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RESEARCH ARTICLE

Observations on the gall rust fungus *Prospodium transformans*, a potential biocontrol agent of *Tecoma stans* var. *stans* (Bignoniaceae) in South Africa

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ABSTRACT

Tecoma stans var. *stans*, a tree originating from the Americas, is emerging as an invasive weed in South Africa. A microcyclic gall inducing rust fungus, *Prospodium transformans*, has been investigated as a biological control agent against this plant. Development of germinating teliospores and symptoms on host plants are described, and the optimum temperature for teliospore germination was found to be 18–22°C. Inoculations of plants grown from seed originating from South Africa and various localities in Mexico and Texas (USA) indicate that there are likely races specific to morphological variants of this widespread and highly variable plant species. Despite readily inducing galls on plants grown under quarantine glasshouse conditions in South Africa, this rust fungus failed to establish in the field upon release. It is suggested that the origin of the form of this plant, which has become invasive in South Africa, needs to be identified to source the correct race of *P. transformans* for release in South Africa.

Key words: Pucciniales, Bignoniaceae, biological control, epidemiology, field releases, infection studies.

INTRODUCTION

Tecoma (Bignoniaceae) is a small genus, mainly occurring in the Neotropics but with two species in Africa (Gentry, 1992; Wood, 2008). The two African species are frequently placed in *Tecomaria* and may be considered subspecies of *T. capensis* (Thunb.) Spach. (Diniz, 1988). *Tecoma stans* (L.) Juss. ex Kunth var. *stans* is a small tree with a widespread natural distribution, occurring throughout Mesoamerica and the Caribbean, as well as much of South America (Gentry, 1992; Wood, 2008). Within this range it is morphologically variable, intergrading in places with three described varieties [*T. stans* var. *velutina* DC., *T. stans* var. *angustata* Rehder (Gentry, 1992) and *T. stans* var. *sambucifolia* (Kunth) J.R.I. Wood (Wood, 2008)].

Tecoma stans var. *stans* is widely naturalized in South Africa, where it invades natural as well as disturbed sites and is therefore a declared weed (Henderson, 2001). Although not yet regarded as a major weed, it is considered to have the potential to invade a large proportion of the country (Nel et al., 2004). It is increasing in abundance, and has been chosen as a target of a biological control programme aimed at preventing it from emerging as a weed of national importance (Olckers, 2004).

Prospodium (Pucciniales, Uropyxidaceae) is a Neotropical genus of about 70 species parasitizing numerous members of the Bignoniaceae, as well as eight species of the Verbenaceae (Carvalho & Hennen, 2010). One species, *P. tuberculatum* (Speg.) Arth., has been introduced into Australia for the biological control of *Lantana camara* L. (Verbenaceae) (Thomas et al., 2006), and another, *P. tumefaciens* Lind. has been proposed as a potential agent for use against *Aloysia gratissima* (Gill. et Hook.) Troncose (Verbenaceae) in the USA (Cordo & DeLoach, 1995).

Six *Prospodium* spp. have been recorded as occurring on *T. stans*, namely the macrocyclic *P. appendiculatum* (G. Winter) Arthur, the microcyclic *P. transformans* (Ellis & Everh.) Cummins and *P. elegans* (J. Schröt.) Cummins (Cummins, 1940), as well as the recently described *P. mexicana* A.A. Carvalho & J.F. Hennen (macrocyclic), *P. abortivum* (Cummins) A.A. Carvalho & J.F. Hennen and *P. aculeatum* (Cummins) A.A. Carvalho & J.F. Hennen (both with only uredinia and telia so far described) (Carvalho & Hennen, 2010). All may be considered as potential biological control agents for use against *T. stans* var. *stans* in South Africa.

In its native range (Caribbean Basin, Guatemala, and Mexico), *P. transformans* has only been recorded from *T. stans* var. *stans* and *T. stans* var. *velutina* (as *T. mollis* Humb., Bonpl. & Kunth) (Cummins, 1940). This rust fungus causes galls up to 3 cm in diameter on petioles, stems, and seed pods, on which initially spermagonia (=pycnia) then telia develop. These are the only two stages of this species' life cycle. The teliospores may germinate as soon as they develop. Two vesicle-like, modified basidiospores are produced by the germinating metabasidium (=promycelium) following germination of a teliospore. Karyogamy

followed by meiosis occurs in the developing metabasidia (Shuttleworth, 1953).

Based on field observations of the impact of galls on the host in the southern U.S.A. and Mexico, and its known limited host range, *P. transformans* was selected for testing as a biological control agent (Madire et al., 2011). Studies were undertaken to assess the suitability of introducing *P. transformans* into South Africa for the biological control of *T. stans* var. *stans*. Results reported here deal with various aspects of the biology of *P. transformans*. Host-specificity testing demonstrated that this rust fungus was highly host specific and safe for release (Wood, 2008). Following permission by the relevant authorities for release of this rust fungus, attempts to establish it in the field were initiated in November 2010 (Madire et al., 2011).

