Experimental determination of the host range of *Puccinia araujiae* Lév. (Pucciniaceae)

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Introduction

Previous studies identified the rust *Puccinia araujiae* Lév. as the most promising pathogen to be introduced to New Zealand as a biological control agent for moth plant, *Araujia hortorum* (Waipara et al. 2006, Delhey et al. 2011, Kiehr et al. 2011). Moth plant belongs to the sub-tribe Oxypetalinae in the family Apocynaceae. The rust has been reported from a subset of other plants in this same sub-tribe, but not from any more distantly related plants (Lindquist 1982). This indicates a moderately high level of specificity. This study tested the hypothesis that the host-range of *P. araujiae* is restricted to plants within the Oxypetalinae sub-tribe. Successful infection of plants outside this sub-tribe in laboratory tests would negate that hypothesis.

Materials and methods

Field work

Field surveys were conducted across the geographical distribution of moth plant, the target weed, in Argentina between autumn 2012 and spring 2013 to collect isolates of *P. araujiae*. Plant material was collected and placed in a plant press for further study in the laboratory. A GPS reading was taken to record the site location. Sites were numbered in the order in which they were recorded. A couple of sites were visited more than once in different seasons.

The nature of the life cycle was already known from both field observations and previous experimental work (Kiehr et al. 2011). Experiments were conducted to confirm such knowledge and to study the infection process in more detail.

Sourcing and maintenance of plants

Araujia hortorum plants from several different locations were grown from seed collected during field trips. Seeds were superficially disinfected by immersion in Sodium hypochlorite in water (3:10) for 2 min followed by a rinse in distilled water. To promote germination, seeds were then placed in Petri dishes lined with wet cotton wool and filter paper and left on a bench in the laboratory at room temperature for 7-10 days. At hypocotyl emergence, seedlings were planted individually into potting mix in plastic seedling trays. At the four-leaf stage, plantlets were transferred to 10-cm-diameter plastic pots containing a 1:1 mixture of potting mix and local soil. Plants were kept until needed on a glasshouse bench (temperature range 16–26ºC).

For host range testing, plants belonging to species within the Oxypetalinae and Asclepiadinae were all grown from seed. Other, more distantly related plants were purchased, fully grown, at local nurseries.

General rust inoculation methodology

An adaptation of the "leaf disc method" (Morin et al. 1993) was used for all inoculation experiments. Small (*ca*. 2.5 mm diameter) discs bearing mature (4-6 weeks after inoculation) telia were cut with a scalpel from diseased *A. hortorum* plants and placed onto the surface of 10% water agar (WA) in 9-cm-diameter Petri dishes. Plates containing 35–40 telial discs were inverted (after the lid was removed) over young, healthy, recently pruned test plants, at a distance of *ca.* 5 cm from the uppermost leaves, by placing them on a wire framework attached to the roof of the inoculation chambers. This allowed spores to fall naturally and inoculate the test plants below. These chambers consisted of cube-shaped polyethylene boxes with the floor lined with water-soaked newspaper to provide around 100% RH (relative humidity), ideal conditions for infection. The surface of inoculated plants was sprayed manually with a fine mist of water. Plants were kept in these chambers and subjected to the inoculum for the first 48 h after which they were removed and kept in controlled environment cabinets at around 75% RH, 18–20ºC and a 12-h dark/12-h light (fluorescent, 1400 l) regime. This inoculation procedure was used in all experiments unless otherwise stated.

Host specificity testing

Puccinia araujiae has not been reported from any plants outside the Apocynaceae family (Lindquist 1982). To confirm this narrow host range, tests were conducted in Argentina. Most of the species were tested in Bahía Blanca where the project is based, with the exception of *Parsonsia heterophylla*, a species exotic to Argentina, and to our knowledge, not present in the country. The latter was tested in a quarantine facility belonging to the Instituto de Microbiología y Zoología Agrícola (IMYZA), INTA, located in Hurlingham, Buenos Aires.

The plants chosen for host specificity testing were selected in accordance with international best practice for weed biocontrol host-range testing (Wapshere 1974, Sheppard et al. 2005, Briese 2005, see Landcare Research 2015c). There are only three species native to New Zealand that are in the same plant family as moth plant, and they all belong to the same genus: *Parsonsia*. The most common of these (*P. heterophylla*) was tested. *Parsonsia* species belong to the sub family Apocynoideae, whereas moth plant belongs to the Asclepiadoideae, so they are not particularly closely related. It was not considered necessary to test any other New Zealand native plants in this study because there are no other native plants in the Apocynaceae family. *Araujia hortorum* plants were included in each test as positive controls. The number of plants tested per species is given in Table 1, and each species was tested on at least two occasions.

Results

Field work

Viable spores of the rust were found and collected at only one site. This site is located near the city of Junín, in the north of Buenos Aires province. Only telia of the rust were ever observed in the field.

