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Information package to support the application to release the rust fungus *Puccinia rapipes* for the biological control of *Lycium ferocissimum* (African boxthorn) in Australia

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Acknowledgements

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Executive summary

Lycium ferocissimum (African boxthorn; Solanaceae) was endorsed as a target for biological control in Australia in August 2016 by the Invasive Plants and Animals Committee (IPAC; now the Environment and Invasives Committee).

The rust fungus *Puccinia rapipes* was identified as a candidate biological control agent for *L. ferocissimum* following a literature review of specialist natural enemies present in the weed's native range of South Africa. The fungus was considered a promising biological control agent as it had only ever been recorded on *L. ferocissimum*, despite the diversity of native *Lycium* species occurring in South Africa.

Accessions of *P. rapipes* were imported into the BC3 Microbiological Area (Approved Arrangement A1280) of the CSIRO Black Mountain Containment Facility in Canberra from November 2016. Cultures of two purified isolates of *P. rapipes* were established on Australian accessions of *L. ferocissimum* for initial studies. A series of host-specificity experiments were then performed using one of the purified isolates (ex. Western Cape, South Africa) to investigate the fungus potential to infect non-target plant species. Test species were selected based on recent molecular phylogenies of the family Solanaceae and comprised a total of 28 representative species closely related to *L. ferocissimum* within this family that occur in Australia (introduced and native). Each species was tested in at least two separate experiments using different accessions of plant material, unless otherwise indicated, with *L. ferocissimum* plants used as positive controls in all experiments.

All of the *Lycium* species non-native to Australia included in host-specificity experiments – the target weed *L. ferocissimum* and the Eurasian goji berry species *L. barbarum* (goji berry), *L. chinense* (goji berry 'chinense') and *L. ruthenicum* (black goji berry) – developed normal uredinia and were rated as highly susceptible or susceptible to *P. rapipes*. In contrast, accessions of the Australian native *Lycium australe* only developed a few, small necrotic spots and were rated as resistant to *P. rapipes*. One accession of *Solanum melongena* also developed such necrotic spots, while a few minuscule, pin-sized uredinia, with non-viable urediniospores, developed on two leaves of *Anthocercis ilicifolia* in one of the experiments. A few of the other non-target species tested developed minor chlorotic flecking and the remaining species did not develop any visible symptoms. The high susceptibility of goji berry (*L. barbarum*) to *P. rapipes* was further confirmed in a study performed under natural conditions in South Africa. Interestingly, three growers of *L. barbarum*, for fruit or plant trade, in South Africa, including the largest producer, indicated that they have never observed symptoms of *P. rapipes* on plants at their production sites.

Goji berry is sold in the nursery and garden trade, although it is an extremely low value and volume plant in Australia. The two main species that are sold, *L. barbarum* and *L. chinense*, have naturalised and are now considered environmental weeds in Australia. Commercial scale production of goji berry for the dried fruit market in Australia is currently inexistent and not forecasted to grow. Consultation with growers, wholesalers and retailers of goji berry revealed that overall, they are not particularly concerned about the possible release of a biological control agent for *L. ferocissimum* that would also affect goji berry, especially if the disease can be managed with fungicide applications. Results from an experiment performed in the biosecurity containment facility showed that damage caused by *P. rapipes* on goji berry (*L. barbarum*) can be mitigated with commercially available fungicides used against other plant diseases in Australia:

AMISTAR® 250 SC (active: azoxystrobin), a systemic fungicide registered for use to control rust diseases on nursery stock and ornamentals and Mancozeb Plus (actives: sulphur and mancozeb), a contact fungicide not yet registered for such use.

Observations made during surveys in South Africa and laboratory studies in the biosecurity containment facility in Australia support that *P. rapipes* would be a potentially effective biological control agent for *L. ferocissimum*, especially on young plants in coastal environments. The level of risk associated with releasing *P. rapipes* in Australia may be acceptable, should stakeholders and regulators be willing to accept potential infection of Eurasian goji berry, which could be managed with fungicide applications if required.

1 Information on the target species in Australia

1.1 Taxonomy

Clade:	Asterids, eudicots
Order:	Solanales
Family:	Solanaceae
Subfamily:	Solanoideae
Clade:	Atropina
Tribe:	Lycieae
Genus:	*Lycium*
Species:	*ferocissimum* Miers
Common names:	African boxthorn, boxthorn, snake-berry (*slangbessie* in Afrikaans)
Synonyms:	*Lycium campanulatum* E.Mey. *ex* C.H.Wright and *Lycium macrocalyx* Domin. *Lycium afrum* L. (misapplied), *Lycium europaeum* L. (misapplied) and *Lycium horridum* Thunb. (misapplied).

1.2 Description

Lycium ferocissimum is a densely branched perennial shrub that can grow up to 5 m high (but more often 2–3 m) and up to 5 m across (though most commonly up to 3 m) (Parsons and Cuthbertson 2001) (Figure 1a). However, in wind-prone situations such as coastal sites, its habit is often quite different. There, it is wind-pruned, very dense and often relatively short, with its shape determined by the predominant wind direction (Noble and Rose 2013; Taylor and Tennyson 1999) (Figure 1b). Stems are silver-grey when young, turning light brown to grey as they mature, and becoming fissured with age. The main stems grow large spines (to 15 cm), with smaller spines on branches. Leaves of *L. ferocissimum* (up to 40 mm long and 20 mm wide) have very short petioles, are slightly fleshy, simple and entire, ovate, obovate to elliptic in shape, glabrous, and clustered at nodes (Figure 1c). Inflorescences are solitary or in pairs, 8–12 mm diameter and 10–12 mm long and formed in the leaf axil (Figure 1c). Flowers have five petals, white to lilac with purple markings toward their inside and 5–6 exerted stamens (Figure 1c). The pedicel is 5–16 mm long and calyx has five unequal sepals. Flowers are present most of the year but are most prolific during summer. The fruit is a smooth round berry, initially green, but ripening to orange-red, up to 12 mm diameter, with a prominent calyx and typically containing between 20 and 70 dull yellow seeds (Figure 1d) (Blood 2001; Green 1994; Muyt 2001; Parsons and Cuthbertson 2001; Purdie et al. 1982).

It is noteworthy that recent morphometric analyses across *L. ferocissimum* and other *Lycium* species in South Africa did not identify any leaf or floral characteristics unique to *L. ferocissimum*, making morphological identification of the species problematic (McCulloch et al. 2020). This is not an issue in Australia, because there are only five other *Lycium* species present, outside of

cultivation, all with restricted distributions: the native *L. australe* and the naturalised *L. barbarum*, *L. chinense*, *L. afrum,* and *L. ruthenicum*.

It is noteworthy that *Grabowskia duplicata*, a species described within Solanoideae tribe Lycieae, had previously been recorded as introduced in Australia (Randall 2007). A revision of the generic circumscription of Lycieae, based on DNA sequence analysis of two nuclear gene regions, resulted in *G. duplicata* being synonymised under *Lycium boerhaviifolium* thus making Lycieae monotypic and only accommodating the genus *Lycium* (Levin et al. 2011). *Lycium boerhaviifolium* occurs in several South American countries including, amongst others, Chile, Argentina, Peru, Bolivia Ecuador, and Paraguay (Agra 2020, GBIF.org 28 May 2021, Levin et al. 2011, 2015). Searches for *G. duplicata* or *L. boerhaviifolium* occurrences in Australia through the Australian Plant Census (APC) and Atlas of living Australia (ALA) did not result in any occurrence records. As such we consider that this species is not present in Australia since it is not included in key databases.

Figure 1 Growth form of *Lycium ferocissimum* (a) in pastures and (b) on the South Australian coastline, where the species is wind-pruned by prevailing winds. (c) Flower and (d) fruit. Photo credits: (a) and (b) Noble and Rose (2013); (c) and (d) Rhodes University, South Africa.

1.3 Native range

Lycium ferocissimum is native to South Africa, with a limited non-native global distribution (Figure 2a). It is widespread in South Africa and native to the Eastern and Western Cape Provinces (Venter 2000). It is recorded further afield, in the Free State, Kwazulu-Natal, Mpumalanga and Northern Cape Provinces of South Africa, and northern Lesotho, where it has been planted as a hedge

(GBIF.org 6 November 2020; Venter 2000; Welman 1993; Welman 2003) (Figure 2b). While *L. ferocissimum* has been recorded as occurring in Namibia (GBIF.org 6 November 2020), the veracity of these records is questionable, especially given the year they were made (1963 and 1976) and difficulties in differentiating *L. ferocissimum* from other *Lycium* species with morphological characters (Levin et al. 2007; Venter 2000). In a recent study, genetic analyses using chloroplast and nuclear markers revealed that some plants putatively identified with morphological characters as *L. ferocissimum* during field surveys for natural enemies in South Africa were different *Lycium* species that did not have sequences in GenBank (McCulloch et al. 2020).

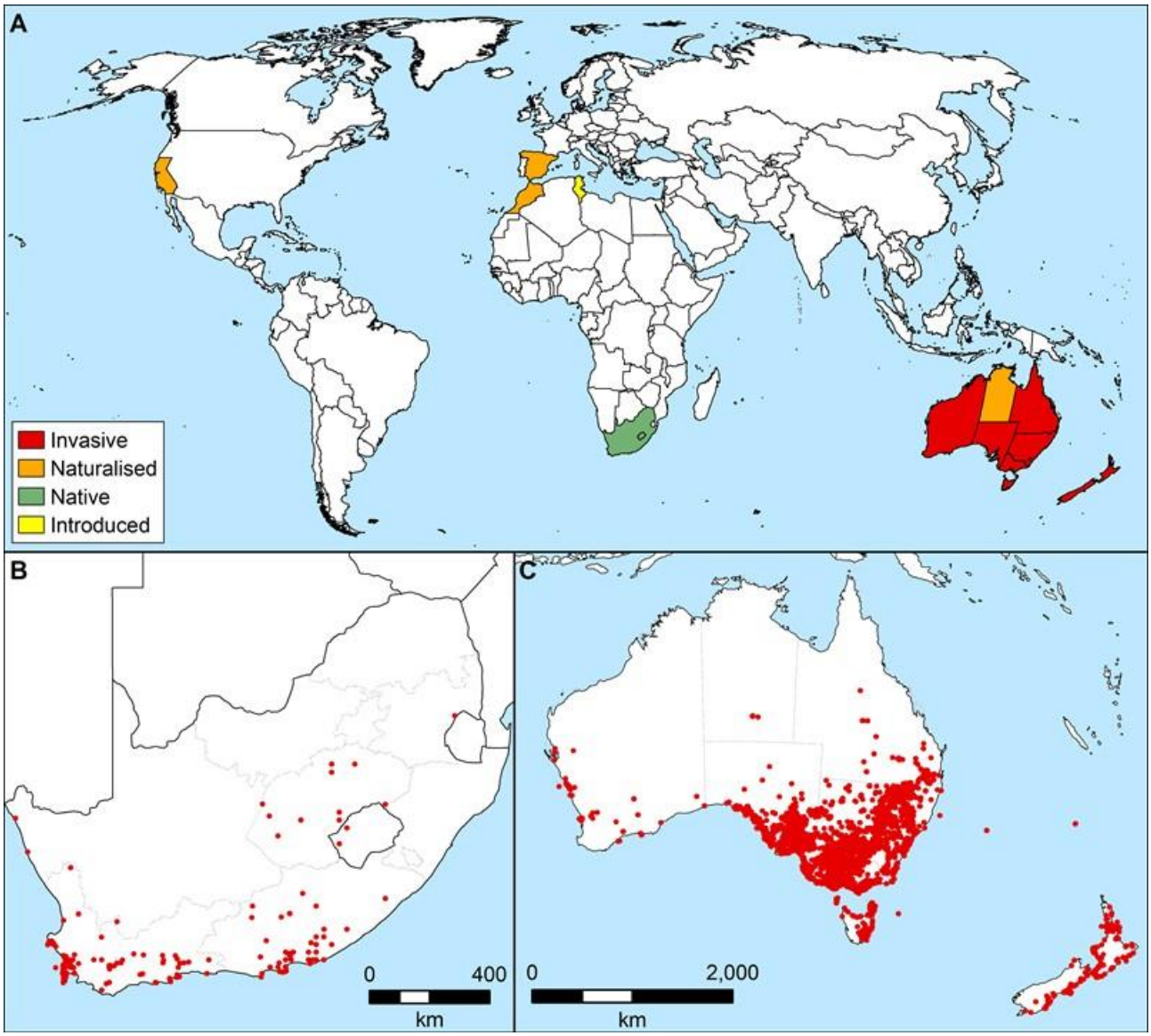

Figure 2 Distribution of *Lycium ferocissimum*: (a) global, (b) in South Africa and (c) Australasia. Reproduced with permission from Noble, Adair and Ireland (in preparation).

1.4 Distribution

Lycium ferocissimum is recorded as introduced to Tunisia (Monastir, Nabeul and Siliana Governorates) (GBIF.org 6 November 2020; Venter 2000), and introduced and naturalized in coastal Morocco (Gharb-Chrarda-Béni Hssen, Marrakech-Tensift-Al Haouz and Rabat-Salé-

Zemmour-Zaer regions) (GBIF.org 6 November 2020; Lambinon and Lewalle 1986; Venter 2000), coastal south-western Spain (Málaga Province, plus a single introduced site in the city of Guadalajara in central Spain) (GBIF.org 6 November 2020; Perez Latorre et al. 2006), and Cyprus (recorded from shores of Lake Akrotiri in the south, to abandoned mine sites in the north-east) (Cetinkaya and Sozen 2011; Gallego 2012; Hand 2000; İlseven and Başta ş 2018; Meikle 1985; Perez Latorre et al. 2006; Peyton and Mountford 2015; Venter 2000) (Figure 2a). The most recent European records of the species, in the southern provinces of Cagliari and Medio Campidano on the island of Sardinia, Italy, are considered to be invasive in nature (Lazzeri et al. 2013). Two unverified records of *L. ferocissimum* have also been made on the Greek island of Crete in 2017 and 2018 (GBIF.org 6 November 2020). In the United States of America, *L. ferocissimum* has been recorded as naturalized, but rare, in California (Calflora 2013; DiTomaso and Healy 2007; USDA NRCS 2020). It is only widespread and troublesome on a national scale in Australia and New Zealand (Parsons and Cuthbertson 2001) (Figure 2c). In New Zealand, it is present on both the North and South Islands, where it is largely restricted to coastal areas; here it persists in large agro-ecosystem distributions as established hedges, with spread also recorded into forest and scrub reserves (Breitwieser et al. 2019; Popay et al. 2010; Timmins and Mackenzie 1995; Timmins and Williams 1991) (Figure 2c).

In Australia, *L. ferocissimum* is widespread in coastal to semi-arid inland habitats and islands of southern Australia, with records from every jurisdiction (GBIF.org 6 November 2020; Noble and Rose 2013; Parsons and Cuthbertson 2001) (Figure 2c). Recent genetic analyses found no evidence of hybridization with any other *Lycium* species (McCulloch et al 2020). One of the two common chloroplast haplotypes of *L. ferocissimum* found across Australia in this study was identified from only two sites in South Africa, both near Cape Town, suggesting that the invasive lineage of the species in Australia may have originated from this region.

Lycium ferocissimum is found predominantly in the southern part of the Australian continent in coastal and island situations (except Queensland). It occurs on islands off the southern half of the Western Australian coastline, along with islands of the Great Australian Bight and Bass Strait, and on Lord Howe Island and Norfolk Island (Erkelenz 1993; Green 1994; Keighery 2010; Keighery et al. 2002; Lawley et al. 2005; Western Australian Herbarium 1998-; Ziegler and Hopkins 2011, as cited by Noble and Rose, 2013). Inland, *L. ferocissimum* is abundant in areas of New South Wales, Victoria and South Australia, where it is a common weed of semi-arid pastures and rangelands, and is often found growing along dry stream beds (Parsons and Cuthbertson 2001). It has a lesser, but significant presence in south-east Queensland, southern Western Australia, and Tasmania. *Lycium ferocissimum* does not occur currently in higher altitude areas of Australia, with no substantial presence in the Victorian, New South Wales and Tasmanian alpine areas.

Distribution modelling of *L. ferocissimum* indicates that the species is already present through much of its projected highly suitable habitat range in Australia (Kriticos et al. 2010; Weed Futures 2014-2019; CSIRO unpublished data) (Figure 3). One exception appears to be south-western Western Australia, where mapping and field experience indicate *L. ferocissimum* is not as widely distributed in highly suitable habitat as it is in other areas of southern Australia. There are several potential explanations for this, including the possibility that *L. ferocissimum* was introduced later to Western Australia and/or that available distribution data are not as comprehensive as elsewhere, making distribution mapping misleading (Noble and Adair 2014). Another possibility is that the absence thus far of an established population of starlings (*Sturnus vulgaris*) and other

dispersers in south-western Western Australia are restricting the distribution of *L. ferocissimum* (Harris and McKenny 1999; Taylor 1968). No matter what the explanation is, indications are that the range of *L. ferocissimum* continues to expand in southern Western Australia (Abbott et al. 2000; Keighery 2010; Keighery et al. 2002).