MATERIAL AND METHODS

Field observations

Rust fungi occurring on *T. stans* in various parts of its native range were collected on an *ad hoc* basis between 2002 and 2011. Specimens were placed between tissue paper in a plant press and dried. They were identified using the key provided by Carvalho & Hennen (2010).

Source and maintenance of cultures and inoculation of plants

Six isolates of *P. transformans* originally collected from Mexico (Table 1), were maintained in the quarantine facilities at ARC-Plant Protection Research Institute, Stellenbosch, South Africa, by repeated inoculation of potted *T. stans* var. *stans* plants. Plants were grown from seed collected in South Africa, as well as seed collected in Chiapas and Baja California Sur, Mexico, and Texas, USA. One of these isolates had been used in previous work [collected SW of Tuxtla Gutierrez, Chiapas, Mexico (Wood, 2008)]. The isolates were kept in separate glasshouses to prevent cross-contamination.

Plants were inoculated by dusting dry teliospores on the petioles and adaxial surfaces of leaflets (pinnae) of immature leaves (approximately half expanded) using a small paintbrush, spraying the leaflets with water using a hand held atomizer until very small droplets were visible to the naked eye, and then sealing the plants within a plastic bag. The inoculated plants were placed in an incubator at 18°C (Shuttleworth, 1953) or 22°C for 48 h and then transferred to quarantine glasshouses with a day/night temperature cycle of 25/20°C.

Teliospores were harvested from the galls that developed on inoculated plants by means of a vacuum pump. This was achieved by cutting a 2ml plastic syringe in half and discarding the back piece, placing a piece of filter paper over the cut end, and inserting this into a length of plastic tubing attached to the vacuum pump. Harvested teliospores were placed in plastic cryo-vials, and kept in a refrigerator (4°C) until used.

Optimal temperature for teliospore germination

Freshly collected teliospores of four different isolates were spread over the surface of water agar (WA, 1.5% *w:v*) in 6.5 cm diameter plastic Petri dishes. Initially three dishes of each isolate were incubated at each of 12, 16, 20, 25 and 30°C, and the percentage that had germinated of 100 randomly observed teliospores per dish was recorded. To determine more accurately the optimal temperature the same procedure was also done at each of 18, 20, 22, and 25°C again for each of four isolates.

Development of germinating spores

Freshly collected teliospores were dusted onto dry glass microscope slides, these were then sprayed with water using a hand held atomizer until very small droplets were visible to the naked eye, and then the slides were sealed in 9 cm diameter plastic Petri dishes. After incubation at 20°C for 2, 4, 6, 9, 12, 18 and 24 hours, the slides were dried carefully over a flame and the spores were mounted in aniline blue in lactophenol and observed with a Nikon E600 microscope.

Host-specificity testing

Limited host range testing was carried out on selected South African plants that are most closely related to *T. stans*, namely *T. capensis* (Thunb.) Lindl. and *Podranea ricasoliana* (Tanfani) Sprague (Olmstead et al., 2009), using two of the isolates that readily infected the invasive form of *T. stans* var. *stans* in South Africa. In addition *Chilopsis linearis* (Cav.) Sweet, *Campsis radicans* (L.) Bureau and *Tabebuia* sp., which had not been available for testing before, were also tested. Three plants of each test species were treated as described above with each of the two isolates and observed for gall development and sporulation for one month after inoculation. For every batch of plants inoculated, a plant of the South African form of *T. stans* var. *stans* was inoculated in the same manner. If sporulating galls did not develop on these check plants, then all plants were re-inoculated. Every plant was inoculated twice, the second time on shoots that had not previously been inoculated.

Field inoculations

Distal ends of actively growing branches of *T. stans* var. *stans* were inoculated as detailed above. These were carried out in late afternoon or early evening once the ambient air temperature fell below 24°C. Plastic bags were removed in the early morning. Inoculations were carried out at three sites in the Durban area (S 29°48'31", E 30°54'53"; S 29°50'08", E 30°51'42"; S 30°12'35", E 30°46'47"), five in the Nelspruit area (S 25°22'26", E 30°59'42"; S 25°30'30", E 30°55'15"; S 25°29'30", E 30°59'47"; S 25°28'34", E 31°00'13"; S 25°28'21", E 31°03'45"), and one in Pretoria (S 25°43"46", E 28°14'06"). Dates of inoculation were: Durban sites - 22 Nov. 2010, and 30-31 Mar. 2011; Nelspruit sites - 14-15 Dec. 2010, 21-22 Jan 2011, 12-13 Oct. 2011, and 8 Nov. 2011; and the Pretoria site - 19

TABLE 1 - Collection localities of *Prospodium* spp. on *Tecoma stans*, including collections maintained under quarantine conditions (marked with an asterix).

