Pathogenicity, life-cycle elucidation and preliminary host-range testing of *Phakopsora jatrophicola* Cummins

Final Report
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KNOWLEDGE FOR LIFE
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1. **Project Objectives:**

1. Conduct survey in México to collect fresh samples of *Phakopsora jatrophicola*, a rust pathogen of *Jatropha gossypiifolia*, to identify potential alternate hosts in the field and to collect and give provisional identification of other pathogens as potential biocontrol agents of *J. gossypiifolia*.

2. Attempt to elucidate the life-cycle of *P. jatrophicola* through teliospore germination and inoculation studies undertaken at the CABI facilities in the UK and, if feasible, at the CSIRO facilities in Veracruz, México.

3. Complete pathogenicity assessments and evaluate the potential impact of *P. jatrophicola* on morphologically distinct *J. gossypiifolia* varieties in Australia using urediniospores in the quarantine facilities at CABI Europe-UK, Silwood, Ascot.

4. Complete preliminary host-range testing of *P. jatrophicola* against six additional plant species using urediniospores, in the quarantine facilities at CABI Europe-UK, Silwood, Ascot.

5. Provide DPI&F with a written report outlining the methods and results on completion of the work.

2. **Objective 1: Field work**

Field survey work was conducted in Veracruz State, México from 29.10. - 4.11.2008 in order to collect fresh spore material of the rust *Phakopsora jatrophicola*, to identify potential alternate hosts of the rust in the field, as well as to collect material of other pathogens present on *Jatropha gossypiifolia*. The survey was undertaken in collaboration with Ricardo Segura and Carlos Pascacio from the CSIRO Field Station, Boca del Río, Veracruz. In total, 10 sites (elevation between 0 - 58 m.a.s.l.) were visited to survey individual *J. gossypiifolia* populations; one additional site was surveyed for presence of the rust and/or other pathogens on *Jatropha curcas*.

The following sites in Veracruz were surveyed for pathogens associated with *J. gossypiifolia*:

- Puerto Lobos II (18 m.a.s.l.)
- Ingenio San Cristóbal (9 m.a.s.l.)
- Ignacio de la Llave (15 m.a.s.l.)
- Tuzales (7 m.a.s.l.)
- El Zapote (32 m.a.s.l.)
- Antón Lizardo (0 m.a.s.l.)
- Tesechoacan (38 m.a.s.l.)
- Villa Azueta (34 m.a.s.l.)
- Casas Viejas (58 m.a.s.l.)
- Paso Doña Juana (0 m.a.s.l.)

The site “Rancho El Martillo”, located between El Zapote and Antón Lizardo was surveyed for pathogens associated with *J. curcas*.

In general, incidence of and damage caused by *P. jatrophicola* on *J. gossypiifolia* was found to be high in the field. Infection of the rust was visible as...
the uredinial stage with abundant urediniospore production. At most sites
_J. gossypiifolia_ plants exhibited a mixture of old and young uredinia indicating a
continuous infection cycle, though at some sites rust infection appeared to be more
recent with only young uredinia present (Fig. 1).

In order to attempt elucidation of the life cycle of _P. jatrophicola_, plant
species commonly growing in association with _J. gossypiifolia_ in the field were
examined for the presence of rust aecia and/or spermogonia which could indicate the
potential status of such a species as an alternate host for this rust. In particular species
belonging to the genera _Ambrosia_, _Cnidoscolus_, _Croton_, _Euphorbia_, _Malva_
(_M. neglecta_), _Phyllanthus_, _Sida_ (_S. acuta, S. cordifolia, S. rhombifolia_) and _Solanum_
were assessed. There were no signs of aecia or spermogonia on the examined
material.

Leaf material of _J. gossypiifolia_ showing potential signs of teliospore
formation of the rust was collected for subsequent detailed macroscopic and
microscopic assessment. This field material was examined in collaboration with Dr.
Gloria Carrión using the facilities of the Instituto de Ecología (IE), Xalapa, Veracruz,
and assessed against herbarium material of _P. jatrophicola_ held at IE including the
type specimen of the rust: _P. jatrophicola_ ex _Jatropha canescens_, Baja California,
México (Fig. 2). While microscopic evaluation of the field material showed potential
initials of teliospores to be present, fully mature teliospores were not detected.
Consequently, inoculation studies using teliospores to elucidate the life-cycle of the
rust could not be undertaken at this point in time. Teliospores constitute the over-
seasoning spore stage in a rust life-cycle, thus teliospore production of *P. jatrophicola* can be assumed to be triggered by drier and slightly cooler conditions commencing at the start of the dry season in México. In 2008, the rainy season in México lasted unusually long until the beginning of November; hence climatic conditions would not have favoured teliospore formation at the time of the field survey. It was agreed, therefore, that field material of the rust would be collected later during the dry season by CSIRO collaborators and sent to the UK for the life-cycle studies.

