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

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**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**

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**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.

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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 Sturgeon, 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 ileostasia and hepatoxotoxicity (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 AS100 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, 2006; Winston et al., 2021). Through natural dispersion, these agents are now found in 65 countries (Winston et al., 2021). Thus far, there have been disappointments 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_ (Spec.) Arthur (Uropylidaceae) from Brazil into Australia (Tomley and Riding, 2002) and New Zealand (Hayes, 2013); the leaf-spot pathogen, _Passalora_ (formerly, _Myccovellia_ lantanae) (Chupp) U. Braun & Crous var. _lantanae_ (Mycosphaerellaceae) from Brazil into South Africa (den Breejen, 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 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 under a stereo microscope (Nikon SMZ-10). 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 under a stereo microscope (Nikon SMZ-10).
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. 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.
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 $\times$ 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

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**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.
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.)
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 (Bruzese 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 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 V. 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 $22 \times 18 \mu m$; whilst the latter measured $28 \times 21 \mu m$.

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 $12 \times 6 \mu m$ (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 $20^\circ C$ (Fig. 6). The minimum temperature for infection was $12^\circ C$, while no infection was observed at $30^\circ C$. At temperatures between $15^\circ C$ and $25^\circ C$, 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

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**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.
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.
(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.

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, \(R^2 = 0.35\) and *P* = 0.006, \(R^2 = 0.49\)) 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, \(R^2 = 0.75\)). Note that the y-axis is on a log scale of the number of telia.
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 supposed systemically-infected tissue but none were observed on L. (Verbenaceae). Microscopic observations were made of the infection sites were observed on two plants (Fig. 11 c-e). These same phenomenon is only observed for suspected systemic infections, where 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.

Infection of the rust on Verbena 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.
the data for 80 mm \( (\text{mean} = 1.25 \pm 0.48) \) were lower than for 40 mm \( (\text{mean} = 5.25 \pm 2.72) \) and 160 mm \( (\text{mean} = 8.75 \pm 3.64) \), (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, autecious 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
uncellular 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 di –

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### 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 |
|---------------|----------------------|---------|
| **Control: Lantana camara** ('Brisbane common pink') | 4-4-4-4-4-4 | Fully susceptible |
| **Phyla nodiflora** | 0-1-0-0-0-0 | Immune/resistant * |
| **Gibaraylux spinosum** | 1-1-1-1 | Resistant |
| **Lippia alba** | 2-2-2-2-2-2 | 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+ | 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 *L. camara* was achieved (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 *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 stem lesions and systemic infection. |
| **Verbena rigida** | - - 0 0 | Immune |
| **Verbena bonariensis** | - - 0 0 0 0 | Immune |
| **Verbena littoralis** | - - 0 0 0 0 | Immune |

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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
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 et al., 2003).
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 germinate 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. 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.
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*. 
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. gauchichauldii 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

| 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 (±66)                                           189 (±27)                                            |
| Low (Dose 4)     | 10                                      | 0                                                   0                                                   |

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.)
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

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.

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McTaggart, 2021) – released in Australia for the control of rubber-vine weed, Cryptostegia grandiflora Roxb. ex R.Br. (Apoxyaceae). The plant test list included a rare Australian native Phylanthera grayi (P.I. Forst.) Venter (formerly, ‘Cryptolepis grayi’), from the same subfamily as rubber-vine (Apoxyaceae: Periplocoideae). At high levels of rust inoculum (1.5 × 10^8 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. partheni-hysterophorae Seier, H.C. Evans & A. Romero (formerly P. ‘melanopodi’) (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 xanthith, 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. (1995) 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. partheni-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 unineucleate 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 forma 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-host 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.

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Appendix A. Supplementary data

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

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