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Insects associated with flowering of *Rhodomyrtus psidioides* (Myrtaceae): Is this a Myrtle Rust (*Austropuccinia psidii*)-induced Plant-pollinator interaction Extinction Event?

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***Abstract*:** The threatened Australian endemic rainforest tree *Rhodomyrtus psidioides* (Myrtaceae) is visited and pollinated by a taxonomically diverse assemblage of mainly small, ecologically unspecialised, insects. Flower structure suggests that it may also be adapted for wind-pollination. However, the recent (2010) invasion by the aggressive fungal pathogen Myrtle rust (*Austropuccinia psidii*) has resulted in the local extinction of both the floral resource and associated plant-insect relationships. Here I table observed insect visitors to the flowers of *Rhodomyrtus psidioides* made before the impact of Myrtle rust - no other records appear to have been published.

Key Words: *Rhodomyrtus psidioides,* Myrtaceae, threatened plants, extinction, subtropical rainforest, Lorien Wildlife Refuge, anthophilous insects, wind-pollination.

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Introduction

In the 1990s, as part of my PhD investigation of the pollination ecology of lowland subtropical rainforests in northern New South Wales (Williams 1995), I undertook the observation and hand collection of insects that visited the flowers of the small myrtaceous tree *Rhodomyrtus psidioides* (G. Don) Benth.). This was one of numerous mass-flowering tree and shrub species I investigated over a three-season period (1990-1993); owing to time constraints observations of *Rhodomyrtus psidioides* were random rather than following a rigorous experimental protocol. Nevertheless, the proximity of several plants at one site (Lorien Wildlife Refuge) allowed opportunity for frequent casual observation, almost on a daily basis during seasonal flowering events in that period.

Rhodomyrtus psidioides in currently proposed for listing in New South Wales as *critically endangered* (Preliminary Determination, NSW Scientific Committee 2017) as it is severely threatened from infection from *Austropuccinia psidii* (Myrtle rust) an introduced pathogen first noted in NSW in 2010. Plants are extremely susceptible with all parts of the plant being affected and populations are threatened with extinction. Quantitative findings of recent very large declines in *Rhodomyrtus psidioides* populations due to *Austropuccinia psidii* infection reported in Carnegie *et al*. (2016) are supported by field botanists who have encountered the species during routine botanical surveys and seed collecting over multiple years (B. Makinson *in litt*. April 2016).

Rhodomyrtus psidioides is a large shrub or small tree endemic to Australia, distributed from Gosford on the central coast of New South Wales to Gympie in southeastern Queensland (Harden, 1991). Populations flower synchronously but flowers on individual trees open sequentially, however, not all regional populations flower each year (Williams 1995). The flowers are large (1.4cm) and usually clustered, individually last for 3-7 days, are bisexual but self-incompatible (Adam & Williams, 2001), fragrant, creamish-white in colour, and with yellow, slightly sticky pollen that is readily expelled from dehiscent anthers (Williams, 1995). Little nectar was evident in the flowers that were microscopically examined (Williams, 1995). The anthers are brush-like, with the stigma extending slightly above, the stigmatic surface being broad and laterally flattened apically (Fig. 1) (Williams, 1995). Flower morphology partly agrees with the wind-pollinated (anemophilous) syndrome (Faegri & van der Pijl, 1979) such that in addition to being adapted for pollination by biotic vectors the floral structure suggests flowers are also facultatively wind-pollinated (Williams & Adam, 2010). The exine sculpture is indistinct – smooth (Williams & Adam, 1999), indicating no special modification for biotic dispersal. Thus the species is considered cryptically ambophilous, a previously poorly recognized biotic-abiotic pollination strategy now considered to be expressed by rainforest angiosperms more widely (Bullock, 1994, Williams & Adam, 2010).

Floral visitor observations were undertaken principally of plants growing on the margin of a subtropical lowland rainforest at Lorien Wildlife Refuge, approximately 3km north of Lansdowne (31°45'00"S, 152°32'30"E). Observations were carried out there during November 1990 and November and December 1992 (occasional observations were continued in subsequent years). At the nearby Lansdowne Nature Reserve (31°47'30"S, 152°32'30"E), a small floodplain rainforest remnant, a single day (19 Nov. 1990) of observations was additionally undertaken. At both sites a small number of mass-netted insect samples were also collected (Williams 1995). These gave indications of the nature of visitor assemblages at particular moments, but are insufficient to allow any statistical analysis.

