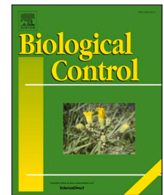
Biological Control 160 (2021) 104688

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon



Assessment of the microcyclic rust *Puccinia lantanae* as a classical biological control agent of the pantropical weed *Lantana camara*

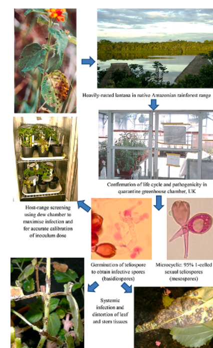
Sarah E. Thomas [a,*], Harry C. Evans [a], Ghislaine Cortat [b], Christina Koutsidou [a], Michael D. Day [c], Carol A. Ellison [a,1]

[a] CABI, Bakeham Lane, Egham, Surrey TW20 9TY, UK
[b] CABI, Rue des Grillons 1, 2800 Delémont, Switzerland
[c] Queensland Department of Agriculture and Fisheries, GPO Box 267, Brisbane, Qld 4001, Australia

HIGHLIGHTS

- The rust fungus *Puccinia lantanae* severely infected *Lantana camara* in Amazonia.
- Symptoms were replicated in greenhouse trials.
- The rust pathotype was highly damaging to a wide range of Australian weed biotypes.
- The pathotype proved to be specific to the *L. camara* complex.
- Infection of several other species in the Verbenaceae was shown to be an artefact.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Biocontrol
Lantana complex
Rust taxonomy
Pathogenicity
Host-range screening
Verbenaceae

ABSTRACT

Lantana camara is a flowering shrub of the family Verbenaceae, native to the Americas which has become a major invasive weed in the Palaeotropics; affecting both natural and agricultural ecosystems. It has been the focus of classical biological control for over a century but has proven to be a problematic target because of its high genetic diversity. Here, we report on an aggressive pathotype of the microcyclic rust *Puccinia lantanae* collected in the Amazonian rainforest, which – based on greenhouse screening – is damaging to a wide range of biotypes of the *L. camara* complex. Host-range testing within the Verbenaceae and related plant families, involving leaf clearing and staining, showed the pathotype to be highly specific to *L. camara sensu lato* but with detectable symptoms in several other verbenaceous species. These results, together with a taxonomic re-appraisal of *Puccinia lantanae*, are discussed in relation to the potential of the rust as a classical biological control agent of *L. camara*. We conclude that this pathotype of *P. lantanae* is a valuable addition to the biological control armoury and posit that it should be especially successful in humid forest situations.

* Corresponding author.
E-mail address: s.thomas@cabi.org (S.E. Thomas).
[1] Deceased.

https://doi.org/10.1016/j.biocontrol.2021.104688
Received 26 February 2021; Received in revised form 3 May 2021; Accepted 8 June 2021
Available online 11 June 2021
1049-9644/Crown Copyright © 2021 Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Lantana camara L. (Verbenaceae) is a neotropical woody shrub with attractive flowers which now has a pantropical distribution due to its horticultural value and ornamental interest. During the 19th and early 20th centuries, it was transported from various countries in the Americas to botanical gardens, mainly in Europe, where this plethora of biotypes was increased by hybridisation (Scott et al., 1997). From these centres, plants selected for their ornamental qualities were disseminated throughout the subtropics and tropics and this well-documented human dispersal is illustrated in Mack et al. (2000). More than 600 cultivars have been named (Howard, 1969), with 29 varieties having been delimited in Australia (Smith and Smith, 1982) and 50 varieties identified in South Africa (Wells and Stirton, 1988). Here, we employ the term biotype – plants that share a specified genotype – to cover all these variants. Subsequently, some of these biotypes have become invasive weeds in their exotic habitats, such that the *L. camara* complex, or *L. camara sensu lato* (Sanders, 1987, 2006; Urban et al., 2011; Goyal and Sharma, 2015), is now considered to rank amongst the world's top 10 most noxious weeds (Holm et al., 1991; Parsons and Cuthbertson, 2001).

The impact of *L. camara* on both natural- and agro-ecosystems has been profound and it can be especially damaging in native forests where the dense weed understorey disrupts succession and decreases biodiversity (Gooden et al., 2009). In East Africa, such lantana thickets also harbour the tsetse fly vectors (*Glossina* spp.) of trypanosomiasis and these refugia have been linked with outbreaks of sleeping sickness (Okoth and Kapaata, 1987; Leak, 1999). It has since been demonstrated that the flies are attracted to *L. camara* by volatiles released from the vegetation (Syed and Guerin, 2004). The situation regarding *L. camara* in East Africa has been highlighted recently and, in particular, the constraints to smallholder agriculture (Shackleton et al., 2017). Amongst the problems caused by weed infestations is their negative impact on livestock health – due to toxins that lead to cholestasis and hepatotoxicity (Sharma et al., 2007) – as well as causing a significant reduction in forage and crop yields due to the production of allelopathic phenolics (Kong et al., 2006; Sharma et al., 2007). However, there is a paucity of information concerning the economic impact of *L. camara* on agriculture, apart from a consultancy study on the grazing industry of Australia (AEC Group, 2007). In this report, it was estimated that the cost to the grazing sector was over A$100 million/per annum – in terms of lost productivity and increased management inputs.

Lantana camara also poses a fire hazard, as has been confirmed in Australia where fire regimes are altered by increasing fuel loads in forests invaded by the weed (Berry et al., 2011). The situation can only get worse as a climate-change study has shown that *L. camara* has the potential to invade new areas in Africa, Asia and Australasia due to global warming (Taylor et al., 2012).

The invasiveness of *L. camara* – as well as other *Lantana* species – and the need to manage the weed in exotic ecosystems have been documented since the 19th century (Lefroy, 1884) and, indeed, this was the first weed ever to be targeted for biological control (Day et al., 2003); making it the longest running of all biological control programmes against alien plant species. More than 40 insect species from the Americas have been released in over 30 countries worldwide, with 28 species having established in at least one country (Thomas and Ellison, 2000; Winston et al., 2021). Through natural dispersion, these agents are now found in 65 countries (Winston et al., 2021). Thus far, the results have been disappointing due to the wide range of biotypes involved and the poor selection of agents – and the causes of such failures have been addressed by Day et al. (2003). However, doubts have been raised on the value of continuing with its management, especially biological control (Zalucki et al., 2007; Bhagwat et al., 2012), whilst others have defended an integrated management strategy (Witt et al., 2012).

With the more recent option of using fungal pathogens for classical biological control of invasive alien weeds, a number of potential agents were identified from *L. camara* in the Neotropics (Evans, 1987; Barreto et al., 1995; Ellison and Evans, 1996). Four of these fungal species have subsequently been released as classical biological control agents, or are still being assessed for introduction: a *Septoria* species (Mycosphaerellaceae) from Ecuador into Hawaii (Trujillo and Norman, 1995); the rust *Prospodium tuberculatum* (Speg.) Arthur (Uropyxidaceae) from Brazil into Australia (Tomley and Riding, 2002) and New Zealand (Hayes, 2013); the leaf-spot pathogen, *Passalora* (formerly, *Mycovellosiella*) *lantanae* (Chupp) U. Braun & Crous var. *lantanae* (Mycosphaerellaceae) from Brazil into South Africa (den Breeÿen, 2003); and the rust, *Puccinia lantanae* Farl. (Pucciniaceae) from Peru into New Zealand (Hayes, 2013). The latter rust is currently being considered for introduction into South Africa (Winston et al., 2021; A.R. Wood, ARC-Plant Health and Protection, pers. comm.). These agents show some preference for particular *L. camara* biotypes; for example, *P. tuberculatum* attacks only pink-flowering biotypes in Australia (Thomas et al., 2006). Details of the biology, pathogenicity and host-range studies involved in evaluating the biological control potential of *P. tuberculatum* have been published previously (Ellison et al., 2006; Thomas et al., 2006). The present paper reports on similar studies involving the microcyclic rust *Puccinia lantanae*, with particular focus on testing invasive weed biotypes from Australia.

2. Materials and methods

2.1. Rust collection

During a survey for fungi associated with cocoa (*Theobroma cacao* L.) in its centre of origin in the Upper Amazon region of Peru, populations of *Lantana camara* heavily attacked by *Puccinia lantanae* were observed in forest clearings (Fig. 1). Such disease severity, including seedling death, had never previously been associated with this rust species (Evans, 1987; Barreto et al., 1995; H.C. Evans, pers. obs.). Leaf samples were collected and stored in a plant press for morphological study. Based on previous experience with embedded microcyclic rusts – which showed that dried material quickly loses viability, with teliospores failing to germinate (Evans and Ellison, 2005) – bare-rooted infected seedlings were also collected for pathogenicity studies. In order to maintain host and therefore rust viability, the roots were wrapped in moist tissues, placed in a small plastic bag, and enclosed within a larger self-sealing bag, which was then inflated and sealed to form a protective, humid bubble during transport.

On arrival in CABI-UK quarantine, seedlings were immediately potted in multipurpose compost and periodically placed in a dew chamber (Mercia Scientific, Birmingham, UK) to stimulate teliospore germination and subsequent re-infection of the plants and thus maintain living cultures of the fungus. Details of the collection were officially documented: on *L. camara*, Tamshiyacu, Upper Amazon, Loreto Region, Peru, 110 m a.s.l., October 1998. The living rust culture used in all the studies reported here, which we designate as a pathotype because of its unique symptomatology – as well as the dried herbarium material –was assigned a deposit number, IMI 398849, and the latter voucher specimen was deposited in the Herb IMI collection, now housed in the fungarium of the Royal Botanic Gardens at Kew, UK.

2.2. Morphological studies

Teliospores were teased from the dense telial pustules embedded within the host tissue (Fig. 2a), using a hypodermic needle and with the aid of a stereo-microscope (Nikon SMZ-10), then transferred to glass slides containing a drop of lactofuchsin or lactophenol and examined using a light microscope (Nikon Optiphot-2). For the biometric data, 50 teliospores were measured at x400 magnification and the mean dimensions of single and two-celled teliospores were calculated.

The germination process was examined using the same technique but with the telia being left overnight in a dew chamber at 20 °C. In

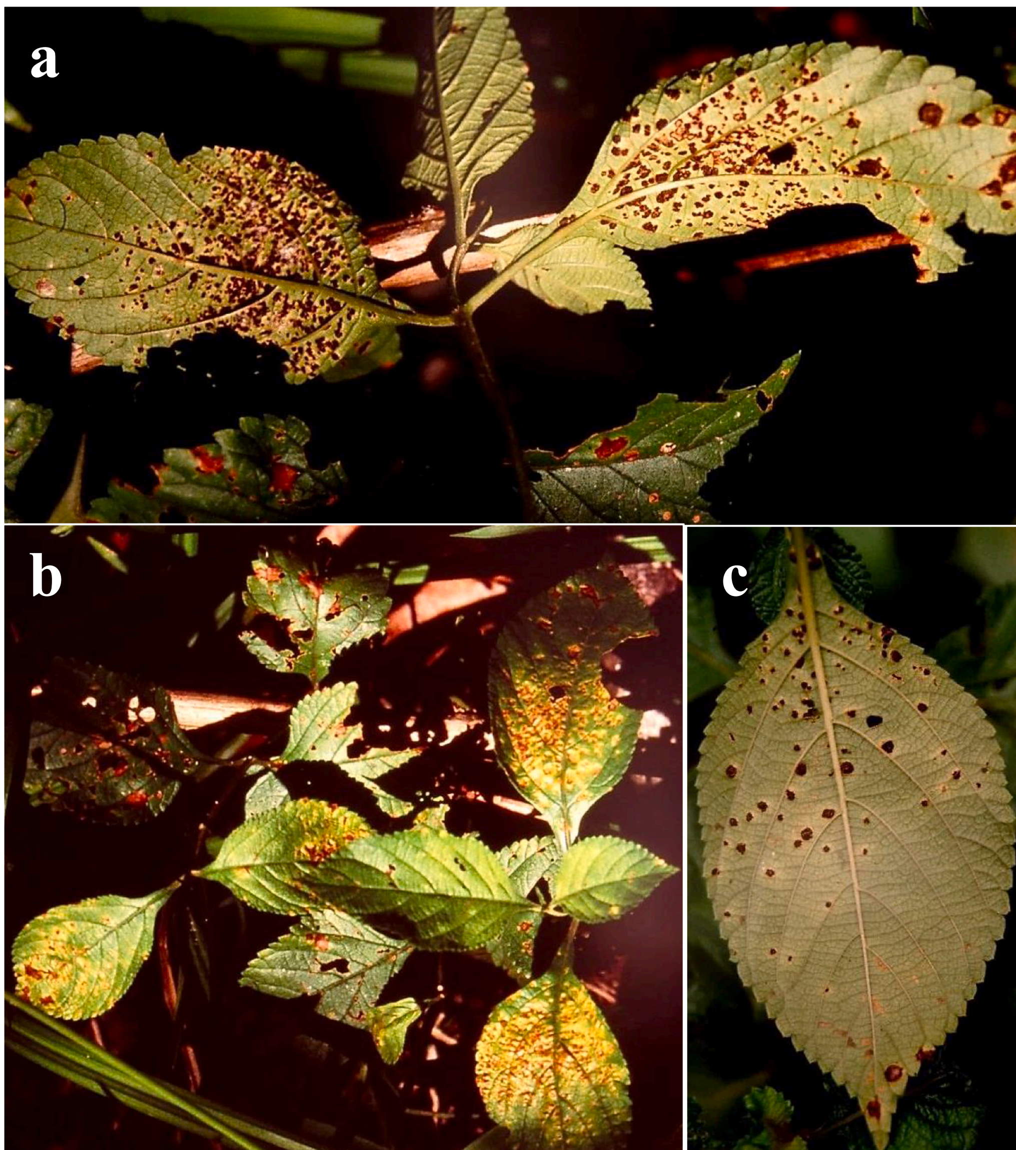


Fig. 1. Collecting site of *Puccinia lantanae* pathotype, IMI 398849, in forest understorey, Upper Amazon, Loreto Region, Peru: a) lower leaf surface of *Lantana camara* showing merging aggregations of telia; b) upper leaves showing severe blistering and necrosis; c) leaf from plant showing minor symptoms either due to resistance within the population or another less aggressive pathotype of the rust.

addition, scanning electron microscopy (SEM) was used to follow the process in more detail. Telial specimens (embedded in *L. camara,* 'Brisbane common Pink') were placed in a dew chamber at 20 °C for 5 hrs. These were rapidly frozen by exposure to liquid nitrogen, followed by sublimation of ice under vacuum. Photographs were taken using a Mamiya camera attached to the electron microscope.

2.3. Plant propagation

All plants used in this study were grown in a 50:50 mixture of two proprietary composts: John Innes No. 2 soil based; and a peat-based compost. Plants were maintained in an air-conditioned quarantine glasshouse, with supplementary lighting on a 12hr light: 12hr dark cycle, with a minimum day temperature of 25 °C and night of 20 °C.

