



Manaaki Whenua
Landcare Research

The host range of Darwin's barberry rust, *Puccinia berberidis-darwinii*, a biological control agent for Darwin's barberry, *Berberis darwinii*

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Summary

- The host range of Darwin's barberry rust (*Puccinia berberidis-darwinii*) was determined in laboratory tests. The rust was sourced from Valdivia, Chile.
- Experiments conducted at Manaaki Whenua – Landcare Research containment facility in Auckland, New Zealand, tested fourteen plants from the order Ranunculales.
- Darwin's barberry rust produced bright yellow-orange pustules on Darwin's barberry.
- The rust did not produce any pustules on any of the other plants tested.
- In the containment facility only aeciospores and teliospores have been observed. However, it has been reported to have a demicyclic life cycle with the production of pycnia, aecia and telia.
- Among the plant species tested, only *Berberis dielsiana* exhibited a hypersensitive response to the rust fungus, with the death of cells in the area surrounding the inoculation site.
- The testing suggests that *Puccinia berberidis-darwinii* is specific to *Berberis darwinii* and will not infect any other plant species.

1 Introduction

This report outlines the history of the biological control programme against Darwin's barberry in New Zealand and presents the results of research to determine the host range of *Puccinia berberidis-darwinii* H.S. Jacks. & Holw., and the direct risk it would pose to valued plants if introduced to New Zealand.

1.1 The biological control programme against Darwin's barberry

Darwin's barberry, *Berberis darwinii* Hook. is an increasingly important environmental weed from central North Island to Stewart Island. A biological control programme was initiated in 2002 to mitigate the effects of the weed and to reduce the rate of spread, because conventional control methods such as herbicide application and physical removal are expensive, damaging to non-target plants, and often impractical.

1.2 Suitability of Darwin's barberry as a target for biological control

The programme began with an assessment of the prospects for biological control as a management tool (McGregor 2002). A survey was subsequently conducted between October 2003 and March 2004 to determine what resident insects and diseases were present on Darwin's barberry in New Zealand, and to assess their role in Darwin's barberry plant ecology (Smith et al. 2004). Twenty-five invertebrate species were identified from Darwin's barberry. Only the barberry aphid *Liosomaphis berberidis* and an unidentified Tortricidae were classified as common on Darwin's barberry. Although *Liosomaphis berberidis* is specific to plants from the genera *Berberis* and *Mahonia*, damage that could be attributed to this aphid and other invertebrates was minimal (Smith et al. 2004). Fourteen fungal species were identified from Darwin's barberry plant tissues, of which only five species were primary pathogens. None of these fungal species were specific. Most disease symptoms observed were attributed to moisture stress, either in wet areas or dry areas (Smith et al. 2004). It was concluded that Darwin's barberry was not subject to any significant biotic pressures, and there were no existing relationships that could influence a biological control programme.

1.3 Selection of potential control agents

The programme took two approaches to management of Darwin's barberry. Manaaki Whenua - Landcare Research (MWLR) was seeking to:

1. limit the survival and/or biomass (pest status) of Darwin's barberry by the introduction of a range of invertebrates and diseases to attack the vegetative parts of the plant.

2. limit the rate at which Darwin's barberry populations establish and grow by introducing invertebrates and diseases that reduce seed production by attacking reproductive structures such as fruits.

Surveys of plants, pathogens and insects were conducted in Chile from Valparaíso to Valdivia between 2005 and 2008 to locate and identify potential agents for classical biocontrol. During these surveys, two weevils causing damage to flowers and fruits and a rust fungus which produces yellow pustules on leaves were found on Darwin's barberry. The rust was responsible for a range of symptoms on the plant from low damage to high levels of infection resulting in chlorotic, premature defoliation and leaf death (Waipara 2007).

The weevils *Anthonomus kuscheli* which targets Darwin's barberry flowers and *Berbericola exaratus* which attacks the weed's fruit and seeds, respectively, were approved by the EPA to be released in New Zealand in 2012. It was believed that a pathogen affecting the plant's biomass would contribute to the damage caused by the weevils. *Berbericola exaratus* was released in 2015 in New Zealand and is well established in Southland (with recent surveys indicating most plants are attacked although impacts on seed production have not been assessed) and has also been observed at a release site in Wellington. *Anthonomus kuscheli* could be released in the future, depending on how effective *B. exaratus* is at reducing seed set but it is recognised that although attacking plant reproductive structures may reduce the rate that Darwin's barberry invades, it is not likely to control dense stands of Darwin's barberry.