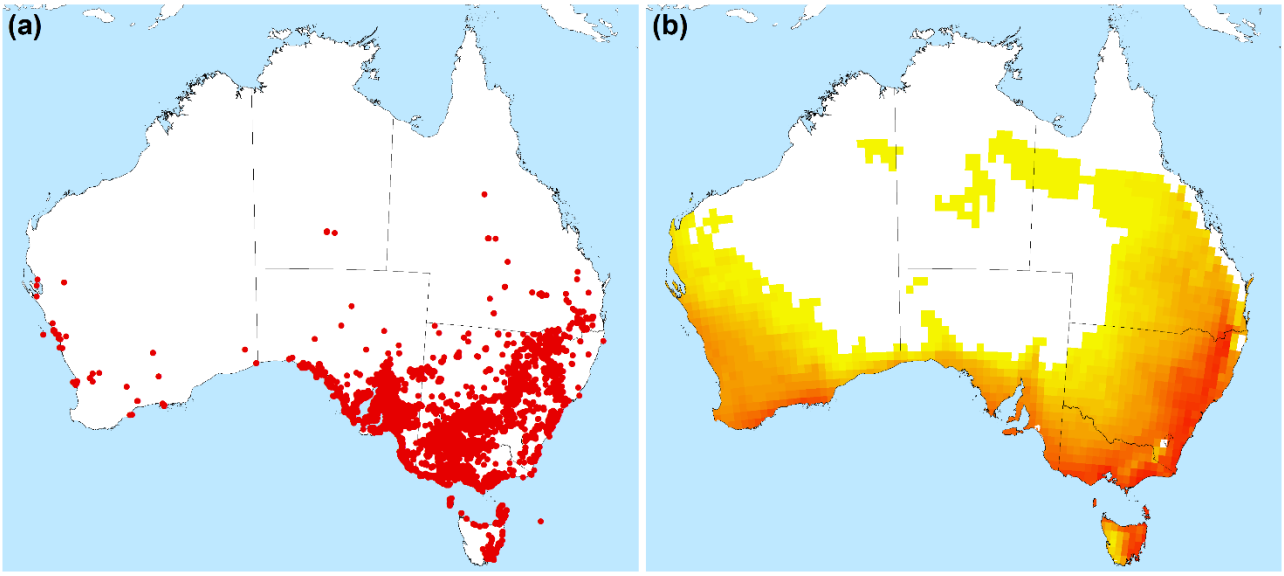

Figure 3 *Lycium ferocissimum* (a) current distribution in Australia (GBIF.org 24th July 2018) and (b) projected climatic suitability, as modelled using CLIMEX with the CliMond dataset of 1981-2010 climate normal (CSIRO unpublished data). Increased intensity of red colour, starting from yellow, indicates higher climatic suitability.

1.5 Current status in Australia, including summary of the economic and environmental losses caused by the target

Lycium ferocissimum is a widespread and significant invasive environmental and agricultural weed in Australia. Stakeholders consider the weed to be both difficult and costly to control (Ireland et al. 2019b). In an environmental context, the weed is known to displace native vegetation and degrade habitats, which negatively impact on native fauna, and possibly facilitates the degradation of cultural heritage sites (Noble and Adair 2014; Noble et al. 2014; Noble and Rose 2013). *Lycium ferocissimum* adversely affects a significant range of native plant species and ecological communities. It is considered a threat in at least two rangeland biodiversity hotspots, Brigalow North and South in Queensland, and the Carnarvon Basin in Western Australia (Martin et al. 2006). It is perceived by natural resource managers as the worst (and most managed) coastal weed in southern Australia (Cousens et al. 2013).

In coastal and island situations it can significantly alter and interfere with native fauna habitats. For example, *L. ferocissimum* can become the only woody plant present, changing the vegetation structure in some small island and coastal dune environments (Lavers 2015; Webb et al. 1988; Ziegler and Hopkins 2011, as cited by Noble and Rose, 2013). In salt marshes where *L. ferocissimum* has become established, bird species assemblages and behavior are altered by the presence of the weed (Carlos et al. 2017). This reduces habitat suitability for some native species and makes these areas more hospitable for pest animals such as starlings or more aggressive

raptor birds. These birds benefit from the increased roosting sites afforded by *L. ferocissimum* to predate on other native and exotic fauna.

A number of native fauna species have been recorded as being adversely impacted by the presence of *L. ferocissimum*. On Althorpe Island off South Australia, the fine dense root mass of *L. ferocissimum* can impede the burrowing efforts of short-tailed shearwaters (*Ardenna tenuirostris*) (Lawley et al. 2005). Where *L. ferocissimum* removal was undertaken, native flora (shrubs and mat plants) recolonized sites, leading to a gradual increase of burrowing opportunities for shearwaters. *Lycium ferocissimum* can ensnare, injure and kill coastal native birds, including the short-tailed shearwater and white-faced storm petrels (*Pelagodroma marina*) (Lohr and Keighery 2016; Noble and Rose 2013; Phillips 2014; Taylor 1968; Ziegler and Hopkins 2011, as cited by Noble and Rose 2013). On islands off South Australia and Western Australia, *L. ferocissimum* displaces the native shrub *Nitraria billardieri,* which is used by seals (*Arctocephalus* spp.) for sheltering pups, disrupting seal and sea lion breeding (Humphries et al. 1994). The weed does not provide the equivalent quality of nursery habitat, leaving pups more vulnerable to predation.

It has also been suggested that the presence of *L. ferocissimum* can degrade cultural heritage sites. Coastal areas in Australia, where *L. ferocissimum* is known to occur (Cousens et al. 2013; Erkelenz 1994; Lohr and Keighery 2016), are likewise likely locations for indigenous heritage such as middens, artefacts and other evidence of occupation (Cann et al. 1991; Veth et al. 2017). These sites may be degraded through increased rabbit (*Oryctolagus cuniculus*) and fox (*Vulpes vulpes*) burrowing associated with *L. ferocissimum*, which may cause direct or indirect damage to artifacts and historical sites (Noble and Adair 2014; Noble et al. 2014; Noble and Rose 2013).

In an agricultural context, *L. ferocissimum* has been associated with reducing access to pasture and water in grazing systems (Brown 1969; Lee 1978), harboring pest animals such as rabbits, foxes and pest birds, and puncturing tires and injuring livestock and people with its thorns (Hoskin 2006; Noble and Adair 2014). It also hosts key pests and diseases of concern to agriculture, including the Queensland fruit fly *Bactrocera tryoni* (Plant Health Australia 2020) and tomato-potato psyllid (TPP) *Bactericera cockerelli* (Vereijssen et al. 2018). The latter is a North American psyllid species that, in its place of origin, overwinters on several native and non-native *Lycium* species (Cooper et al. 2019; Thinakaran et al. 2017). It is a major pest of solanaceous crops, as it is the vector of the plant bacterium *Candidatus* Liberibacter solanacearum (CLso), the causal agent of zebra chip disease in potatoes (Liefting et al. 2009; Munyaneza 2012). While *B. cockerelli* is present in the invaded range of *L. ferocissimum* in Australia, CLso has not yet been reported from Australia (Department of Primary Industries and Regions 2020). Populations of *B. cockerelli* may build up or survive on overwintering hosts such as *L. ferocissimum* and then colonize commercial crops in spring (Vereijssen et al. 2018). Consequently, the presence of *L. ferocissimum* in agricultural areas is likely to be of significant concern when managing populations of the psyllid in these areas.

1.6 Other control methods available

Long-term effective control of *L. ferocissimum* requires a combination of treatments over many years due to the capacity of the species to regenerate from rootstock, stems and seed. Guidance and a planning approach are provided in the *African Boxthorn National Best Practice Manual: Managing African Boxthorn (Lycium ferocissimum) in Australia* (Noble and Rose 2013). A brief summary of control methods available is provided below.

Physical control of *L. ferocissimum* includes winching, pulling (also referred to as 'plucking'), bulldozing, stick raking, blade ploughing and cultivation. These techniques are best used when *L. ferocissimum* plants are not carrying seed (or are carrying minimal seed). Otherwise, fresh seed is likely to be deposited into freshly disturbed soil. Winching and pulling are the lowest impact physical control techniques for situations where disturbance is a concern, such as where *L. ferocissimum* is growing within native vegetation. Bulldozing, stick raking and blade ploughing are suitable in less sensitive landscapes (e.g. pasture), and provide a rapid control method for moderate to heavy infestations.

Successful management of *L. ferocissimum* using the above techniques is dependent on follow-up application of herbicide. Foliar spraying of triclopyr-picloram herbicide mixes, and of triclopyr, picloram and aminopyralid are commonly undertaken for management of *L. ferocissimum*. Glyphosate, glyphosate-metsulfuron-methyl mix, and picloram and 2, 4-D amine-based herbicides can also be foliar sprayed on *L. ferocissimum*. Adjuvants improve herbicide uptake by the plant. Mature *L. ferocissimum* plants are very resilient to foliar spraying, with new foliage readily appearing on plants that had seemingly 'died off' after spraying. In situations where vehicle access is impractical or undesirable (such as coastal and island situations), the cut stump technique is often used. Triclopyr-picloram mix (in diesel), triclopyr (mixed with diesel), picloram and glyphosate (mixed 1:1 with water) are suitable for this technique. Picloram-based herbicides can be used for stem injection; while glyphosate-based herbicides can be used for frilling or stem scrape techniques. Triclopyr, along with triclopyr-picloram based herbicides (mixed with diesel) can be applied to *L. ferocissimum* using the basal bark application technique. Soil-root zone herbicide application is not suitable in environmentally sensitive areas, as there is significant potential for off-target damage. Hexazinone or tebuthiuron-based herbicide is applied to the soil near the drip line of the weed.

1.7 Commonwealth, State and Territory legislative controls of the target species

Lycium ferocissimum is recognized as a Weed of National Significance in Australia (Australian Weeds Committee 2013), and its status varies depending on the jurisdiction (Agriculture Victoria 2020; Biosecurity SA 2020; Business Queensland 2020; Department of Agriculture 2020; Department of Environment and Natural Resources 2019; Department of Primary Industries Parks Water and the Environment 2019; Department of the Environment Climate Change Energy and Water 2009; NSW DPI 2020, Department of Primary Industries and Regional Development 2020) (Table 1).

1.8 Endorsed as a target species for biological control

Lycium ferocissimum was endorsed as a target for biological control in Australia in August 2016 by the Invasive Plants and Animals Committee (IPAC; now the Environment and Invasives Committee; EIC), a cross-jurisdictional sectoral sub-committee of the National Biosecurity Committee.[1]

Table 1 Status of *Lycium ferocissimum* across jurisdictions in Australia

STATE / TERRITORY	LEGISLATION	STATUS
Australian Capital Territory	*Pest Plants and Animals Act 2005*	C2 – pest plant that must be suppressed, and C4 – prohibited pest plant (propagation and supply prohibited).
New South Wales	*Biosecurity Act 2015*	Must not be imported into the State or sold. Management actions prescribed on regional basis.
Northern Territory	*Weeds Management Act 2001*	Schedule Class A/C – to be eradicated if found and not to be introduced to the NT.
Queensland	*Biosecurity Act 2014*	Restricted invasive plant Category 3. Must not be given away, sold, or released into the environment without a permit.
South Australia	*Natural Resources Management Act 2004*	Declared state-wide under Category 2 of the Act. Management actions prescribed on regional basis.
Tasmania	*Weed Management Act 1999*	Zone B (containment) across most of the Tasmanian land area. The importation, sale and distribution of African boxthorn are prohibited in Tasmania.
Victoria	*Catchment and Land Protections Act 1994*	Schedule 2 – regionally controlled.
Western Australia	*Biosecurity and Agriculture Management Act 2007*	Permitted entry to WA.

[1] For confirmation, contact EIC Secretariat eic@agriculture.gov.au.

2 Information on the biological control agent

2.1 Agent name and phylogeny

Order:	Pucciniales
Family:	Pucciniaceae
Genus:	*Puccinia*
Species:	*rapipes* Berndt & E. Uhlmann 2006
Common name:	Boxthorn rust

Voucher specimen: A voucher herbarium specimen will be deposited in the Plant Pathology & Mycology Herbarium of the NSW Department of Primary Industries, Orange, as soon as permission is granted to release *P. rapipes* in Australia.

Various samples of *P. rapipes* have been sequenced (Ireland et al. 2019a). Sequences were deposited in GenBank and representative related herbarium specimens were deposited in the dried herbarium collection (PREM) of the South African National Collection of Fungi, ARC-PHP, Pretoria, South Africa (for details see Table A.4 in supplementary material of Ireland et al. 2019a).

Phylogenetic analysis of sequences from various *P. rapipes* samples collected on *L. ferocissimum* in South Africa showed that they all group into a well-supported clade sister to *Puccinia afra*, in the 'Old World Lineage' of *Puccinia* species on Lycieae (Figure 34) (Ireland et al. 2019a).

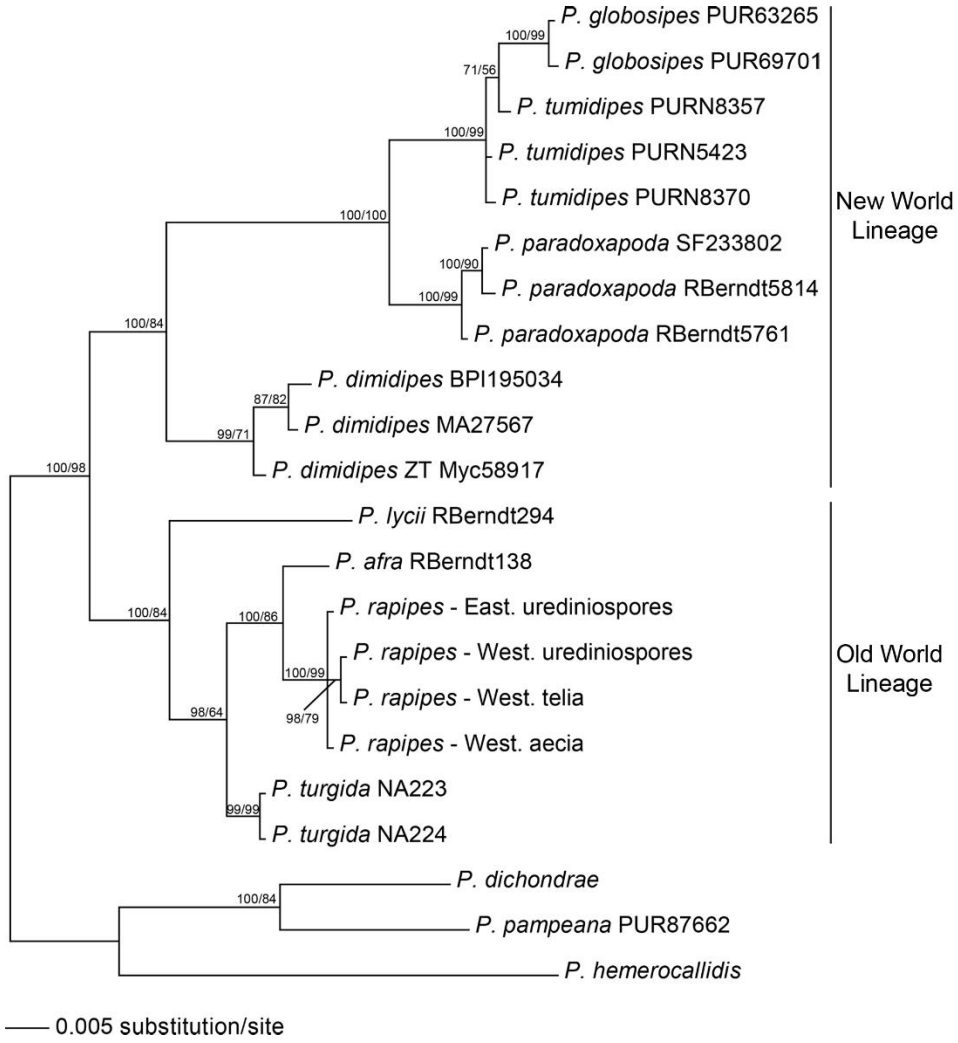

Figure 4 Phylogenetic relationship inferred from the ITS2 and CO3 regions of samples of *Puccinia rapipes* collected from *Lycium ferocissimum* in the field in South Africa to sequences of other *Puccinia* species occurring on Lycieae. Modified from Ireland et al. (2019a).

2.2 Brief description and biology of the agent

Puccinia rapipes was first described in 2006 from *L. ferocissimum* in the Western Cape Province of South Africa (Berndt and Uhlmann 2006). Recent field surveys, laboratory experiments and sequencing confirmed that it is a macrocyclic and autoecious rust fungus (i.e. no alternate hosts) (Ireland et al. 2019a) (Figure 5). Morphological descriptions of all spore types of *P. rapipes* can be found in Berndt and Uhlmann (2006) and Ireland et al. (2019a).

Puccinia rapipes had only ever been reported in the literature from *L. ferocissimum*, despite the wide diversity of *Lycium* species prevalent and in close proximity to the known distribution of *P. rapipes* in South Africa (Berndt and Uhlmann 2006).

Puccinia rapipes produces numerous scattered orange uredinia (0.3–1 mm diam.), which often develop into dark brown/black telia, on both sides of young and old leaves, and occasionally on petioles and green, non-woody thorns but not on fruit (Figure 6a, b, 7b). Under suitable conditions, urediniospores can cycle from germination through to the development of uredinia in approximately 14 days. Teliospores most likely form within uredinia weeks or even months after their development is triggered in response to environmental or abiotic stress. Once fully

developed, neither uredinia nor telia expand in size. Teliospores require a period of dormancy/weathering before germination can occur, and most likely germinate in response to cool, wet conditions (i.e. onset of winter rains) (Ireland et al. 2019a). Teliospores germinate and produce an external basidium and four basidiospores (Figure 6g). Basidiospores germinate readily on plant tissue, providing some moisture is present, and the fungus directly penetrates epidermal cells of susceptible hosts. Few tan-coloured spermogonia (0.1–0.14 mm diam.) develop in small groups, predominantly on the underside of young leaves and sepals, within 14 days of penetration (Figure 6c). Following cross-fertilisation between spermogonia, one to a few aecia develop in rings around each spermogonium (~ 1 mm diam. each aecia, rings up to ~ 5 mm diam.) (Figure 6c, h-l). Aeciospores produced within the aecia are then released under moist conditions and predominantly lead to infection of younger leaves from which uredinia develop within 17–23 days, usually on the opposite side of the leaf from where the aeciospores germinated.