2.4. Inoculation techniques

The inoculation techniques – developed previously for screening the microcyclic rusts attacking another invasive weed, *Mikania micrantha* Kunth (Asteraceae) (Evans and Ellison, 2005) – consisted of suspending pieces of plant material (stems, petioles and leaves) infected with telia over the test plants, under conditions of high humidity, using a dew chamber. The teliospores were from pustules appearing 25 to 40 days after inoculation; those telia produced on the petiole, stem and main leaf vein, remain viable for longer than telia produced on the leaf lamina. Telia were found to remain viable as long as they had living plant tissue around them.

Plants were inoculated in a dew chamber, set at 20 °C, for 48 hrs; however, this was varied for the environmental parameter studies, and details are provided in the methods below. Following the dew period, each piece of inoculum was also checked using a dissecting microscope to assess the level of sporulation that had occurred. This is clearly visible as a white, glistening bloom of basidia and unreleased basidiospores over the surface of the telium, at the end of the dew period (Fig. 2a). Any shoots where the teliospores had not germinated, or the inoculum had fallen off during the dew period, were marked, and these results were not included in the analysis (if no symptoms developed). In addition, for the quantitative experiments, the position of the inoculum in relation to

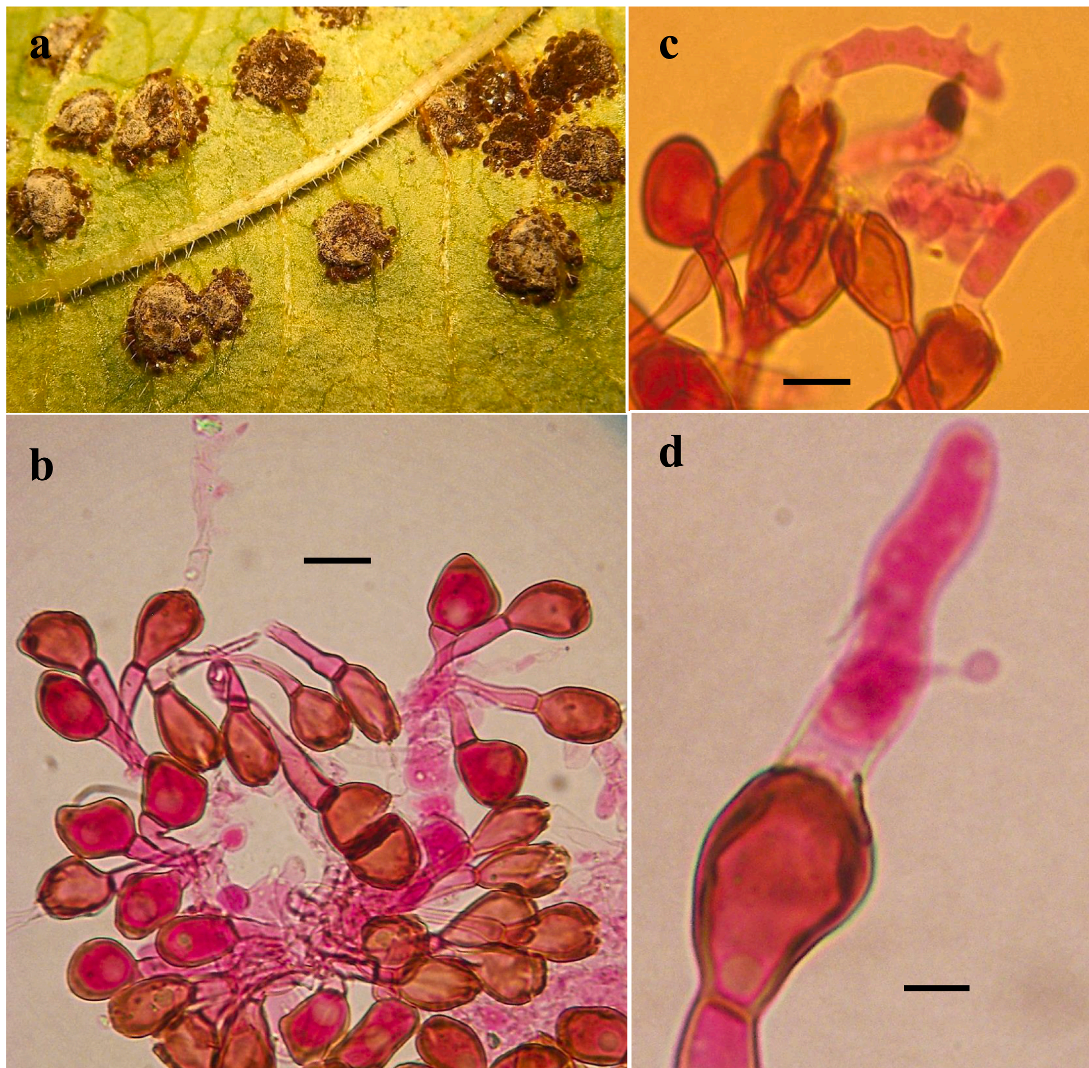


Fig. 2. *Puccinia lantanae*, pathotype IMI 398849: a) telia covered with white bloom with densely-packed, germinating teliospores, after 18 hrs in a dew chamber; b) teliospore squash showing the predominance (~95%) of single-celled spores (mesospores); c) metabasidia, one with four sterigmata in development (top, centre); d) Germinating mesospore with first sterigma and nascent basidiospore developing on the metabasidium. Bar: b) = 15 μm; c) = 12 μm; d) = 6 μm.

the middle shoot was also recorded. Plants where the shoot was not under the inoculum at the end of the dew period were not included in the analysis (if no symptoms developed). Plants were then returned to the quarantine chamber, and monitored for symptoms for at least six weeks. The prolonged dew period and extended monitoring period ensured that any possible delayed infection or latent disease expression would be highlighted. All experiments were repeated. Two methods of inoculation were used, depending on the experiments, as given below.

2.4.1. Qualitative inoculation

This method involved challenging the test plants with large amounts of inoculum at differing concentrations. Pieces of telia, containing at least 5 mm^2 of dense teliospores, were placed, telial side down, directly on the tips of young healthy shoots, and secured with petroleum jelly (Vaseline®); care being taken not to contaminate the teliospores with the Vaseline®, or the meristem directly under the inoculum. Four replicate plants (with a minimum of six shoots) were tested, each with a minimum of three inoculum pieces. This technique ensured that the basidiospores were released from the teliospores directly onto the most susceptible plant tissue and, therefore, removed any risk of the basidiospores missing the target tissue. For plants with fragile shoots, the inoculum was fixed to a 3 cm diameter Petri dish lid using Vaseline®, the lid was attached to a plant support by inserting it into a hole cut towards the edge of the lid, the support was pushed into the soil, and the telia positioned within 1 cm of the plant meristem.

In addition to the inoculum placed directly on the meristem or suspended very close to it, 1–5 leaves containing at least 10 × 5 mm^2 telia (depending on the number and size of the test plant in the test run) were placed on a rack approximately 10 cm above the test plant. This ensured that some of the shoots and older leaves of the test plants received a lower dose of diffuse basidiospores, in order to eliminate any risk of high and dense concentration of basidiospores leading to the plants exhibiting an atypical response. High spore doses can lead to hypersensitivity and to necrosis of the leaves and infection can be masked. In the natural situation, plants are likely to receive a low dose of basidiospores, but it is also important to assess a plant's reaction to high doses.

2.4.2. Quantitative inoculation

This method involved challenging the test plants with a more precise dose of basidiospores, to enable quantitative assessment of the results. Standardised pieces of inoculum consisting of circular leaf telia, approximately 2–3 mm diameter, containing dense teliospores between 25 and 40 days old, were used. These were cut from the leaf, leaving a minimum of 3 mm of leaf lamina around the telia. Plants were also

standardised as far as possible; young (<3-months old), with at least six branches/shoots, and in a vigorous growth phase. One telium was used to inoculate each replicate plant. This was fixed to the centre of a 3 cm diameter Petri dish lid using Vaseline®, the lid was attached to a plant support by inserting it into a hole cut towards the edge of the lid. The support was pushed into the soil, and the telium positioned precisely 3 cm above the middle shoot of the test plant. The middle shoot was kept in position by tying it to the support during the inoculation period. This method ensured that most of the released basidiospores fell onto the middle shoot, since there is an absence of air movement in the dew chamber. However, any basidiospores that were released more widely, infected the other shoots on the plant, and could be included in the results. There was minimal cross-infection between plants in the dew chamber if the plants were placed 30 cm apart. The number of telia that formed on each plant was counted four weeks after inoculation. Any variations in this method are provided in the experimental set-ups below.

2.4.3. Susceptibility score – assessment of infection

The level of susceptibility of the test plants was assessed using the following qualitative scoring system (see Fig. 3):

0 – Immune: no symptoms
1 – Resistant: chlorosis and/or necrosis (no further symptom development)
2 – Weakly susceptible: very sparse, small, restricted telia (<2 mm diameter)
3 – Moderately susceptible: many small, restricted telia (the majority within the 2–3 mm diameter range)
4 – Fully susceptible: large telia when not dense, reduced in size when dense, (the majority in non-dense areas lie within the 4–5 mm diameter range)

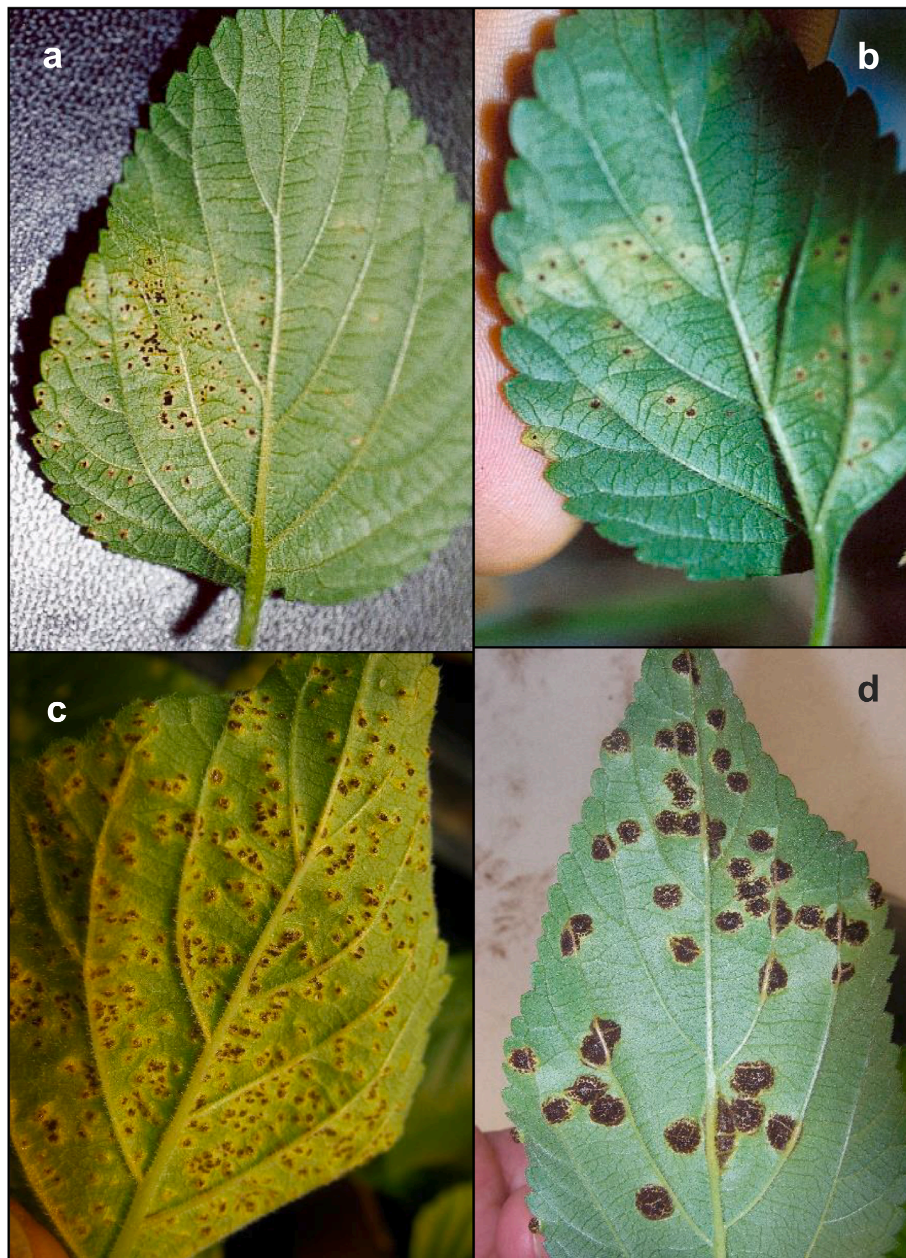


Fig. 3. Susceptibility reactions of different biotypes of *Lantana camara* to *Puccinia lantanae*, pathotype IMI 398849: a) 'Ithaca pink-edged red', score 2; b) 'Kenmore pink' score 3-; c) Lantana ex Ecuador score 3+; d) 'Brisbane common pink' score 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(A '−' and '+' after the score indicate that a result is 'just in' or 'nearly better' than the allocated category. The divisions have been imposed on a continuum of interactions between the test plants and pathogen, from immune to fully compatible, the '+' and '–' are to help indicate this continuum.

2.5. Effect of environmental parameters on infection of Lantana camara by Puccinia lantanae

2.5.1. Effect of temperature on infection

The effect of six temperatures, 12, 16, 19, 20, 24 and 30 °C, on the level of infection of *L. camara* (Brisbane common pink), by *P. lantanae* was investigated using a 48-hr dew period. At each temperature, three to four replicate plants were used with four to 10 shoots per plant. Due to the variation in the plant sizes available for this experiment, plants were divided into three groups (large, medium and small), and at least one plant (randomly selected) from each group was allocated to each temperature. The quantitative method described above was employed. In addition to counting the number of telia that developed on each replicate plant, the diameters of the 10 largest telia were also measured for each plant. The inoculum source for this experiment originated from a group of plants that had been inoculated at the same time. Hence, by the end of the experiment, the inoculum was 12 days older than at the beginning. A final, additional inoculation was undertaken at the optimum temperature, to check that the viability of the spores had not decreased. The data were analysed using the statistical software package R (R Core Team, 2019).

2.5.2. Effect of dew period on infection

The effect of seven dew periods: 5, 8, 11, 14, 20, 26 and 30 hrs was investigated at 19 °C using the same method as described above. Plants were dried rapidly after removal from the dew chamber, so that infection could not continue after the allotted dew period, by placing the plants in front of an air-conditioning unit for about 5 min, until no free water was visible on the leaves. The data were analysed using the statistical software package R (R Core Team, 2019).

2.6. Assessment of the susceptibility of selected biotypes from the Lantana camara complex globally, using a high inoculum concentration of Puccinia lantanae.