A subsequent survey for pathogens was conducted in November 2013 in the Araucanía, Los Ríos and Los Lagos regions of Chile. The rust *Puccinia berberidis-darwinii* was frequently found on Darwin's barberry leaves, with similar symptoms as previous surveys. The rust was first imported into the Beever Plant Pathogen Containment Facility (BPPCF) in Auckland in November 2013 for DNA sequencing. It was followed by successive importations to obtain a viable culture on Darwin's barberry plants and undertake its host range testing.

1.4 Use of rusts as classical biocontrol agents

Most of the fungi used in classical weed biocontrol are rusts (Barton 2004, Morin 2020). These rusts are characterised as obligate parasites. Their high virulence, efficient dispersal capabilities and host specificity make them excellent candidates for weed biocontrol (Barton 2004). Until now, none of the rusts or other fungi released for classical weed biocontrol has caused unforeseen harm to non-target plants (Barton 2004, Morin 2020).

2 Biology and ecology of *Puccinia berberidis-darwinii*

2.1 Taxonomy

Kingdom	Fungi
Phylum	Basidiomycota
Class	Pucciniomycetes
Order	Pucciniales
Family	Pucciniaceae
Genus	<i>Puccinia</i>
Species	<i>berberidis-darwinii</i> H.S. Jacks. & Holw.

The genus *Puccinia* encompasses around 4000 species of rust fungi, all of which are obligate plant pathogens that spread by infecting aerial tissues of hosts (Avasthi et al. 2023).

2.2 Life cycle of typical rust

Rusts life cycles are very complex and can include up to five spore states on two unrelated hosts (Kolmer et al. 2009). A life cycle is considered macrocyclic if it produces all five spore types which are teliospores (in telia), basidiospores (in basidia), pycniospores (in pycnia), aeciospores (in aecia) and urediniospores (in uredinia) (see Figure 1; Barton et al. 2011). A rust can be autoecious if its life cycle is on only one host or heteroecious if the pycnial and aecial stages are on one host while the uredinial and telial stages are on another host (Barton et al. 2011).

Teliospores germinate to produce basidiospores (Figure 1). If these basidiospores can infect the same host, the rust is definitively autoecious (Barton et al. 2011). Conversely, if basidiospores require an alternative host to complete their life cycle, the rust is heteroecious. Heteroecious rusts are not considered candidates for biocontrol as assessing host specificity would require testing species related to both alternate hosts (Morin et al. 2006), which would significantly complicate the process.

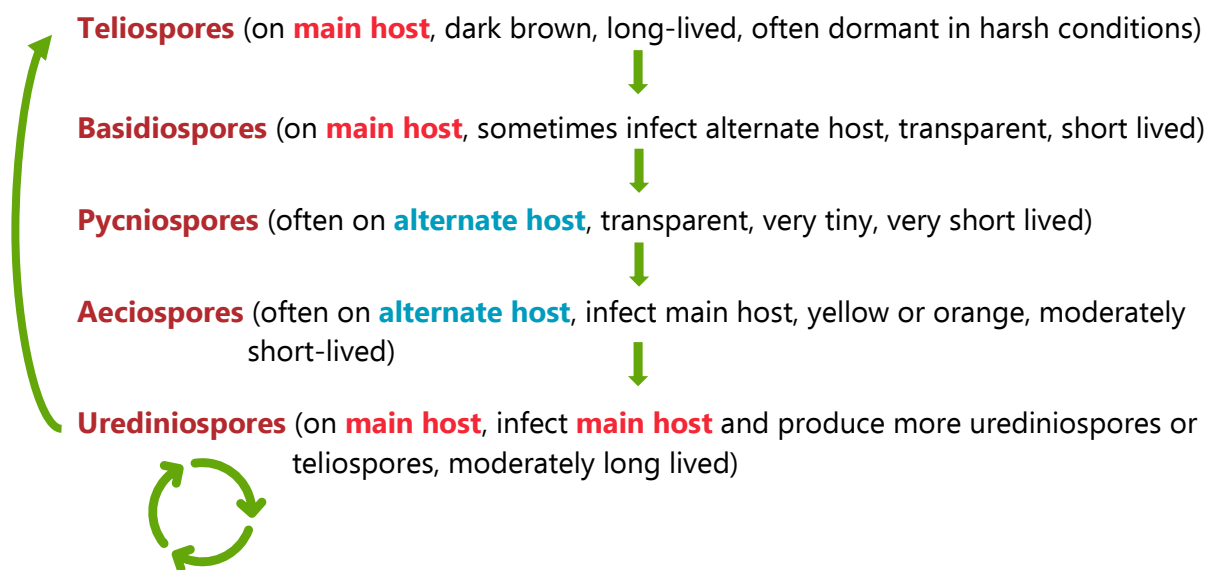


Figure 1. Spore types potentially formed by a rust with a full life cycle (Source: Barton et al. 2011).

