

## TRACKING THE DISTRIBUTION OF *Puccinia psidii* GENOTYPES THAT CAUSE RUST DISEASE ON DIVERSE MYRTACEOUS TREES AND SHRUBS

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### INTRODUCTION

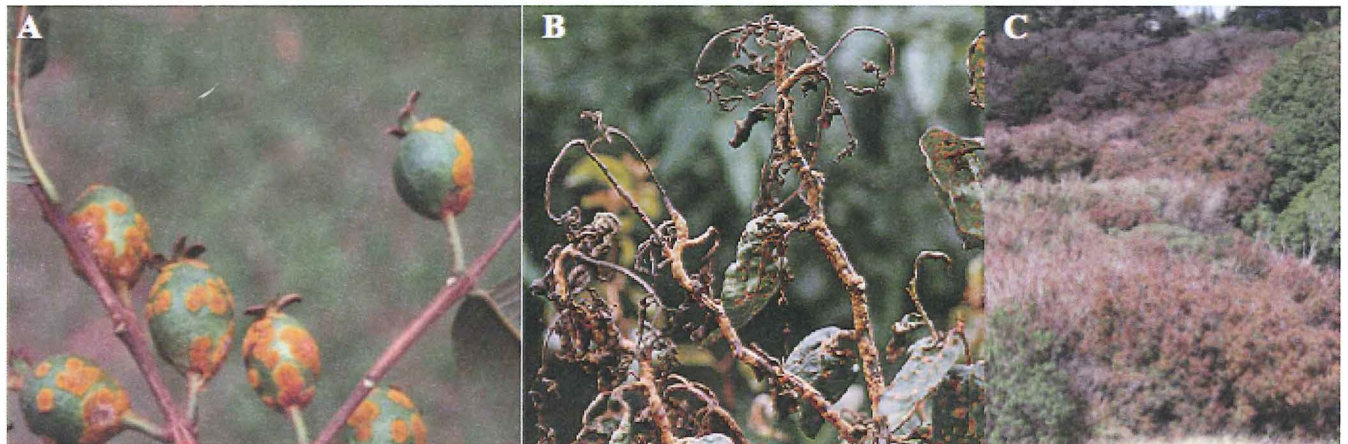
*Puccinia psidii* Winter (Basidiomycota, Uredinales) is a biotrophic rust fungus that was first reported in Brazil from guava in 1884 (*Psidium guajava*; Winter 1884) and later from eucalypt in 1912 (Joffily 1944). Considered to be of neotropical origin, the rust has also been reported to infect diverse myrtaceous hosts elsewhere in South America, Central America, the Caribbean, Mexico, the USA (California, Florida, and Hawaii), Japan, Australia, China, and most recently South Africa and New Caledonia (Figure 1; MacLachlan 1938, Marlatt and Kimbrough 1979, Mellano 2006, Uchida et al. 2006, Kawanishi et al. 2009, Carnegie et al. 2010, Pérez et al. 2011, Zambino and Nolan 2011, Zhuang and Wei 2011, Roux et al. 2013, Rayamajhi et al. 2013). Given the rate at which the pathogen is spreading and its wide host range, the objectives of this study were to estimate genetic diversity within and among populations across the species native range as well as

areas of recent introduction, evaluate possible pathways of spread, and predict geographic areas that are climatically suitable in order to predict risk of invasion.

### METHODS

Six microsatellite loci were scored for *Puccinia psidii* samples derived from diverse hosts in eight countries (Table 1). Samples were assigned to genetic clusters using a Bayesian genetic clustering algorithm implemented in STRUCTURE v2.3.4 (Pritchard et al. 2000). Posterior probabilities were estimated for  $K = 1$  to  $K = 12$  assuming an admixture model. STRUCTURE analyses were performed using clone-corrected data (unique multi-locus genotypes, MGs). Fifty thousand burn-in generations were employed for each of 10 replicate runs of 1,000,000 generations of the MCMC sampler for each  $K$ . The optimal value of  $K$  was inferred using the method of Evanno et al. (2005) implemented in STRUCTURE HARVESTER web v0.6.93 (Earl and von Holdt 2012). Population genetic analyses of all samples (grouped by genetic cluster) were performed using GenAlEx v6.4 (Peakall and Smouse 2006). To examine relationships among MGs, a minimum spanning network was estimated using the genetic distance measure of Bruvo et al. (2004).