Table S1 lists the biotypes of *L. camara* from Australia that were screened, with their provenance, where known. The 29 biotypes tested were those selected by biological control researchers at the Queensland Department of Agriculture and Fisheries (formerly Department of Employment, Economic Development and Innovation), enabling a comprehensive range of biotypes that are problematic in Queensland and New South Wales to be assessed for their susceptibility to *P. lantanae*. An additional 21 biotypes from other parts of the world were also included in the assessment. Each biotype was tested on two occasions, and where possible, four plants were tested, each with at least six shoots. The qualitative method described under 2.4.1 was employed. Flower colour was also recorded for each biotype tested.

2.7. Quantitative assessment of the variable susceptibility of Lantana camara biotypes from Australia using a low inoculum concentration of Puccinia lantanae

The susceptibility of those biotypes that scored 2 or above was quantitatively assessed using the method described under 2.4 Inoculation techniques above. Four plants per biotype were used as replicates, and the number of telia per plant and the mean diameter of the 50 largest telia on each plant were recorded. Whether differences in the number and the size of telia among biotypes occurred was tested using generalised linear models with biotype as explanatory variable and an assumed quasipoisson data distribution. Pairwise comparisons of the means of individual biotypes was done using the least-squares means function in R (R Core Team, 2019), with the Tukey method to adjust P values for multiple comparisons. Significance was assessed at the 0.05 level.

2.8. Host specificity testing of Puccinia lantanae

Plant species were selected within the order Lamiales, focusing on plants from the family Verbenaceae present in Australia and following the centrifugal phylogenetic protocol established and refined by Wapshere (1974, 1989). A total of 19 species from the Verbenaceae and a further 31 species from 14 other families within the Lamiales were screened, including; Acanthaceae, Bignoniaceae, Boraginaceae, Calceolariaceae, Gesneriaceae, Lamiaceae, Lentibulariaceae, Oleaceae, Onagraceae, Orobanchaceae, Phrymaceae, Plantaginaceae, Scrophulariaceae and Tetrachondraceae (see Table S2 for full details). They were tested for their susceptibility to *P. lantanae* using the qualitative method described above. Four replicate plants were used in each test run. A positive control, using *L. camara* ('Brisbane common pink', a biotype known to be fully susceptible to the rust), was included in each test run. All test plants expressing symptoms to the challenge by the rust, but which showed no telial development, were retained for further observation until leaf senescence, in order to confirm that no latent infection had occurred.

In those few plant species on which telia developed, the teliospores were used to inoculate other plants of the same species, as well as fully susceptible *L. camara* biotypes. In addition, a microscopic analysis was undertaken to monitor the interaction between the rust and those test species in which minor symptoms appeared, using a leaf clearing-staining technique (Bruzzese and Hasan, 1983). This procedure was kept to a minimum for health reasons (potential carcinogens) and carried out in a fume cupboard.

2.9. Detailed assessment of the effect of inoculum concentration of Puccinia lantanae on susceptibility of Verbena officinalis subsp. africana

As some infection occurred on *Verbena officinalis* L. subsp. *africana* R. Fernandes & Verdcourt (Verbenaceae), and this plant is considered by some to be native to Australia (Michael, 1995; Munir, 2002), a further investigation of the infection by *P. lantanae* was undertaken to help assess the risk to this species in Australia. Plants were inoculated with four different inoculum concentrations, comprising an increasing number of telia (approximately doubling in number for each concentration). The diameters of the telia used to achieve each concentration were added together; totaling 20 (approximately four telia), 40, 80 and 160 mm. Each group of telia was suspended over individual test plants, using the same method as in 2.4.1., with four replicate plants, each with a minimum of four young shoots. The rust inoculum was 24–33 days old. 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 in total.

2.10. Quantitative assessment of susceptibility of Verbena officinalis var. gaudichaudii

Experiments were conducted to test the effect of inoculating *Verbena officinalis* var. *gaudichaudii* Briq., using four different doses of *P. lantanae*, similar to that conducted on *V. officinalis* subsp. *africana*. Infected, detached *L. camara* leaves ('Brisbane common pink') containing a known number of *P. lantanae* telia were suspended over four *V. officinalis* var. *gaudichaudii* plants (each plant with at least four young shoots) and placed in the dew chamber for 48 hrs as previously described for each inoculum dose.

After the dew period, sporulation was assessed using a dissecting microscope and the inoculation dose (inoculum concentration) was recorded (and adjusted accordingly, if 100% sporulation had not

occurred). Plants were returned to the quarantine chamber and observed for symptoms of infection. After 40 days, the number of pustules formed on each *V. officinalis* var. *gaudichaudii* plant was counted and recorded, noting the number of pustules formed on the leaves and stem. Each experiment was repeated on a separate occasion with the same inoculum dose using fresh plants (more than once if necessary to achieve the same dose). Therefore, a total of eight plants were infected for each *P. lantanae* dose. The viability of any resulting pustules that formed on the plants was checked by re-inoculating both *L. camara* ('Brisbane common pink') and *V. officinalis* var. *gaudichaudii*.

3. Results

3.1. Morphological studies

Pathotype IMI 398849 was found to have 96% single-celled teliospores (mesospores) and only 4% two-celled spores. The mean of the former measured 22 × 18 μm; whilst the latter measured 28 × 21 μm.

The teliospores are embedded in the host tissue (Fig. 2a; 4a) and, under the conditions of high humidity in the dew chamber, these germinate to produce a four-celled metabasidium; each cell forming a single sterigma with a terminal basidiospore, measuring 12 × 6 μm (Fig. 2b-c; 4b). The basidiospores are liberated forcibly from the teliospores which germinate and infect via an appressorium, entering the epidermal cell directly (Fig. 4c). Observations of a raised ridge of plant tissue forming around the appressorium, suggests the involvement of mechanical pressure to penetrate the host tissue (Littlefield and Heath, 1979). In highly susceptible biotypes, such as 'Brisbane common pink' (Fig. 5), it would appear that the infection hypha invades intercellularly and the resultant mycelium then systemically colonises the actively growing or meristematic tissue, as evidenced by frequent hypertrophy, with blistering and swelling, on all vegetative parts of the plant as the tissues expand (Fig. 5c-e), eventually giving rise to chocolate-brown telia which, invariably, coalesce and darken with age (Fig. 5b).

3.2. Effect of temperature on infection

The optimum temperature for the number of telia and their size is just below 20 °C (Fig. 6). The minimum temperature for infection was 12 °C, while no infection was observed at 30 °C. At temperatures between 15 °C and 25 °C, there was an average of ca. 500 telia per plant – with a mean telial diameter of >2.5 mm for the ten largest telia.

3.3. Effect of dew period on infection

There is a positive relationship between dew period and the number of telia (Fig. 7). Even after only 5 hrs of dew, there was some infection recorded, albeit very low. The number of telia continued to increase throughout the experimental period, suggesting that teliospore germination and/or basidiospore production is sequential. No meaningful

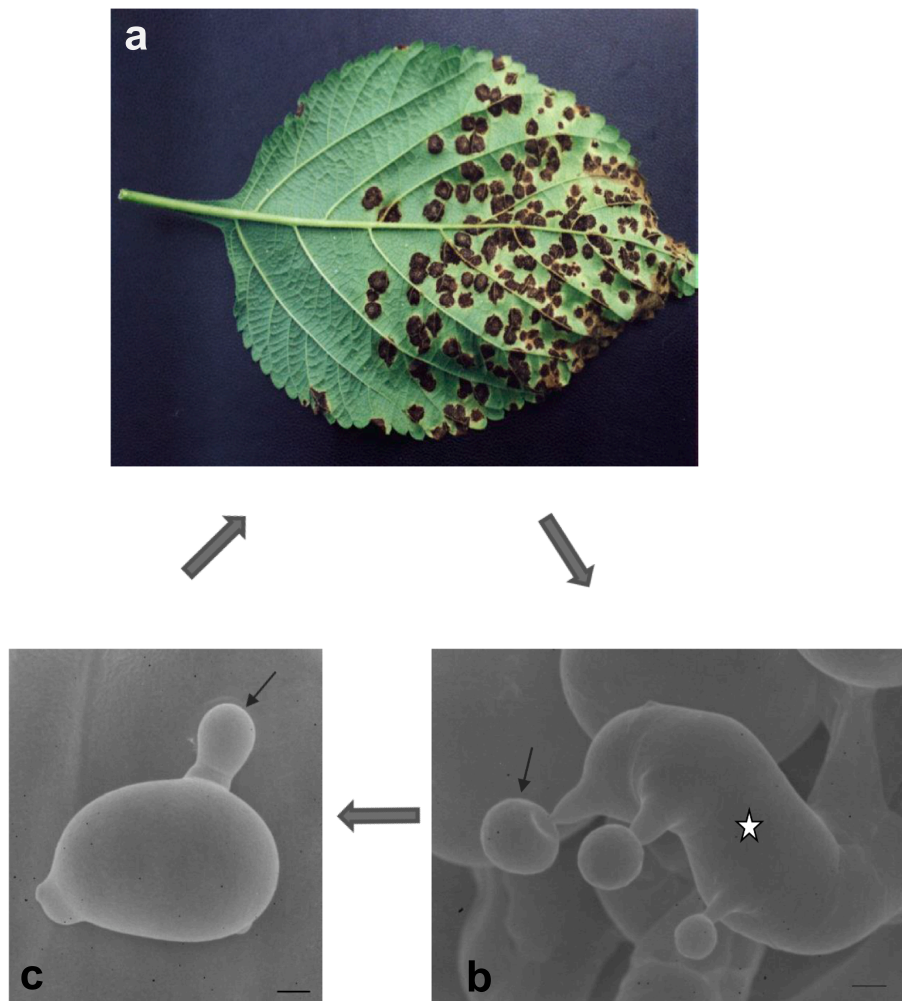


Fig. 4. Life-cycle of *Puccinia lantanae*, pathotype IMI 398849: a) leaf of *L. camara* with embedded telia, b) SEM of basidium (star) with developing basidiospores (arrow) borne on sterigmata, produced from embedded teliospore; c) SEM germinating basidiospore on *L. camara* leaf surface with appressorium (arrow). Bar: b) and c) = 2.1 μm.

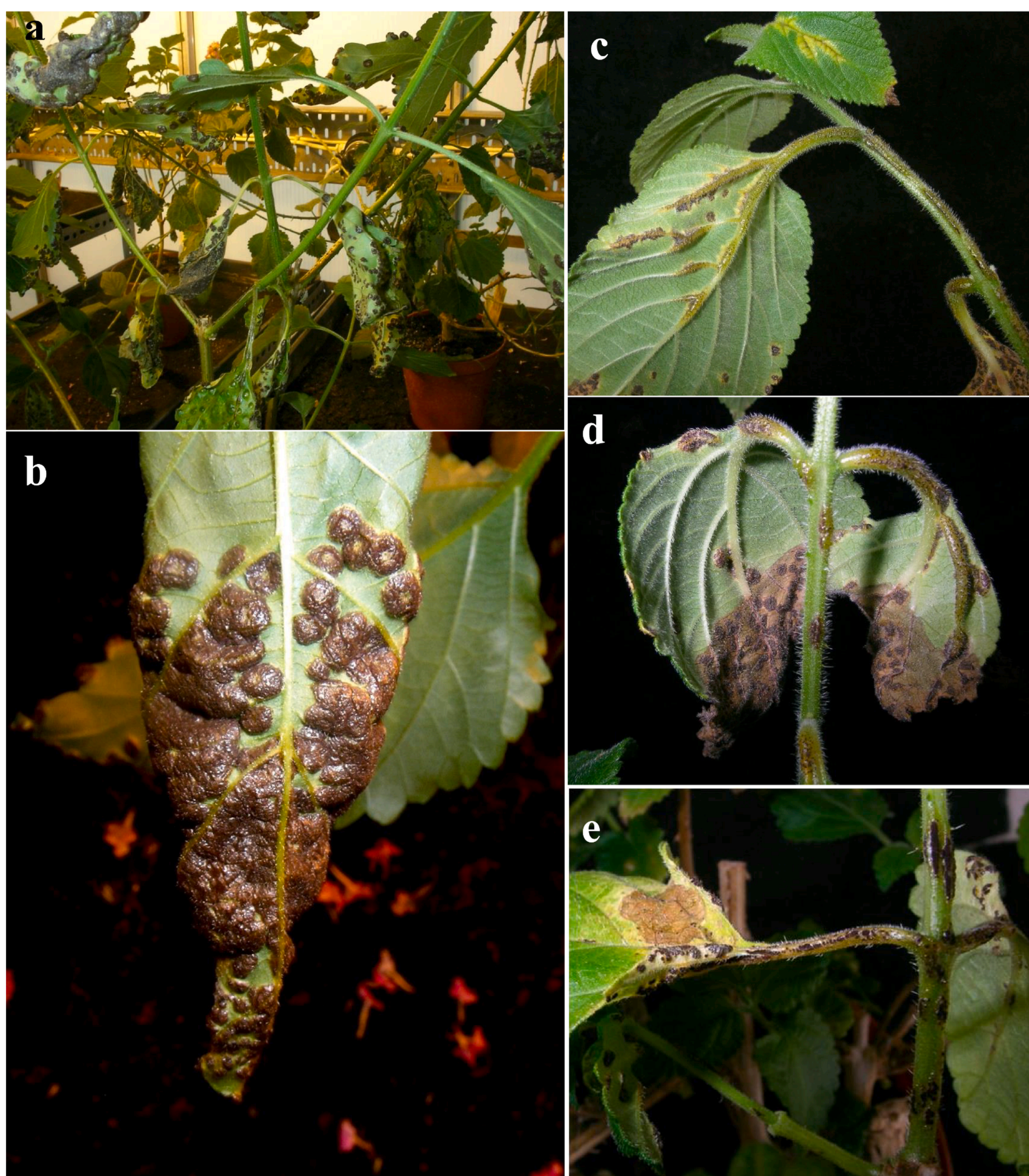


Fig. 5. Fully susceptible symptoms in *Lantana camara* biotype, 'Brisbane common pink', inoculated with *Puccinia lantanae* pathotype, IMI 398849: a) general view of susceptible plants in quarantine greenhouse; b) dense telial production causing blistering and leaf distortion; c-e) young shoots showing swollen leaf midribs, petioles, and stems with apparent systemic movement from leaf lamina via midrib and petiole onto the stem. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

relationship was found between dew period and the diameter of the telia, i.e. no consistent increase or decrease was found, and so the data are not included here.

3.4. Quantitative assessment of the variable susceptibility of Lantana camara biotypes using a low inoculum concentration of Puccinia lantanae

The most susceptible biotypes were 'Biloela pink', 'Brisbane common pink' (control), 'Kempsey pink-edged red', 'Malanda pink-edged red' and 'Richmond pink'. The variability in the susceptibility of the different biotypes can be more accurately defined when low doses of inoculum were used (Table 1 and Fig. 8). The number of telia that developed and their size when low doses of spores are applied, was closely related to their susceptibility score when high doses of spores are applied (Fig. 8). However, variability of the data within each species and relatively low replicate number does reduce the robustness of the conclusions. Only 'Gatton red' did not fit the overall trend.