2.3 Field observations of *Puccinia berberidis-darwinii* in Chile

Puccinia berberidis-darwinii was first collected in a survey in Southern Chile in November 2007. In November 2013, another survey was undertaken in the Araucaria, Los Rios and Los Lagos regions of Chile. Rust pustules on leaves were collected at different sites near Vilcún, Villarica, Melipeuco and Valdivia (Figure 2A). Early rust infections were additionally observed on green Darwin's barberry fruits in Valdivia. Rust leaves were placed in paper bags, dried and were later sent to New Zealand by courier however the parcel was lost in transit. Our collaborator in Chile, Dr. Hernán Norambuena collected leaves with rust pustules from Darwin's barberry in December 2014 from Valdivia, Frutillar and Pucón for DNA sequencing. DNA from different locations showed a 99.3% sequence identity between isolates. Leaves with rust pustules from *Berberis buxifolia* and *Berberis congestiflora* were also collected and their DNA compared to that of rust pustules from Darwin's barberry. DNA sequencing confirmed that the rust found on *Berberis buxifolia* and *Berberis congestiflora*, although identical to each other, was different to the rust isolated from Darwin's barberry (93% DNA sequence identity for the large subunit of the ribosomal ribonucleic acid (LSU rRNA)).

Symptoms observed in the field included chlorotic leaves, premature leaf defoliation and leaf death. Infected Darwin's barberry plants overall appeared unhealthy compared to plants that were not infected, with less foliage. Infected fruits with mature pustules were packed with aeciospores (Figure 2B).

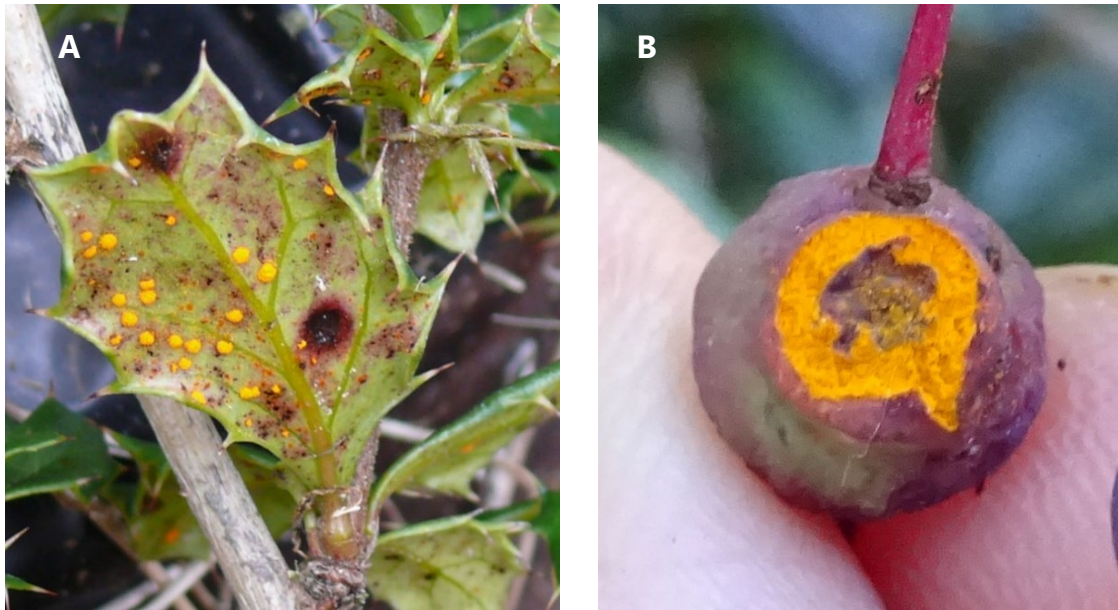


Figure 2. *Puccinia berberidis-darwinii* pustules. **A:** on leaves; **B:** on fruits.

2.4 Description

Puccinia berberidis-darwinii was first described in 1900 as *Caoema berberidis* Diet. & Neger (Dietel & Neger 1900). It was subsequently described as *Puccinia berberidis-darwinii* Jackson & Holway (Jackson 1931). This rust forms 40-64 x 18-24 μm yellow to orange oblong, elongated ellipsoid and sometimes fusiform aeciospores (Figure 3A and 3 B; Dietel & Neger 1900).