Rainforest at Lorien Wildlife Refuge and Lansdowne Nature Reserve represent vegetation communities listed as *endangered ecological communities* (respectively 'Lowland Rainforest in the NSW North Coast and Sydney Basin Bioregions' and **'**Lowland Rainforest on Floodplain in the New South Wales North Coast Bioregion') originally under the NSW *Threatened Species Conservation Act 1995*; this now supplanted by the *Biodiversity Conservation Act 2016*. Both formations are also listed as *critically endangered* under Federal legislation (see 'Lowland Rainforest of Subtropical Australia', *Environmental Protection and Conservation Act 1999*) because of the extent of past agricultural clearing and their now limited extent. Conservation has been a major consideration in their recent management. However, the unforeseen and widespread entry of the South American fungal pathogen 'Myrtle rust' (*Austropuccinia psidii*) (Invasive Species Council, 2011, Makinson, 2018) into the region around 2010 has resulted in the death of all mature *Rhodomyrtus psidioides* trees.

Figure 1: *Rhodomyrtus psidioides* flower showing extended stigma and expanded stigma surface.

Table 1. Insect taxa recorded visiting the flowers of *Rhodomyrtus psidioides* (1990-1992)
(Insects determined to family- Lorien Wildlife Refuge records cited first, Lansdowne Nature Reserve indicated with an asterisk '*'; multiple species given in parentheses; 'sp./spp. = number of species uncertain).

COLEOPTERA-beetles	
Aderidae	*Aderus* sp.
Boganiidae	*Athertonium* sp., **Athertonium* sp.
Cerambycidae	*Syllitus* sp.
Chrysomelidae	*Crepidodera* sp., *Ditropoda* spp. (2), *Monolepta australia, M. ?minuscula, *Crepidodera* sp., *Monolepta* sp.
Cleridae	*Scrobiger splendidus*
Coccinellidae	*Harmonia testudinaria, Rhizobius* sp., *Scymnus* sp.
Corylophidae	*Sericoderus* spp. (2), * *Sericoderus* sp.
Curculionidae	*Cytallia sydneyensis*, undetermined spp. (4), **Cytallia sydneyensis, *Orthorhinus* sp., *undetermined spp. (2)
Dermestidae	*Anthrenus* sp.
Elateridae	*Megapenthes futilis, Microdesmes collaris, *Drymelater* sp., **Megapenthes futilis*
Latridiidae	*Cortinicara* sp.
Melyridae	*Helcogaster* spp. (2), *Neocarphurus ?angustibasis*
Mordellidae	*Mordella inusitata, Mordella* sp., *Mordellistena* sp., **Mordellistena* sp
Nitidulidae	*Notobrachypterus* sp.
Oedemeridae	?*Ischnomera* spp. (2), *Pseudolychus* spp. (2)
Phalacridae	?*Olibroporus* sp.
Ptilidae	*Acrotrichis* sp.
Scarabaeidae	*Diphucephala ?pygmaea, Phyllotocus scutellaris, *Diphucephala ?pygmaea*
DIPTERA-flies	
Bombyliidae	*Geron* spp. (2)
Calliphoridae	?*Calliphora* sp., *Stomorhina* sp.
Dolichopodidae	*Amblypsilopus ?broulensis, Diaphorus* sp.
Drosophilidae	*Drosophila* spp. (2)
Empididae	undetermined sp.
Lauxaniidae	*Melanina* sp., *Steganopsis melanogaster*
Scatopsidae	undetermined sp.
Tachinidae	undetermined spp.
HEMIPTERA-bugs	
?Jassidae	undetermined spp.
Miridae	undetermined sp.
Psyllidae	undetermined sp.
HYMENOPTERA-wasps and ants	
Braconidae	undetermined sp./spp.
Encyrtidae	undetermined sp./spp.
Eulophidae	undetermined sp./spp.
Formicidae	?*Camponotus* sp., **Crematogaster* sp.
Pergidae	?*Neoeurys* sp.
Pteromalidae	undetermined sp./spp.
Vespidae	*Polistes humilis*
HYMENOPTERA/Apiformes-bees	
Apidae	*Amegilla ?pulchra, Apis mellifera, Trigonula carbonaria*
Colletidae	*Hylaeus ?ofarrelli, Hylaeus* sp., *Leioproctus* sp., **Heterapoides* sp.
PSOCOPTERA-book lice, bark lice	
*Caeciliidae	**Caecilius ?lineatus*
Ectopsocidae	*Ectopsocus* sp. near *meridionalis*
THYSANOPTERA-thrips	
Phlaeothripidae	*Haplothrips* sp., **Haplothrips* sp.
Thripidae	*Heliothrips haemorroidalis, Thrips setipennis, Thrips* sp., *?*Thrips* sp.