The lowest scoring *L. camara* biotype in this quantitative assessment, 'Townsville orange' scoring 2 in the qualitative susceptibility assessment, developed no telia when challenged with low spore concentrations. Those biotypes that scored 2+, 3 or 4- ['Ithaca pink edged red', 'Gatton red' and 'Richmond pink-edged red' (2+); 'Helidon white' (3); 'Brookfield orange' (4-)] had similar numbers of telia to all the fully susceptible biotypes, except 'Biloela pink' (i.e. 'Brisbane common pink', 'Kempsey pink-edged red', 'Malanda pink-edged red' and 'Richmond pink'; see Fig. 8a). However, the non-fully susceptible biotypes had smaller diameter telia compared to three of the fully susceptible biotypes ('Biloela pink', 'Brisbane common pink' and 'Malanda pink-edged red'; see Fig. 8b).

3.5. Host-specificity testing of Puccinia lantanae

The results of the host-specificity testing are summarised in Table 2. The control plants ('Brisbane common pink') were fully susceptible

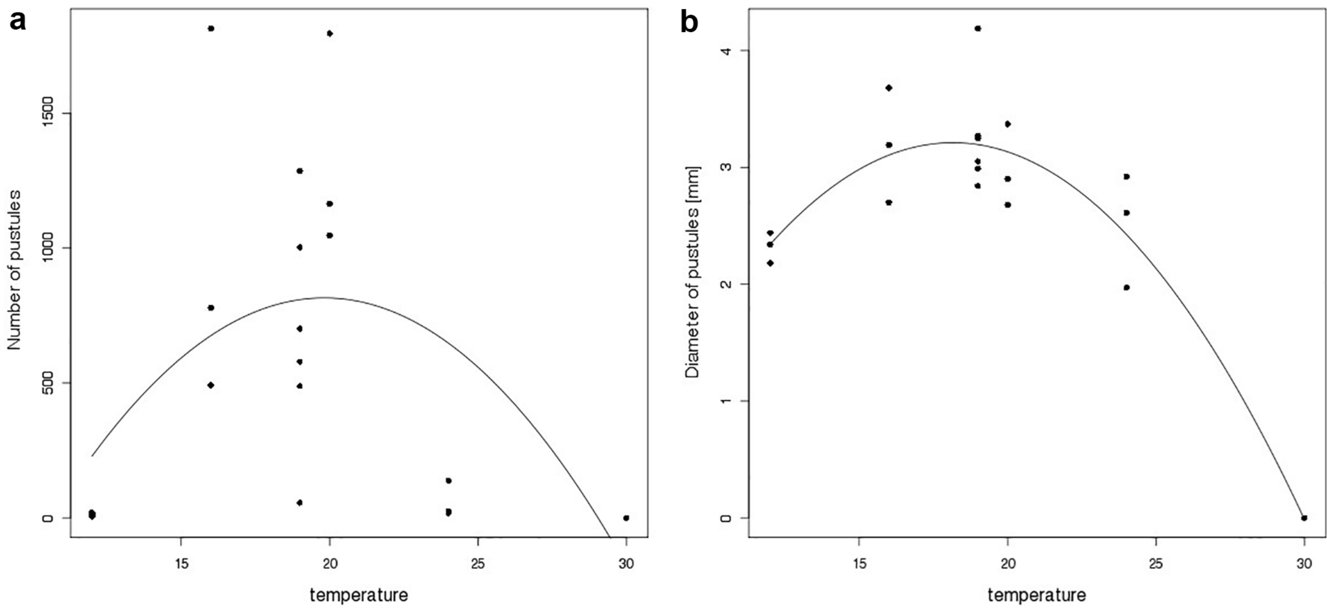


Fig. 6. Effect of temperature on the infection of *Lantana camara* by *Puccinia lantanae*, pathotype IMI 398849; a) number of telia b) diameter of the telia, polygonal regressions were used to describe the relationships between temperature and number of telia a) and the size of telia b), both relationships are significant (P = 0.020, R2 = 0.35 and P = 0.006, R2 = 0.49) for the number of telia and diameter respectively.

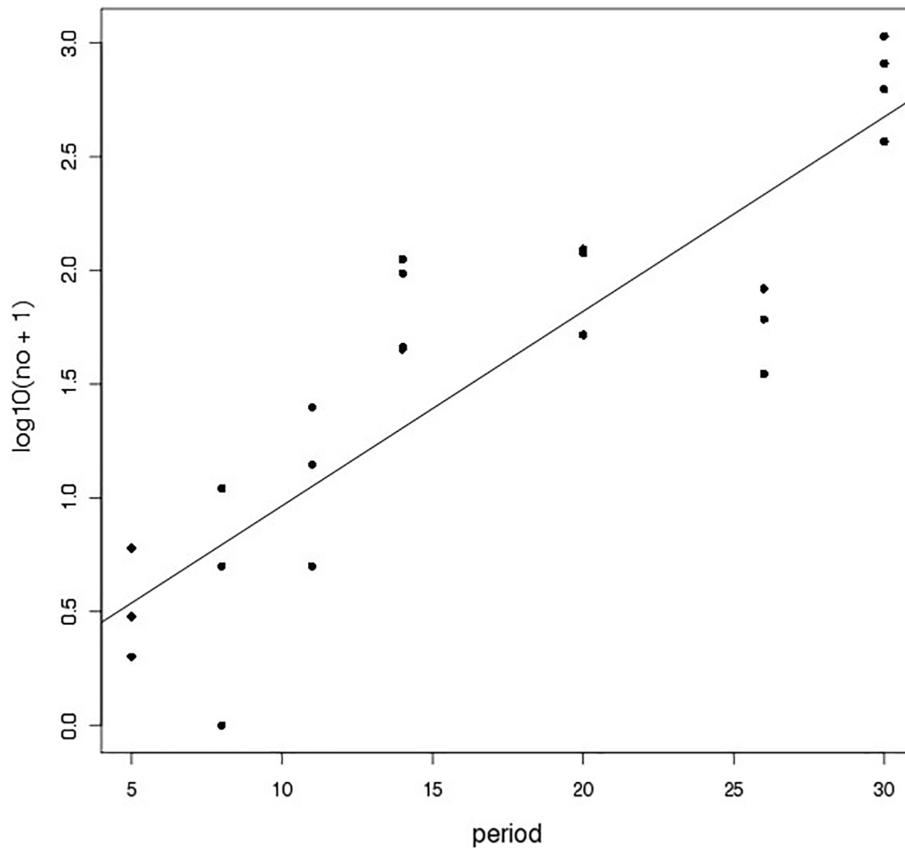


Fig. 7. The effect of dew period on the infection of *Lantana camara* by *Puccinia lantanae*. pathotype IMI 398849. The line indicates a significant relationship (P < 0.001, R2 = 0.75). Note that the y-axis is on a log scale of the number of telia.

(susceptibility score 4) in all the test runs. In contrast, none of the test plants were fully susceptible to *P. lantanae*, although viable teliospores were produced on *Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson, *Phyla canescens* (Kunth) Greene (Verbenaceae) and *Verbena officinalis* subsp. *africana*, albeit in very low numbers. Re-inoculation of the same species with these teliospores did not result in infection. In contrast, re-inoculation of the control, 'Brisbane common pink', with teliospores from *L. camara* led to infection.

Table 1
Results of the host-specificity testing of *Puccinia lantanae* (pathotype IMI 393849) to different biotypes of *Lantana camara* worldwide.

Lantana Biotype description		Flower Colour	Susceptibility Score
a) Australia			
1	Brisbane common pink		4
2	Ithaca pink-edged red	pale yellow throat / orange / dark pink	2+
3	Helidon white	pale yellow throat / white yellow throat / pale pink	3
4	Gatton red	yellow / orange / red	2+
5	Brookfield orange	yellow / orange	4-
6	Biloela pink	pale yellow throat / yellowish / pink	4+
7	Rockhampton pink-edged red	pale yellow throat / yellowish / (pinkish) orange	1-
8	Howard white	pale yellow throat / white	1
9	Rita Island pink	pale yellow throat / white yellow throat / pink	0+
10	Malanda pink-edged red	pale yellow throat / pink	4
11	Townsville orange	yellow / orange / dark orange	2
12	Murwillumbah	pale yellow throat / white yellow throat / pink yellowish	1
13	Grafton pink	pale yellow throat / pink	4
14	Kempsey pink-edged red	pink edged red	4
15	Kempsey red	pale yellow throat / orange / red	0
16	Richmond pink-edge red	pinkish / yellow-orange / red	2+
17	Richmond pink	pink	4
18	Kenmore pink	pale yellow throat / pink	3
19	Yarraman white	white / pale pink	1
20	Kalpowar pink	pale / white yellow throat / pink	4
21	Kalpowar white	pale / white yellow throat / white	0
22	Shute Harbour pink	pale yellow throat / yellowish / pink	4
23	Ingham Hawaiian orange	yellow / orange / dark pink	1
24	Nowra pink	pale / pink	4
25	Ulladulla pink	pale yellow throat / pink	4
26	Tuchekoi pink	pale yellow throat / yellowish / pink	4
27	Emmett Creek pink	pale yellow throat / pale pink / pink	1-
28	Carmila pink	pale yellow throat / pink	2+
29	Common red	pale yellow throat / yellow / pinkish orange	1-
30	Mt Gravatt orange	yellow / orange	4-
b) South Africa			
1	S-Africa 029 white pink	pale yellow throat / white-pink yellow throat	0+
2	S-Africa 021 Total light pink	pale yellow throat / pale pink	1
3	S-Africa 015 white	white yellow throat / white yellow throat	0+
4	S-Africa 018 Dark pink	pale yellow throat / dark pink	0
5	S-Africa 150 Orange small flowers	orange / red	4-
6	S-Africa 163 Light pink	pale yellow throat / yellow / pink	4
c) New Zealand			
7	Kohukohu pink (Northland)	pink	4
8	Tamaki Drive (Auckland)	pink	4
9	Whangaroa (Northland)	pink	4
10	Welcome Bay (Tauranga)	orange	3-
11	Purerua (Northland)	orange	2+
d) Other countries			
12	China	yellow / orange / pink	4
13	Ecuador	yellow / orange / pink	4
14	Hawaii	yellow / orange / pink	1
15	India	pale yellow throat / orange / pink	2
16	Madagascar 1	white yellow throat /yellowish pink	2
17	Madagascar 2	pale yellow throat / pink	1
18	Mexico	yellow / orange / red	1
19	Pakistan	yellow / orange / pink	4
20	Sri Lanka	pale yellow throat / yellow (pinkish)	4
21	Thailand		0

The main reactions observed in the host-specificity screening are shown in Figs. 9–11. In Fig. 9, the upper leaf surface reaction of three test species, in response to the rust (chlorosis and necrosis) are shown. A few teliospores developed in the lesions on the lower leaf surface of both *P. canescens* and *Lippia alba* but none were observed on *Citharexylum spinosum* L. (Verbenaceae). Microscopic observations were made of the plant-rust interaction for *C. spinosum* and *Verbena rigida* Spreng. In the case of *C. spinosum,* there was attempted penetration of the leaf surface by the rust germ tube and possible callus formation around the penetration point, but with no further development inside the leaf (Fig. 9c). For *V. rigida*, attempted penetration of the leaf surface was observed, but there were no obvious macroscopic symptoms and no internal development in the leaf.

Infection of the rust on *Verbena officinalis* subsp. *africana*, from two different areas, is shown in Fig. 10. The reaction of this species to the rust varied between 2 and 3-, suggesting significant genetic variation between and within population of the species. However, an atypical response was observed on the plants sourced from central Queensland, since teliospores were produced on both leaf surfaces. This was not observed to occur on the other host species tested. For *L. camara*, this phenomenon is only observed for suspected systemic infections, where the rust would appear to colonise the vascular tissue, and is not associated with typical leaf infection, composed of single restricted telia.

Typical infection by the rust of *V. officinalis* var. *gaudichaudii*, which is similar to *V. officinalis* subsp. *africana*, is shown in Fig. 11a. However, on one occasion stem infections (Fig. 11b), together with systemic infection sites were observed on two plants (Fig. 11c-e). These same plants had with previous inoculations developed only leaf telia. The teliospores that developed in the supposed systemically-infected tissue (and those on the leaf tissue of these plants) failed to germinate.

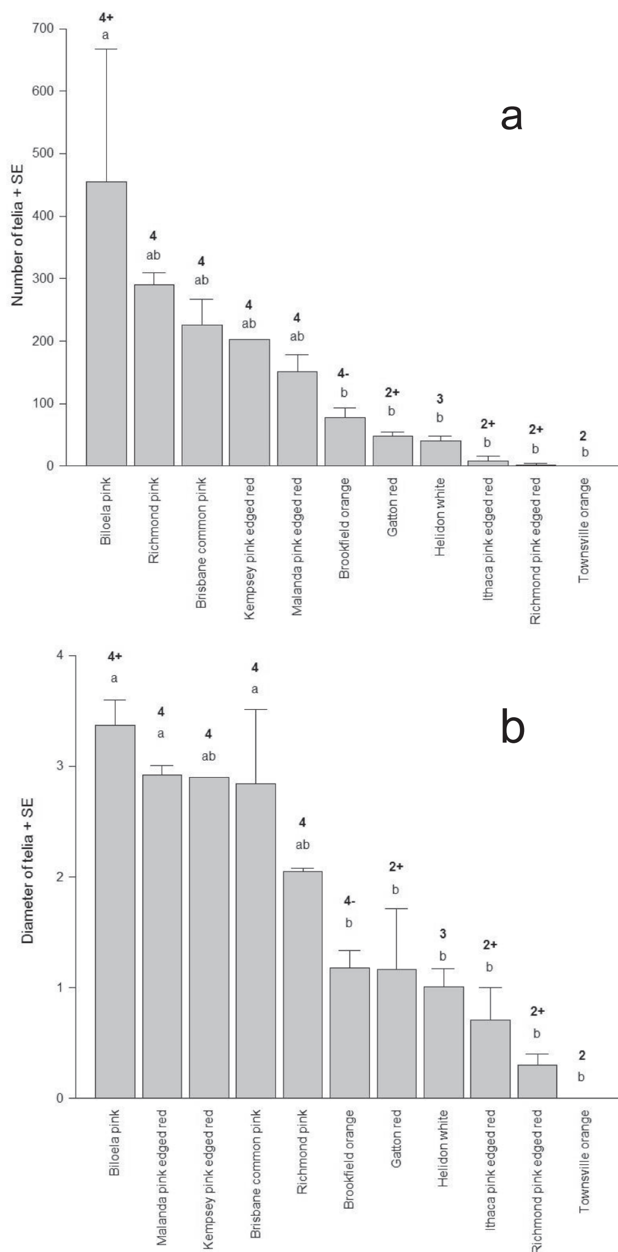


Fig. 8. Effect on *Lantana camara* biotypes of infection by *Puccinia lantanae*, pathotype IMI 398849, after application of low doses of inoculum: a) number of telia; b) size of telia. The number and diameter of telia for the various biotypes are showing different groups of susceptibility. a & b determined with a Tukey HSD test, c was attributed *a priori* to the biotype that did not develop telia in the present experiment. The numbers on each bar are the qualitative susceptibility score for each biotype obtained when high spore doses are applied (see Table 1).