Date collected	Locality	Fungus[a]	Plant[b]
7 Oct 02	Near international airport, Guatemala City, Guatemala	Pt	Tss
*29 Nov 03	SW of Tuxtla Gutierrez, Chiapas, Mexico ; N 16°37', W 93°5'	Pt	Tss
Dec 03	Rio Piedras, Salta, Argentina; S 25°20'78", W 64°56'15.8"	Pap	Tss
5 Dec 03	Caimancito, Jujuy, Argentina; S 23°43'60.6", W 64°36'68.5"	Pap?	Tss
4 Sep 05	W of San Anton io, Baja California Sur, Mexico; N 23°47'44.3", W 110°8'55.6"	Pap?	Tss
10 Sep 05	Edinburg, Texas, USA; N 26°17'30.6", W 98°9'23.3"	Pab	Tss
10 Sep 05	W of Brownsville, Texas, USA; N 26°5'23.1", W 98°10'0"	Pab	Tss
15 Sep 05	W of Santia go, Baja California Sur, Mexico; N 23°26'6.3", W 109°45'9.3"	Pap?	Tss
15 Sep 05	W of San Bartolo, Baja California Sur, M exico; N 23°44'26.8", W 109°45'39.2"	Pap?	Tss
17 Sep 05	Key Lago, Florida, USA; N 25°9'59.7", W 80°22'43.1"	Pap?	Tss
18 Sep 05	Chokoloskee Island, Florida, USA; N 25°48'47.7", W 81°21'27.6"	Pap?	Tss
20 Sep 05	W of San Juan, Puerto Rico; N 18°25'5.9", W 66°14'4.2"	Pab	Tss
22 Sep 05	S of Arecibo, Puerto Rico; N 18°24' 13.6", W 66°41' 33.7"	Pab	Tss
*18 Aug 07	SE of Guadalajara, Jalisco, Mexico; N 20°24' 39.4", W 102°44'36.2"	Pt	Tsv
19 Aug 07	Guadalajara, Jalisco, Mexico; N 20º39'55.5", W 103º24'29.1"	Pt	Tss
*22 Aug 07	N of Zacapu a long Mex -15D, Michoacan, Mexico; N 19°58'4.2", W 101°42'35.2"	Pt	Tsv
*23 Aug 07	N of Chilpancingo, Guerrero, Mexico; N 17°49'16.1", W 99°27'26.5"	Pt	Tss
*24 Aug 07	E of Chilpancingo, Guerrero, Mexico; N 17°33'4.1", W 99°25'38.7	Pt	Tss
27 Aug 07	S of Jalpan, Queretaro, Mexico; N 20°53'35.6", W 99°42'30.6"	Pap	Tss
28 Aug 07	E of Jalpan, Queretaro, Mexico; N 21°10'22", W 99°19'39.4"	Pab	Tsv
3 Sep 07	near Huajuapan, Oaxaca, Mexico; N 17°46'20.8", W 97°47'30.4"	Pt	Tss
4 Oct 07	Santo Domingo, Dominican Republic	Pab?	Tss
7 Oct 08	E of Chilpancingo, Guerrero, Mexico; N 17°33'58", W 99° 46' 27.6"	Pap	Tss
7 Oct 08	W of Tlapa de Comonfort, Guerrero, Mexico; N 17°32'57.8", W 98°41'48.2"	Pap	Tss
11 Oct 08	E of Crucecita, Oaxaca, Mexico; N 15°55'0.6", W 95°48'40.7"	Pap	Tss
19 Oct 08	E of Tuxtla Gutierrez, Chiapas, Mexico; N 16°43'16", W 92°55'16.5"	Pap	Tss
12 Nov 11	SSW of Veracruz, Veracruz, Mexico ; N 18°50'27.1", W 96°22'17.4"	Pap	Tss
12 Nov 11	SE of Cordoba, Veracruz, Mexico ; N 18°47'30.2", W 96°34'12.3"	Pap	Tss
*15 Nov 11	N of Chapala, Jalisco, Mexico; N 20°19'48.1", W 103°11'0.9"	Pt	Tsv
15 Nov 11	S of Tepatit lan de Morelos, Jalisco, Mexico; N 20°37'34.3", W 103°5'48.1"	Pt	Tsv

[a]Pt, *Prospodium transformans*; Pap, *Prospodium appendiculatum*; Pab, *Prospodium abortivum;* ?, no teliospores present therefore identity not certain.
[b]Tss, *Tecoma stans* var. *stans*; Tsv, *Tecoma stans* var. *velutina*.

Jan. 2013. Twenty branches per site on each occasion were inoculated. A mixture of teliospores from SW of Tuxtla Gutierrez, N of Chilpancingo, and E of Chilpancingo (Table 1) were used for all inoculations.

RESULTS

Field observations

Collections of *Prospodium* spp. included *P. transformans*, *P. appendiculatum* and *P. abortivum* (Table 1; Figure 1). *Prospodium transformans* was observed to be damaging to plants at various locations, with numerous galls on individual trees. Both *P. appendiculatum* and *P. abortivum* were common at some localities but appeared to not cause much damage to their host plant as the uredinia and telia were predominantly on mature and older leaves. At all sites where they occurred, pycnial galls of *P. appendiculatum*, perhaps more damaging than the leaf uredinia and telia stages, were always in low numbers and, therefore, associated with little accumulative damage.