Teliospores are 16-20 x 55-80 μm , rounded above and narrow below, clavate spores, with the upper cell around half to three quarter the size of the lower cell (Figure 3A and 3C) (Jackson 1931). The wall thickness is 1.5-2.5 μm , with the apex gradually thickened to 3-3.5 μm . The pedicel is colourless, persistent and has the same length as the spore. The upper cell has an apical pore (Jackson 1931). Teliospores are present in hypophyllous sori which also contain aeciospores.

This rust produces 100-125 x 110-150 μm pycnia that are mostly epiphyllous or occasionally hypophyllous, obovate, or globoid and deep seated (Jackson 1931).



Figure 3. Spores of *Puccinia berberidis-darwinii*. A: teliospores and aeciospores; B: aeciospores; C: teliospores.

2.5 Life cycle of *Puccinia berberidis-darwinii*

In the literature, *Puccinia berberidis-darwinii* forms aecia, telia and pycnia on *Berberis darwinii* (Jackson 1931). As urediniospores were never observed for this rust and aeciospores and teliospores were found in the same sori, Jackson (1931) suggested that this rust species potentially did not form any urediniospores, like *Puccinia senecionis* Lib. Rusts that do not form urediniospores have a life cycle described as demicyclic (Petersen 1974). In containment, *P. berberidis-darwinii* produces profuse number of aeciospores which were used to inoculate Darwin's barberry plants. It usually takes between three to six months to observe new aecia on leaves. Teliospores are also produced in the same sori.

As aeciospores and teliospores are formed on the same host, *Puccinia berberidis-darwinii* is an autoecious rust (only one host).

2.6 *Puccinia* species in New Zealand

Puccinia species are commonly found on Asteraceae, Cyperaceae, Liliaceae and Poaceae in New Zealand (McKenzie 1998). Biota of New Zealand (2023) lists 259 rusts present in New Zealand and 132 are *Puccinia* species. Among these species, 63 are classified as exotic species, 49 as native species to New Zealand and 20 as native to New Zealand and naturally found elsewhere (Manaaki Whenua – Landcare Research 2023).

Most rusts in New Zealand are autoecious (McKenzie 1998). Thirty-eight *Puccinia* species only have one host while 47 only infect plants from the same genus. Thirty-two *Puccinia* species infect plants from the same family and/ or tribe (Manaaki Whenua – Landcare Research 2023).

Only nine species have very different hosts, two of which are endemic to New Zealand. Most heteroecious species are adventive rusts on introduced species (McKenzie 1998). Interestingly, both of the two endemic rusts, *Puccinia caricina* and *Puccinia otagensis*, are found on native and introduced hosts (Manaaki Whenua – Landcare Research 2023).

These observations indicate that most *Puccinia* species in New Zealand have hosts within the same family (88.6%) while very few *Puccinia* species have an alternate host within the country. New adventive (non-native) rusts often arrive into New Zealand through wind currents from Australia, with an average of one new rust per year since 1945 (McKenzie 1998). The recent arrival of *Austropuccinia psidii* (myrtle rust), although specific to the Myrtaceae family, has shown the impact that a rust can have on our environment and the importance of host range testing.

2.7 Evolution of host range

Barton (2004) conducted a review regarding the potential risks associated with fungal pathogens used as classical biocontrol agents, specifically focusing on increased non-target usage, host addition, or host switching through evolution. This study concluded that while the evolution of pathogens does pose some risks of increased target use, a

comprehensive host-range testing can reveal the principal host range of each pathogen which allows for accurate predictions of the extent of such risks (Barton 2004). In the case of *P. berberidis-darwinii*, the pathogen only infects Darwin's barberry, and the only closely related plants in New Zealand are either weeds or exotic ornamental plants (*Berberis* spp. and *Mahonia* spp.), none of which are native to South America. The likelihood of *P. berberidis-darwinii* being able to attack non-target plant species in New Zealand over time is very low.

2.8 Predicted distribution of *P. berberidis-darwinii*

In Chile, *Puccinia berberidis-darwinii* was present across Darwin's barberry's range however its distribution appeared to be influenced by climatic conditions, as it was absent in wind-exposed areas and at high elevations. Microclimates will probably influence the establishment and distribution of this rust in New Zealand. As it thrives in damp conditions, it is very likely to prefer the wetter regions of New Zealand.