3.6. Detailed assessment of the effect of inoculum concentration of *Puccinia lantanae* on susceptibility of *Verbena officinalis* subsp. *africana*

The results of the additional testing with *Verbena officinalis* subsp. *africana*, show that the concentration of rust inoculum has a significant influence on the number of telia that subsequently develop (GLM, $F_{1,\ 14} = 4.75$, $P = 0.047$) (see Fig. 12). A singe telium with a diameter of 20 mm produced insufficient basidiospores to induce any symptoms. A minimum of 40 mm total diameter of inoculum (628 mm^2 of telia) was required for infection. However, the relationship is not linear, because the data for 80 mm (mean = 1.25 ± 0.48) were lower than for 40 mm (mean = 5.25 ± 2.72) and 160 mm (mean = 8.75 ± 3.64), (see Fig. 13).

3.7. Quantitative assessment of susceptibility of *Verbena officinalis* var. *gaudichaudii*

Additional experiments conducted on *V. officinalis* var. *gaudichaudii* with high inoculum doses of inoculum (80, 32 and 16 telia) achieved high levels of infection, although no infection resulted when the dose was lowered to 10 telia, see Table 3 and Fig. 11. When successful infection of *V. officinalis* var. *gaudichaudii* was achieved, the susceptibility reaction varied between 2 (weakly susceptible: very sparse, small, restricted telia) and 3 (moderately susceptible: many small, restricted telia). In general, as inoculum dose increased, infection increased but results were variable. Some very high levels of infection were recorded, for example, on one individual plant infected with a very high dose of inoculum (80 telia), a total of 842 pustules were recorded. In addition to leaf infection, stem tissues occasionally became infected. None of the *V. officinalis* var. *gaudichaudii* plants died and, in all instances, the plants outgrew the infection with *P. lantanae* and completely recovered, even when stem infection occurred. Fig. 14 (a-b) shows typical stem and leaf infection of *V. officinalis* var. *gaudichaudii.* Comparative doses of *P. lantanae* were not applied to *L. camara.*

The viability of the telia produced on *V. officinalis* var. *gaudichaudii* was tested by attempting to re-infect *V. officinalis* var. *gaudichaudii* and *L. camara.* Successful infection was achieved on *L. camara* (Fig. 14 c-d), but not when re-inoculated back onto *V. officinalis* var. *gaudichaudii.*

4. Discussion

Puccinia lantanae is reported in the literature as a microcyclic, autoecious rust species, with a reduced number of spore stages in the life cycle. Only telia, in which the teliospores and basidiospores are produced, have been recorded from the field; spermogonia, aecia and uredinia are unknown (Farlow, 1883; Barreto et al., 1995; Ono, 2002a). This microcyclic life cycle was confirmed here for pathotype IMI 398849 in the pathogenicity and host-specificity screening studies.

The type of *Puccinia lantanae* was described based on material collected in Bermuda by Farlow (1883), who recorded only telia (sexual stage) and noted the absence of uredinia (asexual stage). He further reported the rust to be of common occurrence on *Lantana* '*odorata*' L. (=*L. involucrata* L.), and that it was also parasitised by '*Tubercularia persicina*' Ditmar (=*Helicobasidium purpureum* (Tul.) Sacc.). *Lantana involucrata*, known as common sage in Bermuda, was introduced from the Bahamas towards the end of the 18th century, and: "It is now the pest of Bermuda, over-running woods and pastures, and permitted by the supineness of the inhabitants to render thousands of acres of land valueless" (Lefroy, 1884). Presumably, both the rust and its mycoparasite were introduced together with the living plant host. Since the type description, *P. lantanae* has been recorded on a range of plant species in the Verbenaceae, as well as in the related family Acanthaceae of the Lamiales (Laundon, 1963; Farr and Rossman, 2020).

The genus *Puccinia* accommodates rust fungi in which the dominant teliospore is two-celled. However, *P. lantanae* is atypical in this respect, having predominantly one-celled teliospores (see Fig. 3), and this was highlighted by Laundon (1963), who gave a figure of ~ 95% for single celled teliospores (mesospores) on hosts in the Acanthaceae. This suggests that the rust is closely related to species from the genus *Uromyces* – applied to rusts with single-celled teliospores – and explains why there are several synonyms within this genus (Laundon, 1963; Hennen et al., 2005; Carvalho Júnior et al., 2008). A comparison of the teliospore dimensions recorded for *P. lantanae* isolate IMI 398849 with the original description given by Farlow (1883) shows that the Peruvian isolate has teliospores towards the smaller end of the distribution in the original description. In fact, when compared with the dimensions of teliospores from hosts in the Verbenaceae, reviewed by Barreto et al. (1995), the

Table 2
Results of additional host-specificity testing of *Puccinia lantanae* (pathotype IMI 398849) on plant species on which symptoms appeared.

Plant Species	Score (macroscopic)							Comment
	1st Plant Shipment		2nd Plant Shipment		3rd Plant Shipment		Grown from Seed	
	1st test	2nd test	1st test	2nd test	1st test	2nd test	4 test runs	
Control: *Lantana camara* ('Brisbane common pink')	4	4	4	4	4	4	4	Fully susceptible
Phyla nodiflora	0	1-	1-	0	0	0		Immune/resistant *
Citharexylum spinosum	1	1-	1+	1				Resistant
Lippia alba	2-	2-	2-	2-				Very weakly susceptible. Too few teliospores developed on *L. alba* from the inoculations to be able to realistically test the viability of these teliospores (by attempting to inoculate fresh *L. alba* plants or fully susceptible *L. camara* plants).
Phyla canescens	2+	2+	-	-	0	0		Weakly to moderately susceptible. The teliospores that developed on the *P. canescens* plants were viable, and infection of fully susceptible *L. camara* was achieved. However, no infection of fresh *P. canescens* plant was achieved using these teliospores.
Verbena officinalis subsp. *africana*	2	2	3-	3-	2-	2-		Weakly to moderately susceptible. The teliospores that developed on the *V. officinalis* subsp. *africana* plants were viable and infection of fully susceptible *L. camara* was achieved. However, no infection of fresh *V. officinalis* subsp. *africana* plant was achieved using these teliospores.
Verbena officinalis subsp. *africana*					2+	2+		Weakly to moderately susceptible. The teliospores that developed on the *V. officinalis* subsp. *africana* plants were viable and infection of fully susceptible *Lantana camara,* (score 3) was achieved. However, no infection of fresh *V. officinalis* subsp. *africana* plant was achieved using these teliospores.
Verbena officinalis var. *gaudichaudii*							2+ (3- for 2 plants on one test run of 4 plants)	Weakly to moderately susceptible. The teliospores that developed on the *V. officinalis* var. *gaudichaudii* plants were not found to germinate readily and did not result in infection of either fully susceptible *L. camara* plants, nor fresh *V. officinalis* var. *gaudichaudii* plants. Attempts were made to germinate all the telia that were produced. A score of 2+ on all 4 tests plants was recorded in three of the four test runs. For one test run, a 3- was recorded for 2 plants that showed both stem lesions and systemic infection.
Verbena rigida	-	-	0	0				Immune
Verbena bonariensis	-	-	0	0	0	0		Immune
Verbena litoralis	-	-	0	0	0	0		Immune

unicellular teliospores are in the same range as the smallest of the seven isolates they examined. It is clear that teliospore metrics are highly disparate and that *P. lantanae* may in fact, represent a species complex. A recent publication reinforces this supposition, as the teliospore dimensions for a strain of *P. lantanae* on *Lippia alba* in Brazil (Silva et al., 2017) are significantly longer teliospores than any of those previously described (Barreto et al., 1995); although there is no mention of this evident morphological disparity. Laundon (1963) did draw attention to the "unusual degree of variability [of the collections assigned to *P. lantanae* in the Acanthaceae family], which to a certain extent is correlated with hosts" – adding that – "they all have one important feature in common: the ca. 95% mesospores".

Clearly, an in-depth taxonomic study of *P. lantanae sensu lato*, including molecular data, is needed to resolve the status of the rust collections from the wide spectrum of plant species, in two closely-related families of the Lamiales (Schäferhoff et al., 2010), currently listed as hosts – not only in the Americas but also in Africa and Asia – but this is beyond the remit of this paper and will form the basis of a separate taxonomic publication.

Puccinia lantanae has been recorded from both tropical and subtropical regions in the Americas, from North America (Florida, Mexico) throughout the Caribbean and Central America, and as far south as Argentina, on approximately 55 plant species belonging to the families Acanthaceae (in two genera) and Verbenaceae (in six genera) – including at least 25 *Lantana* species (Table S3). There are two records from Africa (Côte d'Ivoire and Ghana), both on *L. camara*; whilst in Asia, it has been recorded from 15 species, all in the Acanthaceae (in nine genera), with no records from hosts in the Verbenaceae (Table S3). There are two, disparate records from the Americas on *Alternanthera* (Amaranthaceae) and *Hyptis* (Labiatae), but in the light of their rarity these must be viewed as erroneous identifications of either the host plant or the rust species. With this disjunctive host family and geographic distribution, it is tempting to conclude that *P. lantanae sensu stricto* evolved on Verbenaceae hosts in the Americas; whilst the rust fungus reported on Acanthaceae hosts in Asia is a sister microcyclic species evolved from a common full-cycled ancestor.

Puccinia lantanae is commonly reported as a leaf pathogen of *Lantana camara* throughout its neotropical range, where it is described as forming "black leaf spots with a yellow halo" (Evans, 1987), as well as angular, vein-delimited, dark brown, necrotic leaf lesions (Barreto et al., 1995); with similar symptoms being described on other *Lantana* species, as well as on hosts in other genera of the Verbenaceae and Acanthaceae (Laundon, 1963; Barreto et al., 1995; Silva et al., 2017). These leaf-restricted and relatively mild symptoms are in sharp contrast to those observed in the field with the rust pathotype collected in the Upper Amazon region of Peru (IMI 398849), and which have been duplicated on a wide range of *L. camara* biotypes in the greenhouse screening studies reported here.

In his studies of microcyclic rusts, which also included an Asian strain of *Puccinia lantanae*, Ono (2002a, 2002b) concluded that, although the microcyclic derivatives from an ancestral (full-cycled) rust species remain morphologically similar – and, thus, taxonomically conspecific – they may differ in nuclear behaviour. However, the nuclear condition of *P. lantanae* and the life-cycle type was not fully resolved; being either type V, with four diploid, uninucleate basidiospores on a four-celled metabasidium or a variant, type VI, with haploid basidiospores (Ono, 2002b). It is posited that the pathotype of *P. lantanae* assessed here (IMI 398849), is in the haploid, monokaryotic state;

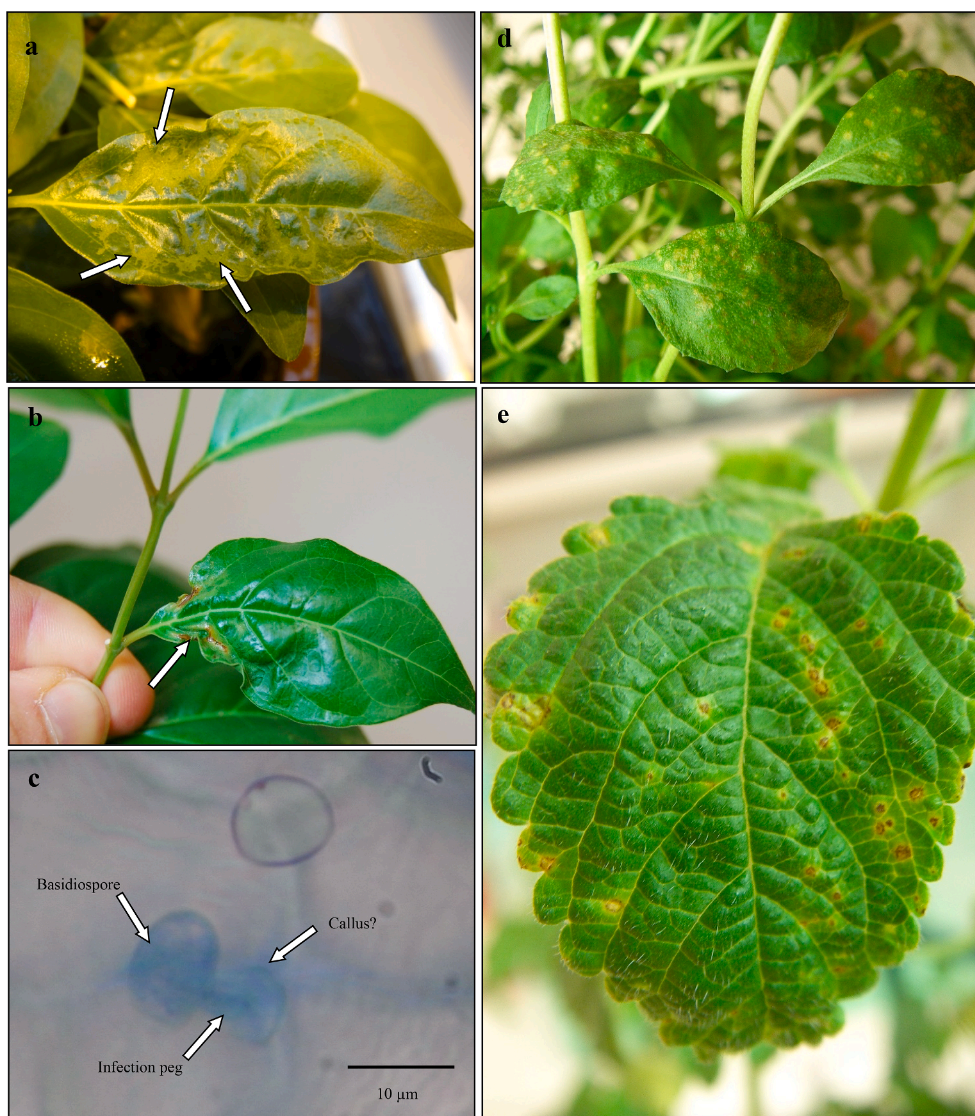


Fig. 9. Reactions of different test plants to high concentrations of *Puccinia lantanae* inoculum (upper leaf surfaces): *Citharexylum spinosum* a) chlorosis (arrows) b) necrosis (arrow) c) close-up of leaf surface showing attempted penetration by basidiospore germ tube, arrows show basidiospore, infection peg and possible callus formation (Bar: 10 µm); d) *Phyla canescens*, showing chlorosis; e) *Lippia alba*, showing chlorosis and necrosis.

enabling it to invade the meristems of its host and to grow intercellularly within the developing tissues; subsequently, inducing hypertrophy and growth abnormalities. In contrast, most *P. lantanae* collections are conjectured to be diploid, dikaryotic pathotypes with limited systemic ability; resulting in the more typical and restricted leaf-spot symptoms.