In: Chadwick, K. Comp. Proceedings of the 61st Annual Western International Forest Disease Work Conference; 2013 October 6-11; Waterton Lakes National Park, Alberta, Canada. <sup>1</sup>Western Forestry and Conservation Association, Portland, OR. See end of article for more affiliations.



**Figure 1A.** *Puccinia psidii* on guava (Brazil); **B.** *P. psidii* on eucalypt (Brazil); **C.** *P. psidii* on rose apple (Hawaii).

**Table 1.** Geographic origin and host of confirmed *Puccinia psidii* genotypes.

Country	Host	CLUSTER*	
Brazil	<i>Eucalyptus</i> spp.	EU/SJ	
	<i>Eugenia uniflora</i>	OTHER	
	<i>Myrciaria cauliflora</i>	OTHER	
	<i>Psidium guajava</i>	OTHER	
	<i>Psidium guineense</i>	OTHER	
	<i>Syzygium cumini</i>	OTHER	
	<i>Syzygium jambos</i>	EU/SJ	
	<i>Callistemon lanceolatus</i>	PANDEMIC	
Costa Rica	<i>Syzygium jambos</i>	PANDEMIC	
Jamaica	<i>Pimenta dioica</i>	PD	
	<i>Syzygium jambos</i>	PANDEMIC	
Mexico	<i>Syzygium jambos</i>	PANDEMIC	
Paraguay	<i>Eucalyptus grandis</i>	EU/SJ	
Puerto Rico	<i>Syzygium jambos</i>	PANDEMIC	
Uruguay	<i>Psidium guajava</i>	OTHER	
	<i>Eucalyptus grandis</i>	EU/SJ	
	<i>Eucalyptus globulus</i>	EU/SJ	
	<i>Myrrhinium atropurpurea</i>	OTHER	
	USA	<i>Eugenia koolauensis</i>	PANDEMIC
		<i>Melaleuca quinquenervia</i>	PANDEMIC
		<i>Metrosideros excelsa</i>	PANDEMIC
		<i>Metrosideros polymorpha</i>	PANDEMIC
		<i>Myrcianthes fragrans</i>	PANDEMIC
		<i>Myrtus communis</i>	PANDEMIC
<i>Rhodomyrtus tomentosa</i>		PANDEMIC	
<i>Syzygium jambos</i>	PANDEMIC		

\*EU/SJ = collections from *Eucalyptus* spp. and *Syzygium jambos* from Brazil, Paraguay, and Uruguay; OTHER = collections from *Psidium guajava*, *Psidium guineense*, *Syzygium cumini*, *Myrciaria cauliflora*, *Eugenia uniflora*, and *Myrrhinium atropurpureum* var *octandrum*; PD = collections from *Pimenta dioica* from Jamaica; and PANDEMIC = collections from diverse hosts from the Caribbean, Mexico, and USA.

Bioclimatic modeling was performed using the documented occurrences of the rust ( $n = 356$ ) and a reduced set of global pandemic occurrence records ( $n = 56$ ) coupled with 19 bioclimatic variables in MaxEnt (Maximum Entropy Species Distribution Modeling) v3.3.3K (Phillips et al. 2006, Phillips and Dudik 2008). This reduced set represents all known occurrences of samples with confirmed genotypes that correspond to the global pandemic biotype. Cross-validation was used to verify results among 10 replicate runs. Quantum GIS (QGIS) was used to create the final output in a cumulative format according to prediction probability.

