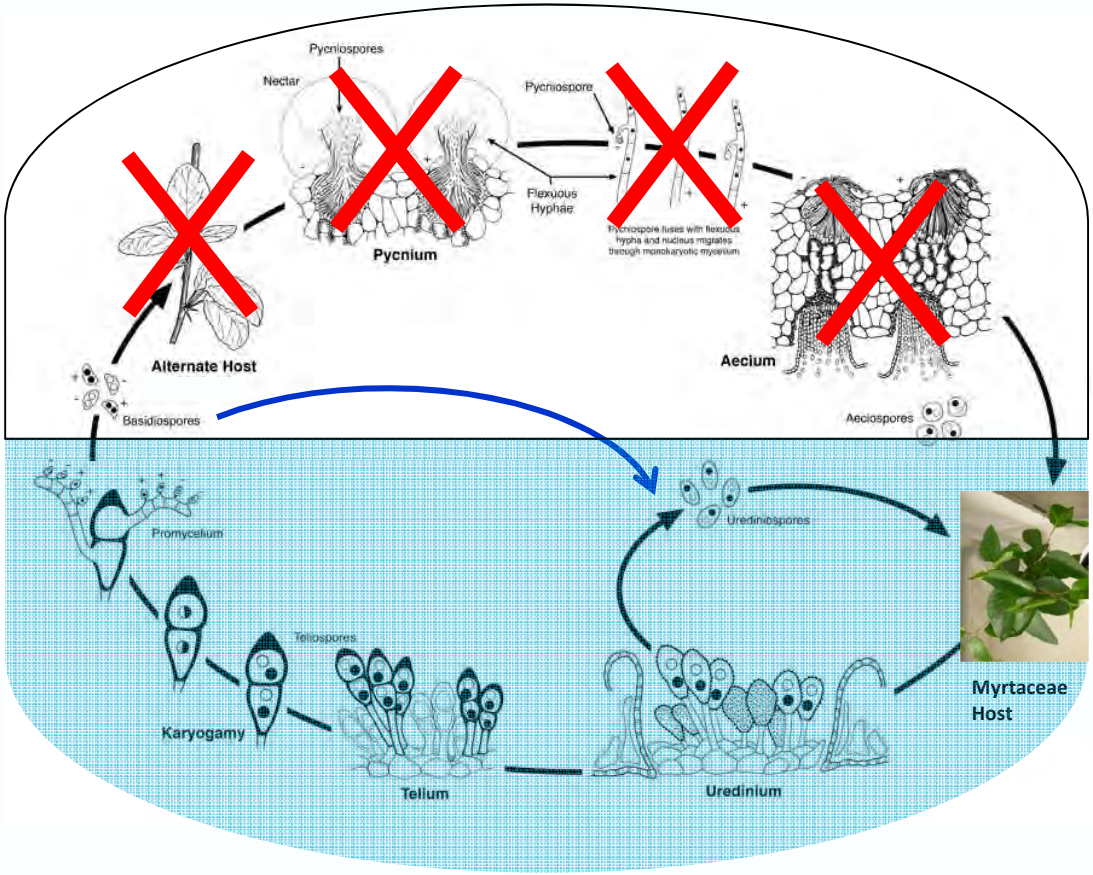
Puccinia graminis (stem rust of cereals)



- Macrocyclic
- Heteroecious
- ➡ Genetic recombination
  - High genotypic diversity
  - High capacity to adapt

http://thescientistgardener.blogspot.com.au/2010/09/orange-mystery-dust.html

Puccinia psidii s. l. life cycle - current conjecture



- Hemicyclic
  - urediniopores & teliospores
- Autoecious
- Basidiospore infections
  - Produce uredinia/urediniospores (= uredinioid aecia)