Besides *P. jatrophicola*, the most common and damaging pathogen on *J. gossypiifolia* was found to be a (or possibly a number of different) cercosporoid pathogen(s). This fungus is most likely *Cercospora* sp., but this would need to be officially identified and confirmed. *Cercospora jatrophicola* has been recorded on *J. gossypiifolia* from Cuba (Farr, D.F. & Rossman, A.Y. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved June 11, 2009, from http://nt.ars-grin.gov/fungal databases/). At some sites damage due to this cercosporoid pathogen was found to be comparable to that caused by the rust (Fig. 3) and frequently both pathogens were found to be associated within one necrotic lesion. Two strains of the potential *Cercospora* sp. have been isolated and are currently kept in culture at CABI E-UK for potential future assessments (W 2479, W 2480; see Table 1 below). Other pathogens identified provisionally from *J. gossypiifolia* are a *Fusarium*-like pathogen, producing white pustules protruding from the stomata, and a powdery mildew, as well as two mycoparasites. *Phakopsora jatrophicola* and a cercosporoid pathogen (*Cercospora* sp.) have also been identified from *J. curcas*.
Upon return to the UK, all field material was assigned an internal CABI W-number and accessed into a reference collection as listed in Table 1. Some of the material was also lodged with the CABI Herbarium and has been assigned an official IMI number.

The field collection of the *P. jatrophicola* strain W 2478 ex *J. gossypiifolia*, El Zapote (see Table 1) is a re-collection of the strain W 2028 collected in 2000 by Ricardo Segura. This rust strain has been evaluated during this first phase of the project with respect to its pathogenicity and host specificity. A voucher specimen of this rust strain (W 2478) has been placed into the CABI herbarium under the reference number IMI 397220.

Further field collections of *P. jatrophicola* on leaf material from *J. curcas* were made by Ricardo Segura and Moises Martinez from two separate locations in México, Mata de Uva, Veracruz (15 m.a.s.l.) and Santa Fe, Veracruz (45 m.a.s.l.) in March 2009. The material was shipped to CABI E-UK in order to evaluate the host specificity of rust strains from *J. curcas*. Urediniospores of both rust strains have been deposited in liquid nitrogen to preserve spore viability and field material was accessed into the internal CABI reference collection (W 2482, W 2483; Table 1). Voucher specimens of the dried plant material have also been lodged in the CABI herbarium under the reference numbers: *P. jatrophicola* ex Mata de Uva – IMI 397097; *P. jatrophicola* ex Santa Fe – IMI 397098.
Table 1: Field material of diseased *J. gossypiifolia* and *J. curcas* accessed into the CABI reference collection

<table>
<thead>
<tr>
<th>Reference number</th>
<th>Location of collection in Veracruz State, México</th>
<th>Host</th>
<th>Pathogens</th>
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<tbody>
<tr>
<td>W 2473</td>
<td>Casas Viejas</td>
<td><em>J. gossypiifolia</em></td>
<td><em>P. jatrophicola, Cercospora sp. (?)</em>, rust mycoparasite</td>
</tr>
<tr>
<td>W 2474</td>
<td>Rancho El Martillo</td>
<td><em>J. curcas</em></td>
<td><em>P. jatrophicola, Cercospora sp.</em></td>
</tr>
<tr>
<td>W 2475</td>
<td>Tesechoacan</td>
<td><em>J. gossypiifolia</em></td>
<td><em>P. jatrophicola, Cercospora sp. (?)</em>, Powdery mildew</td>
</tr>
<tr>
<td>W 2476</td>
<td>Villa Azueta</td>
<td><em>J. gossypiifolia</em></td>
<td><em>P. jatrophicola, Cercospora sp. (?)</em>, Powdery mildew</td>
</tr>
<tr>
<td>W 2477</td>
<td>Antón Lizardo</td>
<td><em>J. gossypiifolia</em></td>
<td><em>P. jatrophicola</em></td>
</tr>
<tr>
<td>W 2478/IMI 397220</td>
<td>El Zapote</td>
<td><em>J. gossypiifolia</em></td>
<td><em>P. jatrophicola</em></td>
</tr>
<tr>
<td>W 2479</td>
<td>Puerto Lobos II</td>
<td><em>J. gossypiifolia</em></td>
<td><em>P. jatrophicola, Sphaerellopsis (Eudarluca)</em>, Cercospora sp.(? 2 types), Fusarium-like sp.</td>
</tr>
<tr>
<td>W 2480</td>
<td>Ingenio San Cristóbal</td>
<td><em>J. gossypiifolia</em></td>
<td><em>P. jatrophicola, Cercospora sp. (?)</em></td>
</tr>
<tr>
<td>W 2482/IMI 397097</td>
<td>Mata de Uva</td>
<td><em>J. curcas</em></td>
<td><em>P. jatrophicola</em></td>
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<tr>
<td>W 2483/IMI 397098</td>
<td>Santa Fe</td>
<td><em>J. curcas</em></td>
<td><em>P. jatrophicola</em></td>
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<tr>
<td>W 2484</td>
<td>Ignacio de la Llave</td>
<td><em>J. gossypiifolia</em></td>
<td><em>P. jatrophicola, Cercospora sp. (?)</em></td>
</tr>
<tr>
<td>W 2485</td>
<td>Tuzales</td>
<td><em>J. gossypiifolia</em></td>
<td><em>P. jatrophicola, Cercospora sp. (?)</em></td>
</tr>
<tr>
<td>W 2486</td>
<td>Paso Doña Juana</td>
<td><em>J. gossypiifolia</em></td>
<td><em>P. jatrophicola, Cercospora sp. (?)</em></td>
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* Mycoparasite

### 3. **Objective 2: Life-cycle elucidation**

As detailed before (Objective 1: Field work), teliospore material could not be collected during the field work conducted in autumn 2008. As agreed, diseased plant material was shipped from México towards the end of March 2009. Collections of rusted *Jatropha gossypiifolia* material had been made from various locations in the Mexican states of Veracruz, Oaxaca and Tabasco by staff of the CSIRO Field Station from December 2008 through to March 2009. Examination of the material has commenced starting with those samples collected in March when the dry season was well advanced. Leaf sections were made for microscopic examination, but presence of
teliospores was not detected. Assessment of the remaining material will continue and this work will form part of the second agreed phase of the project.