Prospodium appendiculatum was widespread, whereas *P. abortivum* was only collected in south-western Texas and the Caribbean, and *P. transformans* was only collected in south-central and southern Mexico and Guatemala. *Prospodium mexicana* known from Veracruz, Mexico, was not found, though several collections were made in that area.

Maintenance of cultures

Three of the six isolates originally from *T. stans* var. *velutina* (from Jalisco and Michoacan, Mexico) could not be maintained on *T. stans* var. *stans* plants originating from South Africa though they were readily maintained on plants originating from Baja California Sur or Texas. These isolates caused necrotic flecking or only produced low numbers of small galls on the South African form of *T. stans* var. *stans* (Figure 3G; Table 2). Three isolates originating from *T. stans* var. *stans* were readily maintained on South African plants. Two of these isolates readily produced galls on plants grown from seed originating from Baja California Sur (Figure 3F) and Texas (Figure 3E) but not from Chiapas. The remaining isolate readily produced galls on all plants but produced the largest (±3–5 cm diam.) and longest lived (>6 months) galls on plants originating from Chiapas as compared to all isolates and plant provenances tested.

Orange spermagonia developed on rapidly expanding galls approximately 12 days following inoculation and produced droplets of spermagonial fluid (Figure 2A; Figure 3A). These were subcuticular with a flat hymenium (Type 7). Induced cross fertilization of spermagonia by means of spermogonial fluid transfer with a fine artist paint brush was observed to enhance subsequent production of teliospores, however, teliospores were still produced from galls in the absence of induced cross fertilization. Teliospores were produced in abundance from galls on both leaves and stems approximately 18 days after inoculation (Figure 2B; Figure 3B-F). It was observed that growth of stems was typically prevented or greatly reduced following the development of galls on those stems, whereas galls on leaflets and petioles resulted in early leaf drop but did not reduce stem growth.

Development of germinating spores

Teliospores germinated at 12 and 25°C, though at very low percentages. The optimum temperature for germination was between 18 and 22°C (Figure 4). Initiation of germination was spread over several hours, and the following descriptions apply to the germinated teliospores that were most advanced in development for that time of incubation. Development occurred as follows: after 2 hr of incubation no metabasidia had begun to develop, but by 4 hr one-celled metabasidia had emerged from either one or both cells of the teliospores; at 6 hr of incubation a septum had developed relatively close to the teliospore, and by 9 hr a second septum had developed and two vesicle-like modified basidiospores had developed; by 12 hr of incubation the metabasidial cell contents had moved into these basidiospores and septa had formed at their bases. The basidiospores germinated immediately upon development, forming long germ tubes by 18hr (Figure 5). By 24 hr of incubation all germinated teliospores were fully developed (Figure 6).

Host range testing

No symptoms occurred after inoculation on any of the 5 species of the Bignoniaceae tested under greenhouse conditions, though the check plants of *T. stans* var. *stans* from South Africa consistently showed rust symptoms.

Field inoculations

Galls readily developed on leaves and sometimes stems of inoculated plants in the field, however on only one occasion (inoculated Dec. 2010) at one site in Nelspruit did teliospores develop. No further infection was observed on any plant at this site. No teliospores were seen on any other occasion (Figure 3H-J). Galls were produced at one of the three Durban sites from both inoculations; galls were produced at two of five Nelspruit sites from the Dec. 2010 and Nov. 2011 inoculations but no gall was seen from the Jan. 2011 inoculation. Galls were also produced at the Pretoria site.

DISCUSSION

Prospodium transformans is one of a complex of closely related species infecting *T. stans*, likely derived from the widespread macrocyclic *P. appendiculatum* (Cummins, 1940). *Prospodium transformans* was the first species of this genus to have its full life cycle proven by inoculation (Shuttleworth, 1953), and *P. bicolor* F.A. Ferreira & J.F. Hennen (Ferreira & Hennen, 1986) and *P. tuberculatum* (Ellison et al., 2006) are the only other species for which this has also been experimentally proven. Of the several species in the rust complex on *Tecoma* , *P. transformans* appeared to