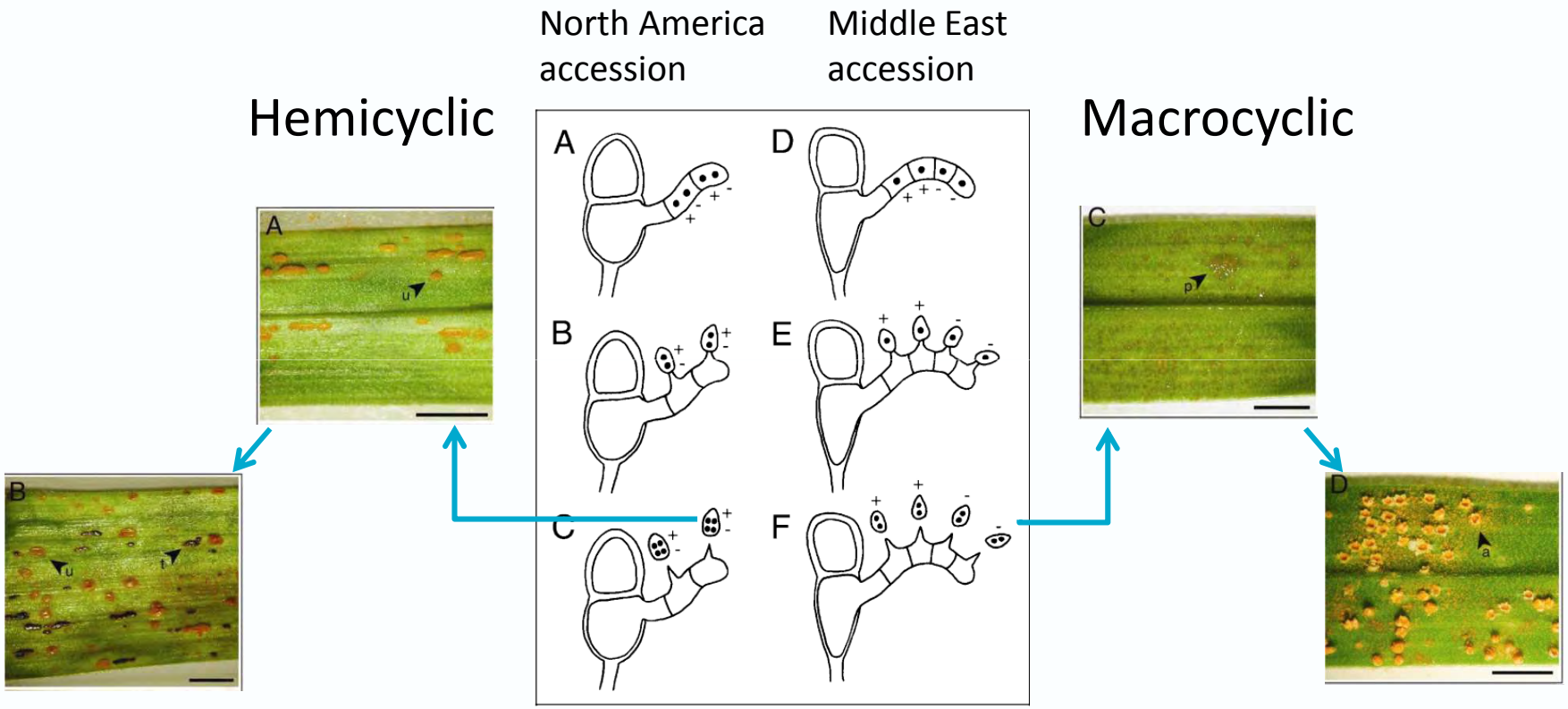
Figueiredo *et al.* (1984)
Alfenas & Zauza (unpublished)

Hemicyclic rust fungi – possible life cycles

- Heteroecious with an undiscovered alternate host
- Teliospores no longer functional and rust solely survives through continued cycling via urediniospores or systemic mycelium in plants
- Basidiospores give rise to uredinia upon host infection
  – conclusively demonstrated in one case…

Hemicyclic rust fungi – an example

- *Puccinia allii* s.l.