4. **Objectives 3 and 4: Pathogenicity assessment and Host-range testing**

The pathogenicity and the preliminary host-range studies were undertaken using the CABI quarantine greenhouse facilities at Silwood, Ascot, Berkshire, UK.

Cuttings of the Australian varieties of *Jatropha gossypiifolia* (‘Queensland Bronze’, ‘Queensland Green’, ‘Queensland Purple’, ‘Darwin Purple’, ‘Katherine Green’, ‘Kunamara Green’ (‘Western Australian Green’)) to be tested for susceptibility to *Phakopsora jatrophicola* were sent by DEEDI (formerly DPF&I) and established in the CABI quarantine greenhouse. The following plant species to be assessed during the preliminary host-specificity testing of the rust were also established in the quarantine greenhouse: *Jatropha multifida*, *Jatropha podagrica* and *Manihot esculentum* from cuttings sent by DEEDI; *Jatropha integerrima* from cuttings obtained from “Jardin Naturel”, Saint Leu, Réunion, France; *Hevea brasiliensis* from cuttings as well as from seeds collected in Brazil; *Jatropha curcas* from seeds sent by DEEDI and by “Jardin Naturel”, Saint Leu, Réunion, France.

Assessments of the pathogenicity and virulence of *P. jatrophicola* towards the different *Jatropha* varieties as well as of its host specificity towards the six closely related non-target species were undertaken using the rust strain W 2028 (identical to W 2478/ IMI 397220) collected ex *J. gossypiifolia* from El Zapote, Veracruz in July 2000 by Ricardo Segura. Urediniospores of this strain have been maintained in liquid nitrogen at CABI E-UK since 2000. Spores were found to be viable upon retrieval from liquid nitrogen and thus this rust strain could be successfully established on *J. gossypiifolia* plants in the quarantine greenhouse at the onset of the first project phase.

During the course of the project it was also attempted to establish an additional strain of *P. jatrophicola* from Venezuela (collector Ricardo Segura, Puente Angosturita II, Bolivar, Venezuela, October 2008; CABI reference number W 2481) in the quarantine greenhouse in order to assess any potential differences in pathogenicity and specificity of the two rust strains. However, due to the limited spore inoculum available on the dried field material, inoculations with this Venezuelan rust strain were unsuccessful and no sporulation was recorded on inoculated plants.

4.1. **Experimental methods**

4.1.1. **Inoculations**

Rust inoculum was prepared by carefully dislodging urediniospores from uredinia on infected leaves of *J. gossypiifolia* plants into a watch glass using a fine needle. Urediniospores were gently mixed with talcum powder at approximate quantities of 1:1 (one needle tip of spores to one paintbrush No 1 tip of talcum powder) and the spore mixture was brushed onto the upper and lower leaf surfaces of *J. gossypiifolia* and test plants using a fine paintbrush (No 1). Three leaves of
different ages (mature, fully expanded, apical) were inoculated per plant. Inoculated leaves were sprayed with a mist of sterile distilled water before plants were incubated in a dew chamber at 20°C for 48hrs. Viability of the urediniospore inoculum was assessed by brushing parts of the talcum-spore mixture onto tap water agar and incubating the Petri dish with the inoculated plants in the dew chamber. Spore germination on agar was assessed after 24 hrs. After removal from the dew chamber inoculated plants were maintained in a designated quarantine greenhouse chamber (natural light conditions with supplement lighting for 15 hrs, 20-30°C) and regularly assessed for any signs of chlorosis and sporulation of the rust.

Previous inoculation studies had shown that using a talcum-spore mixture resulted consistently in high levels of infection and sporulation of the rust. Therefore, this method was used for all of the initial susceptibility assessments of the different varieties of *J. gossypiifolia* towards the selected rust strain, the dew and temperature experiments, as well as for all host-specificity testing in order to provide the most favourable conditions for urediniospore germination and infection.

4.1.2. Dew period
The minimum dew period required by *P. jatrophicola* for host infection was established by inoculating *J. gossypiifolia* (any variety) with the talcum-urediniospore mixture and incubation in the dew chamber as outlined above. Inoculated plants were removed from the dew chamber after 6 hrs, 5 hrs, 4 hrs and 3 hrs with inoculated plants used as controls receiving 24 hrs of dew. All plants were regularly assessed for developing chlorosis and rust sporulation for up to 6 weeks after inoculation. For each dew period assessed 2-3 replicate plants were used.

4.1.3 Influence of temperature on urediniospore infection
An initial assessment of the temperature range required for infection and sporulation of *P. jatrophicola* on its host was undertaken by inoculating plants of *J. gossypiifolia* variety ‘Kunamara Green’ with the talcum-spore mixture and incubating them for 48 hrs in the dew chamber running at 15°C, 20°C and 25°C, respectively. Subsequently, all plants were regularly assessed for symptoms of rust infection. For each incubation temperature, two replicate plants were used. A quantitative assessment of the level of infection for the different incubation temperatures could not be undertaken due to the limited time frame of the first phase of the project.