This contrasting symptomatology of different collections or pathotypes of *P. lantanae* on *L. camara* is uniquely illustrated in the Galápagos Islands. There is a precise date, as well as the circumstances underlying the introduction of *L. camara* into the archipelago; being brought to the island of Floreana by settlers in 1938 as a garden plant (Cruz et al., 1986). These authors warned about the threat posed by *L. camara* to the native flora and fauna of Floreana and recommended its eradication. The weed has since spread or been carried to other much bigger islands, including Santa Cruz, where this predicted threat is now being realised (Renteria and Ellison, 2004), together with invasive *L. montevidensis* (Spreng.) Briq. (Buddenhagen and Tye, 2015). During a survey of the plant pathogenic fungi of the Galápagos, *P. lantanae* was recorded on *L. camara* from Isla Floreana, but not the other islands (Cannon and Evans, 2004); presumably, being brought in with its host. However, the symptoms observed were limited to leaf spots, accompanied by localised necrosis (Fig. 15a). In sharp contrast, *L. camara* plants from the Galápagos (Isla Santa Cruz) when challenged with pathotype IMI 398849 showed symptoms similar to those recorded on the highly susceptible 'Brisbane common pink' (Table 1), with distorted leaves and systemic infection of shoots and stems (Fig. 15a-e). Interestingly, *P. lantanae* was not found on the endemic *L. peduncularis* Andersson or on the alien *L. montevidensis* during the Galápagos survey (Cannon and Evans, 2004), and both these species were found to be resistant in the specificity tests reported here (Table S2), and previously (Renteria and Ellison, 2004).

The temperature requirement of *P. lantanae* during infection was found to be similar to the rust *Pros. tuberculatum*, although the dew period requirement differed significantly: infection was first evident after only five hours of dew for *P. lantanae*, compared to nine hours for *Pros. tuberculatum* (Ellison et al., 2006). The number of telia that developed on the *P. lantanae* plants continued to increase throughout the experimental dew period, with no complete levelling-off of number; suggesting that basidiospores are not all produced and released in synchrony from a telium. This supports the microscopic analysis undertaken by Koutsidou (2000), which showed that the four basidiospores mature and are released sequentially from the basidium. In addition, the microscopic analysis of basidiospore development suggests that the

Fig. 10. Variable reaction of *Verbena officinalis* subsp. *africana* to high concentrations of *Puccinia 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.

teliospores in a telium do not all start germinating at the same time; thus, avoiding wastage if the increased humidity that stimulates their germination falls before completion of basidiospore release and host plant infection.

At night, when infection is most likely to occur during rain or dew formation, having teliospores that germinate at different times is seen as an advantage in terms of biological control as *L. camara* is found in a wide range of climatic areas (Day et al., 2003). Moreover, post-inoculation temperatures of 35 °C experienced in the quarantine glasshouse during the summer did not appear to affect disease expression, following infection under controlled conditions.

Puccinia lantanae in its native range occurs predominantly in tropical climates, whereas *Pros. tuberculatum* is a subtropical rust. They have not been recorded to occur in the same area. However, when both rusts were inoculated at the same time on to susceptible *L. camara* under controlled conditions, no negative interactions were observed. Both rusts infected and sporulated on the plants as they would if inoculated separately (S.E. Thomas, pers. obs.). However, it would be expected that the 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 *Pros. tuberculatum* would favour the subtropical regions of southern Queensland and New South Wales (NSW). Indeed, *Pros. tuberculatum* is well established from southern Queensland to central NSW, with populations in north Queensland at only high altitudes, and the rust appears most effective in the cooler areas, with severe plant defoliation in parts of NSW (Day, 2012).

The genetic diversity of the *L. camara* complex present in Australia was clearly seen by the variation in the disease scores recorded in response to *P. lantanae* infection. This is very different to the rust *Pros. tuberculatum*, where a normal uredinial spore production or an immune response was demonstrated by the biotypes that were tested (Thomas

Fig. 11. Reaction of *Verbena officinalis* var. *gaudichaudii* to high concentrations of *Puccinia lantanae* inoculum: a) typical leaf infection [score 3-]; b) - e) atypical infection, b) stem telia, c) localised infection, meristems continue to develop normally, d) and e) apparent systemic infection of meristem, side shoot meristems fail to elongate normally.

et al., 2006). However, *P. lantanae* is able to infect a wider range of biotypes of *L. camara* which, together with its more damaging disease expression, should make it a more effective biological control agent in the field situation, especially in humid ecosystems. The Peruvian pathotype infects leaves, petioles and stems; leading to apparent systemic infections that result in shoot die-back; whereas *Pros. tuberculatum* mainly forms leaf infections in the field resulting in leaf drop (Day, 2012; Riding et al., 2012).

The results of the host-specificity testing detailed in this study show that only *L. camara sensu lato* is fully susceptible to *P. lantanae* isolate IMI 398849; whilst three related test species were found to be weakly or moderately susceptible to the rust: *V. officinalis* (subsp. *africana* and var. *gaudichaudii*), *Lippia alba* and *Phyla canescens.* In contrast, all of these test plants were shown to be immune to *Pros. tuberculatum* (Thomas et al., 2006). Inoculation of these plant species with high doses of spores invariably resulted in only low numbers of non-infective teliospores being produced. Nevertheless, several plants of *V. officinalis* var. *gaudichaudii* did develop apparent systemic infection sites, but the resultant teliospores failed to geminate and were adjudged to be abnormal and non-viable.

The only species of significance to Australia of these partially susceptible species is *Verbena officinalis.* Both *V. officinalis* subsp. *africana* and var. *gaudichaudii,* are purported to be native to Australia, even though *V. officinalis* L. itself is considered native to Europe. Both *V. officinalis* subsp. *africana* and var. *gaudichaudii* were moderately susceptible to the rust. There was also significant genetic variation between the plants tested of both *V. officinalis* varieties; some of the plants of both varieties were resistant even when inoculated with a high concentration

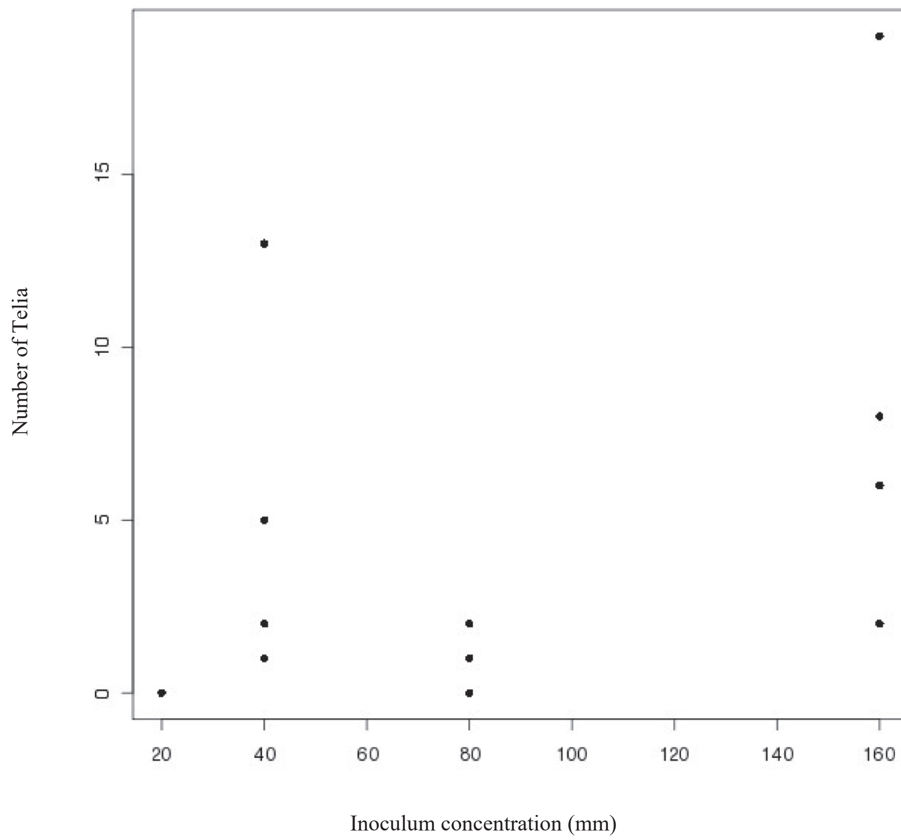


Fig. 12. Effect of inoculum concentration of *Puccinia lantanae* on the number of telia developing on *Verbena officinalis* subsp. *africana* (ex NSW).

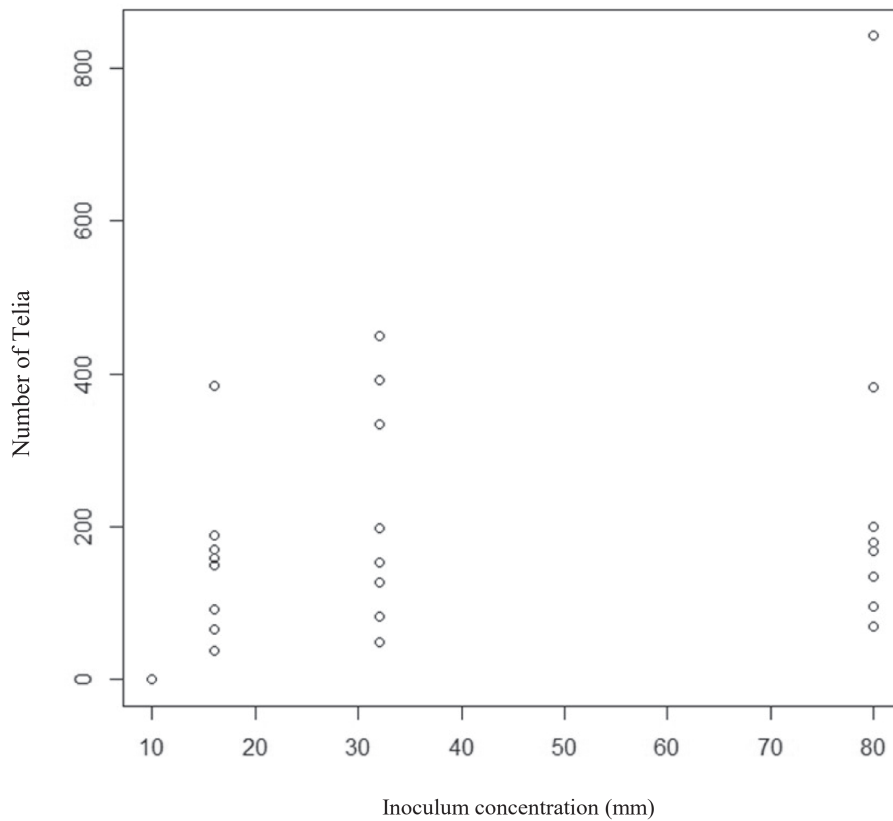


Fig. 13. Number of telia of *Puccinia lantanae* forming on *Verbena officinalis* var. *gaudichaudii*.

Table 3
Effect of dose levels of *Puccinia lantanae* (pathotype IMI 398849) on telia development in *Verbena officinalis* var. *gaudichaudii.*

Dose	Inoculum Concentration (Number of telia)	Average number of pustules formed on *Verbena* (n = 4), standard error in brackets in each trial	
Very high (Dose 1)	80	125 (±28)	393 (±157)
High (Dose 2)	32	233 (±74)	215 (±87)
Medium (Dose 3)	16	147 (±76)	189 (±27)
Low (Dose 4)	10	0	0

of inoculum. This was also demonstrated by the non-linear relationship between inoculum concentration and telial formation. The anomaly could be due, in part, to genetic variation in the plants and variation in susceptibility of the developmental stage of meristems at inoculation; perhaps in combination with variation in inoculum quality. This difference in susceptibility of individual *V. officinalis* subsp. *africana* and var. *gaudichaudii* plants would also help to reduce any potential risk to this species in the unlikely event that infection of *V. officinalis* subsp. *africana* and var. *gaudichaudii* did occur in the field in Australia. Moreover, the probability that rust will not be able to persist on *V. officinalis*

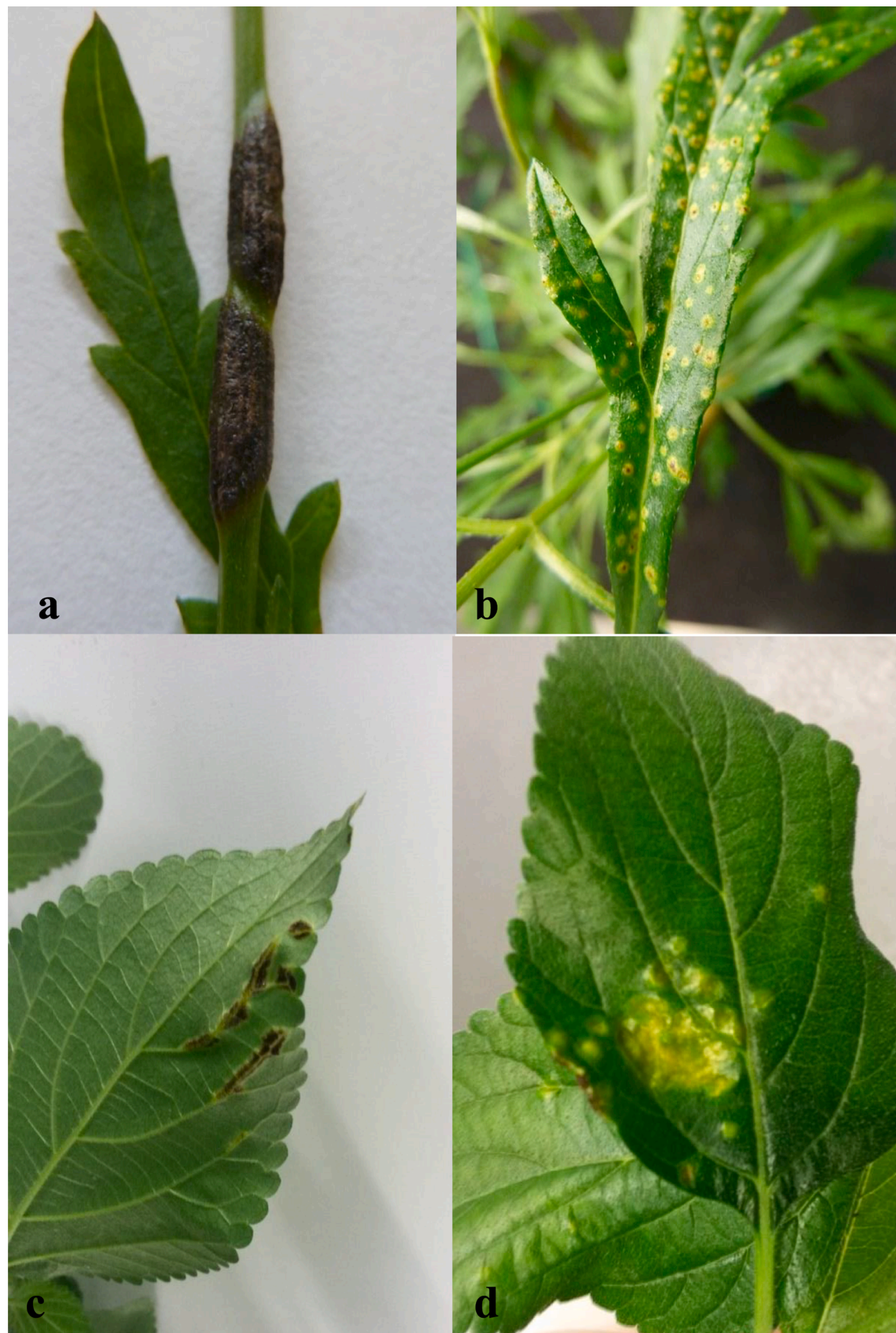


Fig.14. Infection of *Puccinia lantanae*: a) on *V. officinalis* var. *gaudichaudii* stem; b) leaf; c) – d) re-infection on *L. camara* ('Brisbane common pink'). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