Anikster *et al.* (2004)

Study objectives

- Perform a series of inoculations using basidiospores of *P. psidii* s.l.
- Molecularly characterise sori produced
- Investigate the rust's nuclear behaviour at different stages of development

Inoculation experiments – methods

Teliospore source

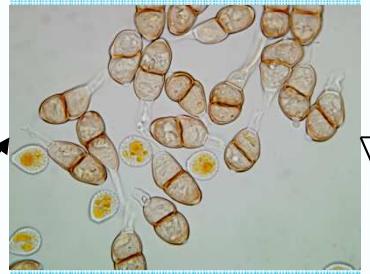
Agonis flexuosa 'Afterdark'

Syzygium francissii

Lindsayomyrtus racemoides

Inoculation method

Teliospore suspension



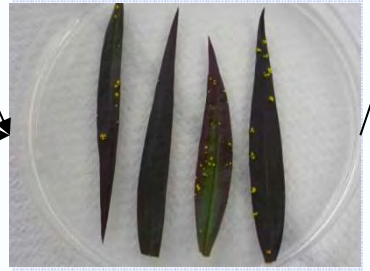
Natural basidiospore discharge

Material inoculated

Agonis flexuosa 'Afterdark'

Attached leaves

Detached leaves



+ microsatellite analysis of single-sorus isolates

+ in vitro germination tests

Inoculation experiments – results

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

Some urediniospores, germinated or not, were observed among teliospores on water agar and occasionally among basidiospores in the *in vitro* germination tests.

* Single-sorus isolates analysed with microsatellites

Microsatellite analysis – results

original single-uredinial sorus isolate from which teliospores were sourced for inoculations

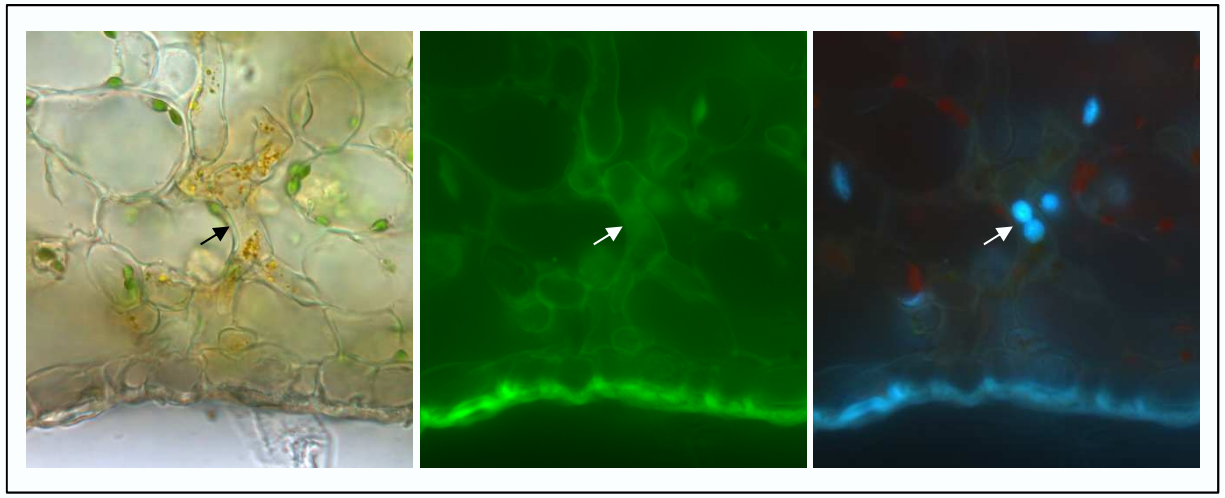
eight single-sorus isolates recovered from inoculations