4.1.4. Assessment of pathogenicity and susceptibility of distinct *J. gossypiifolia* varieties
Following the initial evaluation of the overall susceptibility of Australian varieties of *J. gossypiifolia* to rust strain W 2028, experiments were undertaken to quantify the degree of susceptibility of individual varieties to evaluate the potential impact of the rust on these varieties. The experiments were undertaken by applying defined urediniospore concentrations as spore suspensions in water to the lower leaf surface and counting the number of uredinia in a defined leaf area two weeks after inoculation. Although pre-experiments had shown that infection levels were lower when using urediniospores suspended in water rather than a talcum-spore mixture, for
the purpose of a comparative assessment of the susceptibility of *J. gossypiiifolia* varieties this was of minor importance.

Spore inoculum was prepared by dislodging urediniospores from uredinia and suspending them in sterile distilled water containing 0.01 % Tween 80. The spore suspension was filtered through a 63 µm mesh sieve to eliminate small plant debris. Spore suspensions with concentrations of 3-4 x 10⁵ spores ml⁻¹, as assessed using a haemocytometer, were applied to *J. gossypiiifolia* plants using a 30 ml plastic atomizer. Three leaves of different ages (mature, fully expanded, apical) were inoculated per plant by spraying 1 ml of spore suspension (total of 3-4 x 10⁵ spores) from a distance of *circa* 20 cm onto the lower leaf surface, centred on the central lobe of the leaf. All inoculated plants were incubated under conditions described above and spore germination on agar was assessed after 24 hrs. Fourteen days after inoculation, the number of uredinia per 9 cm² was counted for the leaf area showing the highest density of pustules by using a 9 cm² quadrate of transparent plastic sheet. Three replicate plants were used for each of the varieties ‘Queensland Bronze’, ‘Queensland Green’, ‘Queensland Purple’, ‘Darwin Purple’, ‘Katherine Green’, ‘Kunamara Green’ (‘Western Australian Green’). For each individual test run, three plants of different varieties were used. The data were analyzed using a generalised linear model with variety and leaf age as fixed factors and a Poisson data distribution to test for differences in the number of pustules among varieties and leaves of different ages.

4.1.5. Host-range testing

Host-specificity studies were undertaken using a total of six replicate plants per non-target species in at least two separate test runs. For *J. curcas* a total of 11 replicate plants were assessed (two plants grown from Australian seed shipment received July 2008; eight plants grown from Australian seed shipment received December 2008; one plant grown from seed ex Réunion). Rust inoculations with strain W 2028 were undertaken as outlined before with two to three *J. gossypiiifolia* plants of any of the six Australian varieties used as controls for each test run. One test run for *J. podagrica* was undertaken using a suspension of spores in sterile distilled water, in order to avoid the interference of talcum particles on the plant cuticle during subsequent microscopic examination. Sporulation of *P. jatrophicola* on *J. gossypiiifolia* confirmed the test run to be positive. All test plants were kept for up to 8 weeks after sporulation was recorded on the controls to allow for any late development of symptoms. During this period plants were regularly assessed for macroscopic symptoms of rust development and/or plant defence reactions using a x20 hand lens. After a total of 8 weeks all remaining inoculated leaves were sampled and examined using a stereomicroscope. Small leaf samples were taken for clearing and staining (Bruzzone & Hasan, 1983) and subsequent microscopic evaluation. When sporulation of the rust was recorded on non-target species, viability of the urediniospores produced was tested as spore germination on agar and their infectivity through re-inoculation onto *J. gossypiiifolia* plants and plants of the respective non-target species.

In addition to the host-specificity studies conducted with *P. jatrophicola* strain W 2028 ex *J. gossypiiifolia*, the infectivity of *P. jatrophicola* strain W 2482 ex *J. curcas* towards *J. gossypiiifolia* was evaluated. This assessment was undertaken to further elucidate the potential existence of host-specific strains of the rust.
Inoculations were carried out as outlined before using a talcum-spore mixture and two replicate plants for each, *J. curcas* and *J. gossypiifolia* (varieties ‘Katherine Green’ and ‘Kunamara Green’) were inoculated. Urediniospore inoculum used for this experiment was low due to the limited field material of rusted *J. curcas* leaves available.

4.2. Results

4.2.1. Infection and disease development on the host *J. gossypiifolia*

Under quarantine greenhouse conditions inoculation with urediniospores of *P. jatrophicola* (strain W 2028) consistently led to high infection levels with abundant sporulation on the host plant *J. gossypiifolia* (Fig. 4). Thus inoculum of this rust can be easily maintained and augmented *in planta* in the greenhouse. Plant susceptibility to rust infection tended to decrease from the older to the younger leaves. Higher infection levels were obtained when inoculating lower compared to upper leaf surfaces of the host.

![Figure 4](image)

**Figure 4:** Sporulation of *P. jatrophicola*, strain W 2028, under greenhouse conditions 3 weeks after inoculation

A) *J. gossypiifolia* var. ‘Kunamara Green’; advanced infection and leaf wilting

B) *J. gossypiifolia* var. ‘Queensland Bronze’, close up of uredinia

Under the experimental conditions used successful infection with the rust leads to the development of chlorotic leaf spots on *J. gossypiifolia* 7 - 9 days after inoculation. Urediniospore sporulation of the rust is generally visible 2 - 3 days later with uredinia starting to develop from the centre of the chlorotic lesions. The development of teliospores was never observed in the greenhouse.