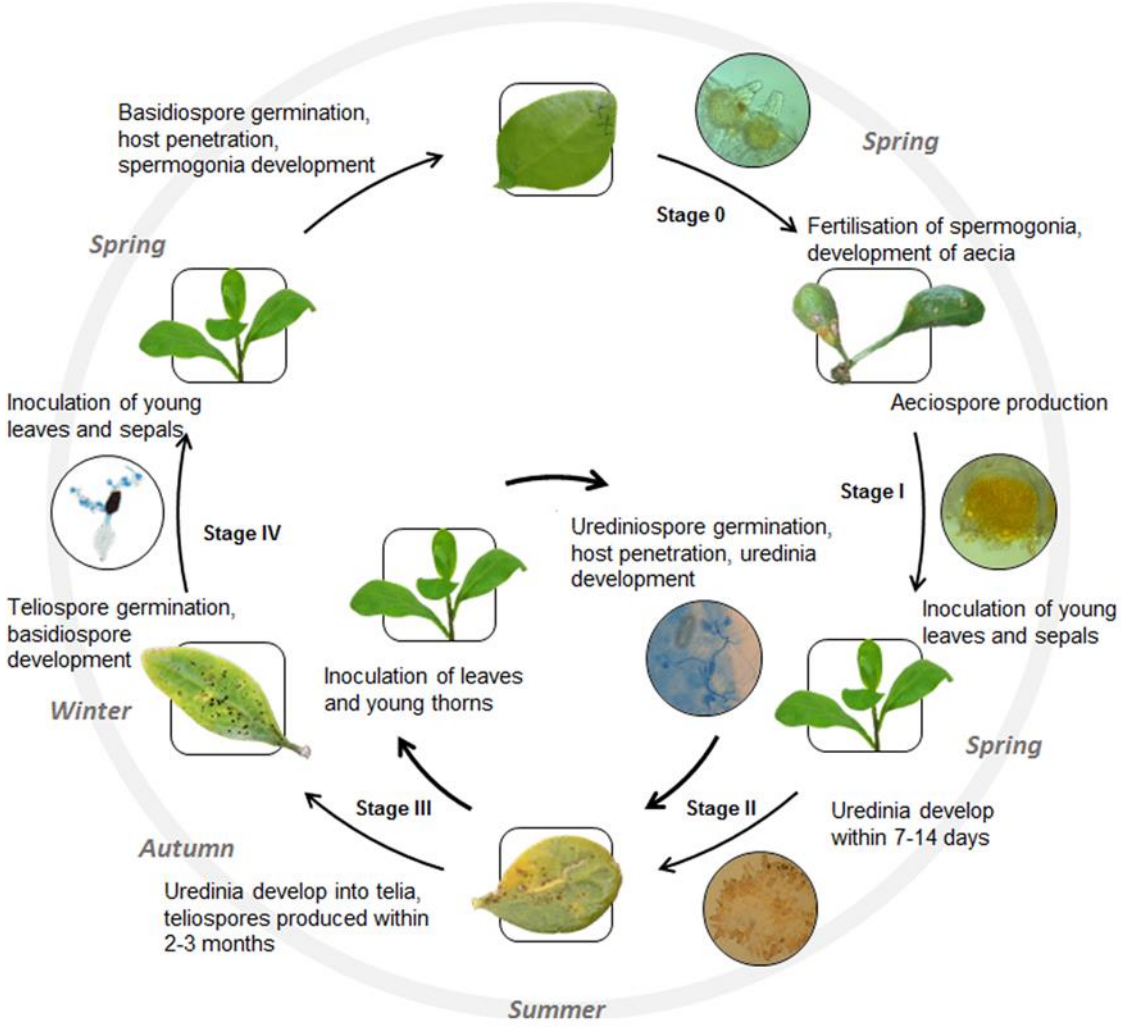

Figure 5 Life cycle of the rust fungus *Puccinia rapipes*, with putative seasonal timeline. Reproduced from Ireland et al. (2019a).

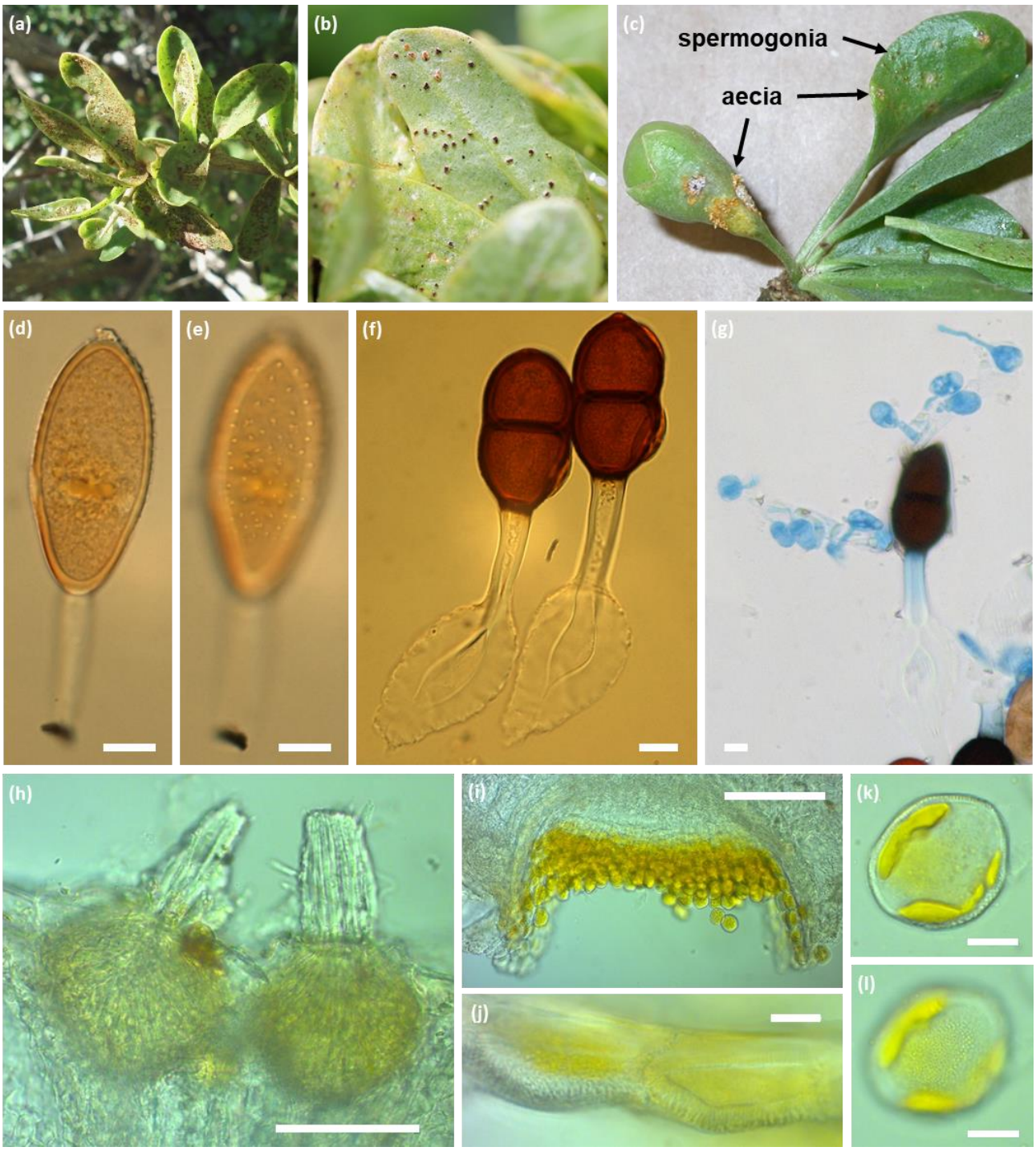

Figure 6 ***Puccinia rapipes*** **on leaves of** ***Lycium ferocissimum*****: (a) symptoms, (b) uredinia (orange) and telia (dark brown), (c) spermogonia (tan) and aecia (yellow), (d–e) urediniospore showing wall ornamentation, (f) teliospores, (g) germinated teliospore with basidia and germinated basidiospores, (h) close-up of two spermogonia, (i) cross-section through an aecium, showing catenulate aeciospores and bounding peridium, (j) peridial cell wall ornamentation and (k–l) aeciospores showing wall ornamentation. Scale bars = 10µm. Reproduced from Ireland et al. (2019a).**

Figure 7 A mature *Lycium ferocissimum* plant infected with *Puccinia rapipes* at Miller's Point, Western Cape, South Africa. (a) Wide view of the whole mature plant and a (b) close-up view, within the canopy, showing leaves bearing many brown/black telia of the rust fungus.

2.3 Native range of the agent

Puccinia rapipes has only been recorded from South Africa (Farr and Rossman 2020), and is present across the Eastern and Western Cape Provinces (Figure 88). It has never been recorded in Australia nor anywhere else in the world.

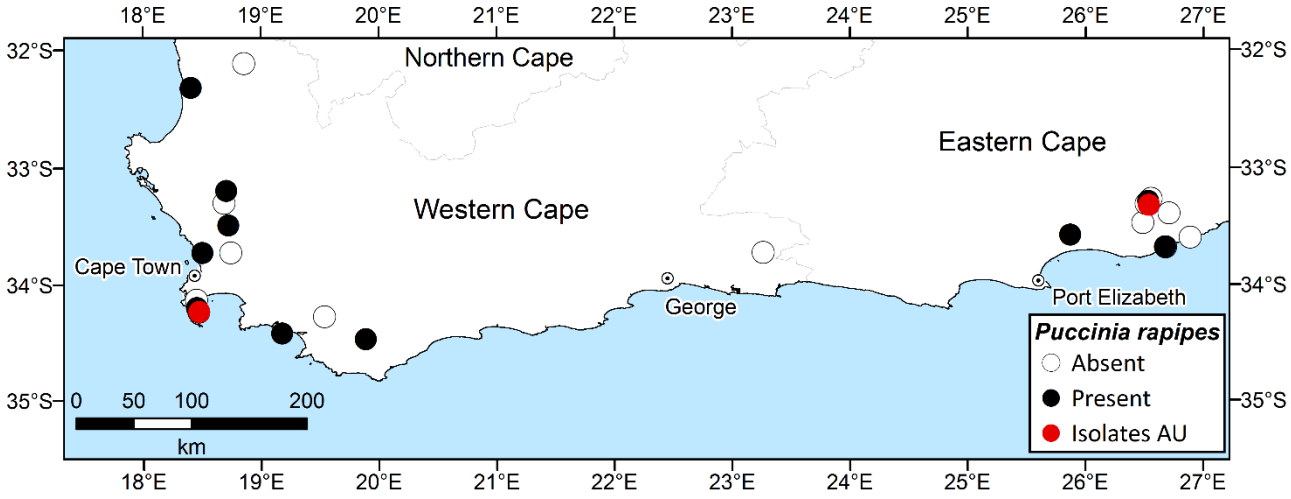

Figure 8 Sites surveyed for *Puccinia rapipes* in South Africa in October 2017. Filled symbols indicate sites where rust symptoms were observed on *Lycium ferocissimum* (black and red circles; 4 sites in the Eastern Cape Province, and 10 sites in the Western Cape Province, 2 of which overlap on the map at this scale). Red circles indicate the locations where the two purified isolates of *P. rapipes* used in our studies originate from, while the open circles indicate *L. ferocissimum* sites where *P. rapipes* was not observed. Modified from Ireland et al. (2019a).

2.4 Species related to the agent and summary of their host ranges

Nine *Puccinia* species have been recognized and described on species within the tribe Lycieae of the Solanaceae family (Otálora and Berndt 2018) (Table 2). These *Puccinia* species form a well-

supported monophyletic group, in which two major lineages are recognised, the New and Old World lineages (Otálora and Berndt 2018). *Puccinia rapipes* is sister to *Puccinia afra*, in the Old World lineage (Figure 4) (Ireland et al. 2019a). No other *Puccinia* species are known to occur on *L. ferocissimum*.

Table 2 Species of *Puccinia* that have been described as occurring on species in the tribe Lycieae by Otálora and Berndt (2018). Host list derived from Otálora and Berndt (2018) and Farr and Rossman (2020).

LINEAGE / *PUCCINIA* SPECIES	HOST(S)
Old World	
P. afra	*Lycium afrum*, *L. barbarum*[1], *L. campanulatum*, *Lycium* sp., *Setaria grisebachii*
P. lycii var. *lycii*	*L. austrinum*, *L. hirsutum*, *L. oxycladum*, *Lycium* sp., *L. tubulosum*
P. lycii var. *bizonata*	*Lycium* sp.
P. lycii [2]	*L. austrimum*, *L. cinereum*, *L. hirsutum*, *L. oxycarpum*, *L. oxycladum*, *Lycium* sp., *L. tubulosum*
P. rapipes	*L. ferocissimum*
P. turgida	*L. europaeum*, *L. oxycarpum*, *Lycium* sp.
New World	
P. globosipes	*L. andersonii*, *L. berlandieri*, *L. berlandieri* var. *parviflorum*, *L. brevipes*, *L. californicum*, *L. carolinianum*, *L. cedrosense*, *L. exsertum*, *L. fremontii*, *L, halimifolium*, *L. minimum*, *L. parishii*, *Lycium* sp., *L. torreyi*
P. paradoxopoda	*Grabowskia duplicata*, *G. obtusa*, *G. schizocalyx*, *G. schlechtendalii*, *Grabowskia* sp., *L. chilense*, *L. ciliatum*, *L. nodosum*, *L. patagonicum*, *Lycium* sp.
P. tumidipes	*L. barbarum, L. berlandieri* var. *parviflorum*, *L. carolinianum*, *L. chilense*, *L. chinense*, *L. halimifolium, L. pallidum*, *L. schaffneri*, *Lycium* sp., *L. torreyi*
P. dimidipes	*Lycium* sp.

[1] Reported on *L. vulgare*, but this host-fungus association is questioned by Otálora and Berndt (2018), due to poor condition of this Spanish specimen.
[2] While *P. lycii* is now considered to comprise two separate varieties in the most recent taxonomic assessment by Otálora and Berndt (2018), it was considered only as *P. lycii* in the prior morphological assessment by Berndt and Uhlmann (2006) and therefore these host associations are included here to allow for complete range of host associations to be recorded.

2.5 Proposed source of the agent

The purified isolate of *P. rapipes* ex. Western Cape, used in all host-specificity experiments performed in the BC3 Microbiological area of the CSIRO Black Mountain Containment Facility in Canberra (Approved Arrangement A1280; Import permit no. 0000921370), is the proposed source of the candidate agent for release. This isolate originated from a sample collected at Miller's Point, south of Simon's Town (34°13'53.18'' S; 18°28'28.35'' E), Western Cape Province, South Africa (Figure 88). This sample was imported into the biosecurity containment facility in Australia in October 2017 (Biosecurity Entry no. Z05546091).

2.6 Agent's potential for control of the target

Puccinia rapipes infects both young and old leaves of *L. ferocissimum*, though it does appear to prefer younger leaves. It obtains nutrients and water from the host plant by establishing an intimate contact with living cells. Through this continuous absorption and diversion of assimilates,

the fungus becomes detrimental to the plant. The sori produced by the fungus on leaves also reduce the photosynthetic surface and capacity of the plant.

Other macrocyclic rust fungi of weeds, such as *Puccinia chondrillina* on skeleton weed (Cullen 2012; Cullen et al. 1973), *Puccinia myrsiphylli* on bridal creeper (Morin and Scott 2012), and *Maravalia cryptostegiae* on rubber vine (Palmer and Vogler 2012) have proved to be very effective biological control agents in Australia. Observations made during surveys in South Africa and laboratory studies in the biosecurity containment facility in Australia support that *P. rapipes* would be a potentially effective biological control agent for *L. ferocissimum*, especially on young plants in coastal environments.

2.7 Information on non-target organisms at risk from the agent

Only six species within the genus *Lycium* occur in Australia: the target weed *L. ferocissimum*, four introduced *Lycium* species of Eurasian origin (*L. barbarum*, *L. chinense*, *L. ruthenicum*, and *L. afrum*) and the native species *L. australe* (Appendix A). There are also in Australia a wide range of native and introduced plant species within the sub-family Solanoideae, to which *L. ferocissimum* belongs (Appendix B). While many of these introduced species are reported as weeds in Australia or elsewhere (Randall 2007), other species were introduced to Australia for horticultural purposes such as tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*) and eggplant (*Solanum melongena*).