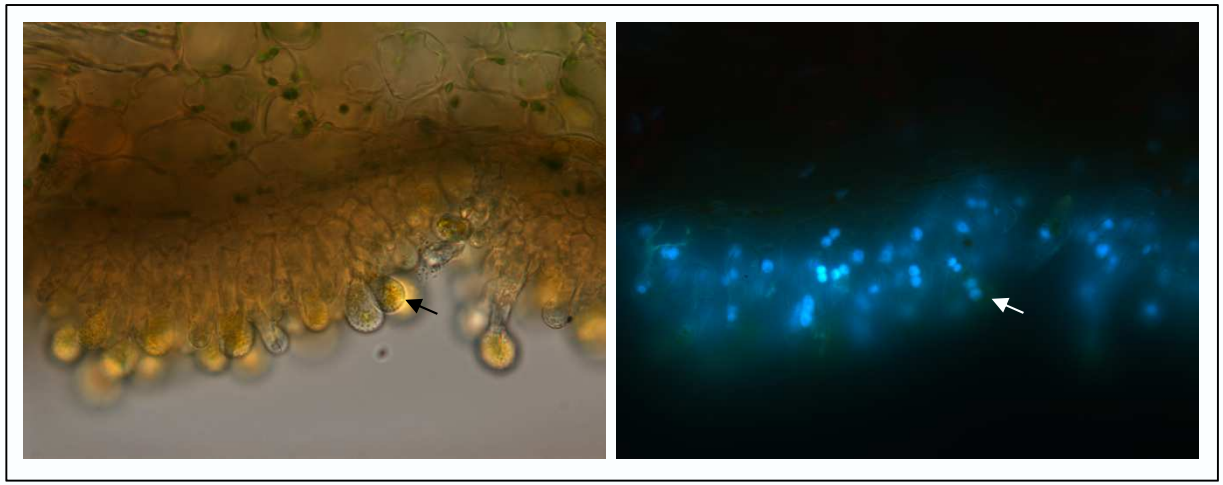
Same microsatellite multilocus genotype

(based on eight loci analysed)

Nuclear behaviour – results

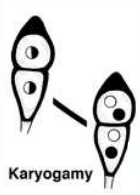
Sections through uredinia stained with WGA (fungal cell wall specific – green) and DAPI (DNA/nucleus specific – blue)