A microscopic assessment of urediniospore-derived infection of *P. jatrophicola* showed that urediniospores germinate with a short germ tube forming an appressorium over an epidermal cell rather than over a stomatal opening. (Fig. 5A). This indicates that penetration of the host is directly via the cuticle. Direct penetration of the host by urediniospores is known for the related rust species *Phakopsora pachyrhizi*, *Phakopsora apoda* and *Physopella zeae* (Bonde *et al.*, 1976, Bonde *et al.*, 1976).
1982, Adendorff & Rijkenberg, 2000). Compared to the indirect penetration by urediniospore germ tubes via the stomatal pore such direct penetration behaviour of urediniospores is far less common. As typical for a compatible pathogen-host reaction haustoria formation and extensive mycelial colonization of the leaf tissue of \emph{P. gossypiifolia} (Fig. 5B,C) lead to the formation of uredial primordia and ultimately to sporulation of the pathogen on this host.

![Figure 5: P. jatrophicola, strain W 2028, on J. gossypiifolia](image)

A) urediniospore (single arrow) germinating and forming appressorium (double arrow), x400
B) internal mycelium with haustoria (arrow), x400
C) extensive colonization of host tissue with internal mycelium, x400

Microscopic observations of the behaviour of \emph{P. jatrophicola} on its host \emph{J. gossypiifolia} formed the basis for the interpretation of the behaviour of the rust on the non-host plants as detailed in the section on host-specificity studies.

4.2.2. Dew period

The minimum dew period required for successful infection and sporulation of \emph{P. jatrophicola} was found to be 4 hrs at an incubation temperature of 20°C. However, after a 4 hr dew period rust infection was found to be inconsistent (sporulation only on one of the two replicate plants) and infection levels were clearly lower than those seen after incubation periods of 5 hrs and longer. No difference was noted regarding the time needed for symptom development of rust infection, i.e. chlorosis and sporulation, between inoculated plants receiving an initial 4 hr dew period and those submitted to longer dew periods. Dew periods above 4 hrs consistently led to high levels of
infection. An initial dew period of 3 hrs was insufficient for \textit{P. jatrophicola} to infect and sporulate on its host.

4.2.3. \textbf{Influence of temperature on urediniospore infection}

The rust was able to infect and sporulate on its host following all three dew period incubation temperatures assessed (15°C, 20°C, 25°C). The initial incubation temperature had no apparent influence on the speed of symptom development, i.e. on chlorosis and uredinia formation. Thus, initial experiments indicate a wide temperature range for spore germination and infection of \textit{P. jatrophicola}. Further inoculations will need to be undertaken in order to establish the full temperature range for infection, and quantitative inoculations will need to be carried out to assess the level of infection in response to incubation temperature for urediniospore germination and infection.

4.2.4. \textbf{Pathogenicity and susceptibility assessment}

An initial assessment of the pathogenicity of \textit{P. jatrophicola} towards the different Australian varieties of \textit{J. gossypiifolia} showed the rust to infect and sporulate on each of the six varieties and thus confirmed their susceptibility towards the strain W 2028.

The subsequent evaluation of the degree of susceptibility of individual \textit{J. gossypiifolia} varieties or, conversely, the virulence of the rust strain towards a specific variety showed the varieties ‘Queensland Purple’ and ‘Kunamara Green’ to be most susceptible to the rust strain, while ‘Queensland Green’ and ‘Queensland Bronze’ seem to be least susceptible (Graph 1). The statistical analysis confirmed that there is a significant difference in the mean number of pustules produced on the different varieties (generalized linear model: $F_{5,36} = 2.75, P = 0.03$) which appears to be based on the higher susceptibility of the varieties ‘Kunamara Green’ and ‘Queensland purple’ (Graph 1).
Graph 1: Mean number of uredinia produced on three leaf ages of distinct varieties of 
*J. gossypiifolia* following application of a defined urediniospore concentration of 
*P. jatrophicola*, strain W 2028

These results give a first indication that the impact of *P. jatrophicola*, strain W 2028, 
could be highest on the two varieties ‘Kunamara Green’ and ‘Queensland Purple’. 
However, this would need to be further assessed under field conditions. It is also 
possible that the susceptibility of individual *J. gossypiifolia* varieties differs towards 
other rust strains of *P. jatrophicola*.

### 4.2.5. Host specificity

Based on the initial assessment of the host specificity of the *P. jatrophicola* 
strain W 2028, the rust appears to be specific to species belonging to the genus 
*Jatropha*. Out of the four non-target *Jatropha* species assessed, sporulation occurred 
on *J. multifida*, *J. curcas* and, in one case, also on *J. integerrima*. Prominent defence 
reactions were observed on rust-inoculated leaves of *J. integerrima* and *J. podagrica*, 
while for *M. esculentum* and *H. brasiliensis* no macroscopic symptoms which could 
be clearly linked to the rust were observed.

Detailed observations of macroscopic and microscopic symptoms on non-
target species in response to inoculation with *P. jatrophicola* urediniospores are as 
follows:

- **Jatropha multifida:** 
  *Jatropha multifida* proved to be susceptible to the *P. jatrophicola* strain 
  assessed showing symptoms comparable to those observed on *J. gossypiifolia*. The 
  rust strain sporulated consistently on *J. multifida* producing viable urediniospores
(shown as spore germination on agar) which were infective to both *J. gossypiifolia* (variety ‘Queensland Green’) and *J. multifida*. Uredinia formation on *J. multifida* was slightly delayed (1-2 days), as well as less prolific, compared to *J. gossypiifolia* (Fig. 6). Leaf age did not have any apparent influence on the symptoms observed for *J. multifida*.