2.8 Similar host-specificity assessments undertaken with the species

Prior to the commencement of the comprehensive host-specificity testing presented in this report, a preliminary host-specificity study using two purified isolates of *P. rapipes*, from the Eastern and Western Cape provinces of South Africa, was performed in a biosecurity containment facility in Australia (Ireland et al. 2019a). The experiments comprised two different chloroplast haplotypes of *L. ferocissimum* identified in Australia (McCulloch et al. 2020) and seven species closely related to the weed that occur in Australia. The *L. ferocissimum* haplotypes and the three *Lycium* species of Eurasian origin tested – *L. barbarum*, *L. chinense* and *L. ruthenicum* – were found to be susceptible to both isolates of *P. rapipes* used, while the Australian native *L. australe* was resistant. The three more distantly related species to *L. ferocissimum* tested were rated as immune to the fungus in this preliminary host-specificity study: *Hyoscyamus albus*, *Hyoscyamus aureus* and *Solanum aviculare*.

Concurrently with the above study, an experiment was performed to determine the susceptibility of accessions of *L. ferocissimum* from different states in Australia to the two purified isolates of *P. rapipes* (CSIRO unpublished data). The *L. ferocissimum* accessions tested were from New South Wales (Scheyville National Park), Victoria (Stratford), Queensland (Toowoomba) and Western Australia (Ravensthorpe). The methods of Ireland et al. (2019a) were used and the experiment was conducted twice, each with two replicates per treatment combination. Both isolates of *P. rapipes* infected all four accessions of *L. ferocissimum*. The first signs of chlorotic flecking on leaves were observed between 7–11 days after inoculation, while uredinia emerged between 13–16 days. All accessions developed similar levels of infection.

2.9 Possible interactions, including conflicts with existing biological control programs

We would expect the release of any biological control agents for *L. ferocissimum* to complement current management strategies, especially at sites which are ecologically or culturally sensitive, or difficult to access.

No biological control agents have been released for *L. ferocissimum* in Australia, and so no conflicts are expected at this stage if *P. rapipes* is approved to be released. However, native range surveys for candidate biological control agents in South Africa have revealed other promising insect agents, such as the leaf-chewing beetles *Cassida distingeuenda* and *Cleta eckloni*, and the leaf-mining weevil *Neoplatygaster serietubertculata* (Chari et al. 2020). Investigations on these candidate agents are at different stages.

Puccinia rapipes and these three insect species are commonly found on the same *L. ferocissimum* plants in South Africa. Damage caused by the insects to leaves can be severe. Interactions between *P. rapipes* and these insects are likely to occur in the field as they all attack leaves of *L. ferocissimum*, in the event of the release of any of these candidate agents in Australia. However, being a biotrophic parasite, *P. rapipes* develops only on healthy growing tissue, which means that it will not infect leaves that are already severely damaged by leaf-feeding insects. Different biological control agents of *L. ferocissimum* may occupy different spatio-temporal niches and thus complement one another's effects over space and time.

2.10 Where, when and how initial releases will be made

Upon obtaining approval to release *P. rapipes* in Australia, mature uredinia on plants used to maintain a live culture of the fungus will be examined with a dissecting microscope to confirm that they are free of any hyperparasites. Urediniospores will then be collected from these uredinia and placed into vials for removal from the biosecurity containment facility (in the presence of relevant officers from the Department of Agriculture, Water and the Environment). Many *L. ferocissimum* plants, grown and maintained in the CSIRO glasshouses at Black Mountain, Canberra, will then be inoculated using these urediniospores in a controlled-environment room. Urediniospore suspensions and/or *L. ferocissimum* plants infected with *P. rapipes* will be used to establish the disease in the field at selected sites across the weed's range (mainly in South Australia, Victoria, New South Wales and Tasmania). Disease development and spread will be closely monitored during the first few growing seasons, provided funding is available.

Redistribution of *P. rapipes* from infected to non-infected sites is unlikely to be necessary on a broad scale because urediniospores are known to travel long distances following wind currents. However, targeted redistribution may be required in some situations and will be assessed on a case-by-case basis.

2.11 Established populations of the agent: where, mode of introduction, spread and any off-target impacts recorded

Puccinia rapipes has not been reported outside of its native range in South Africa.

2.12 Host-specificity testing in the biosecurity containment facility

An isolate of *P. rapipes* from the Western Cape in South Africa found in initial studies to be significantly more pathogenic on *L. ferocissimum* than an isolate from the Eastern Cape (Ireland et al. 2019a) was used for host-specificity testing. The Western Cape isolate is the proposed source of *P. rapipes* for release in Australia (see section 2.5).

The methodology used for host-specificity testing with *P. rapipes* was similar to that used with urediniospores of other macrocyclic rust fungi that have been approved for release for weed biological control in Australia, such as *Phragmidium violaceum* (blackberry rust) (Morin et al. 2011), *Puccinia myrsiphylli* (bridal creeper rust) (Morin 1999), and *Prospodium tuberculatum* (lantana rust) (Thomas et al. 2006).

2.12.1 Test list

The list of non-target plant species used to test the specificity of *P. rapipes* was compiled according to the scientifically-endorsed phylogenetic centrifugal approach, which places greater representation on the more closely-related species to the target weed (Wapshere 1974; Briese 2003) (Table 3). Within this phylogenetic/evolutionary framework, selection of representative test species within each of the relevant genera/tribes/sub-families places an emphasis on endemic species, species of economic importance and those that are likely to overlap biogeographically with the target weed, where possible. When native species could not be sourced, alternative naturalised or weedy species that were more accessible were sourced instead for inclusion in host-specificity testing. No unrelated crop species were included in the test list, since these species do not make any contribution to the delineation of the host range of specialised biological control agents (Briese 2003, Sheppard et al. 2005).

Recent published molecular phylogenies of Solanaceae (Särkinen et al. 2013; Stevens 2001 onwards) and comments from an Australian Solanaceae specialist at the Botanic Gardens and State Herbarium of South Australia, were considered in devising the test list so that species most closely related to *L. ferocissimum* that are present in Australia were given priority. The test list included representatives from tribe Lycieae and clade Atropina (to which *L. ferocissimum* belongs), and representatives from across the other tribes in the sub-family Solanoideae, as well as from the other three subfamilies of Solanaceae (Nicotianoideae, Cestroideae and Pentuniodeae) present in Australia. Despite *Lycium afrum* being recorded as naturalised in Australia, it was not possible to source plant material for propagation.

Table 3 List of plant species used to test the specificity of *Puccinia rapipes* in the biosecurity containment facility in Australia. All species are within the family Solanaceae.

SUBFAMILY[1]	TRIBE	RELATIONSHIP TO TARGET WEED		PLANT SPECIES	STATUS IN AUSTRALIA[2]
Solanoideae	Lycieae	Target weed	1	*Lycium ferocissimum* [3]	Weed
		Same genus	2	*Lycium australe*	Native
			3	*Lycium barbarum*	Ornamental (weed)
			4	*Lycium chinense*	Ornamental (weed)
			5	*Lycium ruthenicum*	Ornamental
	Hyoscyameae	Same subfamily	6	*Hyoscyamus albus*	Ornamental (naturalised)
			7	*Hyoscyamus aureus*	Ornamental
			8	*Hyoscyamus niger*	Ornamental (naturalised)
	Capsiceae		9	*Lycianthes rantonetti*	Ornamental
			10	*Capsicum annum*	Horticultural
			11	*Brugmansia sanguinea*	Ornamental (naturalised)
			12	*Brugmansia x candida*	Ornamental (weed)
	Datureae		13	*Datura inoxia*	Ornamental (weed)
			14	*Datura leichhardtii*	Native (but naturalised beyond its native range within Australia)
			15	*Datura stramonium*	Ornamental (weed)
	Physaleae		16	*Physalis peruviana*	Horticultural (weed)
	Solandreae		17	*Solandra maxima*	Ornamental
	Solaneae		18	*Solanum aviculare*	Native (but naturalised beyond its native range within Australia)
			19	*Solanum lycopersicum*	Horticultural
			20	*Solanum melongena*	Horticultural (naturalised)
			21	*Solanum tuberosum*	Horticultural (naturalised)
	Nicandreae		22	*Nicandra physalodes*	Ornamental (weed)
	Salpichroina		23	*Salpichroa origanifolia*	Ornamental (weed)
Nicotianoideae	Nicotianeae	Same family	24	*Nicotiana velutina*	Native
			25	*Nicotiana forsteri*	Native
	Anthocercideae		26	*Anthocercis ilicifolia*	Native
			27	*Duboisia myoporoides*	Native
Cestroideae	Cestreae		28	*Cestrum nocturnum*	Ornamental (weed)
Petuniodeae	Petunioideae		29	*Petunia nana compacta*	Ornamental

[1] Subfamilies of family Solanaceae.
[2] As recorded in Randall (2007) or the Australian Plant Census (APC) (https://biodiversity.org.au/nsl/services/APC).
[3] All *Lycium* species were identified morphologically in the first instance and confirmed by DNA sequencing (to preliminary haplotype level for *L. ferocissimum*), using three chloroplast and one nuclear marker, by collaborators at the University of Queensland.

2.12.2 Materials and methods

Plant production

Lycium ferocissimum plants used as positive control in each experiment were grown from seed collected at Palmer, South Australia (34°50'22"S, 139°12'59"E) (Appendix C). Seeds were soaked in 500 ppm gibberellic acid for 24 hours to promote germination and sown in seedling potting mix (Plugger 111 Seed raising Mix, Australian Growing Solutions, Tyabb, Victoria).

The different accessions of the non-target plant species tested were propagated from seed or stem cuttings or purchased as whole plants from nurseries (Appendix C). Most seeds were soaked in 250-500 ppm gibberellic acid for 24 hours to promote germination and sown in seedling potting mix (same as above). Stem cuttings were treated with a hormone rooting gel (Yates Clonex Rooting Hormonal Gel Purple, Yates, Clayton, Vic., Australia; 3g L-1 Indole-3-Butyric Acid) or hormone powder (Yates Plant Cutting Powder, Yates, Padstow, NSW; 0.05g/kg Indole Acetic Acid, 0.02g/kg Napthalene Acetic Acid), planted in a 1:1 perlite and vermiculite mixture, and maintained wet with intermittent overhead misting to encourage root development.

For all species, seedlings with true leaves and rooted cuttings were transplanted into a standard potting mix (5:1:1:3 straw-based compost, peat moss, river sand, perlite or 71869 AGS Grow Mix 4Kg GJ Low P[2] from Australia Growing Solutions), in plastic pots of at least 5 cm diam × 5 cm high and transferred to larger pots as necessary to support developing plants. Plants were grown in glasshouses maintained at 16–26 °C, under natural light and/or in 20 °C controlled temperature (CT) rooms with a 14-h photoperiod provided by fluorescent or LED plant growth lights. Actively growing plants were taken into the biosecurity containment facility prior to each experiment.

All plants were fertilised every 1–2 months with slow-release fertiliser (when needed) at the soil surface (Osmocote, Bella Vista, NSW, Australia; NPK 19.4:1.6:5) and fortnightly with Aquasol (Yates, Clayton, Vic., Australia; NPK 23:3.95:14), and treated as necessary with pesticides to reduce pest pressure (never fungicides; most commonly the insecticide Confidor, Yates, Clayton, Vic., Australia [15mg/L Imidacloprid] as a soil drench, or Vertimec, Syngenta, Macquarie Park, NSW, Australia [18 g/L Abamectin] as a spray). All plants treated with pesticides were withheld from experiments for a minimum of two weeks following application to reduce the likelihood of the pesticide interacting with fungal infection.

Production of inoculum

A single-uredinium isolate of *P. rapipes* from a site in the Western Cape Province of South Africa was selected for host-specificity testing. The methods used to generate this purified isolate are outlined in Ireland et al. (2019a). A culture of the isolate was maintained by inoculating *L. ferocissimum* plants at regular intervals to provide fresh urediniospores for the experiments. Plants were inoculated by spraying a suspension of urediniospores in a solution of 0.1 % TWEEN® 80 (Sigma-Aldrich, Castle Hill, NSW, Australia) in deionised water onto the foliage. Inoculated plants were misted with deionised water and placed inside large plastic boxes (80–120 L) with a film of water covering the base within large, clear and sealed plastic bags (moist boxes) in a dark

[2] Comprised of two grades of composted pine bark, coir and fine sand at 5%. Addition of Gypsum, N, TE, Fe in both mid and long term, Mg, Ca, Saturaid and 4Kg Green Jacket Low P (20-1.5-9). Average pH range 5.5 – 6.1 and EC 1000 -1600µS/cm.

CT room set at 20 °C for 24 hours. Plants were then transferred to the bench of the CT room with a 14-h photoperiod. Once uredinia had developed, 2–3 weeks after inoculation, urediniospores were collected twice a week until uredinia ceased producing high levels of urediniospores or had developed into telia. These urediniospores were either used immediately in experiments or dried overnight over silica gel beads and used the following day or stored at –20 °C.

Experimental design

Each non-target plant species (five replicates inoculated and one uninoculated control) was tested in two separate experiments, with different accessions of the species included in different experiments, to account for any possible variation in time and provenance of the plant material, unless stated otherwise. Actively growing plants with new growth (up to 60 cm in height, including pot) were chosen for each experiment. Each experiment consisted of 4–7 species, including *L. ferocissimum* plants as positive controls. Randomized complete block designs at the plant level were used in all experiments to reduce the influence of random factors such as lighting within the room and the quality and consistency of inoculum.

Inoculations

For each experiment, inoculum was prepared by suspending mostly fresh urediniospores (sometimes frozen spores no older than 2 weeks were added to obtain the density required) in a 0.1 % TWEEN® 80-deionised water solution at the beginning of the experiment and was applied within 90 minutes. The density of each suspension was determined using a haemocytometer (Neubauer) and adjusted to 2×10^4 urediniospores/ml. The suspension was first applied with a small camel hairbrush onto the two youngest, fully expanded leaves of each plant replicate, which were marked (Figure 9a). For each non-target species, an extra, young leaf on the first replicate plant was also inoculated with the brush method to provide material for microscopic examination of the rust fungus development (see following section).

At least one dip of the brush in the suspension was used for each side of each leaf and the suspension was spread over the leaf surface with at least three brush strokes, so that the entire surface was visibly inoculated. The suspension was then sprayed onto the entire foliage using a hand-held manual spraying device, ensuring that both the upper and under sides of leaves of the foliage were inoculated (Figure 9b). For each species in each experiment, a 0.1 % TWEEN® 80-deionised water solution without spores was applied in the same way on a separate replicate plant (placed in a separate moist box) to act as a negative control. All plants were then misted with additional deionised water and placed in dark, moist boxes in a CT room (same conditions as above) for approximately 24 hours, before transferring them to the bench of the same CT room.

Figure 9 Methods used to inoculate plants with *Puccinia rapipes*: (a) Brush and (b) spray inoculation methods.

The viability of urediniospores used in each experiment was assessed by applying an aliquot of the spore suspension with a small brush onto the surface of a water agar block placed on a microscope slide, at the beginning and end of the experiment. The slide was placed in a glass Petri dish containing a moist filter paper in the same CT room as for inoculated plants. Germination was assessed after 24 hours using a light microscope. A drop of blue lactoglycerol stain was placed on top of the agar block to stop the germination process and 50–100 urediniospores were assessed.

To account for any variation in inherent susceptibility of *L. ferocissimum* to *P. rapipes* or inoculum used, an experiment was considered valid if at least a third of inoculated *L. ferocissimum* control plant replicates had developed uredinia and assigned a disease rating of 3 or above, and if at least half of young inoculated marked leaves exhibited a disease rating of 3 or above at 28 days after inoculation (Table 4).

Table 4 Disease rating system used to assess visible symptoms on plants, including the control *Lycium ferocissimum*, inoculated with *Puccinia rapipes* in each host-specificity experiment.

RATING	SYMPTOMS
0	No visible symptoms.
1	Chlorotic, purplish or necrotic flecking or spots present.
2	Purplish or necrotic spots, with pin sized uredinia (< 0.5 mm diameter). Limited sporulation.
3	Fully developed, normal size uredinia (0.5—1 mm diameter), covering less than 25% of the leaf surface. Sporulation.
4	Fully developed, normal sized uredinia or large uredinia (> 1 mm diameter), covering more than 25% of leaf surface. Abundant sporulation.

Microscopic examinations

The additional leaf inoculated with the brush method for each non-target species was excised five days after inoculation and cut into small pieces (0.5–1 cm^2). The pieces were cleared and stained in a solution containing aniline blue, ethanol, chloroform, lactic acid, phenol and chloral hydrate for 1–2 days (Bruzzese and Hasan 1983). They were then rinsed in water, placed in a saturated solution of chloral hydrate for 1–2 days and transferred back to water for storage. Prior to microscopic examination, the pieces were placed in blue lactoglycerol stain on a microscope glass slide for 2–5 min. Excess stain was then gently removed with blotting paper and pieces were mounted in water and examined under a light microscope. At least 50 urediniospores per species were examined. Where chlorotic or necrotic lesions developed on some of the two brush-inoculated leaves, samples from other leaves with symptoms were taken at 14 and 21 days after inoculation and processed as above prior to microscopic examination to complement observations made with samples taken at 5 days after inoculation.

Assessment of *Puccinia rapipes* development

The microscopic development of *P. rapipes* and reproduction on test plants were assessed according to 20 categories (Figure 10). Plants were assessed at 14 and 28 days after inoculation for the presence of visible disease symptoms. The overall response of each species/accession to *P. rapipes* was classified according to one of seven categories (Table 5) by considering microscopic observations (Figure 10) and the most severe visible symptoms observed across all replicate plants (Table 4) at the end of the experiment.

