# Application to release *Puccinia lantanae* Farl. (Pucciniales: Pucciniaceae) for the biological control of *Lantana camara* L. (Verbenaceae) in Australia

**Prepared by Jason Callander, Patricia Lu-Irving, and Michael Day**
Biosecurity Queensland, Department of Primary Industries
GPO Box 267, Brisbane QLD 4001
Jason.Callander@dpi.qld.gov.au

Date: December 2025

This publication has been compiled by Jason Callander of Invasive Plants and Animals, Biosecurity Queensland, The Department of Primary Industries.



*The Department of Primary Industries proudly acknowledges Aboriginal people and Torres Strait Islander people as the Traditional Custodians of Country. We recognise their continuing connection to land, sea, waters and sky. We pay our respects to them, their cultures and their Elders past and present, and commit to ongoing reconciliation.*

The Queensland Government is committed to providing accessible services to Queenslanders from all culturally and linguistically diverse backgrounds. If you have difficulty in understanding the Application to release *Puccinia lantanae*, you can contact us for assistance, and we will arrange an interpreter to communicate the report to you.

# Contents

# List of figures

# List of tables

# Summary

Biosecurity Queensland, part of the Queensland Department of Primary Industries, seeks approval for the importation and field release of the fungal rust *Puccinia lantanae* Farl. (Basidiomycotina: Uredinales: Pucciniaceae) for the biological control of *Lantana camara* L. (Verbenaceae), a serious weed in Queensland and New South Wales.

*Lantana camara* (lantana) is a Weed of National Significance in Australia, distributed along coastal and subcoastal areas of eastern Australia, from the Torres Strait islands in the north to the Victorian border in the south. Lantana invades national parks, forestry, grazing lands, and riparian areas where it can from dense thickets. It displaces native vegetation and reduces land productivity and is poisonous to stock. Due to the size and extent of infestations, it is difficult to control effectively using mechanical or chemical means.

Biological control of lantana has been investigated for over 100 years with 19 arthropod species and one fungal pathogen becoming established in Australia, contributing to some seasonal damage of the weed. Despite this, biological control of the plant is not considered adequate. *Puccinia lantanae* (isolate IMI 398849) is a host specific and highly damaging rust pathogen that has potential to complement current management practices of lantana in Australia.

Host-specificity testing of *P. lantanae* was conducted by CABI in the UK. The rust was tested against a wide range of closely related species in the family Verbenaceae, as well as species in 14 other families within Lamiales. Results of the host-specificity testing, detailed in the study by Thomas et al. (2021), show that only *L. camara* is fully susceptible to *P. lantanae* isolate IMI 398849, whilst four related test plant species, *Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson, *Phyla nodiflora* var. *minor* (Gillies & Hook.) N.O'Leary & Múlgura (Syn. *Phyla canescens* (Kunth) Greene), *Verbena africana* (R.Fern. & Verdc.) P.W.Michael (formerly *Verbena officinalis* subsp. *africana*) and *V. gaudichaudii* (Briq.) P.W.Michael (formerly *V. officinalis* var. *gaudichaudii*), in the family Verbenaceae, were found to be weakly or moderately susceptible to the rust. Of the test plant species found to be susceptible, only a low number of viable teliospores were produced, and re-inoculation of the same species with these teliospores did not result in infection.

The findings from surveys conducted in Peru, along with laboratory experiments carried out at CABI-UK (Thomas et al. 2021), provide support for releasing *Puccinia lantanae* pathotype IMI 398849 as a biological control agent for lantana in Australia.

# 1. Information on the target species in Australia

## 1.1. Taxonomy

Division: Angiosperms

Class: Magnoliopsida

Order: Lamiales

Family: Verbenaceae

Genus: *Lantana*

Species: *camara* L.

The family Verbenaceae belongs to the order Lamiales, which is in a state of flux due to ongoing accumulation of molecular data, contributing to the further clarification of species relationships. Recently, 13 small families have been added to the order (Cardoso et al. 2021). The Angiosperm Phylogeny Group (Stevens 2017) suggest that once more data is processed, the families will be condensed into major groups. Previous research ranked the family Lamiaceae as the most closely related family to the Verbenaceae, with many of the species previously allocated to Verbenaceae transferred to Lamiaceae over the last 20 years (Cardoso et al. 2021). However, more recent analyses of family relationships within the order Lamiales, using different molecular datasets to determine dispersal and evolutionary patterns of tropical species, found that the family Verbenaceae was more closely related to the families Bignoniaceae, Martyniaceae, Schlegeliaceae, Thomandersiaceae, Lentibulariaceae, Acanthaceae and Pedaliaceae, and less related to Lamiaceae than previously suggested (Fonseca 2021). Relationships among the families of Lamiales are likely to remain difficult to resolve, due to rapid diversification early in the evolution of this group (Magallón et al. 2019). This is reflected in consistently weak statistical support for the relationships inferred in most studies, including the most recent study (Figure 1; Fonseca 2021). Thus, the difference in degree of relatedness among Verbenaceae and related families is minimal.

Species belonging to *Lantana* Section *Lantana* (from which the invasive lantana species complex has been derived) are taxonomically difficult to define and have further been widely cultivated and hybridised for over 300 years (Howard 1969; Sanders 2006; Sanders 2012). *Lantana camara* L. *sensu lato* includes the neotropical species as first described by Linnaeus, as well as plants derived from interbreeding with other *Lantana* species, creating hundreds of cultivars and hybrids that are the most likely progenitors of the invasive lantana complex (Howard 1969; Stirton 1977). Different cultivars or varieties can be distinguished morphologically (flower size, shape and colour; leaf size, hairiness and colour; stem thorniness; height and branch architecture), physiologically (growth rates, toxicity to livestock) and by their chromosome number and DNA content (Stirton 1977; Gujral and Vasudevan 1983;

Scott et al. 1997). More recent population genetic analysis has revealed that invasive lantana consists of several diverged sub-lineages, with limited gene flow among them (Lu-Irving et al. 2022). These findings provide a framework to re-evaluate previous attempts to classify the invasive *L. camara* complex, and studies are underway to produce a definitive taxonomic treatment aligned with the results of population genomic analysis.

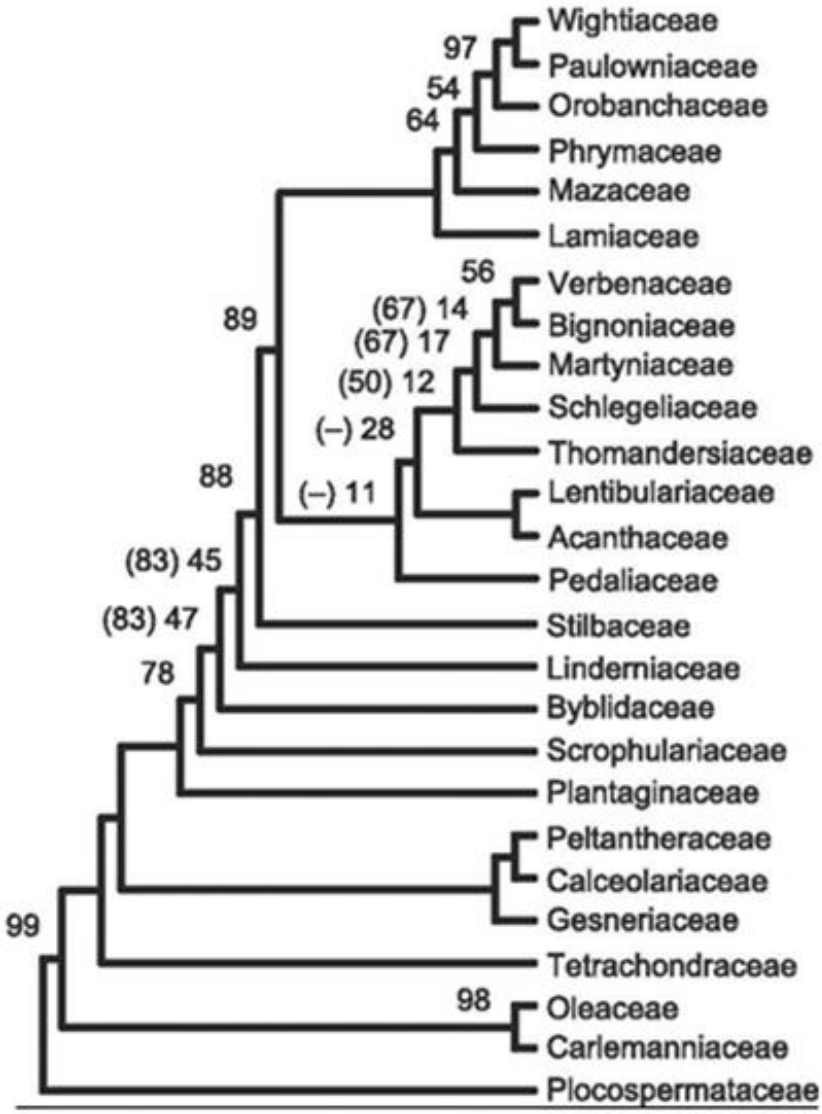



**Figure 1.** Family-level phylogenetic relationships between Lamiales families inferred by Fonseca (2021). Numbers on branches indicate two different measures of bootstrap (statistical) support for the relationship denoted by each branch, from 0 (no support) to 100 (complete support); in general, support values above 90 are considered strong while those below 70 are considered weak.

## 1.2. Description

*Lantana camara* L. *sensu lato* is a perennial shrub, generally reaching a height of 2-4 metres, characterized by its brittle nature, extensive branching, and tendency to form thickets. Young stems have a quadrangular shape, which becomes cylindrical as the plant ages. Stems are hairy and feature stout recurved prickles in weedy forms of the species. The leaves grow in pairs opposite to each other and can be oval, ovate-oblong, or broadly lance-shaped, measuring between 2 to 12 cm in length and 2 to 6 cm in width. The leaves have a finely toothed margin, a bluntly pointed tip, exhibit a rough texture with fine hairs on both surfaces, and are strongly veined on their underside. When crushed, the leaves emit a distinctive and potent fragrance. Inflorescences develop in the junctions of young leaves and are compact, ranging from flat to dome-shaped, with a diameter of 2-4 cm. Each inflorescence consists of 20-40 small, sessile flowers that display a variety of colours, but newly opened flowers typically have yellow throats, while the petals can be white, cream, yellow, pink, orange, red, or purple. Once pollinated, the flowers darken, lose their yellow centre, and eventually fall off. The fruit of *L. camara* is a round, fleshy drupe with the stone containing two embryos (usually one aborted), measuring about 5 mm in width. Initially green, the fruit transitions to purple and then turns blue-black. The root system typically comprises a short taproot with lateral roots that repeatedly divide, forming a root mat (Holm et al. 1991; Swarbrick et al. 1998; Parsons and Cuthbertson 2001).

## 1.3. Native range and centre of origin

*Lantana camara* L*. sensu stricto* is distributed in Mexico, Central America, the West Indies, and northern South America, but is only one of the species that have potentially contributed to forming the invasive lantana complex (Sanders 2006; Sanders 2012). The species of *Lantana* Section *Lantana* in their native range, where they are not usually considered to be a serious pest, are in need of further study. Although the exact origins of invasive varieties are unclear, recent population genetic analyses suggest that some weedy lantana forms found in Australia may have arisen from populations in Mexico and the Caribbean, while others may have arisen from southern Brazil (Lu-Irving et al. 2022). More detailed studies on the relationships of the weedy lantana from a global perspective are currently being conducted.

## 1.4. Introduced distribution

*Lantana camara* has a worldwide distribution, reported in over 80 countries between 35°N and 35°S, and is considered invasive throughout large parts of southern and Southeast Asia, eastern and southern Africa, Oceania, and many island countries in the Indian Ocean. Isolated populations also occur in Europe, particularly Spain and Italy, and invasive populations can be found within the native range of the section in the Americas (Day et al. 2003; Sanders 2006; Sanders 2012; Figure 2).

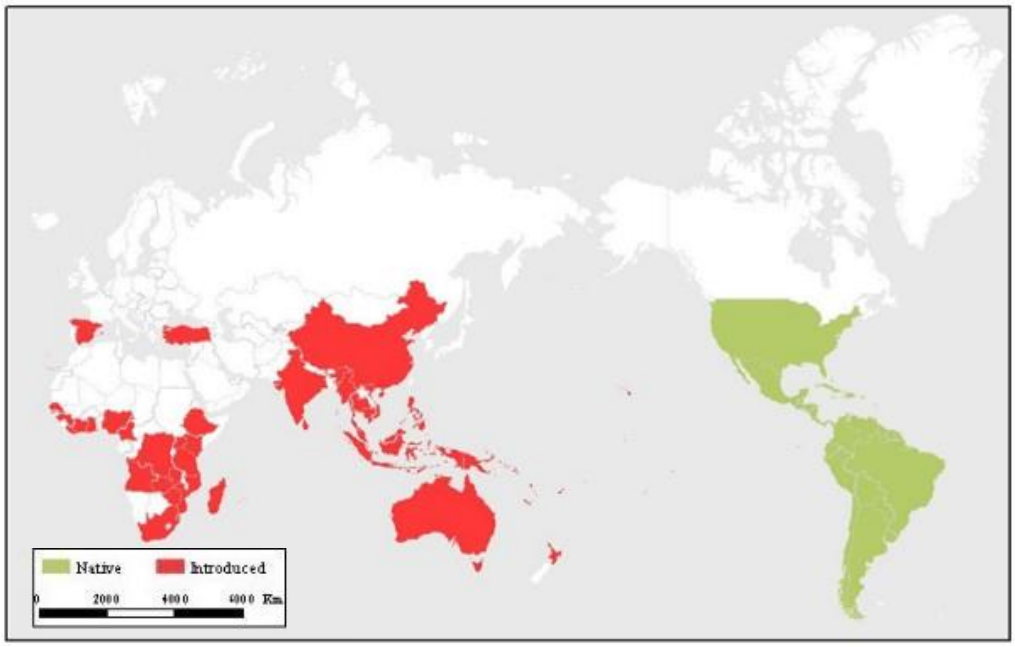



**Figure 2.** Countries in which *L. camara* has been reported. Shading represents presence but not distribution within a country (Day et al. 2003).

In Australia, lantana was first reported in 1841 as a cultivated species in the Adelaide Botanic Gardens. Since then, it has become widely established outside of cultivation and has infested an extensive area in coastal and subcoastal eastern Australia, from as far north as the Torres Strait islands to the Victorian border in the south. It is also present on Norfolk and Lord Howe Islands and as isolated infestations in the Northern Territory and Western Australia (Figure 3). About half of the populations of lantana in Australia are identified as being from two sub-lineages, corresponding broadly with the “common pink” and the "common pink-edged red” varieties described by Smith & Smith (1982; Lu-Irving et al. in prep.).