![Figure 6](image)

Figure 6: Sporulation of *P. jatrophicola*, strain W 2028, on *J. multifida* 13 days after inoculation; Note: sporulation is less prolific compared to sporulation on *J. gossypiifolia* in Figure 4

While *J. multifida* is not a reported host of *P. jatrophicola* (Farr, D.F. & Rossman, A.Y. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved June 11, 2009, from http://nt.ars-grin.gov/fungaldatabases/), the rust species *Uromyces jatrophae* has been recorded on *J. multifida* from México (Gallegos & Cummins, 1981; Herb IMI). *Uromyces jatrophae* has also been recorded on *J. gossypiifolia* from Cuba (Arnold, 1986) and on *Manihot esculenta* from Brazil, Colombia, México and some of the Caribbean islands (Hennen et al., 2005). The rust genus *Uromyces* is characterized by subepidermal, erumpent telia producing teliospores which are born singly on pedicels. The genus *Phakopsora*, on the other hand, has typically subepidermal, non-erumpent telia producing sessile teliospores arranged in crusts (Cummins & Hiratsuka, 2003). Hence the two rust species can easily be distinguished.

- *Jatropha curcas*:

  *Jatropha curcas* showed to be partially susceptible to the *P. jatrophicola* strain W 2028 ex México. Urediniospore inoculation caused initial chlorotic spots on inoculated leaves of *J. curcas*, which subsequently turned necrotic. Necrotic leaf spots
were frequently surrounded by green islands, a typical infection symptom for biotrophic pathogens such as rusts (Fig. 7). Symptoms of rust infection (chlorosis and necrosis) were visible at about the same time on inoculated *J. curcas* plants as on the control plants of *J. gossypiifolia*, while subsequent sporulation appeared to be delayed on *J. curcas* plants. This observation, however, might be partly due to the difficulty in detecting initial, very limited, formation of urediniospores *in vivo* on the plant using a hand lens. Frequently, sporulation could only be confirmed after leaves had abscised and were examined at high magnification using a stereomicroscope. While the rust strain *ex J. gossypiifolia* was able to sporulate on inoculated leaves of *J. curcas*, the number and size of rust pustules as well as the spore production were typically restricted compared to the sporulation observed on *J. gossypiifolia* (Fig. 8).

Overall, mature and fully-expanded leaves of *J. curcas* were shown to be more susceptible to the rust, while apical leaves exhibited less symptoms of rust infection, i.e. less chlorotic/necrotic spotting as well as infrequent sporulation.

Figure 7: Infection of *J. curcas* ex seeds from second Australian shipment and *J. gossypiifolia* variety ‘Kunamar Green’ with *P. jatrophicola*, strain W 2028, 12 days after inoculation; A) *J. curcas*, mature leaf, lower surface, showing necrosis, green islands and restricted sporulation (arrow), B) *J. gossypiifolia* control showing abundant sporulation.
Figure 8: Infection of *J. curcas* ex seeds from second Australian shipment with *P. jatrophicola*, strain W 2028, 40 days after inoculation; restricted sporulation on lower surface of apical leaf (arrowed)

The two *J. curcas* plants grown from the first seed shipment from Australia proved to be less susceptible to the rust strain under evaluation with only one of the two plants showing a single, extremely small rust pustule on each, the mature and the fully-expanded leaf. Susceptibility of *J. curcas* plants from the second Australia shipment was consistent and slightly higher. Symptoms of rust infection on the *J. curcas* plant from Réunion were generally less severe, but restricted sporulation of the rust was recorded on all inoculated leaves.

Urediniospores of *P. jatrophicola* strain W 2028 produced on *J. curcas* plants proved to be viable (shown as spore germination on agar). Inoculation of *J. gossypiifolia* (varieties ‘Katherine Green’ and ‘Queensland Purple’) and of *J. curcas* using urediniospores formed on *J. curcas* resulted in infection and sporulation on both species (Fig. 9). Thus, strain W 2028 ex *J. gossypiifolia* can produced viable urediniospores on *J. curcas* which are infective to both the original host *J. gossypiifolia* and *J. curcas*. However, it is possible that over time urediniospores of W 2028 ex *J. gossypiifolia* cycled exclusively through *J. curcas* might lose infectivity.
Conversely, infectivity studies using *P. jatrophicola* strain W 2482 ex *J. curcas* showed *J. gossypiifolia* to be only partially susceptible to this rust strain. Sporulation of the rust was first recorded on its host *J. curcas* 15 days after inoculation. Infection levels, i.e. the number of uredinia produced, were low due to the low spore concentration in the inoculum used, but spore production of the individual uredinia was normal. Symptom development on *J. gossypiifolia* was noticeably delayed with small necrotic spots visible on one of the inoculated plants 24 days after inoculation. Sporulation was first noted on the older, more susceptible leaf of *J. gossypiifolia* 6 weeks after inoculation for the variety ‘Kunamar Green’ (4 weeks later than *J. curcas* controls) and 9 weeks after inoculation for the variety ‘Katherine Green’ (7 weeks later than *J. curcas* controls). Sporulation on the young leaf was only recorded for the variety ‘Kunamar Green’, 9 weeks after inoculation. In general, sporulation of strain W 2482 on *J. gossypiifolia* was extremely limited in both number of uredinia formed as well as spore production (Fig. 10). Spores produced on *J. curcas* were viable (confirmed as germination on agar); their infectivity could not be assessed as spore production was too low.
Figure 10: Sporulation of *P. jatrophicola* ex *J. curcas*, strain W 2482, on mature leaves of *J. curcas* (A) and on *J. gossypiifolia* variety ‘Kunamara’ Green (B) 7 weeks after inoculation; Note: extremely restricted sporulation on *J. gossypiifolia* (arrowed)