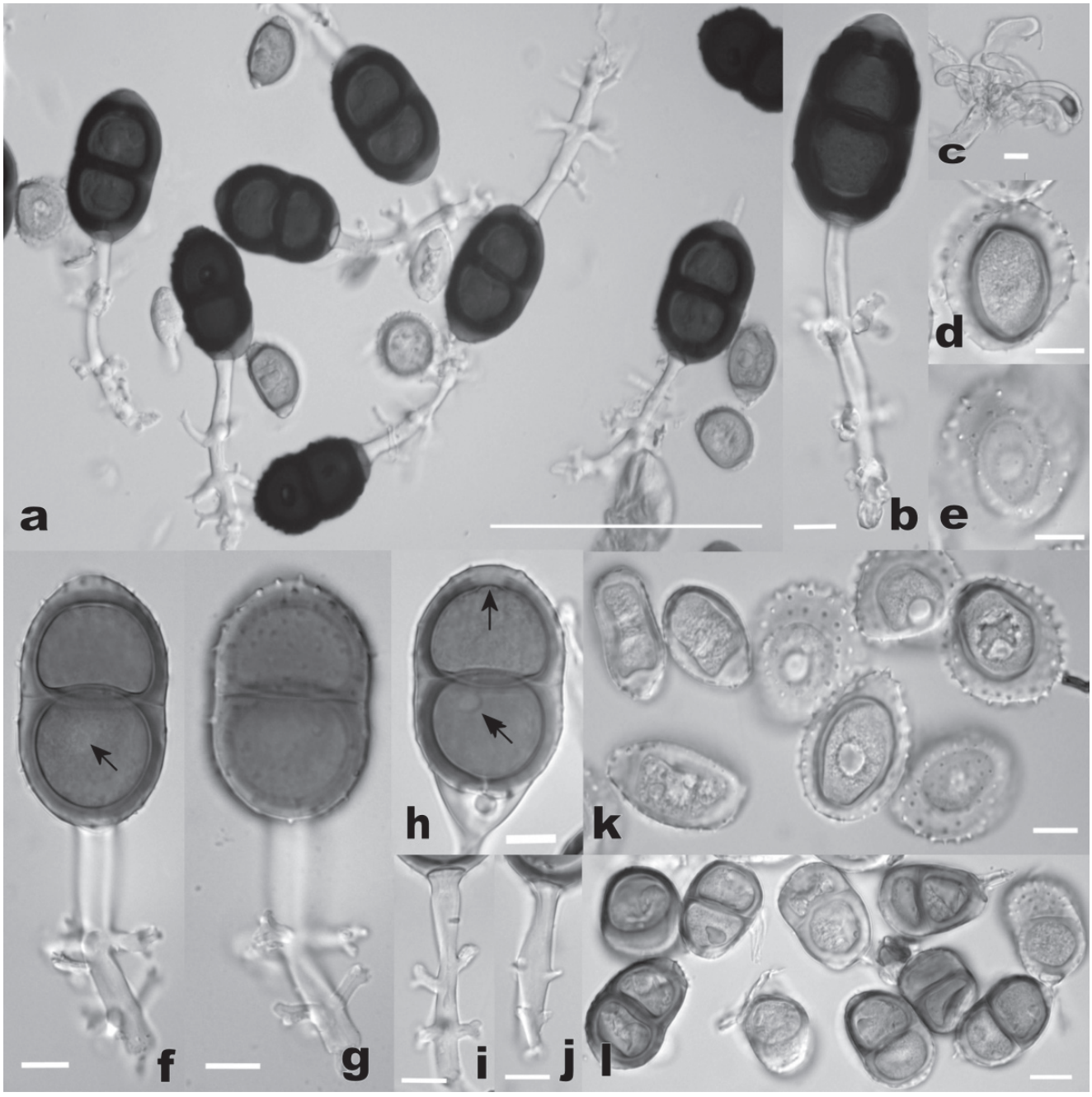

FIGURE 1 - *Prospodium appendiculatum*, *P. abortivum* and *P. transformans*. **A.** Teliospores and urediniospores of *P. appendiculatum*. Scale bar = 100µm; **B.** Teliospore of *P. appendiculatum*; **C.** Peripheral paraphyses from uredinia of *P. appendiculatum*; **D.** Face view of unicapitate urediniospore of *P. appendiculatum*; **E.** Ornamentation of a urediniospore of *P. appendiculatum*; **F-H.** Teliospores of *P. abortivum* (arrows showing position of germ pores); **I, J.** Teliospore pedicels of *P. abortivum* showing appendages; **K.** Urediniospores of *P. abortivum* in face and lateral view; **L.** Teliospores of *P. transformans*. **B-L.** Bar = 10 µm.

be the most damaging in the field. The optimum temperature for germination of teliospores and basidiospore development after germination of several isolates collected from south-west Mexico were found to be similar to that determined by Shuttleworth (1953) for a collection from Florida, USA. This study contributes to the sparse information available on the biology of tropical rust fungi.

Records suggested *P. transformans* is specific to only one species of plant (Cummins, 1940; Farr & Rossman, 2013). Development of infection and sporulation on the host, as well as host specificity testing of 21 plant species in the Lamiales using an isolate from Guatemala City, Guatemala, and one from SW of Tuxtla Gutierrez, Chiapas, Mexico confirmed that *P. transformans* is specific to *T. stans* (Wood,

TABLE 2 - Development of symptoms on different sources of *Tecoma stans* inoculated with five isolates of *Prospodium transformans*[*].

