Mycoscience (2010) 51:149–153
DOI 10.1007/s10267-009-0022-2

SHORT COMMUNICATION

Uromycladium tepperianum, the gall rust fungus from *Falcataria moluccana* in Malaysia and Indonesia

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Received: 9 December 2008 / Accepted: 26 September 2009 / Published online: 22 January 2010

Abstract Batai (*Falcataria moluccana*) is a valuable tree species for forest plantations in Malaysia and Indonesia. Since 1993, a gall rust disease has caused severe damage to all growth stages, from seedlings in the nursery to mature trees in the field. To identify the fungus causing gall rust disease on *F. moluccana* in Malaysia and Indonesia, study of the mode of infection and changes in the anatomy of infected cells were carried out in the anatomy laboratory. The disease in Malaysia and Indonesia is caused by *Uromycladium tepperianum*. The fungus produces three longitudinally ridged teliospores on each head, with spores measuring 13–20 μm wide and 17–28 μm long. The fungus is microcyclic, completing its entire life cycle on *F. moluccana*. This study confirmed that the teliospores themselves cannot infect the host. Under favorable conditions, about 10 h after inoculation, teliospores germinate to produce basidiospores that form penetration pegs about 6 h later, and it is this peg which penetrates the host cells directly through the epidermis. Pycnia, recognized as small brown pustules, break through the epidermis about 7 days after inoculation.

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Keywords Batai · Fabaceae · Gall rust disease · Mode of infection · Pathogenicity test

Falcataria moluccana (Miq.) Barneby & J.W. Grimes (batai), Fabaceae (alt. Leguminosae), previously known as *Paraserianthes falcataria*, *Adenanthera falcataria*, *Albizia falcata*, *A. falcataria*, and *A. moluccana*, is a fast-growing tree frequently planted in the humid tropics (Wagner et al. 1999). This species is best known as a pulp crop (NAS 1979) but is also used for fiber and particleboard, packing cases, boxes, matches, chopsticks, light furniture, lightweight veneer, and plywood and composite products (CABI 2000). It is also suitable for wooden shoes, musical instruments, toys and novelties, and general turnery (Peh and Khoo 1984); it can also can be used for rehabilitation of degraded land or forest (Little and Skolmen 1989) and as a shade tree for coffee, tea, cacao, and cattle because of its favorable thin crown.

From 1990 until 1995, gall rust disease was a devastating disease of *F. moluccana* plantations in the Philippines (Braza 1997). In 1992, the disease spread in Sipitang on the west coast of Sabah, Malaysia (Lee 2004), and in late 1993 the disease had been reported on the east coast of Sabah (Rahayu 2007). In Indonesia, gall rust disease was also found sporadically in Samarinda, East Kalimantan; however, an epidemic outbreak was reported in the plantations at PT INCO Sorowako, South Celebes (Kasno and Hadi 2005). Since 2004, an epidemic outbreak was also initiated in Java Island, including the entire province of East Java; it continued to spread in central Java and is presently spreading in West Java (Rahayu 2008). During spreading of the disease in East Java, in 2006 gall rust disease also observed widely in Bali island, particularly in West Bali, where it has been found at Bangli district

(Rahayu 2008). From another side, in 1997 the disease also occasionally spread in Seram island, Moluccas, and in 1999 an epidemic outbreak was observed in East Timor throughout the coffee-growing districts of the country where *F. moluccana* is planted as shade trees for the coffee plants (Old and Cristovao 2003).