**Figure 3.** Australian distribution of *Lantana camara*. (Left) Current distribution with red dots indicating where the weed has been reported. (Right) Current suitable habitat for the weed (http://www.weedfutures.net/species.php?id=1113, accessed January 2023).

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

Lantana is one of Australia's worst invasive weeds because of its wide-ranging negative effects on the economy, society, and the environment. Its most significant economic impact is on agriculture and livestock, but the weed also poses a serious threat to plantation timber and orchard industries (AEC Group 2007). Lantana forms dense thickets that reduce pasture productivity and limit grazing areas for livestock, and some varieties of lantana found in Australia have leaves rich in alkaloids that are highly toxic to livestock. While the "red" flowered varieties are believed to be the most toxic, certain "white" and "pink" flowered varieties can also be highly toxic. When consumed by cattle and sheep, lantana can cause photosensitivity reactions, diarrhea, jaundice, hepatitis, and poisoning, eventually leading to wasting and death (Seawright 1963; Everist 1974; Culvenor 1985; Sharma et al. 1988; Wells and Stirton 1988; Sharma 1994).

Lantana can also disrupt fire regimes and pasture management due to its volatile oils, which make it highly flammable, and its tendency to form dense thickets can create high fuel loads. These factors contribute to the spread and intensity of wildfires, and areas infested with lantana have an elevated risk of fire outbreaks, endangering both natural and man-made environments (Figure 4a-c). The control and management of lantana infestations demand substantial financial resources and effort. Governments, landowners, and community groups invest significant time and funds into control measures such as mechanical removal, herbicide application, and biological control methods to combat its spread and impact. The Australian grazing industry alone incurs costs of approximately $104.3 million per year due to lost productivity and increased management expenses, with $70.8 million in Queensland and $33.4 million in New South Wales (AEC Group 2007).

Apart from the economic consequences, lantana exerts significant environmental and social impacts. The weed poses a serious threat to disturbed natural ecosystems by outcompeting native plant species, resulting in a decline in biodiversity (Figure 4d). The dense growth of lantana shades out and inhibits growth of native understorey plants, diminishing the quality of habitat for indigenous wildlife. The allelopathic effects of *L. camara* have been extensively studied, with various authors observing reduced recruitment of native seedlings in infested areas. Additionally, the excretion of phenolic compounds by lantana hampers the growth of mature trees, shrubs, and agricultural crops (Lamb 1982; Achhireddy and Singh 1984; Mersie and Singh 1987; Singh and Achhireddy 1987; Sharma et al. 1988; Gentle and Duggin 1997). While quantifying environmental and social impacts can be challenging due to the predominantly qualitative nature of available data, a study conducted by AEC group (2003) revealed that Queensland households were willing to pay an average of about $55 per year to safeguard areas of high conservation value from lantana. This indicates an estimated annual environmental impact of approximately $72.5 million to Queensland alone.

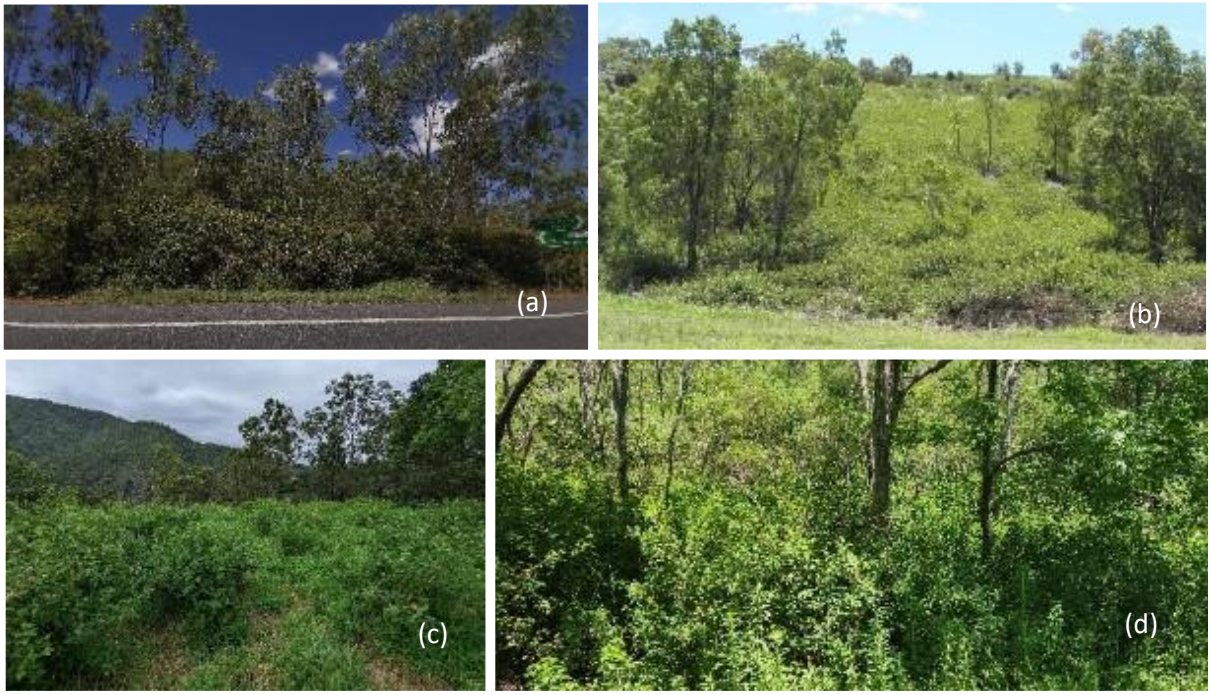



**Figure 4.** *Lantana camara* growth forms (a) along roadsides; (b, c) in pastures; and (d) natural systems.

## 1.6. Control methods available

Long term effective control of lantana requires an integrated management strategy using a combination of treatments over consecutive years. Detailed guidance is provided in the Lantana: Best Practice Manual and Decision Support Tool (Stock et al. 2009) and Lantana: Current management and control options for lantana (*Lantana camara*) in Australia (Van Oosterhout et al. 2004). A summary of effective management methods is provided below.

**Mechanical control** methods can be suitable for extensive infestations, removing large bulk of thickets via slashing, pushing or racking, grubbing, chain pulling or ploughing, and increasing accessibility for other control treatments. Mechanical control methods are only appropriate for flat country or gentle slopes, but is more effective than burning (Bartholomew and Armstrong 1978).

**Burning (or fire)** is sometimes used as pre-treatment before chemical control. It is one of the cheapest methods for controlling *L. camara* and is often used in grazing areas (Day et al. 2003). Fire can reduce the height and density of *L. camara* but will rarely kill the plants in the short-term. Fire also has a minimal effect on the seed bank (Osunkoya et al. 2013). Some authors suggest that fire increases regeneration of *L. camara*, as it can stimulate seeds in the soil to germinate. Additionally, using fire as a control method also risks the destruction of desirable vegetation and exposure of the soil, leading to increased risk of further weed invasion (Raizada and Raghubanshi 2010). If fire is used as a control method, it must be followed by other control methods such as herbicidal foliar spray of regrowth.

**Chemical control** methods for *L. camara* include foliar spray, basal bark and cut stump. Foliar spray is most successful on regrowth. However, spray drift may impact upon nearby plants. Basal bark and cut stump have the least amount of impact on native or desirable species (Diatloff and Haseler 1965; Cilliers 1983; Graaff 1986; Erasmus and Clayton 1992). All methods are most successful when the plants are actively growing (Killilea 1983; Motooka et al. 1991; Hannan-Jones 1998). Follow up treatment is essential, making chemical control more expensive than other methods. However, chemical control is often quite effective on regrowth after fire or mechanical control as herbicides can more efficiently penetrate the young leaves (Hannan-Jones 1998).

**Biological control** offers a sustainable long-term management option for lantana. To date 31 insects and one pathogen have been released in Australia, of which 20 have established. The two most widespread and damaging agents are the sap-sucking bug, *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae), and leaf-mining beetle, *Uroplata girardi* Pic (Coleoptera: Chrysomelidae). Despite a long history of biological control in Australia, lantana remains a significant weed across much of its distribution. Build-up of insect agent populations is often hindered by the weed's tendency to shed its leaves in response to dry conditions, and under favourable conditions (i.e., wet summers) lantana's capacity for rapid growth can often outpace damage inflicted by the increasing populations of insect agents following winter. High impact of established agents is often observed from about mid-summer to autumn and is conditional on the variety of lantana (Day et al. 2003).

Pathogens have great potential as agents for classical biological control of weeds and have received much attention because of their high level of host specificity. *Prospodium tuberculatum* Speg. Arthur. (Pucciniaceae) was the first rust fungus introduced into Australia for biological control efforts against lantana. This pathogen is well suited to more subtropical environments, requiring a long dew period to germinate. It was first released in 2001 and has persisted in environmentally suitable areas of coastal and subcoastal Queensland and New South Wales. Population genomic analyses have shown that *Prospodium tuberculatum* is highly specific to the "common pink" variety of lantana and does not affect other varieties, even some other pink-flowered varieties; Lu-Irving et al. (in prep.).

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

*Lantana camara* is a Weed of National Significance. Lantana is a category 3 restricted invasive plant in Queensland under the Biosecurity Act 2014, is regulated under a general biosecurity duty in New South Wales, a category 3 Declared Pest in Western Australia, a Declared noxious weed (Restricted) in Victoria, and a Class B Declared Weed in the Northern Territory. Across all jurisdictions, at a minimum, landholders

have a biosecurity responsibility to reduce the spread of the weed, and it must not be given away, sold, or released into the environment.

## 1.8. Endorsed as a target species for biological control

Biological control of *L. camara* began in 1902 when 23 insect species were sent from Mexico to Hawai'i. Since then, a total of 44 biological control agents have been deliberately introduced into 33 countries worldwide. Twenty-eight of these agents have established in at least one country. Through natural spread, these agents are now found in 65 countries worldwide (Winston et al. 2023).

The Australian lantana biological control research program commenced in 1914, with the importation of four insect species, which had successfully established in Hawai'i. The Australian Weeds Committee was established in 1983, and weeds, such as lantana, that were targets for biological control prior to its formation were retrospectively approved targets by that committee.

# 2. Information on the agent

## 2.1. Taxonomy

Kingdom: Fungi

Phylum: Basidiomycota

Class: Pucciniomycetes

Order: Pucciniales

Family: Pucciniaceae

Genus: Puccinia

Species: *lantanae* Farl.

Pathotype: IMI 398849

Common name: Lantana blister rust

Voucher specimen: A voucher herbarium specimen has been deposited in the Herb IMI collection, housed in the fungarium of the Royal Botanic Gardens at Kew, UK.

*Puccinia lantanae* has been sequenced (unpublished) with sequences deposited in GenBank (accessions LC799477.1 & LC799476.1).

## 2.2. Brief description and biology of the agent

*Puccinia lantanae* Farl. is a commonly reported leaf rust pathogen of lantana across its neotropical range. Its presence leads to the formation of "black leaf spots with a yellow halo" (Evans 1987) and angular, vein-delimited, dark brown necrotic lesions on the leaves (Barreto et al. 1995). However, these relatively mild leaf-restricted symptoms are in stark contrast with the observed severity of the disease caused by *P. lantanae* pathotype IMI398849 in Peru, and later confirmed through greenhouse screening experiments conducted by CABI-UK using a wide range of *L. camara* varieties.

*Puccinia lantanae* is an autoecious rust pathogen, meaning that it passes through all life stages on the same host, and is classified as a microcyclic, exhibiting a unique life cycle that consists of only two types of spores: basidiospores and teliospores. This was confirmed in the case of *Puccinia lantanae* pathotype IMI 398849, and microscopic examination revealed that the majority (96%) of teliospores were single-celled, while only 4% were two-celled (Figure 5a). This differs from typical *Puccinia* species, where two-celled teliospores are dominant, suggesting a closer relation to species within the genus *Uromyces* (Thomas et al. 2021).

During the infection process, provided there is sufficient moisture on the leaf surface, teliospores embedded within the host tissue (Figure 5b) germinate (Figure 5c) to

produce a four-celled metabasidium (Figure 5d). Each cell of the metabasidium develops a single sterigma with a terminal basidiospore (Figure 5e). Basidiospores are forcefully released from the teliospore, germinate, and infect the host plant by entering the epidermal cell through an appressorium (Figure 5f-g). The presence of raised ridges of plant tissue around the appressorium suggests the involvement of mechanical pressure in penetrating the host plant tissue (Thomas et al. 2021).

In highly susceptible varieties of lantana, the hypha appears to invade intracellularly, leading to the mycelium colonising actively growing tissue and causing blistering and swelling on all vegetative parts of the plant as the tissues expand (Thomas et al. 2021; Figure 6a). The mycelium eventually produces chocolate-brown telia, which merge and darken with age (Figure 6b).

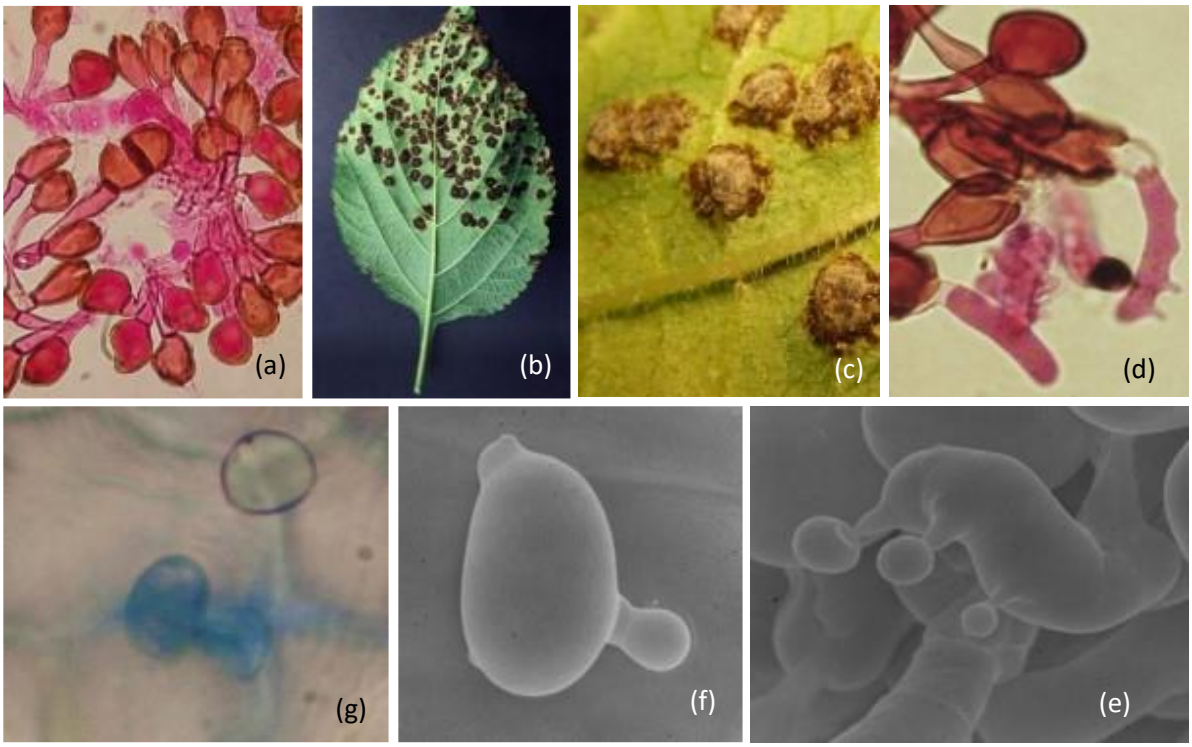



**Figure 5.** Life cycle of *Puccinia lantanae* pathotype IMI398849. a) teliospore squash showing the predominance of single-celled spores (mesospores); (b) *Lantana camara* leaf showing embedded telia; (c) telia covered with white bloom from with densely-packed, germinating teliospores; (d) metabasidia, one with four sterigmata in development; (e) scanning electron microscopy image of a basidium with developing basidiospores attached to sterigmata, originating from an embedded teliospore; (f) scanning electron microscopy image of germinating basidiospore on the leaf surface, accompanied by the presence of an appressorium; (g) close-up of leaf surface showing attempted penetration by basidiospore germ tube.