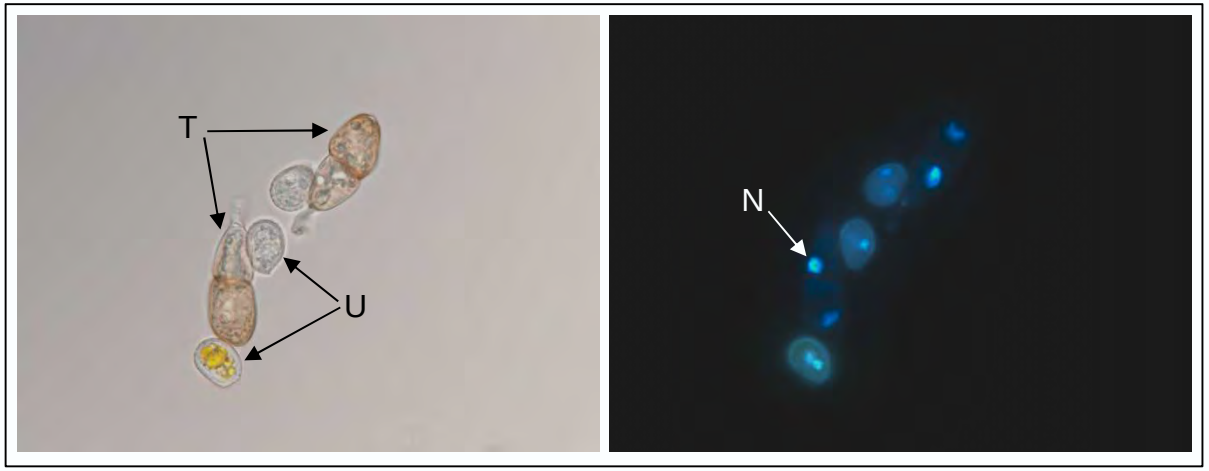




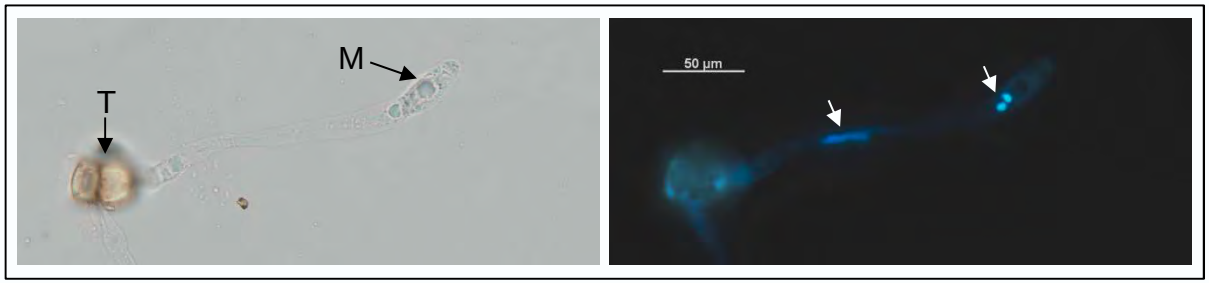
Nuclear behaviour – results

Spores stained with DAPI (DNA/nucleus specific – blue)