2.9 Predicted impact in New Zealand

It is anticipated that the effect of *P. berberidis-darwinii* on Darwin's barberry in New Zealand will be comparable to its impact in its native habitat.

3 Determination of the host range of *P. berberidis-darwinii*

3.1 Taxonomy of *Berberis darwinii* (Darwin's barberry)

Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Order	Ranunculales
Family	Berberidaceae
Genus	<i>Berberis</i>
Species	<i>darwinii</i>

3.2 Selection of plants for host range tests

The process of selecting which plants should undergo host range testing for a classical biocontrol agent typically follows the 'centrifugal phylogenetic' approach suggested by Wapshere in 1974. Wapshere (1974) recommended that the decision of whether to include specific plants in host range tests should be based on two main factors:

- the degree of phylogenetic relatedness to the target weed: this is based on the idea that the more closely related two plant species are, the more likely they share

similarities in morphology and chemistry. Consequently, they are more likely to serve as suitable hosts for the specific pathogen under investigation (Barton 2004).

- the likelihood of the plant being susceptible to the agent being tested: this is to ensure that plants are included on the test list for good reasons. For example, a plant might be included if it has previously been documented in the literature as a potential host for the biocontrol agent, or if it is a particularly desirable species closely associated with the target weed. This prevents the exclusion of plants solely based on their lack of close phylogenetic relation to the target weed (Barton 2004).

The most important consideration for selecting plants for this project is the relatedness of plant species to Darwin's barberry.

Darwin's barberry belongs to the family Berberidaceae which is divided into subfamilies Berberidoideae and Nandinoideae (Wang et al. 2009). Subfamily Berberidoideae includes *Berberis* and *Mahonia* species while subfamily Nandinoideae includes *Nandina*, *Caulophyllum* and *Gymnospermium* species (Figure 4; Wang et al. 2009). Some botanists disagree on whether to recognise *Mahonia* as separate from *Berberis* (Marroquín & Laferrière 1997), however we have chosen to keep them separate in this report.



Figure 4. Phylogenetic tree of the family Berberidaceae. Highlighted in blue: genera with species tested for the specificity of *P. berberidis-darwinii*. (Source: Wang et al. 2009).

Twenty *Berberis* species are listed in Chile and 11 are present in the same native range as Darwin's barberry (Landrum 1999). There are no native *Berberis* species in New Zealand. New Zealand Plant conservation network (2023) lists *B. darwinii*, *B. glaucocarpa* Stapf, *B. soulieana* C.K.Schneid., *B. vulgaris* L. and *B. wilsoniae* Hemsl. as naturalised (Network 2023). Additionally, *Berberis x stenophylla* Lindl. and *B. congestiflora* Gay are found on Flora of New Zealand (Breitwieser et al. 2010–2023). *Berberis x stenophylla* is listed as a hybrid between *B. darwinii* and *B. empetrifolia* and has not been tested for its susceptibility to *P. berberidis-darwinii*.

The Berberidaceae family belongs to the order Ranunculales and the phylogenetic relationship within this order is shown in Figure 5.