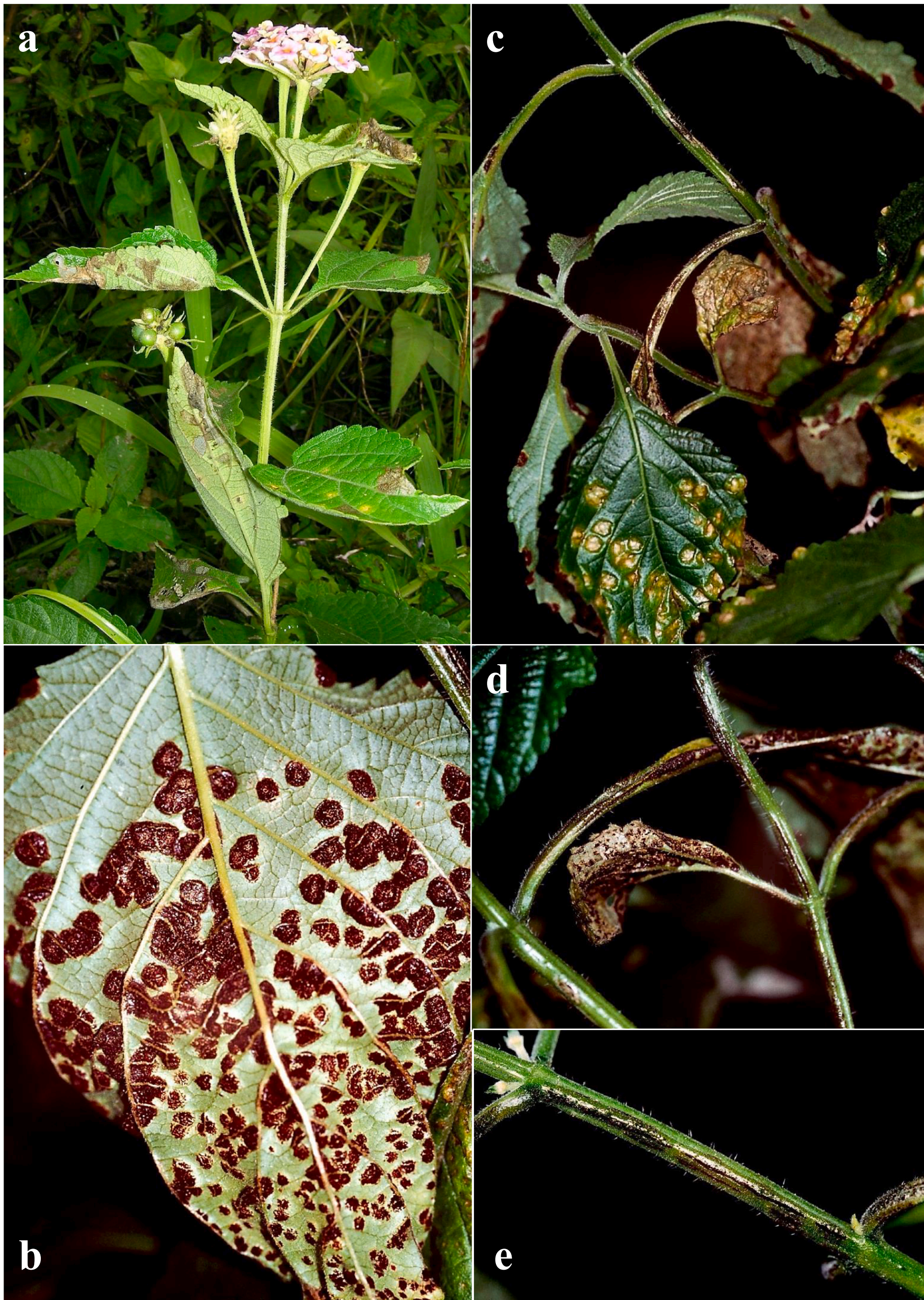


Fig. 15. a) *Lantana camara* on Isla Floreana (Galápagos Islands) infected by *Puccinia lantanae*; b-d) *Puccinia lantanae*, pathotype IMI 398849, on *L. camara* ex Isla Santa Cruz (Galápagos Islands) after inoculation in CABI-UK quarantine, showing leaf (b) and stem (c-d) infection, distortion and blistering; e) close-up of apparent systemic infection of shoots.

subsp. *africana* and *V. officinalis* var. *gaudichaudii*, even if it does become infected, means that plants will always require inoculum from nearby susceptible populations of *L. camara* for infection.

Both *Lippia alba* and *Phyla canescens* are exotic weedy species themselves in Australia; and the latter is also a target for biological control there. Hence, infection by *P. lantanae* should not cause regulatory concern. Indeed, the insect biological control agent *Falconia intermedia* (Distant) (Hemiptera: Miridae), was approved for release, even after testing indicated that *Lippia alba* would be attacked (Day and McAndrew, 2003).

Although it is difficult to predict what will happen in the field situation, experience from other plant-rust biological control projects, suggests that weakly susceptible hosts, which are not coevolved hosts, will not be at risk in the field (Bruckart et al., 1985; Tomley and Evans, 2004). Tomley and Evans (2004) reported on the case of the rust '*Maravalia cryptostegiae*' – now *Uredo cryptostegiae* Vestergr. (Aime and

McTaggart, 2021) – released in Australia for the control of rubber-vine weed, *Cryptostegia grandiflora* Roxb. ex R.Br. (Apocynaceae). The plant test list included a rare Australian native *Phyllanthera grayi* (P.I. Forst.) Venter (formerly, '*Cryptolepis*' *grayi*), from the same subfamily as rubber-vine (Apocynaceae: Periplocoideae). At high levels of rust inoculum (1.5×10^6 spores/ml), fertile pustules appeared on several of the test plants, with some leaves becoming chlorotic and visibly distorted (Evans and Tomley, 1994). Despite the fact that the pustules developed much more slowly and were considerably fewer and smaller than on rubber-vine weed, there was still cause for concern and raised the possibility that the rust would be rejected as a potential biocontrol agent. However, the rust was approved for release by the Australian regulatory authorities and, as predicted from wind-tunnel experiments (Evans and Tomley, 1996), the rust caused only a hypersensitive reaction in *P. grayi* in the experimental garden in Brisbane, and no infection of this species has been reported, thus far, in natural field populations (Tomley and Evans, 2004).

Similarly, Bruckart et al. (1985) reported infection of globe artichoke, *Cynara scolymus* L. (Asteraceae) with the musk thistle rust (*Puccinia carduorum* Jacq.) under greenhouse conditions in the USA. However, the rust has never been found on this crop in Eurasia where musk thistle, *Carduus nutans* L. (Asteraceae) and artichoke are sympatric. Support for their proposal that the musk thistle rust is a *forma specialis* or pathotype, was provided by the fact that an endemic strain of *P. carduorum* on slenderflower thistle, *Carduus tenuiflorus* Curtis (Asteraceae) in California has never been reported on artichoke, which is widely grown in the region. Field trials in the USA supported this hypothesis (Bruckart et al., 1996), after approval for introduction of the rust had been given by USDA Animal & Plant Health Inspection Service, in accordance with previously established protocols (Klingman and Coulson, 1982). Finally, Watson (1985) listed four rust species, which, during greenhouse screening, had infected plant species outside of their normal range. This has been termed induced susceptibility and can be considered to represent artefacts of the screening programme due to abnormally high inoculum loads and optimal conditions for disease expression and falls within the concept of "accepted levels of host susceptibility" (Seier et al., 2013).

There are already two examples where microcyclic rusts are contributing to the control of invasive weeds in Australia: *Puccinia xanthii* Schw., first recorded in Australia in 1975 on Noogoora burr, *Xanthium strumarium* L. (=*occidentale* Bertol.) (Asteraceae); and *Puccinia xanthii* var. *parthenii-hysterophorae* Seier, H.C. Evans & A. Romero (formerly *P.* '*melampodii*') (Seier et al., 2009; 2013), which was introduced in 1999 for the control of *Parthenium hysterophorus* L. (Asteraceae). In the latter case, the rust was released despite its ability to infect some cultivars of *Helianthus annuus* L. (Asteraceae) (sunflower) and *Calendula officinalis* L. (Asteraceae) (marigold). This rust established and has persisted well in northern Queensland with a disease incidence of more than 60% (Dhileepan, 2007; Seier et al., 2013).

Puccinia xanthii, which was accidently introduced, is now widespread on *X. strumarium* in Australia, and in many areas, is having a considerable impact as a biological control agent (van Klinken and Morin, 2012). Morin et al. (1993) tested 24 species for their susceptibility to *P. xanthii* and found that four closely related species in the Noogoora burr complex were fully susceptible to the rust. In addition, 13 cultivars of sunflower were tested and, of these, the rust was found to partially infect eight cultivars; forming abnormal telia, with few teliospores. However, Morin et al. (1996) still emphasised the need to screen any new cultivars of sunflower for their susceptibility to the rust, before commercialisation. Now, 12 years after introduction of *P. xanthii* var. *parthenii-hysterophorae*, and 35 years after the release of *P. xanthii,* there have been no records of them extending their known host ranges or causing any significant damage to sunflower and marigold cultivars in the field in Australia (Dhileepan and McFadyen, 2012; van Klinken and Morin, 2012).

Introduction of the *P. lantanae* pathotype (IMI 398849) is currently being considered for South Africa: encouragingly, the indigenous species *Lantana rugosa* Thunberg, as well as three *Lippia* species, were rated as resistant to the rust in preliminary tests (Seier et al., 2013).

These examples provide circumstantial evidence that non-natural hosts are not at risk in the field. This is further supported on a genetic level for microcyclic rusts by Ono (2002a, 2002b) who considers that: "The evidence seems to support the possibility of multiple origins of microcyclic or clonal rust lineages from the same ancestral rust species". He investigated the life cycle and nuclear behaviour of three microcyclic rusts – including *P. lantanae*, isolated from *Justicia procumbens* L. (Acanthaceae) – and concluded that *P. lantanae* reproduces apomictically, with basidiospore formation resulting from a mitotic rather than a meiotic division and that it is uninucleate throughout the life cycle (Ono, 2002a). Hence, there would be limited genetic variation within individual lineages, and this supports the hypothesis that a number of *formae speciales* (pathotypes), or varieties, exist within the *P. lantanae* complex – possibly similar to the *P. xanthii* complex (Seier et al., 2009) – suggesting that the opportunity for genetic variability with an individual 'clone' of *P. lantanae* is likely to be limited to mutation. Consequently, this restricts the likelihood of the rust increasing its virulence towards the non-natural host *V. officinalis*, in the unlikely event that it does infect this species in the field.

Molecular data and more in-depth taxonomic studies will be necessary to ascertain whether *P. lantanae sensu lato* is a pathotype complex – divided into physiological forms or *formae speciales* – or a species complex – representing distinct taxa with morphological differences separated at the varietal or species level. The considerable circumstantial evidence from the host range tests – in which closely related *Lantana* species proved to be immune – as well as from the field – where rust affected *L. camara* can occur alongside rust-free species of *Lantana* and vice versa – offers compelling support for the working hypothesis that the Peruvian strain of *P. lantanae* from *L. camara* is species specific. The, 'irregularities' in the centrifugal phylogenetic host-range tests, whereby species in other genera of the Verbenaceae – notably, in the genus *Verbena* – were found to be susceptible can best be explained by the evolutionary history of microcyclic rusts such as *P. lantanae*, and eluded to by Ono (2002a, 2002b). *Verbena* would appear to be an ancestral host of a full-cycled rust with a broad host-range within the Verbenaceae; representing an evolutionary throwback. Although *P. lantanae* sporulated on *Verbena officinalis* subsp. *africana* and *V. officinalis* var. *gaudichaudii* during the screening, it was also demonstrated during the additional studies that it cannot complete its life cycle on this plant and thus this would not be a natural host in the field situation.

5. Conclusions

This study demonstrates the critical importance of pathotype selection when evaluating fungal pathogens as classical biological control agents of invasive alien weeds. Initially based on field observations, and confirmed by the screening results presented here, IMI 398849 from the Amazon rainforest displays a wider range of disease symptoms and is significantly more damaging to *Lantana camara* than other pathotypes of *Puccinia lantanae* observed in the field, where only leaf tissues are affected. We conclude that this pathotype has considerable biological control potential, especially against invasions by *L. camara* in forest ecosystems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This project was funded by the Queensland Government through the

Department of Agriculture and Fisheries (DAF). Further and subsequent funding was provided by Agricultural Research Council, Plant Protection Research Institute (South Africa) and Manaaki Whenua, Landcare Research (New Zealand) for the additional host range screening. The authors would like to thank Natasha Riding (DAF, Brisbane) and Jorge Renteria (Charles Darwin Research Station, Galápagos) for their contributions to earlier studies and René Eschen (CABI, Switzerland) for providing statistical support. The rust was imported under Defra plant health licence no. PHL 29/2727 (7/1998).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2021.104688.

References

AEC Group, 2007. Economic Impact of Lantana on the Australian Grazing Industry. Department of Natural Resources, Canberra, Australia, 16 pp.

Aime, M.C., McTaggart, A.R., 2021. A higher-rank classification for rust fungi, with notes on genera. Fungal System. Evol. 7 (1), 21–47.