## RESULTS AND DISCUSSION

Among *Puccinia psidii* sampled from Brazil, Costa Rica, Jamaica, Mexico, Paraguay, Puerto Rico, Uruguay, and the USA, 26 unique MGs were identified. A Bayesian clustering analysis and a minimum spanning network revealed four major genetic clusters among the MGs: (1) PD = collections from *Pimenta dioica* from Jamaica, (2) PANDEMIC = collections from diverse hosts from the Caribbean, Mexico, and the USA, (3) EU/SJ = collections from *Eucalyptus* spp. and *Syzygium jambos* from Brazil, Paraguay, and Uruguay, and (4) OTHER = collections from *Psidium guajava*, *Psidium guineense*, *Syzygium cumini*, *Myrciaria cauliflora*, *Eugenia uniflora*, and *Myrrhinium atropurpureum* var *octandrum* from Brazil and Uruguay (Table 1 and Figures 2 and 4). All loci were polymorphic, with five to 11 alleles detected per locus. Genetic diversity was highest within the OTHER cluster, followed by the PANDEMIC, the PD, and finally the EU/SJ clusters (Table 2).

Similar to previous attempts to assess the geographic invasive potential of the species (Booth et al. 2000, Glen et al. 2007, Magarey et al. 2007, Booth and Jovanovic 2012, Elith et al. 2013, Kriticos et al. 2013), the model using all 356 occurrence records predicted many areas throughout the world as having a suitable climate for *P. psidii* (Figure 3A) whereas the model using only the 56 global pandemic records of occurrence shows a prediction for the western hemisphere of a restricted distribution (Figure 3B).

This work builds on previous research that shows that genetic diversity must be considered when assessing the invasive threat posed by this pathogen to myrtaceous hosts worldwide (Graça et al. 2013). Future work will include occurrence records of *P. psidii* genotypes from Australia, South Africa, and New Caledonia which will allow us to predict the global geographic area that is climatically suitable for the species as a whole and for each individual genetic cluster to better assess pathways of spread and determine areas at risk of invasion.

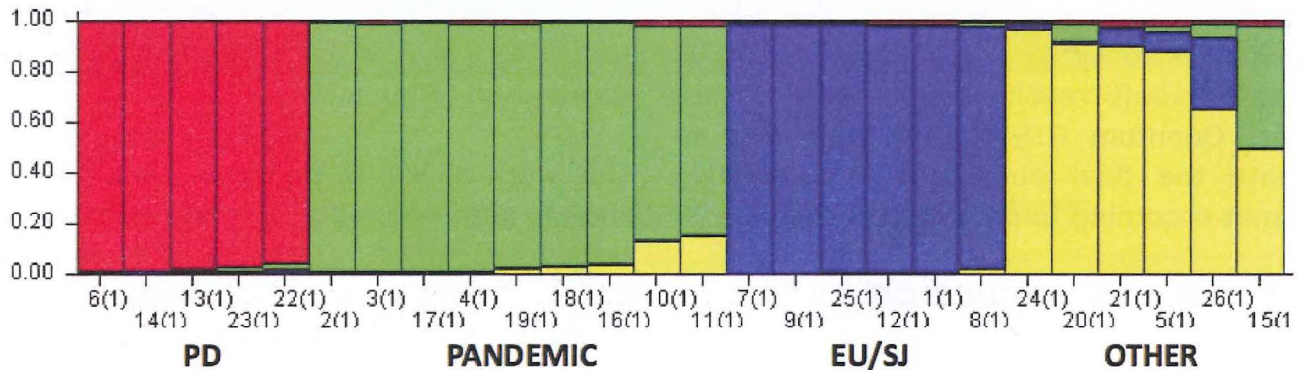
## ACKNOWLEDGEMENTS

Eric Pitman was instrumental in the bioclimatic modeling efforts. This project was partially funded by the Forest Health Protection Special Technology Development Program (R5-2011-04 and R5-2013-05), Western Wildlands Environmental Threat Assessment Center, and USFS Forest Health Protection, Region 5, and Joint Venture Agreements 11-JV-11221633-149 (WFCA) and 06-JV-11221662-22 (WSU).