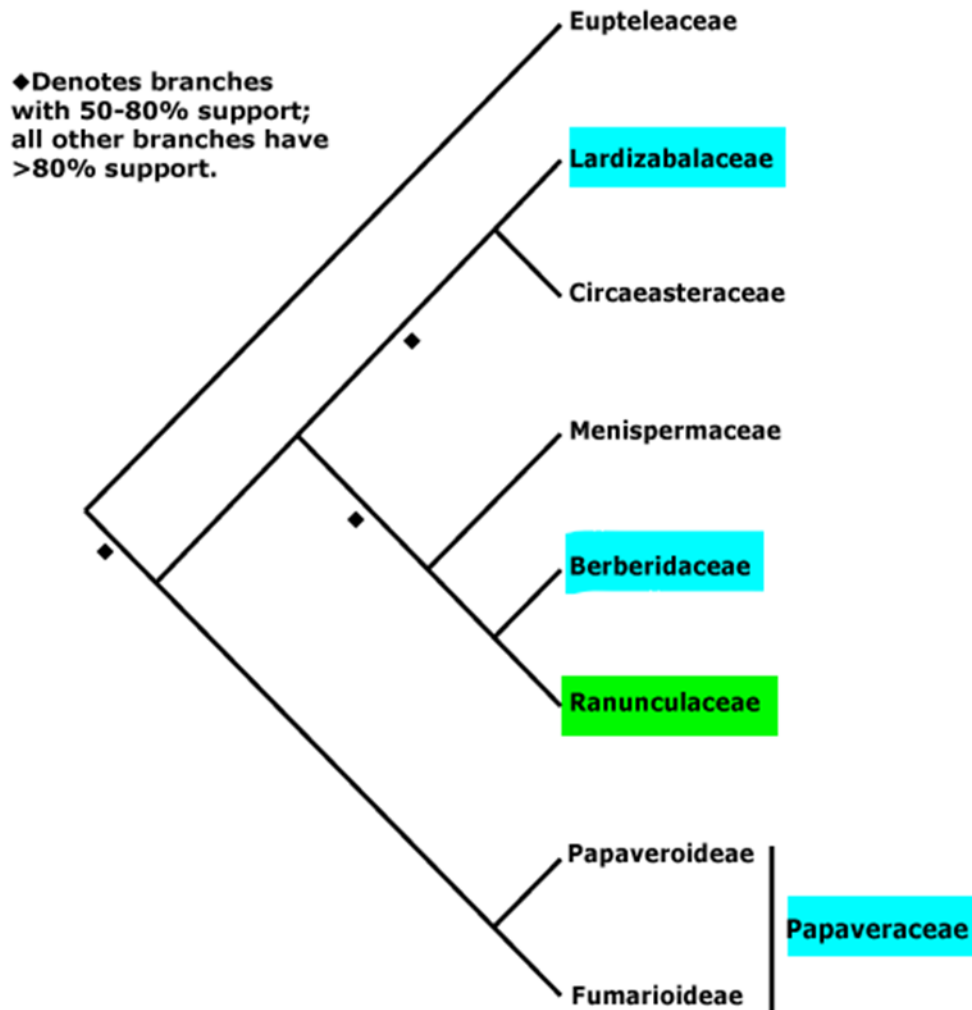


Figure 5. Phylogenetic tree of the order Ranunculales. Highlighted in blue: family with exotic species present in New Zealand; highlighted in green: family with native and exotic species in New Zealand. (Source: <http://www.mobot.org/mobot/research/APweb/>).

The Ranunculales comprises seven families (Hao 2019):

- Berberidaceae with 17 genera and 650 species (including Darwin's barberry)
- Ranunculaceae with over 50 genera and over 2000 species
- Menispermaceae with 65 genera and over 350 species
- Lardizabalaceae with 9 genera and 50 species
- Circaeasteraceae with 2 species
- Papaveraceae with 38 genera and over 700 species
- Eupteleaceae with 2 species.

The closest native species to the family Berberidaceae belong to the family Ranunculaceae and these species show a relatedness level of 5 to *Berberis* species (Figure 6). These include 47 *Ranunculus* species, 9 *Clematis* species, 2 *Caltha* species, *Anemonastrum tenuicaule* and *Ceratocephala pungens*. Plants from families Eupteleaceae, Circaeasteraceae and Menispermaceae are absent from New Zealand (New Zealand Plant Conservation (Network 2023). Plants from families Berberidaceae, Papaveraceae and Lardizabalaceae are all exotic.

Plants selected for the host range testing included species from the genera: *Berberis*, *Mahonia* and *Nandina* within the Berberidaceae family, *Clematis* and *Ranunculus* within the Ranunculaceae family, *Stauntonia* within the Lardizabalaceae family and *Eschscholzia* within the Papaveraceae family. The level of relatedness of genera from the order Ranunculales is indicated in Figure 6.