Table 2. Numbers of individual visiting insects and taxa recorded from selected single *Rhodomyrtus psidioides* tree sampling events (1990-1992)
Samples collected only during the morning at each site (from Williams, 1995)

	No. of individuals	No. of taxa	No. of individuals	No. of taxa
Lorien Wildlife Refuge Nov. 1990	**tree 1**		**tree 2**	
total Coleoptera	47	24	14	11
total Diptera	36	14	51	19
total all Hymenoptera	4	3	6	6
s/total bees	0	0	0	0
total visitors	107	47	103	45
Total <6mm in size	101		103	
Lorien Wildlife Refuge, Nov. 1992	**tree 1**			
total Coleoptera	38	13		
total Diptera	23	17		
total all Hymenoptera	9	9		
s/total bees	0	0		
total visitors	87			
total <6mm	86			
Lansdowne Nature Reserve, Nov. 1990	**tree 1**			
total Coleoptera	467	12		
total Diptera	13	7		
total all Hymenoptera	2	2		
s/total bees	1	1		
total visitors	534	32		
total <6mm in size	532			

Results and Discussion

Sampling and observation results show insect taxa recorded visiting the flowers of *Rhodomyrtus psidioides* in the 1990-1992 period (Table 1) and numbers of individual visiting insects and taxa recorded from selected single *Rhodomyrtus psidioides* tree sampling events (1990-1992) (Table 2). No vertebrates were seen visiting flowers. Table 1 underestimates the numbers of insect species owing to difficulties in identifying to family groups such as small Diptera and microhymenoptera. Insect visitors were predominantly (99%) in the <6mm size class (Table 2, Williams, 1995). Only *Amegilla* ?*pulchra* and the introduced 'honey bee' *Apis mellifera* constituted notable size exceptions. Although individual temporally-discrete sampling events can result in seemingly large numbers of individuals and taxa (Table 2), in general over the period of the study, insects were often few in number at any one time of observation; no taxon exhibited mass attraction responses to open blossoms, most individual blossoms were devoid of insects when observations were made, and even the otherwise ubiquitous *Apis mellifera* was usually absent. This seemed counter-intuitive given that blossoms were massed, conspicuous and fragrant, but might be explained by the small quantity of nectar that individual flowers seemed to offer. No specific visitation patterns were observed. Rather, insects appeared to recruit randomly to flowers throughout each day.

The single day of observations, and the single netted sample collected at Lansdowne Nature Reserve (Table 2), is too small to establish an understanding of the possible full suite of visitors there, however, observations indicated that the assemblage likely mirrors that recorded at Lorien Wildlife Refuge; this being a mixture of 'incidental' visitors (e.g., Dolichopodidae, Psyllidae, microhymenoptera) and potential pollinators dominated by small species, ecologically unspecialised for pollination, that are commonly encountered on a range of mass-flowering rainforest trees and large shrubs elsewhere in the region (Williams, 1995, G. Williams unpubl. records). The few species with specialised morphological adaptations to a floricolous habit were represented by Mordellidae, the scarab *Phyllotocus scutellaris,* apid bees, and the bombyliid fly genus *Geron*. All visitors, regardless of their degree of adaptation to feeding upon floral resources, have the potential to transport pollen loads. In the case of thrips and other minute insects, only single or small numbers of grains are anticipated to be transported, and movements are largely restricted to adjacent flowers and plants; their contribution to out-crossing thus being individually small, though cumulatively over time potentially significant. Only *Amegilla* ?*pulchra* and *Apis mellifera* undertake relatively frequent or long distance foraging movements between dispersed individual plants, with the former known to regularly exhibit 'trap-lining' foraging strategies (see Williams & Adam, 2010, Willmer, 2011).

Myrtle rust has spread globally and was first detected in New South Wales in April 2010. Its infection results in crown dieback, branch death and mortality of sensitive members of the Myrtaceae (Invasive Species Council, 2011, Carnegie *et al*., 2016, Pegg, 2017, Makinson, 2018). Myrtle rust has the potential to fundamentally alter the ecology

of Australia's vegetation communities. At Lorien Wildlife Refuge *Rhodomyrtus psidioides* and the related and highly sensitive *Rhodamnia rubescens* (Benth.) Miq. have been severely attacked but species of the related Myrtaceae genera *Archirhodomyrtus, Corymbia, Eucalyptus, Lophostemon, Syncarpia, Syzygium* and *Tristaniopsis* have been seemingly unaffected. All these genera recruit a taxonomically wide assemblage of putative pollinators, most of which are small in size, that frequent a diversity of mass-flowering shrubs and trees with open insect-adapted floral structures (G. Williams pers. obs.).

Although *Rhodomyrtus psidioides* is able to resprout from root stock, vegetative regrowth over the ensuing years is constantly re-infected, causing gross leaf deformity and tip mortality (Fig. 2). Consequently, sucker growth, persistent as it has been, has not been able to successfully progress to reproductive maturity. This scenario is exhibited by numerous other populations in the region, for example in littoral rainforest at Harrington (G. Williams pers. obs.) and ornamental plantings at Diamond Beach (T. Wright pers. comm.). Although none of the insect visitors recorded constitute species with known obligate plant dependencies, *Rhodomyrtus psidioides* flower – putative pollinator interactions are extinct, at least locally. But should the species be able later to re-establish viable populations, a resident assemblage of pollinators would be present to reconstitute its pollination suite.

Figure 2: Root sucker tip mortality on *Rhodomyrtus psidioides* resulting from Myrtle rust attack.

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