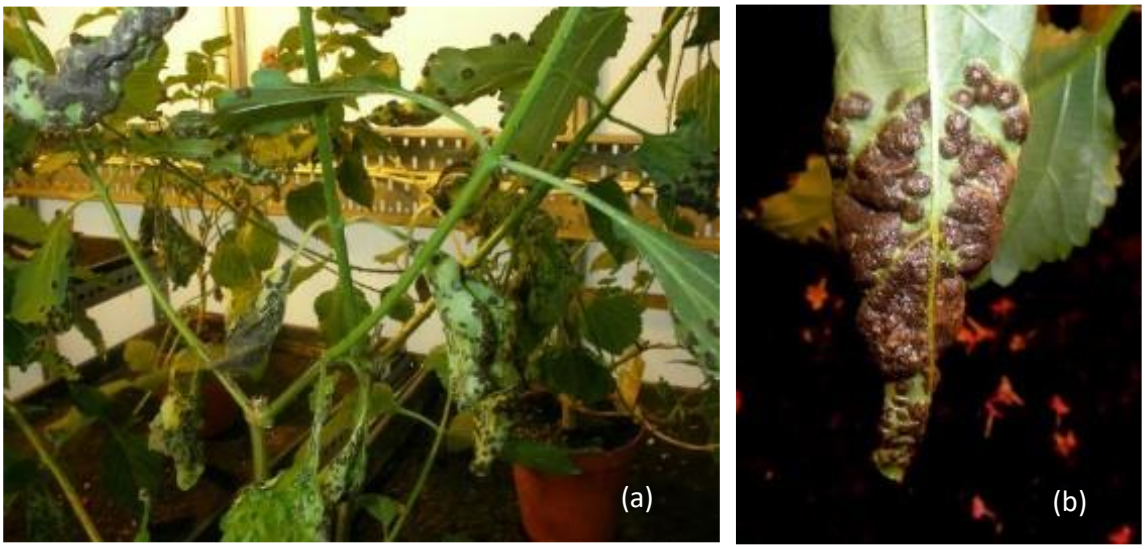



**Figure 6.** Fully susceptible symptoms of *Puccinia lantanae* pathotype IMI398849 infection on *Lantana camara* "Brisbane common pink". (a) Overall view of infect plant in quarantine glasshouse, and (b) closeup view of the dense telia merging, causing swelling and blistering of the leaf.

The development of telia on infected plants continues throughout the dew period, which can be as short as five hours. Teliospore germination and basidiospore production seemingly occur sequentially, with not all spores being produced and released simultaneously from a telium. The optimal temperature for telium formation is just below 20°C, while the minimum temperature for infection is 12°C, and no infection occurring at 30°C. However, temperatures of up to 35ºC, experienced in the quarantine glasshouse during the UK summer, did not affect disease expression in already infected plants. Chlorosis, a yellowing of the plant tissue, becomes noticeable nine days after inoculation, with pustule formation occurring approximately on day 14 (Thomas et al. 2021).

## 2.3. Native range of the agent

*Puccinia lantanae* has been reported in tropical and subtropical regions of the Americas, parts of Africa, and Asia, causing relatively mild and leaf-restricted symptoms on 19 genera across Verbenaceae, Acanthaceae, Amaranthaceae, and Lamiaceae (as summarized in Table 1) (Laundon 1963; Barreto et al. 1995; Silva et al. 2017). The proposed biological control agent, *Puccinia lantanae,* designated pathotype IMI 398849, was discovered heavily infecting populations of lantana in Tamshiyacu, Upper Amazon, Loreto region of Peru. The severity of disease expression in this population, including seedling death, had never previously been associated with this rust species before (Figure 7) (Evans 1987; Barreto et al. 1995; H.C. Evans pers. obs.).

**Table 1**. Records of *Puccinia lantanae* retrieved from Kew (IMI) and USDA-ARS databases

| Genera (no. of species) | Countries or Regions |
|---|---|
| **Family: Acanthaceae** | |
| *Barleria* (1) | Kenya |
| *Dicliptera* (1) | Taiwan |
| *Elytraria* (4) | Central and South America |
| *Hemigraphis* (1) | India |
| *Hypoestes* (1) | Philippines |
| *Justicia* (5) | China, Hong Kong, India, Japan, Nepal, Taiwan |
| *Peristrophe* (2) | India, Indonesia, Malaysia |
| *Phlogacanthus* (1) | Indonesia, Philippines |
| *Ruellia* (1) | USA |
| *Rungia* (1) | India |
| *Strobilanthes* (2) | India, Taiwan |
| **Family: Verbenaceae** | |
| *Aloysia* (1) | Mexico |
| *Lantana* (~25) | North, Central and South America, Cote d'Ivoire, Ghana |
| *Lippia* (~15) | Central and South America |
| *Phyla* (4) | Central America |
| *Priva* (2) | Central America |
| *Stachytarpheta* (1) | Bahamas |
| **Family: Amaranthaceae** | |
| *Alternanthera* (1) | Hong Kong |
| **Family: Lamiaceae (Labiatae)** | |
| *Hyptis* (1) | Dominican Republic |


**Figure 7.** *Puccinia lantanae* (pathotype IMI398849) on lantana in the forest understory, Upper Amazon, Peru: (a) upper leaves showing blistering and necrosis; (b-c) telia on the lower leaf surface.

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

There is some reason to believe that *P. lantanae* might represent a complex of cryptic species. However, resolving this requires a comprehensive taxonomic and molecular investigation. At the very least, there is strong evidence that this species consists of distinct pathotypes specialized on different plant species and, in some cases, on different genotypes within the same plant species. To some extent, host usage appears to be correlated with the highly variable teliospore biometrics recorded across the species. For instance, the teliospore measurements of *P. lantanae* pathotype IMI398849 fall on the smaller end of the size range reported in the original description by Farlow (1883) and are within the range of the smallest teliospores among seven isolates examined by Barreto et al. (1995). *Puccinia lantanae* isolated from *Lippia alba* in Itabuna, Brazil (Silva et al. 2017) were reported to be significantly longer than that from *Lantana* spp. examined by Barreto et al. (1995), and interestingly, from *L. alba* from Lavras, Brazil reported by Lima et al. (2004) (Table 2).

More recently, three isolates of *Puccinia lantanae*, collected from *P. nodiflora* in different provenances in Argentina, have been examined for their potential as biological control agents for *Phyla nodiflora* var*. minor* (syn*. P. canescens* (Kunth) Greene), a serious weed in Australia*.* Of these isolates, two were recommended as having good potential, infecting only *P. nodiflora* var*. minor* and *P. nodiflora* var. *reptans*, and not infecting *P. nodiflora* var. *nodiflora* (putative importance in Australia) (Traversa et al. 2022). Authors reported unicellular teliospore measurements similar

to those reported by Thomas et al. (2021), but bicellular teliospores were more constricted and consistent with those reported by Barreto et al. (1995). It is unclear, however, whether Traversa et al. (2022) took biometric measurements of all three isolates, or just a representative of the collection. It is noteworthy that the two prospective isolates were collected from localities at about 23°S, whilst the unsuitable isolate was collected further south at about 31°S.

**Table 2**. Available biometric data of microcylic *Puccinia lantanae* (in µm*)*

| Puccinia lantanae isolate | Host | Unicellular teliospores | | Bicellular teliospores | |
|---|---|---|---|---|---|
| | | (Length x width) | (mean) | (Length x width) | (mean) |
| Linquist (1982) (ex-Argentina) | *Lantana* sp. | 22 - 28 x 15 - 21 | - | 20 - 42 x 15 - 21 | - |
| Farlow (1983) (ex-USA) | *L. odorata* | 23 - 27 x 15.5 - 20 | - | 26 - 38 x 19 - 26 | - |
| IMI 361062 Barreto et al. (1998) (ex-Rio de Janeiro) | *L. lilacina* | 18 - 32 x 15 - 20 | 25 x 16 | 24 - 32 x 13 - 22 | 28 x 15 |
| IMI 361063 Barreto et al. (1998) (ex-Rio de Janeiro) | *L. lilacina* | 14 - 35 x 11 - 28 | 23 x 17 | 22 - 35 x 14 - 18 | 26 x 15 |
| IMI 345374 Barreto et al. (1998) (ex-Rio de Janeiro) | *L. camara* | 21 - 31 x 41 - 20 | 25 x 17 | 22 - 46 x 14 - 22 | 30 x 17 |
| IMI 38054 Barreto et al. (1998) (ex-Ghana) | *L. camara* | 28 - 35 x 14 - 24 | 32 x 20 | 35 - 49 x 17 - 28 | 38 x 21 |
| IMI 361064 Barreto et al. (1998) (ex-Columbia) | *L. camara* | 28 - 42 x 15 - 22 | 26 x 16 | 36 - 42 x 20 - 28 | 31 x 20 |
| Lima et al. (2004) (ex-Brazil) | *Lippia alba* | 19 - 27 x 17 - 22 | - | 22 - 32 x 15 - 19.5 | - |
| Silva et al. (2017) (ex-Brazil) | *Lippia alba* | 54 - 76 x 19 - 20 | - | 63 - 75 x 16 - 20 | - |
| IMI 398849 * Thomas et al. (2021) (ex-Peru) | *L. camara* | - | 22 x 18 | - | 28 x 21 |
| Traversa et al. (2022) (ex-Argentina) | *Phyla nodiflora* | 17 - 28.5 x 13.5 - 23 | | 23 - 36 x 12 - 23 | |

* *P. lantanae* (pathotype IMI 398849), proposed biological control agent in this report.

## 2.5. Proposed source of the agent

The purified isolate of *P. lantanae* (pathotype IMI 398849), originally collected from heavily infected lantana in Tamshiyacu, Peru, and used in all host-specificity experiments conducted by CABI-UK, is currently held by CABI in the United Kingdom.

## 2.6. Agent's potential for control of the target

Rust pathogens, in general, have several characteristics that grant them high potential for weed biological control. Rust pathogens are obligate parasites, requiring a living plant host to complete development. During infection the pathogen extracts nutrients and other essential elements required for the healthy growth of their plant hosts. This nutrient-drain weakens the plant host and can result in nutrient deficiencies, causing yellowing of leaves (chlorosis) and overall decline in plant health. The characteristic rust pustules that develop on the leaves of plant hosts disrupt the normal functioning of plant cells and tissues, leading to a reduction in photosynthesis, which can result in stunted growth and decreased vigour. As the infection progresses, the pathogen can further damage plant tissues, rupturing the cell walls and weakening the structural integrity of leaves and stems, leading to wilting, leaf drop, and even breakage under severe infection. These physiological stresses can weaken the overall health and defence mechanisms of the plant host, making it more susceptible to other stresses, such as drought, extreme temperatures, or additional diseases and pests. Nineteen species in the genus *Puccinia*, have been utilised for weed biological control against 19 weed species, of which 15 species are in the family Asteraceae (Table 3; Winston et al. 2023).

*Puccinia lantanae* (pathotype IMI 398849) itself, has been demonstrated to be a highly damaging rust pathogen that infects the leaves, petioles, and stems of lantana, resulting in cankering and stem die-back. Systemic infections are often formed within the vascular tissue, resulting in stunting of the shoot, and ultimate death of the whole apex (Figure 6-7). Symptoms such as these are expected to have the most significant impact on seedlings and young plants, contributing to control of lantana by reducing the recruitment, growth, and spread of weed populations. The Peruvian pathotype would be well suited to tropical and subtropical regions in Australia, and in particular the wet tropics where lantana is a serious threat. Biology studies also indicate that teliospores do not all start germinating at the same time, mitigating against wastage if the high humidity that stimulates germination falls before completion of basidiospore release and host plant infection. Moreover, post-infection temperatures of 35$^{o}$C experienced in the quarantine glasshouse during the UK summer did not appear to affect disease expression.

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

Verbenaceae is a family of mainly tropical flowering plants containing trees, shrubs, and herbs, distinguishable by opposite leaves and generally bilaterally symmetrical flowers arranged in often crowded inflorescences. The family Verbenaceae currently includes 34 genera and approximately 800 recognized species (Cardoso et al. 2021; O'Leary et al. 2023). In Australia, Verbenaceae are predominantly introduced ornamental and/or weed species. The genera present in Australia which are most closely related to lantana are (in order of closest to furthest relationship) *Lippia, Phyla, Aloysia, Verbena, Glandularia, Citharexylum, Stachytarpheta,* and *Duranta* (Marx et al. 2010; Atlas of Living Australia).

Notable cultivated species include *Aloysia triphylla* (L'Hér.) Britton (Lemon verbena), *Citharexylum spinosum* L. (Fiddlewood) and *Duranta erecta* L. (of which Geisha girl and Sheena's gold are popular varieties). Some *Duranta* cultivars are capable of self-seeding, and the numerous berries produced by this species are easily spread by birds. As such, *Duranta* has in some areas escaped cultivation and is considered an environmental weed in Queensland and northern New South Wales, and a "sleeper weed" in other parts of Australia. *Stachytarpheta* species are listed on the Australian permitted seed list, although multiple species have already escaped cultivation and are reported as serious environmental weeds along coastal and subcoastal Queensland and the Northern Territory. *Lippia alba*, *Phyla nodiflora* and *Phyla nodiflora* var. *minor* (syn. *Phyla canescens*) are also all introduced plants, which have become invasive in limited regional areas (though some authors have considered local populations of the cosmopolitan *P. nodiflora* to be part of its native distribution; Gross et al. 2017).