Test Plant	Isolate of *Prospodium transformans*[a]					
	Tuxtla Gutierrez	**Guadalajara**	**Zacapu**	**N of Chilpancingo**	**E of Chilpancingo**	**Chapala**
Tecoma stans ex South Africa	large galls[b]	flecks	small galls or flecks[c]	large galls[d]	large galls	no symptoms
Tecoma stans ex Chiapas	large galls	not tested	no symptoms	large galls	no symptoms	not tested
Tecoma stans ex Baja California Sur	large galls	not tested	large galls	large galls[e]	large galls	large galls
Tecoma stans ex Texas	not tested	not tested	large galls	large galls[f]	large galls	large galls

[a]SW of Tuxtla Gutierrez, Chiapas; SE of Guadalajara, Jalisco; N of Zacapu along Mex-15D, Michoacan; N of Chilpancingo, Guerrero; E of Chilpancingo, Guerrero; N of Chapala, Jalisco. See Table 1 for additional details.
[b]Flecks: necrotic flecks; small galls: leaflet galls less than 2 mm in diameter; large galls: leaflet galls usually greater than 3 mm in diameter and larger stem/petiole galls.
[c]See Figure 3G
[d]See Figure 3B-D
[e]See Figure 3F
[f]See Figure 3E
*Inoculations involving three individual plants of each origin inoculated twice with each isolate.

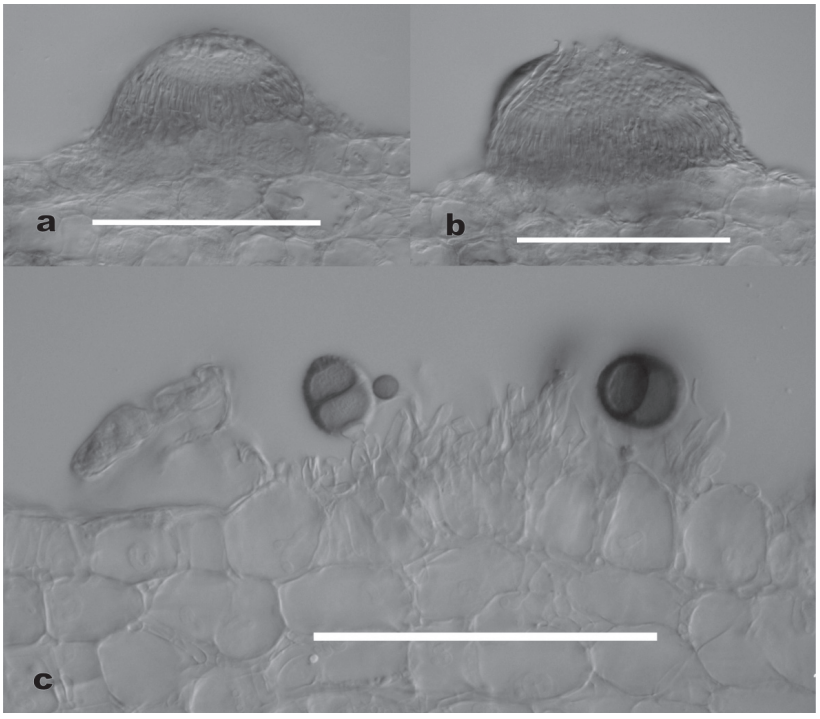

FIGURE 2 - Cross sections of spore structures on galls of *Prospodium transformans*. **A, B.** Spermagonia; **C.** Telium. Bar = 100µm.

2008). The results presented here further confirm this specificity and also indicate that there is variation within the species as to the specific biotypes of the host that individual isolates can infect. It is not yet determined if this variation deserves recognition at the infra-specific level. *Tecoma stans* is a widespread and variable species (Gentry, 1992; Wood, 2008). This plant and the complex of *Prospodium* species attacking it would make an ideal subject for studies on the co-evolution of hosts and pathogens.

Prospodium transformans, being both specific and damaging, remains an ideal biological control agent for use against *Tecoma stans* var. *stans*. However, the origin of the weed in South Africa needs to be determined, and if that population is attacked by *P. tansformans*, then isolates from that population should be released in South Africa. Specificity at lower than the host species level has been commonly observed during searches for suitable pathogens to use as biological control agents against alien invasive

FIGURE 3 - Infection symptoms and structures of *Prospodium transformans* on *Tecoma stans*. **A.** Spermagonia on young developing galls on leaflet of South African form grown in quarantine glasshouse; **B-D.** Galls producing teliospores on leaflets and stems of South African form grown in quarantine glasshouse; **E.** Gall producing teliospores on stem of Baja California Sur form grown in quarantine glasshouse; **F.** Gall producing teliospores on stem of Texas form grown in quarantine glasshouse; **G.** Necrotic flecks and small gall produced on leaflet of South African form grown in quarantine glasshouse; **H-J.** Non-sporulating gall on South African form growing in the field; **A-F.** Isolate from N of Chilpancingo, Guerrero; **G.** Isolate from N of Zacapu along Mex-15D, Michoacan.

plants originating from South and Central America (Morris et al., 1999).