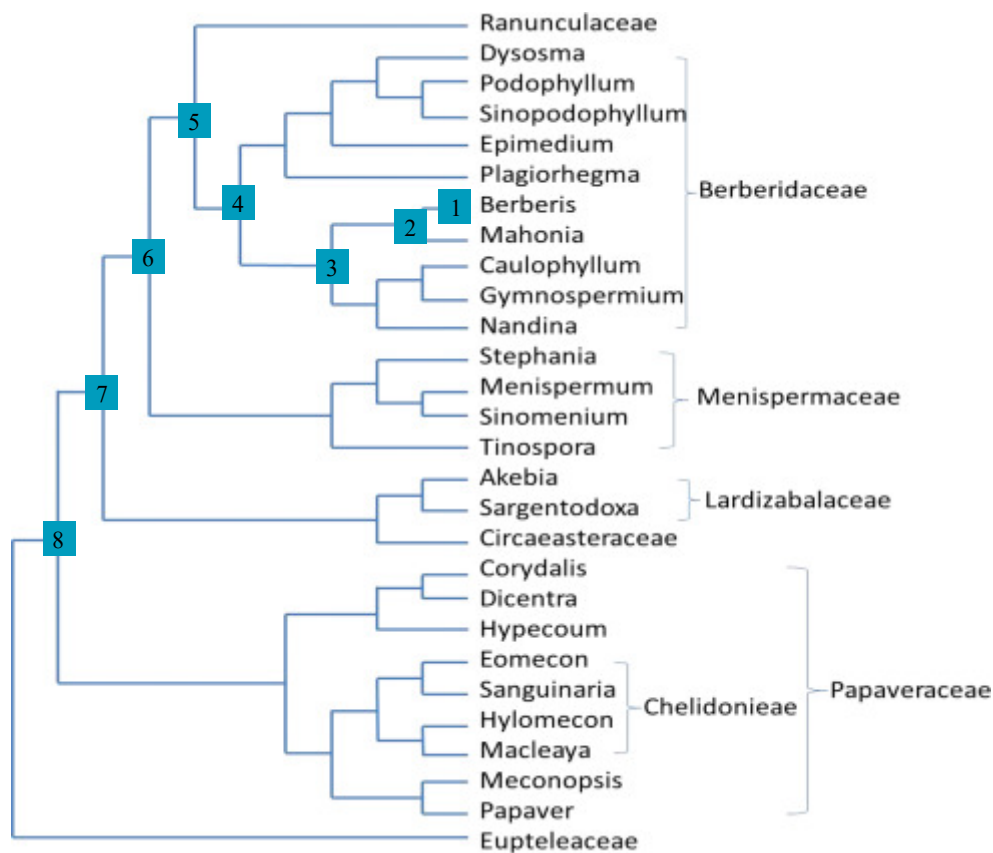


Figure 6. Phylogenetic tree of the Ranunculales order. Highlighted in blue: level of relatedness of different genera. (Source: Wang et al. 2009. © 2009 Rübél Foundation, ETH Zürich).

3.3 Method for host range testing

3.3.1 Plant material

Darwin's barberry plants from New Zealand were obtained from the Manaaki Whenua – Landcare Research site in Lincoln.

Plant species tested were *Berberis glaucocarpa*, *Berberis thunbergii*, *Berberis dielsiana*, *Mahonia bealei*, *Mahonia lomariifolia*, *Mahonia fortunei*, *Nandina domestica*, *Clematis forsteri*, *Clematis montana*, *Ranunculus aocalis*, *Ranunculus repens*, *Stauntonia hexaphylla* and *Eschscholzia californica*. These plants were sourced from nurseries in the North Island while *Ranunculus repens* plants and *Berberis glaucocarpa* cuttings were collected in Auckland.

All plants were placed in a growth room with natural light in the BPPCF in Auckland. The plants are kept in trays at 20°C for 12 h (day) and 18°C for 12 h (night).

3.3.2 Inoculum

Darwin's barberry fruits with mature *Puccinia berberidis-darwinii* pustules were collected from Valdivia, Chile and imported into the BPPCF for host range testing in December 2019 and December 2022. Rust pustules on Darwin's barberry fruits generate hundreds of thousands of aeciospores which were used to conduct host range testing. Between 2020 and 2023, when aeciospores produced on Darwin's barberry leaves in the containment facility were available in high numbers, these were also used for host range testing.

3.3.3 Inoculation

Ten plants or more were tested for each species when possible. For *Mahonia bealei* (synonym *Berberis bealei*), eight plants were tested. A pin needle was used to scarify the abaxial surface of leaves before collecting and spreading aeciospores with the needle onto the abaxial leaf surface. This was performed on 15 young leaves per plant for all species except *Ranunculus aocalis*, *Ranunculus repens* and *Eschscholzia californica* where five leaves per plant were inoculated. The plants were placed into a Perspex box with a humidifier for 48 h (relative humidity 100%). After that time, the plants were kept in the box for a further 48 h before being placed on trays in the growth room. Darwin's barberry plants were used as positive controls and 15 young leaves of 1-4 Darwin's barberry plants were inoculated at the same time as other plant species. All inoculated plants were regularly inspected for external symptoms of infection up to 6 months.

3.3.4 Statistical analysis

Formation of pustules was defined as a binary dependent variable where 0 = no pustules forming and 1 = pustules forming. Test plant species was included as a factor so that the influence of plant species on the formation of pustules could be tested. Due to complete separation of the dependent variable, Firth's bias-reduced penalized-likelihood logistic regression (Heinze & Schemper 2002) was conducted using the `logistf` package in R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

4 Results of host range tests

Results of host specificity testing using *Puccinia berberidis-darwinii* are indicated in Table 1.