Twelve species of *Verbena* have been reported in Australia. Up to five of these taxa have been considered native to Australia (Munir 2002), or treated as varieties of *Verbena officinalis*, which has a distribution that includes Europe, Africa, Asia, and the Americas, and is generally considered exotic to Australia (Munir 2002). The status of all putatively native taxa is not universally accepted among plant taxonomists. Michael (1997) recognises these as native species, whereas Munir (2002) and O'Leary (2010) do not. In Australia, the concepts by Michael (1997) are currently accepted, but the World Flora Online (WFO 2022), follows the most recent taxonomic treatment (O'Leary 2010) and considers all so-called native Australian taxa within the genus *Verbena* to be synonyms of cosmopolitan or American taxa.

**Table 3**. Species of *Puccinia* utilised as weed biological control. All weed species belong to the family Asteraceae except where indicated (adapted from Winston et al. 2023).

| Biological control agent | Isolate/strain/ pathotype | Target weed | Countries established |
|---|---|---|---|
| *Puccinia myrsiphylli* (Thüm.) G.Winter | | *Asparagus asparagoides* (L.) Druce ^ | AU, NZ |
| *Puccinia xanthii* f. sp. *ambrosiae-trifidae* S.W.T.Batra | | *Ambrosia trifida* L. | CN |
| *Puccinia evadens* Harkn. | | *Baccharis halimifolia* L. | AU |
| *Puccinia eupatorii* Dietel | | *Campuloclinium macrocephalum* (Less.) DC. | ZA |
| *Puccinia carduorum* Jacky | Isolate III | *Carduus nutans* L.<br>*Carduus tenuiflorus* Curtis | CA, USA<br>USA |
| *Puccinia cardui-pycnocephali* P.Syd. & Syd. | IT2, FR3 | *Carduus pycnocephalus* L.,<br>*Carduus tenuiflorus* Curtis | AU<br>AU |
| *Puccinia jaceae* var. *diffusae* Savile | | *Centaurea diffusa* Lam. | CA, USA |
| *Puccinia jaceae* var. *solstitialis* Savile | | *Centaurea solstitialis* L. | USA |
| *Puccinia chondrillina* Bubák & Syd. | IT32, IT36, TU788, PC-1, PC-16 | *Chondrilla juncea* L. | AU, AR, CA, USA |
| *Puccinia punctiformis* (F. Strauss) Röhl. | | *Cirsium arvense* (L.) Scop. | NZ, USA |
| *Puccinia spegazzinii* De Toni | Isolate IMI 393075 | *Mikania micrantha* Kunth | CK, FJ, PNG, SOL, TW, VU |
| *Puccinia abrupta* var. *partheniicola* (H.S.Jacks.) Parmelee | | *Parthenium hysterophorus* L. | AU, BT, ET, IN, KE, MU, NP, PK, CN, ZA, TZ |
| *Puccinia xanthii* var. *parthenii-hysterophorae* Seier, H.C.Evans & Á.Romero | | *Parthenium hysterophorus* L. | AU, ZA |
| *Puccinia hieracii* var. *piloselloidarum* (Probst) Jørst. | | *Pilosella officinarum* Vaill. | NZ |
| *Puccinia acroptili* P.Syd. & Syd. | | *Rhaponticum repens* (L.) Hidalgo | CA, USA |
| *Puccinia* xanthii Schwein. | | *Xanthium strumarium* L. | AU, CK, LK, TL |
| *Puccinia komarovii* var. *glanduliferae* R.A.Tanner, C.A.Ellison, L.Kiss & H.C.Evans | India strain<br>Pakistan strain | *Impatiens glandulifera* Royle ~ | ENG |
| *Puccinia arechavaletae* Speg. | | *Cardiospermum grandiflorum* Sw. * | CK, ZA |
| *Puccinia rapipes* Berndt & E. Uhlmann | Ex Western Cape | *Lycium ferocissimum* Miers + | AU - Too early |
| *Puccinia lantanae* Farl. | Pathotype<br>IMI 398849 | *Lantana camara* L.° | NZ - Too early,<br>ZA - Too early |

^ Asparagaceae; ~ Balsaminaceae; * Sapindaceae; + Solanaceae; ° Verbenaceae

AU-Australia; AR-Argentina; BT-Butan; CA-Canada; CK-Cook Islands; CN-China; ENG-England; ET-Ethopia; FJ-Fiji; IN-India; KE-Kenya; LK-Sri Lanka; MU-Mauritius; NP-Nepal; NZ-New Zealand; PK-Pakistan; PNG-Papua New Guinea; SOL-Solomons; TL-Timor Leste; TW-Taiwan; TZ-Tanzania; USA-United States of America; VU-Vanuatu; ZA-South Africa

## 2.8. Possible interactions, including conflict with existing biological control programs

*Puccinia lantanae* in its native range occurs predominantly in tropical climates, whereas *Prospodium tuberculatum* is a subtropical rust. They have not been recorded to occur in the same area in their native ranges. Under controlled conditions, when both rust species were inoculated together onto susceptible *L. camara*, no negative interactions were observed, and they infected and sporulated on the plants as they would if inoculated separately (S.E. Thomas, pers. obs.). In Australia, it is expected that the two rusts will not overlap significantly in the field as they are likely to occur and operate in different climatic niches. *Puccinia lantanae* would be expected to be more prevalent in tropical northern Queensland, and *Prospodium tuberculatum* would favour New South Wales, the subtropical regions of southern Queensland and some higher elevation areas in north Queensland.

## 2.9. Where, when, and how releases will be made

Upon attaining approval to release *P. lantanae* in Australia, a pure laboratory culture of *P. lantanae* spores (pathotype IMI 398849) will be imported into the high security quarantine facility at the Ecosciences Precinct, Brisbane, Australia (QAP No: 2274, QC level 5.3 and QIC level 7.3). Identification of the sample and proof of non-contamination will be provided by a Department of Agriculture, Fisheries and Forestry approved Pathologist and a qualified Queensland Department of Primary Industries Pathologist following arrival.

In the Controlled Environment Room (CER) of this facility, Australian grown *L. camara* “common pink” variety (within a plastic lined cage) will be inoculated with the *P. lantanae* spores to induce infection (as described below in the inoculation methods section). Plants will be maintained in gauzed cages in the adjacent glasshouse for pustules to develop. Once the pathogen has undergone at least one generation under biosecurity containment in Australia, the agent will be released under the supervision of a Department of Agriculture, Fisheries and Forestry biosecurity officer and used to inoculate “common pink” lantana plants, grown outside of biosecurity containment to facilitate field releases.

Initial releases of *Puccinia lantanae* will target the highly susceptible “common pink” and moderately susceptible “common pink-edged red” varieties, and avoid less susceptible and/or less widespread hosts with similar floral colour morphotypes. Given the difficulty in accurately identifying lantana to sub-lineage based on floral colour alone, the distributions of “common pink” and “common pink-edged red” lantana have been modelled based on genetically verified occurrence data (Appendix 1. Supplementary report; Lu-Irving 2023). Prospective releases will be conducted within areas where susceptible hosts are most likely to be distributed (Figure 8), and targeted

populations will be genetically verified as well as morphologically confirmed to match the descriptions.

Release sites will be monitored routinely to record establishment, dispersal and impacts. Biosecurity Officers will inspect plants in all directions from the release plants and record the incidence and severity of the pathogen damage. As the rust pathogen dispersal is wind mediated, the distance from the release location and the distance between survey plants will increase. If the pathogen fails to establish after initial releases, further techniques may be applied to increase the chances of establishment in the field.

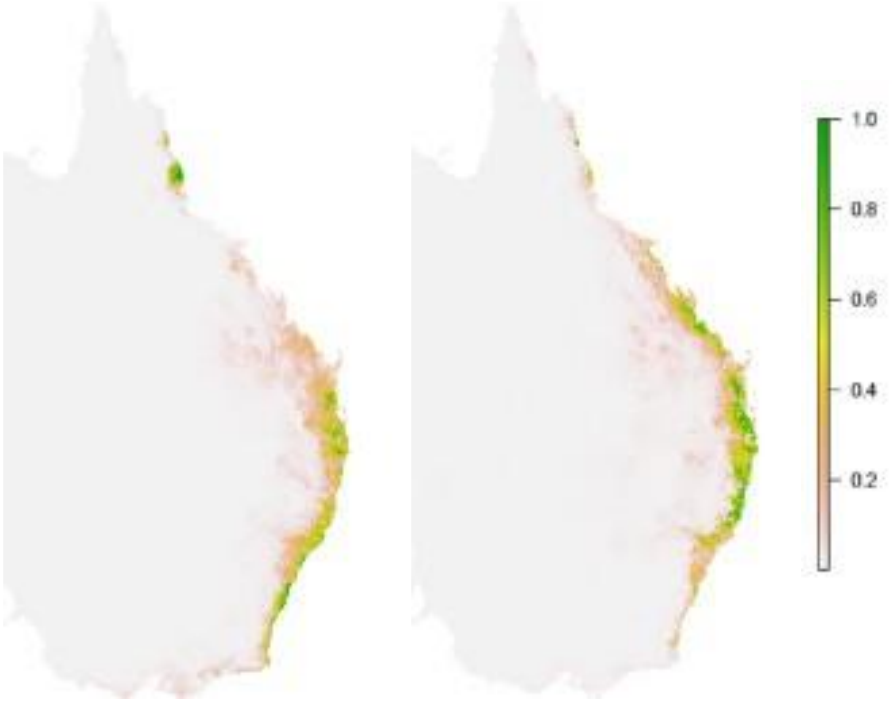



**Figure 8.** Result of MaxEnt species distribution modelling of (a) common pink lantana and (b) common pink-edged red lantana from genetically-verified occurrence data. Green indicates a highly suitable environment for common pink and common pink-edged red, and grey indicates unsuitable environment.

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

*Puccinia lantanae* (pathotype IMI 398849) has been host tested, approved for release, and field released in New Zealand and South Africa. The pathogen was first released in New Zealand in autumn 2015, with subsequent releases following. Establishment has not been confirmed. However, lantana is not as prevalent in New Zealand as it is in Australia and South Africa, and only two sub-lineages pink and orange. New Zealand has a far more temperate climate and may not be climatically suited to the pathogen. In South Africa, a release program has only just commenced.

## 2.11. Host specificity testing in the biosecurity containment facility

A purified isolate of *P. lantanae* (pathotype IMI 398849), originally collected from heavily infected lantana in Tamshiyacu Peru, was used for host specificity testing at CABI in the UK (Genbank accessions LC799477.1 & LC799476.1). The methodology used for host testing *P. lantanae* (pathotype IMI 398849) is fully described in Thomas et al. (2021) and was similar to previously developed methods for screening other microcyclic rusts that have been approved for release for weed biological control in Australia (e.g., *Puccinia spegazzinii* against *Mikania micrantha* Kunth) (Evans and Ellison 2005). A summary of methodology is provided below.

### 2.11.1 Test list

The list of non-target plant species used to test the host specificity of *P. lantanae* (pathotype IMI 398849) was developed based on the modernisation of the centrifugal phylogenetic method (Briese 2006; Gilbert et al. 2012). This method works on the premise that relationships between plants, insects and pathogens are influenced by chemical, morphological and life history traits, which are often phylogenetically conserved. Gilbert et al. (2012) found that the probability that plant species will share insects and pathogens declines as a continuous function of phylogenetic distance between plant species. As such, test plant species are first selected from the same genus as the target species, followed by species from the same tribe or sub-family, followed by a selection of species of more distantly related plants within the same family and fewer plants within closely related families. In the past, host test lists also included species that were phylogenetically very distant from the target species – often because they were economically important. However, this practice has been discontinued as it doesn't assist in determining host-range (Gilbert et al. 2012).

Plant species were all selected within the order Lamiales. A total of 24 species representing seven of the eight genera from the Verbenaceae family, and a further 31 species from 14 other families namely Acanthaceae, Bignoniaceae, Boraginaceae, Calceolariaceae, Gesneriaceae, Lamiaceae, Lentibulariaceae, Oleaceae, Onagraceae, Orobanchaceae, Phrymaceae, Plantaginaceae, Scrophulariaceae and Tetrachondraceae were tested (Table 4). Within the family Verbenaceae, the genera present in Australia which are most closely related to lantana are (in order of closest to furthest relationship) *Lippia, Phyla, Aloysia, Verbena, Glandularia, Citharexylum, Stachytarpheta,* and *Duranta* (Marx et al. 2010; Atlas of Living Australia).

The genus *Glandularia* was not represented in host testing of *Puccinia lantanae.* Prior to taxonomic revision, and at the time of host testing, this species was named *Verbena aristigera* S. Moore. The genus *Glandularia* is represented by only one species in Australia, *Glandularia aristigera* (S. Moore) Tronc., an environmental weed known as Mayne's pest, naturalised in New South Wales, Queensland, and South Australia.

# 2.11.2 Materials and Methods

## 2.11.2.1 Plant propagation

The “Brisbane common pink” lantana plants, which served as positive controls in each experiment, were cultivated from seeds or stem cuttings provided by the Queensland Government (formerly The Department of Employment, Economic Development, and Innovation).

Most non-target test plants were either propagated from seeds or stem cuttings and were obtained from Manaaki Whenua Landcare Research (New Zealand), Agricultural Research Council Plant Protection Research Institute (South Africa), or the Queensland Government (Australia). Every attempt was made to obtain host test plants from more than one source in their respective country of origin. Surrogate and horticultural species were also acquired from nurseries in the UK.

All plants were grown in a mixture of 50% John Innes No.2 soil-based compost and 50% peat-based compost. The plants were carefully maintained in a climate-controlled quarantine glasshouse with a minimum day-time temperature of 25°C, a minimum night-time temperature of 20°C, and a 12-hour light/12-hour dark lighting schedule.

