The viability, pathogenicity and potential impact of the lantana rust *Prospodium tuberculatum*

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Summary The viability, pathogenicity, field release and establishment of the lantana rust *Prospodium tuberculatum* were investigated. In glasshouse trials, semi-dried urediniospores lost viability rapidly at 25°C and were not viable after eight weeks. At -15°C, they maintained viability for over three months while uredinospores stored in liquid nitrogen survived for over one year. Over 220 predominantly pink-flowering lantana plants collected throughout lantana's range in Queensland and New South Wales were inoculated with the rust. Only pink-flowering forms developed rust pustules. However, not all pink-flowering forms did so. Of the 166 pink-flowering forms tested, 127 were susceptible. Glasshouse studies conducted to assess the rust's potential impact on lantana showed that initial premature leaf drop was directly related to inoculum dose rate. Lantana dosed with a high rate (0.1 g rust/100 mL distilled water) exhibited large quantities of premature leaf drop, increased side-shoot growth and outgrew rust infection within two months. Substantial short term defoliation of lantana was also observed in the field where lantana was inoculated by the rust at 0.23 g rust/ 17.5 g talc. A low dose rate (0.025 g rust/100 mL distilled water) caused chronic infection of potted plants which resulted in lower leaf drop but reduced stem and side-shoot growth. Low or chronic infection is mostly observed in the field. It is hoped that the continual presence of *P. tuberculatum* in the field will help suppress lantana growth.

Keywords Lantana, *Lantana camara,* rust, *Prospodium tuberculatum,* biological control.

INTRODUCTION

Lantana camara L. was first introduced into Australia in the mid 1800s and has since spread throughout coastal and subcoastal regions of Queensland and New South Wales. Biocontrol of lantana started in 1914 and 31 agents have since been released, with 18 establishing, causing only seasonal damage. Despite these efforts, lantana is still not under adequate control (Day *et al*. 2003). One of these agents, the rust *Prospodium tuberculatum,* was first released in Australia in 2001 (Tomley and Riding 2002, Ellison *et al.* 2006) and is now widespread in coastal and subcoastal areas of Queensland and New South Wales. As part of the rust project, the storage, susceptibility, efficacy and potential impact of the rust was investigated to determine effectiveness and improve field establishment.

MATERIALS AND METHODS

Viability of *P. tuberculatum* To determine the viability of the urediniospores, freshly collected spores from lantana plants grown in the glasshouse, were semi-dried for 12 hours over silica gel and divided into four equal portions and treated in one of four ways prior to inoculation: stored at -15°C (domestic freezer), stored at 25°C day/15°C night in a Controlled Environment Cabinet (CEC), stored in liquid nitrogen, and mixed with talc at a rate of 0.23 g rust/17.5 g talc and applied immediately to the undersides of 10 tagged leaves on each of 24 potted plants.

Prior to dividing the rust for the above treatments, a small sample of rust was used to inoculate 10 tagged leaves on each of two lantana plants, using a spore suspension in distilled water of 0.1 g rust/100 mL. Another small sample was mixed with talc at 0.23 g rust/ 17.5 g talc (normal field release rate) and applied to the underside of 10 tagged leaves on each of two lantana plants. These plants served as the control for future inoculations.

For each inoculation event, ten leaves on each of two new susceptible lantana plants were inoculated, using the spore suspension in distilled water method at the following frequencies: rust stored at -15°C was tested every four weeks, rust stored in the CEC every week, rust stored in liquid nitrogen after 12 months. Two of the potted lantana plants previously inoculated with the talc mix were selected each week and placed in an incubation chamber.

Inoculated plants were incubated in a perspex cabinet for 24 h at 21°C and 100% humidity prior to being placed in the glasshouse at 25°C day/15°C night, with ambient humidity for rust development. Tagged leaves were monitored for urediniospores and each leaf treated as either negative or positive for rust.

Susceptibility of lantana to *P. tuberculatum* Potted lantana plants originally collected as cuttings from areas with lantana throughout Queensland and New South Wales, were inoculated with a standard

suspension of 0.1 g urediniospores in 100 mL distilled water and subsequently incubated for 24 h at 21°C and 100% humidity to test for their susceptibility to the rust. Development of pustules was monitored over the following three weeks.

Potential impact of *P. tuberculatum* In a glasshouse trial, 82 single-stemmed, similarly grown, potted rust-susceptible pink-flowering lantana plants of similar age were divided randomly into five groups. Plants in one group of 14 were washed, then roots, stems and leaves were dried and weighed separately at the commencement of the experiment. Three of the remaining four groups of 17 plants were inoculated with one of three different rates of inoculum; full strength (0.23 g rust/ 17.5 g talc), half strength and quarter strength. The last group of 17 remained untreated and used as a control.

Plant height was measured and the number of nodes along the single stem of each plant counted weekly. The initiation of growth of side-shoots on this stem and their nodal positions were noted, as was leaf drop from each plant. The number of infected leaves on each plant and the number of pustules on each infected leaf were counted after three weeks.

After seven weeks, all plants were removed from pots and their roots were washed. Their roots, stems and leaves were dried and weighed separately as per the original plant sample.