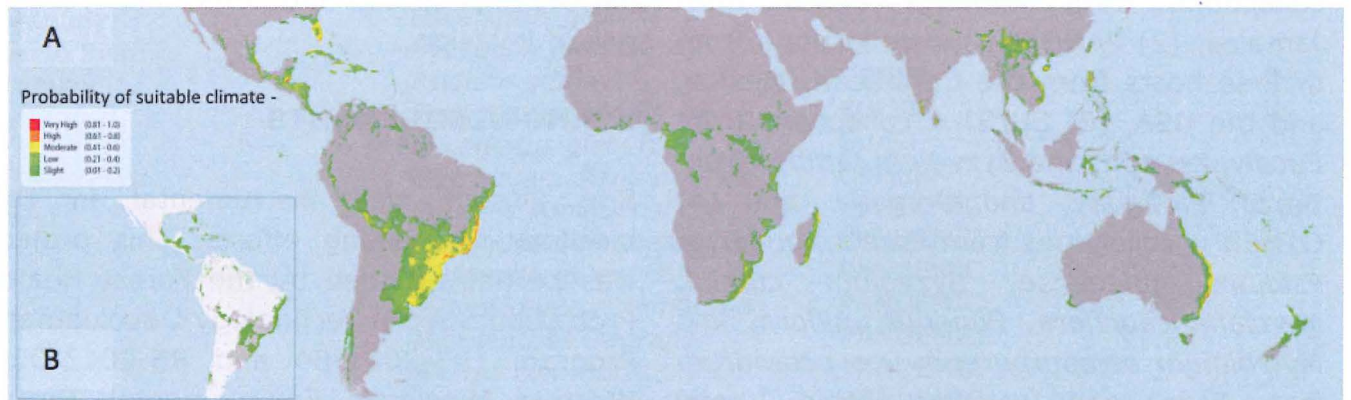
**Table 2.** Summary of genetic variation among clusters of *Puccinia psidii*.

	PD*	PANDEMIC	EU/SJ	OTHER
Alleles per locus	2.50 (0.43**)	3.50 (0.67)	2.33 (0.21)	4.83 (0.60)
Effective alleles per locus	2.26 (0.35)	2.91 (0.45)	1.97 (0.25)	3.40 (0.39)
Private alleles	4	9	1	11
Shannon's info index	0.78 (0.18)	1.07 (0.16)	0.70 (0.12)	1.35 (0.12)
Number of multi-locus genotypes	5	9	6	6
Observed heterozygosity	0.767 (0.158)	1.000 (0.000)	0.69 (0.19)	0.611 (0.127)
Expected heterozygosity	0.487 (0.103)	0.618 (0.050)	0.44 (0.08)	0.685 (0.037)
Fixation index	-0.621 (0.159)	-0.669 (0.128)	-0.388 (0.309)	0.135 (0.164)