The reason for the failure of the isolates used to establish in the field is unknown. One possible explanation is that the environmental conditions following the inoculations were not conducive for the survival of the fungus, for example the summer rainfall region in which the plant is invading experiences high daily temperatures during summer. Long term average of daily maximum temperature in January is 28–30°C for Nelspruit and 26–28 for Durban and Pretoria (Schulze, 1997). High day temperatures reduced the numbers of lesions and number of urediniospores produced by *Phakopsora pachyrrhizi* Syd. & P. Syd. on soybean (Bonde et al., 2013). However, in general, during our field trials, *P. transformans* did not produce teliospores, regardless of temperatures after inoculation. Shuttleworth (1953) suggested that *P. transformans* requires cross fertilization between spermagonia in order to produce teliospores, and it is possible that there were not sufficient insects present in the field to effect cross fertilization. Although cross fertilization by hand was not necessary prior to the development of teliospores in quarantine, it is possible that it occurred via small insects such as aphids. A further possible explanation is that the isolates of *P. transformans* used are not fully compatible under field conditions with the genotype of the weed present in South Africa. A similar situation was found for the rust fungus *Maravalia cryptostegia* (Vestergr.) Y. Ono, where a strain which initially proved pathogenic under quarantine glasshouse conditions performed poorly in the field and died out within a year, whereas another fully compatible strain subsequently released later has been highly successful as a biological control agent (Evans & Tomley, 1996). The last of these three possibilities is considered the most likely explanation.

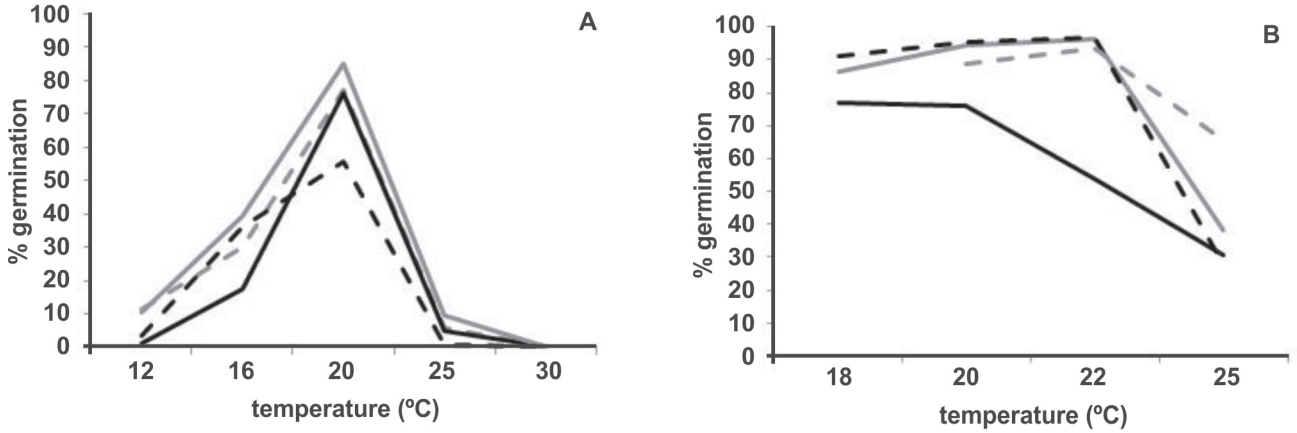

FIGURE 4 - Average percentage germination of teliospores of *Prospodium transformans* at different temperatures after 24 hours of incubation: **a.** at 12, 16, 20, 25 and 30°C; **b.** at 18, 20, 22 and 25°C. Each line represents one of four different isolates: SW of Tuxtla Gutierrez, Chiapas (solid black line); N of Zacapu along Mex-15D, Michoacan (dashed grey line); N of Chilpancingo, Guerrero (solid grey line); E of Chilpancingo, Guerrero (dashed black line). See Table 1 for additional details.