Field release To enhance establishment of the rust in the field, areas with high moisture retention such as creek banks and south facing gullies were selected. Rust was applied to undersides of leaves by one of three means: applying the rust in talc (at a rate of approximately 0.23 g rust/17.5 g talc) on the undersides of leaves, using the 'salt and pepper' shaker method, by employing a manual or electric fan blower to distribute the rust/talc mix or, less often, by suspending the rust in distilled water (0.1 g rust/100 mL) and spraying this suspension out of a 500 ml sprayer. In some cases, the surfactant *Tween* was used to facilitate the suspension of the spores.

RESULTS

Viability of *P. tuberculatum* Rust applied in talc to plants kept at ambient glasshouse temperatures lost viability by the fifth week following inoculation, whilst rust stored at 25°C day/15°C night lost viability by week nine. Rust which had been stored at -15°C for three months still produced spores on 80% of inoculated leaves. Rust stored in liquid nitrogen remained viable for at least 12 months.

Susceptibility of lantana to *P. tuberculatum* Of 221 plants tested, only pink-flowering lantana plants were susceptible. Neither red, pink-edged red, white nor orange-flowering lantana plants could be infected with the rust. Of the 166 pink flowered plants tested, only 127 were susceptible.

Potential impact of *P. tuberculatum* Stem growth rate was lowest on plants inoculated with quarter strength inoculum. Those plants inoculated with full strength inoculum had a slightly higher growth rate than the control but it was not significant (Figure 1).

Leaf drop was greatest on plants inoculated with full strength inoculum, while the control plants had the lowest leaf drop (Figure 2).

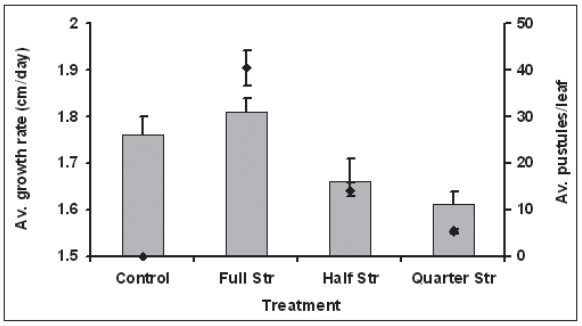

Figure 1. The average growth rate of lantana plants (grey columns) inoculated with full, half and quarter strength inoculum and the average number of pustules per leaf for the four treatments.

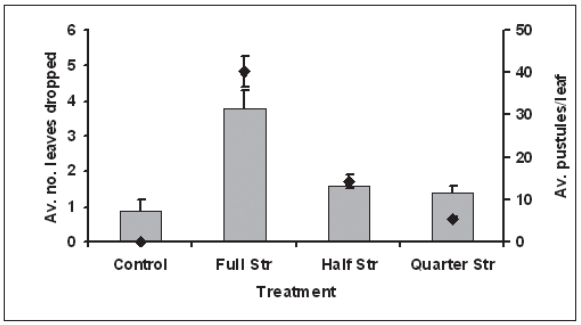

Figure 2. The average number of leaves dropped by lantana plants when inoculated with full, half and quarter strength inoculum and the average number of pustules per leaf for the four treatments.

Field releases Initially most pink-flowering lantana infestations in Queensland and New South Wales were considered suitable for rust release if plants had enough foliage and did not appear wilted. However, as the drought from 2002 to 2008 persisted, lantana at many sites which were viewed as favourable at the time of release, became wilted and thus, the criteria for suitable release sites were revised. By specifically targeting only those lantana infestations in high moisture situations such as along creek beds and south-facing gullies, and by releasing during or just prior to rain, the establishment rate increased from 20% to 60%.

DISCUSSION

Flower colour is the most important criterion in successful field establishment of *P. tuberculatum*. Only pink-flowering lantana plants proved susceptible. However, not all pink-flowering plants were susceptible and it is not possible to determine visually which of these plants would be susceptible. Consequently, it is preferable to test plants prior to release, as there is a chance that plants inoculated opportunistically will fail to develop symptoms if the plants are not susceptible. Following establishment, the rust will spread to all susceptible forms.

Site selection is also important when releasing the rust. Field releases should be undertaken when environmental conditions are favourable, such as impending rain or heavy overnight dew periods, both satisfactory for sporulation. Sites ideally should be in gullies or on south-facing slopes where moisture retention is higher than on exposed slopes.

If conditions are not favourable for immediate field release following harvesting, storage of semi-dried spores in liquid nitrogen would be preferable for long-term storage. Ellison *et al*. (2006) found that rust may survive for at least three years if stored in this manner.

Establishment rates during the first few years of the release program were low due to prolonged periods of drought. However, following attempts to be more selective in release sites and greater rainfall, establishment rates increased to 60%. Field monitoring revealed that teliospores were present at a number of sites in Queensland and New South Wales. Teliospores are the over-wintering stage in the life cycle which allows the rust to survive cold or dry conditions.

Plants treated with full strength rust had the highest average load of rust pustules on leaves and experienced greatest leaf drop. However, they had a higher stem growth rate compared to plants exposed to lower dose rates. This group of lantana plants appeared to outgrow rust infection by dropping leaves. At lower inoculum doses, there were fewer pustules per leaf and a lower leaf drop. However, the average growth rate was also lower, suggesting a more chronic effect. These observations are consistent with field observations where many leaves have low pustule counts.

These results suggest that *P. tuberculatum* can reduce lantana growth and play a role in lantana control. Future releases should be conducted at lower rates to produce chronic infection to avoid initial leaf drop which may affect rust establishment.

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