The disease causes severe damage to all developmental stages of the plant, from seedlings in the nursery to mature trees in the field. However, identification of the fungus causing the gall rust disease of *F. moluccana* has been inconsistent. For example, in Mindanao, the Philippines, Franje et al. (1993) found three distinct stages of *Uromycladium* on batai, i.e., pycniospores, urediniospores, and teliospores. The teliospores germinated, but the infecting unit observed was the urediniospore. Lee (2004) mentioned that the rust fungus forming galls on *F. moluccana* in Sipitang, Sabah, was most likely *Uromycladium tepperianum* (Sacc.) McAlpine. However, the fungus causing gall rust disease of *F. moluccana* in Malaysia and Indonesia has not been previously properly identified or studied. Therefore, this study was conducted to confirm the identification of the pathogen causing gall rust disease on *F. moluccana*, particularly in Sabah, Malaysia, and Java Island, Indonesia. Because infection by *Uromycladium* produces different results on different hosts and the reaction of a particular host species may even differ in different habitats (Burgess 1934), published information on the gall rust symptom was consulted and histopathological studies were also carried out.

To identify and characterize the rust fungus, galls were collected from seven sites in Sabah, included seven infected plantations and two sites in infected nurseries (Table 1). At each site five galls were collected from each of ten trees or seedlings. Young galls were preferred to obtain fresh, viable, uncontaminated spores. To get the best results, samples were examined within 2 days after collection. The samples represented the general distribution of infection in the areas studied. Galls were collected five times for examination in October 2003, February, April, and May 2004, and March 2005 to ascertain the consistency of the pathogen causing the galls. However, in Indonesia galls were collected from four sites in East Java and three sites in Central Java (Table 1). At each site three galls were collected from each of ten trees and sampling was carried out twice, in October 2006 and December 2007.

Spores were used for gall rust fungus identification. A portion of the spore-covered surface of the gall was removed and examined under a transmitted light microscope (Leitzu DMRB; Leica, Nussloch, Germany). The length and width of 50–100 spores from each gall were measured. Spore ornamentation was also noted, and images were acquired using a Leitz DMRB camera. Artificial inoculation on the *F. moluccana* seedlings utilized fresh spores. However, because rust fungi easily form pathogenic races, spores for inoculation were obtained from galls of a single infected *F. moluccana* seedling in the nursery, thereby ensuring that the inoculum originated from only one race. Teliospores were collected by scraping the galls with a flame-sterilized scalpel, and the teliospores were then suspended in sterile distilled water containing Tween 20 (0.2 ml/l).

Six-week-old healthy *F. moluccana* seedlings were used as experimental units. Inoculum density of the first, second, and third inoculation were 23.4×10^6, 13.7×10^6 and 14×10^6 teliospores/ml, respectively. At each time of inoculation, 5 ml of the spore suspension was applied onto each seedling, with 3 ml being dripped onto the stem of the seedling from the terminal shoot tip using a pipette and 2 ml sprayed over the entire seedling using a spray bottle.

Table 1 *Falcataria moluccana* collection sites for gall rust fungus identification

Region, country	Location/site/district	Description
Sabah, Malaysia	Site 87 E	Planted forest, 2-year-old trees
	Site 106 B	Planted forest, 3-year-old trees
	Site 69 F	Planted forest, 4-year-old trees
	Site 109 A	Planted forest, 5-year-old trees
	Site 94 B	Planted forest, 6-year-old trees
	Site 96 J	Planted forest, 7-year-old trees
	Site 80 C	Seedling Seed Orchard (SSO), 15–year-old trees
	Nursery 1	Seedlings, 1–3 months old
	Nursery 2	Overgrown seedlings, 3–6 months old
East Java, Indonesia	Jember, Probolinggo	Community forest with trees 1–5 years old
	Lumajang	Agro forestry system with trees 3–5 years old
	Kediri	Planted forest, 3-year-old trees
Central Java, Indonesia	Temanggung, Kebumen	Community forest with trees 1–3 years old
	Bojonegoro	Community forest with trees 1–5 years old

All seedlings were maintained in the nursery and watered twice a day, up to field capacity.