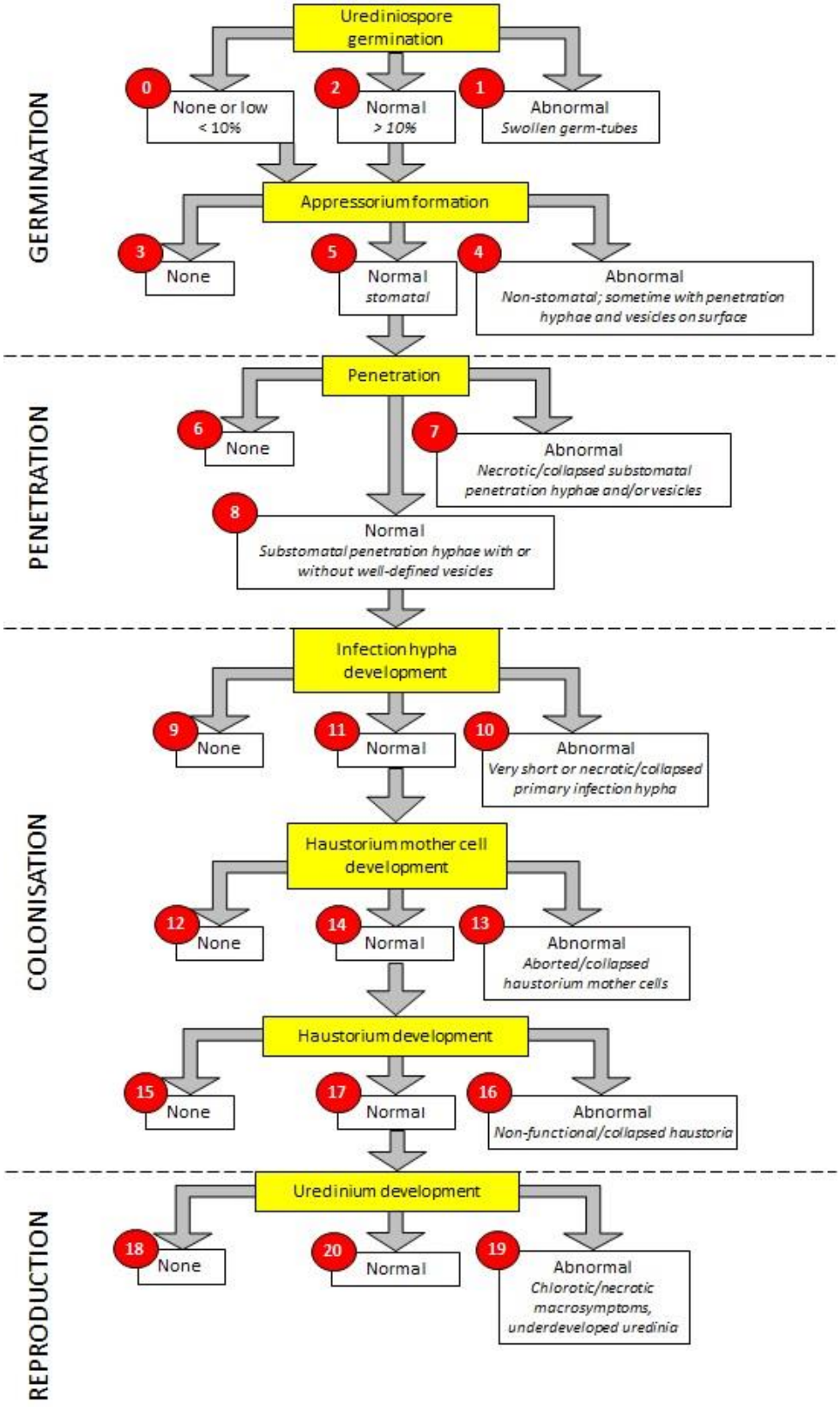

Figure 10 Schematic representation of the categories used to assess the microscopic development of *Puccinia rapipes* on test plant species.

Table 5 Categories used to classify the response of plant species to *Puccinia rapipes*.

CATEGORY	MACRO-SYMPTOMS	TYPICAL DEVELOPMENTAL STAGE OF THE FUNGUS FOR THE CATEGORY [1]
Immune (I)	None	No sign of penetration
Highly resistant (HR)	None	Abnormal penetration (necrotic/collapsed penetration hyphae and vesicle, none or very short or necrotic/collapsed primary infection hyphae); plant defence reaction sometimes visible at the cellular level.
Resistant (R)	Chlorotic or necrotic flecking sometimes present.	Successful penetration and development of some infection hyphae. Haustorium mother cells sometimes developed but generally no haustoria present.
Moderately resistant (MR)	Chlorotic or necrotic spots or blotches present.	Restricted network of infection hyphae developed. Haustoria present, but generally non-functional/collapsed. Plant host cell plasmolysis often present.
Moderately susceptible (MS)	Chlorotic or necrotic spots present. Underdeveloped, non-eruptive uredinia or rare, miniscule uredinia present. No sporulation.	Extensive network of infection hyphae; haustoria abundant but often non-functional/collapsed. Development of uredinia initiated but aborted.
Susceptible (S)	Restricted number of normal uredinia present on less than 25% of leaf surface. Sporulation.	Extensive network of infection hyphae; functional haustoria abundant.
Highly susceptible (HS)	Large number of normal uredinia present on more than 25% of leaf surface. Abundant sporulation.	Extensive network of infection hyphae; functional haustoria abundant.

[1] In some instances microscopic development of the fungus was either less or more advanced than the typical development stage associated with the category used to classify the plant response.

2.12.3 Results

The urediniospores in the suspensions used for inoculation in all 20 host-specificity experiments, were highly viable, with a germination rate of more than 85% on water agar across all experiments.

Microscopic development of *Puccinia rapipes* on tested species

The range of developmental stages of *P. rapipes* observed on the target weed, *L. ferocissimum*, and on each of the test plant species is presented in Table 6. Photographs illustrating the microscopic structures associated with the different development stages are presented in Figure 11. Microscopic examinations revealed that urediniospores of *P. rapipes* germinated normally on all species tested. Following urediniospore germination, *P. rapipes* produced appressoria over stomata on most tested plant species apart from one accession of each of *Brugmansia x candida*, *Datura stramonium*, *Solanum melongena*, and *Salpichroa origanifolia*. In these species/accessions appressoria were categorised as abnormal because they were not positioned over stomata (Table 6). Entry through the stomata, via a penetration hypha produced by the appressorium and in some instances the formation of a substomatal vesicle, was only observed in one or more accessions of 19 of the species tested including: *L. ferocissimum*, *L. australe*, *L. barbarum*, *L. chinense, Hyoscyamus aureus*, *Hyoscyamus niger*, *Capsicum annum*, *Brugmansia x candida*, *Brugmansia sanguinea*, *Datura leichhardtii*, *D. stramonium*, *Solandra maxima*, *S. melongena*, *Nicandra*

physalodes, *Nicotiana velutina*, *N. forsteri*, *Anthocercis ilicifolia*, *Cestrum nocturnum* and *Petunia nana compacta* (Table 6). Normal intercellular infection hyphae within the leaf tissue were only observed in eight species: *L. ferocissimum*, *L. barbarum*, *L. chinense*, *H. aureus*, *D. leichhardtii*, *N. velutina*, *A. ilicifolia* and *P. nana compacta*. Following this stage however, differences in development of *P. rapipes* were observed. Abnormal haustoria were seen in samples from *H. aureus*, *N. velutina*, one accession of *A. ilicifolia* and both accessions of *P. nana compacta,* and normal haustoria in *L. ferocissimum*, *L. barbarum*, *L. chinense*, and the single accession of *A. ilicifolia* in the first experiment it was tested (Table 6).

Development of visible symptoms of *Puccinia rapipes* on tested species

The fungus developed uredinia on *L. ferocissimum* and the three non-target closely related *Lycium* species: *L. barbarum*, *L. chinense* and *L. ruthenicum* (Table 6, Figure 12). All these species were thus categorised as highly susceptible or susceptible to *P. rapipes*. *Puccinia rapipes* also produced a few minuscule, pin-sized uredinia on two leaves of *Anthocercis ilicifolia* (Ant.ili_1) only in one of the two experiments (i.e. the experiment where haustoria were observed in the leaf sample examined) (Figure 13). The species was thus categorised as moderately susceptible in that experiment. Urediniospores from these uredinia did not germinate when placed on water agar. Chlorotic flecking, corresponding to the resistant rating category, was observed on some leaves of accessions of *Hyoscyamus aureus* (Hyo.aur_1), *Capsicum annum* (Cap.ann_5), *Datura leichhardtii* (Dat.lei_2), *Nicotiana velutina* (Nic.vel_1), *Nicotiana forsteri* (Nic.for_1) and *Petunia nana compacta* (Pet2, Pet 3) (Table 6, Figure 13). Some rare necrotic spots on leaves were observed on some replicate plants for both accessions of *Lycium australe* (Lyc.aus_1, Qlyc.aus_2) and on one accession of *Solanum melongena* (Sol.mel_4) and these were categorised as moderately resistant to *P. rapipes* (Figure 13). All other non-target species that were tested did not produce any symptoms following host-testing and were rated as immune or highly resistant to *P. rapipes* (see Table 5 for differences between these two categories).

Table 6 Microscopic development of *Puccinia rapipes* and macro-symptoms observed on each of the test plant species inoculated with the fungus, based on categories described in Figure 10. A positive sign indicates that the category was observed, and a negative sign indicates that the category was not observed or is not applicable because there was no or abnormal development of the fungus in the previous stage. The overall response of each species was classified using categories presented in Table 5.

SPECIES[1]	ACCESSION	EXP. NO. (NO. REPS)	MICROSCOPIC EXAMINATION																			VISIBLE EXAMINATION			OVERALL SPECIES RESPONSE[2]
			GERMINATION						PENETRATION			COLONISATION										REPRODUCTION			
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Lycium ferocissimum	Lyc.fer_24-29	All	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	HS
Lycium australe	Lyc.aus_1	18 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	MR
	QLyc.aus_2	15 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	MR
Lycium barbarum	Lyc.bar_1	7 (5)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	HS
	Lyc.bar_4	2 (5)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	HS
Lycium chinense	Lyc.chin_1	13 (5)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	HS
	Lyc.chin_1	14 (5)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	S
Lycium ruthenicum	Lyc.rut_1	4 (5)	Microscopic development not assessed																		-	-	+	S[3]
	Lyc.rut_2	3 (5)	Microscopic development not assessed																		-	-	+	S[3]
Hyoscyamus albus	Hyo.alb_1	5 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Hyo.alb_2	4 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Hyoscyamus aureus	Hyo.aur_1	17 (5)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+	-	+	-	-	R
Hyoscyamus niger	Hyo.nig_2	2 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Hyo.nig_3	6 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	HR
Lycianthes rantonetti	Lyc.ran_1	1 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Lyc.ran_2	6 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Capsicum annum	Cap.ann_4	6 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	HR
	Cap.ann_5	12 (4)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	R
Brugmansia x candida	Bru_2	12 (5)	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Bru_3	13 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	HR
Brugmansia sanguinea	Bru.san_2	11 (3)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	HR

SPECIES[1]	ACCESSION	EXP. NO. (NO. REPS)	MICROSCOPIC EXAMINATION																			VISIBLE EXAMINATION			OVERALL SPECIES RESPONSE[2]
			GERMINATION						PENETRATION			COLONISATION										REPRODUCTION			
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
Datura inoxia	Dat.ino_2	5 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Datura leichhardtii	Dat.lei_2	2 (5)	-	-	+	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-	R
Datura stramonium	Dat.str_1	4 (5)	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Dat.str_3	16 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	HR
Physalis peruviana	Phy.per_2	9 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Phy.per_3	8 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Solandra maxima	Sol.max_1	18 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Sol.max_2	12 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	HR
Solanum aviculare	Sol.avi_1	5 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Sol.avi_1	7 (5)	Microscopic development not assessed																		+	-	-	I/HR[4]
	Sol.avi_2	14 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Solanum lycopersicum	Sol.lyc_1	7 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Sol.lyc_2	3 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Solanum melongena	Sol.mel_3	6 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Sol.mel_4	14 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	MR
	Sol.mel_5	19 (5)	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Solanum tuberosum	Sol.tub_1	7 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Sol.tub_2	3 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Nicandra physalodes	Nic.phy_1	10 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Nic.phy_2	11 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	HR
Salpichroa origanifolia	Sal.ori_1	16 (5)	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Sal.ori_1	18 (5)	Microscopic development not assessed																		+	-	-	I/HR[4]
Nicotiana velutina	Nic.vel_1	8 (5)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+	-	+	-	-	R
Nicotiana forsteri	Nic.for_1	11 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	R
	Nic.for_2	16 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	HR

SPECIES[1]	ACCESSION	EXP. NO. (NO. REPS)	MICROSCOPIC EXAMINATION																			VISIBLE EXAMINATION			OVERALL SPECIES RESPONSE[2]
			GERMINATION						PENETRATION			COLONISATION										REPRODUCTION			
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Duboisia myoporoides	Dub.myo_2	18 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
	Dub.myo_2	19 (5)	Microscopic development not assessed																		+	-	-	I/HR[4]
Anthocercis ilicifolia	Ant.ili_1	18 (3)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+	-	MS
	Ant.ili_1	20 (5)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+	-	+	-	-	HR
Cestrum nocturnum	Ces.noc_1	13 (5)	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	HR
	Ces.noc_4	16 (5)	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Petunia nana compacta	Pet_2	18 (5)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+	-	+	-	-	R
	Pet_3	17 (5)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+	-	+	-	-	R

[1] Species listed in the same order as in Table 3.
[2] Response of tested plant species to *Puccinia rapipes* at the end of the 28-day experiment. See Table 5 for details of defined categories.
[3] Corresponds to macro-symptoms as described in Table 5.
[4] Based on the categories in Table 5. Without assessing microscopic development, it is not possible to distinguish between immune and highly resistant.

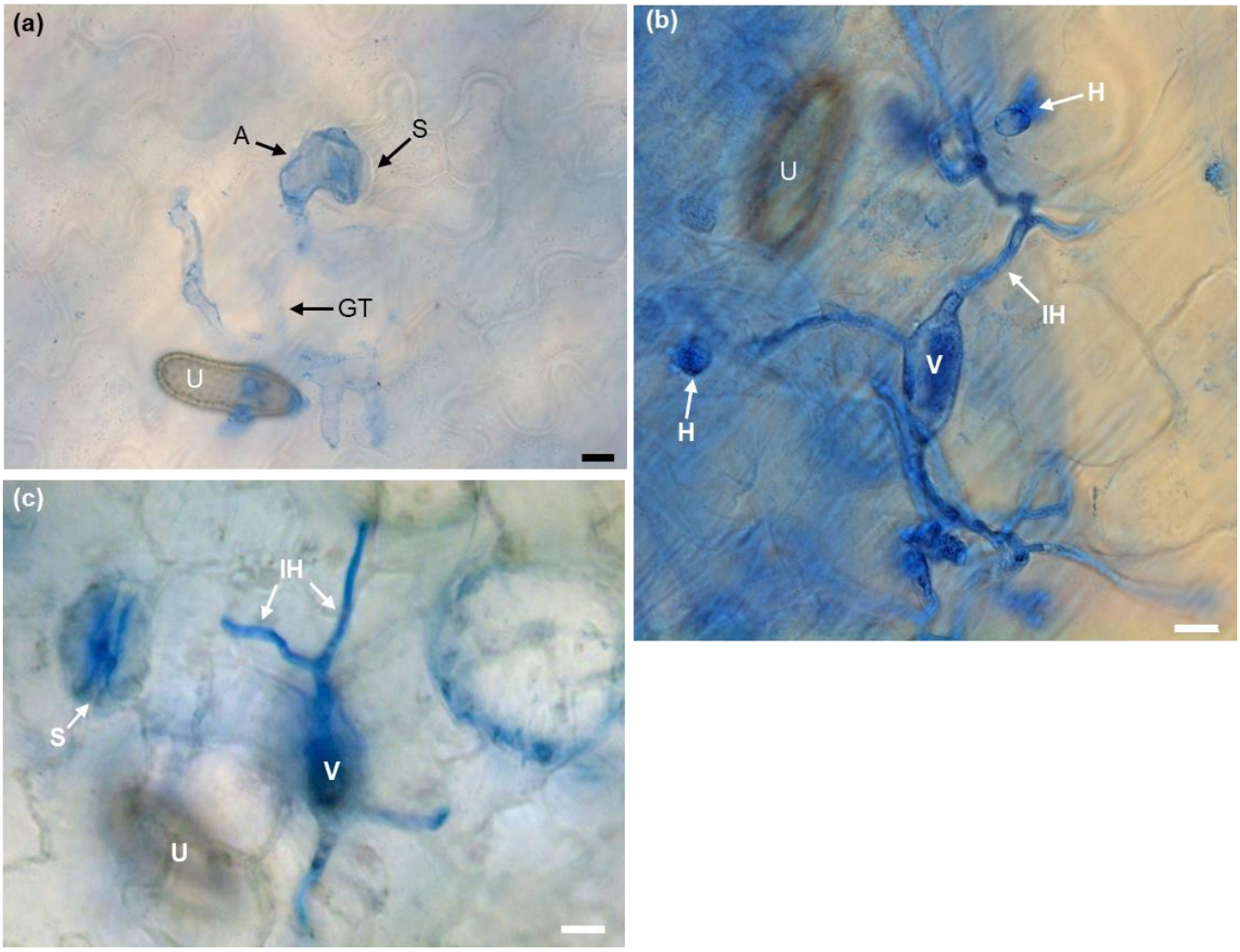

Figure 11 Examples of the various microscopic structures produced by *Puccinia rapipes* on different plant species at 5 days after inoculation. (a) a urediniospore (U) with a germ-tube (GT) that has produced an appressorium (A) over a stomate (S) on a leaf of *Lycium ferocissimum*, (b) following penetration via the stomate a sub-stomatal vesicle (V) has formed from which intercellular infection hyphae (IH) have developed and produced haustoria (H) within cells of *L. ferocissimum*, (c) a sub-stomatal vesicle (V) with short primary infection hyphae (IH) that have stopped developing on *Lycium australe*. Scale bars = 10 µm. Photos (b) and (c) are reproduced from Ireland et al. (2019a).