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. Mycol. Res. 99 (7), 769–782.

Berry, Z.C., Wevill, K., Curran, T.J., 2011. The invasive weed *Lantana camara* increases fire risk in dry rainforests by altering fuel beds. Weed Res. 51, 525–533.

Bhagwat, S.A., Breman, E., Thekaekara, T., Thornton, T.F., Willis, K.J., Traveset, A., 2012. A battle lost? Report on two centuries of invasion and management of *Lantana camara* L. in Australia, India and South Africa. PLoS ONE 7 (3), e32407.

Breeÿen, A. den, 2003. Release strategies for the establishment of the leaf spot pathogen, *Mycovellosiella lantanae* var. *lantanae*, on *Lantana camara* in South Africa., in: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L., Scott, J.K. (Eds.), Proceedings, XI International Symposium on Biological Control of Weeds, CSIRO Entomology, Canberra, Australia, pp. 386-388.

Bruckart, W.L., Politis, D.J., Sutker, E.M., 1985. Susceptibility of *Cynara scolymus* L. (artichoke) to *Puccinia carduorum* Jacq. observed under greenhouse conditions. In: Delfosse, E.S. (Ed.), Proceedings, VI International Symposium on Biological Control of Weeds. Agriculture Canada, Ottawa, Canada, pp. 603–607.

Bruckart, W.L., Supkoff, D.M., Yang, S.M., 1996. Indigenous plant pathogens in evaluation of foreign biological control candidates in the United States of America, in: Moran, V.C., Hoffmann, J.H (Eds.), Proceedings, IX International Symposium on Biological Control of Weeds, University of Cape Town, Cape Town, South Africa, pp. 71-77.

Bruzzese, E., Hasan, S., 1983. A whole leaf clearing and staining technique for host specificity studies of rust fungi. Plant Pathol. 32 (3), 335–338.

Buddenhagen, C.E., Tye, A., 2015. Lessons from successful plant eradications in Galapagos: commitment is crucial. Biol. Invasions 17 (10), 2893–2912.

Cannon, P.F., Evans, H.C., 2004. A Preliminary Plant Pathogen Survey of the Galápagos Archipelago. Unpublished Report: Control of Invasive Species in the Galápagos Archipelago (Project ECU/00/G31), CABI, Egham, UK, 47 pp.

Carvalho Júnior, A.A.d., Hennen, J.F., Hennen, M.M., Figueiredo, M.B., 2008. Fungos causadores de ferrugens (Uredinales) em áreas de Cerrado no estado de São Paulo, Brasil. Rodriguésia 59 (1), 1–55.

Cruz, F., Cruz, J., Laweson, J.E., 1986. *Lantana camara* L., a threat to native plants and animals. Noticias de Galápagos 43, 10–11.

Day, M., 2012. *Lantana camara* L. – lantana. In: Julien, M., McFadyen, R., Cullen, J. (Eds.), Biological Control of Weeds in Australia. CSIRO Publishing, Melbourne, pp. 334–346.

Day, M.D., Broughton, S., Hannan-Jones, M.D., 2003. Current distribution and status of *Lantana camara* and its biological control agents in Australia, with recommendations for further biocontrol introductions into other countries. BNI 24, 63N–76N.

Day, M.D., Mcandrew, T.D., 2003. The biology and host range of *Falconia intermedia* (Hemiptera: Miridae), a potential biological control agent for *Lantana camara* (Verbenaceae) in Australia. Biocontrol Sci. Technol. 13 (1), 13–22.

Dhileepan, K., 2007. Biological control of parthenium (*Parthenium hysterophorus*) in Australian rangeland translates to improved grass production. Weed Sci. 55 (5), 497–501.

Dhileepan, K., McFadyen, R.C., 2012. *Parthenium hysterophorus* L. – parthenium. In: Julien, M., McFadyen, R., Cullen, J. (Eds.), Biological Control of Weeds in Australia. CSIRO Publishing, Melbourne, pp. 448–462.

Ellison, C.A., Evans, H.C., 1996. Amazon-ingly useful fungi- a biological control cornucopia? Mycologist 10, 11-13.

Ellison, C.A., Pereira, J.M., Thomas, S.E., Barreto, R.W., Evans, H.C., 2006. Studies on the rust *Prospodium tuberculatum*, a new classical biological control agent released against the invasive alien weed *Lantana camara* in Australia, I: life-cycle and infection parameters. Australas. Plant Pathol. 35, 306–319.

Evans, H.C., 1987. Fungal pathogens of some subtropical and tropical weeds and the possibilities for biological control. BNI 8, 7–30.

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

Evans, H.C., Tomley, A.J., 1994. Studies on the rust, *Maravalia cryptostegiae*, a potential biological control agent of rubber-vine weed, *Cryptostegia grandiflora* (Asclepiadaceae: Periplocoideae), in Australia. III: Host range. Mycopathologia 126 (2), 93–108.

Evans, H.C., Tomley, A.J.,1996. Greenhouse versus field evaluation of the rubber vine rust, *Maravalia cryptostegiae*, on Madagascan and Australian Asclepiadaceae, in: Moran, V.C., Hoffmann, J.H. (Eds.), Proceedings, IX International Symposium on Biological Control of Weeds, University of Cape Town, South Africa, pp. 165-169.

Farlow, W.G., 1883. Notes on some species in the third and eleventh centuries of Ellis's North American fungi. Proc. Amer. Acad. Arts & Sci. 18, 65–85.

Farr, D.F., Rossman, A.Y., 2020. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. – Published at http://nt.ars-grin.gov/fungaldatabases/ [accessed 15 Dec. 2020].

Gooden, B., French, K., Turner, P.J., Downey, P.O., 2009. Impact threshold for an alien plant invader, *Lantana camara* L., on native plant communities. Biol. Conserv. 142, 2631–2641.

Goyal, N., Sharma, G.P., 2015. *Lantana camara* L. (*sensu lato*): an enigmatic complex. NeoBiota 25, 15–26.

Hayes, L., 2013. The Biological Control of Weeds Handbook: A New Zealand Guide. Landcare Research, Auckland.

Hennen, J.F., Figueiredo, M.B., Carvalho Júnior, A.A., Hennen, P.G., 2005. Catalogue of species of plant rust fungi (Uredinales) of Brazil: http://www.jbrj.gov.br/publica/uredin.

Holm, L.G., Plucknett, D.L., Pancho, J.V., Herberger, J.P., 1991. The World's Worst Weeds: Distribution and Biology, Krieger Publishing, Malabar, Florida.

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

Klingman, D.L., Coulson, J.R., 1982. Guidelines for introducing foreign organisms into the U.S. for biological control of weeds. Plant Dis. 66, 1205–1209.

Kong, C.H., Wang, P., Zhang, C.X., Zhang, M.X., Hu, F., 2006. Herbicidal potential of allelochemicals from *Lantana camara* against *Eichhornia crassipes* and the alga *Microcystis aeruginosa*. Weed Res. 46 (4), 290–295.

Koutsidou, C., 2000. Studies on *Puccinia lantanae*, a potential biological control agent for *Lantana camara*. MSc Thesis, Imperial College, University of London.

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

Leak, S.G.A., 1999. Tsetse Biology and Ecology: Their Role in the Epidemiology and Control of Trypanosomiasis. CABI Publishing, Wallingford.

Lefroy, J., 1884. The botany of Bermuda. Bull. U.S. Nat. Mus. 25, 35-141.

Littlefield, L.J., Heath, M.C., 1979. Ultrastructure of Rust Fungi. Academic Press, London.

Mack, R.N., Simberloff, D., Mark Lonsdale, W., Evans, H., Clout, M., Bazzaz, F.A., 2000. Biotic invasions: causes, epidemiology, global consequences and control. Ecol. Appl. 10 (3), 689–710.

Michael, P.W., 1995. A new name for a widespread and misunderstood species of *Verbena* (Verbenaceae). Telopea 6, 181–183.

Morin, L., Auld, B.A., Brown, J.F., 1993. Host range of *Puccinia xanthii* and post penetration development on *Xanthium occidentale*. Can. J. Bot. 71 (7), 959–965.

Morin, L., Auld, B.A., Smith, H.E., 1996. Rust epidemics, climate and control of *Xanthium occidentale*. University of Cape, Town, Cape Town, South Africa, 385–383.

Munir, A.A., 2002. A taxonomic revision of the genus *Verbena* L. (Verbenaceae) in Australia. J. Adelaide Bot. Gard. 20, 21–103.

Okoth, J.O., Kapaata, R., 1987. A study of the resting sites of *Glossina fuscipes fuscipes* (Newstead) in relation to *Lantana camara* thickets and coffee and banana plantations in the sleeping sickness epidemic focus, Busoga. Uganda. Insect Sci. Appl. 8 (01), 57–60.

Ono, Y., 2002a. Life cycle and nuclear behavior in three rust fungi (Uredinales). Mycoscience 43 (1), 37–45.

Ono, Y., 2002b. The diversity of nuclear cycle in microcyclic rust fungi (Uredinales) and its ecological and evolutionary implications. Mycoscience 43 (5), 421–439.

Parsons, W.T., Cuthbertson, E.G., 2001. Common lantana, in: Parsons, W.T., Cuthbertson, E.G (Eds.), Noxious Weeds of Australia, CSIRO Publishing, Melbourne, pp. 627-632.

R Core Team, 2019. R: A language and environment for statistical computing (3.6.1) [Computer software]. R Foundation for Statistical Computing.

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.

Riding, N., Snow, E.L., Day, M.D., 2012. The variability, pathogenicity and potential impact of the lantana rust *Prospodium tuberculatum*, in Eldershaw, V.:(Ed.), Eighteenth Australasian Weeds Conference, Melbourne, Victoria, Australia, pp. 336-338.

Sanders, R.W., 1987. Taxonomic significance of chromosome observations in Caribbean species of *Lantana* (Verbenaceae). Amer. J. Bot. 74 (6), 914–920.

Sanders, R.W., 2006. Taxonomy of *Lantana* sect. *Lantana* (Verbenaceae): I. Correct application of *Lantana camara* and associated names. SIDA, Contributions to Botany 22, 381–421.

Schäferhoff, B., Fleischmann, A., Fischer, E., Albach, D.C., Borsch, T., Heubl, G., Müller, K.F., 2010. Towards resolving Lamiales relationships: insights from rapidly evolving chloroplast sequences. BMC Evol. Biol. 10 (1), 352.

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 (9), 1560–1563.

Seier, M.K., Morin, L., Van der Merwe, M., Evans, H.C., Romero, A., 2009. Are the microcyclic rust species *Puccinia melampodii* and *Puccinia xanthii* conspecific? Mycol. Res. 113, 1271–1282.

Seier, M.K., Ellison, C.A., Cortat, G., Day, M., Dhileepan, K., 2013. How specific is specific enough? Case studies of three rust species under evaluation for weed

biological control in Australia, in: Wu, Y., Johnson, T., Sing, S., Raghu, S., Wheeler, G., Pratt, P., Warner, K., Center, T., Goolsby, J., Reardon, R. (Eds.), Proceedings, XIII International Symposium on Biological Control of Weeds, USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV, pp. 89-96.

Shackleton, R.T., Witt, A.BR., Aool, W., Pratt, C.F., 2017. Distribution of the invasive alien weed, *Lantana camara*, and its ecological and livelihood impacts in eastern Africa. Afr. J. Range Forage Sci. 34 (1), 1–11.

Sharma, O.P., Sharma, S., Pattabhi, V., Mahato, S.B., Sharma, P.D., 2007. A review of the hepatotoxic plant, *Lantana camara*. Critical Rev. Toxicol. 37 (4), 313–352.

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.

Smith, L.S., Smith, D.A., 1982. The naturalised *Lantana camara* complex in eastern Australia. Queensland Botany Bull. 1, 1–26.

Syed, Z., Guerin, P.M., 2004. Tsetse flies are attracted to the invasive plant *Lantana camara*. J. Insect Physiol. 50 (1), 43–50.

Taylor, S., Kumar, L., Reid, N., Kriticos, D.J., Cannon, A.J., 2012. Climate change and the potential distribution of an invasive shrub, *Lantana camara* L. PLoS ONE 7 (4), e35565.

Thomas, S.E., Ellison, C.A., Tomley, A.J., 2006. Studies on the rust *Prospodium tuberculatum*, a new classical biological control agent released against the invasive alien weed *Lantana camara* in Australia, II: host range. Australas. Plant Pathol. 35, 321–328.

Tomley, A.J., Riding, N., 2002. *Prospodium tuberculatum*, lantana rust, a new agent released for the biocontrol of the woody shrub *Lantana camara*. In: Spafford Jacob, H., Dodd, J., Moore, J.H. (Eds.), Proceedings, XIII Australian Weeds Conference. Plant Protection Society of Western Australia, Perth, pp. 389–390.

Tomley, A.J., Evans, H.C., 2004. Establishment of, and preliminary impact studies on, the rust, *Maravalia cryptostegiae*, of the invasive alien weed, *Cryptostegia grandiflora* in Queensland. Australas. Plant Pathol. 53, 475–484.

Trujillo, E.E., Norman, D.J., 1995. *Septoria* leaf spot of lantana from Ecuador: a potential biological control for bush lantana in forests of Hawaii. Plant Dis. 79, 819–821.

Urban, A.J., Simelane, D.O., Retief, E., Heystek, F., Williams, H.E., Madire, L.G., 2011. The invasive '*Lantana camara* L'. hybrid complex (Verbenaceae): a review of research into its identity and biological control in South Africa. African Entomol. 19 (2), 315–348.

van Klinken, R., Morin, L., 2012. *Xanthium occidentale* Bertol.– Noogoora burr. In: Julien, M., McFadyen, R., Cullen, J. (Eds.), Biological Control of Weeds in Australia. CSIRO Publishing, Melbourne, pp. 591–600.

Wapshere, A.J., 1974. A strategy for evaluating the safety of organisms for biological weed control. Ann. Appl. Biol. 77 (2), 201–211.

Wapshere, A.J., 1989. A testing sequence for reducing rejection of potential biological control agents for weeds. Ann. Appl. Biol. 114 (3), 515–526.

Watson, A.K., 1985. Host specificity of plant pathogens in biological weed control, in: Delfosse, E.S. (Ed.), Proceedings, XI International Symposium on Biological Control of Weeds, Agriculture Canada, Ottawa, Canada, pp. 577-586.

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

Witt, A.B.R., Day, M.D., Urban, A.J., Sankaran, K.V., Shaw, R., 2012. Lantana: the battle can be won. BNI 33, 13N–15N.

Zalucki, M.P., Day, M.D., Playford, J., 2007. Will biological control of *Lantana camara* ever succeed? Patterns, processes and prospects. Biol. Control 42, 251–261.

Winston, R.L., Schwarzlander, M., Hinz, H.L., Day, M.D., Cock, M.J.W., Julien, M.H. Eds. 2021. 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 January 2021].