## 2.11.2.2 Inoculation methods

*Puccinia lantanae* pathotype IMI 398849, which was obtained from infected lantana plants in Peru, was used in all the host testing. Plant material (stems, petioles and leaves) infected with teliospores (between 25 and 40 days old) were suspended over the test plants, under conditions of high humidity, using a dew chamber (Fig. 9). Two inoculation methods were employed:

1) **Qualitative method**: All test plants were challenged with inoculum containing dense teliospores measuring at least 5 mm². This was achieved by either directly placing the inoculum onto new shoot tips and securing it with petroleum jelly or suspending the inoculum within 1 cm of the meristem. Multiple pieces of inoculum were used per plant to ensure the release of basidiospores onto the most susceptible plant tissue. Additionally, 1-5 leaves containing at least 10 x 5 mm² telia were suspended approximately 10 cm above the test plants on a wire rack.
2) **Quantitative method**: In addition to the qualitative method, *Verbena africana and V. gaudichaudii* test plants were challenged with four different inoculum concentrations, comprising an increasing number of telia (approximately doubling in number for each concentration). Slightly different methods were used to obtain inoculum concentrations and for testing of the two *Verbena* species (described below).

**Table 4.** List of all plant species used to test the specificity of Puccinia lantanae (pathotype IMI 398849) in biosecurity containment facility in CABI UK. All species are within the order Lamiales.

| Family | Species | Status in AU | Source |
|---|---|---|---|
| **Verbenaceae** | *Lantana camara* L. (Brisbane common Pink^) | Target weed | QDPI Brisbane |
| | *Lantana lilacina* Desf. | | Peru |
| | *Lantana montevidensis* (Spreng.) Briq. | Naturalised weed | QDPI |
| | *Lantana peduncularis* Andersson | | Galapagos |
| | *Lantana rugosa* Thunb. | | ARC-PPRI |
| | *Aloysia triphylla* (L'Hér.) Britton | Horticultural | Perhill Plants, UK |
| | *Aloysia citriodora* Paláu | Horticultural | ARC-PPRI |
| | *Citharexylum spinosum* L. | Horticultural | QDPI – SEQ |
| | *Duranta erecta* L. | Horticultural | Burncoose Nurseries, UK & QDPI |
| | *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson | Naturalised | QDPI – CQ |
| | *Lippia javanica* (Burm F) Spreng | | ARC-PPRI |
| | *Lippia rehmannii* H. Pearson | | ARC-PPRI |
| | *Lippia scaberrima* Sond. | | ARC-PPRI |
| | *Lippia wilmsii* H. Pearson | | ARC-PPRI |
| | *Lippia* sp. A | | ARC-PPRI |
| | *Lippia* sp. B | | ARC-PPRI |
| | *Phyla nodiflora* L. Greene | Naturalised/ Putative native | Perhill Plants, UK & QDPI |
| | *Phyla nodiflora* var. *minor* (Gillies & Hook.) N.O'Leary & Múlgura (syn. *Phyla canescens* (Kunth) Greene) | Naturalised weed | ARC-PPRI & QDPI |
| | *Priva meyeri* Jaub. & Spach | | ARC-PPRI |
| | *Stachytarpheta australis* Moldenke | Naturalised weed | QDPI |
| | *Verbena africana* (R.Fern. & Verdc.) P.W.Michael | Putative native | QDPI |
| | *Verbena gaudichaudii* (Briq.) P.W.Michael | Putative native | QDPI |
| | *Verbena rigida* Spreng. | Naturalised weed | QDPI |
| | *Verbena bonariensis* L. | Naturalised weed | QDPI |
| | *Verbena litoralis* Kunth | Naturalised weed | QDPI |
| **Lamiaceae** | *Glossocarya hemiderma* (F.Muell. ex Benth.) Benth. ex B.D.Jacks | Native | QDPI |
| | *Gmelina leichardtii* F.Muell. ex Benth. | Native | QDPI |
| | *Mentha cunninghamii* Benth. | Congener | Landcare, NZ |
| | *Plectranthus argentatus* S.T. Blake | Native | QDPI |
| | *Plectranthus parviflorus* Willd. | Ornamental | QDPI |
| | *Scutellaria novae-zelandiae* Hook.f. | Congener | Landcare, NZ |
| | *Teucrium parvifolium* (Hook.f.) Kattari et Salmaki | Congener | Landcare, NZ |

| | | | |
|---|---|---|---|
| | *Vitex triflora* Vahl | Native | QDPI |
| **Bignoniaceae** | *Tecomanthe hillii* (F. Muell.) Steenis | Native | QDPI |
| **Acanthaceae** | *Acanthus mollis* L. | Ornamental | Chiltern Seeds, UK |
| | *Avicennia marina* (Forssk.) Vierh. | Native | Landcare, NZ |
| | *Graptophyllum excelsum* (F.Muell.) Druce | Native | QDPI |
| | *Hypoestes sanguinolenta* (Van Houtte) Hook. F. | Naturalised weed | Chiltern Seeds, UK |
| **Boraginaceae** | *Cordia dichotoma* G. Forst | Native | QDPI |
| **Calceolariaceae** | *Jovellana violacae* (Cav.) G.Don | Ornamental | Constantine Garden Nursery, UK |
| **Gesneriaceae** | *Saintpaulia ionantha* H. Wendl. | Ornamental | Longacres Nursey, UK |
| **Lentibulariaceae** | *Utricularia dichotoma* Labill. | Native | Hewitt-Cooper carnivorous plants, UK |
| **Oleaceae** | *Nestegis lanceolata* (Hook.f.) L.A.S.Johnson | | Landcare, NZ |
| **Onagraceae** | *Calylophus serrulatus* (Nutt.) P.H.Raven | | Chiltern Seeds, UK |
| **Orobanchaceae** | [+]*Euphrasia officinalis* L. | Congener | Herbiseed, UK |
| **Phrymaceae** | *Glossostigma elatinoides* (Benth.) Hook.f | Native | Landcare, NZ |
| | [+]*Mazus novae-zelandiae* R.Br. | Congener | Landcare, NZ |
| | [+]*Mimulus repens* R.Br. | Native | Landcare, NZ |
| **Plantaginaceae** | *Callitriche muelleri* Sond. | Native | Landcare, NZ |
| | *Gratiola sextendata* A.Cunn. | Congener | Landcare, NZ |
| | *Hebe pinguifolia* Cockayne & Allan | Ornamental | Saville Garden Nursery, UK |
| | *Ourisia coccinea* (Cav.) Pers | | Kevock Gardens, UK |
| | [+]*Plantago masonae* Cheeseman | Congener | Landcare, NZ |
| **Scrophulariaceae** | *Limosella lineata* Glück syn *L.australis* R. Br. | Native | Landcare, NZ |
| | *Myoporum laetum* G. Forst. | Congener | Landcare, NZ |
| **Tetrachondraceae** | *Tetrachondra hamiltonii* Petrie ex Oliv. | | Landcare, NZ |

[+]Unresolved name; QDPI – Queensland Department of Primary Industries; ARC-PPRI – Agricultural Research Council, Plant Protection Research Institute.

### 2.11.2.3 Host specificity testing of *Puccinia lantanae*

In addition to the non-target test plant species, 29 lantana floral colour morphotypes were tested for susceptibility to *P. lantanae* using the qualitative method (Figure 9). Each test run included four replicate plants and a positive control (Brisbane common pink variety, known to be fully susceptible to the rust). Every effort was made to standardize the plants used in the testing process, ensuring that they were young (less than three months old), had at least six branches or shoots, and were in a vigorous growth phase.

All test plants were inoculated using the qualitative method, in a dew chamber maintained at a temperature of 20°C for 48 hours. After this period, the inoculum was inspected to evaluate the level of sporulation, and the test plants were examined to confirm their position under the inoculum. The test plants were then returned to the chamber and monitored for symptoms for a duration of six weeks. If, during the initial inspection, the inoculum had not sporulated or the plant shoots had not remained under the inoculum during the inoculation period, and no symptoms were observed after six weeks, these plants were excluded from the analysis.

Microscopic analysis, using a leaf clearing-staining technique (Bruzzese and Hasan 1983), was only undertaken to investigate the interaction between *P. lantanae* and the non-target test species where minor symptoms appeared (i.e. macro-symptom score of 1). On the few plant species on which sporulation was achieved and telia had developed (i.e. macro-symptom scores of 2 and above), the teliospores were used to inoculate other plants of the same species, as well as a lantana control plant (Brisbane common pink).

### 2.11.2.4 Quantitative assessment of susceptibility of *Verbena* species

*Verbena africana* and *V. gaudichaudii*, two non-target species of proposed importance to Australia, were further tested using the quantitative method. For *V. africana*, diameters of the telia used to achieve each concentration were added together totalling 20 mm (approximately four telia), 40 mm, 80 mm, and 160 mm. Each group of telia was suspended over individual test plants, as described above, with four replicate plants, each with four young shoots. For *V. gaudichaudii,* detached infected lantana leaves containing a known number of *P. lantanae* telia (10, 16, 32, and 80) were suspended above four test plants, each with a minimum of four young shoots. Following the dew period, sporulation was determined using dissecting microscope and if needed was adjusted. The number of resulting telia was recorded after 40 days and the whole experiment was repeated twice, using new plants (i.e. eight different plants were tested in total).

**Figure 9.** Dew chamber used for rust infection in all experiments. (left) qualitative method of inoculation of host test plants; (right) quantitative assessment of susceptibility of *Verbena gaudichaudii.*

## 2.11.2.4 Assessment of infection

Non-target test plants were assessed for susceptibility to *Puccinia lantanae* using a qualitative scoring system (Table 5; Figure 10.). A "+" and "-" denoted after the score indicates that a result is "just in" or "nearly better" than the allocated category.

**Table 5**. Categories used to classify the response of the test plants to *Puccinia lantanae*.

| Score | Susceptibility | Macro-Symptoms |
|---|---|---|
| 0 | Immune | None |
| 1 | Resistant | Necrosis and/or chlorosis on inoculated leaves – no further development |
| 2 | Weakly susceptible | Very sparse, small, restricted telia (<2 mm in diameter) |
| 3 | Moderately susceptible | Many small, restricted telia (2-3 mm in diameter) |
| 4 | Fully susceptible | Large telia when not dense (4-5 mm diameter), reduced size when dense. |

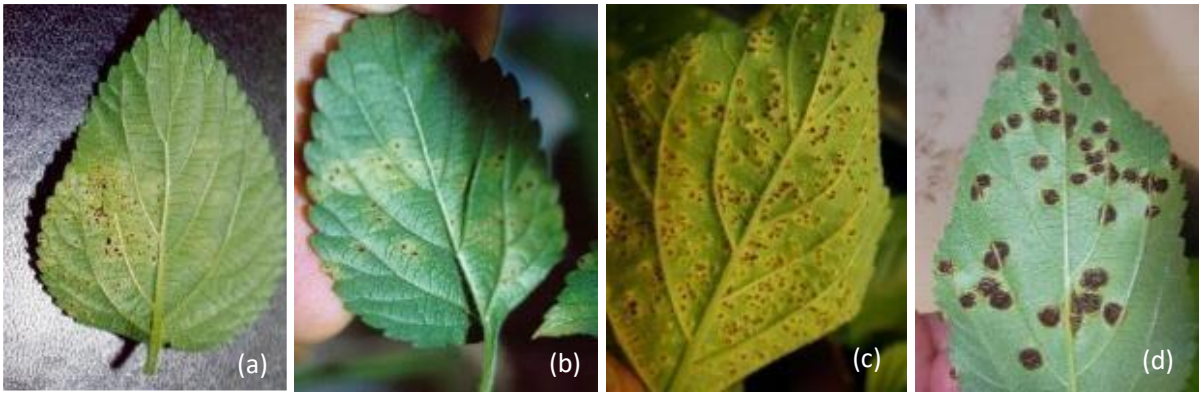



**Figure 10.** Susceptibility symptoms of different forms of lantana to *P. lantanae.* (a) Ithaca pink-edged red, score 2; (b) Kenmore pink, score 3-; (c) lantana species ex Ecuador, score 3+; (d) Brisbane common pink, score 4.

## 2.11.3 Results

The detailed findings of the host-specificity testing can be found in the research conducted by Thomas et al. (2021) (provided in Appendix 2). The latent period for disease expression is about 5-7 days (Renteria and Ellison 2004). However, this will vary depending on the susceptibility of the plant species and most importantly the temperature the plants are held at after infection (S. Thomas, pers comm. 2024).

Susceptibility of lantana flower colour morphotypes ranged from fully susceptible to resistant. In general, “common pink” lantana was demonstrated be highly susceptible to *Puccinia lantanae*, “common pink-edged red” was found to be at least moderately susceptible, and other (less widespread) varieties varying from susceptible to resistant (Appendix 1).

During the host specificity testing, only *L. camara* exhibited complete susceptibility to *P. lantanae* pathotype IMI 398849. Among the non-target species tested, four non-target species, *Verbena africana* (R.Fern. & Verdc.) P.W.Michael, *V. gaudichaudii* (Briq.) P.W.Michael*, Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson*,* and *Phyla nodiflora* var*. minor* (Gillies & Hook.) N.O'Leary & Múlgura, were classified as weakly or moderately susceptible to the rust pathogen (macro-symptom scores of 2 to 3; Table 6). Successful sporulation on these species produced only a small number of viable teliospores, which when re-inoculated onto different plants of the same species, failed to induce further infection.

*Puccinia lantanae* produced some small necrotic areas and/or very small chlorotic spots on four other non-target test species, *Lantana rugosa* Thunb., *Citharexylum spinosum* L., *Phyla nodiflora* L. Greene (ex QDPI), and *Priva meyeri* Jaub. & Spach. The pathogen did not develop any further on these plants, and they were determined to be resistant (macro-symptom score of 1). Microscopic examination revealed attempted penetration of the leaf surface by the rust germ tube. However, no further internal development occurred beyond this initial stage.

There was significant variation in the susceptibility of both *V. africana* and *V. gaudichaudii,* with plants of both species ranging from being resistant to moderately susceptible (Figure 11-12). *Verbena africana* sourced from central Queensland displayed an atypical response to the pathogen, developing teliospores on both the upper and lower surface of the leaves, indicative of a suspected systemic infection. A few plants of *V. gaudichaudii* did develop apparent systemic infection sites, but the resultant teliospores failed to geminate and were adjudged to be abnormal and non-viable (Thomas et al. 2021).

A quantitative assessment of susceptibility of both *Verbena africana* and *V. gaudichaudii* determined that, in general, as the amount of inoculum increased, so too did the level of infection, but the results were variable. In all situations, telia produced on either species failed to infect the same species when reinoculated, despite being able to infect the control lantana plants. A medium dose of inoculum (about 40mm in diameter, or about 16 telia) was required to induce any infection on the two verbena species. At this dose, 147 (±76) telia were produced on *V. gaudichaudii* plants, whereas only 5.25 (±2.72) were produced on *V. africana* plants, suggesting that the former species may be more susceptible to the rust pathogen. However, none of the *V. gaudichaudii* plants died during the experimentation and in all cases, plants outgrew their infection and completely recovered.