*Phakopsora jatrophicola* is commonly found on both *J. gossypiifolia* and *J. curcas* in the field in México and both species are reported hosts of this rust (Farr, D.F. & Rossman, A.Y. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved June 11, 2009, from http://nt.ars-grin.gov/fungaldatabases; Herb IMI). The results of the cross-inoculation studies conducted using rust strain W 2028 ex *J. gossypiifolia* and rust strain W 2482 ex *J. curcas* strongly indicate the existence of distinct populations of this rust specialized on individual host species. Furthermore, there are indications of different levels of susceptibility towards the *P. jatrophicola* strain ex *J. gossypiifolia* within the *J. curcas* plants assessed (i.e. from the first and second seed shipment from Australia). This could be due to different biotypes or varieties of these plants involved.

- *Jatropha integerrima*:

Out of the six *J. integerrima* plants assessed, the rust was only able to sporulate on one plant. The other five plants showed strong defence reactions following rust inoculation. For all plants, macroscopic symptoms first became visible as chlorotic and necrotic spots on rust-inoculated leaf areas. Symptom development was typically slightly delayed with chlorotic/necrotic lesions appearing *circa* three to four days after they were first noted on the *J. gossypiifolia* controls. Over time necrotic leaf spots became
raised exhibiting a black centre and the surrounding leaf tissue reddened (Fig. 11). For the majority of inoculated plants no sporulation within these lesions was observed. Symptoms were consistently more pronounced on the mature leaves and least on the apical leaves. For all leaf ages, stronger defence reactions were observed when urediniospores had been inoculated onto lower leaf surfaces compared to inoculations of the upper leaf surfaces (Fig. 12).

Figure 11: Macroscopic defence reactions of *J. integerrima* (‘non-hirsute leaf type’) one months after inoculation with urediniospores of *P. jatrophicola*, strain W 2028
A) fully expanded leaf at time of inoculation, lower leaf surface showing necrotic spotting and reddening of surrounding tissue
B) close-up of necrotic lesions and reddened leaf tissue, fully expanded leaf at time of inoculation, lower leaf surface
Figure 12: Degree of defence reactions in relation to leaf age of *J. integerrima* (‘non-hirsute leaf type’) 2 months after inoculation with *P. jatrophicola*, strain W 2028; From left to right: mature leaf, fully expanded leaf, apical leaf at time of inoculation, A) upper leaf surface, B) lower leaf surface; Note: for all leaf ages, defence reactions were more pronounced on the leaf half which had been inoculated from the lower leaf surface (arrowed, showing the symptoms on upper leaf surface) compared to the other leaf half inoculated on the upper surface.

In the one case where *P. jatrophicola* sporulation was noted on *J. integerrima*, uredinia formation was slightly delayed compared to the controls and sporulation was restricted both in number and size of uredinia with very low spore production (Fig. 13). Viability of the uredinospores produced on *J. integerrima* was confirmed as spore germination on agar, inoculation of *J. gossypiifolia* (varieties ‘Katherine Green’ and ‘Darwin Purple’) resulted in sporulation, thus confirming their infectivity. Due to the limited amount of spore inoculum available spore infectivity could not be tested against *J. integerrima*. 
Figure 13: Infection of *J. integerrima* (‘hirsute leaf type) with *P. jatrophicola*, strain W 2028, 2 weeks after inoculation; necrotic lesions with commencing restricted sporulation on lower surface of mature leaf (arrowed). Note: no plant defence reactions macroscopically visible at this stage

Microscopic assessment of leaf areas of *J. integerrima* showing macroscopic symptoms revealed the presence of internal fungal hyphae which were consistently seen to be necrotic. It can thus be concluded that urediniospores are able to penetrate this host, but subsequent internal development of the rust is severely compromised (Fig. 14) compared to the development in its host *J. gossypiiifolia* (compare Fig. 5).
The six *J. integerrima* plants ex Réunion differed in their leaf morphology: four plants had non-hirsute, shiny leaf surfaces, the leaves of one plant were strongly hirsute, the leaf of the remaining plant was also hirsute, but to a lesser degree. Since the *J. integerrima* plant showing strongly hirsute leaves was the only one susceptible to *P. jatrophicola*, this plant might represent a different, more susceptible biotype or maybe even a different variety.

*Jatropha integerrima* has been recorded as a host of *P. jatrophicola* in a report of the Division of Plant Industry, Florida Department of Agriculture and Consumer Services (Leahy, 2004). However, the rust is not listed on *J. integerrima* in the official databases of Herb IMI or the Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA.