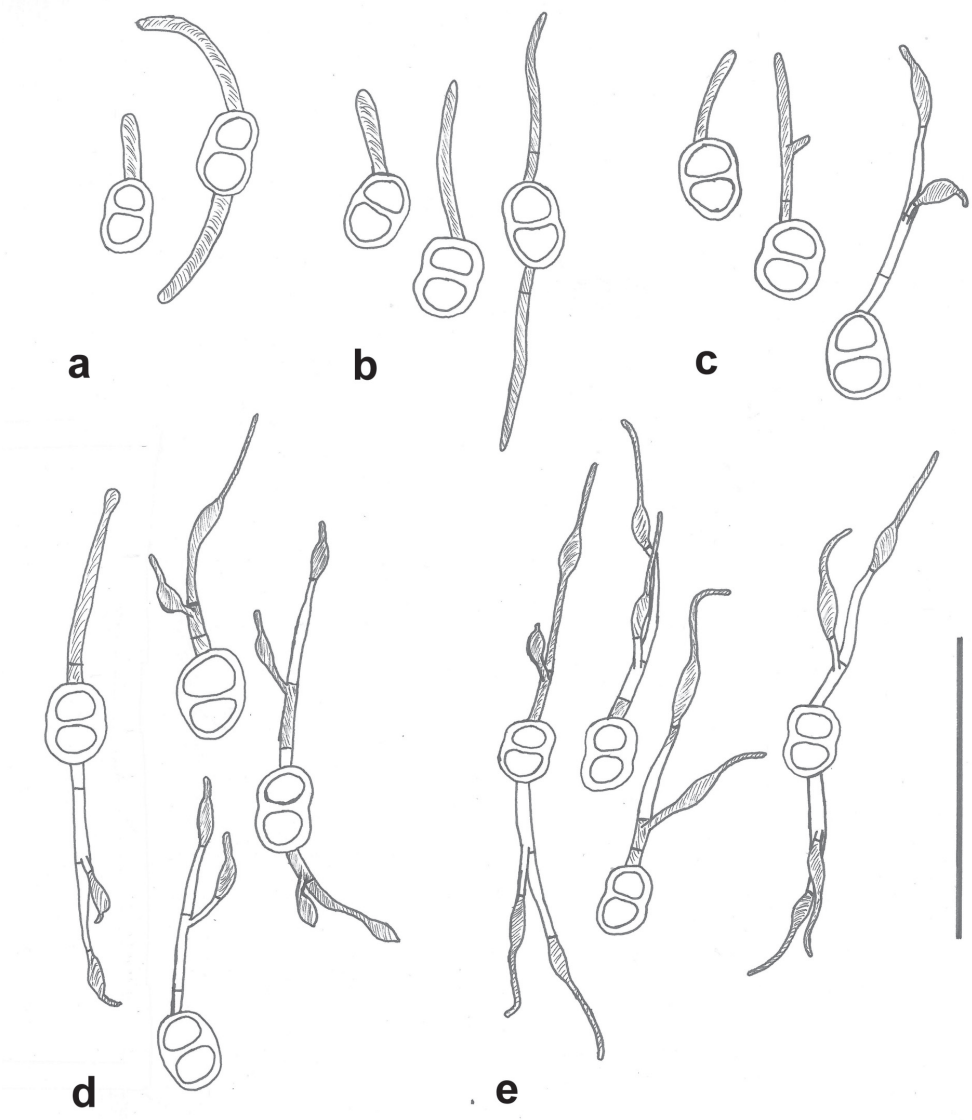

FIGURE 5 - Camera lucida drawings of germinating teliospores of *Prospodium transformans* at different time intervals incubated at 20°C. **A.** After 4 hours; **B.** 6 hours; **C.** 9 hours; **D.** 12 hours; **E.** 24 hours. Shading indicates cytoplasm. Bar = 100µm.

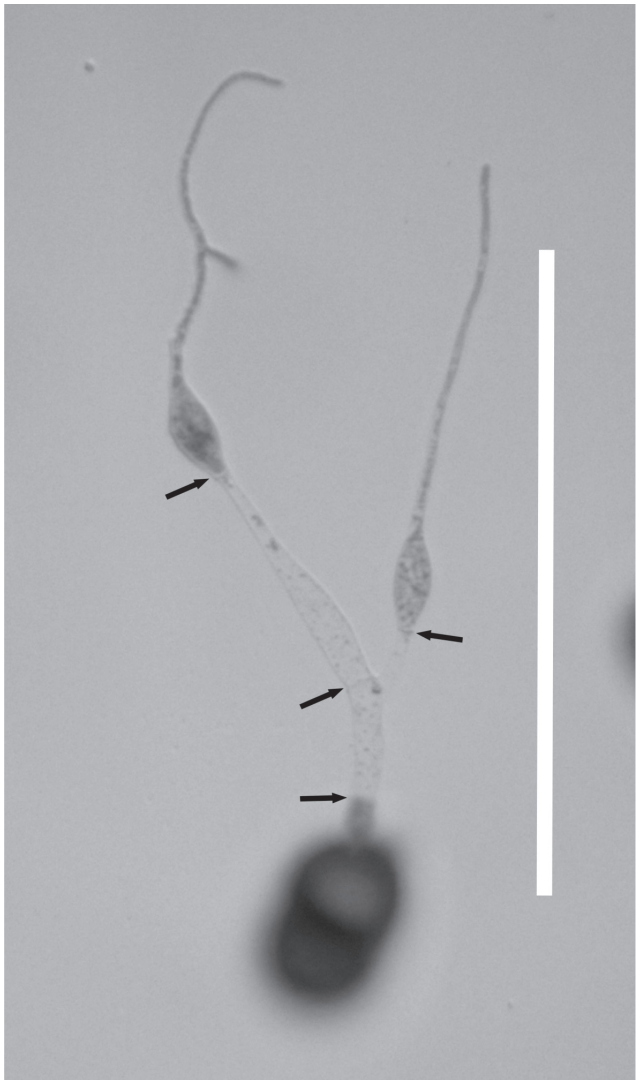

FIGURE 6 - Germinated teliospore of *Prospodium transformans* after incubation at 20°C for 24 hours showing two germinated modified vesicle-like basidiospores, position of septa (arrows) and concentration of cytoplasm in the basidiospores. Bar = 100 µm.

Information on the biology, particularly on the complete life cycle and epidemiological parameters for infection, efficacy and host specificity are the usual requirement for permission to release a potential biological control agent to be granted (Berner & Bruckart, 2005). The information provided here and in Wood (2008) indicates that *P. transformans* is suitable for release as a biological control agent and the search for fully compatible isolates should continue.

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