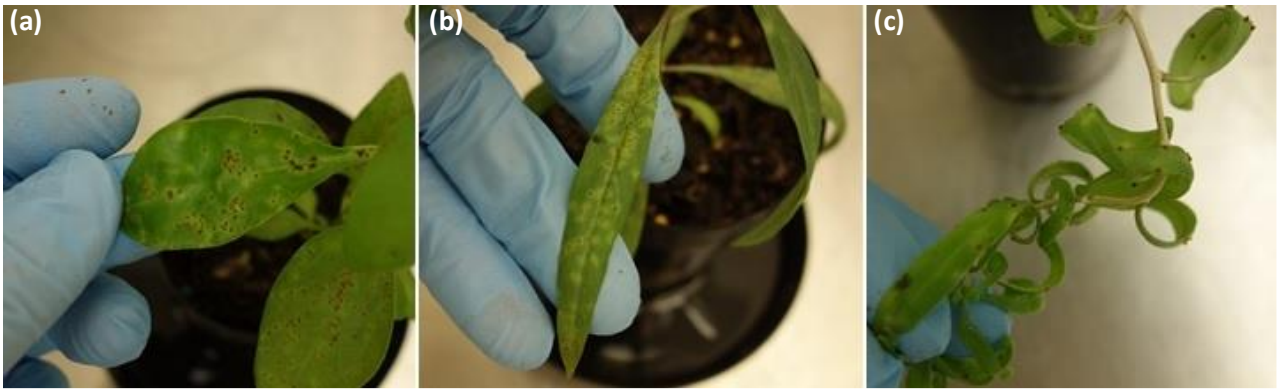

Figure 12 Sporulating uredinia of *Puccinia rapipes* on (A) *Lycium ferocissimum*, (B*) Lycium barbarum* and (C) *Lycium ruthenicum* at 28 days after inoculation.

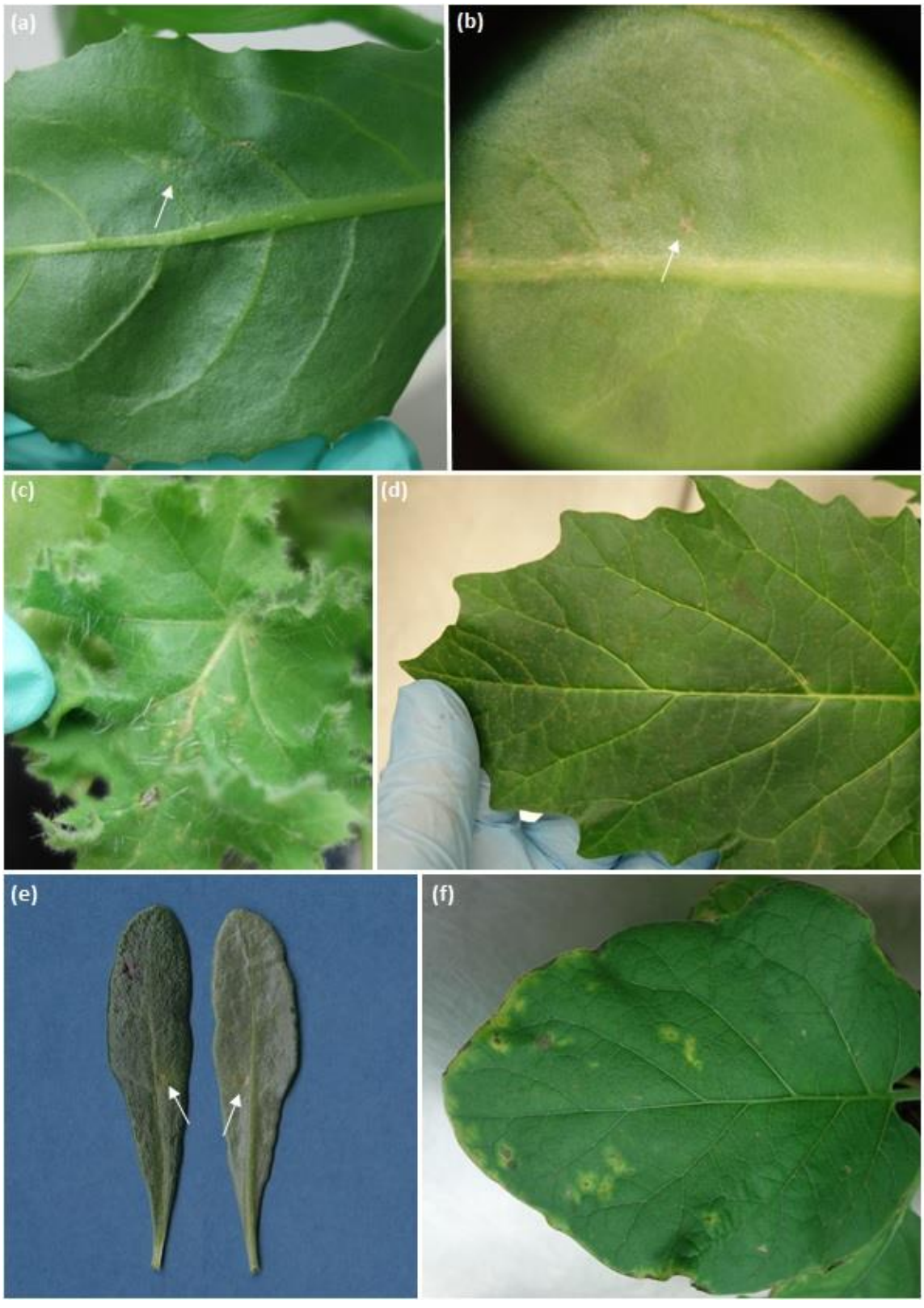

Figure 13 Examples of various responses of non-target species 28 days after inoculation with *Puccinia rapipes*. (a, b) A miniscule uredinium with non-viable urediniospores (white arrow) on different leaves of *Anthocercis ilicifolia*. Chlorotic flecking on leaves of (c) *Hyoscyamus aureus* and (d) *Datura leichhardtii*. Adaxial and abaxial view of a necrotic spot (white arrows) on a leaf of (e) *Lycium australe* and necrotic spots on (f) *Solanum melongena*.

2.13 Field host-specificity study

2.13.1 Materials and Methods

A study was conducted in the field in South Africa by collaborators from Rhodes University to investigate the potential for *P. rapipes* to naturally infect and cause disease symptoms on *Lycium* species other than *L. ferocissimum*. The study was initiated following confirmation of the susceptibility of the Eurasian goji berries *L. barbarum*, *L. chinense* and *L. ruthenicum* to *P. rapipes* in the preliminary host-specificity experiments performed in the biosecurity containment facility (Ireland et al., 2019a) (section 2.8). These experiments were performed under ideal conditions (20°C constant temperature, 14-h artificial photoperiod, very high inoculum densities and long exposure to moist conditions conducive to infection following inoculation) that were highly favourable for severe infection to occur, which may not reflect the level of disease symptoms that would develop under field conditions. The methods used in this field study and detailed results are presented in Appendix D.

2.13.2 Results

Results from this field study showed that *P. rapipes* can naturally spread from infected *L. ferocissimum* plants and readily infect other *L. ferocissimum* plants as well as plants of the goji berry species *L. barbarum* and the southern African native *L. oxycarpum* when they are placed in proximity (1–1.5 m). This is the first record of infection of these hosts by *P. rapipes* under natural field conditions in South Africa. These results supported those obtained during host-specificity testing in the biosecurity containment facility in Australia which found *L. barbarum* to be highly susceptible to *P. rapipes* (Ireland et al. (2019a) and section 2.12.3).

Three growers of *L. barbarum*, for fruit or plant trade, in South Africa were also approached and none have ever observed symptoms of *P. rapipes* on plants at their production sites. These growers included the largest producer of *L. barbarum* plants, 'Berries for Africa', in South Africa. All three production sites are in regions outside of *L. ferocissimum* distribution, and so natural spread of *P. rapipes* to these areas may rarely occur.

2.14 Importance of goji berry and stakeholder reactions to possible off-target effects of *P. rapipes*

Highlights from a review of the importance of non-native *Lycium* species propagated and sold within Australia (*L. barbarum*, *L. chinense*, *L. ruthenicum*) are (Appendix E):

- Goji berry plants are sold in the nursery and garden trade, although it is an extremely low value and volume plant in Australia.
- The main species of goji berry plants sold in Australia, *L. barbarum* and *L. chinense*, have naturalised and are considered environmental weeds in Australia and elsewhere. *Lycium ruthenicum* is a relatively new species in the nursery and garden industry market, and thus its capacity to naturalize and become invasive is not known.

- Commercial scale production of goji berry for the dried fruit market in Australia is currently inexistent and not forecasted to grow. Economic viability of such a production is too low since Australian producers are in direct competition with producers in China where labour costs are low (berries require hand-picking even in commercial production).

We also consulted growers, wholesalers and retailers of goji berry to identify possible concerns they would have if a biological control agent for *L. ferocissimum*, that could infect their goji berry plants, was to be released in Australia (Confidential report to the Department of Agriculture, Water and the Environment). Overall, respondents were not particularly concerned about possible off-target effects on goji berry if *P. rapipes* is released in Australia, especially if the disease can be managed with fungicide applications.

2.15 Efficacy of fungicides to control *P. rapipes* on goji berry

2.15.1 Materials and Methods

The efficacy of the systemic fungicide AMISTAR® 250 SC (active: azoxystrobin, Syngenta, Macquarie Park, NSW, Australia; registered for use to control *Puccinia* spp. on nursery stock and ornamentals) and contact fungicide and miticide Mancozeb Plus (actives: sulphur and mancozeb, Yates, Clayton, Vic., Australia; not currently registered for a use pattern consistent with that suggested here) to protect goji berry (*L. barbarum*; Lyc.bar_1) plants from *P. rapipes* was tested in an experiment performed in the biosecurity containment facility. The two fungicides were applied once to plants at different time-points: (i) 3 days before plants were inoculated with *P. rapipes*, (ii) 7 days after inoculation when the first visible signs of infection (chlorotic flecks) were observed, and (iii) 14 days after inoculation when uredinia had begun to erupt on leaves (which is not a registered use pattern for AMISTAR® 250 SC as per the label, in part due to the capacity of fungi to develop fungicide resistance to this active chemistry in short periods of time). For each fungicide treatment and time point, five replicate plants were inoculated. For a positive control, an additional five plants were inoculated with *P. rapipes* but not treated with any fungicide.

The same inoculation methods as those used for host-specificity experiments (described above) were used. The two fungicides were applied at the prescribed label rates and a fresh batch of each fungicide was prepared for each time point application. The fungicides were sprayed onto adaxial and abaxial leaf surfaces of the foliage using a hand-held manual sprayer. To prevent fungicide cross contamination, plants sprayed with each fungicide were placed in closed plastic boxes for 24 hours and then placed on the bench of the CT room for the duration of the experiment. All plants were assessed for visible disease symptoms at 28 days after inoculation using the rating system described above (Table 4).

2.15.2 Results

Both AMISTAR® 250 SC and Mancozeb Plus protected plants from infection by *P. rapipes* when applied 3 days before inoculation with the fungus. No disease symptoms developed on any of these plants except for a single uredinium on one leaf of one of the replicate plants sprayed with Mancozeb Plus (Figure 14a, d).

AMISTAR® 250 SC applied at 7 and 14 days after inoculation with *P. rapipes* successfully arrested the development of the fungus (Figure 14b, c). Chlorotic flecks were still present by the end of the experiment on plants sprayed 7 days after inoculation and thus these plants were given a rating of 1. By the end of the experiment, small necrotic spots, some associated with pin-sized uredinia with limited sporulation, were observed on plants sprayed 14 days after inoculation (Rating of 2, Figure 14c). This indicated that the fungicide had killed the fungus or severely restricted its capacity to develop mature uredinia. In contrast, Mancozeb Plus sprayed on plants at 7 and 14 days after inoculation did not stop development of *P. rapipes*, and all plants developed abundant sporulating uredinia by the end of the experiment (Rating of 3–4, Figure 14e).

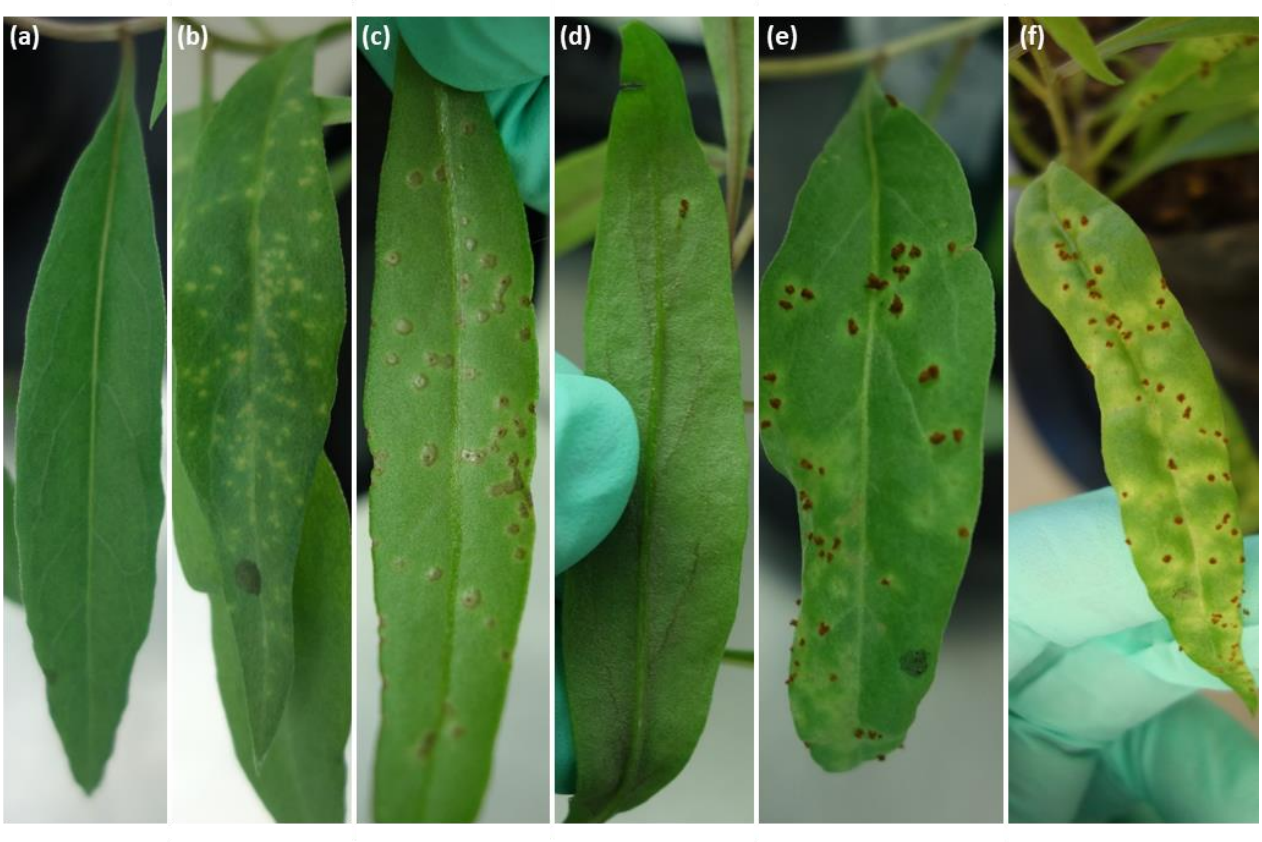

Figure 14 Examples of disease symptoms observed at the end of the fungicide experiment, on leaves of goji berry (*Lycium barbarum*) plants sprayed with the fungicides AMISTAR® 250 SC or Mancozeb Plus at different time points before or after inoculation with *Puccinia rapipes* (a-e) compared with symptoms on inoculated plants not sprayed with fungicide (control; f). Plants sprayed with AMISTAR® 250 SC; (a) 3 days before inoculation with *P. rapipes*, (b) 7 days after inoculation and (c) 14 days after inoculation. Plants sprayed with Mancozeb Plus; (d) 3 days before inoculation with *P. rapipes* and (e) 14 days after inoculation (note that similar symptoms were observed on plants sprayed with the fungicide at 7 days after inoculation). (f) Control – not sprayed with fungicide.

3 Discussion

Puccinia rapipes is only known from *L. ferocissimum* in South Africa, where uredinia and telia were the only stages observed on leaves when the species was first discovered (Berndt and Uhlmann 2006). Detailed experimentation as part of a study by Ireland et al. (2019a) has elucidated the complete lifecycle of the fungus and confirmed *P. rapipes* to be a macrocyclic, autoecious species that also forms spermagonia and aecia on *L. ferocissimum*.