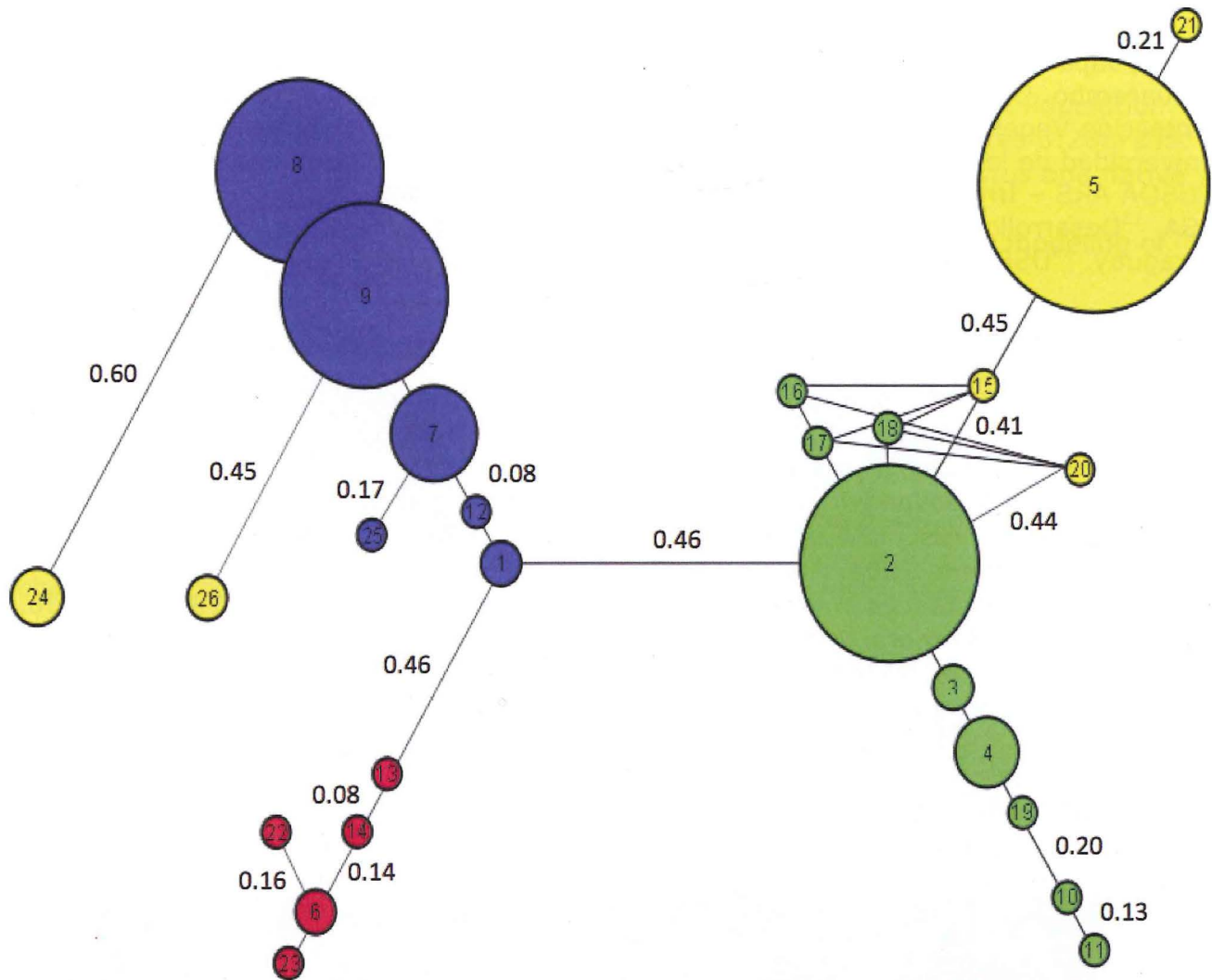
\*genetic clusters are described in Table 1; \*\*standard error



**Figure 2.** Population structure inferred using a Bayesian clustering algorithm implemented in STRUCTURE v2.3.4 at  $K = 4$ . PD = *Pimenta dioica* (Jamaica), PANDEMIC = global pandemic cluster (which includes collections from diverse hosts from the Caribbean, Mexico, and USA), EU/SJ = collections from *Eucalyptus* spp. and *Syzygium jambos* (Brazil, Paraguay, and Uruguay), OTHER = other hosts (collections from *Psidium guajava*, *Psidium guineense*, *Syzygium cumini*, *Myrciaria cauliflora*, *Eugenia uniflora*, and *Myrrhinium atropurpureum* var *octandrum*).



**Figure 3A.** Prediction of suitable climate space for *Puccinia psidii* using all 356 records of occurrence in MaxEnt (Maximum Entropy Species Distribution Modeling) and 19 bioclimatic variables (worldclim.org). Dark green represents predicted suitable climate space for *P. psidii* with light green, yellow, orange, and red indicating increasing probabilities of suitable climate; **B.** Predictions of suitable climate space in the western hemisphere for *Puccinia psidii* using all records of occurrence (gray) and 56 records of global pandemic occurrences (green).



**Figure 4.** Minimum spanning network of multilocus genotypes (MGs) estimated using Bruvo distances (Bruvo et al. 2004). Red circles represent collections from *Pimenta dioica* (Jamaica), blue represents collections from *Eucalyptus* spp. and *Syzygium jambos* (Brazil, Paraguay, and Uruguay), green from the global pandemic cluster (which includes collections from diverse hosts from the Caribbean, Mexico, and USA), and yellow from other hosts (collections from *Psidium guajava*, *Psidium guineense*, *Syzygium cumini*, *Myrciaria cauliflora*, *Eugenia uniflora*, and *Myrrhinium atropurpureum* var *octandrum*). Sizes of circles are proportional to MG frequency and connections are labeled with distances if different from 0.06, which corresponds to one mutational step at one locus. Loops in the network indicate multiple tied minimum spanning trees.

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