**Figure 11.** Reaction of *Verbena gaudichaudii* to high concentrations of *P. lantanae* inoculum. (a) typical leaf infection, score 3-. (b - e) Atypical infection; (b) stem telia, (c) localised systemic infection, and (d-e) systemic infection of meristem.

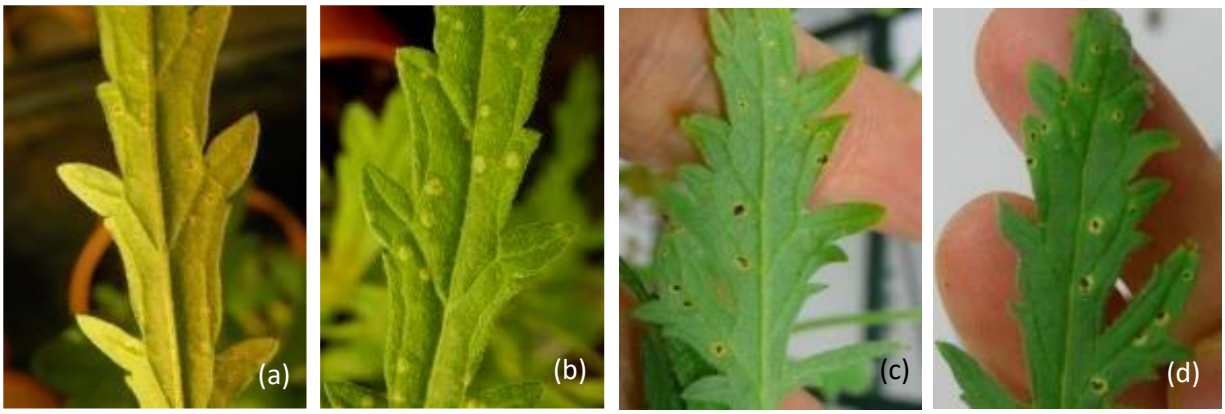



**Figure 12.** Variable reaction of *Verbena africana* to high concentrations of *P. lantanae* inoculum: ex NSW [score 2] (a) lower leaf surface, (b) upper leaf surface; ex central Qld [score 2+] (c) lower leaf surface, (d) upper leaf surface. The dark brown areas visible in the lesions (chlorotic areas) are small telia containing viable teliospores.

**Table 6**. Results of host specificity testing of *Puccinia lantanae* (pathotype IMI 398849) in biosecurity containment facility in CABI-UK. Highest susceptibility score across all replicates was used to determine overall susceptibility of plant species.

| Family | Species | Highest score | Susceptibility | All scores |
|---|---|---|---|---|
| Verbenaceae | *Lantana camara* (Brisbane common pink) | 4 | Fully susceptible | 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4 |
| | *Lantana lilacina* | 0 | Immune | 0 |
| | *Lantana montevidensis* | 0 | Immune | 0 |
| | *Lantana peduncularis* | 0 | Immune | 0 |
| | *Lantana rugosa* | 1 | Resistant | 0, 0, 1,1, 1, 1, 1, 1, 1 |
| | *Aloysia triphylla* | 0 | Immune | 0 |
| | *Aloysia citriodora* | 0 | Immune | 0 |
| | *Citharexylum spinosum* | 1+ | Resistant | 1-, 1-, 1, 1, 1, 1, 1+, 1+ |
| | *Duranta erecta* (QDPI) | 0 | Immune | 0 |
| | *Duranta erecta* (UK) | 0 | Immune | 0 |
| | *Lippia alba* | 2- | Weakly susceptible | 2-, 2-, 2-, 2-, 2-, 2-, 2- |
| | *Lippia javanica* | 0 | Immune | 0 |
| | *Lippia rehmannii* | 0 | Immune | 0 |
| | *Lippia scaberrima* | 0 | Immune | 0 |
| | *Lippia wilmsii* | 0 | Immune | 0 |
| | *Lippia* sp. *A* | 0 | Immune | 0 |
| | *Lippia* sp. *B* | 0 | Immune | 0 |
| | *Phyla nodiflora* (QDPI) | 1- | Resistant | 0, 0, 0, 0, 1-, 1-, 1- |
| | *Phyla nodiflora* (UK) | 2 | Weakly susceptible | 1, 2, 2, 2, 2, 2, 2, 2, 2 |
| | *Phyla nodiflora* var. *minor* (QDPI) | 2+ | Weakly susceptible | 0, 0, 2+, 2+, 2+ |
| | *Phyla nodiflora* var. *minor* (ARC-PPRI) | 2 | Weakly susceptible | 2, 2, 2, 2, 2, 2, 2, 2 |
| | *Priva meyeri* | 1 | Resistant | 1, 1, 1, 1, 1, 1, 1, 1 |
| | *Stachytarpheta australis* | 0 | Immune | 0 |
| | *Verbena africana* (NSW) | 3- | Moderately susceptible | 2-, 2-, 2, 2, 3-, 3- |
| | *Verbena africana* (QLD) | 2+ | Weakly susceptible | 2+, 2+ |
| | *Verbena gaudichaudii* | 3- | Moderately susceptible | 2+, 2+, 2+, 2+, 2+, 2+, 2+, 2+, 2+, 2+, 2+, 2+, 2+, 2+, 2+, 3-, 3- |
| | *Verbena rigida* | 0 | Immune | 0 |
| | *Verbena bonariensis* | 0 | Immune | 0 |
| | *Verbena litoralis* | 0 | Immune | 0 |
| Lamiaceae | *Glossocarya hemiderma* | 0 | Immune | 0 |

| Family | Species | Highest score | Description | Susceptibility scores |
|---|---|---|---|---|
| Lamiaceae | *Gmelina leichardtii* | 0 | Immune | 0 |
| | *Mentha cunninghamii* | 0 | Immune | 0 |
| | *Plectranthus argentatus* | 0 | Immune | 0 |
| | *Plectranthus parviflorus* | 0 | Immune | 0 |
| | *Scutellaria novae-zelandiae* | 0 | Immune | 0 |
| | *Teucrium parvifolium* | 0 | Immune | 0 |
| | *Vitex triflora* | 0 | Immune | 0 |
| Bignoniaceae | *Tecomanthe hillii* | 0 | Immune | 0 |
| Acanthaceae | *Acanthus mollis* | 0 | Immune | 0 |
| | *Avicennia marina* | 0 | Immune | 0 |
| | *Graptophyllum excelsum* | 0 | Immune | 0 |
| | *Hypoestes sanguinolenta* | 0 | Immune | 0 |
| Boraginaceae | *Cordia dichotoma* | 0 | Immune | 0 |
| Calceolariaceae | *Jovellana violacae* | 0 | Immune | 0 |
| Gesneriaceae | *Saintpaulia ionantha* | 0 | Immune | 0 |
| Lentibulariaceae | *Utricularia dichotoma* | 0 | Immune | 0 |
| Oleaceae | *Nestegis lanceolata* | 0 | Immune | 0 |
| Onagraceae | *Calylophus serrulatus* | 0 | Immune | 0 |
| Orobanchaceae | *+Euphrasia officinalis* | 0 | Immune | 0 |
| Phrymaceae | *Glossostigma elatinoides* | 0 | Immune | 0 |
| | *+Mazus novae-zelandiae* | 0 | Immune | 0 |
| | *+Mimulus repens* | 0 | Immune | 0 |
| Plantaginaceae | *Callitriche muelleri* | 0 | Immune | 0 |
| | *Gratiola sextendata* | 0 | Immune | 0 |
| | *Hebe pinguifolia* | 0 | Immune | 0 |
| | *Ourisia coccinea* | 0 | Immune | 0 |
| | *+Plantago masonae* | 0 | Immune | 0 |
| Scrophulariaceae | *Limosella lineata,* syn *L.australis* | 0 | Immune | 0 |
| | *Myoporum laetum* | 0 | Immune | 0 |
| Tetrachondraceae | *Tetrachondra hamiltonii* | 0 | Immune | 0 |

[+]Unresolved name

# 3. Discussion

*Puccinia lantanae* is a microcyclic, autoecious rust pathogen commonly reported on *Lantana* spp. across its neotropical range, and documented on other genera in Verbenaceae, Acanthaceae, Amaranthaceae, and Lamiaceae throughout tropical and subtropical regions of the Americas, parts of Africa, and Asia (Laundon 1963; Barreto et al. 1995; Silva et al. 2017). Typical symptoms have tended to be mild and restricted to the leaves.

*Puccinia lantanae* pathotype IMI 398849 was discovered heavily infecting populations of *Lantana camara* in Tamshiyacu, Loreto region of Peru. The severity of disease expression, which includes high mortality of seedlings, is in stark contrast to known associations with this rust species (Evans 1987; Barreto et al. 1995; H.C. Evans pers. obs.). The severity of disease expression was confirmed through comprehensive greenhouse experiments conducted by CABI-UK using a wide range of *L. camara* varieties.

Host specificity testing was undertaken on a comprehensive set of representative non-target plant species covering a broad range of diversity within Verbenaceae, as well as closely related families within the Order Lamiales. Among the tested species, only *L. camara* was fully susceptible to *P. lantanae* pathotype IMI 398849 and four non-target species, *V. africana, V. gaudichaudii, L. alba,* and *Phyla nodiflora* var. *minor* (syn. *P. canescens*), were classified as weakly or moderately susceptible. No species outside Verbenaceae showed any symptoms of infection and were all considered immune to *P. lantanae*.

*Lippia alba* is an aromatic plant native to Mesoamerica. The species is exotic to Australia, with localised distributions reported in central and north Queensland and in the Northern Territory. *Phyla nodiflora* var. *minor* is native to South America and considered a serious environmental and agricultural weed in sub-tropical and temperate Australia. In Queensland, this species is considered a well-established weed in the Condamine and Border Rivers catchments, as well as posing serious threat to the Murray-Darling River system. In New South Wales, *P. nodiflora* var. *minor* poses a major threat to Lachlan River and Murrumbidgee River floodplains, *Phyla nodiflora* var. *minor* is an approved target for biological control in Australia.

Among the species examined, *V. africana* and *V. gaudichaudii* have implications for Australia. These species are widely distributed in New South Wales and Queensland, and there is a belief that they are native to these regions (Michael 1997). However, the claim of their native status is not universally accepted by plant taxonomists who consider them to be variations of cosmopolitan or American taxa, specifically *Verbena officinalis* (Munir 2002, O'Leary 2010). Nevertheless, both species have been found to be weakly to moderately susceptible to the rust pathogen under optimal conditions for infection. Interestingly, there was considerable variation in susceptibility among the individual plants tested, with some plants displaying resistance even when exposed to high concentrations of the pathogen under these optimal conditions. This suggests

significant genetic diversity within populations of these species. In every case, *V. gaudichaudii* plants outgrew and fully recovered from infection by the *P. lantanae* pathotype IMI 398849 (Thomas et al. 2021).

The presence of genetic variation and differential susceptibility among individual plants of *V. africana* and *V. gaudichaudii* would reduce the potential risk posed by the rust pathogen to these species. Furthermore, since the rust pathogen cannot persist on either species, it would require a continuous source of inoculum from nearby susceptible populations of *L. camara* for subsequent infections to occur. However, the opportunity for such is rather limited as *V. africana* and *V. gaudichaudii* have a more inland distribution in Queensland and New South Wales, and a much-reduced occurrence in coastal and subcoastal areas (Figure 13). Lantana, on the other hand, is primarily distributed in coastal and subcoastal regions, with limited penetration further inland (Figure 13-14). Some areas of overlap do exist, particularly in some coastal cities and further inland around Toowoomba.

Modelled distributions of “common pink” and “common pink-edged red” lantana, based on genetically verified occurrence data, indicate that Toowoomba is not particularly suitable for either of these highly susceptible lantana lineages (Figure 14). This, in combination with the higher elevations of the Toowoomba range, which may not be particularly suitable for the rust pathogen, mean that concerted releases and or establishment of *P. lantanae* pathotype IMI 398849 would be unlikely in this region.

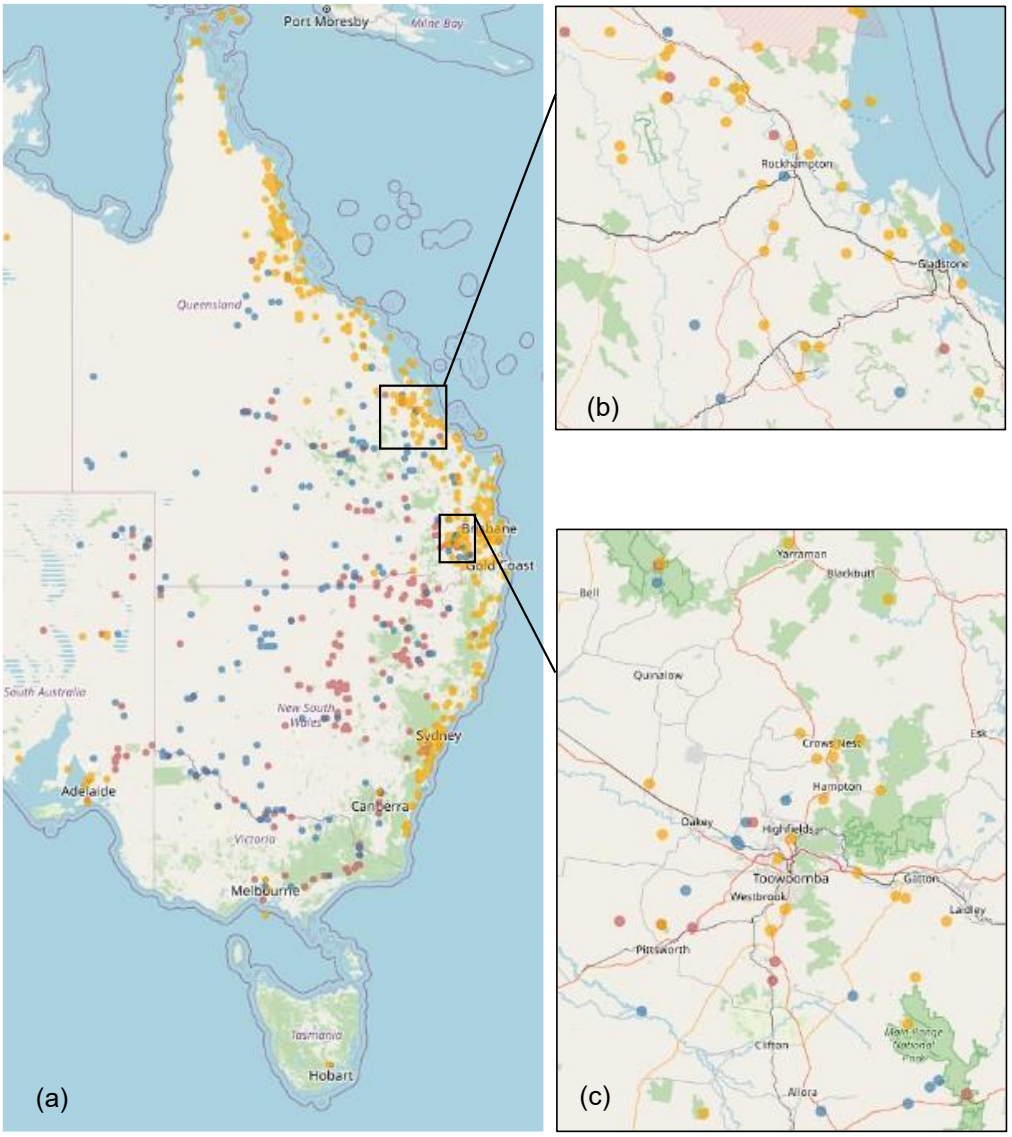



**Figure 13.** (a) Distribution of *Verbena africana* (blue), *V. gaudichaudii* (red), and lantana (yellow) in eastern Australia. (b) Zoomed in view of the overlapping distribution of the two *Verbena* species and lantana, in central Queensland around Rockhampton. (c) Zoomed in view of the furthest western overlapping distribution of the two *Verbena* species and lantana, around Toowoomba. Occurrence data was obtained from Atlas of Living Australia and was filtered by points containing preserved specimens from 1970 to 2020 (The Atlas of Livings Australia's Spatial Portal; Atlas of Living Australia occurrence download 10.26197/ala.1784384a-a68d-4f40-8bce-ebb1b41f6469 Accessed 28 June 2023).