- *Jatropha podagrica*:

  *Jatropha podagrica* showed strong defence reactions following inoculation with the rust strain under evaluation. Symptoms were first visible as chlorotic spots turning necrotic with time. Necrotic spots generally had a dark brown to black centre frequently surrounded by a darkened ring (Fig. 15). Necrotic tissue was often raised
but sporulation of the rust was never observed on *J. podagrica*. Leaf age did not have any apparent influence on the symptoms observed.

Figure 15: Macroscopic defence reactions of *J. podagrica*, fully expanded leaf, in form of necrotic spots with darkened centre, 10 weeks after inoculation with urediniospores of *P. jatrophicola*, strain W 2028.

Microscopic examination of stained and cleared leaf material showed germinating urediniospores forming an appressorium over epidermal cells or cell junctions (Fig. 16A). Commonly, a penetration hole could be seen as a lightly blue stained ring below the appressorium (Fig. 16A,B). Internal hyphae were always found to be short and encased (Fig. 16C). Extensive internal mycelium was never observed. Thus, the clearly visible plant defence reactions successfully prevent the development of the rust in the very early stages.
Hevea brasiliensis did not express any macroscopic symptoms following inoculation with *P. jatrophicola*, which could be interpreted as defence reactions or as signs of rust infection. The rust never sporulated on any of the inoculated leaves. Occasionally, chlorotic and/or necrotic spots were recorded, particularly on the apical leaves of *H. brasiliensis*. Microscopic assessments of selected inoculated leaf samples showed urediniospores with long germ tubes which frequently induced cell necrosis in underlying tissues (Fig. 17). The formation of appressoria, successful penetration or internal development was not observed.
Manihot esculentum

As for *H. brasiliensis*, no clear symptoms were observed on *M. esculentum* in response to inoculation with *P. jatrophicola*. The rust never sporulated on this non-target species. Any chlorotic or necrotic spotting noted on inoculated leaves was generally also observed on non-inoculated leaves. Microscopic examination of selected inoculated leaf samples showing such symptoms revealed *P. jatrophicola* spores germinating either with long germ tubes forming appressoria, or appressoria formed adjacent to the spore (‘sessile appressoria’) inducing plant defence responses in the form of necrosis and cell wall thickening (Fig. 18). Successful penetration with internal development of the rust was not observed.
5. **Summary and Conclusions**

Field work conducted in México at the end of October/beginning of November 2008 showed widespread infection of *Jatropha gossypiifolia* with *Phakopsora jatrophicola* with urediniospores being the prevalent spore stage. Teliospores were not detected on infected field material at this time of the year. Later collections of diseased field material from México and shipped to the UK also failed to show the telial stage, but to date not all of the material has been examined. The second most damaging pathogen on *J. gossypiifolia* in the field in México was found to be a cercosporoid fungus, *cf Cercospora.*
Initial studies of the biology and host specificity of *P. jatrophicola* conducted under quarantine greenhouse conditions proved that the strain **W 2028** ex *J. gossypiifolia* evaluated is pathogenic to all Australian varieties of *J. gossypiifolia* assessed. Differences were established in the degree of susceptibility of individual varieties, with the two varieties ‘Kunamara Green’ and ‘Queensland Purple’ being the most susceptible ones. The rust needs a short dew period of 4 hrs for spore germination and infection and evaluation of the temperature window for host infection showed this to be wide. Initial host-specificity assessments proved the evaluated strain **W 2028** to be specific to species within the genus *Jatropha*. Results for inoculations of *J. curcas*, as well as cross-inoculation studies undertaken with a rust strain ex *J. curcas* (**W 2482**), indicated that host specific populations of *P. jatrophicola* exist which are adapted to its various reported hosts. Hence, it was decided to focus further studies on the evaluation of additional rust strains ex *J. gossypiifolia*, in order to identify a strain, which is both virulent to *J. gossypiifolia* as well as highly specific to this host, for further comprehensive host-range studies.

6. **Taxonomy**

The combination *Phakopsora jatrophicola* Cummins was published by Cummins (1937) as a transfer of *Uredo jatrophicola*, the anamorph name for the asexual (uredinial) stage, to the teleomorph (sexual telial stage) genus *Phakopsora*. However, in his original publication Cummins described the telia in English rather than in Latin, as would have been required by the code. In a later publication Cummins (1956) published *Phakopsora jatrophicola* Cummins as a new species in Latin based on the type on *Jatropha canescens* Muell.-Arg from México, Baja California, **M. E. Jones-24531**. Since the combination *Phakopsora jatrophicola* had already been taken, this name could not be used for a new species (Hennen et al., 2005). As Cummin’s combination *Phakopsora jatrophicola* (Arthur) Cummins (1937) technically only refers to the anamorph (or ‘uredinial’ stage) Buriticá (1994) published a new name for this species:


≡ *Phakopsora jatrophicola* Cummins, Mycologia 48: 604. 1956. TYPE on *Jatropha canescens* Mueller from México, Baja California: Laguna Mountains, east of Todos los Santos, Feb.21, 1928, **M. E. Jones-24531**.

Anamorph


≡ *Uredo jatrophicola* Arthur, Mycologia 7: 331. 1915. TYPE on *Jatropha curcas* Linnaeus from Puerto Rico, Hormigueros, 14 Jan 1914, **F. L. Stevens-220**.

To date, the name *P. arthuriana* has not been taken up in the ‘Index Fungorum’, and the species is still referred to as *P. jatrophicola*.
7. References


8. **Contacts**

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