Host-specificity testing was undertaken on a comprehensive set of representative non-target species covering a sufficiently broad genetic diversity of the Solanaceae. The test list comprising 28 species was devised after considered analyses of current phylogenetic standings of the Solanaceae (Särkinen et al. 2013; Stevens 2001 onwards) combined with guidance from a botanist with expertise on the Solanaceae. We have purposefully tested a greater number of non-target species phylogenetically closely related to *L. ferocissimum*; 22 representative species across 9 tribes of the Solanoideae subfamily. Testing was also undertaken on horticulturally important representatives within this subfamily including tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*) and potato (*Solanum tuberosum*), all of which proved to be immune or resistant to *P. rapipes*.

Results from the host-specificity experiments support the findings of Ireland et al. (2019a) that *P. rapipes* can also infect *L. barbarum*, *L. chinense* and *L. ruthenicum*. Most other *Puccinia* species that are phylogenetically, closely related to *P. rapipes* and known from plant species in the tribe Lycieae, can also infect more than a single *Lycium* species.

Lycium australe, the only native *Lycium* species in Australia, proved resistant to *P. rapipes* in our host-specificity experiments, as previously discovered during the preliminary testing performed by Ireland et al. (2019a). Microscopic examinations of leaf samples from these plants indicated that the fungus development is halted after the initial infection hyphae are formed within the leaf. Based on DNA sequence data from three chloroplast loci, *L. australe* clustered between a European *Lycium* clade accommodating *L. chinense*, *L. barbarum* and *L. ruthenicum* and an African clade containing *L. ferocissimum* (Fukuda et al. 2001; Li et al. 2020). These phylogenetic differences may explain why *L. australe* is not a host for *P. rapipes*.

Outside the Lycieae tribe, *P. rapipes* did not develop uredinia on any of the other species tested, except for rare, pin-sized uredinia on two leaves of *Anthocercis ilicifolia* in one experiment. The few urediniospores produced in these uredinia did not germinate when placed on water agar. Considering these results obtained under optimal conditions for infection by *P. rapipes*, it is extremely unlikely that the fungus would be able to maintain a population on this plant species in the field.

The review of the literature and internet searches performed in parallel to the experimental work presented here provided evidence that goji berry species (predominantly *L. barbarum* and *L. chinense*) are primarily planted in home gardens but also recorded as environmental weeds (Appendix E). The negative effects that *P. rapipes* could have on invasive populations of goji berry, should it be released in Australia, would be beneficial to reduce impact and spread of these weeds, as well as that of the main target weed *L. ferocissimum*.

Our extensive consultation of growers, wholesalers and retailers of goji berry revealed that it is not a high volume/sale plant in the nursery and garden trade and has rarely been grown commercially for fruit production in Australia because it is not economically viable (Confidential

report to the Department of Agriculture, Water and the Environment). While the possible off-target effects of *P. rapipes* on goji berry would be inconvenient for commercial growers of this plant, most of them indicated that they would simply adapt their operations and manage infections as they do for diseases of other plant species. Results of the experiment we performed in the biosecurity containment facility to test the efficacy of fungicides to control *P. rapipes* showed that AMISTAR® 250 SC and Mancozeb Plus, systemic and contact fungicides used to control rust diseases on other plants, respectively, can protect goji berry against the fungus and/or arrest its development in infected plants. Both these fungicides are readily available and easily accessible to growers and home gardeners and thus could be incorporated into their existing pest control regimes to control the fungus on young or mature goji berry plants. We do recognise however, that Mancozeb Plus is not yet registered for use to control rust diseases in nursery stock and ornamentals. It also has limited effectiveness because its active ingredients, mancozeb and wettable sulphur, only have a contact fungicidal action that prevents entry of plants by the fungus but cannot stop development of already established infections.

Observations made during surveys in South Africa and laboratory studies in the biosecurity containment facility in Australia (Ireland et al. 2019a) support that *P. rapipes* would be a potentially effective biological control agent for *L. ferocissimum*. The fungus produces many urediniospores from uredinia on leaves of susceptible hosts that are easily and widely dispersed by wind. Although we do not have quantitative data on the distance that urediniospores of *P. rapipes* would be able to travel, it is well known that urediniospores of rust fungi are very effectively dispersed by wind over large distances (thousands of kilometres in some instances) during favourable environmental conditions (Helfer 2014). In moist weather, particularly in the coastal distribution of *L. ferocissimum* in Australia, or after rain showers, recurrent infections of plants and production of abundant urediniospores should occur, providing temperatures are suitable for the fungus development. *Puccinia rapipes* is most likely to have the greatest impact on *L. ferocissimum* seedlings and young plants by reducing their photosynthetic ability and causing defoliation. It is difficult to predict what its impact will be on older, larger, and well-established *L. ferocissimum* plants.

The level of risk associated with releasing *P. rapipes* in Australia may be acceptable, should stakeholders and regulators be willing to accept damage to the Eurasian goji berry grown in Australia, which could be managed with fungicide applications if required.

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Any of these references can be provided in electronic form if requested. Contact Gavin Hunter (gavin.hunter@csiro.au; Ph: (02) 6218 3658)

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Appendix A *Lycium* species documented in Australia and their current status

SPECIES	COMMON NAME	ORIGIN	ALA[A]	SOCIO-ECONOMIC SIGNIFICANCE IN AUSTRALIA	WEED STATUS IN AUSTRALIA	GLOBAL WEED STATUS[B]
L. afrum	Kaffir boxthorn	Africa	Y (38) Tas., Vic. 2013	Prior nursery and garden? No record of current propagation.	Environmental weed Naturalised, not common	**Low** Casual alien, Naturalised, Weed
L. australe	Australian boxthorn	Australia	Y (2572) NSW, SA, Vic., WA 2020	Biodiversity values	Native	
L. barbarum	Goji berry, Chinese boxthorn	Eurasia	Y (170) ACT, NSW, Qld, SA, Tas., Vic., WA 2019	Nursery and garden	Cultivation escape Environmental weed Naturalised	**Extreme** Agricultural weed, Casual alien, Contaminant, Cultivation escape, Environmental weed, Invasive species, Naturalised, Quarantine weed, Weed
***L. chinense* (inc. var. chinense)**	Goji berry 'chinense'	Eurasia		Nursery and garden	Environmental weed	**Medium** Agricultural weed, Casual alien, Cultivation escape, Invasive species, Naturalised, Quarantine weed, Weed
L. ferocissimum	African boxthorn	Africa	Y (109,324) ACT, NSW, NT, Qld, SA, Tas., Vic. 2020	**Weed of National Significance Biological control target**	Agricultural weed Cultivation escape Environmental weed Invasive species Noxious weed	**Medium** Agricultural weed, Contaminant, Cultivation escape, Environmental weed, Invasive species, Noxious weed, Quarantine, Weed
L. ruthenicum	Black goji berry	Eurasia		Nursery and garden	Unknown, likely recent introduction	Agricultural weed
Uncommon species or misapplied names in Australia						
L. cestroides		South America				
L. chilense		South America				**Low** Weed
L. europaeum	European boxthorn	Europe				**Low** Agricultural weed, Naturalised, Ruderal, Weed
L. horridum		Africa				
L. pallidum	Pale wolfberry	Central America				

[a] Y indicates observations recorded in the Atlas of Living Australia (ALA 2020), with the number of these observations given in parentheses. States where the species has been recorded, where ACT = Australian Capital Territory, NSW = New South Wales, NT = Northern Territory, Qld = Queensland, SA = South Australia, Tas. = Tasmania, Vic. = Victoria and WA = Western Australia. Year of last record follows this.

[b] Global weed risk rating (Randall 2017) in bold, and all statuses as recorded by Randall (2007).

Appendix B Members of the Solanaceae sub-family Solanoideae (excluding tribe Lycieae) present in Australia

TRIBE[1]	SUBTRIBE	GENUS	SPECIES	STATUS[2]
Capsiceae		*Capsicum*	*Capsicum annuum*	Naturalised
			Capsicum annuum var. *glabriusculum*	Naturalised
			Capsicum baccatum	Naturalised
			Capsicum chacoense	Naturalised
			Capsicum chinense	Naturalised
			Capsicum fastigiatum	Naturalised
			Capsicum frutescens	Naturalised and Weed
			Capsicum pubescens	Introduced
		Lycianthes	*Lycianthes biflora*	Native
			Lycianthes rantonnetii	Naturalised
			Lycianthes shanesii	Native
Datureae		*Brugmansia*	*Brugmansia x candida*	Naturalised
			Brugmansia sanguinea	Naturalised
			Brugmansia suaveolens	Naturalised
			Brugmansia versicolor	Introduced
		Datura	*Datura ferox*	Naturalised
			Datura inermis	Weed
			Datura innoxia	Naturalised
			Datura leichhardtii	Native, but naturalised beyond its native range within Australia
			Datura leichhardtii subsp. *leichhardtii*	Native, but naturalised beyond its native range within Australia
			Datura metel	Naturalised and Weed
			Datura stramonium	Naturalised and Weed
			Datura wrightii	Naturalised and Weed
Hyoscyameae		*Atropa*	*Atropa bella-donna*	Introduced
		Hyoscyamus	*Hyoscyamus albus*	Naturalised
			Hyoscyamus niger	Naturalised
			Hyoscyamus aureus	Introduced
		Scopolia	*Scopolia physaloides*	Introduced

TRIBE[1]	SUBTRIBE	GENUS	SPECIES	STATUS[2]
Jaboroseae		*Jaborosa*	*Jaborosa integrifolia*	Introduced
Juanulloeae		*Juanulloa*	*Juanulloa mexicana*	Introduced
Mandragoreae		*Mandragora*	*Mandragora officinarum*	Introduced
Nicandreae		*Nicandra*	*Nicandra physalodes*	Naturalised and Weed
Nolaneae		*Nolana*	*Nolana humifusa*	Introduced
			Nolana paradoxa	Introduced
Physaleae	Iochrominae	*Acnistus*	*Acnistus arborescens*	Introduced
			Acnistus breviflorus	Introduced
		Dunalia	*Dunalia fasciculata*	Introduced
			Dunalia tubulosa	Introduced
		Iochroma	*Iochroma australe*	Introduced
			Iochroma coccinea	Introduced
			Iochroma cyanea	Introduced
			Iochroma cyaneum	Introduced
			Iochroma fuchsioides	Introduced
			Iochroma grandiflorum	Introduced
			Iochroma warscewiczii	Introduced
	Physalinae	*Physalis*	*Physalis alkekengi*	Naturalised and Weed
			Physalis angulata	Naturalised
			Physalis cinerascens	Naturalised
			Physalis crassifolia	Naturalised
			Physalis crassifolia var. *versicolor*	Naturalised
			Physalis franchetii	Introduced
			Physalis hederifolia	Naturalised
			Physalis ixocarpa	Naturalised and Weed
			Physalis lanceifolia	Naturalised
			Physalis longifolia	Naturalised
			Physalis micrantha	Naturalised and Weed
			Physalis minima	Naturalised and Weed
			Physalis peruviana	Naturalised and Weed
			Physalis philadelphica	Naturalised and Weed
			Physalis pubescens	Naturalised
			Physalis virginiana	Naturalised and Weed
			Physalis viscosa	Naturalised and Weed
		Witheringia	*Witheringia coccoloboides*	Introduced
	Withaninae	*Withania*	*Withania aristata*	Introduced
			Withania frutescens	Introduced
			Withania riebeckii	Introduced

TRIBE[1]	SUBTRIBE	GENUS	SPECIES	STATUS[2]
			Withania somnifera	Naturalised
Salpichroina		*Salpichroa*	*Salpichroa origanifolia*	Naturalised and Weed
Solandreae		*Solandra*	*Solandra grandiflora*	Introduced
			Solandra guttata	Introduced
			Solandra longiflora	Introduced
			Solandra maxima	Introduced
		Solanum	Approximately 265 species of *Solanum* have been recorded in Australia. Of these, 185 are native, 34 are introduced, 21 are naturalised and a further 25 species are naturalised and known to have been recorded as a weed in Australia[2].	

[1] Tribes of Solanoideae occurring in Australia arranged in alphabetic order.
[2] Status in Australia as described in Randall (2007) and the Australian Plant Census (APC) (https://biodiversity.org.au/nsl/services/APC).

Appendix C Source of accessions of each plant species tested

PLANT SPECIES	ACCESSION ID	SOURCE	MATERIAL	LOCATION OR PROVIDER NAME	STATE
Lycium ferocissimum[1]	Lyc.fer_24-29	Field collection	Fruit	Palmer	SA
Lycium australe	Lyc.aus_1	Field collection	Plants	Botanic Garden	SA
	QLyc.aus_2	Field collection	Plants	Kalgoorlie	WA
Lycium barbarum	Lyc.bar_1	Commercial	Fruit	Belconnen Markets	ACT
	Lyc.bar_4	Commercial	Seed	eBay	VIC
Lycium chinense	Lyc.chin_1	Commercial	Plants	Mudbrick Cottage Herbfarm	QLD
Lycium ruthenicum	Lyc.ruth_1	Commercial	Seed	Fairdinkum Seeds	QLD
	Lyc.ruth_2	Commercial	Seed	eBay	VIC
Hyoscyamus albus	Hyo.alb_1	Commercial	Seed	Herbalistics	QLD
	Hyo.alb_2	Commercial	Seed	eBay	QLD
Hyoscyamus aureus	Hyo.aur_1	Commercial	Seed	Herbalistics	QLD
Hyoscyamus niger	Hyo.nig_2	Commercial	Seed	eBay QLD	QLD
	Hyo.nig_3	Commercial	Seed	All Rare Herbs	QLD
Lycianthes rantonetti	Lyc.ran_1	Commercial	Plants	Garden Express	VIC
	Lyc.ran_2	Commercial	Plants	Devon Tubestock and Rare Plants	VIC
Capsicum annum	Cap.ann_4	Commercial	Plants	Bunnings	ACT
	Cap.ann_5	Commercial	Seed	Mr Fothergrill's	ACT
Brugmansia sanguinea	Bru.san_2	Commercial	Seed	Northern Rivers Seeds	NSW
Brugmansia x candida	Bru_2	Commercial	Plants	Garden Express	VIC
	Bru_3	Commercial	Plants	Garden Express	VIC
Datura innoxia	Dat.ino_2	Commercial	Seed	Fairdinkum Seeds	QLD
Datura leichhardtii	Dat.lei_1	Commercial	Seed	Herbalistics	QLD
Datura stramonium	Dat.str_1	Field collection	Seed	Field collection, Canberra	ACT
	Dat.str_3	Commercial	Seed	Fairdinkum Seeds	QLD
Physalis peruviana	Phy.per_2	Commercial	Seed	Eden Seeds	QLD
	Phy.per_3	Commercial	Seed	The Seed Collection	VIC
Solandra maxima	Sol.max_1	Commercial	Plants	Fairdinkum Seeds	QLD
	Sol.max_2	Commercial	Plants	All Rare Herbs	QLD
Solanum aviculare	Sol.avi_1	Field collection	Seed	Biosecurity SA	SA
	Sol.avi_2	Commercial	Plants	Daleys Fruit Tree Nursery	NSW
Solanum lycopersicum	Sol.lyc_1	Commercial	Seed	Eden Seeds	QLD

PLANT SPECIES	ACCESSION ID	SOURCE	MATERIAL	LOCATION OR PROVIDER NAME	STATE
	Sol.lyc_2	Field collection	Seed	Field collection, Canberra	ACT
Solanum melongena	Sol.mel_3	Commercial	Plants	Diggers, Bunnings	ACT
	Sol.mel_4	Commercial	Seed	Herbalistics	QLD
	Sol.mel_5	Commercial	Seed	Johnsons World Kitchen	NSW
Solanum tuberosum 'Nadine'[2]	Sol.tub_1	Commercial	Tubers	Garden Express	VIC
Solanum tuberosum 'Salad Rose'[2]	Sol.tub_2	Commercial	Tubers	Garden Express	VIC
Nicandra physalodes	Nic.phy_1	Commercial	Seed	Fairdinkum Seeds	QLD
	Nic.phy_2	Commercial	Seed	Etsy	Serbia
Salpichroa origanifolia	Sal.ori_1	Field collection	Plants	Red Hill Reserve	ACT
Nicotiana velutina	Nic.vel_1	Field collection	Seed	Biosecurity SA	SA
Nicotiana forsteri	Nic.for_1	Commercial	Seed	Herbalistics	QLD
	Nic.for_2	Commercial	Seed	Fairdinkum Seeds	QLD
Anthocercis ilicifolia	Ant.ili_1	Commercial	Seed	Herbalistics	QLD
Duboisia myoporoides	Dub.myo_2	Commercial	Plants	Firewheel Rainforest Nursery	NSW
Cestrum nocturnum	Ces.noc_1	Commercial	Plants	Mudbrick Cottage Herbfarm	QLD
	Ces.noc_4	Commercial	Plants	Plants in a Box	QLD
Petunia nana compacta	Pet_2	Commercial	Seed	Eden Seeds	QLD
	Pet_3	Commercial	Seed	The Seed Collection	NSW

[1] All accessions used correspond to chloroplast haplotype 5 previously used in Ireland et al. (2019a), which have been referred to the AU common haplotype in McCulloch et al. (2020).

[2] Varieties of *Solanum tuberosum* included in host-specificity testing.