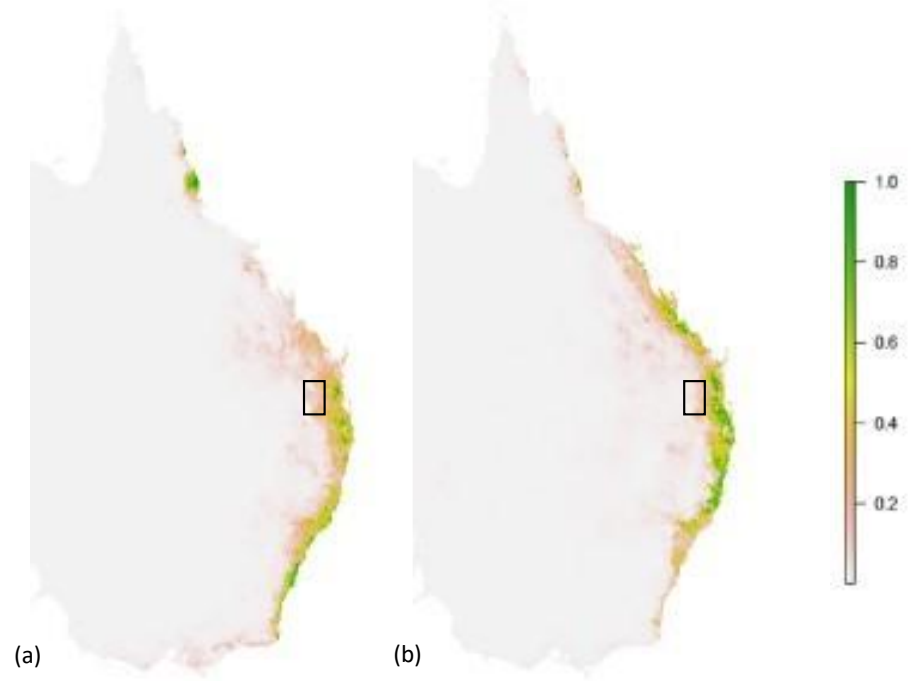



**Figure 14.** Result of MaxEnt species distribution modelling of (a) common pink lantana and (b) common pink-edged red lantana from genetically-verified occurrence data. Green indicates a highly suitable environment for common pink and common pink-edged red, and grey indicates unsuitable environment. Box indicates Toowoomba region where occurrence data for *Verbena africana* and *V. gaudichaudii* exists (see Figure 13c).

# 4. Conclusion

The findings from surveys conducted in Peru, along with laboratory experiments conducted at CABI-UK (Thomas et al. 2021), provide support for considering *P. lantanae* pathotype IMI 398849 as a potentially effective biological control agent for lantana in Australia. This proposed agent demonstrates the ability to cause severe damage to highly susceptible lantana varieties such as "common pink" and "common pink-edged red", which comprise a the majority of lantana populations in Queensland and New South Wales. In regions with moist weather conditions, particularly along the eastern coast, or following rainfall, it is anticipated that there will be repeated infections of plants and the production of abundant teliospores. While the effects of *Puccinia lantanae* pathotype IMI 398849 on older, larger, established plants is difficult to predict, it is expected to have the most significant impact on seedlings and young plants, contributing to control of lantana by reducing the recruitment, growth, and spread of weed populations. There is a level of risk to *V. africana* and *V. gaudichaudii* plants growing in close proximity to susceptible lantana varieties. However, neither of these species were fully susceptible to the rust, and both species are primarily distributed in areas that are not suitable for either the rust pathogen or the susceptible lantana varieties. Therefore, the risk to these species associated with releasing *P. lantanae* pathotype IMI 398849 in Australia would be minimal.

# 5. References

Achhireddy, N. R., & Singh, M. (1984). Allelopathic effects of Lantana (*Lantana camara*) on milkweedvine (*Morrenia odorata*). *Weed Science, 32*, 757-761.

AECgroup. (2003). Economic assessment of environmental weeds in Queensland. Retrieved from Brisbane.

AECgroup. (2007). Economic impact of lantana on the Australian grazing industry. Retrieved from Brisbane.

Barreto, R. W., Evans, H. C., & Ellison, C. A. (1995). The mycobiota of the weed *Lantana camara* in Brazil, with particular reference to biological control. *Mycological Research, 99*(7), 769-782.

Bartholomew, B. L., & Armstrong, T. R. (1978). A new look at lantana control. *Queensland Agricultural Journal, July-August*, 339-344.

Briese, D.T. (2006). Host specificity testing of weed biological control agents: Initial attempts to modernise the centrifugal phylogenetic method. Paper presented at the Fifth Annual California Conference on Biological Control (CCBC V), 25–27 July 2006, Riverside, California.

Bruzzese, E., & Hasan, S. (1983). A whole leaf clearing and staining technique for host specificity studies of rust fungi. *Plant Pathology, 32*, 335-338. https://doi.org/10.1111/j.1365-3059.1983.tb02841.x

Cardoso, P. H., O'Leary, N., Olmstead, R. G., Moroni, P., & Thode, V. A. (2021). An update of the Verbenaceae genera and species numbers. *Plant Ecology and Evolution, 154*(1), 80-86. Retrieved from https://www.jstor.org/stable/27000385

Cilliers, C. J. (1983). The weed, *Lantana camara* L., and the insect natural enemies imported for its biological control into South Africa. *Journal of the Entomological Society of Southern Africa, 46*(1), 131-138.

Culvenor, C. C. J. (1985). Economic loss due to poisonous plants in Australia. In Proceedings of the Australia-U.S.A. Poisonous Plants Symposium (pp. 3-13). Queensland Department of Primary Industries. Brisbane, Australia.

Day, M. D., Wiley, C. J., Playford, J., & Zalucki, M. P. (2003). Lantana: current management status and future prospects. *ACIAR Monograph No. 102*. ACIAR, Canberra, Australia.

Diatloff, G., & Haseler, W. H. (1965). Varietal differences in lantana. In *Australian Weeds Conference*, Toowoomba, Queensland (pp. 11-12).

Erasmus, D. J., & Clayton, J. N. G. (1992). Towards costing chemical control of *Lantana camara* L. *South African Journal of Plant Soil, 9*(4), 206-210.

Evans, H. C. (1987). Fungal pathogens of some subtropical and tropical weeds and the possibilities for biological control. *Biocontrol News and Information, 8*, 7-30.

Evans, H. C., & Ellison, C. A. (2005). The biology and taxonomy of rust fungi associated with the neotropical vine Mikania micrantha, a major invasive weed in Asia. *Mycologia, 97*(4), 935-947.

Everist, S. L. (1974). *Poisonous Plants of Australia*. London: Angus and Robertson Publishers.

Farlow, W. G. (1883). Notes on some species in the third and eleventh centuries of Ellis's North American Fungi. *Proceedings of the American Academy of Arts and Sciences, 18*, 65-85.

Fonseca, L. H. M. (2021). Combining molecular and geographical data to infer the phylogeny of Lamiales and its dispersal patterns in and out of the tropics. *Molecular Phylogenetics and Evolution, 164*, 107287.

Gentle, C. B., & Duggin, J. A. (1997). *Lantana camara* L. invasions in dry rainforest - open forest ecotones: The role of disturbances associated with fire and cattle grazing. *Australian Journal of Ecology, 22*, 298-306.

Gilbert, G. S., Magarey, R., Suiter, K., & Webb, C. O. (2012). Evolutionary tools for phytosanitary risk analysis: phylogenetic signal as a predictor of host range of plant pests and pathogens. *Evolutionary Applications, 5*(8), 869-878.

Graaff, J. L. (1986). *Lantana camara*, the plant and some methods for its control. *South African Forestry Journal, March*, 26-30.

Gross, C. L., Fatemi, M., Julien, M., McPherson, H., & Van Klinken, R. (2017). The Phylogeny and biogeography of *Phyla nodiflora* (Verbenaceae) reveals native and invasive lineages throughout the world. *Diversity, 9*(20).

Gujral, G. S., & Vasudevan, P. (1983). *Lantana camara* L., a problem weed. *Journal of Scientific and Industrial Research, 42*, 281-286.

Hannan-Jones, M. A. (1998). The seasonal response of *Lantana camara* to selected herbicides. *Weed Research, 38*, 413-423.

Holm, L. G., Plucknett, D. L., Pancho, J. V., & Herberger, J. P. (1991). *Lantana camara* L. Verbenaceae, verbena family. In L. G. Holm, D. L. Plucknett, J. V. Pancho, & J. P. Herberger (Eds.), *The world's worst weeds: distribution and biology* (pp. 299-302). Krieger Publishing Company, Malabar, Florida.

Howard, R. A. (1969). A check list of cultivar names used in the genus *Lantana*. *Arnoldia, 29*(11), 73-109.

Killilea, D. M. (1983). Chemical control of *Lantana camara* L. 2. Foliar spray applications of various arboricides. *Zimbabwe Journal of Agricultural Research, 21*, 59-67.

Lamb, R. (1982). Some effects of *Lantana camara* on community dynamics of Eucalypt woodland. In *52nd ANZAAS Congress* (pp. 304-305).

Laundon, G. F. (1963). Rust fungi 1: on Acanthaceae. *Mycol. Papers, 89*, 1–89.

Lima, C. S., Souza, P. E., & Botelho, A. O. (2004). Fungos da família Pucciniaceaecausadores de ferrugem em plantas medicinais. *Fitopatologia. Brasileira, 29*, 499-503.

Lu-Irving, P., Encinas-Viso, F., Callander, J., Day, M. D., & Le Roux, J. (2022). New insights from population genomics into the invasive *Lantana camara* L species complex. In C. Brodie, J. Emms, L. Feuerherdt, S. Ivory, R. Melland, & S. Potter (Eds.), *Proceedings of the 22nd Australasian Weeds Conference* (pp. 45-47). Weed Management Society of South Australia Inc., Adelaide.

Marx, H. E., O'Leary, N., Yuan, Y. W., Lu-Irving, P., Tank, D. C., Mulgura, M. E., & Olmstead, R. G. (2010). A molecular phylogeny and classification of Verbenaceae. *American Journal of Botany, 97*(10), 1647-1663. doi:10.3732/ajb.1000144

Mersie, W., & Singh, M. (1987). Allelopathic effect of lantana on some agronomic crops and weeds. *Plant and Soil, 98*, 25-30.

Michael, P. W. (1997). Notes on *Verbena officinalis* sensu stricto and *V. macrostachya* (Verbenaceae) with new combinations in two closely related taxa. *Telopea, 7*(3), 293-297.

Motooka, P., Ching, L., & Nagai, G. (1991). Mesulfuron efficacy on *Lantana camara* with different surfactants. Paper presented at the *Proceedings of the 13th Asian-Pacific Weed Conference*, Brisbane, Australia.

Munir, A. A. (2002). A taxonomic revision of the genus *Verbena* L. (Verbenaceae) in Australia. *Journal of the Adelaide Botanical Gardens, 18*(1), 21-103.

O'Leary, N., Lu-Irving, P., Moroni, P., Salimena, F.R.G., dos Santos Silva, T.R., Cardoso, P.H., Mirra, F., Schaefer, J., Bedoya, A.M., Viccini, L.F., Bitencourt, C., Thode, V.A., & Olmstead, R.G. (2023). Making Lantaneae (Verbenaceae) taxonomy useful: A phylogenetic classification. *TAXON, 72*, 572-589. https://doi.org/10.1002/tax.12934

O'Leary, N., Múlgura, M. E., & Morrone, O. (2010). Revisión taxonómica de las Especies del Género Verbena L. (Verbenaceae) II: Serie Verbena. *Annals of the Missouri Botanical Garden, 97*(3), 365-424.

Osunkoya, O. O., Perrett, C., Fernando, C., Clark, C., & Raghu, S. (2013). Modelling population growth and site-specific control of the invasive *Lantana camara* L. (Verbenaceae) under differing fire regimes. *Population ecology, 55*(2), 291-303.

Parsons, W. T., & Cuthbertson, E. (2001). *Noxious weeds of Australia*. CSIRO publishing.

Raizada, P., & Raghubanshi, A. S. (2010). Seed germination behaviour of *Lantana camara* in response to smoke. *Tropical Ecology, 51*(2), 347-352.

Renteria, J. L., & Ellison, C. A. (2004). Potential biological control of *Lantana camara* in the Galápagos using the rust *Puccinia lantanae*. *SIDA, Contributions to Botany, 21*, 1009-1017.

Sanders, R. W. (2006). Taxonomy of Lantana Sect. Lantana (Verbenaceae): I. Correct application of Lantana camara and associated names. *Sida, 22*, 381-421.

Sanders, R. W. (2012). Taxonomy of Lantana sect. Lantana (Verbenaceae): II. Taxonomic revision. *Journal of the Botanical Research Institute of Texas, 6*(2).

Scott, L. J., Graham, G. C., Hannan-Jones, M., & Yeates, D. K. (1997). DNA profiling resolves the limited importance of flower colour in defining varieties of *Lantana camara*. *Electrophoresis, 18*, 1560-1563.