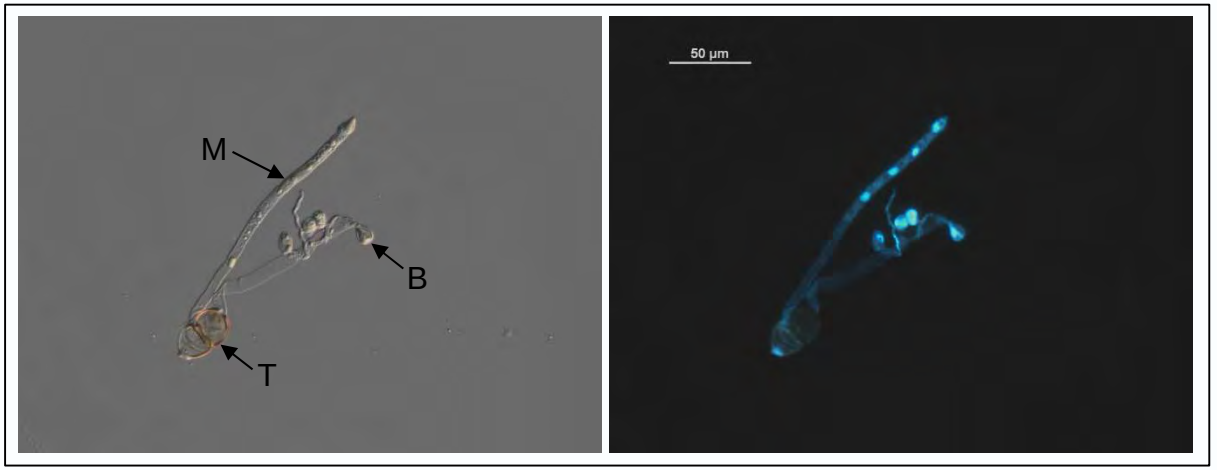


Development of metabasidium from germinated teliospore

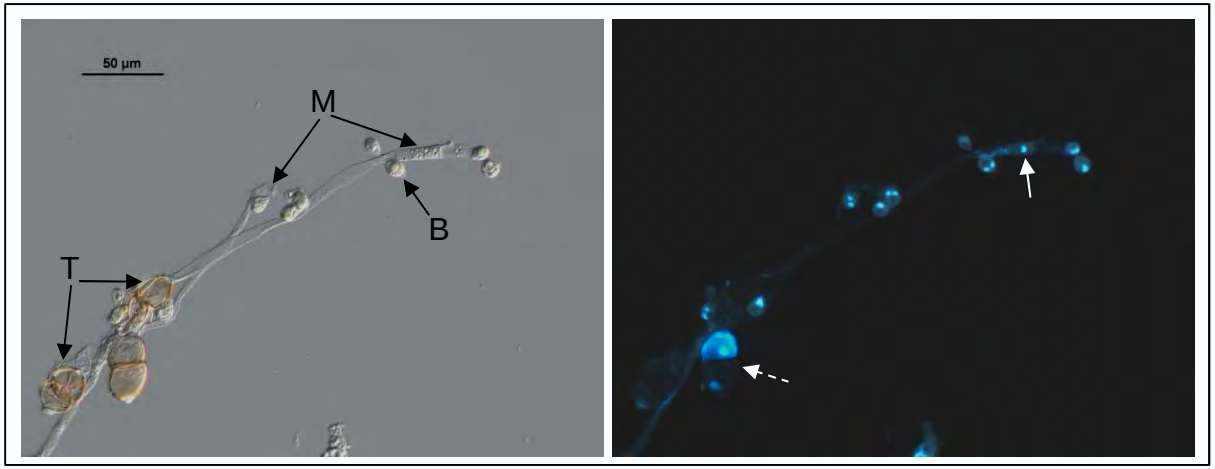


Nuclear behaviour – results

Metabasidia at different stages of development

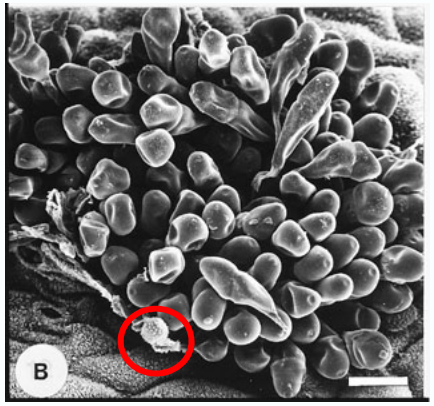


Binucleate basidiospores



Inoculation experiments – discussion

- Inoculated leaves never developed spermogonia (=pycnia).
- Doubt that uredinial-like sori observed were the result of basidiospore infections.
  - Urediniospores were present in some of the *in vitro* tests of experiments with naturally discharged basidiospores
- Not possible to be totally certain that telia are completely free of urediniospores prior to use in experiments.



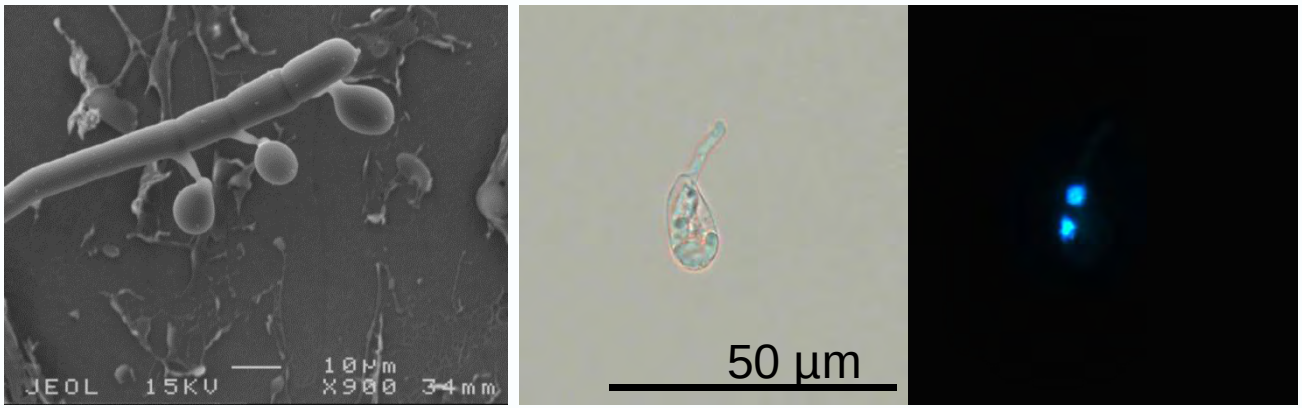
Coutinho *et al.* (1998)

Microsatellite analysis – discussion

- Microsatellite loci used are heterozygous in the rust population present in Australia (Glen *unpublished*).
- Would have expected to detect some variation in microsatellite genotype if sori had originated from basidiospore infections.
  - considering meiotic divisions lead to basidiospore development
- Independence of microsatellite loci not verified:
  - Limited knowledge of genomic structure of *P. psidii*
  - Number of chromosome unknown
- ... Nonetheless apparent lack of detectable sexual recombination in these isolates support that sori most likely originated from urediniospore infections.