Appendix D Methods and results of field host-specificity study in South Africa

Methods

The study was conducted by collaborators from May to July 2019 at the Centre for Biological Control, Waainek research facility, Rhodes University, Makhanda (formerly Grahamstown), in the Eastern Cape Province of South Africa (33.314091 S; 26. 519683 E; altitude 585m). One mature *L. ferocissimum* plant, ~1.5 meters tall, estimated to be at least four to five years old, naturally infected with *P. rapipes* (i.e. uredinia on leaves) was located on the grounds of the research facility. A potted *L. ferocissimum* plant (Eastern Cape provenance), approximately two years old, that had become naturally infected near the large, established plant, was also used in the study.

The study comprised two experimental set ups:

- Open-field conditions: potted *Lycium* plants placed around the naturally infected, mature *L. ferocissimum* plant, in the open and exposed to prevailing westerly winds, and
- Shade cloth (10%) conditions: potted *Lycium* plants placed around the large, naturally infected potted *L. ferocissimum* plant transferred under a shade cloth, with protection from prevailing winds but with swirling airflow.

Under each condition, three replicate, medium-sized (25 - 50 cm tall), plants of *L. barbarum*, *L. oxycarpum* and *L. ferocissimum* (Western Cape provenance) in pots were placed in a circle at 1–1.5 m distance from the infected *L. ferocissimum* plant (Figure A.1). Three replicate *L. ferocissimum* plants (Eastern Cape provenance) and a single replicate plant of *Lycium cinereum* in pots were also included with the other plants under the shade cloth. All plants in pots had been propagated in shade and polyhouses at the research facility and were pest and disease free at the outset of the study.

Plants were watered regularly, but no foliage watering was used to encourage infection. The study was conducted during winter, with dominant westerly winds, day lengths of 10–11 hours, average temperatures of 18.8–20 °C, and temperature extremes of 35 and 6.2 °C recorded at the site (Table A.1).

Plants were assessed for presence of uredinia on a weekly basis for the first six weeks and a final assessment was made at 11 weeks before the experimental setups were dismantled. A qualitative estimate of the percentage of all leaves on each plant infected with *P. rapipes* was recorded at each assessment.

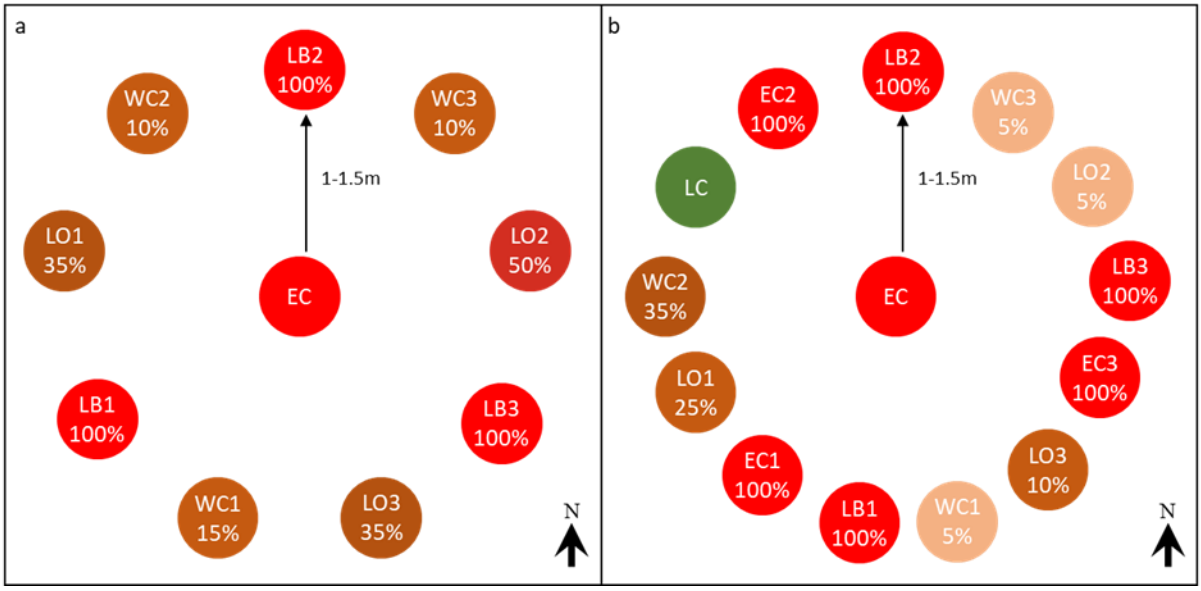

Figure A.1. Experimental setups and level of infection caused by *Puccinia rapipes* on *Lycium* species exposed to inoculum produced by a central, naturally infected *Lycium ferocissimum* plant (ex. Eastern Cape; EC) for 11 weeks under open-field (a) and shade cloth (b) conditions. LB = *Lycium barbarum*, LC = *Lycium cinereum*, LO = *Lycium oxycarpum* and WC = *Lycium ferocissimum* ex. Western Cape. Numbers indicate the estimated percentage of leaves of the plant with uredinia. Green indicates no infection by *P. rapipes*, while beige, to light brown, dark brown and red indicates increasing disease incidence measured for those plants.

Table A.1. Monthly weather data collected during the study by the Geography Department, Rhodes University, Makhanda, South Africa, located 1.2 km from the Waainek research facility. Data was collected using Davis Vantage Pro2 plus equipment.

	Temperature (c°)					Humidity (%)			Rainfall (mm)	Wind (km/hr)		
Month	Max	Min	Mean	High	Low	Mean	Min	Max	Total	Mean	High	Dominant direction
May	27.5	16.2	20.6	35.4	11.1	71	20	97	15.0	6.5	54.7	W
June	24.8	13.6	18.8	31.0	6.9	55	13	97	4.2	8.1	78.9	W
July	26.1	13.7	19.3	34.2	6.2	50	9	95	9.0	7.7	86.9	W

Results

Throughout the study both naturally infected *L. ferocissimum* plants used as the source of inoculum under the two experimental setups had 100% of their leaves infected with *P. rapipes*. Three weeks after the commencement of the study, *P. rapipes* was recorded on *L. ferocissimum* ex. Eastern Cape and *L. barbarum* plants in pots (Table A.2). Except for the single *L. cinereum* plants, all plants became infected to some degree under both conditions by the end of the study, with the percentage of leaves becoming infected gradually increasing over the course of the study (Table A.2). *Lycium ferocissimum* and *L. barbarum* were observed to have the highest disease incidence under both conditions, while *L. oxycarpum* had the lowest.

While not tested explicitly, *P. rapipes* infection under the open-field conditions was recorded on plants in the direct line of the prevailing winds from the central infected plant, while this pattern was not observed under the shade cloth conditions (Figure A.1).

Table A.2. Estimated percentage of leaves with uredinia of *Puccinia rapipes* on plants in each of the experimental setups (open-field or shade cloth conditions) assessed in the first 6 weeks and at the end (11 weeks) of the study. Replicate numbers indicated below each species name, with Eastern Cape (EC) and Western Cape (WC) provenances of *L. ferocissimum* indicated. The inoculum source column refers to the naturally infected *L. ferocissimum* plant used as the primary source of inoculum in each experimental setup.

	Lycium ferocissimum							***L. cinerium***	***L. barbarum***			***L. oxycarpum***		
Assessment (week no.)	**Inoculum source**	**EC1**	**EC2**	**EC3**	**WC1**	**WC2**	**WC3**	**LC1**	**LB1**	**LB2**	**LB3**	**LO1**	**LO2**	**LO3**
Open-field conditions														
1	100	–	–	–	0	0	0	–	0	0	0	0	0	0
2	100	–	–	–	0	0	0	–	0	0	0	0	0	0
3	100	–	–	–	0	0	0	–	0	0	5	0	0	0
4	100	–	–	–	5	0	0	–	20	60	40	10	0	0
5	100	–	–	–	10	5	5	–	40	95	100	20	5	10
6	100	–	–	–	10	5	5	–	55	95	100	35	50	35
11	100	–	–	–	15	10	10	–	100	100	100	35	50	35
Shade cloth (10%) conditions														
1	100	0	0	0	0	0	0	0	0	0	0	0	0	0
2	100	0	0	0	0	0	0	0	0	0	0	0	0	0
3	100	5	5	0	0	0	0	0	5	5	18	12	0	0
4	100	50	20	17	0	0	0	0	20	50	26	20	0	0
5	100	60	55	50	5	30	0	0	90	85	90	20	0	0
6	100	100	100	100	5	30	5	0	100	100	100	25	5	10
11	100	100	100	100	5	35	5	0	100	100	100	25	5	10

Appendix E Importance of goji berry in Australia

A review performed by Kylie B. Ireland, Michelle A. Rafter and Louise Morin in 2019. Links of internet resources presented were verified and updated in October 2020.

Commercial production of goji berries

Goji berries have become increasingly popular in the health food, grocery and food flavour sectors globally in the past ten years, and are often marketed as a "super food" (Decker and Kurnik 2018). The majority of dried goji berries sold in Australia originate in China and are most commonly from *L. barbarum*, though berries from *L. chinense* may also be sold under the same name (Decker and Kurnik 2018). The two species can be confused, and both are known to be marketed as goji berries. No reports or websites for commercial *L. ruthenicum* berry production could be found for Australia or globally.

Despite being included in extended lists of new and emerging industries or products in reports commissioned by the Rural Industries Research and Development Corporation (now trading as AgriFutures Australia) in the past ten years, goji berry does not seem to have warranted much additional industry development (Decker and Kurnik 2018; RIRDC 2010). While sales of goji berry plants continue, commercial sales of the fruit have not been realised (Wainwright 2015). Harvesting the berries at commercial scale is likely to be difficult, as they require hand-picking and thus goji berry may not be economically viable for most farms to invest in, especially if in direct competition with China, where the plant is native and labour is cheaper (Wainwright 2015).

While references to potential commercial crops of goji berry in Australia litter the internet, it would still seem the industry is in its infancy for commercial berry production, if anything at all.

Example websites about the commercial prospects for goji berries (*L. barbarum*, *L. chinense*) in Australia:

http://www.abc.net.au/news/rural/2015-09-30/rural-nsw-Goji/6814060

~~http://www.abc.net.au/site-archive/rural/tas/content/2010/02/s2826664.htm~~ [3]

~~http://www.abc.net.au/sitearchive/rural/tas/content/2011/02/s3133192.htm~~

Example websites about the health benefits of goji berries (including *L. ruthenicum* for an Australian audience:

https://www.naturimedica.com/Goji-berries-can-be-grown-in-australia/

~~https://liveability.com.au/liveabilityguides/growing-superfoods-organic-garden-Goji-berries/~~

[3] Strikethrough indicates that the resource was not available anymore on the internet as of October 2020.

https://permaculturenews.org/2013/07/20/top-five-edible-shrubs-for-the-backyard-food-forest-garden-canberra-australia/

https://www.mrsupplement.com.au/Goji-berries

https://www.gardenworld.net.au/2009/03/go-the-Goji.html

~~https://www.ebay.com.au/itm/Organic-Natural-Wild-Black-Goji-Berry-Dried-Lycii-Wolfberry-Lycium-Ruthenicum-/152665891351~~

https://www.ebay.com.au/b/Goji-Berry-Herb-Botanical-Supplements/19260/bn_35751792

https://au.dhgate.com/wolfberry-berry-australia.html

https://au.dhgate.com/wolfberry-seeds-australia.html

Goji berries sale sites in Australia, demonstrating products originating in China:

https://www.royalnutcompany.com.au/dried-fruit/all-dried-fruit/Goji-berries

https://thesourcebulkfoods.com.au/shop/dried-fruit/organic-Goji-berries/

https://forestsuperfoods.com.au/product/Goji-berries-organic/

Commercial production of goji berry plants

Goji berry plants (*L. barbarum*, *L. chinense*, *L. ruthenicum*) are widely sold in the nursery and garden trade in Australia, with large scale sellers such as Bunnings, many bespoke nurseries and even home gardeners on Gumtree selling plants across the country. In an interview with the ABC in 2015 a goji berry plant producer Mr Mark Beaumont was quoted as saying that "his main customer base had shifted from bulk orders for farmers to smaller orders for the average home grower" (Wainwright 2015).

Example of nursery and garden websites selling goji berry plants:

https://www.bunnings.com.au/johnsons-world-kitchen-Goji-berry-wolfberry-vegetable-seeds_p2961823

https://australiangardener.net.au/?s=Goji

~~http://www.Gojiplantsaustralia.com.au/~~

https://www.diggers.com.au/shop/fruit/berries-other/Goji-berry/wGoji/ [goji berry was not available anymore at this nursery in October 2020]

https://guildfordgardencentre.com.au/services/information/articles-factsheets/Goji-berries/

https://guildfordgardencentre.com.au/product/Goji-berry/

~~http://www.faceysnursery.com.au/pickmeedibles/pick-me-edibles/Goji-berry~~ [replace by this link in October 2020] https://www.faceysnursery.com.au/catalogue/lycium-barbarum-goji-berry/

https://www.nurseriesonline.com.au/plant-index/trees-shrubs/fruit-trees-berry-fruit/Goji-berries/

~~http://www.woodbridgenursery.com.au/fruit-and-edibles/1336-Goji-berry.html~~

https://fairdinkumseeds.com/products-page/ethnobotanical-or-medicinal-plants/black-goji-berry-wild-lycium-ruthenicum-seeds/

https://www.daleysfruit.com.au/buy/Goji-Berry-Black-Fruit-Tree.htm

Invasive status of goji berry species

Lycium barbarum

Lycium barbarum has escaped cultivation, is naturalised and considered an environmental weed in Australia (Randall 2007). Globally, it is recorded as an escapee from cultivation, a weed of the natural environment and agriculture, or an invasive species (Randall 2017). According to Randall (2007) the latter "… is the most serious criterion that can be applied to a plant and is generally used for serious high impact environmental and/or agricultural weeds that spread rapidly and often create monocultures."

Websites and quotes referencing *Lycium barbarum* as a current or potential weed in Australia:

https://weeds.brisbane.qld.gov.au/weeds/african-boxthorn - "…introduced weedy relative"

http://www.weedfutures.net/species.php?id=192 – Weed Futures

From https://keyserver.lucidcentral.org/weeds/data/media/Html/lycium_barbarum.htm

"Widely naturalised in eastern Australia (i.e. in south-eastern Queensland, eastern New South Wales, Victoria, Tasmania and south-eastern South Australia)."

"Chinese boxthorn (*Lycium barbarum*) is regarded as an environmental weed in Victoria and Tasmania. This garden escape has mainly become naturalised in coastal and sub-coastal districts in south-eastern Australia. It is often found growing in disturbed sites and waste areas, but also invades riverbanks and native bushland (e.g. Yarra Bend Park in Victoria).

Chinese boxthorn (*Lycium barbarum*) is very similar to African boxthorn (*Lycium ferocissimum*) and its distribution and impact in Australia may be under-estimated as a result of it being confused with this species. Like African boxthorn (*Lycium ferocissimum*), it is dispersed into natural areas by birds and other animals that eat its fruit and may cause similar environmental impacts (e.g. form dense thickets along waterways to the detriment of native species)."

~~From https://liveability.com.au/liveabilityguides/growing-superfoods-organic-garden-goji-berries/ (Kearney 2018)~~

~~"Even though, at this stage, there is no indication the goji Berry variety is dangerous to our Australian ecosystem, if commercial quantities are grown in Australia this may change (see more information at the end of this post). To be on the safe side we recommend planting your goji berry plants in pots."~~

Lycium chinense

Lycium chinense is naturalised and considered an environmental weed in Australia (Randall 2007). Globally, it is recorded as an escapee from cultivation, a casual alien, a quarantine weed, an agricultural weed and an invasive species (Randall 2017).

Websites and reports referencing *Lycium chinense* as a current or potential weed in Australia:

Due to the confusion in identity between *L. barbarum* and *L. chinense*, these weed names may have been misapplied in the past and so it would be prudent to consider that *L. chinense* may likely be the culprit weed in some of the literature and websites related to *L. barbarum* above.

Lycium ruthenicum

As a relatively new species in the nursery and garden industry market, far less seems to be known about this species at present. The species does not have any records of observation in the Atlas of Living Australia (2018), indicating that it may not have naturalised as yet. There is only two references in Randall (2017) that the plant has been identified as an agricultural weed in other countries, indicating it may have some potential to naturalise and become a weed in Australia.

References

ALA (2018) Atlas of Living Australia website at http://www.ala.org.au. Accessed 4 December 2018.

Decker C, Kurnik B (2018) Scan of new and emerging agricultural industry opportunities and market scoping. AgriFutures Australia Publication No 18/040. AgriFutures Australia. Available at https://www.agrifutures.com.au/wp-content/uploads/2018/10/18-040.pdf Linked checked on 16 October 2020

~~Kearney P (2018) Growing Goji Berries. The Center for Livability Real Estate, Available at: https://liveability.com.au/liveabilityguides/growing-superfoods-organic-garden-goji-berries/ Accessed 4 May 2018~~

Randall RP (2007) The introduced flora of Australia and its weed status. CRC for Australian Weed Management, Department of Agriculture and Food, Western Australia. Available at: https://www.une.edu.au/__data/assets/pdf_file/0019/52372/2007.-The-introduced-flora-of-Australia-and-its-weed-status.pdf Link checked on 16 October 2020

Randall RP (2017) A global compendium of weeds. Ed.3 edn. Perth, Australia: R. P. Randall, Perth, Australia. Available at http://www.hear.org/gcw/ Link checked on 16 October 2020

RIRDC (2010) RIRDC Annual Report 2009-10. Australian Government Rural Industries Research and Development Corporation. Available at https://www.agrifutures.com.au/wp-content/uploads/publications/10-196.pdf Link checked on 16 October 2020

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