Seawright, A. A. (1963). Studies on experimental intoxication of sheep with Lantana camara. *Australian Veterinary Journal, 39*, 340-344.

Sharma, O. P. (1994). Plant toxicosis in north-western India. In S. M. Colgate & P. R. Dorling (Eds.), *Plant-associated Toxins: Agricultural, Phytochemical and Ecological Aspects* (pp. 19-24). CAB International. Wallingford, U.K.

Sharma, O. P., Makkar, H. P. S., & Dawra, R. K. (1988). A review of the noxious plant *Lantana camara*. *Toxicon, 26*(11), 975-987.

Silva, C. S. d., Pereira, M. B., & Pereira J. (2017). Report of *Puccinia lantanae* on *Lippia alba* in the state of Bahia, Brazil *Agrotrópica, 29*(2), 173-176.

Singh, M., & Achhireddy, N. R. (1987). Influence of lantana on growth of various citrus rootstocks. *Hortscience, 22*(3), 385-386.

Smith, L. S., & Smith, D. A. (1982). The naturalised *Lantana camara* complex in eastern Australia. *Queensland Botany Bulletin, 1*, 1-26.

Stirton, C. H. (1977). Some thoughts on the polyploidy complex *Lantana camara* L. (Verbenaceae). In *Proceedings of the Second National Weeds Conference of South Africa* (pp. 321-340). Stellenbosch, South Africa.

Stock, D., Johnson, K., Clark A., & Van Oosterhout, E. (2009). Lantana: best practice manual and decision support tool. Department of Employment, Economic Development and Innovation, Brisbane, Qld, Australia.

Swarbrick, J. T., Willson, B. W., & Hannan-Jones, M. A. (1998). *Lantana camara* L. In F.D. Panetta, R.H. Groves, & R.C.H. Shepherd (Eds.), *The Biology of Australian Weeds* (pp. 119-140). R. G. & F. J. Richardson, Melbourne.

The Atlas of Livings Australia's Spatial Portal. (n.d.). *Atlas of Living Australia occurrence download*. 10.26197/ala.1784384a-a68d-4f40-8bce-ebb1b41f6469. Retrieved from http://www.ala.org.au. Accessed on 28 June 2023.

Thomas, S. E., Evans, H. C., Cortat, G., Koutsidou, C., Day, M. D., & Ellison, C. A. (2021). Assessment of the microcyclic rust *Puccinia lantanae* as a classical biological control agent of the pantropical weed *Lantana camara*. *Biological Control, 104688*.

Traversa, G., Sosa, A., Chantre, G. R., & Bianchinotti, M. V. (2022). Biological studies of *Puccinia lantanae*, a potential biocontrol agent of "Lippia" (*Phyla nodiflora* var. *minor*). *Agronomía Colombiana, 40*, 383-394. 10.15446/agron.colomb.v40n3.103562.

Van Oosterhout E., Clark A., Day M. D., & Menzies E. (2004). Lantana Control Manual. Current Management and Control. Options for Lantana (*Lantana camara*) in Australian State of Queensland. Department of Natural Resources, Mines and Enegry, Brisbane, Qld, Australia. Available from URL: http://www.nrm.qld.gov.au/pests/wons/Lantana. Accessed 23 November 2004.

Wells, M. J., & Stirton, C. H. (1988). *Lantana camara* a poisonous declared weed. *Farming in South Africa, Weeds (A.27)*, 1-4.

Winston R.L., Schwarzländer M., Hinz H.L., Day M.D., Cock M.J.W., & Julien M.H. (Eds.). (2023). *Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds*. Based on FHTET-2014-04, USDA Forest Service, Forest Health Technology Enterprise Team. Available online at https://www.ibiocontrol.org/catalog/. Accessed 12 July 2023.

World Flora Online. (n.d.). *Published on the Internet*. http://www.worldfloraonline.org. Accessed on 05 Jul 2023.

# Susceptible host characterisation and recommendations to promote successful release of *Puccinia lantanae*

*This report was prepared as part of the project "Weed Genomics for Improved Lantana Biocontrol" supported by the Department of Agriculture, Forestry, and Fisheries (activity ID 4-FY9KQZ3)*

April 2023

Research Centre for Ecosystem Resilience
Botanic Gardens of Sydney

The introduced species complex commonly known as lantana (*Lantana camara* L *sensu lato*) is a Weed of National Significance in Australia, causing substantial economic and environmental impacts despite decades of control efforts. Biocontrol has many potential benefits as a control solution for this weed, and lantana biocontrol is an active area of research and development. A national-scale study of lantana population genomic variation was undertaken to support biocontrol programs to achieve optimal outcomes. One goal was to identify genetic sub-lineages within the species complex, and characterise their susceptibility to biocontrol agents with previously observed host preferences.

The rust *Puccinia lantanae* is an agent which has shown variable success on different target host individuals during host-specificity trials (Thomas *et al*. 2021; Table 1). However, patterns of host susceptibility did not covary consistently with floral colour morphotype. To predict the susceptibility of specific wild lantana populations prior to the agent's release, additional information must be considered. Here, lantana sub-lineage identity and distribution, as characterised by population genomic analysis, are reported on to maximise efficiency of targeted release by voiding non-susceptible host populations.

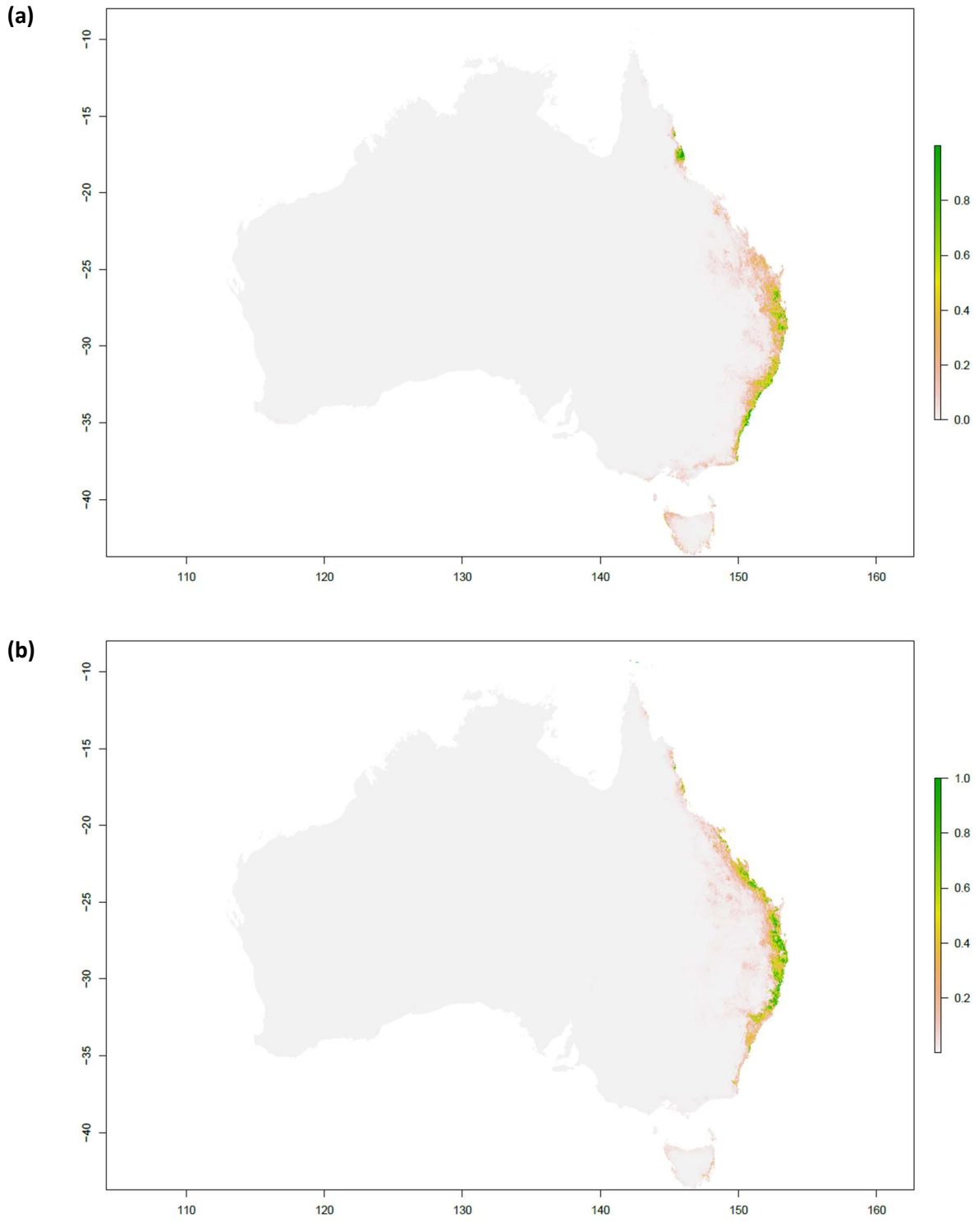
Invasive lantana consists of several divergent sub-lineages, and gene flow among them is limited, consistent with the notion that the complex consists of multiple species and hybrids (Lu-Irving *et al*. 2022). It is thus counterproductive to refer to the weed as a single species, with more accurate identification to lower taxa expected to facilitate better management. Two widespread sub-lineages are estimated to comprise at least half of the populations in Australia (Lu-Irving *et al*., in prep.); these correspond broadly with the "Common Pink" and "Common Pink-Edged Red" varietes described by Smith & Smith (1982). Common Pink lantana is highly susceptible to *Puccinia lantanae*, and Common Pink-Edged Red appears to be at least moderately susceptible, with other (less widespread) varieties varying from susceptible to resistant (Table 1).

Thus, initial releases of *Puccinia lantanae* should target the highly susceptible Common Pink and moderately susceptible Common Pink-edged Red varieties, and avoid less susceptible and/or less widespread hosts with similar floral colour morphotypes. Given the difficulty in accurately identifying lantana to sub-lineage based on floral colour alone, modelled distributions of Common Pink and Common Pink-Edged Red lantana based on genetically verified occurrence data are provided (Fig. 1). We recommend that prospective releases be conducted within areas where susceptible hosts are most likely to be distributed, and target populations be genetically verified as well as morphologically confirmed to match the descriptions included with this report.

**Table 1.** Results of *Puccinia lantanae* host specificity trials (reproduced and modified from Thomas *et al*. 2021), including details of predicted identity of individual host plants based on population genomic analysis.

| Host plant description | Susceptibility | Sub-lineage assignment | Confidence in assignment |
|---|---|---|---|
| Biloela pink | 4.5 | common pink | moderate |
| Brisbane common pink | 4 | common pink | moderate |
| Malanda pink-edged red | 4 | common pink-edged red | moderate |
| Grafton pink | 4 | common pink | low |
| Kempsey pink-edged red | 4 | common pink-edged red | low |
| Richmond pink | 4 | common pink | moderate |
| Kalpowar pink | 4 | common pink | moderate |
| Shute Harbour pink | 4 | other | low |
| Nowra pink | 4 | common pink | high |
| Ulladulla pink | 4 | common pink | moderate |
| Tuchekoi pink | 4 | common pink | moderate |
| Brookfield orange | 3.5 | other | very high |
| Mt. Gravatt orange | 3.5 | other | moderate |
| Helidon white | 3 | Helidon white | high |
| Kenmore pink | 3 | common pink | low |
| Ithaca pink-edged red | 2.5 | common pink-edged red | moderate |
| Gatton red | 2.5 | other | high |
| Richmond pink-edged red | 2.5 | common pink-edged red | moderate |
| Carmila pink | 2.5 | other | low |
| Townsville orange | 2 | other | low |
| Howard white | 1 | Helidon white | moderate |
| Murwillumbah pink | 1 | common pink | moderate |
| Yarraman white | 1 | Helidon white | very high |
| Ingham Hawaiian orange | 1 | other | low |
| Rockhampton pink-edged red | 0.5 | other | moderate |
| Rita Island pink | 0.5 | other | moderate |
| Emmett Creek pink | 0.5 | other | low |
| Common red | 0.5 | other | low |
| Kempsey red | 0 | other | low |
| Kalpowar white | 0 | other | low |

**Figure 1.** Result of MaxEnt species distribution modelling of (a) Common Pink lantana and (b) Common Pink-Edged Red lantana from genetically-verified occurrence data.



# Descriptions of the two major varieties of lantana in Australia

***Lantana camara* L *sensu lato* var. "Common Pink"**
Revised description following Smith & Smith 1982

Shrubs with prickly stems; foliage often with a yellowish hue (particularly in exposed habitats); leaves generally ovate to elliptic, sometimes twisted towards the apex, size highly variable; leaf adaxial surfaces flat to slightly rugose, usually slightly scabrous, more or less dull; leaf margins finely serrate-crenate; corollas changing colour as they age with young (central) flowers cream to yellow with darker centre, mature (marginal) flowers usually rose to fuschia with orange centre; distributed chiefly within 150 km of the coast from Rockhampton in QLD south to Narooma in NSW but also with a disjunct presence in northern QLD from the Daintree to the Atherton tablelands.

***Lantana camara* L *sensu lato* var. "Common Pink-Edged Red"**
Revised description following Smith & Smith 1982

Shrubs with prickly stems (may sometimes lack prickles on new growth); foliage usually dark green; leaves generally ovate to elliptic to lanceolate, typically with apex acuminate, size highly variable; leaf adaxial surfaces rugose, scabrous, and usually shiny; leaf margins serrate, often coarsely so; corollas changing colour as they age with young (central) flowers usually bright yellow, becoming orange, and mature (marginal) flowers usually red to coral or magenta, sometimes fading to pink; distributed chiefly within 150 km of the coast from St Lawrence in QLD south to Sydney in NSW, but also with a disjunct presence in northern QLD from the Daintree to the Atherton tablelands.

# References

Lu-Irving P, Encinas-Viso F, Callander J, Day MD, Le Roux JJ (2022) New insights from population genomics into the invasive *Lantana camara* L species complex. In: Proceedings of the 22nd Australasian Weeds Conference. Council of Australasian Weed Societies pp. 45–47.

Lu-Irving P, Encinas-Viso F, Callander J, MacMaster E, Day MD, Le Roux JJ (in preparation) Population genomic characterisation of the invasive *Lantana camara* complex in Australia.

Smith, L.S. & D.A. Smith. 1982. The naturalised *Lantana camara* complex in Eastern Australia. Queensland Botany Bulletin 1: 1–26.

Thomas SE, Evans HC, Cortat G, Koutsidou C, Day MD, Ellison CA (2021) Assessment of the microcyclic rust *Puccinia lantanae* as a classical biological control agent of the pantropical weed Lantana camara. Biological control: theory and applications in pest management 160:104688