Life cycle

Inoculation experiments performed in the laboratory confirmed field observations with respect to the life-cycle of *Puccinia araujiae* and were in accordance with previous work (Kiehr et al. 2011). Teliospores germinated without a resting period to produce basidiospores which were able to infect all vegetative parts of *A. hortorum* plants, giving rise to new telia and teliospores. Thus, these experiments confirmed that the rust is microcyclic and completes its life-cycle on a single host (i.e. the rust is autoecious).

Sourcing plants

Two of the species that had to be tested, *Parsonsia heterophylla* and *Oxypetalum caeruleum*, are not known to be present in Argentina and therefore needed to be tested in quarantine. The only authorised facility is located around 700 km northeast of Bahía Blanca, in Hurlingham, Buenos Aires Province. Seed belonging to these two species were imported directly to the quarantine facility in July 2011 and a batch of plants of each was obtained and prepared for inoculation soon after. Unfortunately the only samples of the rust available at that time, obtained from artificial inoculations performed by Rolf Delhey and Mirta Kiehr, proved to have lost viability precluding the testing of these species as planned. The plants were kept and maintained by staff at the quarantine facility thereafter. New funding allowed a second stage of the project to begin in May 2012. New field surveys were then conducted in search for sources of viable spores for experimentation. By the time a new rust culture was established in the laboratory, only five individuals belonging to *P. heterophylla* had survived in the quarantine facility. Efforts were made to grow new plants of this species from the remaining seed without success. In addition, all further attempts to propagate the other species *Oxypetalum caeruleum,* commonly called tweedia, an ornamental species grown in New Zealand gardens, also failed and so this species could not be tested.

Host range tests

All but two of the 51 positive control plants developed disease symptoms. The two plants that did not develop symptoms were not in the same batch and every batch had at least some *Araujia hortorum* plants that showed disease symptoms.

All four test plants in the genera *Araujia* and *Morrenia* developed pustules (Figure 1). These belong to the same tribe (Oxypetalinae) as the target weed (Table 1). None of the individuals belonging to the other tested genera developed symptoms typical of infection by *Puccinia araujiae*.

Table 1. Results of host specificity tests. Plant taxonomy/relatedness also shown.

Subfamily	Tribe	Subtribe	Plant species	Nº plants tested	% of plants developing pustules
Asclepiadoideae	Asclepiadeae	Oxypetalinae	*Araujia hortorum (positive controls)*	**51**	**96%**
			A. angustifolia	**6**	**100%**
			Morrenia odorata	**7**	**86%**
			M. brachystephana	**6**	**100%**
		Asclepiadinae	*Asclepias curassavica*	**12**	**0%**
			Gomphocarpus physocarpus	**16**	**0%**
	Marsdenieae		*Hoya carnosa*	**9**	**0%**
Apocynoideae	Nerieae		*Nerium oleander*	**8**	**0%**
	Mesechiteae		*Mandevilla laxa*	**8**	**0%**
			Mandevilla sanderi	**2**	**0%**
	Echiteae		*Parsonsia heterophylla*	**5**	**0%**
Rauvolfioidae			*Vinca major*	**8**	**0%**

The only damage recorded on plants outside the *Oxypetalinae* was as follows: *Gomphocarpus physocarpus*: three leaves were observed to have chlorotic specks, but these were most likely due to infection by powdery mildew; and, *Nerium oleander*: two leaves had chlorotic specks of unknown origin.

Figure 1. *Puccinia araujiae* infection of a *Morrenia odorata* leaf.

Discussion

Viable spores of *Puccinia araujiae* were found at only one site in the field, and that was 550 km from where the project is based. This was unfortunate because the great distance between the field site and the laboratory precluded close observation of the effect of the rust on its host in the field.

Experimental results confirmed previous work and field observations on the nature of the life cycle, and also provided evidence that the disease can seriously damage plants by inducing premature senescence and defoliation.

The validity of this testing regime and interpretation of the results has been peer-reviewed. That review has been provided to EPA.

The plants tested were exposed to a high load of inoculum under conditions that were highly favourable for infection. Despite this, none of the plant species tested that belong outside the sub-tribe Oxypetalinae supported pustule development in this rust. Other symptoms observed (a few chlorotic specks) were probably attributable to other pathogens and were too minor to have impacted plant health. Each of the three test plants tested that do belong to the sub-tribe appear to be equally susceptible to the rust. The test results support the field observation that *P. araujiae* is restricted to hosts within the sub-tribe Oxypetalinae (Lindquist, 1982).

The ornamental plant tweedia (*Oxypetalum caeruleum*) also belongs to this sub-tribe. This species was not tested. It must be assumed that this species would be susceptible to *P. araujiae* if the rust was introduced to New Zealand.

Three other ornamental plants that are prized by gardeners in New Zealand were amongst the test plants that were not damaged by the rust. These are *Asclepias curassavica* (bloodflower), *Gomphocarpus physocarpus* (swan plant, preferred food of monarch butterfly caterpillars) and *Parsonsia heterophylla* (New Zealand jasmine).

As the host range of *P. araujiae* is confined to species within the sub-tribe Oxypetalinae, and as no New Zealand native species belong to this sub-tribe, the tests confirm that no native plant species are at risk from this rust.

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