Nuclear behaviour – discussion

- This type of nuclear cycle:
  - Commonly occurs in macrocyclic and demicyclic species
  - Less frequent in microcyclic species
- No indication that teliospores were non-functional
- Four-celled (not two-celled) metabasidium developed
- Tri- or tetra-nucleate basidiospores never observed

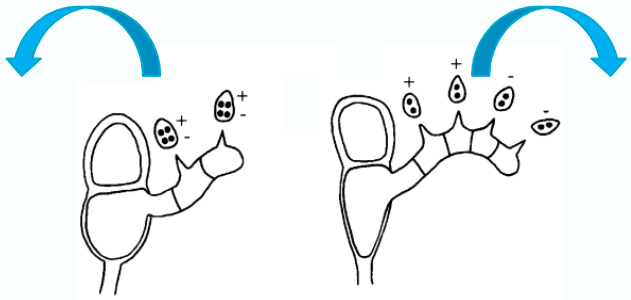


Infection by basidiospore – possibilities

telia
(e.g. *Puccinia mesnieriana*)

aecia
(e.g. *Uromyces christensenii*)

uredinia
(e.g. *Puccinia allii*)



telia
(e.g. *Puccinia malvacearum*)

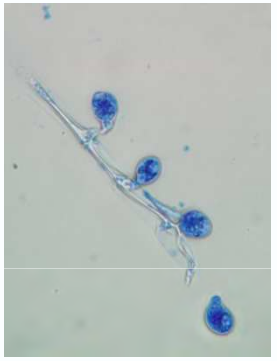
aecia
WHY NOT?????

uredinia
WHY NOT?????

spermogonia
(e.g. *Puccinia graminis*)

Additional work recommended by reviewers

- Investigate the fate of basidiospores on leaf surface:
  - Do they germinate? Does penetration and colonisation occur?
  - Examine a large number
  - If infection detected then…

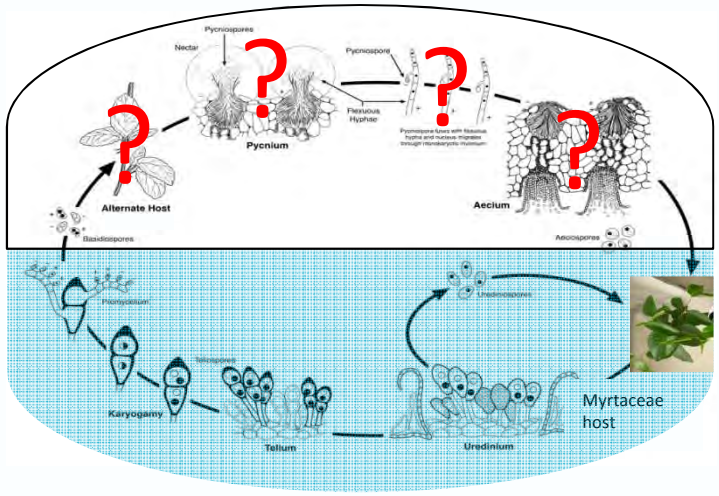


- Inoculation with single basidiospores:
  - Challenging
  - Consider inoculation with single germinated teliospores
  - A large number would be required
  - If uredinia developed = proof of no alternate host in life cycle

Conclusion I

- Proving the nonexistence or permanent loss of spore stages is a challenge.

- Experimental inoculations to confirm atypical life cycle and investigations of nuclear cycle have been published for very few rust fungi.
  - Hemicyclic rusts have rarely been investigated



Conclusion II

- A recent population genetic study in Brazil revealed a clonal population structure for *P. psidii* s.l.
  - teliospores are infrequently operational, or
  - putative alternate host is extremely rare or even extinct in the native range.
- A single genotype observed in each of the recent incursions in Hawai'i and Australia
  - teliospores are infrequently operational, or
  - putative alternate host probably not present in the introduced range.
- *P. psidii* s.l. populations may be primarily maintained via continued asexual reproduction, with mutation as the key process for emergence of new genotypes.

Thank you

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