Whether an inoculation resulted in the formation of pustules varied significantly according to plant species (Likelihood ratio test=262.2854 on 13 df, $p=0$, $n=266$). All 68 inoculations on *B. darwinii* resulted in the formation of pustules but no pustules formed on any of the test plant species. Leaves of *Berberis dielsiana* developed a hypersensitive response to the rust causing localised cell death when aeciospores were smeared on the abaxial leaf surface (Figure 7).



Figure 7. *Berberis dielsiana* leaves after inoculation with *P. berberidis-darwinii*.

It is important to note that the environmental conditions for host range testing were optimal for the rust and unnaturally high numbers of aeciospores were applied to plants that were tested. In natural conditions, *Berberis dielsiana* will not exhibit large surfaces of dead cells on leaves as observed on Figure 7. It is more likely that localised dots on leaves would be observed where a single rust spore would have landed.

Table 1. Results of host range tests

Level of relatedness	Latin name (synonym)	Common name	Family	Status in New Zealand	Pustules formed	Comments
Target	<i>Berberis darwinii</i>	Darwin's barberry	Berberidaceae	weed	yes	
1	<i>Berberis glaucocarpa</i>	Barberry	Berberidaceae	weed	no	
1	<i>Berberis thunbergii</i>	Japanese barberry	Berberidaceae	exotic, ornamental	no	
1	<i>Berberis dielsiana</i> (<i>Berberis dasystachya</i>)	Diel's barberry	Berberidaceae	exotic, ornamental	no	Hypersensitive response from plant with localised cell death where spores were smeared on leaf surface.
2	<i>Mahonia fortunei</i> (<i>Berberis fortunei</i>)	Chinese mahonia, Fortune's mahonia or holly grape	Berberidaceae	exotic, ornamental	no	
2	<i>Mahonia bealei</i> (<i>Berberis bealei</i>)	Leatherleaf mahonia or Beale's barberry	Berberidaceae	exotic, ornamental	no	
2	<i>Mahonia lomariifolia</i> (<i>Berberis oiwakensis</i> subsp. <i>lomariifolia</i> , <i>Berberis lomariifolia</i>)	Chinese holly grape	Berberidaceae	exotic, ornamental	no	
3	<i>Nandina domestica</i>	Sacred bamboo	Berberidaceae	exotic, ornamental	no	
5	<i>Clematis forsteri</i>	Forster's Clematis	Ranunculaceae	native	no	
5	<i>Clematis montana</i>	Mountain clematis	Ranunculaceae	exotic, ornamental	no	
5	<i>Ranunculus aocalis</i>	Shore buttercup, dune buttercup or sand buttercup	Ranunculaceae	native	no	
5	<i>Ranunculus repens</i>	Creeping buttercup	Ranunculaceae	weed	no	
7	<i>Stauntonia hexaphylla</i>	Japanese staunton vine or sausage vine	Lardizabalaceae	exotic, ornamental	no	
8	<i>Eschscholzia californica</i>	California poppy	Papaveraceae	exotic, ornamental	no	

5 Discussion

Puccinia berberidis-darwinii does not pose a threat to New Zealand's native plants. The rust was unable to infect and produce spores on the plants used in the host range testing, with only *Berberis dielsiana* showing dead patches on leaves, resulting from a hypersensitive response from the plant to the rust.

None of the native *Berberis* species from Chile have been tested however, according to the literature and from the field surveys conducted by our collaborators in Chile, *P. berberidis-darwinii* has only been observed on Darwin's barberry (Dietel & Neger 1900; Jackson 1931). Rusts collected by our collaborator Dr. Hernán Norambuena on *Berberis* species growing next to Darwin's barberry in Chile were different from *P. berberidis-darwinii* and only shared 93% DNA sequence identity for the LSU rRNA, with 92 different base pairs and 36 gaps. Observation of aeciospores, teliospores and pycnia on Darwin's barberry were recorded in the literature (Jackson 1931) and aeciospores and teliospores were observed in during host range testing indicating that *P. berberidis-darwinii* is autoecious, completing its life cycle on a single host.

The hybrid *Berberis x stenophylla* was not tested and it is unknown whether *P. berberidis-darwinii* will be able to infect this hybrid. However, it has naturalised and could potentially become a threat to New Zealand's environment if it behaves like its parent, Darwin's barberry.

6 Acknowledgements

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