To ascertain the infection point, infection process, and subsequent development of the gall, observations began as soon as the seedlings were inoculated, by making sequential sections of the infected tissue. Transverse sections 25–35 μm thick were made of the stems of seedlings with fresh gall rust symptoms. The sections were stained with 0.25% lactophenol cotton blue prepared in tap water, a differential stain for hyphae. The stain was left for approximately 2 min and sections were then floated in water for approximately 1 min to remove excess stain. As a basis for comparison, and to help with the identification of tissues in the gall, sections of uninfected stems were also made. The general morphology of the galls on the stems of infected plants was examined using a compound light microscope (Leica MZ8) whereas stem sections were examined using a transmitted light microscope (Leitz DMRB).

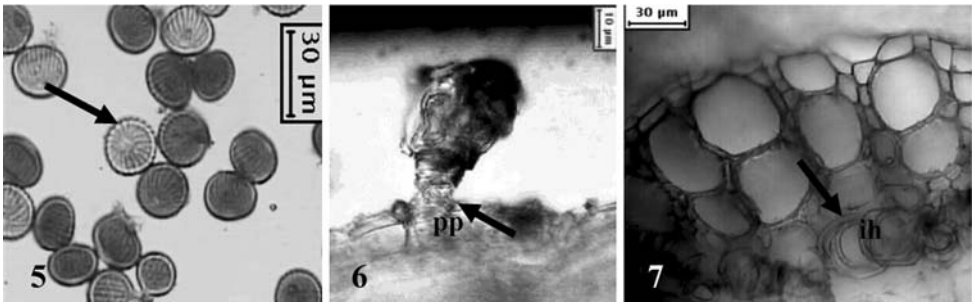
The most characteristic early symptom of infected seedlings, both in Sabah, Malaysia and Java, Indonesia, was a stiff, slightly bent stem or shoot (Fig. 1), with or without a dark red necrotic lesion. This symptom has not been mentioned before, probably because it is indistinct and easily overlooked. Subsequently, a white stripe (Fig. 2) with white pustules or a reddish-brown necrotic lesion formed on the stem. At this stage, teliospores may or may not be produced on the stem surface. Depending on the response of individual seedlings to the gall rust fungus, pustules or other infected cells may develop further galls. Gall size, number of galls, and time to maturity varied, depending on the interaction of the gall rust fungus, host response, and environmental conditions. The disease developed rapidly in infected seedlings transferred from the nursery to the field, probably because of favorable environmental conditions. Diseased *F. moluccana* trees in Tawau, Sabah, Malaysia and in Java, Indonesia produced symptoms similar to those reported elsewhere (Franje et al. 1993; Braza 1997; Old and Cristovao 2003). The range of symptoms included development of large chocolate-brown, irregularly shaped, cauliflower-like or whip-like galls on the stem (Fig. 3), branch, petiole, shoot, pod, seed (Fig. 4), or flower stalk. Cinnamon-colored spores generally covered the surface of mature galls. As the galls age, they become reddish brown, eventually blackening, and are frequently invaded by tunneling insects.

Susceptible trees may bear hundreds of branch galls, which affect the tree form as a consequence of rust-induced repeated branching. The infected terminal foliage can die from girdling of the shoot, and severely infected trees may die. Generally, most galls are found on the smaller branches of the canopy, and these would, presumably, have little effect on tree growth. However, because rust spores are wind dispersed, they are potential inocula for infecting seedlings or young trees located nearby. In addition, the sight of many galls hanging on the trees is not aesthetically pleasing, and heavily infested branches are prone to frequent wind damage and breaking.

Figs. 1–4 Gall rust disease symptom. **1** Early symptom shown as stiff, slightly bent stem or shoot. **2** White stripe on the infected stem surface. **3** *Chocolate-brown* galls on stem of young tree. **4** Mature gall on seed pods covered with *cinnamon-colored* spores

Based on the results of identification, the fungus causing gall rust of *F. moluccana* possesses teliospores with distinct ridged longitudinal striations (Fig. 5), with three spores on each head. Teliospores from Malaysian collections were 13–18 μm wide and 17–26 μm long whereas those from Indonesia were 14–20 μm wide and 17–28 μm long. These dimensions concur with the teliospores of *Uromycladium tepperianum* reported from *Acacia* (14–17 μm wide and 18–25 μm long) (McAlpine 1906). Therefore, the pathogen causing gall rust disease of *F. moluccana* in Sabah, Malaysia and Java, Indonesia is the fungus *Uromycladium tepperianum* (Sacc.) McAlpine (family Pileolariaceae, order Pucciniales, class Pucciniomycetes).

During infection of *F. moluccana*, the landing teliospore germinates to produce a basidiospore on the host surface. Under favorable conditions, high relative humidity (RH ≥90%), the basidiospore forms about 10 h after inoculation, and 6 h later forms a penetration peg, which penetrates the host cells directly through the epidermis (Fig. 6) (Rahayu 2007). Teliospores of other microcyclic and



Figs. 5–7 Teliospores. **5** Teliospores with ridged longitudinal striations. **6** Mature basidiospore with penetration peg (*pp*) directly penetrating the epidermis. **7** Intracellular haustoria (*ih*) in the xylem of infected cells in the stem

endocyclic rusts do not produce basidiospores on germination; rather, branches or vesicles at the end of the germ tube take over the role of host penetration (Hodges and Gardner 1984; Morris 1987). However, in *Acacia saligna*, the tree is infected through natural openings such as lenticels and stomata (Serdani 2001). According to Burgess (1934), Dick (1985), and Morris (1987), the germ tube of a *U. tepperianum* teliospore is capable of direct penetration of the host by the formation of a penetration peg. Our results are the first to show that the penetration peg in *U. tepperianum* develops from a basidiospore that germinates from the landing teliospore.

After successful penetration through the epidermis, the hyphae and mycelia of the fungus spread inter- and intracellularly. As a result, the periderm and phloem cells become misshapen while xylem cells become twisted or die. Sometimes giant cells were present and surrounded by well-developed vascular bundles. Hence, there was also formation of circular vessels in the parenchyma. Generally, parenchyma cells become larger, or become highly organized in tissue differentiation, resulting in gall formation. In addition, the fungus also forms intracellular haustoria in the xylem (Fig. 7) and may develop into haustorial mother cells (hmc).

About 7 days after inoculation, pycnia are formed. These are small brown pustules that eventually erupt from under the epidermis. The dark brown pycnia are scattered over the galls and swellings or on the green stem and bear telia that produce the teliospores. No urediniospores were observed throughout the study. Franje et al. (1993) reported both urediniospores and teliospores of a *Uromycladium* causing gall rust disease on *F. moluccana* in Mindanao, the Philippines. However, it is not known if the rust was *U. tepperianum*.

As the gall rust fungus is an obligate parasite, development of fungus haustoria will stop when the *F. moluccana* host cells die. A similar pattern is displayed by some colonies of *Puccinia graminis* var. *tritici* race C17 (56), which stop growing about 60 h after inoculation on wheat cultivars carrying the Sr6 gene, an intermediate resistance gene. However, on wheat cultivars without the resistance gene, the fungus continues to grow until it produces pustules (Skipp and Samborski 1974). To acquire supporting information for a gall rust disease resistance program in the future, the mechanism of haustorium development in *F. moluccana* seedlings from various seed sources needs to be further investigated.

As sporulation of this fungus was observed at 7 days after infection, the interval between infection and sporulation is rather short. Consequently, (i) fresh teliospores are always abundant as a source of inoculum, and (ii) the infection process can occur at any time under favorable conditions (Rahayu 2007). This situation results in the gall rust disease of *F. moluccana* becoming severe over a wide area whether in the nursery or in the field. In addition, because *F. moluccana* seedlings are only transferred to the field at the age of about 2 months, serious losses of seedlings in the nursery can result. Consequently, more intensive disease management must be undertaken in the nursery for the control of gall rust disease of *F. moluccana.*

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